

**Effects of age on reproduction and chick rearing in bridled
terns (*Onychoprion anaethetus*) at Penguin Island, Western
Australia**

This thesis is presented for the degree of
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Submitted by

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Declaration

I declare that this thesis is my own account of my research and contains as its main content work that has not previously been submitted for a degree at any tertiary education institution

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Abstract

Effects of age on reproduction in seabirds have been widely documented and need to be taken into account when devising management strategies. The purpose of this research was to: 1) establish a general context by determining which age-related processes affect reproduction in seabirds; and 2) in a specific case of a population which has undergone exponential growth associated with changes in oceanic conditions and increased food supply, determine whether age has an effect on the reproduction of bridled terns *Onychoprion anaethetus* at the Penguin Island colony in Western Australia. To investigate the latter, I attempted to use a molecular method for aging bridled terns. To determine whether age affected breeding in bridled terns, I used two different approaches: one was based on relating the age of breeding pairs to several breeding variables using cross-sectional and longitudinal analyses, and one was based on determining whether there were differences in the diet fed to chicks by parents of different ages using faecal DNA meta-barcoding techniques. I found that effects of age on the reproduction of seabirds were wide-spread and that breeding experience and breeding senescence were the main processes driving these effects. I found that it was not possible to age bridled terns using skin collagen-bound pentosidine as a bio-marker, so I had to use banding information instead. There did not appear to be an effect of age on the post-laying breeding performance in bridled terns and there was no clear difference in the diet of chicks from younger and older parents. Such findings suggest that the only effect of age on reproduction in this species occurs pre-laying because younger birds did not lay eggs, and that the favourable environmental conditions in Western Australia and/or unidentified physiological attributes negate effects of aging post-laying. However, small sample sizes and possible bias in aging birds were major limitations. My research suggests that bridled terns may be differently impacted by aging compared to other seabird species.

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To my beloved bridled terns: I would not have continued with this project if it was not for these endearing birds. Waiting on the jetty every spring night and seeing the first birds come back from migration kept me wide awake with anticipation. Seeing fledglings take their first flights and crashing into bushes gave me hope that one day I might be able to submit this thesis. Seeing parents depart with their fledglings to travel to warmer seas brought the promise that one day, after graduation, I would also go on holidays. But like bridled terns, I will always come back to Penguin Island, and I promise my beloved birds that will work hard to protect their home so they can return every spring.

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Overview of manuscripts

Two chapters of this thesis are under review and three are being submitted to journals. I undertook the main role in each manuscript, the co-authors participated by critically reviewing the manuscripts and Michael Calver provided assistance with the statistical analyses.

The manuscripts below form the basis for parts of this thesis:

1. Under review (Marine Ecology Progress Series): Aurélie M. T. Labbé, James N. Dunlop, Michael Calver, Jill M. Shephard, Joseph B. Fontaine, Mike van Keulen. Age effects on breeding in seabirds – a review. (Chapter 2)
2. To be submitted (Marine Ornithology): Aurélie M. T. Labbé, Anna T. Le Souëf, James N. Dunlop, Michael Calver, Jill M. Shephard, Mike van Keulen. Skin pentosidine does not correlate with age in a long-lived seabird. (Chapter 4)
3. To be submitted (Marine Ornithology): Aurélie M.T. Labbé, James N. Dunlop, Michael Calver, Jill M. Shephard, Mike van Keulen. Effects of age on the reproduction of a long-lived tropical seabird: the bridled tern. (Chapter 5)
4. To be submitted (Molecular Ecology): Aurélie M T Labbé, James N Dunlop, Michael Calver, Jill M Shephard, Mike van Keulen. Diet analysis using faecal DNA metabarcoding techniques in seabird chicks. (Chapter 6)
5. In press (Journal of the Royal Society of Western Australia): A M T Labbé, J N Dunlop, M Calver, J M Shephard, M van Keulen. Restoration of native vegetation and re-introduction of *Malva preissiana* on Penguin Island – preliminary findings

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1. Introduction

1.1 The ecological significance of seabirds

In recent years, the state of the world's oceans has become a major concern as the effects of pollution, over-fishing and climate change have become noticeable, particularly on marine megafauna populations (Hooker and Gerber, 2004; Lewison *et al.*, 2014). Marine megafauna includes higher marine predators that range from cetaceans to polar bears and from sharks to seabirds (Hooker and Gerber, 2004; Sleeman *et al.*, 2007). Recent studies have shown that health and population size of large predators are crucial elements of ocean condition evaluation (Croxall *et al.*, 1984; Hooker and Gerber, 2004; Lewison *et al.*, 2014). The loss of marine megafauna appears to accompany significant changes in ecosystem functions and processes and thus can serve as an indicator of marine ecosystem disturbance and degradation (Lewison *et al.*, 2014). Therefore, marine megafauna are being used as indicators of the state of the marine environment (Furness *et al.*, 1993; Sleeman *et al.*, 2007; Lewison *et al.*, 2014).

Seabirds rely on the marine environment to survive so they display extreme life history characteristics reflecting evolutionary adaptations to a saline environment, and do not rely on freshwater sources to live (Schreiber and Burger, 2002; Taylor and Friesen, 2012). Some species travel great distances to feed and reproduce, and their spatial distribution encompasses large areas, sometimes across hemispheres (Croxall *et al.*, 1984; Schreiber and Burger, 2002). Seabirds are long-lived iteroparous animals (Clutton-Brock, 1984; Schreiber and Burger, 2002); when not breeding, they spend most of their time foraging and travelling (Schreiber and Burger, 2002). However, over 93% of seabird species breed in colonies so their breeding grounds and the areas where they forage are spatially separated in space and sometimes in time (Orians and Pearson, 1979; del Hoyo *et al.*, 1992; Schreiber and Burger, 2002). Yet despite a lack of inter-species spatial segregation, different species of seabirds show great degrees of speciation and divergence (Taylor and Friesen, 2012). For these reasons, seabirds have been the subject of research on evolution, ecology and conservation in order to refine conservation guidelines on a species basis since not all species of seabirds have the same requirements for survival (Taylor and Friesen, 2012).

1.2 The tern family

The tern family (order Charadriiformes, family Sternidae), is a group of long-distance migratory seabirds (with a few exceptions such as the crested terns, *Thalasseus bergii*, which disperses outside of its breeding season but does not migrate) that has a world-wide distribution, from polar to tropical habitats (Gochfeld and Burger, 1996). One of the longest annual migratory routes for vertebrates was recorded in the Arctic tern (*Sterna paradiseae*) with a return journey of over 80,000 km between its breeding grounds in the boreal zone of Greenland to the Southern Ocean (Egevang *et al.*, 2010). Terns can have a lifespan of several decades (Gochfeld and Burger, 1996); for example, a 25-year-old common tern (*Sterna hirundo*) is still being monitored in a colony in the German North Sea (Zhang *et al.*, 2015). Generally, terns nest in colonies and form monogamous pairs that breed annually (Gochfeld and Burger, 1996). They rely on coastal and marine food sources to live, and although they have webbed feet, they do not swim (Gochfeld and Burger, 1996). Such life history traits and evolutionary features make terns valuable indicators of the vast spatio-temporal environment in which they live.

1.3 Bridled terns

In the tern family, bridled terns (*Onychoprion anaethetus*, previously *Sterna anaethetus*, Bridge *et al.*, 2005) are medium-sized (average body mass of 140g) tropical to sub-tropical migratory seabirds that have a worldwide distribution (Figure 1.1; Haney, 1986; Gochfeld and Burger, 1996). They have adapted to feeding over oligotrophic waters that have low salinity and low productivity (Dunlop and Rippey, 1998). They generally feed and rest over floating *Sargassum* and macro-algal reefs where small fish and other marine organisms find shelter (Haney, 1986; Dunlop and Rippey, 1998). Hence, they do not rely on areas of high marine productivity such as up-welling zones to feed and instead forage in areas of lower productivity, thereby avoiding inter-species competition (Dunlop, 1997; Dunlop, 2009). Because of their high global population and extensive distribution, they are listed as a species of Least Concern in the IUCN Red List of Threatened Species. Therefore, bridled terns are marine birds that are benefitting from climate change as warmer sea temperatures provide favourable conditions for the expansion of their feeding and breeding grounds, as seen in Western Australia (Dunlop, 2009).



Figure 1.1 Bridled tern (*Onychoprion anaethetus*) with band, guarding nest tube and its egg on Penguin Island, Western Australia.

1.4 Particularities of oceanographic conditions in Western Australia and implications for bridled terns

In Western Australia, many tropical and sub-tropical marine organisms are able to live further south than their usual range in other parts of the southern hemisphere thanks to unusual oceanographic features and climate change (Hutchins and Pearce, 1994; Waite *et al.*, 2007; Dunlop, 2009). The Leeuwin Current is a warm oligotrophic current that flows southwards along the coast of Western Australia and suppresses the upwelling of the colder and nutrient-rich West Australian Current along the coast (Godfrey and Ridgway, 1985; Pearce and Phillips, 1988). Because the Leeuwin Current takes its source from the Indonesian Through-Flow, it is more dependent on the Southern Oscillation Index than the Indian Ocean Dipole (IOD) (Pearce and Phillips, 1988). Therefore, the current is less strong during El Niño but still allows the dispersion and survival of tropical marine organisms in south Western Australia, so that even hard coral reefs are firmly established in the Houtman Abrolhos Islands located at 28°S (Pearce and Phillips, 1988; Pearce and Walker, 1991). Moreover, there has been a

significant increase in continental shelf sea surface temperatures (SST) over the past decades, brought about by climate change (Pearce and Feng, 2007). Such unusual oceanographic conditions provide an opportunity for tropical and sub-tropical marine organisms to live on the western coast of Australia, which contrasts with other highly productive ecosystem on the western coasts of Africa and South America; this makes Western Australia unique in the southern hemisphere (Pearce and Phillips, 1988; Dunlop, 2009).

Together with other tropical seabird species, bridled terns have expanded their breeding range southwards (Dunlop, 2009; Dunlop and Surman, 2012). Dunlop (2009) demonstrated the rapid growth of newly established colonies of tropical seabird species, including red-tailed tropic birds (*Phaethon rubricauda*) and sooty terns (*Onychoprion fuscatus*), at frontier colonies in Western Australia over the past 80 years. The redistribution of tropical seabirds in this region is linked with broad-scale changes in marine productivity and areas of prey availability so that newly established colonies consist of young recruits that did not return to their northern natal colonies and instead chose to breed on coastal islands further south where competition for nesting spots is probably lower (Dunlop, 2009). Therefore, changes in the unique oceanographic conditions in Western Australia are reflected in tropical seabird population expansions, and bridled terns are a distinctive example of this. Indeed, in 1843 bridled terns were not recorded breeding further south than the Houtman Abrolhos, but in 1889 breeding pairs were recorded on Rottnest island (420 km away from the Abrolhos Islands) and in 1956 they were observed on the islands at Cape Leeuwin (Figure 1.2; Dunlop, 2009). Finally, in 2007 bridled terns were recorded breeding on Termination Island in the Recherche Archipelago (Dunlop and Surman, 2012). This represents a range extension of about 1,400 km in just over 160 years, but with a significant acceleration over the past three decades, for a species of seabird which has a wingspan of less than one meter (Figure 1.2).

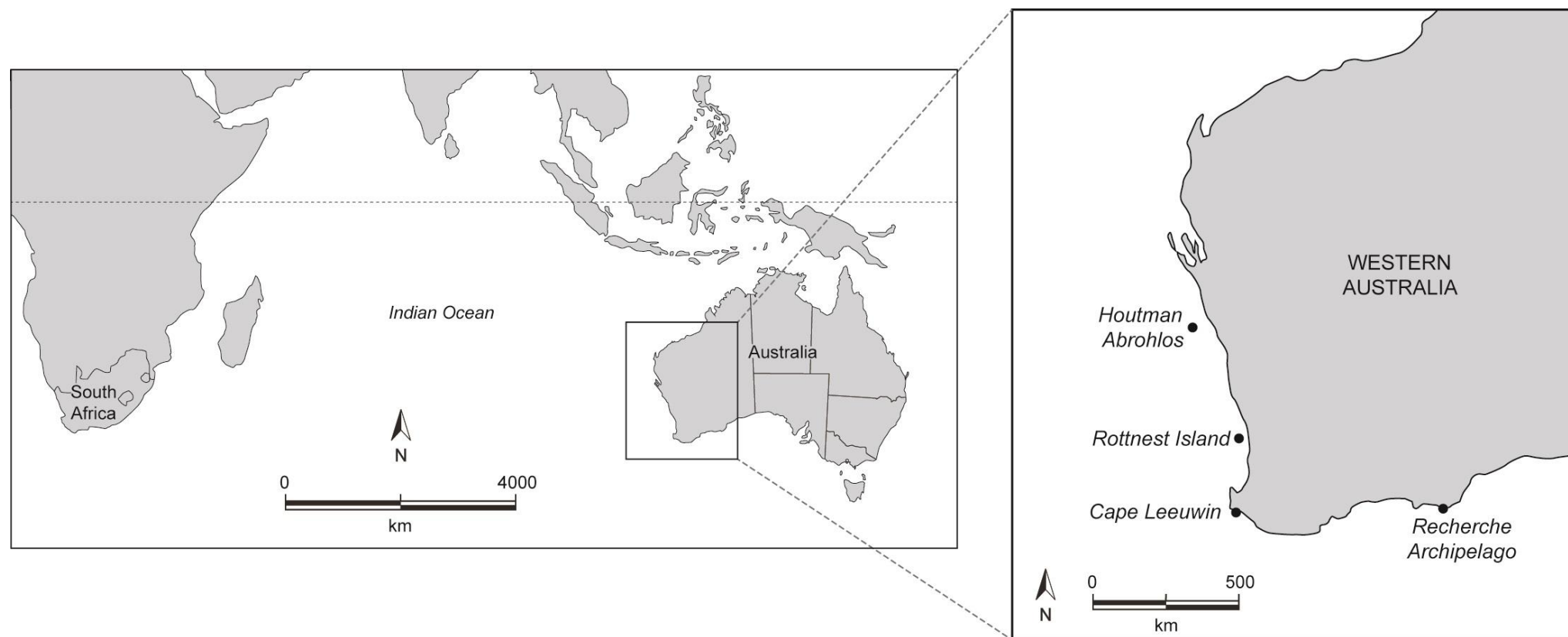


Figure 1.2 Breeding range extension of the bridled tern (*Onychoprion anaethetus*) in Western Australia between 1843 and 2007, from the Houtman Abrolhos islands to the Recherche Archipelago.

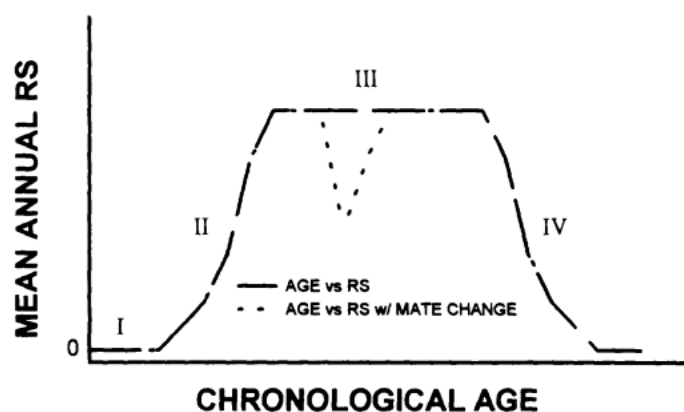
1.5 Bridled terns on Penguin Island

The population of bridled terns breeding on Penguin Island (WGS84 32° 18' 19" S, 115° 41' 28" E) has undergone particularly drastic transitions (Dunlop, 2009; Dunlop and Surman, 2012). The first breeding pairs settled between 1940 and 1942 and the population underwent an exponential growth which was particularly marked during the 1980s when the population grew from 200-300 pairs to 1,000-2,000 pairs and again to 3,000-4,000 pairs in summer 2006/2007 (Garavanta and Wooller, 2000; Dunlop, 2009). The first pairs of bridled terns nested on the rocky limestone cliffs on the north-eastern and southern ends of Penguin Island (Dunlop and Jenkins, 1994; Dunlop, 2009). Today, the whole island's grounds are used by bridled terns during the breeding season, including highly-vegetated areas as well as human constructions (Dunlop, 2009). Over the past three decades, egg-laying appears to occur progressively later during the year which is thought to be in response to a shift in the seasonal peak of sea surface temperature in the waters surrounding Penguin Island (Dunlop, 2012). Furthermore, this population of bridled terns seems to have reached its population density plateau as there is virtually no nesting space left for young breeding birds to recruit (Dunlop, 2009). Hence, the bridled tern population on Penguin Island is among the largest recorded in the world (Dunlop, 2009).

1.6 Bridled terns as a case study

The colony of bridled terns breeding on Penguin Island has now been studied for over 30 years with an emphasis on the species' diet, increasing population size and biology (Dunlop and Jenkins, 1992; Dunlop and Jenkins, 1994; Dunlop, 1997; Garavanta and Wooller, 2000). The results of those studies most likely reflect changes in oceanographic and ecological conditions surrounding Penguin Island, as discussed above, but they may also reflect changes in the species' population demography since this colony has been established for over 70 years and appears to have gone through an extensive demographic transition (Dunlop, 2009). This demographic transition could have a major impact on feeding and breeding variables recorded in this particular population because it has been shown that in seabirds, older birds become better breeders than younger birds through the process of ontogenesis (see Chapter 2).

The effect of age-related processes on breeding parameters has been demonstrated in 42 seabird species (Chapter 2). In most species, there appears to be an improvement in breeding performance following recruitment in a breeding colony and a pair's first breeding attempts (Figure 1.3; Fowler, 1995; Bradley and Safran, 2014). Breeding performance then reaches a plateau of peak performance in middle ages before declining in birds of older ages through a process known as breeding senescence (Figure 1.3; Fowler, 1995; Nisbet, 2001). Breeding experience has been shown to explain this initial improvement in breeding performance, but selective disappearance of younger seabirds which have lower phenotypic qualities and hence poorer reproductive outputs occasionally occurs (Curio, 1983; Bradley and Safran, 2014).



Stages of age-related reproductive success (RS) in birds. I.) Delayed Breeding. II.) Improving performance after onset of breeding. III.) Experienced adulthood. IV.) Senescence. Not all species exhibit all four stages. Dotted line indicates the RS of pairs that have changed mates in many species that maintain long-term pair bonds.

Figure 1.3 Effect of chronological age on the breeding performance of seabirds (from: Fowler, 1995).

Such a pattern between age and reproductive performance can be explained in the light of the life-history theory which stipulates that there is a trade-off between current reproduction, future reproduction and survival (Pianka and Parker, 1975; Curio, 1983; Clutton-Brock, 1984; Bradley and Safran, 2014). Hence, if the cost of current reproduction is higher than that of future reproduction and survival, then critical resources that are time-intensive (such as incubation of the eggs, caring for and guarding the chicks, defending nesting sites) and resource-intensive (such as chick provisioning and finding prey for chicks) may be diverted (Pianka and Parker, 1975;

Clutton-Brock, 1984). In fact, young seabirds which have a long life-span and an associated high probability of survival together with many future opportunities to breed may not invest as much time and energy in current reproduction (Pianka and Parker, 1975; Clutton-Brock, 1984; Bradley and Safran, 2014). There even appears to be a “mature” age at which seabirds start to breed, for example bridled terns do not breed until they are at least three years of age on Penguin Island, so that prior to this critical age, no resources are allocated to reproduction (Dunlop and Jenkins, 1992; Garavanta and Wooller, 2000). As seabirds age, their chances of reproducing in the future diminish and therefore they invest more in current reproduction (Clutton-Brock, 1984). At very old ages, breeding senescence is observed together with actuarial senescence so that decreased physiological functions prevent older birds from investing many resources into current reproduction (Clutton-Brock, 1984; Anderson and Apanius, 2003). Therefore, the population demography of a seabird colony needs to be known and understood before interpreting its breeding parameters and foraging habits in relation to its habitat and food supply.

1.7 Aims of the thesis

The over-arching hypothesis of this thesis is: in the breeding population of bridled terns on Penguin Island, there is an effect of age on reproduction. In other words, this thesis aims to answer the question: are older bridled terns better breeders than younger birds?

To test this hypothesis, I used two different approaches with the following questions:

- Do older bridled terns breed earlier, lay bigger eggs, incubate their eggs for shorter periods of time, have chicks that grow bigger, faster and fledge earlier and have a higher breeding success than younger birds?
- Do older bridled terns feed their chicks less diverse prey items (and hence feed their chicks a more targeted diet) than younger birds which would explain potentially different chick growth rates, and do younger bridled terns feed their chicks less often?

These questions are separately investigated in chapters 5 and 6. Additionally, Chapter 2 provides an extensive review of the literature of the effect of age on breeding in seabirds. Chapter 4 shows that a new technique for aging birds using pentosidine levels in skin samples cannot be used to estimate the age of bridled terns.

The effects of age in reproduction have yet to be investigated in bridled terns. Furthermore, determining whether age does affect reproduction and how, are necessary steps before using a species of seabirds as a proxy for the health of its environment (see Chapter 2). Finally, it is important to gain a greater understanding of this species of marine bird for conservation and management purposes, not only because they are becoming more prominent in their environment and establishing new colonies in southern parts of Australia, but also because they attract attention from the members of the public who want information on these seabirds (particularly on Penguin Island).

Additionally, Appendix 1 shows that the restoration of native vegetation and the re-introduction of *Malva preissiana* on Penguin Island is possible and that it may help recover nesting habitats for bridled terns that has been lost to introduced weeds.

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2. Age effects on breeding in seabirds – a review

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This chapter is under review for the journal of Marine Ecology Progress Series. To maintain consistency with the rest of the thesis, the acknowledgements, key words, author affiliations and running page head were removed but the references were kept in the journal's style. Otherwise, the text is identical to that of the manuscript.

2.1 Abstract

Several age-related processes affect marine birds' breeding performance, including: selective appearance/disappearance, breeding experience, parental effort and reproductive senescence. We identified 103 relevant peer-reviewed research papers published between 1949 and June 2016. Ninety-eight per cent found evidence for age-related processes affecting breeding performance. Twenty-three studies investigated selective disappearance; 12 found evidence to support it. In contrast, every study of the effect of experience on breeding performance found a positive relationship between accumulated experience and enhanced reproductive output ($n=66$). Twelve studies investigated the link between reproductive effort and breeding performance; only three of those found some evidence to support its occurrence. Finally, of the 46 studies that researched reproductive senescence, 43 found that it occurred in marine birds. We conclude that there is a definite effect of age-related processes on breeding performance across a range of marine birds. The strongest evidence is for breeding experience, selective appearance/disappearance and reproductive senescence affecting breeding in seabirds.

2.2 Introduction

Research over the last 65 years has noted a positive relationship between age and breeding success in marine birds (reviewed in Pianka & Parker, 1975; Curio 1983; Fowler, 1995; Bradley & Safran, 2014). In many studies, older breeders (that were not senescent) performed better than younger breeders because of: higher breeding probability, higher quality fledglings with chicks that have a higher probability of survival into adulthood and recruitment in a breeding colony, and the ability to maintain their body condition which increased their chance of survival until the next breeding event (Pianka & Parker 1975; Curio *et al.*, 1983; Nol & Smith, 1987; Erikstad *et al.*,

1998). The overall pattern reported is an increase in reproductive success following a seabird's first breeding attempt, which then plateaus over subsequent breeding events (Fowler, 1995). For species observed breeding over an extended period of time (e.g. a colony of black-browed albatrosses on the Kerguelen islands which has been studied over 40 years, Pardo *et al.*, 2013), reproductive success decreases with the parents' age following the plateau phase (Ollason & Dunnet, 1978).

Several studies link reproductive variables in marine birds to the apparent health and status of their environment (e.g. Furness & Camphuysen, 1997; Parrish & Zador, 2003; Parsons *et al.*, 2008). However, such studies omit the effect of the birds' ages on their breeding performances, which could be a possible drawback to correlating reproductive data with environmental variables/changes. If indeed older birds are better breeders than younger birds, then the ages of the seabirds sampled in a colony need to be known prior to correlating their reproductive variables with environmental condition to remove any influence of age on birds' breeding parameters. This would ensure that trend detection is linked to environmental drivers rather than effects of age on the birds' reproduction. This is particularly important because legal action (e.g. impact of tourism or fisheries on marine birds) needs a sound basis, and expert opinions and a lack of rigorous experimental design are unlikely to hold up in court as inferences between seabirds' reproductive variables and environmental changes will be weak (Calver, 2005, p. 236). Therefore, it is necessary to take into account seabirds' ages in monitoring studies.

In studies that investigated association of age and seabird reproduction (either at the population level and/or the individual level), four main age-related processes have been identified. These include: selective appearance/disappearance (the gain/loss of individuals from the colony with high/low reproductive outputs), breeding experience (which includes previous breeding experience, established nest territory and mate familiarity), an increase in breeding effort triggered by increasing age (e.g. number of nesting attempts, chick feeding frequency, incubation shift length), and breeding senescence: the deterioration of a bird's condition and its reproductive ability with age (e.g. Ollason & Dunnet, 1986; Aebischer & Coulson, 1990; Komdeur, 1996; Rebke *et al.*, 2010). Such studies have highlighted the interaction between these variables, and have demonstrated that disentangling the effects of age on breeding is complex but achievable (Forslund & Pärt, 1995; Bradley & Safran, 2014). Additionally, Curio (1983) postulated two hypotheses to explain this phenomenon: 1) either younger birds

are able to reproduce as well as older birds but they choose not to, the restraint hypothesis; or 2) young birds are not physically and physiologically ready to perform as well as older birds so their survival and breeding skills are yet to be fully developed, the constraint hypothesis.

Here we review the current knowledge surrounding the effect of age-related processes on breeding performance in marine birds. Using a quantitative methodology, we determined the extent to which age-related processes (namely selective appearance/disappearance, breeding experience, breeding effort and breeding senescence) have been studied in populations of marine birds.

2.3 Materials and methods

For the purpose of this study, Furness' & Monaghan's (1987) definition of a seabird was used, so that birds that obtained most of their food from the sea were included but birds such as seaducks (Merginae), grebes (Podicipedidae), phalaropes (Scolopacidae) and divers (Gaviiformes) were excluded.

Following the approach of Pickering & Byrne (2014), we systematically searched the peer-reviewed literature using Google Scholar, Web of Science and Science Direct electronic databases for original empirical journal articles that investigated the effect of age of marine birds on breeding. Research papers were identified using the terms 'marine bird' or 'seabird' and a combination of the following: 'age', 'breeding success', 'ontogeny', 'breeding', 'primiparity', 'iteroparity', 'breeding experience', 'residual reproductive value', 'terminal investment', 'senescence', 'reproductive cost' and 'breeding performance'. Studies from journals published between 1949 and June 2016 were used in this review. Research papers on seabirds that were breeding inland were excluded so that papers such as Pugesek's (1981) research on California gulls *Larus californicus* were not included in this review. Additionally, the reference lists of these papers and those of relevant book chapters and review papers were used to cross-check the gathered reference list for any omissions.

