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1.1 Thesis aim

The study of penguin vocalisations dates back nearly fifty years, covering a wide range of species, numerous call types and a range of social contexts. Primarily, the aim of these studies was to understand how recognition functions in the noisy (visually and vocally) colonial environment in which most penguins breed. The Adélie penguin (*Pygoscelis adeliae*) (which nest in some of the largest known colonies) is the only species however to have two distinct display calls, the Ecstatic Display Call (EDC) and Loud Mutual Display (LMD) call. The EDC is also said to be sex specific (male), but apart from general structure (syllable organisation) and function this call remains largely unstudied. Hence, the general aim of this thesis is to broaden the knowledge regarding the EDC; specifically its characteristics, functions, stability and potential variations (both spatial and temporal). More specifically, there are four aims to this thesis, firstly to examine the variation in male EDCs with regard to individuality, sex differences and variation in call parameters between the EDC and LMD call. Secondly, variation in EDCs is investigated with respect to both spatial (colony and sub-colony location) and temporal (between years and across the breeding season) stability. Thirdly, the use of the EDC as an honest cue of male condition for female mate choice is assessed, including the effects of arrival and nest location and ultimate breeding success of focal males. Lastly, the factors affecting breeding success in a focal sub-colony (at three stages) during a year of inclement environmental conditions is examined by trialing the use of logistic regression models. Nest and individual specific factors are also compared to the condition of chicks in successful nests. This dissertation will enhance the wealth of knowledge regarding Adélie breeding behaviour and expand our understanding of communication in and the vocal repertoire of this species. Additionally, there will also be implications for mate choice signalling theory, and the evolution and stability of vocal variation at different spatial and temporal scales.

1.2 Communication theory

The diversity and evolution of communication systems has fascinated ethologists for many years. Communication as a definition can be termed the provision of information by a sender to a receiver, using an information vehicle known as a signal (Bradbury & Vehrencamp 1998). Diversity of communication signals can be segregated into four different realms: mechanistic (e.g. neural, physiological and psychological), ontogenetic (e.g. genetic/environmental factors that guide the development of a trait), functional (e.g. fitness consequences of a trait) and phylogenetic (e.g. the evolutionary history of both species and traits) (Hauser 1996). In order for "true communication" to occur the signal must conform to two major criteria; firstly, the provision of information is not accidental and should benefit the sender (Bradbury & Vehrencamp 1998). Where signals are not beneficial to the sender they are often termed cues (Seeley 1989), and if a receiver exploits the signals given by the sender they are termed to be eavesdropping (Bradbury & Vehrencamp 1998). The second criterion is that the receiver must also benefit by having access to the signal information (Bradbury & Vehrencamp 1998).

1.2.1 Sexual selection and honest signalling

One of the mechanisms by which communication is thought to evolve is through sexual selection (Hauser 1996). Sexual selection, first termed by Darwin (1871), arises from differences in reproductive success caused by competition over mates (Andersson 1994). Thus competition can lead to the evolution of communication both within and between the sexes. The role of sexual selection and signal evolution has been reviewed by Maynard Smith & Harper (2003). They state that signals (given during courtship) can occur in a number of different contexts and depend to some extent on what the female receives from the male (i.e. paternal care, sexy sons, good genes or nothing). Theories regarding the evolution and stability of signals (especially concerning the reliability of the information) have been the focus of much debate. Of the theories examining the evolution of honest (reliable) signals, the conditional handicap model, predicts that sexually selected traits are reliable signals of quality because they are both condition dependent and costly to produce or maintain (Zahavi 1975; Zahavi 1977). Recently, Maynard Smith and Harper (1995) proposed the concept of index signals, which are reliable indicators of quality, but are not necessarily costly. Distinguishing between handicap and index signals is not always clear-cut; especially if the costs associated with the signal are unknown (Maynard Smith & Harper 2003). This is particularly true if the 'efficacy costs' (costs of unambiguous transmission; Guilford & Dawkins 1991) and 'strategic costs' (costs required to ensure honesty; Grafen 1990) are unclear (Maynard Smith & Harper 1995). Regardless of signal type, females selecting mating partners on the basis of honest sexual traits are expected to benefit either directly through choosing benefits for themselves or their offspring (Price *et al.* 1993), or indirectly by choosing "good genes" for their offspring (Møller & Alatalo 1999).

1.3 Avian vocalisations

1.3.1 Theory

The majority of animals communicate through a combination of visual, acoustic and chemical signals (Slabbekorn 2004). Undoubtedly vocal signals are an important medium for signalling and communication in birds. Many bird species have evolved songs that are complex in structure and are used for both intra- and inter-sexual communication (Catchpole 1980; 1982; Catchpole & Slater 1995). Avian vocalisation (song) likely evolves through sexual selection to increase success in male-male competitions or in obtaining mates (Lambrechts 1996). Consequently, mate choice and the evolution of mate preferences is area which has been studied extensively in birds (Searcy & Yasukawa 1996). The evidence that links the functions of bird song (male songs attract females and repels rival males) has been reviewed by Kroodsma and Byers (1991) and further summarised by Collins (2004). However, given the huge diversity of vocal communication systems the evolution of vocalisations must be considered in the light of all the selective forces and constraints acting on the transmission, production and detection of the signals (Ryan & Brenowitz 1985). Additionally, whether the song is learned or inherited seems to play a part in the diversity and plasticity observed in avian species (Kroodsma 2004). For example, it is generally believed that learned songs (as given by some hummingbirds, some parrots and songbirds) show greater variation with regard to song structure, complexity and repertoire size (Kroodsma 2004).

The reason why many avian species rely on vocalisations rather than visual or chemical signals to attract mates or defend territories likely has something to do with the features of sound i.e. it can be transmitted over long distances, is multidirectional, is not constrained by whether it is day or night and to some degree is less affected by obstacles in the landscape (Slabbekorn 2004). In order to understand why certain acoustic signals are favoured above others it is necessary to assess selection factors associated with variation on species, social context and the habitat in which the signal is given (Slabbekorn 2004). Sound transmission in the environment is complicated by several factors, including; attenuation, degradation, obstacles, turbulence, reflection, ambient noise and interference (Slabbekorn 2004). Additionally, constraints on sound production are associated with phylogeny, mass and body size of the species in question (Ryan & Brenowitz 1985).

1.3.2 Mechanics of avian sound production

The mechanics of sound production in avian species has been studied extensively over a wide variety of species (Greenewalt 1968). The sound-producing organ (syrinx) is considered to vary in complexity and increase with higher taxonomic orders; however, the basic vocal structure in birds is essentially the same (Greenewalt 1968). The syrinx is located in the pleural cavity and surrounded by the inter-clavicular air sac (Catchpole & Slater 1995). The exact location and structure of the syrinx varies with species and can be classified into three different types: the tracheal syrinx (e.g. parrots & pigeons), the bronchial syrinx (e.g. penguins & oilbirds) and the tracheobronchial syrinx (e.g. songbirds) (Suthers 2004). The tracheaobronchial syrinx (Figure 1.1) represents the classic syrinx form found in many birds and has been used by most researchers to examine sound production. It is generally agreed that vocalisations are generated when the tympaniform membranes (Figure 1.1; label f & g) vibrate as air is passed over them (Catchpole & Slater 1995). Several pairs of syrangeal muscles (Figure 1.1; label b) also control the membrane tension and thus vary the quality of the sounds produced (Catchpole 1979). As a general rule the greater the number of syringeal muscles the greater the complexity of the song produced (Suthers 2004). Additionally, due to the location of the syrinx at the junction of two bronchi, birds are able to produce two harmonically related sounds simultaneously, known as the “two-voice” theory (Greenewalt 1968).

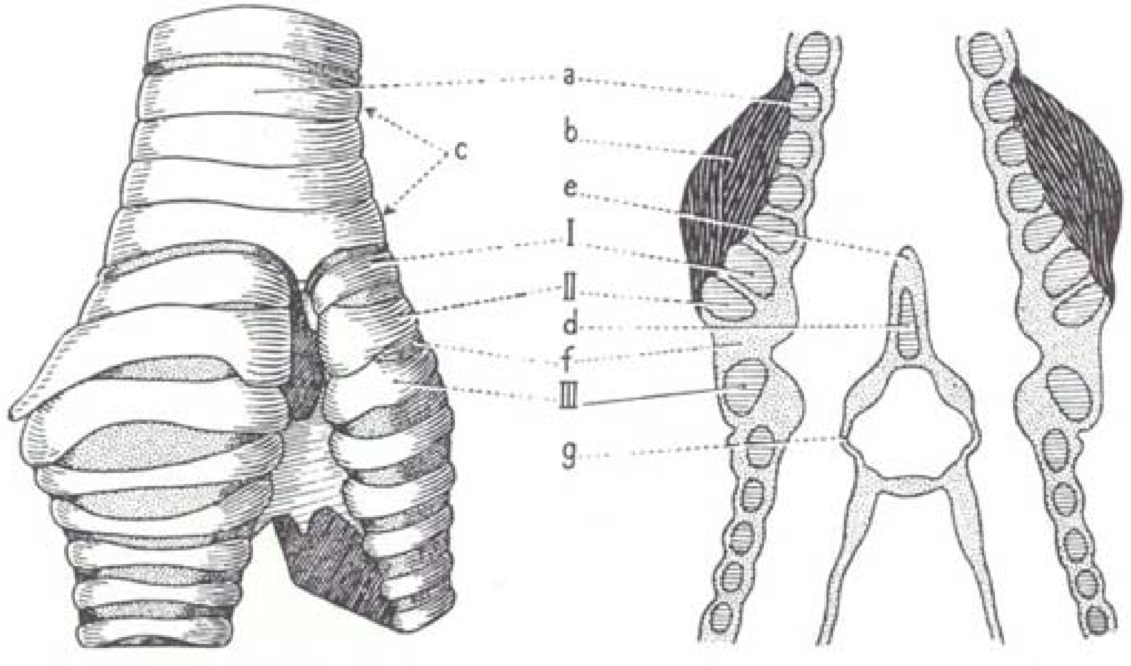

Figure 1.1: Tracheobronchial syrinx. On the left - the syrinx viewed externally; on the right - the syrinx with the ventral side removed. **Key:** a = tracheal ring; b = muscles of the syrinx; c = tympanum; d = pessulus; e = semilunar membrane; f = external tympaniform membrane; g = internal tympaniform membrane; I, II & III represent the first bronchial rings (Adapted from Greenewalt 1968 page 26).

1.4 The general penguin

1.4.1 Taxonomy and classification

Penguins belong to the Class: Aves (all birds); Order: Sphenisciforms (all penguins); Family: Spheniscidae (six genera); Genus: *Spheniscus* (four species), *Eudyptula* (one species), *Megadyptes* (one species), *Eudyptes* (six species), *Aptenodytes* (two species) and *Pygoscelis* (three species): 17 species in total (Jouventin 1982; Müller-Schwarze 1984), 18 if you include the separation of the rockhopper into two sibling species: *Eudyptes chrysocome* and *E. moseleyi* (Jouventin *et al.* 2006). All the known extinct penguins belong to the same order and family. With greater fossil evidence and analysis it may be possible to clarify distinct families (Simpson 1976). In terms of taxonomy in principle it is Schlegel's taxonomy of 1876 that is still used today.

1.4.2 General description

Penguins constitute the largest family of completely flightless birds (Sparks & Soper 1987). They have a proportionally large head, short neck, an elongate body, with a wedge shaped tail with 14-18 stiff rectrices, short, stout legs with webbed feet and a vestigial hind claw (Williams 1995). Penguins are stocky, flightless and aquatic; their wings are reduced to short hard flippers covered with scale-like feathers (Williams 1995). Penguins "fly" through the water using their wings for propulsion and their feet and tails for steering (Watson 1975). All species can be distinguished with some degree of accuracy by head patterning alone (Jouventin 1982). The sexes are monomorphic in plumage in all species (Jouventin 1982); though males are usually heavier than females (Davis & Speirs 1990) and often have a larger bill size and longer flipper lengths (Ainley & Emison 1972; Davis & Speirs 1990; Kerry *et al.* 1992; Murie *et al.* 1991; Williams 1995). The degree of sexual dimorphism in penguins has been associated with latitude, with the extreme low (e.g. emperor *Aptenodytes forsteri*) and high (e.g. Galapagos *Spheniscus mendiculus*) latitude penguins being least sexually dimorphic and the mid latitude (e.g. rockhopper) penguins being the most (Agnew & Kerry 1995). There is a considerable size variation within the Spheniscidae, the smallest being the 40 cm high, little blue *Eudyptula minor*, weighing only 1-1.2 kg, and the largest the 1 m tall emperor, which has an average weight of 30 kg (Freethy 1982).

This "flightless seabird" shares many features with other seabirds including salt glands and colonial breeding (Müller-Schwarze 1984). Penguins breed mostly on sheltered beaches, exposed headlands, ice- free areas, or in the case of the emperor on fast shelf ice (Watson 1975). The sexes take turns in incubating two eggs (only one for *Aptenodytes*), on grasses and pebbles (*Megadyptes, Eudyptes* and *Pygoscelis*), on their feet (*Aptenodytes*) or in burrows (*Spheniscus* and *Eudyptula*) (Jouventin 1982). Most species attempt to breed annually during the austral summer, the exception being the emperor that breeds during the winter, the little blue penguin that breeds late winter and the king (*Aptenodytes patagonicus*) which can, depending on previous years breeding success, either breed during the summer or winter. At the cessation of breeding each year most species fully moult their feathers before the onset of winter, the exceptions include the king, Galapagos and African (*Spheniscus demersus*) penguins, which moult prior to breeding (Williams 1995). Adult survival at 70-90% per annum is low compared to other sea birds. This however may be inversely related to the relatively high breeding success (Williams 1995). All penguins are largely monogamous with pair-bonds persistent over several years (Williams 1995). Due to the dense nesting situation penguins have developed a complex repertoire of visual and vocal signals that aid in defence and mate acquisition, recognition and chick rearing (Jouventin 1982). Penguins are long-lived birds and most delay the onset of breeding until they are several years old (Reilly 1994).

1.4.3 Worldwide distribution

Penguins range from the Equator to 78° S (the Ross Sea region), and swim in waters ranging from 20° C to below zero (Gaskin & Peat 1991) (Figure 1.2). Physiological constraints (adaptations to cold climate) and prevailing Antarctic currents are likely to explain the failure of penguins to expand their ranges across the equator and into the northern hemisphere (Gaskin & Peat 1991). In terms of concentrations of penguins, the greatest number can be found around the Antarctic and inhabiting the sub-Antarctic islands, an area where due to the Antarctic convergence there is an upwelling of nutrient rich polar waters (Reilly 1994). Greatest species numbers however, are found between 45-60° S, with New Zealand having the greatest diversity of species (seven) (Williams 1995).

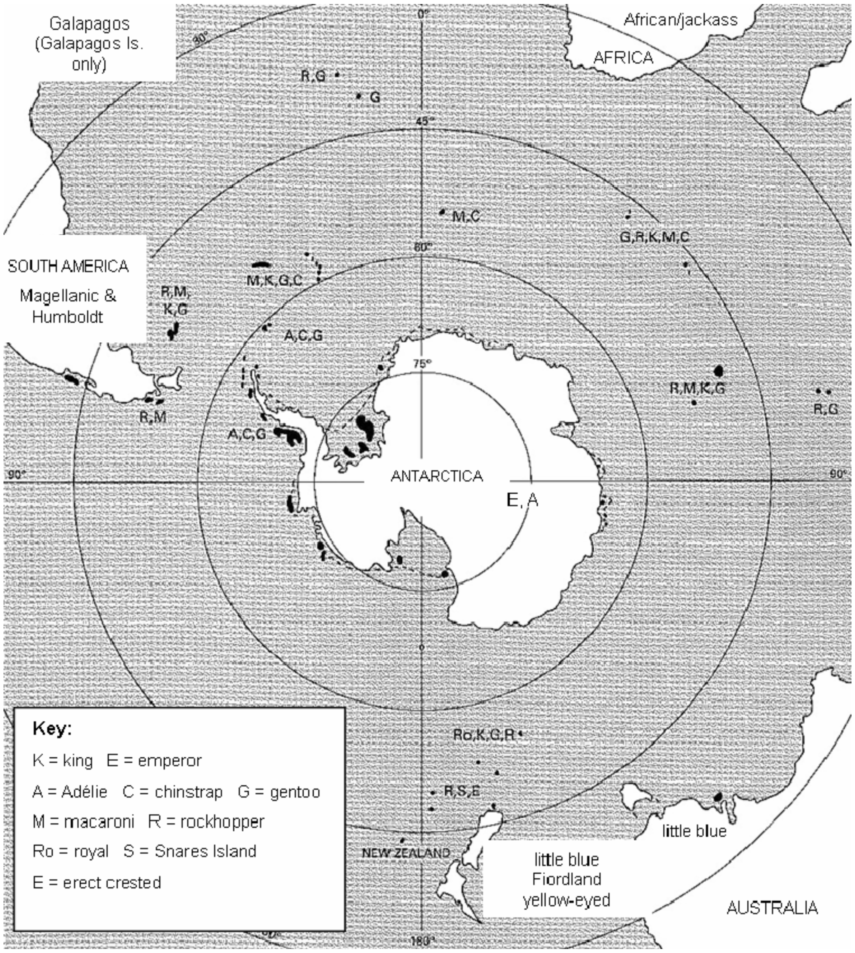

Figure 1.2: Worldwide distribution of penguins (Adapted from Williams 1995 page 5).

1.5 General biology

Several unique anatomical and physiological characters are present in penguins, which make them highly adapted to life at sea. These include; reduction in size but increase in number of contour feathers; the complete loss of the flight feathers; reduced articulation of the wing; flattening of the wing elements; thickening of the walls of the long bones, and the loss of 12 flight muscles (Livezey 1989). Furthermore, the prominent keel of the breastbone is highly developed and huge pectoral muscles have developed to power the wings (Freethy 1982). The toes have strong nails, which aid grip when walking over ice floes and the upright gait is made possible by legs set well back on the body (Freethy 1982).

A short, streamlined body is necessary for efficient diving and swimming. Underwater, the neck is shortened and head pulled back to enhance a hydrodynamic shape and legs and webbed feet trail for use as rudders in conjunction with the stiff tail feathers (Williams 1995). Penguins are close to optimal shape for swimming as their maximum body width is one-third from the front (Sparks & Soper 1987). To aid in submersion penguin flipper bones are thicker and denser than bones of flighted birds (Bannasch 1995).

The generic bill of a penguin is robust and hooked for aquatic prey capture (Williams 1995). Lacking teeth, penguins swallow prey whole. As penguins do not have crops to aid the breakdown of food most penguins swallow stones that lodge in their stomachs (Gaskin & Peat 1991). To aid in prey capture the mouth is lined with keratinous backward facing spines (Williams 1995). Penguins prey upon euphausiid shrimps, crustaceans, fish and squid. In turn, they are preyed upon by giant fulmars (*Macronectus spp.*), sheathbills (*Chionis spp.*) and skuas (*Catharacta spp.*) on land, and leopard seals (*Hydrurga leptonyx*) and sharks (Order: Selachii) in water (Watson 1975).

The penguin eye is adapted for vision both in and out of water (Reilly 1994). Penguins have an almost flat cornea; therefore focusing is left primarily to the lens (Howland & Sivak 1984). Penguins have the ability to flatten their lenses and reduce the pupil to approximately one millimetre in diameter, thus images are always sufficiently focused and detailed (Howland & Sivak 1984). Also it appears penguins' retinas show remarkable sensitivity to violet, blue and green light and consequently they are able to distinguish fine scale changes in the colours specifically associated with aquatic life (Sparks & Soper 1987). For example, the king penguin has comparatively large eyes and a unique pupil type which in daylight contracts to a square-shaped pinhole but dilates to a circular aperture in darkness (Martin 1999). This change allows for 300-fold retinal illumination. It appears that the king penguin has a retina adapted to the low light conditions the bird would encounter during mesopelagic dives (Martin 1999).

Penguins are endothermic homeotherms (Williams 1995). They are restricted to the southern hemisphere and have several adaptations for living and breeding in cold climates. A layer of blubber beneath the skin cuts down heat loss and there is also a dense overcoat of feathers, those on the body are curved, and overlap making them resilient to ruffling by wind, beneath these are the down feathers (Freethy 1982). Pre-breeding emperors have a 2-3 cm layer of blubber to withstand the winter temperatures (Williams 1995). Oil applied to feathers during preening is crucial in maintaining waterproofing as it prevents water entering the feathers (Kooyman *et al.* 1976). Small muscles associated with each feather, allow each feather position to be altered (Williams 1995). The feathers can be held erect on land, trapping a layer of air for warmth. In water, by contrast, the feathers can be flattened creating a thin watertight layer (Sparks & Soper 1987). In the flippers, feet and nasal passages, a counter-current heat exchange system is present, which in conjunction with vasoconstriction, are adaptations minimise heat loss to the environment (Williams 1995). Behavioural strategies on land to reduce heat loss include holding the flippers tight against the body, standing on the heels with most of the foot lifted off the ice (Kirkwood & Robertson 1999) and a behaviour known as huddling (Le Mayo *et al.* 1976). Penguins can reduce heat loss by up to 50% by huddling (Fothergill 1994). Heat can also be generated by shivering, a method employed by all Antarctic penguins (Baudinette *et al.* 1986).

Penguin chicks are poikilothermic when hatched; i.e. body heat is supplied by the parent (Taylor 1986). Chinstrap penguin (*Pygoscelis antarctica*) chicks, for example, become homeothermic 15 days after hatching (Barre 1984). Emperor chicks huddle for warmth and have a natal down that has greater insulation capacity than adult feathers (Chappell *et al.* 1989). Adélie adults (*Pygoscelis adeliae*) are so good at insulating themselves that if the temperature rises and approaches zero they begin to show signs of heat stress including ruffling their feathers thus breaking the insulating air layer (Sparks & Soper 1987) and by panting (Chappell & Souza 1988).

1.6 The Adélie penguin (*Pygoscelis adeliae*)

1.6.1 Description and habitat

There are three species of *Pygoscelis* or “brush-tailed” penguins, the gentoo (*Pygoscelis papua*), chinstrap and Adélie (Jouventin 1982). The Adélie penguin, although smaller than the gentoo, stands approximately 70 cm tall and weighs between 3-6 kg (Ainley *et* *al.* 1983). Adélie penguins are long lived seabirds that delay the onset of breeding until several years of age (Spurr 1975a). Male and female Adélie penguins show some size dimorphism with males being on average slightly larger (Ainley & Emison 1972) but considerable overlap in size exists (Kerry *et al.* 1992). Both sexes are classically black above and white below and the species can easily be identified by their distinctive white eye ring (Williams 1995) (Figure 1.3 – adult on the left of the picture). The bill appears short as is half covered by feathers (Williams 1995). The feet are pale white to pink and black on the soles (Williams 1995). Juveniles are easily distinguished as they are pale under the throat, have no white eye ring (Williams 1995) (Figure 1.3) and also when first fledged, feathers are more dark blue than black (*pers. obs.*). The white eyelids develop after the first year and the chin becomes black after moult (Watson 1975).

Figure 1.3: Adélie adult and juvenile during a feeding chase, note pattern and colour variations in plumage between adult and fledged chick (Photo by E. Marks).

Adélie penguins are circumpolar in distribution and are rarely found north of 50° S (Williams 1995) (Figure 1.4). During the non-breeding season they have a largely unknown pelagic range (Williams 1995). The main predator at sea is the leopard seal; on land the south polar skua (*Catharacta maccormicki*), giant petrel (*Macronectes giganteus*) and sheathbills (*Chionis alba*) are all both hunters (of eggs and small chicks) and scavengers (Müller-Schwarze 1984).

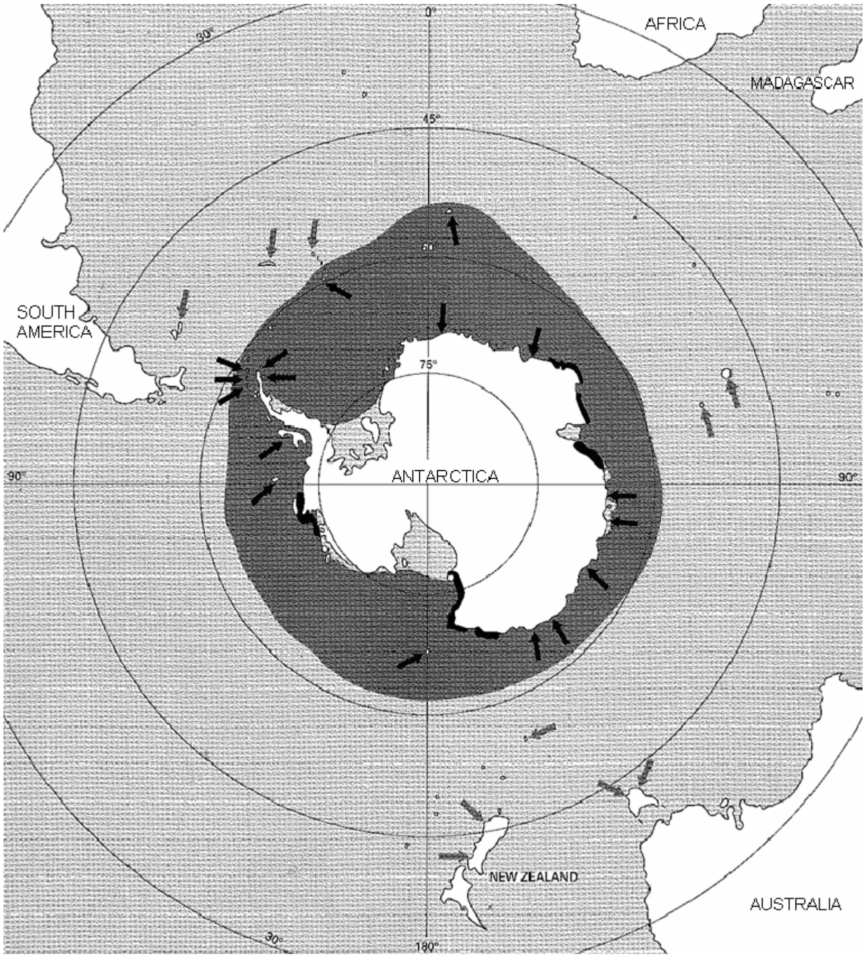

Figure 1.4: Distibution of breeding locations (black arrows) and sightings (grey arrows) of the Adélie penguin (Adapted from Williams 1995 page 170).

1.6.2 Foraging and diet

The foraging trips of smaller species of penguins (e.g. little blue penguin) may last from six hours to a few days, and foraging dives are typically less than 50 m in depth and 1-2 minutes in duration (Williams 1995). The larger species of penguin (e.g. king penguin) in contrast may forage from 6-14 days (Charrassin *et al.* 1999). Summer foraging ranges and diet of male and female Adélie penguins on Bechervaise Island were studied and considerable variation in trip lengths and diet was found throughout the season and with phase of breeding (Kerry *et al.* 1995). Additionally, the body weights of Adélie penguins prior to trips of long duration were significantly lower than those of short trips, which suggests that choice of trip type is regulated by adult body condition (Clarke 2001). Foraging decisions in the Adélie penguin therefore results from a trade off between allocation of food to chicks and storage of parental body reserves (Clarke 2001; Watanuki *et al.* 2002). This is seen in many seabirds, for example, the wandering albatross (*Diomedea exulans*) (Clarke 2001). Continental Antarctic penguins' (Adélie & emperor) behaviour is highly sensitive to the changing sea ice conditions and thus patterns of foraging have to alter accordingly and this can be daily as well as seasonally (Kato *et al.* 2003; Rodary *et al.* 2000; Watanuki *et al.* 1993; Watanuki *et al.* 1997; Wienecke *et al.* 2004). It has also been shown that Adélie penguins in the Ross Sea forage close to their colonies but also that foraging ranges expand and shift (especially for large colonies) as the season progresses as the available food is depleted (Ainley *et al.* 2004). Sex differences in foraging behaviour have also been noted at two locations (Bechervaise Island & Edmonson Point) in Antarctica, where females during the guard stage were observed to forage for less time and closer to the colony. In addition, fish made up a greater proportion of the diet in females than in males across the breeding season (Clarke *et al.* 1998). During incubation on Ross Island however, Davis *et al.* (1988) showed birds travelled up to 100 km from shore, in order to forage for the more pelagic krill (*Euphausia superba*).

Whilst some penguin species (king & macaroni) show distinctly diurnal diving patterns (Kooyman 2002; Puetz *et al.* 1998), Adélie penguins at Dumont D'Urville have been observed to be more frequent divers at midnight (possibly due to the nightly vertical migration of *E. crystallorophias*) (Rodary *et al.* 2000). Chappell *et al.* (1993) investigated the diving behaviour of Adélie penguins near Palmer Station and found dives were primarily diurnal and varied between three and 98 m with a mean diving depth of 26 m, with most dives lasting between 60-90 seconds. Diving strategies are also affected by local sea-ice conditions as dives without sea-ice at Dumont D'Urville were almost twice as deep and longer (40 m/102 s) than with sea-ice (22 m/78 s) (Rodary *et al.* 2000). Studies by Wienecke *et al.* (2000) have also shown that Adélie penguins at Shirley and Petrel Island preferentially foraged over the continental shelf or shelf break and not in oceanic waters and that nearly 70% of dives were to 35 m. The maximum depth to which Adélie penguins dive to at Hope Bay was 170 m but less than 25% of birds exceeded 100 m (Wilson 1989).

The Adélie penguin diet consists mainly of euphausiid crustaceans (>70%) with some fish and cephalopod prey caught (Williams 1995). Emslie and McDaniel (2002) note however, that as most studies of diet occur during the breeding season the role of squid and other species may be under-represented. Diet was also seen to be effected by the degree of ice-cover both within and between years (Rombolá *et al.* 2003). Additionally, it has been shown in the Ross Sea region that fish is a more energetically valuable than krill (Ainley *et al.* 2003), but changes in diet observed were not due to colony location but occurred with temporal changes across the breeding season and with the amount of sea-ice cover (Ainley *et al.* 1998; Ainley *et al.* 2003). Coria *et al.* (1995) found that during the post-hatching phase at Esperanza Bay, Antarctic Peninsula that euphausiids (predominantly *E. superba*) made up 96% of the diet, with the remaining 4% consisting of fish (mostly *Pleuragramma antarcticum*). The proportion of Antarctic krill in the diet of breeding Adélie penguins at Signy Island (1997-2001) was even higher with 99% of the diet being krill (Lynnes *et al.* 2004). In contrast at Shirley Island, fish and krill were represented in roughly equal proportions during the early chick rearing phase (Kent *et al.* 1998). Historically, changes in temperature have been shown to affect Adélie penguin diet, with fish being seen in greater proportions during cooler periods and more squid being represented in the diet during warmer periods (Emslie & McDaniel 2002). Fish otoliths and squid beaks found in colonies up to 6000 years old (Lagoon Island) also show that Adélie penguins preferentially select prey (excluding krill) in the size range between 95-117 mm (McDaniel & Emslie 2002).

1.6.3 Breeding biology

Adélie penguins breed from Cape Royds (77° S) in the Ross Sea, along the coast of the Antarctic continent, the west coast of the Antarctic Peninsula, on the islands of the Scotia Arc, and north to the South Sandwich, South Orkney and Bouvetoya Islands (54° S) (Williams 1995). They breed on ice-free exposed ridges and outcrops of available land, in large colonies (Oelke 1975) (up to 200 000 pairs; Williams 1995) composed of discrete sub-colonies (Ainley *et al.* 1983). In several areas (Antarctic Peninsula, South Orkney and South Shetland Islands), the three Pygoscelid species can be found nesting together (Watson 1975). The breeding behaviour of the Adélie penguin has been well studied in several areas around the Antarctic continent, from Cape Crozier (Ainley *et al.* 1983; Oelke 1975), Cape Royds (Stonehouse 1963), Cape Bird (Davis 1982a; Davis 1982b; Davis & McCaffrey 1986; Davis & McCaffrey 1989), to Signy Island (Sladen 1958) and Bechervaise Island (Clarke 2001, 2002; Irvine *et al.* 2000).

Birds begin arriving into the colonies to breed between late September and early October (Ainley *et al.* 1983). Older, more experienced breeders arrive earlier in the season, with males arriving (up to four days) earlier than females in order to establish a territory and begin nest building (Ainley *et al.* 1983). Males establish a territory based upon natal site, experience during pre-breeding wanderings, timing of arrival and available space (Ainley *et al.* 1983). A study by Penney (1968) near Wilkes station showed nesting territories averaged 0.75 m^2 and distances between nests averaged 69 cm. Males showed a 93% fidelity to their nest site in successive seasons, females if they did not reunite with their partner from a previous season would change territory but not sub-colony. Return of breeders to their colonies and sub-colonies was over 99% (Penney 1968).

Adélie penguins are classically described as socially monogamous; however several studies (Davis & Speirs 1990; Hunter & Davis 1998; Hunter *et al.* 1995; Pilastro *et al.* 2001) have shown the occurrence of extra-pair copulations (EPCs), fertilisations (EPFs) and mate switching within and between seasons. Davis and Speirs (1990) found that males and females were equally likely to change mate, having 2.0 and 2.1 mates over a four year period. Adélie penguins (specifically from the Ross Sea region) have low levels of mate retention with only approximately 49% of birds retaining their mate from the previous season (Ainley *et al.* 1983). One of the reasons they postulated for mate change was asynchronous arrival into the colony. Hunter *et al.* (1995) found that 14.9% of females during the pre-laying period switched mate within a season and that all of these females successfully copulated with both initial and final males. A further 9.8% of females engaged in EPCs over this same period. Pilastro *et al.* (2001) found 10% of offspring studied were not sired by the attending male. A further study showed that females may solicit EPCs from solo males in return for nest material, five different females were seen to solicit courtship from an extra-pair male in return for stones, and in none of these cases aggression towards the female by the dupe was seen (Hunter & Davis 1998).

Nests consist of a depression scraped into the ground and a collection of pebbles arranged into a bowl shape. Pebbles range in size from 1-5 cm in diameter and are collected from the surrounding area, or if poorly guarded, the neighbours nest (Taylor

1962). Each nest is a "work in progress" and pebbles may be added and arranged throughout pairing, incubation and guard stage. After pairing both male and female participate in nest building behaviours (Müller-Schwarze 1984).

Egg-laying commences between the end of October and November (Williams 1995), the clutch size is normally two, with the first egg being laid 1-4 days before the second (Ainley *et al.* 1983). A third egg can be laid if the first is lost within 24 hours (Taylor 1962). Replacement clutches are not laid due to the short nature of the breeding season (Williams 1995). Incubation begins only after the second egg is laid and both male and female will incubate the eggs until hatching, between 31-35 days later (Ainley *et al.* 1983). Males take the first incubation shift (mean 19.02 ± SD 3.35 days) (Davis 1988), thus fasting from arrival into the colony until relieved by their mates after incubation; this can be 4-6 weeks (Ainley *et al.* 1983). Sladen (1958) noted that males lost up to 40% of their body weight during this prolonged fast. The chicks hatch 1-2 days apart and can take up to 48 hours to hatch (Taylor 1962). Hatching success varies from 50-80% depending on study, location and climatic variables (Williams 1995). Causes of egg failure include infertility, breakage, nest desertion and predation (Williams 1995).

Chicks are 80-90 grams upon hatching, nidiculous, and have soft grey down covering the body and black down covering the head (Müller-Schwarze 1984). Chicks are guarded for about one month before they begin to form crèches (Williams 1995). During the crèche phase adults return from sea every 2-3 days to feed the chicks (Ainley *et al.* 1983). Through vocal recognition (Davis & McCaffrey 1989) and feeding chases, adults ensure they only feed their chicks (Thompson 1981). Boersma and Davis (1997) also believe feeding chases function to partition food allocation between siblings. By forming crèches Adélie chicks reduce the risk of predation by skuas and also increase their thermoregulatory abilities during inclement weather (Davis 1982a; Lawless *et al.* 2001). Chicks fledge (end of January/early February) at 40-60 days of age and approximately 70% of adult weight (Williams 1995).

Breeding success in this species is dependent on a number of factors, most of which have been examined in detail. The role of age and experience (both male and female) was investigated by Ainley (1983), who found that older birds, with more breeding experience have greater breeding success. Both age and experience are also related to foraging efficiency and timing of nest reliefs and arrival, which have also been related to breeding success (Clarke *et al.* 1998; Clarke *et al.* 2001; Davis 1982; Davis 1988). The location of the nest is crucial for successful breeding in this species (Davis & McCaffrey 1986; Tenaza 1971) as nests at the edges of sub-colonies are more vulnerable to predation (Young 1994). Not only location but also nest quality is important as poorly constructed nests are more susceptible to flooding and predation (Moreno *et al.* 1995; Tenaza 1971; Young 1994). Additionally, environmental factors such as food availability, weather and sea-ice abundance are also influential (Ainley & LeResche 1973; Clarke *et al.* 2002; Rombolá *et al.* 2003; Trathan *et al.* 1996).

Post-breeding the adults moult (January-March). The process takes approximately two weeks and occurs on sea-ice or land (Watson 1975). Adélie penguins begin to breed between 4-6 years of age (females breed earlier on average than males) (Ainley *et al.* 1983). They have an annual survivorship of approximately 60% and have been estimated to live to about 16 years of age (Ainley & Demaster 1980).

1.6.4 Visual and vocal signals

Amongst the *Pygoscelis* penguins a similar series of visual and vocal signals have evolved. The Adélie penguin has the most complex and ritualised displays followed by the chinstrap and then the gentoo, which as the most peaceful of the three has a simpler set of behaviours (Jouventin 1982).

1.6.4.1 Visual signals

The Adélie penguin has evolved a series of highly ritualised behaviours (Jouventin 1982). These behaviours have been studied, defined and re-defined by several authors (Ainley 1975; Jouventin 1982; Penney 1968; Sladen 1958; Spurr 1975b). Most behaviours can be categorised into four classes; displacement, agonistic, appeasement and sexual. Adélie penguins according to Jouventin (1982), exhibit no unique displacement activities however, they have generic head shaking, stretching, yawning, scratching and shaking behaviours common to all penguins. The only appeasement behaviour seen in the Adélie penguin is the slender walk as described by Spurr (1975b). When travelling through the colony the Adélie penguin elevates the head, sleeks back the feathers (along the head and neck especially), raises the flippers from the body and walks on tip toes swiftly through the crowded nesting area (Spurr 1975b).

Of all the penguins, the Adélie penguin has perhaps the most numerous and varied repertoire of agonistic behaviours (Penney 1968). The most extreme being "the attack", which is described by Penney (1968) as a bird rushing an opponent, keeping the flippers by the side until contact is made. The aggressor, whilst either pecking or holding onto the opponent with the bill, uses the flippers to pummel the adversary rapidly and forcefully. Bouts of fighting can last between 10-15 minutes. However, often a quick pecking across territorial lines is the limit of the attack. Also noted by Spurr (1975b), during a severe attack the penguins will use their chests to push one another and throughout the encounter the aggressor will have the crest raised and both parties are likely to have the whites of their eyes showing.

Of less severity is "the charge", which precedes the attack, but does not always lead to an attack (Spurr 1975b). Here the penguin moves toward the stimulus a few steps or a short run, the body is upright, head feathers erect, head held forward from the body, eyes rolled downwards and flippers held out from the body. The bill may either be open or closed (Spurr 1975b).

Jouventin (1982) describes a behaviour known as the "tête-à-tête" which encompasses the "point", "gape" and "peck" described separately by Spurr (1975b) and Ainley (1975). Penney (1968) included both the "point" and "gape" in one display named the "direct stare". Essentially these behaviours entail an escalation of actions, from the "point" where the bird inclines the body towards the stimulus, beak closed, flippers usually by the side, the occipital feathers are erect and the eyes rolled to expose the white sclerae. Often following this is the gape, where although the body posture is similar to the "point" the beak is held wide open and is often accompanied by a harsh call. If this is not sufficient warning to the stimulus the bird may then "peck" or attempt to grab and twist the beak of the opponent as described in the "tête-à-tête".

There are three slightly more passive agonistic displays seen in this species. The "bill-to-axilla" was first described by Sladen (1958). It entails either sex of Adélie stooping forward to an angle of approximately 45° with the head turned to one side of the body so the bill points toward the axilla of one flipper. The head is then rolled up and down several times in one direction; it may be then be rolled on the other side of the body or in front of the body. At full intensity the behaviour is accompanied by a repetitive growl. During the display the crest is raised and eye whites exposed. This behaviour is given predominantly by birds solo at the nest and occurs most frequently in males after an "Ecstatic Display" or when other males are giving an "Ecstatic Display" (Spurr 1975b). The "sideways stare" was first named by Spurr (1975b) but has also been termed the "fixed one sided stare" by Ainley (1975) and can be given either standing or lying. It involves the bird moving the head in a sideways orientation, where it is held, so that one eye stares directly at the intruder. The feathers are erect and the eyes rolled. Females also perform this behaviour when approaching bachelor males, the difference being the feathers are sleeked, eyes not rolled, the body is more hunched, head lower, and often she approaches the nest in a sideways manner (Ainley 1975). The final agonistic behaviour is the "alternate stare" which was first described by Penney (1968), this behaviour is similar in position to the "sideways stare" but the head is moved from side to side so both eyes are presented to the intruder. The flippers are also waved slowly up and down, and the bird may emit a soft growl. It is a stronger response to persistent unwanted intrusion than the "bill-to-axilla" or the "sideways stare".

The final group of behaviours is sexual displays. There are four main types. Firstly, the "Ecstatic Display" (Figure 1.5a), described by (Sapin-Jaloustre & Bourliere 1952), in which the penguin stands upright, with the feet slightly apart, the head and bill are raised vertically and neck is fully extended. The behaviour begins when chest thrust out; the bird emits a series of beats that emanate from the chest. The initial pumping sound builds to a climax, when the bird lets out a harsh cry. During this display the flippers are rhythmically beaten back and forward. The crest is erect and the eyes rolled. This is primarily a male territorial display and can be repeated a number of times, it is also contagious to surrounding males (Spurr 1975b). Secondly, there is the "Bow Display" which often occurs when two birds (of the opposite sex) face each other in the nest and one or both will incline the head approximately half way to the ground. There is no sound and the crest and eyes may or may not be erect or rolled (Sladen 1958). Thirdly, the "Loud Mutual Display" (Figure 1.5b) described by Penney (1968), is usually given by a pair of birds standing at the nest, the birds stand facing each other and with bills pointed high, crests erect, eyes rolled, flippers by the side, they rapturously emit a series of loud sonorous notes through the open bill whilst waving their heads from side to side. The display can be repeated many times; and is given in a number of contexts including: mate reuniting, between parent and crèched chick, by a lone male on finding his territory again, by a parent towards eggs/chicks and during fights between adults (Spurr 1975b). The fourth and final display is the “Quiet Mutual Display” (Figure 1.5c) which again involves two birds (or an adult with chicks) facing each other in the nest (Spurr 1975b). The birds firstly lower their heads sideways and bills are pointed toward each other, then the heads are raised and both birds wave their heads out of phase with each other whilst emitting a soft humming noise (Spurr 1975b). The bill is kept closed and flippers are next to the body; crest is erect and eyes are rolled (Spurr 1975b). This display can be given by one bird in response to movement by the mate, and is often performed by the female when her mate is performing either a “Loud Mutual Display” to the eggs/chicks or an “Ecstatic Display” at the nest (Spurr 1975b). Ainley (1975) also believes this display has an appeasement function.

Figure 1.5: a) “Ecstatic Display”, **b)** “Loud Mutual Display” & **c)** “Quiet Mutual Display” (Photos by E. Marks).

1.6.4.2 Vocal signals

There are three main calls emitted by the Adélie penguin. The Ecstatic Display Call (EDC), the Loud Mutual Display (LMD) call and the contact call (Williams 1995). The agonistic and passive sounds have not been described, although they may be informative with regards to motivation and possibly body size, as they only represent very simplified sounds that are not thought to be used for either mate choice or recognition. Jouventin (1982) notes however, that these sounds vary greatly depending on individual and social context and are likely to represent fragments of the display song. These sounds deserve greater study; however they were outside the scope of this dissertation. The first of three well defined calls given by the Adélie penguin is the contact call. This call is a short monosyllabic bark and has been described as *aark* (Williams 1995). The call rises to approximately 2000 Hz and is up to 0.3 seconds in duration; it is most often given by birds at sea or birds arriving on to the shore/beach (Jouventin 1982). The Adélie penguin is the only penguin species with two distinct display songs, the EDC and LMD call (Jouventin 1982). First described by Sladen (1958) and then by Penney (1968), the EDC begins with a series of pumping/clapping sounds, the call then climaxes in a series of rasping pulses (Penney 1968) that are said to resemble the LMD call (Ainley 1975). The call is mainly given by lone males as a mate attractant or territorial statement (Penney 1968). The LMD call is variable in length depending on motivation and consists of a series of repeated phrases (Jouventin 1982). The call has sound that pulsates throughout the phrase and often consists of a series of several pulses followed by a tone of constant or changing frequency with multiple harmonics (Jouventin 1982). The call is most often heard at the nest during pair-formation, nest reliefs, or when a bird returns to feed chicks (Müller-Schwarze & Müller-Schwarze 1980). The call is highly characteristic of the individual (Jouventin 1982). It is stated by Jouventin (1982) that redundancy of syllables and calls probably facilitates recognition, similar to that of *Aptenodytes* chick calls which are always uttered in series of threes. Ainely (1975) has separated LMD calls given solo or by a bird alone at the nest and named them Locomotory Hesitance Vocalisations. These calls can be given to chicks/eggs or when a bird approaches their territory.

Adélie chick calls at hatching are simple weak “peep” sounds. The calls are highly variable within individuals up to 10 days of age. However, the song becomes more stable from 20 days onwards (Jouventin 1982). Once the chicks begin to leave the nest the call again becomes more plastic until at fledging the adult call begins to develop (Jouventin 1982). Syllables of the adult song seem to originate from modulations in chick calls (Jouventin 1982).

1.7 Colonial bird and penguin vocal studies

1.7.1 Studies of vocal behaviour in colonial birds

Studies examining vocal recognition in colonial birds are growing, although they are not as in depth or specific as the literature on penguin vocal behaviour. In seabirds, there has been a series of papers examining the vocal recognition capabilities of gulls (*Larus* spp.), for example: the ring-billed gull, *L. delawarensis*, (Evans 1970; Evans 1980; Miller & Conover 1983), the laughing gull, *L. atricilla*, (Beer 1970; Beer 1979; Impekoven 1972), the black-headed gull, *L. ridibundus*, (Bremond & Aubin 1992; Charrier *et al.* 2001b; Mathevon *et al.* 2003), the black-tailed gull, *L. crassirostris*, (Park & Park 1997) and the black-billed gull, *L. bulleri*, (Evans 1970). A related species, the kittiwake, *Rissa tridactyla* L., has also shown the potential for calls to encode for individuality (Wooler 1978).

The calls of the south polar skua (*Catharacta maccormicki*), an Antarctic seabird nesting in loose colonies and often in cohabitation with penguins, were examined by Charrier *et al.* (2001a). They found that both the courtship and contact calls of the skua had potential to code for individual identity. Another group of seabirds, which have had their vocal characteristics studied in some detail, are the Procellariiformes (petrels). This diverse group of seabirds demonstrates calls that can vary individually (Bretagnolle *et al.* 1998), by species (Bretagnolle 1996), sex (Bretagnolle 1989; Bretagnolle & Genevois 1997; James 1984), with body size/condition (Barbraud *et al.* 2000; Genevois & Bretagnolle 1994) and geographical location (Bretagnolle 1989; Bretagnolle & Genevois 1997).

Another colonial (but not sea-) bird investigated by Mathevon (1996; 1997) is the greater flamingo (*Phoenicopterus ruber*). The flamingo also breeds in large numbers (a noisy environment), and the chicks also form large crèches. Individual recognition was studied by analysing the call parameters, much like the studies done on the emperor penguin. The results indicated a "two-voice" signal was used with most of the individual call components being related to frequency. To improve call identity flamingos also encoded individuality into several call parameters, both temporal and frequency related (Mathevon 1997). Not all colonial birds are non-passerines; the highly colonial cliff swallow (*Hirundo pyrrhonota*) has also been shown to have a sophisticated recognition system (Beecher *et al.* 1985; Loesche *et al.* 1991; Medvin *et al.* 1992; Medvin *et al.* 1993; Stoddard & Beecher 1983).

1.7.2 Studies of vocal behaviour in penguins

The evolution of a highly individual call is crucial for successful breeding in colonial species (like penguins) as it enables both mate and chick identification (Medvin & Beecher 1986). Much of the work in this field has been done on *Aptenodytes* (emperor and king) and *Pygoscelis* (gentoo, Adélie and chinstrap) vocalisations; however, there is a growing body of work on the less available species.

1.7.2.1 *Aptenodytes* penguins

Aptenodytes penguins are unique amongst penguins as they are non-nesting, instead incubating and raising their small chicks on their feet: thus are mobile (Derenne *et al.* 1979). Consequently, they have evolved highly complex vocal behaviours.

