PENGUIN PARENTING: ASSORTATIVE MATING, NEST ATTENDANCE AND SEX-SPECIFIC CHICK PROVISIONING IN THE AFRICAN PENGUIN (*SPHENISCUS DEMERSUS*)

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What outlandish beings are these?

…Their bodies are grotesquely misshapen, their bills short, their feet seemingly legless; while the members at their sides are neither fin, wing, nor arm. And truly neither fish, flesh, nor fowl is the penguin; as an edible, pertaining neither to Carnival nor Lent; without exception the most ambiguous creature yet discovered by man. Though dabbling in all three elements, and indeed possessing some rudimental claims to all, the penguin is at home in none. On land it stumps; afloat it sculls; in the air it flops.

...Nature keeps this ungainly child hidden away at the ends of the earth…

~ Herman Melville ~

***The Encantadas* (1854)**

ABSTRACT

Animal behaviour is especially sensitive to environmental variability and prey availability during the breeding season, and this is particularly true for non-volant, central place foragers such as the endangered African Penguin (*Spheniscus demersus*). Individual sex and morphology, as well as the level of assortment within mated pairs can influence both the behaviour and the reproductive success of species exhibiting biparental care. This study made use of a large biometric database and nest attendance video footage to determine the influence of intrinsic (assortative mating, brood size and chick age) and extrinsic (environmental conditions, anthropic disturbance) factors on breeding behaviour and performance of African Penguins on Bird Island, Algoa Bay, during peak breeding (March - July) in 2013.

While sexual dimorphism in African Penguins is subtle, the colony-specific discriminant functions presented here provide an accurate sexing tool when only bill and flipper length are available. Despite the premise that selection of a large, high-quality mate in this long-lived, monogamous seabird governs lifetime fitness, only low levels of assortative mating were found, and this only for earlier breeders, when larger females (but not males) bred. The 2013 season was a particularly successful one, coinciding with above-average sardine and anchovy abundance, and almost 80% of monitored nests were double-brooded, with very low levels of mortality. A- and B-chicks of double broods and singleton chicks grew at similar rates and exhibited similar body condition indices. In these conditions, chick developmental rates were independent of parental size, assortment or provisioning behaviour. Females raising a double brood were significantly lighter and in poorer body condition than those raising a single chick, although the same trend was not evident in males. Offspring sex ratio in 2013 (2.27:1) favoured male chicks, suggesting that there is potential to over-produce the larger sex when resources are plentiful.

Peak nest arrival and departure times of parents did not change over the course of monitored breeding attempts (March-June), nor were they different for disturbed and undisturbed nests or for a single or double brood. The increase in CCTV-observed provisioning rate as chicks grew larger was best explained by brood size, at-sea chlorophyll *a* concentration, and maximum air temperature, but was unrelated to parental morphology or assortative index. Importantly, parental absenteeism commenced earlier and was markedly greater in nests frequently handled by researchers than in undisturbed nests. Both the time spent together by parents, and absenteeism were measurably affected by maximum afternoon air temperatures, the effects of which are expected to be exacerbated by poorer foraging conditions and climate change.

A third of manually-monitored nests shared chick-guarding duties unequally, although this phenomenon was independent of parental sex or morphology. The adaptive benefits of mating patterns and division of labour during chick-rearing may only become apparent in a year of below-average food availability and it is highly recommended that this study be repeated in a year of scarce food resources. These findings augment past foraging ecology studies and demonstrate that investigator disturbance and environmental conditions can affect the nesting behaviour of this highly threatened seabird.

Keywords: African Penguin, assortative mating, morphometrics, nest attendance, provisioning rate, disturbance.

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GLOSSARY AND LIST OF ABBREVIATIONS

AI: Assortative Index; the smaller the index, the more similar the pair is for a particular trait.

ASI: Absolute Size Index; the combination of the morphometric measurements of a pair for a particular trait.

Assortative mating: A non-random pattern of mate choice which can be positive or negative; positive assortative mating is the selection of a mate with a similar phenotype to itself.

BC: Body condition (mass divided by flipper length).

BCI: Body condition Index; a measure of chick health attained by relating its mass at an unknown age to its structural size, which is relatively standard over chick development.

Brood size: Denotes the number of offspring, i.e. one or two. The term is applied here to chicks of all ages.

Chick age classes with approximate ages in days:

- **P0 (0-7 days):** Freshly hatched chick, weak and thermally dependent on parents.
- **P1 (8-18 days):** Eyes have opened but still completely hidden under the parent.
- **P2 (19-39):** Small to medium downy chicks that have reached thermal independence.
- **P3 (40-60 days):** Large downy chicks.
- **P4 (61-81 days):** Similar size to adults, and starting to shed down.

Chl *a*: Chlorophyll *a* (concentration).

Clutch: The number of eggs laid by a female at one time.

Double-brood/ed: Used in this thesis to denote a breeding attempt, or nest, with two chicks.

Nest-day: One 24-hour period of data collected from a single nest**.**

Single-brood/ed: Used in this thesis to denote a breeding attempt, or nest, with one chick.

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CHAPTER 1

GENERAL INTRODUCTION

1.1 THESIS STRUCTURE

This thesis consists of six chapters. The first is an introduction to the distribution, conservation status and reproductive biology of the study species and outlines the aims of this study. The second chapter gives a brief overview of the study site in terms of its history, location, climate and its important fauna and flora. The next two chapters address the purpose of the study, detailing the undertaking of field and laboratory work and discuss the results of two aspects of the African Penguin's breeding biology: assortative mating and its influence on breeding performance, and nest attendance patterns of breeding penguins with regard to timing, brood size, disturbance and environmental parameters. A synthesis of findings is presented in the fifth chapter. For ease of access or recall, all cited literature has been referenced fully in Chapter 6.

1.2 INTRODUCTION

Seabirds, and penguins in particular, have been heralded as sentinel species; birds which have been reliably used as indicators of ocean health and the status of marine ecosystems (Boersma 2008). As they are wholly dependent on marine resources for their survival, are colonial (gathering densely at breeding grounds) and are relatively easily censused, handled and measured, penguins are easily enumerated denizens of an otherwise invisible underwater habitat (Piatt *et al.* 2007). Changes in their demographics and individual well-being reflect oceanic variations that stem from a number of causes, such as climate change, fishing pressures and human disturbance. Understanding how their reproductive output changes with the variation in food availability is underpinned by the response in energy expenditure by provisioning parents (Barlow & Croxall 2002). Provisioning of chicks is an essential duty for parents of altricial offspring and is most commonly shared between the sexes through biparental care (Clutton-Brock 1991). Seabirds exhibit greatly variable success in relation to the availability of prey during the breeding season and understanding how central place foragers allocate their time to foraging while raising offspring is a central question in seabird ecology (Croxall *et al.* 1988, 1999).

Marine ecosystems have provided man with an untold wealth of natural resources and ecosystem services for thousands of years and, until recently, were thought to be inexhaustibly self-sustainable, seemingly boundless and immune to the demands placed on them by man (Roberts 2007). But in light of recent global fish stock collapses and seabird declines and with a better understanding of how climate change affects oceans, we have realized that our ever-expanding population and grossly over-equipped fishing vessels have pressed the oceans to the limits of their sustainability (Pauly *et al.* 2002, Roberts 2007). Due to its sheer vastness and its inaccessibility, scientific research of the ocean and our understanding of its multi-dimensional relationships has lagged behind that of terrestrial ecosystems, despite the extraordinary technological advances achieved in recent decades (Costanza 1999, Jelinski 2015). Ecosystems have undergone fluctuations in structure and climate throughout earth's history and it is the response to global change in the form of favourable life history traits and adaptations that determine the evolutionary success or failure of a species (Vitousek 1994, Jiguet *et al.* 2007). By improving our understanding of the drivers and effects of these responses, we are better able to predict the outcomes of environmental variability and refine pre-emptive conservation approaches (Jiguet *et al.* 2007).

Eleven of the seventeen species of penguin found worldwide are now recognised as threatened or endangered, with even the more abundant species (Macaroni *Eudyptes chrysolophus* and Rockhopper *E. chrysocome* Penguins) undergoing population declines (IUCN 2013). It is clear that all is not well. Understanding how animals respond to environmental heterogeneity is an important ecological topic and many studies have addressed how foraging seabirds, in particular, adjust their movements in light of landscape heterogeneity (Pinaud & Weimerskirch 2005, 2007). The breeding penguin is a central place forager (Crawford *et al.* 2011), as it is constrained by the demands of provisioning its offspring at regular intervals (Taylor *et al.* 2002) and for a breeding attempt to be successful, this sets an upper limit on the travelled distance and the duration of their foraging bouts. The African Penguin forages on a much finer scale than offshore foragers such as the King Penguin which can travel 100-1000km using meso-scale oceanic conditions to locate food (Charrassin & Bost 2001). While predator-prey relationships are spatially complex on a meso-scale (Bost *et al.* 2009), complexity at finer scales is amplified further. For this reason, little is known about the short-range foraging behaviour of the African Penguin in relationship to local environmental factors (van Eeden 2012).

Inshore-foraging penguins, such as the African Penguin, face highly unpredictable availability of prey, with the number of eggs laid representing the maximum number of chicks that can be raised in a season when food is plentiful (Forbes 1990, Croxall & Davis 1999). Characteristics which are strongly developed in most of the inshore foragers include hatching asynchrony and a mismatch in egg size and weight, which generally delivers an advantage to the first-hatched chick that extends throughout the rearing period (Williams & Cooper 1984, Ramírez *et al*. 2015). As a monogamous seabird, mate choice in the penguin is especially important, as lifetime fitness will depend on the selection of a suitable partner. However, mating patterns and the potential resultant effects in seabirds have been poorly elucidated (Teather & Nol 1997, Catry *et al.* 1999, Forero *et al.* 2001).

Two previously unaddressed aspects of the breeding biology of the endangered African Penguin (*Spheniscus demersus*) are the subject of this study. Through the use of CCTV technology and standard morphometric measurements and sexing techniques on a relatively large sample of the population of penguins on Bird Island (Algoa Bay, South Africa), assortative mating, sex-specific parental investment and nest attendance characteristics over the course of one peak breeding season were investigated.

1.3 THE SEABIRD SYNDROME

"The seabird syndrome" is a term that describes the K-selected life history traits (low fecundity, coloniality, extended lifespans and minimal sexual dimorphism) that characterize the lives of birds feeding on highly variable marine food webs (Pianka 1970, Ricklefs 1990, Gaston 2004). Lack (1968) postulated that the parental attributes that attend the high costs of foraging at sea (low chick-feeding rates, reduced clutches and slow rates of chick growth) have selected for biparental care and monogamy in many seabirds, since it is almost impossible for the average parent to feed both its chicks and itself concurrently.

Besides deserting current offspring for the purpose of re-mating, perhaps with a higher-quality mate, parents most often desert their eggs or chicks when their own body condition is poor (Székely *et al.* 1996). This is often the case for incubating penguins and other long-lived seabirds; the choice to desert is often manifested when the toll of caring for offspring on already low body reserves threatens the survival of the parent (Székely *et al.* 1996, Dearborn 2001, Bijleveld & Mullers 2009). As long lived species, seabirds are “prudent parents” (Drent & Daan 1980) and tend to favour their own survival and future reproductive attempts over their current breeding attempt (Lloyd 1987, Saraux *et al.* 2011). Parental care in penguins may also be terminated prematurely to prepare for their annual moult during the non-breeding season (Székely *et al.* 1996).

Several factors affect the trade-off between brood care and self-preservation, namely brood size (Székely & Cuthill 2000), body condition of the parents (Chaurand & Weimerskirch 1994) and the quality of the offspring (Erikstad *et al.* 1997, Griggio *et al.* 2004). In polygamous species, the prevalence of desertion tends to be greater for the sex favoured by the operational sex ratio (OSR: ratio of available females to available males) (Griggio *et al.* 2004), however, the African Penguin is monogamous and only occasionally undergoes a second breeding attempt (Hockey *et al.* 2005), and it can be assumed that the decision to desert is thus a physiological one.

1.4 DESCRIPTION OF STUDY SPECIES

1.4.1 *Physical appearance*

The scientific name for the African Penguin, *Spheniscus demersus* (Linnaeus 1758), stems from the Greek word *spen*, diminutive for ‘a wedge’, and the Latin word *demersus* for ‘plunging’ (Hockey 2001). This description, of a “little plunging wedge” portrays the streamlined body structure and habits of this non-volant, marine top predator. Formerly known as the Jackass, Cape or Black-footed Penguin, the African Penguin (see inset, right) is Africa’s only endemic penguin. The average adult African Penguin stands 55-65cm tall and weighs between 2.1 and 3.7kg (Hockey 2001). Sexes are alike in plumage, and on average, males are slightly larger and possess longer and deeper bills than females (Hockey 2001). However, sexes may overlap, with large females physically bigger than the smallest males (Randall 1989).

1.4.2 *Distribution of the African Penguin*

The African Penguin is endemic to the nutrient rich waters of the greater Benguela upwelling ecosystem along the south-western coast of Africa (Frost *et al.* 1976, Crawford *et al.* 2011). It breeds primarily at 27 colonies between Hollamsbird Island, Namibia, and Bird Island in Algoa Bay (South Africa, Figures 1.2 and 1.2), but was probably displaced by human disturbance, prey shortages and outcompeted for space by the Cape Fur Seal (*Arctocephalus pusillus*) at a further 10 locations (Williams & Cooper 1984, Crawford *et al.* 1995a, Hockey *et al.* 2005). As of 2000, only 14 of the 27 breeding colonies contained more than 1000 adult penguins. The African Penguin currently breeds in three regions, namely southern Namibia and South Africa's Western and Eastern Cape provinces (Crawford *et al.* 2008). The regions span 2000km of coastline and are separated from each other by ca. 500km.

The distribution of the African Penguin is roughly confined to the cold waters of the Benguela Current where upwelling occurs, bringing nutrients to the surface as a result of strong offshore winds (Hockey 2001). The Algoa Bay population of approximately 10 500 pairs represents 40% of the global total (Crawford *et al.* 2011) and also occurs in the vicinity of a smaller, intermittent upwelling system near Cape Padrone (Lutjeharms *et al.* 2000).

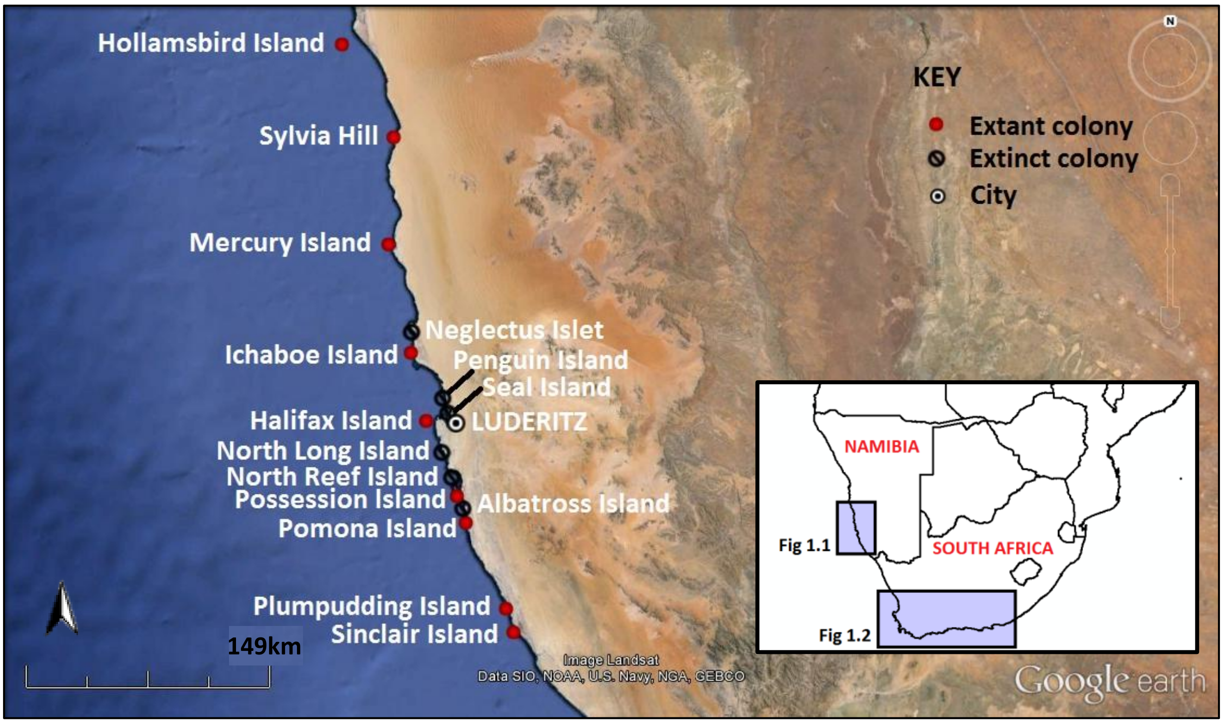

Figure 1.1: Distribution of extant and extinct African Penguin breeding colonies in Namibia.

Figure 1.2: Distribution of extant and extinct African Penguin breeding colonies in South Africa. Bird Island, along with five other breeding sites, is situated within Algoa Bay, the easternmost bay shown here.

1.4.3 *Population trends*

The African Penguin has undergone a radical population decline in the 20th Century (La Cock *et al.* 1987, Crawford *et al*. 2011) and was declared a species vulnerable to extinction in 1996. It has been listed by the International Union for Conservation of Nature as endangered since 2010 (IUCN 2013). Comprehensive colony censuses began in South Africa in 1956 and indicated that there were at least 300 000 breeding birds and juveniles on all breeding colonies (Crawford *et al.* 1990, Crawford 1998). Between 2001 and 2009, the South African population collapsed by 60%, with a loss of 35 000 breeding pairs (Crawford *et al.* 2011). In Algoa Bay alone, the breeding population more than halved between 2001 and

2003, from 21 000 to 10 000 pairs (Crawford *et al.* 2011). This left the global population total at an estimated 26 000 breeding pairs including the Namibian breeders. The rapidity of the decline provided support for the decision to urgently downgrade the species' Red List status and increase protection for this iconic marine bird (Crawford *et al.* 2011, Pichegru 2013).

Breeding in Algoa Bay occurs on six islands forming two groups of three islands each, and since the 1990's, the St Croix group has consistently been recorded as having greater numbers of active nests than the Bird Island group (Ralph 2008). During the last decade, both populations have remained relatively stable apart from occasional poor breeding seasons. Overall, however, the trend is one of decline. The recent exceptional decline in the global African Penguin population has been linked principally to several anthropogenic effects. Initially, human disturbance caused by egg harvesting and guano collection, (particularly from west-coast islands) caused severe population crashes (Kemper 2006). Oil spills and on-going competition for food with the ever-growing fishing industry continue to be serious burdens on a species in distress (Crawford *et al*. 2006).

1.5 DIET

1.5.1 *Prey composition and foraging habits*

The diet of the African Penguin varies considerably on both temporal and spatial scales, but consists primarily of pelagic schooling fish (Randall & Randall 1986, Hockey *et al.* 2005). It includes sardine (*Sardinops sagax*), anchovy (*Engraulis encrasicolus*), horse mackerel (*Trachurus capensis)*, round herring (*Etrumeus whiteheadii*), southern mullet (*Liza* *richardsonii*), cephalopods, crustaceans, polychaetes and crab larvae (Hockey *et al.* 2005). Penguins forage at sea alone or in small groups and are often seen feeding with Cape Gannets, cormorants, gulls and terns (Hockey *et al.* 2005). Prey fish of between 10 and 300mm are generally caught from beneath and (except for the biggest meals) swallowed underwater (Hockey 2001, Hockey *et al.* 2005).

The average adult African Penguin requires approximately 300g of fish per day, excluding food caught for offspring (Hockey 2001), while the average chick requires approximately 26kg of anchovy prior to successful fledging (Randall 1989). Squid meals take a longer period to digest than fish (Wilson *et al.* 1985), and are also not as nutrient-rich, and chicks fed on a diet dominated by squid do not grow as rapidly as those fed on fish-diets (Randall 1989, Hockey 2001).

Penguins forage more frequently in daylight hours than during the night; this is especially true for the Macaroni Penguin (Barlow & Croxall 2002), Rockhopper Penguin and the African Penguin (Wilson *et al.* 1993), and it is at night that they spend more time in the colony. African Penguins that were shot at sea at dawn were found to have little, if any, food in their stomachs (Rand 1960), suggesting that time spent at sea during the night is not devoted to foraging. It is unclear how penguins locate the prey they do catch at night or at greater depths, however many pelagic cephalopods, fish and crustaceans are bioluminescent and this must be relevant (del Hoyo *et al.* 1992).

1.5.2 *Prey dynamics*

Years of above average breeding failure have been linked to scarcity of prey species which may in turn be linked to increased fisheries pressure (Hockey *et al.* 2005, Crawford *et al.* 2011). Prey availability is highly variable in the marine ecosystems in which penguins forage, and these seabirds are particularly sensitive to these changes during their breeding attempts as central place foragers (Ancel *et al.* 2013). It is very likely that the pre-breeding body condition of the penguin as well as the decision to reproduce is heavily influenced by the food availability in the waters surrounding breeding colonies (Crawford *et al.* 2011, Ancel *et al.* 2013). For example, significant relationships have been found between the number of African Penguin chicks fledged per breeding pair and spawner biomass of anchovy, and between the proportion of breeding adults and sardine biomass (Crawford *et al.* 1995b).

Worldwide, in areas where populations of sardine (*Sardinops sagax*) and anchovies (*Engraulis* spp.) co-occur, regimes of the relative abundance of the two species alternate (Crawford 1998). This has an effect on ecosystem ecology, affecting both higher (predator) and lower (prey) trophic levels (Schwartzlose *et al.* 1999). Recent distribution shifts of sardines and anchovy spawners have been observed off the coast of South Africa since the mid-1990s; a progressive eastwards movement of these small pelagic species has undoubtedly altered the foraging patterns of their predators. It has been suggested that this shift at least partly accounts for the decline of other top marine predators on the west coast, the Cape Gannet *Morus capensis* and the Cape Cormorant *Phalacrocorax capensis* (Schwartzlose *et al.* 1999, Pichegru *et al.* 2007, Crawford *et al*. 2014).

Regime shifts in prey species (sardine to anchovy and vice versa) have coincided with marked reductions in African, Galápagos and Humboldt Penguin (*Spheniscus humboldti*) numbers (Boersma 1974, Crawford 1998, Le Maho & Durant 2011). Competition between the fisheries and marine seabirds is exacerbated when overlapping target species are scarce, and seabirds are often the losers in this scenario. However, the life history traits of seabirds have evolved to buffer their populations against environmental stochasticity such as shifts in target species abundances and unpredictability in food availability (Crawford 2004, Pichegru *et al.* 2007).

Industrial fisheries deplete marine stocks and produce vast amounts of waste that many seabirds have learnt to rely upon (Pichegru *et al.* 2007). However, unlike the Cape Gannet and many others seabirds, African Penguins do not scavenge and thus do not benefit from the fish remains that are discarded from fishing boats.

1.5.3 *Foraging dynamics*

Inequality in parental duties may correlate with a spatial and temporal discrepancy in the manner in which prey aggregations are exploited by the two sexes of a pair (Pichegru *et al.* 2013). Males tend to dive deeper than females, probably due to the greater physiological capabilities of bigger bodies linked to allometry (Walker & Boersma 2003, Cook *et al.* 2007, Raya Rey *et al.* 2012). In addition, a larger bird may subdue and consume larger prey, even exploiting different species, thus suggesting possible niche partitioning between the sexes (Cook *et al.* 2007, Zavalaga *et al.* 2007). Intra-specific dietary differences in the Magellanic penguin (*S. magellanicus*) and a greater maximum diving depth of the (larger) male

Humboldt Penguin have been observed (Taylor *et al.* 2002), albeit only under certain circumstances. Although it is unclear why there would be differences in foraging characteristics (swimming speed, trip duration, dive depth and dive frequency, etc.) in some species and not others, the Adélie (*Pygoscelis adeliae*), Emperor (*Aptenodytes forsteri*), African and Little (*Eudyptula minor*) Penguins all show some degree of sex-specific differences (Chappell *et al.* 1993, Bethge *et al.* 1997, Wienecke & Robertson 1997), while the Gentoo (*Pygoscelis papua*), Yellow-eyed (*Megadyptes antipodes*), Royal (*Eudyptes schlegeli*) and Rockhopper Penguins do not (Croxall *et al.* 1988, Seddon & van Heezik 1990, Hull 2000).

1.6 REPRODUCTION

Parental care by means of natural selection leads to optimization of the balance between the quality and the number of young produced over an individual's lifetime (Lack 1954). Since the number of progeny per lifespan of all seabirds is limited, and reproductive investment is potentially made at the expense of future breeding attempts, parents must allocate resources to their offspring so as to maximize their fitness (Trivers 1972, Forbes 1990, Schreiber & Burger 2002). The strategy of reducing the number of offspring produced (or choosing not to breed at all) is manifested most intensely under conditions of food scarcity (Lack 1954). All penguins, except for those in the genus *Aptenodytes* typically lay a clutch of two eggs that may or may not exhibit obligate brood reduction. The reproductive biology of the African Penguin is now discussed in detail.

1.6.1 *Incubation and chick rearing*

The monogamous African Penguin has a prolonged breeding season, although peak breeding in South Africa occurs from February to September (Crawford *et al.* 1990, Hockey *et al.* 2005). In a study assessing mate and nest site fidelity, 80-92% of the birds whose mate returned in subsequent years, nested with the same partner the following breeding season at St Croix Island, with 89% of males and 78% of females returning to the same nest location (Hockey *et al.* 2005). There is evidence that penguins which have separated from their partner of the previous season have lower chances of breeding that season compared to pairs which reunite for the subsequent breeding attempt (Croxall & Davis 1999).

Age at first breeding for the African Penguin is between 3 and 6, but generally 4 or 5 years (Hockey 2001, Hockey *et al.* 2005, Whittington *et al.* 2000). Prior to breeding, the male arrives first at the colony, followed by the female about four days later. The pair then reinforces their bond, mate repeatedly and prepare a nest; no defined pre-laying exodus occurs. Nests are often made by burrowing into guano or soil, or are established in rocky crevices or artificial structures. Nests are lined with pebbles, vegetation, feathers and debris. Birds nesting in the open are known to have a higher breeding failure rate than rock- or burrow-nesting birds, primarily due to the increased risk of flooding, exposure (insolation and risk of hypothermia), and predation, mainly due to Kelp Gulls (*Larus dominicanus*; Seddon & van Heezik 1991a, Pichegru 2013). Approximately three weeks after the pair comes ashore, two eggs are laid. Incubation commences with the laying of the first egg, which hatches 38-41 days later (Hockey *et al.* 2005). Fewer than 1% of clutches consist of a single egg, and the mean interval between the laying dates is 3 days; hatching dates are an average of 2.1 days apart (Williams & Cooper 1984, Seddon & van Heezik 1991a, Hockey *et* *al.* 2005). The first-laid egg, or the A-egg, is on average 4.6g heavier than the second-laid, or B-egg (Williams & Cooper 1984, Seddon & van Heezik 1991a). If eggs are lost to flooding or predators, replacement clutches may be laid with a frequency that varies from colony to colony; a second clutch is rare on Robben Island, whereas up to four clutches have been laid by Bird Island (Algoa Bay) pairs (Hockey 2001). Penguin chicks are semi-altricial, the only precocial characteristic being that they hatch with sooty-grey down already present (O'Connor 1984). Chicks encourage their parents to disgorge food by incessant calling and pecking at the adult's bill and throat; they are subsequently fed by incomplete regurgitation (Seddon & van Heezik 1991).

Newly hatched chicks weigh approximately 70g, and their eyes open at 6-10 days. Between 11-15 days, secondary down appears, bringing white feathers to the face and belly and full control over thermoregulation (Hockey *et al.* 2005). Contour feathers begin to grow beneath the down at 31-35 days and the juvenile plumage is fully formed at 61-65 days. Fledging occurs between 60 and 130 days and adults will feed their young as long as they are still in the colony (Hockey 2001). Unlike many other seabird species, no starvation period precedes fledging (Randall 1989). When fledglings leave for the sea, they do so alone and may spend 12-23 months without coming ashore until they are forced to do so to moult into their adult plumage (Hockey 2001).

If food supply is limited in the initial phase of chick development, parents selectively feed the A chick, which already has a competitive advantage over its smaller sibling. The B chick succumbs to starvation once its energy reserves are depleted, the parents avoiding wastage of food by provisioning a chick that would probably perish in any event (Williams & Cooper 1984). First-hatched chicks generally complete their feather development and attain independence earlier than their siblings, although once receiving all the food from its parents after its sibling has gone to sea, the B chick fledges at a weight equal to, or in excess of, the A chick (Williams & Cooper 1984). This extension of the chick-rearing period may enable penguins to raise two chicks when food is limited (although not below an undetermined threshold of prey scarcity). Death of chicks after 40 days of age (the commencement of peak food demand) is most likely a result of starvation, with B chicks the more susceptible victims (Seddon & van Heezik 1991a).

While breeding success is a useful measure of productivity, coarse-scale information does not provide insight into the quality of the offspring produced. Chick quality has been shown to be highly variable within and between years (Sherley 2010). The chick-rearing period is the most energetically demanding phase of the reproductive cycle and chick-provisioning is thus potentially an extremely useful tool for assessing ecosystem quality (Sherley 2010).

1.6.2 *Components of offspring mortality*

Predation of both eggs and nestlings is often the most important mortality factor for small birds. Kelp Gulls have been shown to have a major impact on penguin numbers on Bird Island (Pichegru 2013). Nest flooding is also a significant source of mortality for young chicks (Seddon & van Heezik 1991a) as they are unable to thermoregulate independently before about two weeks of age (Hockey 2005).

Nestling starvation is relatively frequent among birds. Two hypotheses are proposed to account for the phenomenon; 1) growing offspring present increasing energetic and nutritional demands on their parents, thus mortality increases with offspring age, or 2) mortality due to starvation occurs as a by-product of adaptive brood reduction of oversized brood to match that of food supply (O’Connor 1984). The hatching asynchrony of African Penguins provides a way for an age, and thus size, difference to be manifested between the chicks of a double brood. If food becomes limited, the competitive advantage conferred to the A-chick will ensure that it is fed preferentially. Were both chicks of similar stature, both might succumb to starvation if food availability declined (Clarke & Wilson 1981, O’Connor 1984). Thus, hatching asynchrony slightly spreads the peak food demand between chicks (Seddon & van Heezik 1991 and references therein relating to explanatory hypotheses).

Birds are more likely to desert a breeding attempt at the incubation stage than at the chick-guarding stage either due to greater dangers associated with this initial period, or due to greater territoriality or nest site tenacity in later stages (Ricklefs 1969). Longer-lived species are also more likely to desert than those with short longevity, as the “seabird syndrome” would predict (Clutton-Brock 1991).

1.7 THREATS FACING THE AFRICAN PENGUIN

1.7.1 *Historical guano collection and egg harvesting*

Penguins once burrowed into a thick layer of guano (nitrogen- and phosphate-rich seabird droppings accumulated over centuries) that formed the crust of their breeding islands, which has since been removed by man (Craig 1964). Early sealers and whalers reported penguins almost covering islands entirely off the South West African (now Namibian) coast, even on islands which today are barely inhabited by the birds. Reports of guano deposits 30-40 foot high ignited the “guano rush” of the 1840s, where this “white gold” was used primarily as fertilizer, but also in the cosmetic and gunpowder industries (Kemper 2006;

Snyders 2011). Schwartzlose *et al.* (1999) reported a peak guano harvest of 6 000 tonnes from Namibian islands in 1924, and 4 000 tonnes from South African islands in 1926.