For each paper, the following information was recorded: authors, year of publication, country that undertook the research, climatic zone and hemisphere, journal in which the paper was published, whether the study was a cross-sectional (i.e. compared individuals across different age classes) or longitudinal study (i.e. followed development of individual birds over time), species and order examined, whether the study used an

experimental or observational design, variables recorded, age-classes which were investigated, if the study gave evidence for the restraint/constraint hypotheses, whether the research looked into the processes of selective appearance/disappearance, experience, reproductive effort and reproductive senescence, and if their findings were in accordance with these age-related processes.

The definitions of the restraint/constraint hypotheses, selective appearance/disappearance, breeding experience and reproductive effort given in the introduction were used, and only those studies which had significant results to support those and provided reasoning for attributing their observed trends to any of those hypotheses/processes were included in the following analyses. If a study had found strong evidence for an effect of age on reproduction but did not have an experimental design which allowed for determining which factor influenced such findings, it was not included in any of the categories for subsequent analyses and was only included in the ‘supports effect of age on breeding’ category.

2.4 Results

2.4.1 Overview

A total of 103 peer-reviewed publications was identified (see Appendix 2). The identified publications included four orders and 43 species of seabird (Table 2.1, Table 2.3). No studies investigating for effects of age-related processes on breeding outputs on the fifth order of seabirds, the Phaethontiformes (tropicbirds), were found. Birds aged between 2-7 years (Great skuas *Catharacta skua* Ratcliffe *et al.*, 1998) and 5-44 years (Wandering albatrosses *Diomedea exulans* Pardo *et al.*, 2014) were the topic of the studies included in this review, so a range of age classes was covered. Sixty-six studies were conducted in the northern hemisphere, 36 in the southern hemisphere and one in both hemispheres. Nine studies were strictly longitudinal in design, so that they followed the breeding performance of the same individuals over several breeding seasons, while 56 were cross-sectional and 38 were both longitudinal and cross-sectional. Four studies were strictly experimental (e.g. exchanging eggs of northern fulmars *Fulmarus glacialis* of different ages, Ollason & Dunnet, 1986), 89 were observational, and the remaining ten studies were both experimental and observational (Table 2.2).

Table 2.1 List of seabird species studied in research papers investigating the effects of age-related processes on their breeding parameters and associated number of studies carried out on those species.*

Order	Species	Number of studies
Charadriiformes	Arctic skua (<i>Stercorarius parasiticus</i>)	1
	Arctic tern (<i>Sterna paradisaea</i>)	1
	Audouin's gull (<i>Larus audouinii</i>)	1
	Black-legged kittiwake (<i>Rissa tridactyla</i>)	12
	Thick-billed murre (<i>Uria lomvia</i>)	3
	Brown skua (<i>Stercorarius antarcticus</i>)	1
	Cassin auklet (<i>Ptychoramphus aleuticus</i>)	3
	Common guillemot (<i>Uria aalge</i>)	3
	Common gull (<i>Larus canus</i>)	1
	Common tern (<i>Sterna hirundo</i>)	16
	Glaucous-winged gull (<i>Larus glaucescens</i>)	1
	Great skua (<i>Stercorarius skua</i>)	2
	Heermann's gull (<i>Larus heermanni</i>)	1
	Herring gull (<i>Larus argentatus</i>)	2
	Lesser black-backed gull (<i>Larus fuscus graellsii</i>)	1
	Oystercatcher (<i>Haematopus ostralegus</i>)	2
	Red-billed gull (<i>Larus novaehollandiae scopulinus</i>)	1
	Ring-billed gull (<i>Larus delawarensis</i>)	1
	Roseate tern (<i>Sterna dougallii</i>)	1
	Western gull (<i>Larus occidentalis</i>)	2
Procellariiformes	Antarctic fulmar (<i>Fulmarus glacialis</i>)	1
	Black-browed albatross (<i>Thalasarche melanophris</i>)	3
	Blue petrel (<i>Halobanea caerulea</i>)	1
	Grey-headed albatross (<i>Thalassarche chrysostoma</i>)	1
	Laysan albatross (<i>Phoebastria immutabilis</i>)	1
	Leach's storm petrel (<i>Oceanodroma leucorhoa</i>)	2
	Manx shearwater (<i>Puffinus puffinus</i>)	1
	Mediterranean storm petrel (<i>Hydrobates pelagicus melitensis</i>)	1
	Northern fulmar (<i>Fulmarus glacialis</i>)	4
	Scopoli's Shearwater (<i>Calonectris diomedea</i>)	1
	Short-tailed shearwater (<i>Puffinus tenuirostris</i>)	3
	Snow petrel (<i>Pagadroma nivea</i>)	3
	Wandering albatross (<i>Diomedea exulans</i>)	8
Sphenisciformes	Adélie penguin (<i>Pygoscelis adeliae</i>)	3
	Little penguin (<i>Eudyptula minor</i>)	1
	Yellow-eyed penguin (<i>Megadyptes antipodes</i>)	2
	King penguin (<i>Aptenodytes patagonicus</i>)	1
Suliformes	Australasian gannet (<i>Morus serrator</i>)	2
	Blue-footed booby (<i>Sula nebouxii</i>)	5
	Cape gannets (<i>Morus capensis</i>)	1
	European Shag (<i>Phalacrocorax aristotelis</i>)	2
	Great cormorant (<i>Phalacrocorax carbo</i>)	1
	Nazca booby (<i>Sula granti</i>)	1

*Note: two journal articles included more than one species in their research so the total number of articles in this table is over 103.

Table 2.2 Published studies (1949 – June 2016) that investigated the effects of age-related processes on marine birds' reproductive performance.*

Category	Total	Evidence for effect of age-related processes on breeding	No evidence for effect of age-related processes on breeding
All studies	103	92	2
Method			
Observational	89	82	2
Experimental	4	2	0
Both	10	9	0
Study design			
Cross-sectional	56	50	0
Longitudinal	9	8	1
Both	38	35	1
Country undertaking the research			
Australia	6	5	0
Belgium	1	1	0
Canada	5	4	0
Denmark	1	1	0
Estonia	1	1	0
France	21	20	1
Germany	13	11	1
Mexico	6	5	0
Netherlands	2	2	0
New Zealand	3	3	0
Seychelles	1	1	0
South Africa	1	1	0
Spain	3	3	0
United Kingdoms	22	20	0
United States of America	17	15	0

*Note: not all studies specifically investigated the effects of age-related processes on reproductive performance, instead some studies focused on determining the details of selected processes that had been shown to influence breeding performance in seabirds in previous studies. Therefore, the totals in this table do not add up

Table 2.3 Published studies (1949 – June 2016) that investigated the effects of age-related processes on marine birds' reproduction. Numbers represent the number of peer-reviewed articles that found evidence to support each category out of the total number studies that investigated each process.

	Support effect of age on reproduction	Support for constraint (C) or restraint (R) hypotheses	Support for selective appearance or selective disappearance	Support for breeding experience	Support for increased reproductive effort with age	Support for breeding senescence
Charadriiformes	50/51	17C+1R/18	7/15	36/36	17/5	21/23
<i>Larus</i>	11/11	5C/5	1/5	10/10	0/3	4/5
<i>Sterna</i>	15/16	9C/9	2/4	12/12	-	6/6
<i>Haematopus</i>	2/2	-	0/1	2/2	-	1/1
<i>Uria</i>	5/5	-	-	2/2	17/2	2/2
<i>Ptychoramphus</i>	3/3	1C/1	-	2/2	-	2/2
<i>Rissa</i>	11/11	1C+1R/2	4/5	4/4	-	5/6
<i>Stercorarius</i>	4/4	1C/1	-	4/4	0/1	1/1
Procellariiformes	26/27	12C+2R/13	4/6	20/20	1/5	15/15
<i>Fulmarus</i>	4/4	3C/3	-	3/3	-	3/3
<i>Thalasarche</i>	4/4	3C/3	1/2	3/3	-	3/3
<i>Halobanea</i>	1/1	-	1/1	1/1	-	-
<i>Phoebastria</i>	1/1	-	-	-	-	-
<i>Oceanodroma</i>	2/2	1C/1	1/1	1/1	0/2	1/1
<i>Puffinus</i>	3/3	1R/1	1/1	4/4	0/1	-
<i>Hydrobates</i>	1/1	-	-	-	-	1/1
<i>Calonectris</i>	1/1	1C/1	-	1/1	-	-
<i>Pagodroma</i>	3/3	1C and R/1	-	2/2	-	2/2
<i>Diomedea</i>	7/8	3C/3	0/1	5/5	1/1	6/6
Sphenisciformes	7/7	4C/4	-	4/4	-	2/2
<i>Pygoscelis</i>	3/3	2C/2	-	2/2	-	1/1
<i>Eudyptula</i>	1/1	1C/1	-	1/1	-	1/1
<i>Megadyptes</i>	2/2	-	-	-	-	-
<i>Aptenodytes</i>	1/1	1C/1	-	1/1	-	-
Suliformes	10/10	3C/3	1(+1?)/1	6/6	2/2	6/6
<i>Sula</i>	5/5	1C/1	17/1	2/2	1/1	5/5
<i>Morus</i>	3/3	1C/1	-	2/2	1/1	-
<i>Phalacrocorax</i>	2/2	1C/1	1/1	2/2	-	1/1

2.4.2 Assessing the effects of age on breeding performance

Of the 95 studies which specifically assessed the effects of age on breeding performance, 98% found evidence for an effect of age-related processes on reproduction (Table 2.2). Only two studies found no evidence for an effect of age on breeding (Table 2.2, Table 2.3). In the first, Pardo *et al.* (2014) examined a colony of wandering albatross over several decades and discovered that the breeding population on Île de la Possession in the Crozet Islands was partitioned into two groups. One of the groups (94% of the breeding population on Île de la Possession) consisted of individuals with consistent bi-annual breeding cycles and a virtually unchanging breeding output over their lifetime, so that for this group there appeared to be little effect of age on breeding performance (Pardo *et al.*, 2013; Pardo *et al.*, 2014). However, in the remaining 6% of the population, which bred annually, there was a strong effect of age-related processes on their breeding outputs (Pardo *et al.*, 2014). In the second study that found no effect of age on breeding performance, Wendeln & Becker (1999) investigated the effects of parental quality and effort in common terns in a colony in Wilhelmshaven in the German North Sea. They included birds ranging from three to 16 years of age in a five-year longitudinal study which showed that the birds' "individual quality" (using body condition and bird mass as proxies) and reproductive effort, rather than age, were the main determinants of breeding success. However, it is worth noting that subsequent studies did find an effect of age on the breeding performance in this particular colony (Limmer & Becker, 2010; Rebke *et al.*, 2010).

2.4.3 The constraint/restraint hypothesis

In the 38 studies which looked at the constraint/restraint hypotheses, 92% found evidence for the constraint hypothesis (Table 2.2, Table 2.3). Only three studies found evidence for the restraint hypothesis (out of the eight studies which specifically investigated the restraint hypothesis). Of these, Wooller *et al.* (1990) investigated the reproductive success of short-tailed shearwaters *Puffinus tenuirostris* on Fischer Island in Australia and found that birds which started breeding at a later age had a slightly better reproductive output at their first attempt than birds which started breeding earlier, but that there was no notable difference in their breeding performance over their lifetime. Effectively these results suggested that short-tailed shearwaters could refrain from breeding at an earlier age. In the second study that found evidence for the restraint hypothesis, Angelier *et al.* (2007) found evidence for both the constraint and restraint

hypotheses in their studies of snow petrels in Terre Adélie in Antarctica. They investigated the hormonal response (corticosterone and prolactin) of breeding adults aged between eight and 36 years and found that males were physiologically constrained, whereas younger females restrained from breeding due to their ability to alter their hormonal levels (prolactin, which is induced by stress). Finally, Elliott *et al.* (2014a) found terminal restraint in black-legged kittiwakes so that very old birds refrained from breeding. Hence the majority of studies (35 of 38) found no support for the restraint hypothesis or did not investigate its occurrence.

2.4.4 Studies of selective appearance/disappearance and breeding experience

Of the 23 studies which looked at the selective disappearance of lower quality breeders at an early age and the selective appearance of higher quality breeders at older ages, ten found no evidence for this process whereas 12 found some evidence of its occurrence and one was inconclusive (Table 2.3). They used long-term datasets and models that linked the disappearance/appearance of the individuals to their breeding performance to investigate this process (e.g. black-legged kittiwakes, Cam & Monnat, 2000).

All 66 studies which investigated the effect of breeding experience on breeding success found that previous breeding experience significantly improved reproductive output.

2.4.5 Reproductive effort

Only 12 studies investigated the effects of reproductive effort (e.g. number of nesting attempts, chick feeding frequency) on reproductive output (e.g. clutch size, fledging success). Seven found no evidence linking reproductive effort with outcome whereas two found that increased reproductive effort with age enhanced breeding success (Pyk *et al.*, 2007; Pardo *et al.*, 2014), and one found some evidence that it occurred when thick-billed murres *Uria lomvia* overcame their intrinsic energy ceilings to invest more in offspring (Elliott *et al.*, 2014b). Furthermore, one study by Wooller *et al.* (1990) had insufficient evidence to determine if increased breeding effort yielded better reproductive outcomes in short-tailed shearwaters. Therefore, the majority of the research (seven of twelve) linking breeding effort to breeding outcome found no evidence of a relationship - more effort did not produce more offspring - in marine birds.

2.4.6 Reproductive senescence

Finally, 43 of the 46 studies which looked at reproductive senescence found that it occurred in 23 species of seabirds across four orders (see Appendix 2). As discussed previously, Pardo *et al.* (2014) found that there was little evidence for reproductive senescence in the wandering albatross population breeding on Île de la Possession. Furthermore, Sydeman *et al.* (1991) found that western gulls breeding on Southeast Farallon Island, aged between three and 21 years, did not exhibit reproductive senescence. Finally, Coulson and Fairweather (2001) showed that black-legged kittiwakes breeding in North Shields exhibited terminal illness but not senescence.

2.5 Discussion

Nearly all of the studies reviewed have reported an improvement in breeding variables following the first breeding attempts in marine birds. Furthermore, the effects of age-related processes appeared to be uniform across the four orders of marine birds, although fewer studies were conducted on Sphenisciformes and Suliformes than on Charadriiformes and Procellariiformes (Table 2.3). While for some species an improvement in breeding performance may take several attempts and hence a number of years to reach a plateau at which maximum breeding performance is achieved (e.g. the black-browed albatross *Thalasarche melanophris*, Angelier *et al.*, 2007), in other species the first breeding attempt seems to be sufficient to reach this plateau (Pyle *et al.*, 1991). Hence there is broad evidence for an effect of age on breeding performance in marine birds.

2.5.1 Processes involved in breeding performance related with age

2.5.1.1 Selective disappearance/appearance

One factor that enhances reproductive success would be that individuals of lower quality, which reproduce less well, are selected against over time; the overall colony's breeding improvement with age may therefore be a reflection of the removal of lower quality individuals (Curio, 1983; Nol & Smith, 1987). Conversely, individuals of higher quality could be entering the colony, which would also lead to an improvement in the colony's breeding variables. This selective disappearance/appearance (Nol & Smith, 1987; Pyle *et al.*, 1991) was shown to take place in 12 of 23 studies, however the magnitude of the effect of this process was often described as negligible (e.g. Pyle *et al.*, 1991; Rebke *et al.*, 2010; Pardo *et al.*, 2014).

If young and lower-quality breeders are not selected against on a large scale, then two distinct hypotheses can explain differential breeding outputs between younger and older birds: the constraint and the restraint hypotheses (Curio, 1983). As Curio (1983) suggested, these hypotheses are not mutually exclusive, however the vast majority of the work which has been reviewed here has found more evidence for the constraint hypothesis; that is, younger birds' breeding skills are not fully developed because of physical and physiological constraints.

There is little evidence for the restraint hypothesis, which is mostly due to the fact that very few studies have compared the physiological and physical capabilities of younger and older birds, which would show whether younger breeders are physically capable of breeding as more mature breeders (although see Angelier *et al.*, 2006; 2007). Most of the work investigating the relationship between age and breeding performance has focused on breeding skills (a portion of the constraint hypothesis).

2.5.1.2 Breeding experience

Quantifying the role of breeding experience in reproductive output is difficult (Weimerskirch, 1990; Wooller *et al.*, 1990; Aebischer & Coulson, 1990; Pyle *et al.*, 1991). Breeding experience sits within the constraint hypothesis and there is overwhelming evidence to support the existence of breeding experience (all 66 studies which investigated its occurrence found evidence to support it). The experience hypothesis, which comprises of a range of behaviours including breeding, foraging, nesting site selection, nest/chick guarding, and mate familiarity, purports that birds can refine their foraging, breeding, incubating and parenting skills over time through a learning process (Curio, 1983). Unfortunately, disentangling age and experience in field studies is difficult because birds gain experience as they age. Bradley's & Safran's (2014) review of proximate factors influencing reproduction in all species of birds outlined a number of ways in which experience and age can be teased apart.

A number of studies were able to consider experience-related factors separately by conducting extensive field-based studies in which great numbers of chicks were marked so that they had a large pool of known-age birds to monitor over time (Weimerskirch, 1990; Angelier *et al.*, 2006). By ensuring that no bird banded as a chick recruited in other colonies to either breed or prospect (as they could potentially gain skills before coming back to their natal colonies to breed), the authors were able to follow the development of breeding skills over several years. Some of the studies recorded the

breeding stages at which younger birds failed and examined whether either partner in a breeding pair had previous breeding experience (Ollason & Dunnet, 1986; Weimerskirch, 1990; Pyle *et al.*, 1991). Some studies with longitudinal designs were powerful in identifying the individual improvements in breeding as seabirds gained experience over time (e.g. Limmer & Becker, 2010). All of these studies concluded that breeding experience was indeed a major factor in ensuring breeding success, thereby providing overwhelming evidence of age-experience driven increases in breeding success in marine birds.

2.5.1.3 Increased Reproductive Effort

Pianka & Parker (1975) explained that as birds grow older, their chances to reproduce and pass on their genes are reduced due to the low number of breeding opportunities remaining before they die. In some cases, old age may drive seabirds to breed even if this cost may reduce their own chance of survival (Pianka & Parker, 1975). Increased reproductive effort at older ages sits within the restraint hypothesis and is difficult to assess in field studies, and defining variables linked to reproductive effort and measuring them is problematic and controversial (Pyle *et al.*, 1991; Wendeln & Becker, 1999). For example, can chick feeding frequency be used as a measure of reproductive effort or would it correlate to prey availability? Is frequent feeding an indication of good parenting skills and increased reproductive effort? Reproductive effort has been predicted to increase with age (Williams, 1966; Charlesworth, 1981) but there is insufficient evidence to support its occurrence: only three out of twelve studies found solid evidence for increased reproductive effort with age.

2.5.1.4 Senescence

Reproductive senescence is linked to a physiological decline in the birds' breeding condition as they age. Catry *et al.* (2006) demonstrated that in the case of the grey-headed albatross *Thalassarche chrysostoma*, a decline in breeding performance was linked with a decline in foraging performance which was likely due to a deterioration in the birds' physical abilities. Furthermore, the effect of reproductive senescence at the population level was demonstrated in common terns by Rebke *et al.* (2010). Using a longitudinal dataset, they modelled the reproductive parameters of the common terns as they aged while taking into account natural mortality. Our review found that 96% of the 46 studies which investigated reproductive senescence in marine birds support its occurrence, so there is strong evidence for the occurrence of this age-related process in

long-lived species of seabirds. However, as Coulson & Fairweather (2001) noted, care must be taken not to confound senescence with terminal illness when very low reproductive performance is observed in the breeding attempt preceding a bird's death.

2.5.2 Further research

As identified in this review, there is a lack of information about the effects of age on the breeding performance of seabirds from the Phaethontiformes (tropicbirds). Tropicbirds have a world-wide tropical to sub-tropical distribution, but their populations are decreasing (Bird Life International, 2016). It would be useful to determine whether this could be due to a decline in the recruitment rates of younger birds into breeding populations and/or if more global threats such as pollution and the introduction of predators are affecting tropicbirds of all ages.

Furthermore, most of the literature gathered in this review was led by European countries and the United States of America with a lack of information on the topic from Russian, South American, African, and Asian countries which are home to some of the biggest seabird colonies in the world. The World Seabird Union's (Seabirds.net, 2016) repertory of seabird colonies can be used to identify colonies that are yet to be included in studies that focus on the effects of age on breeding performance. Advancing knowledge on seabird species that breed in colonies in these countries will greatly help refine conservation strategies by identifying the needs of breeding marine birds. However, facilities for long-term studies, including adequately trained and supported local scientists, are critical for such projects.

Additionally, there was a limited number of experimental studies on the subject. However, clear trends were identifiable in studies that used cross-sectional and longitudinal designs, based on observable breeding parameters such as breeding success. So while experimental studies are necessary to understand the mechanisms involved in the effects of age on reproduction, they are not necessary to determine their existence in a species.

It is also worth noting that small datasets can reduce statistical power and this would therefore hinder the detection of the occurrence of trends such as increased reproductive effort in older ages and restraint in young in old ages. Furthermore, few studies were specifically designed to investigate the restraint hypothesis and reproductive effort, so although there is a lack of evidence to support these, it cannot be assumed that they do

not occur in seabirds. Moreover, although only two studies found no evidence for an effect of age on the reproduction of common terns and wandering albatrosses, it cannot be excluded that other species and populations may also not show signs of ontogenesis. Further research could identify populations where abundant and reliable food sources as well as a lack of predators and adverse weather would remove any effect of age on breeding, particularly those associated with experience and senescence, as Le Vaillant *et al.* (2016) noted that favourable environmental conditions may remove any effect of age on reproduction in some populations of marine birds.

2.6 Conclusion

Based on the surveyed literature, there is a definite effect of age-related processes on breeding performance across a range of marine birds. While there is evidence to suggest that breeding experience, selective appearance/disappearance and reproductive senescence affect breeding in seabirds, there is less support for the occurrence of increased reproductive effort with age.

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3. Site description, general methods and baseline of the study

3.1 Overview

To study the effect of parental age on reproduction in the population of bridled terns breeding on Penguin Island in Western Australia, a sample of bridled terns that nest on the north-east end of Penguin Island was chosen and their breeding attempts were observed over three consecutive breeding seasons. The three breeding seasons occurred over summer 2013/2014, summer 2014/2015 and summer 2015/2016 (southern hemisphere December – February), when bridled terns came back to Penguin Island from their winter migration to the Celebes Sea, as determined from band recoveries (Figure 3.1; Dunlop *et al.*, 1988; Dunlop and Johnstone, 1994). This was possible thanks to the birds' high nest site fidelity and monogamous relationships over their lifespans (del Hoyo *et al.*, 1996).

The study focused on pairs of breeding birds along the limestone cliff as well as those breeding inside nest tubes that were installed in winter 2013. Artificial nest tubes were installed where the vegetation has been highly degraded by weeds, and no longer offers shelter. The research undertaken in this study began shortly after a successful rat eradication project that took place in 2012/2013. An entire cohort of chicks was decimated and a proportion of breeding adults was lost in the 2012/2013 bridled tern breeding season due to a booming population of black rats (*Rattus rattus*) that was introduced on the island early in 2012. Bridled terns in this study were banded for individual recognition, as recommended by the Australian Bird and Bat Banding Scheme, and had their age and sex determined in order to research possible ontogenic changes in their reproduction over three markedly different breeding seasons brought about by changing climatic and oceanic conditions.

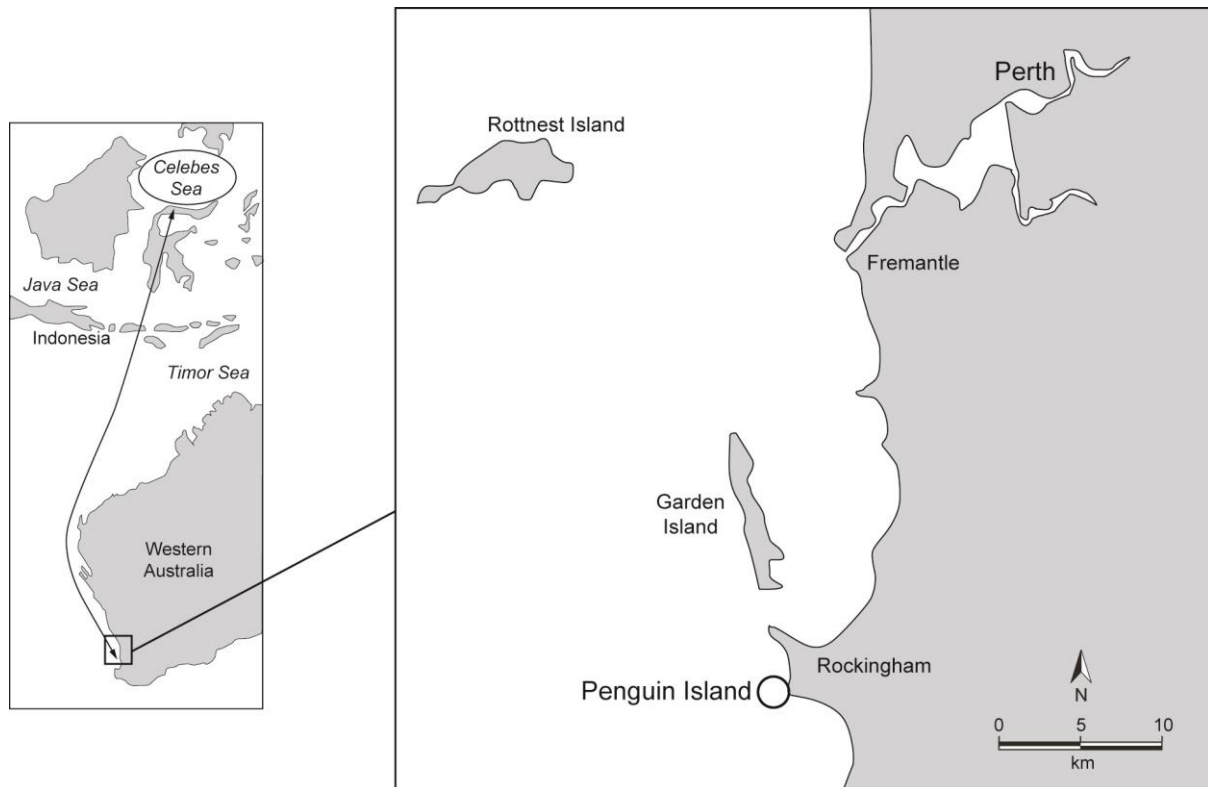


Figure 3.1 Map showing the location of the wintering area of bridled terns (*Onychoprion anaethetus*) in the Celebes Sea and their summer breeding location on Penguin Island, Western Australia.