The emperor penguin has the most extreme of all penguin breeding habits and as such has the evolved one of the most multifaceted systems of vocal recognition. Jouventin *et al.* (1979) established it was the "Mutual Display" call that was used for individual identification, and since then many authors (Aubin *et al.* 2000; Bowles 1994; Bremond *et al.* 1990; Guillottin & Jouventin 1979; Jouventin 1972; Jouventin *et al.* 1979; Lengagne *et al.* 1997; Robisson 1990; Robisson 1991; Robisson 1992b; Robisson *et al.* 1993) have tried to establish which aspects of the call are specifically used for recognition. Robisson (1990), through manipulation of the calls and recognition responses in subject penguins, demonstrated that although the temporal pattern of syllables had a part in recognition the syllable structure was more important. Robisson (1991) then investigated the broadcast distance (the distance over which the information is carried) of the "Mutual Display" call. The broadcast distance was determined by measuring sound amplitude, sound attenuation, the ratio of signal to background noise and the degradation of the signal structure due to a scattering medium i.e. penguin bodies. The distance calculated was 4-7 m; this indicates that the call is transmitted over short-medium range. This corresponds closely to the observed pattern of calls that are given by a returning adult who stops and calls repeatedly when looking for his/her chick.

Both king and emperor produce a signal that consists of a continuous pattern of two harmonically related bands of different frequencies (Aubin *et al.* 2000). The two fundamental frequencies of the call produce a beat phenomenon known as a "two voice" signal, which likely facilitates recognition in a noisy environment (Robisson 1992b). The sound structure of emperor calls was analysed by Robisson *et al.* (1993) to determine individual-specific cues of the call. Of the temporal and frequency specific variables quantified, analyses showed that several parameters were highly individual and that beat was important in recognition as was the amplitude modulation which was also less likely to be degraded by the environment (Robisson *et al.* 1993).

Robisson (1992a) also studied the discrimination of mate calls in the king penguin using two single parameters, syllable duration and pitch; both are thought to be involved in individual recognition. The behavioural variation in response to changes in these parameters demonstrated that alterations in duration of the syllable up to 20% did not hamper recognition. By contrast, alteration of pitch did change responses. Hence frequency parameters were deduced to be more important in individual recognition than temporal parameters (Robisson 1992a). The distance at which king chicks should recognise their parents call above the background noise was calculated by Aubin & Jouventin (1998) to be 8-9 m. However, in reality chicks were shown to be able to distinguish adult calls at an even greater distance, a term named the "cocktail-party effect". The "cocktail-party effect" was first described in humans (Cherry 1966) and can be defined as the ability to discriminate a specific call from extraneous calls (Aubin & Jouventin 1998). This effect has been found in several animal species (Busnel 1977; Wiley & Richards 1982). Jouventin *et al.* (1999) then showed that king chicks are able to recognise their parents call even when peak energy values were altered, thus chicks used frequency rather than amplitude modulation for recognition. This same experiment also demonstrated that the first half of a syllable and three harmonics were sufficient to elicit recognition. Like the emperor, it has been shown that acoustic communication in kings works most efficiently at the short-medium range (8.8 m) (Lengagne *et al.* 1999). Adult birds also respond only to the calls of mates, not neighbours or strangers, and the birds attend to the frequency modulation of the call, and not the amplitude modulation or absolute frequency (Lengagne *et al.* 2000). As well as frequency modulation, Lengagne *et al.* (2001) showed that, like emperors, kings use the beat frequency generated by the "two voice" system to assess individual identity. Aubin & Jouventin (2002) then examined the function of the syllabic organisation, harmonic structure and amplitude modulations of call syllables, as previous research (Jouventin *et al.* 1999; Lengagne *et al.* 1997; 2000; 2001 & Robisson 1992a) had indicated they were not necessary for identification. They found that although not critical for identification these call features were used to help localise the signal of the caller, and the redundant nature of the syllabic organisation helped reduce the masking effect of background noise.

1.7.2.2 *Pygoscelis* penguins

In nesting species most work has been done on *Pygoscelis* penguins, specifically Adélie penguins. Adélie penguin chicks have been shown to recognise the calls of their parents, and also recall accurately the location of their natal territory (Penney 1968). Upon hearing their parents' voices Adélie chicks act as if they anticipate a reunion with their parents on that natal ground (Penney 1968). During the guard stage, chicks and parents (usually one at a time) are in constant contact and therefore conditions are favourable for learning parental calls (Penney 1968). Discrimination abilities of Adélie penguins were studied by Davis & McCaffrey (1989) by cross-fostering eggs within two days after laying, chicks five days after hatching, chicks 11-15 days old and chicks 17-21 days of age. Results showed that Adélie adults have no innate mechanism for recognising their offspring. Discrimination by adults was only apparent after 17-21 days of age; this corresponds with the end of the guard stage and the beginning of the crèche phase. By contrast the chicks may have learned to discriminate adults by 11-15 days of age (Davis & McCaffrey 1989).

Adult Adélie calls are individual (Jouventin 1982) and as approximately 50% of Adélie penguins retain the same mate over at least two consecutive breeding seasons (Ainley *et al.* 1983), mate retention may be aided or enhanced by the individual recognition of each others calls (Speirs & Davis 1991). Playback experiments were conducted by Speirs & Davis (1991) during the incubation period to test the responses of male and female penguins to recordings of the LMD calls of their partners, neighbours and strangers. The results demonstrated that both males and females discriminate between the calls of their mates and strangers. However, only males discriminated between the calls of neighbours and strangers. The greater fidelity of males to the nest site (Davis & Speirs 1990) probably results in a more stable "vocal environment", and therefore males have a greater opportunity to learn the calls of their neighbours (Speirs & Davis 1991).

A study by Jouventin & Aubin (2002) compared the nesting gentoo and Adélie species' calls with the non-nesting *Aptenodytes* species and found that Adélie and gentoo chicks respond to signals with amplitude modulation retained but with frequency modulation removed. They also appeared to use the harmonic structure to analyse the call and were sensitive to shifting the frequency by more than 25 Hz (Jouventin & Aubin 2002). One full syllable was also required to elicit recognition. In both species, chicks reacted more to the lower part of the frequency spectrum (Jouventin & Aubin 2002). This is probably due to the fact these frequencies are less attenuated by distance (Wiley & Richards 1982). Furthermore, the Adélie chick could detect a signal at the same level as the ambient noise, thus demonstrating the "cocktail-party effect" (Jouventin & Aubin 2002). In comparison to non-nesting species, it appears the signal to noise ratio is higher in nesting species, thus their ability to detect calls below ambient noise is reduced by comparison (Jouventin & Aubin 2002). Also non-nesting species tolerated greater changes in frequency than nesting species. In the temporal domain all species demonstrate redundancy in the calls, but non-nesting penguins require greater redundancy (Jouventin & Aubin 2002).

The two remaining *Pygoscelis* species (the gentoo & chinstrap) have had minimal attention paid to their vocal displays. The gentoo, like the Adélie penguin, has a "Mutual Display" call that likely facilitates recognition between partner and offspring (Jouventin 1982). Only basic acoustic structure and function of the chinstrap calls have been studied (Bustamante & Marquez 1996), and it is believed this species also possesses an individual display call used for recognition (Jouventin 1982).

1.7.2.3 Other penguins

The calls of *Spheniscus* penguins have received little study; however it is known that these northerly living penguins have a similar complement of calls to the rest of the Spheniscidae family (Eggleton & Siegfried 1979). It was hypothesised that due to their less dense colonies and nesting habitat that allows for numerous landmarks, variation and type of calls may have diminished (Thumser & Ficken 1998). The results of a study by Thumser & Ficken (1998) showed that several calls were common to all *Speniscus* species (haw, bray, courtship bray and peep), and the potential for individual recognition was apparent in the sonograms of the Humboldt penguins (*Speniscus humboldti*) for the haw call, and all species showed individual variation in the bray call. Seddon (1993) has also experimentally demonstrated the recognition capabilities of the African penguin. Additionally, a study by Thumser *et al.* (1996) has demonstrated that through discriminant and cluster analysis the bray call of the African and Magellanic penguins (*Spheniscus magellanicus*) were more similar than the Humboldt; this vocal congruence mirrors phylogenetic allozyme data for this genus.

As a genus the *Eudyptes* have had several studies devoted to their vocal characteristics. Like the Adélie penguin, a study on royal penguins (*Eudyptes schlegeli*) also found birds reacted more strongly to calls of birds that were mates and neighbours than birds from other colonies, thus were able to recognise these calls upon hearing them (Waas *et al.* 2000). The macaroni penguin (*Eudyptes chrysolophus*) has been shown to have a unique calling system, which uses a double signal like that of *Aptenodytes* penguins (Searby & Jouventin 2004), for effective parent-chick recognition at the nest site (Dobson & Jouventin 2003). The same double signal was also then discovered in the rockhopper; hence it appears to be a calling trait linked to all crested penguins (Searby & Jouventin 2005). Both Snares Island (*Eudyptes robustus*) and Fiordland crested penguins (*Eudyptes pachyrhynchus*) have also been shown to have an effective parent-chick recognition system (Proffitt & McLean 1991; Studholme 1994).

Studies using little blue penguins have demonstrated individuality of calls (Jouventin 1982) and also a multi-level communication system that is effective at different ranges and through different mediums (Jouventin & Aubin 2000). As a burrow dweller, for example, the territorial function of the call encodes information at the lower end of the spectrum, as these frequencies are more resistant to degradation (Jouventin &

Aubin 2000). It has also been shown that (like the royal penguin), vocal displays can facilitate sexual encounter rates (Waas 1988), and the social repertoire of the species can depend on the breeding habitat (Waas 1990). Additionally, in this species the variation between individual male calls can be correlated with body weight, and therefore potentially used as an indicator of quality to prospective females, and may also enhance mate fidelity (Miyazaki & Waas 2002; Miyazaki & Waas 2003b; Miyazaki & Waas 2003a; Miyazaki & Waas 2005).

1.8 Summary of aims

To date, the focus of the majority of penguin vocalisation studies has been to try to understand how recognition functions in the acoustically cluttered environment in which most penguins breed. The Adélie penguin (which nest in some of the largest colonies) is the only species however, to have two distinct display calls, the Ecstatic Display Call (EDC) and Loud Mutual Display (LMD) call. But apart from general structure and function the EDC remains largely unstudied. Hence, the aim of this dissertation is to broaden the knowledge regarding the EDC and specifically its characteristics, functions, stability and potential variations (both spatial and temporal). Hence the following four aims are the focus of this study:

1. To examine variation in EDCs of Adélie penguins with regard to individuality and differences from the Loud Mutual Display (LMD) call. Additionally, sex differences in EDC parameters will be examined, as this call has been specifically associated with male behaviours such as mate choice and territory defence. The prediction being that EDC parameters will be individual but distinct from the LMD and given males and females perform the EDC the parameters will be similar in both sexes.

2. To investigate the variability in the male EDC at a variety of spatial and temporal scales. Adélie calls from disparate colonies will be analysed to see whether local geographic variation has evolved (both between and within colony localities), and also whether these colonies exhibit either seasonal (over a single breeding season) or yearly (over different breeding seasons) variations in vocal characteristics. Additionally, the breeding success and overall condition of the colonies will be examined to see if this correlates with some aspects of the EDC. As primarily the male EDC will be recorded all

birds that have their calls analysed will also have their sex confirmed through genetic analysis of feathers.

3. To assess the legitimacy of the EDC as an honest signal, some aspect of the EDC should signal male condition, and therefore be a useful cue for early season mate choice. Male condition should also change throughout the breeding season, and this should be reflected in the EDC. Both male condition and one or more call parameters should also predict breeding success. Finally, males arriving early into the colony will be in better condition and have greater breeding success.

4. To study which specific factors led to nest success at three stages (pair formation, egg incubation & chick fledging period) during the 2002-2003 austral breeding season at Cape Bird, using logistic regression models. The predictive ability and constancy over the breeding stages, of both nest and individual penguin characteristics will also be examined. Due to extensive sea-ice, it was predicted that breeding success would be poor, consequently although many birds will succeed at the first and maybe second breeding stage, only the most experienced males are expected to succeed in raising chicks to fledging. Male quality (early arrival and weight), nest location or quality may also have an effect on the condition of chicks in focal nests.

1.9 Thesis structure

The thesis includes six chapters. The first chapter introduces the theoretical background to communication and also the study species. Additionally, it covers the background literature plus outlines the main aims. Chapter 2 describes the main behavioural and recording methods that apply to all of the results chapters (3 to 5), as well as detailing the method of call analyses used. The initial results regarding EDC individuality, differences between LMD and EDC call parameters and any sex specific differences in this primarily male call are also described. The three main results chapters (3 to 5) have been written as separate papers and therefore contain some repetition. However, chapter 2 provides details on the general methods used that apply to all of the results chapters thus minimising repetition. Chapter 3 investigates variation in Adélie calls on a large geographic scale, and looks for local geographic call variation, as well as seasonal and annual variation in EDC calls from disparate colonies. Chapter 4 examines the use of the male EDC as an honest signal used by females during mate choice to assess male condition and the likelihood of a successful breeding attempt. The final data chapter, 5, assesses breeding success using male and nest specific factors potentially linked to breeding success during a poor breeding season. This chapter trials and evaluates the use of nominal logistic regression in order to predict Adélie breeding success at three critical stages of the breeding season. The final chapter, 6, is a general conclusion.

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2

2.1 Introduction

This chapter focuses on the general methodology used throughout the study. It also contains the results for the first aim of the thesis (section 1.8). The results from the first aim were required prior to assessing the larger objectives of the study (chapters three-five). These include: the results of the DNA sexing study; the Ecstatic Display Call (EDC) parameter stability assessment; clarification of the EDC as an individual call; the structure (syllable organisation) and acoustical differences between the Loud Mutual Display (LMD) call and EDC; and lastly whether the assumption that the EDC is a male specific call is justified.

The study was carried out at two spatial scales, at the largest spatial scale, three Ross Island colonies (Crozier, Royds and Bird) were investigated mainly for broadscale differences in calls, weight and breeding success. At the smaller scale, a single focal sub-colony (at Cape Bird North) was used to examine individual changes in calls, with weight and included estimates of nest specific parameters and breeding success. Methods similar at both scales have been described together. Where the methods are specific to either scale they have been described separately. Methods unique to data chapters three-five can be found in subsequent methods sections.

2.2 Study area

2.2.1 Ross Island breeding colonies

On Ross Island there are three main areas where Adélie penguins (*Pygoscelis adeliae*) breed (Figure 2.1a), these are Cape Crozier (77 °30’S, 169°22’E), the largest of the colonies with an estimated 120 000 breeding pairs (Barton K *pers. comm.* – Landcare Research, New Zealand). Next in size is the Cape Bird (77°14’S, 166°28’E) breeding colony, which is itself comprised of three discrete colonies (Figure 2.1b), Cape Bird North (≈35 000 breeding pairs in 2000; Ainley *et al.* 2004), Middle (≈5 000 breeding pairs) and South (≈20 000 breeding pairs) (Barton K *pers. comm.* – Landcare Research, New Zealand). The smallest and most southerly of all Adélie breeding colonies is Cape Royds (77 °33’S, 166°10’E), which is comprised of approximately 8 000 breeding pairs of birds (Barton K *pers. comm.* – Landcare Research, New Zealand).

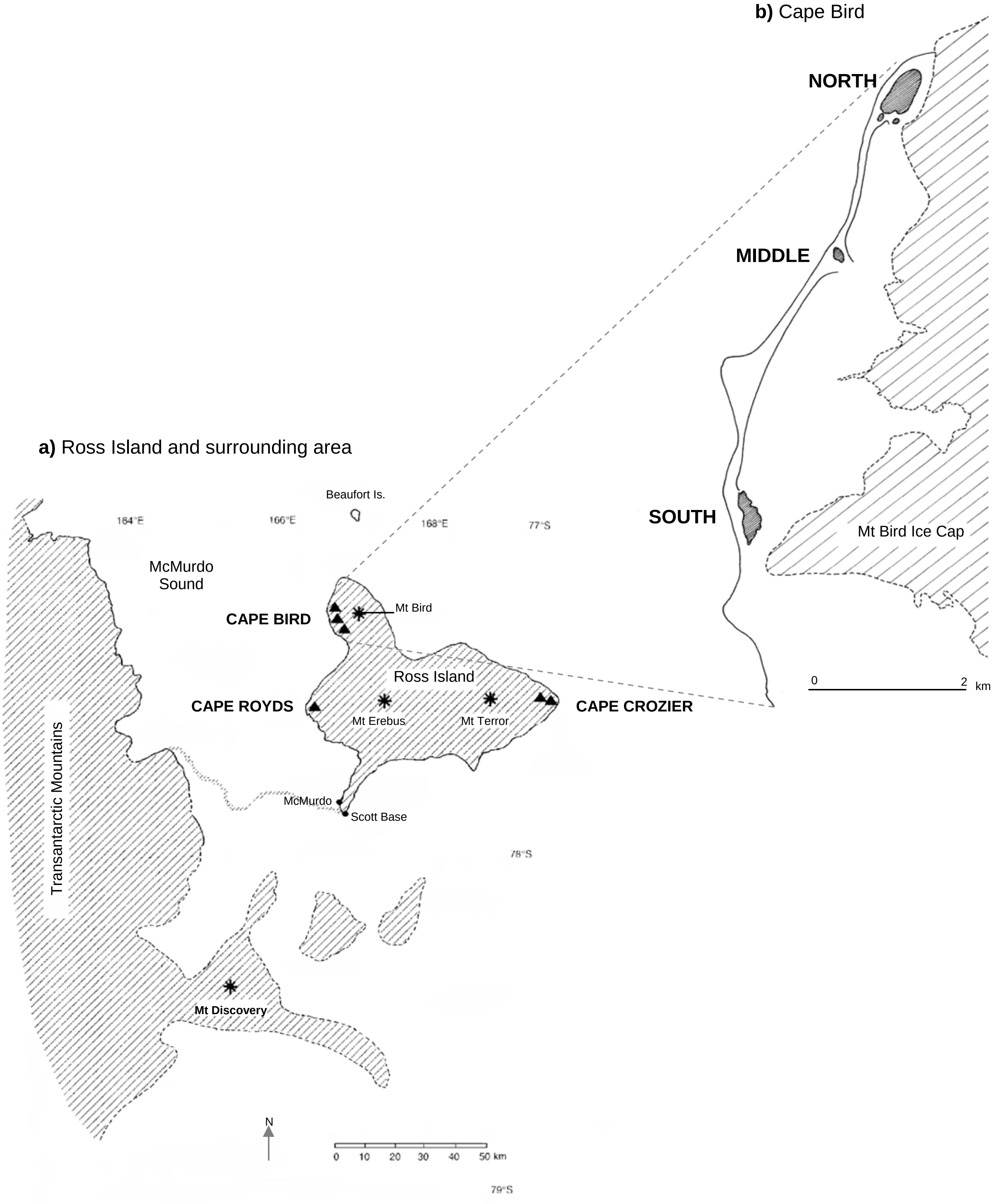

Figure 2.1: a) Map of Ross Island, with three Adélie breeding areas (Cape Royds, Crozier and Bird) highlighted and **b)** insert of the three Cape Bird colonies (North, Middle and South) (Figures adapted from Young (1994) page 21 & 23).

The study was carried out during two breeding seasons, the first between 9th December 2000 and 28th January 2001, and the second between 23rd October 2002 and 27th January 2003. Data were collected from all three Ross Island Adélie breeding areas, with Cape Crozier (28th – 30th December 2002) and Cape Royds (19th November 2002) only being visited during single sampling period in the 2002/3 breeding season. Due to logistic reasons Cape Bird was able to be visited for longer and during both field seasons thus more extensive data were able to be collected at these colonies. Bird handling only occurred at Cape Bird. A total of 370 adult male Adélie penguins were handled at Cape Bird North (n = 200), Middle (n = 50) and South (n = 120) during the early breeding season (23rd October – 18th November 2002) and again in the late breeding season (9th January – 27th January 2003). In addition, the same proportion of fledging chicks were handled at each of the Cape Bird colonies at the end of the breeding season (total n = 370). Permits to enter sites of special scientific interest (SSSI) including Cape Crozier (SSSI 4) and Cape Royds (SSSI 1) were approved by Antarctica New Zealand.

2.2.2 Focal sub-colony (Cape Bird North)

Focal Adélie penguin recordings and observations were carried out at North Colony, Cape Bird (77°13’10”S, 166°28’30”E), Ross Island between 23rd October 2002 and 27th January 2003. The focal sub-colony of approximately 100 breeding pairs was situated in the center of North Colony. The sub-colony was elongated in shape, with an approximate distance of three meters from center to edge (Figure 2.2). A total of 51 randomly selected focal males were used in the study. Only one focal sub-colony was used as this maximised the amount of time that could be spent observing and recording focal males. Although replication of sub-colonies used and nest locations sampled may have been advantageous it was decided that the focal sub-colony used accurately reflected a representative area for breeding within North Bird colony and therefore the results would reflect this.

Figure 2.2: Photograph of focal sub-colony at Cape Bird North (by E. Marks).

2.3 Study subject

2.3.1 The Adélie male

The male Adélie penguin was the target for all sound and behavioural analyses, and only adult males that were considered mature were used; birds displaying signs of immaturity with regards to breeding behaviour (for example, if during the pairing phase they failed to make a nest of any kind or if they did not remain at a their nest site to attract a mate despite having a nest) were excluded from analyses. The study was approved by the University of Auckland Animal Ethics Committee (AEC N905 & AEC/08/2002/R71).

2.3.2 Sexing techniques

In focal sub-colony studies Adélie penguins were sexed using a combination of behavioural cues (including copulatory position and nest building behaviour – such as persistent scraping and stone collecting - until some form of nest was evident), and arrival time. On a larger scale where birds from disparate colonies were being recorded, each bird sampled had up to three feathers plucked (using metal pliers) from the chest area for DNA sex identification. Feather samples were stored in uniquely identifiable zip-lock bags and kept below freezing temperature at all times. To minimise the possibility of sound recording females during the early breeding season, any bird exhibiting sex linked behavioural cues (i.e. having dirty feet marks on the back therefore likely to have been in the female copulatory position) were excluded. During the late breeding season, there were no reliable behavioural cues observed therefore all birds giving an Ecstatic Display Call (EDC) were assumed to be male. In part due to the cost of DNA sex analyses and in part due to the uncertainty of behavioural sexing in the late breeding season it was decided that ¼ of the samples (*n* = 92/370) during the early breeding season were to be DNA analysed, as opposed to approximately ¾ of the samples (*n* = 275/370) during the late breeding season. All DNA sexing of feathers was carried out commercially using standard laboratory protocols (Griffiths *et al.* 1998) at the Allen Wilson Centre for Molecular Ecology and Evolution, Massey University.

Results of DNA analyses showed that, for the early breeding season, only one female (from the North colony) of the 92 samples (North *n* = 50, Middle *n* = 12 and South *n* = 30) was recorded. During the late breeding season 271 of 275 individuals were successfully assigned a sex, and 37 (13.7%) of these were females (16.3%, 24/147 in North, 13.5%, 5/37 in Middle and 9.2%, 8/87 in South colony). The proportion of females in the early breeding season recorded by mistake was very small, and unlikely to significantly affect EDC comparisons. In the late breeding season, even though there was a larger proportion of females sampled, recordings of these females were removed from EDC analyses. The proportion of females in the samples that were not DNA sexed therefore probably constituted between 1.8-3.4% hence were also unlikely to affect EDC analyses. A comparison of the EDC parameters between the males and females can be found in section 2.11.

2.4 Behavioural methods

All Adélie males were caught using a hand net, and then held securely under the arm, whilst either having bands fitted, wing lengths measured, or having feathers plucked for DNA analyses. Bird handling was kept to a minimum with each animal handled only once (in large scale study) or twice (in focal animal study) throughout the study period. Adult male weights were measured using a strop/breathable black bag and 10 kg Pesola scale, wing lengths were measured using a custom metal ruler. All males were caught at their nest site and then removed to outside their sub-colony for manipulation. Birds were released outside their sub-colony but near their nesting area. All birds were seen to return to their nests. During the early breeding season capture of males was done prior to egg laying therefore there was no potential for skua predation or loss of breeding potential. At the end of the breeding season when males were being re-weighed or sampled for the first time, chicks were protected from skua predation by placing a large box over the nest. No chicks were left exposed until the male had returned to the vicinity of the nest. Although there was some disturbance to the males, chicks and surrounding birds, it appeared to be of short duration and unlikely to have either affected their ultimate breeding success or the results observed in this study. All recordings of calls were made prior to handling and therefore were not affected by human disturbance.

All chicks sampled were handled only once and (depending on their size) were either caught by hand or using a handheld net and weighed using a breathable bag and either a one or five kilogram Pesola scale. They also had their wing lengths measured (using a custom metal ruler). All chicks were sampled away from their nest or sub-colony but either returned to their nest or (if later in the season) the crèche they were taken from. Disturbance to the sampled chicks and nearby birds was of short duration and it was considered unlikely to have resulted in predation or loss of condition. Chicks were not marked as it was deemed unnecessary. In the focal study they were weighed (at two weeks of age) before they began the crèche phase and moved away from the marked nests. They could still be identified later in the breeding season (for assessment of focal nest breeding success) by daily monitoring of their changing fledging patterns and by observing feeding bouts with banded males. During large scale measurements taken across Ross Island colonies, chicks were only observed and weighed once and location of the chicks was not noted as identity was not important.

In focal studies, for individual identification, each male had a uniquely coded (PA26401-PA26450) permanent metal flipper band (34x17 mm) attached (U.S.A.R.P. Bird Banding Program, Sladen and LeResche, 1970). One male was banded from a previous study (PA27062). Although flipper bands have been documented to decrease annual survivorship and breeding success in several penguin species (Jackson & Wilson 2002), at present there are not many widely available alternatives, especially for observational studies (Stonehouse 1999). Velcro tags (which are not permanent) were not considered as the length of time needed for the birds to be easily identifiable was relatively long (October – end of January) and it was feared the Velcro would not be durable enough. Additionally, the use of standard metal bands means that these focal birds can, in future, be used for further studies and also included in a long term research project on Ross Island that looks at the migration and survivorship of the Ross Island Adelie populations.

Aspects of male breeding recorded (depended on whether focal or large scale study) included arrival date and timing (early/late; Table 2.1), sub-colony nest location (centre/middle/edge; Table 2.1), and nest quality (good/poor/average; Table 2.1). Breeding stages achieved by males were categorised three ways, firstly if they formed a stable pair bond (Table 2.1), then once eggs were laid the male was categorised as having reached the incubation stage (Table 2.1). Lastly, breeding success (Table 2.1) was assigned at the end of January before researchers left the study site. Observations of all focal male breeding behaviours and categories were made daily (often twice daily). Due to logistic constraints observations could not be made during the guard period as researchers departed the Cape Bird study site on the 19th November and returned on the 30th of December. Recording sessions at the colony lasted for a minimum of one hour and during that time all focal male breeding behaviours were noted. Both nest location and quality were assessed throughout the early breeding season to ensure nest location and quality was correctly assigned. Due to the variable nature of Adélie nests which alter size and shape (especially during the early part of the season when males are still arriving at the colony and actively nest building), all quantitative measures taken were deemed too innacurrate. Consequently, a qualitative estimate was assigned by comparing all sub-colony male nest sizes, shapes and structures. A final decision regarding both location and quality was made once focal males had begun the egg incubation stage. The timing of early versus late arriving birds (14 days) was determined by assessing the number of birds arriving daily into the focal sub-colony. When the sub-colony was deemed to be two-thirds occupied (based on previous years nest scrapings) the length of time corresponed to 14 days after the first male had arrived and all males arriving after that day were categorised as late arrivals.

Table 2.1: Definitions of behavioural categories and terms used during male Adélie observations.

Behavioural categories	**Definition**
Arrival	**Early** arrivals were males that arrived in the sub-colony within 14 days of the "first" male arrival. All other males were defined as **Late** arrivals.
Nest location	**Centre** nests were two or more nests from the edge. **Middle** nests were one complete nest circumference from the nearest edge nest. **Edge** nests had some part of their nest circumference exposed to the sub-colony perimeter (Davis & McCaffrey 1986).
Nest quality	Qualitatively described as either **Good** (large size, round shape and with a bowl structure), **Average** (often of large size but lacking shape or bowl structure, or **Poor** (only a few stones collected and placed haphazardly on top of a scraping in the ground).
Breeding stage	
1) Pair bond	Males were considered paired if they maintained a mate for three consecutive days or more. Unpaired males occupied territories but failed to procure a mate for the required minimum length of time.
2) Incubation stage	Males that reached the incubation stage (one or two eggs) but were not necessarily successful in fledging chicks were considered to have reached the incubation stage. Males that failed to achieve a pair bond which laid eggs did not reach the incubation stage.
3) Breeding success	Birds were categorised as successful breeders when they raised at least one chick to independence (Spurr 1974).

2.5 Ecstatic Display Calls

The EDC is described as a series of staccato pulses followed by repetitive hoarse rasps often climaxing in a syllable resembling the Loud Mutual Display (Ainley 1975). For a full description of both visual and vocal elements concerning the EDC see Chapter 1 (section 1.6.4). In this study the EDC has been divided into three audibly distinct components for analysis; the initial staccato pulses (introduction beats), the repetitive rasps (short repeteated syllables) and the final climactic syllable (the long syllable) (Figure 2.3).

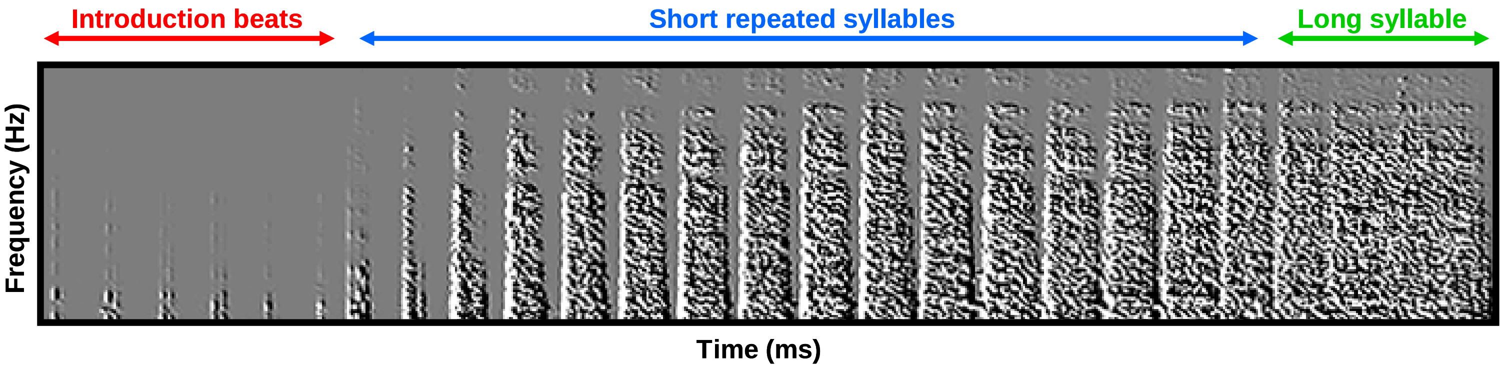

Figure 2.3: Spectral derivative of an Ecstatic Display Call (EDC), the three call sections investigated are highlighted in red (introduction beats), blue (short repeated syllables) and green (long syllable).

2.6 Recording methods

All EDCs (regardless of breeding season) were recorded during two main periods: 1) the early breeding season during nest building and mate choice (28th October -19th November) and 2) the late breeding season during the chick rearing stage (28th December-24th January). All calls were digitally recorded with a Sony (Sony Corporation, Tokyo, Japan) portable minidisc recorder (MZ-R909-sampling frequency 44100 Hz, frequency response 20-20000 Hz ± 3 dB) and a handheld omni directional microphone, either Sennheiser (Sennheiser Inc. Québec, Canada) ME66 (frequency response 50-20000 Hz ± 2.5 dB) or Nakamichi (Nakamichi Corporation, Tokyo, Japan) CM-300 (frequency response 20-16000 Hz ± 3.5 dB). Although the minidisc uses an "ATRAC" (Adaptive Transform Acoustic Coding) system which compresses digital acoustic information, it does not do so within the range of human hearing (Sony Minidisc Manual - www.minidisc.org). Penguin calls fall within the range of human hearing (16-24 000

Hz; Meyer 1986); therefore penguin call data are not likely to have been affected by the minidisc recording system. Wind noise was minimised with custom microphone socks. Birds were recorded from outside the selected sub-colony at a distance of one to three metres. At each sub-colony where birds were recorded the observer stood still and silently during recording and for approximately five minutes before recording began. The birds all appeared acclimatised to observer presence before recording sessions began as behaviours observed prior to observer presence had resumed. Disturbance to the sampled birds and neighbouring birds was kept as brief as possible and as stated in section 2.4 was unlikely to have caused any long term negative effects. Recording was conducted across the 24 hr daylight period, but only when weather conditions were favourable.

2.7 Sound analyses

Calls were digitised to computer (using 16-bit accuracy and a sampling rate of 44100 Hz) using Canary 1.2 (Mitchell *et al.* 1995) and then converted to waveform files (.wav) using Goldwave 4.21 (Craig 2000).

Calls were analysed in the frequency versus time domain using Sound Analysis Pro 1.0 (Swigger *et al.* 2004). Sound Analysis Pro 1.0 allows the user to state the required time resolution and it calculates the appropriate Fast Fourier Transform (FFT) size and degree of overlap in Fourier bins. In this study the temporal resolution was set at 20 ms with 3.02 ms advances, which equates to 84.9% overlap, and a 43.1 Hz frequency resolution using an FFT of 882 samples. A relatively long time window was used, which maximised frequency resolution in order to capture the broad scale structure of the frequency modulations across the calls (Tchernichovski O, *pers. comm.* – The City College of New York). This decreased the time resolution; however the nature of a FFT dictates a time-frequency compromise (Beecher 1988). The duration of the introduction beats were too short (mean length 22.7 ms) to be analysed using an FFT of 882 bins as this sampling frequency cannot accurately represent the time-frequency course of the signal and were therefore not used in this aspect of the study. The average duration of the short repeated syllables were 100.48 ms; however only 4-9% of the syllables were represented by fewer than four unique consecutive Fourier windows (i.e. had a duration less than 80 ms). These data however, were shown not to affect the statistical outcome of any analyses, therefore were included in the study. The long syllables were on average 1214.81 ms and always represented by 17 or more unique Fourier bins. A "Highpass" filter (<200 Hz) was used to remove low frequency background noise. The mimimum frequency estimate recorded for any part of the EDC or LMD was 566 Hz, and therefore a filter removing sounds below 200 Hz was unlikely to have removed any acoustic information. Additionally, a random sample of calls were analysed with and without the filter and no difference in any of the frequency estimates was observed.

Calls were visualised using multi-taper spectral derivatives, which represent the change of power in a time frequency plane and allow the user to see the frequency traces and modulations more clearly than the traditional sonogram (Tchernichovski & Mitra 2004). Two (out of the three) sections of all EDCs (short syllables and long syllable) were measured. The introduction beats and short syllables could be visually separated as introduction beats represent a highly simplistic sound without visible internal structure. As only short syllables were being quantified any syllable less than 40 ms in duration was excluded as it may have represented a form of introduction beat. Calls were quantified using six independent sound parameters (Table 2.2). A more comprehensive description of the call parameters can be found in Tchernichovski *et al*. (2000) and Tchernichovski and Mitra (2004).

Table 2.2: Sound parameter measurements taken from Sound Analysis Pro for analyses of EDC sections (short syllables and long syllable).

Call parameter	Description
Duration	Length of highlighted sound **(mS).**
Pitch	A measure of period oscillation **(kHz)**. In a pure tone the frequency (1/period) is the pitch, in a multi-harmonic tone the pitch is the fundamental frequency (defined by the separation between successive harmonics, and median difference between consecutive contours) (Tchernichovski *et al.* 2000).
Mean Frequency	An estimate of frequency **(kHz)**, calculated using time and frequency derivatives thus following the centre of derivative power across a highlighted sound.
Frequency modulation (FM)	Visually, FM is an estimate of the (absolute) slope of frequency traces in reference to the horizontal line **(degrees)**. Calculated as a ratio between the time and frequency derivatives of the spectrum. A sound rapidly changing in frequency across time has a high FM value, and a sound with constant pitch will have a low FM value.
Amplitude modulation ($AM^{\wedge 2}$)	Overall time-derivative power across all frequencies within a range **(1/ms).**
Entropy	An index of white noise (1) to pure tone (0) measured on a logarithmic scale (white noise: *log*1=0; complete order: *log*0=minus infinity) across highlighted sound.No units are applicable.

The mean call parameters, for a single EDC can be visualised in Figure 2.4. Note the similarity between the pitch and mean frequency estimates in this example. Also the FM clearly drops in the middle section of this EDC. Furthermore, estimates of $AM^{\wedge 2}$ and entropy vary considerably throughout the call.

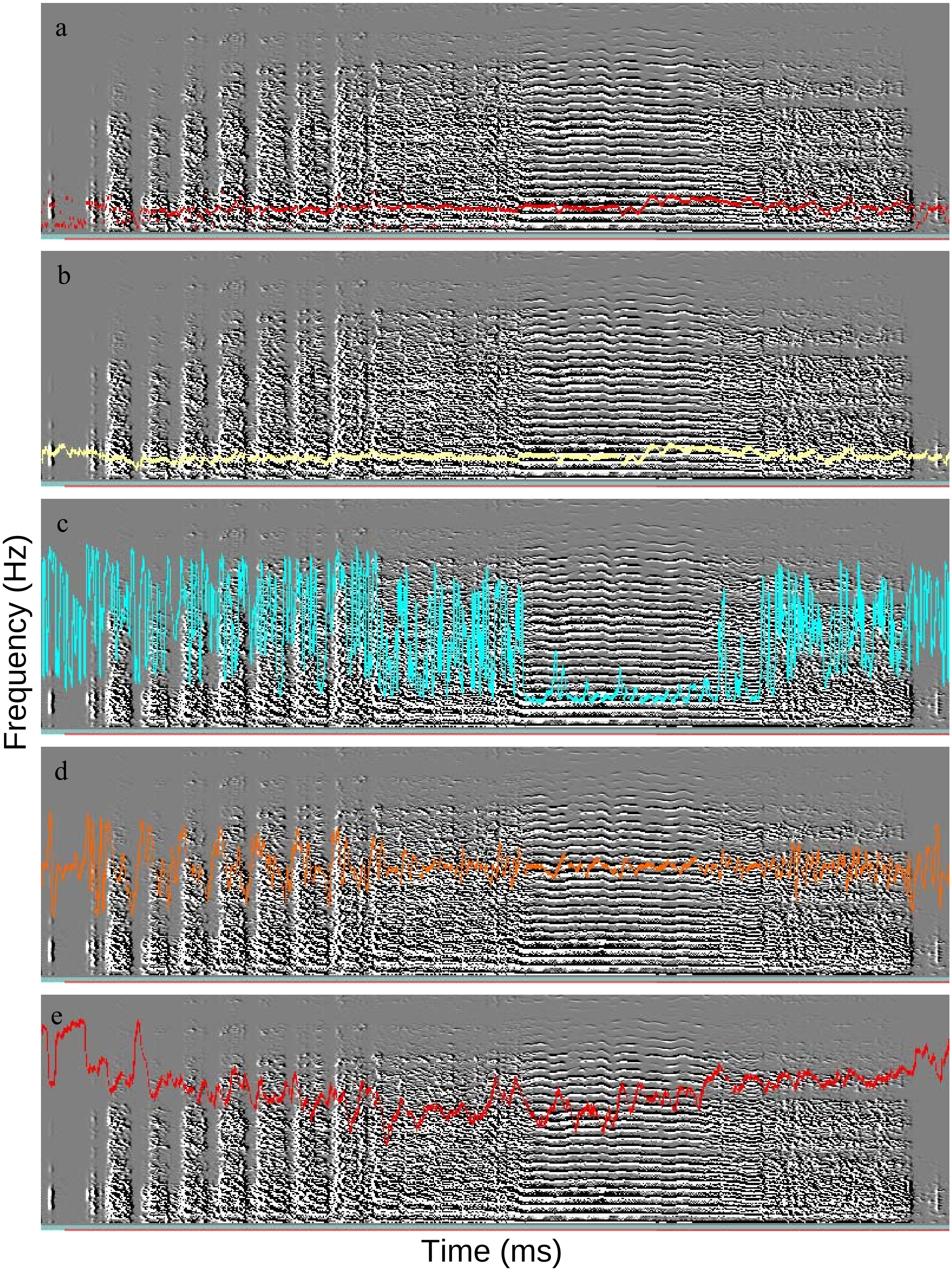

Figure 2.4: Spectral derivative (from Sound Analysis Pro) of a male Ecstatic Display Call showing the mean traces of **a)** pitch, **b)** mean frequency, **c)** FM, **d)** AM^2 and **e)** entropy.

To establish the stability (at the 20 ms time window) of the sound parameter measurements recorded within calls, between individuals and across the season. Initial analyses were repeated on a random sample of calls using time windows of 4.01 ms (0.5 ms advance), 6.46 ms (0.86 ms advance), 9.27 ms (1.36 ms advance), 15.01 ms (2.27 ms advance) and 20 ms (3.02 ms advance). It was found that there was a similar trend for all call parameters (Figure 2.5). Additionally, correlations between individual male weights and all six call parameters were analysed using a time window of 4.01 ms (0.5 ms advance) and a similar trend was observed at either end of the season and across the season. Hence, a 20 ms time window was used in all subsequent analyses.

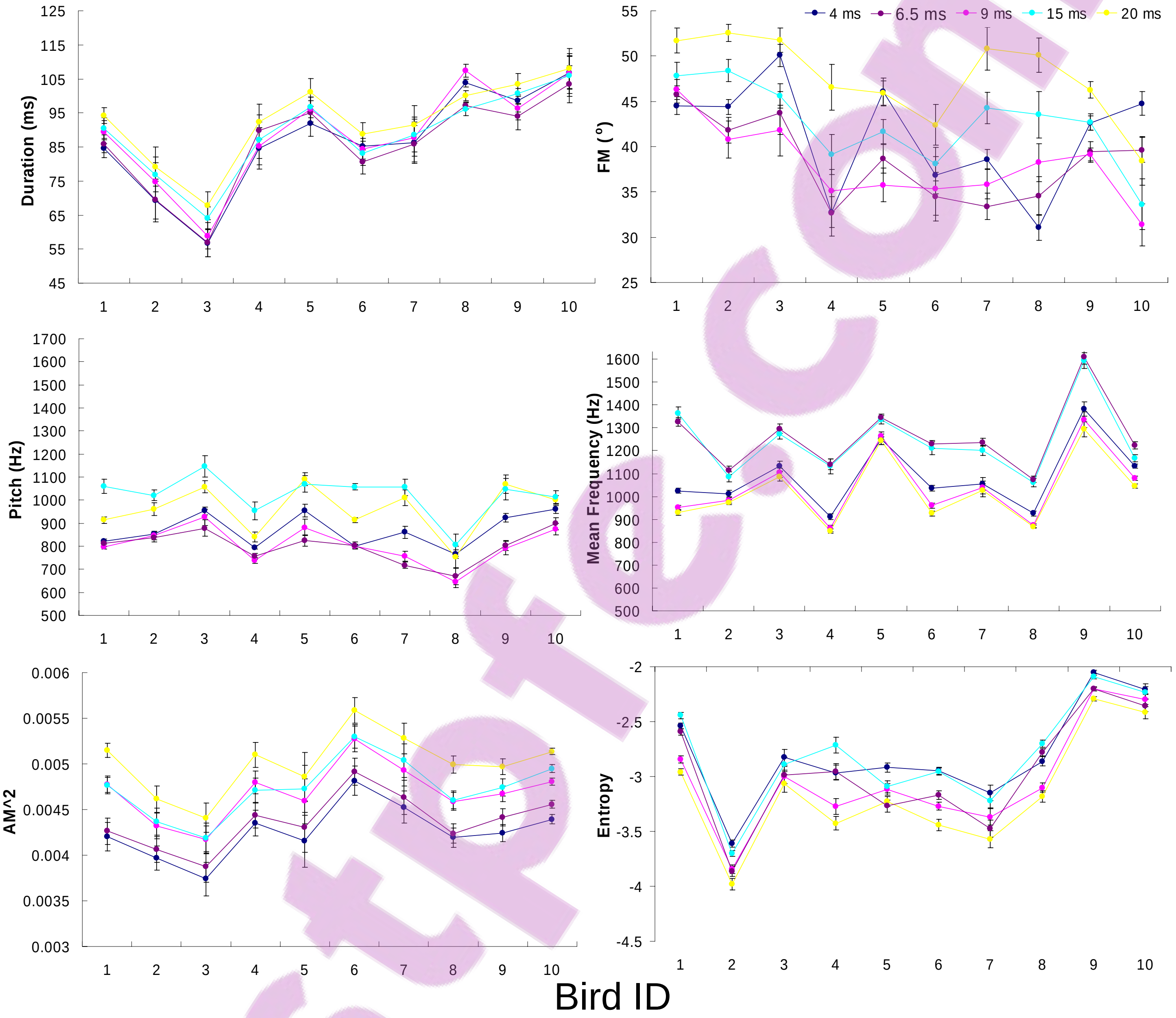

Figure 2.5: Comparison of mean (± SE) EDC parameter stability in Sound Analysis Pro 1.0 using measurements from ten different birds and five time windows of varying size. Key of time window sizes and colours is located in the top right of the figure.

During the focal sub-colony study between one and a maximum of 49 (median 19.5) call replicates per focal bird were available for analysis. Visual inspection of the variances for the six call parameters measured for each of the available call replicates showed a steady decline to relative stability in the variances at three to four call replicates. Penguin calls (Jouventin 1982; Robisson *et al.* 1993; Lengagne *et al.* 1997; Jouventin *et al.* 1999; Jouventin & Aubin 2000), much like blue petrel calls (Genevois & Bretagnolle 1994), show little variation between individual repetitions but vary considerably individually, therefore only individuals that had a minimum of three replicate calls per individual were included in statistical analyses. Due to the scope (number of calls) and inherent recording time limitations in the study of EDCs between Ross Island colonies a single call per bird was recorded and analysed.

2.8 Statistical analyses

All data were checked for normality and heterogeneity of variance and General Linear Models (GLM) were used when these assumptions were met. GLMs were used to compare group (colony, sub-colony, nest specific parameters, arrival and breeding stage) and male effects (weight and call parameters). Least square means (LSM) of call parameters were calculated for analyses. When group size was unbalanced and sample sizes small or the assumption of variance heterogeneity was not met the Welch ANOVA was used (Zar 1999). Small sample sizes (N<20) when comparing call parameters, weight change and mate choice meant non-parametric Spearman's Rank Correlation coefficients were calculated. Nominal logistic regression was used to compare focal male individual and nest parameters and breeding stage achieved. Categorical data were analysed using Chi-squared analyses, when observed frequencies were low; *P* values were calculated with an Exact Contingency Table using online software (http://www.physics.csbsju.edu/stats/). Fisher's Exact (two tailed) analyses were performed on data comparing individual male weight change and call parameters, using a 2x2 contingency table and GraphPad Software (GraphPad Software Inc. San Diego, USA). All other statistical analyses were performed in JMP 5.1 (SAS Institute, 2004). Data analysed in JMP 5.1 that were unbalanced was assigned an averaged denominator degree of freedom, which was reported with one decimal place where appropriate. Unless otherwise stipulated, all data cited in text are mean ($\overline{X}$) ± standard deviation (SD) and all tests used a level of significance of $\alpha = 0.05$.

2.9 Individual identification using EDC parameters

To confirm the validity of the assumption that EDCs are more varied between individuals than within individuals (Jouventin 1982), a sample of n = 29 birds were recorded, and three calls per bird were analysed for differences between birds for each of the five call parameters for the short and long syllables in the EDC; the whole call was tested for duration and number of syllables. Additionally, the short repeated syllables were analysed to find out whether each call within an individual varied significantly. All calls were recorded from a single colony (Cape Bird North), during the same part of the season (2002/3 late breeding season), to minimise any potentially confounding effects of colony and season. Results from all sections (short, long and whole) of the EDC clearly show the individual nature of this call with all call parameters being highly significant (Table 2.3 & 2.4a & b). Additionally, an individual birds' calls for the short repeated syllables also varied significantly for each call parameter (Table 2.3). Figure 2.6 shows the variation in structure in three individual EDCs.

