



THE UNIVERSITY OF  
**WAIKATO**  
*Te Whare Wānanga o Waikato*

Research Commons

<http://researchcommons.waikato.ac.nz/>

## Research Commons at the University of Waikato

### Copyright Statement:

The digital copy of this thesis is protected by the Copyright Act 1994 (New Zealand).

The thesis may be consulted by you, provided you comply with the provisions of the Act and the following conditions of use:

- Any use you make of these documents or images must be for research or private study purposes only, and you may not make them available to any other person.
- Authors control the copyright of their thesis. You will recognise the author's right to be identified as the author of the thesis, and due acknowledgement will be made to the author where appropriate.
- You will obtain the author's permission before publishing any material from the thesis.

**Relationships among male size, reproductive  
success, female choice and male advertising  
calls in Little Penguins (*Eudyptula minor*)**

A thesis  
submitted in partial fulfilment  
of the requirements for the Degree  
of  
Doctor of Philosophy in Biological Sciences  
at the  
University of Waikato  
by  
**Masamine Miyazaki**



The  
**University  
of Waikato**  
*Te Whare Wānanga  
o Waikato*

**2002**

This thesis is dedicated to my parents and friends.

## **Declaration**

I declare that this thesis is a record of my own work.

Masamine Miyazaki

# Abstract

The role of male acoustic features in the process of mate choice was investigated in Little Penguins (*Eudyptula minor*). Male call features and the calling behaviour may reveal important information on male attributes that could affect female choice. Larger males showed more aggressive behaviour to simulated nest intruders than smaller males. Chicks of larger males also grew at a faster rate than those of smaller ones, possibly because of differences in the quality of food delivered to chicks or paternal genetic effects on chick growth. However, there were no differences in fledging age, mass or success between larger and smaller males. Larger males also appeared to occupy or select better nest site; they occupied low and middle elevation nesting sites in comparison to smaller males, probably because the sites reduce energetic costs of walking on land. Larger males also mated earlier in the breeding season than smaller ones, suggesting that females may prefer larger males. Thus, male body size may play an important role in the attraction of potential mates in Little Penguins. Acoustic signals can influence female responses to males particularly in nocturnal seabirds. Larger males were found to produce lower-pitched call elements in their exhalation phrases than smaller males. In playback experiments with two loudspeakers, females did not distinguish low from high-pitched calls, although they responded vocally only to low-pitched calls. In another experiment, using current mate and stranger calls, females were more likely to approach their partners' call and responded vocally only to their current partners, regardless of the stranger's call pitch and, therefore, apparent size. Females may recognise their partners' call and maintain mate fidelity even when larger, potentially more successful, strangers are presented. In addition to the effect of call pitch, calling patterns of males could influence female choice. By conducting experiments with a dual-speaker design in which calls from one speaker consistently overlapped those from the other speaker, I found that females approached overlapping advertising calls more than overlapped calls or a silent control speaker. The 'masking effects' of call overlap may obscure information that females require for mate choice. Alternatively, females may simply be able to locate overlapping calls more easily. It is possible

that call overlap is associated with dominance or other quality indicators of the callers; thus, females may approach overlappers more often because they represent higher quality mates. My results indicate that acoustic signals can reflect the qualities of the males and that female Little Penguins may prefer larger males to enhance their reproductive potential.

# Acknowledgements

I wish to thank my supervisor Joseph R. Waas for providing me with the opportunity to pursue this work in New Zealand and for his restless encouragement. Let me also acknowledge my co-supervisors, Ian Hogg and Kim King. I am grateful to Pauline Reilly, John Darby, Peter Dann, Pierre Jouventin, Lloyd Davis, Terry Aubin, Tony Hocken, Dave Houston and the late Mike Cullen and their valuable comments on my study. To all those people, Shinichi Nakagawa, Dai Morgan, Laura Molles, discussed ideas and shared a wealth of knowledge with me. I thank Chris Challies for invaluable help during this study. I am grateful to Conrad Pilditch for making suggestions on the statistical analyses. I thank the staff of the Biological Sciences Department, particularly Dudley Bell, Chris Hill and Gavin Reynolds for their help. Toshiyuki Motoda, Yuko Mame Suzuki, Hayao Izuhara, Atsushi Watanabe and Mihoko Numata provided invaluable assistance for this study. I am particularly indebted to Noriko Aoyanagi and the late Masahiro Aoyanagi for their encouragement throughout my studies. Special thanks must go to Barbara and Ray Walter, and Dave Houston who helped me in the field. The research was supported by the Penguin Fund Japan and grants from the Department of Biological Sciences, University of Waikato. A permit to work was supplied by the Department of Conservation, New Zealand. The University of Waikato Animal Ethics Committee approved the experimental protocol. Finally, I wish to thank Gao Liangliang. I am thankful for her support and encouragement to finish this study.

# Table of Contents

Declaration	ii	
Abstract	iii	
Acknowledgements	v	
Table of Contents	vi	
List of Figures	viii	
List of Tables	ix	
<b>Chapter 1</b>	<b>General Introduction</b>	<b>1</b>
<b>Chapter 2</b>	<b>Correlations between body size, defensive behaviour and reproductive success in male Little Penguins <i>Eudyptula minor</i>: implications for female choice</b>	<b>14</b>
Abstract		15
Introduction		15
Methods		17
Results		20
Discussion		25
Acknowledgements		29
References		29
<b>Chapter 3</b>	<b>Influence of parental body size on nest site elevation and food provisioning in Little Penguins <i>Eudyptula minor</i></b>	<b>33</b>
Abstract		34
Introduction		34
Methods		35
Results		37
Discussion		39
Acknowledgements		43
References		43

<b>Chapter 4</b>	<b>Acoustic properties of male advertisement and their impact on female responsiveness in Little Penguins</b>	<b>47</b>
	<i>Eudyptula minor</i>	
	Abstract	48
	Introduction	48
	Methods	50
	Results	52
	Discussion	55
	Acknowledgements	57
	References	58
<b>Chapter 5</b>	<b>Effects of male call pitch on female behaviour and mate fidelity in Little Penguins</b>	<b>61</b>
	Abstract	62
	Introduction	62
	Methods	63
	Results	66
	Discussion	68
	Acknowledgements	71
	References	71
<b>Chapter 6</b>	<b>‘Last word’ effects of male advertising calls on female preference in Little Penguins</b>	<b>74</b>
	Abstract	75
	Introduction	75
	Methods	77
	Results	80
	Discussion	82
	Acknowledgements	85
	References	85
<b>Chapter 7</b>	<b>Conclusions</b>	<b>88</b>

# List of Figures

## Chapter 2

Figure 1. The timing of 1st egg laying, in relation to male head length.

Figure 2. Chick growth rate (g/day) in relation to male head length.

Figure 3. The relationship between peak frequency of growl and head length.

## Chapter 4

Figure 1. Sonagram of the advertising call of a male Little Penguin, *Eudyptula minor*, showing seven call properties that were analysed (DF = dominant frequency, HF = highest frequency, LF = lowest frequency and PD = phrase duration).

## Chapter 5

Figure 1. The number of females approaching playback of their own mate and a stranger (shaded, entering the burrow; open, occupying the 'approach area').

## Chapter 6

Figure 1. Example of call overlap on the playback tapes.

# List of Tables

## Chapter 2

Table 1. Principal component analysis of five morphometric characters of males and females, and a sexual dimorphism index.

## Chapter 3

Table 1. Body size of birds nesting at low, middle and high sites (mean  $\pm$  SD,  $N = 11$  per elevation).

## Chapter 4

Table 1. Comparison of inter- and intra-individual coefficients of variation (CV), along with the corresponding CV ratios.

Table 2. Pearson correlation coefficients between seven call properties and overall body size (PC1) and body mass ( $N = 12$ ).

Table 3. Responses of 8 females that vocalised during the playback of Low, Medium and High calls.

## Chapter 5

Table 1. Average location scores and the likelihood of being within approach areas (%) associated with Low and High Calls. The 95 % confidence interval (CI) is also shown.

## Chapter 6

Table 1. The mean  $\pm$  SD number of birds found within each treatment arena.

# Chapter 1

## General Introduction

This Ph.D study is concerned with relationships among male size, reproductive success, female choice and male advertising calls in Little Penguins (*Eudyptula minor*). The principal aim of this General Introduction is to provide a background to some of the factors that may influence female choice. Male body size and acoustic signals are examined in detail as traits that females may be attentive to. This chapter finishes with a summary of Little Penguin ecology and behaviour, followed by the aims and the structure of the thesis.

### Mate choice

Mate choice has grown into a major subdiscipline of behavioural and evolutionary ecology. Darwin (1871) believed that male ornaments and male weapons evolve through sexual selection by female choice and male-male contests over females. Fisher (1930) elaborated sexual selection theory and suggested that a sexual preference of a certain kind may lead to a reproductive advantage. In recent studies, there is much evidence that females actively select their mates, and that female choice favours conspicuous male traits (reviewed by Andersson 1994). When a female selects a mate, she may use a number of biological cues to assess mate quality. There are many different kinds of benefit that individuals may obtain by selecting certain mates over others. Aspects of male behaviour (or other characteristics) that provide information on age, parental care, reproductive experience and body size may be particularly important to choosing females because these traits may have a significant effect on female fitness (Andersson 1994). In many empirical studies of sexual selection, male calls and male size are the most commonly investigated traits in a variety of species such as insects, fishes, amphibians, birds and mammals (probably because these traits can often be readily quantified). In particular, calls in many cases are easier to manipulate and synthesize than other traits like visual or chemical signals (reviewed by Andersson 1994).

### *Body size*

Body size is an important trait during male-male competition and in the context of female choice because body size can be strongly correlated with physiological and fitness characters (Peters 1983). The influence of male body size on reproductive success has been thoroughly investigated in a wide range of taxa. Male body size is positively correlated with reproductive success (e.g. Ewing 1961, Borgia 1981, Kraak *et al.* 1999). Large males may be stronger and therefore more successful during male-male competitions (e.g. Arak 1983, Rowland 1989), provide better parental care (e.g. Brown 1981, Côte & Hunte 1989), or acquire food more efficiently (e.g. Ryan 1980, Wiklund & Kaitala 1995). For example, diving ability in seabirds has been shown to vary allometrically with body size (Costa 1991). Thus, smaller birds may have difficulty taking less accessible prey in deep water or may have more difficulty obtaining high quality prey as a result of poorer plunging capabilities (Barbraud *et al.* 1999). As a consequence, this ability may also affect chick growth and survival. Thus, male size may be an important trait in female choice (e.g. Andersson 1994, Andersson & Iwasa 1996).

Body size is highly heritable and positively correlated between parents and their young (Price & Grant 1984, Simmons 1987, Barbraud *et al.* 1999). Because larger males may grow faster than smaller ones, rapid growth may be passed on to offspring (Arak 1983). Females may obtain the benefits of enhanced growth rates for offspring by mating with a large male.

In seabirds, parental body size may be positively related to the meal size fed to chicks (e.g. Chinstrap Penguins, *Pygoscelis antarctica*, de León *et al.* 1998; Snow Petrels, *Pagodroma nivea*, Barbraud *et al.* 1999). de León *et al.* (1998) suggested that larger birds can carry larger amounts of food in a foraging trip either because of foraging efficiency or a larger stomach capacity.

Penguins are sexually dimorphic in body size. Males are generally heavier and larger than females (Agnew & Kerry 1995). Larger males in Adélie Penguins (*Pygoscelis adeliae*) have advantages in male-male competition during territorial interactions (Ainley & Emison 1972) and can fast longer than smaller males during the incubation period (Davis & Speirs 1990). Larger male Adélie Penguins start breeding earlier than smaller ones, suggesting that females select larger males (Davis & Speirs 1990).

## *Acoustic signals*

### Call properties

Female choice based on differences in the parameters of male calls has been investigated in many species (e.g. insects, anurans, birds). Acoustic signals can provide a good estimate of body size in anurans; larger males usually produce lower-pitched calls (Ryan 1980, Robertson 1986, Márquez 1995). Females may prefer low-pitched calls (which suggest large male size), because larger size in anurans can lead to greater fertilization success and rapid growth in offspring (Arak 1983). In birds, negative correlations between song-pitch and the size of the birds also occur (Wallschläger 1980, Ryan & Brenowitz 1985, Tubaro & Mahler 1998, Barbraud *et al.* 2000). Davis and Speirs (1990) found that lower-pitched calls of male Adélie Penguins reflect the large body size of the caller and may be selected preferentially by females.

In some species of seabirds, body condition or body size at the time of pair formation may reflect information on foraging abilities, so body size appears to be an important criterion of mate choice (Bried & Jouventin 2001). Pair formation in burrow- and crevice-dwelling seabirds such as petrels and shearwaters is generally based on vocalisations (Nelson & Baird 2001). In nocturnal seabirds, in particular, information about males available to females is thus contained in acoustic signals (Genevois & Bretagnolle 1994). For example, Genevois and Bretagnolle (1994) found correlations between body size and some acoustic parameters in Blue Petrels (*Halobaena caerulea*). Female Blue Petrels may use male calls as an indicator of the caller's size and quality; therefore, male calls may be used as an important criterion in mate choice.

### Call timing

Animal communication is an interactive process; a signal leads to a response by the receiver and the response may affect the behaviour of the signaller. Although there are some acoustic signals that do not depend on interactions (e.g. those associated with species and individual recognition), interaction is regarded as one of the important aspects of signalling (McGregor *et al.* 1992). Ficken *et al.* (1974) suggested that the timing of bird song may be altered to avoid the 'jamming' of signals.

Recent studies using interactive playback experiments have shown that singing interactions such as overlapping may have signal value and can reveal differences in social status or quality between the interacting signallers (Hultsch & Todt 1982, Dabelsteen *et al.* 1996, 1997). Previous overlapping experiments using territorial songbirds demonstrated that overlapping songs may indicate aggressive motivation and a willingness to escalate territorial contests. For example, male Robins (*Erithacus rubecula*) responded more intensity to overlapping songs than alternating songs (Dabelsteen *et al.* 1997). Dabelsteen *et al.* (1997) suggested that overlapping songs may signal a dominant status, and a range of listeners may collect information from such contests by eavesdropping. Naguib and Todt (1997) conducted overlapping playback experiments using Nightingales (*Luscinia megarhynchos*) and suggested that overlapping songs could attract females. Signallers who get overlapped by other birds may become less attractive to females because information in an overlapped song that is important for female choice may be masked or obscured (Naguib & Todt 1997). Overlapping songs may signal dominant status and dominant males may be higher quality partners (Naguib & Todt 1997). Thus, dominant males during call interactions may be preferred by females.

## **Little Penguin description**

### *Taxonomy and names*

Little Penguins are the only species in the genus *Eudyptula*. Other names include Little Blue Penguins, Blue Penguins and Fairy Penguins. I refer to the species as Little Penguins throughout this thesis because this name is used most commonly in international journals and recent books (Marchant & Higgins 1990, Reilly 1994, Williams 1995).

### *Description and habitat*

Little Penguins are the smallest of all penguins at 40 cm long and weight about 1 kg. The species breeds on the coastal mainland and offshore islands of southern Australia and New Zealand. The nests of Little Penguins are often found between boulders on rocky shores and in grasslands. Dense vegetation and caves are also frequently used. The research in this thesis was conducted on breeding colonies on

Tiritiri Matangi Island (4 km off Auckland, the North Island of New Zealand), Motunau Island (1.5 km off the east coast of the South Island's Banks Peninsula) and the Oamaru Blue Penguin Colony (the east coast of the South Island's Otago District).

### *Sexual dimorphism*

Agnew and Kerry (1995) discussed sexual dimorphism in penguins; males are generally larger than females in terms of bill size, body mass and flipper size (Agnew & Kerry 1995). According to their analyses, sexual dimorphism of bill depth is greatest in most species of penguins. The dimorphism of the bill depth in Rockhopper Penguins (*Eudyptes chrysolophus*) is the greatest of all body size parameters in their analyses. Little Penguins appeared to show the fifth greatest dimorphism in bill depth of the 15 penguin species (Agnew & Kerry 1995). Bill depth is the most significant dimension for identifying gender and developing sex discrimination formulas in Little Penguins (Kinsky & Falla 1976, Gales 1988, Renner & Davis 1999). Bill size may be related to feeding ability and intraspecific conflicts (e.g. they often use their bills in territorial behaviours to intruders; Waas 1990).

The development of sexual dimorphism is often attributed to contests over mates and female choice (Andersson 1994). Larger male Adélie Penguins have advantages in male-male competition during territorial interactions (Ainley & Emison 1972) and can fast longer than smaller ones during the incubation period (Davis & Speirs 1990). Larger male Adélie Penguins start breeding earlier than smaller ones, suggesting that females choose larger males as a partner first (Davis & Speirs 1990). If sexual dimorphism in penguins is facilitated by a female preference for larger males (and advantages associated with choosing large males), it should be male size, not female size, that accounts for most size-success correlations.