3.2 Site description: Penguin Island

3.2.1 Geomorphology and currents surrounding Penguin Island

Penguin Island is a small coastal island in Western Australia that lies less than 800 m from the mainland, 40 km south of Perth (latitude: 32°305'S, longitude: 115°690'E). Penguin Island is on the partly submerged Garden Island Ridge, which is an Aeolian dune of Tamala limestone that was submerged about 130,000 years ago (Haig, 2002). The geomorphology of the area includes a chain of small coastal islands with intertidal limestone preceding the mainland's sandy shore (Haig, 2002). Between the ridge and the mainland, the submerged substrate is less exposed to currents and swells so that seagrass meadows and macro-algal beds are able to grow in the area where refracted and diffracted swell dominates the water movements (Haig, 2002). The Garden Island Ridge has been eroding and a tombolo has formed between Penguin Island and the mainland which becomes almost entirely exposed during low-tide events (Haig, 2002). The island is about 12.5 hectares in surface area and has a maximum elevation of about 20 meters. Seasonal sand accretion and erosion driven by the dominating Leeuwin Current in late summer and the Capes Current in spring and early summer

result in very mobile beaches and eroding limestone cliffs (Haig, 2002; DEC and MPRA, 2007). Therefore the geomorphology of Penguin Island is very dynamic and subjected to seasonal oceanographic features as well as local weather patterns.

3.2.2 Flora on Penguin Island

The geomorphology, substrate and climatic conditions on Penguin Island have shaped the flora communities that grow there (DCALM, 1992). Native plants growing on Penguin Island display sclerophyllous and xeromorphic characteristics that are typical of coastal and island plants (DCALM, 1992). The western side of the island is generally more exposed to the salt spray of breaking waves and onshore winds, and harbours *Carprobrotus* and low-lying bushes including *Tetragonia*, *Frankenia* and *Rhagodia baccata* assemblages (DCALM, 1992). The eastern side of Penguin Island, however, is less exposed to wind and salt spray and harbours *Spinifex*, *Nitraria billardierei* and taller *Rhagodia*, *Scaevola* and *Acacia rostellifera* assemblages (DCALM, 1992). Human activities starting in the early 1900s have impacted and contributed to the degradation of Penguin Island's flora, particularly following the construction of shacks until 1987 when the island's lease was bought by the Department of Parks and Wildlife (DPaW, formerly the Department of Conservation and Land Management, DCALM; Brown *et al.*, 2015a). Furthermore, the booming population of scavenging birds such as silver gulls (*Chroicocephalus novaehollandiae*), Australian pelicans (*Pelecanus conspicillatus*) and feral pigeons have also impacted Penguin Island's flora by contributing to the introduction of weeds from the mainland and disturbing the vegetation when nesting (Brown *et al.*, 2015a). The 'Weed Management and Restoration Plan for Penguin Island' (Bettink *et al.*, 2009) specifies that the weeds that are of main concern for conservation purposes are the Geraldton carnation weed (*Euphorbia terracina*), *Melaleuca nesophila*, introduced *Malva* plants and buffalo grass (*Megathyrsus*) which compete with the native vegetation. Therefore, while Penguin Island's native vegetation is adapted to growing in coastal conditions, it became severely degraded but is now being managed by DPaW (see Appendix 1).

3.2.3 Fauna and tourism on Penguin Island

Penguin Island has over 30 species of birds, some of which are migratory and protected under the Australian, Japan and Chinese Migratory Bird Agreements (DCALM, 1992). Both tropical (bridled terns) and temperate (little penguins

Eudyptula minor) species breed on the island so that it has become a famous bird-watching destination and wildlife interaction hotspot. Tourism has been driven by the presence of the marine mega fauna that resides in and around the Garden Island Ridge and includes a population of Australian sea lion (*Neophoca cinerea*) males, bottle nose dolphins, various rays, sharks and the colonies of little penguins on Garden and Penguin Island. As a result, an estimated 150,000 tourists visit Penguin Island alone between September and June each year when the island is open to the public (Brown *et al.*, 2015a). In an effort to preserve the bird sanctuary zones on the island, DPaW maintains a visitors' centre where rehabilitated little penguins are displayed, together with a picnic area under introduced Norfolk pine trees, toilets, a research station, an office and stretches of boardwalk. However, due to the high inflow of visitors during summer, Penguin Island's wildlife is exposed to very high numbers of tourists during day-light hours, when the island is open to the public (Barter *et al.*, 2008). Therefore, Penguin Island does not have a pristine habitat and is still being significantly altered by anthropogenic activities.

3.2.4 Black rats on Penguin Island

Anthropogenic activities led to the introduction of black rats on Penguin Island early in 2012. Under the 'Environment Protection and Biodiversity Conservation Act 1999', predation by introduced exotic rats is listed as a major threat to Australian wildlife and flora, so a permit was issued to the Department of Parks and Wildlife to eradicate black rats on Penguin Island using the potent rodenticide X-Verminator (brodifacoum). In January 2013, this anti-coagulant poison was distributed across 300 baiting stations across a 20 m grid over the island to specifically target black rats and exclude Penguin Island's native fauna, such as the king skinks (*Egernia kingii*; Figure 3.2). This resulted in the eradication of black rats from Penguin Island, with very little secondary poisoning observed as the carcasses of the poisoned black rats were collected several times a week. However, no bridled chick fledgling was observed at the end of the 2012/2013 breeding season, and a proportion of adult bridled terns was found either dead, or dying from injuries inflicted by rats. Furthermore, the program failed to eradicate house mice (*Mus musculus*) that also occasionally predate on bridled tern eggs. A number of little penguins also died from black rat predation and the population of king skinks suffered significant losses. The present research project

began in summer 2013/2014 after most black rats were eliminated through the eradication project.



Figure 3.2 Example of a baiting station that was used to target and poison introduced black rats (*Rattus rattus*) on Penguin Island, Western Australia, during the eradication program in January 2013. Rats (top-left photo) were able to access the bait inside the station through one of the two protruding sleeves, but king skinks *Egernia kingii* (top-right photo) and other wildlife such as bridled terns *Onychoprion anaethetus* (bottom photo) were not be able to access the bait.

3.3 Study site

The study site for this project was located on the north-east end of Penguin Island (Figure 3.3) so that birds breeding both along the cliff and on top of the cliff were included in this study. This particular site was chosen in an effort to include older bridled tern pairs as this part of the island was one of the very first areas used by bridled terns for nesting (Dunlop and Jenkins, 1994; Figure 3.3). Bridled terns display very high nest site fidelity, so it was hoped that older birds could be included in this study in order to test the main hypothesis. Furthermore, the vegetation on this part of the island has been greatly impacted by weeds so that the native vegetation cover used by bridled terns for nesting has diminished (Appendix 1). Therefore 50 artificial nest tubes (see Section 3.4) were installed in the areas that are bare in summer when the weeds die off to provide shelter and alternative nesting locations for bridled terns. It was hoped that younger bridled terns would recruit in those nesting boxes as they constituted “unclaimed territory” by other bridled terns that would have bred there

previously. Indeed, it has been shown in a number of seabird colonies that younger and less experienced pairs nest at the edge of the colony, in “unclaimed” areas that can sometimes be considered as being of lesser quality (Gochfeld, 1980). Furthermore, this particular location had the advantage of being accessible through the beach at low tide, and from the northern boardwalk on top of the cliff, while being sheltered from the prevailing south-westerly winds in summer (Rosman *et al.*, 1980). Bridled terns are able to rapidly take off by using wind-generated lift, therefore choosing a sheltered location assisted with the capture of the birds in this study.

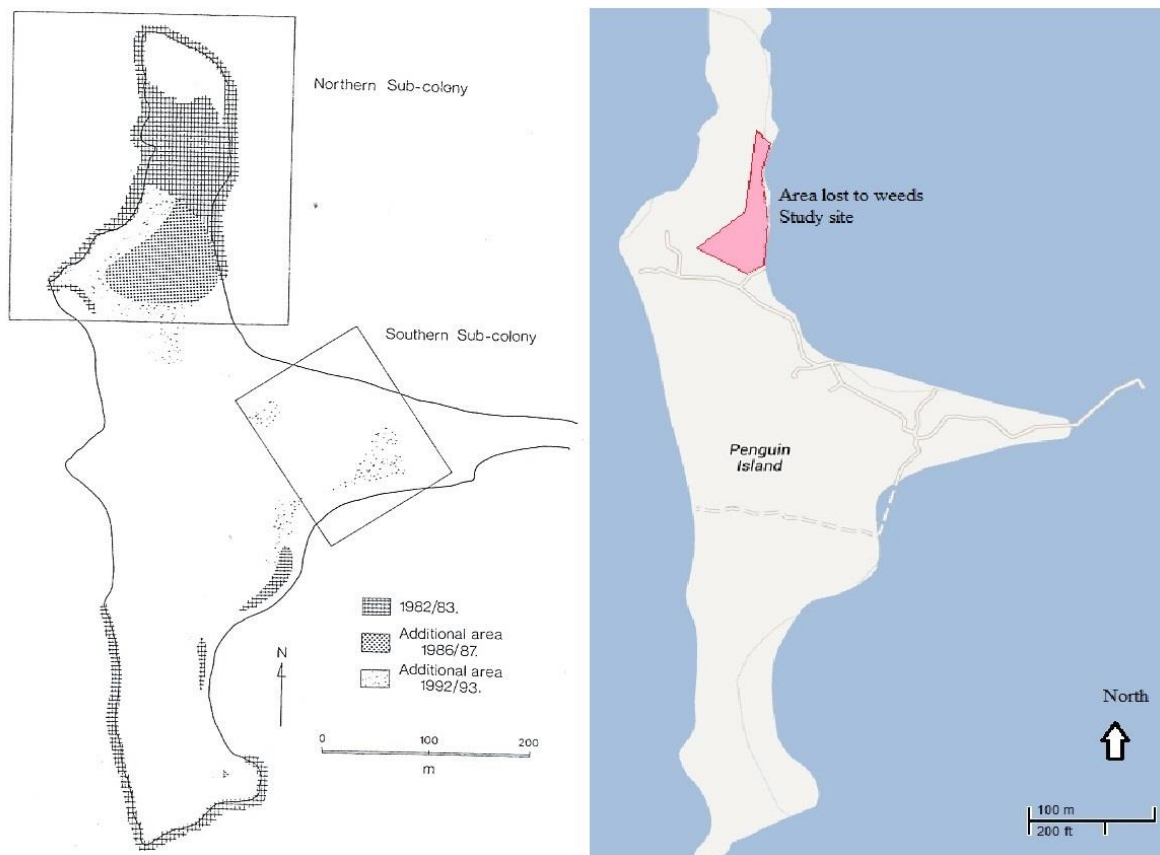


Figure 3.3 Map of Penguin Island, Western Australia, showing the distribution of the breeding population of bridled terns in 1982/1983, 1986/1987 and 1992/1993 (left, adapted from Dunlop and Jenkins, 1994), and the study site of this study (adapted from Google Maps).

3.4 Artificial nest tubes

Fifty artificial nest tubes were built out of a storm drain PVC pipe (diameter 30cm). The pipe was cut length-wise to obtain 2 dome-shaped half pipes and sectioned in 50cm long pieces (Figure 3.4). The artificial nest tubes were then painted with limestone-coloured paint in order to give them a more natural appearance (Green, 2004). The resulting artificial nest tubes were installed on the north-east side of

Penguin Island on patches that were cleared of weeds, and were identified with unique numbers (e.g. AN7 for Artificial Nest number 7; Plate 3.5; Figure 3.6). The nest tubes were laid about 4m apart as bridled terns usually nest 1-5m apart from each other (del Hoyo *et al.*, 1996). The nests were installed in early September 2013 so that for the 2013/2014 breeding season, bridled terns that came back from their winter migration as early as the end of September would have time to prospect the new nesting tubes. The design of the artificial nest tubes meant that they were easily transportable, and excluded larger birds so that silver gulls were not able to use them for shelter or for breeding, which would have generated inter-species competition for nesting spots. The artificial nest tubes also provided a smooth landing area for bridled terns where they could roost when not incubating their egg or looking after their chick (Figure 1.1). Because the nest tubes did not cover the ground, bridled terns were able to use natural substrate to lay their eggs. They also provided exit points at each end of the tube so that the nesting bridled terns would not be cornered inside the tube if predators such as black rats attempted to prey on incubating birds. Bridled terns had previously been observed breeding inside broken equipment and fences around the rangers' shed, therefore the artificial nature of the nest tubes was not believed to be a deterrent for breeding bridled tern pairs.

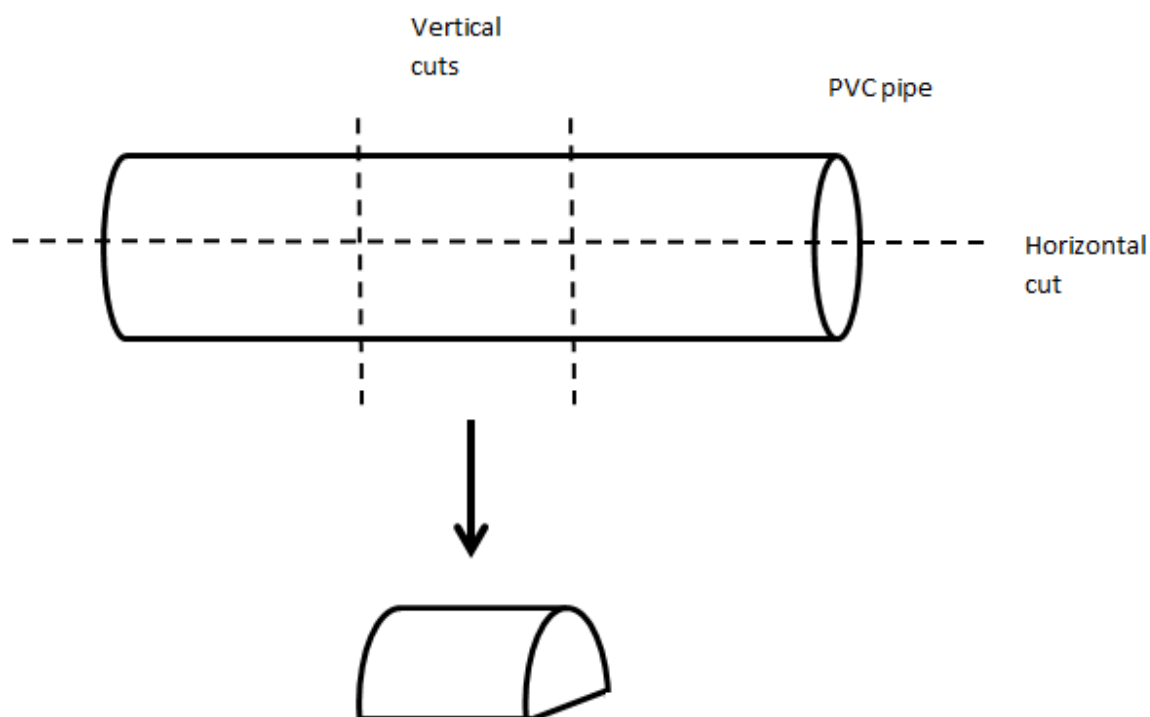


Figure 3.4 The cutting of a storm drain pipe to make artificial nest tubes for bridled terns (*Onychoprion anaethetus*) breeding on Penguin Island, Western Australia.



Figure 3.5 Artificial nest tubes installed in an area lost to weeds on Penguin Island, Western Australia.

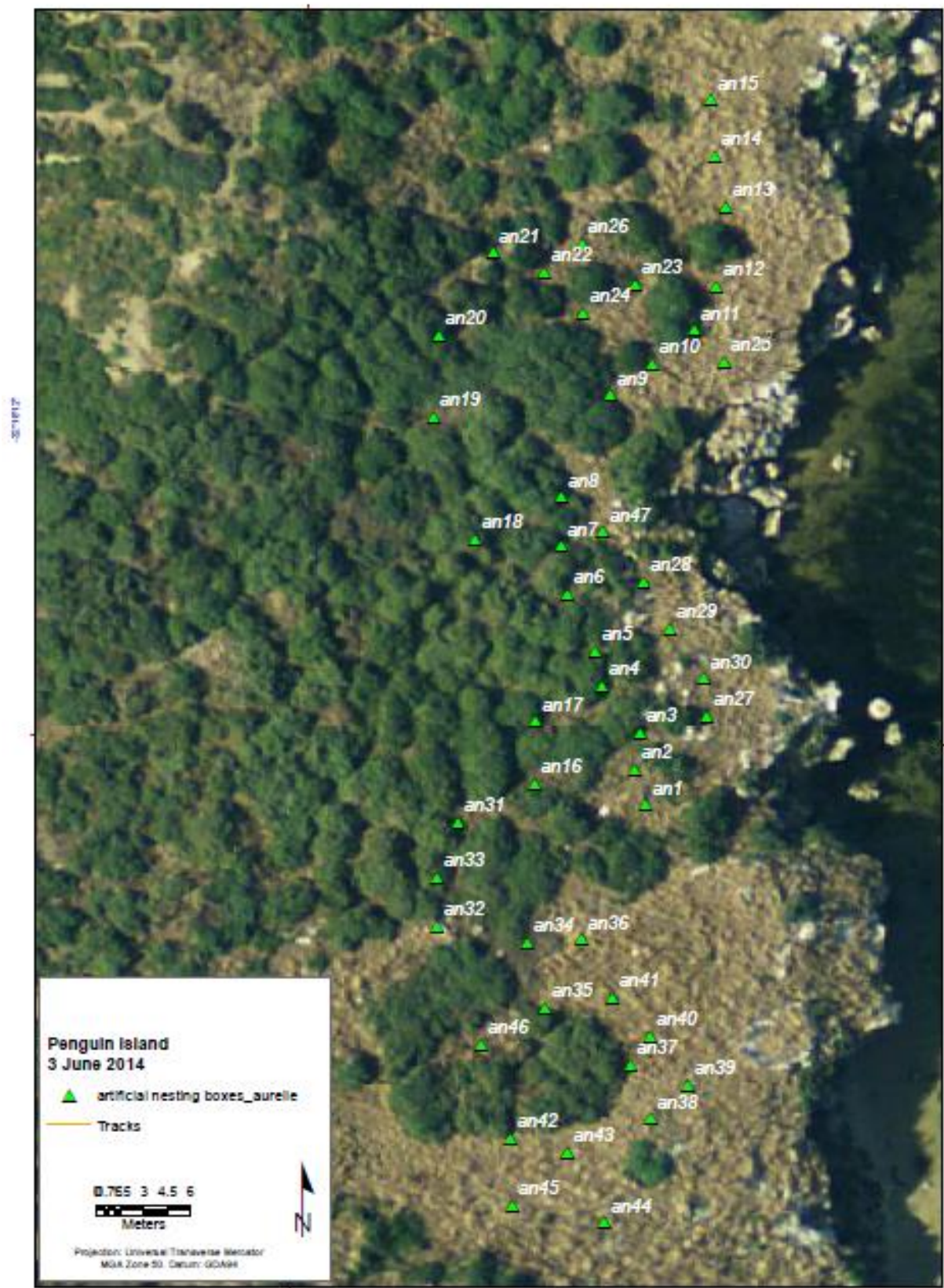


Figure 3.6 Locations of the bridled tern (*Onychoprion anaethetus*) nest tubes installed on Penguin Island, Western Australia in winter 2013.

3.5 Natural nests selection

Thirty natural nests on the north-eastern limestone cliff facing east were selected. Those nesting spots were identified using unique numbers (e.g. NN7 for Natural Nest number 7, Figure 3.7) that were written on the limestone near the incubating site using markers. While those nesting sites were inside limestone cavities or under bushes, all nesting spots had a sandy substrate where the bridled terns would lay their eggs. The natural nests were easily accessible from the beach and were fenced off in 2014 so that visitors would not disturb the breeding birds or harvest eggs.



Figure 3.7 Example of natural nest sites for bridled terns (*Onychoprion anaethetus*) breeding on Penguin Island in Western Australia. Inside a limestone cavity, two bridled terns (circled in red) are incubating their eggs, one on the left-hand side and one on the bottom right corner of the photo, and one feral pigeon (circled in yellow) is incubating its eggs on the top right corner of the photo.

3.6 Revegetation of the study area

Starting in early 2014, and in partnership with the Department of Parks and Wildlife, a revegetation program was trialled on the northern end of Penguin Island, specifically in the study area of this project, in an effort to minimise the spread of weeds and re-establish native vegetation cover (Brown *et al.*, 2015a). The overall aim of this project was to re-establish native vegetation cover over the artificial nest tubes so that those could be removed at the end of this research project while the vegetation cover that grew over those boxes would remain (Figure 3.8). In winter 2014, there was also an attempt to re-introduce a native coastal plant that had disappeared from Penguin Island in the 1970s: the Australian Hollyhock *Malva preissiana*, which has a life cycle that is tightly linked with nesting seabirds (Brown *et al.*, 2015b). More information on this revegetation project is given in Appendix 1. Monthly weeding efforts, together with the planting around 40 bridled tern artificial nest tubes of young plants grown from

seeds of sea spinach (*Tetragonia decumbes*) and seaberry saltbush (*Rhagodia baccata*), resulted in an overall decrease in weed cover and an increased cover of the pre-existing seaberry salt bush. The *M. preissiana* seeds that were planted around 10 artificial nest tubes yielded 9 plants which survived over 2 years and produced many fruits. Efforts to determine techniques for increasing the survival of young native plants and reducing weed cover are ongoing. Recovering native vegetation cover will greatly help breeding bridled terns regain nesting grounds that were lost to weeds on Penguin Island.



Figure 3.8 Growing re-introduced Australian Hollyhock *Malva preissiana* over an artificial nest tube for bridled tern (*Onychoprion anaethetus*) on Penguin Island, Western Australia.

3.7 Capture and banding of bridled terns

Adult bridled terns and fledglings were caught using long-handled nets that had a fine and soft mesh so that the birds would not become injured or have their feathers bent inside the mesh. Chicks were caught by hand following the protocol in sections 4.8 and 4.9 of ‘The Australian Bird Bander’s Manual’ (Lowe, 1989). After trapping, the birds’ legs were checked for bands that bear a unique identifying code. In instances where bridled terns did not have a band, they were ringed using alloy bands provided by the Australian Bird and Bat Banding Scheme (ABBBS; Lowe, 1989). Chicks were banded between 1 to 4 days of age as their legs are almost fully developed when they hatch, so that the bands fit and do not pose a risk of injury to the chick but are not

overly loose so they would not fall from the legs (section 5.8 of ‘The Australian Bird Bander’s Manual’, Lowe, 1989). Adult bridled terns that were not banded were ringed following the protocol in section 5.1 of ‘The Australian Bird Bander’s Manual’ (Lowe, 1989).

Chicks were caught early in the mornings so they were easy to locate, trap and measure (see Chapter 5). This ensured that chicks would not have received their first meal of the day (first feed tended to occur after 7.30 am, personal observations) and would not regurgitate their food when being handled. However, adult bridled terns were caught at night time using a white or red light head torch which would temporarily attract the birds’ attention. Poot *et al.* (2008) showed that migratory seabirds were attracted to red wave-lengths to some extent, so using this technique provided an opportunity to trap adult birds that would otherwise be difficult to catch once they see incoming researchers, particularly during day time. Adults were caught at least one hour after dusk so that the chicks would have received their meal before their parents were caught (last feed tended to occur before 9 pm, personal observations).

3.8 Justifications

Gregory *et al.* (2004) explained that colonial nesting birds, such as seabirds, were better studied by selecting a sample of the colony and extrapolating the results so that only a small part of the breeding population would be exposed to stress induced by researchers entering the colony. They also showed that it was a more practical option than surveying an entire breeding colony which can have large numbers of birds scattered over extensive areas, which is the case of bridled terns breeding on Penguin Island.

The methods used for catching bridled terns and their chicks/fledglings were simple and had been successfully implemented numerous times before this study.

Furthermore, using the catching methods described above reduced the likelihood of birds becoming injured and stressed, so that birds trapped in devices such as mist nets would not be left exposed to predators and temperature changes or rain (Gregory *et al.*, 2004). Indeed, breeding birds need to incubate their eggs and guard their chicks, so they had to be caught and processed over very short periods of time to ensure that it would not impact their chick’s development and survival (Gregory *et al.*, 2004).

Moreover, using long-handled nets ensured that no bird which was not part of this study was caught, so this selective catching approach was chosen over other methods such as mist nets, noose traps and canon nets. Using this method also meant that no trapping equipment would be forgotten on site, such as concealed noose traps (Gregory *et al.*, 2004).

The ringing of birds in this study was necessary to follow breeding pairs and their chicks throughout the breeding seasons. It ensured the accuracy of the data collected (Gregory *et al.*, 2004) so that a chick's growth rate was attributed to the right parents for example (see Chapter 5).

3.9 Bridled tern age determination

In order to investigate the main hypothesis of this study, it was essential to be able to reliably age bridled terns. Unfortunately, once seabirds reach sexual maturity, they display no anatomical marker of their age (Nisbet, 2001). Plumage and moult patterns can be used to determine the age of immature birds (Lowe, 1989); however, seabirds can have long life expectancy (sometimes of several decades) and techniques to estimate their age after they have reached maturity are critical not only to establish population dynamics in colonies, but also to be able to determine the susceptibility of birds of different ages to their changing environment. Therefore, being able to reliably age birds is a crucial component to answering many ecological questions (Nisbet, 2001).

In the case of bridled terns, not only is there a lack of physical dimorphism between the sexes, but there are also no physical cues that could indicate their age (del Hoyo *et al.*, 1996). Therefore, in the context of this study, an emerging molecular technique was trialled to determine if it were possible to age bridled terns using levels of pentosidine (a bio-marker of oxidative damage that affects proteins and which is related to telomere length in some species of birds) in small patagial skin samples (Cooey *et al.*, 2010; see Chapter 3). Unfortunately, no correlation was found between the amount of pentosidine in the skin samples and the bridled terns' ages ($n=13$), which may possibly be due to the fact that in seabirds physiological and chronological age may be decoupled (Rattiste *et al.*, 2015). So instead, the birds' band numbers were used as an estimation of their ages:

- If an adult bridled tern had been banded as a chick, then its accurate age was known.
- If an adult bridled tern was un-banded, then it was considered as a young recruit and its age was estimated to be equal to the minimum age at first breeding in the bridled tern colony breeding on Penguin Island (3 years).
- If a bridled tern was previously banded as an adult, then the numbers of years elapsed since it was first captured (and banded) was added to the minimum age at first breeding in the Penguin Island colony (3 years) to obtain an estimate of its real age. For example, if an unbanded bridled tern was caught 5 years prior to being caught in the present study and was banded at the time, then its estimated age would be 8 years old (5 years + 3 years).