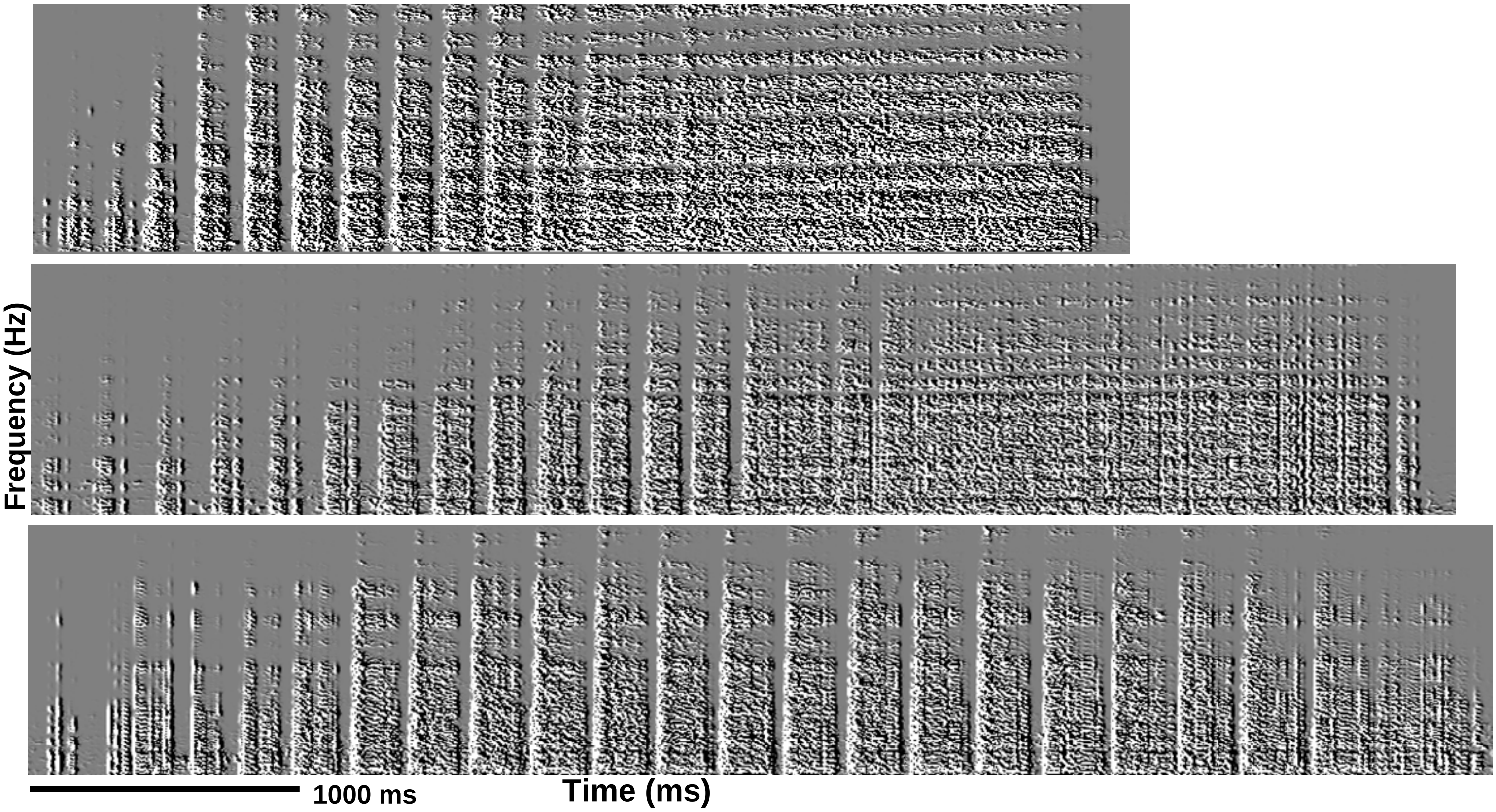

Figure 2.6: Comparison of three individual EDCs visualised using spectral derivitives (from Sound Analysis Pro 1.0).

Table 2.3: Individual bird call parameters (*n* = 29) of the short repeated syllables of the EDC.

Short syllables		R^2	*df*	*F*	*P*
Duration		0.552			
	Bird ID		28,59.1	12.365	**< 0.0001**
	Call ID		58,742	2.24	**< 0.0001**
Pitch		0.592			
	Bird ID		28,58.6	6.914	**< 0.0001**
	Call ID		58,742	4.341	**< 0.0001**
Mean Freq		0.835			
	Bird ID		28,58.2	7.292	**< 0.0001**
	Call ID		58,742	14.534	**< 0.0001**
FM		0.411			
	Bird ID		28,59.1	5.595	**< 0.0001**
	Call ID		58,742	2.429	**< 0.0001**
AM$^{\wedge2}$		0.735			
	Bird ID		28,58.3	6.54	**< 0.0001**
	Call ID		58,742	8.115	**< 0.0001**
Entropy		0.747			
	Bird ID		28,58.2	3.869	**< 0.0001**
	Call ID		58,742	13.35	**< 0.0001**

Note: Significant *P* values are highlighted in **bold**. Units of measurement - see Table 2.2.

Table 2.4 a & b: Individual bird and call parameters (*n* = 24) of the long end syllable and whole call parameters of the EDC.

a) Long syllable	R^2	*df*	*F*	*P*
Duration	0.752	23,48	6.339	**< 0.0001**
Pitch	0.715	23,48	5.226	**< 0.0001**
Mean Freq	0.779	23,48	7.351	**< 0.0001**
FM	0.877	23,48	14.826	**< 0.0001**
AM$^{\wedge2}$	0.696	23,48	4.784	**< 0.0001**
Entropy	0.798	23,48	8.255	**< 0.0001**
b) Whole call				
Duration	0.781	23,48	7.42	**< 0.0001**
No. syllables	0.808	23,48	8.778	**< 0.0001**

Note: Significant *P* values are highlighted in **bold**.Units of measurement - see Table 2.2.

Pair-wise correlations for each call parameter within each call section were compared to see how these factors varied in the EDC as a whole. The short repeated and long end syllable showed some similar patterns of correlations (Table 2.5). The two frequency parameters (pitch and mean frequency) were unsurprisingly positively correlated; they also were both positively correlated with entropy, which relates to an increase in the noise in the calls with increased pitch and mean frequency. In the short syllables both these parameters were also correlated with duration, however this pattern was not observed in the long syllable. In the short syllables there was less amplitude modulation in syllables of shorter duration. Lower AM^2 was also linked with higher entropy (noisier calls). The long syllable also had a negative relationship with AM^2, this time it was with FM. The whole call parameters showed a significant positive correlation between total call duration and number of syllables (*r* = 0.596, *n* = 24, *P* = < 0.0001).

Table 2.5: Pair-wise correlations for both short (across; *n* = 29) and long (down; *n* = 24) syllables of the EDC, significant *P* values are highlighted in bold, correlation coefficients (*r*) and direction of correlation is signified by colour (red = positive, blue = negative). Units of measurement - see Table 2.2.

Short > **V Long**	**Duration**	**Pitch**	**Mean Freq**	**FM**	**AM^2**	**Entropy**
Duration	-	+ 0.168 **< 0.0001**	+ 0.184 **< 0.0001**	- 0.036 0.301	- 0.13 **0.0002**	+ 0.005 0.881
Pitch	- 0.036 0.764	-	+ 0.794 **< 0.0001**	- 0.026 0.459	- 0.058 0.098	+ 0.229 **< 0.0001**
Mean Freq	+ 0.045 0.706	+ 0.75 **< 0.0001**	-	- 0.015 0.658	+ 0.031 0.375	+ 0.25 **< 0.0001**
FM	+ 0.008 0.949	- 0.134 0.261	- 0.068 0.573	-	- 0.051 0.143	- 0.051 0.145
AM^2	- 0.013 0.916	- 0.058 0.627	+ 0.149 0.212	- 0.321 **0.006**	-	- 0.217 **< 0.0001**
Entropy	- 0.173 0.147	+ 0.453 **0.0001**	+ 0.385 **0.0008**	+ 0.018 0.882	- 0.075 0.534	-

General statistics describing the EDC can be seen in Table 2.6. The EDC has on average 12 repeated syllables (of roughly 100 ms), followed by a longer syllable (usually over one second in length), giving a total average length of call of nearly three seconds. The total length of EDCs recorded during this analysis concurs with sonograms measured by Penney (1968). The pitch of the call varies greatly but is on average 965 Hz in the short syllables and slightly lower in the long end syllable (914 Hz). Mean frequency values were similar to pitch estimates for both sections of the EDC, they were however on average slightly higher, which may reflect the method by which this parameter is calculated (section 2.7; Table 2.2). FM, AM$^{\wedge 2}$ and entropy have not been described for the EDC; in this analysis the short syllables were shown to have higher FM in the short syllables as compared to the long, but both sections of the EDC have similar AM$^{\wedge 2}$ and entropy measures.

Although no specific call parameters could be deduced from the introduction beats, as the sound analyses used were not adapted for such short segments of sound (section 2.7), the mean length of these sounds was calculated to be 22.71 ± 5.62 ms (n = 28). The introductory beats are given at a uniform in rate (7-8 pulses per second); and last approximately 3-7 seconds (Penney 1968).

Table 2.6: General characteristics of the EDC (n = 29 – short, n = 24 long and whole) and LMD (n = 25), EDCs have been coloured red and LMD calls black. Units of measurement - see Table 2.2.

EDC section	Mean ± SD	Minimum	Maximum	Range
Short syllables				
Duration	100.48 ± 14.28	57.30	180.95	123.65
	104.54 ± 17.56	72.38	159.84	87.46
Pitch	965.54 ± 157.66	566	2209	1643
	1172.34 ± 261.99	640	2243	1603
Mean Freq	1042.15 ± 200.99	671	2418	1747
	1523.91 ± 339.35	891	2943	2052
FM	48.88 ± 6.64	22.1	67.7	45.6
	45.24 ± 7.83	22.9	62.9	40
AM$^{\wedge 2}$	0.0047 ± 0.0008	0.0001	0.006	0.0059
	0.0055 ± 0.0004	0.004	0.0065	0.0025
Entropy	-2.57 ± 0.44	-4.34	-1.39	2.95
	-1.77 ± 0.46	-3.51	-0.6	2.91
Long syllable				
Duration	1214.81 ± 488.44	343.81	2738.41	2394.6
	597.18 ± 172.81	325.71	1043.49	717.78
Pitch	914.63 ± 105.7	671	1192	521
	1045.61 ± 214.2	671	1540	828
Mean Freq	990.08 ± 128.29	773	1286	513
	1540.57 ± 255.07	1007	2178	1171
FM	39.39 ± 10.11	10.8	56.1	45.3
	34.25 ± 9.12	14.3	50.1	35.8
AM$^{\wedge 2}$	0.005 ± 0.0005	0.0033	0.00585	0.0026
	0.0055 ± 0.0004	0.0042	0.00614	0.002
Entropy	-2.67 ± 0.44	-3.63	-1.7	1.93
	-1.78 ± 0.52	-3.51	-1.07	2.44
Whole call				
Duration	2587.45 ± 694.06	980.16	4535.87	3555.71
	1093.99 ± 292.98	630.32	2231.75	1601.43
No. syllables	12.08 ± 3.99	4	22	18
	3.95 ± 1.79	1	10	9

2.10 LMD call versus EDC

Due to the fact the LMD call and the EDC resemble each other with respect to call structure and acoustic parameters (Ainley 1975) and may in fact represent an equivalent call given at different intensities (e.g. EDC – low & LMD call – high intensity); a comparison of recorded EDC and LMD calls was made to see how different these calls were. Visually, the differences between the EDC and LMD call can be seen in Figure 2.7. Results clearly show that an individual birds' LMD call has distinctive call properties (Table 2.7 & 2.8a & b) which concurs with studies by Jouventin (1982). It is also unsurprising given the belief that this call specifically is used for mate and chick recognition (Ainley 1975; Penney 1968; Spurr 1975). Notably, for the short syllables the variation in calls from a single bird shows less variation than for the EDCs with both duration and FM having all three calls with essentially the same values.

Table 2.7: Nested random effects (ID & Call ID) ANOVA of individual bird call parameters (n = 25) of the short repeated syllables of the LMD call. A Bonferroni correction of 0.05/6 = 0.008 applies therefore only calculated P values below this threshold are considered significant.

Short syllables		R^2	*df*	*F*	*P*
Duration		0.577			
	Bird ID		24,51	13.352	**< 0.0001**
	Call ID		50,220	0.789	0.84
Pitch		0.455			
	Bird ID		24,50.9	2.483	**0.003**
	Call ID		50,220	1.683	**0.006**
Mean Freq		0.784			
	Bird ID		24,50.2	3.055	**0.0004**
	Call ID		50,220	6.412	**< 0.0001**
FM		0.581			
	Bird ID		24,51	6.39	**< 0.0001**
	Call ID		50,220	1.535	0.02
AM$^{\wedge 2}$		0.782			
	Bird ID		24,50.3	4.808	**< 0.0001**
	Call ID		50,220	4.806	**< 0.0001**
Entropy		0.782			
	Bird ID		24,50.2	3.047	**0.0004**
	Call ID		50,220	6.561	**< 0.0001**

Note: All significant P values are highlighted in **bold**. Units of measurement - see Table 2.2.

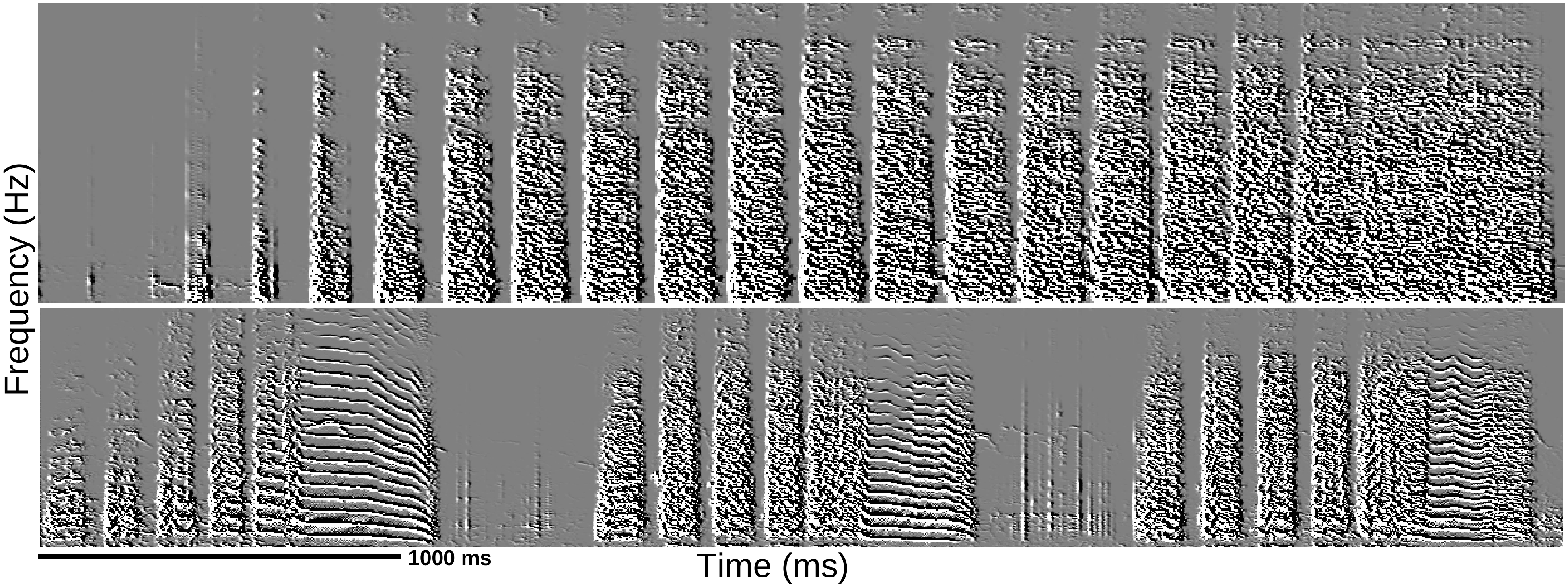

Figure 2.7: Spectral derivatives (from Sound Analysis Pro) of **a)** a single Ecstatic Display Call and **b)** a Loud Mutual Display call (repeated three times).

Table 2.8: Univariate ANOVA of individual variation in the long syllable and whole call of the LMD (n = 25). A Bonferroni correction applies to the long syllable (0.05/6 = 0.008) and whole call (0.05/2 = 0.01) data. Only calculated P values below these thresholds are considered significant.

a) Long syllable	R^2	*df*	*F*	*P*
Duration	0.88	24,50	15.29	**< 0.0001**
Pitch	0.776	24,50	7.226	**< 0.0001**
Mean Freq	0.682	24,50	4.472	**< 0.0001**
FM	0.8	24,50	8.342	**< 0.0001**
AM$^{\wedge 2}$	0.844	24,50	11.274	**< 0.0001**
Entropy	0.837	24,50	10.712	**< 0.0001**
b) Whole call				
Duration	0.873	24,50	14.256	**< 0.0001**
No. syllables	0.885	24,50	16.041	**< 0.0001**

Note: Significant *P* values are highlighted in **bold**. Units of measurement - see Table 2.2.

Table 2.9: Univariate ANOVA of LMD (n = 25) and EDC parameters (n = 29 short, n = 24 long & whole) for the short, long and whole call sections. A Bonferroni correction of 0.05/6 = 0.008 applies therefore only calculated P values below this threshold are considered significant.

Call section	R^2	*df*	*F*	*P*	**EDC >/< LMD**
Short syllables					
Duration	0.88	1,52	6.321	0.015	
Pitch	0.763	1,52	31.539	**< 0.0001**	EDC < LMD
Mean Freq	0.819	1,52	74.697	**< 0.0001**	EDC < LMD
FM	0.785	1,52	10.913	**0.002**	EDC > LMD
AM$^{\wedge 2}$	0.84	1,52	40.167	**< 0.0001**	EDC < LMD
Entropy	0.825	1,52	71.447	**< 0.0001**	EDC < LMD
Long syllable					
Duration	0.866	1,47	44.841	**< 0.0001**	EDC > LMD
Pitch	0.795	1,47	9.251	**0.004**	EDC < LMD
Mean Freq	0.896	1,47	124.735	**< 0.0001**	EDC < LMD
FM	0.852	1,47	4.038	0.05	
AM$^{\wedge 2}$	0.816	1,47	19.056	**< 0.0001**	EDC < LMD
Entropy	0.899	1,47	43.79	**< 0.0001**	EDC < LMD
Whole call					
Duration	0.932	1,47	119.459	**< 0.0001**	EDC > LMD
No. syllables	0.936	1,47	101.639	**< 0.0001**	EDC > LMD

Note: Significant *P* values are highlighted in **bold**. Direction of difference between EDC and LMD is signified by </> symbols. Units of measurement - see Table 2.2.

Also evident from this study is that the LMD call differs markedly from the EDC in several aspects (Table 2.9). To begin with the EDC has greater numbers of short syllables and although no difference in the short syllable lengths, greater length long syllables giving a greater call duration in total. The pitch and mean frequency is consistently higher in the LMD call, and both sections also have higher entropy scores, which equates to noisier calls than found in the EDC. The amplitude modulation is also greater in the LMD call for short and long syllables, the FM was however only lower in the LMD call in the short syllables and not found to be different in the long syllable. Although the EDC and LMD call resemble each other structurally by having repeated short syllables followed by a longer syllable, in fact these calls have quite different call parameters. As amplitude modulation is important for recognition (Jouventin & Aubin 2002) in the Adélie penguin the higher AM$^{\wedge 2}$ in the LMD may aid in recognition.

The differences between the LMD call and EDC are summarised in Table 2.6, which shows the mean and standard deviation plus the range of values for each call parameter. What becomes clear from these data is that the EDC is on average over twice the length of an LMD call, however the LMD can be repeated many times and according to Penney (1968) each “song” may last 1-10 seconds (depending on motivation). Penney (1968) also using sonograms measured what he called “phrases”, here termed the LMD call, and found each phrase to last 1-2 seconds, which concurs with findings in Table 2.6, which reports a mean duration of 1093.99 ± 292.98 ms for the LMD call. Although pitch values are consistently lower in the EDC, the ranges of frequencies are similar for both call types, in both sections of the call. Mean frequency is also consistently lower for the EDC call but also occurs over a smaller range of frequencies than the LMD call. Jouventin (1982) measured the frequency parameters of 15 individuals for the LMD call and found the mean frequency of the calls to be 1432 Hz. This is similar to what was reported in this study (short – 1523.91 ± 339.35, long – 1540.54 ± 255.07 Hz). Although Jouventin’s estimates are lower for frequency they fall within the standard deviation range reported in this study. The maximum AM$^{\wedge 2}$ for both call types is similar (≈ 0.0065) but the minimum for the short syllables in the EDC is considerably less (0.0001), which means essentially no AM$^{\wedge 2}$ was found in some short syllables. FM was not as different between the call types, there was however a trend for both types to have lower FM in the long syllable as compared to the short repeated syllable. Although the entropy scores were less negative in general in the LMD call, both call types had a similar range of scores, noticeably the noisiest (score closest to zero) call section was found in the short syllables of the LMD call (-0.6).

2.11 Sex differences in the EDC

Given the EDC is reported by several researcher to be given by both male and female Adélie penguins (Ainley 1975; Jouventin 1982; Penney 1968; Spurr 1975), it is not surprising that in Section 2.1.2 there were several females sampled giving this call. In the late breeding season there were a total of 37 females recorded, which could then be compared with a sample of male calls to see if the calls differed in any way. To see if the female Adélie penguins were producing calls that were also individual, a sample of three calls per bird were checked for each call section for each call parameter. Results show clearly that like the male Adélie (Section 2.8) the EDC given by the females is able to be classified as an individual call (Table 2.10 & 2.11a & b).

Table 2.10: Nested random effects (ID & Call ID) ANOVA of individual female bird call parameters (n = 15) of the short repeated syllables of the EDC. A Bonferroni correction of 0.05/6 = 0.008 applies therefore only calculated P values below this threshold are considered significant.

Short syllables		R^2	*df*	*F*	*P*
Duration		0.557			
	Bird ID		14,30.5	14.508	**< 0.0001**
	Call ID		30,308	1.572	0.032
Pitch		0.52			
	Bird ID		14,30.4	11.462	**< 0.0001**
	Call ID		30,308	1.701	0.015
Mean Freq		0.778			
	Bird ID		14,30.3	23.021	**< 0.0001**
	Call ID		30,308	2.924	**< 0.0001**
FM		0.504			
	Bird ID		14,30.5	11.89	**< 0.0001**
	Call ID		30,308	1.585	0.03
AM^2		0.697			
	Bird ID		14,30.5	29.624	**< 0.0001**
	Call ID		30,308	1.573	0.032
Entropy		0.822			
	Bird ID		14,30.2	23.448	**< 0.0001**
	Call ID		30,308	3.93	**< 0.0001**

Note: Significant P values are highlighted in **bold**. Units of measurement - see Table 2.2.

Table 2.11: Univariate ANOVA of individual female variation in the long syllable and whole call of the EDC (n = 13).

a) Long syllable	R^2	*df*	*F*	*P*
Duration	0.777	12,30	7.689	**< 0.0001**
Pitch	0.689	12,30	4.183	**0.0007**
Mean Freq	0.834	12,30	11.963	**< 0.0001**
FM	0.729	12,30	6.028	**< 0.0001**
AM$^{\wedge 2}$	0.851	12,30	13.629	**< 0.0001**
Entropy	0.827	12,30	11.933	**< 0.0001**
b) Whole call				
Duration	0.856	12,30	11.224	**< 0.0001**
No. syllables	0.7165	12,30	5.892	**< 0.0001**

Note: Significant *P* values are highlighted in **bold**. Units of measurement - see Table 2.2.

Interestingly, the female EDC for the short repeated syllable shows a greater degree of consistency within each individual compared to the male (Table 2.3) with most parameters (except for entropy and mean frequency) being not significantly different between calls (Table 2.10). Once the individuality of the female EDC was established a sample of only one call (to increase effective sample size) per female was then compared to a sample of male EDCs. As females were recorded in all three Bird colonies, the model also incorporated the effect of colony and the interaction of colony and sex. If not reported in the table these effects were non-significant. The results for the short, long and whole call parts of the EDC show that there were no significant differences in any of the call parameters between male and female EDCs (Table 2.12). Additionally, the weights at the end of the breeding season were not different between the sexes (male $\overline{X}$ = 4.25 ± 0.43 kg: female $\overline{X}$ = 3.99 ± 0.51 kg; R^2 = 0.132, $F_{1,70}$ = 1.495, P = 0.226). However, males had significantly longer wing lengths than females (male $\overline{X}$ = 18.94 ± 0.62 cm: female $\overline{X}$ = 18.39 ± 0.67 cm; R^2 = 0.198, $F_{1,70}$ = 7.939, P = 0.006). So although males appear to have larger skeletal frames than females, at the end of the breeding season there appears to be no difference in weights or call parameters in the EDCs.

Table 2.12: Univariate ANOVA of sex differences in the short, long and whole sections of EDC (male *n* = 39, female *n* = 36). A Bonferroni correction of 0.05/6 = 0.008 applies for both short and long syllable data therefore only calculated *P* values below this threshold are considered significant. Whole call data has an adjusted *P* value of 0.05/2 = 0.025.

Call section	R^2	*df*	*F*	*P*
Short syllables				
Duration	0.661	1,70.7	3.168	0.079
Pitch	0.561	1,71.1	0.665	0.418
Mean Freq	0.819	1,70.3	0.342	0.56
FM	0.503	1,71.4	0.058	0.811
AM^2	0.732	1,70.5	2.405	0.125
Entropy	0.788	1,70.4	1.521	0.222
Long syllable				
Duration	0.179	1,70	3.573	0.063
Pitch	0.09	1,70	0.061	0.806
Mean Freq	0.041	1,70	0.837	0.363
FM	0.013	1,70	0.076	0.783
AM^2	0.179	1,70	2.557	0.114
Entropy	0.083	1,70	3.532	0.064
Whole call				
Duration	0.141	1,70	4.366	0.04
No. syllables	0.054	1,70	1.281	0.262

Note: Significant *P* values are highlighted in **bold**. Units of measurement - see Table 2.2.

2.12 References

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3

3.1 Abstract

The Adélie penguin (*Pygoscelis adeliae*) is unique amongst penguins by having two distinct display calls, the mutual display (used for individual recognition) and the Ecstatic Display Call (EDC, used for mate choice and territory defence). These two call types may show different patterns of geographical variation. Although there are low rates of migration in Adélie, no geographic variation has been found in the mutual display call. However, the potential for geographic variation in the EDC has not yet been studied in detail and is the focus of this chapter. The possibility that the EDC possesses geographic variation because of its duality of function and its freedom from the ties of recognition was investigated in three colony areas of Ross Island, Antarctica during two breeding seasons (2000/1 and 2002/3). The stability of the call was examined across a single breeding season, between colonies and concurrently with estimates of colony breeding success. Results indicated that some call parameters (pitch, Frequency Modulation, Amplitude Modulation, entropy and total call duration) varied between Ross Island colonies and that these parameters varied with both geographical location and colony size. In conjunction with variation in call parameters, the breeding success of the colonies was predictably different; larger colonies with better access to open water and potential foraging grounds had greater success and produced chicks in better condition. The stability of call parameter differences requires further study as data from Cape Bird indicated that not only did the parameters that separate colonies alter between breeding years, but that they also altered during a single breeding season. The observed variation in call parameters may be akin to variation in breeding success and thus possibly linked to environmental conditions, sub-colony nesting position, and condition of the colonies. The EDC whilst retaining its individuality appears remarkably changeable, consequently before geographic variation can be ascertained in this species more studies over time, with varying environmental conditions, between more disparate colonies need to be undertaken.

3.2 Introduction

3.2.1 Variation in song

Bird vocalisations are enormously varied as a result of a diverse set of influences and selection pressures. These include phylogeny, body size, sound function and habitat (Becker 1982), as well as sexual selection (Catchpole & Slater 1995). Vocal variation occurs across a range of spatial and temporal scales, thus calls can vary individually, geographically, seasonally, and yearly, with the function and evolution of these signals being of great interest (Catchpole & Slater 1995). Three main hypotheses have been proposed to describe observed avian vocal variation. Firstly, ecological adaptation may drive the evolution of song (Mayr 1963). However, adaptation to divergent ecologies has typically explained only minimal proportions of variation in song structure (Badyaev & Leaf 1997; Buskirk 1997). Secondly, changes in morphology may explain variation between species (Podos 2001) by being an indirect result of adaptation to differing ecologies. Lastly, species recognition may promote vocal variation whereby calls that have evolved in closely related species occurring sympatrically might be expected to be dissimilar to reduce the effects of hybridisation (Miller 1982). These three hypotheses have shown to be potentially responsible for observed vocal variation in some species (e.g. 163 species of sub-oscine antbirds; Seddon 2005). However, there is little consensus in the literature, and causes of call variation remain to be conclusively understood (Boughman 2002).

Geographic vocal variation can be thought of on micro- and macro- scales. A dialect (also known as micro-geographic variation) usually refers to variation in calls between local populations that could interbreed, and geographical variation (often referred to as macro-geographic variation) tends to suggest long distance variation in calls associated with populations that tend not to interbreed (Catchpole 1979). By definition, only geographic differences that arise through learning can be considered a dialect (Kroodsma 2004). Although vocal learning is thought to have arisen independently three times during the evolution of birds (Kroodsma 1982), most work has focused on passerines, specifically the oscines (Mundinger 1982); for example the much studied song of the white-crowned sparrows of the genus *Zonotrichia* (Cunningham & Baker 1987; Nelson 1998; MacDougall-Shackleton & MacDougall-Shackleton 2001; MacDougall-Shackleton *et al.* 2002; Nelson & Soha, 2004 and Soha *et al.* 2004). Far fewer studies concerning dialects have been undertaken amongst the parrots or hummingbirds (Kroodsma *et al.* 1996: but see Baker 2003; Bond & Diamond

2005; Bradbury 2001; Gaunt *et al.* 1994; Kleeman & Gilardi 2005; Wanker & Fischer 2001; Wiley 1971; Wright 1996 and Wright & Dorin 2001), but even less research has been devoted to species that are not thought to learn their calls but also exhibit vocal geographic variation. Several examples can be found in the seabird literature however; e.g. the snow petrel - *Pagodroma nivea* (Barbraud *et al.* 2000), blue petrel - *Halobaena caerulea* (Bretagnolle & Genevois 1997) and Wilson's storm petrel - *Oceanites oceanicus* (Bretagnolle 1989). Penguins, a group of highly specialised, widely ranging, aquatic seabirds have not been studied in detail with regards to vocal geographic variation.

While geographic variation has been studied in a range of species (Catchpole 1979), yearly variation in individual acoustic signals has been poorly studied, especially in long lived species (Lengagne 2001). Compounding this, results are contradictory, Wooler (1978) established the constancy of calls in kittiwakes (*Rissa tridactyla* L.) between years by examining spectrograms and Peak *et al.* (1998) found corncrake (*Crex crex*) calls to be similar across a two year period. Additionally, Lengagne (2001), found the eagle owl (*Bubo bubo*) to have similar call parameters across the two years of recording. Conversely, call parameters that identified individual bald eagles (*Haliaeetus leucocephalus*) varied between years (Eakle *et al.* 1989). Like geographic variation, how species acquire their songs is important. For song learning species, songs and song types can vary considerably between years and over time (Catchpole & Slater 1995); for example in the yellow-rumped caciques (*Cacicus cela*), up to 78% of songs varied from one year to the next (Trainer 1989). In species where there is no adult song learning the rate of change between years is considerably slower as new song types can only arise through immigration or inaccurate replication of songs by immature birds (Catchpole & Slater 1995). Consequently, the stability of most species calls over time still requires investigation, especially when estimating geographical variation and species specific call characteristics.

Importantly, most studies relating avian vocalisations to geographic variation occur during the breeding season (Nowicki 1983). In several species changes in call characteristics within a single breeding season have been examined, but often only in regards to changes in breeding status and energetics associated with breeding (e.g European starling – *Sturnus vulgaris*: Henry *et al.* 1994; vermilion flycatcher – *Pyrocephalus rubinus*: Chelen *et al.* 2005; and hoopoe – *Upupa epops*: Martin-Vivaldi *et al.* 1998). It is possible however, that changing call characteristics may be affecting patterns of geographical variation. Additionally, calls that vary spatially may not persist outside the breeding season even in species that call throughout the year (Warren 2003).

This is a possibility that has not been investigated in depth (DeWolfe & Baptista 1995; Nowicki 1983; Warren 2003). It is therefore pertinent to establish whether patterns of call variation are stable within the breeding season and (if possible) between years before ascribing vocal differences to populations of birds.

3.2.2 Penguin calls and variation

While geographic variation in vocalisations of species that develop an inherited call has received some attention, the spatial and temporal variation in penguin vocalisations (which are not believed to learn their calls) is still largely unstudied. Interspecific differences in penguin calls have been analysed across groups of penguins (*Spheniscus* penguins: Thumser *et al.* 1996; Thumser & Ficken 1998), and in conjunction with different life history traits and recognition requirements of particular species (Jouventin & Aubin 2000; Jouventin & Aubin 2002; Robisson 1990; Robisson 1992). A large body of work has also examined the adaptations of particular species calls to extreme environments, for example the noisy, windy, emperor (*Aptenodytes forsteri*) and king (*Aptenodytes patagoniscus*) colonies (Aubin & Jouventin 1998; Aubin & Jouventin 2002; Jouventin *et al.* 1999; Lengagne *et al.* 1999a; Lengagne *et al.* 1999b; Lengagne *et al.* 2000; Robisson 1991; Robisson *et al.* 1993). Species variation has mainly been examined for vocal repertoires; most notably the work of Jouventin (1982) described the basic call parameters and function of calls for most penguin species. That same study also examined geographic variation within intra-specific calls, when differences were found they were most often ascribed by the separation of sub-species (e.g. king penguins from Macquarie, Kerguelen and Crozet Island (*A. p. patagonicus*) have shorter calls with fewer syllables than Falkland Island penguins (*A. p. halli*), which corresponds to the suggested location of the two sub-species). However, for the gentoo penguin (*Pygoscelis papua*) which also has two sub-species, *P. p. papua* and *P. p. ellsworthi*, the differences found in the call parameters and the distribution of sub-species did not directly overlap. Therefore although taxonomic clarification has been used to explain most vocal variation in penguins it is not a completely compelling argument.

3.2.3 Adélie display calls

The Adélie penguin (*Pygoscelis adeliae*) is likely the most common penguin species (Ainley *et al.* 1983) and also one of the most intensively studied. The Adélie penguin has been shown to have low rates of migration (Ainley 2002) and high rates of natal colony site fidelity (Ainley *et al.* 1983; Ainley 2002), therefore it is possible that even on a single island where birds can walk between colonies (e.g. Cape Bird), that the vocal environment is stable enough to allow the potential for a colony to evolve different call parameters. Despite behavioural studies showing low rates of migration, genetic studies have shown surprisingly little genetic dissimilarity between Adélie penguin colonies (Roeder *et al.* 2001). However, recent work using ancient DNA compared to current population DNA has shown considerable variation in microsatellite nuclear gene frequencies (hence micro-evolutionary change) over a relatively short timescale (≈6 000 years) (Shepherd *et al.* 2005). Therefore, notwithstanding the high degree of genetic variability in Adelie populations, genetic drift and the relatively high mutation rates (Shepherd *et al.* 2005) may yet be having an effect on the evolution of Adélie penguin calls.

The vocal characteristics of this species have mainly been investigated in relation to mutual display calls associated with individuality and recognition of mate and chick (Davis & McCaffrey 1989; Jouventin 1982; Jouventin & Aubin 2002; Lengagne *et al.* 1997; Müller-Schwarze & Müller-Schwarze 1980; Speirs & Davis 1991). The little work concerning geographic variation has shown that calls of birds from Caird Coast and Adélie Land (on opposite sides of the continent) did not differ (Jouventin 1982). However, a relatively small sample of calls was used (South Orkneys $n = 12$ and Adélie Land $n > 50$) and analyses were performed on the "Mutual Display" call. Unique to the Adélie penguin is the presence of a second distinct display call the male Ecstatic Display Call (EDC) (Jouventin 1982). The EDC has two functions, firstly to defend the nesting territory of the male and secondly, to attract a mate (Penney 1968; Ainley 1975; Spurr 1975). Unlike the "Mutual Display", the EDC is not generally considered important in pair bond maintenance or parent-chick recognition (Jouventin 1982). This duality in function and independence from recognition requirements gives rise to a possibility for greater variability and therefore the potential for change not just in the individual but also between populations. It is this question of stability in the EDC that has yet to be answered. Performance parameters (i.e. how often a male calls or the timing of calling bouts) may also be relevant to assessing variation in EDC calls; however they were outside the scope of this study. To my knowledge no study published has examined the stability of variation in call parameters within a breeding season and between colonies over two seasons using the EDC of the Adélie penguin.

3.2.4 Aims

The EDC has only previously been studied in terms of its functionality and individuality; the stability of the EDC parameters and variation between geographical areas over time has yet to be investigated and is the focus of this chapter. Specifically, the aims of this chapter are to investigate the variability in the male EDC at a series of spatial and temporal scales.

At a large spatial scale it is predicted that due to the low rates of migration between colonies on Ross Island that the EDC parameters would show some geographical variation. Is is also predicted that colony location and size might have an effect on EDC parameters, with the hypothesis being that proximate colonies would be more similar than distant colonies and that smaller colonies would have different call parameters compared to larger colonies. Likewise, both colony location and size are predicted to affect condition (male weight) and breeding success; with smaller, more southerly colonies, having males in worse condition with poorer breeding success (as measured by chick condition and frequency of chick fledging stage achieved).

At a smaller spatial scale, sub-colony nest location, male weight and breeding stage are also predicted to vary with EDC parameters. It is hypothesised that the position of the nest could reflect male quality (weight) with older more experienced males (that are good foragers hence of high weight) tending to prefer the centre of sub-colonies. Males of high quality with centre nests are also predicted to have greater breeding success.

At a temporal scale the colonies are investigated across a single breeding season (2002/3) and between non-consecutive breeding seasons (2000/1 & 2002/3). It is hypothesised that during the breeding season the EDC parameters might vary with changes in male condition (weight), with nest location and breeding stage. Additionally, if the EDC is affected by male condition and/or environmental variables, a comparison of early season EDC parameters over two years is predicted to show variation; as the two years represent a "good" year for Adélie breeding (2000/1), and due to extensive sea-ice, a "poor" year (2002/3) for Adélie breeding. Prior to assessing the main aims of this chapter an evaluation of the individuality of the EDC (section 3.3.6.1) and the requisite number of calls per colony needed to examine geographical variation (section 3.3.6.2) will be carried out.

3.3 Methods

3.3.1 Subjects and study area

This study was carried out during two breeding seasons, the first between 9th December 2000 and 28th January 2001, and the second between 23rd October 2002 and the 27th January 2003. Data were collected from all three Ross Island Adélie breeding areas (Chapter 2; Figure 2.1a), with Cape Crozier and Royds only being visited during single sampling period in the 2002/3 breeding season. Cape Bird North, Middle and South (Chapter 2; Figure 2.1b) were visited for longer and during both field seasons.

3.3.2 Ecstatic Display Calls

The EDC was described in detail visually and vocally in Chapter 1 (section 1.6.4). The EDC was divided into two audibly distinct components for analysis, the short repeated syllables and the final climactic syllable (the long syllable) (Chapter 2; section 2.5). Due to the focus on broad scale variation in EDCs, aspects of the whole call, such as total duration and number of syllables were also included.

3.3.3 Recording methods

EDCs were recorded at all three Adélie breeding locations on Ross Island using the same method. To avoid biased sampling, sub-colonies across the whole colony area were sampled. Due to time constraints and the sizes of some of the colonies (particularly Cape Crozier), if there was no clear call given at a selected sub-colony within the first few minutes of recording a new sub-colony nearby was targeted. Once the observer thought a clear call had been given it was noted and a new sub-colony was sampled. At Cape Bird where behavioural measurements were also included, often several birds were recorded at one sub-colony, which were then individually coded. Large numbers of birds were sampled across the spread of each colony. EDCs were recorded not in successive breeding seasons but sampled during the 2000/1 breeding season and again during the 2002/3 breeding season. These breeding seasons represent a good year for Adélie breeding (2000/1) and a poor breeding year (2002/3) as conditions were considerably harsher during the latter breeding season. To compare variation in call parameters between these seasons, samples of calls were taken from Cape Bird North, Middle and

South at a comparable time period; the latter stages of the breeding season. To compare variation in EDC parameters between Ross Island colonies, data was only taken from the 2002/3 season and during the early part of the breeding season (28th October 2002 – 30th Dec 2003). Further recording methods (including how disturbance was minimised), equipment and specifications used for recording can be found in Chapter 2 (section 2.6).

3.3.4 Sound analyses

All sound analyses follow the protocol specified in Chapter 2 (section 2.7). Calls were quantified using six standard independent sound parameters, descriptions of which can be found in Chapter 2 (Table 2.2). As adult male Adélie penguins were the target of the study, all birds identified as female during DNA sex analyses (Chapter 2; section 2.3.2) were removed from this study, however a comparison of the EDC between the sexes can be found in Chapter 2 (section 2.11).

3.3.5 Behavioural data

At Cape Crozier (28th Dec – 30th Dec 2002), Royds (19th Nov 2002) and Cape Bird (2000/1) the primary information collected were EDC recordings, however additionally at each sub-colony where recordings were made a Global Positioning System (Garmin eTrex) saved a position fix. At Cape Bird (North, Middle and South) during the 2002/3 field season data were collected over two separate time periods, firstly during the early breeding season (23rd Oct – 18th Nov 2002) and secondly during the late breeding season (9th January – 27th January 2003). During both sampling periods as well as GPS readings, each male recorded was weighed (refer to Chapter 2, section 2.4 for method). Birds were weighed after their calls had been recorded. If multiple birds were recorded from the same sub-colony, all birds were recorded first before any were weighed and the colony disturbed. Only a single weight measure was recorded for each male as the individual variations in male weights was of secondary interest to the colony scale variations in weight and EDC parameters that were required to investigate vocal and behavioural spatial and temporal variation. Data that investigates the intra-individual effects of weight on EDC parameters can be found in Chapter 4.

The location of the nest (that the target male was associated with) was also noted during both sampling periods, and categorised as “edge”, “middle” or “centre” (see Chapter 2; Table 2.1 for a full description of terms). Additionally, each bird had his or her breeding status noted; in the early breeding season (28th Oct-10th November) this was recorded as either "solo" (having no mate at the nest site during the time of recording) or "paired" (when a mate was present throughout the recording session). At the end of the breeding season (9th January-23rd January) each bird was assigned a breeding status of "failed" if there were no chicks at the nest, or having "one chick" or "two chicks" at the nest. However, the chicks had to be clearly associated with the nest/adult in question to be given this classification. Recordings were made prior to onset of the crèche period, therefore the majority of chicks were still at their nest site and clearly associated with the focal male being recorded. Each adult sampled was given a unique code, which was later used to collate all of the information for analysis including the EDC recorded and the sex of the bird when established.

Chick condition across Cape Bird colonies was assessed at the end of the breeding season (late January) after most chicks had formed crèches. The sampling regime was randomised using the following method: one person identified a focal chick in a crèche but did not disclose its location or identity to the second observer. The second person then chose a number between one-ten and also a direction (either left, right, forward or backward). The first person then calculated a new focal chick, from the position of the original chick, which was then sampled. Each focal chick was weighed (using a breathable bag and either a one or five kilogram Pesola scale), and had their wing measured (using a custom metal ruler). Chicks were sampled across the entire colony and were not categorised by their location thus did not need to be marked. For each focal chick, an index of condition was calculated by dividing the wing length (mm) by the linear component of weight ($g^{1/3}$). This is the simplest and most straightforward method used to estimate condition as it assesses the influence of linear skeletal growth rate versus a volumetric measure of weight. It has been determined that in the little blue penguin, chick flipper growth follows a standard growth curve pattern and was relatively predictable over the development of the chick (Wienecke *et al.* 2000), whereas overall body weight can vary greatly depending on size and fat reserves; which is an indication of food availability (Klomp & Wooller 1988). Therefore an index that incorporates these two factors was applied to the focal Adélie chicks used in this study.

Additionally, each chick sampled was categorised into a fledging category, "Not begun" signified the down was intact, "Begun" referred to when small patches of down were gone (especially under the wings), "Partially" was designated when less than half of the down was gone, "Mostly" refers to when more than half of the down is removed but there remains patches on the back and head, finally "Totally" is when there is no down remaining and juvenile plumage is complete. Sampling was spread across the total colony area where chicks were present. Chick condition data for Cape Crozier and Cape Royds were supplied by Kerry Barton (Antarctic Event No. K122), with permission from David Ainley (Antarctic Event No. B031).

3.3.6 Statistical analyses

General linear models (GLMs) were used to compare group (colony, season, sub-colony, breeding success) and male effects (weight and call parameters). Linear regressions were used to assess the relationship between male weights and call parameters. Categorical data (including breeding success of males in the early and late breeding season by colony location and sub-colony nest location) were analysed using Chi-squared analyses. An analysis of covariance (ANCOVA) was performed when male weight was included in the model effects. All biologically appropriate interaction terms were included in initial models; where non-significant they are removed and main effects models reported. When interactions are significant, models include interactions and are reported as such, these data sets were further analysed by splitting the model effects.

Broad scale differences in call parameters between the Ross Island colonies were visually assessed by principle component analysis (PCA) for both the short and long syllable call sections sampled. To see how each PC varied by colony a series of univariate analyses were run. Eigenvectors greater than 0.5 are highlighted in bold in the tables to indicate which of the call parameters are contributing most to each of the PCs. As only two whole call parameters were measured they were graphed as least squared means with standard error bars to show colony differences. Trends in significant call parameters by colony size and position on Ross Island by longitude were also graphed as least squared means with standard error bars. Post hoc Tukey's groups were used to separate the differences between the colonies, sub-colonies and breeding stages for each of the significant call parameters or principle components for each section of the call analysed.

Data analysed in JMP 5.1 (SAS Institute 2004) that were unbalanced was assigned an averaged denominator degree of freedom, which was reported with one decimal place where appropriate. Chick condition was analysed using GLMs. The fledging category assigned to focal chicks, was analysed for colony effects, using Chi-squared analyses. Unless otherwise stipulated, all data cited in text are mean ($\overline{X}$) ±

standard deviation (SD) and all tests used a level of significance of α = 0.05. When multiple comparisons were calculated an alpha adjustment using the Bonferroni correction was applied, and specified in the results.

3.3.6.1 Individuality of the EDC at Cape Bird

The first step before analysing seasonal variation in the EDC at Cape Bird was to confirm individuality in the short repeated syllables between calls sampled. Table 3.1 shows that each of the EDCs recorded had significantly different call parameters. Following this analysis the repeated syllables within a single bird call were averaged giving a single value for each call parameter per call. The averaged data set was then analysed for differences across the breeding season, between the colonies, sub-colony nest locations and with male weight as a covariate. The long syllable data model does not include individual variation as only one syllable is given per call and only one call per bird was recorded. In order to examine the effect of breeding status on EDC call parameters the data were divided into early and late breeding season and analysed for breeding status, colony, sub-colony and weight effects.

Table 3.1: Individual bird ID for the short repeated syllables of the Ecstatic Display call at Cape Bird North, Middle and South (*n* = 331), tested by random effects univariate ANOVA. Units of measurement - see Table 2.2.

Bird ID	Call parameter	R^2	$F_{325, 3555}$	P
Short syllables	Duration	0.478	9.462	**< 0.0001**
	Pitch	0.562	12.775	**< 0.0001**
	Mean Freq	0.796	39.201	**< 0.0001**
	FM	0.417	7.571	**< 0.0001**
	$AM^{\wedge 2}$	0.515	10.606	**< 0.0001**
	Entropy	0.842	53.928	**< 0.0001**

3.3.6.2 Estimate of EDC variation on Ross Island

To estimate the number of unique calls required to accurately depict the variation in call parameters between the Ross Island (all five colonies) and Cape Bird colonies (three colonies) a sample of ten randomly chosen calls per colony were used to run a series of univariate analyses of call parameter by colony and then for each call parameter the least significant number (LSN) was calculated to assess the required number of calls needed to achieve significant differences between the colonies.

Results of the LSN analyses can be found in Table 3.2 for the Ross Island colonies and Table 3.3 for the Cape Bird colonies. The minimum number of calls per colony required for the short syllable Ross Island data set was 12 (mean frequency) and the maximum was 56 calls (FM). For the long syllable data the minimum was six calls (pitch) and the maximum was 234 calls (duration). The whole call data set required a minimum number of calls for duration of 12 calls and the maximum number the calls for the number of syllables was 15 calls. The mean number of calls required per colony for all parameters for the short syllables was 29, for the long syllable data was 49, and for the whole call data was 14 calls.

For the Cape Bird colonies short syllable data, the minimum number of calls required was 14 calls (mean frequency) and a maximum number of calls was 107 (duration). The long syllable data set had a minimum number of calls of seven (mean frequency) and a maximum number of calls required of 870 (duration). The whole call data set required a minimum number of calls for duration of 11 calls and a maximum number of calls for the number of syllables of 14 calls. The mean number of calls required per colony for all Bird parameters for the short syllables was 51, for the long syllable data was 158, and for the whole call data was 13 calls.

Due to the time required to record and analyse each call, the mean number of calls analysed per colony for the Ross Island data set was 61 calls with the minimum number of calls from Middle colony being 36. For the Cape Bird colony data set the mean number of calls per colony analysed across the breeding season for the short syllable data set was 110 calls and for the long syllable and whole call data set were 105 calls. Consequently, the number of calls analysed for each data set exceeded the minimum number of calls required for each call parameter except for duration in the short and long syllable data set which required numbers which exceeded the scope of this study.

Table 3.2: Calculation of least significant number (LSN) of number of calls per colony required to detect geographical differences between colonies based on an alpha=0.05 from a pilot sample of ten calls from each of the five Ross Island Adélie colonies.

Call type	Call parameter	Sigma	Delta	LSN (total)	LSN (per colony)
Short syllables	Duration	44.18	11.01	158.14	31.63
	Pitch	402.59	148.34	79.43	15.89
	Mean Freq	293.01	154.84	57.37	11.47
	FM	19.34	3.59	279.26	55.85
	AM^2	0.002	0.0004	222.68	44.54
	Entropy	0.88	0.34	74.82	14.96
Long syllable	Duration	518.8	46.81	1169.27	233.85
	Pitch	108.85	66.09	29.95	5.99
	Mean Freq	152.3	64.6	56.71	11.34
	FM	7.22	3.94	35.99	7.2
	AM^2	0.0005	0.0003	34.53	6.91
	Entropy	0.56	0.15	130.3	26.06
Whole call	Duration	596.85	250.05	58.03	11.61
	No. syllables	4.05	1.51	72.43	14.49

Note: LSN (per colony) is calculated by dividing LSN (total) by the number of colonies sampled (=5). Units of measurement - see Table 2.2.