### *Pair bond*

Little Penguins are monogamous. Mate fidelity is strong. The average rate of divorce has been reported as 17 % (Reilly & Cullen 1981). About 50 % of two-year-old birds attempt to breed and most birds attempt to breed by three years of

age (Dann & Cullen 1990). Little Penguins breed more successfully as they age (Dann & Cullen 1990). Little Penguins can live for a considerable time, some penguins surviving to the age of 24 years (Dr. C. N. Challies, *pers. comm.*). Their average life expectancy is approximately 6.5 years (Stahel & Gales 1987). There is no difference in mortality between males and females (Stahel & Gales 1987).

### *Breeding cycle*

The start of the breeding season is variable between regions. It begins in late June or early July on the North Island of New Zealand. Males usually obtain a nest site first, and then pair with a female (Reilly 1994). Unmated females may keep company with a number of males before finally deciding on a mate (Waas 1991). The breeding biology of Little Penguins was studied by Kinsky (1960). They normally lay two egg clutches. Both parents share parental duties about equally and alternate to incubate eggs for 33 – 43 days. The incubation shift is normally 1 – 2 days. After hatching, parents guard chicks for three weeks. During the first 10 – 14 days of this stage, one parent returns to the nest at night and feeds the offspring. Parents change roles regularly every evening. After the chick guard stage, both parents leave the chicks in the nest during the day while they forage at sea to obtain food for themselves and their offspring. Chick development depends on the capacity of the adults to supply food to their growing offspring. Their diet varies but usually consists of small schooling fish, cephalopods and crustaceans (Montague & Cullen 1988). Fledging can take approximately eight weeks (Reilly & Balmford 1975). Fledging success varies between different populations and different years (i.e. depending on food availability, sea surface temperature etc.) (Reilly 1994). Parental age and reproductive experience (e.g. the individual's foraging abilities) can also affect breeding success (Dann & Cullen 1990).

### *Vocal communication*

Vocalisations in Little Penguins function as signals to attract mates, maintain pair-bonds and defend or obtain territories (Stahel & Gales 1987). Little Penguins are wholly nocturnal on land, arriving on shore after dusk. They do not have ornamentation around the head like other species of penguins. Therefore, their vocal signals may be very important during interactions such as male-male

competition and mate choice. Their vocalisations are separated into three main types: (1) contact calls, (2) agonistic calls, and (3) sexual calls (Jouventin 1982). Contact calls are mainly used to advertise an individual's location both on land and at sea (Jouventin 1982). 'Growl' is a common vocalisation produced as the bird exhales; this grades into low bray, medium bray and full bray (Waas 1990). Each call is normally composed of 3 – 10 exhalation/inhalation sets. These bray calls are used in agonistic contexts such as defending territories and may indicate a willingness to escalate conflicts (Waas 1991). The mutual display between partners is performed as a greeting ceremony when a partner returns to the nest from the sea (sexual mutual display) (Stahel & Gales 1987); such displays facilitate vocal responses from other birds living nearby (territorial mutual display) (Stahel & Gales 1987, Waas 1988). Advertising calls are similar to full brays, but the phrase length of inhalation and exhalation is longer than most bray calls. Solitary males use advertising calls to attract females during the early stages of breeding (Waas 1988). Males normally use this sexual call in the nesting areas and on the shorelines. This call may present information on the quality of the caller. Males of many species may perceive advertising signals of other males as an indicator of female presence (Farr 1976, Waas 1988). Playback of male Little Penguin advertising calls can facilitate advertising calls of other males which are attempting to attract a potential partner to their area (Waas 1988).

### **Aim of thesis**

In this thesis, I have investigated the role of male acoustic features in the process of mate choice in Little Penguins. Little Penguins may rely considerably on acoustic signals to communication due to their nocturnal habits on their breeding grounds. I predicted that male call features and calling behaviour may reflect valuable information on male attributes that could provide important information during the process of female choice. I provide direct evidence for female call preference for larger males by conducting several playback experiments. I also demonstrate that a relationship exists between male size and male call features, and reveal the importance of male size in reproductive success.

## **Structure of thesis**

This thesis represents work commenced in August 1999 under the supervision of Dr. Joseph R. Waas, Dr. Ian D. Hogg and Dr. Carolyn M. King. Most of the research chapters have been accepted by or submitted to various international journals. As a result, some background information may be repeated in more than one chapter. The main body of the thesis is composed of five research chapters. The first two research chapters deal primarily with ecological issues relating to the influence of male size on reproduction; the importance of male size on female success is also presented. In the third research chapter, the relationship between male size and the parameters of their sexual calls is examined. A correlation between size and call pitch was found. The last two research chapters show how male calls affect female choice. It is suggested that male calls may be an important cue for female choice in Little Penguins. The specific research chapters in this thesis are as follows:

**Chapter 2**, *Correlations between body size, defensive behaviour and reproductive success in male Little Penguins (Eudyptula minor): implications for female choice*, examined how male size affected reproductive parameters. Because larger males were shown to have chicks with faster growth rates and showed more intense defensive behaviour, females may prefer larger males as breeding partners to improve their own reproductive potential.

**Chapter 3**, *Influence of parental body size on nest site elevation and food provisioning in Little Penguins (Eudyptula minor)*, reported how parental body size was related to nest site elevation and chick food provisioning in Little Penguins. In Chapter 2, I found a difference in chick growth rates between larger and smaller males. However, the reason why chicks of larger males grew faster was not considered. This question was dealt with in Chapter 3. In addition, a difference in nest site elevation between males of various sizes was found. Large males occupied better breeding sites.

**Chapter 4**, *Acoustic properties of male advertisement and their impact on female responsiveness in Little Penguins (Eudyptula minor)*, described the relationship

between the acoustic properties of male advertisement and male size. There was a negative correlation between male size and some properties of their sexual calls. I suggested that male calls, therefore, could reveal information on the size of the caller. Furthermore, I conducted playback experiments to determine whether females discriminate between male vocalisations on the basis of the call pitch. Most females did not respond to the playbacks vocally. The vocal minority responded vocally to low and medium calls. The importance of vocal cues for female choice in Little Penguins was discussed in detail.

**Chapter 5, *Effects of male call pitch on female behaviour and mate fidelity in Little Penguins***, examined how females responded to male calls of various call pitches. I conducted two speaker choice tests to examine female behaviour to stranger males and their current mates. Females responded vocally only to low-pitched calls. However, there was no difference in the number of approaching females between low- and high-pitched calls. However, in the experiment broadcasting current mate calls and stranger calls, most females approached the calls of their current partners, regardless of the call pitch of the stranger playbacks.

**Chapter 6, *'Last word' effects of male advertising calls on female preference in Little Penguins***, examined how advertising call overlap between males influenced other birds. Call overlap can cause masking effects and influence the behaviour of the listeners. I found overlapping calls were more likely to draw females to speakers in two-choice tests. The reason why receivers responded in different ways to playbacks was discussed.

These chapters represent papers co-authored with my primary supervisor, Dr. Joseph R. Waas. I performed most of the data collection, data analyses and writing reported within these papers. The fieldwork reported in Chapter 6 was carried out by Dr. Joseph R. Waas and myself.

## REFERENCES

- Agnew, D.J. & Kerry, K.R. 1995. Sexual dimorphism in penguins. In Dann, P., Norman, F. I. & Reilly P. (eds) *The Penguins: Ecology and Management*: 299-318. Sydney: Surrey Beatty & Sons.
- Ainley, D.G. & Emison, W.B. 1972. Sexual size dimorphism in Adélie Penguins. *Ibis* 114: 267-271.
- Andersson, M.B. 1994. *Sexual Selection*. Princeton: Princeton University Press.
- Andersson, M.B. & Iwasa, Y. 1996. Sexual selection. *Trends Ecol. Evol.* 11: 53-58.
- Arak, A. 1983. Sex differences in choosiness: Male-male competition and mate choice in anuran amphibians. In Bateson, P. (ed.) *Mate Choice*: 181-210. Cambridge: Cambridge University Press.
- Barbraud, C., Weimerskirch, H., Robertson, G. & Jouventin, P. 1999. Size-related life history traits: insights from a study of Snow Petrels (*Pagodroma nivea*). *J. Anim. Ecol.* 68: 1179-1192.
- Barbraud, C., Mariani, A. & Jouventin, P. 2000. Variation in call properties of the Snow Petrel, *Pagodroma nivea*, in relation to sex and body size. *Aust. J. Zool.* 48: 421-430.
- Borgia, G. 1981. Mate selection in the fly *Scatophaga stercoraria*: female choice in a male-controlled system. *Anim. Behav.* 29: 71-80.
- Bried, J. & Jouventin, P. 2001. Site and mate choice in seabirds: an evolutionary approach. In Schreiber, E.A. & Burger, J. (eds) *Biology of Marine Birds*: 263-305. Boca Raton: CRC Press.
- Brown, J. 1981. Patterns of female choice in Mottled Sculpins (*Cottidae*, *Teleostei*). *Anim. Behav.* 29: 373-382.
- Costa, D.P. 1991. Reproductive and foraging energetics of high latitude penguins, albatrosses and pinnipeds: implication for life-history patterns. *Am. Zool.* 31: 111-130.
- Côte, I.M. & Hunte, W. 1989. Male and female mate choice in the Redlip Blenny: why bigger is better. *Anim. Behav.* 38: 78-88.

- Dabelsteen, T., McGregor, P.K., Shepherd, M., Whittaker, X. & Pedersen, S.B. 1996. Is the signal value of overlapping different from that of alternating during matched singing in Great Tits? *J. Avian Biol.* 27: 189-194.
- Dabelsteen, T., McGregor, P.K., Holland, J., Tobias, J.A. & Pedersen, S.B. 1997. The signal function of overlapping singing in male Robins. *Anim. Behav.* 53: 249-256.
- Dann, P. & Cullen, J.M. 1990. Survival, patterns of reproduction and lifetime reproductive output of Little Penguins *Eudyptula minor* on Phillip Island, Victoria, Australia. In Davis, L.S. & Darby, J.T. (eds) *Penguin Biology*: 63-84. San Diego: Academic Press.
- Darwin, C. 1871. *The Descent of Man, and Selection in Relation to Sex*. London: John Murray.
- Davis, L.S. & Speirs, E.A.H. 1990. Mate choice in penguins. In Davis, L.S. & Darby, J.T. (eds) *Penguin Biology*: 377-397. San Diego: Academic Press.
- Ewing, A.W. 1961. Body size and courtship behaviour in *Drosophila melanogaster*. *Anim. Behav.* 9: 93-99.
- Farr, J.A. 1976. Social facilitation of male sexual behaviour, intrasexual competition, and sexual selection in the guppy, *Poecilia reticulata* (Pisces: Poeciliidae). *Evolution* 30: 707-717.
- Ficken, R.W., Ficken, M.S. & Hailman, J.P. 1974. Temporal pattern shifts to avoid interference in singing birds. *Science* 183: 762-763.
- Fisher, R.A. 1930. *The Genetical Theory of Natural Selection*. Oxford: Clarendon Press.
- Gales, R. 1988. Sexing adult Blue Penguins by external measurements. *Notornis* 35: 71-75.
- Genevois, F. & Bretagnolle, V. 1994. Male Blue Petrels reveal their body mass when calling. *Ethol. Ecol. Evol.* 6: 377-383.
- Hultsch, H. & Todt, D. 1982. Temporal performance roles during vocal interactions in Nightingales (*Luscinia megarhynchos*). *Behav. Ecol. Sociobiol.* 11: 253-260.
- Jouventin, P. 1982. *Visual and Vocal Signals in Penguins, Their Evolution and Adaptive Characters*. Berlin & Hamburg: Verlag Paul Parey.

- Kinsky, F.C. 1960. The yearly cycle of the Northern Blue Penguin (*Eudyptula minor novaehollandiae*) in the Wellington Harbour area. *Rec. Dom. Mus. Wellington* 3: 145-218.
- Kinsky, F.C. & Falla, R.A. 1976. A subspecific revision of the Australasian Blue Penguin (*Eudyptula minor*) in the New Zealand area. *Rec. Nat. Mus. NZ.* 1: 105-126.
- Kraak, S.B.M., Bakker, T.C.M. & Mundwiler, B. 1999. Sexual selection in Sticklebacks in the field: correlates of reproductive, mating, and paternal success. *Behav. Ecol.* 10: 696-706.
- de León, A., Fargallo, J.A. & Moreno, J. 1998. Parental body size affects meal size in the Chinstrap Penguin (*Pygoscelis antarctica*). *Polar Biol.* 19: 358 -360.
- McGregor, P.K., Dabelsteen, T., Shephard, M. & Pedersen, S.B. 1992. The signal value of matched singing in Great Tits: evidence from interactive playback experiments. *Anim. Behav.* 43: 987-998.
- Marchant, S. & Higgins, P.J. 1990. *Handbook of Australian, New Zealand and Antarctic Birds. Vol.1A.* Melbourne: Oxford University Press.
- Márquez, R. 1995. Female choice in the Midwife Toads (*Alytes obstetricans* and *A. cisternasii*). *Behaviour* 132: 151-161.
- Montague, T.L. & Cullen, M. 1988. The diet of the Little Penguin *Eudyptula minor* at Phillip Island, Victoria. *Emu* 88: 138-149.
- Naguib, M. & Todt, D. 1997. Effects of dyadic vocal interactions on other conspecific receivers in Nightingales. *Anim. Behav.* 54: 1535-1543.
- Nelson, J.B. & Baird, P.H. 2001. Seabird communication and displays. In Schreiber, E.A. & Burger, J. (eds) *Biology of Marine Birds*: 307-357. Boca Raton: CRC Press.
- Peters, R.H. 1983. *The Ecological Implications of Body Size.* Cambridge: Cambridge University Press.
- Price, T.D. & Grant, P.R. 1984. Life history traits and natural selection for small body size in a population of Darwin's Finches. *Evolution* 38: 483-494.
- Reilly, P.N. 1994. *Penguins of the World.* Australia: Oxford University Press.
- Reilly, P.N. & Balmford, P. 1975. A breeding study of the Little Penguin *Eudyptula minor* in Australia. In Stonehouse, B. (ed) *The Biology of Penguins*: 161-187. London: Macmillan.

- Reilly P.N. & Cullen, J.M. 1981. The Little Penguin *Eudyptula minor* in Victoria, II. Breeding. *Emu* 81: 1-19.
- Renner, M. & Davis, L.S. 1999. Sexing Little Penguins (*Eudyptula minor*) from Cook Strait, New Zealand, using discriminant function analysis. *Emu* 99: 74-79.
- Robertson, J.G.M. 1986. Male territoriality, fighting and assessment of fighting ability in the Australian Frog *Uperoleia rugosa*. *Anim. Behav.* 34: 763-772.
- Rowland, W.J. 1989. The effects of body-size, aggression and nuptial coloration on competition for territories in male Threespine Sticklebacks, *Gasterosteus aculeatus*. *Anim. Behav.* 37: 282-289.
- Ryan, M.J. 1980. Female mate choice in a neotropical frog. *Science* 209: 523-525.
- Ryan, M.J. & Brenowitz, E.A. 1985. The role of body size, phylogeny, and ambient noise in the evolution of bird song. *Am. Nat.* 126: 87-100.
- Simmins, L.W. 1987. Heritability of a male character chosen by females of the Field Cricket, *Gryllus bimaculatus*. *Behav. Ecol. Sociobiol.* 21: 129-133.
- Stahel, C. & Gales, R. 1987. *Little Penguins: Fairy Penguins in Australia*. Kensington, New South Wales: New South Wales University Press.
- Tubaro, P.L. & Mahler, B. 1998. Acoustic frequencies and body mass in New World Doves. *Condor* 100: 54-61.
- Waas, J.R. 1988. Acoustic displays facilitate courtship in Little Blue Penguins, *Eudyptula minor*. *Anim. Behav.* 36: 366-371.
- Waas, J.R. 1990. Intraspecific variation in social repertoires: evidence from cave- and burrow-dwelling Little Blue Penguins. *Behaviour* 115: 63-98.
- Waas, J.R. 1991. Do Little Blue Penguins signal their intentions during aggressive interactions with strangers? *Anim. Behav.* 41: 375-382.
- Wallschläger, D. 1980. Correlation of song frequency and body weight in passerine birds. *Experientia* 36: 412.
- Wiklund, C. & Kaitala, A. 1995. Sexual selection for large male size in a polyandrous butterfly: the effect of body size on male versus female reproductive success in *Pieris napi*. *Behav. Ecol.* 6: 6-13.
- Williams, T.D. 1995. *The Penguins*, Spheniscidae. Oxford: Oxford University Press.