This was only possible thanks to an ongoing yearly capture-mark-recapture project that has been carried out on Penguin Island since 1982 on the population of bridled terns (see Dunlop *et al.*, 1988).

3.10 Sex determination of bridled terns

As explained previously, there is no evident sexual dimorphism between adult bridled terns. In some instances, the male of a pair may be slightly bigger than the female, but this difference can be difficult to observe in the field (del Hoyo *et al.*, 1996).

Therefore, molecular sexing methods were used instead. One adult of each pair had a sample of chest feathers taken from the edge of the brood patch when it was incubating the egg (in bridled terns, parents share incubating shifts and chick feeding/guarding shifts). The birds were held in clean cotton bags while the feather was removed using clean forceps and placed inside a paper envelope that was immediately frozen at -15°C. At the end of the breeding seasons, the feathers were sent to DNA Solutions Pty Ltd where the sex of the birds were determined using standard molecular analysis for birds (using the CHD-1 gene which is present on both the W and Z bird chromosomes, Jensen *et al.*, 2003). Hence, the feathers were not stored for longer than 4 months at a time. The sex of the other birds of the pairs that were not sampled were inferred from the results. Using this technique insured the reliability of the sexing results while causing minimum stress to the birds. Indeed, it was believed that the removal of a chest feather close to the bare brood patch would not cause pain to the birds as they are not vascularised and the calamus of the feather is held in the outer layer of the skin (Lowe, 1989). Therefore, removing a chest feather did not cause

any bleeding in brooding bridled terns and did not impair the birds' flying abilities or insulation.

3.11 Climatic and oceanic conditions

The monthly averages for maximum air temperatures, minimum air temperatures, sea surface temperatures (SST), as well as the monthly total precipitations, were retrieved from the Australian Bureau of Meteorology's (BoM) website for the duration of this study (Figure 3.9). Values for the maximum air temperatures, minimum air temperatures and total precipitation were taken from the Garden Island HSF meteorological station, which is the closest meteorological station to Penguin Island, and the SST were obtained from the Rottneest Island offshore logger, which is also the closest floating station from Penguin Island. The breeding season of bridled terns breeding on Penguin Island coincides with the warmest months and lowest rainfall records between October and May each year (Figure 3.9).

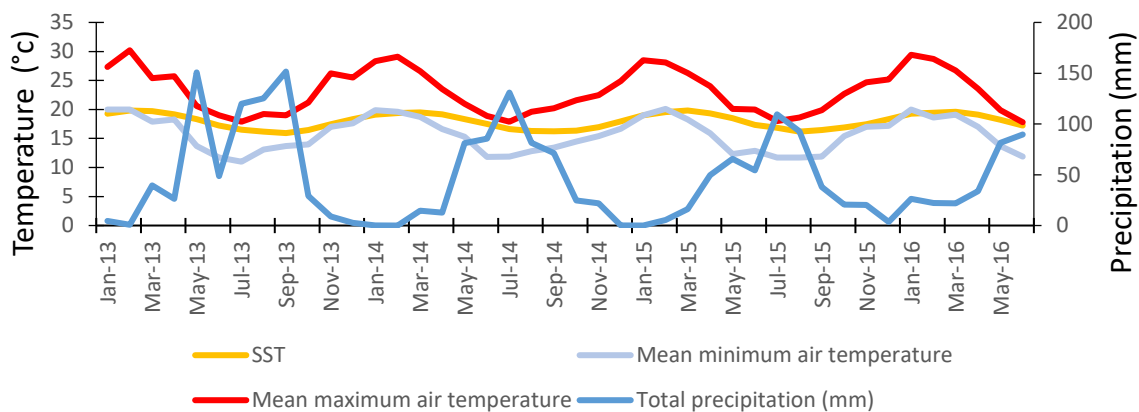


Figure 3.9 Monthly averages for air (maximum and minimum) temperatures, sea surface temperatures (SST) and total precipitation (as measured at the Garden Island HSF meteorological station and the Rottneest Island offshore data logger) from January 2013 until June 2016. Data retrieved from the Australian Bureau of Meteorology (www.bom.gov.au).

The Integrated Marine Observing System (IMOS) noted that since June 2014, the Southern Oscillation Index (SOI) had been in El Niño phase, and a “Godzilla El Niño” took place in 2015 (IMOS, 2016; marked by consecutively low SOI values, Figure 3.10) with a record percentage of the world's oceans displaying record high SST in December 2015 (IMOS, 2016).

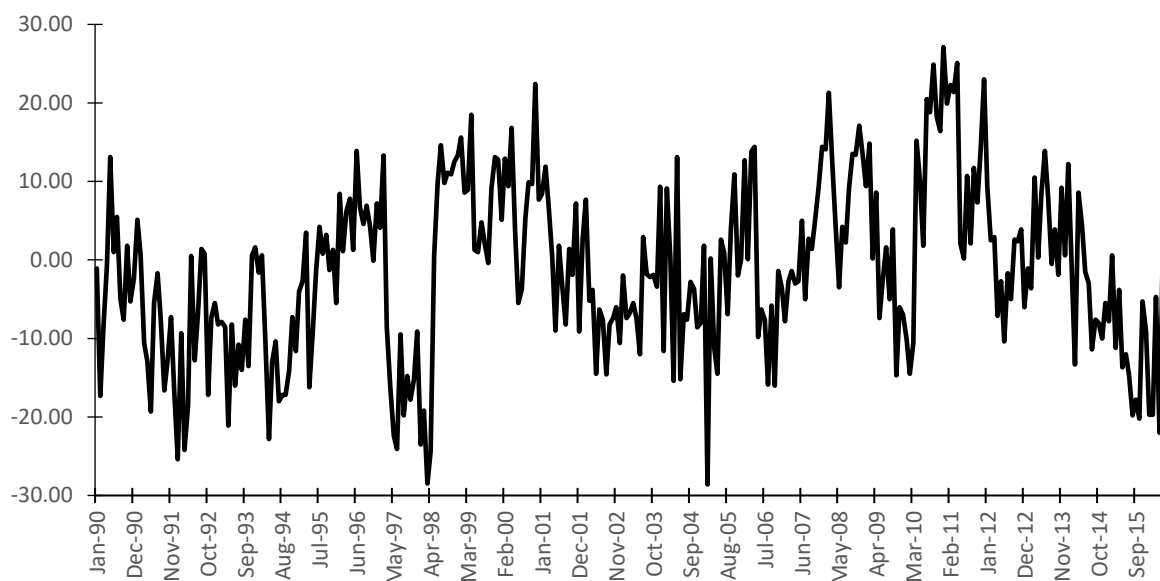


Figure 3.10 Southern Oscillation Index values from January 1990 until June 2016. Data retrieved from the Australian Bureau of Meteorology (www.bom.gov.au).

The Leeuwin current typically strengthens in winter or during La Niña when there is a sharper difference between the SST off the coast of Western Australia and Indonesian waters (IMOS, 2016). However, in November 2013, IMOS noted a diversion in the Leeuwin current along the northern coast of Western Australia due to strong eddies near the continental slope so that there was an overall decrease in the amount of tropical water flowing towards south-western Australia at the start of the 2013-2014 breeding season of bridled terns on Penguin Island.

3.12 Permits and licences

This study was undertaken in compliance with university, state and federal permits (Table 3.1).

Table 3.1 Permits and licences applying to this study.

Permit/licence/registration	Department/institution
Risk Assessment Murdoch Project	Murdoch University
Animal Competency and Experience Registration	Murdoch University
Animal Ethics Committee Permit (R2603/13)	Murdoch University
Banding Authority Number 2943	Australian Bird and Bat Banding Scheme
Regulation 4 (Authority to enter CALM land and/or waters): CE005025, CE004529, CE004213	Department of Parks and Wildlife
Regulation 17 (Licence to take fauna for scientific purposes): SF009937, SF009561, SF010526	Department of Parks and Wildlife
Regulation 23 (Licence to take and mark fauna for research purposes): BB003316, BB003167	Department of Parks and Wildlife
Animal Research & Ethics Committee Approval 2014-3	Perth Zoological Parks Authority
Wildlife Trade Permit WTP-1536889-1695	Australian Government Department of the Environment
Integrated Cargo System Registration	Australian Customs Service
Licence to use animals for scientific purposes	Department of Agriculture and Food

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4. Skin pentosidine does not correlate with age in a long-lived seabird

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This chapter is to be submitted to the journal of Marine Ornithology. To maintain consistency with the rest of the thesis, the acknowledgements, key words, and authors' affiliations were removed but the references were kept in the journal's style. Otherwise, the text is identical to that of the manuscript.

4.1 Abstract

The ability to age organisms is important for investigating ecological processes. In particular, seabirds, which form a major part of the coastal and marine ecosystems, may use and respond to their environment differently based on their age. This study aimed to determine whether pentosidine, a biological marker of age which has previously been shown to accumulate in other avian species, could be used to age bridled terns (*Onychoprion anaethetus*) breeding on Penguin Island, Western Australia. It was found that patagial skin collagen-bound pentosidine cannot be used to determine the age of bridled terns because of low collagen levels in skin samples and that overall, there was a low recapture rate of birds born on the island (less than 5% of the birds trapped in this study). Bridled terns' diet and life at sea may explain their low skin collagen levels but some molecular adaptations and antioxidants from their diet may help them resist oxidative challenge. Furthermore, it is postulated that bridled terns born on the island may recruit in newly established colonies further south.

4.2 Introduction

In order to investigate the ecological processes associated with the population demographics of organisms, it is essential to be able to determine the age of their individuals. For example, the ability to age organisms will help address questions relating to intraspecific resource partitioning among the different age classes of a population (Young et al. 2010, Kappes et al. 2011). Such knowledge will help articulate better-informed population management and conservation strategies (Chaney et al. 2003, Fallon et al. 2006).

In particular, shorebirds and seabirds, which rely on aquatic environments, have life strategies that can include elements of migration, dispersal and colonial breeding (del

Hoyo et al. 1992). Such strategies are age-dependent and it is therefore crucial to be able to determine how individuals of various ages use their environment. Some of the outstanding questions in seabird population dynamics relate to the locations where immature birds disperse before they recruit into either their natal colony or a different colony to breed (Votier et al. 2011). Another key question includes whether newly established breeding colonies of seabirds consist of older individuals that were displaced from other breeding grounds or of young birds in search of unclaimed territory where they can establish their nests (Dunlop, 2009). Such questions have been difficult to answer because both shorebirds and seabirds display no anatomical marker of their age once they reach sexual maturity (Nisbet, 2001). Therefore, while some immature birds may have some plumage characteristics and differing moult patterns that can be used to distinguish their sexual maturity, it is often not possible to infer the age of adult seabirds (Lowe, 1989).

The main method used to infer a bird's age is banding (Fallon et al. 2006). Banding a juvenile bird with a leg band that has a unique identifying code and/or colour bands not only allows ornithologists to identify individual birds but also allows them to reliably age them upon recapture/resighting (Lowe, 1989). However, unless a bird was banded the year it was born, it is not possible to determine its exact age. An adult bird's real age can only be inferred as the number of years since it was first caught and banded (as an adult) plus the minimum age at which adult plumage is developed, which is often the age at first breeding in seabirds (Reed et al. 2008). While this method can be reliable and useful to age birds, it has limited applications in studies which focus on bird populations with few or no banded individuals.

The aim of this study was to determine if pentosidine, a biological marker of age, could be used to infer the age of bridled terns (*Onychoprion anaethetus*), a long-lived species of seabird that breeds on islands off the coast of Western Australia. Pentosidine is a naturally occurring biological product that accumulates over time in living organisms (Chaney et al. 2003). It has been shown to be a successful biomarker of age in several species of land birds and is a result of glycative and oxidative damage to proteins that occurs over their lifetime (Chaney et al. 2003, Fallon et al. 2006, Cooey 2008). Both diet and physical activity have been shown to influence pentosidine accumulation in the skin of birds, however, young birds generally have less epidermal collagen-bound pentosidine than older birds and the pattern of pentosidine accumulation in avian skin is

either linear, quadratic or exponential depending on the species (Iqbal et al. 1997, Chaney et al. 2003). Therefore, pentosidine accumulation offers a unique opportunity to age birds (Iqbal et al. 1997, Chaney et al. 2003). A number of known-age individuals can be sampled to determine their respective epidermal pentosidine levels and an age curve can thus be constructed (Chaney et al. 2003, Fallon et al. 2006). Subsequently, individuals of unknown-age can be sampled and their age determined using the amount of pentosidine in their skin and extrapolating chronological age from the curve (Chaney et al. 2003, Fallon et al. 2006).

The objective of this study was to determine if such an age curve could be constructed by using known-age individuals in a breeding population of bridled terns on Penguin Island in south-western Australia. This population has been studied continuously since 1982, so that during each breeding season (in summer) a number of chicks were banded. Therefore, it was assumed that a proportion of the population breeding on Penguin Island would consist of known-age individuals that have come back to their natal colony to breed; 2,552 chicks have been banded since 1991 and the current breeding population is estimated to be about 8,000 birds (Dunlop, 2009).

4.3 Materials and Methods

4.3.1 Trapping of birds

Between the summer months of November 2014 and March 2015, adult bridled terns were caught during night time using head torches and long-handled nets. The whole island was searched for known-age birds which had been banded as chicks in previous years using alloy bands provided by the Australian Bird and Bat Banding Schemes. If a bird had a band number that was on the list of bridled terns banded as chicks, then it was brought back to the field station for sampling.

4.3.2 Skin sampling

When a known-age bridled tern was trapped, a patagium sample from its left wing was taken following the sampling protocol refined by Cooley (2008). The bird's head was placed in a clean cotton bag to reduce stress and the bird was then placed on its back with its left wing extended and its right wing tucked against its body. The area of the inner patagium was cleaned using alcohol swabs and any feathers covering the area to be sampled was removed using forceps. A subcutaneous injection of lignocaine was administered to lift the epidermis and to also anaesthetise the local area (0.1 mL

lignocaine diluted 50:50 with saline). A 4 mm² biopsy punch was then used to outline the area of skin to be sampled. Clean forceps and scissors were used to cut the delimited sample area and the skin sample was placed in distilled water and immediately frozen. The patagium was then dried using gauze and the area of the biopsy closed using 3M® Vetbond Tissue Adhesive before releasing the bird.

4.3.3 Sample analysis

The samples were sent to the University of West Virginia for analysis. They were thawed and prepared (scraped and minced) before using the standard processing procedures from Cooley et al. (2010) which involves delipidation, rehydration, acid hydrolysis, acid evaporation, rehydration and filtering. To determine the collagen content in the samples, 30 µg sub-samples were assessed through spectrophotometric hydroxyproline analysis.

4.4 Results

Only 13 birds out of the 274 bridled terns that were caught during the sampling periods were of known-age, so that less than 5% of the birds that were caught were of known-age. The samples were taken from birds that ranged between four and 23 years.

Because the samples contained very little hydroxyproline and different sample amounts did not yield higher results, the samples were not run in the reverse-phase high-performance liquid chromatography machine to determine pentosidine concentration as this would have yielded skewed and unreliable results (Cooley, pers. comm., Rattiste et al. 2015). There was very little collagen in the 30 µg aliquots of each sample (mean=0.006814 mg, s.e.= 0.002005; Fig 4.1).

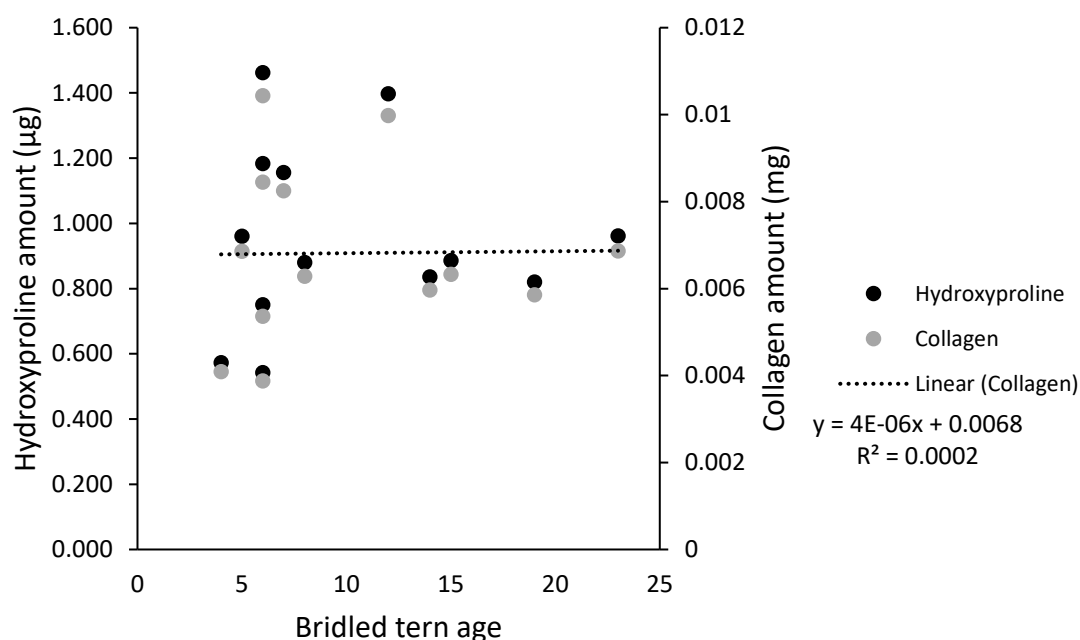


Figure 4.1 Hydroxyproline and collagen amounts in 30 µL aliquots of patagial skin samples in relation to age from bridled terns (*Onychoprion anaethetus*) breeding on Penguin Island, Western Australia.

4.5 Discussion

4.5.1 Pentosidine in bridled terns

The low collagen readings were unexpected since this had not been an issue in previous studies. The standard curves for the hydroxyproline analysis indicated that the machine functioned properly and there was no issue with the standards used in the laboratory analysis (Cooey, pers. comm.). All avian species that had previously been included in pentosidine studies yielded results that could be used to age individual birds (Chaney et al. 2003, Fallon et al. 2006, Cooey et al. 2010), with the exception of common gulls (*Larus canus*) from Estonia (Rattiste et al. 2015).

Both bridled terns and common gulls are seabirds of the Charadriiforme order. However, other species of seabirds had previously been included in pentosidine research and showed a linear increase between skin pentosidine concentration and age; these include double-crested cormorants (*Phalacrocorax auritus*) and California gulls (*Larus californicus*) (Chaney et al. 2003, Fallon et al. 2006). Rattiste et al. (2015) suggested that physiological and chronological age may be uncoupled in common gulls since they found no correlation between telomere length, skin pentosidine concentration and age in common gulls. Yet, they found strong evidence for breeding senescence in their study

population which included birds aged between two and 30 years. Such an explanation could also apply to bridled terns.

The low skin collagen content in the samples could be explained by the smaller sizes of the biopsies used in this study, the birds' diet, high UV exposure, or timing of moult. However, Cooley's (2008) research indicated that similar results were obtained whether a 4 mm² biopsy was taken or a standard 6 mm², so the smaller size of the biopsies used in this study was not believed to have contributed to the low skin collagen content of the samples. Instead, the diet of bridled terns may explain this result. Bridled terns breeding on Penguin Island primarily feed on small fish (particularly mullid larvae and small clupeids), crustaceans (Columbus crab megalopae) and cephalopods (Dunlop, 1997). Such a diet will result in high blood glucose levels which is typical of seabird species (Holmes et al. 2001), and this in turn can negatively affect the amount of skin collagen. Furthermore, exposure to UV radiation, which is prevalent in tropical and sub-tropical marine environments, and to salt spray, may also contribute to lower collagen content in the skin; particularly as the small white feathers covering the inner patagium may not provide much protection against UV radiation (Eyre et al. 1984; Gabrijelčič et al. 2009). It is also possible that the amount of collagen in the skin of the patagium may vary in relation to moult.

Interestingly, high blood glucose levels and pro-oxidants such as UV radiation would also contribute to increased levels in advanced glycation end products, including pentosidine (Holmes et al. 2001). However, some avian physiological traits could be playing a role in reducing and mitigating those effects. Holmes et al. (2001) proposed that birds might produce fewer reactive oxygen species and have shown that avian cells appeared to be more resistant to oxidative stress, probably with higher aminoguamide levels which prevent the production of advanced glycation products, and also by having a diet high in omega-3 and anti-oxidants. Therefore, bridled terns' diet and lifestyle in the marine environment could contribute to low collagen content in the skin of the patagium and reduced pentosidine levels in that collagen.

It would be beneficial to conduct a similar study in another long-lived seabird species to determine if similar results are found.

4.5.2 Low recapture rate of known-age individuals

The low recapture rate of individuals born on Penguin Island compares with Dunlop and Jenkins's (1994) recapture of 53 known-age individuals out of 603 captures (about 9% recapture rate). This could either indicate a high mortality rate in individuals that were born on the island and/or that Penguin Island acts as a "source" population with young birds that recruit to other colonies to breed. The latter is likely to be the case since Penguin Island is now fully occupied by established bridled tern pairs, which increases the probability of dispersal of new recruits to other areas. Furthermore, a significant breeding range expansion of bridled terns was recorded in south-western Australia over the past decades (Dunlop, 2009). To confirm whether this is the case, it would be necessary to recapture banded individuals at those new southern breeding colonies and see if some of those birds were banded as chicks on Penguin Island.

It is also worth noting that the number of fledglings banded each year represented a small proportion of the total number of fledglings across the island.

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5. Effects of age on the reproduction of a long-lived tropical seabird: the bridled tern

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5.1 Abstract

The effect of age on the breeding performance of marine birds has been demonstrated in many species but had yet to be studied in bridled terns *Onychoprion anaethetus*. Bridled terns breeding at the Penguin Island colony in Western Australia, where this species has been expanding its breeding range because of changing climatic conditions and increased prey availability, were observed over three consecutive breeding seasons and had their breeding parameters recorded. Three different analyses (two cross-sectional and one longitudinal) found no effect of age on the breeding variables that were considered except breeding success. This could be because: younger birds are still refining their foraging skills and do not have the endogenous reserves to produce an egg, there is no effect of age on the post-laying breeding performance in this species of seabird, or individual variability blurred any effect of age on breeding, and bigger sample sizes would be needed to disentangle this effect. Laying date was significantly influenced by the breeding season and associated oceanic climate. Additionally, there may be an indication for a shortening in the time taken for a chick to fledge at the colony level, which could be in response to changing oceanic conditions and associated prey availability. Prey availability may be an important factor in age-related breeding success at this colony and possibly more generally.

5.2 Introduction

The effects of age on reproduction in seabirds have been the topic of extensive research for 60 years (Fowler 1995, Bradley & Safran 2014). Because most species of seabirds are long-lived with multiple reproductive cycles over their lifetime, they have several opportunities to breed before they die (Pianka & Parker 1975, Curio 1983). In many species of marine birds, ornithologists have noted an improvement in breeding

performance (ability to successfully fledge a chick) with age and a subsequent decrease associated with senescence (Curio 1983, Fowler 1995). It has been postulated that once a seabird becomes sexually mature and recruits into a breeding colony (sometimes several years after it fledged), its initial breeding performance is poorer than that of older breeding birds, but following the first breeding attempt, breeding performance improves to reach a performance plateau (Curio 1983, Fowler 1995, Bradley & Safran 2014). When the seabird becomes senescent, its breeding performance declines shortly before the bird dies (Fowler 1995, Rebke *et al.* 2010).

Such changes in breeding performance can mostly be explained by three major processes occurring at either the population or at the individual level. The first is gaining breeding experience over several breeding attempts (Curio 1983, Bradley & Safran 2014). This occurs in most species of marine birds and may be the main driver of observed improvements in breeding (Bradley and Safran 2014). Breeding skills are refined and there is better coordination between partners for care of the chicks and foraging (Limmer & Becker 2010). Another process occurring at the individual level is breeding senescence; that is, the deterioration in body condition and ability to reproduce with age (Fowler 1995, White *et al.* 2008, Rebke *et al.* 2010). This is mostly observed in long-lived species such as black-browed albatrosses *Thalassarche melanophris*, and occurs towards the end of a bird's life when a physiological decline in its breeding condition prevents it from investing resources into breeding (Pardo *et al.* 2013). The third process, which influences breeding performance at the population level, is the selective disappearance/appearance of low/high quality individuals from a colony over time (Curio 1983, Pyle *et al.* 1991). Individuals of lower quality that reproduce less well are selected against whereas individuals of high quality that reproduce better may enter the colony, leading to a notable improvement in a colony's reproductive performance over time. Finally, there is also some evidence that as some seabirds get older and their chances to reproduce in the future decrease, they will increase their reproductive effort which would ultimately increase their reproductive performance (Pianka & Parker 1975, Pyle *et al.* 1991, Velando *et al.* 2006). However, reproductive effort in seabirds is difficult to measure in the field (Pyle *et al.* 1991, Wendeln & Becker 1999).

Age effects on breeding performance in seabirds have been studied in many ways over the past decades. Initial studies investigated the link between birds' ages and directly observable breeding parameters, and in particular breeding success (whether a seabird

pair produced a live fledgling following a breeding attempt). Field-based studies used directly measurable parameters such as laying date, clutch size, egg weight, egg size, hatching success, chick weight, fledging success, habitat quality and pair bond duration (for example, Richdale 1949, Coulson 1966, Bogdanova *et al.* 2006). However, longitudinal databases and models are increasingly used to study the links between seabirds' ages and their breeding performances/lifetime reproductive success. These studies estimate breeding probability, survival probability, fledging rates and yearly breeding success (for example, Cam & Monnat 2000, Rattiste 2004). They are also useful to study trans-generational effects, to determine if a seabird's age will affect the viability and recruitment probability of the descendants of its own progeny (Bouwhuis *et al.* 2015). Other studies take a biochemical approach by relating physiological parameters to seabirds' ages and their breeding performance. For example, hormones such as corticosterone and prolactin, as well as blood glucose, metabolites, and chick telomere length, are used to understand the physiology of the mechanisms underlying the effects of age on breeding (Angelier *et al.* 2007, Elliott *et al.* 2014, Heidinger *et al.* 2016). Finally, recent studies apply behavioural approaches. For example, wandering albatrosses' personalities (boldness/shyness) were assessed and related to the birds' ages and breeding performances (Patrick & Weimerskirch 2015). Therefore, there is a multitude of ways to investigate how age affects seabirds' breeding performances.

While age effects on seabirds' breeding have been studied in over 40 species (Chapter 2), it is yet to be investigated in bridled terns *Onychoprion anaethetus*. Bridled terns are long-lived tropical to sub-tropical seabirds with a global distribution (Gochfeld & Burger 1996). Bridled terns breeding on Penguin Island in south-western Australia recruit in the colony at three years of age and have a life expectancy of over 20 years with one of the oldest breeding terns captured at 28 years of age (Dunlop & Jenkins 1992, Dunlop 2009). They breed annually in summer (their breeding season on Penguin Island takes place between October and April) and lay one egg (Dunlop & Jenkins 1992, Garavanta & Wooller 2000). The Penguin Island population is of particular interest because it has undergone remarkable growth since the first breeding pairs settled in the 1960s, and this is believed to reflect an increase in prey availability within foraging range of the island brought about by changing climatic and oceanic conditions (Dunlop 2009).