Table 3.3: Calculation of least significant number (LSN) of number of calls per colony required to detect geographical differences between Cape Bird colonies based on an alpha=0.05 from a pilot sample of ten calls per colony taken from Cape Bird North, Middle and South.

Call type	Call parameter	Sigma	Delta	LSN (total)	LSN (per colony)
Short syllables	Duration	45.3	7.12	320.3	106.77
	Pitch	387.09	139.17	67.86	22.62
	Mean Freq	276.81	177.83	42.54	14.18
	FM	18.4	3.46	225.26	75.09
	$AM^{\wedge 2}$	0.002	0.0003	202.38	67.46
	Entropy	0.75	0.33	52.48	17.49
Long syllable	Duration	554.8	26.6	2609.56	869.85
	Pitch	116.12	65.62	22.05	7.35
	Mean Freq	127.35	72.64	21.71	7.24
	FM	9.02	4.19	30.92	10.31
	$AM^{\wedge 2}$	0.0005	0.0002	38.67	12.89
	Entropy	0.51	0.12	114.22	38.07
Whole call	Duration	633.8	284.77	32.87	10.96
	No. syllables	4.68	1.81	43.25	14.42

Note: LSN (per colony) is calculated by dividing LSN (total) by the number of colonies sampled (=3). Units of measurement - see Table 2.2.

3.4 Results

3.4.1 Geographic variation in the EDC

Univariate analysis of the short repeated syllable, the long end syllable and whole call duration and number of syllables per call between Ross Island Adélie colonies reveals that there are significant differences in vocal parameters between these colonies (Table 3.4).

Table 3.4: Univariate ANOVA analyses of vocal differences (with Tukey's groups) between Ross Island Adélie colonies for the short (n = 320), long (n = 303) and whole (n = 303) call parameters. A multiple comparison Bonferroni correction (0.05/6) was applied to the short and long syllable data thus the calculated *P* value had to fall below a threshold of 0.008 before being considered significant.

Colony	Call parameter	R^2	*df*	*F*	*P*	Tukey's Groups
Short syllables	Duration	0.443	4, 323.5	1.307	0.267	
	Pitch	0.533	4, 321.4	4.921	**0.001**	(S (R) N M C)
	Mean Freq	0.788	4, 316.9	2.893	0.022	
	FM	0.411	4, 325.1	4.013	**0.003**	(M R (S N) C)
	AM$^{\wedge 2}$	0.479	4, 322.9	5.217	**0.0004**	(N (S (M) C) R)
	Entropy	0.827	4, 316.5	3.844	**0.005**	(R C (S M) N)
Long syllable	Duration	0.005	4, 298	0.345	0.848	
	Pitch	0.092	4, 298	7.572	**< 0.0001**	(S (M) R N C)
	Mean Freq	0.026	4, 298	2.004	0.0940	
	FM	0.065	4, 298	5.213	**0.0005**	(R (M (S) N) C)
	AM$^{\wedge 2}$	0.09	4, 298	7.383	**< 0.0001**	(N S C (M) R)
	Entropy	0.072	4, 298	5.733	**0.0002**	(R (C (M) S) N)
Whole call	Duration	0.045	4, 298	3.508	**0.008**	(R (M S N) C)
	No. Syllables	0.049	4, 298	3.869	**0.004**	(R (S M N) C)

Note: Significant *P* values are highlighted in **bold**.Tukey's groups are ordered from highest LSM value (left) to lowest (right) and coded as C=Crozier, R=Royds, N=North, M=Middle and S=South colonies; coloured parentheses separate groups that are significantly different at α = 0.05. Units of measurement - see Table 2.2.

The short repeated syllable has a higher pitch at the South colony as compared to the North, Middle and Cape Crozier colonies with Cape Royds being intermediary. At Bird Middle and Cape Royds there is higher FM than at Cape Crozier with South and North Bird colonies being intermediary. The AM$^{\wedge 2}$ of North colony is higher than at Cape Crozier or Cape Royds, with the South and Middle colonies falling between these extremes in AM$^{\wedge 2}$. The final significant parameter for the short syllables is entropy, which is highest (closer to zero thus noisier) at Cape Crozier and Cape Royds and lowest (more negative thus more tonal) at Cape Bird North, the remaining Middle and South colonies have intermediary entropy values.

Similar differences between the colonies were observed for the long syllable of the EDC (Table 3.4). The same call parameters were significant and similar although not identical patterns of differences were seen (Table 3.4). Duration of the short and long syllables was not significantly different between colonies; however, the total duration of the EDCs was (Table 3.4). The Cape Royds calls were significantly longer than the calls recorded at Cape Crozier; the Cape Bird colonies had intermediary length calls. Given there was no difference in short or long syllable duration the remaining factor measured was number of syllables emitted per call, which was significantly different (Table 3.4). The Cape Royds birds gave calls with a higher number of syllables per call on average than the Cape Crozier calls and again the Cape Bird colonies performed calls with an intermediary number of syllables.

Principle Component (PC) scores for the Ross Island colonies for both short and long syllable call sections sampled can be seen in Table 3.5 & 3.6. For the short repeated syllables PC1 is significant, explaining 36.4% of the total variation and is most strongly associated with pitch and mean frequency. PC3 was also significant, explaining 16.6% of the total variation, and is strongly associated with AM$^{\wedge 2}$. PC4 is weakly significant, explaining 12.9% of the total variation and is strongly associated with entropy. The final significant PC was PC5, which explained 10.6% of the total variation and was strongly associated with the combined parameters of duration and FM. Tukey's groupings highlight the pattern of colony differences for each significant PC (Table 3.5).

Table 3.5: Short syllable call parameter principle components, including univariate ANOVA of colony differences and Tukey's groups, n = 320, principle components used in Figure 3.1a are highlighted in **bold**.

Short syllable	PC1	PC2	**PC3**	**PC4**	**PC5**	PC6
Eigenvalue	2.185	1.247	0.998	0.772	0.635	0.163
Percent	36.4	20.8	16.6	12.9	10.6	2.7
Cum Percent	36.4	57.2	73.8	86.7	97.3	100
Eigenvectors						
Duration	-0.164	**0.725**	0.076	0.026	**0.663**	-0.039
Pitch	**0.574**	0.224	0.147	-0.397	-0.066	**0.661**
Mean Freq	**0.613**	0.205	0.122	-0.146	-0.124	**-0.729**
FM	0.237	**-0.578**	0.449	0.063	**0.636**	-0.01
AM$^{\wedge 2}$	-0.215	0.192	**0.853**	0.228	-0.369	0.032
Entropy	0.407	0.105	-0.17	**0.875**	-0.02	0.171

Colony	R^2	$F_{4,315}$	P	**Tukey's Groups**
PC1	0.042	3.446	**0.009**	(S (R C M) N)
PC2	0.021	1.645	0.163	
PC3	0.098	8.592	**< 0.0001**	(S N (M) (R) C)
PC4	0.032	2.615	**0.035**	(C (R M N) S)
PC5	0.065	5.431	**0.0003**	(R (M (N) S) C)
PC6	0.008	0.64	0.635	

Note: All call parameters with an eigenvector greater than 0.5 are highlighted in **bold**. Significant univariate analyses of colony by each principle component with a P value less than α = 0.05 are **bold**. Tukey's groups are ordered from highest LSM value (left) to lowest (right) and coded as C=Crozier, R=Royds, N=North, M=Middle and S=South colonies; coloured parentheses separate groups that are significantly different at α = 0.05. Units of measurement - see Table 2.2.

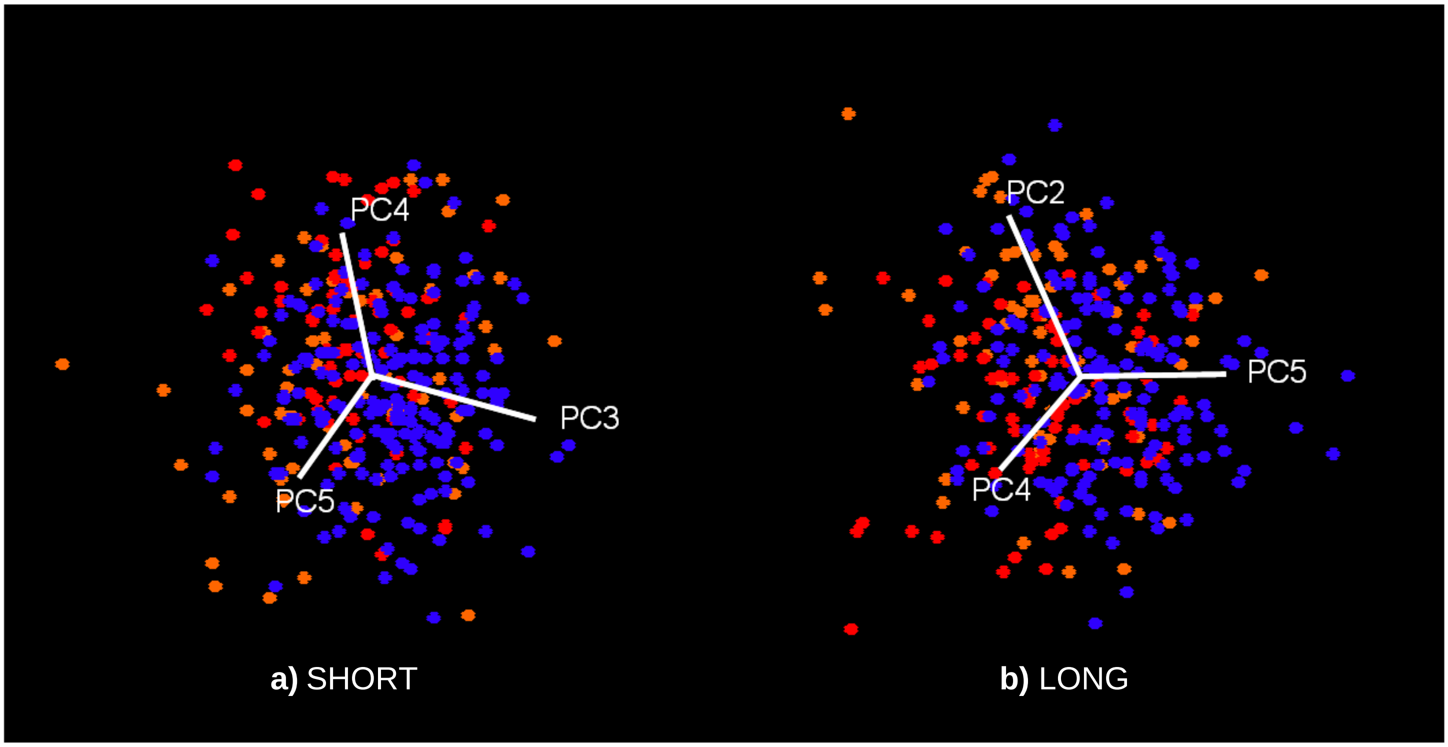
Table 3.6: Long syllable call parameter principle components, including univariate ANOVA of colony differences and Tukey's groups, *n* = 303, principle components used in Figure 3.1b are highlighted in **bold**.

Long syllable	PC1	**PC2**	PC3	**PC4**	**PC5**	PC6
Eigenvalue	1.954	1.161	1.021	0.886	0.714	0.264
Percent	32.6	19.4	17.0	14.8	11.9	4.4
Cum Percent	32.6	51.9	68.9	83.7	95.6	100
Eigenvectors						
Duration	-0.035	0.101	**0.954**	-0.092	-0.263	0.036
Pitch	**0.526**	0.368	-0.053	-0.47	0.125	**0.59**
Mean Freq	**0.607**	0.345	0.039	0.075	0.068	**-0.708**
FM	0.253	**-0.644**	0.252	-0.109	**0.667**	-0.03
AM$^{\wedge 2}$	-0.321	**0.549**	0.143	0.359	**0.661**	0.103
Entropy	0.432	-0.14	0.046	**0.79**	-0.171	0.372

Colony	R^2	$F_{4,298}$	P	**Tukey's Groups**
PC1	0.0714	5.665	**0.0002**	(R (S (M) C) N)
PC2	0.074	5.959	**0.0001**	(R (M) C N S)
PC3	0.021	1.612	0.171	
PC4	0.087	7.092	**< 0.0001**	(C (R) N M S)
PC5	0.045	3.501	**0.008**	(S (M N R) C)
PC6	0.011	0.829	0.508	

Note: All call parameters with an eigenvector greater than 0.5 are highlighted in **bold**. Significant univariate analyses of colony by each principle component with a *P* value less than α = 0.05 are **bold**. Tukey's groups are ordered from highest LSM value (left) to lowest (right) and coded as C=Crozier, R=Royds, N=North, M=Middle and S=South colonies; coloured parentheses separate groups that are significantly different at α = 0.05. Units of measurement - see Table 2.2.

Due to an observed pattern similarity in short syllable EDC parameters between Cape Bird colonies, the three PCs to be graphed were PC3, 4 & 5; as they separated the Cape Bird (North, Middle and South) colonies away from Cape Crozier and Royds and therefore could be coded with a single colour on the graph (blue). Cape Royds was coloured orange and Cape Crozier was coloured red. As can be seen from Figure 3.1a the Cape Bird colonies are clustered to the right of the plot, closer to PC3 (AM^^{2}), whereas the Cape Crozier colony calls scatter more on the left of the plot and towards PC4 (entropy) and PC5 (duration and FM). Cape Royds data are relatively evenly scattered although show some trend towards PC4 and PC5.



a) SHORT

b) LONG

Figure 3.1:Principle component graph illustrating geographic variation between colonies for both **a)** the short and **b)** long syllable parts of the Ecstatic Display call. Colonies are coded as **BLUE** = Cape Bird North, Middle and South, **ORANGE** = Cape Royds and **RED** = Cape Crozier. The call parameters strongly associated with principle components used in the graph can be found in Table 3.5 & 3.6.

Principle component analysis of the long syllable again highlighted colony differences (Table 3.6), with PC1 being highly significant, explaining 32.6% of the total variation, and being most strongly associated with the combined parameters of pitch and mean frequency. PC2 was also significant, explaining 19.4% of the variation, and being positively associated with AM$^{\wedge 2}$ and negatively associated with FM. PC4 was highly significant, explaining 14.8% of the total variation, and was associated with entropy. Finally, PC5 was significant, explaining 11.9% of the total variation and being most associated with FM and AM$^{\wedge 2}$. Tukey's groupings highlight the pattern of colony differences for each significant PC (Table 3.6).

The three PCs to be graphed were PC2, 4 & 5 as these (much like for the short syllables) appeared to group the Cape Bird colonies away from the Cape Crozier and Royds colonies and hence coloured similarly to the short syllable graph. Geographic variation between the colonies for the PCs chosen can be seen in Figure 3.2b. Much like the differences observed for the short syllable data, the Cape Bird colony data falls largely to the right of the plot and the Cape Crozier data falls to the left of the plot. Again the Cape Royds data seems to be more scattered, however there may be a trend for the data to be clustering near PC2 (FM and AM$^{\wedge 2}$). The Cape Crozier data also seems to be clustering near PC4 (entropy), with the Cape Bird data concentrated largely by PC5 and perhaps PC2 (both PC5 and PC2 represent FM and AM$^{\wedge 2}$).

For whole call parameters there are similar differences observed for both parameters (Table 3.4), with the highest duration and number of syllables found in the Cape Royds colony and the lowest duration, hence number of syllables, in the Cape Crozier colony. Cape Bird North, Middle and South colonies show relatively similar measures of duration and number of syllables in their EDCs (Figure 3.2).

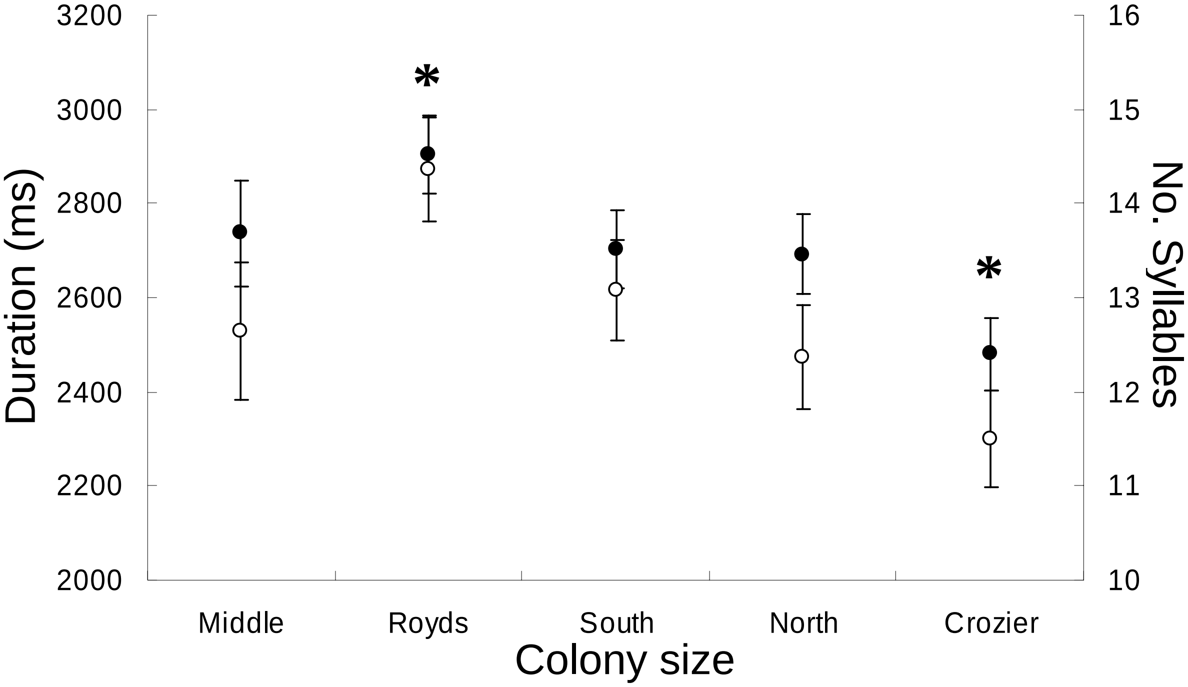


Figure 3.2: Geographic variation in Ecstatic calls measured using mean (± SE) of whole call duration (●) and number of syllables (o). Colonies are ordered by size with smallest on the left to largest on the right. Significant differences between colonies were classified using Tukey's grouping (α = 0.05) and significantly different colonies are marked with an asterisk.

Only FM differed significantly with colony size (Figure 3.3a & b). With the smaller colonies (Cape Bird Middle and Cape Royds) having higher FM and the larger colonies (Cape Crozier and Cape Bird North) having lower FM. Also of note is the much lower FM in the long syllables as compared to the short syllables; however the trend with colony size is similar for both syllable types. Entropy was the only call parameter to show differences with longitude position on Ross Island, and although the trend is stronger in the short syllables (Figure 3.4a) compared to the long syllables (Figure 3.4b), there is still an evident tendency in the data for more northerly colonies (Cape Bird North) to have higher entropy (thus more tonal calls) than more southerly colonies (Cape Royds and Crozier) which had have lower entropy scores (thus noisier calls).

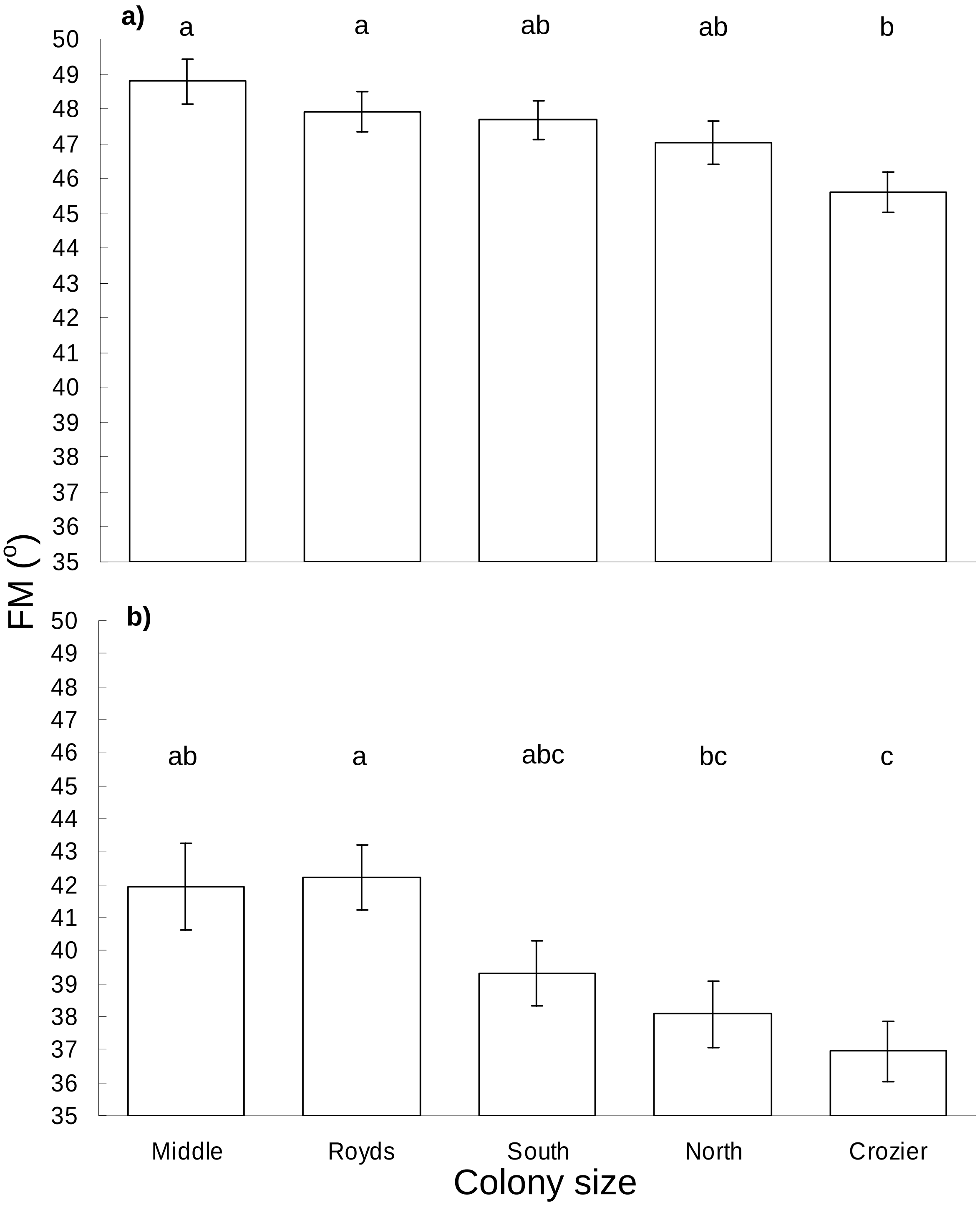


Figure 3.3: Variation in mean (± SE) Frequency Modulation (FM) by Ross Island colony location for **a)** short repeated syllables and **b)** the long end syllable. Colonies are ordered by size with smallest on the left to largest on the right. Colonies separated by different letters are significantly different using Tukey's group assignments.

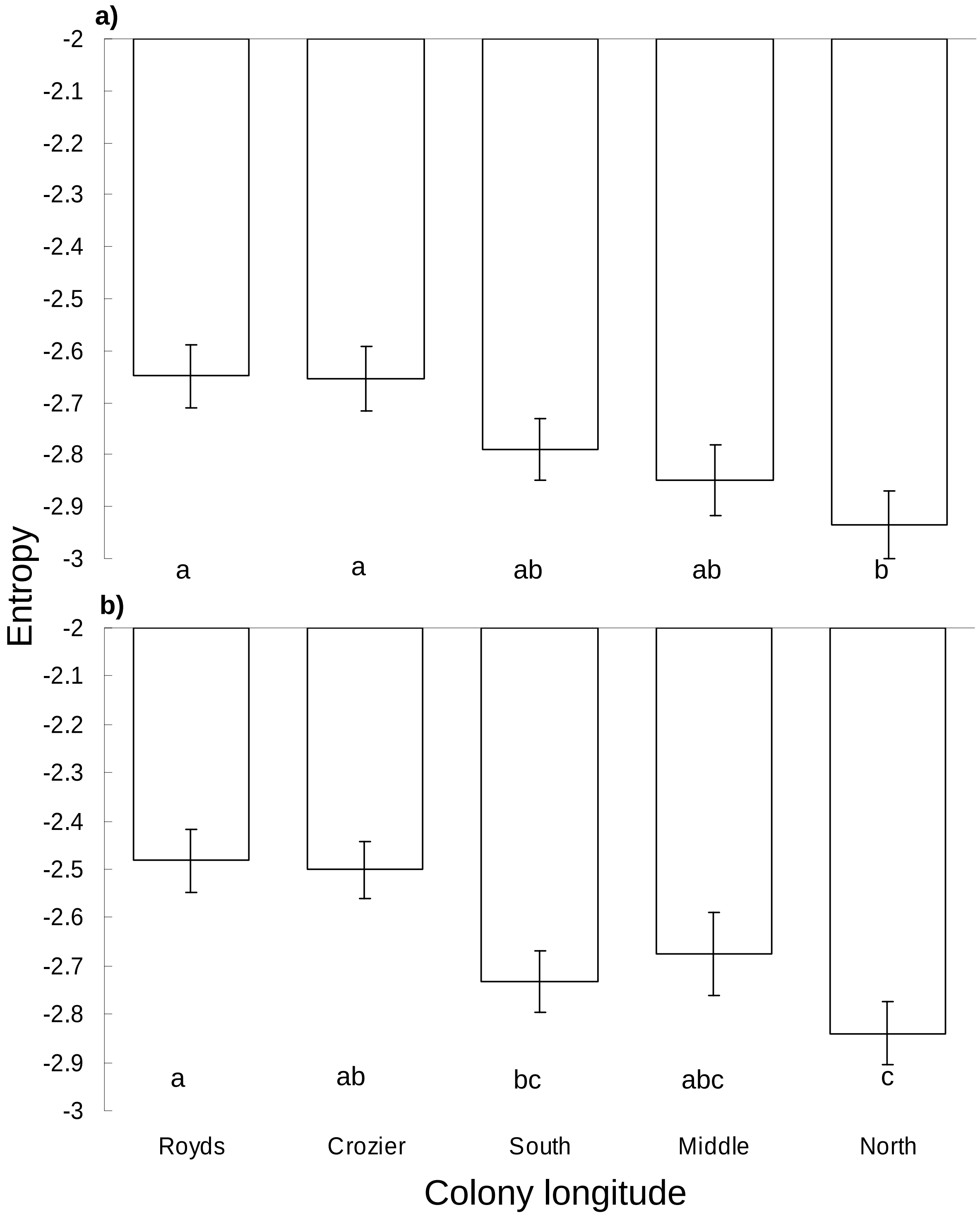


Figure 3.4: Variation in mean (± SE) entropy by Ross Island colony location for **a)** short repeated syllables and **b)** the long end syllable. Colonies are ordered by longitude with most southerly on the left and most northerly on the right. Colonies separated by different letters are significantly different using Tukey's group assignments.

3.4.2 Chick condition between Ross Island colonies

One further way the colonies on Ross Island have been investigated for variation is through their breeding success, measured as the condition of the fledging chicks at the end of the breeding season. Univariate analysis across the colonies for chick condition was significant ($R^2 = 0.0681$, $F_{4,496} = 9.065$, $P = < 0.0001$), with Cape Crozier (the largest colony) having the chicks in the best condition, followed by Bird North, Middle, South and then Cape Royds (Figure 3.5).

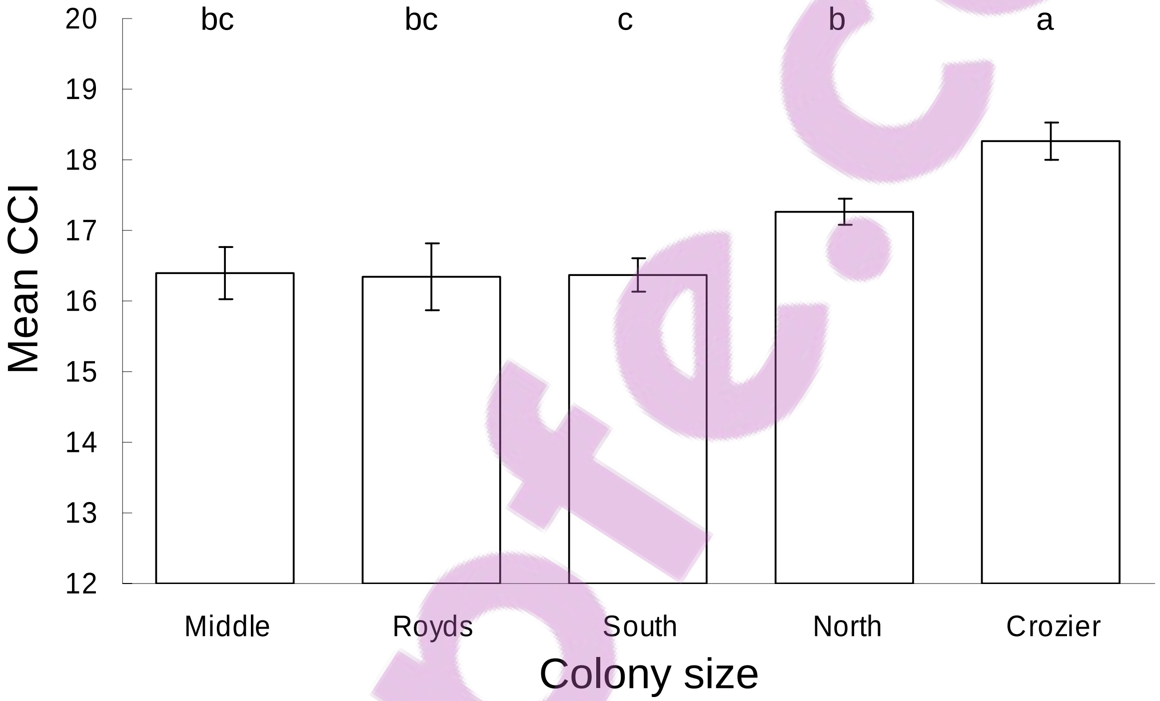


Figure 3.5: Mean (± SE) chick condition index (CCI) measured at the end of the breeding season between all major colonies on Ross Island ($n = 501$). Colonies are ordered by size with smallest on the left to largest on the right. According to Tukey's groups colonies not connected by same letter are significantly different.

3.4.3 Yearly EDC parameter variation at Cape Bird

Results show that there are individual, yearly and colony differences in the short syllables of the EDC parameters (Table 3.7). All call parameters are significant for the individual (Table 3.7) but duration is only significantly different at an individual level (Table 3.7). Both pitch and mean frequency have significant interaction terms (Table 3.7) therefore the data were re-analysed for each separate breeding season with the results showing significantly higher pitch and mean frequency recorded for Bird North than South during the 2000/1 data (pitch - $R^2 = 0.646$, $F_{2,137.4} = 6.191$, $P = 0.003$; mean frequency - $R^2 = 0.871$, $F_{2,135.6} = 4.853$, $P = 0.009$; Table 3.8; Figure 3.6). Middle colony had intermediary pitch and mean frequency recordings during the 2000/1 breeding season. There was no colony variation in either of these parameters during the 2002/3 breeding season (Table 3.9). With regards to yearly variations, Cape Bird North had significantly greater pitch and mean frequency recordings during 2000/1 than 2002/3 (pitch - $R^2 = 0.646$, $F_{1,155.1} = 44.297$, $P = < 0.0001$; mean frequency - $R^2 = 0.881$, $F_{1,152.8} = 56.259$, $P = < 0.0001$; Figure 3.6). There were no yearly variations at Bird Middle and only mean frequency showed significantly higher recordings in 2000/1 than 2002/3 for Bird South ($R^2 = 0.804$, $F_{1,93} = 15.65$, $P = 0.0001$; Figure 3.6). FM was not shown to be different between the colonies but was significantly higher in 2002/3 than in 2000/1 (Table 3.7). There were both significant colony and yearly effects for AM^2 (Table 3.7), with North colony having significantly greater values of AM^2 than Middle colony. Significantly greater AM^2 was also recorded across the colonies between the years, with 2002/3 having greater AM^2 than previously recorded (Table 3.7). Entropy was also more negative (thus more tonal) in 2002/3 as compared to 2000/1 (Table 3.7) and colony differences were significant also with Bird North and Middle having higher (more noise) entropy values compared to Bird South (Table 3.7).

Table 3.7: Random effects (ID) univariate ANOVA of short repeated syllable call parameters recorded for individuals, over years, between Cape Bird North, Middle and South (n = 328), including the potential interaction effect of yearly*colony data. A multiple comparison Bonferroni correction (0.05/6) was applied to the data thus the calculated P value had to fall below a threshold of 0.008 before being considered significant.

Call Parameter		***R*^{2}**	***df***	***F***	***P***	**+/-**	**Tukey's Group**
Duration		0.58					
	Bird ID		322,2966	12.59	**< 0.0001**		
	Colony		2,329.6	0.541	0.583		
	Yearly		1,329.6	1.594	0.208		
	Yearly*Colony		2,329.6	0.121	0.886		
Pitch		0.641					
	Bird ID		322,2966	12.894	**< 0.0001**		
	Colony		2,329.4	8.032	0.0004		
	Yearly		1,329.4	36.524	< 0.0001		
	Yearly*Colony		2,329.4	5.061	**0.007**		
Mean Freq		0.877					
	Bird ID		322,2966	47.2049	**< 0.0001**		
	Colony		2,324	5.3965	0.0049		
	Yearly		1,324	52.3686	< 0.0001		
	Yearly*Colony		2,324	6.1473	**0.002**		
FM		0.467					
	Bird ID		322,2966	7.6379	**< 0.0001**		
	Colony		2,334.6	0.7785	0.4599		
	Yearly		1,334.5	11.6102	**0.0007**	+	
	Yearly*Colony		2,334.6	3.4019	0.0345		
AM$^{\wedge 2}$		0.702					
	Bird ID		322,2966	12.999	**< 0.0001**		
	Colony		2,329.4	6.011	**0.003**		(N (S) M)
	Yearly		1,329.3	195.394	**< 0.0001**	+	
	Yearly*Colony		2,329.4	0.925	0.398		
Entropy		0.892					
	Bird ID		322,2966	48.042	**< 0.0001**		
	Colony		2,324	5.185	**0.006**		(N M) (S)
	Yearly		1,324	118.917	**< 0.0001**	-	
	Yearly*Colony		2,324	3.972	0.02		

Note: Significant P values are highlighted in **bold**. Where interaction terms between yearly and colony data are significant the values have been marked as grey as these factors cannot be interpreted independently. The direction of change from 2001/2 – 2002/3 (Yearly) data where significant is marked with a +/-. Tukey's groups are ordered from highest LSM values on the left to lowest on the right, and groups significant at an alpha level of less than 0.05 are separated by coloured parentheses. Units of measurement - see Table 2.2.

Table 3.8: Univariate ANOVA analyses of colony differences between Cape Bird North, Middle and South during the 2000/1 breeding season for the short repeated syllables, long end syllable and whole call parts of the Ecstatic Display Call (n = 138). A multiple comparison Bonferroni correction (0.05/6) was applied to the short and long syllable data thus the calculated P value had to fall below a threshold of 0.008 before being considered significant.

Call parameter		R^2	*df*	*F*	*P*	**Tukey's Groups**
Short syllables	Duration	0.536	2,138.1	0.284	0.754	
	Pitch	0.632	2,137.4	6.191	**0.003**	(N (M) S)
	Mean Freq	0.871	2,135.6	4.853	0.009	
	FM	0.422	2,140	1.447	0.239	
	AM^2	0.581	2,137.7	2.393	0.095	
	Entropy	0.885	2,135.5	5.399	**0.006**	(N (M) S)
Long syllable	Duration	0.038	2,135	2.65	0.074	
	Pitch	0.035	2,135	2.435	0.091	
	Mean Freq	0.045	2,135	3.169	0.045	
	FM	0.018	2,135	1.267	0.285	
	AM^2	0.1	2,135	7.489	**0.0008**	(N) (S M)
	Entropy	0.039	2,135	2.767	0.067	
Whole call	Duration	0.024	2,135	1.639	0.198	
	No. Syllables	0.004	2,135	0.239	0.788	

Note: Significant P values are highlighted in **bold**. Tukey's groups are ordered from highest LSM value (left) to lowest (right) and coded as N=North, M=Middle and S=South colonies; coloured parentheses separate groups that are significantly different at α = 0.05. Units of measurement - see Table 2.2.

Table 3.9: Univariate ANOVA analyses of colony differences between Cape Bird North, Middle and South during the 2002/3 breeding season for the short repeated syllables, long end syllable and whole call parts of the Ecstatic Display Call (*n* = 190). A multiple comparison Bonferroni correction (0.05/6) was applied to the short and long syllable data thus the calculated *P* value had to fall below a threshold of 0.008 before being considered significant.

Call parameter		R^2	*df*	*F*	*P*	Tukey's Groups
Short syllables	Duration	0.601	2,189.9	0.441	0.644	
	Pitch	0.562	2,190.5	2.632	0.075	
	Mean Freq	0.785	2,188.2	2.255	0.108	
	FM	0.48	2,191.9	2.968	0.054	
	AM$^{\wedge 2}$	0.627	2,189.7	5.49	**0.005**	(S N) (M)
	Entropy	0.815	2,188	2.144	0.12	
Long syllable	Duration	0.007	2,187	0.625	0.536	
	Pitch	0.009	2,187	0.863	0.424	
	Mean Freq	0.023	2,187	2.174	0.117	
	FM	0.015	2,187	1.422	0.244	
	AM$^{\wedge 2}$	0.031	2,187	3.032	0.051	
	Entropy	0.04	2,187	3.871	0.023	
Whole call	Duration	0.004	2,187	0.385	0.681	
	No. Syllables	0.0002	2,187	0.02	0.99	

Note: Significant *P* values are highlighted in **bold**. Tukey's groups are ordered from highest LSM value (left) to lowest (right) and coded as N=North, M=Middle and S=South colonies; coloured parentheses separate groups that are significantly different at $\alpha = 0.05$. Units of measurement - see Table 2.2.

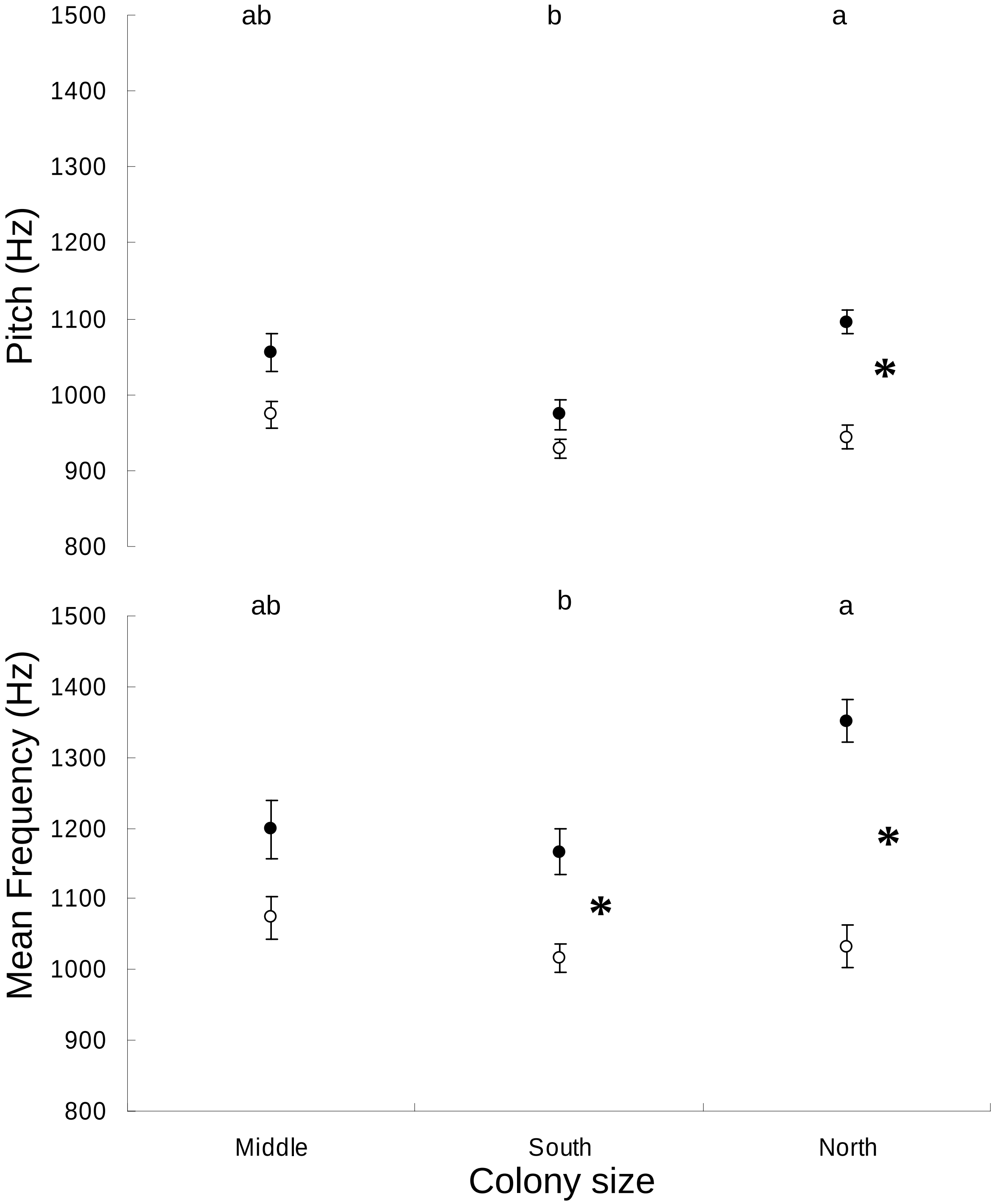


Figure 3.6: Mean (± SE) change in short syllable pitch and mean frequency between 2000/1 (●) and 2002/3 (o) between North, Middle and South Cape Bird colonies (*n* = 328). Significant differences between breeding years are highlighted with asterisks. Colonies are ordered by size with smallest on the left to largest on the right. Significant Tukey's groups that separate colony differences for the 2000/1 data are highlighted with letters above the respective colonies.

Long syllable data also shows significant yearly and colony differences for the EDC parameters (Table 3.10). Duration shows a significant yearly effect with duration of the long syllable being greater in the 2002/3 breeding season but showing no variation between Bird colonies (Table 3.10). Pitch and mean frequency both show a significant decrease between breeding seasons and no colony variations (Table 3.10). FM is not significantly different between breeding seasons recorded or between Cape Bird colonies (Table 3.10). There was a significant interaction between yearly and colony data for AM^2 therefore the data were analysed for the separate breeding season and for each colony. A significant difference between the colonies was seen only in the 2000/1 data (R^2 = 0.1, $F_{2,135}$ = 7.489, P = < 0.0008), with Cape Bird North having significantly greater AM^2 than either Cape Bird Middle or South (Figure 3.7). There was however significantly higher AM^2 across the three colonies between the 2000/1 and 2002/3 data set (North - R^2 = 0.112, $F_{2,152}$ = 19.127, P = < 0.001; Middle - R^2 = 0.46, $F_{2,78}$ = 66.52, P = < 0.001; South - R^2 = 0.457, $F_{2,92}$ = 77.436, P = < 0.001; Figure 3.7). Entropy was significantly more tonal in 2002/3 than 2000/1 (Table 3.10); Cape Bird Middle and North also had significantly less tonal calls than Cape Bird South (Table 3.10).

The whole call duration and number of syllables per call were also tested for differences between Bird colonies and between the two breeding seasons sampled. There was a significant increase in the duration of calls between the 2000/1 and 2002/3 season (Table 3.10); there was no difference in the number of syllables between the seasons. There were no colony differences for either whole call parameters (Table 3.10).

Table 3.10: Univariate ANOVA analyses of the long syllable and whole call parameters recorded for over years and between Cape Bird North, Middle and South (n = 328), including the potential interaction effect of yearly*colony data. A multiple comparison Bonferroni correction (0.05/6) was applied to the long syllable data thus the calculated P value had to fall below a threshold of 0.008 before being considered significant.

Call Parameter		R^2	***df***	***F***	***P***	**+/-**	**Tukey's Group**
Long syllable							
Duration		0.037					
	Colony		2,322	0.367	0.693		
	Yearly		1,322	10.211	**0.002**	**+**	
	Yearly*Colony		2,322	2.541	0.080		
Pitch		0.069					
	Colony		2,322	3.971	0.02		
	Yearly		1,322	9.132	**0.003**	**-**	
	Yearly*Colony		2,322	1.317	0.27		
Mean Freq		0.213					
	Colony		2,322	4.092	0.018		
	Yearly		1,322	46.835	**< 0.0001**	**-**	
	Yearly*Colony		2,322	3.234	0.041		
FM		0.017					
	Colony		2,322	0.733	0.481		
	Yearly		1,322	0.073	0.787		
	Yearly*Colony		2,322	2.117	0.122		
AM^2		0.306					
	Colony		2,322	8.383	0.0003		
	Yearly		1,322	133.412	< 0.0001		
	Yearly*Colony		2,322	7.847	**0.0005**		
Entropy		0.34					
	Colony		2,322	5.877	**0.003**		(M N) (S)
	Yearly		1,322	119.491	**< 0.0001**	**-**	
	Yearly*Colony		2,322	0.583	0.559		
Whole call							
Duration		0.044					
	Colony		2,322	0.454	0.635		
	Yearly		1,322	13.32	**0.0003**	**+**	
	Yearly*Colony		2,322	1.595	0.205		
No. syllables		0.013					
	Colony		2,322	0.212	0.809		
	Yearly		1,322	3.457	0.064		
	Yearly*Colony		2,322	0.123	0.884		

Note: Significant P values are highlighted in **bold**. Where interaction terms between yearly and colony data are significant the factors have been marked as grey as they cannot be interpreted independently. The direction of change from yearly data where significant is marked with a +/-. Tukey's groups are ordered from highest LSM values on the left to lowest on the right, and colonies (N=North, M=Middle & S=south) significant at an alpha level of less than 0.05 are separated by coloured parentheses. Units of measurement - see Table 2.2.

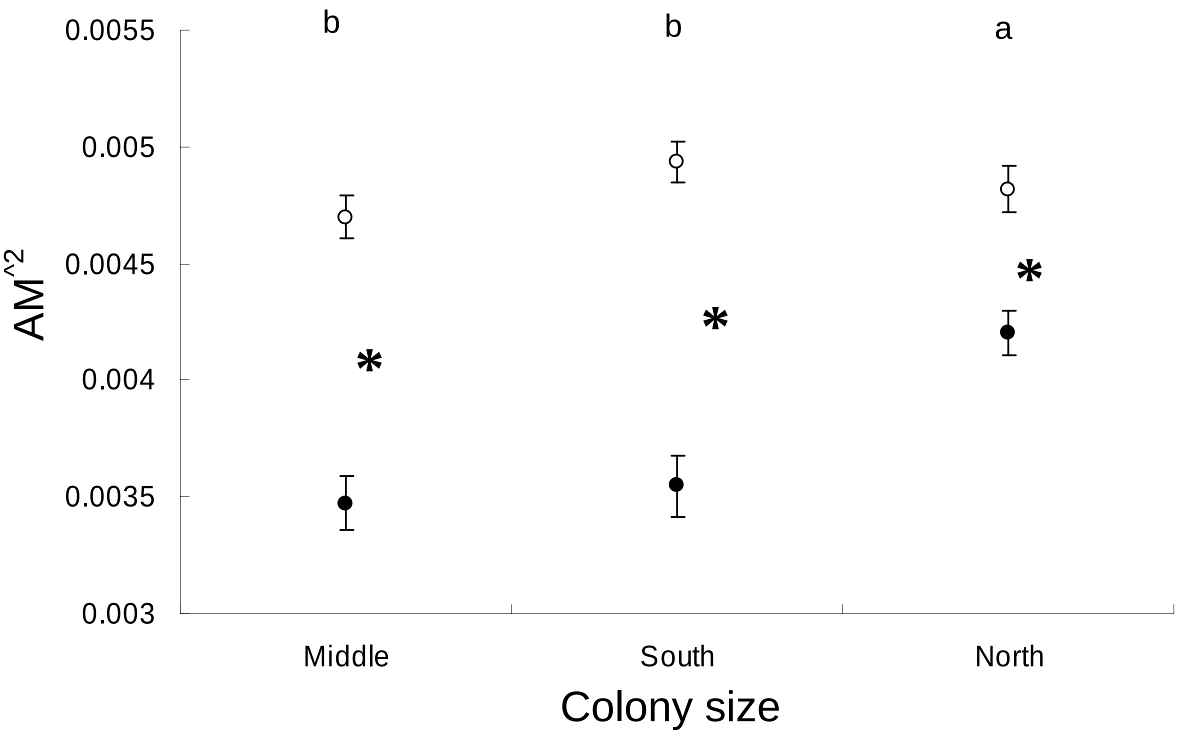


Figure 3.7: Mean (± SE) change in long syllable mean amplitude modulation^2 between 2000/1 (●) and 2002/3 (o) between North, Middle and South Cape Bird colonies (*n* = 328). Colonies are ordered by size with smallest on the left to largest on the right. Significant differences between breeding years are highlighted with an asterisk. Significant Tukey's groups that separate colony differences for the 2000/1 data are highlighted with letters above the respective colonies.