The negative effects of the guano trade still impact penguins today, since removal of suitable burrowing substrate forces the birds to make use of surface nests (Pichegru 2013). While guano burrows provide protection from aerial predators and wind as well as providing a thermally buffered microclimate, birds in surface nests are subjected to inclement weather, harsher insolation and increased predation risk by Kelp Gulls and Sub-Antarctic Skuas (*Catharacta antarctica*; Pichegru 2013). Fortunately, appropriately designed artificial burrows in conjunction with predator-culling practices on Bird Island have been shown to improve chick survival rates (Pichegru 2013) and thus slightly ameliorate the effect of historical guano removal.

Large-scale harvesting of penguin eggs, said to be very palatable (Randall 1989), began soon after Europeans settled in the Cape; the population of African Penguins on Robben Island was extinct as earlier as 1800, indicating heavy exploitation (Frost *et al.* 1976). Egg crop data for the African Penguin are said to be among the most accurate for any species (Frost *et al.* 1976) Between 1900 and 1930, a total of approximately thirteen million eggs was harvested but records are likely to be an underestimation of the true exploitation, since well-incubated eggs were destroyed in order to induce brooding adults to re-lay (Frost *et al.* 1976).

1.7.2 *Oiling*

The southern African coast lies adjacent to a globally important shipping route and as such, is subjected to the risk of contamination of its coastal waters by oil slicks from passing vessels (Moldan & Dehrman 1989). The ecological catastrophes that result from major oil spills are well recognized and publicized, although smaller-scale, yet on-going illegal discharge of oil from vessels other than tankers is comparatively poorly regulated and under-reported (Moldan & Dehrman 1989). As flightless foragers, penguins spend a large proportion of their time at sea on the surface, especially during the non-breeding season when they range widely, and are greatly susceptible to oiling when they surface to breathe (Adams 1994). These birds may then come ashore at locations that are distant from their breeding colonies, unable to swim due to matted plumage and with reduced ability to thermoregulate due to the loss of the waterproofing properties of their feathers (Fry & Lowenstine 1985). Oil ingested by preening birds also has a wide array of toxicological and pathological effects (Randall *et al.* 1980; Fry & Lowenstine 1985).

Despite no major oil spill having occurred in the area in the 3 ½ year period between June 1976 and February 1980, Randall *et al.* (1980) reported the leading cause of death of 295 adult penguins on St Croix Island as being oiling-related. On a much greater scale, the Treasure oil spill of June 2000 oiled approximately 19 000 African Penguins, endangering the lives of close to 40% of the world population (Kemper 2006). This statistic highlights the unnatural and unprecedented impact of the hazards of oiling facing the penguin population.

1.7.3 *Competition with the fishing industry*

South Africa possesses a well-developed fishing industry, particularly on the west-coast of the country. Purse-seine fisheries, mid-water and bottom (demersal) trawling, as well as line fishing have become firmly established commercial trades (Badenhorst 1997). Anchovy was first exploited by purse seine fisheries off southern Africa in the 1960s (coinciding with the sardine collapse at this time) and by 1966 it was the species that contributed most to the these catches; this continued until 1995 (Crawford 1998). Declines in sardine and anchovy recruitment reduce fisherman's catch per unit effort, resulting in economic difficulties and social pressures on a dependent populace. This in turn increases the pressure on fisheries managers to overlook the risks associated with short-term overfishing in order for the industry to continue (Badenhorst 1997). Overfishing is arguably the biggest threat facing the African Penguin, and many studies (Crawford *et al.* 2011, Pichegru *et al.* 2010, 2012, Weller *et al*. 2014) have pointed to competition with the purse seine industry as a major cause of their rapid decline over the past decades. Fish species exploited by man have been empirically shown to be more variable in abundance than unexploited species, and this increased unpredictability over and above natural variations can override even the best buffering defences of the "seabird syndrome" (Hsieh *et al.* 2006, Crawford *et al.* 2011). When the spawner biomass of combined fish prey was estimated using hydro-acoustic surveys to be lower than 2 million tonnes, breeding pairs on Robben Island fledged on average 0.46 chicks annually, compared with the 0.73 chicks fledged per pair per year when greater than 2 million tonnes of prey were available (Crawford *et al.* 2006). Based on survival estimates and the age at first breeding, successful reproduction at the lower bound is insufficient to sustain the population of African Penguins (Crawford *et al.* 2006). In addition to the competition for food with fisheries, penguins also form part of the industry's by-catch, and may become entangled in fishing gear or debris, although this is rare (Randall 1989, Underhill *et al.* 2006).

1.7.4 *Climate change*

Climate change models predict that an increase in the intensity, frequency and duration of extremely hot conditions will be experienced in the future (IPCC 2007). The predicted increases in maximum air temperature relate directly to a larger increase in evaporative water loss required by homeothermic animals to maintain a constant body temperature (McKechnie & Wolf 2010). While rates of mass-specific evaporative water loss decrease with increasing body size (McKechnie & Wolf 2010), African Penguins possess a layer of subcutaneous fat that insulates them from the cold waters of the Benguela system (Dawson & Whittow 2000). This adaptation to counter the cold works against them when breeding ashore in much warmer conditions than their more southern congenerics (Luna-Jorquera 1996). The dark dorsal plumage of the African Penguin and the fat reserves accumulated prior to the breeding season confer heat stress on birds during hot, humid, windless and cloudless days. Incubating adults not able to sufficiently dissipate heat by panting may abandon clutches or young chicks temporarily to cool down in the ocean and prevent further dehydration (Frost 1976). Exposed eggs and chicks then become vulnerable to overheating and predation by gulls, especially in open nests (Kemper 2006, Pichegru 2013).

Variations in marine productivity caused by El Niño events constitute the most extreme cases of climate change. Not only do they lead to severe decreases in the numbers of adult birds, they may also cause total breeding failures in the most vulnerable penguin species, namely the Humboldt and Galapagos Penguins (del Hoyo *et al.* 1992). Although warm-water events (when upwelling is reduced or ceases completely) in the Benguela upwelling system are not as severe as those in the Pacific, they have been linked to mass mortalities of seabirds as well as general breeding failure (La Cock 1986). Strong El-Nino events pose a threat to penguins through the anchovy recruitment failures which may ensue, such as happened off the coast of Namibia during 1983-84 (Boyd *et al.* 1998).

1.7.5 *Parasites, diseases and predators*

Apart from subclinical infestations of fleas, ticks and cestodes, outbreaks of bacterial infections have caused the death of African Penguins at rehabilitation centres in South Africa; *Escherichia coli* and *Staphylococcus aureus* (the causative agent of Bumblefoot) have been implicated in these outbreaks (Randall *et al.* 1980). Chicks and fledglings at St Croix Island have been killed by severe infestations of the intestinal trematode *Cardiocephaloides physalis* in the past (Randall & Bray 1983). Avian cholera (*Pasteurella multocida*) and piroplasm (*Babesia peircei*) have also been recorded (Crawford *et al*. 1992, Graczyk *et al*. 1996). Overcrowding has been known to result in Aspergillosis (a fungal lung infection) as well as outbreaks of Viral Newcastle Disease, a highly pathogenic and contagious infection (Whittington *et al.* 2000). Subclinical avian malaria (*Plasmodium relictum*) is common within the wild population, although in rehabilitation centres the disease is often found in conjunction with *Babesia,* and together with stress, the disease may fatally compromise the bird’s immune system (Schultz & Whittington 2005). Diseases contracted by rehabilitated birds may be spread to wild conspecifics upon release; this problem is exacerbated when large numbers of penguins are kept in high densities in rehabilitation centres following mass oiling events.

Other threats to the African Penguin include predation by feral cats and dogs and the large-spotted genet (*Genetta tigrina*) on mainland colonies, and competition with the Cape fur seal (*Arctocephalus pusillus pusillus*) for breeding space on particular islands (Underhill *et al.*

2006). In addition to the predation threat posed by gulls, Sacred Ibis (*Threskiornis aethiopicus*) and Great White Pelicans (*Pelecanus onocrotalus*) also feed upon unattended eggs and chicks (Hockey 2001). Seals and white sharks (*Carcharodon carcharias*) also predate upon penguins, or actively pursue and attack them as aggressive intraguild displays (Johnson *et al.* 2006). Seals kill approximately 2 – 2.5% of the adult population of breeding adult penguins per year and since the loss of one adult represents the loss of its clutch, the predation impact is intensified (Johnson *et al.* 2006, Makhado *et al*. 2013).

1.8 RATIONALE FOR THIS STUDY

The threats facing the African Penguin have shifted somewhat in the past 50 years, as humans no longer collect millions of eggs per year, nor harvest guano in any appreciable quantity. However, the effects of suboptimal surface nests in lieu of preferred guano burrows persist, posing an added hindrance to chick survival. In addition, oil pollution, climate change and ever-increasing fisheries pressures have exacerbated the decline in the penguin population, and numbers continue to decrease steadily (Crawford *et al.* 1995b, 2011). Although efforts are underway to curb fish stock collapse and oil pollution, it is imperative that breeding success exceeds mortality to counteract the demise of this top marine predator.

Assortative mating patterns in the African Penguin have not been studied or considered in terms of their consequences for reproductive success. This study addresses this lacuna. In addition, detailed long-term nest attendance patterns of individual breeding attempts in 2013 are reported and compared to environmental conditions both on land and at sea.

This study is based on morphometric data collected from parents and chicks during March-July 2013, encompassing a large portion of the African Penguin’s peak breeding season (March - September) on Bird Island. Measurements from the birds of monitored nests were collected by a number of collaborating penguin researchers throughout the aforementioned breeding period, and were pooled for analyses. All fieldwork and data collection was performed under the ethics clearance reference PICL578 issued by the Department of Agriculture, Forestry and Fisheries (DAFF).

CHAPTER 2

STUDY SITE: BIRD ISLAND, ALGOA BAY

2.1 ALGOA BAY AND ITS ISLAND GROUPS

2.1.1 *Physical oceanography*

Algoa Bay is one of the largest log-spiral bays on the south coast of South Africa and extends for about 80km from the headlands of Cape Recife at the western boundary to the less prominent Cape Padrone to the east (Figure 2.1; Goschen *et al.* 2012). Port Elizabeth, the largest city in the province, is located to the north of Cape Recife, while the Alexandria Coastal Dunefield (the largest and least degraded coastal dunefield in the country) extends for 50km between Sundays River and Woody Cape (Illenberger & Rust 1988, Kerley & Boshoff 1997). While the western shores of the bay are heavily influenced by urbanisation, the eastern shores are sparsely populated, forming part of the Greater Addo Elephant National Park.

The warm Agulhas Current is the dominant oceanographic feature of this coastline. This oceanic current is a deep, swift-moving branch of the Indian Ocean's wind-driven transportation of warm tropical and subtropical water in a southwards direction and has a quantifiable effect on the climate and weather (particularly rainfall) of the contiguous landmass (Schumann *et al.* 2005, Lutjeharms 2006). Algoa Bay faces the southwest Indian Ocean, sloping gently towards the south-southeast with a maximum depth of approximately 70m (Goschen & Schumann 2011). The continental shelf here is narrow, extending no more than 25km between the shore and the 200m isobath (Lutjeharms 2006). The bay is fed by shallow, short-lived columns of freshwater from the Swartkops, Coega and Sundays Rivers (Goschen & Schumann 2011, Stewardson *et al.* 2012) of which the Swartkops and Sundays Rivers are the most important, providing important nursery habitats for many species of juvenile fish (Beckley 1984).

2.1.2 *The St Croix and Bird Island groups*

There are two island groups within Algoa Bay, namely the St Croix group, comprising Brenton Rock, Jahleel and St Croix islands, and the Bird Island group, comprising Black Rocks and Stag, Seal and Bird Islands. The St Croix group is close to a recently established deep-water port at the mouth of the Coega river, while the Bird Island group is situated 53km due east of Port Elizabeth and 8 - 9km from the nearest landfall at Woody Cape Nature Reserve. These are the only notable islands along the 1770km from Cape Agulhas to Inhaca Island in Mozambique (BirdLife South Africa 2012).

The St Croix and Bird Island groups are breeding sites for approximately 7 500 and 3 000 pairs of African Penguins respectively, together constituting 40% of the world, and half of the South African, population (Crawford *et al.* 2011). The population within the bay has declined since the early 2000s, but is now stable (Crawford *et al.* 1995b, Pichegru *et al.* 2013, Oceans and Coasts unpubl. data).

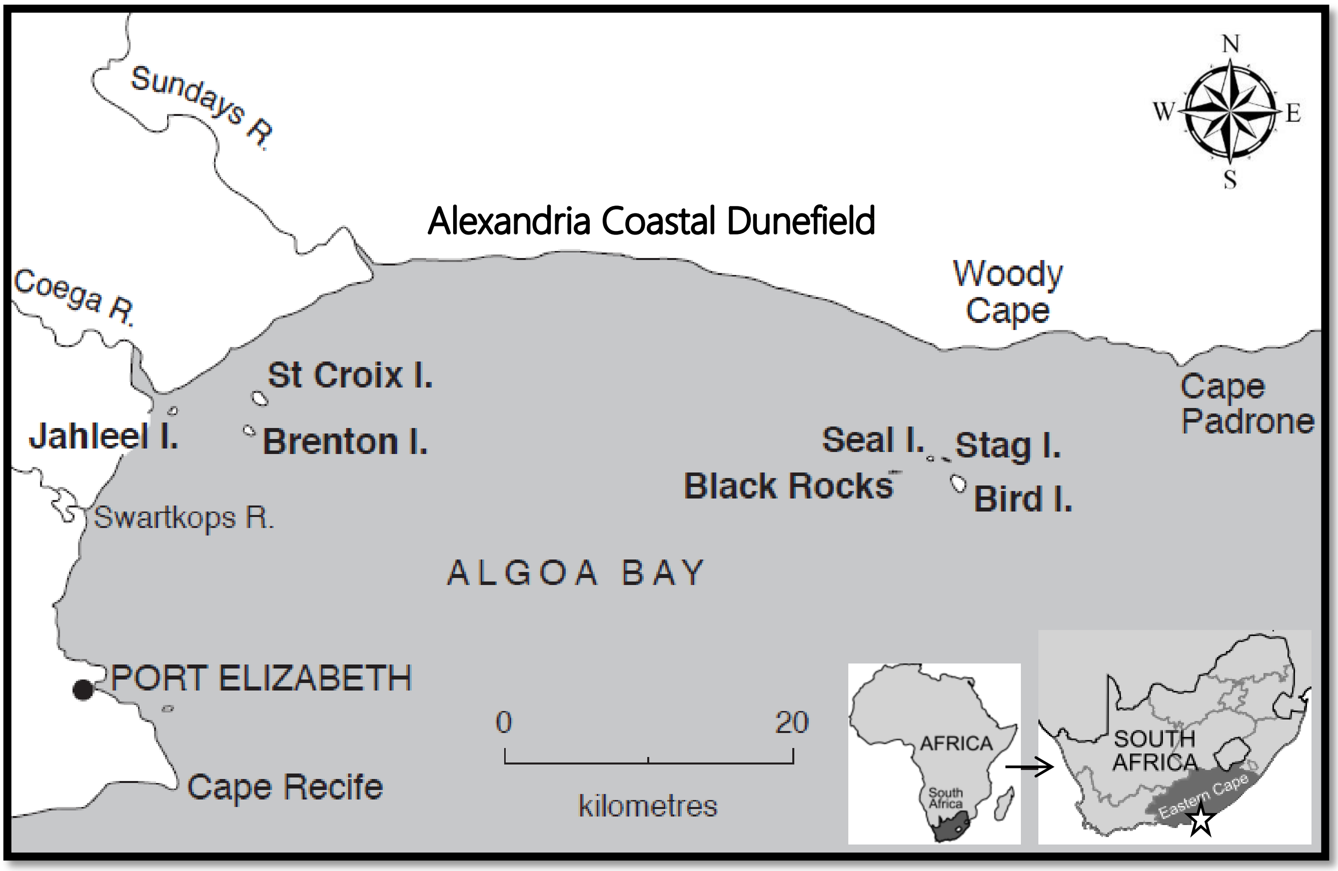

Figure 2.1: Algoa Bay, showing the two island groups; the St Croix group to the west, the Bird Island group to the east.

2.2 BIRD ISLAND, ALGOA BAY

2.2.1 *Physical description*

Bird Island (33°50’29’’S 26°17’13’’E) is the largest island in Algoa Bay, with an area of 19ha, and rising to only 9m above sea level (Figure 2.2). The islands of this group, unprotected by any headlands, have been isolated from the mainland since the last major sea level rise, about eight to ten thousand years ago, and have probably been occupied by seabird colonies ever since (Urquhart & Klages 1996). Geologically, the Bird Island group is composed of quartzitic Ordovician sandstones which rise from a smooth bed of Quaternary sand and form part of the Peninsula Formation, in turn forming part of the Table Mountain

Group and Cape Supergroup (Goschen & Schumann 2011, Stewardson *et al.* 2012). Due to millennia of seabird occupation, the island's soils are generally ornithogenic in nature (Urquhart & Klages 1996).

Figure 2.2: Google Earth image of Bird Island. The light grey area to the northeast of the island is the gannetry. A = newly-constructed jetty, B = island infrastructure (ranger and researcher accommodation, storehouse, etc.), C = lighthouse.

2.2.2 *Climate*

The climate of the greater Port Elizabeth district is remarkably variable and is generally described as transitional between subtropical and temperate, with mild summer and winter temperatures, and typically windy conditions (Stone 1988). Algoa Bay experiences the influence of the southeast coast's wet and humid summers, and weather more strongly influenced by the tail end of fronts from the west in winter (Stone 1988). Mean annual rainfall is 600 - 680mm (Mucina & Rutherford 2006) and falls mostly in summer (Stewardson *et al.* 2012), but mean monthly rainfall on Bird Island varies considerably between years. Significant rainfall events of more than 100mm in a short period occur irregularly on the island. These downpours flood nests and drown many young penguins and gannets or may lead to hypothermia if they coincide with low temperatures (Stewardson *et al.* 2012). Intervention in these extreme cases has become common practice, with rangers removing chicks from water-logged nests, drying them as best as possible and sending them to rehabilitation centres *en masse* for rearing (H. Visser, SANParks section ranger, *pers. comm.*).

2.2.3 *Winds, coastal upwelling and sea temperature*

Bird Island experiences a bimodal wind regime, prevailing east-northeasterly and west-southwesterly winds blow parallel to the coastline’s general orientation (Goschen & Schumann 2011). Westerly winds occur year-round, while easterlies are more common in summer (Stewardson *et al.* 2012). Easterly winds are responsible for upwelling at Cape Recife, the western boundary of Algoa Bay, while westerly winds may carry this cold, upwelled water further into the bay (Schumann *et al.* 1988, Goschen & Schumann 2011). A semi-permanent upwelling system occurs near Port Alfred, approximately 60km east of Bird Island and this influences conditions within the bay, as advected upwelled water reduces temperatures and enhances productivity by delivering nutrients to the surface (Lutjeharms *et al.* 2000, Lutjeharms 2006). Sea surface temperatures (SST) in this region are more variable than anywhere else along the length of the Agulhas Current (Lutjeharms 2006) and, within Algoa Bay, vary seasonally, with a maximum in summer of ca. 26°C, decreasing by 3 - 9°C in winter (Schumann 1987).

2.2.4 *Fauna and flora*

2.2.4.1 Vegetation of Bird Island

The vegetation of the Bird Island group forms part of the Algoa (or, interchangeably, Albany) Dune Strandveld biome (Mucina & Rutherford 2006). Bird Island is covered by sparsely growing mixed plants dominated by *Mesembryanthemum aitonis* (fig marigold)*, Tetragonia fruticosa* (duneweed), and *Chenopodium* (goosefoot) spp (Urquhart & Klages 1996, Mucina & Rutherford 2006). Local sea winds carrying salty spray ensure that the flora is under perpetual stress and vegetation remains low (Mucina & Rutherford 2006).

2.2.4.2 Cape Gannets

The island represents one of only six breeding sites in the world for the Vulnerable-listed Cape Gannet, *Morus capensis* (Figure 2.3; IUCN 2013), and is the largest gannetry in the world (Crawford *et al.* 2007). The number of breeding pairs on Bird Island increased from 50 000 to 65 000 during the period 1986 – 1999 and again to 98 000 pairs by 2006 (Crawford *et al.* 2007). Attendant decreases on the west coast of southern Africa (largely due to sardine collapses) coupled with the capability of this bird of flying very long distances support the theory that Cape Gannets (particularly juveniles) are immigrating to Bird Island from the West Coast (Oschadleus & Brooks 2006, Crawford *et al.* 2007).

The island's adult gannets and penguins do not actively encroach onto one another's territories, however, due to foraging inexperience, many gannet fledgings grow weak and wander through (or land in) the penguin colony (Oatley *et al.* 1992). Recently, interspecies aggression has been documented, with African Penguins killing the gannet fledglings by biting and flipper slapping (McInnes *et al.* 2014).

2.2.4.3 Kelp Gulls

Kelp Gulls (*Larus dominicanus*), which breed predominantly on Stag and Seal Islands, have been implicated in the predation of unsustainable numbers of penguin eggs and chicks (Pichegru 2013). They have learnt to capitalize on the effects of disturbance, exploiting temporarily abandoned eggs and small chicks in the event of human presence (Voorbergen *et al.* 2012). Kelp Gulls are also natural predators of other non-burrowing birds such as the Cape Cormorant, Cape Gannet and the Roseate Tern which are presently all of conservation concern (IUCN 2013). As a result, SANParks management officials have authorized the culling (by shooting) of these gulls and the regular pricking of gull eggs on their breeding islands (Seal and Stag Islands) to reduce population growth (H. Visser, SANParks section ranger, *pers. comm.*). This action, in addition to supplying breeding African Penguins with suitable artificial nests that obscure unguarded offspring from these aerial foragers, has successfully increased penguin chick survival on Bird Island (Pichegru 2013).

2.2.4.4 Roseate Terns and other important avifauna

The islands of Algoa Bay are the only places in southern Africa where the regionally endangered Roseate Tern (*Sterna dougallii*) is known to breed regularly (Gochfeld & Burger 1996). While the species is not considered to be threatened globally, little is known about the taxonomy, migration or true population size of *S. dougallii* in the southwestern sector of the Indian Ocean (Tree 2005, IUCN 2013). Efforts are made to reduce human presence on the eastern shore of the island during the winter months when this species is nesting (*pers. obs*).

Other important avifauna in the region includes African Black Oystercatcher (*Haematopus moquini*), Cape Cormorant (*Phalacrocorax capensis*) and large numbers of Antarctic Terns (*Sterna vitatta*). Ruddy Turnstones (*Arenaria interpres*), Whimbrels (*Numenius phaeopus*), White-fronted Plovers (*Charadrius marginatus*), Cape Wagtails (*Motacilla capensis*) and Little Egrets (*Egretta garzetta*) are commonly sighted on the island (*pers. obs*). Vagrant records include Barn Owl (*Tyto alba*), Sooty Tern (*Onychoprion fuscatus*), Australasian Gannet (*Morus serrator*), and Wedge-tailed Shearwater (*Puffinus pacificus*) (BIRP 2014).

2.2.4.5 Mammals

Bird Island is free of rats and other larger mammalian predators, however house mice (*Mus musculus*) are relatively common, although these are not known to prey on eggs or chicks on the island (Ralph 2008).

Black Rocks is home to approximately 6 000 Cape fur seals (Stewardson *et al.* 2012, Oceans and Coasts unpubl. data), the numbers of which are kept in check by storm surges that drown young pups, and by the local great white shark population (Urquhart & Klages 1996). Appropriately trained SANParks officials have also been instructed to cull rogue seals which habitually attack and often fatally wound penguins around the island (H. Visser, SANParks section ranger, *pers. comm.*).

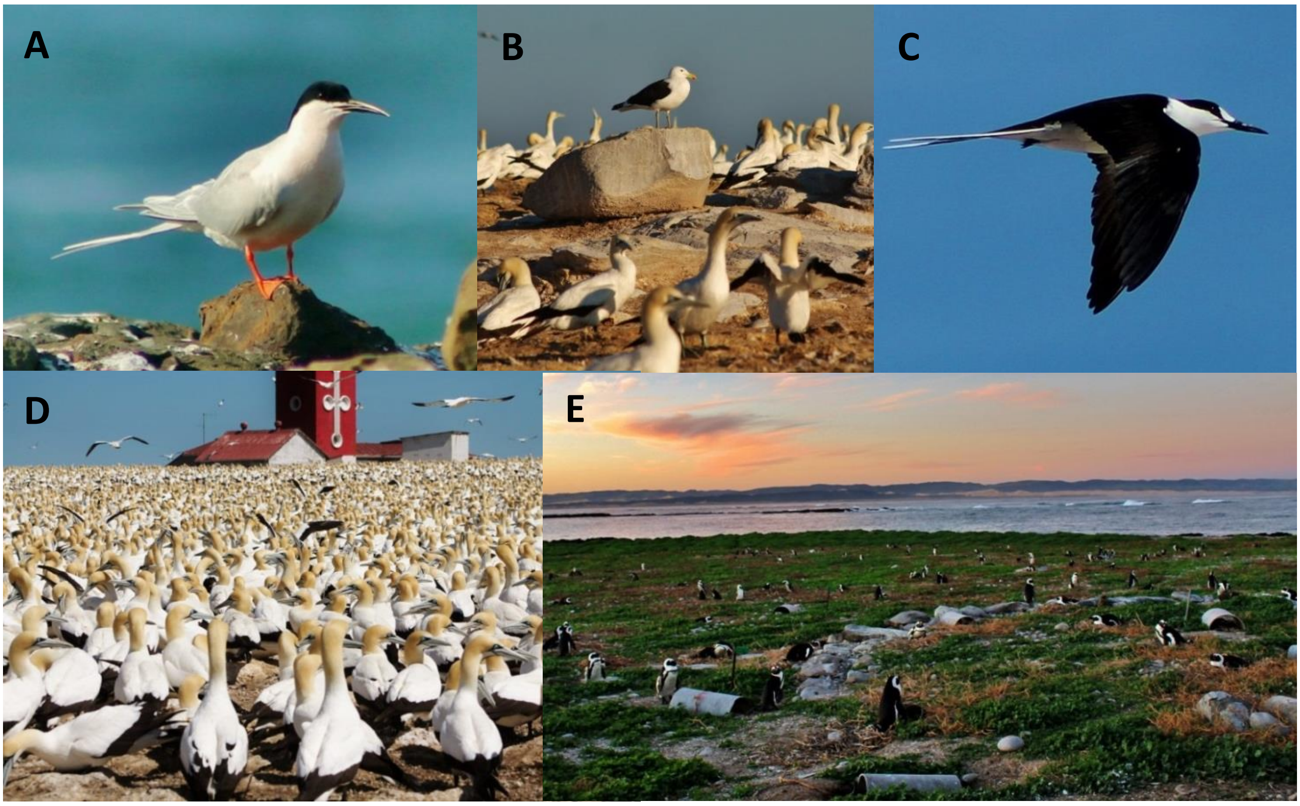

Figure 2.3: A: Roseate Tern (*Sterna dougallii*) in breeding plumage with a pinkish wash to its breast. B: Kelp Gull (*Larus dominicanus*), an opportunistic predator of abandoned or vulnerable gannet and penguin chicks seen amidst roosting Cape Gannets. C: Vagrant birds are often seen on Bird Island, like this Sooty Tern (*Onychoprion fuscatus*) sighted in September 2013. D: The largest gannetry in the world, Bird Island hosts up to 98 000 breeding pairs of Cape Gannets (*Morus capensis*) in the austral summer. E: Bird Island looking to the northeast, with nesting African Penguins (*Spheniscus demersus*) in the foreground. Parts of Stag Island are seen offshore, and Woody Cape Nature Reserve appears on the mainland. Photos: D.L. Smith.

2.3 A BRIEF HISTORY OF THE ALGOA BAY ISLANDS

In terms of their avifauna, the Algoa Bay islands are now a shadow of their former glory. Bartholomeu Dias, the first European explorer to document these islands in 1488, noted that St Croix Island was simply teeming with seabirds and seals (Urquhart & Klages 1996).

From historical photos and lighthouse keeper reports from the late 1800s and early 1900s (Urquhart & Klages 1996), it is clear that just a century ago Bird Island was once a bustling seabird colony, with penguin numbers perhaps two orders of magnitude greater than today.

With the arrival of European settlers to this part of the world in the 1820s, immediate exploitation of fisheries and harvesting of penguin eggs began, and much of Bird Island's guano was removed between 1850 and 1950 (Figure 2.4; Pichegru 2013). The guano industry, particularly on Bird Island where deposits were thought to be of lesser quality than the West Coast colonies, was plagued by lawlessness, oversubscription of guano licences and the absence of true control by authorities (Snyders 2011). The discovery of cheaper synthetic fertilizer alternatives eventually caused guano prices to plummet and Atlas Organic Fertiliser decided in 1989 not to renew their concession rights for the island (Urquhart & Klages 1996). Due to the much lower population density and ever-decreasing numbers of the resident seabirds, the historical state of these islands will probably never be recovered.

Figure 2.4: A mountain of guano harvested after the breeding season (circa 1930) waiting to be dried, bagged and shipped. Photo: C.H. Hannabus in Urquhart & Klages (1996).

Bird Island's treacherous seas are the site of at least 29 shipwrecks, including the *Doddington*, which ran aground in July 1755 after striking Doddington Rock. With many guano tugboats calling on the island and with vessels at anchor off of the island for up to a month at a time in bad weather, the unpredictable currents and submerged reefs elicited the necessity of a guiding light in the bay, and a wooden lighthouse was erected in 1852, which was later built of stone in 1873 (Urquhart & Klages 1996, Kerley & Boshoff 1997). Now entirely automated, thus eliminating the need for permanent lighthouse-keepers, the lighthouse remains fully functional (Figure 2.5).

Figure 2.5: The Bird Island lighthouse was first built of wood in 1852, and later of stone in 1873. The gannetry with many airborne Cape Gannets is seen in the foreground. Photo: D.L. Smith.

2.4 CONSERVATION OF THE ALGOA BAY ISLANDS

BirdLife South Africa (2012) lists the Algoa Bay islands as a globally Important Bird Area (IBA) and as such, it is at the forefront of significant conservation programme initiatives. The 7 000ha Bird Island Marine Protected Area (MPA) was declared in 2004, and was incorporated into Addo Elephant National Park (AENP) in 2005 (WWF SA 2009). The island is not only important for significant numbers of bird species, but is now protected for its ecologically unique subtidal ecosystems that are home to numerous endemic invertebrates and seaweeds (WWF SA 2009).

SANParks rangers maintain a constant presence on Bird Island, and numerous researchers and other groups visit periodically, contributing to sustained levels of human disturbance. At the time of writing, plans are afoot to enlarge the Bird Island MPA by a further 12 000ha to encompass the Port of Ngqura as well as Cannon Rocks off Alexandria. This will increase protection to a distance of 20km offshore. In addition, talks are currently underway to restore the dilapidated Atlas Organic Fertilizers house near the lighthouse to provide overnight accommodation for paying tourists. At present, no tourism-based operations occur on the islands themselves, and while the revenue would be welcome, it must be offset against the undesired effect of further increasing the levels of human disturbance.