## **Chapter 2**

**Correlations between body size, defensive behaviour and reproductive success in male Little Penguins *Eudyptula minor*: implications for female choice**

## ABSTRACT

Field studies exploring relationships among body size, defensive behaviour (including vocalisations) and reproductive success in male Little Penguins (*Eudyptula minor*) were conducted to identify behavioural and physical attributes that might provide females or opponents with information on male quality. Head length provided a simple estimator of body size in both sexes. Nests defended by larger males produced eggs and chicks earlier in the breeding season, and contained chicks that grew more quickly than chicks in nests defended by smaller males. However, there were no differences in fledging parameters (mass, age and success) between larger and smaller males. In contrast, female body size did not affect any of the reproductive parameters we measured. The pitch of male defensive calls varied significantly with male size, so females (or opponents) could potentially gauge male size by assessing the calls. Large males were also more likely to respond vocally to simulated nest intrusions. As a result, by 'keeping company' with prospective partners early in the breeding season, females can obtain information about males that would allow them to select better partners and improve their own reproductive potential.

## INTRODUCTION

Theoretical models of sexual selection have focused on the evolution of female mate choice (Andersson 1994). In particular, the relationship between male body size and female preference has been most thoroughly investigated, because male body size can show strong positive correlations with reproductive success in a wide range of species (e.g. Ewing 1961, Borgia 1981, Kraak *et al.* 1999). Large males are more successful during male-male competitions (e.g. Arak 1983, Rowland 1989), court more frequently (e.g. Partridge *et al.* 1987, Abell *et al.* 1999), and acquire food more efficiently (Ryan 1980, Wiklund & Kaitala 1995) possibly making them superior parents (e.g. Downhower & Brown 1980, Côte & Hunte 1989). Thus, body size should be an important component of female mate choice.

Davis and Speirs (1990) suggested that female Adélie Penguins (*Pygoscelis*

*adeliae*) may prefer larger mates. Penguins are sexually dimorphic in body size, with males being slightly larger than females (Agnew & Kerry 1995, Williams 1995). Larger than average male Adélie Penguins were found to breed earlier than smaller ones, suggesting that larger males were preferred by females (Davis & Speirs 1990). Penguins normally have three pre-breeding stages: courtship, a pre-laying exodus and a pre-laying stage. In Adélie Penguins, males then fast for around 10 days immediately following egg-laying, to incubate the eggs while females replenish their fat reserves at sea. Davis and Speirs (1990) argue that the main advantage of selecting large males is that 'fat birds can fast longer', thus reducing the chances of nest desertion and the costs of re-nesting. However, in many penguins, the post-laying fast is only 1 – 2 days long (e.g. Gentoo Penguins *Pygoscelis papua*, Trivelpiece *et al.* 1983, Jackass Penguins *Spheniscus demersus*, Williams & Cooper 1984); temperate and tropical penguins also tend to have shorter incubation shifts than Adélie Penguins (Williams 1995). As a result, 'fast endurance' may not always be the key factor promoting sexual dimorphism or a female preference for large males. On the other hand, size could contribute to the success of penguins in a number of other ways (e.g. male-male competition, feeding ability). However, the ways in which male size might influence the reproductive success of female penguins have not yet been explored in any detail.

The burrow-dwelling Little Penguin (*Eudyptula minor*) is an excellent subject for investigating links between male attributes and female reproductive success for several reasons: (1) Early in the breeding season, unmated females 'keep company' with different males in burrows (Waas 1991a), allowing them to obtain accurate information on mate size and other characteristics. (2) Because the males play a lead role in instigating most courtship activities including copulation (Waas 1988), they may have a major influence on the timing of reproduction. (3) Once paired, the males have a very short incubation fast (2 days, Stahel & Gales 1987, 3 – 4 days, Chiaradia & Kerry 1999), so the risk of nest desertion is unlikely to be the main factor promoting a female preference for large males. (4) Males are more aggressive than females, and have evolved a wide range of well-defined defensive activities to protect the breeding burrow (Waas 1991b), so the male's role in nest defence can be quantified easily.

Here we examine whether the size of male Little Penguins influences nest

defence, the timing of reproduction and the growth rates of their chicks. It could be expected that, if sexual dimorphism in penguins is 'driven' by a female preference for large males, it should be male size, not female size, that accounts for most size-success correlations (although female size might increase as an artifact of selection for large size in males). To examine these ideas, we quantified the contribution that male and female size made to the measures of reproductive success that we recorded.

Our results indicate that the size of males, but not females, was correlated with defensive activities, the timing of breeding and chick growth rates of Little Penguins. Thus, we provide evidence that female Little Penguins can improve their own reproductive potential by selecting larger males as breeding partners.

## **METHODS**

### **Study area and period**

The study was conducted on a population of Little Penguins on Tiritiri Matangi Island (36°36'S, 174°53'E), 4 km off Auckland, New Zealand, during the 1999 breeding season (August – December). At night, we walked along the shoreline and main pathways around the island and found nests by listening for sexual and aggressive calls (see Waas 1988 and 1990 for descriptions of calls). The Little Penguins nested mainly in grasslands, where soil depth allows burrowing, and amongst boulders on the rocky shores around the island.

### **Measurements**

Forty-one breeding pairs, spaced sporadically along approximately 1 km of shoreline, were investigated. To assess body size, we measured bill length, bill depth and head length with digital calipers (following Jones 1978). Head length was measured from the tip of the bill to the back of the skull. Flipper and foot sizes were measured by tracing the outspread appendage on paper and later measuring them to the nearest 1 mm. Flipper size was estimated by measuring from the elbow to the tip of the outstretched flipper. Foot size was estimated by measuring the distance between the base of the first digit and the tip of the claw of the third digit. Because body weights were extremely variable (i.e. they could

vary dramatically before and after feeding trips) during the breeding season, weight was not used to estimate body size.

For each pair's nest, the date of egg laying, chick hatching, chick deaths and chick fledging were recorded (nest contents were checked every day). Chicks from each pair's nest were placed in a cloth bag of known mass and weighed every 5 days with a 500-g or 1.5-kg Pesola scale from hatching to fledging. If chicks were not located for 10 days in succession, they were assumed to have died. All observed chick deaths occurred within 18 days of hatching. Only chicks reaching 50 days of age were considered to have fledged (Williams 1995).

The recordings that were used for nest defense experiments (see below) were made with a Digital Audio Tape-corder (DAT, model TCD-D7, Sony Corporation) and an AKG shotgun microphone (head, model C460B; body, model CK68-ULS). During the pairing period, five good low to medium bray calls were recorded from five different males that were not otherwise used in the study. After removing a male and female from their nest, the male was released into the nest. In this context, most males produced a low or medium bray call. Once satisfactory recordings of the male were obtained, the female was also released into the nest.

### **Sex determination**

We used Gales' (1988) formula for determining the sex of Little Penguins by bill measurements. The formula sexes the adults with 94 % accuracy (Gales 1988). We studied only breeding pairs in nest burrows.

### **Body size**

A Principal Component Analysis (PCA) was used to identify the best parameter (chosen from head length, bill length, bill depth, foot size and flipper size) to use in body size estimates. The parameter explaining most variation in Principal Component 1 (PC1) was selected for analyses (see Manly 1986 for a description of PCA).

### **Design of nest defense experiment**

Little Penguins call most frequently after dusk and before sunrise (Waas 1988). To examine the relationship between a given male's response to intruders and its

body size, we conducted the following experiment. During the daytime, we scouted for nests that contained only a single male. These nests were then visited again during the night. All playback experiments at the 41 nests that we sampled were carried out on calm evenings between 1800 and 2000 h, and between 0500 and 0700 h. A speaker (Sony, model SRS-77G) was used with a cassette player (Aiwa, model HS-SP550) for playback (the DAT recordings described above were transferred to cassette tapes using an Aiwa cassette recorder, model HS-JX970). The speaker was placed approximately 1 m in front of the nest burrow entrance; the bray call of a single male was then broadcast at a level equivalent to the amplitude of a displaying bird. Each bray call had three consecutive exhalation-inhalation phrases (see Waas 1990). After playing the bray call of the simulated male intruder, we remained still and recorded any vocal responses for 30 s (any sounds were recorded to DAT). After the 30 s silent period, we played back the same bray call again and then listened for another 30 s. In total, three playback-silence sets were presented to each subject. After waiting a further 30 s, we moved a penguin model composed of blue clay moulded into the shape of a penguin's head to within 30 cm of the burrow occupant to simulate the approach of a stranger; the model remained there for 30 s. Any responses to the model were then recorded. Following the presentations, we rechecked the identity of the bird (to ensure that only the single male was present). We conducted a total of 111 experiments on the 41 males through the breeding season (each male was tested 1 – 6 times during the breeding season, but consecutive tests on a given male were always at least 2 days apart). By rotating which of the five intruder calls we used with each subject, we limited habituation to simulated intruders. We calculated the percentage of experiments in which each penguin performed each of three activities (remained silent, vocalised or hissed) in response to playback or the model presentation. To determine whether there was any relationship between male size and the response to the playback/model, we used a linear regression analysis.

Female Little Penguins show little in the way of defensive behaviour unless accompanied by their mate (Waas 1991a), so it was not possible to quantify their defensive behaviour independently.

## **Growl measurements**

We examined the calls that males produced in response to playback. 'Growl' is the most common vocalisation used by Little Penguins, in both defensive and sexual contexts. This low-pitched sound, produced as the bird exhales, is used both before and after pairs engage in mutual displays (Waas 1988). We analysed the growls of 26 male subjects by measuring: (1) the peak frequency (Hz); (2) the highest frequency (Hz); (3) phrase duration (s); and (4) the number of syllables per second. Growl responses were analysed by producing sonagrams with Canary 1.2.4 software on a Macintosh computer. A one-way ANOVA was conducted to obtain an  $F$  ratio for each call feature. By comparing the  $F$  ratios, we identified the call features that showed the greatest inter- versus intra-individual variation (Campbell 1989). After that, we looked at the relationship between the call features and male body size by using a linear regression analysis.

## **The relationship between body size and reproductive parameters**

Associations between the parameter that best estimated body size, and the reproductive parameters we measured (egg laying date, chick hatching date, chick growth rate and chick fledging mass and age), were examined by using a regression analysis. The chick growth pattern approximated a linear function against development time between the ages of 1 and 15 days ( $R > 0.95$ ,  $N = 25$ ,  $P < 0.05$  for all chicks), so individual growth rates were estimated by using a linear regression between these ages. Hatching and fledging success were each independently classified on a scale of 1 – 3 (1 = no hatching/no fledging chick, 2 = one hatching/one fledging chick, 3 = two hatching/two fledging chicks); success and the mean body size of parents (both males and females) were then compared. With respect to the breeding scores, the influence of mean body size or reproductive success was examined by using a one-way ANOVA.

## **RESULTS**

### **Body size**

The results of our PCA of body size measures for Little Penguins on Tiritiri Matangi Island are shown in Table 1. The variation in body size accounted for

over 53 % of the variance (Table 1). Head length was the most important PC1 parameter, and was therefore used as a quick and simple estimator of body size for both males and females. We went on to examine correlations between head length, reproductive parameters, and responses during the nest defense experiments. In general, Little Penguins show sexual dimorphism in a number of features, and males are slightly larger than females (Alexander & Nicholls 1918, Nicholls 1918, Richdale 1940, Reilly & Balmford 1975, Jones 1978). In all five morphometric characters measured, males were found to be larger than females (Table 1). There was no significant correlation between males and their partners in terms of the head length ( $R = 0.17$ ,  $N = 39$ ,  $P = 0.28$ ) or PC1 score ( $R = 0.04$ ,  $N = 37$ ,  $P = 0.83$ ).

### Breeding parameters

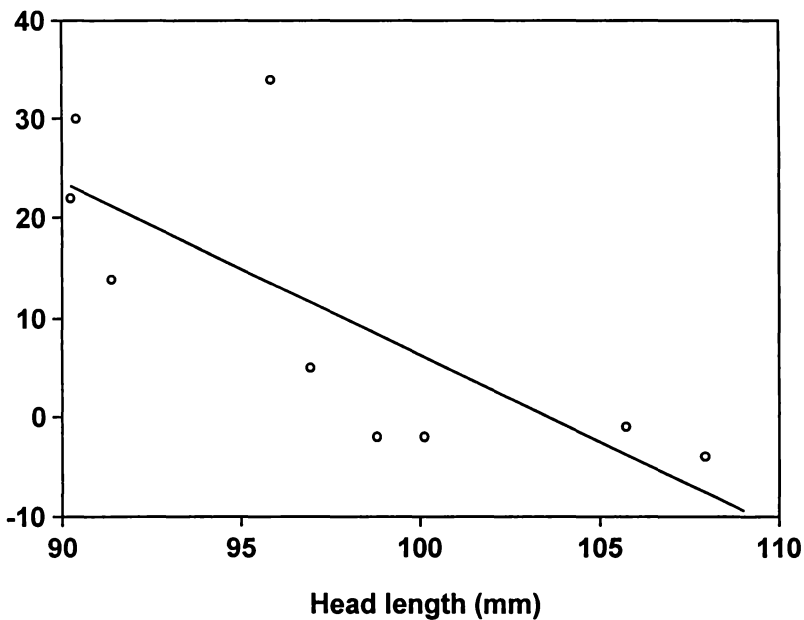
The relationship between body size (estimated by head length) and reproductive parameters was examined separately for males and females. The first eggs fathered by larger males appeared significantly earlier in the breeding season than those of smaller males (Fig. 1;  $R = 0.72$ ,  $N = 9$ ,  $P < 0.05$ ). The first chicks of larger males also hatched significantly earlier than those of smaller males ( $R = 0.46$ ,  $N = 20$ ,  $P < 0.05$ ). In birds that had two chicks, both the first and the second

**Table 1.** Principal component analysis of five morphometric characters of males and females, and a sexual dimorphism index. The percentage of explained variance is also shown ( $N = 74$ ).

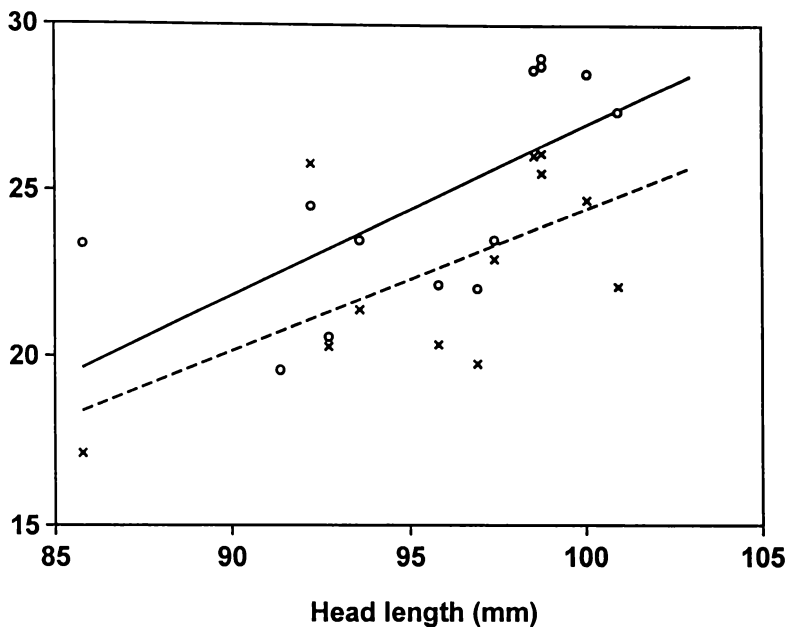
Variable	PC1	PC2	Dimorphism (male/female)
Head length	-0.527	-0.122	1.09
Bill length	-0.484	-0.143	1.08
Bill depth	-0.384	-0.668	1.14
Foot size	-0.432	0.320	1.04
Flipper size	-0.392	0.645	1.06
Percent of total variance	53.2	18.1	

chicks of larger males grew at a faster rate than those of smaller males (Fig. 2; first chick,  $R = 0.66$ ,  $N = 13$ ,  $P < 0.05$ ; second chick,  $R = 0.61$ ,  $N = 12$ ,  $P < 0.05$ ), although male size did not influence the mean fledging mass (first chick,  $R = 0.10$ ,  $N = 10$ ,  $P = 0.78$ ; second chick,  $R = 0.22$ ,  $N = 10$ ,  $P = 0.54$ ) or the mean fledging age (first chick,  $R = 0.36$ ,  $N = 10$ ,  $P = 0.31$ ; second chick,  $R = 0.56$ ,  $N = 10$ ,  $P = 0.10$ ). There were no significant differences in the mean body sizes of males in terms of the three levels of hatching or fledging success identified ( $F_{2, 33} = 0.36$ ,  $P = 0.70$  and  $F_{2, 33} = 0.27$ ,  $P = 0.76$ , respectively).

There were no significant correlations between female body size (estimated by head length) and any reproductive parameter (first egg laying date,  $R = 0.19$ ,  $N = 9$ ,  $P = 0.94$ ; first chick hatching date,  $R = 0.01$ ,  $N = 20$ ,  $P = 0.96$ ; chick fledging age, first chick,  $R = 0.12$ ,  $N = 10$ ,  $P = 0.73$ , and second chick,  $R = 0.19$ ,  $N = 10$ ,  $P = 0.59$ ; chick growth rate, first chick,  $R = 0.15$ ,  $N = 13$ ,  $P = 0.62$ , and second chick,  $R = 0.10$ ,  $N = 12$ ,  $P = 0.76$ ; chick fledging mass, first chick,  $R = 0.24$ ,  $N = 10$ ,  $P = 0.51$  and second chick,  $R = 0.05$ ,  $N = 10$ ,  $P = 0.89$ ; hatching success,  $F_{2, 33} = 2.24$ ,  $P = 0.12$ ; fledging success,  $F_{2, 33} = 2.31$ ,  $P = 0.12$ ).