3.4.4 Cape Bird seasonal variation and breeding success

There were no significant interactions found for any of the call parameters for the short syllables (Table 3.11). Differences across the breeding season were found in the duration of the syllables, with length increasing over the season, entropy was also significantly higher (therefore noisier) at the end of the breeding season compared to the beginning (Table 3.11). There was also a difference between colonies with the South and North colony having significantly higher AM$^{\wedge 2}$ than the Middle colony (Table 3.11). The was a significant effect of sub-colony nest location on the mean frequency of the EDCs, with males in the centre nests having higher frequencies in their short syllables than either middle or edge nesting males (Table 3.11). There were no significant effects of male weight on any of the short syllable call parameters.

Table 3.11: Univariate ANCOVA of changes in short repeated syllable call parameters of the Ecstatic Display call at Cape Bird North, Middle and South over the breeding season with changing weight and sub-colony nest location (n = 331). All interaction terms between season, colony, sub-colony and weight were non-significant therefore only main effects are displayed. A multiple comparison Bonferroni correction (0.05/6) was applied to the data thus the calculated P value had to fall below a threshold of 0.008 before being considered significant.

Call Parameter		R^2	*df*	*F*	*P*	**+/-**	**Tukey's Group**
Duration		0.062					
	Season		1,324	13.952	**0.0002**	+	
	Colony		2,324	0.233	0.792		
	Sub-colony		2,324	1.641	0.196		
	Weight		1,324	4.948	0.027		
Pitch		0.064					
	Season		1,324	5.126	0.024		
	Colony		2,324	0.858	0.425		
	Sub-colony		2,324	4.048	0.018		
	Weight		1,324	0.07	0.791		
Mean Freq		0.051					
	Season		1,324	0.223	0.637		
	Colony		2,324	0.451	0.638		
	Sub-colony		2,324	7.12	**0.0009**		(C) (E M)
	Weight		1,324	0.033	0.856		
FM		0.03					
	Season		1,324	0.1239	0.7250		
	Colony		2,324	4.2289	0.0154		
	Sub-colony		2,324	0.9507	0.3875		
	Weight		1,324	0.0208	0.8855		
AM$^{\wedge 2}$		0.098					
	Season		1,324	5.852	0.016		
	Colony		2,324	8.299	**0.0003**		(S N) (M)
	Sub-colony		2,324	4.564	0.011		
	Weight		1,324	0.775	0.379		
Entropy		0.075					
	Season		1,324	14.342	**0.0002**	+	
	Colony		2,324	1.023	0.361		
	Sub-colony		2,324	3.254	0.04		
	Weight		1,324	0.0001	0.992		

Note: All significant P values are marked in **bold**. The direction of change across the season or with the covariate weight where significant is marked with a +/-. Tukey's groups are ordered from highest LSM values on the left to lowest on the right, and groups significant at an alpha level of less than 0.05 are separated by coloured parentheses. Colonies are labelled as N=North, M=Middle, S=South and sub-colonies as C=Centre, M=Middle and E=Edge. Units of measurement - see Table 2.2.

The long syllable data also showed significant effects of breeding season, colony and sub-colony location on the EDC call parameters (Table 3.12). Both pitch and entropy significantly decreased, thus long syllables were noisier but with lower pitch at the end of the breeding season (Table 3.12). Entropy also showed an effect of nest location in the sub-colony with birds in the centre having significantly higher entropy (more noise) in their calls than either the edge or middle nesting birds (Table 3.12). Mean frequency also exhibited an effect of sub-colony nest location with birds in the centre having higher mean frequencies than either edge or middle nesting males (Table 3.12). This pattern was also observed in the short repeated syllables. There were no significant effects found in the long syllable for FM or AM$^{\wedge 2}$, and none of the call parameters co-varied with male weight.

Duration in the long syllable had significant interactions between season*colony and colony*weight (Table 3.12) therefore the data were separated by colony and season to examine the effect of duration on these factors. The effect of both season and colony can be seen clearly in Figure 3.8, with there being significantly smaller duration in the long syllable in the South colony between the early and late breeding season ($R^2 = 0.144$, $F_{1,113} = 14.329$, $P = 0.0002$) but no effect of season in either the Middle or North colonies. Visually, the interaction between weight and duration by colony can be seen in Figure 3.9. Middle colony showed a trend linking decreased weight and an increase in duration in the long syllable over the breeding season, however the South colony was significantly changed over the season; North colony shows no effect of changing weight on duration. Neither of the whole call parameters (duration or number of syllables) had any significant interaction effects nor were any of the model effects significant (Table 3.12).

Table 3.12: Univariate ANCOVA of changes in long and whole call parameters of the Ecstatic Display Call at Cape Bird North, Middle and South over the breeding season with changing weight, sub-colony nest location and appropriate interactions (*n* = 316). A multiple comparison Bonferroni correction (0.05/6) was applied to the data thus the calculated *P* value had to fall below a threshold of 0.008 before being significant.

Call Parameter		R^2	*df*	*F*	*P*	**+/-**	**Tukey's Group**
Long syllable							
Duration		0.077					
	Season		1,305	3.565	0.06		
	Colony		2,305	0.143	0.867		
	Sub-colony		2,305	0.131	0.878		
	Weight		1,305	0.366	0.545		
	Season*Colony		2,305	6.522	**0.002**		
	Colony*Weight		2,305	8.226	**0.0003**		
Pitch		0.051					
	Season		1,309	7.042	**0.008**	-	
	Colony		2,309	1.01	0.366		
	Sub-colony		2,309	1.702	0.184		
	Weight		1,309	0.962	0.328		
Mean Freq		0.057					
	Season		1,309	0.004	0.951		
	Colony		2,309	1.061	0.347		
	Sub-colony		2,309	8.06	**0.0004**		(C) (E M)
	Weight		1,309	0.224	0.636		
FM		0.033					
	Season		1,309	0.23	0.632		
	Colony		2,309	3.98	0.02		
	Sub-colony		2,309	0.88	0.416		
	Weight		1,309	0.105	0.746		
AM^2		0.065					
	Season		1,309	1.115	0.292		
	Colony		2,309	2.206	0.112		
	Sub-colony		2,309	3.406	0.034		
	Weight		1,309	3.706	0.055		
Entropy		0.103					
	Season		1,309	8.296	**0.004**	-	
	Colony		2,309	2.493	0.084		
	Sub-colony		2,309	5.468	**0.005**		(C) (E M)
	Weight		1,309	2.533	0.113		
Whole call							
Duration		0.019					
	Season		1,309	0.012	0.914		
	Colony		2,309	0.232	0.794		
	Sub-colony		2,309	2.357	0.096		
	Weight		1,309	0.101	0.751		
No. syllables		0.023					
	Season		1,309	1.041	0.309		
	Colony		2,309	0.098	0.907		
	Sub-colony		2,309	3.093	0.047		
	Weight		1,309	0.867	0.352		

Note: Where interaction terms between Season, Colony and Weight data are significant the factors have been marked as grey as they cannot be interpreted independently. The direction of change from season and weight data where significant is marked with a +/-. Tukey's groups are ordered from highest (left) LSM values to lowest (right), and significantly different colonies (N=North, M=Middle & S=south) or sub-colonies (C=Centre, M=Middle, E=Edge) are separated by coloured parentheses. Units of measurement - see Table 2.2.

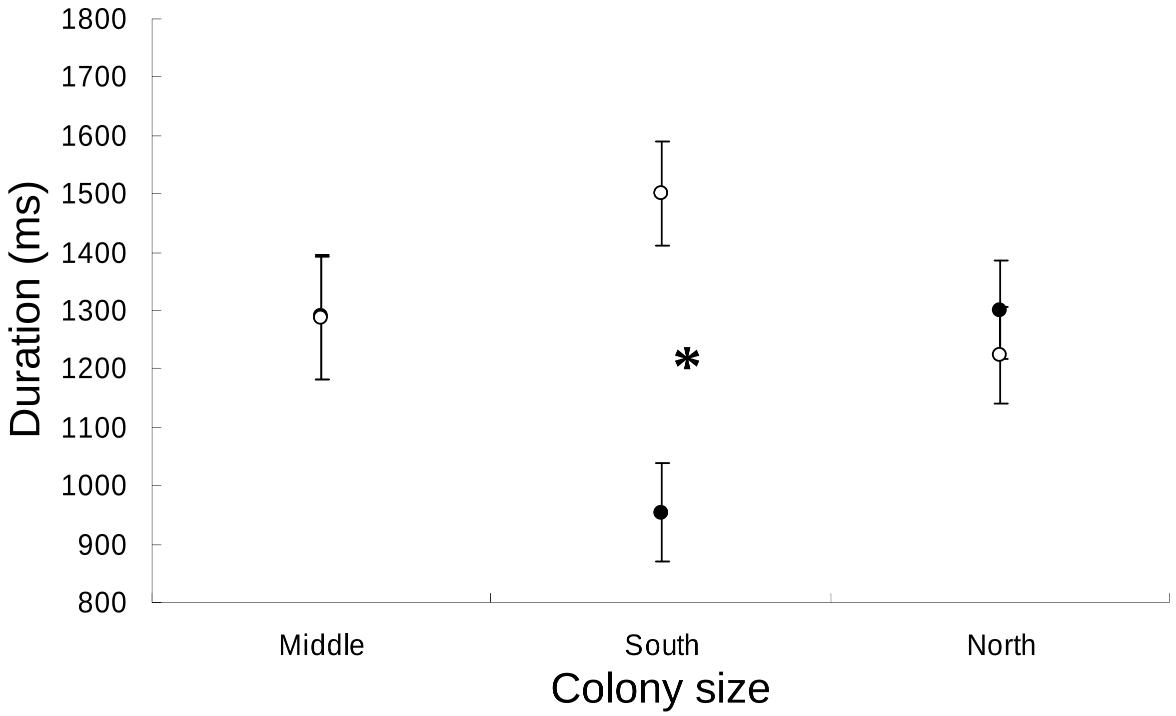


Figure 3.8: Mean (± SE) colony differences in the duration of the long syllable of the Ecstatic Display call between Cape Bird North (n= 126), Middle (n = 72) and South (n = 118) across the breeding season. Early season = (●), late season = (o). Colonies are ordered by size with smallest on the left to largest on the right. Significant differences between early and late season data are highlighted with an asterisk.

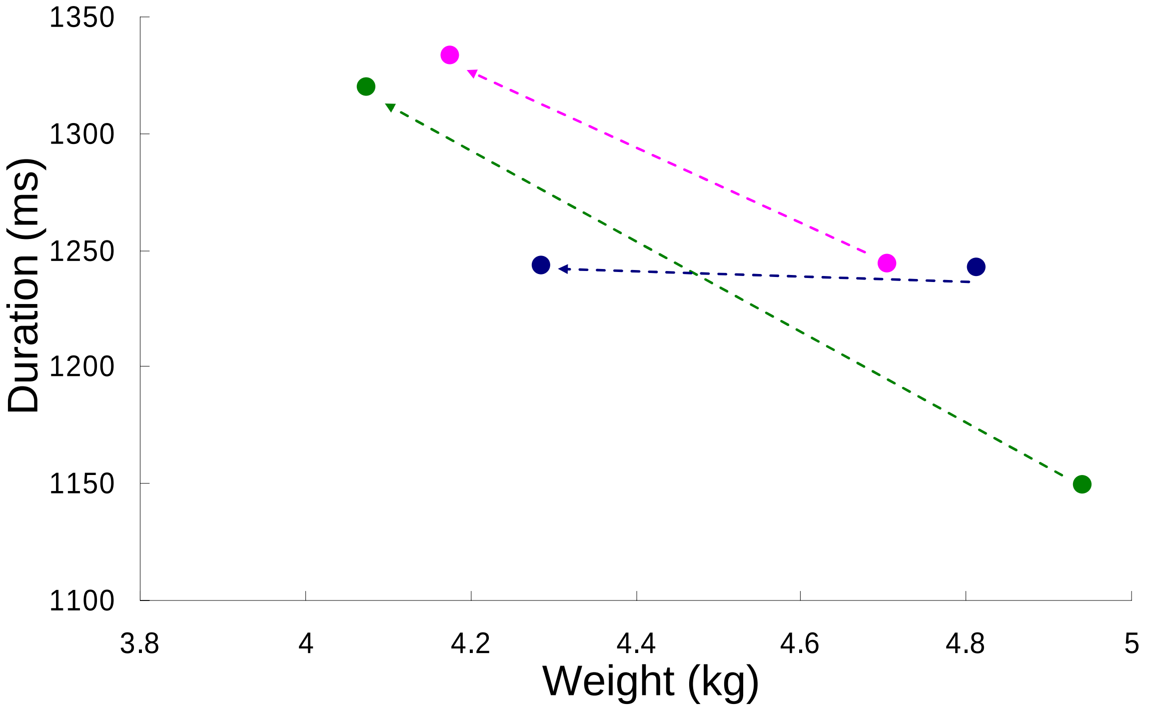


Figure 3.9: Scatterplot of long syllable duration measured against weight of Cape Bird North (n = 126), Middle (n = 72) and South (n = 118) over the breeding season. Colonies are coded as North=blue, Middle=pink, South=green. Direction from early to late season is signified by a line with an arrow for each of the colonies. Note, due to the nature of the graph no standard error of weight or duration is included, and the effect of season on duration was significant only for the South colony (R^2 = 0.144, $F_{1,113}$ = 14.329, P = 0.0002).

None of the short repeated syllable call parameters showed any significant interactions or main effects during the early breeding season (Table 3.13), except for EDC duration, which had a significant interaction between sub-colony nest location and weight (Table 3.13). Although all three sub-colony locations had a similar trend of weight loss and increased EDC duration over the season, the effect was much less pronounced in the edge nests as compared to middle and centre nests (Figure 3.10). When sub-colonies were separated it was the centre nests that showed a significant correlation between early breeding season EDC duration and weight (Figure 3.11).

Table 3.13: Univariate ANCOVA analyses of early season short repeated syllable call parameters across Cape Bird North, Middle and South between sub-colonies and with individual male weight, breeding status (n = 168). Apart from duration all other call parameter interaction terms between breeding, colony, sub-colony and weight were non-significant therefore only main effects were included in the model. A multiple comparison Bonferroni correction (0.05/6) was applied to the data, thus the calculated P value had to fall below a threshold of 0.008 before being considered significant.

Call Parameter		R^2	*df*	*F*	*P*
Duration		0.154			
	Breeding		1,152	0.539	0.464
	Colony		2,152	0.916	0.402
	Sub-colony		2,152	1.776	0.173
	Weight		1,152	1.493	0.224
	Sub-colony*Weight		2,152	5.213	**0.007**
Pitch		0.092			
	Breeding		1,161	5.755	0.012
	Colony		2,161	1.69	0.188
	Sub-colony		2,161	1.656	0.194
	Weight		1,161	0.01	0.922
Mean Freq		0.07			
	Breeding		1,161	2.296	0.132
	Colony		2,161	0.91	0.405
	Sub-colony		2,161	2.899	0.058
	Weight		1,161	0.051	0.822
FM		0.073			
	Breeding		1,161	5.512	0.02
	Colony		2,161	2.823	0.062
	Sub-colony		2,161	1.631	0.199
	Weight		1,161	0.004	0.949
AM^2		0.107			
	Breeding		1,161	1.155	0.284
	Colony		2,161	4.153	0.017
	Sub-colony		2,161	3.457	0.034
	Weight		1,161	3.385	0.068
Entropy		0.036			
	Breeding		1,161	0.645	0.423
	Colony		2,161	0.795	0.454
	Sub-colony		2,161	1.368	0.258
	Weight		1,161	0.319	0.573

Note: All significant *P* values are marked in **bold**. Where the interaction term between sub-colony*weight for the call parameter duration was significant the factors have been marked as grey as they cannot be interpreted independently. Apart from duration all other call parameter interaction terms between breeding, colony, sub-colony and weight were non-significant therefore the final model could be run without these terms. Units of measurement - see Table 2.2.

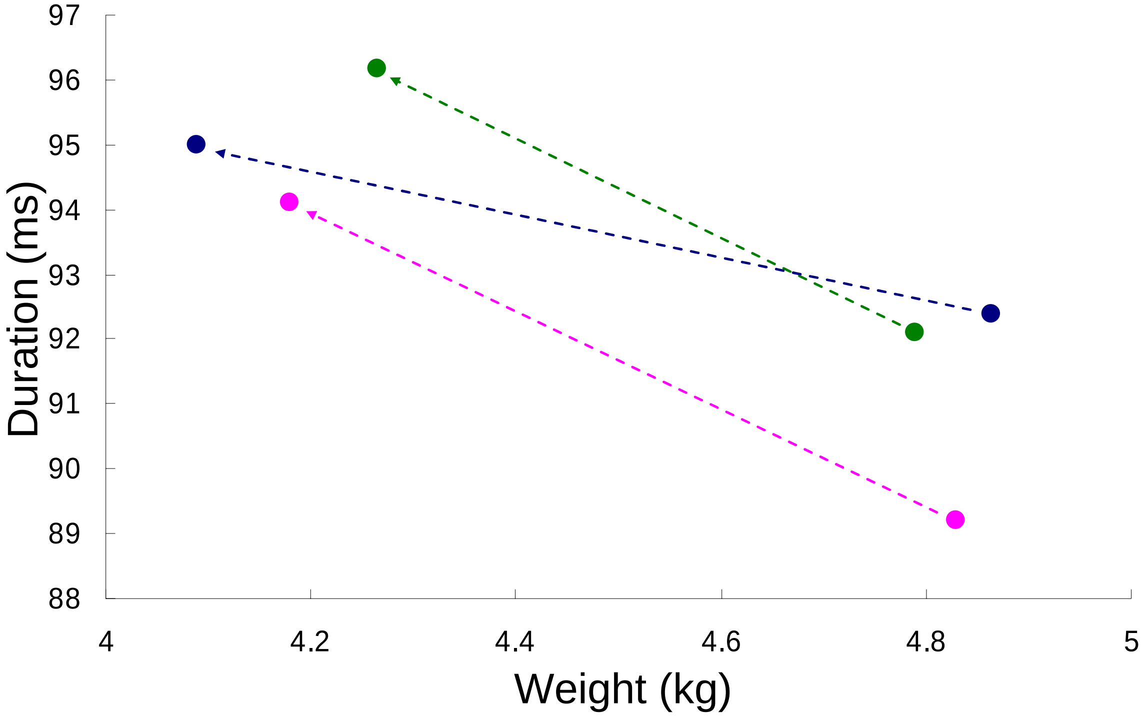


Figure 3.10: Scatterplot of short repeated syllable duration measured against weight of Cape Bird sub-colony nest locations over the breeding season. Sub-colonies are coded as Centre=pink (*n* = 65), Middle=green (*n* = 124) and Edge=blue (*n* = 142). Direction from early to late season is signified by a line with an arrow for each of the sub-colonies. Note, due to the nature of the graph no standard error of weight or duration is included, and the effect of breeding season on duration was significant only for the centre sub-colony nests.

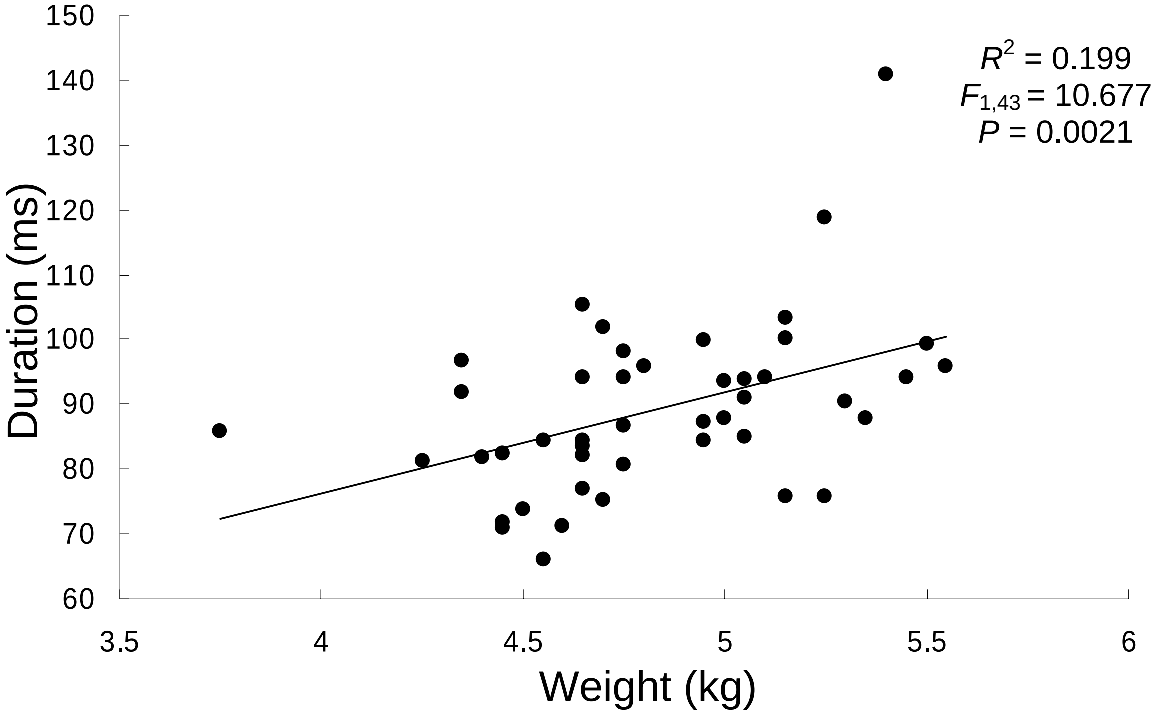


Figure 3.11: Linear regression of short syllable duration against weight in the centre sub-colonies of Cape Bird at the beginning of the breeding season (*n* = 45).

The long syllables had no significant interactions or main effects with any of the EDC parameters, as for the whole call parameters, duration was not significantly related to any of the model effects. However, the number of syllables was, with birds having paired having significantly more syllables in their calls than those that were still without a mate (Table 3.14). There was also an effect of sub-colony nest location on the number of syllables, with birds in the middle of the colony having higher numbers as compared to centre nesting birds, with edge nesting birds having an intermediary number of syllables (Table 3.14).

Table 3.14: Univariate ANCOVA analyses of early season long and whole call parameters across Cape Bird North, Middle and South between sub-colonies and with individual male weight and breeding status (n = 153). All interactions between breeding, colony, sub-colony and weight were not significant thus only main effects were included in the model. A multiple comparison Bonferroni correction (0.05/6) was applied to the long syllable data, thus the calculate P value had to fall below 0.008 before being considered significant.

Call Parameter		R^2	*df*	*F*	*P*	**P/S**	**Tukey's Group**
Long syllable							
Duration		0.026					
	Breeding		1,146	0.019	0.892		
	Colony		2,146	0.429	0.652		
	Sub-colony		2,146	1.358	0.261		
	Weight		1,146	0.023	0.88		
Pitch		0.088					
	Breeding		1,146	1.764	0.186		
	Colony		2,146	2.476	0.088		
	Sub-colony		2,146	2.3	0.104		
	Weight		1,146	0.071	0.79		
Mean Freq		0.089					
	Breeding		1,146	1.155	0.284		
	Colony		2,146	1.486	0.23		
	Sub-colony		2,146	4.834	0.009		
	Weight		1,146	0.183	0.669		
FM		0.043					
	Breeding		1,146	0.852	0.358		
	Colony		2,146	2.113	0.125		
	Sub-colony		2,146	0.07	0.933		
	Weight		1,146	0.447	0.505		
AM^2		0.09					
	Breeding		1,146	6.325	0.013		
	Colony		2,146	0.993	0.373		
	Sub-colony		2,146	2.255	0.109		
	Weight		1,146	1.643	0.202		
Entropy		0.071					
	Breeding		1,146	0.2	0.655		
	Colony		2,146	1.462	0.235		
	Sub-colony		2,146	3.284	0.04		
	Weight		1,146	1.24	0.267		
Whole call							
Duration		0.05					
	Breeding		1,146	2.99	0.086		
	Colony		2,146	0.271	0.763		
	Sub-colony		2,146	1.348	0.263		
	Weight		1,146	0.03	0.864		
No. syllables		0.102					
	Breeding		1,146	5.886	**0.017**	P>S	
	Colony		2,146	0.197	0.822		
	Sub-colony		2,146	4.332	**0.015**		(M (E) C)
	Weight		1,146	0.084	0.773		

Note: All significant P values are marked in **bold**. Breeding status (P=Pair, S=Solo) and direction of difference is denoted by </> symbols. Tukey's groups are ordered from highest (left) LSM values to lowest (right), and significantly different colonies (N=North, M=Middle & S=south) or sub-colonies (C=Centre, M=Middle, E=Edge) are separated by coloured parentheses. Units of measurement - see Table 2.2.

At the end of the breeding season there were again no main or interaction effects for the short syllable EDC call parameters, except for duration, which had a significant interaction (between breeding status and colony) (Table 3.15). When the colonies were separated the effect of breeding status on duration could be seen more clearly (Figure 3.12a & b). The South colony in the early breeding season has significantly shorter syllables for birds that have achieved a mate ($R^2 = 0.077$, $F_{1,58} = 4.821$, $P = 0.032$; Figure 3.12a). In the late breeding season it was birds that had failed to breed in the South colony that had shorter syllables than birds with either one or two chicks ($R^2 = 0.121$, $F_{2,53} = 3.661$, $P = 0.032$; Figure 3.12b). Additionally, at the Middle colony the same pattern is observed with the failed breeders having significantly shorter repeated syllables than birds with either one or two chicks ($R^2 = 0.329$, $F_{2,34} = 8.342$, $P = 0.001$; Figure 3.12b). There were no colony differences in duration for either early or late breeding season short syllable data.

Table 3.15: Univariate ANCOVA analyses of late season short repeated syllable call parameters across Cape Bird North, Middle and South between sub-colonies, with individual male weight and breeding status (*n* = 163). Apart from duration all other call parameter interaction terms between breeding, colony, sub-colony and weight were non-significant therefore only main effects were included in the model. A multiple comparison Bonferroni correction (0.05/6) was applied to the data, thus the calculated *P* value had to fall below a threshold of 0.008 before being considered significant.

Call Parameter		R^2	*df*	*F*	*P*
Duration		0.237			
	Breeding		2,141	4.605	0.012
	Colony		2,141	4.919	0.009
	Sub-colony		2,141	2.936	0.056
	Weight		1,141	1.358	0.246
	Breeding*Colony		4,141	3.897	**0.005**
Pitch		0.068			
	Breeding		2,155	1.149	0.32
	Colony		2,155	0.905	0.407
	Sub-colony		2,155	3.314	0.039
	Weight		1,155	0.253	0.616
Mean Freq		0.08			
	Breeding		2,155	0.664	0.516
	Colony		2,155	1.256	0.288
	Sub-colony		2,155	3.281	0.04
	Weight		1,155	1.044	0.308
FM		0.093			
	Breeding		2,155	5.013	0.008
	Colony		2,155	1.566	0.212
	Sub-colony		2,155	0.344	0.709
	Weight		1,155	0.211	0.647
AM^2		0.094			
	Breeding		2,155	2.375	0.096
	Colony		2,155	3.929	0.022
	Sub-colony		2,155	1.539	0.218
	Weight		1,155	0.088	0.767
Entropy		0.053			
	Breeding		2,155	0.227	0.797
	Colony		2,155	1.949	0.146
	Sub-colony		2,155	1.312	0.272
	Weight		1,155	1.05	0.307

Note: All significant *P* values are marked in **bold**. Where the interaction term between breeding*colony for the call parameter duration was significant the factors have been marked as grey as they cannot be interpreted independently. Units of measurement - see Table 2.2.

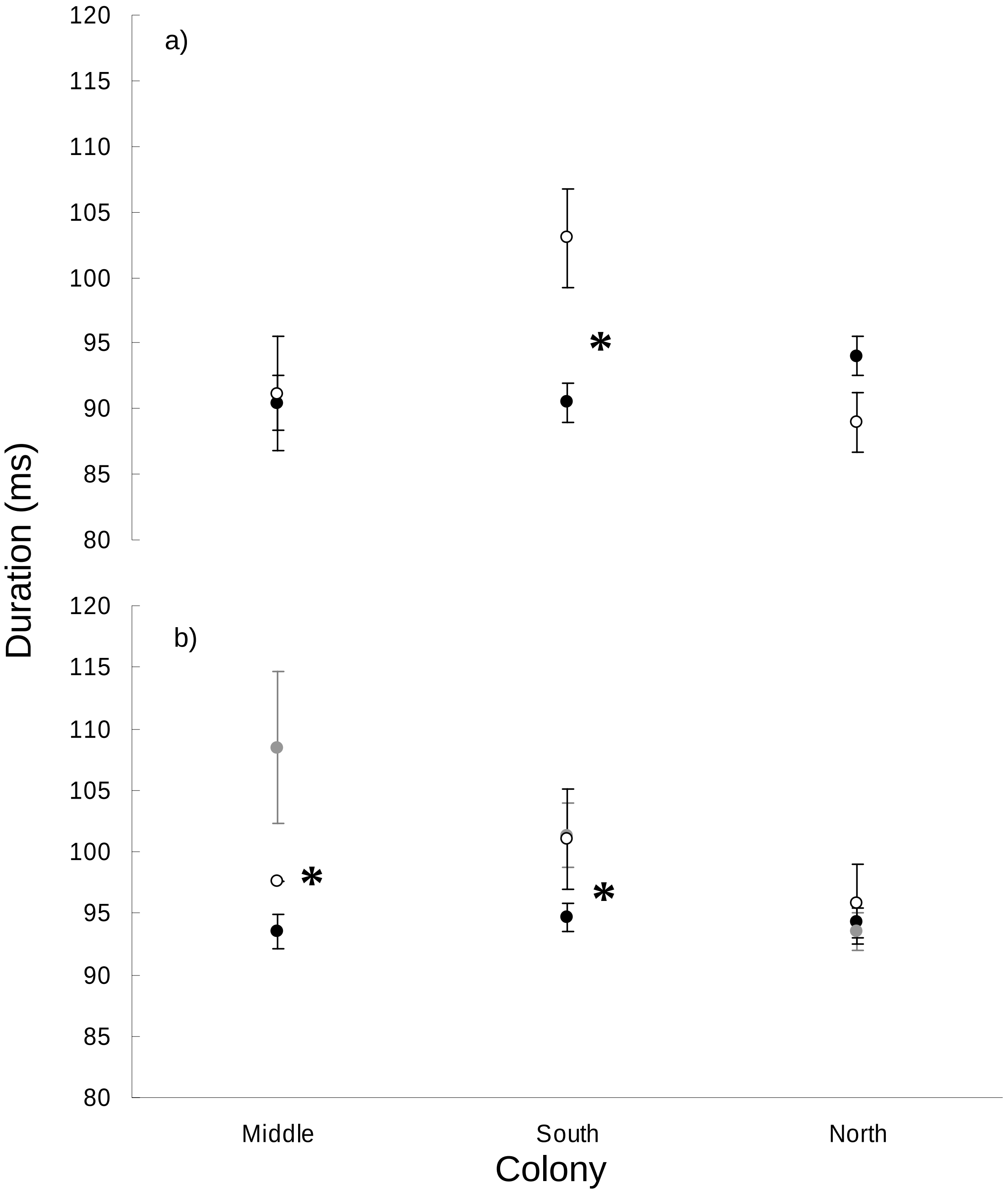


Figure 3.12: Mean (± SE) short repeated syllable duration between Cape Bird North (*n* = 126), Middle (*n* = 87) and South (*n* = 118) categorised by **a)** early and **b)** late season breeding status achieved. Colonies are ordered by size with smallest on the left to largest on the right. For early season status **solo** (●) refers to a male at nest with no female present and **pair** (o) was assigned to a male is a female was present throughout the recording period. Late breeding status is defined as having a double chick nest (o), a single chick nest (closed grey o), and fail (●) where no chick was observed in the vicinity of the male/nest. Significant differences in duration between breeding status achieved within a colony are highlighted with an asterisk.

The long syllables at the end of the breeding season also showed no significant main effects or interactions for any of the EDC parameters except for duration, which again had a significant interaction, this time between colony and weight (Table 3.16). When duration was separated by colony there was a significant positive correlation with weight found in the South colony (Figure 3.13). The whole call parameters at the end of the breeding season showed that the EDC duration was significantly longer in birds that had failed to breed than birds that had one chick, with birds with two chicks having intermediary length calls (Table 3.16). There were no differences in the number of syllables in EDCs at the end of the breeding season for any of the main effects (Table 3.16).

Table 3.16: Univariate ANCOVA analyses of late season long and whole call parameters across Cape Bird colonies between sub-colonies and with individual male weight and breeding status (*n* = 163). Apart from duration all other interactions between breeding, colony, sub-colony and weight were not signficant thus only main effects were included in the model. A Bonferroni correction (0.05/6 = 0.008) adjusts the critical *P* value.

Call Parameter		R^2	*df*	*F*	*P*	**Tukey's Group**
Long syllable						
Duration		0.195				
	Breeding		2,141	3.457	0.034	
	Colony		2,141	0.104	0.902	
	Sub-colony		2,141	0.808	0.448	
	Weight		1,141	0.335	0.563	
	Colony*Weight		4,141	5.441	**0.005**	
Pitch		0.051				
	Breeding		2,155	2.446	0.09	
	Colony		2,155	0.365	0.695	
	Sub-colony		2,155	0.314	0.731	
	Weight		1,155	2.074	0.152	
Mean Freq		0.076				
	Breeding		2,155	1.219	0.299	
	Colony		2,155	1.325	0.269	
	Sub-colony		2,155	2.753	0.067	
	Weight		1,155	0.564	0.454	
FM		0.054				
	Breeding		2,155	0.267	0.766	
	Colony		2,155	2.207	0.114	
	Sub-colony		2,155	1.761	0.175	
	Weight		1,155	0.065	0.8	
AM^2		0.118				
	Breeding		2,155	3.792	0.025	
	Colony		2,155	2.859	0.06	
	Sub-colony		2,155	2.467	0.088	
	Weight		1,155	2.923	0.089	
Entropy		0.089				
	Breeding		2,155	1.046	0.354	
	Colony		2,155	3.531	0.032	
	Sub-colony		2,155	1.684	0.189	
	Weight		1,155	2.123	0.147	
Whole call						
Duration		0.075				
	Breeding		2,155	5.21	**0.007**	(F (T) O)
	Colony		2,155	0.69	0.503	
	Sub-colony		2,155	0.007	0.993	
	Weight		1,155	0.121	0.728	
No. syllables		0.047				
	Breeding		2,155	1.395	0.251	
	Colony		2,155	0.121	0.886	
	Sub-colony		2,155	1.344	0.264	
	Weight		1,155	0.226	0.635	

Note: Where the interaction term between colony*weight for the call parameter duration was significant the factors have been marked as grey as they cannot be interpreted independently. Tukey's groups are ordered from highest (left) LSM values to lowest (right), and significantly different colonies (N=North, M=Middle & S=south), sub-colonies (C=Centre, M=Middle, E=Edge) or breeding status (T=Two chicks, O=One chick, F=Fail) are separated by coloured parentheses. Units of measurement - see Table 2.2.

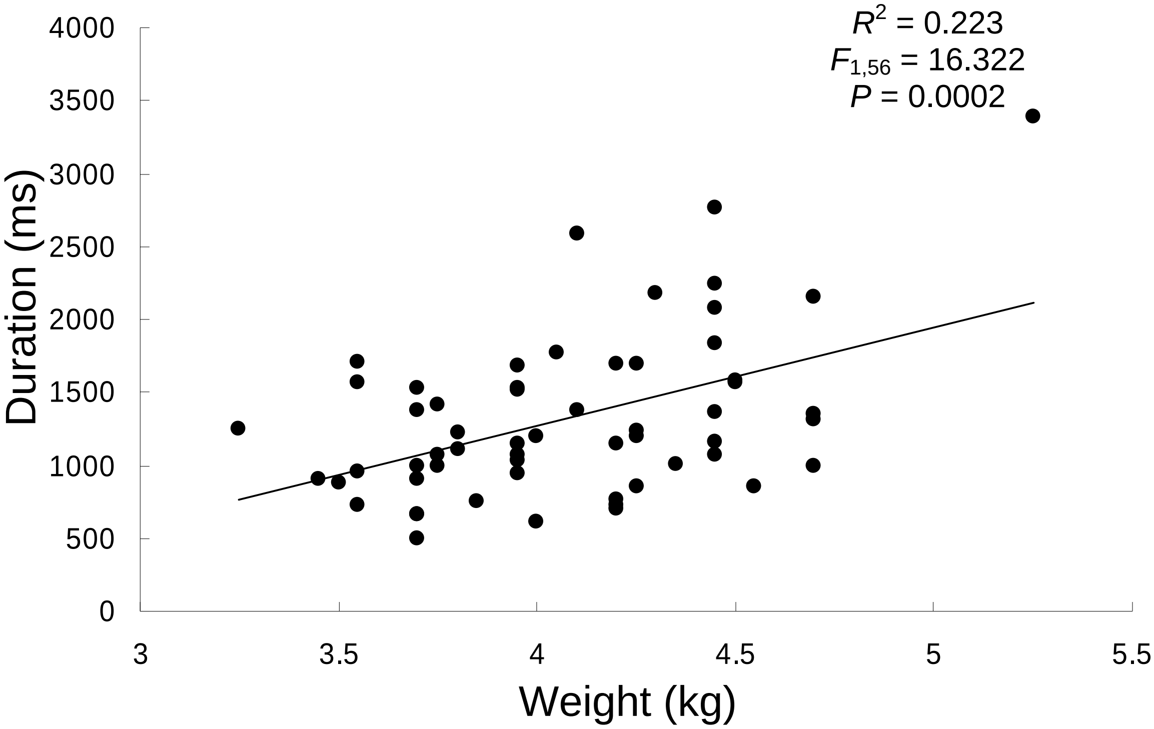


Figure 3.13: Linear regression of long syllable duration against weight for Cape Bird South colony at the end of the breeding season ($n = 58$).

Variation in male weight between colonies (Table 3.17a) shows that there was a significant interaction between colony and breeding season for weight. The data were segregated by colony and re-analysed (Table 3.17b). Results indicate that for both the North and Middle colony there was a significant decrease in weight over the season, and no effect of sub-colony location (Table 3.17b). Figure 3.14 demonstrates this change in weight over the season between the colonies. There was a significant interaction between sub-colony nest position and season at the South colony, therefore the data were once again segregated (Table 3.17c). The results show that in the early season there is no effect of sub-colony location on male weight, however at the end of the breeding season birds with nests in the middle of the sub-colony weighed more than birds at the edge, and centrally nesting birds had intermediary weights (Table 3.17c). South colony also had significant weight loss over the season (centre – $R^2 = 0.603$, $F_{1,64} = 97.381$, $P = < 0.0001$; middle - $R^2 = 0.597$, $F_{1,88} = 130.476$, $P = < 0.0001$; edge - $R^2 = 0.388$, $F_{1,74} = 46.98$, $P = < 0.0001$; Figure 3.14). The whole model also found a significant association between weight and sub-colony nest location with birds in middle nests being heavier on average than those in edge nests (Table 3.17a). The central nests were again intermediary in weight (Table 3.17a).

Table 3.17: Univariate ANOVA analyses of male weight across the breeding season, between Cape Bird North, Middle and South for differing sub-colony nest locations and with changing breeding status of the males (*n* = 703). Part **a)** refers to the whole model ANOVA used to compare season, colony and sub-colony data. Due to the significant interaction between colony and season the colonies have been separated in part **b)** to investigate season and sub-colony effects. Due to the significant interaction between season and sub-colony in the South colony part **c)** looks at the effect of sub-colony location on weight in both the early and late breeding season. All models were run with appropriate interactions, which if not significant were removed from the model, thus only main effects are shown.

		R^2	*df*	*F*	*P*	**+/-**	**Tukey's Groups**
a) *Whole*		0.381					
	Season		1,695	291.25	< 0.0001		
	Colony		2,695	7.179	0.0008		
	Sub-Colony		2,695	3.212	**0.041**		(M (C) E)
	Colony∗Season		2,695	6.117	**0.002**		
b) *Colony*							
North		0.276					
	Season		1,370	133.63	**< 0.0001**	-	
	Sub-Colony		2,370	2.504	0.083		
Middle		0.349					
	Season		1,93	47.783	**< 0.0001**	-	
	Sub-Colony		2,93	0.85	0.431		
South		0.557					
	Season		1,231	254.694	< 0.0001		
	Sub-Colony		2,231	0.477	0.621		
	Season∗Sub-Col		2,231	4.58	**0.011**		
c) *South*							
		0.026					
Early	Sub-Colony		2,119	1.552	0.216		
		0.063					
Late	Sub-Colony		2,111	3.675	**0.029**		(M (C) E)

Note: Significant results are marked in **bold**. Significant interaction effects have been coloured grey to signify they cannot be interpreted independently. Direction of weight change over the season is signified by a +/- symbol. Significant differences determined by Tukey's groups between colony and sub-colony categories are ordered from highest LSM value (left) to lowest (right) and separated by coloured parentheses. They are coded for colony as N=north, M=Middle, S=South and by sub-colony C=Centre, M=Middle and E=Edge.

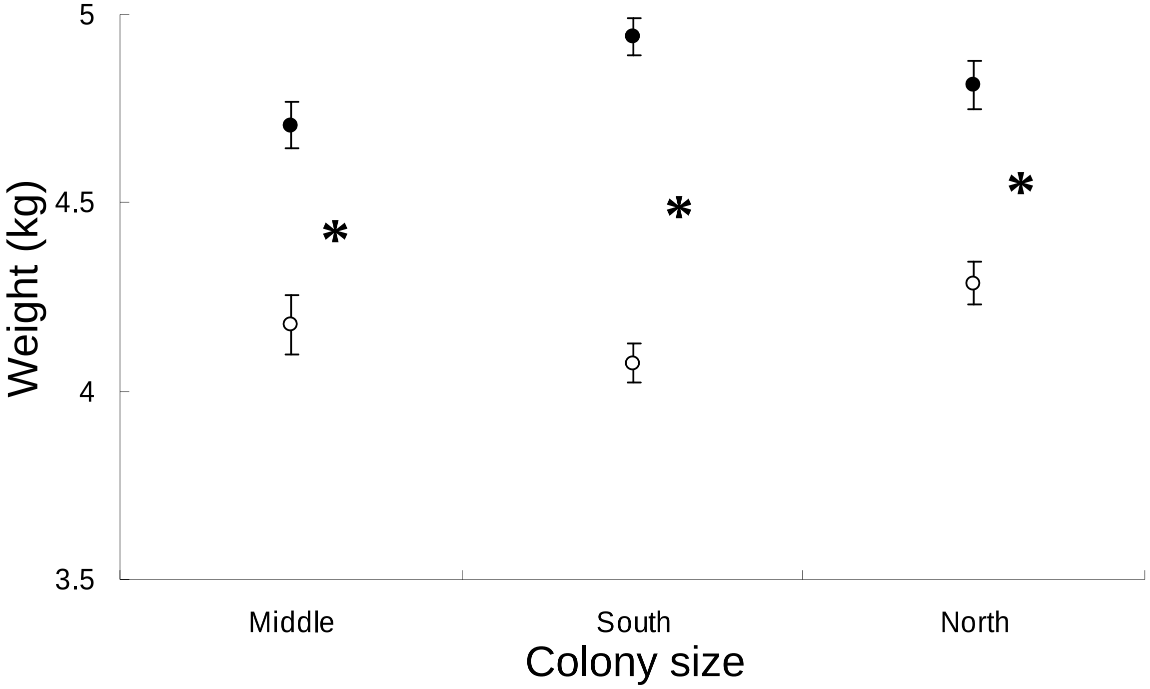


Figure 3.14: Mean (± SE) male weight changes over the breeding season between Cape Bird North, Middle and South (*n* = 703). Early season = (●), late season = (o). Colonies are ordered by size with smallest on the left to largest on the right. Significant differences between seasonal weights are highlighted with an asterisk.

To investigate the relationship between breeding status and male weight across the breeding season the data were separated by season (Table 3.18a). The early season data shows no difference in weight in birds that are paired compared to those still solo, and no effect of sub-colony nest position on male weight (Table 3.18a). There was however, a weakly significant effect of weight between colonies (Table 3.18a), the Tukey's groups could not separate the colonies, but looking at Cape Bird colonies alone, South colony has slightly greater weights than the North or Middle colony males (Figure 3.14). The late season data also shows no effect of sub-colony location on male weight (Table 3.18a). There was however a significant interaction between colony and breeding status (Table 3.18a); therefore the data were separated by colony (Table 3.18b). The North colony shows a significant effect of breeding status, with males of greater weight having succeeded to breed and having either one or two chicks, and failed breeders being of lesser weight (Table 3.18b). The Middle colony is significant for neither breeding status nor sub-colony location (Table 3.18b). The South colony is not significant for breeding status, but males in the middle of the sub-colony weighed significantly more than males nesting at the edge of the sub-colony; centre nesting males were of intermediary weight (Table 3.18b).

Table 3.18: Univariate ANOVA analyses of male weight during the early (*n* = 369) and late (*n* = 334) breeding season, between Cape Bird North, Middle and South for differing sub-colony nest locations and with changing breeding status of the males. Part **a)** refers to the whole model ANOVA used to compare colony, sub-colony and breeding data. Due to the significant interaction between breeding status and colony during the late season the colonies have been separated in part **b)** to investigate breeding and sub-colony effects. All models were run with appropriate interactions, which if not significant were removed from the model, thus only main effects are shown.

		R^2	*df*	*F*	*P*	Tukey's Groups
a) *Season*						
Early		0.018				
	Breeding Status		1,363	0.05	0.823	
	Colony		2,363	3.089	**0.047**	NS
	Sub-Colony		2,363	0.256	0.774	
Late		0.185				
	Breeding Status		2,323	3.27	0.039	
	Colony		2,323	13.893	< 0.0001	
	Sub-Colony		2,323	2.49	0.085	
	Colony*Breeding		4,323	5.814	**0.0002**	
b) *Late*						
North		0.208				
	Breeding Status		2,170	20.976	**< 0.0001**	(O T) (F)
	Sub-Colony		2,170	1.034	0.358	
Middle		0.063				
	Breeding Status		2,42	1.038	0.363	
	Sub-Colony		2,42	0.206	0.815	
South		0.069				
	Breeding Status		2,107	0.354	0.703	
	Sub-Colony		2,107	3.968	**0.022**	(M (C) E)

Note: Significant results are marked in **bold**. Significant interaction effects have been coloured grey to signify they cannot be interpreted independently. Significant differences determined by Tukey's groups between colony, sub-colony and breeding categories are ordered from highest LSM value (left) to lowest (right) and separated by coloured parentheses. They are coded for colony as N=North, M=Middle, S=South; by sub-colony C=Centre, M=Middle and E=Edge and by breeding T=Two chicks, One=One chick and F=Fail.

In the early breeding season there was a significant difference in the frequency of males paired versus those solo between the colonies (R^2 = 0.021, $\chi^2_{2,366}$ = 7.843, P = 0.02) and with nest location within the sub-colonies (R^2 = 0.029, $\chi^2_{2,366}$ = 10.92, P = 0.004). Figure 3.15 shows that proportionally there were fewer paired males at the South colony than at either Middle or North colonies. The pattern of paired versus solo males between the sub-colonies clearly shows that a greater proportion of birds on the edge of the sub-colony were solo, with middle nests and centre nests having more paired males (Figure 3.16). At the end of the breeding season there was again a difference in frequency of breeding status categories and both the colony (R^2 = 0.025, $\chi^2_{4,328}$ = 13.786, P = 0.008) and sub-colony location (R^2 = 0.056, $\chi^2_{4,328}$ = 31.271, P = < 0.0001) of the males sampled. Colony differences and breeding status patterns show a greater proportion of two chick nests in the North colony compared to either Middle or South colonies, and also a greater proportion of birds that failed to breed in the Middle and South colonies (Figure 3.17). Sub-colony nest location also shows and interesting pattern of breeding stages achieved by sampled males, with centre nests having more two chick nests, followed by middle nests and lastly the edge nests, which also failed in a much greater proportion than the middle and centre nests (Figure 3.18).

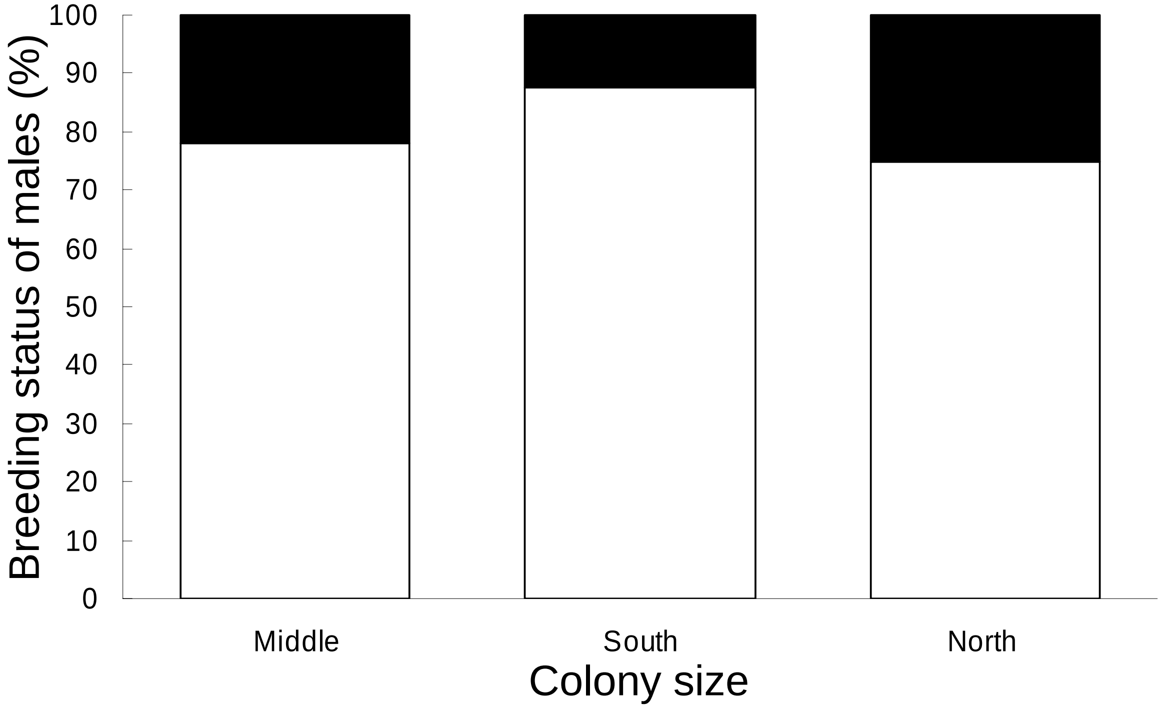


Figure 3.15: Breeding status (■= pair, ☐= solo) of Cape Bird North (*n* = 199), Middle (*n* = 50) and South (*n* = 120) birds during the early breeding season represented as a percentage of total birds sampled per colony. Colonies are ordered by size with smallest on the left to largest on the right.