CHAPTER 3

PHENOTYPIC ASSORTATIVE MATING IN THE MONOGAMOUS AFRICAN PENGUIN AND ASSOCIATED BREEDING PERFORMANCE

3.1 INTRODUCTION

Assortative mating, or the selection of a partner on the basis of phenotypic similarity relative to self, is the most frequently described mating pattern within the vertebrates (Burley 1983, Crespi 1989, Jiang *et al.* 2013). The strength of assortative mating differs among the higher taxa; it is particularly strong in fish, but comparatively weaker in birds (Jiang *et al.* 2013). Non-random mating patterns are widespread phenomena amongst long-lived, monogamous birds (Ludwig & Becker 2008) and have been found in many avian species for a number of traits including overall size (Catry *et al.* 1999, Einoder *et al.* 2008), mass (Chardine & Morris 1989, Wendeln 1997, Forero *et al.* 2001), bill length (Coulter 1986), bill depth (Wagner 1999, Einoder *et al.* 2008), bill colour (Møller *et al.* 2007), tarsus length (Jones & Montgomerie 1991, Helfenstein *et al.* 2004), age/breeding experience (Coulson & Thomas 1983, Nisbet *et al.* 1984, Jouventin *et al.* 1999, Fasola *et al.* 2012), plumage colour (Davis & Donald 1976, Jones & Montgomerie 1991, MacDougall & Montgomerie 2003), moult stage (Bridge & Nisbet 2004) and tail streamer length (Boland *et al.* 2004). In spite of the prevalence and presumed effects of assortative mating, the demographical, behavioural and physiological mechanisms driving the phenomenon are poorly understood (Crespi 1989, Harari *et al.* 1999, Ludwig & Becker 2008). Nevertheless, detecting assortative mating is important; it is often associated with sexual selection and can influence the genetics and demographics of whole populations (Boag 1983, Parker & Partridge 1998). Indeed, assortative mating can preserve genetic variation (negative assortative mating for the major histocompatibility complex is one such example, (Fisher 1958, Mays & Hill 2004, Knafler *et al*. 2012), influence fecundity (Findlay *et al.* 1985) and even drive reproductive isolation (Bolnick & Kirkpatrick 2012) and, ultimately, speciation (Udovic 1980, Harari *et al.* 1999).

Seabirds are typically monogamous species that share the responsibilities of raising their offspring and are thus considered to be excellent candidates for studies of mutual mate selection (Boland *et al.* 2004). Often however, their relative inaccessibility and lack of sexual ornamentation have limited such research, especially in the Sphenisciformes (Davis & Speirs 1990, Forero *et al.* 2001). While penguin species typically lack obvious secondary sexual characteristics or sex-linked plumage differences, most of them, including the endangered African Penguin (*Spheniscus demersus*), exhibit at least minor sexual dimorphism (Forero *et al.* 2001, Pichegru *et al.* 2013), with the male being physically sturdier, and possessing a deeper and longer bill (Cooper 1972). Distinguishing between the sexes is essential to many seabird studies, although field ornithologists are presented with a problem when both sexes appear externally similar. Many studies have been conducted discriminating between the sexes of penguins using a number of different methods, such as copulation observations in the breeding season, post mortem gonadal examination, ultrasonography, chromosome analysis (karyotyping), cloacal endoscopy or post-laying cloacal examination, polymerase chain reaction (PCR) techniques and discriminant analyses of structural morphology (Renner *et al.* 1998 and references therein). Of these methods, PCR and analyses of morphometric data are among the cheapest and most efficient means to sex birds. Discriminant Function Analysis (DFA) is particularly useful in populations where sexual dimorphism is reduced to that of a statistical nature (Weidinger & van Franeker 1998). DFA produces a formula using several morphometric measurements from sexed individuals to distinguish between the sexes based on a combination of the traits' discriminatory powers.

Despite the rich literature devoted to sex discrimination, sexual dimorphism and the consequences of potential assortative mating on reproductive output have been poorly described (Forero *et al.* 2001, Ludwig & Becker 2008). Here, the degree of sexual dimorphism and the assortative mating patterns of a random sample of African Penguin pairs breeding on Bird Island were examined, as well as the effects of parent biometric characteristics on offspring development.

3.1.1 *Sexual selection*

Sexual selection is the active choice for certain sexual traits in members of the opposite sex for the purpose of reproduction Trivers 1972) and is based on sexual dimorphism. In the case of penguins, this generally pertains to size.

Three main hypotheses for the existence of this sexual dimorphism have been proposed:

a) Intrasexual competition has led to selection for larger males that can compete successfully for females or good nest sites (Trivers 1972).

The intrasexual competition hypothesis posits that the larger sex competes for the limiting resource: members of the smaller sex that are mates of good reproductive calibre (Olsen & Cockburn 1993). This phenomenon is widely accepted as the explanation for mammalian sexual dimorphism (Jarman 1983). Hence, the size of the smaller sex is determined by natural selection, while the size of the larger sex is an artefact of intrasexual competition for mating opportunities with suitable partners.

b) By utilizing different dive depths and prey size due to female size constraints, males and females have evolved foraging niche partitioning through sexual dimorphism as a means of reducing intersexual competition. This is the intersexual food competition hypothesis (Davis & Speirs 1990).

According to Pichegru *et al.* (2013), sexual dimorphism in the African Penguin may have evolved as a means of foraging niche partitioning, thus decreasing the overlapping use of available prey size and depth exploited by the two sexes. Foraging parameters determined by GPS time-depth recorders have revealed that males dive deeper and for longer periods than females during the breeding season, providing evidence to support this hypothesis (Walker & Boersma 2003, Raya Rey *et al.* 2012, Pichegru *et al.* 2013).

c) Sexual selection is due to discriminating females (Andersson 1994).

The preference of females for large males may be based on the greater probability that he has above average fat reserves to withstand the fasting periods while she is at sea (Davis & Speirs 1990). However, the reverse reasoning could also be applied; that males select larger females that could theoretically see out the lengthy initial incubation shifts without deserting their young. It has been observed that female Adélie penguins initiate the pairing process (Davis & Speirs 1990), although similar evidence for the African Penguin is lacking, providing uncertainty for the “discriminating female” model.

3.1.2 *Assortative mating*

Among the mammals and birds (with a few exceptions of reversed size dimorphism, Catry *et al.* 1999), males tend to be the structurally larger sex (Fairbairn & Shine 1993). This sexual dimorphism often leads to assortative mating, either positive or negative (Forero *et al.* 2001). Positive assortative mating (or homotypic mating) occurs when individuals of similar phenotype pair for breeding more frequently than expected by chance, whereas negative assortative mating (or heterotypic mating) occurs when dissimilar individuals mate significantly more often than expected (Burley 1983). Lack of preference for a particular phenotype in a partner constitutes atypical selection. It is often concluded when mated pairs exhibit similar or dissimilar characteristics that “like attracts like” or that “opposites attract”, respectively, and that the female is the more selective sex (Ridley 1994). However, according to theories of sexual selection, both sexes of species which invest equally in offspring rearing should select good quality mates, as this choice will affect lifetime fitness (Trivers 1972, Burley 1977, 1983). Theoretically, high quality individuals should be more selective in terms of partners (Burley 1977), resulting in the pairing of high quality mates (or large males and large females), leaving the lower quality individuals (or small males and small females) to pair amongst themselves, not because of homotypic preferences, but due to their shortcomings and restricted access to the best mates. The selection for the most desirable mate (or for any specified trait) exhibited by the population as a whole is known as a “type preference” and results in a pattern of mating which is indistinguishable from one in which each phenotype preferentially mates with itself (Burley 1983).

Superior reproductive output has been reported for most colonial seabirds that re-pair with the previous season’s mate compared to those that establish new pair bonds (Rowley 1983).

Choosing a mate is costly both in terms of time and risk of disease and thus the consequences of choosing a good mate will impact the rest of a first-time breeder's reproductive lifespan. As maximization of fitness is the major driver of mate selection, it was hypothesized that the best assorted African Penguin pairs in this study would on average rear the most number of offspring of the best condition as indicated by high growth rates and body condition indices. It was also hypothesised that the relative differences in the combined size of pairs would have detectable effects on reproductive output.

3.1.3 *Chick development*

Lack (1947, 1948) hypothesised that avian clutch size in altricial species evolved through natural selection toward producing the optimal number of surviving offspring. There exists a trade-off between the quality and the number of young that can be raised successfully (Magrath 1991). The idea that the breeding performance of seabirds is indicative of food availability, and thus the state of marine ecosystems, has become widely accepted and penguins, especially, have been heralded as marine sentinels (Williams & Croxall 1990, Boersma 2008). Chick growth rates in particular are useful to such studies as they are sensitive to fluctuations in food provision which is in turn sensitive to prey availability and environmental conditions (Williams & Croxall 1990, Lubbe *et al.* 2014). The African Penguin almost invariably produces two eggs per breeding attempt, representative of the maximum brood size that can be raised when conditions are favourable (Hockey 2005), although offspring survival varies greatly with year and in relation to fish stocks, as for many marine predators (Barlow & Croxall 2002). Pre-fledging mass and growth rates have been strongly correlated with juvenile survival and recruitment in numerous bird species (Hunt & Hunt 1976, Coulsen & Porter 1985, Magrath 1991, Schmutz 1993).

3.1.4 *Offspring sex ratio*

Natural selection predicts that parents should favour offspring of the sex which confers to them the maximum returns of fitness (Trivers & Willard 1973). The offspring of sexually dimorphic species which already display sexual size dimorphisms may incur different energetic demands on their parents based on their sex. For example, larger male offspring might be more costly to rear, requiring a larger quantity of food and higher feeding rates (Beaulieu *et al.* 2009). As a result, parents may adjust their investment into the reproductive attempt in terms of foraging trip length and food provisioning. The sex allocation theory, as most famously put forward by Trivers and Willard (1973), has been the focus of much attention since the advent of molecular sexing techniques, which rapidly expanded the opportunities for investigating sex-linked parental investment (Pike & Petrie 2003, Ewen *et al.* 2004). As males are typically the larger and more robust sex, often relying on physical strength to outcompete rivals for mating opportunities, a high-quality son has more reproductive value than a poorer-quality son, which may not mate at all, or may be matched with a poorer-quality mate, leaving fewer grandchildren (Trivers & Willard 1973). A mother in good condition and able to provide sufficient resources to her offspring would do best to use a period of above-average food resources to raise high-quality sons instead of high-quality daughters. However, a mother in poor condition or suffering unpredictable or scarce resources would raise more grandchildren through her daughters (if indeed she was able to successfully reproduce at all) rather than through low-quality sons. Many studies have shown skewed offspring sex ratios in favour of investment in sons when mothers are in good condition, or under favourable environmental states (Pike & Petrie 2003, Merkling *et al.* 2012). The opposite has also been reported; i.e. more daughters are produced than sons under stressful situations or when the mother is not in good condition (Nager *et al.* 1999). In spite of these findings, sexual allocation is one of the most controversial and equivocal theories in evolutionary biology, confronted with mixed empirical results, sometimes even within the same species (Ewen *et al.* 2004, Merkling *et al.* 2012).

3.1.5 *Aims*

The first aim of this aspect of the study was to assess the degree of sexual dimorphism in breeding pairs of African Penguins and to test for assortative mating based on several body size measurements using individuals sexed by molecular methods or, where genetic procedures failed, assigned to a predicted sex based on a discriminant function analysis.

The second aim of this study was to determine if earlier-breeding penguins paired more selectively than later-breeding birds, as a result of the preponderance of potential mates at the beginning of the breeding season. Later-breeding birds may be forced to compromise on mate quality for lack of both available and desirable mates, and assortment of pairs was thus also assessed on a temporal scale.

Lastly, the biometrics of individual parents and the assortment of breeding pairs were related to reproductive performance as judged by aspects of their offspring-raising capabilities, including brood size, chick growth rate and chick body condition index and the variability therein. The offspring sex ratio and sex-specific development were also assessed for a subset of the population.

3.2 MATERIALS & METHODS

3.2.1 *Field-based data collection*

Data on breeding African Penguins and their chicks were collected from nests marked with numbered stones during March to July 2013 on Bird Island, Algoa Bay. Wherever possible, morphometric measurements were collected from every bird handled during fieldwork; both chicks of double clutches were measured, as were both parents of each monitored nest. An important note made here is that the term “double brood”, in this study refers to a nest of two chicks, rather than the act of two successive breeding attempts. Likewise, “single brood” refers to a next containing only one chick. Nests with appropriately-aged chicks (P1 – P3 stage, see Glossary) that were selected for monitoring were chosen at random from within a radius of about 200m of the SANParks rangers’ house, although care was taken to avoid very dense nesting zones, as accessing these areas on a regular basis would have created excessive disturbance. To distinguish between partners from each nest, members of the pair were marked with either blue or orange wax markers mounted at the end of a 1m long wooden broomstick. Marks faded with each foraging bout, so adults were re-marked every three to five days. Chicks were differentiated by means of a quick-drying, brightly coloured swine marker (Porcimark ©, Kruuse) which was sprayed on the underside of the flippers. Chicks were re-marked during each measurement since the marks faded with feather development.

Morphometric measurements taken from adult penguins included bill length, bill depth, flipper length and the mass of the bird. Chick measurements comprised bill length, total head length (measured from the tip of the bill to the bulge of the occipital condyle), flipper length (measured from wing tip to the base of the humerus) and body mass. Where possible, both parents of monitored nests were measured at similar stages of fasting, i.e. after equal periods on the nest. Bill length and depth, as well as the total head length of chicks were measured to the nearest 0.1mm with digital Vernier callipers, while flipper length was measured to the nearest millimetre with a stopped metal ruler. The body mass of chicks was measured by placing them into a material drawstring bag suspended by an electronic balance, while adult birds were suspended by a strap securely tightened around the breast and beneath the flippers. Making sure to collect the roots, down was collected from every chick in monitored nests, along with up to eight head feathers from one of the parents. Feathers and down were stored in small, labelled Ziploc bags at ambient temperature, and kept in the dark to prevent UV degradation, and later used for sexing analyses. DNA analysis based on feather samples eliminated the need for stressful blood-drawing or tissue biopsy and represented a rapid, painless and minimally invasive sampling method for molecular examination.

Chick growth was measured every five days. If measurements were due on a rainy day, they were instead taken on the following day or the soonest possible moment when the chicks were no longer wet, to prevent thermal stress.

All adult birds responded well to handling and returned to their nests where they immediately resumed incubating or guarding duties. When chicks strayed from their nests after measurements were taken, they were carefully placed under the parent again or observed until they returned to their parent.

3.2.2 *DNA-based analyses*

Birds differ from mammals in that the male is the homogametic sex, possessing a set of two identical chromosomes, while the female is heterogametic, with two different sex chromosomes (Birkhead 2008). Avian sex chromosomes are denoted Z and W; ZZ-bearing birds are male, ZW-bearing birds are female (Birkhead 2008). In order to determine the sex of individually monitored penguins, a molecular sexing method was undertaken, using an ISOLATE II Genomic DNA isolation kit (Bioline), followed by amplification using standard polymerase chain reaction (PCR) protocol and product separation by agarose electrophoresis.

Molecular sexing of birds using the amplified chromo-helicase-DNA-binding 1 (CHD1) gene has been well established since the mid-1990s (Griffiths *et al.* 1996, Fridolfsson & Ellegren 1999). These methods entail the annealing of two primers to conserved regions of the exon and then amplify a less conserved non-coding intron with a different length to that of the CHD1 genes (Costantini *et al.* 2008). The resultant PCR products of different sizes allow differentiation between male and female birds. This approach is non-invasive and reliable for many non-ratite species (Fridolfsson & Ellegren 1999, Costantini *et al.* 2008).

3.2.2.1 DNA Extraction and isolation

Using sterile laboratory practices for each DNA extraction, a 0.2 - 0.4cm segment was cut from the base of four or five individual root-bearing feathers or down that were collected from monitored birds. Tissue was finely diced with a clean surgical blade to release as much cellular material as possible. To eliminate the loss of tissue particles owing to static electricity in the atmosphere (which caused feathers to cling onto the outside of plastic

Eppendorf tubes), dicing was often carried out in a laboratory fume hood with a humidifier. All tissue was then placed into a sterile 1.5ml microfuge tube, and stored at -20°C until further analysis. Blades and glassware were washed thoroughly between samples using the sterile technique.

The standard kit protocol for purifying and isolating DNA from animal tissue was then followed, as set out in the manufacturer's guidelines (see Appendix).

3.2.2.2 PCR protocol

Molecular sexing of birds is based on the PCR amplification of two sex-linked introns on the conserved CHD (chromo-helicase-DNA-binding) genes located on all non-ratite avian sex chromosomes (Fridolfsson & Ellegren 1999). The *CHD-Z* gene is located on the Z chromosome common to both avian sexes, while the *CHD-W* gene is unique to female sex chromosomes. Homologous portions of both genes including introns of differing lengths are amplified using the primer pair 2550F (5' – GTTACTGATTCGTCTACGAGA – 3') and 2718R (5' – ATTGAAATGATCCAGTGCTTG – 3'). Upon standard agarose electrophoresis, male birds present one amplified intron, while females are distinguished by a second amplification product stemming from their unique W-chromosome.

PCR amplification was conducted on a final volume of 25µl per sample, constituted by 12.5µl PCR MasterMix (2X, Promega), 1µl of each primer (2550F and 2718R), 3µl magnesium chloride (25mM, Promega), 5.5µl nuclease-free water (Promega) and 2µl isolated DNA. Samples were subjected to the thermal cycling profile set forth in Fridolfsson & Ellegren (1999), with slight modifications, using a BIO RAD T100™ Thermal Cycler. The touch-down procedure was adjusted to begin at 58°C (instead of 60°C) and decreased by 1°C per cycle for ten cycles. Thereafter, the annealing temperature remained at 48°C for the next 30 cycles.

Amplified DNA (5µl) was bound with 2µl loading dye (6X, Promega) and SYBR® Safe DNA gel stain (Invitrogen) and separated electrophoretically on a 1.5% agarose gel next to a 100bp DNA ladder (Promega). Electrophoresis was conducted in 0.5% TBE buffer at 80V for 40-50 minutes. Bands were visualised using a molecular imager (BIO RAD Gel Doc™ XR+ with Image Lab™ Software). When bands were feint or absent, individual assays were repeated.

3.2.3 *Assortative mating*

A total of 123 pairs of breeding African Penguins were measured at their nests between March and July 2013 on Bird Island. A subset of adults was sexed using feather samples as described above.

By assessing four morphometric characteristics of these genetically sexed birds, (bill depth and length, flipper length and body mass) a backwards stepwise discriminant function analysis was performed in STATISTICA 12 (StatSoft) to determine which trait (or traits) best discriminated between the sexes. An additional parameter, “body condition”, was calculated by dividing the mass of each bird by its flipper length (Lewis *et al.* 2006). Pearson’s correlation analysis was then used to determine the level of assortment between members of a pair; a significant positive or negative correlation would indicate potential homotypic (positive) or heterotypic (negative) assortative mating respectively.

The Sexual Dimorphism Index (SDI) of Forero *et al.* (2001) assesses the degree of dissimilarity between members of a pair. SDI is simply calculated as the female measurement divided by the male measurement and then multiplied by 100. A value of 100% is achieved when traits are identical between the sexes, while increasingly smaller values indicate greater sexual dimorphism (with males being the larger sex).

An Assortative Index (AI) was calculated separately for each biometric trait where:

$$\mathbf{AI = |100 - DI|}$$

The AI indicated the degree to which the pair was assorted; lower values were indicative of better assortment.

The Absolute Size Index (ASI) of each pair was subsequently calculated by summing measurements for the same trait for both members of the pair. This index was calculated to investigate if the overall size of the pair had any bearing on their proclivity to mate assortatively, i.e. did larger birds pair assortatively more often than smaller birds?

3.2.4 *Chick growth rates*

Growth (change in mass) of African Penguin chicks follows a von Bertelanffy curve (Ricklefs 1967) and for this reason, chicks were categorised into the average developmental stage (P0 – P4, as described by Seddon & van Heezik 1993) they exhibited over the period of measurement. This was achieved by calculating the average mass of the chicks throughout the monitoring period and comparing it to Ralph's (2008) list of weights for chicks of known age. Chicks with an average weight of less than 300g were P0 chicks, while between 300g and 900g, stage P1 was assigned. Chicks between 900g and 1500g were P2 stage, while chicks weighing more than 1500g were P3. No chicks with an average weight more than 2500g (P4) were handled. Unfortunately, since the ages of the chicks being handled in this study were mostly unknown, it was not possible to construct and compare best fit growth curves (Ricklefs 1967, Ralph 2008).

Where more than one set of measurements was available per chick, the growth rate was determined simply by dividing the change in mass by the number of days between measurements.

$$\textbf{Growth rate} = (\textbf{mass}_2 - \textbf{mass}_1) / (\textbf{time}_2 - \textbf{time}_1)$$

The average growth rate was then calculated for the duration of observation and the standard deviation between measurements was indicative of the variability in growth.

3.2.5 *Chick body condition indices*

Body mass development in birds is known to vary more strongly and rapidly in response to variation in food provisioning than does structural growth, such as head, bill and flipper characteristics (O'Connor 1984, Robinson *et al.* 2002). In penguin chicks, the systemic growth of the head, culmen and flippers is best described by a Gompertz equation (Barlow & Croxall 2002, Lubbe *et al.* 2014). Mass increases most linearly with head length (>75mm) in African Penguin chicks and thus an index of body condition can be attained by relating the weight of a chick of unknown age to its structural properties (Lubbe *et al.* 2014). Chicks younger than about 20 days are excluded by the limit of a head length greater than 75mm. However, the delay allows for the development of an observable accumulation or depletion of body reserves in the chick.

Lubbe *et al.* (2014) describes the calculation of an African Penguin chick body condition index (BCI) as:

BCI = (observed mass – predicted minimum) / (predicted maximum – predicted minimum)

where the predicted minima and maxima are calculated by the quantile regression equations (see below) from the 0.05 and 0.95 quantiles from a dataset of 125 chicks successfully fledged on Robben Island in 2004.

0.05 quantile: Mass = -2472.1629 + 42.4157 * total head length

0.95 quantile: Mass = -3499.0741 + 60.1852 * total head length

Chicks with data points between the two quantiles had BCIs ranging from 0 – 1, chicks with data points below the 0.05 quantile had a negative BCI, while those above the 0.95 quantile had BCIs > 1. In other words, the BCI describes the condition of penguin chicks relative to where their weights fall between the upper and lower quantiles for their total head length (Lubbe *et al.* 2014).

Penguin siblings share both genes and provisioning rates, and for this reason growth rate and body condition are generally correlated between chicks within a nest (this study: $r = 0.288$, $p = 0.006$, $n = 89$ and $r = 0.556$, $p < 0.001$, $n = 75$ respectively). Where applicable, the within-nest averages for these parameters were calculated to avoid pseudoreplication.

3.2.6 *Parental phenotypes and breeding performance*

A model-building approach using multiple linear regressions was used to tease apart potential relationships between parental morphology and chick development. Analyses were conducted using “Spatial Analysis in Macroecology“ (SAM) v4.0. Aspects of chick growth rate and body condition and the variation in these parameters (determined as standard deviation) were assessed individually for each monitored chick and in the case of a double brood, the within-nest average for each trait and the size difference between A- and B-chicks were calculated and compared to their parent’s morphologies. Separate linear regressions were generated for each of the five morphometric traits of individual parents as well as all possible permutations of combined traits after ensuring these combinations did not correlate (Pearson’s correlation). This was repeated for AI and ASI values. To correct for potential Type II errors without enforcing the stringent significance restrictions of Bonferroni correction, a Benjamini-Hochberg step-up False Discovery Rate (FDR) procedure was used (Garcia 2004).

African Penguins on Bird Island attempt to breed as late into the season as October but for the purposes of this study, “early” and “late” breeding attempts refer to the periods March to April and May to July respectively. These periods were chosen as similar numbers of nests were monitored in each. All tests were two-tailed and significance was defined at the $p < 0.05$ level. Because some measurements were lacking for certain nests, sample sizes differed between analyses. Unless otherwise stated, analyses were performed using STATISTICA 12 (StatSoft).

3.3 RESULTS

3.3.1 *Sexual dimorphism and assortative mating*

Of the 123 pairs of nesting African Penguins measured on Bird Island between March and July 2013, a subset of 30 individual birds were successfully sexed using CHD primers. Assuming that homosexual nesting behaviour of this species is absent or negligible, the partner was assigned the opposite sex to its mate.

The biometric data met the required assumptions for discriminant analysis (multivariate normality, homogeneity of variance/covariance and independence). All within-sex correlation coefficients were small ($r < 0.5$), indicating that multicollinearity was not problematic. Using the 30 sexed pairs and a further five pairs of unsexed birds that were clearly sexually dimorphic, a backwards stepwise discrimination function analysis selected bill length (BL) and flipper length (FL) as the most important indicators of sex and produced the following classification functions:

$$\mathbf{C1 = -625.778 + (4.543\ BL) + (5.592\ FL)}$$

$$\mathbf{C2 = -699.271 + (5.068\ BL) + (5.828\ FL)}$$

The two computed scores calculated above were compared; where C1 > C2, the bird was female, whereas if C2 > C1, the bird was male. The Wilk's lambda values in Table 3.1 are the values for the overall model if the respective parameter were removed.

Table 3.1: Result of the backwards stepwise discriminant function analysis (performed using four morphometric traits from 35 pairs) used to discriminate the sex of African Penguins on Bird Island, Algoa Bay. Overall Wilk's lambda = 0.465, $F_{2,67}$ = 38.61, $p < 0.0001$.

Predictive trait	**Wilk's lambda**	**F - remove (1,67*)***	**p**

Bill length	0.565	14.445	< 0.001
Flipper length	0.553	12.752	< 0.001

When cross-validated with the birds of known sex, the DFA correctly predicted the sex of 91% of females and 86% of males. The DFA was subsequently applied to the remaining 88 pairs. When the same sex was assigned to both members (n = 14 pairs), manual corrections were performed to correct this. Males were denoted as the partner with larger measurements for at least three of the four morphometric parameters. When there was uncertainty (n = 2 pairs), the data were removed.

Mean values of the measurements of all five traits were calculated separately for each sex. Table 3.2 shows a summary of the morphometric distinctions between the sexes, with males structurally larger than females for all traits (Student’s t-tests, all p < 0.001). Each trait exhibited overlap between the sexes, with the largest females being larger than the smallest males (Figure 3.1).

Coefficients of variation (CVs) revealed that body mass and body condition were more variable than the structural-size measurements (bill and flipper characteristics) and that variability in these traits was generally slightly higher in females than in males (Table 3.2). Flipper length was the least variable trait in both sexes (male: CV = 2.8, female: CV = 3.0) and was also the least dimorphic (SDI = 95.3%). Body mass was both the most variable trait (male: CV = 9.4, female: CV = 8.7) as well as the most dimorphic (SDI = 87.4%). Flipper length and bill length (male: CV = 3.7, female: CV = 3.6, SDI = 92.4%), were the least dimorphic characters and the traits used to discriminate between the sexes in the initial DFA.

Table 3.2: t-tests on the morphometric parameters of breeding African Penguins (*Spheniscus demersus*). n = 121 pairs. Sexual Dimorphism Index (SDI) is expressed as a percentage of the female trait over the male trait. Coefficients of variation (CV) are given for each parameter for both sexes. Samples include birds sexed by discriminant function analysis.

Parameter	Males			Females			SDI (%)	t	p
	Mean ± SD	Range	CV	Mean ± SD	Range	CV			
Mass (g)	3517.0 ± 330.6	2730 – 4570	9.4	3051.9 ± 266.0	2480 – 3845	8.7	87.4	-13.11	< 0.001
Bill length (mm)	60. 1± 2.2	52.8 – 65.3	3.7	55.5 ± 2.0	51.2 – 61.2	3.6	92.4	-17.50	< 0.001
Bill depth (mm)	24.7 ± 1.1	21.7 – 27.1	4.3	22.4 ± 1.0	19.7 – 27.5	4.8	91.0	-16.38	< 0.001
Flipper length (mm)	187.7 ± 5.3	176 – 200	2.8	178.7 ± 5.3	168 – 190	3.0	95.3	-13.62	< 0.001
Body condition	18.73 ± 1.7	14.7 – 23.7	8.9	17.08 ± 1.4	14.0 – 20.9	8.1	91.7	-9.17	< 0.001

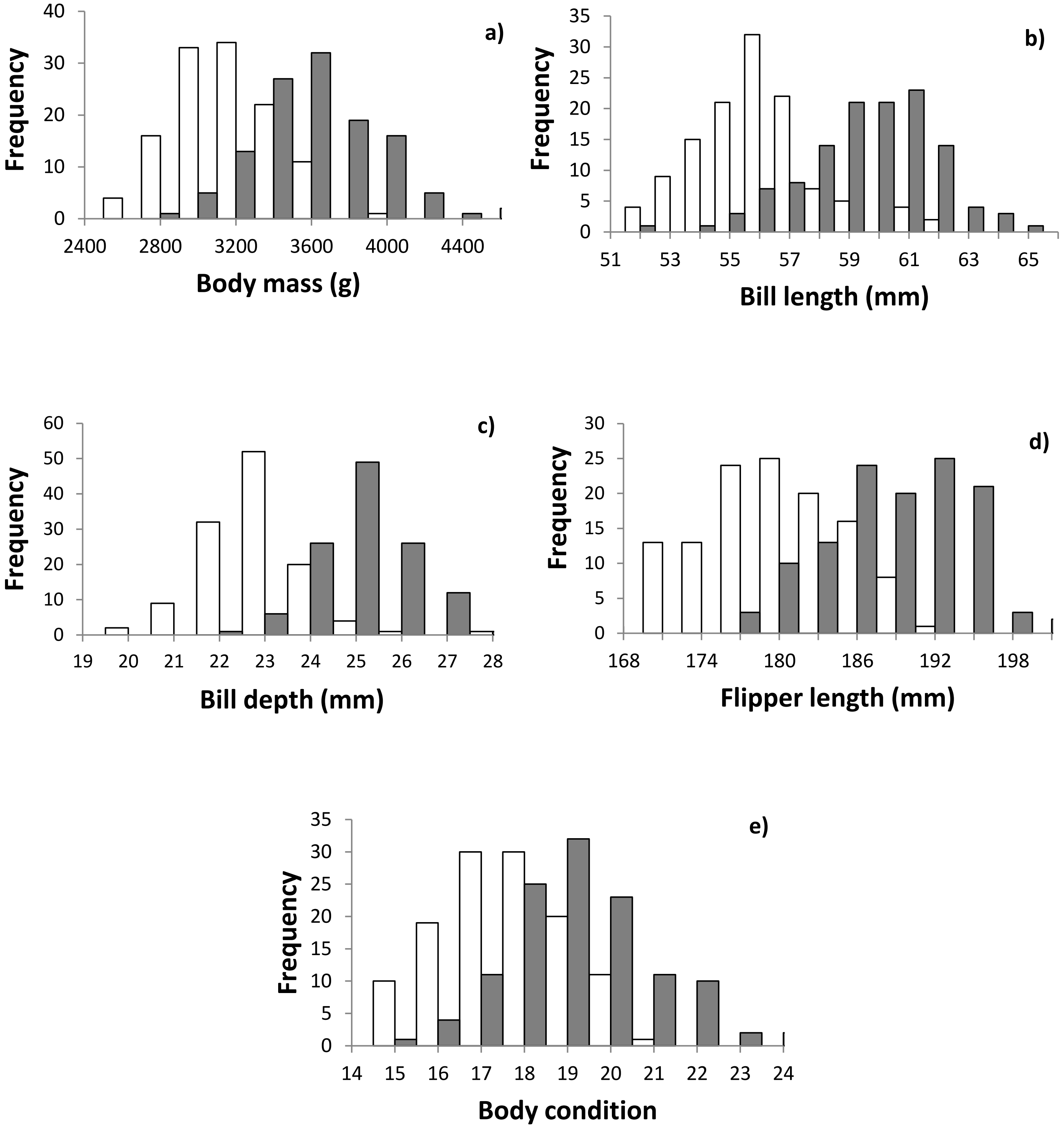

Figure 3.1: Frequency distributions of adult African Penguin mass a), bill length b), bill depth c) flipper length d) and body condition e) according to sex (white bars = female, grey bars = male, n = 121 pairs).

Although sexual dimorphism was found (Table 3.2, Figure 3.1), no phenotypic assortative mating was found for any of the five morphometric parameters, or for any combination of these traits. This was indicated by absence of significant Pearson correlations for any combination of the morphometric traits of adult penguins (Table 3.3). However, when mating patterns were assessed separately for pairs breeding earlier (March and April) in the season compared to those breeding later (May to July), an interesting result emerged. Positive assortative mating was found between female body condition (r = 0.273, p = 0.036 and, tentatively, female mass, r = 0.248, p = 0.058) and male mass for early breeders only, while disassortative mating was found in later-breeders for female bill depth and male bill and flipper length (Figures 3.2 and 3.3).