**Figure 1.** The timing of 1st egg laying, in relation to male head length. The date of 1 September corresponds to the zero value on the y-axis.



**Figure 2.** Chick growth rate (g/day) in relation to male head length. The growth rates of first chicks are shown by circles while those of second chicks are shown by crosses. The solid line shows the regression line of the first chick's growth.

### Vocalisations

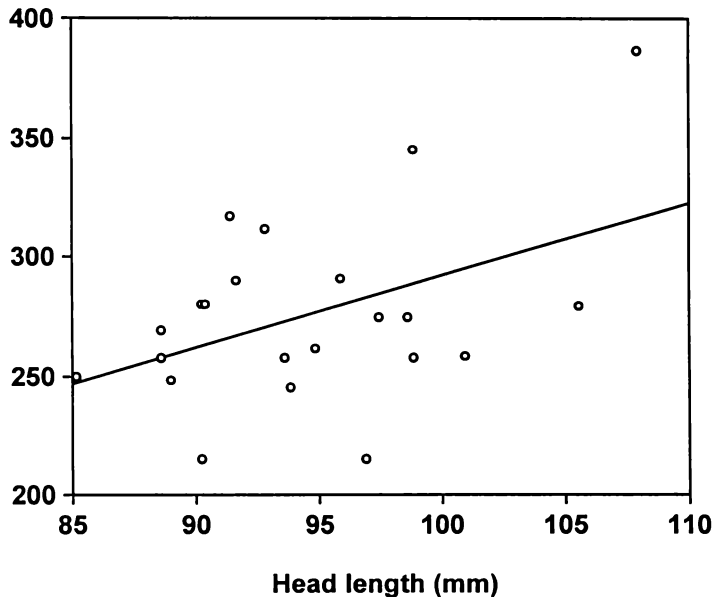
When playback was presented to males at their nests, they either remained silent, or vocalised (growled or brayed). Larger males were no more likely to vocalise in response to playback than smaller males ( $R = 0.04$ ,  $N = 38$ ,  $P = 0.81$ ). Response data for each male were also analysed separately with respect to the stage of the breeding cycle when the tests were conducted (incubation, guard and post-guard stage). In the post-guard stage (2 weeks after hatching), larger males were more likely to call in response to playback than smaller males ( $R = 0.70$ ,  $N = 14$ ,  $P < 0.01$ ), although there were no significant differences between size and response to playback during incubation ( $R = 0.05$ ,  $N = 32$ ,  $P = 0.77$ ) or the guard stage ( $R = 0.16$ ,  $N = 21$ ,  $P = 0.50$ ).

When a model was moved towards their burrow, males either remained silent, vocalised (growled or brayed) or hissed (a display where the performer lunges forward and expels a short burst of air; Waas 1990). There was no significant difference in response to the model stimulus between larger and smaller males ( $R = 0.04$ ,  $N = 38$ ,  $P = 0.80$ ). There were also no significant differences between larger and smaller males in response to the model with respect to the stage of the

breeding cycle (incubation,  $R = 0.05$ ,  $N = 32$ ,  $P = 0.80$ ; guard,  $R = 0.11$ ,  $N = 22$ ,  $P = 0.64$ ; post-guard,  $R = 0.20$ ,  $N = 16$ ,  $P = 0.46$ ).

Growls included frequencies up to 4620 Hz. The mean peak frequency was 148.6 Hz (range 10 – 290 Hz). An ANOVA conducted across the males sampled indicated that the only significant differences between individuals were in peak frequency ( $F_{6, 34} = 4.09$ ,  $P < 0.01$ ) and the number of syllables per second ( $F_{5, 30} = 2.63$ ,  $P < 0.05$ ). Differences between individuals in phrase duration ( $F_{6, 37} = 0.86$ ,  $P = 0.53$ ) and highest frequency ( $F_{6, 37} = 2.21$ ,  $P = 0.06$ ) were not significant.

Larger males growled at higher peak frequencies (Hz) than smaller males (Fig. 3;  $R = 0.44$ ,  $N = 22$ ,  $P < 0.05$ ). Differences between larger and smaller males were not apparent in the number of syllables per second ( $R = 0.07$ ,  $N = 18$ ,  $P = 0.78$ ).



**Figure 3.** The relationship between peak frequency of growl and head length.

We also analysed all the relationships mentioned above by using PC1 scores. All the trends in the relationship between male PC1 scores and reproductive parameters, responses to playback (and models) and vocalisations were the same as those identified between male head length and each parameter. There were significant differences in egg-laying date ( $R = 0.73$ ,  $N = 9$ ,  $P < 0.05$ ), in response

to playback in the post-guard stage ( $R = 0.61$ ,  $N = 13$ ,  $P < 0.05$ ) and in peak frequency ( $R = 0.67$ ,  $N = 21$ ,  $P < 0.001$ ) between larger and smaller males. No significant relationships between female PC1 scores and reproductive parameters could be detected.

## **DISCUSSION**

Our results show that, in Little Penguins, larger males bred earlier than smaller ones and that chicks of larger males grew more rapidly than those of smaller males. A female's body size, however, did not affect her reproductive success. Large males also used higher pitched aggressive calls and were more likely to vocalise in response to an intruder's call, but only during the post-guard stage. This vocal information could be used by opponents as well as females to gauge male attributes.

### **Nest defense behaviour**

The agonistic behaviour of Little Penguins has been investigated in detail by Waas (1988, 1990, 1991ab). He reported that several aggressive displays, including calls, are used when penguins are confronted by strangers, especially when both the male and the female are present in the burrow. A lone bird in the nest, which might have difficulty deterring an intruder, tended to remain silent and avoid advertising the presence of a burrow (Waas 1991a).

There was no significant difference in response to playback between larger and smaller males. However, when we examined the response with respect to each breeding stage (incubation, chick-guard and post-guard), larger males were found to have responded more often than smaller males in the post-guard stage. Aggressive displays may reveal information on the performer's willingness to escalate during aggressive encounters (Waas 1991a). Larger males in the post-guard stage appeared to be more willing to defend or protect their offspring than smaller males, presumably because the value of chicks peaks during the post-guard stage (just before fledging).

No differences between larger and smaller males were found in response to a penguin model. There can be serious costs associated with using aggressive

displays (Waas 1991a). In particular, a physical response to a potential stranger has higher risks or costs than responding vocally from within the burrow. We expected larger males to be more willing to bear the costs of escalating conflicts, but this was not the case. Perhaps there is a significant ‘positional’ advantage associated with occupying a burrow that leads both large and small males to adopt a ‘wait and see’ strategy. In our experiment, the model stimulus was presented to each subject for 30 s and then withdrawn. In this context, the intruder may have been seen by both large and small males as backing down, preventing further escalation (Waas 1991a).

The growls of Little Penguins are low-pitched pulsed sounds produced as the bird exhales. Individuals differed little in both the variation in the highest frequency and the length of calls they produced, so these features may not be useful for revealing individual-specific information. In contrast, the peak frequency and the number of syllables per second varied considerably between individuals (see also Jouventin 1982). However, there were no significant correlations between the number of syllables per second of the males’ calls and their body size. In playback experiments conducted by Jouventin (1982), the removal of the highest and lowest frequencies of calls had little effect on a Little Penguin’s response. The *F* ratios generated by our ANOVAs showed that the greatest inter- versus intra-individual variation was in the peak frequencies. Thus, peak frequencies might be expected to be useful in distinguishing between males.

The peak frequency of the male defensive ‘growl’ was positively correlated with male body size. When the PC1 score was used as a body size index instead of head length, the relationship became much stronger ( $R = 0.67$ ,  $P < 0.001$ ). Therefore, various body features that might have been better measured by the PC1 score (e.g. lung size, chest size) appeared more strongly associated with call features. ‘Growl’ is the most common aggressive vocalisation used by Little Penguins. Their calls are graded (from growl to low, medium and finally full brays; Waas 1990). The pitch of the calls (both within and between the four ‘levels’) increases with the intensity of the response. Thus, large males may have had higher pitched growls because they were responding more strongly to the standardized stimulus, perhaps indicating a greater willingness to escalate conflicts. We suggest that large males producing higher pitched growls may be

preferred by females because they may be better, or more willing, defenders of the nest site.

### **Timing of reproduction**

On Tiritiri Matangi Island, Little Penguins lay one clutch per year (Jones 1978). Large males fathered their first eggs and first chicks significantly earlier in the breeding season than did small males. This suggests that large males may have mated earlier than small males, assuming the pairing dates can be backdated from known egg laying or hatching dates using a mean incubation period of 35 days (Reilly & Balmford 1975). Female Adélie Penguins may choose males on the basis of characteristics that correlate with body size (Davis & Speirs 1990). Large male Adélie Penguins were thought to breed first because of a female preference for large males. Assuming that early egg laying and hatching dates are valid indicators of the mate preferences of females (Catchpole 1980, Searcy 1984, Davis & Speirs 1990), our findings suggest that mate choice by female Little Penguins could be influenced by male body size. Another reason why females may prefer large males is that the growth rates of the chicks may be improved.

### **Parental care**

The growth rate of the chicks of large males was higher than that of small males. Little Penguin parents take turns to feed and guard their chicks on alternate nights from immediately after hatching (the guard stage; Hursthouse 1939). Adult weight is at a minimum during the 2 – 3 week guard stage that follows hatching (Chiaradia & Kerry 1999), suggesting that adults may find it difficult to obtain enough food when they have to begin providing for both their chicks and themselves (a goal that can sometimes be achieved by feeding closer to the colony during the guard stage; Weavers 1992). Many birds can not cope with the sudden increase in demand, especially if food availability is a problem, so the mortality rate of chicks is highest during the early guard stage (Renner 1998). Thus, the parent's feeding ability during the guard stage is an extremely important factor in determining reproductive success. In our study, chicks of large males grew significantly faster between the ages of 1 and 15 days (the guard stage) than those of small males; this may simply be a consequence of hatching earlier, if food is

more plentiful early in the season. Alternatively, larger males might be better foragers or providers; females that select larger males may themselves be better providers. Because there were no significant relationships between female body size and chick growth, it may be male body size that most influences chick growth, and females should use information on male size to obtain the best mates. However, there were no significant differences between large and small males in the fledging age or mass of chicks (although, on average, chicks of large males fledged earlier and heavier). Fledging mass can affect chick survival in seabirds. Thus, male quality should be related to the size of fledglings, but this was not the case in this study. Food availability can have profound influences on chick fledging mass (i.e. even parents with poor foraging abilities can obtain enough food for chicks in rich food years). Therefore, one year's data would not be adequate to fully consider relationships between fledging mass and parental quality.

Age has been known to affect breeding success in Little Penguins (Dann & Cullen 1990) and probably affects the timing of pair reunions (which will, in turn, have implications for the success and timing of breeding). In our study, without knowing the age of our males, it is not possible to comment on the possibility that our relationships simply reflect differences in success of young (small) birds (e.g. 2 – 3 years old) and older (more experienced) birds. However, from the female's perspective this distinction may be immaterial — by selecting a large male, she improves her own reproductive success, regardless of whether male size or age is responsible for the effect.

## **Conclusions**

Females mated with large male Little Penguins produced eggs and chicks earlier in the breeding season. This may be because large males courted more, had a superior ability to obtain resources, or were more successful during male-male competition. Also, large males may mate earlier than small males because females prefer and select them first. This preference may occur because chicks of large males grow faster than those of small males. Large males were more likely to respond to an intruder to protect their offspring, at least during the post-guard stage when the value of chicks would be expected to peak. In contrast, there were

no significant correlations between female body size and any reproductive parameter. Female Little Penguins can improve their own reproductive potential by selecting larger males for mates. However, we would expect there to be a 'ceiling' on selection for larger males, assuming that costs associated with large size (e.g. heat stress, loss of maneuverability) will eventually outweigh the benefits. Females may select males of an optimum size, not necessarily just the largest males.

## ACKNOWLEDGEMENTS

We thank Barbara and Ray Walter on Tiritiri Matangi Island for invaluable help during this study. We are grateful to C. Pilditch and S. Nakagawa for making suggestions on the statistical analyses. S. Nakagawa also provided support in the field. The manuscript was further improved by useful comments from C. M. King, I. D. Hogg and two anonymous referees. Our research was supported by the Penguin Fund Japan and grants from the University of Waikato. A permit to work on Tiritiri Matangi Island was supplied by the Department of Conservation, New Zealand. The University of Waikato Animal Ethics Committee approved our experimental protocol. This paper is dedicated to the memory of Masahiro Aoyanagi and of Mike Cullen.

## REFERENCES

- Abell, A.J., Cole, B.J., Reyes, R. & Wiernasz, D.C. 1999. Sexual selection on body size and shape in the Western Harvester Ant, *Pogonomyrmex occidentalis* Cresson. *Evolution* 53: 535-545.
- Agnew, D.J. & Kerry, K.R. 1995. Sexual dimorphism in penguins. In Dann, P., Norman, F. I. & Reilly P. (eds) *The Penguins: Ecology and Management*: 299-318. Sydney: Surrey Beatty & Sons.
- Alexander, W.B. & Nicholls, B. 1918. A study of Australian specimens of the Little Penguin (*Eudyptula minor*, Foster). *Emu* 18: 50-57.
- Andersson, M.B. 1994. *Sexual Selection*. Princeton: Princeton University Press.

- Arak, A. 1983. Sex differences in choosiness: Male-male competition and mate choice in anuran amphibians. In Bateson, P. (ed.) *Mate Choice*: 181-210. Cambridge: Cambridge University Press.
- Borgia, G. 1981. Mate selection in the fly *Scatophaga stercoraria*: female choice in a male-controlled system. *Anim. Behav.* 29: 71-80.
- Campbell, R.C. 1989. *Statistics for Biologists*. Cambridge: Cambridge University Press.
- Catchpole, C.K. 1980. Sexual selection and the evolution of complex songs among European warblers of the genus *Acrocephalus*. *Behaviour* 74: 149-166.
- Chiaradia, A.F. & Kerry, K.R. 1999. Daily nest attendance and breeding performance in the Little Penguin *Eudyptula minor* at Phillip Island, Australia. *Mar. Ornithol.* 27: 13-20.
- Côte, I.M. & Hunte, W. 1989. Male and female mate choice in the Redlip Blenny: why bigger is better. *Anim. Behav.* 38: 78-88.
- Dann, P. & Cullen, J.M. 1990. Survival, patterns of reproduction and lifetime reproductive output of Little Penguins *Eudyptula minor* on Phillip Island, Victoria, Australia. In Davis, L.S. & Darby, J.T. (eds) *Penguin Biology*: 63-84. San Diego: Academic Press.
- Davis, L.S. & Speirs, E.A.H. 1990. Mate choice in penguins. In Davis, L.S. & Darby, J.T. (eds) *Penguin Biology*: 377-397. San Diego: Academic Press.
- Downhower, J.F. & Brown, L. 1980. Mate preferences of female Mottled Sculpins, *Cottus bairdi*. *Anim. Behav.* 28: 728-734.
- Ewing, A.W. 1961. Body size and courtship behaviour in *Drosophila melanogaster*. *Anim. Behav.* 9: 93-99.
- Gales, R. 1988. Sexing adult Blue Penguins by external measurements. *Notornis* 35: 71-75.
- Hursthouse, E.W. 1939. Some observations on the breeding habits of the Little Penguin. *Emu* 39: 15-17.
- Jones, G. 1978. *The Little Blue Penguin (Eudyptula minor) on Tiritiri Matangi Island*. MSc Thesis, University of Auckland, New Zealand.
- Jouventin, P. 1982. *Visual and Vocal Signals in Penguins, Their Evolution and Adaptive Characters*. Berlin & Hamburg: Verlag Paul Parey.