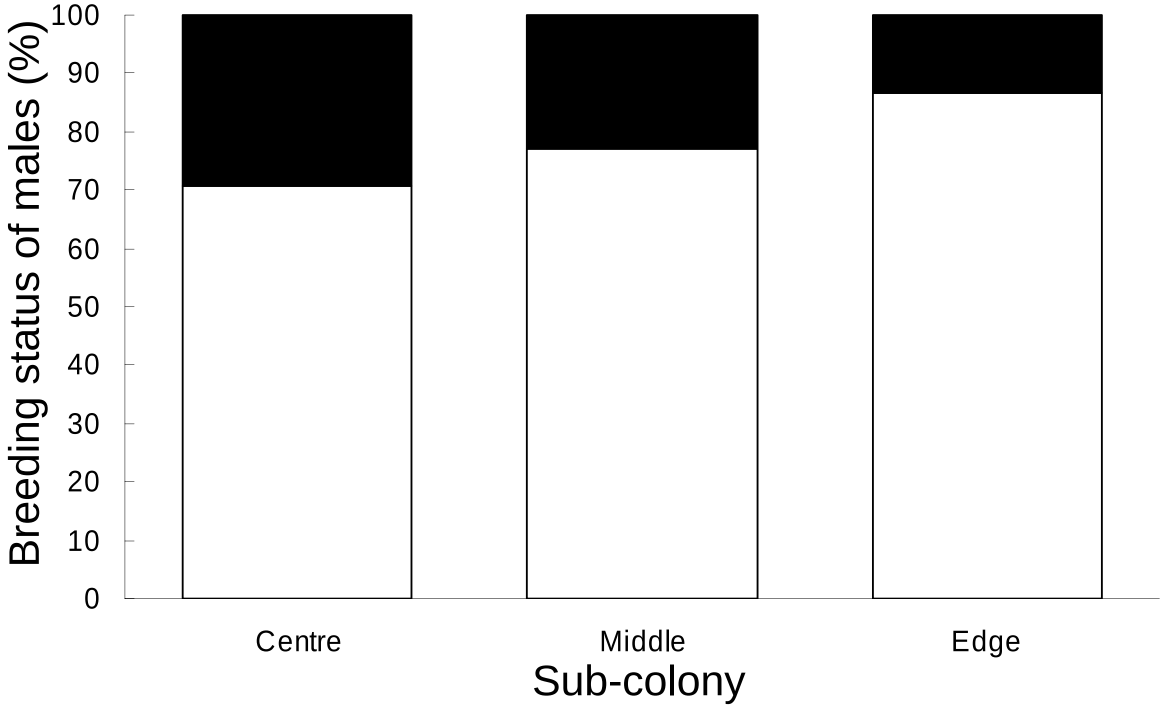


Figure 3.16: Breeding status (■ = pair, ☐ = solo) of males associated with sub-colony nest locations (Centre *n* = 113, Middle *n* = 91 and Edge *n* = 165) across Cape Bird colonies during the early breeding season with data represented as a percentage of total birds sampled per sub-colony type.

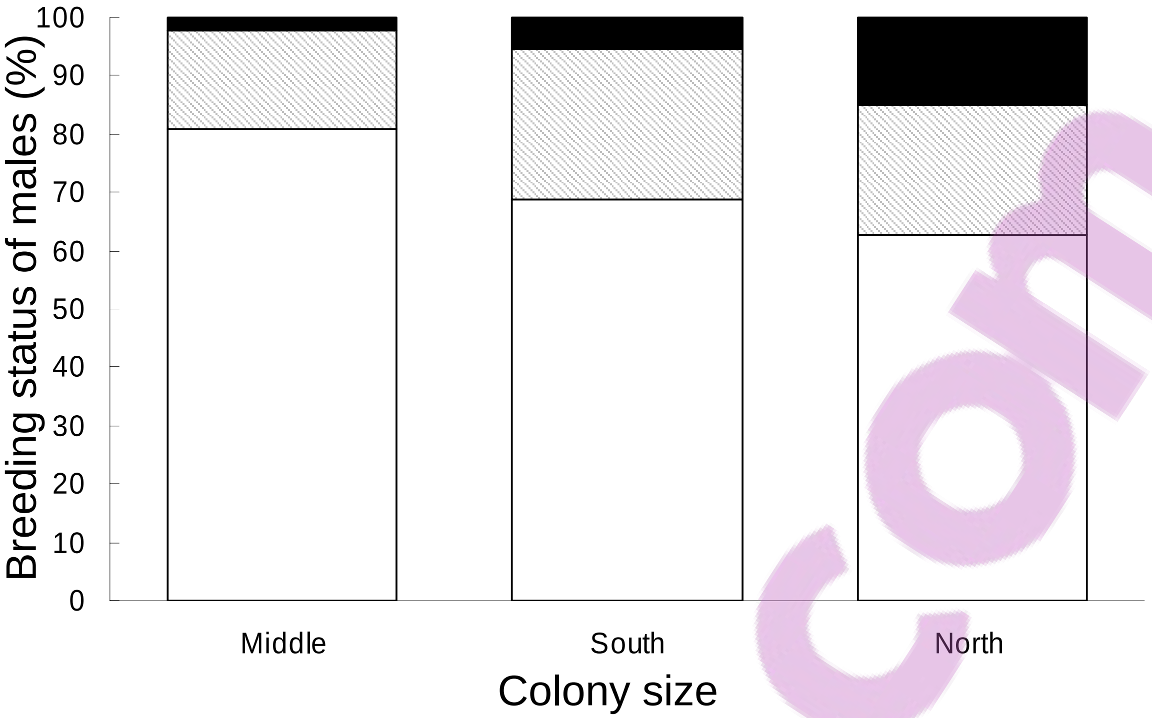


Figure 3.17: Breeding status (■= double chick nest, ▨= single chick, ☐= fail) of Cape Bird North (*n* = 175), Middle (*n* = 47) and South (*n* = 112) birds during the late breeding season represented as a percentage of total birds sampled per colony. Colonies are ordered by size with smallest on the left to largest on the right.

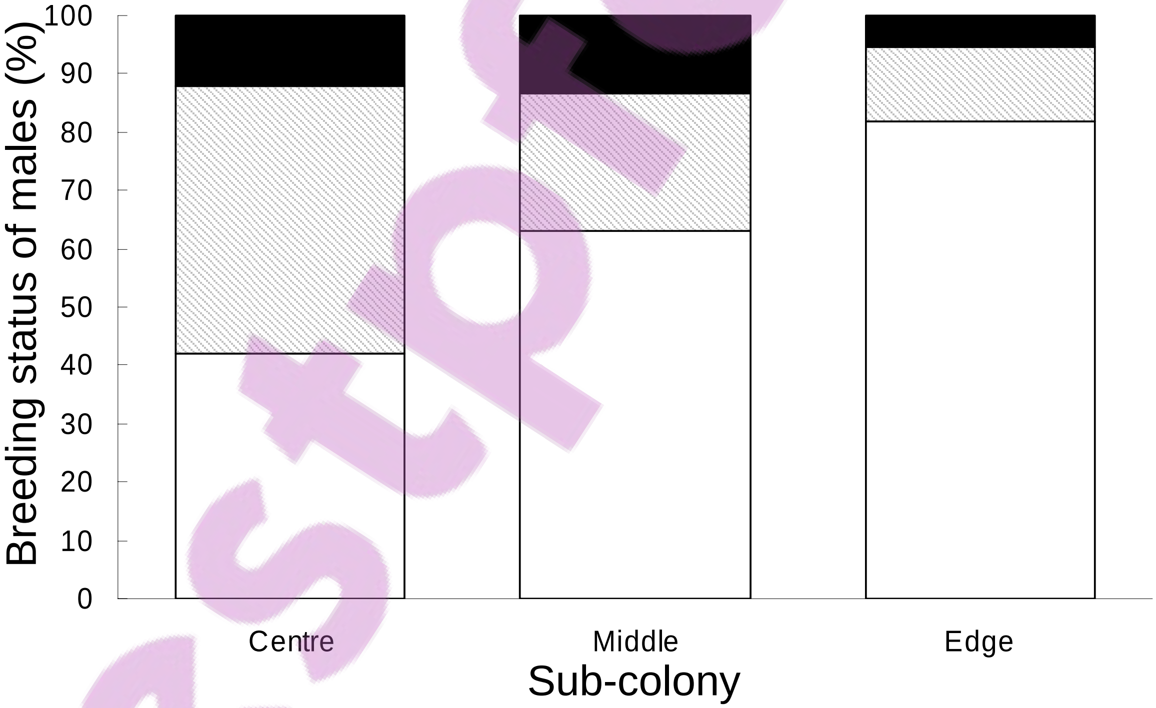


Figure 3.18: Breeding status (■ = double chick nest, ▨= single chick, ☐= fail) of males associated with sub-colony nest locations (Centre *n* = 50, Middle *n* = 151 and Edge *n* = 133) across Cape Bird colonies during the late breeding season with data represented as a percentage of total birds sampled per sub-colony type.

3.4.5 Chick condition at Cape Bird

To complement the differences found in Cape Bird call parameters between colonies and across the season it must also be noted that there were differences in the breeding success (measured as a condition index) between the colonies. An additional estimate of the breeding state of the three colonies was the observed frequency of chick fledging stages between the colonies and how that was related to the condition index (weight and wing length estimate) of the chicks at those stages.

Firstly, chicks from Cape Bird North were in significantly better condition than chicks in either Bird Middle or South ($R^2 = 0.317$, $F_{2,312} = 8.178$, $P = 0.0003$; Figure 3.19). When fledging stage between colonies was examined it was shown that again there was a significant difference in the frequency of fledging stages observed between the colonies ($R^2 = 0.317$, $F_{4,312} = 30.821$, $P = < 0.0001$; Figure 3.20), with Cape Bird Middle having greater numbers of chicks nearly finished fledging. Cape Bird North (the largest colony), despite having chicks in better condition than Cape Bird Middle, had fewer chicks that had finished the fledging process. Figure 3.20 also shows that approximately 25% of the chicks sampled from Cape Bird South had not begun to fledge yet.

To see the relationship between fledging stage and chick condition the data between colonies was combined and a univariate analysis of chick condition by fledging stage was performed. Results show that there is a difference in condition between fledging stages ($R^2 = 0.02$, $\chi^2_{6,316} = 15.945$, $P = < 0.0001$) and that chicks which have nearly finished fledging, were in the best condition, and chicks that had finished fledging were in similar or poorer condition (Figure 3.21). Those same chicks that had finished fledging were in similar condition to those partially fledged or those just beginning to fledge (Figure 3.21). The chicks in the poorest condition were those that had yet to begin the fledging process (Figure 3.21).

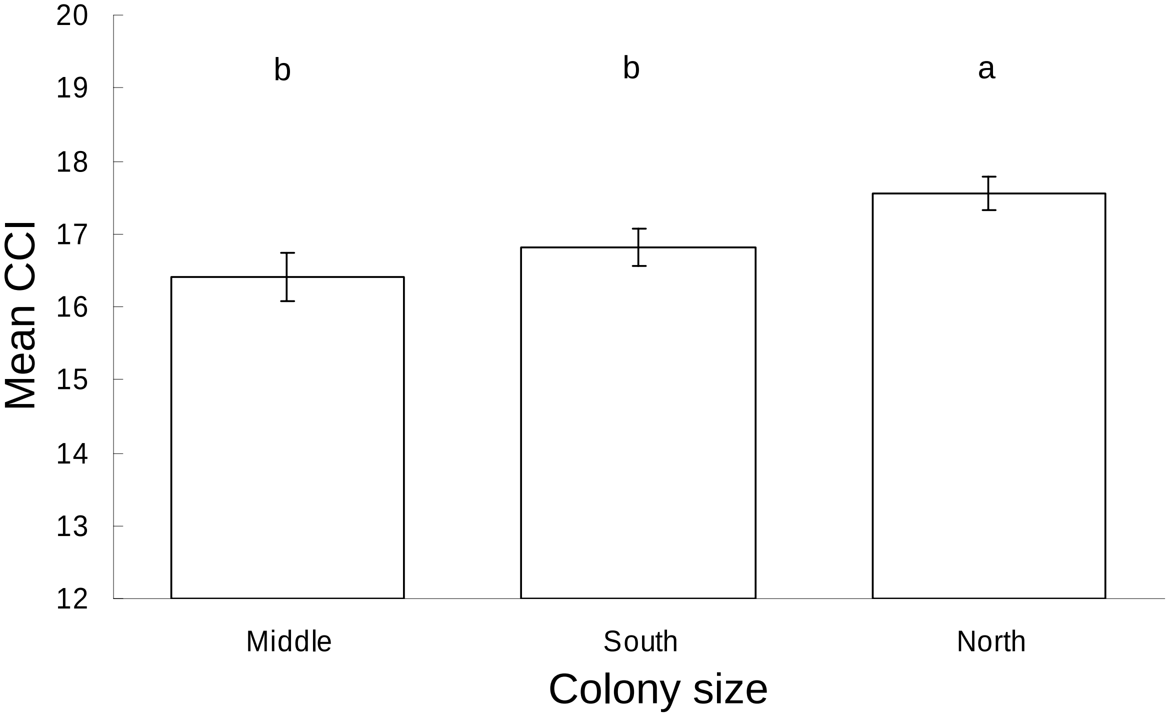


Figure 3.19: Mean (± SE) chick condition index (CCI) measured at the end of the breeding season between Cape Bird colonies on Ross Island (n = 319). Colonies are ordered by size with smallest on the left to largest on the right. According to Tukey's groups colonies not connected by same letter are significantly different.

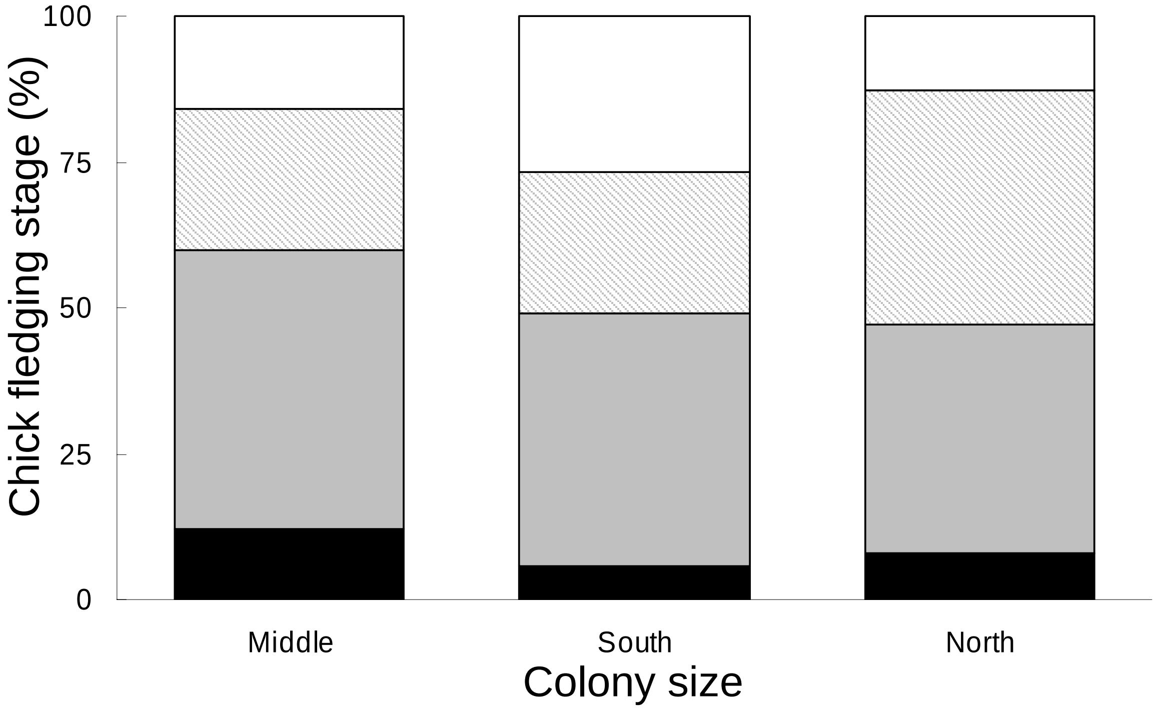


Figure 3.20: Percentage of chicks sampled at different fledging stages between Cape Bird North (n = 149), Middle (n = 50) and South (n = 120) colonies on Ross Island. Stage "Mostly" and "Totally" have been combined to increase inter-colony frequencies for contingency analyses. Fledging stage "Mostly+Total" = ■, "Partially" = ▒, "Begun" = ▨ and "Not begun" = □ . Colonies are ordered by size with smallest on the left to largest on the right.

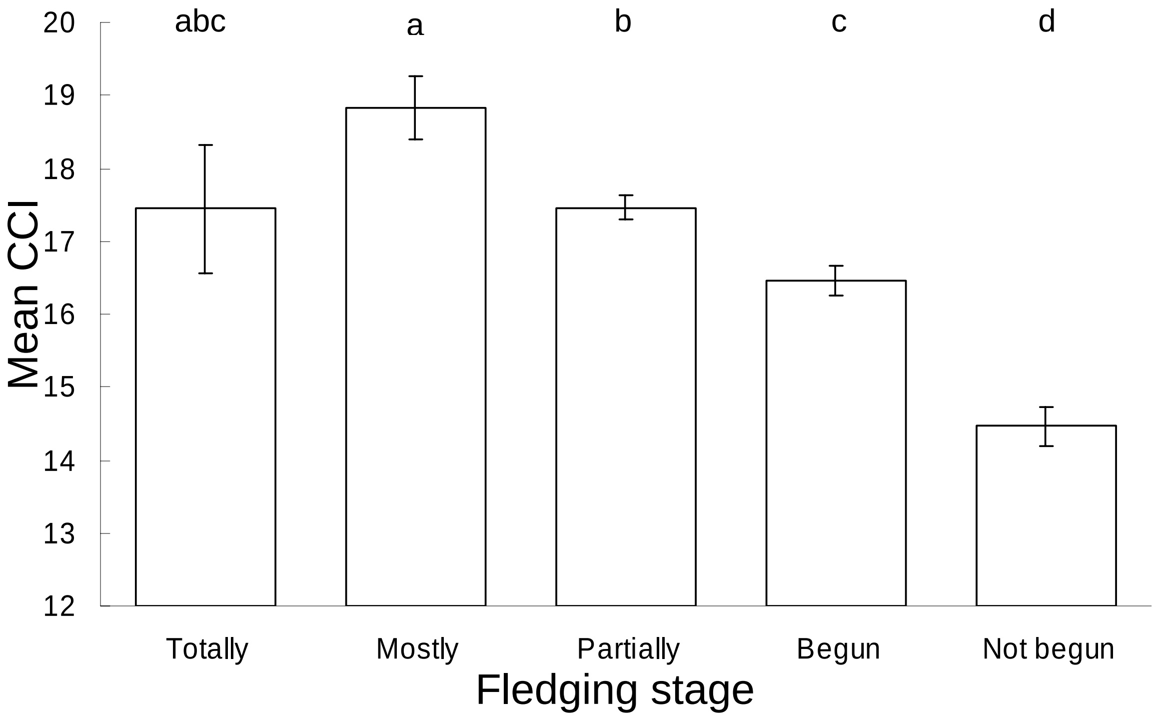


Figure 3.21: Mean (± SE) chick condition index (CCI) measured at the end of the breeding season as compared to chick fledging stage across Cape Bird colonies (n = 319). According to Tukey's groups, stages not connected by same letter are significantly different.

3.5 Discussion

The results of this study have found male EDC parameters that vary by geographic location. This variation appears to be structured by both colony size (FM), and location (entropy). EDC parameters also varied by sub-colony nest position (especially pitch and entropy), by weight (duration) and with breeding stage (paired males tended to have calls with greater numbers of short syllables and successful breeders had longer short syllables). Colony size and location also influenced male weight and breeding success (chick condition). Lastly, at Cape Bird, the effects of both year and season were shown to alter the acoustic parameters of the EDC.

3.5.1 Spatial variation in the EDC

At the largest spatial scale several of the EDC parameters (pitch, FM, AM^2 and entropy) were shown to vary by geographical location during the early breeding season on Ross Island. Similar trends in call parameters were observed between colonies for both the short repeated and long end syllables of the EDC. Indicating geographical differences between the colonies are similar for both sections of the call. Colony differences for both short and long syllables also appeared to be comparable, with colonies most proximate to each other, for example the Cape Bird colonies, often having similar call properties, thus when graphed using principle components (PC) these birds separated from the Cape Crozier colony, and partially from the Cape Royds colony. Cape Bird South also had significantly higher pitch for both long and short syllables than other colonies. Larger colonies appeared to have lower FM than the smaller colonies and how southerly the colony is related to the entropy (noise) in the calls with the most southerly colonies having more noisy calls than more northerly colonies.

The degree of variability in the PC plot from Cape Royds suggests a more diverse vocal environment (seen in the high frequency and amplitude modulations), which may be due to its size and marginality (being the most southerly on Ross Island and in the world: Müller-Schwarze 1968). This apparent vocal instability may also explain why this colony had the longest calls, due to their increased number of syllables, as this may be an attempt to increase the signal transmission quality in such a vocally variable environment (Slabbekoorn 2004). King penguins have been shown to increase their call lengths and number of syllables in windy, noisy conditions (Lengagne *et al.* 1999a). Therefore, although not measured directly, the environmental noise at Cape Royd may have been higher compared to other Ross Island colonies when recordings were made early in the breeding season. This colony was also shown to have higher pitch and entropy (a less tonal quality) in both short and long syllables.

Factors such as background noise, the amount of wind at a colony, or altitude may be having an effect on the EDC parameters between colonies but these factors were beyond the scope of the study. In future, it would be interesting to quantify these features to see if they give some structure to the variations recorded in the EDCs on Ross Island. The are several possible hypotheses for altitude having an effect on calls; for example, preferential nesting sites may be lower in the colony and thus older, more experienced birds, with potentially differing calls would likely nest there leaving the higher less favourable sites for the younger birds. Alternatively, the higher altitudes may be subjected to increased winds (levels of exposure) and therefore call parameters may be altered so birds can communicate more effectively during inclement weather conditions.

On a smaller spatial scale at Cape Bird the effect of sub-colony nest location and mean frequency were the same for short and long syllables with both having higher values in the centre as compared to middle and edge nest positions. As sound at low frequency is less attenuated than at high frequency (Pierce 1981) and as the penguin colony is a noisy place filled with dense penguin bodies; most penguins call with their beak in the air (Jouventin 1982) (to reduce sound wave disruption by the ground; Catchpole & Slater 1995) and give calls of generally low frequency with repeated syllables (Aubin & Jouventin 1998; Robisson 1991). To increase the distance at which a call could be heard, from the centre of a sub-colony, the frequency of the call would ideally be lower (although the validity of this theory has yet to be tested in Adélie penguins); this appears not be the case for the EDC. Entropy in the long syllable was also less tonal in the centre of the sub-colony compared to the middle and edge nests.

Why centre nesting birds and Cape Royds birds are giving calls that are noisy and higher in frequency is perplexing. One way in which birds can attempt to increase the distance travelled of their calls is to increase the volume, a phenomenon known as the Lombard effect (Slabbekoorn 2004). From field observations, louder calls are given when the beak is opened wider, and these calls also tended to be of higher frequency (*pers. obs.*). One explanation is that centre nesting Adélie penguins and birds that breed at Cape Royds are giving a louder, less tonal call of higher frequency that may actually have better sound transmission than a quieter, low frequency calls. This possibility has not been explored in this study. Other birds species have been shown to increase the amplitude of their calls with increased noise (e.g. tree swallow nestlings, *Tachycineta bicolor*, Leonard & Horn 2005; blue-throated hummingbirds, *Lampornis clemenciae*, Pytte *et al.* 2003 & male territorial nightingales, *Luscinia megarhynchos*, Brumm 2004). Additionally, the ability of birds to increase the pitch of their song due to environmental noise has been found in the passerine literature; for example a study on great tits (*Parus major*), in urban environments demonstrated a positive correlation between the minimum frequency of their calls and ambient noise (Slabbekoorn & Peet 2003). Experimentally the effect of background noise/wind could be manipulated in playback experiemts to look for a change in EDC parameters. The hypothesis being that birds calling when background noise was increased or when the environment was windier would have higher pitch, noisier calls that were longer in duration (either by increasing syllable length or number of syllables).

The effect of male weight on EDC call parameters was not observed except in the short syllables which correlated in the early breeding season with sub-colony nest location and call duration (centre nests had positively correlated weights and durations). In the late breeding season, long syllable weight and duration were correlated in the South colony (greater weights correlated with longer syllables). The link between weight and call duration is not clear, it may pertain to energy levels, and in larger, heavier birds the call lengths may simply be a function of the birds being able to produce longer syllables. It is assumed song production is costly (Oberweger & Goller 2001), therefore when attracting a mate or defending a territory, it may be advantageous to produce calls with longer syllable duration. The effect of weight on birds' song has been established in the literature (Ryan & Brenowitz 1985; Wallschläger 1980), however is largely unstudied in penguins. Miyazaki and Waas (2005) found a relationship in little penguins between the pitch of calls (but not duration) and the size and weight of birds. Alternatively, the correlation between weight and duration may be a function of older, more experienced males (who tend to be heavier and habitually nest in the centre of sub-colonies) producing longer short syllables to increase the broadcast information (Wiley & Richards 1982) in their calls during mate choice and nest defence. However, this hypothesis does not explain the South colony having long syllables of greater length at the end of the breeding season. In order to test these hypotheses an experiement that assesses EDC parameters with weight, age and experience along with nest location needs to be carried out.

An increasing number of syllables in the early breeding season was also linked to both sub-colony nest location (birds in the middle had more syllables) and breeding status (paired birds had more syllables than solo birds). Not only did paired birds have more syllables, in the South colony, they also had longer short syllables than those birds that remained solo. Successful birds were also more likely to be of greater weight and nest in the centre or middle of the sub-colony. Given the previous finding of higher frequency in the centre of the sub-colonies it is also likely these birds were giving louder, noisier calls. This finding supports the hypothesis that increased background noise in the centre of the sub-colony (through increased numbers of males calling in the near vicinity creating a masking effect) may be forcing the males to produce calls of longer duration and increased numbers of syllables to attract a mate (in the early breeding season) and defend their nesting territory. In the late breeding season, in the Middle and South colony, birds that bred successfully had short syllables of greater length however a lesser total call duration, which can be attributed to a trend in decreasing length of long syllables (although not significant once the Bonferroni correction was applied). Once the mate selection period is over, the sole purpose of the EDC is as a territorial warning, thus the successful males (that likely nested in the centre of a sub-colony), may be increasing their signal tranmission by giving calls with increased short syllable length. Again manipulation of the number of competing males calling in a sub-colony concurrently with a focal male using playback experiments across the season may elucidate the ability of the male to alter the EDC parameters with season and background noise.

3.5.2 Colony condition and breeding success

Factors such as colony location, size, and sub-colony nest position not only influenced EDC parameters, but also the weights and breeding success of males. It was not always possible to separate colony location and size from weight and breeding success given that male weight/condition is evidently a variable that affects breeding success in the Adélie penguin (Chappell *et al.* 1993). Colony and sub-colony variability in weights were predictably linked to breeding success estimates. In addition, the issue of foraging efficiency, which has clear consequences for breeding success and male weight, could not be assessed in this study but is likely to co-vary with both colony location and size.

During the early 2002/3 breeding season there was extensive sea-ice (until late December: *pers. obs.*) which meant birds had to travel long distances (approximately 90 km: Kerry Barton *pers. comm.*) across ice to reach the breeding colony. Sea-ice was also slow to break out thus off-shore open water for foraging was not available until early January (*pers. obs.*). It was plainly demonstrated that the Cape Bird colonies birds lost weight over the breeding season. Small colony size was likely to have resulted in Bird

Middle beginning the season with relatively low male weights and having poor breeding success with low numbers of successful nests and chicks in poor condition. It is probable that Middle colony is less desirable as a breeding location (due to limited available breeding areas) and therefore less experienced, younger birds nest there. These birds are known to have poorer breeding success (Ainley 1983). Small marginal colonies (which are often newer in terms of establishment – Young 1994) also suffer from greater predation pressure (Young 1994), in conjunction with this; birds that establish new breeding areas are often younger less experienced breeders (Ainley 1983). The effect of colony location with regard to distance from the sea-ice edge at Cape Bird Middle was probably less of a factor as Cape Bird Middle (and North) was (on Ross Island) as close to the sea-ice edge as possible, so there was no delay in breeding (similar numbers to Bird North were paired rather than solo at the beginning of the breeding season). The potential advantages of nesting in a large colony with respect to enhanced breeding success have been reviewed by Danchin & Wagner (1997) who found birds in larger colonies (on average) experienced greater breeding success. Additional evidence that it was size not location that resulted in Bird Middle having such poor success comes from Cape Bird South, a much larger colony that began the season with the highest mean male weights (4.9 kg). This colony was further from the sea-ice edge (approximately 3.3 km further than Middle – measured from N.Z.M.S. 173/4) and not only had far fewer males paired at the beginning of the season but had large numbers of birds fail to breed and chicks in poor condition with delayed fledging. With such a short austral breeding season, any delay in breeding, hindered males at Bird South, this in conjunction with large distances to travel in order to forage meant Bird South males could not raise chicks to sufficient condition in order to fledge. They also lost the most weight across the breeding season (0.8 kg) and in fact ended the season with the lowest mean male weights (4.1 kg). Cape Bird North was both the largest Cape Bird colony and was closest to the sea-ice edge; therefore not surprisingly males from North began the season with relatively high weights and had the highest proportion of males with a mate early in the breeding season. At the end of the breeding season this colony had the best breeding success with chicks in better condition than Middle (the smallest) or South (the farthest from the sea-ice edge).

On Ross Island the effects of colony size and location on chick condition was also evident with the largest colony (Cape Crozier) having highest chick condition and the lowest values being recorded at Cape Royds. Cape Royds is a small colony on the east side of Ross Island and was the farthest breeding colony from open water foraging grounds therefore there was a dramatic but unsurprisingly negative effect on the condition of chicks. This pattern clearly reflects both colony size and location, with larger colonies (Cape Crozier & Cape Bird North) having chicks in good condition, and more southerly colonies (Cape Bird South), having chicks in poorer condition. Cape Royds, a small and southerly colony had chicks that were in the poorest condition.

In terms of condition of chicks at various fledging stages, the chicks in the best condition had almost finished fledging. Chicks that had finished fledging were often in poorer condition than those nearly finished. It is likely the adults had decreased feeding frequency in those chicks and so their condition was actually declining (Williams 1995). Chicks in the poorest condition had not yet begun to fledge, and it was unlikely they would survive the fledging process.

Regardless of colony location or size the position of the nest within the sub-colonies also affected breeding stages achieved, with more males being paired in the middle and centre of the sub-colonies than at the edge. Likewise, at the end of the breeding season, the centre and middle of the colonies had greater numbers of one and two chick nests than edge nests which had failed in far greater numbers. Adélie penguins are known to have poorer breeding success at the margins of sub-colonies (Tenaza 1971) due to the combined effects of increased predation risk (Young 1994) and birds of lesser breeding experience (and often poorer condition) tending to nest there (Ainley *et al.* 1983). Nest location and breeding success has been correlated in other penguin species (e.g. the chinstrap, *Pygoscelis antarctica*; Barbosa *et al.* 1997) and also other colonially nesting species (e.g. the kittiwake, *Rissa tridactyla*; Aebischer & Coulson 1990). In order to separate the effects of increased predation risk and breeding experience on breeding failure, experiments that eliminate one of the factors (i.e. by excluding major predators) would need to be carried out. The hypothesis that breeding success would be greater in centre or middle nests compared to edge nests as these birds are more experienced breeders and are better at timing nest relief, have higher nest quality and are more efficient foragers could then be tested.

3.5.3 Temporal variation in the EDC

If the only data collected with regard to EDC parameters and colony health and breeding success was spatial, you would be tempted to assign both vocal and behavioural geographic differences to the Ross Island colonies. However, when temporal variations in EDC parameters are included a slightly more complicated pattern of vocal colony dissimilarity evolves. Variation in EDC parameters at the three Cape Bird colonies was investigated at two temporal scales, both within and between breeding years. Firstly of note is that regardless of year (2000/1 or 2002/3), or whether early or late in the breeding season all birds retained individually distinct EDC call parameters in their short repeated syllables. Therefore despite temporal changes, the calls functionality is likely to be preserved.

The largest temporal scale compared two breeding seasons at Cape Bird and showed there were clearly differences in EDC call parameters. Pitch and mean frequency in the short syllable in the North colony was greater in 2000/1 than 2002/3. This pattern was repeated in the long syllable where all three colonies had lower pitch in 2002/3. FM was higher in the short syllables in 2002/3 than 2000/1 but showed no variation in the long syllables. Both AM^2 and entropy were different between the two seasons, with calls in short and long syllable EDCs being less noisy but more modulated in the 2002/3 season. The general state of the EDCs in 2002/3 seemed to indicate they were more variable and perhaps of poorer quality given the frequencies and amplitudes changed more, at least within the short syllables. Also of note is the total duration of the calls was longer in 2002/3, which relates not to the short syllable length or number of syllables but to longer end syllables in the calls. Given calls were recorded in similar quantities at a similar time of the year, the only factor changing was the year, and the main difference between these years was in 2002/3 there was persistent sea-ice and poorer climatic conditions, which has already shown to have negatively effected the condition of the males and decreased the percentage of birds successfully breeding and condition of fledging chicks. Comparative studies of annual Adélie breeding attempts clearly show the negative effects of abundant sea-ice (Ainley & LeResche 1973; Ainley *et al*. 1998; Irvine *et al*. 2000).

At a smaller temporal scale (across the 2002/3 breeding season) there were also interesting changes in EDC parameters and the effects of colony and nest location within sub-colonies. Over the season males increased the length of their short syllables, and had increased noise in both their long and short syllables. The long syllable was however lower in pitch at the end of the breeding season and for the South colony longer in duration. It appears that even seasonally the calls are not acoustically stable as aspects of their length, frequency and tonal quality shifted. Of note, is that the changes observed across a single breeding season (2002/3) and the changes in EDC parameters between the "good" and the "poor" breeding seasons show similar EDC characteristics. For example late season calls were lower in pitch in the long syllables, as were 2002/3 calls. The calls also had aspects of the duration of the calls altered. In 2002/3, the total call duration was longer due to an increase in long syllable length and in late season data the short syllables were increased in length. Perhaps, these changes in call parameters are indicating the general state of the colony. In 2002/3, the colony was in poorer condition compared to 2000/1 (as mentioned earlier) and late in 2002/3 they were in poorer condition than at the beginning of that same breeding season. How and why changing condition may affect EDCs is currently unknown. Potentially factors such as changing weather, breeding circumstance, colony noise levels, motivation and energy levels of the birds may be causing this shift in vocal parameters. It is recognised that both abiotic (such as weather; Slabbekorn 2004) and biotic sources (such as conspecific vocal competition; Galeotti *et al.* 1997, bird health; Appleby & Redpath 1997; Saino *et al.* 1997 and breeding status; Martin-Vivaldi *et al.* 1998) can impact on quality, frequency and duration of bird calls. Additional research examining how changes in abiotic factors, such as temperature, and biotic factors such as motivation, also need to be undertaken. Focal animals repeatedly sampled over a range of temperatures and also with changes in breeding circumstance may elucidate how the change in season affects the EDC. As well as this factors such as number of calls given (and when) and the energetic costs of calling need to be examined.

3.5.4 Conclusion

In summary, EDCs do vary geographically; however these calls were not acoustically stable between two years with very different environmental conditions. Moreover, the calls varied across a single breeding season, which again may be due to changing conditions (both of callers and their acoustic environment). It is also possible the EDC is vocally malleable enough to be modified to adapt to certain environments, for example the denser nesting positions within sub-colonies. The EDC although retaining it's individuality in all conditions appears remarkably changeable, and maybe this is because it is not essential for pair or chick recognition and therefore has a less rigid set of vocal characteristics. Furthermore, the fact that the EDC is used for both mate attraction and nest defence also means that the signals importance and clarity may change over the breeding season, especially once the mate selection period is over as the frequency of calls decreases (*pers. obs.*). Continued nest defence means the call is given throughout the breeding season just perhaps with different behavioural motivation, and this may be the source of some of the end of season vocal changes. Also clear from this study was that colonies were different in terms of breeding success, chick weights, and the change in weights of males over the season. A more comprehensive study of the effects of male weight, breeding success and changes in the EDC with environmental variables such as season and colony location over successive years is now required to see if the EDC is predictable or stable in any of its vocal characteristics.

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4

4.1 Abstract

The Adélie penguin (*Pygoscelis adeliae*) breeds in large, vocally challenging (i.e. noisy) Antarctic colonies and has evolved a communication system of complex intra- and intersexual visual and vocal behaviours. The theory of honest signalling predicts that only signals that are costly to produce or maintain (a conditional handicap) or unable to be manipulated (an index) are evolutionarily stable as they provide reliable information to a potential mate or competitor. Honest signalling has been examined in many species and as the Ecstatic Display Call is used for both mate choice and territory defence the potential validity of this call was assessed. This study examines the association between the Ecstatic Display Call and male condition (estimated using body weight). Spectral analyses provide support for the hypothesis that Ecstatic Display Calls could be an honest signal of condition used during mate choice. Early in the breeding season, heavier males had significantly lower Frequency Modulation in the short repeated syllables of their calls than lighter males. Furthermore, a male's breeding success could be predicted during the early breeding season by the Frequency Modulation, with lower Frequency Modulation being associated with higher breeding success. In addition, Frequency Modulation altered predictably with changing male weight. Late arriving poor male breeders tended to increase in weight over the season and gave calls that decreased in Frequency Modulation. In contrast, successful males tended to lose weight and gave calls with greater Frequency Modulation. Early arrival time into the colony was also a good predictor of breeding success. However, of the early arriving males those with lower Frequency Modulation calls obtained mates more quickly, despite competing with a greater number of males. Therefore, female Adélie penguins may reliably use Frequency Modulation of the Ecstatic Display Call as an honest signal of early season male weight/condition and the likelihood of a successful breeding outcome due to the long incubation fasts in this species. Whether Frequency Modulation is evolutionarily stable, as either an index or a handicap signal, has yet to be studied.

4.2 Introduction

Of the theories examining the evolution of honest signals, the conditional handicap model, predicts that sexually selected traits are reliable signals of quality because they are both condition dependent and costly to produce or maintain (Zahavi 1975; Zahavi 1977). Recently, Maynard Smith and Harper (1995) proposed the concept of index signals, which are reliable indicators of quality, but are not necessarily costly. Distinguishing between handicap and index signals is not always straightforward, especially if the costs associated with the signal are unclear (Maynard Smith & Harper 2003). This is particularly true if the ‘efficacy costs’ (costs of unambiguous transmission; Guilford & Dawkins 1991) and ‘strategic costs’ (costs required to ensure honesty; Grafen 1990) are unknown (Maynard Smith & Harper 1995). Regardless of signal type, females selecting mating partners on the basis of honest sexual traits are expected to benefit either directly through choosing benefits for themselves or their offspring (Price *et al.* 1993), or indirectly by choosing “good genes” for their offspring (Møller & Alatalo 1999). There is considerable support for honest signalling (for review, see Johnstone 1995) in species ranging from the pied flycatcher (*Ficedula hypoleuca*) (Lampe & Saetre 1995) and shelduck (*Tadorna tadorna*) (Ferns & Lang 2003), to the gray tree frog (*Hyla versicolor*) (Doty & Welch 2001), drumming wolf spider (*Hygrolycosa rubrofasciata*) (Mappes *et al.* 1996) and red deer (*Cervus elaphus*) (Clutton-Brock & Albon 1979).

Undoubtedly acoustic signals are an important medium for signalling and communication in birds. Many bird species have evolved songs that are complex in structure and are used for both intra- and inter-sexual communication (Catchpole 1980; 1982; Catchpole & Slater 1995). Relationships between song and body weight were first established in passerine species by Wallschlager (1980); since then correlations between acoustic signals and body condition have been established in several non-passerine species e.g. tawny owls, *Strix aluco* (Appleby & Redpath 1997). Mountjoy and Lemon (1997) suggest that traits such as body size and condition may be reliable indicators of future male parental investment if they reflect an individual’s energy reserves which in turn may influence breeding success. Specifically, in species exhibiting bi-parental care, the ability to reliably assess male condition and predict breeding success prior to mate choice is advantageous to females.

Historically the bioacoustics of non-passerine species has received less attention than passerines (Bretagnolle *et al.* 1998), but given many are species that exhibit complex vocal behaviours and bi-parental care, the literature regarding mate choice and honest signalling of non-passerines is growing. Genevois and Bretagnolle (1994) were the first authors in the seabird literature, to examine the relationship between body weight and vocalisations with regards to mate choice and breeding success. They found that the rhythm of the blue petrel (*Halobaena caerulea*) call was related to condition of the birds, with heavier birds producing quicker calls, which could potentially be used by females during mate choice. More recent work by Miyazaki & Waas (2003a; 2003b; 2005) on little blue penguins (*Eudyptula minor*) found that the dominant frequency of male calls to be negatively associated with body size and therefore could potentially influence female choice.

Both Spurr (1975b) and Ainley (1975b) have given extensive descriptions of the displays and vocalisations of Adélie penguins (*Pygoscelis adeliae*). In particular they describe the structure and behavioural context of the Ecstatic Display Call (EDC) also known as the Ecstatic Vocalisation (Ainley 1975b). The EDC serves two primary functions, firstly as a territorial claim to a nest site directed at other males and secondly for mate attraction during the early breeding season (Ainley 1975b; Penney 1968; Spurr 1975b). The EDC is contagious in nature and given throughout the breeding season. Also reported by several researchers, including observations noted during this study, the EDC can be given by females at the nest.

Adélie penguins (specifically from the Ross Sea region) have low levels of mate retention with only approximately 49% of birds retaining their mate from the previous season (Ainley *et al.* 1983), consequently at the beginning of each breeding season there is likely to be a large proportion of prospective breeders choosing a new mate. However, birds that eventually retain their mate still call at the beginning of the breeding season and compete for prospective partners as the females return is not guaranteed. If the female does return, the male may not have used the call to attract a new mate but it has been used to defend his territory. Mate choice in the Adélie penguin follows a relatively simple sequence; a female must choose a male, at a constructed nest, at a certain location and time, within a breeding colony of penguins (Ainley 1975b; Spurr 1974). More specifically, Davis and Speirs (1990) suggest females partly base their initial mate choice on the EDC of males. They hypothesise that a fat male can potentially fast longer, reducing potential for nest desertion and improving breeding success, and that fat males may demonstrate their good condition through the EDC. What, (if any), aspects of the EDC that females may use to assess potential mates are currently unknown. The assumption that pre-season fat reserves (arrival weight) and EDC parameters may be linked to honest signalling and female mate choice is predicated on the idea that the male of this species has to undergo this long period of fasting at the beginning of the season, thus it may be important to females during mate choice. If throughout the season the maintenance of condition was more important to breeding success the use of the EDC as an honest signal would be of little value.

The current study examines the association between vocal displays, mate choice and male condition in the Adélie penguin, which breed in acoustically cluttered (i.e. noisy) and environmentally challenging Antarctic colonies. Adélie penguins have evolved highly complex individual calls enabling both mate and chick recognition (Davis & McCaffrey 1989; Jouventin & Aubin 2002; Penney 1968). Male Adélie penguins are also able to distinguish between strangers and neighbours (Speirs & Davis 1991).

The aim of this study is to assess the validity of the EDC as an honest signal based on the following hypotheses; 1) the EDC may signal male condition and therefore provide a useful cue for female mate choice; 2) male condition will change during a breeding season, and this will correspond to a change in one or more EDC parameters; 3) one or more call parameters will predict success at three critical stages during the breeding season (pair bond – including pair latency, incubation & fledging success); 4) male condition should likewise predict breeding stage success; and 5) males arriving early into the colony will be in better condition, and have greater breeding success.

4.3 Methods

4.3.1 Subjects and study area

Adélie penguin recordings and observations were carried out at the North Colony, Cape Bird (77°13'10"S, 166°28'30"E), Ross Island, Antarctica between 23rd October 2002 and 27th January 2003. The focal sub-colony of approximately 100 breeding pairs was situated in the centre of North Colony (≈35 000 breeding pairs in 2000; Ainley *et al.* 2004). The sub-colony was elongated in shape, with an approximate distance of three meters from centre to edge. Focal Adélie penguins were behaviourally sexed (see Chapter 2; section 2.3.2) and a total of 51 focal males were used in the study. Individual identification was achieved with the use of permanent metal flipper bands (method of attachment, specifications and ethics approval documentation in Chapter 2; section 2.3.1 & 2.4).

4.3.2 Ecstatic Display Calls

Descriptions of the Ecstatic Display can be found in Chapter 1 (section 1.6.4). Vocally the EDC represents a series of staccato pulses (introduction beats) followed by repetitive hoarse rasps often climaxing in a syllable resembling the Loud Mutual Display (Ainley 1975b). In this study, the EDC has been divided into two audibly distinct components for analysis; the repetitive rasps (short repeated syllables) and the final climactic syllable (the long syllable) (see Chapter 2; section 2.5). Additionally, whole call duration and number of syllables were analysed for changes across the breeding season.

4.3.3 Recording methods

EDCs of focal males were recorded daily during two periods: 1) the nest building and mate choice period immediately prior to the onset of male incubation (28th October -18th November) and 2) the chick rearing stage with chick ages from two weeks to fledging (28th December-15th January). Recording sessions lasted a minimum of one hour. Additional recording methods, equipment and specifications can be found in Chapter 2 (section 2.6).

4.3.4 Sound Analyses

All sound analyses follow the protocol specified in Chapter 2 (section 2.7). Calls were quantified using six independent sound parameters, descriptions of which can be found in Chapter 2 (Table 2.2).

4.3.5 Behavioural and condition data

Behaviour of focal males was recorded on their arrival at the breeding sub-colony and at the initiation of nest building. Observations included arrival date and timing (Chapter 2; Table 2.1), weight upon arrival (for methods see Chapter 2; section2.4), right flipper length (measured using a custom metal wing ruler), date of pair bond (Chapter 2; Table 2.1), and egg laying (first and second) dates. At the end of the breeding season focal males were re-weighed. Breeding success (Chapter 2; Table 2.1) was determined at the end of January before researchers departed from the study site. Only adult males were used in the study. Only focal males were used to examine associations between mate attractiveness and calls. Results regarding patterns of mate choice were inferred from focal males within the whole sub-colony. Male attractiveness was assessed by calculating the time taken from arrival until a mate was achieved (pair latency – days) and also the time taken when compared to how many other males where free to pair within the sub-colony. This was only calculated for the early arriving males and when compared to all available males it represented the number of non-paired or free males at the time of recording, so although many males mates had yet to arrive from sea and they did eventually obtain a mate, at the time of observation, their mate had not arrived and theoretically they were available to pair.

4.3.6 Statistical analyses

Linear regressions were used to assess the relationship between wing length and weight measurements; they were also used to study the relationship between early and late season calls, body weight and pair latency (length of time from arrival until a stable pair bond was achieved, measured in days). General linear models (GLM) were used to compare group (arrival, season and breeding stage) and male effects (weight and call parameters). Least square means (LSM) of call parameters were calculated for analyses. Spearman's Rank Correlation coefficients were calculated for call parameters, weight change and early season mate choice when sample sizes were less than 20. Fisher's Exact (two tailed) analyses were performed on data comparing individual male weight change and call parameters, using a 2x2 contingency table and GraphPad Software (GraphPad Software Inc. San Diego, USA). Male weight loss for successful and unsuccessful breeders was compared using an independent sample student t-test. All other statistical analyses were performed in JMP 5.1 (SAS Institute 2004). Data are presented as mean ± standard deviation ($\overline{X} \pm SD$) and unless otherwise stipulated, all tests used a significance level of $\alpha = 0.05$.

4.4 Results

Weight measures and wing length did not correlate in either early or late season data (linear regression: early $F_{1,28} = 0.011$, $R^2 < 0.001$, $P = 0.917$; late $F_{1,20} = 0.422$, $R^2 = 0.022$, $P = 0.524$). In addition, the weight variation of early and late season birds was similar for the three call sampling regime (two tailed Fisher's Exact: $P = 0.101$). Of the 51 focal males, 29 were recorded during the early breeding season, 21 were recorded at the end of the breeding season. Sixteen birds were recorded across the breeding season for the short syllables, 11 were recorded for the long syllable, and eight for the whole call parameters of the EDC.