Table 3.3: Pearson correlation matrix between phenotypic traits of male and female African Penguins within pairs from March - July (n = 121 pairs).

		Male				
		Mass	**Bill length**	**Bill depth**	**Flipper length**	**Body condition**
Female	**Mass**	r = 0.158 p = 0.084	r = 0.069 p = 0.451	r = 0.062 p = 0.500	r = 0.063 p = 0.490	r = 0.146 p = 0.109
	Bill length	r = 0.062 p = 0.500	r = 0.044 p = 0.634	r = -0.034 p = 0.715	r = 0.058 p = 0.525	r = 0.049 p = 0.592
	Bill depth	r = -0.088 p = 0.340	r = -0.149 p = 0.102	r = -0.014 p = 0.876	r = -0.119 p = 0.193	r = -0.056 p = 0.543
	Flipper length	r = 0.031 p = 0.735	r = 0.066 p = 0.470	r = -0.076 p = 0.405	r = 0.612 p = 0.505	r = 0.012 p = 0.895
	Body condition	r = 0.161 p = 0.079	r = 0.046 p = 0.620	r = 0.094 p = 0.308	r = 0.046 p = 0.619	r = 0.155 p = 0.089

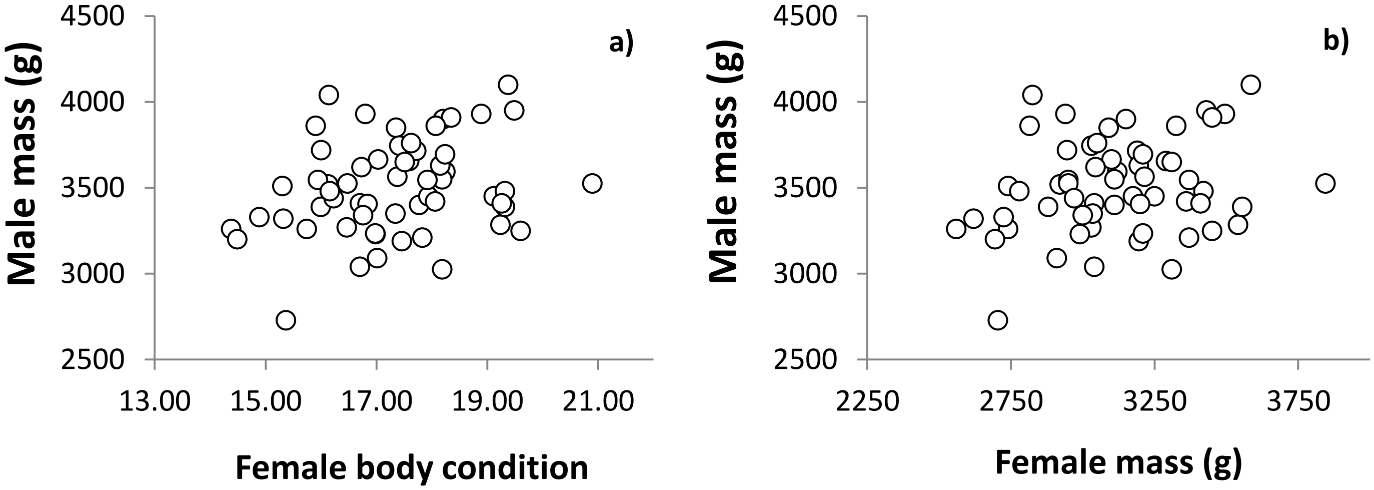

Figure 3.2: Assortative mating patterns in early-breeding African Penguin pairs (Mar-Apr, n = 59): a) Female body condition positively correlated with male mass (r = 0.273, p = 0.036) and b) male and female masses closely approach significant correlation (r = 0.248, p = 0.058).

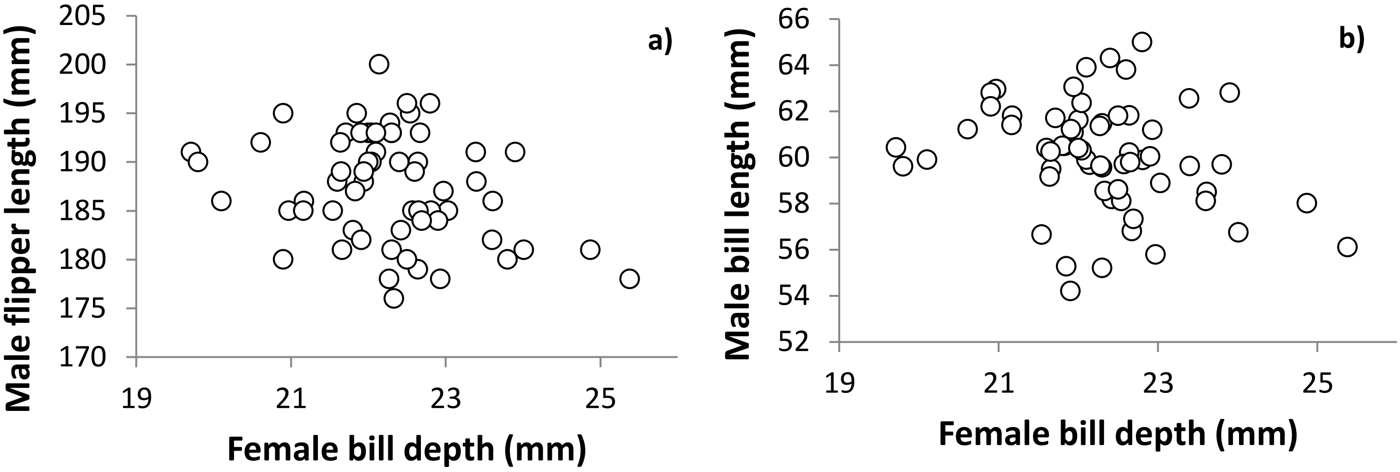

Figure 3.3: Assortative mating patterns in later-breeding African Penguin pairs (May-July, n = 62): a) female bill depth and male flipper length (r = -0.282, p = 0.027) were negatively correlated while b) female bill depth and male bill length (r = -0.248, p = 0.052) were marginally non-significantly negatively correlated in later-breeding birds, indicating negative assortative mating.

Table 3.4: Summary of Assortative Indices (AI) and Absolute Size Indices (ASI) for five phenotypic traits measured in the African Penguin (n = 121 pairs).

	Assortative Index (AI)			Absolute Size Index (ASI)		
	Mean ± SD	**Range**	**CV**	**Mean ± SD**	**Range**	**CV**
Mass	13.86 ± 8.33	0.00 – 34.94	60.1	6568.8 ± 455.8	5435 – 7685	6.9
Bill length	8.08 ± 3.89	0.35 – 18.15	48.1	115.6 ± 3.1	107.1 – 124.2	2.6
Bill depth	9.60 ± 4.96	0.57 – 23.57	51.7	47.1 ± 1.5	44.1 – 52.3	3.2
Flipper length	5.20 ± 3.14	0.00 – 12.31	60.4	366.4 ± 7.7	349.0 – 384.0	2.1
Body condition	10.71 ± 7.19	0.07 – 30.94	67.1	35.8 ± 2.3	30.1 – 41.0	6.5

Assortative Indices (AIs) were smallest for the morphometric traits that were the least variable (flipper and bill length and bill depth, Table 3.4), showing that pairs were on average more assorted with regard to structural size. Both AIs and Absolute Size Indices (ASIs) were generally strongly correlated among each other, (Table 3.5 and 3.6) suggesting the traits exhibited by individuals pairing together varied in much the same manner. Body mass, especially, was strongly correlated with all the other variables.

No correlation was found between AIs and ASIs when all pairs breeding from March - July were analysed together (Pearson correlations; Table 3.7), showing that larger birds were not more assorted than smaller ones, i.e. the strength of mate choice was independent of size. When early and late breeders were considered separately, however, a positive relationship emerged between ASI and AI for mass in later-breeders. Thus, from May – July, smaller pairs were more assorted than larger ones. Overall, body condition was the most assorted trait between all pairs (r = 0.161, p = 0.079, Table 3.3); body mass was the second most assorted (r = 0.158, p = 0.084), though neither showed a significant relationship. Analysed temporally, a significant pattern of positive assortment was evident in early breeders for male mass and female body condition (r = 0.273, p = 0.036, Figure 3.2a) and closely approached significance for male mass with female mass (r = 0.248, p = 0.058, Figure 3.2b).

Table 3.5: Pearson correlation matrix of Assortative Indices (AI) for all five phenotypic traits measured in the African Penguin (n = 121 pairs). Significant results in red.

	AI Mass	**AI Bill length**	**AI Bill depth**	**AI Flipper length**
AI Bill length	**r = 0.191** **p = 0.036**	-	-	-
AI Bill depth	**r = 0.374** **p < 0.001**	**r = 0.326** **p < 0.001**	-	-
AI Flipper length	**r = 0.260** **p = 0.004**	r = 0.152 p = 0.097	**r = 0.221** **p = 0.015**	-
AI Body condition	**r = 0.852** **p < 0.001**	r = 0.161 p = 0.078	**r = 0.253** **p = 0.005**	r = -0.062 p = 0.500

Table 3.6: Pearson correlation matrix of Absolute Size Indices (ASI) for all five phenotypic traits measured in the African Penguin (n = 121 pairs).

	ASI Mass	**ASI Bill length**	**ASI Bill depth**	**ASI Flipper length**
ASI Bill length	**r = 0.287** **p = 0.001**	-	-	-
ASI Bill depth	**r = 0.338** **p < 0.001**	**r = 0.202** **p = 0.027**	-	-
ASI Flipper length	**r = 0.356** **p < 0.001**	**r = 0.256** **p = 0.005**	r = 0.097 p = 0.292	-
ASI Body condition	**r = 0.951** **p < 0.001**	**r = 0.224** **p = 0.014**	**r = 0.331** **p < 0.001**	r = 0.051 p = 0.578

Table 3.7: Pearson correlations between Assortative Indices (AIs) and Absolute Size Indices (ASIs) for each phenotypic trait in African Penguins.

	Early (Mar-Apr) n = 59		Late (May-July) n = 62		All (Mar-July) n = 121	
Parameter	**r**	**p**	**r**	**p**	**r**	**p**
Mass	0.027	0.839	**0.313**	**0.013**	0.169	0.064
Bill length	-0.043	0.748	0.210	0.102	0.040	0.662
Bill depth	0.193	0.144	0.113	0.381	0.133	0.146
Flipper length	-0.152	0.249	0.089	0.490	0.022	0.815
Body condition	0.091	0.494	0.148	0.252	0.119	0.193

When comparing temporal assortative patterns (i.e. early versus late breeders), birds paired more assortatively by mass in March and April than later in the season (Table 3.8). When assessing Pearson correlations of morphometric traits separately within pairs for early and later breeders, pairs breeding earlier were found to be better assorted, although not significantly so, for all traits except bill depth. Early breeders could almost be said to have exhibited assortative mating for mass (r = 0.248, n = 59, p = 0.058, Figure 3.2b). It is important to note that early-breeding females weighed more (p = 0.007), had longer flippers (p = 0.017) and were also in better body condition (p = 0.045) than later-breeding females, while no such temporal difference existed in males (Table 3.8). When comparing the ASIs between pairs from early and late nesting periods, no differences were found, apart from early-breeding pairs having (combined) longer flippers than later-breeding pairs (p = 0.042).

Table 3.8: Temporal comparison of parental morphometrics for earlier (Mar-Apr) and later (May-July) breeders.

		Mean			
		March – April (n = 59)	**May – July (n = 62)**	**t**	**p**
Female	Mass	**3118.05**	**2988.97**	**2.74**	**0.007**
	Bill length	55.57	55.38	0.52	0.610
	Bill depth	22.53	22.27	1.33	0.185
	Flipper length	**179.88**	**177.60**	**2.42**	**0.017**
	Body condition	**17.33**	**16.83**	**2.02**	**0.045**
Male	Mass	3505.17	3528.23	-0.38	0.703
	Bill length	60.21	60.05	0.39	0.700
	Bill depth	24.77	24.56	1.06	0.290
	Flipper length	188.02	187.45	0.58	0.560
	Body condition	18.65	18.81	-0.52	0.602
AI	Mass	**12.085**	**15.556**	**-2.332**	**0.021**
	Bill length	7.878	8.267	-0.549	0.584
	Bill depth	9.578	9.620	-0.046	0.963
	Flipper length	4.834	5.555	-1.264	0.209
	Body condition	9.676	11.701	-1.558	0.122
ASI	Mass	6623.220	6517.097	1.283	0.202
	Bill length	115.773	115.429	0.618	0.538
	Bill depth	47.300	46.835	1.719	0.088
	Flipper length	**367.898**	**365.048**	**2.051**	**0.042**
	Body condition	35.985	35.642	0.813	0.418

Figure 3.4 shows both male and female measurements plotted against their AI value for their pair. For every trait, the birds belonging to the best assorted pairs were smaller males and larger females. Progressively larger males and smaller females belonged to pairs less similar for each trait. Thus, mates with average physical aspects were assortatively mated, while sexual dimorphism drove the disparity in AI at the extremes of the minimum (female) and maximum (male) size continuum.

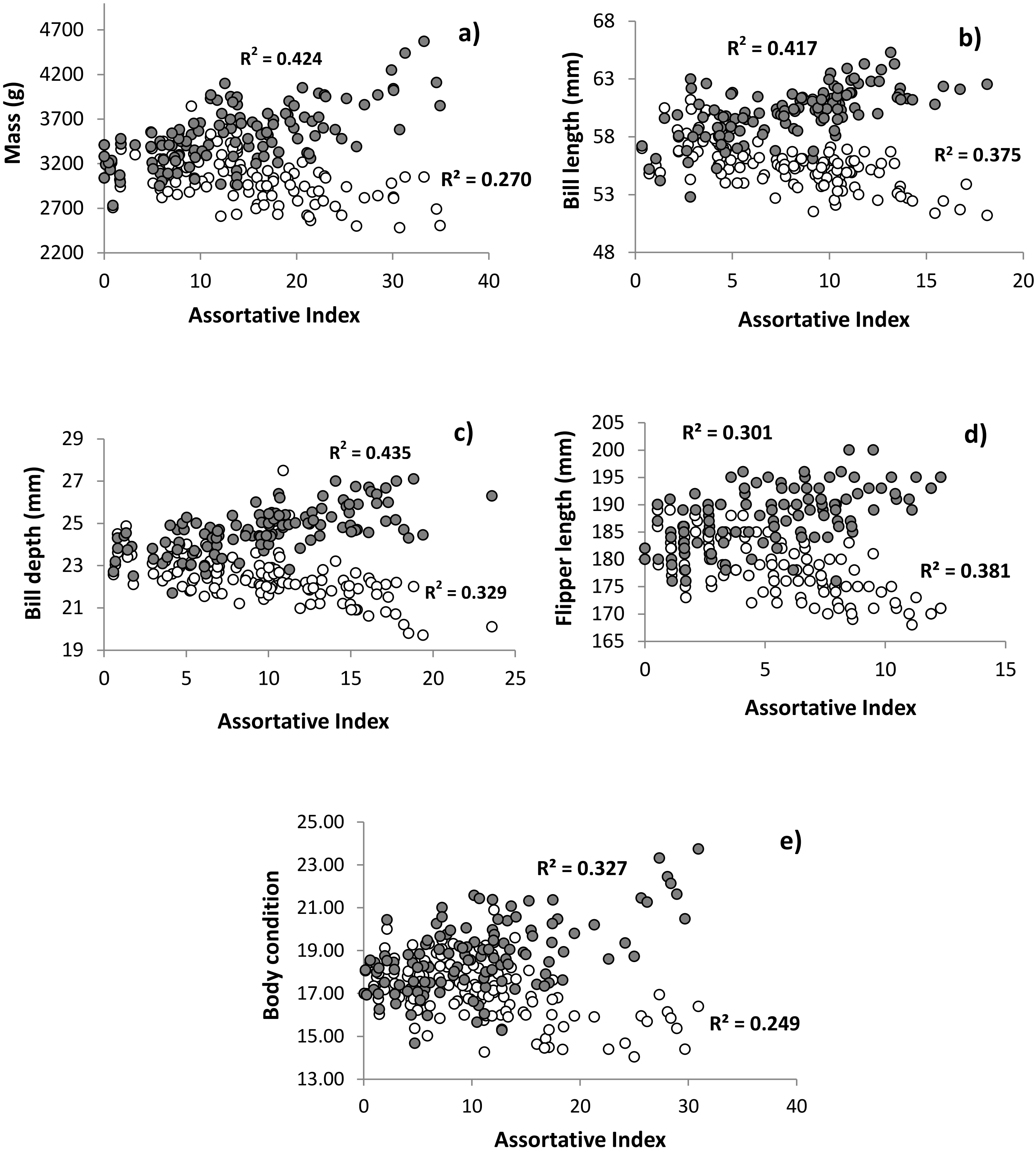

Figure 3.4: Male (grey circles) and female (white circles) measurements versus the Assortative Index of the pair for all five traits (all $p < 0.001$).

3.3.2 *Chick growth*

This study took place in a year (2013) of above-average food availability (McInnes unpubl. data) and chick growth (Table 3.9) was greater and less variable than during the same period in the previous year (average growth rate for 2012: 37.4 ± 26.2 g/day, n = 48, variability: 26.8 ± 15.8, n = 26, L. Pichegru, unpubl. data). African Penguin chick growth rate follows a Gompertz or von Bertelanffy curve and is not linear throughout development. P1 chicks generally grew slightly more slowly (39.2g/day, n = 34 nests) than P2 chicks (46.1g/day, n = 76 nests, t = -2.35, p = 0.021) in this study, although no difference in body condition index was observed for these two developmental stages (P1: 0.348, P2: 0.342, t = -0.070, p = 0.944). For this reason, aspects of chick growth rate and parental morphometric regressions were calculated separately for each age category (see 3.3.3). A- and B-chicks had similar growth rates (Paired t-tests, A: 43.98g/day, B: 42.13g/day, t = 0.904, p = 0.368) and body condition indices (A: 0.34, B: 0.33, t = 0.192, p = 0.848) and did not differ in the variability of their growth rates, although variability in body condition indices was lower for B-chicks (A: 0.23, B: 0.19, t = 2.12, p = 0.035). When chick development was assessed for temporal differences between early (Mar-Apr) and late (May-July) broods, no difference was found between them in terms of growth rates (early: 46.11g/day, late: 41.17g/day, t = 1.847, p = 0.067), body condition indices (early: 0.38, late: 0.30, t = 1.631, p = 0.106) or within-nest variability for these traits (Table 3.10).

The averages for within-nest growth rate and body condition were strongly positively correlated, as would be expected (Pearson's correlation, r = 0.52, p < 0.001, n = 108) as a fast-growing chick would presumably be in better condition than a slow-growing one. Brood size had no bearing on within-nest growth rates (Student's t-test, single brood: 44.07g/day, double brood: 43.24g/day, t = 0.25, p = 0.800) or body condition indices (single brood: 0.32, double brood: 0.34, t = -0.32, p = 0.750, Table 3.11), although variability in body condition differed between singleton chicks and the A-chicks of double broods; body condition varied more in single broods. Conversely, mass gain was more variable in chicks of double broods, although not significantly so.

Table 3.9: Aspects of breeding performance (clutch size, chick growth, condition and variability) of African Penguin chicks on Bird Island in 2013.

Brood parameter	
Brood size	1.8 ± 0.4 (n = 117; 79.5% double brood)
Chick growth (g/day)	
A-chick	43.98 ± 15.5 (n = 117)
Range	-18.0 – 90.0
B-chick	42.13 ± 14.1 (n = 93)
Range	8.0 – 94.0
Body condition index (BCI)	
A-chick	0.34 ± 0.26 (n = 109)
Range	-0.93 – 1.07
B-chick	0.33 ± 0.32 (n = 77)
Range	-0.42 – 1.41
Variability in chick growth	
A-chick	19.88 ± 12.17 (n = 107)
B-chick	18.83 ± 11.40 (n = 80)
Variability in body condition index (BCI)	
A-chick	0.23 ± 0.13 (n = 93)
B-chick	0.19 ± 0.13 (n = 66)

Table 3.10: Chick growth comparison of early and late broods (sample size in brackets).

	Early brood (Mar-Apr)	Late brood (May-July)	t	p
Growth rate (g/day)	46.11 (53)	41.17 (64)	1.847	0.067
Variability in growth rate	17.77 (46)	19.82 (62)	-1.024	0.308
Body Condition Index	0.38 (51)	0.30 (57)	1.631	0.106
Variability in BCI	0.23 (41)	0.22 (52)	0.579	0.564

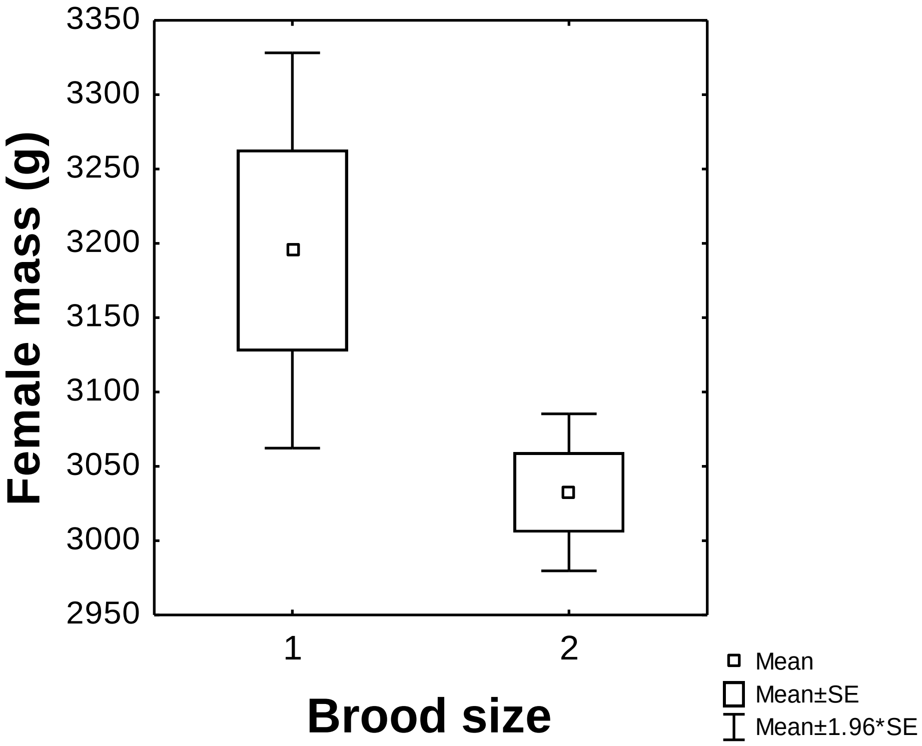

Figure 3.5: Box and whisker plot of adult female African Penguin mass for single and double broods of similar age (P1). Mean mass is centred within box depicting SE of the mean. Whiskers show mean ± SD (single brood n = 20, double brood n = 60).

The body mass of female parents of double broods (3031.5g on average) was significantly less than the body mass of female parents of single broods (3195.2g on average, t = 2.57, p = 0.011, Figure 3.5). The same was true for the body condition (which was calculated from mass and flipper length) of adult females (17.8, 17.0, t = 2.57, p = 0.010). No other phenotypic parameter differed between parents of either single or double broods when calculated separately for male and female parents, or when calculated for AIs and ASIs.

Table 3.11: Effects of brood size on chick development parameters.

Parameter	**Brood size** **1** **(n = 24)**	 **2** **(n = 93)**	**t**	**p**
Growth rate of singleton-chick vs A-chick of double brood (g/day)	44.07	44.00	0.031	0.98
Within-nest average growth rate (g/day)	44.07	43.24	0.25	0.80
BCI of singleton-chick vs A-chick of double brood	0.32	0.35	-0.42	0.67
Within-nest average BCI	0.32	0.34	-0.32	0.75
Variation in growth rate of singleton-chick vs A-chick of double brood	16.03	20.87	-1.68	0.10
Within-nest average variation in growth rate	16.03	19.69	-1.49	0.14
Variation in BCI of singleton-chick vs A-chick of double brood	**0.31**	**0.21**	**2.94**	**0.004**
Within-nest average variation in BCI	**0.31**	**0.20**	**3.83**	**< 0.001**

Table 3.12: Growth parameters of African Penguin chicks (females: n = 15, males: n = 34).

Parameter	Female	Male	t	p
Growth rate (g/day)	40.294	43.413	-0.710	0.481
Variation in growth rate	22.258	18.390	1.334	0.189
BCI	0.212	0.269	-0.779	0.440
Variation in BCI	0.262	0.195	1.543	0.132

Chick growth rate and body condition index did not differ between the sexes for the 49 chicks that were successfully sexed by molecular methods (Table 3.12). Although males grew faster, had a higher body condition index and varied less in these parameters than female chicks, the differences were not statistically significant. All chicks of both sexes belonged to the average size class P2, eliminating age as a confounding factor.

It is noteworthy that the sex ratio of the chicks for which gender was determined was 2.27:1; a substantial deviation from parity (χ^2 = 6.61, DF = 1, p = 0.01, Yates correction applied). While the sample size of sexed chicks was small (n = 49), this points towards male offspring bias (15 females = 30.6% to 34 males = 69.4%). Of the 13 double broods for which the sex of both chicks was determined, 10 (77%) were a male-female pair, and hatching order was random with regards to sex. The other three (23%) were male-male pairs. No double broods containing only females were discovered.

3.3.3 *Parental phenotypes and breeding performance*

Of all the models used to identify possible relationships between parental morphology and chick growth, only three parental parameters explained two chick developmental parameters, after correcting for false discovery rates. These were the variation of within-nest body condition index of all chick sizes, and the variation of within-nest growth rate of P1 chicks (Table 3.13). The body condition index of chicks of all sizes was more variable when female parent mass and body condition was larger. These two parental aspects had to be considered separately owing to autocorrelation. Within-nest variation in chick growth rate also varied directly with the ASI for parental flipper length, indicating that variability in brood growth was greatest in the largest pairs for this trait.

Table 3.13: Significant relationships between parental phenotype and chick development after Benjamini-Hochberg step-up FDR was performed on multiple regression models.

Chick development parameter	Parental parameter	n	r	p
Within-nest variation in BCI*	Female mass	85	**0.317**	**0.003**
Within-nest variation in BCI*	ASI body condition	85	**0.315**	**0.003**
Within-nest variation in growth rate of P1 chicks	ASI flipper length	27	**0.548**	**0.003**

* BCI not considered separately for P1 and P2 chicks as this index remained constant across ages.

3.4 DISCUSSION

3.4.1 *Sex determination and sexual dimorphism in the African Penguin*

Most species of penguin are difficult to sex in the field owing to the lack of any sex-linked plumage differences or obvious secondary sexual characteristics (Renner *et al.* 1998). While observing copulatory positions and measuring cloacal width are effective tools for identifying the sex of individuals (Pichegru *et al.* 2013), their efficacy is limited to a short window of time during pre- and post-laying periods. A simple and reliable method has been presented for future sex discrimination of adult African Penguins on Bird Island using only two biometric parameters: bill and flipper length. These biometric features were the least dimorphic and the least variable of the five measured traits (SDI: 92.4% and 95.3%, CV: 3.6 and 3.0 respectively). The classification functions described (in 3.3.1) have eliminated the need to measure body mass, which is known to vary greatly with season and stage of breeding, to sex African Penguins (Davis & Speirs 1990, Hull 1996) and thus allow accurate gender determination in the field at any stage of the year.

Classification functions presented in Pichegru *et al.* (2013) used bill depth instead of flipper length (this study) to predict sex with an accuracy of ca. 90%; however, these functions only predicted 59% of the males from the present study. When a species is widely distributed, geographic variation in morphology between members of different colonies can occur; this is particularly common for philopatric seabirds such as penguins (Renner *et al.* 1998, Einoder *et al.* 2008). For this reason, care should be taken to apply discriminant functions only to the individuals of the specific locality from which they were derived, unless there is reason to assume they can be applied to members from other colonies. Inter-observer bias in data collection in this study may have led to subtle variations in recorded morphology, yet the relatively large number of adults sexed using genetic methods should have buffered against such discrepancies. This study used biometric data from Bird Island penguins only, whereas the classifying predictors in Pichegru *et al.* (2013) were calculated from a combination of penguins from both Bird Island and St Croix Island (approximately 50km apart). It is unclear how accurately the classification functions presented here would predict the sex of African

Penguins on the West Coast or from Namibia, although preliminary analyses reveal there is little difference in phenotypic traits between even distant colonies (L. Pichegru, unpubl. data). As a particularly under-studied phenomenon in this species, it would be beneficial to conduct separate DFA analyses during future studies of sexual dimorphism and compare the results with Pichegru *et al.* (2013) and this study, for both distant colonies and different years.

3.4.2 *Assortative mating*

Large changes in the body masses of both male and female penguins occur before, during and after the breeding season and subsequent moulting period (Croxall 1995). Consequently, breeding penguins potentially exhibit a range of weights depending on timing. Although efforts were made to attain at least two measurements of mass (post-feeding) in this study, mass was the most inherently variable parameter. In spite of the large potential range of dimorphism indices, mass and body condition (derived from mass and flipper length) were the best contenders for positive assortative mating on Bird Island, with pairs (relatively weakly) assorted for these traits early in the season, but not later. Later-breeding birds exhibited negative assortative mating for female bill depth and male bill and flipper length. This may have been linked to the effect of larger females (but not smaller males) breeding at the start of the season. The Assortative Index and Absolute Size Index for body mass significantly correlated with all other Assortative and Absolute Size Index parameters, suggesting that body mass was the best indicator of overall body size, despite being the most variable trait. The Assortative Index also positively correlated with Absolute Size Index in the later-breeders, indicating that during May to July, smaller pairs were more assorted by mass than larger pairs, although the consequences of this are unclear. As a general rule across seabird species and populations in general, the later the onset of breeding within a season, the poorer the reproductive success is likely to be (Reilly & Cullen 1981, Weimerskirch *et al.* 1992, Svagelj & Quintana 2011). This was not the case for the period of monitoring in this study, however, and breeding performance was similar for early and late breeders.

Sexual selection through the mechanism of intrasexual competition for mates is one of the best supported explanations for the phenomenon of avian sexual dimorphism (Hedrick & Temeles 1989, Forero *et al.* 2001). Many species of penguins, including the African Penguin, use their bills and flippers as weapons during territory disputes with conspecifics during the breeding season, with males arriving at the colony first and securing this resource (Hockey *et al.* 2005). Furthermore, bills and flippers are also used during courtship rituals (Hockey *et al.* 2005) and it would be expected that these traits may be prone to sexual dimorphism (Williams 1995). Nonetheless, bill and flipper measurements were the phenotypic traits displaying the lowest level of sexual dimorphism and the least amount of variability between the sexes, yet no assortative mate choice was evident for these traits as would be expected if the largest males competed for the largest females. For the intrasexual competition hypothesis to be validated in this study, better breeding performance would need to have been demonstrated for larger males. This was not the case, and no advantage in terms of larger brood size or faster chick growth rate or body condition index was conferred on males (or females, for that matter) that were larger for any biometric trait.

It was suggested by Davis and Speirs (1990) and later demonstrated by Marks *et al.* (2010) that the Adélie Penguin's ecstatic call can be used as an honest signal of a male's condition;

larger, better quality males consistently produce ecstatic calls of a lower frequency and pitch than smaller, poorer quality males. Both sexes of African Penguins also produce the ecstatic call during the breeding season, and this may be a means by which to mutually assess the condition of potential mates. The “discriminating female” hypothesis cannot be ruled in this species out as there may be long-term fitness consequences acting on mate choice in the population that may not have been discovered in this short-term study.