- Kraak, S.B.M., Bakker, T.C.M. & Mundwiler, B. 1999. Sexual selection in Sticklebacks in the field: correlates of reproductive, mating, and paternal success. *Behav. Ecol.* 10: 696-706.
- Manly, B.F.J. 1986. *Multivariate Statical Methods A PRIMER*. London: Chapman & Hall.
- Nicholls, B. 1918. An introduction to the study of the penguins of the Nobbies, Phillip Island, Western Port, Victoria. *Emu* 18: 118-144.
- Partridge, L., Ewing, A. & Chandler, A. 1987. Male size and mating success in *Drosophila melanogaster*: the roles of male and female behaviour. *Anim. Behav.* 35: 555-562.
- Reilly, P.N. & Balmford, P. 1975. A breeding study of the Little Penguin *Eudyptula minor* in Australia. In Stonehouse, B. (ed) *The Biology of Penguins*: 161-187. London: Macmillan.
- Renner, M. 1998. *Survival of Little Penguin chicks*. MSc. Thesis, University of Otago, New Zealand.
- Richdale, L.E. 1940. Random notes on the genus *Eudyptula* on the Otago Peninsula, New Zealand. *Emu* 40: 180-216.
- Rowland, W.J. 1989. The effects of body-size, aggression and nuptial coloration on competition for territories in male Threespine Sticklebacks, *Gasterosteus aculeatus*. *Anim. Behav.* 37: 282-289.
- Ryan, M.J. 1980. Female mate choice in a neotropical frog. *Science* 209: 523-525.
- Searcy, W.A. 1984. Song repertoire size and female preferences in Song Sparrows. *Behav. Ecol. Sociobiol.* 14: 281-286.
- Stahel, C. & Gales, R. 1987. *Little Penguin: Fairy Penguins in Australia*. Kensington, New South Wales: New South Wales Univ. Press.
- Trivelpiece, W.Z., Trivelpiece, S.G., Volkman, N.J. & Ware, S.H. 1983. Breeding and feeding ecology of pygoscelid penguins. *Antarctic J. United States.* 18: 209-210.
- Waas, J.R. 1988. Acoustic displays facilitate courtship in Little Blue Penguins, *Eudyptula minor*. *Anim. Behav.* 36: 366-371.
- Waas, J.R. 1990. Intraspecific variation in social repertoires: evidence from cave- and burrow-dwelling Little Blue Penguins. *Behaviour* 115: 63-98.

- Waas, J.R. 1991a. Do Little Blue Penguins signal their intentions during aggressive interactions with strangers? *Anim. Behav.* 41: 375-382.
- Waas, J.R. 1991b. The risks and benefits of signalling aggressive motivation: a study of cave-dwelling Little Blue Penguins. *Behav. Ecol. Sociobiol.* 29: 139-146.
- Weavers, B.W. 1992. Seasonal foraging ranges and travels at sea of Little Penguins *Eudyptula minor*, determined by radiotracking. *Emu* 91: 302-317.
- Wiklund, C. & Kaitala, A. 1995. Sexual selection for large male size in a polyandrous butterfly: the effect of body size on male versus female reproductive success in *Pieris napi*. *Behav. Ecol.* 6: 6-13.
- Williams, T.D. 1995. *The Penguins*, Spheniscidae. Oxford: Oxford University Press.
- Williams, A.J. & Cooper, J. 1984. Aspects of the breeding biology of the Jackass Penguin, *Spheniscus demersus*, *Proc. of the Fifth Pan-African Ornithological Conference*: 841-853. Johannesburg: Southern African Ornithological Society.

## **Chapter 3**

**Influence of parental body size on nest site elevation and food provisioning in Little Penguins *Eudyptula minor***

## ABSTRACT

Field studies were conducted to investigate how parental body size was related to nest site elevation and chick food provisioning in Little Penguins (*Eudyptula minor*). The nests of large males were more commonly found at low to middle elevations, possibly because these are better breeding sites (there was no relationship between female body size and nest site elevation). Males delivered larger amounts of food to chicks than females. No difference in meal size delivered to chicks was found between large and small males, or large and small females. However, chicks of large males grew more quickly than those of small ones; female body size did not correlate with chick growth rate. A previous study of this species has shown that larger males mated earlier in the season than smaller ones, suggesting that females prefer large males. By selecting large males, females may improve their own reproductive potential by gaining better nest sites and obtaining better growth rates for their offspring. Male body size may play an important role in the attraction of potential mates in Little Penguins.

## INTRODUCTION

Nest site selection in burrow-dwelling seabirds can influence reproductive success; the physical structure of nesting habitat can provide shelter from kleptoparasitism (Miyazaki 1996), direct exposure to egg-predators (Nettleship 1972) and strong rainfall (Renner & Davis 2001). Reproductive success also may be affected by other nest site characteristics like distance to foraging sites, along with important parental attributes like age and reproductive experience (Dann & Cullen 1990).

Body size can be related to major life-history traits such as age (Jones 1994). In seabirds, parental body size may be positively related to the meal size fed to chicks (e.g. Chinstrap Penguins, *Pygoscelis antarctica*, de León *et al.* 1998; Snow Petrels, *Pagodroma nivea*, Barbraud *et al.* 1999). de León *et al.* (1998) suggested that larger birds can carry larger amounts of food in a foraging trip either because of foraging efficiency or a larger stomach capacity. Because males are typically larger than females in most birds, the relationships between male body size and

reproductive performance has attracted particular attention (Forero *et al.* 2001).

Penguins are sexually dimorphic in body size. Males are generally heavier and larger than females (Agnew & Kerry 1995). Larger male Adélie Penguins (*Pygoscelis adeliae*) have advantages in male-male competition during territorial interactions (Ainley & Emison 1972) and can fast longer than smaller males during the incubation period (Davis & Speirs 1990). Larger male Adélie Penguins start breeding earlier than smaller ones, suggesting that females prefer larger males (Davis & Speirs 1990).

Little Penguins (*Eudyptula minor*) breed on the coastal mainland and islands of New Zealand and Australia (Marchant & Higgins 1990). They have various nest types (e.g. Kinsky 1960, Marchant & Higgins 1990) and nest features; nest type can influence chick survival (Renner & Davis 2001). For example, chicks from nests with dense covering have greater survival rates (Renner & Davis 2001). In terms of nest site placement, the distance between a Little Penguin's nest and the sea may be particularly important, and is very likely to be influenced by age and reproductive experience.

The objective of this study was to examine how parental body size was related to nest site elevation and chick provisioning in Little Penguins. Little Penguins are monogamous and share parental duties (e.g. incubation and chick guarding) equally (Dann & Cullen 1990). In a previous study, Miyazaki and Waas (2003) found that chicks of larger males grew at a faster rate than those of smaller males; in contrast, a female's body size did not affect chick growth or fledging success. Because larger males may be more experienced and better competitors (Miyazaki & Waas 2003), we predicted that large males would occupy breeding sites that provided easier access to the sea. We also tested the hypothesis that parental body size might be related to the size of food deliveries and, ultimately, to chick growth.

## **METHODS**

### **Study area and period**

The study was conducted on a population of Little Penguins on Tiritiri Matangi Island (36°36'S, 174°53'E), 4 km off Auckland, New Zealand from September to

October 2001. Little Penguins nested mainly between boulders just above the rocky shore or in grasslands higher above the western shorelines of the island (Miyazaki & Waas 2003).

### **Sex determination**

We used Gales' (1988) formula for determining the sex of Little Penguins by bill measurements. This formula sexes the adults with 94 % accuracy (Gales 1988). We investigated only breeding pairs in established nest burrows.

### **Differences in body size of nesting birds among nest sites**

The island rises to a maximum height of 60 m above sea level. The study area on the island had gentle and evenly sloped contours. Thus, the elevation above sea level was highly correlated with the distance from the sea. Little Penguins nested up to 40 m above sea level and 500 m inland, among boulders on the rocky shores, under New Zealand Flax (*Phormium tenax*) roots at low to middle sites and under Wire-netting Bush or Korokio (*Corokia cotoneaster*) at high elevation sites. Thirty-three breeding pairs at low (5 – 10 m), middle (20 – 25 m) and high (35 – 40 m) elevation sites (11 pairs at each elevation) were investigated. Elevation was measured with an altimeter (Casio, Protrek, model PRT-41-BJ-3; with 5 m accuracy). To assess body size, we measured bill length, bill depth, head length, flipper length and foot size (see Miyazaki & Waas 2003 for details). A principal component analysis (PCA) was used to calculate the first principal component (PC1) score for each individual based on those measurements (Rising & Somers 1989). We used PC1 scores to index body size in all analyses. Differences in the body size of nesting birds among these three sites were detected using one-way ANOVA and Fisher's multiple comparison tests.

### **Adult body size and chick growth rate**

A total of nine breeding pairs (that each produced two chicks) nesting at a range of elevations (low – high) were investigated. Chicks from each pair's nest were weighed with a Pesola scale at 0700 – 0730 h every day during the chick guard stage (1 – 15 days). Chick growth rates were estimated by using a linear regression between weight and chick age (Miyazaki & Waas 2003). Associations

between the body size of each parent and their chicks' growth rates were also examined by using a linear regression analysis.

### **Mass of food delivered to chicks**

Parents of Little Penguins change incubation duties regularly (i.e. every c. 24 h) and take turns to feed their chicks during the chick guard stage (Kinsky 1960). Exchanges take place early in the evening when the partner comes in from the sea (Kinsky 1960). We measured the mass of food delivered to chicks by weighing chicks at 24 h intervals; therefore, the meal size delivered to chicks was estimated from the sum of positive mass increments recorded over 24 h intervals throughout the chick guard stage. Because chicks can lose mass through respiration and excretion during the 24 h intervals between weighings, the mass change over the interval would have underestimated meal size (Ricklefs *et al.* 1985). Little Penguins feed their chicks only at night (Kinsky 1960). Their arrivals and departures are synchronised, returning to nests 1 – 2 h after sunset and departing before sunrise (Klomp & Wooller 1991). To determine the rate of chick mass loss, we weighed chicks twice: at 0700 h (after adult departure) and at 1700 h (before adult return). The differences in the mass of food delivered to chicks between males and females, and between small and large birds were examined.

## **RESULTS**

### **Nesting birds at low, middle and high sites**

The body size parameters of birds nesting at low, middle and high sites are shown in Table 1. Smaller males tended to nest at high sites while larger males tended to nest at low to middle sites ( $F_{2, 30} = 5.28, P < 0.05$ ). The 95 % confidence interval for males nesting at low sites (-0.37 — 0.65) overlapped much that for males nesting at middle sites (-0.32 — 1.34). Birds at high sites had shorter bills ( $F_{2, 30} = 3.53, P < 0.05$ ) and shallower bill depths ( $F_{2, 30} = 5.32, P < 0.05$ ), compared with birds at low and middle sites (Table 1). In contrast, there was no significant difference in the size of females among nesting elevations ( $F_{2, 30} = 0.43, P = 0.64$ ). Furthermore, there was no correlation between males and their partners in terms of body size at any elevation ( $P > 0.1$  for low, middle and high sites).

**Table 1.** Body size of birds nesting at low, middle and high sites (mean  $\pm$  SD,  $N = 11$  per elevation).

		Head Length	Bill Length	Bill Depth	Flipper size	Foot size	PC1 score
Low	male	95.91 $\pm$ 2.91	36.80 $\pm$ 1.70 <sup>A</sup>	14.78 $\pm$ 0.62 <sup>A</sup>	79.36 $\pm$ 2.83	52.54 $\pm$ 2.38	0.14 $\pm$ 0.76 <sup>A</sup>
	female	89.82 $\pm$ 1.96	33.74 $\pm$ 1.60	12.91 $\pm$ 0.55	76.27 $\pm$ 3.77	49.36 $\pm$ 3.38	- 0.02 $\pm$ 1.01
Middle	male	95.52 $\pm$ 3.18	37.02 $\pm$ 1.23 <sup>A</sup>	15.01 $\pm$ 0.65 <sup>A</sup>	81.55 $\pm$ 3.91	53.82 $\pm$ 3.25	0.51 $\pm$ 1.24 <sup>A</sup>
	female	89.66 $\pm$ 2.79	34.24 $\pm$ 1.54	13.33 $\pm$ 0.47	77.00 $\pm$ 3.22	50.82 $\pm$ 2.36	0.21 $\pm$ 1.19
High	male	93.49 $\pm$ 3.10	35.62 $\pm$ 0.87 <sup>B</sup>	14.15 $\pm$ 0.64 <sup>B</sup>	79.81 $\pm$ 2.52	51.81 $\pm$ 1.40	- 0.68 $\pm$ 0.48 <sup>B</sup>
	female	89.46 $\pm$ 1.68	34.14 $\pm$ 1.10	12.71 $\pm$ 0.89	75.09 $\pm$ 3.23	50.45 $\pm$ 2.01	- 0.19 $\pm$ 0.80

Values (in columns) corresponding to each sex and not sharing the same letter in superscript were significantly different (ANOVA and Fisher's test,  $P < 0.05$ ).

## **Adult body size and chick growth**

Chick growth rates were highly correlated with chick age (between 1 and 15 days) ( $N = 18$ ,  $R > 0.95$ ,  $P < 0.01$  for all chicks). Principal components were constructed so that PC1 (69 % of variation) accounted for most of the variation in body size-related variables. Chick hatching masses were not related to parental body size (males:  $R = 0.21$ ,  $N = 6$ ,  $P = 0.68$ ; females:  $R = 0.49$ ,  $N = 6$ ,  $P = 0.32$ ). The relationship between adult body size and chick growth rates was examined separately for males and females. Chicks of larger males grew at a faster rate than those of smaller males ( $R = 0.73$ ,  $N = 9$ ,  $P < 0.05$ ). In contrast, female size did not influence chick growth rates ( $R = 0.27$ ,  $N = 9$ ,  $P = 0.47$ ).

## **The relationship between body size and meal size**

Meal size estimated by mass increments over 24 h averaged 28.60 g/day. We obtained data on chick mass loss from 6 – 13 days of age. The rate of chick mass loss averaged 2.55 g/h. Thus, the estimated meal size was 89.80 g/day on average. There was no significant relationship between the rate of mass loss and the original mass of chick ( $R = 0.51$ ,  $N = 9$ ,  $P = 0.15$ ) or chick age ( $R = 0.17$ ,  $N = 9$ ,  $P = 0.65$ ). When we used mass increments over 24 h to estimate meal size, we found males delivered larger meals to chicks than females (males:  $93.30 \pm 10.65$ g; females:  $84.27 \pm 8.83$ g; paired  $t$ -test:  $t_8 = 2.39$ ,  $P < 0.05$ ). No significant correlation was found between the body size of parents and the size of the meal delivered to chicks (males:  $R = 0.17$ ,  $N = 9$ ,  $P = 0.65$ ; females:  $R = 0.08$ ,  $N = 9$ ,  $P = 0.82$ ). There was no increase in meal size delivered to chicks over the guard stage by either males ( $R = 0.004$ ,  $N = 11$ ,  $P = 0.99$ ) or females ( $R = 0.36$ ,  $N = 11$ ,  $P = 0.27$ ) (note, however, that the amount of delivered during first few days was obviously smaller).

## **DISCUSSION**

Our results suggested that large males occupied low to middle sites on the island probably because these are better breeding sites (i.e. the energetic costs of walking on land would be lower for birds nesting at lower elevations). Male Little Penguins carried a larger amount of food to chicks than females. No difference in

meal size delivered to chicks was found between larger and smaller males (or between larger and smaller females). However, chicks of large males still grew at a faster rate than those of small ones (female body size did not correlate with chick growth rates).

The structure of nests can provide protection from climatic factors, which can influence breeding success of Little Penguins (Bull 2000, Renner & Davis 2001). Our results show that the distance to the sea may also be an important nest site attribute. Walking on land makes high energy demands upon Little Penguins in comparison with the energy expended while swimming (Pinshow *et al.* 1977). This energy expenditure may affect offspring growth (e.g. feeding frequency). Foraging parents need to capture food to meet both their own energy requirements and those of their offspring (Gales & Green 1990). Therefore, if parents nest at low to middle sites, they can potentially spend less energy on themselves and more on chicks, than birds nesting at high sites.

In our study, males nesting at high sites were smaller than those at low to middle nest sites. In contrast, there was no significant difference in female body size among nest sites. Reilly and Cullen (1981) believe that male Little Penguins obtain a nest first, then search for mates and take them there. It has also been found that larger males mate earlier than smaller ones (Miyazaki & Waas 2003). Thus, larger males may obtain low to middle sites early in the mating season because they obtain females earlier and then take the preferred sites for breeding. In Little Penguins, bill size (depth) increases with age, certainly until breeding age (Hocken & Russell 2002). It is unknown at what age bills stop growing but bill growth has been shown to continue for up to nine years in other species of seabirds (Coulson *et al.* 1981). Males nesting at high sites may be younger birds because they have smaller bills. If experienced or established pairs (i.e. older pairs) occupy breeding colonies earlier, and prefer sites near the sea, this could explain why larger birds occurred at lower elevations. However, the size-elevation relationship only existed for males, suggesting that our results are better explained as the result of large males obtaining mates (of any size) earlier than small males and then selecting preferred low elevation nests.