The aim of this study was to determine if age affects breeding performance in bridled terns in the Penguin Island colony. The following questions were investigated: Do older bridled terns breed earlier, lay bigger eggs, incubate their eggs for shorter periods of time, have chicks that grow bigger, faster and fledge earlier and have a higher breeding success than younger birds?

Furthering our knowledge on this species of marine bird would also help inform conservation and management strategies, not only for Penguin Island, but for other populations of bridled terns as well.

5.3 Methods

5.3.1 Data collection

This study was conducted on Penguin Island in south-western Australia (32°305'S, 115°690'E) where a colony of bridled terns first settled in the 1960s, and a yearly banding program has been taking place since 1982 (Dunlop 2009). The study site was on the north-east side of the island (where some of the oldest birds were believed to be located, Dunlop & Jenkins 1994) over three consecutive breeding seasons: summer 2013/2014, summer 2014/2015 and summer 2015/2016, when bridled terns were back from their migration to the Sulawesi Sea during the austral winter (Dunlop 2009).

In September 2013, 50 artificial nest tubes were installed on top of the cliff where the native succulent shrubland cover had been lost to introduced grasses. It was assumed that such nest tubes would attract young breeding pairs to unclaimed territories as most of the island has been claimed by breeding bridled tern pairs that have high nest site fidelity (Dunlop & Jenkins 1992). Additionally, natural nesting spots along the cliff were identified and included in this study.

Breeding adults inside natural and artificial nests were trapped at night using long-handled nets and head-torches to determine their age and sex. A chest feather adjacent to the brood patch was sampled for one adult in each breeding pair to identify its sex using standard molecular analysis for avian sex determination (see Jensen *et al.* 2003). To determine the birds' ages, their band numbers were checked. If a bird did not have a band, then it was banded upon capture and its age was inferred to be at least 3 years old (the youngest age at which bridled terns recruit to the Penguin Island colony, Dunlop & Jenkins 1992). If a bridled tern already had a band and was banded as a chick, then its true age was known. However, if a bridled tern was banded as an adult, then its age was

inferred to be the minimum age of recruitment plus the number of years elapsed since it was banded. All alloy bands used were supplied by the Australian Bird and Bat Banding Schemes (Lowe 1989).

The justifications for using this aging technique were: a molecular technique based on the amounts pentosidine in birds' skin samples was trialled for aging bridled terns but did not work (Chapter 4; Rattiste *et al.* 2015), therefore this study had to rely on band numbers instead. Similar studies also used band numbers to infer seabirds' ages and 'time elapsed since first capture' plus the minimum age at first breeding was found to be a reliable method for estimating the birds' ages and investigating the effects of age on breeding performance (for example, Crespín *et al.* 2006, Reed *et al.* 2008). Because less than 5% of the breeding birds were adults that were banded as chicks, it was not possible to compare the age distributions between known-age birds and birds that were banded as adults. However, since 1986, over 7,000 bridled terns were banded. The colony on Penguin Island is estimated to be between 6,000 and 8,000 birds (Dunlop & Rippey 2006), and more than 60% of the bridled tern colony on Penguin Island appears to be banded; consequently, the error in estimating age should be low. Nevertheless, instead of using individual birds' ages for the subsequent analyses, the ages of the adults of a pair were averaged for each pair (as there appeared to be age-assortative mating in this colony as explained below), and three age groups were used to reduce potential errors in estimating bridled terns' ages.

Throughout the breeding seasons, nest sites were visited either daily or every second day to determine whether the nesting spots showed signs of occupancy (bridled tern actively defending nesting sites and/or scratches/hollows at the nesting spots or inside the nest tubes) and the following variables were recorded for each nest following protocols established by the Australian Bird and Bats Banding Schemes (Lowe 1989, Table 5.1)

Table 5.1 Reproductive variables recorded for assessing the effects of age on bridled terns' breeding attempts and associated methods of measurements.

Variable	Recording method
Laying date	The presence of an egg in a nest was recorded and it was assumed to have been laid on the day it was observed since early in the breeding seasons nests were inspected daily
Egg weight	The egg weight was measured using a digital field scale on the day it was laid (precision ± 0.01 g)
Egg length	The egg length was measured using digital calipers (precision ± 0.01 mm)
Egg width	The egg width was measured using digital calipers (precision ± 0.01 mm)
Hatching date	The hatching date was recorded as the date on which a newborn chick was observed in the nest
Hatching chick weight	The newborn chick's weight was measured by placing the chick in a clean cotton bag and weighing it using a Pesola scale (precision ± 1 g)
Hatching chick wingspan	The newborn chick's hatching wingspan was measured by placing the chick on its back on a ruler and measuring the wings from tip to tip (precision ± 0.1 cm)
Hatching chick head and bill	Using butted digital calipers, the newborn chick's head and bill were measured (precision ± 0.1 mm)
Hatching chick tarsus	Using the sliding ruler of the digital calipers, the newborn chick's right tarsus was measured (precision ± 0.1 mm)
Date of appearance of flight feathers	The date on which pin feathers on the edges of a chick's wings were observed was recorded
Fledging date	For the purpose of this study, the date on which a chick was able to fly more than 10m away from an oncoming researcher without gliding was recorded as the fledging date
Fledgling weight	The fledgling's weight was measured by placing the chick in a clean cotton bag and weighing it using a Pesola scale (precision ± 1 g)
Fledgling wingspan	The fledgling's hatching wingspan was measured by placing the chick on its back on a ruler and measuring the wings from tip to tip (precision ± 0.1 cm)
Fledgling head and bill	Using butted digital calipers, the fledgling's head and bill were measured (precision ± 0.1 mm)
Fledgling tarsus	Using the sliding ruler of the digital calipers, the fledgling's right tarsus was measured (precision > 0.1 mm)
Breeding success	A bridled terns pair breeding success was recorded as whether they successfully produced a live fledgling or not (binary)
Incubation time	The number of days between egg laying and hatching was recorded as the incubation time
Lag for appearance of flight feathers	The number of days between hatching and the appearance of flight feathers was recorded
Raising time	The number of days between hatching and fledging was recorded as raising time
Growth rates	The growing chicks' weight, wingspan, tarsus and head and bill were measured weekly using methods described above. Regression curves were fitted to each variable in IBM SPSS 24 for Microsoft (quadratic for weight and head and bill, cubic for wingspan, and linear over the first 10 days of tarsus growth – all regression curves had R^2 values over 0.9) and the growth coefficients and constants were recorded for each chick

To determine whether the data gathered over the three different breeding seasons could be combined in the following analyses, a log-linear analysis for a three-way contingency table was performed in Vassarstats using overall breeding success/failure observations and two age categories (under six years of age and over six years of age) as well as breeding season as layers (Appendix 3). No effect of year was found on breeding success ($G^2_{AC,2} = 0.84, p=0.657$ and $G^2_{BC,2}=0.9, p=0.638$, where C corresponds to the breeding seasons, A the age category, and B breeding success/failure, Appendix 3) and therefore the data were combined for subsequent analyses.

5.3.2 Cross-sectional analysis

Egg and chick variables were plotted against the parents' averaged ages to visualise any effect of age on those variables. There only appeared to be a slight effect of age on laying date, wingspan growth and fledging weight with a possible small improvement before the age of 6 and a slight decline after the age of 12; therefore for the purpose of this analysis, parents were classified into three groups: under 6 years, between 6 and 12 years, and over 12 years.

Pairs that were observed over more than one breeding season were only included once in subsequent analyses so that samples were independent.

Four one-way MANOVAs were performed in IBM SPSS 24 for Windows to answer the following questions: Do younger birds lay smaller eggs and later during the breeding season? Do younger birds incubate their chicks for longer and hatch smaller chicks? Do older parents have chicks that grow faster? Do older parents fledge bigger chicks? To do this, all variables were range-standardised between 0 and 1 because they had different numerical scales and this could have affected the MANOVA results. All of the Box's tests for equality of covariance matrices were not significant with $p>0.001$, so this assumption held for the analyses (Tabachnick & Fidell 2001). The descriptive statistics for each variable are given in Appendix 2.

MANOVA analyses were chosen over other statistical analyses because plotting variables against the parents' averaged ages did not reveal any clear trend, so it was hoped that by combining the pairs in age categories, any difference in the breeding variables would be detected by having bigger sample sizes per group. Furthermore, four MANOVAs were carried out instead of one, otherwise the number of cases in the

groups would have been less than the total number of variables which is not recommended to perform this type of analysis (Field *et al.* 2012).

5.3.3 Longitudinal analysis

To test whether there was improvement in the breeding variables at the individual level, a general linear model analysis for repeated measurements (GLMRM) was performed in IBM SPSS 24 for Windows using bridled tern pairs that were observed over two consecutive breeding seasons and which were under six years of age ($n=12$). Breeding experience obtained over two breeding seasons was set as the ‘within subject factor’ (with two levels for the two consecutive seasons) and the reproductive parameters of individual pairs were set as the ‘between subject factors’. The assumption of sphericity was met for all tests ($p \geq 0.106$).

5.4 Results

5.4.1 Overview

A total of 73 bridled tern pairs (25 of which nested inside artificial nest tubes) were included. The youngest birds were least three years old (they were banded as adults and had breeding plumage with dark cap feathers). The oldest bird was a 28-year-old male. Only five adults were banded as chicks and hence their exact ages were known. A total of 15 pairs were observed over three consecutive breeding seasons (ages varying from three to 12 years) and 12 pairs under the age of six were observed over two consecutive breeding seasons. There was no significant difference between the ages of the adults in the pairs (paired t-test $p > 0.847$, $n=41$), so there was evidence for age-assortative mating.

The breeding success across the three breeding seasons and all age classes was 47% (including pairs that did not lay eggs but guarded nesting spots). Average egg weight at laying was 25.0 g (± 2.2 g, s.e.), average egg width was 3.2 cm (± 0.1 cm), average egg length was 4.6 cm (± 0.2 cm). Incubation time was on average 30 days (± 2 days) and the mean chick hatching weight was 17.2 g (± 2.5 g), hatching wingspan 9.6 cm (± 0.6 cm), hatching head and bill 34.0 mm (± 1.2 mm) and hatching tarsus 17.1 mm (± 1.1 mm). It took on average 10 days for pin feathers to appear (± 2 days) and 52 (± 5) days for bridled terns to raise their chicks until they fledged.

Chicks’ weight growth curves and head and bill length growth curves were quadratic (all R^2 values > 0.9 ; Figure 5.1) and followed the following regression equations:

$$Y_1 = 12.785 (\pm 4.530) + 5.181 (\pm 1.145) x - 0.074 (\pm 0.116) x^2,$$

$$Y_2 = 34.766 (\pm 1.258) + 1.290 (\pm 0.103) x - 0.012 (\pm 0.002) x^2$$

where Y_1 is the chick's weight (g), Y_2 is the chick's head and bill length (mm), and x the number of days since hatching.

Chicks' wingspan growth curves were cubic (all R^2 values > 0.9 ; Figure 5.1) and followed the following regression equation:

$$Y_3 = 9.378 (\pm 0.751) + 0.653 (\pm 0.322) x + 0.020 (\pm 0.074) x^2 + 0.001 (\pm 0.006) x^3$$

where Y_3 is the chick's wingspan length (cm) and x the number of days since hatching.

Chicks' tarsus growth rates over the first ten days were linear (all R^2 values > 0.9 ; Figure 5.1) and followed the following regression equation:

$$Y_4 = 16.991 (\pm 1.047) + 0.721 (\pm 0.137) x$$

where Y_4 is the chick's tarsus length (mm) during the first ten days following hatching and x the number of days since hatching.

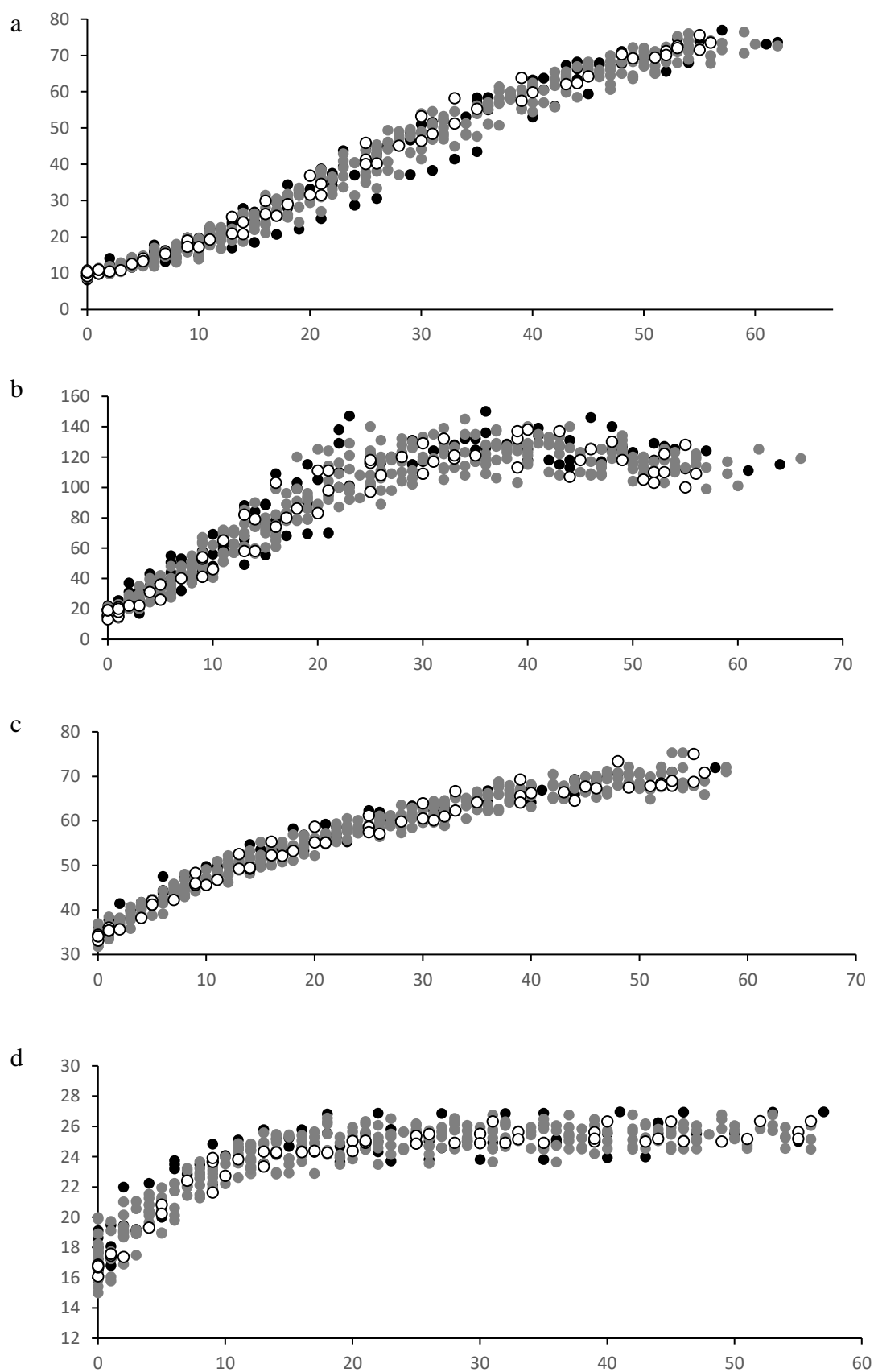


Figure 5.1 Bridled tern *Onychoprion anaethetus* chick growth curves. X-axis represents the number of days since hatching, a) wingspan (cm), b) chick weight (g), c) head and bill length (mm), d) tarsus length (mm). Black circles are for chicks from parents that were under 6 years

of age, grey circles are for chicks from parents that were between 6 and 12 years of age, white circles are for chicks from parents that were over 12 years of age.

On average, chicks' fledging weight was 118.1 g (± 8.6), fledging wingspan was 70.4 cm (± 3.2), fledging tarsus length was 25.5 mm (± 0.8) and head and bill length at fledging was 68.6 mm (± 2.8).

5.4.2 Cross-sectional analysis

The overall breeding success of bridled terns was greater for older birds: $G^2_{AB,1}=13$, $p=0.0003$ and $G^2_{AB(C),3}=13.88$, $p=0.0031$ so that even when the effect of year was removed, the difference in breeding success between the age groups was significant. However, this difference was only due to three-year-old pairs that did not lay an egg but were in full breeding plumage and were guarding nesting spots, so that they failed at breeding.

The one-way MANOVAs showed that there were no significant differences in the egg and chick parameters of parents of different ages. For determining whether there were differences in egg parameters and laying dates between the different age classes, a one-way MANOVA returned $F_{8,94}=1.065$, Wilks' $\lambda=0.841$, $p=0.395$. To investigate whether there were differences between the incubation time and chick hatching parameters between the age classes, a one-way MANOVA returned $F_{10,42}=0.493$, Wilks' $\lambda=0.801$, $p=0.885$. For determining whether there were differences in the chicks' growth rates between the age classes, a one-way MANOVA returned $F_{14,28}=0.834$, Wilks' $\lambda=0.498$, $p=0.630$. Finally, to investigate whether there were differences between the chicks' fledging parameters between the age classes, a one-way MANOVA returned $F_{8,34}=0.992$, Wilks' $\lambda=0.657$, $p=0.460$.

5.4.3 Longitudinal analysis

There did not appear to be any effect of breeding experience on the breeding parameters recorded in this study (Table 5.2). However, there appeared to be some indication that the laying date may have been the only variable influenced by breeding experience (Table 5.2), therefore a further GLMRM including 15 pairs that had been observed over three consecutive breeding season and whose ages ranged from 3 to 12 years of age (so not just young birds were included) was performed using breeding season as the within subject factor (with three levels for the three consecutive years) and laying date as the between subject factor. This revealed a significant difference in laying dates ($F=7.710$, $p=0.002$) with a significantly earlier laying date in the 2015/2016 breeding season than

the two previous breeding seasons for those bridled tern pairs (that included birds over six years of age).

Table 5.2 Effect of experience gained over two consecutive breeding seasons on breeding variables in bridled terns *Onychoprion anaethetus* breeding on Penguin Island, Western Australia (n=12 and pairs were under six years of age), tested using general linear models with repeated measurements in IBM SPSS 24 (Microsoft).

Parameter	Within subject factor	<i>F</i>	d.f.	Mean square	<i>p</i>
Laying date (Julian days)	Breeding experience	4.697	1,11	782.042	0.053
Egg width	Breeding experience	0.741	1,11	0.010	0.408
Egg length	Breeding experience	1.941	1,11	0.015	0.191
Egg weight	Breeding experience	0.992	1,11	1.276	0.341
Incubation time	Breeding experience	0.140	1,11	12.042	0.716
Chick hatching weight	Breeding experience	0.741	1,11	25.647	0.416
Chick hatching wingspan	Breeding experience	0.168	1,11	1.654	0.690
Lag for appearance of flight feathers	Breeding experience	0.181	1,11	3.375	0.679
Weight growth B1	Breeding experience	0.006	1,11	0.030	0.940
Wingspan growth B1	Breeding experience	0.052	1,11	0.014	0.823
Wingspan growth B2	Breeding experience	1.234	1,11	0.007	0.290
Raising time	Breeding experience	0.263	1,11	96.000	0.618
Fledging weight	Breeding experience	0.087	1,11	132.545	0.774
Fledging wingspan	Breeding experience	0.310	1,11	200.104	0.589

5.5 Discussion

No study had previously investigated the effect of age on the breeding parameters of this species of seabird. Surprisingly, this study found no effect of age on the breeding parameters of bridled terns besides breeding success, differing from published results on other seabirds.

5.5.1 Overview and comparisons with other studies

The low overall recapture rate of known-age bridled terns (birds banded as chicks on Penguin Island) is comparable to those of Dunlop & Jenkins's (1992) which ranged from 0% in October 1989 to 14% in March 1990. Averaged over the breeding season this recapture rate is 5.9% (compared with 3.4% of known-age birds included in this study), and this rate had increased to 9% in 1993 (Dunlop & Jenkins's 1994). This low recapture rate of known-age individuals could be due to the low number of chicks and fledglings banded per breeding season (generally under 300 fledglings per year for a population of 3,000-4,000 breeding pairs; Dunlop 2009), a high mortality rate in fledglings born on Penguin Island, and/or dispersal of chicks born on the island to recruit in southern colonies where there is less competition for nesting spots (Dunlop 2009, Feare & Doherty Jr 2011).

The oldest breeding bird in this study was a 28 year old male, which is older than the previous oldest individuals of 23 years (estimated age) and 19 years (real age, Dunlop & Pedelty, 2007). Such longevity has been observed in other tern species. For example, Caspian terns *Hydroprogne caspia* have a longevity record of 26+ years (del Hoyo *et al.* 1996), a 28 years old common tern *Sterna hirundo* was recorded on Bird Island in the U.S.A. (Heidinger *et al.* 2006), Arctic terns *Sterna paradisaea* have a longevity record of 34 years (del Hoyo *et al.* 1996), and a 32 year old sooty tern *Onychoprion fuscatus* was also recorded (del Hoyo *et al.* 1996). Bridled terns may have longer lifespans than the one recorded in this study, however only an intensive recapture program on Penguin Island could confirm whether there are older birds still breeding on the island.

Nevertheless, this shows that bridled terns have a long lifespan and a yearly breeding cycle, so they have many opportunities to breed. Hence an effect of age on breeding performance was expected in this study as it has been previously found in many species of long-lived seabirds.

There was also no significant difference in the parents' ages, suggesting that bridled terns pair with partners of similar ages, as previously shown (Dunlop & Rippey 2006). Age assortative mating is also found in other species of marine birds such as common terns (Ludwig & Becker 2008) and Arctic terns (Coulson & Horobin 1976). Age assortative mating would make age effects on breeding performance more apparent since older and more experienced individuals would pair with other older and more experienced partners, thereby leaving younger and inexperienced birds to find partners

among their own age classes (Ludwig & Becker 2008). Without any differences in the age and breeding experience between parents, the age effects on a pair's breeding performance would become more evident.

The breeding success of bridled terns breeding on Penguin Island over three breeding seasons (47% including birds that did not lay eggs but guarded nesting spots, and 66% excluding these birds) compares with a minimum breeding success of 35% that was previously observed on the northern end of Penguin Island (Garavanta & Wooller 2000). Garavanta & Wooller (2000) noted that their recorded breeding success may be lower than the actual breeding success as they may have lost track of a few chicks before they fledged and recorded them as dead. We are confident in our observed breeding success, because fieldwork was carried out every day or every second day and no chick disappeared. Broken egg shells in the nests and recovered carcasses of chicks were used to determine breeding failure. Eggs were preyed on by king skinks *Egernia kingii*, fell out of nests in limestone alcoves and cracked, or were abandoned and left cold for over five days. Chicks that died were preyed on by Australian ravens *Corvus coronoides* or in two cases appeared to have died from starvation. Although this study's breeding success was higher than the one recorded by Garavanta and Wooller (2000), it remains lower than the breeding success of bridled terns in other colonies such as the one on One Tree Island on the Great Barrier Reef which appeared to have a breeding success of 77% (Hulsman & Langham 1985). Bridled terns on Penguin Island face pressures from ravens, king skinks, mice *Mus musculus* and particularly human disturbances linked with the high number of tourists visiting the island when it is open to the public in summer (corresponding with the breeding season of bridled terns). These factors could contribute to low breeding success in this species of seabird (Garavanta & Wooller 2000). However, this was not believed to impair the ability of this study to determine effects of age on breeding performance in bridled terns as numerous breeding variables including all the stages of the breeding cycle were measured (from egg variables to fledging parameters); even if an egg was preyed on, at least its measurements could be compared with the eggs of other birds. Furthermore, the pressures experienced by bridled terns during their breeding season appeared to be uniform across age classes since breeding success was not significantly different between 3-5 year olds, 6-12 year olds and 13+ year olds when excluding birds that did not lay eggs.

The average egg and chick parameters used in this study are comparable with those measured in other studies but indicate that the chicks may put on weight faster and fledge earlier than those of other bridled tern colonies. Garavanta & Wooller (2000) had similar egg variables (egg width=3.22 cm, egg length=4.54 cm and egg weight=23.7 g compared with egg width=3.2 cm, egg length=4.6 cm and egg weight=24.9 g in this study). The incubation time was also similar (27-33 days for Garavanta and Wooller 2000, 28-30 days for Hulsman & Langham 1985, and 30 ± 2 days in this study).

However, chick weight gain was faster in this study (half of the asymptotic weight was reached at 10.5 days compared with 14 days previously recorded on Penguin Island, Garavanta & Wooller 2000, and 13.6 days on One Tree Island, Hulsman & Langham 1985). Time to fledging was also shorter in this study (51.9 days compared with 53-63 days previously recorded, Garavanta & Wooller 2000, and 55-60 days on the Great Barrier Reef, Hulsman & Langham 1985). This difference was not believed to be the result of using different regression equations for the weight curves (both Garavanta & Wooller 2000, and Hulsman & Langham 1985 used Gompertz equations whereas this study used quadratic regressions) as both methods had $R^2 > 0.9$ and residual plots were random. Techniques to determine time of fledging were also very similar and therefore were not believed to lead to differences in the results. Therefore, there appeared to be a shortening of the time taken for chicks to fledge in recent years in the Penguin Island colony. Rapid initial growth in chicks is believed to be a survival means for hiding from predators (Garavanta & Wooller 2000) and therefore could explain why bridled tern chicks on Penguin Island, which are facing multiple threats, are growing slightly faster. However, this faster growth rate could also reflect changes in food availability in recent years in the waters surrounding Penguin Island (Dunlop 2009).

5.5.2 Age and breeding performance in bridled terns

The lack of effect of age on the post-laying breeding performance of bridled terns breeding on Penguin Island is in sharp contrast with the majority of studies on other seabird species (Chapter 2). The only significant difference was the lower breeding success of birds under the age of six when birds that did not lay eggs (and therefore had a breeding success of 0) were considered. Such birds were not banded but were in full breeding plumage and displayed courtship behaviour as well as nest guarding behaviours and had scratched hollows inside their nesting spots. All of these pairs, apart from two (out of 20 non-laying pairs), guarded newly installed nest tubes. These nest

tubes were considered to act as unclaimed nesting spots which were believed to attract young recruits, as most of Penguin Island's ground cover is already used by established pairs of bridled terns with high nest site fidelity (Dunlop 2009). However, the nest tubes could also have been nesting locations of lower quality that would have attracted bridled tern pairs of 'lower quality', and which would not necessarily be dependent on age, but on individual quality (Cam & Monnat 2000). This could explain the lack of egg-laying in these unbanded pairs; however, seven of the artificial nest tubes also hosted banded pairs aged between 6.5 and 24.5 years that successfully bred.