An alternative advantage to the benefits derived from mating assortatively is suggested by the hypothesis that maintaining sexual dimorphism reduces intersexual competition (Hedrick & Temeles 1989, Pichegru *et al.* 2013). Spatial segregation of foraging habitats between the sexes is widespread in seabirds and is often not limited to the breeding season (Wearmouth & Sims 2008). The feeding niche hypothesis has been supported in studies addressing feeding niche separation between the sexes in diving birds; the larger sex tends to be capable of deeper and longer dives (Cook *et al.* 2007, 2012; Pichegru *et al.* 2013) and has been documented to prey on larger size classes of prey than the smaller sex (Cook *et al.* 2007, Zavalaga *et al.* 2007). Besides the observed sexual segregation in foraging locations in some species of seabird (Weimerskirch *et al.* 1997, Xavier *et al.* 2004), differences in diet composition (Hunter & Brooke 1992, González-Solis 2004) and foraging effort (Wanless *et al.* 1995, Kato *et al.* 2000) accompany this segregation, sometimes even on a temporal scale (Bernstein & Maxson 1984, Kato *et al.* 2000). While it may be the avoidance of intersexual competition that drives and maintains sexual dimorphism in the African Penguin, it may not be the only possible explanation (Wearmouth & Sims 2008).

3.4.3 *Chick development*

3.4.3.1 Brood size, survival and sex ratio

Double-brooded nests were more common in 2013 (this study, 79.5%) than in both 2011 (54.4%) and 2012 (40.5%, L. Pichegru, unpubl. data). During nest monitoring, only one brood failed completely and five B-chicks either died or went missing. Chick survival up to the P2-stage was high in 2013, and surpassed previous years of monitoring, although fledging success rates are unknown. During May especially, small piles of anchovies were regularly seen on the penguins' landing beaches and jetties, signs of plentiful forage fish surrounding the island that made regurgitation necessary prior to the birds walking back to the nest (*pers. obs.,* McInnes *et al.* in prep.).

When conditions become unfavourable, seabird parents may actively discriminate between offspring; either preferentially provisioning the larger sibling since it is the most likely to survive and more energy has already been expended on it, or alternatively, favouring the smaller sibling since returns on expenditure are greater (Clutton-Brock 1991). In light of this, foraging conditions must have been favourable indeed, for no difference was found between A- and B-chick growth rates or body condition.

The greater variability in body condition index observed in singleton chicks compared to the A-chick of double broods may be indicative of inexperienced parents or parents of poorer foraging capabilities. While provisioning of one chick intuitively demands less of parents than provisioning a double brood, and since it is in single broods that death has already occurred in the nest (either pre-or post-hatching), it is possible that the greater variability in food delivery is considerably detrimental to chick survival, and that this increased variability continues post-brood-reduction.

3.4.3.2 Chick sex ratio and parental investment

Adjustment of the sex of offspring has been observed to occur in many species of birds (Pike & Petrie 2003) although the mechanisms which drive this adaption have been poorly elucidated (Rutkowska & Badyaev 2008, Pinson *et al.* 2011). Almost half of all avian orders contain at least one species able to manipulate the primary sex ratio (Pike & Petrie 2003). It is almost certain that the female bird determines the primary sex ratio, as she is the heterogametic sex, contributing either a Z or W chromosome in the fertilized egg (Wiebe & Bortolotti 1992, Pinson *et al.* 2011). The dimorphism of avian sex chromosomes in terms of their shape, size, protein bodies and position during meiosis is able to facilitate the formation of non-random offspring sex ratio bias in conjunction with the influence of maternal hormones (Gam *et al.* 2011, Pinson *et al.* 2011) and epigenetic drivers (Rutkowska & Badyaev 2008). If environmental conditions change post-laying, parents may opt to bias the secondary sex ratio in favour of the optimal sex. This may be achieved by preferentially provisioning the desired progeny, although may also be achieved passively from the parent's inability to provision the more expensive sex (Wiebe & Bortolotti 1992).

Although the sample size was low in this study, a marked male-biased sex ratio (2.27:1) was found. This may indicate rather rapid and strong sex chromosome allocation to favour production of the sex that might be more expensive to raise (male chicks grew slightly faster, and had a marginally higher body condition than females, although the differences were not significant) during a year of above-average food availability (Trivers & Willard

1973). While primary sex ratios are determined in a Mendelian manner in birds and despite empirical evidence being limited to a relatively small number of species, a few mechanisms have been suggested by which clutch sex ratios may be adjusted through non-random segregation of sex chromosomes (Breitwisch 1989). These include the order in the clutch, clutch size, egg weight, habitat quality, season of birth or paternal breeding success (Clutton-Brock 1986, Pike & Petrie 2003). Alternatively, avian secondary sex ratios may be adjusted through differential mortality, as the heterogametic sex (females) may be more susceptible to embryonic and nestling death, analogous to lower survival in heterogametic male mammals (McMillen 1979, Breitwisch 1989). It is unclear how parent penguins provision their chicks according to their sex, although Teather (1992) reported a difference in food solicitation (begging) between male and female nestlings.

Male-biased sex ratios in adult populations of wild birds are more common than previously thought but the margin of disparity is often greater than expected in threatened populations (Donald 2007). Owing to their smaller size and frequently higher levels of parental investment, female penguins are more susceptible to death from starvation, parasitism and disease than males (Reis *et al.* 2011, Pichegru & Parsons 2014), and this differential mortality is further exacerbated when food is scarce (Boersma 1998, Olsson & van der Jeugd 2002). Pichegru & Parsons (2014) recently reported significantly higher mortality rates (>60%) for adult female and juvenile African Penguins from the Western Cape, although mortality was similar in both sexes for chicks. While interpretation of the current study's results requires caution, skewing of the sex ratio could originate as early as the nestling stage. Male-biased sex ratios present a major concern to small populations: increased intrasexual competition has been linked to infanticide and aggressive behaviour towards nesting females by unpaired male Humboldt Penguins (Taylor *et al.* 2001) and may serve to intensify the effects of inbreeding and further reduce the potential number of breeding pairs (Donald 2007).

Overall there was no strong evidence of assortative mating of the African Penguin in this study. Apart from the possibility that African Penguins simply do not exhibit assortative mating, a possible explanation stems from the likelihood of a skewed tertiary sex ratio (Pichegru & Parsons 2014). In this scenario, females would be mated regardless of their size, since they are the limiting resource, while mated males would be among the largest in the population if intrasexual competition were important (Harari *et al.* 1999). Given the urgency of conservation efforts for the African Penguin, further sex ratio and sex-specific investment studies are warranted.

3.4.4 *Parental investment*

Unequal investment in offspring care in the form of increased female foraging effort has been documented in the Gentoo (*Pygoscelis papua*, Lescroël *et al.* 2009) and African (Pichegru *et al.* 2013) Penguins. Breeding female Adélie Penguins have also been shown to lose more body reserves than males, and this in a species which exhibits very little sexual dimorphism (Beaulieu *et al.* 2009). Similarly, female Manx Shearwaters adjust their provisioning rates in response to their chick's body condition, while males do not (Quillfeldt *et al.* 2004). While the mass and body condition of penguins on Bird Island did not differ between males raising either one or two chicks, females raising double broods were significantly lighter and in poorer body condition than females raising a single chick. It thus appears that female African Penguins invest more into their reproductive attempts than do males. In light of their higher mortality rate as adults (Pichegru & Parsons 2014), female African Penguins may be prone to investing more resources into a breeding attempt than males, may breed earlier, and may also be more flexible in modifying chick provisioning. When the developmental parameters of chicks were assessed in terms of their parent's morphometrics and assortment, only female mass and body condition sufficiently explained variation in chick body condition indices. Variation in chick body condition, but not body condition itself, increased with increasing female size and condition. That chick body condition remained unaffected by the mother's size, but with greater variability in the case of larger females, suggests that the larger a female is, the more flexible she is in ensuring that her brood is well provisioned.

Variability in P1 chick growth rates was directly affected by, and increased with, increasing combined parental flipper length. Again, larger birds, but this time for the combined size of the pair, could possibly adjust offspring provisioning depending on the size of a very important diving tool: the flipper. Given the large distances covered daily by foraging penguins during the chick-rearing stages (60 to 100km, Pichegru *et al.* 2010), the flipper is a critical locomotive device. Conclusive evidence for the connection among higher food delivery capacity, brood size and larger flipper size has been found in the Chinstrap and Magellanic Penguins (de Leon *et al.* 1998, Forero *et al.* 2001). While inconclusive, the results of this study may support the suggestion that the importance of flipper length for optimal foraging results in low levels of sexual dimorphism in this trait. Flipper length in African Penguins on Bird Island was indeed the least sexually dimorphic characteristic measured.

3.4.5 *Assumptions and limitations*

While it is not impossible that the penguins of Bird Island may engage in extra-pair (heterosexual) copulations and then proceed to raise a family with a member of the same sex (Young *et al.* 2008), this has not been documented in this wild population, and the phenomenon was assumed to be absent. Homosexual behaviour in captive penguins deprived of potential mates, however, has been documented in Chinstrap (Smith 2004), Humboldt (BBC News 2004), Magellanic (SFGate 2009) and African Penguins (CBC News 2011, Huffington Post 2011) where same-sex pairs allo-preen, engage in sexual activities and may incubate rocks in lieu of fertilized eggs. These pairs often remained bonded even when suitable mates were provided.

The performance of the DNA-sexing protocol in this study was poor; unequivocal PCR products (clear single or double bands) were produced in less than 50% of attempts, improving to ca. 60% after adjustment of PCR conditions and re-analysis. It may have been preferable to freeze feather samples post-plucking instead of storing them at ambient temperatures (Dr. Lisa Nupen, UCT post-doctoral researcher at UCT, *pers. comm.*). However, successful sexing has previously been accomplished from feathers stored at ambient temperature (Morin *et al.* 1994; Dr. Maëlle Connan, post-doctoral researcher at NMMU, *pers. comm.*) and lack of reliable freezer facilities due to intermittent electricity on Bird Island prohibited freezing.

It might be argued that the genetic sex determination was unreliable on the basis of poor performance, and that birds were misidentified as male due to failure of the W-linked female sex chromosome to amplify, especially in the case of the greatly skewed sex-ratio in favour of males. However, chick and adult tissue was analysed side by side and when manually comparing the genetically determined results of the adult birds with their morphometric data, outcomes were corroborated in nearly every case (95%). Ambiguous pairs were removed prior to discriminant function analyses.

3.4.6 *Concluding remarks*

The African Penguin has suffered an extraordinary population decline since the late 19th Century (Crawford *et al.* 2011). If the species is to avoid extinction and recover, it is imperative that chick survival and thus fledging success exceed the mortality rate in spite of the numerous threats faced, including oil pollution, climate change and, most importantly, competition with fisheries (Pichegru *et al.* 2012). Breeding success was high in this study, at least for the brief period during nest monitoring; a high percentage of nests were double-brooded and remarkably few deaths were recorded. As marine sentinels, penguins serve as indicators of environmental condition and this is especially true during offspring provisioning (Boersma 2008). No assortative mating was found when all pairs of breeding birds were assessed, and only weak positive assortment was found in pairs with small chicks early in the season. Surprisingly, better assortment and larger size did not confer any breeding performance advantages to parents. While speculative, the plentiful food resources in Algoa Bay during the peak breeding season in 2013 may have buffered against the benefits of mating assortatively; in a year of abundance, even poorly matched pairs or first-time breeders may be able to raise a healthy brood successfully. A tentative male-bias in chick sex ratio was observed, which could bode poorly for a species in which female-biased mortality has been recently confirmed (Pichegru & Parsons 2014). Further insight into sex-specific reproductive strategies and performance of penguins is especially urgently warranted in Algoa Bay, a comparatively under-studied population.

CHAPTER 4

NEST ATTENDANCE PATTERNS OF THE AFRICAN PENGUIN IN A CHANGING ENVIRONMENT

4.1 INTRODUCTION

Seabirds are important consumers of marine resources that can be highly mobile and distributed in a patchy manner, necessitating cooperation by members of a pair to guarantee chick survival (Weimerskirch 2007). Biparental care is the most common chick-rearing strategy in birds, observed in 80-90% of all species (Lack 1968, Cockburn 2006), yet males and females may not invest equally in a breeding attempt (Creelman & Storey 1991, Bradley *et al.* 2002, Quillfeldt *et al.* 2004). Disproportionate investment within a pair may require compensatory behaviour by a partner to maximize fitness (Bijleveld & Mullers 2009, Saraux *et al.* 2011). The costs of survival and undertaking a reproductive attempt are balanced against future breeding potential, with trade-offs existing between self-preservation and potential long-term fecundity (Trivers 1972, Stearns 1989). Individual adults in poor condition as a result of low prey availability may need to spend longer periods foraging to support themselves, at the cost of their offspring's provisioning. Time allocation strategies in long-lived, biparental species are thus dependent on both the current state of the individual (and the quality of the pair) and environmental conditions (Rishworth *et al.* 2014).

Marine ecosystems are shaped by numerous, largely unpredictable environmental conditions such as upwelling, chlorophyll concentrations and sea surface temperature (SST), the effects of which on top predators are still poorly understood (Simmonds & Isaac 2007). Satellite data of marine conditions such as SST and surface chlorophyll concentrations have been widely used to assess primary productivity in marine ecosystems and serve as a predictor of foraging behaviour in seabirds, despite their limitations and the fact that seabirds feed on prey at much higher trophic levels, far removed from primary production (Grémillet *et al.* 2008). Whilst volant seabirds are capable of travelling great distances in search of prey, flightless central place foragers such as penguins are more constrained during their chick-rearing periods and cannot greatly increase their foraging ranges (Booth 2011, Sherley *et al.* 2013). The population dynamics of these seabirds are thus largely regulated by the availability of food within their relatively small foraging ranges (Anderson *et al.* 1982, Pichegru *et al.* 2010). As the foraging partner is the determiner of shift length, the conditions at sea and foraging success are thought to be strong predictors of incubation/chick-guarding shift dynamics.

It is of key importance for any breeding performance study to investigate how the investment and expenditure of time and energy by parents influences reproductive success, especially for endangered keystone species, such as the African Penguin. Provisioning behaviour in this seabird is one such proxy of parental input, with the length of nest attendance bouts and the frequency of feeding acting as indicators of food abundance and resource delivery rates respectively (Saraux *et al.* 2011). It is well established that the success of marine avifauna can be highly variable in consecutive years and that success is strongly reliant on sufficient ecosystem-wide resources (Schreiber & Burger 2002, Agenbag *et al.* 2003). It is important that we establish baseline information on incubation and chick-guarding attendance and provisioning rates in both years of plenty and years of paucity to better understand the behavioural thresholds of reproductive success or failure.

4.1.1 *Foraging shift dynamics of the African Penguin*

Foraging dynamics of the African Penguin can generally be divided into three phases, namely 1) night time swimming, 2) day foraging and 3) return swimming (Randall 1989). Night time swimming involves birds relieved from their partners leaving the colony after dark, and swimming in a more or less straight line towards a feeding area which they reach at dawn. Day foraging includes a complex pattern of variable diving depth and duration for the purpose of prey capture (Pichegru *et al.* 2013). Return swimming to the island occurs in the afternoon, with birds swimming rapidly and directly towards the colony. Occasionally, breeding birds will overnight at sea (Randall 1989). Descriptions of foraging behaviour from the literature tend to be generalised, and, being largely based on at-sea observations, cannot be related to the characteristics of the breeding pair or their offspring. Here, instead of studying the at-sea foraging dynamics of this bird directly, a land-based approach was taken where its general nesting behaviour and offspring provisioning rates were addressed in relation to individual and brood characteristics.

Seabird nest attendance patterns in many species have been used as a proxy to assess prey availability in the foraging habitat (Gaston & Nettleship 1982, Montevecchi & Myers 1995). Poor feeding conditions may require seabirds to cover greater distances at sea and/or spend longer periods away from the nest, while short foraging bouts may be indicative of plentiful prey close to the colony (Seddon & van Heezik 1991a). As the length of nest attendance (bout duration) is generally determined by the absent partner, the length of incubation or chick-guarding shifts is reflective, to an extent, of the feeding partner's foraging success.

One of the easiest ways of adjusting chick-provisioning is for parents to alter their foraging bout lengths. Longer foraging bouts are thought to benefit the feeding adult at the expense of the waiting offspring, whereas shorter feeding forays allow the parent, sometimes to its own detriment, to feed the chicks at a higher rate (Beaulieu *et al.* 2009). It has been shown that successfully-breeding penguins generally have shorter incubation shifts and more frequent foraging trips than failed breeders (Chiaradia & Kerry 1999, Collins *et al.* 1999). Indeed, shorter foraging trips imply higher feeding frequency of the chicks.

Penguins use energetically costly means of locomotion to forage for both themselves and their offspring and thus their activity budgets are important indicators of their food requirements and their investment into a breeding attempt (Cairns *et al.* 1987). Cohen (2013) documented a positive correlation between prey abundance and the percentage of time both Cape Gannet parents spent together at their nest site. Likewise, as time spent ashore equates to missed foraging opportunities for one parent, the potential exists for the time spent together by partners at the nest site to be used as an indicator of the level of stress in the African Penguin. The same can be said for parental absenteeism, which generally increases with chick age (Davis 1982). As chick energy demands increase with age, parents start engaging in simultaneous foraging bouts and chick crèching behaviour commences. However, the time at which this behaviour commences may also be an indication of physiological stress, with their prolonged fasting periods during the breeding attempt having resulted in rapid weight loss and the need to trade comparative offspring safety and nest defence for increased feeding opportunities (Harding *et al.* 2007).

4.1.2 *The impact of human disturbance on nesting birds*

The negative impacts of human disturbance on wildlife populations have long been recognized and can be constituted by recreational activities as well as the efforts of conservationists and researchers when closely investigating or handling members of a population (Liddle & Scorgie 1980, Boyle & Sampson 1985, Hockin *et al.* 1992). Research in the past 30 years has shown that colonially nesting seabirds are more sensitive to the presence of humans than was previously thought and that even the most well-meaning conservation studies can have detrimental effects (Boyle & Samson 1985, Regel & Pütz 1997). Colonial nesters often flush from their nests when approached by people, in an attempt to either flee from perceived danger or to intimidate the intruder, and in the process, nest contents may be damaged or become subject to the elements and/or predators (Anderson & Keith 1980, Burger 1982). The reported effects of human disturbance also include more subtle, longer-term responses on covert demographical (Bolduc & Guillemette 2003), physiological (Nimon *et al.* 1996) and hormonal levels (Fowler 1999). Penguins were once thought to be largely immune and indifferent to human proximity, but when examined more closely, investigator presence and handling can create significant increases in heart rate (Wilson *et al.* 1991, Nimon *et al.* 1996), body temperature (Regel & Pütz 1997) and corticosterone stress responses (Walker *et al.* 2005). Human disturbance of nesting penguins may also lead to changes in activity budgets which could confound the results of attendance behaviour studies and the use of the behaviour of frequently handled birds as a proxy for prey conditions (Belanger & Bedard 1989, Hockin *et al.* 1992). The effect of researcher proximity and handling on penguin nest attendance patterns was thus an important factor to assess, given the potential for disturbance to affect behavioural studies in which some nests are more frequently disturbed than others.

4.1.3 *Aims*

Unfavourable disturbance imposes limitations on the frequency with which nest attendance can be recorded in densely breeding colonial birds (Chiaradia & Kerry 1999). The use of remote monitoring using video surveillance technology overcomes this restriction, and it was the aim of this study to improve our understanding of the nest attendance patterns of breeding African Penguins on Bird Island, Algoa Bay, using continuous fine-scale activity-budget data provided by remote video camera surveillance. Aspects such as bout duration, the number of shift changes per day (hereafter referred to as the provisioning rate), and time spent by both partners at the nest together as well as the time the nest was left unattended were investigated and related to brood size and age, pair quality (measures of individual and pair body condition) and environmental conditions such as SST and chlorophyll *a*. Additionally, a large number of nests were manually monitored to determine how equally pairs shared their daytime nest-guarding duties. The influence of handling of birds by researchers on nest attendance patterns was also investigated by a comparison of the behaviours of disturbed (handled) versus undisturbed (unhandled) nests.

4.2 MATERIALS & METHODS

4.2.1 *Nest monitoring and camera surveillance*

A total of 2063 nest-days (one full 24-hour period of monitoring per nest) of incubation and chick-rearing was manually monitored at 85 nests on Bird Island, ranging from 11 to 49 days at individual nests during the peak of the 2013 breeding season. Surface nests were preferentially chosen for monitoring purposes, as partners were more easily identifiable from a distance than birds in artificial burrows, both for manual and video observations. Flipper banding as an identification tool is no longer routinely carried out on the African Penguin population, as the bands have been shown to cause wounds of varying severity and alter hydrodynamics during diving, resulting in greater foraging effort and contributing to scientific bias as well as conservation concerns (Jackson & Wilson 2002, Le Maho *et al.* 2011). Therefore, monitored birds were individually identified by means of a bright blue or orange animal dye. So as to avoid direct handling, birds were marked from a distance with the dye taped onto the ends of a wooden broom handle. Every two hours between 07h00 and 17h00, the identity of the attending parent was visually recorded to assess the degree to which daytime incubation or guarding duties were shared between members of a pair. The morphometrics of two thirds of these manually monitored pairs (n = 56) were also recorded.

In addition to the manually monitored nests, in order to monitor exact partner arrival and departure times as well as the length of time spent at the nest site, three weather-proof CCTV security cameras (CC203-1 Series Colour Cameras) were installed near the rangers' house (for power supply). The cameras were set to record continuously between March and

July 2013, and 29 nests were found to be suitably located within the video frames for long-term surveillance. The cameras were connected to two truck batteries and an Uninterrupted Power Supply (UPS) system for alternating between electricity provided by generator, truck battery and main power supplies. Video footage (see Figure 4.1) was stored by means of a 4- and 8-channel DVR (Digital Video Recorder), backed up to a Transcend 3TB HDD and accessed via Clip Player software on a standard laptop computer.

Electricity was not available throughout the day on the island. The island generators were only switched on at about 16h00 each day, and the truck batteries only held a charge for approximately five hours. Cameras were thus set to film from 12h00 until 08h00 each day, using infrared technology to film at low light intensity and at night. All arrival and departure times of parents were recorded during film analysis, as well as the number of shifts per day and the length of time spent together by the pair and the length of time the chicks spent unattended. Chicks generally began to exhibit crèching behaviour during the late P3-P4 stage and it often became impossible to determine their nest identities. Thus, once crèching commenced, monitoring of the nest was terminated and the breeding attempt was considered successful.

Figure 4.1: Examples of good quality video footage. Still captures of the nests under Camera 3 (undisturbed location) during the day (left) and at night (right).

Despite twice daily cleaning of the camera lenses, heavy sea sprays and fog sometimes clouded the video footage and severely limited visibility. Additionally, the island's generator was not switched on every day and if the truck batteries did not hold their charge until the mainland power was switched on in the evenings, an hour or two of recording time was lost. The return of the foraging partner was invariably followed by ritual ecstatic calls and almost immediate commencement of regurgitation of fish for the offspring, which continued intermittently for about half an hour. Thus, even if gaps in the video footage were present, a shift swap could be recorded up to 30 minutes after the relieved partner left the nest, and arrival time of the new partner could be estimated. Alternating between foraging and chick-guarding duties always constituted a meal for the offspring from the returning parent, thus the number of shift change-overs was a direct indication of the provisioning rate by the parents.

4.2.2 *Environmental data*

Sea surface temperature (SST, °C) data were obtained from the moderate-resolution imaging spectroradiometer (MODIS) Aqua satellite. Daily records (level 2 data) were extracted from the SeaWiFS server (http://oceancolor.gsfs.nasa.gov/SeaWiFS.html accessed 2013). Primary productivity was analysed using chlorophyll *a* (chl *a*) concentrations also obtained from MODIS/Aqua Ocean Color (NAS, Washington, USA). Daily concentrations (mg/m^3) were compiled on a 1km grid scale. Average environmental conditions from March to July 2013 were extracted from the MODIS database using 9 coordinates (Table 4.1) or the nearest available data to these points from within a 20km radius of Bird Island. Owing to cloud cover or incomplete satellite coverage of the region, data were missing for certain days or periods (Weeks *et al.* 2006). An average of 19 days of data per month were available, with a daily standard deviation of 2.4/day for chl *a,* and 0.74/day for SST.

Table 4.1: Coordinates used for chl *a* and SST data extraction.

Site	**Coordinates**	
	Latitude	**Longitude**
1	-33.8000°	26.2862°
2	-33.8408°	26.0862°
3	-33.8408°	26.1862°
4	-33.8408°	26.3862°
5	-33.8408°	26.4862°
6	-33.9039°	26.1959°
7	-33.9000°	26.2862°°
8	-33.8995°	26.3972°
9	-34.0000°	26.2862°

Daily air temperature and wind speed maxima recorded at the Bird Island weather station were kindly provided by the South African Weather Service. Moon phase data for the monitoring period were taken from a standard calendar; days during new moons were assigned a value of zero, denoting no light, while full moons were assigned a value of 15 for maximum light. Intermediate days (waxing and waning phases alike) were assigned values of 1, 2, 3 etc.

4.2.3 *Level of disturbance*

To determine if on-going researcher investigations affected nest attendance patterns, a comparison was made between nests under two cameras which were visited each day at a distance (to determine partner identity) and from which chicks were handled frequently (every 5 days, see Chapter 3, i.e. "Disturbed" condition; Figure 4.2), and a third nest site which was not disturbed in any way apart from a short fortnightly nest-contents check ("Undisturbed" condition; see also Figure 4.1). All three camera locations were situated adjacent to the island's demarcated pathways which share approximately equal volumes of human traffic (SANParks rangers and/or management staff and visitors). "Disturbance" thus constituted regular handling by researchers, and not the mere presence of passers-by. "Disturbed" nest sites were visited six times daily to establish the identity of the incubating or chick-guarding partner, but researchers only stepped off the pathway and walked among the nests on average twice a day, especially to check the identity of the small number of monitored birds occupying artificial pipe-burrows. In addition, both members of the pairs were caught, weighed, measured and marked once each, and re-marked from a distance every third to fifth day with a wax animal marker attached to a 1m pole. Chicks from nests under the cameras were measured every fifth day, requiring repeated visits among the nests for about one hour. As far as possible, chick-measuring was limited to the same day within a certain area. Chick ages were not precisely known, as monitoring usually commenced post-hatching, but stages were denoted as P0 – P5. A general description of the behavioural and morphological characteristics for identifying these stages is given in the "Glossary and Abbreviations" section.

An important ethical note made here is that investigators did not disrupt nest sites of the "disturbed" category unnecessarily (in order to produce an unnaturally disturbed state); measures were taken to avoid handling during any period that could lead to thermal stress (i.e. after 10h00 or during extreme heat or cold), and nests were visited as infrequently as possible while still gathering the required data. Care was taken to move quietly and slowly within the nesting areas and handling times were kept to a minimum (Brown & Morris 1994).

Figure 4.2: Surveillance Cameras 1 (background) and 2 (foreground) situated alongside the demarcated walkway. SANParks rangers and researchers use these pathways daily, constituting similar human presence at a distance for all nest sites.

4.2.4 *Statistical methods*

4.2.4.1 Arrival and departure times, the effects of disturbance, chick age and brood size, and the sharing of duties by the sexes

Frequency histograms of arrival and departure times were constructed using all video surveillance data. It is noted here that the term “provisioning rate” may be used synonymously with “rate of returns to the nest” and may be used to refer to behaviour that does not result in a meal for offspring, i.e. for pre-laying and incubating pairs. Owing to their bimodality, frequency tables of arrival and departure times were analysed separately for parents returning once or twice per day for differences across the stages of chick development using Friedman ANOVAs for multiple dependent samples. Wilcoxon matched pair tests were used to determine the effect of disturbance and brood size on arrival and departure times using tables of hourly bin proportions. Spearman correlations were used to assess potential relationships between arrival and departure times, and environmental parameters (chl *a*, SST, maximum air temperature, maximum wind speed and moon phase).

Given that for the pre-laying stage brood size does not apply and for the incubation stage, brood size is always two, it was not possible to run a three-way ANOVA to test the effect of breeding stage, disturbance and brood size on the mean time spent together by parents. Rather than run a three-way ANOVA on a reduced subset where all levels were present, separate two-way factorial ANOVAs were used. The first tested the effects of disturbance (fixed, 2 levels) and breeding stage (fixed, 7 levels, including pre-laying and incubation stages). The second two-way ANOVA tested the effects of brood size (fixed, two levels) and chick age (fixed, five levels, excluding pre-laying and incubation stages) on this same parameter. These analyses were also repeated using parental absenteeism, determined by the length of time the nest was unattended by either parent each day, in place of time spent together. ANOVAs were followed by Tukey Honest Significant Difference (HSD) post-hoc tests when necessary. Relationships between environmental parameters and the daily time spent together by parents and the length of bouts of absenteeism were assessed by means of Spearman or Pearson correlations.

Chi-square analyses of the total length of guarding duties were constructed for breeding pairs for which nest attendance was manually recorded during daylight hours, to determine how equally the pairs, and the sexes, shared daytime guarding. Morphological characteristics (mass, bill length, bill depth, flipper length and body condition) between unequal (male- and female-dominant) and equal guarders were compared using one-way ANOVAs. The protocol for sexing adult penguins has been detailed in Chapter 3. Similar comparisons of total attendance times per partner were not attempted with surveillance data, as partners could have changed duties during the periods when cameras were offline (between 08h00 and 12h00 or during lack of power) or when heavy sea sprays occasionally obscured observations at night. While bout durations and provisioning rates may have been affected by occasional missed partner swaps, this effect would have been minimal and would not have influenced the overall description of behaviour. Missing a single partner swap would have compromised comparison of behaviour between the sexes, however, as the assumed identity of the unmarked birds would be incorrect.

The mean provisioning rate, bout duration and the time parents spent together and absent from each nest were separately collated for the period of time spanning the measurements of their offspring's growth (between 5 to 36 days). Pearson correlations were used to determine if the parents' nest attendance behaviour had any bearing on chick development. As chicks of a double brood share genes and experience similar parental care, within-nest averages of growth rates and body condition indices were used. Unless otherwise specified, analyses were completed in STATISTICA 12 (Statsoft).

4.2.4.2 Provisioning rate and bout duration modelling

Graphs of the nest attendance bout durations were right-skewed for all nests, with most bouts being of shorter duration, therefore the data were log-transformed prior to being incorporated into a general linear mixed-effects model (LMM; "lmer" in the "lme4 package") which was fitted by restricted maximum likelihood (REML) in R (R 3.0.3, R Foundation for Statistical Computing, Vienna, Austria). Provisioning rate data were Poisson distributed and thus incorporated into a generalized linear mixed-effects model (GLMM, “glmer”) with a log-link function. Chick age, brood size and disturbance level (as categorical factors), the AI (assortative index) and ASI (Absolute Size Index) for parental body condition and environmental aspects such as the daily chlorophyll *a* concentration, SST, maximum air temperature, maximum wind speed and moon phase were set as continuous predictors. The models included the fixed effect interactions of disturbance level and brood size, and chick age with: maximum air temperature, maximum wind speed and moon phase. Where environmental data were missing for particular days, the data were removed. All permutations of non-correlated predictor variables were modelled separately and Akaike Information Criterion (AIC) scores extracted. The lowest AIC score yielded the most parsimonious model, although any scores having ΔAIC ≤ 2 were also considered. Nest identity (which accounted for potential similarities in the behaviour of paired adults) was specified as a random effect to ameliorate the effects of repeated measures (pseudoreplication). Normality of the residuals of the model was visually assessed. A significance level of $\alpha = 0.05$ was used, with all results presented as mean ± 95% CI of the mean.

4.3 RESULTS

4.3.1 *Patterns of arrival and departure at nests*

In addition to the total of 2063 nest-days of manual nest monitoring, 1658 nest-days’ worth of fine-scale nest attendance behaviour was recorded at 29 nests under the three surveillance cameras (n = 17 in the disturbed areas and n = 12 in the undisturbed location), ranging from 13 to 90 days for individual nests. This included 1778 observed attendance changes between the end of March and the beginning of July 2013. Only one breeding attempt under the cameras failed; the foraging mate did not relieve its incubating mate and the nest was abandoned on the day the eggs hatched. Data for this nest were removed as they were considered as outliers. During the period of monitoring, 12.1% of nest-days exhibited no change-over, while on 64.8% of nest-days, parents exchanged guard duties once, with the remainder (23.1%) of nest-days displaying a double swapping of duties. In no case was a third change-over observed within a 24-hour period. To my knowledge, no detailed, long-term arrival and departure schedule has previously been described for breeding African Penguins; a detailed description of nest attendance is therefore presented here.