The mortality rate of chicks is highest during the early guard stage (Renner & Davis 2001). Thus, the feeding ability of both parents during the guard stage is an

extremely important factor in determining reproductive success (Miyazaki & Waas 2003). Miyazaki and Waas (2003) suggested that larger male Little Penguins have chicks with faster growth rates. In the present study, we investigated chicks from nest sites that differed in terms of elevation. Regardless of elevation, chicks of larger males grew at a faster rate. However, the actual meal size delivered by larger males was similar to that delivered by smaller males. Also, female body size did not influence the size of meals delivered to chicks. Body size is highly heritable and positively correlated between parents and their young in birds (Price & Grant 1984). Barbraud *et al.* (1999) suggested that chicks of Snow Petrels inherit the body size of their parents. Our study may indicate genetic effects of paternity on chick growth, though female body size was not related to the chick growth rate. The heritability of growth rates between chicks and parents would be worth pursuing further in future research. The difference in body size between individuals or populations may also result from direct influences of food availability (Barbraud *et al.* 1999). We did not find a correlation between male size and meal size delivered to chicks, although we can not exclude the possibility of a difference in foraging ability. Future research is required with greater sample sizes to examine this possibility. Alternately, larger males (and the mates they pair with) might bring better quality food for chick growth. The quality of the diet given to growing birds can affect growth rate (Aubin *et al.* 1986). Diving ability in seabirds has been shown to vary allometrically with body size (Costa 1991). Thus, smaller birds may have difficulty taking less accessible prey in deep water or may have more difficulty obtaining high quality prey as a result of poorer plunging capabilities (Barbraud *et al.* 1999). The species composition of the Little Penguin diet is highly variable (reviewed by Williams 1995). Squid is one of the common items of the diet of many penguin species (Cullen *et al.* 1992). Chicks grow more slowly on a squid diet than on a fish diet in Jackass Penguins (*Spheniscus demersus*) (Heath & Randall 1985). If this is true in Little Penguins and the difference in body size can affect feeding ability, variation in the quality of delivered food may explain why there is a difference in chick growth between large and small males. Additional research is needed to assess adequately the difference in diet between larger and smaller males.

Parents need to obtain enough food for their offspring and themselves during

the chick rearing stage. Because large birds require more food to support their large size (Blanckenhorn 2000), they should theoretically be capable of capturing more food than small birds. Thus, we can not exclude the possibility that large birds obtain more food than small birds, although we did not find the difference in the individual meal sizes fed to chicks between large and small males in this study. No chick deaths occurred during the guard stage, suggesting that breeding conditions and food availability in the study year were good. When feeding conditions are good, differences in the foraging ability of larger and smaller birds could be masked.

In some species of seabird, males deliver larger meals and provision chicks more frequently than females (e.g. Wandering Albatrosses, Weimerskirch *et al.* 2000, Berrow & Croxall 2001). This may be because there are differences in body size and/or feeding behaviour between males and females. In our study, male Little Penguins delivered larger meals to their chicks than females. Therefore, differences in body size may have some influences on provisioning. However, because there was no difference in meal size delivered between larger and smaller birds when sexes are examined separately, differences in feeding tactics between sexes may have a greater influence on meal size. Sex differences in foraging behaviour have been found in Adélie Penguins (Chappell *et al.* 1993; Clarke *et al.* 1998) and in Gentoo Penguins, *Pygoscelis papua* (Williams 1991). It has been suggested that sexual dimorphism may reduce intersexual competition in the exploitation of food resources (Adélie Penguins, Ainley & Emison 1972; Eudyptid penguins, *Eudyptes* spp., Warham 1975). In Little Penguins, there are differences in swimming speeds, diving depths, diving duration and foraging areas between the sexes (Bethge *et al.* 1997), which may result in the differences we observed in provisioning between males and females.

The breeding success of Little Penguins is positively related to the age and experience of males, but not of females (Dann & Cullen 1990). Male body size may be an important factor in female choice in Little Penguins (Miyazaki & Waas 2003). If females select larger males as partners, they may obtain better breeding sites and improve conditions for their offspring. Therefore, females may prefer larger males in order to improve their own reproductive potential.

## ACKNOWLEDGEMENTS

We thank Barbara and Ray Walter on Tiritiri Matangi Island for invaluable help during this study. The manuscript was further improved by useful comments from P. Reilly and L. Molles. Our research was supported by the Penguin Fund Japan and grants from the University of Waikato. A permit to work on Tiritiri Matangi Island was supplied by the Department of Conservation, New Zealand. The University of Waikato Animal Ethics Committee approved our protocol.

## REFERENCES

- Ainley, D.G. & Emison, W.B. 1972. Sexual size dimorphism in Adélie Penguins. *Ibis* 114: 267-271.
- Agnew, D.J. & Kerry, K.R. 1995. Sexual dimorphism in penguins. In Dann, P., Norman, F.I. & Reilly, P. (eds) *The Penguins: Ecology and Management*: 299-318. Sydney: Surrey Beatty & Sons.
- Aubin, A.E., Dann, E.H. & Macinnes, C.D. 1986. Growth of Lesser Snow Geese on Arctic breeding grounds. *Condor* 88: 365-370.
- Barbraud, C., Weimerskirch, H., Robertson, G. & Jouventin, P. 1999. Size-related life history traits: insights from a study of Snow Petrels (*Pagodroma nivea*). *J. Anim. Ecol.* 68: 1179-1192.
- Berrow, S.D. & Croxall, J.P. 2001. Provisioning rate and attendance patterns of Wandering Albatrosses at Bird Island, South Georgia. *Condor* 103: 230-239.
- Bethege, P., Nicol, S., Culik, B.M. & Wilson, R. P. 1997. Diving behaviour and energetics in breeding Little Penguins (*Eudyptula minor*). *J. Zool.* 242: 483-502.
- Blanckenhorn, W.U. 2000. The evolution of body size: what keeps organisms small? *Q. Rev. Biol.* 75: 385-407.
- Bull, L.S. 2000. Factors influencing Little Penguin *Eudyptula minor* egg success on Mitiu-Somes Island, New Zealand. *Emu* 100: 199-204.
- Chappell, M.A., Janes, D.N., Shoemaker, V.H., Bucher, T.L. & Maloney, S.K. 1993. Reproductive effort in Adélie Penguins. *Behav. Ecol. Sociobiol.* 33: 173-182.

- Clarke, J., Manly, B., Kerry, K., Gardner, H., Franchi, E., Corsolini, S. & Focardi, S. 1998. Sex differences in Adélie Penguin foraging strategies. *Polar Biol.* 20: 248-258.
- Costa, D.P. 1991. Reproductive and foraging energetics of high latitude penguins, albatrosses and pinnipeds: implications for life-history patterns. *Am. Zool.* 31: 111-130.
- Coulson, J.C., Duncan, N., Thomas, C.S. & Monaghan, P. 1981. An age-related difference in the bill depth of Herring Gulls *Larus argentatus*. *Ibis* 123: 499-502.
- Cullen, J.M., Montague, T.L. & Hull, C. 1992. Food of Little Penguins *Eudyptula minor* in Victoria: comparison of three localities between 1985 and 1988. *Emu* 91: 318-341.
- Dann, P. & Cullen, J.M. 1990. Survival, patterns of reproduction and lifetime reproductive output of Little Penguins *Eudyptula minor* on Phillip Island, Victoria, Australia. In Davis, L.S. & Darby, J.T. (eds) *Penguin Biology*: 63-84. San Diego: Academic Press.
- Davis, L.S. & Speirs, E. A. H. 1990. Mate choice in penguins. In Davis, L.S. & Darby, J.T. (eds) *Penguin Biology*: 377-397. San Diego: Academic Press.
- Forero, M.G., Tella, J.L., Donazar, J.A., Blanco, G., Bertellotti, M. & Ceballos, O. 2001. Phenotypic assortative mating and within-pair sexual dimorphism and its influence on breeding success and offspring quality in Magellanic Penguins. *Can. J. Zool.* 79: 1414-1422.
- Gales, R. 1988. Sexing adult Blue Penguins by external measurements. *Notornis* 35: 71-75.
- Gales, R. & Green, B. 1990. The annual energetics cycle of Little Penguins (*Eudyptula minor*). *Ecology* 71: 2297-2312.
- Heath, R.G.M. & Randall, R.M. 1985. Growth of Jackass Penguin chicks (*Spheniscus demersus*) hand reared on different diets. *J. Zool.* 205: 91-105.
- Hocken, A.G. & Russell, J.J. 2002. A method for determination of gender from bill measurements in Otago Blue Penguins (*Eudyptula minor*). *New Zeal.J. Zool.* 29: 63-69.

- Jones, I.L. 1994. Mass changes of Least Auklets *Aethia pusilla* during the breeding season: Evidence for programmed loss of mass. *J. Anim. Ecol.* 63: 71-78.
- Kinsky, F.C. 1960. The yearly cycle of the Northern Blue Penguin (*Eudyptula minor novaehollandiae*) in the Wellington Harbour area. *Rec. Dom. Mus. Wellington* 3: 145-218.
- Klomp, N.I. & Wooller, R.D. 1991. Patterns of arrival and departure by breeding Little Penguins at Penguin Island, Western Australia. *Emu* 91: 32-35.
- de León, A., Fargallo, J.A. & Moreno, J. 1998. Parental body size affects meal size in the Chinstrap Penguin (*Pygoscelis antarctica*). *Polar Biol.* 19: 358-360.
- Marchant, S. & Higgins, P.J. 1990. *Handbook of Australian, New Zealand and Antarctic Birds, Vol. 1A*. Melbourne: Oxford University Press.
- Miyazaki, M. 1996. Vegetation cover, kleptoparasitism by diurnal gulls, and timing of arrival of nocturnal Rhinoceros Auklets. *Auk* 113: 698-702.
- Miyazaki, M. & Waas, J. R. 2003. Correlations between body size, defensive behaviour and reproductive success in male Little Blue Penguins *Eudyptula minor*: implications for female choice. *Ibis* 145: 98-105.
- Nettleship, D.N. 1972. Breeding success of the Common Puffin (*Fratercula arctica* L.) on different habitats at Great Island, Newfoundland. *Ecol. Monogr.* 42: 239-268.
- Pinshow, B., Fedak, M.A. & Schmidt-Nielsen, K. 1977. Terrestrial locomotion in penguins: It costs more to waddle. *Science* 195: 592-594.
- Price, T.D. & Grant, P.R. 1984. Life history traits and natural selection for small body size in a population of Darwin's Finches. *Evolution* 38: 483-494.
- Reilly, P.N. & Cullen, J.M. 1981. The Little Penguin *Eudyptula minor* in Victoria, II. Breeding. *Emu* 81: 1-19.
- Renner, M. & Davis, L.S. 2001. Survival analysis of Little Penguin *Eudyptula minor* chicks on Motuara Island, New Zealand. *Ibis* 143: 369-379.
- Ricklefs, R.E., Day, C.H., Huntington, C.E. & Williams, J.B. 1985. Variability in feeding rate and meal size of Leach's Storm-Petrel at Kent Island, New Brunswick. *J. Anim. Ecol.* 54: 883-898.
- Rising, J.D. & Somers, K.M. 1989. The measurement of overall body size in birds. *Auk* 106: 666-674.

- Warham, J. 1975. The crested penguins. In Stonehouse, B. (ed) *The Biology of Penguins*: 189-269. London: Macmillan.
- Weimerskirch, H., Barbraud, C. & Lys, P. 2000. Sex differences in parental investment and chick growth in Wandering Albatrosses: fitness consequences. *Ecology* 81: 309-318.
- Williams, A.J. 1991. Foraging ecology and diet of Gentoo Penguins *Pygoscelis papua* at South Georgia during winter and an assessment of their winter prey consumption. *Ibis* 133: 5-13.
- Williams, T.D. 1995. *The Penguins*, Spheniscidae. Oxford: Oxford University Press.

## **Chapter 4**

**Acoustic properties of male advertisement  
and their impact on female responsiveness  
in Little Penguins *Eudyptula minor***

## ABSTRACT

Acoustic signals can play an important role in the attraction of potential mates particularly in nocturnal seabirds, because their visual abilities may be limited under low light conditions. In previous studies, a negative relationship between body mass and call pitch has been commonly documented. However, the relationship between male call properties and the responsiveness of females has not been explored in any detail. We examined the relationship between the acoustic properties of male advertisement and male body size in Little Penguins (*Eudyptula minor*). Larger males were found to produce lower-pitched vocal elements in their exhalation phrases. Playback experiments were conducted to assess female responsiveness to low, average and high-pitched calls of males. Females were more likely to respond vocally to lower pitched male calls. Most females did not respond vocally to the playbacks. The minority that did respond vocalised to low and medium calls. Responding females might have interest in larger males as potential partners. Alternately, females may be responding territorially to larger, but not smaller, intruders. Lone females, whether paired or not, distinguish size differences of males on the basis of call pitch.

## INTRODUCTION

Call frequencies in songbirds are shown to be constrained by body size because the mass of a vibrating structure affects the pitch (Wallschläger 1980, Ryan & Brenowitz 1985). Several studies have demonstrated a negative relationship between body mass and call properties in birds (Badyaere & Leaf 1997, Tubaro & Mahler 1998). Larger birds tend to have calls with lower frequencies (Morton 1977, Wallschläger 1980, Badyaere & Leaf 1997). Barbraud *et al.* (2000) showed that other indicators of body size (e.g. bill size) have a negative relationship with call frequencies in the Snow Petrel (*Pagodroma nivea*).

In nocturnal seabirds, acoustic signals play an important role in the attraction of potential mates because their visual abilities may be limited under low light conditions (Brooke 1978a, James 1984, Storey 1984). For example, Genevois and Bretagnolle (1994) reported that acoustic properties of sexual calls of male Blue

Petrels (*Halobaena caerulea*) are related to body mass. Genevois and Bretagnolle (1994) suggested that females could use call properties to obtain information on the body mass or condition of males.

Davis and Speirs (1990) suggested that larger than average male Adélie Penguins (*Pygoscelis adeliae*) have lower-pitched sexual calls and breed earlier than smaller ones, suggesting that larger males were preferred by females. However, the ways in which male calls may influence the female's responsiveness in penguins have not been explored in any detail.

Little Penguins (*Eudyptula minor*) occupy a wide range of nesting habitats along the coastlines of New Zealand and Australia and are nocturnal, breeding in burrows. Their acoustic behaviour has been frequently investigated (Waas 1988, 1990a,b, 1991a,b, Jouventin & Aubin 2000, Nakagawa *et al.* 2001, Miyazaki & Waas 2003) because their courtship and agonistic activities have loud and obvious vocal elements. These activities occur mainly at night when their visual abilities may be restricted. Therefore, the loud braying calls used by solitary males during the mating season are very likely to be important for female choice. Males produce advertising calls frequently during the mating season, but the call properties and their significance to females have received little attention (Miyazaki & Waas 2003).

The objective of this study was to determine if male attributes of Little Penguins, that might be expected to be important during mate choice (e.g. body size, age), correlate with the call properties of advertising males. This study involved the following activities. (1) The properties of recorded sexual calls of males of various sizes were analysed. (2) Measurements associated with body size (i.e. bill length, bill depth, head length, flipper length and foot size) and body weight were obtained. (3) Possible relationships between these properties and call measurements were examined. (4) The response of females to played back male advertising calls was investigated. We examined the possibility that the structural parameters of calls can provide information on body size in penguins, which may be an important factor in female mate choice. Little is known on the process of mate choice in seabirds, especially in penguins. As a result, this study also provides important data to advance our general understanding of female choice.

## **METHODS**

### **Study area and period**

The study was conducted on a population of Little Penguins from 10 to 30 May 2001 in Oamaru (45°07'S, 170°58'E), New Zealand. This colony contains approximately 500 breeding pairs (D. Houston, Department of Conservation, New Zealand, *pers. comm.*). Little Penguins in this area start to lay eggs in August or September every year.

### **Recordings of male advertising calls**

Male Little Penguins produce advertising calls to attract mates (see Waas 1988) during the pairing stage of the breeding season (May to July in the study area). They usually call from shorelines before sunrise and after sunset (Waas 1988). In our study area, most males called within nesting areas rather than along the shoreline. The advertising calls of 12 males were recorded from the Oamaru Creek area after sunset (between 1800 and 2100 h) and before dawn (between 0400 and 0700 h). When we found a calling male, we quietly approached the bird by slowly crawling until positioned in front of it at a distance of 2 m. An AKG shotgun microphone (head, model C460B; body, model CK68-ULS; frequency response 20-22,000 Hz  $\pm$  2 dB) was pointed at the bill and the calls were recorded using a Sony Digital Audio Tape-Corder (DAT; model TCD-D7, sampling frequency 44.1 kHz, frequency response 20-22,000 Hz  $\pm$  1 dB). After we recorded calls, we captured the bird and measured bill length, bill depth and head length with digital calipers to assess body size (see Miyazaki & Waas 2003 for details). After that, the bird was placed in a cloth bag of known mass and weighed with a 3-kg Pesola scale. Previous studies have used body mass as an indicator of body size (Badyaere & Leaf 1997, Tubaro & Mahler 1998). Warham (1990) suggested that body mass of seabirds is extremely variable, so other parameters of body size (bill size etc.) are better indicators. We estimated their body size by using body mass and other parameters of body size separately. A Principal Component Analysis (PCA) was used to identify the best parameter (chosen from head length, bill length, bill depth, foot size and flipper size) to use in body size estimates. The parameter exhibiting most variation in Principal Component 1 (PC1) was selected

for analysis (see Manly 1986 for a description of PCA). We could then determine how male attributes (PC1, weight etc.) correlated with the measured acoustic properties of the calls. Gender was determined by bill measurement as described by Hocken and Russell (2002): the formula sexes the adults with 97 % accuracy.