Therefore, three possibilities arise: the design of this study was flawed with small sample sizes and age detection using alloy bands not being reliable, bridled terns may only need one breeding attempt to become experienced breeders, which has been observed in other species of seabirds (e.g., western gulls *Larus occidentalis*, Pyle *et al.* 1991), or there is no effect of age on the post-laying breeding performance of bridled terns, which has also been observed in a few species (e.g., common terns, Wendeln & Becker 1999). Age determination was not believed to be an issue because other studies had used 'time since first capture' as a proxy for real age and concluded that it was a reliable technique (Crespin *et al.* 2006). Furthermore, molecular methods of determining the chronological age of bridled terns were attempted with no result (Chapter 4) and there may be evidence to suggest that physiological and chronological age is de-coupled in these birds (Rattist *et al.* 2015). However, despite the extensive number of breeding parameters considered in this study, sample sizes could still be too small to overcome the possible issue of individual variability that would affect the recorded breeding variables. Additional sampling and an extensive banding program would be necessary to ascertain whether there is indeed no effect of age on the post-laying breeding in bridled terns, or if only one breeding attempt is necessary for bridled terns to gain the necessary skills for successful breeding.

Nevertheless, if the main difference between young and old breeding birds lies in the ability to produce an egg, as suggested by the results of this study, then the effect of age may occur at the pre-breeding stage. Egg production relies on the females' endogenous reserves and diet (Williams, 2005), so that if a bird does not have reserves and is unable to compensate by increasing foraging effort, then either it will not lay an egg or it will produce an egg later in the breeding season when food becomes more abundant. In some species of seabirds, young birds were found to be poorer foragers than older birds

(e.g. European shags *Phalacrocorax aristotelis*, Daunt *et al.* 2007). Therefore, younger bridled terns could still be refining their foraging skills when they recruit in a colony and this may impact their ability to produce an egg during the breeding season. This may be the only stage at which there is a clear effect of age on breeding in this species. Future research focussing on adult body mass and dietary analysis in pre-laying birds would help determine whether this is the case or not. A lower body mass in young pairs that did not lay an egg and a more diverse diet that does not include nutritious prey would support this explanation. Similar investigations in other species of seabirds could also help explain participation rates and how it relates to the age of the birds.

Furthermore, it would be useful to compare Penguin Island's bridled tern colony with Cousin Island's colony in the Seychelles. Bridled terns in the Seychelles have a sub-annual breeding cycle which overlaps with their moulting cycle, and they do not migrate (Diamond 1976). It is possible that in this particular colony, age has an effect on breeding in a similar way to the wandering albatrosses *Diomedea exulans* breeding on Île de la Possession in the Crozet Islands. In this colony of wandering albatrosses, birds that breed bi-annually show virtually no effect of age on reproduction, whereas birds that breed annually show a strong effect of age on breeding because they are not able to restore their body conditions and replace worn flight feathers before the next breeding attempt (Pardo *et al.* 2014). Hence, such a comparison between Penguin Island's and Cousin Island's bridled tern colonies would be useful to determine whether this is the case.

Finally, this study found a significant effect of breeding season on laying date (all age classes were included so that it was not an effect of age on laying date) with earlier laying in the 2015/2016 breeding season (average laying date was on the 15 November 2015) than in the two previous years. This finding had already been reported by Dunlop & Surman (2012) who found that the first egg-laying date of bridled terns at the Penguin Island colony was correlated with the three-year rolling mean of the Southern Oscillation Index as observed over a 26 years period. This shows that bridled terns are able to time their reproduction with oceanic climate conditions and associated changes in their food sources. The effect of breeding season was not observed on any other breeding variable recorded in this study. Similar findings in three tern species breeding at the Houtman Abrolhos in Western Australia showed that lesser noddies *Anous tenuirostris*, brown noddies *Anous stolidus* and sooty terns *Onychoprion fuscata* could

advance or delay breeding based on climate variability and particularly the strength of the Leeuwin Current, even if the species was migratory (Surman & Nicholson 2009, Surman *et al.* 2012). In particular, the strength of the ENSO was not only linked with delayed breeding, but also lower participation rates and reduced breeding success (Surman & Nicholson 2009). It would be useful to investigate whether lower participation rates in these species were mostly found in young pairs to see if it compares with the present study. If this is not found to be the case, then the difference between the tern species breeding at the Houtman Abrolhos and bridled terns breeding further south on Penguin Island could reside in the different foraging strategies of bridled terns. Bridled terns forage over oligotrophic surface waters, particularly around *Sargassum* rafts, and avoid competition (Dunlop & Rippey 2006, Dunlop & Surman 2012). Therefore, if younger pairs are still refining their foraging skills, then this would explain their lower participation rates, but older birds that are able to compensate for food shortages with their foraging skills and by avoiding competition are able to participate and breed successfully.

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6. Diet analysis using faecal DNA metabarcoding techniques in seabird chicks

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This chapter is to be submitted to the Journal of Molecular Ecology. To maintain consistency with the rest of the thesis, the acknowledgements, key words, and authors' affiliations were removed but the references were kept in the journal's style. Otherwise, the text is identical to that of the manuscript.

6.1 Abstract

DNA barcoding of faeces is increasingly popular in studying seabird diets because it provides a low-disturbance, non-invasive qualitative approach to identifying prey diversity. The diet of bridled tern chicks (*Onychoprion anaethetus*) was determined using mitochondrial DNA 16S primers. The feeding rates of chicks were compared between adults of different age groups using camera trapping to investigate the effect of parental experience on provisioning in this species of seabird. Using DNA barcoding techniques provided a novel approach to investigating the effects of age on breeding in this species of marine birds. We were able to amplify degraded prey DNA in the faeces of chicks following pre-digestion by the parents and full digestion by the chicks, although very small amounts of amplifiable DNA fragments were isolated. There was no clear difference in the diversity of fish fed to chicks from parents under seven years of age and over seven years of age (using presence/absence data) and there was no difference in the feeding rates of chicks. One surprising finding was that bridled tern chicks which died before fledging had more amplifiable DNA in their faeces than chicks that fledged. Heat stress or diseases could have contributed to their death rather than starvation. Low amounts of amplifiable prey DNA is a common issue in seabirds' faeces and this could be the result of very efficient digestion, and because of a potential amplification bias in the primer sets used, it is recommended that future work uses a combination of techniques to study seabird diet.

6.2 Introduction

Despite our advancing knowledge in ecosystem functions and species biology, many food web links remain obscure (Deagle *et al.* 2007; Deagle *et al.* 2010). Threatened species and biodiversity conservationists have highlighted the need to gain a greater

understanding of intricate food web links to help understand how a species lives in its natural habitat and what food sources it needs to survive (Deagle *et al.* 2010; Oehm *et al.* 2011). An enhanced knowledge of species interactions, as well as a better understanding of variability in prey availability over time, is necessary to understand how a species may rely on a particular prey type and whether it may switch food source if its main prey was to disappear (Deagle *et al.* 2007; Oehm *et al.* 2011). This would also help identify whether anthropogenic activities have either a direct or indirect impact on a species' food sources, and recommend management strategies to mitigate existing impacts (Deagle *et al.* 2007).

Marine birds' diets are particularly challenging to study because they forage at sea where it can be difficult to identify prey (Barrett *et al.* 2007). Seabirds are mostly observable during their breeding season when they come back on land to nest and raise their chicks (Barrett *et al.* 2007; Oehm *et al.* 2011). This provides an opportunity to study their diet. However, several species of seabirds do not carry whole prey back to their nests; instead they regurgitate pre-digested food items which can be difficult to identify (Barrett *et al.* 2007; Cherel & Ridoux 1992). Furthermore, seabirds may not feed their chicks the same prey that they feed on themselves, and some species feed on different prey when not breeding (Cherel & Ridoux 1992; Labbé *et al.* 2013). For example, king penguins (*Aptenodytes patagonica*) target particular prey to feed their chicks; specifically, two species of myctophid fish and squid (Cherel & Ridoux 1992). Therefore, the chicks are provided with nutritious meals that are necessary to their growth (Cherel & Ridoux 1992). Cherel and Ridoux (1992) also found that non-breeding king penguins have a different diet composition to breeding birds, and there is a notable difference in the diversity of prey they catch. Hence it can be difficult to observe what seabirds feed on at different stages of their life cycle and yet this is essential knowledge for managing threats to their environment (Deagle *et al.* 2007).

Several methods have been used to study seabirds' diets and identify individual prey items (Barrett *et al.* 2007; Deagle *et al.* 2007). These include: killing individual birds and classifying gut contents of carcasses (Barrett *et al.* 2007), identifying remaining hard parts of prey in faeces or regurgitated pellets (Barrett *et al.* 2007), stomach flushing (Barrett *et al.* 2007; Deagle *et al.* 2007), water offloading (Neves *et al.* 2006), observing (with visual aids such as binoculars) prey species/sizes when parents feed chicks at the colony (Barrett *et al.* 2007), and a range of biochemical methods including serological

and electrophoretic analysis (Walter & O'Neill 1986), fatty acid signatures (Stewart *et al.* 2014), and stable isotopes (Constantini *et al.* 2017).

All of these methods rely on catching birds or using carcasses, and have ethical drawbacks ranging from killing birds, particularly if they are endangered, to shocked and starved birds following stomach flushing, regurgitation or blood sampling (Barrett *et al.* 2007; Deagle *et al.* 2007). Moreover, there are inherent biases in some observational studies because of differential digestion, and also because what the birds may ingest, they may or may not digest (Barrett *et al.* 2007; Deagle *et al.* 2007).

One way to overcome these issues is to use DNA meta-barcoding techniques combined with Next Generation Sequencing (NGS) technology on faecal samples (Deagle *et al.* 2010; Oehm *et al.* 2011). Remaining prey DNA in the faeces can be isolated (even minute quantities), amplified and sequenced in automated NGS sequencers to identify the taxa to which they belong by using DNA reference libraries (Lerner & Fleischer 2010; Oehm *et al.* 2011; Shokralla *et al.* 2012). The technique offers a less invasive method of studying diet and does not rely on catching wild birds (Valentini *et al.* 2009; Deagle *et al.* 2010).

While DNA meta-barcoding aims to identify prey taxa by using a standardised DNA region that is based on the persistence of DNA fragments in the environment, it can be achieved using two different approaches (Valentini *et al.* 2009; Pompanon *et al.* 2012; Shokralla *et al.* 2012). The first approach is to use group-specific primers (short DNA sequences that are used to amplify DNA fragments) that will amplify a targeted group of prey DNA that is present in a sample (cephalopod DNA for example, Deagle *et al.* 2010). To do this, one must have existing information on the diet of the species of interest and the DNA sequences of the prey species must be available in a DNA library to design such primers (Valentini *et al.* 2009; Pompanon *et al.* 2012). The other approach is to use universal primers that will amplify any prey DNA present in a sample such as the mitochondrial 16S rDNA primer sets used by Deagle *et al.* (2007) (Jarman *et al.* 2004; Valentini *et al.* 2009; Pompanon *et al.* 2012).

However, there are drawbacks to this technique. Digestion itself can be affected by the birds' physiology, age, gut flora, parasites, and the nature of the prey themselves (Deagle *et al.* 2010; Oehm *et al.* 2011). For example, prey size can affect how much DNA persists through the digestive process, so smaller prey types could potentially

leave less DNA than bigger prey types (Deagle *et al.* 2007). Therefore, while meta-barcoding and NGS methods can help uncover seabirds' diet, the results must be considered in the context of digestion-related processes (Oehm *et al.* 2011).

The purpose of this study was to use meta-barcoding methods with a universal primer set on faecal samples to analyse the diet of bridled tern chicks on Penguin Island in Western Australia. There were four aims to this project. The first was to determine if it were possible to isolate and amplify prey DNA that survived pre-digestion by parents that regurgitate prey items to feed their chicks, and subsequent digestion by the chicks themselves. The second aim was to determine whether older bridled tern adults feed their chicks different prey items compared to younger parents. This offered a novel approach to test the parental experience hypothesis in this species of seabird; that is, whether parental age affects their reproductive performance. We hypothesised that older bridled tern adults feed specific, high-energy prey items to their chicks whereas younger bridled tern parents may feed a more diverse diet to their chicks. The third aim was to compare the amount of prey DNA found in the faeces of chicks that survived and fledged and chicks that died before fledging. Finally, the fourth aim to was determine if there was a difference in chick feeding frequency between parents of different ages.

During the breeding season on Penguin Island, bridled terns become central place foragers that feed over *Sargassum* rafts present in the mid and outer continental shelf waters within 70 km of the colony (Dunlop 1997; Labbé *et al.* 2013). Bridled terns are contact-dipping foragers that feed in oligotrophic surface waters (Dunlop 1997; Dunlop 2011). Using regurgitate samples, Dunlop (1997) determined that during the breeding season, bridled terns feed on small fish (particularly mullid larvae and small clupeids), crustaceans (Columbus crab *Planes minutus* megalopae) and cephalopods. An inventory of bridled tern prey types was compiled for the Penguin Island colony by Dunlop (1997), so the purpose of this study was not to determine the bridled terns' diet during the breeding season, but rather to test hypotheses regarding parental experience

6.3 Methods

6.3.1 Study site

This study was carried out on the north-east end of Penguin Island in Western Australia where a colony of 3,000-4,000 bridled tern pairs breed annually (Garavanta & Wooller 2000; Dunlop 2009). An intensive banding program has been underway for the past 30

years, so it was possible to identify individual birds and infer their age from their band numbers (Dunlop 2009). It was possible to determine the exact age of adults that were banded as chicks, but the age of birds that were banded as adults was inferred to be the minimum age of recruitment into the Penguin Island colony (three years, Dunlop & Jenkins 1992), plus the number of years elapsed since they were banded. Therefore, adult birds that were banded for the first time in this study were inferred to be at least three years old. This aging technique had been shown to be reliable to infer the age of seabirds in other species (e.g. Reed *et al.* 2008). Nevertheless, in order to limit the effect of possible errors in age determination in the subsequent analyses, the age of the parents was averaged for each pair and they were then categorised into two groups: pairs that were seven years old or under, and pairs over seven years of age. Previous work pointed at a possible difference in the breeding performance of bridled terns between these age groups (Chapter 5) so similar age groups were used in this study.

6.3.2 Faecal sample collection and storage

Between December 2015 and March 2016, faecal samples from chicks aged between three and 45 days were collected inside cotton bags directly after the chicks were weighed. Chicks were weighed in separate cotton bags that were thoroughly cleaned and bleached before use. No bag was used for weighing more than one chick during each visit to the study site, minimising cross-contamination between faecal samples. Samples were immediately transferred into Eppendorf tubes, frozen at -18°C, and stored away from light sources until analysed. These precautions were taken to increase the possibility of detecting prey DNA as suggested by Oehm *et al.* (2011), who recommended collecting avian faeces as soon as possible from clean and smooth surfaces that are not exposed to rain and sunlight, and to avoid collecting faeces from soil surfaces as these would enhance DNA degradation through decomposition and may yield more PCR inhibitory elements that would prevent DNA amplification. Because samples were collected from chicks between hatching and fledging, any prey DNA in the faeces samples should reflect prey items fed by their parents; bridled tern chicks fledge between 50 and 65 days of age and do not forage prior to fledging (Garavanta & Wooler 2000).

6.3.3 DNA metabarcoding analysis

In total, 48 samples were sent to the Australian Genome Research Facility for DNA extraction, amplicon preparation, sequencing and diversity profiling. Mitochondrial 16S

rDNA1F/2R-degenerate primer sets were used to identify fish, cephalopods and crustaceans without the use of blocking primers for bridled tern DNA as it was deemed unnecessary because of a nucleotide mismatch between the primer sequence and the corresponding bird mitochondrial sequence (Deagle *et al.* 2007).

Following extraction and PCR amplification using the 16S1F and 16S2R primers, a QC gel revealed that only 22 samples generated visible bands. Additional dilutions did not improve the results so PCR inhibition was unlikely to contribute to this result.

Therefore, all the samples were used in the subsequent barcoding (following Deagle *et al.* 2007) and quantification procedures. DNA concentrations in the samples were determined using picogreen fluorometry. Because the amplicon sizes were on average 100bp longer than expected, 2x250bp PE chemistry had to be used for sequencing.

Image analysis was performed in real time using MiSeq Control Software v2.6.2.1 and Real Time Analysis v1.18.54. The samples were run in the Illumina bcl2fastq 2.18.0.12 pipeline. The generated sequences had their paired-end reads assembled by aligning the forward and reverse reads using PEAR1 v0.9.5. Primers were identified and trimmed and the resulting sequences were processed using Quantitative Insights into Microbial Ecology 1.8, USEARCH version 8.0.1623, and UPARSE software.

Resulting sequences were quality filtered and full length duplicate sequences were removed and sorted by abundance. Singletons or unique reads were discarded.

Sequences were clustered followed by chimera filter. To obtain number of reads in each Operational Taxonomic Unit (OTU), reads were mapped back to OTUs with a minimum identity of 97%. Taxonomy was then assigned using the Blast database.

OTUs that were of mammalian origin such as human and goat were discarded (assumed contamination), together with those from bacteria, archaea, fungi, algae and sponges, as those were assumed to be either prey of prey or incidental catches when bridled terns fed over *Sargassum* rafts at sea.

6.3.4 Feeding frequencies

Reconyx HC500 HyperFire Lo-Glow Semi-Covert IR cameras were placed at 14 nests locations in the study area to determine the feeding frequency of chicks by parents of different ages. Chicks were between two and eight days old when the recordings were made. The number of meals given to the chicks over a 24h period was recorded to test the hypothesis that younger parents may feed their chicks less often than older parents.

6.3.5 Statistical analyses

6.3.5.1 DNA concentrations in samples and chick survival

Because the amounts of DNA generated through preliminary PCR in the samples were low, it was hypothesized that chicks that had little DNA in their faeces were chicks that died before fledging as a result of starvation. This was tested in IBM SPSS 24 for Microsoft Windows (t-test, equal variances).

6.3.5.2 Prey diversity and adults' ages

The diversity profiling database was converted to presence/absence of OTUs and analysed in PRIMER v6 for Microsoft Windows. This approach was chosen over using percentages in order to avoid possible bias from preferential amplification. Samples were categorised using factors: age groups for the parents (three to seven years old, and eight to 23 years old; bridled terns do not breed until three years of age and may take a few breeding attempts to perfect their foraging skills), and DNA amount in samples (whether the sample had less than 0.35 ng/ μ L of DNA, or over 0.35 ng/ μ L). A Bray-Curtis similarity matrix was generated and an MDS plot was produced from the resulting matrix. Two one-way ANOSIMs (using 9999 permutations) were carried out to test differences within each factor (age group and DNA amount).

6.3.5.3 Chick feeding rates and adults' ages

The feeding frequencies of chicks by adults under seven years of age, and adults over seven years of age were compared in IBM SPSS 24 for Microsoft Windows (t-test, equal variances).

6.4 Results

6.4.1 Sample DNA amounts and chick survival

Chicks that died had on average significantly more DNA in their faeces samples that could be amplified (\bar{x} =0.89 ng/ μ L, s.e.=0.48) than chicks that survived and fledged (\bar{x} =0.40 ng/ μ L, s.e.=0.28) (t_{30} =-2.739, p =0.010).

6.4.2 Prey diversity fed to chicks and adults' ages

In total, 51 OTUs were identified in the faecal samples (Table 6.1). The most common were leatherjackets (Monacanthidae) found in all the samples, frigate tuna (*Auxis thazard*) found in 29 samples, trevally (*Pseudocaranx*) found in 27 samples, whiting (*Sillago*) found in 22 samples, spotted pipefish (*Stigmatopora argus*) found in 20 samples, goatfish (*Upeneichthys*) found in 19 samples, yellowtail kingsfish (*Seriola*

lalandi) found in 17 samples, anchovies (*Engraulis*) found in 14 samples, ribboned pipefish (*Haliichthys taeniophorus*) found in 14 samples, mackerel (*Trachurus*) found in 14 samples, and Australian sardines (*Sardinops sagax*) found in 13 samples (Table 6.1). All other OTUs were found in less than seven samples.

Table 6.1 Percentages of operational taxonomic units (prey DNA) recovered from faecal samples using metabarcoding techniques for investigating differences in prey species fed to chicks from parents under seven years of age, and over seven years of age, in bridled terns (*Onychoprion anaethetus*) breeding on Penguin Island, Western Australia.

Prey DNA	Chicks fledged	Chicks died	Chicks from parents under seven years of age	Chicks from parents over seven years of age
<i>Scobinichthys granulatus</i>	20.42%	19.45%	25.01%	17.24%
<i>Acanthaluteres spilomelanurus</i>	10.17%	27.15%	6.87%	15.27%
<i>Haliichthys taeniophorus</i>	0.00%	11.41%	0.00%	1.97%
<i>Auxis thazard</i>	3.39%	0.29%	5.52%	1.46%
<i>Meuschenia australis</i>	3.22%	0.33%	2.51%	3.18%
<i>Trachurus japonicus</i>	2.16%	0.00%	4.87%	0.01%
<i>Engraulis japonicus</i>	1.81%	0.00%	4.05%	0.03%
<i>Stigmatopora argus</i>	1.74%	0.30%	2.63%	0.91%
<i>Sillago aeolus</i>	2.07%	0.14%	0.01%	3.09%
<i>Upeneichthys stotti</i>	0.03%	4.06%	0.03%	0.73%
<i>Brachaluteres jacksonianus</i>	1.44%	0.00%	3.27%	0.00%
<i>Seriola lalandi</i>	1.72%	0.13%	0.30%	2.37%
<i>Pseudocaranx dentex</i>	1.60%	0.16%	0.08%	2.35%
<i>Upeneichthys lineatus/porosus</i>	0.14%	2.19%	0.01%	0.57%
<i>Eubalichthys mosaicus</i>	0.61%	0.25%	0.53%	0.61%
<i>Macquaria ambigua</i>	0.00%	1.61%	0.00%	0.28%
<i>Sardinops sagax</i>	0.56%	0.00%	1.26%	0.00%
<i>Pseudocaranx wrighti</i>	0.61%	0.08%	0.64%	0.50%
<i>Phyllognathia ceratophthalma</i>	0.00%	1.37%	0.00%	0.24%
<i>Acanthaluteres brownii</i>	0.48%	0.16%	0.36%	0.49%
<i>Nelusetta ayraudi</i>	0.43%	0.00%	0.74%	0.15%
<i>Siphonognathus argyrophanes</i>	0.18%	0.00%	0.40%	0.00%
<i>Malassezia restricta</i>	0.13%	0.00%	0.22%	0.05%
<i>Chaetodermis penicilligerus</i>	0.08%	0.08%	0.00%	0.13%
<i>Stigmatopora sp.</i>	0.09%	0.00%	0.00%	0.13%
<i>Etrumeus teres</i>	0.04%	0.00%	0.10%	0.00%
<i>Pictilabrus laticlavius</i>	0.04%	0.00%	0.09%	0.00%
<i>Astacopsis gouldi</i>	0.00%	0.10%	0.00%	0.02%
<i>Anoplocapros lenticularis</i>	0.03%	0.00%	0.07%	0.00%
<i>Acentrogobius janthinopterus</i>	0.03%	0.00%	0.07%	0.00%

There was no difference in prey diversity between the samples that had generated more than 35 ng/ μ L of DNA and those that did not ($R=0.025$, $p=0.293$).

There was no apparent difference in prey composition in the samples from chicks that had parents under seven years of age and over seven years of age on the MDS plot (Figure 6.1, 2D stress<0.2): the samples from both groups are clustered together except for two samples from chicks with parents that were under seven years of age that lay outside of the main cluster. No significant difference in prey composition was found in the ANOSIM test, although the test approached significance ($R=0.123$, $p=0.053$).

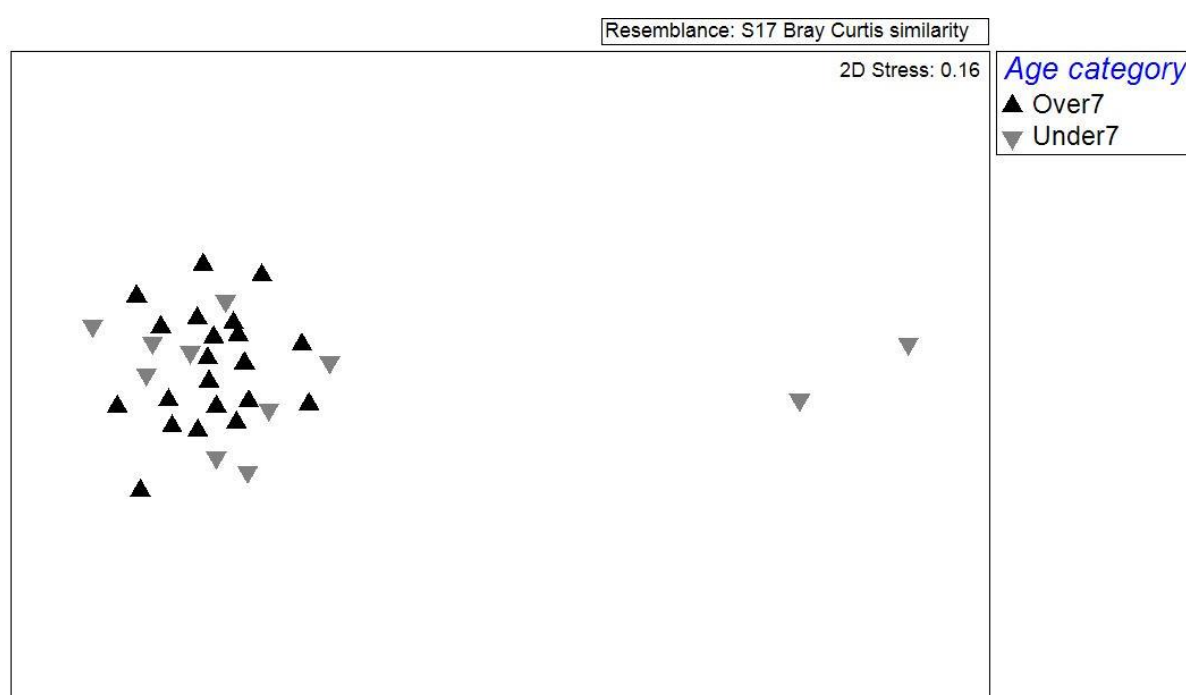


Figure 5.1 Non-metric multidimensional scaling ordination (MDS) plot showing the relative 2-dimensional disposition of faecal samples used for investigating differences in prey species fed to chicks from parents under seven years of age, and over seven years of age, in bridled terns (*Onychoprion anaethetus*) breeding on Penguin Island, Western Australia.