No difference between hourly arrival and departure times was found between pre-laying and incubating birds, and birds rearing chicks (Wilcoxon matched pair tests: arrival times, $Z = 0.406$, $n = 22$, $p = 0.685$, departure times, $Z = 0.456$, $n = 23$, $p = 0.648$) and for this reason arrival and departure times were pooled for all stages of breeding. Figures 4.3 and 4.4 show the general pattern of arrival and departure times (in hourly bins) for all breeding penguins under surveillance cameras throughout the period of monitoring. Birds returning to the nest twice daily showed peak arrival times between 12h00 and 15h00 and then again between 19h00 and 21h00. Peak arrival for birds returning once per day was between 18h00 and 19h00. Fewer than 3% of all returns by foraging mates occurred between 02h00 and 09h00. Departure times exhibited strong bimodality primarily due to double-provisioning parents, although two less pronounced peaks of departure also occurred for single-provisioners between 16h00 and 17h00 and again between 02h00 and 04h00. Overall, the fewest departures were recorded between 18h00 and 20h00. By 08h00, very few partners remained together before the cameras were temporarily switched off, and following manual inspection, 95% of partners were noted to have departed to forage by 09h00.

Arrival times of birds returning once per day and for the second time when returning twice per day were positively correlated with chl *a* concentration (Spearman correlation: r = 0.146, n = 630, p < 0.001 and r = 0.126, n = 241, p = 0.049 respectively). There was a slight tendency for birds returning once per day to do so earlier on windier days (r = -0.066, n = 1000, p = 0.038). Arrival times were also influenced by moon phase in single-provisioners returning after 19h00, with birds arriving at the nest later when the moon provided more light (i.e. closer to full moon than new moon, r = 0.163, n = 314, p = 0.004). Departure times were influenced by maximum daily temperature; adult birds left the nest earlier after a hot day was experienced by partners being relieved for the only time that day (r = -0.130, n = 504, p = 0.004), although this effect was only noticeable for birds departing after midnight. SST did not appear to influence the timing of arrivals or departures in any way.

Friedman's ANOVAs for multiple dependent samples indicated that arrival and departure times were similar across all stages of chick development, from pre-laying to P4 (Table 4.2). Arrival and departure times were also similar between disturbed and undisturbed nests, and between broods of one or two chicks, for parents returning once or twice per day (Table 4.3) as determined by Wilcoxon matched pair tests on the proportion of arrival times arranged into one-hour bins.

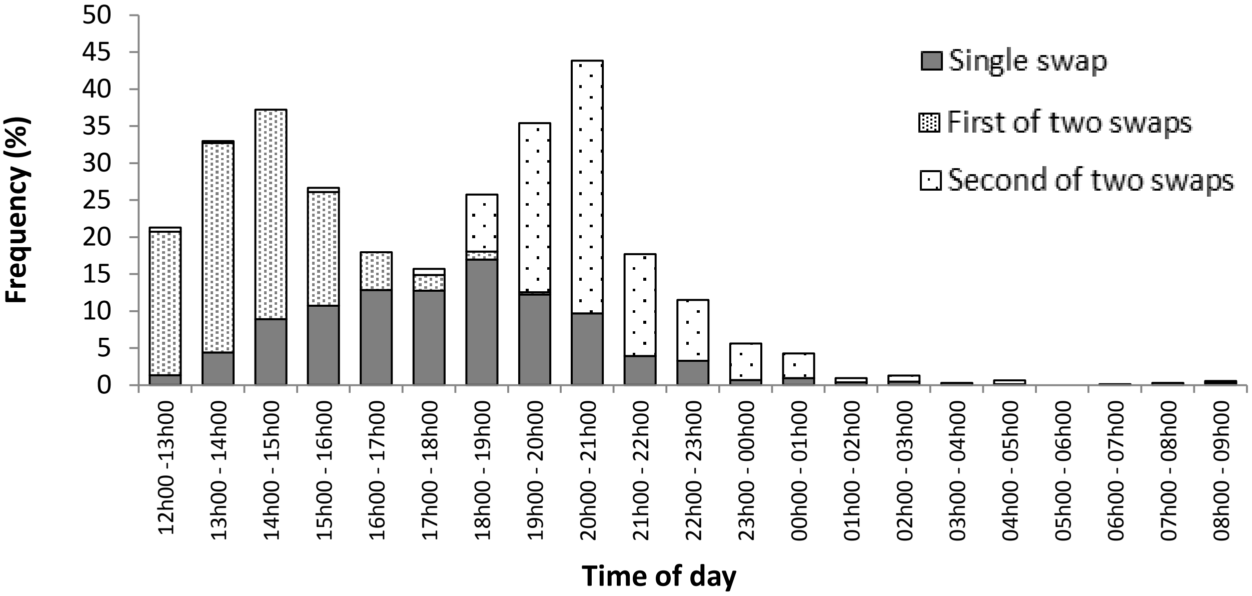

Figure 4.3: Arrival times of all returning parents under camera surveillance. The proportion of arrival times is shown separately for parents provisioning chicks once per day (n = 1022 nest-days) and twice per day (first of two arrivals: n = 371, second arrival: n = 363).

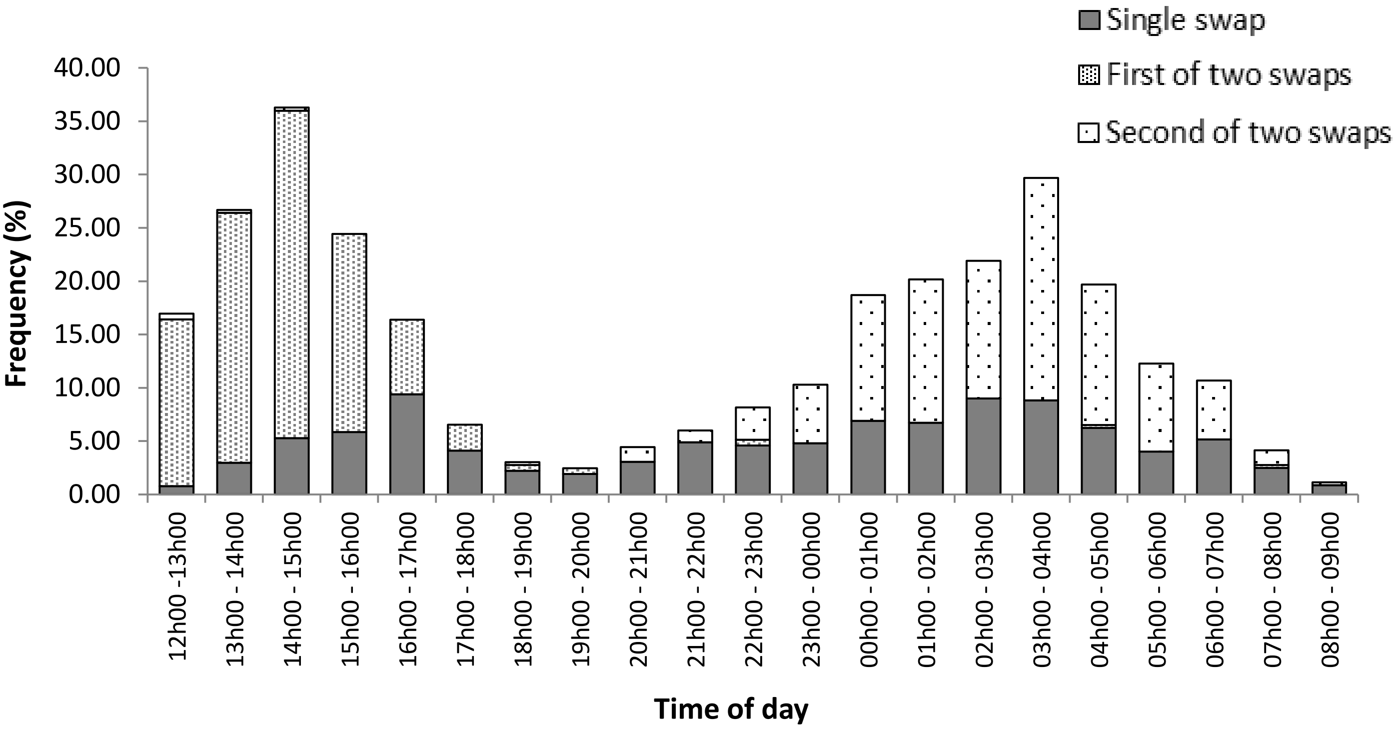

Figure 4.4: Departure times for all parents under camera surveillance. The proportion of departure times is shown separately for parents provisioning chicks once per day (n = 1022 nest-days) and twice per day (first of two departures: n = 371, second: n = 363).

Table 4.2: Results of Friedman ANOVAs comparing arrival and departure times of parents across chick development (pre-laying to P4).

	Arrival times				Departure times			
	χ^2	df	n (hourly bins)	p	χ^2	df	n (hourly bins)	p
Single return per day	8.752	6	20	0.188	3.716	6	20	0.715
First return of two	1.141	5	20	0.950	2.893	5	20	0.716
Second return of two	4.468	5	20	0.484	2.270	5	20	0.811

Table 4.3: Comparison of arrival and departure times of disturbed and undisturbed nests and nests containing single and double broods, using Wilcoxon matched pair tests.

	Arrival times			Departure times		
	Z	n (hourly bins)	p	Z	n (hourly bins)	p
Comparison of disturbance levels						
Single return per day	0.523	19	0.601	1.120	22	0.263
First return of two	0.059	9	0.953	0.622	11	0.534
Second return of two	0.024	17	0.981	0.024	12	0.981
Comparison of brood sizes						
Single return per day	0.282	19	0.778	0.179	22	0.858
First return of two	0.059	9	0.953	0.533	11	0.594
Second return of two	0.497	17	0.619	0.260	17	0.795

4.3.2 *Time spent together by partners*

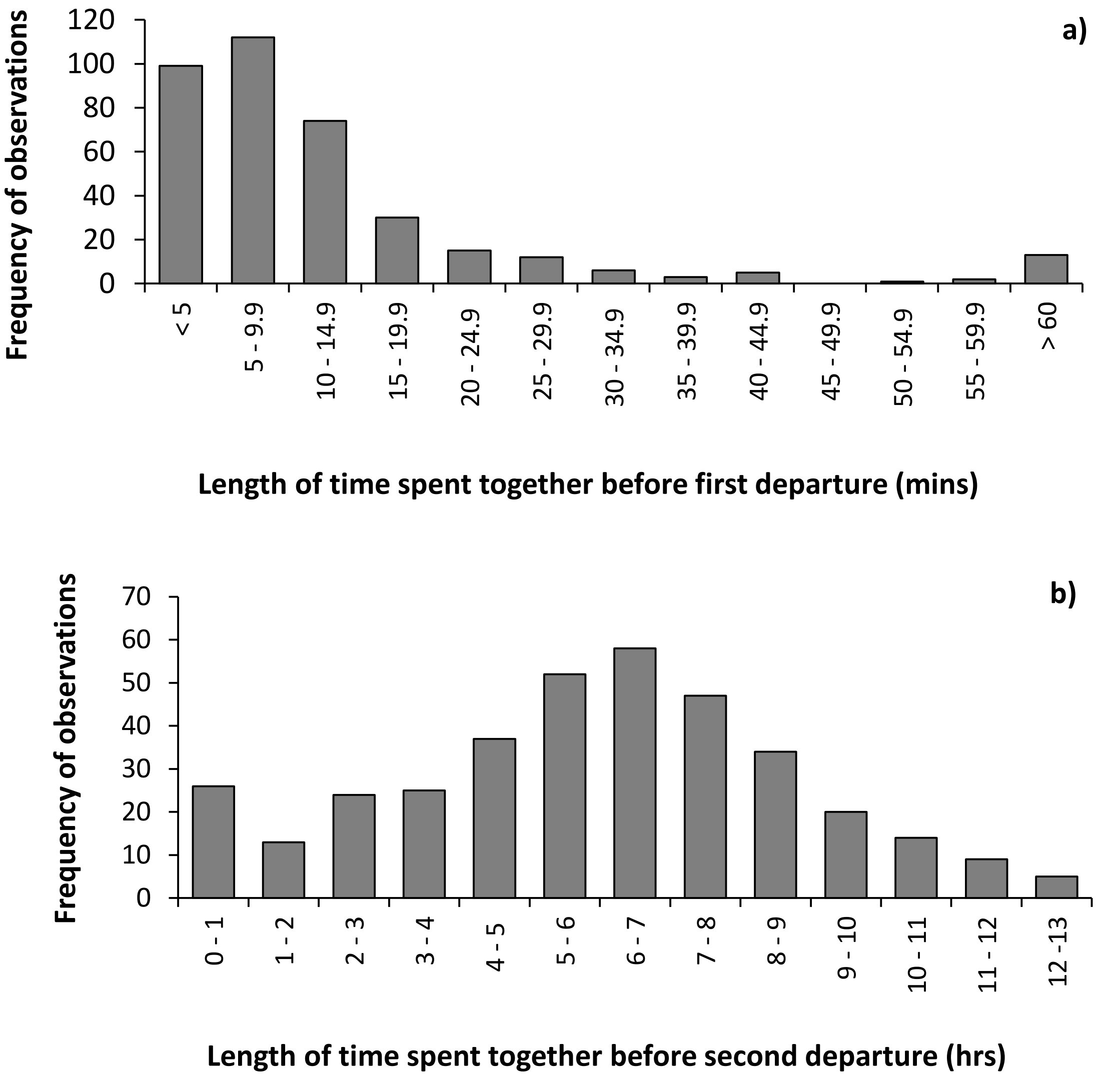

Figure 4.5: Frequency histograms depicting the length of time during the first (n = 371 nest-days) (a) and second (b) periods both parents spent ashore together at the nest when parents provisioned their chick/s twice per 24-hour period (n = 363 nest-days).

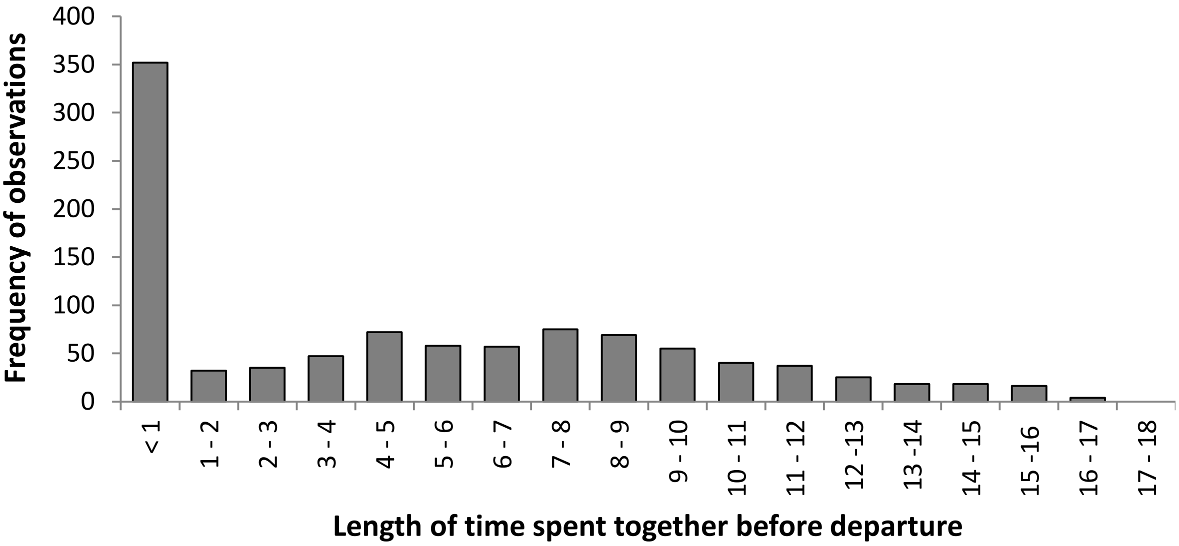

Figure 4.6: Frequency histogram of length of time spent together by partners provisioning their chicks once a day (n = 1022 nest-days).

The first of a double shift change was almost invariably more rapid than the second (Figure 4.5). The first shift change took on average 18 minutes (± 2.9 min, SE), while for the second return of a partner, pairs spent on average 355 (± 18.6) minutes, or 5.9 hours together. On average, birds that returned to the nest once per day exhibited a similarly bimodal pattern of time spent together, which was either less than one hour (mean: 15.6 ± 12.7 min, SD) or considerably longer (mean: 7.6 ± 3.6 hrs, Figure 4.6). In general, incubating or chick-guarding birds left the nest very shortly after their partners returned from foraging during the afternoon, but pairs remained together for longer periods if the foraging mate returned after dusk (Spearman correlation: r = 0.086, n = 1389, p = 0.001).

Table 4.4: Two-way factorial ANOVA to test the effect of fixed factors breeding stage (pre-laying to P4) and disturbance level (disturbed or undisturbed) on the time (minutes) both parents spent together at the nest site each day.

Source	d.f.	M.S.	F	p
Breeding stage	6	2213859	36.568	**< 0.001**
Disturbance	1	65127	1.076	0.300
Stage*Disturbance	6	460795	7.611	**< 0.001**
Error	1538	60541		

Table 4.5: Two-way factorial ANOVA to test the effect of fixed factors chick age (P0 - P4) and brood size (single or double brood) on the time (minutes) both parents spent together at the nest site each day.

Source	d.f.	M.S.	F	p
Chick age	4	614026	11.954	**< 0.001**
Brood size	1	1839043	35.803	**< 0.001**
Chick age*Brood size	4	220922	4.301	**0.002**
Error	1149	51366		

The mean daily time spent together differed between parents of disturbed and undisturbed nests only at the pre-laying and P1 stages of breeding, however a significant interaction between the level of disturbance and breeding stage was apparent ($F_{6,1538}$ = 7.611, p = 0.002; Table 4.4, Figure 4.7). Prospecting pairs in disturbed areas spent more time at the nest site together than did prospecting pairs in the undisturbed area, while parents of P1 chicks in the undisturbed area spent more time together than disturbed parents of P1 chicks. A significant interaction between brood size and chick age was also observed: mean time spent together was statistically similar for the early stages of chick development, but increased for parents with a single chick, while decreasing for parents with two chicks ($F_{4,1149}$ = 4.301, p = 0.002; Table 4.5, Figure 4.8). No comparison of the average time spent together with different brood sizes is given for parents incubating eggs, as the brood size for this developmental stage was invariably two.

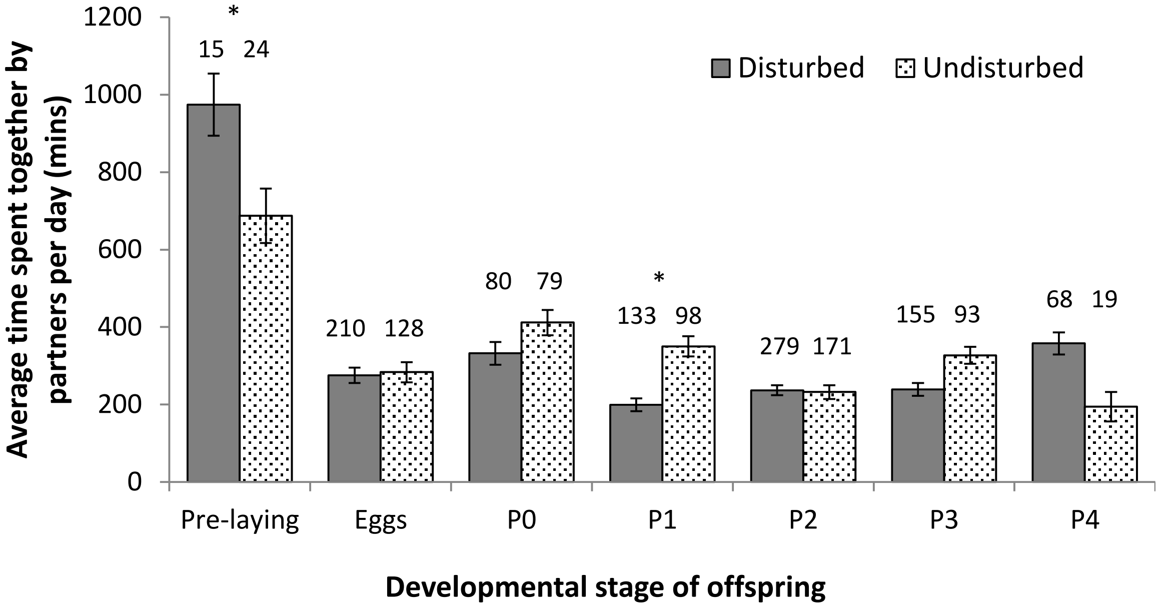

Figure 4.7: Mean time spent together at the nest (±SE) for disturbed and undisturbed nests at all breeding stages. Asterisks denote statistical differences between disturbed and undisturbed groups within breeding stages (Tukey post-hoc: $p < 0.05$). Sample sizes are shown above bars.

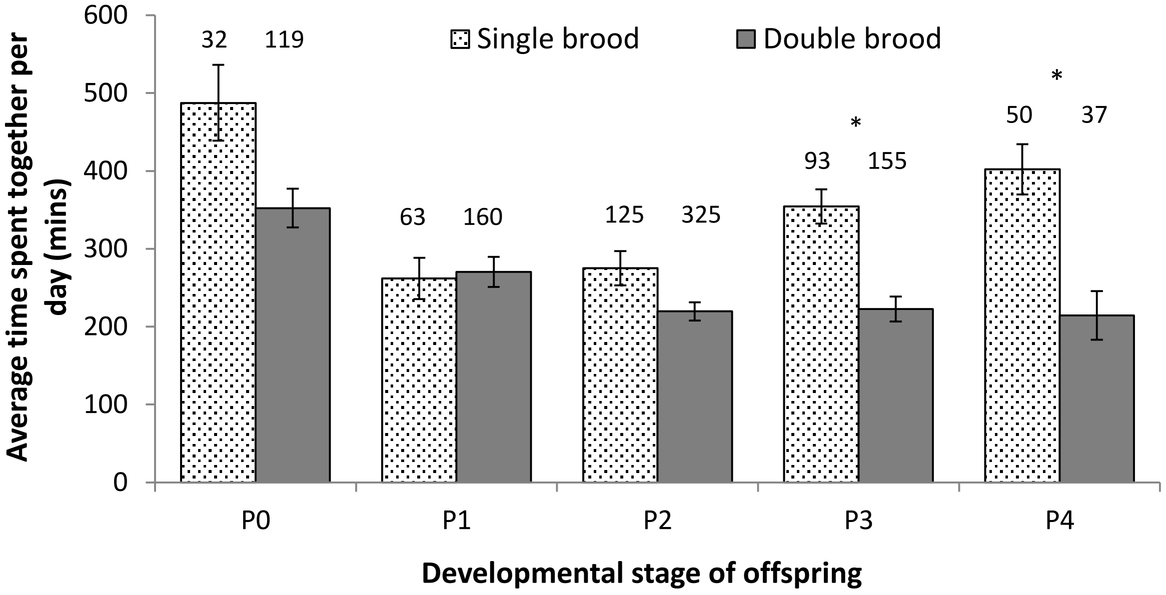

Figure 4.8: Mean time spent together at the nest per day (±SE) for single and double broods at all stages of offspring development. Asterisks denote statistical differences (Tukey post-hoc: $p < 0.05$) between single and double broods within age groups, sample sizes are shown above bars.

The length of time partners spent together at the nest in the afternoon (during a shift-change period of less than 30 minutes) upon return of the foraging partner was significantly negatively correlated with maximum daily temperatures above 20°C (Spearman correlation: $r = -0.171$, $n = 315$, $p = 0.002$; Figure 4.9). Although daily maximum temperature and maximum wind speed were inversely related, maximum wind speed had no bearing on the time spent together by parents at the nest site (Pearson's correlation, $r = 0.019$, $p = 0.735$). Pairs spent significantly more time together when the sea surface temperature was lower that day, however (Spearman correlation: $r = 0.274$, $n = 857$, $p < 0.001$; Figure 4.10). This was true for parents returning once per day and for the time spent together after the first of two returns per day.

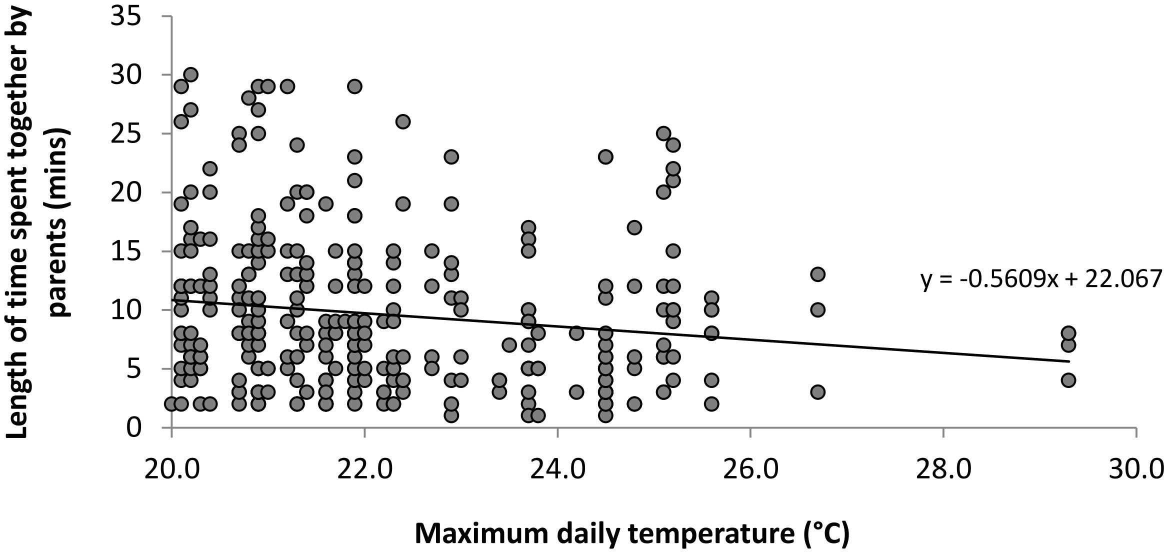

Figure 4.9: Time spent together by parents during shift changes shorter than 30 minutes in the afternoon (12h00 – 16h00, n = 315) plotted against maximum daily temperature (°C, p = 0.002).

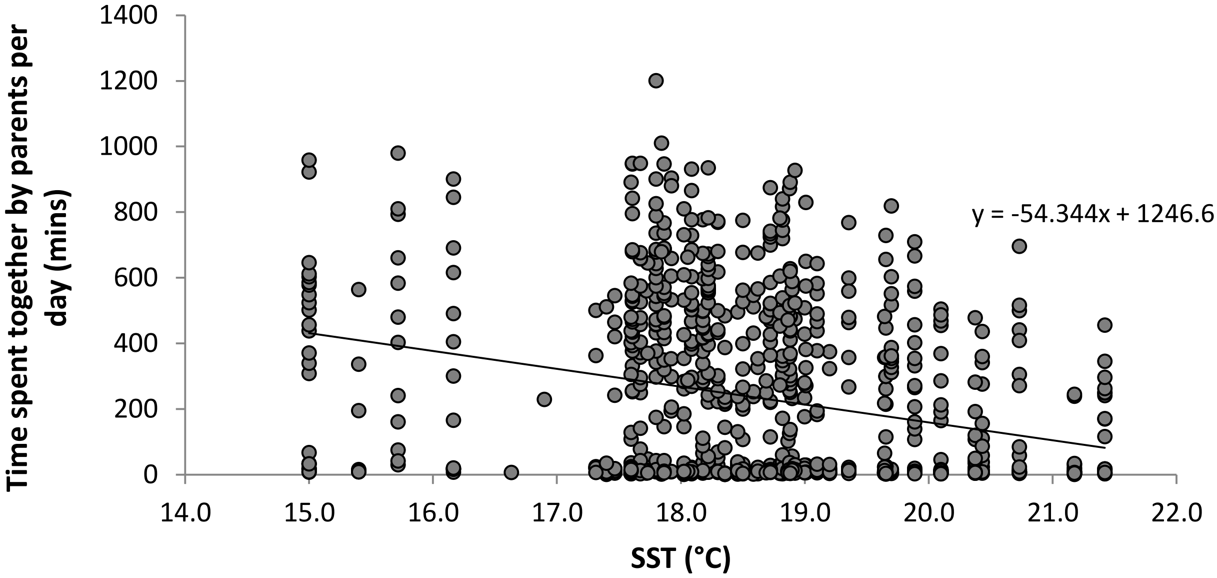

Figure 4.10: Daily total time spent together by parents against the sea surface temperature (SST) in °C for that day, for parents returning once per day and for the first of two shift swaps (n = 857, p < 0.001).

4.3.3 *Parental absenteeism*

The average length of time that chicks were left unattended (with no parent guarding them, usually in the crèche stage) was not correlated with the total time spent together by pairs (Spearman correlation: $r = 0.021$, $n = 1388$, $p = 0.428$). That is to say, pairs that spent longer periods at the nest site together did not necessarily leave their chicks unattended for short periods and vice versa. Absenteeism in both disturbed and undisturbed locations was independent of age for single-brooded nests (Kruskal-Wallis (disturbed): $\chi^2 = 0.797$, df = 2, $p = 0.671$; Figure 4.11). Double broods, however, were on average left unattended for longer periods each day as the chicks matured, in both disturbed and undisturbed locations (Kruskal-Wallis, (disturbed): $\chi^2 = 31.054$, df = 2, $p < 0.001$, undisturbed: $\chi^2 = 57.438$, df = 2, $p < 0.001$). At the P2 and P3 stages for both single and double broods, and the P4 stage of single broods, disturbed nests experienced longer periods of time unattended compared to undisturbed nests, (Mann-Whitney U-tests, all $p < 0.05$). Only double-brooded nests of P4 stage did not differ in parental absence between disturbance levels (Mann-Whitney $U = 135.5$, $n = 31{,}9$, $p = 0.894$).

When parental absenteeism was recorded, the length of time that monitored nests were left unattended was positively correlated with maximum daily temperature, although the coefficient of determination was low (Spearman correlation: $r = 0.289$, $n = 100$, $p = 0.003$; Figure 4.12). The longest period of absenteeism (12h00 - 08h00) was recorded in a disturbed nest that consisted of one P2 chick, which skewed the data somewhat. This long period of absenteeism did not result in chick mortality. Measures of the other environmental parameters (chl *a* concentration, SST, maximum wind speed and moon phase) did not correlate with the length of absenteeism.