### **Call measurements**

Recorded calls were analysed by producing sonagrams with Canary 1.2.4 software on a Macintosh computer. The sampling frequency was set at 44,000Hz with 16-bit accuracy. We analysed the advertising calls of 12 males by measuring: (1) the dominant frequency (DF; kHz); (2) the highest frequency (HF); (3) the lowest frequency (LF); and (4) phrase duration (PD; s). A coefficient of variation (CV) within individuals was calculated for each call property of each individual. The ratio of inter- to intra-individual CV was used as a measure of call individuality (see Jouventin 1982). After that, we looked at the relationship between these call properties and male body size by using a linear regression analysis.

### **Playback tapes**

After analysing the recorded calls of advertising males, we conducted playback experiments to assess female responses. The recordings used for the experiments were made with a DAT.

We determined the average frequency of the 12 recorded calls. After that, we selected the five best calls and made three auditory treatments for each call using Peak 2.5 software to change call pitch without altering other call features. The three auditory treatments were: (1) an advertising call with an average dominant frequency (Medium call); (2) an advertising call with a dominant frequency 30 % higher than average (High call); and (3) an advertising call with a dominant frequency 30 % lower than average (Low call). Each playback tape consisted of the three versions (Low, Medium and High) of a given male's call (each of three treatments consisted of five exhalation/inhalation sets). We rotated the order of treatments (1), (2) and (3) on each tape, and composed a total of six tapes for each of the five males (i.e. total 30 tapes from five males). We selected a tape from the 30 tapes for each test randomly but never used the same tape more than once.

## **Playback experiments**

To examine the response of females to male advertising calls with different dominant frequencies, we conducted the following playback experiment. All experiments at the 29 nest boxes that we sampled were carried out on calm evenings and mornings between 1800 and 0700 hours. An amplified speaker (Sony, model SRS-77G; frequency response 80-20,000 Hz  $\pm$  1 dB) was used with a DAT. The speaker was placed at a distance of approximately 1 m in front of the nest box entrance; the advertising call of a single male was then broadcast at a level equivalent to the amplitude of a displaying bird. Throughout the experiment, the female's vocal responses were recorded using a DAT with an AKG shotgun microphone. Following each experiment, the sex of the burrow occupant was checked using the flipper band number and bill measurements. We conducted a total of 29 experiments on 29 females through the pre-pairing phase of the breeding season (each of the 29 test females was tested only once.).

Each playback experiment started with a 10-min settling period, and then the subject was presented with three acoustic treatments separated by 10-min silent periods during which birds could recover from their exposure to each treatment.

## **Female response measurements**

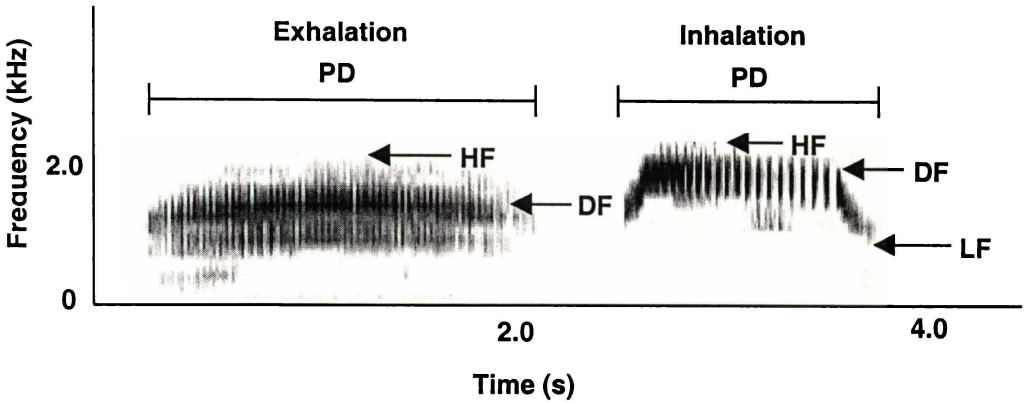
Females produced two call types (growl and bray; see Waas 1990a) in response to played back male calls or remained silent. We analysed female responses by measuring: (1) whether there was any response vocally; (2) the type of call used when vocalisations occurred; and (3) which of three stimuli (Low, Medium and/or High calls) elicited a response.

## **RESULTS**

### **Male advertising calls**

The dominant frequency of exhalations we sampled was 1.78 kHz  $\pm$  0.28 (mean  $\pm$  SD) with a range between 1.27 kHz and 2.15 kHz ( $N = 12$ ). An example of an advertising call is shown in Fig. 1. Advertising calls were composed of repeated exhalation and inhalation phrases. The seven acoustic properties we measured are

also shown in Fig. 1. Because the lowest frequency of an exhalation is always zero, we did not include this property for the following analysis.



**Figure 1.** Sonagram of the advertising calls of male Little Penguins, *Eudyptula minor*, showing seven call properties that were analysed (DF = dominant frequency, HF = highest frequency, LF = lowest frequency and PD = phrase duration).

**Intra- and inter-individual variation of calls**

The between- and within-individual CVs are shown in Table 1. All inter-individual CVs were greater than intra-individual CV. Thus, each bird has an individually distinctive advertising call (see Jouventin 1982 for details). The greatest inter- versus intra-individual variability was found in the maximum exhalation frequency (Table 1).

**Table 1.** Comparison of inter- and intra-individual coefficients of variation (CV), along with the corresponding CV ratios.

	Exhalation			Inhalation			
	DF	HF	PD	DF	HF	LF	PD
Inter-individual (%)	15.6	11.4	23.3	5.6	4.6	6.2	19.7
Intra-individual (%)	5.1	2.6	17.3	2.1	2.7	2.0	6.5
CV ratio	3.1	4.4	1.3	2.7	1.7	3.1	3.0

## Male body size and call properties

Principal components were constructed so that PC1 (32 % of variation) accounted for most of the variation in body size related variables. The relationship between call parameters and body size indicators (both PC1 and body mass) are shown in Table 2. The DF of inhalation of larger males in terms of PC1 was significantly lower than that of smaller males. The relationship between PC1 and the other call parameters was not significant. When we used body mass as an indicator of body size, the DF and HF of exhalations correlated negatively with body mass. The PD of heavier males was longer in both exhalation and inhalation. Bill size was negatively correlated with PD of exhalations ( $R = -0.71$ ,  $N = 12$ ,  $P < 0.05$ ) and DF of inhalations ( $R = -0.68$ ,  $N = 12$ ,  $P < 0.05$ ).

**Table 2.** Pearson correlation coefficients between seven call properties and overall body size (PC1) and body mass ( $N = 12$ ).

Call parameter	PC1	Body mass
exhalation		
DF	-0.277	-0.720**
HF	0.051	-0.913***
PD	-0.036	0.659*
inhalation		
DF	-0.618*	-0.054
HF	0.204	-0.109
LF	0.208	-0.380
PD	-0.001	0.684*

\* $P < 0.05$ , \*\* $P < 0.01$ , \*\*\* $P < 0.001$

(DF: dominant frequency, HF: highest frequency, LF: lowest frequency, PD: phrase duration)

## Female response to the playbacks

During experiments, eight females responded vocally to playback (Table 3) and twenty-one females remained silent. Six of eight females responded to Low and/or Medium call playback. Two females responded to High call playbacks; these females also responded to Low and Medium call playbacks. When a female responded by growling, the number of call repetitions was small (Table 3). On the contrary, several bray calls were given per response (Table 3).

**Table 3.** Responses of 8 females that vocalised during the playback of Low, Medium and High calls.

	No. of females	Mean Growl per response	Mean Bray per response
Low only	2	1	4.5
Medium only	3	1	4.5
High only	0	0	0
Low and Medium	1	3	0
Low and High	0	0	0
Medium and High	0	0	0
Low, Medium and High	2	0	8.8

## DISCUSSION

Previous studies have demonstrated the existence of a negative relationship between call frequencies in bird songs and body mass (e.g. Wallschläger 1980). Our analysis also indicates that body mass is correlated with the call properties of advertising calls produced by male Little Penguins. Females appeared more likely to respond vocally to the medium and low-pitched calls. However, that trends were not very strong and only eight females responded to playbacks vocally. The non-responding females might have already had fixed partners and so were not interested in other males. The vocal minority may therefore reflect unpaired young or ‘divorcees’.

The relationship between body size and acoustic features has been reported in many species (e.g. anurans, Castellano *et al.* 1999; humans, Collins 2000). In previous studies investigating correlations between body size and call properties in songbirds, body size indicators have been divided into two main categories: body mass and other body size features (e.g. bill proportions). According to our regression analysis, the relationships between PC1 and most call properties were weak. On the contrary, body mass was negatively correlated with many call properties. In particular, larger males used lower frequencies in the exhalation phrase. Furthermore, larger males exhaled for a longer time than smaller ones. There are important constraints on the production and detection of animal acoustic signals. For example, the mass of the vibrating structure that produces the sound

influences the frequency of the sound. In a number of songbirds, the mass of the structure is correlated with body size (Wallschläger 1980). In Little Penguins, some internal organ weights are correlated with the body mass (Hocken 2000). Our result may be consistent with the finding that body size is positively correlated with the mass of the song-producing structures in birds (Ryan & Brenowitz 1985). In addition, large body size may reveal nutritional condition (i.e. fat stores). Good fat stores may reflect a successful forager. The CV ratio of exhalation HF was high and the correlation between the HF and body mass was strong. This shows exhalation HF may be a very good indicator of male size in Little Penguins.

Hocken and Russell (2002) suggested that bill depth of Little Penguins increases with age, but it is not known at what age maximum bill size is achieved. Palacios and Tubaro (2000) suggested that the beak length of woodcreepers (*Dendrocolaptinae*) is correlated with call frequencies. In our study, bill size was negatively correlated with inhalation DF ( $R = -0.68$ ,  $N = 11$ ,  $P < 0.05$ ) and exhalation PD ( $R = -0.72$ ,  $N = 11$ ,  $P < 0.05$ ). It is possible that call properties change as the bill size grows. Further work needs to be done on the yearly change of bill dimensions and call properties.

Female Little Penguins are more likely to remain silent than call when alone at a nest (Waas 1991a). In our experiments, 27.6 % (8/29) of females responded to playbacks vocally. Little Penguins vary in their acoustic responses. Growl and bray calls are common vocalisations in both defensive and sexual contexts; growl calls grade into bray calls (Waas 1990b). In response to playback, females repeated bray calls more than growl calls. Perhaps their bray calls signal more 'interest' in the male producing the advertising calls. If a female is interested in forming a sexual relationship, calling would therefore facilitate a union. Because Little Penguins often respond vocally to intruders in their territories (Miyazaki & Waas 2003), the responses in our study may have been territorial. However, our study was conducted very early in the mating season (no eggs and no chicks). If a female that has a mate spends a night on land alone, it will sometimes accept the company of any other male even for one night (Kinsky 1960). In addition, we did not play back male territorial calls; instead, we played back advertising calls that males use in the nesting areas and on the shoreline to attract females. In the

mating season, females respond to male advertising calls by calling back presumably to maintain contact, followed by movement towards males (*pers. obs.*). Thus, female vocal responses in our experiments probably demonstrate ‘interest’ in the advertising calls.

According to our results, calls with lower frequencies reveal larger body size of the callers. Thus, females might use call properties as a key parameter to assess male size. In some species of seabird, male body mass can be a good indicator of male qualities because size may increase with age and/or breeding experience (Brooke 1978b, Weimerskirch 1992). Miyazaki and Waas (2003) have suggested that chick growth of Little Penguins is associated with the body size of males. Chicks of larger males grew at a faster rate than those of smaller males (Miyazaki & Waas 2003). Most of the responding females in the present study replied to Low and/or Medium pitched playbacks. Only two birds responded to High pitched playback, but these birds responded to Low and Medium calls as well. Therefore, the two females might have been very sensitive to all playbacks. Responding females might be interested in larger males as partners to improve their own reproductive potential.

In conclusion, our results show that male advertising calls of Little Penguins vary significantly across individuals, particularly in the exhalation phrase. Call parameters of the exhalation could be a reliable signal of male body size. Females might discriminate, on the basis of calls, between large and small males and may have more interest in the calls of larger males. Thus, acoustic signals may be extremely important during the process of mate choice in nocturnal seabirds like Little Penguins.

## **ACKNOWLEDGEMENTS**

We are grateful to Pauline Reilly, Tony Hocken, Dave Houston and Shinichi Nakagawa for their help and valuable comments on an earlier version of this manuscript. Our research was supported by the Penguin Fund Japan and grants from the University of Waikato. A permit to work in Oamaru was supplied by the Department of Conservation, New Zealand. The University of Waikato Animal Ethics Committee approved our experimental protocol.

## REFERENCES

- Badyaev, A.V. & Leaf, E.S. 1997. Habitat associations of song characteristics in *Phylloscopus* and *Hippolais* warblers. *Auk* 114: 40-46.
- Barbraud, C., Mariani, A. & Jouventin, P. 2000. Variation in call properties of the Snow Petrel, *Pagodroma nivea*, in relation to sex and body size. *Aust. J. Zool.* 48: 421-430.
- Brooke, M.de L. 1978a. Sexual differences in the voice and individual vocal recognition in the Manx Shearwater (*Puffinus puffinus*). *Anim. Behav.* 26: 622-629.
- Brooke, M.de L. 1978b. Weights and measurements of the Manx Shearwater, *Puffinus puffinus*. *J. Zool.* 186: 359-374.
- Castellano, S., Rosso, A., Doglio, S. & Giacoma, C. 1999. Body size and calling variation in the Green Toad (*Bufo viridis*). *J. Zool.* 248: 83-90.
- Collins, S.A. 2000. Men's voices and women's choices. *Anim. Behav.* 60: 773-780.
- Dann, P. & Cullen, J.M. 1990. Survival, patterns of reproduction and lifetime reproductive output of Little Penguins *Eudyptula minor* on Phillip Island, Victoria, Australia. In Davis, L. S. & Darby, J. T. (eds) *Penguin Biology*: 63-84. San Diego: Academic Press.
- Davis, L.S. & Speirs, E.A.H. 1990. Mate choice in penguins. In Davis, L.S. & Darby, J.T. (eds) *Penguin Biology*: 377-397. San Diego: Academic Press.
- Genevois, F. & Bretagnolle, V. 1994. Male Blue Petrels reveal their body mass when calling. *Ethol. Ecol. Evol.* 6: 377-383.
- Hocken, A.G. 2000. Internal organ weights of the Blue Penguin *Eudyptula minor*. *New Zeal. J. Zool.* 27: 299-304.
- Hocken, A.G. & Russell, J.J. 2002. A method for determination of gender from bill measurements in Otago Blue Penguins (*Eudyptula minor*). *New Zeal. J. Zool.* 29: 63-69.
- James, P.C. 1984. Sexual dimorphism in the voice of the British Storm Petrel *Hydrobates pelagicus*. *Ibis* 126: 89-92.
- Jouventin, P. 1982. *Visual and Vocal Signals in Penguins, Their Evolution and Adaptive Characters*. Berlin & Hamburg: Verlag Paul Parey.

- Jouventin, P. & Aubin, T. 2000. Acoustic convergence between two nocturnal burrowing seabirds: experiments with a penguin *Eudyptula minor* and a shearwater *Puffinus tenuirostris*. *Ibis* 142: 645-656.
- Kinsky, F.C. 1960. The yearly cycle of the Northern Blue Penguin (*Eudyptula minor novaehollandiae*) in the Wellington Harbour area. *Rec. Dom. Mus. Wellington* 3: 145-218.
- Manly, B.F.J. 1986. *Multivariate Statical Methods A PRIMER*. London: Chapman & Hall.
- Miyazaki, M. & Waas, J.R. 2003. Correlations between body size, defensive behaviour and reproductive success in male Little Blue Penguins *Eudyptula minor*: implications for female choice. *Ibis* 145: 98-105.
- Morton, E.S. 1977. On the occurrence and significance of motivational structural rules in some bird and mammal sounds. *Am. Nat.* 111: 855-869.
- Nakagawa, S., Waas, J.R. & Miyazaki, M. 2001. Heart rate changes reveal that Little Blue Penguin chicks (*Eudyptula minor*) can use vocal signatures to discriminate familiar from unfamiliar chicks. *Behav. Ecol. Sociobiol.* 50: 180-188.
- Palacios, M.G. & Tubaro, P.L. 2000. Does beak size affect acoustic frequencies in Woodcreepers? *Condor* 102: 553-560.
- Ryan, M.J. & Brenowitz, E.A. 1985. The role of body size, phylogeny, and ambient noise in the evolution of bird song. *Am. Nat.* 126: 87-100.
- Storey, A.E. 1984. Function of Manx Shearwater calls in mate attraction. *Behaviour* 89: 73-89.
- Tubaro, P.L. & Mahler, B. 1998. Acoustic frequencies and body mass in New World Doves. *Condor* 100: 54-61.
- Waas, J.R. 1988. Acoustic displays facilitate courtship in Little Blue Penguins, *Eudyptula minor*. *Anim. Behav.* 36: 366-371.
- Waas, J.R. 1990a. An analysis of communication during the aggressive interactions of Little Blue Penguins (*Eudyptula minor*). In Davis, L. S. & Darby, J. T. (eds) *Penguin Biology*: 345-376. San Diego: Academic Press.
- Waas, J.R. 1990b. Intraspecific variation in social repertoires: evidence from cave-and burrow-dwelling Little Blue Penguins. *Behaviour* 115: 63-98.