All six call parameters for both short and long sections of the EDC had significantly greater inter- than intra-individual variation. Additionally, whole call duration and number of syllables also showed significant inter-individual variation. The six call parameters for the short and long sections of the EDC were compared separately for a relationship between weight and season. Results indicate that, the short repeated syllable had significantly lower Frequency Modulation (FM) for larger males early in the breeding season (linear regression: $F_{1,28} = 7.938$, $R^2 = 0.227$, $P = 0.009$) (Figure 4.1) but not at the end of the breeding season ($F_{1,20} = 1.174$, $R^2 = 0.058$, $P = 0.292$). No other call parameters measured in the short syllables were significantly related to weight either during the early or late season. Additionally, neither the long syllable, nor the whole call parameters, showed any significant relationship with weight either early or late in the breeding season.

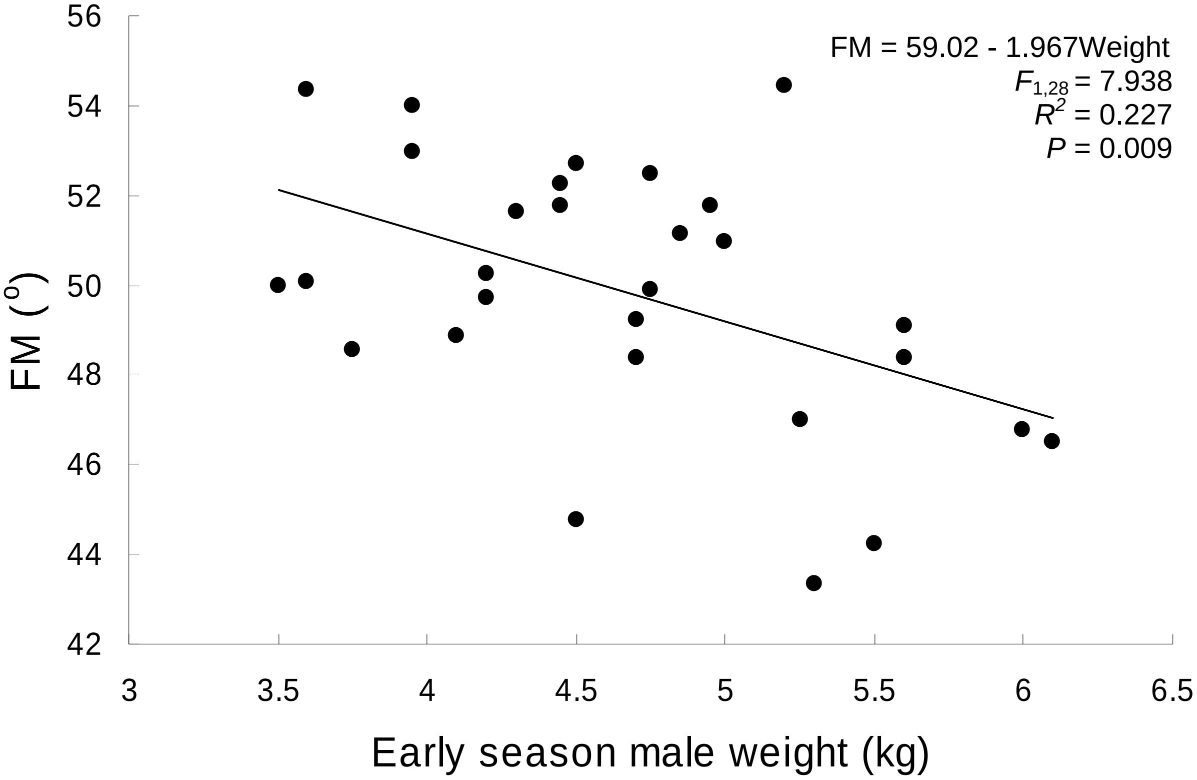

Figure 4.1: Early season negative correlation of focal male weight and the least square mean (LSM) of Frequency Modulation (FM), n = 29.

FM in the short syllable was also shown to significantly alter with changes in male weight. For the males that were followed throughout the breeding season, weight changes (calculated as a percentage) were negatively correlated with changes in FM (Spearman's rank correlation: r_s = -0.581, n = 16, P = 0.015) (Figure 4.2). Mean individual changes in FM also correlated with weight change over the season (two tailed Fisher's Exact: P = 0.011) (Figure 4.3).

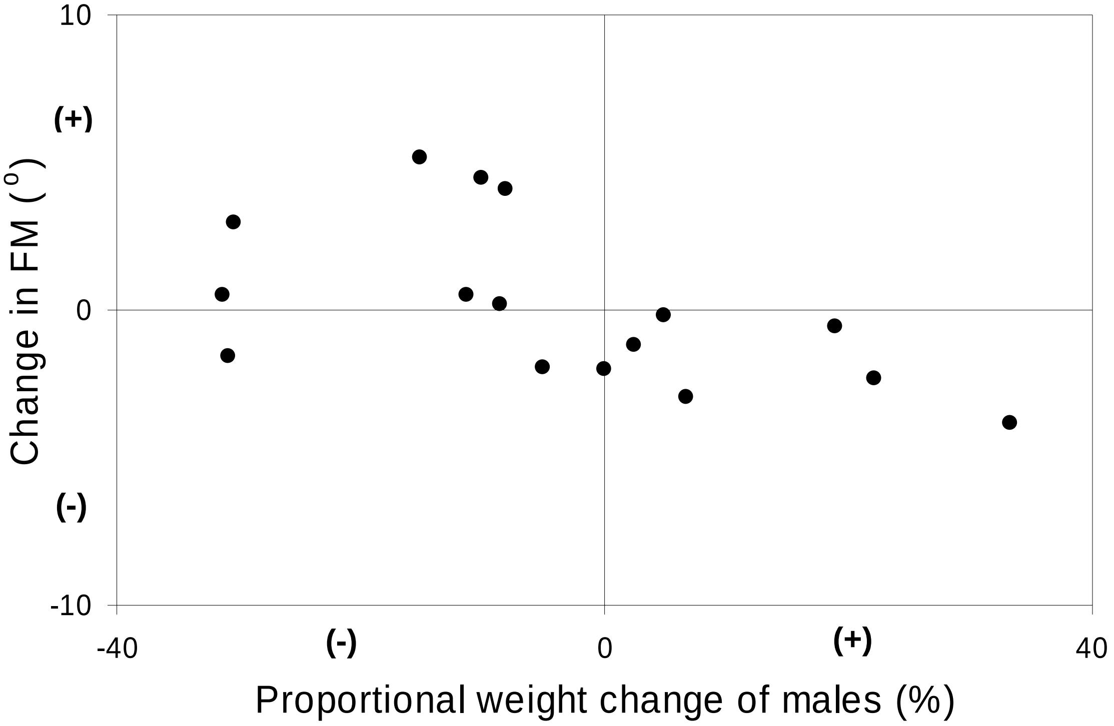

Figure 4.2: Mean seasonal change of Frequency Modulation (FM) plotted against percentage weight change for focal males, n = 16.

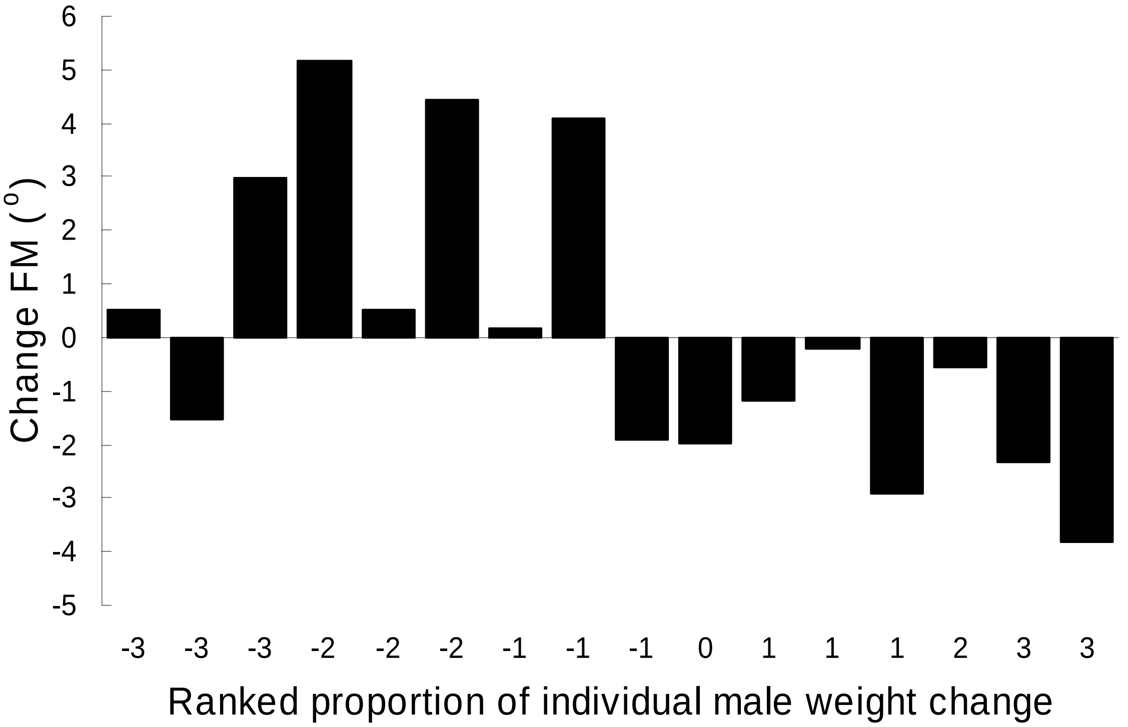

Figure 4.3: Individual focal male weight change across the breeding season, ranked as a percentage proportion (gain **3** = >30%, **2** = 10-29%, **1** = 1-9%, **0** = 0%; loss **-1** = 1-9%, **-2** = 1—29%, **-3** = > 30%) measured against the least square mean (LSM) of FM change, n = 16.

The predictive potential of FM (recorded during the early season mate choice), was then tested at three critical breeding stages (Chapter 2; Table 2.1). While FM did not significantly predict a male's ability to form a stable pair bond (one-way ANOVA: $F_{1,28}$ = 0.008, P = 0.929) or reach the egg incubation stage ($F_{1,28}$ = 3.24, P = 0.083), it did significantly predict ultimate breeding success ($F_{1,28}$ = 6.876, P = 0.014). Although the pair bond and egg incubation stage showed no significant differences in FM, there was a decreasing trend in FM from the pair, to the egg, to the breeding success stage (Figure 4.4a).

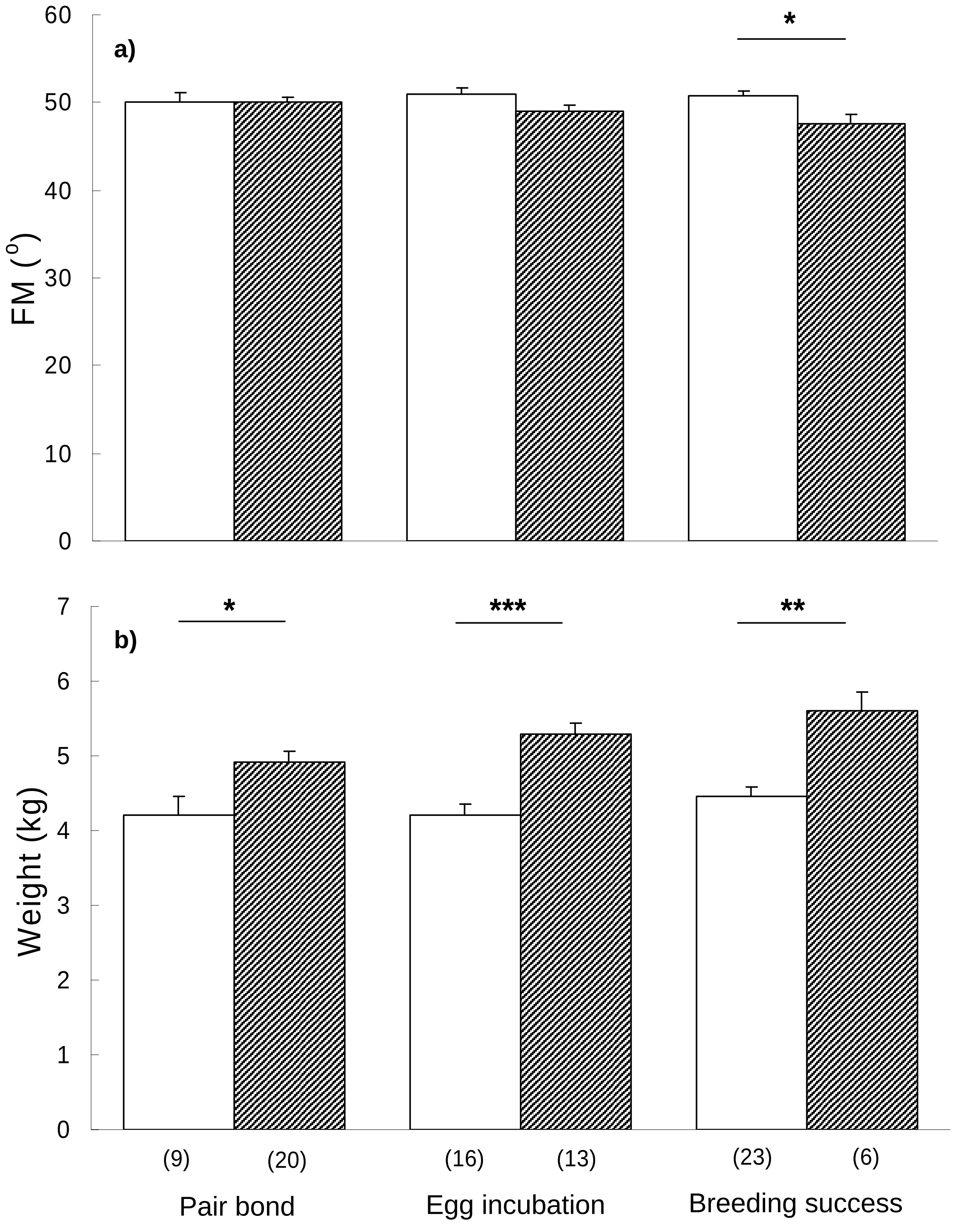

Figure 4.4: Univariate ANOVAs of least square mean (LSM) of **a)** Frequency Modulation (FM) and **b)** mean weight taken from early season focal males against breeding stage attained (pair bond, egg incubation stage and breeding success). Failure =☐ success = ▨. Significant results ($P < 0.05$) are denoted by an asterisk, $P < 0.05$ *, $P < 0.01$ ** and $P < 0.001$ ***, bars represent standard error (SE). Sample sizes for each breeding stage are in parentheses. Total focal birds $n = 29$.

The breeding stage reached by each male was then compared to its weight at the beginning of the breeding season. Males achieving stable pair bonds (one-way ANOVA: $F_{1,28} = 6.27$, $P = 0.019$), reaching the egg stage ($F_{1,28} = 28.21$, $P < 0.0001$), or breeding successfully ($F_{1,28} = 16.758$, $P = 0.0003$), had significantly higher body weight at the start of the season than unsuccessful males (Figure 4.4b).

Attractiveness and ability to secure a female by early arriving males were further investigated to see how male availability, FM and weight correlated with the latency of pairing. It was found that the greater the percentage of available males the shorter the latency of pairing (linear regression: $F_{1,24} = 25.264$, $R^2 = 0.523$, $P < 0.0001$) (Figure 4.5a). Those early arriving birds that were chosen most quickly also had lower mean FM (Spearman's rank correlation: $r_s = 0.673$, $n = 11$, $P = 0.023$) (Figure 4.5b). In addition, early arriving male weights were not significantly correlated with latency of pairing ($r_s = -0.316$, $n = 11$, $P = 0.343$), although there was a trend for heavier males to have a shorter latency than light males. In particular, males weighing six kilograms or over had a median latency of pairing of four days, as compared to males five kilograms or under which had a median latency of eight days.

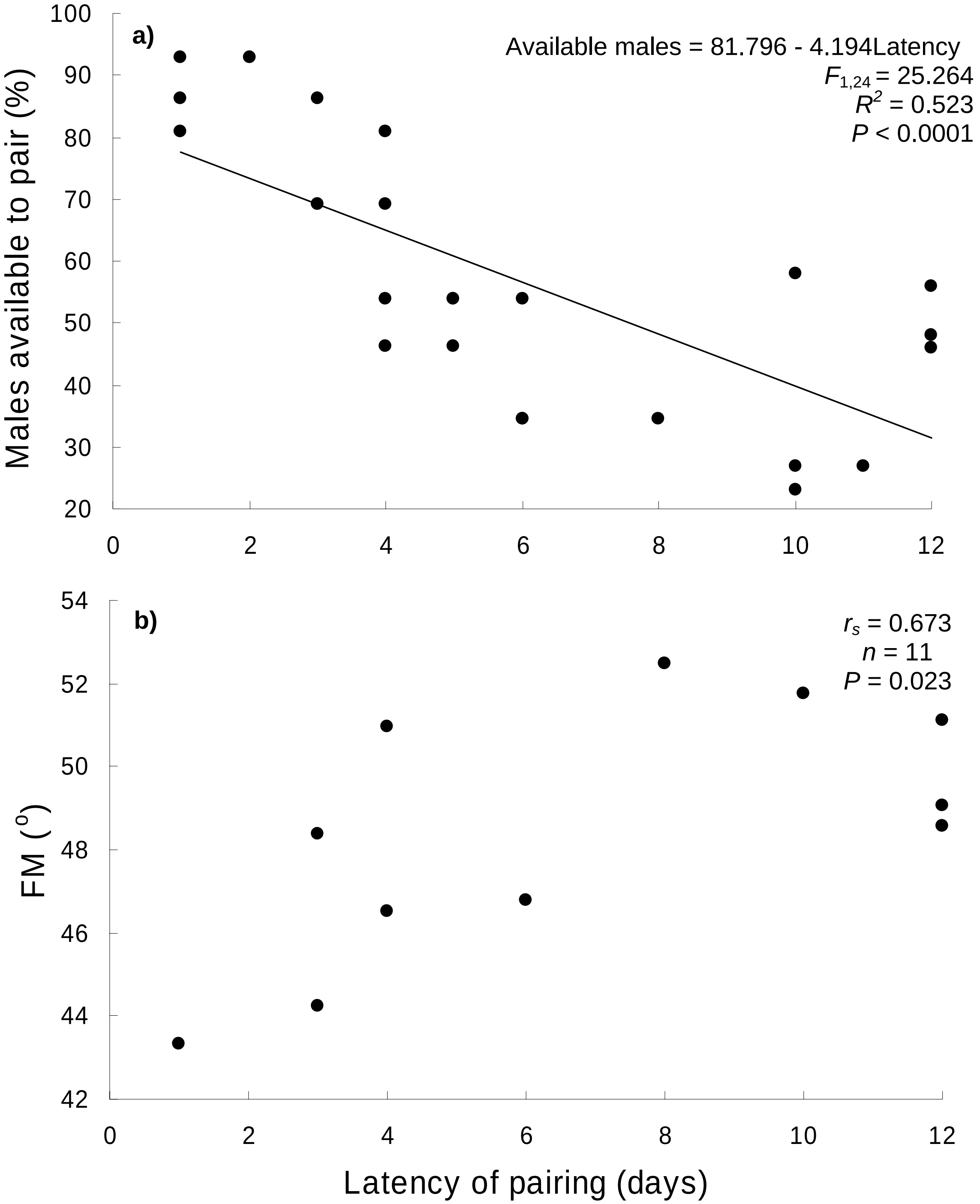

Figure 4.5: a) Negative linear regression of number of early arriving focal males free to pair, against the time taken for focal males to make a stable pair bond (latency) **b)** Positive Spearman's Rank correlation of mean frequency modulation (FM) against latency of pairing. Note there is no trend line as data are non-parametric.

Males arriving early (Chapter 2; Table 2.1) into the colony were of greater weight ($\overline{X}$ ± SD early = 5.38 ± 0.58 kg vs. late = 4.35 ± 0.42 kg; one-way ANOVA: $F_{1,28} = 23.813$, $P < 0.0001$). Similarly early arriving birds had significantly lower mean values of FM ($\overline{X}$ ± SD early = 48.48 ± 3.03° vs. late = 50.98 ± 2.46°; one-way AVOVA: $F_{1,28} = 5.94$, $P = 0.022$) than late arriving males. This better early condition was reflected in breeding, all but two (92.6%) of the focal males that arrived early in the season reached the egg incubation stage compared to 5/24 (20.8%) of males that arrived later in the breeding season. Only two of the males that arrived later in the breeding season ($n = 24$) managed to raise chicks as opposed to 14/27 (51.9%) of males who arrived earlier in the breeding season. The two males that arrived early and failed to reach the egg incubation stage also failed to find a mate, whereas only 13/24 (54.2%) late arriving males managed to find a mate.

Males that successfully bred lost significantly more weight ($t = 3.789$, $df = 41$, $P = 0.001$) than those that failed to breed (as a percentage proportion of body weight: -19.3 ± 3.4%, $n = 16$, and -1.4 ± 2.7%, $n = 27$, respectively), and early arriving birds lost more weight and were also more successful than males that arrived later into the colony (Table 4.1).

Table 4.1: Mean weight changes of focal groups of males over the breeding season (difference between early and late).

Weight changes	**Mean (kg)**	**SD**	***n***
Overall focal birds	**-0.5**	**0.88**	**51**
Early arrival	-0.84	0.89	27
Late arrival	0.02	0.57	24
Successful breeder	-1.11	0.8	16
Failed breeder	-0.14	0.73	35
Early successful breeder	-1.2	0.77	14
Late successful breeder	-0.41	0.9	2
Early failed breeder	-0.41	0.86	13
Late failed breeder	0.08	0.53	22

4.5 Discussion

This study examines the potential validity of the EDC as an honest signal. Several authors have described the EDC as a tool used in female mate choice (Ainley 1975b; Ainley *et al.* 1983; Davis 2001; Davis & Speirs 1990; Jouventin 1982; Spurr 1975b; Williams 1995). However, this study is the first to find any evidence that the EDC is linked to male weight and that females choosing a mate with lower FM can hope to gain greater breeding success. FM was found to be an unambiguous predictor of breeding success. Females choosing a mate in the early breeding season could use the level of FM in the EDC to gauge both male condition and the probability of a successful breeding outcome. Also shown clearly in this study is that early arriving males were in better condition, had lower FM, and were more successful at achieving stable pairings, reaching the egg incubation stage and successfully fledging chicks. For males, breeding was energetically demanding and successful males lost far more weight than failed breeders. The results also show that the EDC call parameters were not informative of weight later in the breeding season.

To establish whether the EDC could act as an honest signal, the data were analysed in three ways. Firstly, by establishing which call parameters correlate with male weight during the early breeding season. FM of the short repeated syllables of the EDC was the only parameter that correlated with weight and was lower for birds with greater weights. Secondly, FM was shown to be predictably altered by changing weight. Males that gained weight had decreased FM, and males that lost weight had increased FM. Finally, both lower FM and higher weight were good predictors of breeding success. Heavier males were also more likely to pair and reach the egg incubation stage. Not significant in the results was the correlation between FM and likelihood of pairing or reaching the egg incubation stage, this was not unexpected given the intra-individual variation in FM, and that despite differences in arrival and condition most focal males eventually achieved a pair bond, and many reached the egg incubation stage even though the majority of these poorer quality birds were ultimately unsuccessful in breeding. Additionally, by examining the early arriving males, it was shown that when greater numbers of males were available for pairing, males that achieved a stable pair bond the quickest also had the lowest FM values. FM appeared to be a more precise predictor of pair latency than weight. Why this relationship was not observed for weight may be an effect of sample size and decreased inter-individual variances compared to FM.

Mountjoy and Lemon (1997) postulated that a trait which reflects an individuals energy reserves may predict the individual level of parental investment. This supposition could be particularly relevant to the Adélie penguin as males assume the first incubation shift and therefore fast considerably longer, and loose more weight on average than females (Chappell *et al.* 1993). Only a male in good condition will be able to sustain the long fast and therefore, male parental investment and condition are inherently linked in this species. Male condition is critical for successful breeding and hence imperative in Adélie mate choice. In conjunction with male condition many other factors are required for successful breeding, including female quality (condition and experience) and the foraging efficiency of both male and female, these factors were however outside the scope of this study, but would be an interesting area for future research.

Using morphometric analyses Ainley and Emison (1972) and Kerry *et al.* (1992) found wing length to be relatively uninformative with regards to skeletal size in Adélie penguins. Several studies on *Pygoscelis* species have shown that bill depth and length may be better estimates of skeletal size (Ainley & Emison 1972; Kerry *et al.* 1992; Minguez *et al.* 2001; Reilly & Kerle 1981) and if the study were to be repeated addition of these morphological characters would be advised. Given the high degree of variation in weight (assessed here using a percentage coefficient of variation) across the season and between individuals (CV% early = 14.8, n = 51, late = 10.9, n = 43), as compared to wing length (CV% = 3.1, n = 43), gross weight alone was considered an informative proxy for male condition. Hence this study used body weight to estimate fat reserves early in the breeding season thus approximating male condition. Studies by Ainley and Emison (1972) and Ainley (1975a) demonstrated that arrival time and initial body weight significantly correlated with subcutaneous fat deposits from the axilla and incubation patch areas. Moreover, Chappell *et al.* (1993) demonstrated males began the breeding season heavier than females and lost both more weight and fat than females.

Potentially, weight has a more direct association with arrival time and breeding stage achieved than FM. However, weight alone would seem to be a poor visual signal, as Burley (1981), in conjunction with work investigating morphology (monomorphism) and social organisation (monogamous colonial breeders) and behavioural studies in pigeons (*Columba livia*) has categorised most penguin species as sexually indistinguishable (n = 9) or barely distinguishable (n = 1). It is therefore considered unlikely that female Adélie penguins visually determine the weights of their prospective mates (Davis & Speirs 1990). Additionally, during the EDC the posture (extended chest and erect feathers) of the male may make true size evaluation difficult (*pers. obs.*). It is also unlikely that an Adélie female arriving early in the breeding season randomly chooses an available male. Even amongst the early arriving heavier males there is active selection for lower FM. In all cases FM correlated with weight and therefore, FM is likely a potentially useful cue available to females for choosing a successful male early in the breeding season.

The articulatory mechanism linking FM and weight in the Adélie penguin is currently unknown. If the EDC is a handicap signal some aspect of call production should have high 'strategic costs'. It is commonly accepted that acoustic communication in birds is energetically demanding (Oberweger & Goller 2001), whether these costs in Adélie penguins are simply associated with the 'efficacy' of producing the call however is also not known. One possible 'strategic cost' of the production of FM in the short syllables of EDC may be muscle contractions creating modulation of airflow through the syrinx, hence, larger males in better condition may be able hold their frequencies over the short syllables (creating lower levels of FM). However, without further physiological studies it cannot be determined that changing FM is more active than keeping FM constant (Tchernichovski O, *pers. comm.*). Alternatively, there may be no 'strategic cost' associated with modulating the frequency of the calls and the signal may be more of an index. For example, fat surrounding the syrinx may increase attenuation by absorbing some frequencies (Davis 2001). It is also possible that the size of the bird may have an effect on the modulation of the calls and that larger birds with bigger chests may have a greater capacity for absorption of reverberations, this may also be enhanced by excess fat accumulation, thus altered with changing weight. As the energetic costs of the EDC were not examined in this study, and the articulatory mechanism of FM production is unknown, at this stage the results of this research can point to neither of these hypotheses directly.

Jouventin and Aubin (2002) found that Adélie and gentoo chicks recognise signals with amplitude modulation retained but frequency modulation removed. They concluded that individual recognition in these species involved the frequency values of the harmonics and spectral profile of syllables, but not frequency modulation. This is not necessarily inconsistent with the findings, as call parameters such as amplitude modulation (AM$^{\wedge 2}$) and pitch or fundamental frequency (FF) may be constrained for use in individual recognition in the EDC. As FM appears to vary with condition it would not be a useful cue for individual recognition.

Only the short repeated syllable showed any potential for being an honest signal. One explanation for this is that the central section of the call may be particularly important in the noisy arena of a colony as birds increase the detectability of their signals by repeating phrases or syllables (Wiley & Richards 1982). The most repeated phrase of the EDC (and the largest proportion of the call) is the short repeated syllable. The long syllable at the end of the EDC is not repeated, varies in length, and may be truncated.

Further testing of the EDC as an honest signal requires manipulation of FM in playback experiments such as experiments of female gray tree frog mate choice using altered male calls Gerhardt *et al.* (2000) and Nolan & Hill's (2004) work on female house finch (*Carpodacus mexicanus*) song preferences. Such experiments could demonstrate whether FM is used in mate choice and also whether mate choice can be manipulated and females directed into choosing males of differing quality and then examine any subsequent breeding outcomes.

Evidence supporting the evolution of an honest signal that females can use to assess potential mates is becoming more common, especially amongst birds (Searcy & Yasukawa 1996). Given their extreme breeding conditions and short breeding season, the female Adélie penguin clearly requires a reliable method for mate choice and a good predictor of breeding outcome. In addition to simply chosing last years successful mate (a tactic employed by most penguin species) an honest auditory cue would likely be advantageous when searching for a mate for the season, especially given that weight/condition (quality) of a male may be difficult to visually assess. This study demonstrates quantitatively the potential for female Adélie penguins to use the EDC for mate choice based on the condition of males.

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5

5.1 Abstract

Breeding success is traditionally derived by calculating success (as a ratio of successful to total number of focal nests observed) during pertinent points across the breeding season. Additionally, the use of logistic regression has become an increasingly popular tool in avian literature for identifying the influential factors that predict nest and/or individual breeding success. The aim of this study is to trial the use of logistic regression models on factors affecting Adélie penguin (*Pygoscelis adeliae*) breeding success during the inclement season of 2002-2003 austral summer, when the presence of icebergs and extensive sea-ice meant overall breeding success of Adélie penguins was low (16/51 focal males fledged chicks). Logistic regression models for the early breeding season showed that good/average nest quality and central/middle nest location within the sub-colony were most important in predicting the likelihood of pair formation of males. Later, during incubation males that arrived at the colony earlier and were heavier upon arrival were more successful when nest quality was good/average. During the final stage of fledging, the combined parameters of heavier male weight, early arrival time and good nest quality was shown to be most influential in predicting success. The logistic regression method also showed that the parameters measured varied in their predictive ability as the season progressed. Furthermore, logistic models made precise predictions of breeding events across all breeding stages. The effect of extensive sea-ice and in particular iceberg B-15A was again shown to be highly detrimental to Adélie breeding efforts and although breeding parameters important to successful breeding are not likely to radically differ, in future seasons with improved conditions further evaluations of the relative importance of parameters important for breeding success would be particularly interesting. In conjunction with further estimates of breeding success using logistic regression models a comparison of traditional versus logistic methods in estimating breeding success would be insightful.

5.2 Introduction

Standard definitions of breeding or nesting success are usually derived from calculating success during the egg laying, incubation or brood rearing stages, and the simplest estimate involves calculating a ratio of successful nests to total number of focal nests observed (Aebischer 1999). Although there are many additional methods for estimating breeding success (Aebischer 1999; Bart & Robson 1982; Hensler & Nichols 1981; Johnson 1979; Johnson & Shaffer 1990; Mayfield 1975; Miller & Johnson 1978) none of these methods help explain why particular nests or individuals are more successful than others. Logistic regression (Hosmer & Lemeshow 1989), more commonly used for modelling habitat preferences and nest site usage (Benoit & Askins 2002; Jeganathan *et al.* 2004; McLeod *et al.* 2000; Osnas 2003; Saab *et al.* 2004; Venier *et al.* 2004; Westphal *et al.* 2003), has recently become popular for predicting nest or breeding success in terms of individual and nest-specific explanatory parameters (Shaffer 2004), for example Bisson & Stutchbury (2000); Blums *et al.* (2002); Hinsley *et al.* (1999); Sedinger *et al.* (2004) and Tarvin & Garvin (2002). The use of logistic regression can perhaps be seen as an advance in how breeding success estimates can be used to make suggestions about the causes/correlates of success and their relative importance. If the model is sufficiently accurate it is then possible to make suggestions about which factors may accurately predict success in future breeding attempts.

Colonial seabirds (which make up 98% of all marine birds; Wittenberger & Hunt 1985) present an interesting topic for studying breeding success as finding and daily monitoring of nests for an entire breeding season is less problematic than estimating success of cryptically nesting birds. Factors such as nest site characteristics, location, timing of arrival and parental quality can all be studied at close range, with readily available comparisons. Seabird studies using logistic regression to explain habitat use, nest site selection and breeding success are also current in the literature, for example Duriez *et al.* (2000); Isacch & Martínez (2003); Hernandez-Matias *et al.* (2003) and Meyer & Miller (2002).

Adélie penguins (*Pygoscelis adeliae*) breed in large colonies (Oelke 1975) composed of discrete sub-colonies on the Antarctic continent. The onset of breeding is delayed until several years of age (Spurr 1975). Older, more experienced breeders arrive earlier in the season and males arrive earlier than females to establish a territory and begin nest building (Ainley *et al.* 1983). Females typically lay a single clutch of two eggs, 2-3 days apart (Ainley *et al.* 1983). Both males and females coordinate incubation (≈34 days), guard duties (≈21 days), and chick feeding until fledging occurs at approximately 50-60 days of age (Williams 1995).

As penguins are marine predators, which breed on land but feed at sea their foraging ecology is particularly important when assessing their breeding biology. Continental Antarctic penguins' (Adélie & emperor) behaviour is highly sensitive to the changing sea ice conditions and thus patterns of foraging have to alter accordingly and this can be daily as well as seasonally (Kato *et al.* 2003; Rodary *et al.* 2000; Wienecke *et al.* 2004). Summer foraging ranges and diet of male and female Adélie penguins on Bechervaise Island were studied and considerable variation in trip lengths and diet was found throughout the season and with phase of breeding (Kerry *et al.* 1995). In general, during incubation birds make trips of longer duration (9-25 days) and up to 100 km from the colony (Davis *et al.* 1988; Davis & Miller 1990). However, during the chick rearing period foraging trips are significantly shorter (less than two days) and closer to the colony (within 12 km) (Clarke & Kerry 1992). Additionally, the body weights of Adélie penguins prior to trips of long duration were significantly lower than those of short trips, which suggests that choice of trip type is regulated by adult body condition (Clarke 2001). Foraging decisions in the Adélie penguin therefore results from a trade off between allocation of food to chicks and storage of parental body reserves (Clarke 2001). The Adélie penguin diet consists mainly of euphausiid crustaceans (>70%) with some fish and cephalopod prey caught (Williams 1995). Emslie and McDaniel (2001) note however, that as most studies of diet occur during the breeding season the role of squid and other species may be under-represented. Diet was also seen to be effected by the degree of ice-cover both within and between years (Rombolá *et al.* 2003).

Penguins, specifically the Adélie penguin, have had most aspects of their general breeding biology studied at a variety of breeding locations around the Antarctic continent (Ainley & Demaster 1980; Clarke *et al.* 2002; Davis & McCaffrey 1986; Lishman 1985b; Oelke 1975; Penney 1968; Spurr 1974; Spurr 1975; Tenaza 1971; Wilson 1990; Yeates 1968; Lishman 1985a). Researchers have also examined aspects of Adélie breeding characteristics including mate choice and incubation duties (Davis 1982b;

Davis 1988; Hunter *et al.* 1995), breeding energetics and physiology (Astheimer & Grau 1985; Chappell *et al.* 1993; Culik 1994; Vleck *et al.* 2000; Vleck & Vleck 2002), parental foraging and diet (Ballard *et al.* 2001; Clarke *et al.* 1998; Clarke 2001; Irvine *et al.* 2000; Lynnes *et al.* 2004; Rombolá *et al.* 2003; Watanuki *et al.* 2002), chick growth and behaviour (Aoyanagi 1988; Davis 1982a; Janes 1997b; Salihoglu *et al.* 2001; Watanuki *et al.* 1994; Janes 1997a), and the effects of changing sea-ice conditions (Ainley & LeResche 1973; Ainley *et al.* 1998; Irvine *et al.* 2000; Rombolá *et al.* 2003; Trathan *et al.* 1996; Watanuki *et al.* 1993; Watanuki *et al.* 2002; Wilson 1990; Wilson *et al.* 2001). Breeding success in this species is influenced by many factors, both biotic and abiotic. Biotic factors include: breeding experience, condition, arrival time, nest position and quality, foraging efficiency and food abundance. Abiotic factors include: weather conditions, the presence of sea-ice and breeding habitat.

The use of logistic regression to examine breeding success has not yet been applied to Adélie penguins, however it has been used in the rockhopper penguin (*Eudyptes chrysocome*), by Hull *et al.* (2004). Noted in their study was that during the three breeding seasons of field research the reproductive success was high and an assessment of factors affecting breeding success during a poor year would be instructive. Adélie breeding efforts in the Ross Sea have been poor since the iceberg B-15 (≈10 000 km^2) calved from the Ross Ice Shelf in March 2000 and subsequently fragmented into nine sections. One of which B-15A (≈6 400 km^2) grounded near Ross Island at the face of the Ross Ice Shelf (Arrigo *et al.* 2002) (Figure 5.1), greatly restricting the northwest drift pattern of pack ice (Arrigo *et al.* 2002). As a result, sea-ice concentration remained heavy throughout November and December 2000, delaying the maximum open water area by approximately two months (Arrigo *et al.* 2002) A dramatic effect on the phytoplankton bloom was recorded with primary production in the area being reduced by about 40%, altering the abundance of euphausiid species observed in the diet of the Adélie penguins (Arrigo *et al.* 2002). Other studies have also shown dietary shifts and changes in foraging patterns with heavy sea-ice, usually resulting in decreased annual breeding success (Ainley & LeResche 1973; Ainley *et al.* 1998; Kato *et al.* 2002; Watanuki *et al.* 1993; Watanuki *et al.* 1997; Watanuki *et al.* 2002; Wilson 1990; Wilson *et al.* 2001; Yeates 1968). During the 2002-2003 austral summer not only was B-15A still grounded off the coast of Ross Island but in May 2002, a further iceberg, C-19 (200 km long and 35 km wide) calved off the Ross Ice Shelf thus sea-ice cover was extensive until late December, and distances to open water in order to forage were large. Consequently, seasonal breeding success was expected to be low.

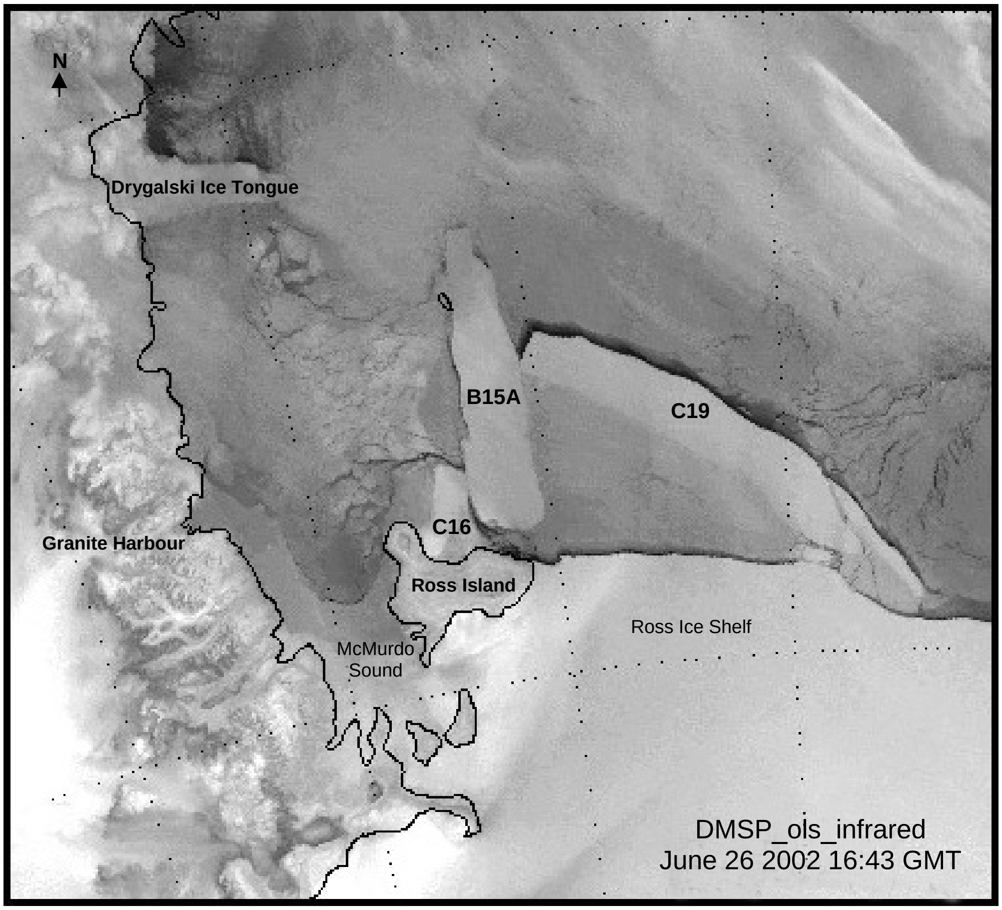

Figure 5.1: Satellite image of Ross Island showing the location of icebergs C16, B15A and C19 during the winter of 2002. (Adapted from a Defense Meteorological Satellite Program image - www.ngdc.noaa.gov).

Most colonial seabird studies of breeding success are complicated by the complexity with which so many factors affect breeding success (Davis & McCaffrey 1986). In this study, a logistic regression modelling approach is trialed in an attempt to predict the factors affecting breeding success at three nesting stages (pair formation, egg incubation and chick fledging) during the 2002-2003 austral breeding season at Cape Bird, Ross Island, when extensive sea-ice cover was persistent. During "poor" breeding years it is expected that factors predicting breeding success will be more stringent in terms of breeding behaviours as compared to when conditions are less inclement. Additionally, nest and individual penguin characteristics are examined with respect to their predictive ability and constancy over the breeding stages. The three breeding stages capture an estimate of breeding success at crucial stages of breeding. Since breeding success was likely to be poor, it is predicted that although many birds will succeed at the first and possibly second stages, the cumulative effects of an increased breeding effort will mean that only the most experienced males will succeed in raising chicks to fledging. A further prediction is that some or all aspects of male quality (early arrival and weight), nest location, and/or nest quality will have an effect on the condition of chicks in focal nests. In addition, it is expected that chicks in two-chick nests will be in poorer condition, due to sibling competition, given the scarcity of food resources.

5.3 Methods

5.3.1 Subjects and study area

This study was carried out between 23rd October 2002 and 27th January 2003 at North Colony, Cape Bird (77°13'10"S, 166°28'30"E), Ross Island, Antarctica. The focal sub-colony (see Chapter 2, Figure 2.2) of approximately 100 breeding pairs was situated in the centre of North Colony (≈35 000 breeding pairs in 2000; Ainley *et al.* 2004). The sub-colony was elongated in shape, with an approximate distance of three meters from centre to edge. Focal Adélie penguins were behaviourally sexed (see Chapter 2; section 2.3.2) and a total of 51 randomly chosen focal males were used in the study. Individual identification was achieved with the use of permanent metal flipper bands (method of attachment, specifications and ethics approval documentation in Chapter 2; section 2.3.1 & 2.4).

5.3.2 Breeding/nesting parameters measured

The arrival date and timing of focal males (early versus late – see Chapter 2, section 2.4 and Table 2.1 for details) to the sub-colony and the initiation of nest building was recorded. Weight upon arrival (measured using a strop/breathable black bag and 10kg Pesola scale), nest location, nest quality, date of pair bond, and egg laying (first and second) dates were also recorded. The latency between achieving a pair bond from arrival (days), pair bond to first egg laid (days), arrival to first egg laid (days) and length of time between first and second egg laid (days) was calculated for each focal male. Additionally, the approximate length of time each male fasted was calculated as the difference between the date of arrival and 17 days after the start of incubation (corresponding to the end of the males' first incubation shift; Davis, 1982b). Once eggs were laid in a focal nest the male was categorised as having reached the incubation stage. Observations of breeding behaviours and nest specific characteristics were made daily (further detail regarding focal nest observations can be found in Chapter 2, section 2.4). At the end of the breeding season, focal males were re-weighed and breeding success was assigned. All definitions of breeding behaviour (including arrival time and nest specific characteristics) and breeding success are defined in Chapter 2 (Table 2.1).

Using an average incubation length of 34 days (33.7 ± 1.1 day, range 32-38 days, n = 192; Davis 1982b), dates of hatching were estimated and chicks from focal nests measured at approximately two weeks of age. Dates of hatching had to be estimated as researchers were not present between the 20th of November and the 30th of December 2002. This may have added additional variation to both estimates of hatching and thus chick weight at two weeks of age but this estimate was unavoidable and affected both early and late arriving birds and so was considered unlikely to have significantly altered the results observed. Weights were measured using a breathable bag and either a one or five kilogram Pesola scale, and wing length measured using a custom metal ruler. Chick wing length was measured so a condition index comparing weight could be calculated. Additionally, a direct comparison of wing length and weight can offer information about the nutritional conditions of the focal chicks as a proportionally longer wing compared to weight implies a lack of regular food and a shorter wing compared to weight implies an abundance of food (Kerry Barton *pers. comm.* – Landcare Research).

5.3.3 Statistical analyses

The relationships between breeding success and the parameters measured at each nest were first analysed using univariate statistics. Data were checked for normality and heterogeneity of variance and general linear models (GLM) were used when these assumptions were met. When group size was unbalanced and sample sizes small or the assumption of variance heterogeneity was not met, the Welch ANOVA was used (Zar

1999). Categorical data were analysed using Chi-squared analyses, when observed frequencies were low; *P* values were calculated with an Exact Contingency Table using online software (http://www.physics.csbsju.edu/stats/). All other statistical analyses were performed in JMP 5.1 (SAS Institute 2004). Unless otherwise stipulated, all data cited in text are mean ($\overline{X}$) ± standard deviation (SD) and all tests used a level of significance of $\alpha = 0.05$. Tests used and associated data are outlined in Table 5.1.

Table 5.1: Summary of statistical methods used and associated data.

Statistical method	Type	Associated data
GLM	Linear regression	Chick growth
	Nominal logistic regression	Male breeding stage predictions
	ANCOVA	Chick condition parameters
non-GLM	Welch ANOVA	Focal male pair, male weight, incubation and hatching factors
Contingency table	Chi-squared	Nest and site specific parameters
	Exact Analysis	Nest and site specific factors – with small frequency distributions

A series of mixed model stepwise nominal logistic regressions were performed to examine the importance of nest specific parameters with regard to breeding stage achieved. Due to the high degree of variable correlation between male arrival into the colony and individual male weight (r = 0.715, n = 51, P = < 0.0001) and consequential lack of independence between these factors, principle component scores were created. The first principle component (PC1) had a cumulative percentage eigenvalue of 85.7%, and therefore this combined variable was used to represent these two factors in subsequent logistic analyses. The logistic stepwise procedure includes all parameters (PC1, nest quality and nest location) in the analysis but only those parameters that significantly ($\alpha < 0.05$) contributed toward the whole model were retained and run in the final model. When there is no significant difference in influence between categorical nest variables (i.e. good/average versus poor nest quality or centre/middle versus edge nest location) the data are pooled in the subsequent logistic model. A Receiver Operating Curve (ROC) (Metz 1978) provides a graphical method for assessing the discrimination capability of a model over a range of threshold probabilities and was used to examine the discrimination ability of the models. A model that has no discrimination ability will create an ROC curve that follows a 45° line and have an index value of 0.5, a model with perfect discrimination will have a value of one (Pearce & Ferrier 2000). Additionally, sensitivity and specificity scores were calculated for each regression model.

An analysis of covariance (ANCOVA) was performed on factors that may have influenced chick condition (calculated by dividing the wing length (mm) by transformed weight ($g^{1/3}$)) at two weeks of age; these were clutch size, nest location, male weight at the beginning of the season (covariate) and the interaction terms of early male weight, nest location and clutch size. Factors such as nest quality or arrival time of the male into the colony were unable to be included in the model as sample sizes were too small and unbalanced.

5.4 Results

5.4.1 Pair bond

Of the 51 focal males in the study sub-colony, 27 arrived early in the breeding season and 24 males arrived late (Figure 5.2a). The average weight of the 51 males when they arrived at the sub-colony was 4.9 ± 0.72 kg, but varied with arrival time (early 5.38 ± 0.58 kg, late 4.35 ± 0.42 kg; $F_{1,50} = 51.175$, $P = < 0.0001$). The mean date by which males established a pair bond was the 7th of November (SD 2nd – 12th); however, earlier arriving males tended to form pairs earlier than late arriving males (Figure 5.2b). Earlier males were also more likely to form pair bonds (92.6%) than late arriving males (54.2%) but took longer than later males to form these bonds (early 5.92 ± 3.66, late 3.92 ± 1.49 days; Welch ANOVA: $F_{1,37} = 5.525$, $P = 0.025$).

Focal males showed no clear preference for nesting in the centre of the sub-colony ($\chi^2_2 = 5.272$, $P = 0.072$) with only 27.5% males choosing to nest there (of those males however the proportion of early males choosing to nest there was 40.7% as compared to 12.5% of late arriving males). One third (33.3%) of the focal males chose to nest in the middle of the colony (early 29.6% vs. late 37.5%) and a slightly larger percentage chose to nest at the edge of the colony (39.2%), however, a greater proportion of the edge nesting birds were late arriving males (early 29.6% vs. late 50%). Note, nest location was determined after the sub-colony was fully occupied, thus results regarding arrival time and choice of nest location were not influenced by early season changes in nest location due to competition for space or males that had not yet returned to the sub-colony. Male weight did not predict nest location (Welch ANOVA: $F_{2,50} = 1.283$, $P = 0.286$).