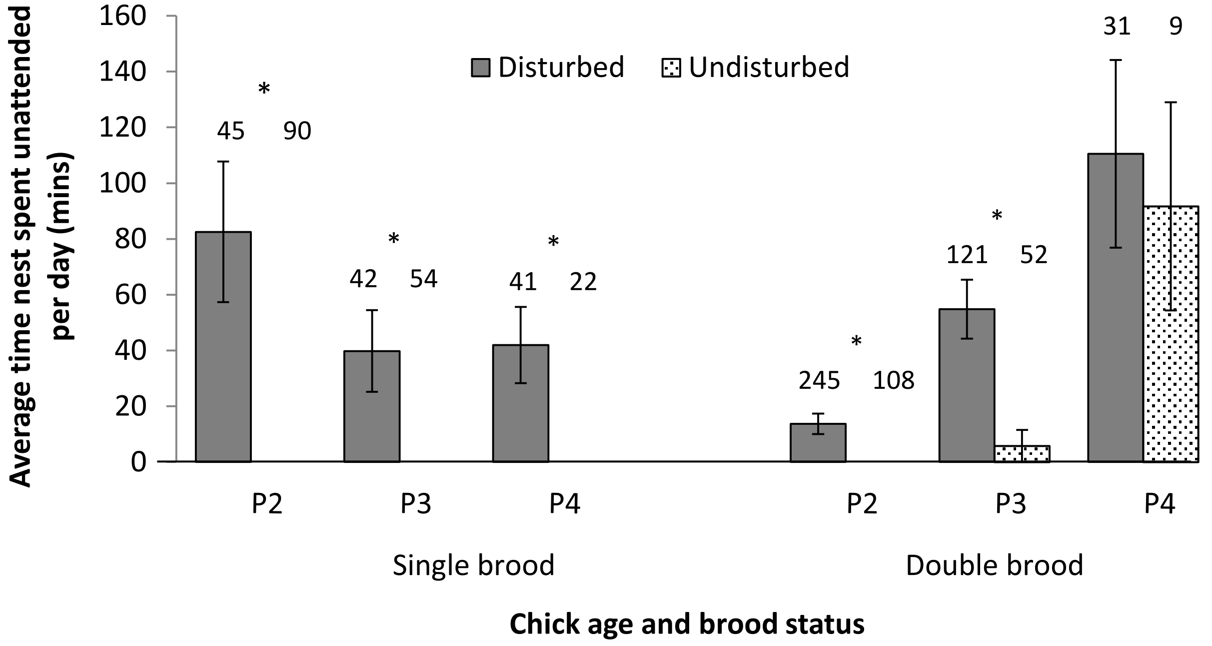

Figure 4.11: Mean time per day (±SE) that nests with broods of one or two chicks aged P2 – P4 were left unattended in disturbed and undisturbed locations. Number of nest-days given above, asterisk denotes statistical difference between disturbance levels.

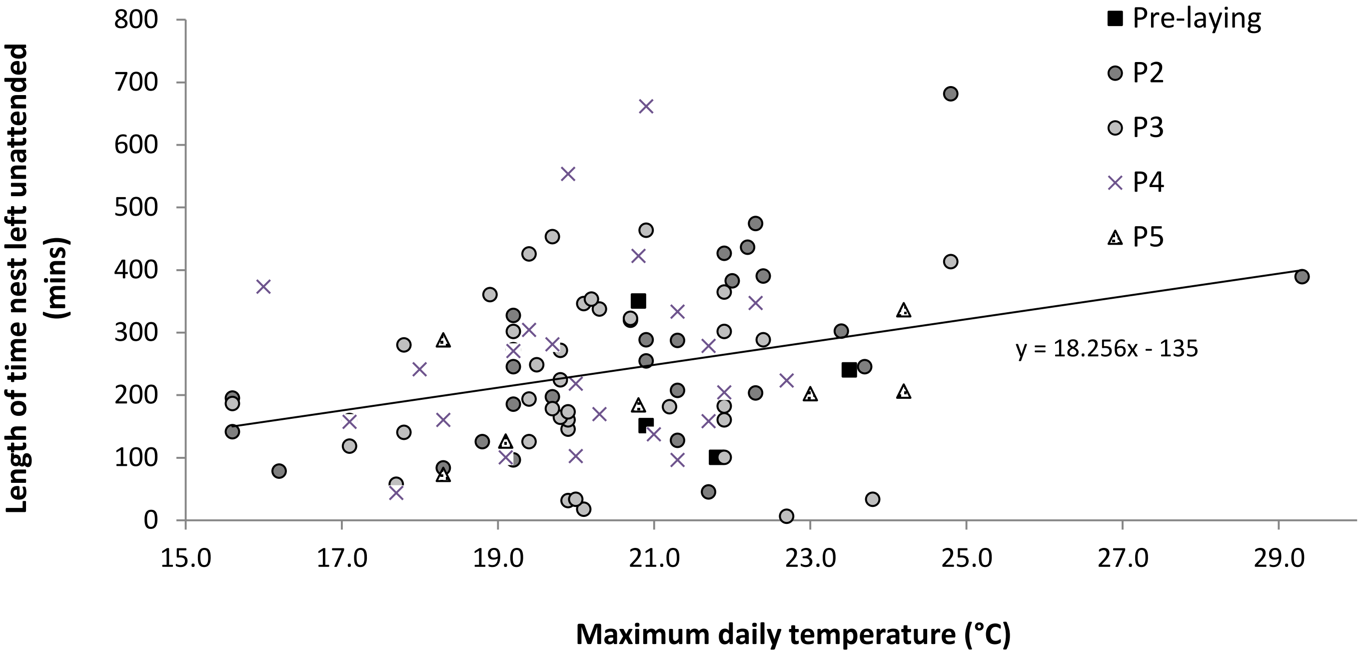

Figure 4.12: Length of time disturbed and undisturbed nests were left unattended by both parents plotted per breeding stage against the maximum air temperature experienced that day (n = 100).

4.3.4 *Provisioning rate and bout duration*

The daily provisioning rate of offspring was best predicted by the combination of brood size, chl *a*, maximum air temperature and maximum wind speed (ΔAIC ≤ 2, Model PR1 and 2; Table 4.6). Bout duration, inversely correlated with provisioning rate ($r = -0.416$, $n = 1344$, $p < 0.001$), was best explained by chick age and the level of disturbance (Model BD1; Table 4.6). Parental body condition indices were not included among the most parsimonious models.

Provisioning rate was greater in single than double broods with the difference being more marked in the proportion of twice-daily returns ($F_{1,1918} = 48.501$, $p < 0.001$; Figure 4.13). Provisioning rate was also higher when chl *a* concentrations (Spearman correlation $r = -0.138$, $n = 1252$ nest-days, $p <0.001$), as well as maximum temperatures ($r = -0.072$, $n = 1939$ nest-days, $p = 0.001$) were lower. The rate of returns to the nest site increased with the developmental stage of the offspring (Figure 4.14). As soon as chicks hatched, parents immediately responded by increasing the number of times they returned to the nest each day.

The length of nest attendance bouts decreased with increasing chick age ($F_{5,1723} = 72.56$, $p < 0.001$; Figure 4.15) and was shorter for parents from disturbed locations than from the undisturbed area ($F_{1,1723} = 20.67$, $p < 0.001$). This was particularly evident during the period immediately following hatching, when chicks were of the P0 – P1 age. The difference was less pronounced as chicks matured further.

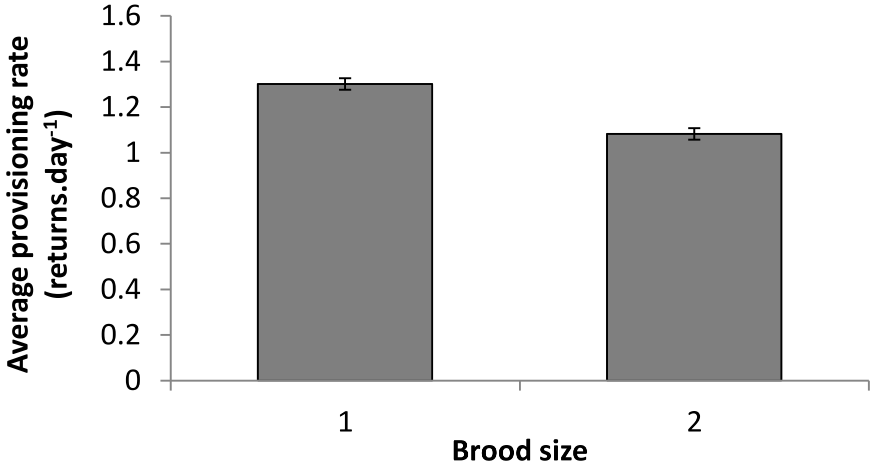

Figure 4.13: Mean provisioning rate (±SE) of parents with a brood size of one and two.

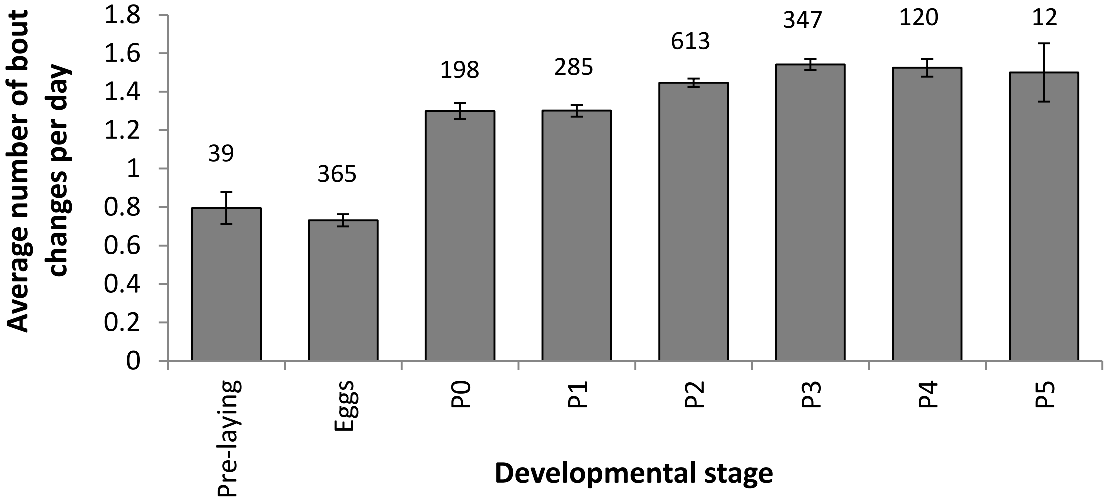

Figure 4.14: Mean number of bout changes per day (±SE) per developmental stage of a breeding attempt. Nest-days are sample sizes.

Table 4.6: Linear mixed effects models of African Penguin behavioural parameters. Models were constructed using provisioning rate (PR) and log-transformed attendance bout duration (BD) as response variables and the following fixed effects: chick age (CA), disturbance (Dist.) and brood size as categorical factors, and chlorophyll *a* (Chl *a*), SST, maximum air temperature (Max Temp) and maximum wind speed (Max Wind) as continuous factors. The models included the following interactions: disturbance level and brood size (Dist*Brood), and chick age with: maximum air temperature (CA*MaxT), maximum wind speed (CA*MaxW) and moon phase (CA*Moon). Aikaike Information Criterion (AIC) scores and the difference in AIC score (ΔAIC) from the most parsimonious model are given.

Model	Chick age (CA)	Dist.	Brood size	Dist*Brood	Chl *a*	SST	Max Temp	Max Wind	Moon	CA*MaxT	CA*MaxW	CA*Moon	AIC	ΔAIC
						Provisioning rate (returns.day^{-1})								
PR1			*		*		*						2708.0	0
PR2			*		*			*					2709.4	1.4
PR3	*				*		*						2731.4	23.4
PR4	*				*			*					2732.8	24.8
PR5			*			*		*					2733.8	25.8
PR6	*				*	*		*					2733.9	25.9
PR7			*			*		*	*				2735.2	27.2
PR8				*		*		*					2736.7	28.7
						Bout duration (hrs)								
BD1	*	*											-883.1	0
BD2	*												-880.4	2.7
BD3										*			-853.4	29.7
BD4											*		-850.5	32.6
BD5												*	-836.2	46.9
BD6			*				*						-498.7	384.4
BD7				*									-497.0	386.1
BD8			*	*									-492.4	390.7

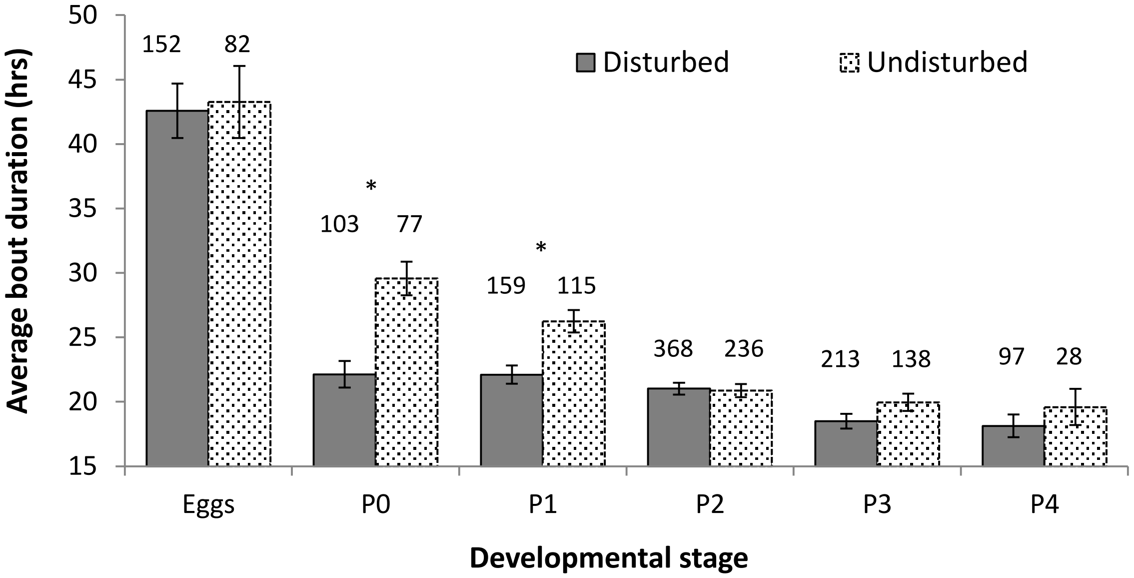

Figure 4.15: Mean bout length (± SE) for disturbed and undisturbed nests across all stages of offspring development. Sample sizes given above bars. Asterisks denote statistical differences within age groups.

4.3.5 *Sharing of daytime guard-duties by the sexes*

A third of the 85 monitored nests (28 nests, 32.9%) did not exhibit equal chick-guarding duties during daylight hours (Table 4.7). The partner attending the nest for the longer period was present on average 53.1% (± 4.5%) longer at the nest than its mate. Of the nests for which adult sexes were known and significant differences were recorded (n = 18), half (n = 9) were attended predominantly by female parents during daytime, while the other half (n = 9) were predominantly male-guarded during the day. Of the unequal partnerships, males were recorded for 2632 daylight hours and females for 2718 daylight hours ($\chi^2 = 1.350$, d.f. = 1, n = 18 pairs, p = 0.245). Overall, the sexes guarded chicks equally (Wilcoxon matched pairs test, Z = 0.903, p = 0.366); males contributed a total of 5232 hours of care, while females attended chicks for a total of 5140 hours. No difference in the morphometric or assortative properties between pairs undertaking unequally long daytime guarding duties could be found, nor were there morphometric or assortative differences between equal and unequal pairs, as determined by one-way ANOVA analyses (all $p < 0.05$, Table 4.8).

Table A1 (Appendix) details the within-nest means of chick growth rates and body condition indices (calculations described in Chapter 3) of offspring from nests under the cameras in the disturbed areas. Parental attendance parameters extracted from the interval between the first and last days of chick measurement were summarized and Pearson correlations used to determine if a higher provisioning rate or shorter bout duration resulted in faster chick growth or better body condition (Table 4.9). No such relationships were apparent, and chick development also appeared to be independent of the time parents spent together, and the time nests were left unattended (all $p < 0.05$).

Table 4.7: Within-nest comparisons of partners' nest attendance durations (hours) during daylight hours (07h00-17h00). Significant differences shown in red.

Nest	Partner 1		Partner 2		Proportion of difference (%)	χ^2	p
	Hours	Sex	Hours	Sex			
GC5	318	Male	234	Female	**35.9**	**12.480**	**<0.001**
C2N15	270	Male	228	Female	18.4	3.376	0.066
GC6	262	Male	236	Female	11.0	1.255	0.263
C2N10	256	Male	248	Female	3.2	0.097	0.755
GC8	254	Male	232	Female	9.5	0.907	0.341
C2N17	248	Male	188	Female	**31.9**	**7.984**	**0.005**
C1N2	232	Male	234	Female	0.9	0.002	0.963
H-M	206	Male	188	Female	9.6	0.734	0.392
P-M	198	Male	138	Female	**43.5**	**10.360**	**0.001**
I-M	194	Male	184	Female	5.4	0.214	0.643
C-M	192	Male	208	Female	8.3	0.563	0.453
F-M	188	Male	206	Female	9.6	0.734	0.392
C2N13	186	Male	126	Female	**47.6**	**11.157**	**<0.001**
J-M	184	Male	146	Female	**26.0**	**4.148**	**0.042**
R-M	184	Male	138	Female	**33.3**	**6.289**	**0.012**
B-M	182	Male	174	Female	4.6	0.138	0.711
E-M	182	Male	218	Female	19.8	3.063	0.080
62	180	Male	138	Female	**30.4**	**5.286**	**0.021**
C2N6	178	Male	204	Female	14.6	1.638	0.201
L-M	178	Male	152	Female	17.1	1.894	0.169
C2N16	172	Male	194	Female	12.8	1.205	0.272
C1N1	166	Male	166	Female	0.0	0.003	0.956
M-M	164	Male	170	Female	3.7	0.075	0.784
63	162	Male	148	Female	9.5	0.545	0.460
S-M	160	Male	176	Female	10.0	0.670	0.413
66	158	Male	136	Female	16.2	1.500	0.221
D-M	156	Male	194	Female	**24.4**	**3.911**	**0.048**
C1N4	152	Male	234	Female	**53.9**	**16.998**	**<0.001**
C2N5	144	Male	168	Female	16.7	1.700	0.193
C1N3	130	Male	198	Female	**52.3**	**13.686**	**0.002**
65	122	Male	158	Female	**29.5**	**4.375**	**0.036**
C2N20	118	Male	98	Female	20.4	1.671	0.196
A-M	112	Male	166	Female	**48.2**	**10.104**	**0.001**
C2N1	110	Male	98	Female	12.2	0.582	0.446
G-M	106	Male	168	Female	**58.5**	**13.580**	**<0.001**
Q-M	104	Male	112	Female	7.7	0.227	0.634
C1N5	98	Male	194	Female	**98.0**	**30.908**	**<0.001**
C2N21	98	Male	110	Female	12.2	0.582	0.446
67	82	Male	88	Female	7.3	0.147	0.701

K-J	76	Male	40	Female	**90.0**	**10.560**	**0.001**
C2N22	74	Male	132	Female	**78.4**	**15.772**	**<0.001**
57	72	Male	62	Female	16.1	0.604	0.437
E-J	70	Male	46	Female	**52.2**	**4.560**	**0.033**
L-J	66	Male	46	Female	43.5	3.223	0.073
A-J	64	Male	48	Female	33.3	2.009	0.156
D-J	62	Male	46	Female	34.8	2.083	0.149
I-J	60	Male	54	Female	11.1	0.219	0.640
G-J	58	Male	58	Female	0.0	0.008	0.926
J-J	56	Male	56	Female	0.0	0.009	0.923
D10	54	Male	52	Female	3.8	0.009	0.923
V-J	54	Male	40	Female	35.0	1.798	0.180
B-J	52	Male	56	Female	7.7	0.083	0.773
C-J	52	Male	62	Female	19.2	0.711	0.399
F-J	50	Male	64	Female	28.0	1.482	0.223
M-J	48	Male	58	Female	20.8	0.764	0.382
H-J	38	Male	80	Female	**110.5**	**14.246**	**<0.001**
51	200	Unknown	170	Unknown	17.6	2.273	0.131
50	200	Unknown	170	Unknown	17.6	2.273	0.131
53	190	Unknown	170	Unknown	11.8	1.002	0.317
C2N23	190	Unknown	192	Unknown	1.1	0.003	0.959
52	190	Unknown	172	Unknown	10.5	0.798	0.372
54	182	Unknown	160	Unknown	13.8	1.289	0.256
61	136	Unknown	172	Unknown	**26.5**	**3.978**	**0.046**
C1N26	130	Unknown	120	Unknown	8.3	0.324	0.569
82	122	Unknown	120	Unknown	1.7	0.004	0.949
R10	100	Unknown	70	Unknown	**42.9**	**4.947**	**0.026**
R1	98	Unknown	64	Unknown	**53.1**	**6.722**	**0.010**
R2	98	Unknown	76	Unknown	28.9	2.534	0.111
K-M	94	Unknown	86	Unknown	9.3	0.272	0.602
55	92	Unknown	188	Unknown	**104.3**	**32.232**	**<0.001**
R9	90	Unknown	82	Unknown	9.8	0.285	0.594
N-M	88	Unknown	56	Unknown	**57.1**	**6.674**	**0.010**
R7	82	Unknown	94	Unknown	14.6	0.688	0.407
R5	74	Unknown	102	Unknown	**37.8**	**4.142**	**0.042**
R8	74	Unknown	82	Unknown	10.8	0.314	0.575
C2N11	74	Unknown	78	Unknown	5.4	0.059	0.808
O-M	72	Unknown	62	Unknown	16.1	0.604	0.437
R6	70	Unknown	104	Unknown	**48.6**	**6.259**	**0.012**
R3	68	Unknown	92	Unknown	35.3	3.306	0.069
R4	68	Unknown	104	Unknown	**52.9**	**7.122**	**0.008**
56	66	Unknown	106	Unknown	**60.6**	**8.843**	**0.003**
T-M	66	Unknown	78	Unknown	18.2	0.840	0.359
O-J	66	Unknown	42	Unknown	**57.1**	**4.898**	**0.027**
P-J	50	Unknown	54	Unknown	8.0	0.087	0.769
U-J	38	Unknown	54	Unknown	42.1	2.446	0.118

Table 4.8: One-way ANOVAs comparing morphology and assortment of African Penguin parents with equal and unequal daytime guarding duties. Means of the three categories (eg: females with longer shifts, females with shorter shifts and females with equal shifts to males) were compared.

		Female				Male		
	d.f.	M.S.	F	p	d.f.	M.S.	F	p
Mass	2	56814	0.88	0.420	2	100835	0.89	0.415
Error	49	64358			52	112842		
Bill length	2	2.5	0.69	0.505	2	7.7	1.33	0.272
Error	49	3.6			52	5.8		
Bill depth	2	0.9	0.96	0.389	2	0.8	0.90	0.414
Error	49	1.0			52	0.9		
Flipper length	2	32.0	1.22	0.303	2	70	2.64	0.081
Error	49	26.0			52	26		
Body condition	2	0.9	0.55	0.582	2	0.8	0.28	0.759
Error	49	1.7			52	2.9		
		Assortative index				**Absolute Size Index**		
Mass	2	68.4	0.90	0.413	2	1.3	0.63	0.536
Error	49	75.9			49	2.1		
Bill length	2	1.7	0.12	0.892	2	18.5	2.72	0.076
Error	49	14.7			49	6.8		
Bill depth	2	4.4	0.19	0.825	2	3.9	2.33	0.108
Error	49	22.7			49	1.7		
Flipper length	2	7.8	0.68	0.511	2	165.0	3.13	0.053
Error	49	11.5			49	53.0		
Body condition	2	11.0	0.18	0.836	2	0.7	0.14	0.872
Error	49	60.9			49	5.3		

Table 4.9: Pearson correlations of within-nest chick development (growth rate and body condition indices - BCI) and parental nest attendance behaviour during the period of chick growth monitoring at nests beneath cameras in disturbed areas.

Parental parameter	Growth rate (g/day)			Body condition index (BCI)		
	r	n	p	r	n	p
Mean provisioning rate (returns.day^{-1})	0.272	16	0.308	0.287	14	0.319
Mean bout duration (hrs)	-0.247	16	0.356	-0.368	14	0.196
Mean time parents spent together (min)	0.398	16	0.127	0.219	14	0.451
Mean time unattended (min)	-0.010	16	0.969	0.296	14	0.305

4.4 DISCUSSION

Feeding predominantly on sardine and anchovy (Hockey *et al.* 2005) in a restricted foraging range of approximately 20-40km during the breeding season (Pichegru *et al.* 2010, 2013), the African Penguin is more sensitive to brood requirements and environmental changes than more mobile, generalist predators such as the volant Cape Gannet (Sherley *et al.* 2013, Crawford *et al.* 2014). Analysis of long-term, continuous and fine-scale attendance patterns of breeding African Penguins using video camera surveillance on Bird Island, Algoa Bay, in 2013 provided detailed data regarding the nature of behavioural patterns during incubation and chick-guarding duties. Very low levels of desertion were recorded for 2013, with only 2 out of 40 (5%) breeding attempts in areas being monitored by cameras noted to have failed completely, one each in a disturbed and undisturbed area. A further 3 out of 90 (3.3%) monitored breeding attempts outside of the range of the cameras failed completely.

4.4.1 *Intrinsic influences on nest attendance patterns*

4.4.1.1 Arrival and departure times

Peak arrival of returning partners was not similar for birds provisioning their offspring once or twice per day. Birds switching foraging and guarding duties once per day exhibited peak arrival at the colony later in the day (18h00 - 19h-00) than birds provisioning their offspring twice in a 24-hour period (first arrival peak: 13h00 - 15h00). This is presumably because if mates return earlier, the relieved bird can perform its own (shorter) foraging bout before dark.

Foraging birds were recorded returning in appreciable numbers as late into the night as 01h00, which still resulted in a meal for the offspring. The fact that returning parents were seen feeding chicks after a relatively short foraging bout in the late afternoon/evening challenges the idea that penguins do not hunt actively under low-light conditions or at night (Wilson *et al.* 1993, Jansen *et al.* 1998). Alternatively, it suggests that forage-fish abundance was very favourable during the period of monitoring in 2013. To return within a few hours and feed chicks indicates that bait fish (sardine and/or anchovy) were schooling in close proximity to the island. This is confirmed by concomitant small pelagic fish surveys that were conducted around Bird Island that year (McInnes, unpubl. data).

Wilson and Wilson (1990) found a slight tendency for *Spheniscus* parents to return earlier in the afternoon when they had smaller chicks (<500g), although no trend in return time could be deduced for larger chicks. This study found no difference in the return (or departure) times per provisioning rate across all stages of African Penguin breeding attempts. Moreover, arrival and departure times of parents did not differ between single and double broods or between disturbed or undisturbed nest sites. It thus appears that arrival and departure times are largely determined by the provisioning rate rather than the chick age or brood size, as well as by extrinsic factors which will be discussed shortly.

4.4.1.2 Absenteeism and time spent together at the nest by both parents

Parents relieved by their returning mates departed from the nest considerably sooner during the first of two shift swaps, or, for parents changing duties once per day, in the afternoon, compared to the second shift change-over or after dusk. The length of time spent together was strongly influenced by the developmental stage of the offspring; as the breeding attempt progressed, parents of double broods spent less and less time together, whereas parents of single broods increased the time spent together as their chick approached fledging. The decrease in the time spent together by parents at the nest site during the latter stages of chick-rearing most likely reflects an increase in time devoted to foraging in order to provide chicks with sufficient nutrition, as well as to compensate for their own declining condition (Benvenuti *et al.* 2002, Harding *et al.* 2007). The reason for single-brooded parents increasing time spent together towards the fledging stage of breeding is unclear. Small pelagic fish abundance can be highly variable on a monthly scale (Agenbag *et al.* 2003) and the above result may indicate that feeding conditions improved slightly in the latter period of the breeding season, although not enough to warrant a similar response in parents of a double brood.

4.4.1.3 Provisioning rate and bout duration

Contrary to the findings of Williams & Rothery (1990) for Gentoo Penguins, Tremblay & Cherel (2005) and Raya Rey *et al.* (2007) for Northern and Southern Rockhopper Penguins and Ainley (2004) for Adélie penguins, the duration of foraging trips by chick-rearing adult Macaroni Penguins decreases with the age of their offspring (Barlow & Croxall 2002). Interspecific differences are thought to stem from the different foraging strategies adopted by inshore versus offshore foragers (Barlow & Croxall 2002). The African Penguin, an inshore forager, was shown to increase its provisioning rate as the breeding attempt progressed. This is likely to be due to the need to provision offspring at a higher rate as their nutritional demands increase before fledging (Benvenuti *et al.* 2002, Harding *et al.* 2007). The provisioning rate was slightly lower in a double brood than a single brood, presumably because parents traded increased frequency of meal delivery for prolonged foraging bouts to provide for a larger brood. As singleton chicks approached fledging, their parents spent more time, on average, together; this could also have contributed to the disparity.

Incubating birds have been demonstrated to maintain a more constant rate of attendance across a wider range of prey availabilities than birds rearing chicks, confirming that chick-rearing parents are more sensitive to resource accessibility than incubating parents (Harding *et al.* 2007). For this reason, time budget measures of parents raising chicks, rather than incubating birds, may be more informative of environmental or prey dynamics. Additionally, if conditions worsen, birds in good body condition may be able to tolerate longer incubation shifts, but once hatched, chicks will require regular provisioning and presumably only the fittest or most experienced parents will be able to provide adequate care for their brood. No apparent link between adult body condition and parental behaviour was found in this study, but this might have been due to high food availability which allowed even smaller parents or parents in low body condition to provide successfully for their offspring.

Chiaradia and Kerry (1999) found that Little Penguins that bred earlier in the season were more successful than later breeders, and that during the chick-guard phase, pairs which undertook more frequent foraging bouts had higher breeding success. While no difference in reproductive success was found between early and late breeders in this study (see also Chapter 3), parents of single broods provisioned their chick more frequently than parents of a double brood, but there was no indication that chicks from these double broods were at a disadvantage, as suggested by the similar growth rates and body condition indices of A- and B-chicks of double broods and singleton chicks (Chapter 3). Importantly, nest attendance behaviour (the rate of provisioning, bout duration, absenteeism and time spent together by the pair) was not found to influence the growth rate or the body condition index of offspring. Neither, in turn, did the body condition (AI and ASI) of pairs have any quantifiable effect on nest attendance behaviour, presumably due to the small sample size of individual pairs and the overarching phenomenon of 2013 being a plentiful year (McInnes unpubl. data). As very low levels of assortative mating were found in the population, the probability of finding a relationship between this phenomenon and nesting behaviour in a reduced subset of nests was very low. Similarly, Rishworth *et al.* (2014) found no correlation between adult body condition and provisioning rates in Cape Gannets on Bird Island, although birds in better body condition did spent longer periods at the nest.

4.4.1.4 Sharing of daytime guarding duties by sexes

The long-term manual nest checks performed on a large number of nests in this study showed that nest attendance during the day is not always shared equally between partners. A third of the monitored nests experienced unequal daytime attendance, although from the results presented here, it appears that the inequality is generally unrelated to gender. Since it is known that pairs often spend long periods of time ashore together during the night, it is unlikely that the foraging partner offsets its time spent at sea by increasing its solo chick-guarding duties during the night. The implications of this unequal guarding are confusing; why would one parent be the predominant forager, and one the predominant incubator or chick-guarder?

A sexually distinct nest attendance pattern has been recorded in the colonial-nesting Blue-eyed Shag (*Phalacrocorax atriceps*), where males attend the brood from midnight until midday, and females from midday until midnight, citing reduction of intersexual competition as a reason (Bernstein & Maxson 1984). Differences in offspring provisioning are generally thought to be caused by disparities in individual quality or sexual dimorphism and its associated foraging abilities and intraspecific competition strategies (Saraux *et al.* 2011). No such explanation was obvious when comparing the morphometrics within and between pairs, only that for a considerable number of monitored pairs, one mate was the predominantly attending partner during the day. Perhaps the adaptive value of this disparity may only become apparent in a year of average or poor prey availability.

Seddon and van Heezik (1991b) reported equal shift lengths by incubating male and female African Penguins on Dassen Island in 1989, as did Chiaradia and Kerry (1999) for Little Penguins breeding on Phillip Island, Australia. However, it is unclear if data for each sex from these studies were pooled and reported on as a general across-colony trend, or if attendance was assessed for individual pairs. Information regarding the division of labour during chick-guarding is also lacking. While it may be true that males and females share duties equally on a colony-level scale, high inter-nest variation may occur, as evidenced by the results of this study; the drivers of this inequality remain unknown.

4.4.2 *Extrinsic influences on nest attendance behaviour*

4.4.2.1 Arrival and departure times

Nutrient-rich waters within the euphotic zone provide good conditions for photosynthetic production, thus providing the primary base for subsequent trophic levels. Particularly at the fine scale examined in this study, considerable lags may exist between physical oceanic processes, such as upwelling, resultant productivity and the response by intermediate levels of the food chain according to the match-mismatch hypothesis (Durant *et al.* 2007). The decrease in provisioning rate and delayed return to the nest site observed at higher chlorophyll *a* concentrations in breeding African Penguins may be an example of the temporal discontinuity between top predator and small pelagic fish availability (Grémillet *et al.* 2008).