6.4.3 Chick feeding rates and adults' ages

Bridled tern pairs that were under seven years of age fed their chicks on average 4.8 (± 2.2 s.e.) meals per 24 hours, and pairs that were over seven years of age fed their chicks on average 3.9 (± 1.3 s.e.). There was no difference between the feeding rates of chicks from adults under seven years of age and adults over seven years of age ($t_{12}=0.914$, $p=0.379$).

6.5 Discussion

6.5.1 Diversity profiling

6.5.1.1 Overview

The amounts of DNA amplicons generated through initial PCR amplification were mostly under the 1 ng/μL threshold which is used in DNA profiling studies (Gerwing *et al.* 2016). Only two samples (out of 46) generated concentrations slightly higher than the threshold. Additional dilutions did not improve these results which suggests that PCR inhibitors were not an issue. Low DNA concentrations in seabird faecal samples had previously been noted in macaroni penguins (*Eudyptes chrysolophus*, Deagle *et al.* 2007) and semipalmated sandpipers (*Calidris pusilla*, Gerwing *et al.* 2016). Therefore, either there were low amounts of prey DNA in the faecal samples, or the extraction procedure did not effectively isolate remaining prey DNA. Because bridled tern adults feed their chicks regurgitated/pre-digested prey and chicks grow rapidly in the days following hatching, it is possible that extensive digestion of prey items occurs in their digestive tracts, which would result in low levels of remaining prey DNA in their faeces.

6.5.1.2 Prey diversity

Using DNA meta-barcoding techniques on faecal samples revealed 51 taxonomic units, compared with 20 using adult regurgitates and visual prey identification at the same colony (Dunlop 1997). However, while it can be inferred that meta-barcoding reveals a larger variety of prey than conventional diet analyses, the meta-barcoding results must be considered carefully as DNA of the prey of prey (secondary ingestion) could possibly be present (Sheppard *et al.* 2005). Some of the taxonomic units found in this study were presumed to be larval and post-larval stages that were associated with flotsams since bridled terns can only catch and ingest small prey items. Nevertheless, the results from both studies are comparable as most taxonomic units identified using the barcoding techniques were also identified in regurgitate studies (e.g. goatfish, leatherjackets, sardines, trevally, seahorses, crabs and insects). Some groups, such as squid and *Hyporhamphus*, which were identified in Dunlop's (1997) regurgitate study were not found in the present study. This could be due to either the temperature specificity of the primer sets used in this study or the actual absence of squid in the diet of bridled terns during the 2015/2016 breeding season. The primer sets 16S rDNA1F/2R-degenerate may not amplify DNA from certain taxonomic groups because

of the difference in the binding efficiency caused by the variety of melting temperatures for the primers to match the binding sites of some taxonomic groups (Deagle *et al.* 2007). Therefore, this would result in differential PCR amplification as fish DNA is preferentially amplified (Deagle *et al.* 2007). Furthermore, differences in prey availability throughout the breeding season as well as inter-annual fluctuations would also explain the differences between the results of Dunlop's (1997) study and the present study.

6.5.2 Prey diversity and relation to parental age

No difference in prey species fed to chicks from parents under seven years of age, and parents over seven years of age was found. Although there were two clear outliers on the MDS plot (chick faecal samples from parents under seven years of age, Figure 6.1), the ANOSIM analysis is not sensitive to outliers and returned a marginal p value. The prey types fed to chicks by parents from both age groups were very similar and predominantly included leatherjackets, trevally and pipefish. However, because there was potential bias in detecting prey diversity due to the shifting primers' specificity depending on temperature, it cannot be concluded that there was no difference in diet fed to chicks from parents of different ages. Additional analyses focussing on detecting cephalopod DNA would help determine whether this is the case.

The wide variety of prey found in the diversity profile was surprising given that the ecological niche of bridled terns narrows significantly during the breeding season (Labbé *et al.* 2013). Based on stable isotope analyses from feathers, bridled terns appear to restrict their diets during the breeding season when energy demands are high (Labbé *et al.* 2013). While a wide prey variety can be explained as secondary ingestion (which is a known issue in meta-barcoding studies), it is possible that bridled terns do in fact feed their chicks a diverse diet, but that all the prey that they bring back to their nests come from the same stable isotope niche. Bridled terns feed over floating *Sargassum* rafts in offshore waters during their breeding season on Penguin Island and hence the prey that they feed on lived and fed in association with those floating rafts. Therefore, while the stable isotope signatures of bridled tern chicks' feathers appeared to be narrow compared to that of adults' feather outside of their breeding season, it does not reflect the variety of prey that they were fed because such prey would have similar isotopic signatures as they lived on the same floating rafts.

6.5.3 Chick survival and DNA amounts in faeces

One surprising and counter-intuitive finding in this study was that there was a significant difference in the amounts of amplifiable prey DNA in the faeces of chicks that died compared to chicks that fledged. Chicks that died had higher amounts of amplifiable prey DNA while chicks that fledged had lower amounts. A possible explanation for this result could be that the chicks that did not survive may have been unable to fully digest prey items prior to dying, so that prey in their digestive tracts were not extensively digested prior to defecation. If this was the case, then the cause of death may not have been starvation (which would be consistent with the field observations that those chicks died of unknown causes). Other causes of death may have been heat stress or disease.

6.5.4 Feeding rates

Although there was no difference found in the chick feeding frequency between parents of different ages, the frequency found in this study was higher than the frequency reported in a previous study. Garavanta and Wooller (2000) reported that bridled terns fed their chicks two to three meals a day. The observation techniques used in both studies were different, however. Bridled tern chicks in the present study were monitored using field cameras, which allowed the recording of activities at the nests for uninterrupted periods of time while also minimising stress caused by a human presence close to their nesting sites. Furthermore, the chicks in this study were younger than those observed in Garavanta and Wooller's (2000) study and may have required more frequent feeding. Overall, there did not appear to be a difference in the feeding frequency between younger and older parents.

6.5.5 Considerations for using 16S rDNA primer sets

Despite a mismatch in the primer sets designed by Deagle *et al.* (2007), bridled tern DNA was found in 23 samples (out of 48), albeit in small quantities (less than 6% of the amplified DNA). While DNA amplification was not meant to occur because of a mismatch between the forward primer and the birds' own DNA, it is possible that bridled terns' DNA was amplified regardless of the mismatch since the primers' specificity will depend on the annealing temperature used in the cycling and the strength of the flanking sequence (Kwok *et al.* 1990). Furthermore, once the extension of the DNA strand from the bound primer has taken place, the resulting product (and its complement) can be amplified without restrictions as they will be fully matched to the

primers (Kwok *et al.* 1990). Therefore, further studies on bridled terns' diet may require bird DNA blocking primers to ensure that there is no amplification of avian DNA.

6.6 Conclusions and further research

This study showed that it is possible to amplify prey DNA in the faeces of seabird chicks despite preliminary digestion by the parents. Diversity profiling of prey DNA using faecal DNA metabarcoding techniques had also been used in little penguin chicks (*Eudyptula minor*), although Deagle *et al.* (2010) were unable to determine which samples came from adults/chicks since they collected them from nests. Therefore, this study provides a clear example of the feasibility of using chicks' faeces for DNA barcoding studies.

Seabirds appear to have very efficient digestive systems as there generally is little prey DNA remaining in their faeces as found in this study and previous studies (Deagle *et al.* 2007; Gerwing *et al.* 2016). Because seabirds' life at sea requires high energetic inputs, they have very efficient digestive systems that enable them to rapidly use the nutrition and energy derived from their prey (Jackson & Ryan 1986; Roby 1991).

There was no clear difference in the prey diversity fed to chicks between young and old parents, and there was no difference in chick feeding frequency between the two groups. Therefore, additional sampling is required to assert whether there is indeed no effect of age and experience on breeding and foraging performance in bridled terns, as the results from this study were ambiguous and possibly biased. In terms of chick feeding frequency, similar results were observed in common terns (*Sterna hirundo*; Limmer & Becker 2009). While common terns' chick feeding rates were not linked to experience (and the birds' ages), young parents were found to feed their chicks prey that had low energy content more often than experienced parents (Limmer & Becker 2009). However, this slight difference in chick provisioning was linked to a lower breeding success in common terns, which was not found to be the case in this study.

While faecal DNA metabarcoding can be used in seabirds to investigate their diet, care must be taken to ensure that the generated diversity profiles do not give biased results resulting from differential amplification of prey DNA. Deagle *et al.* (2007) mitochondrial 16S rDNA1F/2R-degenerate primer sets were useful in identifying the fish and crustacean components of bridled tern chicks' diet but failed to reveal cephalopods. Therefore, future work should focus on using more than one primer set to

detect all prey, or should use a combination of faecal DNA meta-barcoding methods and conventional dietary analyses.

6.7 References

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7. Discussion, conclusions and further research

Age effects on breeding in seabirds have been found in 42 species (Chapter 2). Such effects are found in many different breeding variables such as breeding success, clutch size, feeding frequency, and transgenerational effects on chick survival. Knowledge on whether this occurs in a species of seabirds, and how age might affect its breeding parameters, is necessary before using a species as an indicator for its environment as well as for determining demographic transitions in colonies. The aim of this thesis was to determine whether there was an effect of age on breeding in bridled terns at the Penguin Island colony in Western Australia. This was investigated using two approaches, one based on directly-measurable breeding variables (Chapter 5), and one based on using DNA techniques to determine the types of prey fed to chicks from parents of different ages (Chapter 6). Surprisingly, this study found no difference in the breeding parameters of bridled terns of different ages, except for breeding success between young birds that did not lay an egg and older birds that bred, and there was no clear difference in the diet of chicks from younger and older parents. These results were unexpected as age has been found to have an effect on breeding in 98% of the studies on seabirds (Chapter 2). While possible biases in the determination of the bridled terns' ages and small sample sizes could have affected the results, it is possible that the location of Penguin Island and the associated changes in climatic and oceanic conditions in recent years may be favourable to this species of seabird, and that the only effect of age on breeding occurs pre-laying when females need to acquire enough food to produce an egg.

7.1 Effects of age on reproduction in bridled terns

Despite using cross-sectional and longitudinal analyses on multiple breeding parameters, no effect of age on post-laying breeding performance was found in bridled terns. Older pairs did not breed earlier, lay bigger eggs, incubate their eggs for shorter periods of time, have bigger chicks that grow faster and fledge earlier than younger birds. The only possible effect of age on breeding could be found in younger pairs not laying an egg. There was evidence for age assortative mating and overall the breeding success of the colony was around 66% (excluding young birds that did not breed). While these findings differ from the majority of the research on other species of marine birds (Chapter 2), they were similar to those found in another study on a different tern

species: the common tern *Sterna hirundo* (Wendeln and Becker, 1999). However, further studies on this particular colony of common terns which breeds in the German Sea demonstrated that an effect of age on breeding did exist and particularly a trans-generational effect of parental age on the breeding of their progeny (Bouwhuis *et al.*, 2015). Therefore, the results of the present study diverge from the findings from most of the research on the topic of age effects on reproduction in marine birds.

7.2 Effects of parental age on bridled tern chicks' diet

This study found no clear difference in the prey diversity fed to young chicks from parents under seven years of age and over seven years of age. There only appeared to be a slight disparity in the 'additional fish prey' types fed to chicks between the two age groups, but because the primers used in this study did not amplify cephalopod DNA, it could not be ascertained whether there was a real difference in chick feeding between the two age groups. Therefore, it is possible that younger bridled tern parents are still refining their foraging skills to feed their chicks but if this was the case, then it did not appear to impact their chicks' development and survival. Other studies found that younger pairs fed a more diverse diet to their chicks (Daunt *et al.*, 2007; Limmer and Becker, 2009; Patrick and Weimerskirch, 2015) however such findings were linked to a lower breeding success, unlike this study. Furthermore, this study did not find any difference in the chick feeding rates between parents of different ages, but this had also been observed in common terns (Limmer and Becker, 2009). Therefore, this study did not find a clear difference in prey items fed to chicks which would have suggested a possible effect of age on breeding in bridled terns.

7.3 Aging in bridled terns

While this study found no evidence for an effect of age on the breeding performance of bridled terns on Penguin Island, it is also interesting to note that it was not possible to determine the age of those birds using pentosidine as a bio-marker (Chapter 4). Because of the low collagen contents in the skin of bridled terns, the levels of pentosidine could not be determined. This had not been an issue in other species of land birds and coastal-dwelling birds, but low collagen levels were also an issue in another seabird species: common gulls *Larus canus* in Estonia (Rattiste *et al.*, 2015). Interestingly, even telomere length in Estonian common gulls could not be used to infer their age (Rattiste *et al.*, 2015). The results of this study and other studies on aging seabirds may suggest that there could be a decoupling of biological and chronological age in some species of

marine birds (Juola *et al.*, 2006; Foote *et al.*, 2011; Rattiste *et al.*, 2015). Seabirds may have some metabolic and physiological attributes that help them resist stress and oxidative challenges from the environment in which they live (such as high exposure to UV radiation, high salinity and diet). Therefore, a possible decoupling of biological and chronological age in bridled terns and a lack of adequate evidence for an effect of age on post-laying breeding performance suggest that this species of seabird may not be susceptible to aging and maturing after they recruit in the Penguin Island colony. The environmental conditions in Western Australia could provide an ideal setting for this species to thrive and not suffer from external stresses that could lead to biological aging, and younger birds may not need to refine their breeding and foraging skills to reproduce as well as older birds. The observed expansion in the breeding range of bridled terns in Western Australia would support this (Chapter 1, Dunlop, 2009).

Because less than 5% of the population of bridled terns on Penguin Island are known-age birds that were banded as chicks, and the birds' ages could not be determined using pentosidine as a bio-marker, there could be potential bias in the ages of the birds in this study. Most birds in this study were either banded as adults, or were not banded, so their true ages were not known. Hence, to approximate the age of breeding bridled terns, the minimum recruitment age (3 years old) was assumed for un-banded birds, and the minimum recruitment age plus time elapsed since first capture were used to approximate the age of banded adults (Chapter 3). Such a technique in studies investigating the effects of age on breeding in common guillemots (*Uria aalge*) found that using time elapsed since first capture added to recruitment age as a proxy for age worked, and they were successful in identifying effects of age on breeding in this species (Crespin *et al.*, 2006; Reed *et al.*, 2008). However, because the colony of bridled terns on Penguin Island may not be a closed population and there is an expansion in the breeding range of this species in Western Australia, recruitment age on Penguin Island may be heterogeneous as younger individuals may recruit in southern colonies instead of Penguin Island (Dunlop, 2009). Therefore, accurate aging of bridled terns in this study could have been a limitation. Studying the effects of age on the reproduction of seabirds in colonies that have very few known-age individuals can be difficult and therefore may only be possible in closed colonies that undergo extensive banding/marking programs over long periods of time.

Nevertheless, in order to minimise possible errors in aging bridled terns, the average age of a pair was used instead of the individuals' ages, and the birds were classified into three age groups. Seventy-three breeding pairs were included in this study (including pairs that did not lay an egg), but the three age groups used in the cross-sectional analyses may have been small and this could have decreased the possibility of identifying age-related differences in breeding performance (Chapter 5). The three groups used for the cross-sectional analyses were: 18 pairs under the age of six years, 23 pairs between the age of six and 12 years, and five pairs over the age of 13 years. However, while most studies studying the effect of age on reproduction in seabirds used larger overall sample sizes (over 100), a few studies were still able to demonstrate an effect of age on breeding using sample sizes under 50 pairs (e.g. Australasian gannets *Morus serrator* Bunce *et al.*, 2005). Nevertheless, because of the issue of individual variability, small sample sizes could have hindered the detection of effects of age on the reproduction of bridled terns in the post-laying phase.

It is also worth noting that the different age categories used for analyses in Chapter 5 and Chapter 6 were also due to small sample sizes. While the age groups in Chapter 5 were based on the preliminary observations of the possible effect of age on a few breeding variables, the same age classes could not be used in the diet study as the faeces samples used in Chapter 6 were collected whenever available, and there were few samples from chicks of bridled tern pairs aged under 6 years compared to chicks of bridled terns aged over 7 years of age. Therefore, the same age categories could not be used for analysis as this would have affected the assumptions for statistical analyses, but they were very similar.

7.4 Impact of climatic conditions on this study

Studies investigating the effects of age on breeding in seabirds found that in poorer environmental conditions the difference in the breeding performance of younger and older seabirds was more visible (Barbraud and Weimerskirch, 2005; Pardo *et al.*, 2013). Despite having three markedly different breeding seasons in this study with summer 2013/2014 and summer 2014/2015 being less favourable than summer 2015/2016, such results were not found in bridled terns breeding on Penguin Island (Chapter 3 and Chapter 5). The only effect of breeding season on the breeding of this species was found in the egg laying date, with an earlier laying date in summer 2015/2016. Hence, if there is indeed no effect of age on the reproduction of bridled terns (besides the production of

an egg), then their breeding performance, and particularly their laying date, could potentially be used to infer the state of their environment without taking into account their ages, in conjunction with other oceanographic observations as well as the breeding performance of other seabird species that breed on Penguin Island.

7.5 Management implications and further research

The low recapture rate of known-age individuals and the high number of un-banded bridled terns on Penguin Island indicate that the colony on Penguin Island is not a closed population and that it may act as a source population for other colonies of bridled terns breeding in Western Australia. Therefore, if threats to this population such as predation by black rats, loss of breeding habitat and disturbance by human activities (and particularly tourists) are not managed, then not only would the population of bridled terns on the island suffer, but satellite colonies may also be indirectly impacted. Appendix 1 showed that the restoration of native vegetation cover was possible and this would help regain habitats that were lost to introduced weeds for bridled terns to breed, and also for other species of seabirds. Therefore, care must be taken to ensure that disturbances are managed.

7.6 Further research

Further research on developing techniques for aging seabirds is necessary as not all colonies of marine birds have high proportions of banded individuals and banding in colonies that comprise of thousands of individuals require time and resources that may not be available. Although pentosidine could not be used as a bio-marker in this study, this technique may work in other species of seabirds. Other molecular approaches that do not require the killing of individuals to determine their age would be useful and would likely be widely implemented in seabird colonies that also have low proportions of banded individuals. Such techniques would help determine the longevity of seabirds, their mortality rate, and they would also help study the effects of age on reproduction.

Future studies on bridled terns should focus on the pre-laying performance of breeding birds. Determining whether younger birds are able to sequester resources to produce an egg during the breeding season would be necessary to understand why younger bridled terns do not lay eggs. Dietary work using a combination of DNA techniques for identifying prey items in faeces and other techniques based on the visual identification of prey would help determine whether younger bridled terns feed on the same prey

types as older birds. This in turn would help determine whether younger birds are able to forage as well as older birds so that a difference in breeding participation would point at the constraint rather than the restraint hypothesis (Chapter 2; Curio, 1983).

Although most of the literature suggests that there is an effect of age on breeding performance in seabirds, the results of this study differed. Therefore, either methodological problems hindered the detection of such effects on the post-laying performance of bridled terns, or the only effect of age on breeding in this species occurs before egg laying, but once a pair is able to produce an egg then its breeding performance will not be affected by age because the climatic and oceanic conditions surrounding Penguin Island are favourable to this species. To determine whether this is the case, other populations of marine birds should be identified that are established in areas that provide abundant food and a favourable climate with low predation pressures and their breeding performance should be monitored to be compared with the results of this study and see if they also lack an effect of age on breeding. An alternative experiment in colonies that show an effect of age on breeding could involve supplementary feeding and predator removal to determine if this would negate the effects of age on breeding performance. Such studies could parallel attempts to remove some of the biases at the Penguin Island colony. However, additional observations on breeding parameters and larger sample sizes are necessary to further investigate the effects of age on breeding in bridled terns on Penguin Island.

If there is in fact no effect of age on the breeding performance of bridled terns breeding on Penguin Island, then this species could be used to monitor its environment without having to take into account age bias in breeding variables, and particularly laying date.

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Appendix 1. Restoration of native vegetation and re-introduction of *Malva preissiana* on Penguin Island – preliminary findings

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This chapter is in press with the journal of the Royal Society of Western Australia. To maintain consistency with the rest of the thesis, the acknowledgements, key words, author affiliations and running page head were removed but the references were kept in the journal's style. Otherwise, the text is identical to that of the manuscript.

A1.1 Abstract

Penguin Island's vegetation in southwestern Australia has been degraded by anthropogenic activities and breeding silver gulls (*Chroicocephalus novaehollandiae*). However, native vegetation cover is important for breeding seabirds such as bridled terns (*Onychoprion anaethetus*). This pilot project aimed to restore native vegetation cover around artificial nest tubes for bridled terns using tubestock of berry salt bush (*Rhagodia baccata*) and bower spinach (*Tetragonia implexicoma*), and to re-introduce the Australian hollyhock (*Malva preissiana*), an ornithocoprophilic coastal plant that disappeared from Penguin Island in the 1970s due to competition with introduced *Malva* spp. and other nitrophilous weeds (Rippey *et al.* 2002). A total of 80 *R. baccata* or *T. implexicoma* seedlings and 980 *M. preissiana* seeds were planted, but less than 4% of the tubestock survived and less than 1% of the *M. preissiana* seeds germinated. Disturbance by breeding silver gulls was identified as a major issue, therefore seedling protection from these birds is now being used for ongoing restoration projects.

A1.2 Introduction

The vegetation on Penguin Island, a small (12.5 ha) coastal island in Western Australia 40 km south of Perth (32°305'S, 115°690'E), has been shaped by its geology and the predominant climatic conditions in the region. Penguin Island is an A-class reserve located in the Shoalwater Islands Marine Park and lies on the Garden Island ridge which is an aeolian dune of Tamala limestone (Haig, 2002). It has a Mediterranean climate and is subjected to a coastal sea breeze which carries salt spray (DCALM, 1992). Therefore, the native vegetation is adapted to growing in these conditions and displays sclerophyllous and xeromorphic characteristics (DCALM, 1992).

Numerous seabird colonies such as the little penguin (*Eudyptula minor*), Caspian terns (*Hydroprogne caspia*), crested terns (*Thalasseus bergii*) and bridled terns (*Onychoprion anaethetus*) rely on Penguin Island's native vegetation cover to nest (DEC and MPRA, 2007). However, anthropogenic activities have degraded the island's vegetation, especially through the construction of shacks until 1987 when the Department of Parks and Wildlife bought the island's lease and undertook restoration work (Brown *et al.*, 2015a). Furthermore, in the 1960s silver gulls (*Chroicocephalus novaehollandiae*) started breeding on Penguin Island during the winter months and contributed to the degradation of its native vegetation through trampling, picking material for nest-building, dispersing seeds from weeds and depositing significant amounts of guano that alters soil composition (Hogg & Morton, 1983; Brown *et al.*, 2015a; Otero *et al.*, 2015). Hence, disturbance from human activities and gulls, as well as pelicans and cormorants to some extent, has contributed to the continued degradation of the island's vegetation so that in winter, weeds grow and compete with the native vegetation, and in summer bare soil remains where the weeds grew (Brown *et al.*, 2015a). The overall decline in native vegetation cover has led to the loss of nesting habitats for seabirds. It is probable that these disturbances also contributed to the disappearance of the native Australian hollyhock (*Malva preissiana*) on Penguin Island in the 1970s (Brown *et al.*, 2015b).

The aims of this study were:

to determine if native vegetation cover could be re-established around bridled tern nesting boxes by planting tubestock of berry salt bush (*Rhagodia baccata*) and bower spinach (*Tetragonia implexicoma*), and

to determine if the Australian hollyhock could be grown from seeds around bridled tern nesting boxes.

A1.3 Methods

A1.3.1 Study site

The study site was located on the north-east end of Penguin Island which has been a known breeding area for bridled terns for the past 60 years (Dunlop & Jenkins, 1994). A large portion of the vegetated area has been lost to weeds, such as weedy annual grasses including *Lolium* spp., *Bromus* spp. and Brassicaceae (Bettink *et al.*, 2009; Brown *et al.*, 2015a). The area had also previously been flagged as a hotspot for tree mallow (*Malva dendromorpha*) which is a weed of main concern growing on Penguin Island (Bettink *et*

al., 2009). The remaining native bushes include *R. baccata* and *T. implexicoma* and are used by bridled terns for cover for nesting in summer.

In an effort to recover nesting grounds for bridled terns, 50 artificial nest tubes were installed in spring 2015 on weeded patches in the study area. The nest tubes were built out of a storm drain PVC pipe (diameter 30 cm) that was cut length-wise to obtain two dome-shaped half pipes and sectioned in 50 cm long pieces. They were then painted with limestone-coloured paint to give them a more natural appearance. The nest tubes were laid about 4 m apart on bare ground as bridled terns usually nest 1-5 m apart from each other (del Hoyo *et al.*, 1996).

A1.3.2 Restoration techniques

Seeds from *R. baccata* growing on Penguin Island and cuttings from *T. implexicoma* were collected and grown in pots offsite. The resulting tubestock of *R. baccata* and *T. implexicoma* were planted around 40 artificial nest tubes in June 2014. Before planting, weeds that were growing within a 1.5 m radius of the nest tubes were removed. Two seedlings of either *R. baccata* or *T. implexicoma* were planted on either side of the nest tube.

Additionally, *M. preissiana* fruits were collected early in 2014 from plants cultivated in Perth that had been grown from seeds that originated from Shag Rock in the Shoalwater Islands Marine Park (650 m North of Penguin Island). The seeds were isolated using sieves and filtered through Selecta aspirator/gravity separator zig-zag ZZ1 – Machinefabriek B.V. The fruit cases were removed to separate the seeds and for half of the seeds, a small portion of the upper seed coat was removed (“nicked” seeds) to determine if this would enhance germination. The germination rate for both the “nicked” and “not nicked” seeds was determined to be 100% using standard testing procedures on agar plates (Carlson *et al.*, 1982).

The seeds were sown around ten bridled terns’ artificial nest tubes in June 2014. The areas around the nest boxes were weeded before using a seeding board to plant the seeds. The boards were placed on both sides of the nest tubes (0.5 m in length) to plant an equal number of “nicked” and “not nicked” seeds (Figure A.1). The seeds were sown at a depth of about 2 mm.

The germination and survival of *M. preissiana* seedlings was determined by visiting the sites every four weeks and the plots were also weeded during those visits.