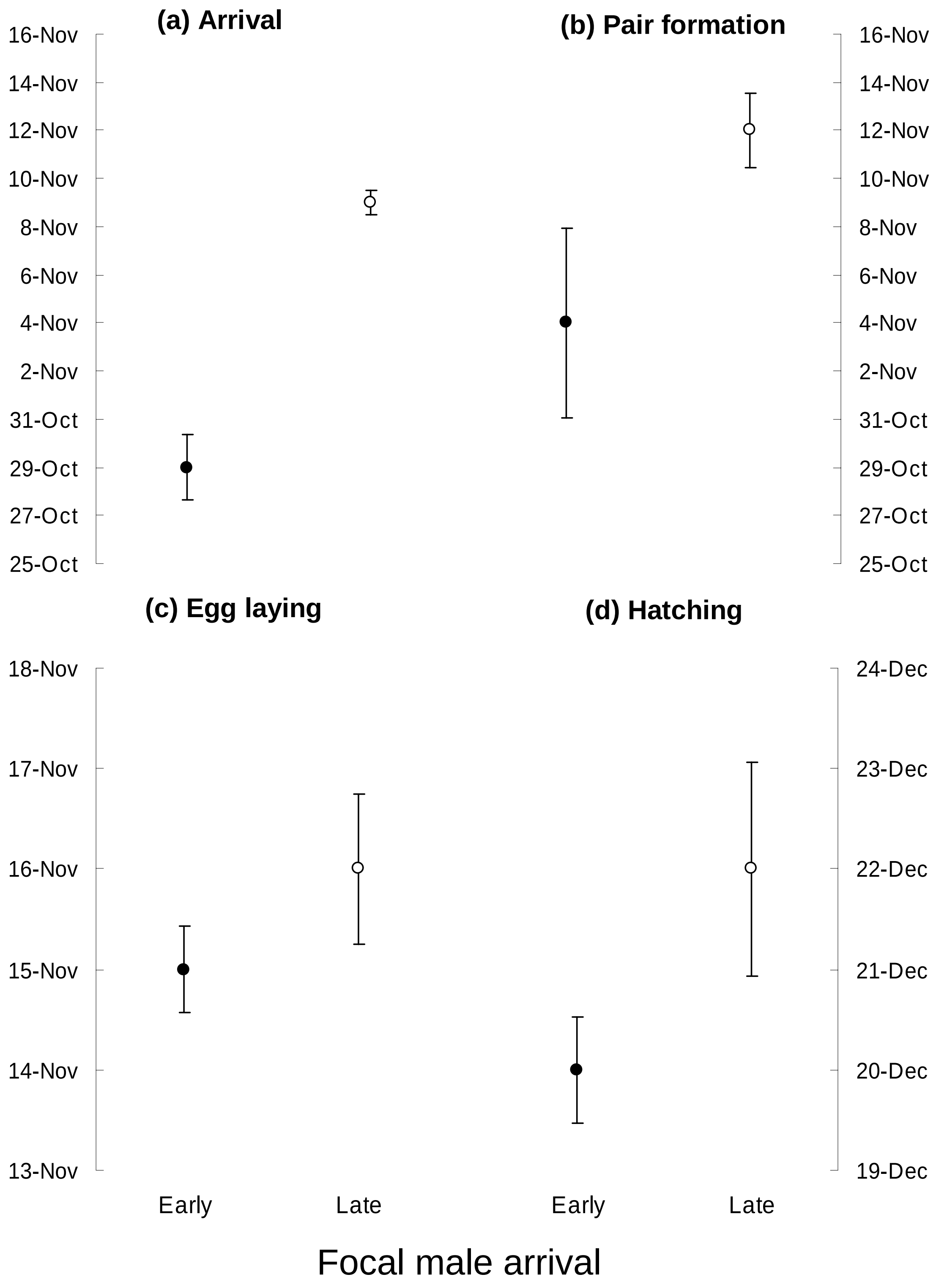

Figure 5.2: Mean observed dates (± SE) for breeding stages (**a** – Arrival, **b** – Pair formation, **c** – Egg laying and **d** – Hatching date) of early (*n* = 27) and late (*n* = 24) arriving focal Adélie males.

Nest quality varied considerably with male arrival time ($\chi^2_2 = 14.772$, $P = 0.001$) and nest location (3x3 Exact Contingency Table $P = 0.024$). Additionally, heavier males were more likely to build average and good nests rather than poor nests (Welch ANOVA: $F_{2,50} = 7.545$, $P = 0.002$) (Figure 5.3). Nearly half of all nests were qualified as good (41.2%), whereas a roughly equal proportion were either average (27.5%) or poor (29.4%). Again arrival time was important, as early arriving males built a greater proportion of good nests (good = 63%, average = 29.6% and poor = 7.4%) whereas the majority of nests built by late arriving males were poor (54.2%). Of the 22 good nests, nine were in the centre (40.9%), seven were in the middle (31.8%) and six were at the edge (26.3%). The average (n = 14) nests had roughly equal percentages in the centre, middle and edge (35.7%, 35.7% and 28.6% respectively). One third of the 15 poor nests were located in the middle of the sub-colony but 66.7% were located at the edge, while there were no poor nests in the centre of the sub-colony (Figure 5.4).

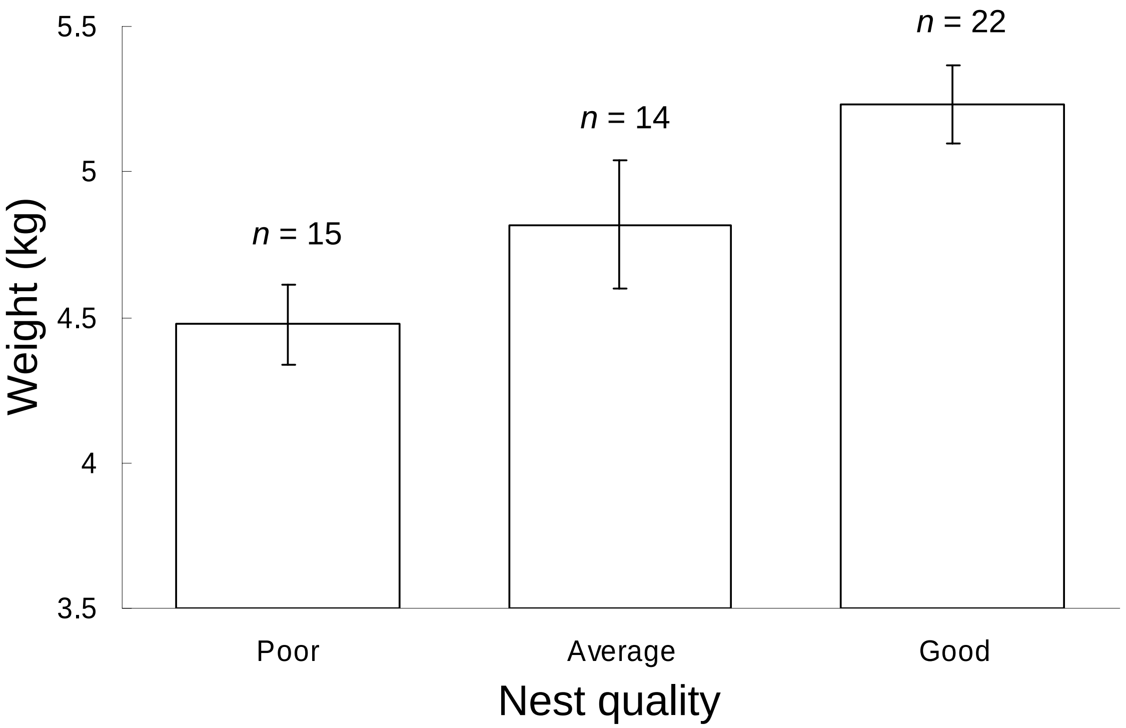

Figure 5.3: Nest quality of focal males (n = 51) based on their mean (± SE) arrival weights (kg).

Figure 5.4: Graphical representation of the quality of nests located within centre, middle or edge of the sub-colony, the size of each sphere approximates a percentage.

Logistic regression parameters that significantly influenced whether a male obtained a pair bond were nest quality (poor - average & good), and nest location (edge - middle & centre) (Table 5.2a). Nest quality explained a greater proportion of model variation than nest location (nest quality R^2 = 0.314, nest location R^2 = 0.078). Thus average and good nests were similar in predictive pairing ability and both were different to poor nests; additionally middle and centre nests were similar but both were different from edge nests. Therefore, an average or good nest in the middle or centre of the sub-colony significantly increased the probability of a male obtaining a stable pair bond (Table 5.2a). The ROC value was 0.882, which indicates the model had reasonable discrimination ability (Table 5.2). The final model used was examined for its sensitivity and specificity (Table 5.3a). The results demonstrate the model was correct with its predictions 86.3% of the time, the proportion of correctly classified events (birds that paired) in this model was greater than the proportion of correctly classified non-events (non-paired birds); therefore predictions regarding birds that did pair were more accurate than for birds that did not pair. This is corroborated by the fact that the model made more false negative than false positive errors (the model predicted fewer birds to pair than actually did).

Table 5.2: Nominal logistic regression of **a)** pair bond, **b)** egg stage and **c)** breeding success using a mixed stepwise procedure, only significant ($\alpha < 0.05$) predictor effects were included in the final model. Sample size of focal males for each analysis was $n = 51$.

Predictor	ß	SE ß	Wald's χ^2	*df*	*P*	
a) Pair Bond						
Intercept	-0.905	0.441	4.222	1	**0.040**	
Nest {Poor-Average & Good}	1.395	0.433	10.367	1	**0.001**	
Nest Loc{Edge-Middle & Centre}	0.905	0.441	4.222	1	**0.040**	
Whole model			χ^2	*df*	*P*	R^2
Log Likelihood ratio test			22.677	2	**<0.0001**	0.392
b) Egg Stage						
Intercept	0.171	0.700	0.060	1	0.807	
PC1 {Early weight & Arrival}	-1.847	0.618	8.933	1	**0.003**	
Nest{Poor & Average-Good}	2.193	0.839	6.831	1	**0.009**	
Whole model			χ^2	*df*	*P*	R^2
Log Likelihood ratio test			45.596	2	**<0.0001**	0.660
c) Breeding Success						
Intercept	1.733	0.679	6.520	1	**0.011**	
Nest {Poor-Average & Good}	1.908	0.601	10.079	1	**0.002**	
PC1 {Early weight & Arrival}	-1.109	0.459	5.839	1	**0.016**	
Whole model			χ^2	*df*	*P*	R^2
Log Likelihood ratio test			34.934	2	**<0.0001**	0.551

Note: Discrimination ability of model assessed using Receiver Operating Curve (ROC): pair bond = 0.882 (0.7-0.9) indicates reasonable discrimination), egg stage = 0.969 and breeding success = 0.946 (>0.9 indicates very good discrimination).

Table 5.3: Observed and predicted frequencies for Adélie male penguins to achieve **a)** pair bond **b)** incubation stage and **c)** breeding success using stepwise nominal logistic regression with a cut-off of 0.50.

a) Pair Bond	Predicted		
Observed	Yes	No	% Correct
Yes	36	$2^{t\,t}$	94.74^{*}
No	5^{t}	8	61.54^{**}
Overall % correct			86.28

b) Egg Stage	Predicted		
Observed	Yes	No	% Correct
Yes	28	$2^{t\,t}$	93.33^{*}
No	1^{t}	20	95.24^{**}
Overall % correct			94.12

c) Breeding Success	Predicted		
Observed	Yes	No	% Correct
Yes	10	$3^{t\,t}$	61.54^{*}
No	7^{t}	31	86.84^{**}
Overall % correct			74.55

Note: *Sensitivity; ** Specificity; ^{t} False positive ;$^{t\ t}$ False negative

5.4.2 Incubation stage

Not all of the males that achieved a stable pair bond reached the incubation stage. Of the 38 males classified as paired, only 30 successfully began the incubation stage. The remaining eight males were all late arriving males. Of the 30 birds reaching the incubation stage, 25 were early males, and five were late arriving males. The egg laying period took place between the 10th and 21st of November. Peak egg laying occurred on the 15th November (SD 12th – 18th), the first eggs in a two clutch nest were laid on the 13th of November (SD 10th – 16th) and the second on the 17th November (14th – 20th). Early arriving males and late arriving males had relatively similar laying dates (Figure 5.2c).

The time between arrival to laying of the first egg was significantly different depending on time of arrival; early arriving males had a longer latency than later arriving males (early 15.52 ± 2.29, late 6.4 ± 1.95 days; Welch ANOVA: $F_{1,29} = 85.712$, P = <0.0001; Figure 5.5a). Similarly, latency between pairing to first egg laid was longer for early arriving males (early 9.64 ± 2.16, late 3.6 ± 1.52 days; Welch ANOVA: $F_{1,29} = 56.45$, P = <0.0001; Figure 5.5b). However, the delay between laying the first and second egg was not significantly different between the two arrival groups (early 3.16 ± 0.37, late 2.6 ± 0.55 days; Welch ANOVA: $F_{1,29}$ = 4.781, P = 0.083; Figure 5.5c). The fasting period (arrival – end of first incubation shift) is determined by the time between arrival and first egg layed and thus males that arrived early fasted longer than males that arrived later in the breeding season (early 35.7 ± 2.2 vs. late 26 ± 2.5 days). The maximum time any one male spent fasting was 40 days (an early arriving male) and the minimum time was 24 days (a late male).

The proportion of males proceeding from pair bond to incubation stage seemed to be correlated with nest quality. Only one of the five (20%) poor nests that obtained a stable pair bond also reached the incubation stage, whereas nine out of 12 (75%) of the average nests reached the incubation stage and only one of the 21 (4.8%) paired birds with a good nest failed to reach the incubation stage. Nest location seemed to be less relevant, as similar proportions of males, regardless of nest location (centre 28.6%, middle 14.3% and edge 20%), failed to proceed from the pair bond to incubation stage.

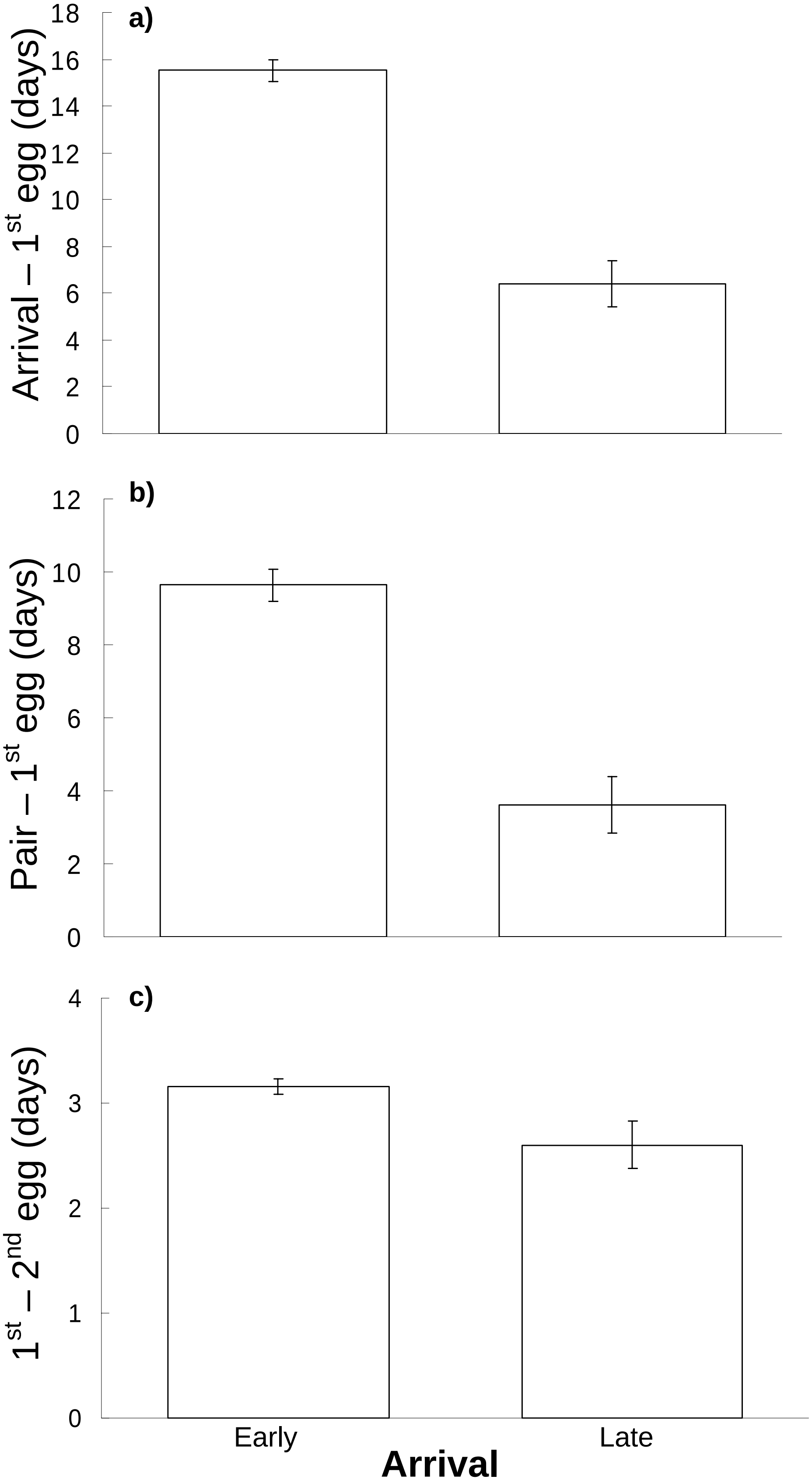

Figure 5.5: Mean arrival time (± SE) of focal males against **a)** time taken from arrival into the colony until first egg is laid, **b)** time taken once stable pair bond is formed until first egg laid and **c)** time between first and second egg being laid. All measured in days. Early males *n* = 27, late males *n* = 24.

Compared to the pair bond logistic model, a slightly different set of parameters predicted the probability of a male reaching the incubation stage (Table 5.2b). The primary parameter included in the model was the first principle component (PC1) of arrival time and early male weight; with lower PC1 values (corresponding to late arrival and lower weight) predicting significantly fewer males would reach the incubation stage. Nest quality (poor – average & good) was also a significant factor. Again the average and good nests were grouped away from the poor nests. In the final model, PC1 explained more variation than nest quality (PC1 R^2 = 0.469, nest quality R^2 = 0.191). Hence, males with the highest probability of reaching the incubation stage should arrive early, be heavy and build either an average or good nest. Models at the incubation stage were more precise than at the pair bond stage (94.2% vs. 86.3%; Table 5.3b). However, the sensitivity and specificity of the model was similar, indicating the predictive ability of the model for birds to either succeed or fail to reach the incubation stage was similar (Table 5.3b). The best model was conservative with more false negative predictions than false positives. Finally, the ROC demonstrated that the discrimination ability of the incubation model was greater than the model predicting a stable pair bond (0.969 vs. 0.882) and was classified as having very good discrimination ability (Table 5.2).

5.4.3 Breeding success

Breeding success was poor, with only 16 (53.3%) of the 30 pairs that reached the incubation stage (25 early males and five late males), successfully fledging at least one chick. One further pair re-laid but the sole hatching chick did not survive past two weeks of age. Of the 16 successful males, five nests fledged a single chick and 11 fledged two chicks. The estimated hatching date (based on an average 34 day incubation) for all possible chicks was 21st December (SD 18th – 24th); on average early nest chicks were scheduled to hatch on 20th December (SD 17th – 23rd), and late chicks just two days later on the 22nd December (SD 20th – 24th) (Figure 5.2d). Consequently, due to differences in the length of the pair bond stage, there was no significant difference between arrival time of the males and mean hatching date (Welch ANOVA: $F_{1,29}$ = 1.148, P = 0.321).

The mean weight for the 27 chicks from 16 nests was 905.74 ± 249.9 g. The mean wing length was 82.54 ± 15.86 mm. The early nests had 14 successful nests (nine 2-chick and five 1-chick nests), with an average weight of 881.74 ± 230.99 g and wing length of

81.39 ± 15.35 mm. The late arrival males had two successful nests both of which had two chicks, the average weight of those four chicks was 1043.75 ± 346.64 g and had an average wing length of 89.13 ± 19.57 mm. There was a strong positive relationship between body weight and wing length for both 1-chick and 2-chick nests ($F_{1,26}$ = 65.361, R^2 = 0.723, P = <0.0001) (Figure 5.6).

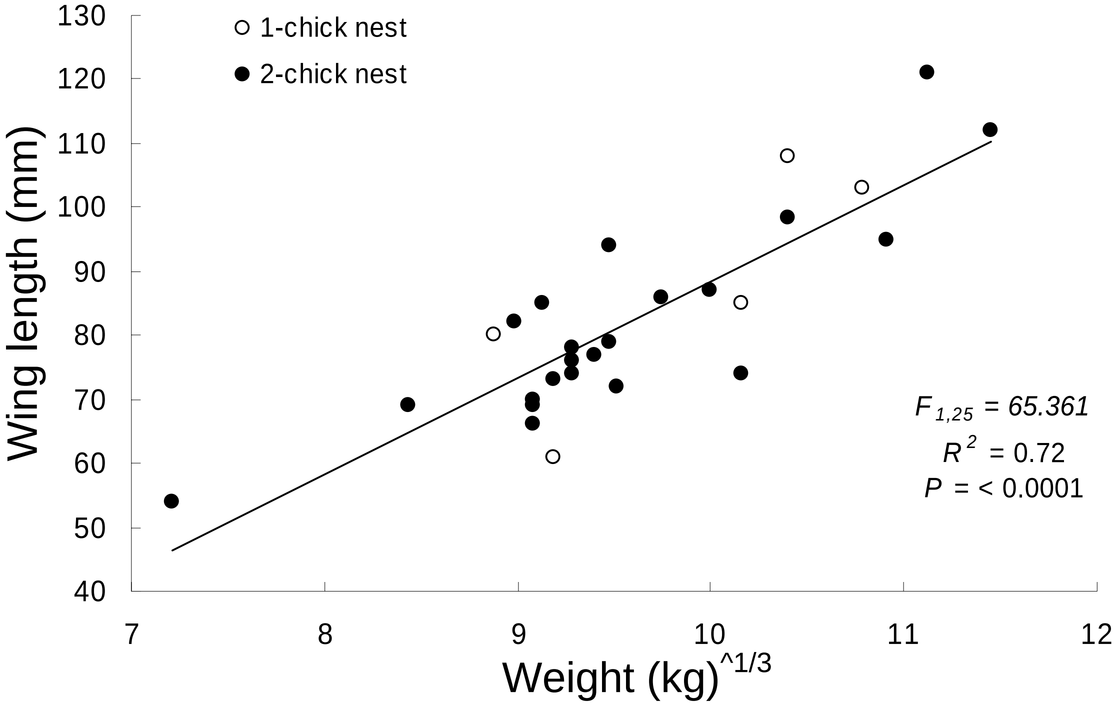

Figure 5.6: Relationship between weight of chicks (cube rooted to be linear) and wing length (mm). Nest brood size: ● = 2 - chicks (n = 11 nests) and o = 1 – chick (n = 5 nests).

The logistic model predicting end of season breeding success shows a similar set of predictive parameters (nest quality R^2 = 0.429, PC1 R^2 = 0.122; Table 5.2c), however, nest quality has greater explanatory power than in previous models. To predict a positive breeding outcome the nest quality has to be good, as poor and average nests clump together in the analysis. A greater PC1 value (early arrival and high early male weight) also predicts greater breeding success. Overall ability of the model to predict breeding success was 74.6%, the sensitivity and specificity of the model demonstrates a better ability to predict non-events (nest failure), and a poorer ability to predict nest success (Table 5.3c). The ROC value of the model (whilst still categorised as having very good discrimination capacity) is correspondingly lower than that for the incubation model but still higher than the model predicting pair bond achievement (pair = 0.882 < success = 0.946 < egg = 0.969) (Table 5.2).

Results of the ANCOVA show that none of the factors included in the model significantly influenced chick condition ($F_{7,26} = 0.68$, $R^2 = 0.2$, $P = 0.688$). Chicks in one or two chick nests were not dissimilar in condition ($F_{1,26} = 0.096$, $P = 0.761$) and nest location did not influence condition ($F_{2,26} = 0.409$, $P = 0.67$). Early weight (as an indication of male condition) was not relevant to chick condition during the guard stage ($F_{1,26} = 0.737$, $P = 0.401$). Likewise, the number of successful nests in the centre, middle and edge of the sub-colony were not different (centre = 6, middle = 5, and edge = 5; $\chi^2_2 = 0.621$, $P = 0.733$).

Brood size did not vary significantly with location (3x2 Exact Contingency Table; $P = 0.176$). Nest quality however, appeared to affect breeding success, with only one of the poor nests reaching the incubation stage, and that nest failed to fledge any chicks. Similarly, of the nine average nests reaching the incubation stage only one was successful (11.1%), in contrast 15/20 (75%) of the good nests that reached the egg stage were successful.

Weight changes across the breeding season showed focal males loosing on average 0.5 ± 0.88 kg. Early arriving males lost on average 0.84 ± 0.89 kg (which equates to approx. 14% body weight; Figure 5.7). Late arriving males gained 0.02 ± 0.57 kg (approx. 1% body weight; Figure 5.7). The males that lost the most weight were early arriving successful males, which lost approximately 21 % of their body weight (Figure 5.7). Birds that were affected least throughout the breeding season were late arriving failed breeders, which actually increased their weight slightly 2.3 ± 12.3% over the breeding season (Figure 5.7).

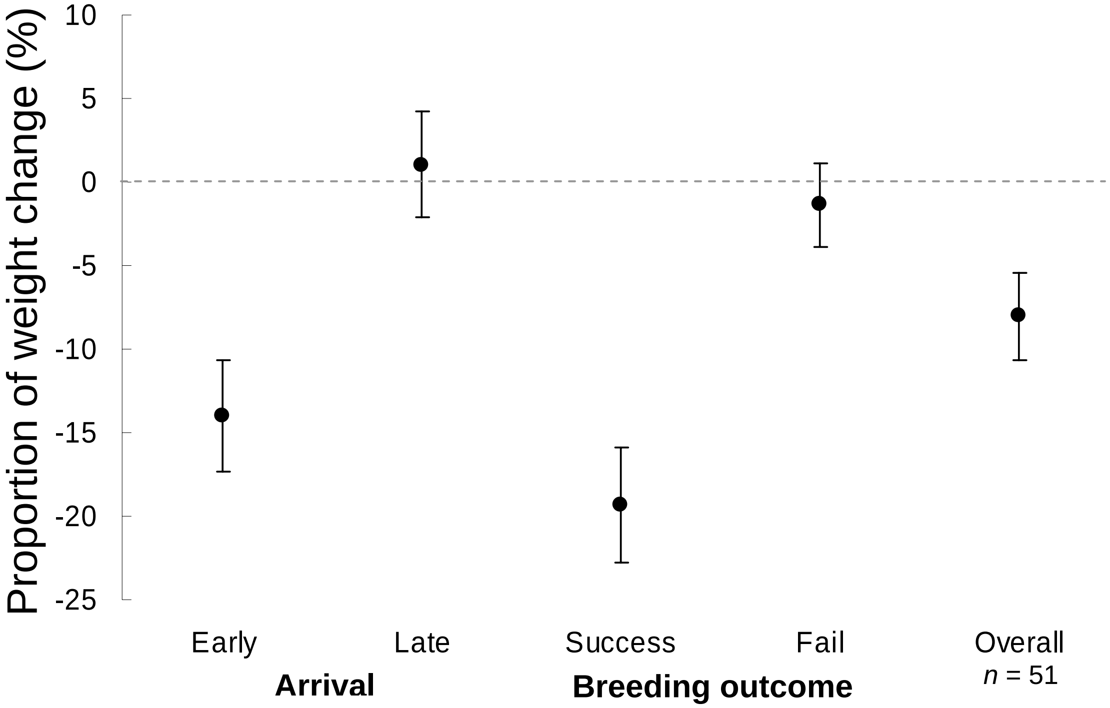

Figure 5.7: Arrival of focal males (early or late), breeding outcome (success or fail) and total number focal males (overall) plotted against mean (± SE) proportion of body weight change over the breeding season (%).

5.5 Discussion

The most significant contributing factors to successful Adélie breeding attempts during the 2002-2003 breeding season were nest quality, male arrival and weight. As with most biological systems, it was a combination of biological factors which predicted success across the breeding season. Factors that were influential during the pair bond stage (i.e. nest location) became non-significant as the breeding season progressed. It is also likely that in a breeding season when climatic conditions were unfavourable the importance of some factors were likely to be exacerbated, whilst others minimised. For example, nest quality in a year without severe storms and snow melt might mean an average nest will suffice during incubation or when chicks are still in the guard stage.

Early arriving Adélie males were more likely to pair but took longer on average to do so, perhaps because they arrived before the majority of the females (Davis 1982b), and more time was spent establishing nesting territories and nest building. Later arriving males, with fewer central territories available were more likely to build poorer nests, (possibly as materials were scarcer; Wittenberger & Hunt 1985) at more exposed locations. Logistic regression models were able to accurately predict the probability of male pairing using nest location and nest quality; a poor nest, especially on the edge of a sub-colony would appear not to be an attractive proposition, however an average or good nest even at the edge of the sub-colony was still likely to attract a female. In terms of finding a mate an average male, in an average nest, in an average location appears to suffice. The logistic model for this early breeding stage was also somewhat conservative as indeed more males obtained a mate than were predicted. As there is usually a male bias in Adélie colonies (Davis & Speirs 1990), females that arrived later into the colony would be faced with fewer choices, and therefore would likely be less choosy than an early arriving female.

As the breeding season advanced, significantly fewer of the paired males successfully began incubation duties. Nonetheless, the late arriving males compensated for their later return by shortening the interval between pair bonding and egg laying. Thus no difference was found in hatching dates between early and late males. This flexibility in breeding chronology has also been shown in king (*Aptenodytes patagoniscus*) (Weimerskirch *et al.* 1992) and gentoo (*Pygoscelis papua*) penguins (Böst & Jouventin 1991). Later arriving males were of significantly lower weight, and given the high energetic costs of incubation (average males loose 52g/day whilst fasting Chappell *et al.* 1993), it is unlikely that these late males could withstand a long fast. Using regression models it was found the best predictors of males fledging chicks were arrival time and weight; however, successful incubation was best explained by nest quality in average or good nests. The incubation phase is critical in terms of male weight and arrival time as it is during this phase that the male is solely relying on post winter fat reserves. A male with poorer fat reserves paired with a female slow to return from the first incubation shift is likely to abandon the nest (Davis 1982b; Davis 1988; Davis & McCaffrey 1986). Nest desertion clearly affects hatching success with as many as one-third of eggs lost at this point in the breeding cycle (Davis 1982b; Davis & McCaffrey 1986). During inclement weather and sea-ice conditions, nest desertion was likely to have been the greatest factor affecting breeding success. Davis (1982b), found that very early and late nesting birds were the most likely to desert, as very early nesting males depleted their fat stores before the female could return, while late nesters (most likely inexperienced breeders) were less likely to coordinate their incubation shifts. In abandoning a nest a life history trade off between current reproductive success and future reproduction and survival is made (Olsson 1997).

Nest quality also became crucial during incubation, as weather conditions were often inclement during early summer and severe storms followed by snow melt meant eggs in poorly built nests became waterlogged and perished (*pers. obs.*). A study by Moreno *et al.* (1995) showed flooding affected 31% of chinstrap (*Pygoscelis antarctica*) nests and the loss of 14% of eggs/hatchlings; small nests were also more prone to flooding. Predation pressure was also greater during this stage as unguarded or poorly defended eggs were quickly and easily taken by south polar skua (*Catharacta maccormicki*). Tenaza (1971) postulated that poorer peripheral nests were more vulnerable to stone stealing, and the inability to maintain stones would lead to poorer construction and more eggs being accidentally rolled from the nest.

Breeding success was poor during the 2002-2003 austral summer. The presence of extensive sea-ice not only meant that fewer eggs survived the incubation phase (most nests were likely abandoned before chicks hatched), but also the ability of the male and female to feed chicks and coordinate guard shifts would have been severely tested. Nearly half of the males that began incubation (13/30) failed either during incubation or when chicks were small. The number of fledged chicks that resulted from total number of eggs laid was 27/60 (45%) or 0.9 chicks fledged per pair. This is less than the average recorded for this species (51 ± 2%; Ainley 2002) and considerably less than the estimates recorded at Cape Bird during years with favourable sea-ice conditions (66% in 1967 & 64% in 1970; Ainley 2002). Chicks left unguarded, or vulnerable in an average or poor nest, were easy prey for skua. Small chicks are also unable to regulate their body temperature (Williams 1995) and therefore dry shelter provided by a good nest would be essential.

Once the chicks were measured at two weeks they were homeothermic (Williams 1995) and essentially too large to be easily taken by skua (*pers. obs.*). Sea-ice was also less abundant; consequently distances to open water were less (*pers. obs.*). Potentially this meant all surviving chicks regardless of nest location or clutch size were of similar condition. Results (although sample sizes were small) indicated a male in good condition, with a female of similar quality was likely to raise two chicks. The model predicting fledging success was less accurate than the incubation model largely because many early arriving males, with reasonably high weight and good nests, failed to breed. What separated success from failure in birds with outwardly similar characteristics, likely includes parameters such as age and breeding experience, mate fidelity and nest site fidelity which were not within the scope of this study. Additionally, other factors such as weather and food availability (which were not assessed) may have been causing the negative impacts on breeding success therefore the results seen may not solely reflect sea-ice conditions. It is however generally accepted that extensive sea-ice has detrimental effects on Adélie penguin breeding success (Ainley 2002).

An interesting alternative strategy for males beginning the breeding season in poorer condition may be to arrive late (so as to minimise weight loss), and construct a good nest on the edge of the sub-colony. Coordination of feeding and guarding would then be contemporaneous with the early arriving males and in this study the chicks should be able to crèche and fledge safely along with chicks produced by the early arriving males. At the end of this study season only two late arriving males (who began the season with below average weights) had been successful, but both had lost far less weight than the early males and therefore potentially were in a better position to build up fat stores, moult and survive the harsh winter to breed again the next season. The king penguin is known for its adaptive and variable breeding strategies (Jiguet & Jouventin 1999; Olsson 1996; Olsson 1997; Van Heezik *et al.* 1994) and it has been recorded that experienced breeders with lower body reserves can compensate by being more proficient foragers than inexperienced breeders in better condition (Olsson 1997). Therefore, it is possible late arriving but experienced Adélie males might also be able to compensate for poorer condition by foraging effectively and still being able to raise chicks to fledging. One other strategy not discussed previously is the decision not to breed, which for a male in poor condition (due to poor winter foraging) may be a beneficial strategy as it has been established that in the Adélie penguin annual mortality is greater in breeding than non-breeding birds (Ainley 2002). The theory of reproductive skipping (due to potentially poorer likelihood of survival) could be a strategy that would enhance lifetime reprodutive success (Schaffer 1974). This strategy has been seen in several bird species including kittiwakes, *Rissa tridactyla* (Cam *et al.* 1998), shearwaters, *Puffinus tenuirostrus* (Wooller *et al.* 1990) & eider ducks, *Somateria mollissima* (Coulson 1984), but has not yet been examined in Adélie penguins.

It is always difficult to determine whether variation in breeding success in different areas is due to either individual or site parameters or a combination of both (Bunin & Boates 1994). The use of multivariate analyses such as logistic regression can help determine the relative significance of individual and nest specific parameters over the breeding season. In conclusion (during the 2002/3 season), for a male Adélie to pair it had to build a nest of average or good quality, preferably in the middle or centre of the sub-colony. The probability of reaching incubation was increased if a heavy male arrived early and built and average or good nest. Finally, fledging success was highest for heavy, early arriving males with good nests. The key points for improving chances of fledging chicks in the 2002-2003 breeding season were early arrival, heavy, centrally (middle or centre) located males with good nests. Clearly this data only represents a single breeding attempt by these focal birds and conclusions about nest or individual parameters that increased the probability of success only apply to this season. Logistic regression modelling did however prove to be an accurate predictor of breeding parameters and therefore a useful tool for use in further research. Not only would additional comparison breeding seasons with higher probabilities of success (now that iceberg B15A has moved away) be of interest but also an evaluation of logistic regression techniques compared to more traditional breeding success estimates could be undertaken.

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6

6.1 This research's contribution to understanding the acoustic behaviour of Adélie penguins

This dissertation on the EDC and Adélie penguin breeding has not only enhanced the wealth of knowledge regarding Adélie breeding behaviour and expanded our understanding of the vocal repertoire of this species, but also has implications for mate choice signalling theory (honesty), and the stability of vocal variation across time and with geographic location.

The ability to understand the complex mechanisms by which species communicate has been a focus of study for many years (Catchpole & Slater 1995; Kroodsma & Byers 1991; Slater 2003). Colonial birds in particular have to compete vocally with surrounding birds and often the environment in order to effectively communicate with their partners and offspring. The ability to attract a mate from a multitude of similar looking birds has always lead researchers to believe there was some aspect of the Adélie penguin EDC that was "attractive". Until now however, it has always been assumed to be the pitch of the call, with larger males giving lower pitch calls. This study has shown not only that larger males generally do not give calls of lower pitch (centrally nesting males actually gave higher pitch calls), but that frequency modulation was the only factor linked to weight and breeding success. The concept that this call parameter may be an index signal is one not suspected before. If the EDC is shown to be an honest index signal it may enhance our understanding of mate choice and honest signalling in not only birds, but in all species where females exercise mate choice and males compete (visually or vocally) to attract a mate.

The potential for geographic variation to have evolved in this species, also documents the importance of recording calls not just of one type. The possibility that

EDC parameters can relate information regarding health and potential breeding success at a colony level may also have implications for penguins that have already shown vocal variation at a population level. Especially when colony location, size and environmental conditions may result in geographical variation in vocalisations in a species that was previously thought not to have any variation. This study both adds to geographic variation studies in non-passerines but also highlights the need to examine stability in calls, outside the breeding season (although not possible in penguins), between years and during the breeding season.

The EDC is something of an anomaly in the penguin vocal repertoire not only because it is functionally unique, but also due to the scale of vocal malleability observed, and the potential for birds to adjust call parameters such as pitch and amplitude when the environment dictates (much like the king penguin which has been shown to increase call length in windy, noisy conditions; Lengagne *et al.* 1999). The use of Sound Analysis Pro to categorise orthogonal call parameters in separate sections of the EDC (including entropy which has not been used before in penguin studies), has also added greater understanding of the quality and constancy of calls. The ability to directly compare male and female calls may result perhaps in the phrase "male Ecstatic Display Call" diminishing from the literature, as clearly although not given as frequently, females give the same call. Given the territorial and contagious nature of the EDC, nest defence and territoriality should also perhaps be more strongly associated with female Adélie penguin behaviour.

The energetic costs of breeding in the male Adélie penguin are well documented. The multitude of often correlated factors that impact on the likelihood of breeding successfully however, often precludes a clear understanding of the relative importance of each of these factors. The use of logistic regression is one way in which some clarification regarding factors affecting breeding success can be applied, and then evaluated not just within a breeding season, but also over time with changing breeding conditions. This method was successfully applied in this dissertation to the Adélie penguin but can also be applied to other colonially breeding birds, and any species that requires certain individual and habitat characteristics to breed successfully.

6.2 Adélie vocalisations and breeding success

6.2.1 EDC parameter variation

Jouventin (1982) demonstrated greater inter-individual than intra-individual variation for the syllable duration and main frequencies in the EDC. Results from the present study concur with Jouventin's (1982) finding as all parameters measured regardless of season, colony, or year showed highly significant individual variation (Chapter 2). Therefore, the individuality of this call is unquestionably stable. Also noted by Jouventin (1982) was the similarity in syllabic organisation between the EDC and the LMD. This is however, the first time a detailed comparison between the two calls has been made and, although structurally the calls may look similar, the duration of the short repeated syllables was the only parameter measured that was non-distinct between these two calls. Overall the EDC is longer (with more short syllables and greater long syllable duration), lower in pitch/mean frequency, more tonal (i.e. calls contain less noise), and has higher frequency and amplitude modulations (Chapter 2). Although almost always described as a male vocal behaviour (Ainley 1975b; Jouventin 1982; Penney 1968; Spurr 1975b), even to the extent that Jouventin (1982) describes the EDC as potentially able to differentiate the sexes, the present study not only showed that significant numbers of females gave this call (13.7% of the birds sexed at the end of the breeding season), but there were no discernable differences in EDCs given by males or females (Chapter 2). This is despite females being generally smaller (having decreased wing lengths) and at the beginning of the season, of lesser weight (Ainley & Emison 1972).

6.2.2 Spatial and temporal variation in the EDC

Studies examining geographical variation in bird calls are numerous. The literature on variation in bird calls that do not show dialects is less abundant, and there are even fewer studies documenting variation in penguin calls. Jouventin (1982) has shown the disparity in intra-species call characteristics within the king, gentoo, rockhopper and macaroni penguin. These differences however, have largely been attributed to the segregation of species into sub-species, which inhabit separate islands. The Adélie penguin, after initial inspection (by Jouventin 1982) of the LMD calls, showed no potential for geographic variation but the EDC had yet to be studied. The findings from this thesis showed not only did the EDC vary by colony (on Ross Island), but also that these differences were potentially associated with colony location and size (Chapter 3). However, the stability of the EDC was also shown to be fragile, as not only did the calls vary between years, they also varied within colonies across the breeding season. The changes in call parameters seemed to be associated with the overall changes in colony health (estimated by adult weight and chick condition), as there was a similarity between the 2002/3 calls (a poor breeding season) and calls recorded at the end of the breeding season (when male condition had declined). The Ross Island colonies further from the sea-ice edge in 2002/3 (Cape Royds) also showed signs of stress, which was demonstrated in both vocal variability and low condition of chicks. Within colonies the EDC appeared to be linked to sub-colony nest position, which is in turn linked to male size, breeding success and chick condition. Contrary to the central tenet that larger/heavier birds should have lower pitch (Ryan & Brenowitz 1985; Wallschläger 1980), this study showed that larger males in the centre of the sub-colony have calls with a higher pitch. This may be a method to increase signal transmission as these calls were less tonal, but perhaps, louder and longer. The potential for both male specific characteristics and external environmental conditions (such as surrounding noise) to be affecting the EDC is a possibility that has not been recorded before.

6.2.3 The EDC, mate choice, honest signalling and success

The effect of male health or condition on the EDC has implications for its use in mate choice. The EDC, although used in early season mate choice (Penney 1968; Ainley 1975b; Spurr 1975b), has not been studied with regards to what factors females may be using to determine mate choice decisions. Although previously assumed to be related to male size, hence decreased pitch (Davis & Speirs 1990), the present study showed that male weight (a proxy for condition) was not associated with pitch or mean frequency (Chapter 4). It was instead the modulations in frequency (FM) in the short repeated syllable that correlated with weight, with larger males having lower FM. Not only did FM change predictably with weight across the season, it was also linked with males finding a mate earlier in the season resulting in breeding success. The link between weight, fat storage and FM is not clear. Two possibilities were hypothesised in this dissertation; firstly, that the higher fat deposits during the early breeding season directly affected the sound production (an index signal; Maynard Smith & Harper 1995, 2003). The second hypothesis is that there may be some active method whereby males of greater weight controlled the FM in their calls and this was energetically too expensive for males of lesser weight to achieve. These hypotheses need to be investigated, but both essentially support the idea that the EDC may be an honest signal of male condition that females may use to select males of good condition and thus increase their chances of breeding successfully.

6.2.4 Predicting factors that affect breeding success using logistic regression

The use of logistic regression models to examine nest and male specific factors has only been applied to rockhopper penguins (*Eudyptes chrysocome*) (Hull *et al.* 2004). Although many studies have examined Adélie penguin breeding behaviour, none have looked at the relative importance of factors (such as male arrival, weight, nest location within a sub-colony and nest quality) with regard to predicting success; and the potential for those factors to change with changing breeding stage (mate choice, egg incubation and the fledging of chicks). Results presented in Chapter 5 clearly showed the importance of male condition and arrival time (heavy and early), with regard to overall breeding success. This analysis also showed the importance of having a good nest that reduces the risks of eggs being flooded, and protects chicks from the elements or being predated upon. The relative importance of nest quality was reduced earlier in the breeding season, as average nests were equally likely to be successful and nest location was only relevant during the pair bond phase (with middle and central nests being preferred). The factors important in predicting success are unlikely to be different in a breeding season where conditions are favourable however the importance of the nest site or nest qualities may be more flexible. Males that had succeeded in raising chicks to two weeks of age also succeeded in fledging those chicks. The condition of chicks in these nests was not affected by any parameter measured (brood size (1 or 2), nest location & male weight), but notably these males were almost all early arriving males in good condition (high weights) with good nests. Additionally, the use of logistic regression proved to be an accurate method by which to assess breeding success in this species.

6.3 Limitations of the study

There were several limitations to the study that hindered the conclusions that could be reached. One of the major disadvantages of studying a species, which spends more than half its life at sea, is that vocal recording of birds outside the breeding season is impossible. This means that assumptions regarding the stability of calls and variation between colonies can only be examined during the breeding season. Additionally, the limited time frame (two seasons) of this study and the locations of the study sites (Ross Island) meant that call variation could only be examined across a relatively short time frame and between colonies that were not very geographically distant. Seasonal and geographic differences found in the EDC that were potentially related to environmental factors such as altitude (level of exposure), background noise and wind strength were also not able to be assessed thus the reasons the EDC may vary with location could only be speculated on. Limited time frame also impeded the understanding of factors that affected breeding success, as mate retention, age and breeding experience could not be examined. Foraging efficiency/ecology of the male and female were also outside the scope of the study. Only males were used in the study as it is males that primarily give the EDC and it is assumed that in Adélie penguins' females are the "choosy" sex thus estimates of honesty in the EDC would be related to the males. By excluding the females from the study however, their input into achieving a successful breeding attempt (through timing, nest relief and foraging capabilities) was not examined, thus the factors assessing breeding success are biased towards the males. As the study seasons were not consecutive and honesty was assessed in the final season mate choice could also not be followed across seasons. Additionally, only approximately 50% of mates seek a new partner each season so the relevance of the EDC as an honest signal would be less important to birds that paired with their previous season's partner. This potentially dims the picture of honest signalling, however it does not diminish the findings in this study, as honest signalling in order to be evolutionarily stable, should persist even when the call is not required to be an honest signal in the short term. Finally, as permission to undertake play-back experiments were unable to be approved prior to the field season, investigation into the honesty of EDC parameters was limited.

6.4 The function of the EDC and future directions for study

The EDC is an individual call that cannot reliably be described as a male display as females give (albeit less frequently) a call that is acoustically and behaviourally equivalent. The inter-individual similarities with the LMD also appear to be solely related to the syllabic organisation (structure) as almost every call parameter differed between the calls. Given the uniqueness of the EDC and the lack of knowledge about penguin call variation in general, the call parameter differences found between disparate colonies is less surprising. The ability of the penguins to alter the EDC depending on their physical condition, over time and with geographic location and yet retain the individuality of the call is nonetheless remarkable. The EDC seems to be linked with male health as condition varied with colony, year, season and nest position within the sub-colony, as did the EDC. The link between male condition and breeding in the Adélie penguin is well established (Ainley 1975a; Ainley *et al.* 1983; Davis 1982; Davis & McCaffrey 1986; Davis & Speirs 1990; Spurr 1975a; Stonehouse 1975), as the short austral summer and extreme fasts necessitates only those in good condition can succeed. Given the link between weight and breeding it is perhaps logical that a call that females use for mate choice is also linked to weight. Variations observed within the colony may not be stable with regards to EDC call parameters being fixed in the population. They may however reflect their location, i.e. small southern colonies are always likely to endure harsher conditions and suffer decreased health and breeding success, and this may be consistent in EDC parameters. The use of logistic regression to predict factors affecting breeding success also highlighted the importance of male arrival, weight, nest location and quality, but also that the relative importance of those factors shifts across the breeding with changes in breeding stages.

There are many facets uncovered in this research that deserve further study. For example the function and frequency of EDCs given by females should be studied, especially given the paucity of knowledge regarding female vocal function and variation not just in penguins but in most bird species.

To understand the effects of geographical variation in the Adélie penguin, EDC studies on more colonies, over more seasons are required. The stability of this call and factors affecting stability (i.e. condition) also need to be investigated more thoroughly.

Environmental parameters such as altitude (level of exposure), wind stength, background noise and nest density should also be included in future estimates of vocal variation. It would also be interesting to see whether the LMD call also varied with regard to changes with condition and season.

The mechanistic link between weight, condition, and the EDC also needs further study to see how changing weight may be affecting FM and whether this signal is an index of condition or related to the energetics of sound production. Factors such as the frequency and timing of calls and changes in mate choice over time also need to be included in futher studies. Additionally, playback experiments are required to see if, by altering call parameters, female mate choice can be manipulated, along with aspects of breeding success.

Finally, the use of similar logistic regression models may be applied to predicting breeding success in years where higher levels of breeding success are expected at Cape Bird. A more direct comparison of traditional breeding success estimates versus logistic modelling would also be instructive. Also of interest would be a comparison of factors affecting breeding success in different colonies of varying size, location and over a series of breeding seasons.

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