In the absence of the cooling effect of moderate to strong winds, birds may spend longer periods at the periphery of the island or remain in the water for longer after foraging bouts for thermoregulatory purposes. In windy conditions, however, birds may be more willing to return to their partners directly after foraging bouts. Indeed, parents returned to their nests earlier in the day on windier days. It is also possible that higher wind speeds provide olfactory cues to foraging birds; African Penguins have been shown to be attracted to dimethyl sulphide at sea, a chemical released when phytoplankton is being grazed by zooplankton (Wright *et al.* 2011).

Moon phase, although not assessed concurrently with cloud cover, affected the arrival times of birds returning to the nest after 19h00. Arrival times became progressively later with increased lunar illumination, and may give credence to the hypothesis that penguins could utilize the improved light intensity provided by a fuller moon to hunt, if at low levels, at night. It would be interesting to expand on this finding.

4.2.2.2 Absenteeism and time spent together by adults

While it appears that typical arrival times of adults were governed by the provisioning rate, chl *a* concentrations and the moon phase (when returning after dark), departure times seemed to be determined, only slightly, by maximum air temperature, with birds leaving their nests earlier after they experienced warmer days while incubating or guarding chicks during the day. SST did not appear to influence the timing of attendance patterns or the time spent together by adults in any way. van Eeden (2012) documented little evidence of at-sea habitat preference of breeding Bird Island penguins based on SST, but longer bottom times associated with higher SST. Instead, the structure and strength of the thermocline when present in the water was a better overall indicator of foraging behaviour (van Eeden 2012) and it may be the dynamics of this feature within the water column that are a much better gauge of penguin hunting success and subsequent foraging bout length than the SST.

As maximum daily temperatures peaked in the mid-afternoon, (coinciding with the majority of rapid shift change-overs) and the time spent together is determined by the departing bird, evidence is presented here that the terrestrial environment can affect the attendance behaviour of incubating or chick-guarding parents. For days with maximum temperatures exceeding 20°C, afternoon shifts swaps were shown to become more rapid as temperatures increased. This, coupled with observations of heat-stress behaviours in the field, indicates that the attendance patterns of chick-guarding penguins can be markedly influenced by environmental conditions and that they may serve as an indicator of physiological stress. By mid-morning, particularly on cloudless, still days, incubating or chick-guarding parents were observed in a standing position, shielding their eggs or small chicks from direct sun and exhibiting signs of heat-induced stress such as panting. Heat stress affects parental behaviour by decreasing the time partners spend together, and increasing the period of time chicks spend unattended when parents have to cool off in the sea (Pichegru 2013).

Historical removal of tonnes of guano from Bird Island now necessitates nesting in the open, subject to intense solar radiation, often without the microclimate of a suitable, if artificial, burrow (Craig 1964, Frost *et al.* 1976, Lei *et al.* 2014). It is with some urgency that mitigation measures are implemented; heat stress *is* having a quantifiable effect on breeding behaviours in this endangered seabird. Current climate change predictions project that maximum air temperatures, and the frequency with which uncomfortably hot conditions occur, will increase (IPCC 2007, McKechnie & Wolf 2010). The thick, subcutaneous layer of fat which the African Penguin possesses to protect it against prolonged periods at sea may become an increasing handicap against terrestrial heat during hotter breeding seasons. In light of this and the results of this study, predicted outcomes of breeding behaviour may include a protraction of the breeding season towards the winter months, a decrease in time spent together at the nest site (with consequences for pair-bonding activities) and an increase in brood desertions coinciding with midday temperature maxima. These phenomena should be more apparent when prey availability is low and parents are in poor condition, factors conducive to a lower provisioning rate and decreased tolerance to fasting.

4.2.2.3 Provisioning rate and bout duration

Data from this study support the hypothesis that environmental parameters influence the provisioning rate of offspring by parents: maximum air temperature and chlorophyll *a* concentrations were predictors of provisioning rate. As chlorophyll *a* concentrations and maximum air temperatures increased, the frequency of returns per day by parents was markedly lower. It thus stands to reason that hot conditions and high primary productivity promote longer foraging periods at sea, the effects of which are enhanced when parents are raising a double brood. Greater energy expenditure on foraging for self and offspring may be required under high chl *a* concentrations due to a spatial mismatch between zones of high primary productivity and pelagic fish (as found by Grémillet *et al.* 2008), the effects of which were detectable on a daily scale. It is probable that hormonal cues act in tandem with environmental cues to influence the length of the penguins' foraging bouts (Chiaradia & Kerry 1999); resolving this relationship would further enhance our understanding of time allocation strategies in the species.

Measures of marine primary productivity are widely used to track the predicted movements of seabirds even though they do not feed directly on this trophic level of the food chain, but rather on several levels higher (Grémillet *et al.* 2008). A spatial match of high primary productivity zones and seabird habitat use have been recorded in the Cape Gannet, despite the predator's pelagic prey showing habitat preferences away from zones of high primary productivity (Grémillet *et al.* 2008). This result highlights the restrictions of equating high measures of primary productivity with the profitability of the foraging grounds, but nevertheless, a match between high phytoplankton levels and prolonged foraging behaviour were found in this study. The use of provisioning behaviour in African Penguins with chicks of known age as a proxy for indirect prey availability at sea is thus a plausible proposal, were the caveat of increased yearly sample size and concurrent acoustic-based fish-stock assessments satisfied.

4.4.3 *Disturbance*

Human disturbance in the form of visits by educational groups, fishermen, tourists and researchers alike can be severely disruptive for colonial-nesting seabirds and may result in nest desertions (Anderson & Keith 1980, Hockey & Hallinan 1981, Carney & Sydeman 1999). It is important for the conservation of vulnerable species that the effects of disturbance by people on nest attendance patterns or breeding success is kept to a strict minimum. While Magellanic Penguins appear to be remarkably tolerant of close contact with human visitors (Yorio & Boersma 1992; but see Fowler 1999), return to resting heart rate after a simple human approach can take up to half an hour in Humboldt Penguins (Ellenberg *et al.* 2006). Response to human presence may thus be species-specific. Disturbance-induced stress on penguins may not be manifested by behavioural responses such as threat displays, nest desertion or delayed return of foraging partners to the nest location, but also by physiological changes (Hockey & Hallinan 1981, Ellenberg *et al.* 2006). Such stresses may only be expressed in depressed fitness; Humboldt Penguins, for example, exhibit poorer than normal reproductive output when human visitation is frequent (Ellenberg *et al.* 2006). It is thus important from both a conservation and an academic standpoint to know if frequent handling of the penguins on Bird Island has an effect on breeding pairs. The easiest way to ascertain this is through observations of behavioural changes, which is the least invasive and most easily quantifiable approach, although additional physiological measurements of stress would increase our understanding of the extent of the consequences of disturbance on penguins.

No effect of disturbance was noticeable in changes in arrival and departure times, although attendance bout durations were longer for undisturbed parents of P0 and P1 chicks. However, perhaps the strongest indicator of human disturbance negatively affecting the behaviour of breeding penguins was to be found in the results of the comparison of parental absenteeism in disturbed and undisturbed locations. While no bouts of absenteeism were observed in single-brooded nests in the undisturbed state, the same could not be said for disturbed single broods. Absenteeism was recorded for longer durations for nests with P2 – P4 chicks in disturbed double broods, compared to undisturbed double broods. Here, absenteeism increased with chick age for both disturbance levels, as predicted, but was greater for P2 and P3 chicks from disturbed locations. No difference in breeding success was observed between disturbed and undisturbed areas, but such differences may appear only during times of lower food availability. It is possible that stress from human disturbance as well as lower body condition will have synergistic negative effects on breeding success in a year of below average food abundance (Yorio & Boersma 1994).

Disturbance level had no bearing on the overall time spent together by parents at the nest site. Although disturbed nests exhibited longer periods of attendance by both partners during the pre-laying phase, this trend was reversed only for the P1 stage of chick development when parents from undisturbed nests spent longer periods together.

Prospecting parents may remain together at the nest for longer periods than at undisturbed areas due to the perceived need to defend the nest site more strongly before eggs are laid. Longer attendance bouts by undisturbed birds during the early stages of chick development were attributed to their longer times spent together at the nest site than disturbed birds.

4.4.4 *Assumptions and limitations*

One of the main limitations of this study was that the meal size provided to the chicks could not be quantified. This would have provided clarity on the nature of the success of foraging bouts. Provisioning rate in this study thus describes the number of meals provided to offspring. Variation in individual bird's foraging success may be an important determinant of the meal size. Another drawback was that the age or breeding experience of parents was indeterminable. First-time breeders are known to exhibit lower reproductive success than more experienced pairs (Clutton-Brock 1991). The difference between the behaviours of all nests under surveillance or manual observations may have been age-related, particularly with regards to the provisioning rate, as older penguins may have acquired better knowledge of profitable foraging grounds or diving techniques (MacLean 1986, Pyle *et al.* 1991).

It must also be noted that satellite-based sensors only provide data on the conditions at the sea surface (SST and chl *a*) or within a very limited depth, and may be in poor agreement with conditions deeper in the water column (Barlow *et al.* 2002, Grémillet *et al.* 2008). For this reason, relationships between oceanic conditions and foraging behaviour may have misrepresented actual conditions experienced by the penguins.

No comparison was possible between aspects of reproductive success and parental abandonment, since only two nests (one each in a disturbed and undisturbed area) were recorded to be abandoned. The attending parents simply deserted their newly hatched chicks and two eggs after a bout length of 15.2 days and 6.1 days respectively, during which time no sign of the partners could be seen. Breeding inexperience or death at sea are possible explanations for the foraging partner's desertion.

Daily gaps in surveillance data prohibited partner identification in unmonitored nests, as shift swaps between 08h00 and 12h00 would have resulted in incorrect identity. Improved accuracy of nest attendance data could be provided in the future with uninterrupted 24-hour video recording if a suitable power source could be found that provides cheap and reliable power to continuously run the cameras.

4.4.5 *Concluding remarks*

The relationship between reproductive output and food availability in natural populations is a complex one and understanding how factors such as the provisioning rates, bout durations and their patterns are affected by intrinsic and extrinsic factors is an important undertaking (Raya Rey *et al.* 2007). In addition to their importance relating to conservation, studies of seabird behaviour can provide invaluable information on changes in relative prey abundance and distribution that could serve to be a cost effective means by which to survey the marine environment (Monaghan 1996). Reduction in provisioning rate, albeit on a much larger scale, was documented in Gentoo Penguins when significant decreases in their favoured prey type, krill, were recorded (Croxall *et al.* 1999). African Penguins also appear to respond relatively rapidly and strongly to the demands of their offspring's requirements and to certain measures of environmental variability, such as temperature maxima and primary production. Therefore, this seabird may indeed be a useful tool for assessing the availability of its major prey classes in proximity to their breeding colonies during reproduction. This study highlighted the importance of improved knowledge of food web spatial relationships and the implications of foraging strategies in marine top predators, and suggests that the nest attendance behaviour of the African Penguin could be used as a proxy for the health of fish stocks. Birds with older chicks and birds raising a double brood should preferentially be used for this end, as the demands placed on them to supply adequate provisioning were demonstrated to be greater than in incubating birds.

While some information is lost when nests are not examined frequently, less frequently visited nests may provide more meaningful data as human disturbance affects behaviour, as shown in this study, and has been shown to affect the physiology in other species of penguin (Giese 1996). When measures are taken to lessen disruption by investigators, such as walking slowly, keeping low and limiting contact times to cool periods, the detrimental effects of nest-monitoring activities can be reduced (Brown & Morris 1994, 1995) but sub-behavioural responses may remain. It is possible that adult penguins with the lowest tolerance for human proximity do not nest along the island's walkways, preferring more undisturbed localities, thereby lowering the average stress response under all cameras. Nevertheless, differences attributed to investigator handling were found, and wherever possible, human disturbance should be emphatically kept to a minimum. Human traffic should be minimised between the hours of 13h00 and 15h00 in the afternoon as this is the peak arrival time of partners returning to the nest site during the day and coincides with the hottest part of the day when birds are most heat-stressed.

A paradox exists for the outcome of future African penguin conservation efforts on Bird Island. While the income and interest created through proposed Bird Island ecotourism would be to the benefit of the species' plight (by promoting rehabilitation efforts and public awareness of stranded birds, etc.), increased human presence in a relatively insular population has the potential to depress the time spent together by parents as well as increase the general absenteeism rate. That these effects were noted in a year of above average food availability (regardless of the lack of evidence of decreased fitness) serves to warn that the consequences can only be more dire when the life history strategies of these long-lived seabirds promote self-preservation above a breeding attempt when food is scarce (Schreiber & Burger 2002).

A long-term study combining the knowledge of sex-specific parental investment with the monitoring of the abundance and availability of prey is necessary to unravel the network of complex relationships between members of a pair and their responses to their ever-changing environment. A study such as this could (and should) be extended to incorporate relative fledging success and mortality rates from years of both above and below average prey abundance. Continuous monitoring of nesting attendance patterns and reproductive output combined with GPS tracking technology and acoustic environmental assessments could provide an important management tool for the sustained future of the African Penguin population.

CHAPTER 5

SYNTHESIS

Seabirds have evolved a set of life history traits, such as delayed sexual maturity, long lifespans, low fecundity and biparental care with long-term pair bonds (Pianka 1970, Stearns 1989, Gaston 2004), that have been shaped by interactions with the spatio-temporally unpredictable marine environment (Ricklefs 1990, Fauchald 2009). The innate longevity of the African Penguin (which serves to buffer particularly unproductive seasons or environmental heterogeneity) allows for multiple breeding attempts, but may be threatened by past and present anthropogenic and climate threats. These include the lingering effects of historical egg collection (Frost *et al.* 1976) and guano removal (Snyders 2011), the threat of on-going, unreported, illegal oiling events (Adams 1994, Whittington 2002), predicted increase in temperature maxima (McKechnie & Wolf 2010), and competition with fisheries for sufficient food (Crawford 2004, Crawford *et al.* 2006). Once the most abundant seabird in the waters surrounding southern Africa, barely 26 000 pairs remain and improved chick survival is now of utmost importance; to halt the unprecedented decline in the population, survival of new recruits must exceed mortality (Whittington 2002, Crawford *et al.* 2011). Better insight into the breeding biology of the African Penguin with regards to patterns of mate selection and its adaptive functions are thus critically important in understanding how its reproductive strategies will change in a changing environment. Moreover, understanding how resource availability affects breeding behaviour in seabirds is of great importance to the holistic study of marine ecosystems and fisheries management, as it can be used as a tool for monitoring the state of prey (Harding *et al.* 2007, Piatt *et al.* 2007).

Non-random mating patterns are found in many avian taxa (Catry *et al.* 1999, Boland *et al.* 2004, Ludwig & Becker 2008) and serve to improve fitness and preserve genetic variation (Mays & Hill 2004). African Penguins typically share chick-rearing responsibilities with a long-term partner, and make good candidates for mutual mate selection studies. The species exhibits reduced sexual dimorphism, however, which has limited sex-specific studies in the past (Davis & Speirs 1990), although we now have the molecular basis to determine the gender of these birds from feather samples. Discriminant function analysis of morphometric data from a large number of pairs provide colony-specific classification functions for predicting gender when only bill and flipper lengths are known.

Positive or negative assortative mating is the phenomenon whereby pairs select for traits in their partners that are similar, or dissimilar to their own, respectively (Burley 1983). Positive assortative mating for body mass and bill depth has been found in the congeneric Magellanic Penguin and this has been shown to be related to improved reproductive success compared to randomly-mated pairs (Forero *et al.* 2001). The advantages of positive assortment within the genus *Spheniscus* may thus have important evolutionary significance, although no such studies have been undertaken on its prevalence and implications in the African Penguin population. Pairs in 2013 exhibited no assortative mating when the morphometric measurements (body mass, bill length, bill depth and flipper length) for 121 pairs were assessed for March-July, although when early breeders and late breeders were assessed separately, early breeders were found to be slightly positively assorted by body mass, the most dimorphic and variable of morphometric parameters (SDI: 87.4%, CV males: 9.4, CV females: 8.7) and by female body condition and male mass. No such pattern was evident for later breeders (May-July), although during these months, larger pairs tended to be more assorted than smaller ones. Males and females of average size, and larger females and smaller males belonged to the most assorted pairs, whereas the largest males and the smallest females belonged to the least assorted pairs. With regards to the low levels of assortative mating patterns found in this study, the effect of the population bottleneck in this endangered seabird should not be ignored as a potential explanation (Crawford *et al.* 2011); the currently reduced pool of available mates compared to the natural state may force the pairing of mismatched mates.

The majority of randomly chosen breeding attempts monitored in 2013 were double-brooded (79.5%) and, importantly, within-nest growth rates and body condition indices and the variability in these parameters did not differ between single and double broods. There was also no difference in chick development between early and late breeding attempts, despite later attempts being undertaken by significantly lighter females. Female weight and body condition also differed between brood sizes; mothers of single broods were significantly heavier and in poorer condition than mothers raising a brood of two. The smaller size of female African Penguins results in less efficient diving capabilities than in males (Pichegru *et al.* 2013), and the added physiological burden of producing the eggs, as well as undertaking energetically-demanding fasting and provisioning bouts during incubation and chick-rearing elevate their chances of starvation once the breeding season is completed, if sufficient body condition is not regained before moulting commences (Wolfaardt *et al.* 2009).

The rate of chick growth and the body condition of offspring were independent of parental biometrics in 2013, although the variability in development was greatest when mothers were heavier, and when parents had combined greater body condition and longer flipper lengths. Larger females and pairs in combined better condition may possibly be able to alter their foraging behaviour to a larger degree in relation to the begging intensity of their offspring, which would account for the disparity, although this is speculative. Chick growth also appeared to be unrelated to the average provisioning rate, bout duration of parents, time left unattended or the time spent together by parents during the course of growth measurements.

The chromosomally-determined offspring sex ratio of 2.27 males to 1 female, although determined from a small sample size (n = 49), indicates a fairly strong bias towards sons. In light of the demonstrated reduction of female, but not male, weight with increased reproductive demands, and the documented female-biased mortality among adults (Pichegru & Parsons 2014), the potential exists for there to be a skewed tertiary sex ratio in favour of males. In this case, competition for females would ensure that the majority of females would be mated, leaving a surplus of less desirable males. Whether the offspring sex ratio approaches parity or female-bias during years of average or below-average food availability respectively, as is the case for a number of taxa (Nager *et al.* 1999, Pike & Petrie 2003, Merkling *et al.* 2012), remains to be investigated.

The typical arrival and departure times of pre-laying, incubating and chick-rearing birds were similar throughout the monitoring period (March-June) but nest attendance bout durations decreased as the increasing nutritional demands of growing chicks were met by more frequent returns to the nest. As chicks grew, parents decreased the length of time they remained together at the nest site, and increased the time the brood was left unattended. As time spent together translates to missed foraging opportunities for one of the mates, these parameters are easily-assessed proxies of stress. Providing further evidence for this statement was the finding that parents with double broods spent even less time together, particularly nearing fledging, and longer periods both away from the nest than parents with a single chick. Disturbance in the form of frequent researcher presence and handling exacerbated this effect, lending support for the recommendation that the effect of human presence be given serious reconsideration in the later stages of chick development.

A combination of intrinsic (brood size) and extrinsic (daily maximum air temperature, chlorophyll *a* concentrations, and to a lesser extent, maximum wind speed) factors affected the provisioning rate by parents. Fewer returns to the nest per day were recorded when measures of chlorophyll *a* and maximum air temperatures were higher, indicating a potential mismatch of high primary productivity and good foraging conditions. Departures of relieved mates during the afternoons were more rapid when air temperature maxima were higher, the effect of which is predicted to become stronger, with temperature maxima set to increase as a result of climate change (IPCC 2007, McKechnie & Wolf 2010).

Overall, males and females guarded their offspring equally during daylight hours but high intra-nest variability was found regarding the sex of the predominant daytime chick-guarding parent. For one third of nests (28 of 85 or 32.9%) monitored every two hours over many days, one parent was recorded on average 53.1% longer than its partner, although this inequality was unrelated to sex. The inequality was also apparently unrelated to the morphometric characteristics of the predominant guarders and predominant foragers. The benefits and implications of this division of labour were unclear, but they may stem from an age- or experience-related foraging advantage of one mate over the other, allowing the less efficient partner more time at sea to replenish reserves and provision the offspring.

Alternatively, the relationship could be one of compensatory behaviour by one member of an “unequally yoked” pair that increases their foraging effort to compensate for poor performance on behalf of the mate (Wright & Cuthill 1989, Griggio *et al.* 2004, Bijleveld & Mullers 2009).

The African Penguin is a highly charismatic species that could serve to facilitate the conservation of whole marine ecosystems by means of its flagship and umbrella status (Simberloff 1998, Sergio *et al.* 2008). Paradoxically, although the proposition to allow low-level tourism operations on Bird Island could potentially generate conservation interest, boost awareness and revenue, the increased human presence on the island could negatively affect the reproductive success of the very population managers are attempting to protect (Hockey & Hallinan 1981, Carney & Sydeman 1999). The implications of disturbance on the attendance patterns of African Penguins may vary from year to year in relation to prey abundance, and the “seabird syndrome” would predict that physiologically stressed individuals would desert their breeding attempts more readily than the results of a disturbance experiment in a good year would suggest (Drent & Daan 1980, Schreiber & Burger 2002). The adaptive benefit of larger size, assortative mating patterns and attendance behaviours may not necessarily be evident during conditions of above-average food availability, yet it is crucial for conservation managers to be provided with baseline data at the individual- and pair-levels across the full spectrum of prey abundance. The period of monitoring (March to July 2013) coincided with an abundant small pelagic fish resource surrounding Bird Island, as determined by acoustic surveys (McInnes unpubl. data). The high prevalence of double-brooded nests during the monitoring period, coupled with the similar growth rates and body condition documented for asynchronously-hatched A-and B-chicks serve to highlight the reproductive success that is possible under near-optimal conditions. The results of this study are unlikely to be similar if repeated in a year with significantly lower prey availability. Indeed, the implications for reproductive success (particularly brood size and development rates) of the timing of breeding, degree of assortative mating and division of labour within pairs may only become evident when resources are less plentiful and the adaptive benefits of these strategies become clear. When food is plentiful, such as in 2013, it is possible for pairs of all sizes and assortative properties, and pairs exhibiting strong division of labour to breed successfully without obvious consequences for chick development. Linking a study such as this with longer-term acoustic survey data on prey abundance and sex-specific foraging across a range of prey abundances would be the logical next step forward.

CHAPTER 6

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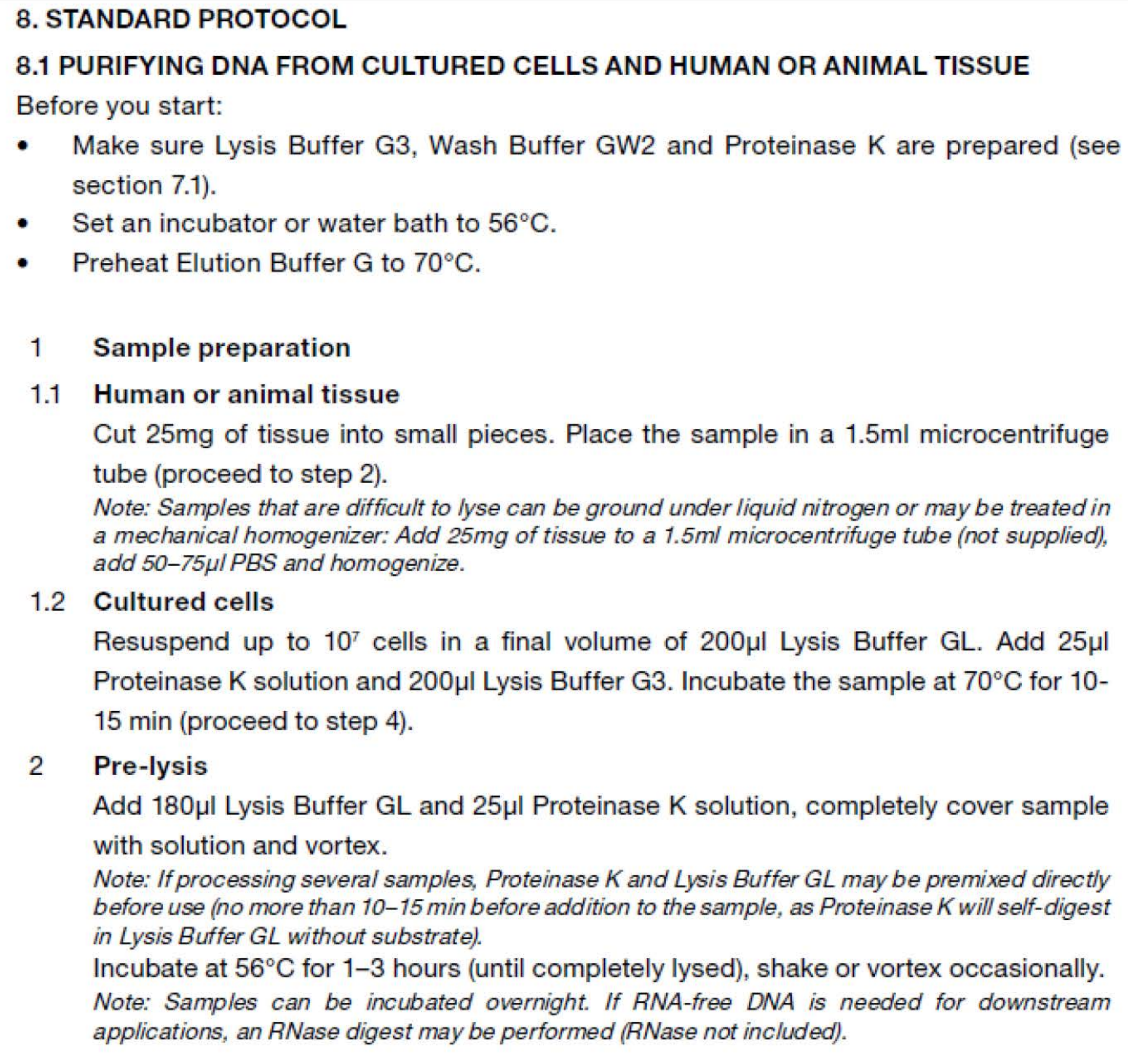
APPENDIX

ISOLATE II Genomic DNA kit (Bioline) - ***Instructions for isolating DNA from feathers.***



8. STANDARD PROTOCOL

8.1 PURIFYING DNA FROM CULTURED CELLS AND HUMAN OR ANIMAL TISSUE

Before you start:

- Make sure Lysis Buffer G3, Wash Buffer GW2 and Proteinase K are prepared (see section 7.1).
- Set an incubator or water bath to 56°C.
- Preheat Elution Buffer G to 70°C.

1 **Sample preparation**

1.1 **Human or animal tissue**

Cut 25mg of tissue into small pieces. Place the sample in a 1.5ml microcentrifuge tube (proceed to step 2).

Note: Samples that are difficult to lyse can be ground under liquid nitrogen or may be treated in a mechanical homogenizer: Add 25mg of tissue to a 1.5ml microcentrifuge tube (not supplied), add 50–75µl PBS and homogenize.

1.2 **Cultured cells**

Resuspend up to 10^7 cells in a final volume of 200µl Lysis Buffer GL. Add 25µl Proteinase K solution and 200µl Lysis Buffer G3. Incubate the sample at 70°C for 10-15 min (proceed to step 4).

2 **Pre-lysis**

Add 180µl Lysis Buffer GL and 25µl Proteinase K solution, completely cover sample with solution and vortex.

Note: If processing several samples, Proteinase K and Lysis Buffer GL may be premixed directly before use (no more than 10–15 min before addition to the sample, as Proteinase K will self-digest in Lysis Buffer GL without substrate).

Incubate at 56°C for 1–3 hours (until completely lysed), shake or vortex occasionally.

Note: Samples can be incubated overnight. If RNA-free DNA is needed for downstream applications, an RNase digest may be performed (RNase not included).

3 **Lyse sample**

Vortex sample briefly and add 200µl Lysis Buffer G3. Vortex vigorously and incubate at 70°C for 10 min.

Note: If insoluble particles are visible, centrifuge for 5 min at high speed and transfer the supernatant to a new microcentrifuge tube.

4 **Adjust DNA binding conditions**

Vortex briefly and add 210µl ethanol (96-100%) to the sample. Vortex vigorously.

Note: After addition of ethanol a stringy precipitate may become visible. This will not affect the DNA isolation.

5 **Bind DNA**

For each sample, place an ISOLATE II Genomic DNA Spin Column into a Collection Tube. Add all of the sample to the column and centrifuge for 1 min at 11,000 x g. Discard the flow-through and reuse Collection Tube. Repeat at a higher g force if samples are not completely filtered through matrix.

6 **Wash silica membrane**

- Add 500µl Wash Buffer GW1. Centrifuge for 1 min at 11,000 x g. Discard flow-through and reuse Collection Tube.
- Add 600µl Wash Buffer GW2 to the column and centrifuge for 1 min at 11,000 x g. Discard flow-through and reuse Collection Tube.

7 **Dry silica membrane**

Centrifuge 1 min at 11,000 x g, to remove residual ethanol. Place the ISOLATE II Genomic DNA Spin Column in a 1.5ml microcentrifuge tube (not supplied).

8 **Elute DNA**

Add 100µl preheated Elution Buffer G (70°C) directly onto the silica membrane. Incubate at room temperature for 1 min. Centrifuge 1 min at 11,000 x g.

Note: For alternative elution procedures see section 7.1.

Table A1: Within-nest chick development (growth rate and body condition indices - BCI) and parental nest attendance behaviour during the period of chick growth monitoring at nests beneath cameras in disturbed areas.

Nest	Brood size	Mean chick growth rate (g/day)	Mean chick BCI	Number of days	Provisioning rate (returns.day^{-1})		Bout duration (hrs)		Time spent together per day (min)		Time absent per day (min)	
					Mean	±SE	Mean	±SE	Mean	±SE	Mean	±SE
1	1	46.84	-	25	1.07	0.10	21.04	1.58	265.21	38.44	167.5	48.2
2	2	44.75	0.44	20	1.62	0.11	16.94	0.91	310.90	37.08	0.0	0.0
3	2	31.73	0.64	27	1.26	0.11	19.44	1.72	148.70	31.10	6.9	6.9
4	2	40.72	0.29	24	1.00	0.06	20.29	1.89	113.05	33.48	81.2	26.9
5	1	67.22	0.25	15	1.31	0.12	20.84	1.71	214.38	36.12	0.0	0.0
6	2	43.6	0.23	25	1.54	0.10	16.22	0.88	302.77	42.04	11.5	6.6
7	2	28.78	0.00	31	1.28	0.09	20.52	1.28	222.60	42.57	39.5	21.9
8	2	36.2	0.35	25	1.35	0.10	19.7	1.38	147.15	35.63	7.0	7.0
9	2	26.81	0.19	26	1.07	0.07	20.43	1.35	204.96	41.03	47.4	21.7
10	2	35.00	0.07	26	1.53	0.12	20.83	1.55	325.53	33.42	0.0	0.0
11	2	30.91	0.01	35	1.04	0.06	23.81	1.46	80.31	26.90	0.0	0.0
12	2	55.13	-	21	1.32	0.10	18.77	1.03	208.00	39.00	0.0	0.0
13	2	47.34	0.32	30	1.00	0.00	23.99	1.42	113.59	28.42	20.0	9.8
14	2	37.97	0.15	30	1.23	0.08	17.29	1.21	165.43	38.62	74.5	25.6
15	2	52.13	0.61	27	1.64	0.10	16.49	0.90	469.13	29.37	0.0	0.0
16	2	21.00	0.41	5	1.17	0.17	21.81	2.65	84.17	31.12	0.0	0.0