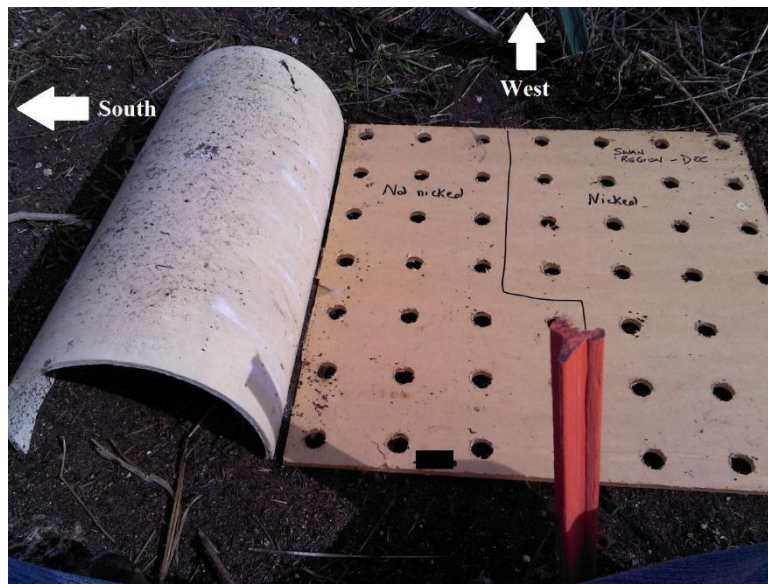


Figure A.1 Planting board (0.5 x 0.5 m) for *Malva preissiana* seeds for germination around bridled tern artificial nest tubes on Penguin Island in June 2014. The “nicked” slots were for seeds with a small part of the seed coat removed.

A1.4 Results

A total of 80 *R. baccata* or *T. implexicoma* seedlings were planted around the bridled tern nest tubes. All except for three plants were removed by breeding silver gulls within a day. The remaining three plants (all *R. baccata*) were still growing in June 2016, two years after planting. Therefore, less than 4% of the planted tubestock survived.

A total of 980 *M. preissiana* seeds were planted around ten artificial nest tubes. Germination was recorded in July 2014 (one month after planting) and a total of eight plants grew around seven of the nest boxes. Therefore, less than 1% of the seeds that were sown had developed by the time of the visit. Seven of the seeds that grew were “not nicked”. By November 2014, one of the *M. preissiana* plants was flowering. A year after planting, only five plants survived and in July 2015 those five plants were flowering and producing fruits. In particular, one plant reached 1.5 m in height and nearly 2 m in diameter. All plants died in summer 2015/2016.

A1.5 Discussion

Breeding gulls (Laridae) are known to be major disturbances to the vegetation where they nest (Hogg & Morton, 1983; Otero *et al.*, 2015). While our trial showed that planted tubestock was removed by breeding gulls, it also showed that *M. preissiana* grown from seeds were not affected. Therefore, it is important to protect planted

tubestock, and ongoing revegetation trials on Penguin Island are showing successful growth of seedlings when protected with cages (see Brown *et al.*, 2015a).

While the field germination rate of *M. preissiana* seeds was very low (less than 1% compared with 100% germination rate in the laboratory test), the plants that grew and survived for nearly two years produced abundant fruits. Furthermore, nicking of the seeds was unnecessary for enhancing germination rate. The sites where those plants grew are being monitored to detect any sign of germination from the dropped fruits. However, in spring 2015 many of the flowers and fruits were eaten by insects, so it is unknown whether the seeds from the plants that dropped on the soil were viable or not.

It is interesting to note that the nest tubes around which the *R. baccata* plants grew, and four of the nest tubes around which *M. preissiana* grew, were taken up by bridled terns during their breeding season in summer (out of the ten artificial nest tubes that were occupied during the summer). Therefore, a total of 70% if the shaded nest tubes were taken up by bridled terns compared with 7.5% of the unshaded nest tubes. The natural shade cover provided by the growing plant was a likely factor in drawing the breeding pairs to the restoration site. It is believed that the guano that falls on either side of the nest tube when bridled terns roost on top of them will help *M. preissiana* plants grow. Indeed, bridled terns are true seabirds and feed almost exclusively on fish, squid and crustacea during their breeding season (Dunlop & Jenkins, 1994). Therefore, since *M. preissiana* has a life cycle that is reliant on guano deposit (Rippey *et al.*, 2002), it is believed that the birds' droppings are beneficial to the plants.

Overall, while native vegetation can be re-established around bridled tern nest tubes on Penguin Island, it is necessary to protect young plants. Therefore, the restoration of native vegetation on coastal islands in Western Australia would be possible provided that weeds are removed and tubestock of native plants are protected from breeding silver gulls.

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Appendix 2. List of 103 original empirical journal articles on the topic of age and breeding in seabirds published between 1949 and June 2016

Reference number	1	2
Cross-sectional/longitudinal study	Both	Both
Experiment/observational	Observational	Observational
Species	Yellow-eyed penguin (<i>Megadyptes antipodes</i>)	Yellow-eyed penguin (<i>Megadyptes antipodes</i>)
Order	Sphenisciformes	Sphenisciformes
Country	New Zealand	New Zealand
Hemisphere	Southern	Southern
Age range	2-11	2-17
Support effect of age on breeding	yes	yes
Constraint/restraint hypothesis	-	-
Selective disappearance	-	-
Experience	-	-
Reproductive effort	-	-
Reproductive senescence	-	-

3	4	5	6
Cross-sectional	Cross-sectional	Both	Cross-sectional
Observational	Observational	Observational	Observational
Black-legged kittiwakes (<i>Rissa tridactyla</i>)	Black-legged kittiwakes (<i>Rissa tridactyla</i>)	Brown skua (<i>Stercorarius antarcticus</i>)	Adélie penguin (<i>Pygoscelis adeliae</i>)
Charadriiformes	Charadriiformes	Charadriiformes	Sphenisciformes
United Kingdom	United Kingdom	United Kingdom	United States of America
Northern	Northern	Southern	Southern
2-10	2-11.5	1-7	3-6+
yes	yes	yes	yes
-	-	-	-
-	-	-	-
-	-	yes	-
-	-	-	-
-	-	-	-

7	8	9	10
Cross-sectional	Cross-sectional	Cross-sectional	Cross-sectional
Observational	Observational	Observational	Observational
Adélie penguin (<i>Pygoscelis adeliae</i>)	Red-billed gull (<i>Larus novaehollandiae scopulinus</i>)	Herring gull (<i>Larus argentatus</i>)	Arctic skua (<i>Stercorarius parasiticus</i>)
Sphenisciformes	Charadriiformes	Charadriiformes	Charadriiformes
United States of America	New Zealand	United Kingdom	United Kingdom
Southern	Southern	Northern	Northern
3-8	2-10	6-10+	3-5+
yes	yes	yes	yes
Constraint	-	-	-
-	-	-	-
yes	yes	-	yes
-	-	-	-
-	-	-	-

11	12	13	14
Cross-sectional	Both	Cross-sectional	Cross-sectional
Observational	Observational	Observational	Observational
Arctic tern (<i>Sterna paradisaea</i>)	Manx shearwater (<i>Puffinus puffinus</i>)	Fulmar (<i>Fulmarus glacialis</i>)	Ring-billed gull (<i>Larus delawarensis</i>)
Charadriiformes	Procellariiformes	Procellariiformes	Charadriiformes
United Kingdom	United Kingdom	United Kingdom	Canada
Northern	Northern	Northern	Northern
3-29	4-16+	6-19	2-7
yes	yes	yes	yes
-	-	Constraint	-
-	-	-	-
-	yes	yes	yes
-	-	-	-
-	-	yes	yes

15	16	17	18
Cross-sectional	Cross-sectional	Cross-sectional	Cross-sectional
Observational	Experimental	Both	Observational
Great skua (<i>Stercorarius skua</i>)	Fulmar (<i>Fulmarus glacialis</i>)	Glaucous-winged gull (<i>Larus glaucescens</i>)	Black-legged kittiwakes (<i>Rissa tridactyla</i>)
Charadriiformes	Procellariiformes	Charadriiformes	Charadriiformes
United Kingdom	United Kingdom	United States of America	United Kingdom
Northern	Northern	Northern	Northern
6-10+	-	4-23	3-20
yes	yes	yes	yes
-	Constraint	Constraint	-
-	-	no	no
yes	yes	yes	-
-	-	no	-
-	-	yes	-

19	20	21	22
Cross-sectional	Cross-sectional	Cross-sectional	Cross-sectional
Observational	Observational	Observational	Observational
Short-tailed shearwater (<i>Puffinus tenuirostris</i>)	Antarctic fulmar (<i>Fulmarus glacioides</i>)	Short-tailed shearwater (<i>Puffinus tenuirostris</i>)	Western gull (<i>Larus occidentalis</i>)
Procellariiformes	Procellariiformes	Procellariiformes	Charadriiformes
Australia	France	Australia	United States of America
Southern	Southern	Southern	Northern
2-10+	6-26	5-32	3-9
yes	yes	yes	yes
-	Constraint	Restraint	Constraint
-	-	yes	no
yes	yes	yes	yes
-	-	maybe	-
-	-	-	-

23	24	25	26
Cross-sectional	Both	Both	Both
Observational	Observational	Observational	Observational
Western gull (<i>Larus occidentalis</i>)	Wandering albatross (<i>Diomedea exulans</i>)	Wandering albatross (<i>Diomedea exulans</i>)	Cassin auklet (<i>Pychooramphus aleuticus</i>)
Charadriiformes	Procellariiformes	Procellariiformes	Charadriiformes
United States of America	United Kingdom	France	United States of America
Northern	Southern	Southern	Northern
3-21	7-30	5-27+	3-10+
yes	yes	yes	yes
Constraint	-	Constraint	-
no	-	-	-
yes	yes	yes	yes
no	-	-	-
no	-	-	yes

27	28	29	30
Cross-sectional	Cross-sectional	Cross-sectional	Both
Observational	Observational	Both	Observational
Oystercatcher (<i>Haematopus ostralegus</i>)	Short-tailed shearwater (<i>Puffinus tenuirostris</i>)	Thick-billed murre (<i>Uria lomvia</i>)	Great skua (<i>Stercorarius skua</i>)
Charadriiformes	Procellariiformes	Charadriiformes	Charadriiformes
Netherlands	Australia	Canada	United Kingdom
Northern	Southern	Northern	Northern
-	20+	4-7	5-29
yes	-	yes	yes
-	-	-	Constraint
-	-	-	-
yes	yes	yes	yes
-	-	-	no
-	-	-	yes

31	32	33	34
Longitudinal	Cross-sectional	Cross-sectional	Cross-sectional
Observational	Observational	Observational	Observational
Common terns (<i>Sterna hirundo</i>)	Kittiwake (<i>Rissa tridactyla</i>)	Kittiwake (<i>Rissa tridactyla</i>)	Black-legged kittiwakes (<i>Rissa tridactyla</i>)
Charadriiformes	Charadriiformes	Charadriiformes	Charadriiformes
Germany	France	France	United Kingdom
Northern	Northern	Northern	Northern
3-16	2-14+	2-14+	4+
no	yes	yes	yes
-	-	-	-
-	yes	-	-
-	-	-	yes
-	-	-	-
-	-	-	no

35	36	37	38
Both	Cross-sectional	Longitudinal	Cross-sectional
Observational	Observational	Observational	Observational
Cassin auklet (<i>Ptychoramphus aleuticus</i>)	Common tern (<i>Sterna hirundo</i>)	Black-legged kittiwake (<i>Rissa tridactyla</i>)	Nazca booby (<i>Sula granti</i>)
Charadriiformes	Charadriiformes	Charadriiformes	Suliformes
United States of America	United States of America	France	United States of America
Northern	Northern	Northern	Both
2-14	6-9 and 18+	-	4-30
yes	yes	yes	yes
Constraint	-	-	-
-	-	yes	-
yes	-	-	-
-	-	-	-
yes	-	yes	yes

39	40	41	42
Both	Both	Both	Cross-sectional
Observational	Observational	Observational	Observational
Leach's storm petrel (<i>Oceanodroma leucorhoa</i>)	Common gull (<i>Larus canus</i>)	Common tern (<i>Sterna hirundo</i>)	Common tern (<i>Sterna hirundo</i>)
Procellariiformes	Charadriiformes	Charadriiformes	Charadriiformes
Canada	Estonia	Germany	United States of America
Northern	Northern	Northern	Northern
1-25+	1-16	1-15	3-21
yes	yes	yes	yes
-	-	Constraint	-
yes	no	no	-
-	yes	yes	-
no	maybe	-	-
-	yes	yes	-

43	44	45	46
Both	Both	Cross-sectional	Cross-sectional
Observational	Observational	Observational	Observational
Blue petrel (<i>Halobanea caerulea</i>)	Common tern (<i>Sterna hirundo</i>)	Australasian gannet (<i>Morvus serrator</i>)	Common tern (<i>Sterna hirundo</i>)
Procellariiformes	Charadriiformes	Suliformes	Charadriiformes
France	Germany	Australia	United States of America
Southern	Northern	Southern	Northern
?	2-6+	4-16	4-28
yes	yes	yes	-
-	Constraint	Constraint	Constraint
yes	-	-	-
yes	yes	yes	-
-	-	-	-
-	-	-	-

47	48	49	50
Cross-sectional	Both	Longitudinal	Both
Both	Observational	Observational	Both
Herring gull (<i>Larus argentatus</i>)	Great cormorant (<i>Phalacrocorax carbo</i>)	Common guillemot (<i>Uria aalge</i>)	Oystercatcher (<i>Haematopus ostralegus</i>)
Charadriiformes	Suliformes	Charadriiformes	Charadriiformes
United Kingdom	Denmark	United Kingdom	Netherlands
Northern	Northern	Northern	Northern
3-3+	2-12	8-28	3-25+
yes	yes	yes	yes
Constraint	Constraint	-	-
-	yes	-	no
yes	yes	-	yes
-	-	-	-
-	-	yes	yes

51	52	53	54
Cross-sectional	Both	Cross-sectional	Cross-sectional
Observational	Both	Observational	Observational
Grey-headed albatross (<i>Thalassarche chrysostoma</i>)	Blue-footed booby (<i>Sula neboxii</i>)	Wandering albatross (<i>Diomedea exulans</i>)	Australasian gannet (<i>Morrus serrator</i>)
Procellariiformes	Suliformes	Procellariiformes	Suliformes
United Kingdom	Mexico	France	Australia
Southern	Southern	Southern	Southern
10-44	3-13	7-39	5-12+
yes	yes	yes	yes
-	-	Constraint	-
-	-	-	-
-	yes	yes	yes
no	yes through terminal investment	-	yes
yes	yes	yes	-

55	56	57	58
Cross-sectional	Cross-sectional	Both	Cross-sectional
Experimental	Both	Observational	Observational
European Shag (<i>Phalacrocorax aristotelis</i>)	Snow petrel (<i>Pagadroma nivea</i>)	Common tern (<i>Sterna hirundo</i>)	Black-browed albatross (<i>Thalassarche melanophrys</i>)
Suliformes	Procellariiformes	Charadriiformes	Procellariiformes
United Kingdom	France	Germany	France
Northern	Southern	Northern	Southern
2+	8-36	2-21	8-14+
yes	yes	-	yes
-	Both	Constraint	Constraint
-	-	no	yes
yes	yes	yes	yes
-	-	-	-
-	-	-	-

59	60	61	62
Cross-sectional	Cross-sectional	Cross-sectional	Both
Experimental	Observational	Observational	Observational
Blue-footed booby (<i>Sula nebouxi</i>)	Black-legged kittiwake (<i>Rissa tridactyla</i>)	Black-browed albatross (<i>Thalassarche melanophrys</i>)	Common murre (<i>Uria adlgae</i>)
Suliformes	Charadriiformes	Procellariiformes	Charadriiformes
Mexico	France	France	United States of America
Southern	Northern	Southern	Northern
3-10+	2-8+	5-23	4-15+
-	yes	yes	yes
-	Constraint	Constraint	-
-	yes	no	-
-	yes	yes	-
-	-	-	-
yes	yes	yes	-

63	64	65	66
Both	Cross-sectional	Cross-sectional	Cross-sectional
Observational	Observational	Observational	Experimental
Common tern (<i>Sterna hirundo</i>)	Laysan albatross (<i>Phoebastria immutabilis</i>)	Cape gannet (<i>Morus capensis</i>)	Black-legged kittiwake (<i>Rissa tridactyla</i>)
Charadriiformes	Procellariiformes	Suliformes	Charadriiformes
Germany	United States of America	South Africa	United States of America
Northern	Northern	Southern	Northern
2-18	7-32	5-22	-
yes	yes	yes	-
-	-	-	-
-	-	-	-
yes	-	-	-
-	-	-	-
-	-	-	yes

67	68	69	70
Both	Both	Both	Both
Observational	Observational	Observational	Observational
Common guillemot (<i>Uria aalge</i>)	Heermann's gull (<i>Larus heermanni</i>)	Common tern (<i>Sterna hirundo</i>)	Black-legged kittiwake (<i>Rissa tridactyla</i>)
Charadriiformes	Charadriiformes	Charadriiformes	Charadriiformes
United Kingdom	Mexico	Germany	France
Northern	Northern	Northern	Northern
10-26	4-13	3-16	3-22
yes	yes	yes	yes
-	-	Constraint	-
-	-	-	yes
yes	yes	yes	yes
-	-	-	-
yes	-	-	yes

71	72	73	74
Cross-sectional	Both	Both	Both
Observational	Observational	Observational	Observational
Northern fulmar (<i>Fulmarus glacialis</i>)	Snow petrel (<i>Pagadroma nivea</i>) and southern fulmar (<i>Fulmarus glacialis</i>)	Little penguin (<i>Eudypula minor</i>)	Common tern (<i>Sterna hirundo</i>)
Procellariiformes	Procellariiformes	Sphenisciformes	Charadriiformes
United Kingdom	France	Australia	Germany
Northern	Southern	Southern	Northern
30+	5-36 and 6-36	2-22	3-20
-	yes	yes	yes
-	-	Constraint	Constraint
-	-	-	-
-	-	yes	yes
-	-	-	-
yes	yes	yes	-

75	76	77	78
Both	Cross-sectional	Cross-sectional	Cross-sectional
Observational	Both	Observational	Observational
Common tern (<i>Sterna hirundo</i>)	Snow petrel (<i>Pagadroma nivea</i>)	Common tern (<i>Sterna hirundo</i>)	Adelie Penguin (<i>Pygoscelis adeliae</i>)
Charadriiformes	Procellariiformes	Charadriiformes	Sphenisciformes
Germany	France	Germany	United States of America
Northern	Southern	Northern	Southern
2-12	6-44	3-21	3-10
yes	yes	yes	yes
Constraint	-	Constraint	Constraint
-	-	yes	-
yes	yes	yes	yes
-	-	-	-
-	yes	yes	yes

79	80	81	82
Cross-sectional	Both	Longitudinal	Both
Observational	Observational	Observational	Observational
Black-legged kittiwake (<i>Rissa tridactyla</i>)	Blue-footed booby (<i>Sula nebouxii</i>)	Blue-footed booby (<i>Sula nebouxii</i>)	Cassin auklet (<i>Pychooramphus aleuticus</i>)
Charadriiformes	Suliformes	Suliformes	Charadriiformes
France	Mexico	Mexico	United States of America
Northern	Southern	Northern	Northern
3-16	1-15	2-18	2-15
yes	yes	yes	yes
-	-	Constraint	-
-	maybe	-	-
yes	yes	-	-
-	-	-	-
-	yes	yes	-

83	84	85	86
Cross-sectional	Both	Both	Both
Observational	Observational	Observational	Observational
Roseate tern (<i>Sterna dougalii</i>)	Common tern (<i>Sterna hirundo</i>)	Leach's storm petrel (<i>Oceanodroma leucorhoa</i>)	Lesser black-backed gull (<i>Larus fuscus grællsii</i>)
Charadriiformes	Charadriiformes	Procellariiformes	Charadriiformes
Seychelles	Germany	Canada	Belgium
Southern	Northern	Northern	Northern
2-7	3-21+	4-24+	1-13
yes	yes	yes	yes
-	-	Constraint	-
-	-	-	-
yes	yes	yes	yes
-	-	no	-
-	yes	yes	-

87	88	89	90
Both	Cross-sectional	Longitudinal	Both
Observational	Observational	Observational	Both
Wandering albatross (<i>Diomedea exulans</i>)	Black-browed albatross (<i>Thalassarche melanophrys</i>)	Wandering albatross (<i>Diomedea exulans</i>)	Thick-billed murre (<i>Uria lomvia</i>)
Procellariiformes	Procellariiformes	Procellariiformes	Charadriiformes
France	France	United Kingdom	Canada
Southern	Southern	Southern	Northern
6-40	5-35	7-37	4-23
yes	yes	yes	-
-	Constraint	Constraint	-
-	-	no	-
-	yes	yes	-
-	-	-	no, but terminal investment
yes	yes	yes	-

91	92	93	94
Cross-sectional	Both	Cross-sectional	Cross-sectional
Both	Observational	Observational	Both
Blue-footed booby (<i>Sula nebouxii</i>)	Wandering albatross (<i>Diomedea exulans</i>)	Audouin's gull (<i>Larus audouinii</i>)	Black-legged kittiwakes (<i>Rissa tridactyla</i>) and Thick-billed murre
Suliformes	Procellariiformes	Charadriiformes	Charadriiformes
Mexico	France	Spain	United States of America
Northern	Southern	Northern	Northern
3-18	5-44	3-25	3-22 and 3-29
yes	no	yes	yes
-	-	Constraint	Terminal restraint
-	-	yes	-
-	yes	yes	-
-	yes	-	-
-	both	yes	yes

95	96	97	98
Longitudinal	Both	Longitudinal	Both
Observational	Observational	Observational	Observational
Scopoli's Shearwater (<i>Calonectris diomedea</i>)	Common tern (<i>Sterna hirundo</i>)	Common tern (<i>Sterna hirundo</i>)	Wandering albatross (<i>Diomedea exulans</i>)
Procellariiformes	Charadriiformes	Charadriiformes	Procellariiformes
Spain	Germany	Germany	France
Northern	Northern	Northern	Southern
5-23	2-5+	2-21	7-42
yes	yes	yes	yes
Constraint	-	-	-
-	yes	-	-
yes	yes	-	-
-	-	-	-
-	yes	yes	yes

99	100	101	102
Cross-sectional	Cross-sectional	Longitudinal	Longitudinal
Observational	Observational	Observational	Observational
Common tern (<i>Sterna hirundo</i>)	King penguin (<i>Aptenodytes patagonicus</i>)	Mediterranean storm petrel (<i>Hydrobates pelagicus</i>)	Wandering albatross (<i>Diomedea exulans</i>)
Charadriiformes	Sphenisciformes	Procellariiformes	Procellariiformes
Germany	France	Spain	France
Northern	Southern	Northern	Southern
1-25	4-11	3-14	6-41
yes	yes	yes	yes
Constraint	Constraint	-	-
-	-	-	-
yes	yes	-	-
-	-	-	-
yes	-	yes	yes

103
Cross-sectional
Observational
European Shag (<i>Phalacrocorax aristotelis</i>)
Suliformes
United Kingdom
Northern
2-22
-
-
-
-
-
yes

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Appendix 3Table A3.1 Breeding success of bridled terns (*Onychoprion anaethetus*) on Penguin Island, Western Australia over three breeding seasons.

Breeding season	Adult age classes	Breeding success	Breeding failure
2013/2014	Under 6 years	3	11
	Over 6 years	8	6
2014/2015	Under 6 years	2	8
	Over 6 years	9	4
2015/2016	Under 6 years	4	8
	Over 6 years	7	2

The log-linear analysis for a three-way contingency table was performed in Vassarstats using age (factor A), breeding success (factor B) and breeding season (factor C – layer). Because the expected frequencies were on average greater than six, the test could be conducted.

Table A3.2 Results of the log-linear analysis for a three-way contingency table on the breeding success/failure of bridled terns (*Onychoprion anaethetus*) on Penguin Island, Western Australia using age (factor A), breeding success (factor B) and breeding season (factor C – layer).

Source	G^2	Degrees of freedom	p
ABC	15.62	7	0.0288*
AB	13	1	0.0003*
AC	0.84	2	0.657
BC	0.9	2	0.638
AB(C)	13.88	3	0.0031*
AC(B)	1.72	4	0.7871
BC(A)	1.78	4	0.7761

* shows significance ($p < 0.05$)

Appendix 4

Table A4 Breeding parameters in bridled terns (*Onychoprion anaethetus*) from Penguin Island, Western Australia recorded over three breeding seasons from summer 2013/2014 to summer 2015/2016 (results shown as mean \pm standard error).

	Parents' age classes			All age classes
	Under 6 years of age	Between 6 and 12 years of age	Over 12 years of age	
Laying date (Julian days)	332 (± 17)	341 (± 21)	342 (± 22)	327 (± 62)
Egg weight (g)	25.2 (± 1.9)	25.0 (± 2.0)	23.7 (± 3.2)	24.95 (± 2.18)
Egg length (cm)	4.54 (± 0.15)	4.59 (± 0.20)	4.42 (± 0.26)	4.55 (± 0.22)
Egg width (cm)	3.24 (± 0.07)	3.22 (± 0.15)	3.20 (± 0.11)	3.22 (± 0.12)
Incubation time (days)	29 (± 2)	30 (± 2)	30 (± 2)	30 (± 2)
Hatching chick weight (g)	17.3 (± 2.4)	17.4 (± 2.2)	15.7 (± 3.1)	17.2 (± 2.5)
Hatching chick wingspan (cm)	9.7 (± 0.7)	9.5 (± 0.5)	9.7 (± 0.7)	9.6 (± 0.6)
Hatching chick head and bill (mm)	34.6 (± 1.1)	33.7 (± 1.2)	33.8 (± 1.3)	34.0 (± 1.2)
Hatching chick tarsus (mm)	17.4 (± 1.2)	17.1 (± 1.2)	16.7 (± 0.5)	17.1 (± 1.1)
Lag for flight feathers appearance (days)	9 (± 2)	10 (± 2)	9 (± 1)	10 (± 2)
Weight growth coefficient	5.43 (± 1.06)	4.93 (± 1.33)	5.62 (± 0.39)	5.13 (± 1.22)
Wingspan growth coefficient B1	0.64 (± 0.44)	0.36 (± 0.28)	0.79 (± 0.13)	0.65 (± 0.32)
Wingspan growth coefficient B2	0.00 (± 0.11)	0.03 (± 0.05)	0.02 (± 0.02)	0.02 (± 0.07)
Head and bill length growth coefficient	1.29 (± 0.07)	1.30 (± 0.12)	1.25 (± 0.08)	1.29 (± 0.10)
Tarsus growth coefficient	0.79 (± 0.14)	0.70 (± 0.13)	0.71 (± 0.14)	0.72 (± 0.14)
Raising time (days)	52 (± 7)	52 (± 4)	52 (± 2)	52 (± 5)
Fledgling weight (g)	120.1 (± 11.2)	117.5 (± 6.9)	116.3 (± 10.5)	118.1 (± 8.6)
Fledgling wingspan (cm)	71.1 (± 2.8)	70.0 (± 3.6)	71.3 (± 1.1)	70.4 (± 3.2)
Fledgling tarsus (mm)	25.6 (± 1.0)	25.4 (± 0.8)	25.6 (± 0.5)	25.5 (± 0.8)
Fledgling head and bill (mm)	68.1 (± 3.7)	68.5 (± 2.6)	69.6 (± 2.3)	68.6 (± 2.8)