- Waas, J.R. 1991a. Do Little Blue Penguins signal their intentions during aggressive interactions with strangers? *Anim. Behav.* 41: 375-382.
- Waas, J.R. 1991b. The risks and benefits of signaling aggressive motivation: a study of cave-dwelling Little Blue Penguins. *Behav. Ecol. Sociobiol.* 29: 139-146.
- Wallschläger, D. 1980. Correlation of song frequency and body weight in passerine birds. *Experientia* 36: 412.
- Warham, J. 1990. *The Petrels: Their Ecology and Breeding Systems*. London: Academic Press.
- Weimerskirch, H. 1992. Reproductive effort in long-lived birds: age-specific patterns of condition, reproduction and survival in the Wandering Albatross. *Oikos* 64: 464-473.

## **Chapter 5**

# **Effects of male call pitch on female behaviour and mate fidelity in Little Penguins**

## ABSTRACT

Playback experiments with two loudspeakers were conducted to examine how female Little Penguins (*Eudyptula minor*) respond to bray calls of males that varied in: (1) pitch (low versus high) and (2) familiarity (current partner versus stranger). In the first experiment, although there was no difference in the number of female approaches to playbacks of Low and High Calls, females responded vocally only to Low Calls. Our earlier research has demonstrated that the dominant frequency of male advertising calls was negatively related to male body size and large males show better breeding performance. Female vocal responses may show interest in Low Calls. However, they did not appear to discriminate between the two types of calls in terms of their approaching behaviours. In the second experiment, using calls from the current mate and a stranger call, females were more likely to approach their own mates' call and responded vocally only to their current partners, regardless of the pitch of the stranger's call. We conclude that female Little Penguins may recognise their partners' call (or at least familiar male calls) and appear to maintain mate fidelity even when larger, potentially more successful, strangers are on offer.

## INTRODUCTION

Acoustic features of male calls can reflect the quality of the caller and affect female choice in some species (e.g. in frogs, Ryan 1980, Márquez & Bosch 1997; in birds, Catchpole 1980, Eens *et al.* 1991). In nocturnal seabirds, in particular, acoustic signals play an important role in the attraction of potential mates because their visual abilities are restricted under low light conditions (Brooke 1978, Storey 1984). For example, female Thin-billed Prions (*Pachyptila belcheri*) increase their vocal activities when they hear playback of male calls, suggesting that male calls may play an important role in mate attraction (Bretagnolle *et al.* 1998). Genevois and Bretagnolle (1994) found that the duration and rhythm of male Blue Petrel (*Halobaena caerulea*) calls were correlated with body mass. Thus, female Blue Petrels can use male call parameters to obtain information on male body size (Genevois & Bretagnolle 1994).

Little Penguins (*Eudyptula minor*) are nocturnal on land, depart and return to/from the sea solely during darkness. Their acoustic behaviour has been extensively investigated (Waas 1988, 1990, 1991a,b, Jouventin & Aubin 2000, Miyazaki & Waas 2002, 2003). Vocalisations may play an important role in pair-bonding and in the process of mate choice (Miyazaki & Waas 2002, 2003). Male Little Penguins produce advertising calls to attract females early in the mating season (Waas 1988). Little Penguins have individually distinctive calls, identified by dominant frequency and a number of other parameters (Jouventin 1982); the dominant frequency of male advertising calls appears to be negatively correlated with body size (Miyazaki & Waas, *in press*). Because male body size of Little Penguins is positively correlated with reproductive parameters that are associated with reproductive success (Miyazaki & Waas 2003), females may display a preference for large males. Thus, females may be attentive to the dominant frequency of male calls as it may be a good indicator of the caller's size. Investigating the effects of male call pitch on female choice provides important empirical information to advance our understanding of mate selection in Little Penguins.

The first objective of the present study was to investigate whether female Little Penguins discriminate lower-pitched male advertising calls from higher-pitched calls. We conducted this experiment early in the mating season, when mate attraction and selection was occurring. Next, we tested the prediction that recently paired females would be more likely to investigate advertising strangers, if the stranger's call was lower in pitch than her partner's call. To do this, we compared paired females' responsiveness to their current partners' calls with that to a stranger's call with more/less 'attractive' acoustic parameters.

## **METHODS**

### **Study area and recordings**

Little Penguins on Tiritiri Matangi Island (36°36'S, 174°53'E), New Zealand were studied in 2000 and 2001. Little Penguins nested mainly in grasslands and between boulders just above the rocky shores around the island (Miyazaki & Waas 2002).

We first recorded the braying advertising calls of males for the playback experiments. These braying calls are the most common vocalisations used by Little Penguins, in both defensive and sexual contexts. Brays are used both before, during and after pairs engage in mutual displays (Waas 1988), and grade from low to medium and finally to full brays (Waas 1990). Recordings were made with a Digital Audio Tape-corder (DAT; model TCD-D7, Sony Corporation, sampling frequency 44.1 kHz, frequency response 20-22,000 Hz  $\pm$  1 dB) and an AKG shotgun microphone (head, model C460B; body, model CK68-ULS; frequency response 20-22,000 Hz  $\pm$  2 dB). Gales' (1988) formula for the sexing of Little Penguins was used, which classifies adults with 94 % accuracy (Gales 1988).

### **Experiment 1: Low versus High Calls**

During the early pairing period, six advertising calls from six different males were obtained from shorelines around the Island. We measured the dominant frequency (kHz) of the exhalation phrase by producing sonagrams with Canary 1.2.4. software. The range of variation within this population was  $\pm$  20 % from the average dominant frequency. We then changed the call pitch of the six calls we sampled without altering any of the other call parameters by using Peak 2.5 software. We made six 'Low Calls' and six 'High Calls' from the original six calls. The six Low Calls had a dominant frequency 20 % lower than average, while the six High Calls had a dominant frequency 20 % higher than average. Each tape consisted of a single male's call presented in 20-s vocal segments separated by 60-s silent periods. The total length of a tape was 45-min. We used one Low Call and one High Call in each test. High and Low playback tapes were selected randomly before each test.

We conducted the first experiment by using a field-based experimental arena between 1900 and 2100 h from June to July during the early courtship period in 2001. It was dark when our experiments were started at this point in the season. The natural distance between calling penguins varies depending on numbers of birds ashore, but averaged approximately 5 to 10-m on shorelines of this island (*pers. obs.*). In a bush area near the shoreline, a 5.00  $\times$  2.50-m arena was created using temporary garden fencing (0.95-m tall). There were no breeding nests within the enclosure area. An artificial nest burrow (33  $\times$  33  $\times$  90-cm) was placed

in each corner of the rectangular enclosure. Two of the four burrows, diagonally opposite one another, had speakers placed 50-cm inside the burrow entrance (Sony, model SRS-77G; frequency response 80-20,000 Hz  $\pm$  1 dB). No speakers were placed in the other two burrows. In the middle of the enclosure, a lone female recently captured on the shoreline was placed in a circular plastic cage (56-cm in diameter) that allowed her to see outside, but not to leave.

After a 10-min setting period, the 45-min tapes were broadcast to the female. Firstly, three exhalation/inhalation sets (20-s) of Low Call were broadcast. After an interval of 30-s, three exhalation/inhalation sets (20-s) of High Call were played back, followed by another 30-s silent interval. Thus, Low and High Calls did not overlap one another, but alternated systematically. The type of playback broadcast first (Low or High Calls) was changed between tests. Five minutes after the first broadcast call, the female was released from the cage, using a remotely operated device to open the cage. The amplitude of playback was well above background noise levels. The volume control settings were standardized to 85 dB (the amplitude of a natural penguin advertising call) at 1-m from the speaker with a sound level meter (Techcessories, model 33-2050).

During the tests, we recorded how the female responded vocally to the playback. We also recorded her location in the enclosure every 10-min after releasing her from the cage. We conducted a total of 15 experiments on 15 different females. Females were recorded as approaching one of the four burrows if they moved to within 1-m of the burrow entrance (the 'approach area'). The distance between a burrow and the subject was further classified on a scale of 0 to 3 (0 = out of the approach area, 1 = between 50-cm and 1-m of the burrow, 2 = within 50-cm of the burrow, 3 = within the burrow). The four location scores we obtained (from the 10-min samples) were averaged for the 40-min observation period of each experiment; differences in the average scores in response to Low and High Call playback burrows were examined by using Wilcoxon signed rank tests. The percentage of samples we simply found the female within the approach area during the four observations per test were also examined between Low and High Call playback with a Wilcoxon signed rank test. We stopped counting approaches when the subject entered a burrow at any time and regarded her entrance as a final choice.

## **Experiment 2: Mate versus stranger calls**

The second experiment was conducted between 1900 and 2100 h from September to October (late in the courtship period) in 2000. Ten breeding pairs were investigated. Bray calls from the male of each pair were recorded using the methods described above. Five bray calls for use as stranger calls were recorded from five additional males. We measured the dominant frequency (kHz) of all the male calls we sampled. The 10 females of the selected pairs were individually tested to determine how they reacted when presented their mates' call in conjunction with a stranger's call. The experimental design was the same as described for the first experiment except the following playback tapes were used: (1) the bray call of the subject's partner; and (2) the bray call of a stranger. We used recorded calls without altering any call feature. We conducted a total of 15 experiments on the 10 females. Five of the 10 females were tested twice (once with a lower than average pitched call, and once with a higher pitched stranger call). Consecutive tests on a given female were always at least one day apart. Overall, eight females were tested by using a stranger's call lower in pitch than their partner's call pitch. Seven females were tested using a stranger call higher in pitch than their partner's call pitch. We did not include data from one female that did not adapt well to the enclosure (i.e. she moved quickly around and tried to get out of the enclosure).

## **RESULTS**

### **Experiment 1**

The dominant frequency of exhalations of the six advertising calls we sampled averaged  $1.30 \text{ kHz} \pm 0.11$  (mean  $\pm$  SD). The inter-individual coefficient of variation (CV; 10.0 %) was greater than the intra-individual CV (5.8 %) (see Jouventin 1982).

Ten of the 15 females approached Low Calls, and three of them entered the burrow. The remaining five females approached High Calls, and two entered the burrow. No females entered burrows in corners without playback. Overall, females were not more likely to approach Low Calls ( $\chi^2 = 1.06$ ,  $df = 1$ ,  $P = 0.30$ ).

Further, there were no differences in the averaged location score ( $P = 0.11$ ) or the likelihood of being in approach areas ( $P = 0.13$ ) associated with the playback of Low and High Calls (Table 1). During the 10-min observations periods, females also tended to be found in the approach area of Low Calls more often than that of High Calls (Table 1). There was no difference in the time subjects first entered the approach area between Low Calls and High Calls (Mann-Whitney U test,  $U = 16.0$ ,  $P = 0.26$ ). In four cases, females responded vocally to Low Calls with a single contact call (overlapping playback or just after playback), whereas no bird responded vocally to High Calls.

**Table 1.** Average location scores and the likelihood of being within approach areas (%) associated with Low and High Calls. The 95 % confidence interval (CI) is also shown.

Playback	Location score		95% CI	Being approach area		95% CI
	Mean	SD		Mean	SD	
Low Call ( $N = 15$ )	1.13	1.15	0.84 - 2.11	45.00	41.40	22.07 - 67.92
High Call ( $N = 15$ )	0.45	0.96	-0.08 - 0.98	18.33	34.67	-0.86 - 37.52

## Experiment 2

The 10 bray calls of subjects' mates we sampled had a mean dominant frequency of  $0.74 \text{ kHz} \pm 0.41$  (mean  $\pm$  SD). All three low-pitched stranger calls had a dominant frequency of  $0.34 \text{ kHz}$ , while two high-pitched stranger calls both had a dominant frequency of  $1.21 \text{ kHz}$ .

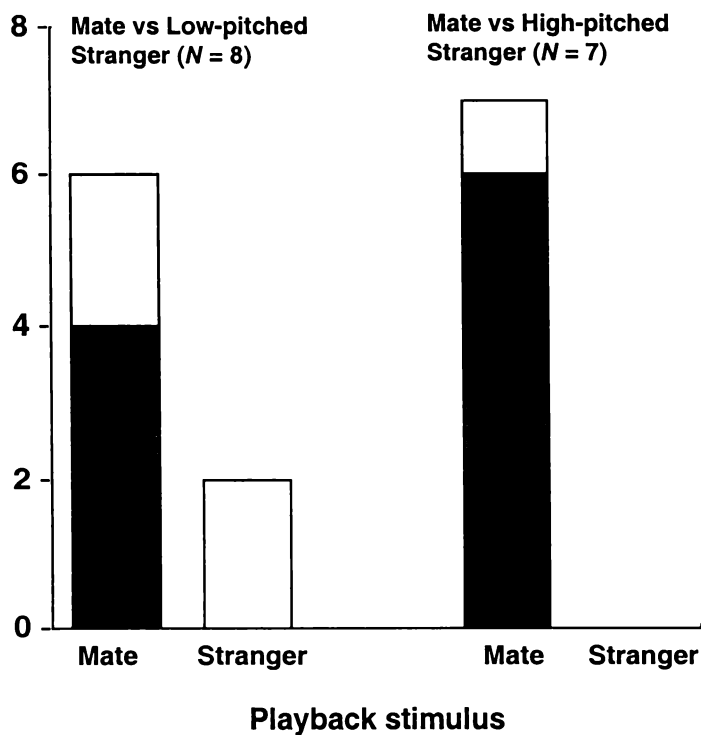
### *Mate versus low-pitched stranger calls*

Females tended to approach their mates' calls more often than low-pitched stranger calls, but the effect fell well short of statistical significance (Binominal test,  $P = 0.14$ ,  $N = 8$ , Fig. 1). In two of the eight tests, females responded vocally to their mate's playback. They called just after the mate's playback segments ended. In one trial, the female responded to the playback of her mate's call with a series of four contact calls over a period of 17-min (each contact call came just after her partner's call was broadcast). During the other trial, the female

responded to her mate's calls with a series of two contact calls over a period of 10-min (again, only after her partner's call was broadcast).

### *Mate versus high-pitched stranger calls*

Females approached their mates' calls and no birds approached the burrow broadcasting high-pitched stranger calls (Binominal test,  $P < 0.01$ ,  $N = 7$ , Fig. 1). In one of the seven tests, a female responded vocally to her mate's playback call. She called just after the mate's playback segments ended (two contact calls over a period of 7-min).



**Figure 1.** The number of females approaching playback of their own mate and a stranger (shaded, entering the burrow; open, occupying the 'approach area').

## **DISCUSSION**

Our results show that female Little Penguins responded vocally only to Low Calls, although there were no differences in the average location scores or the likelihood of being with the approach areas associated with Low and High Calls. However,

in playback experiments broadcasting current mate calls and stranger calls, many females approached the call of their own mate, but there was no statistically significant difference in the number of approaching birds between mate and low-pitched stranger calls.

### **Experiment 1**

Songbirds can detect very small differences in call pitch (reviewed by Ratcliffe & Weisman 1992). Nelson (1988) suggested that song pitch is the most important acoustic parameter in song recognition. In most species of penguins, the dominant frequency of calls varies considerably between individuals and penguins use this acoustic parameter for individual recognition (Jouventin 1982). The dominant frequency of male sexual calls is negatively correlated with body size in Little Penguins (Miyazaki & Waas, *in press*). Female Little Penguins may have more interest in lower-pitched male calls (Miyazaki & Waas, *in press*). We played back advertising calls, normally used by male Little Penguins to attract females. In the mating season, female Little Penguins often respond to male advertising calls by calling back, probably to maintain contact and attract the attention of the male; calls are generally followed by movement towards the male caller (*pers. obs.*). If a female is interested in forming a pair bond, calling would, therefore, facilitate a union. Thus, female vocal responses in our experiments may demonstrate ‘interest’ in the lower-pitched advertising calls (that may in turn indicate larger body size of the caller). However, the number of females that actually approached Low and High Calls did not differ statistically.

Larger males start to breed earlier than smaller ones, suggesting that they may acquire mates first because females prefer larger males and select them first (Miyazaki & Waas 2003). Chicks of larger males also grow at a faster rate than those of smaller males (Miyazaki & Waas 2003). Large males also have advantages in male-male competition during nest defense. As a result, larger males may obtain better breeding sites. Thus, female Little Penguins can improve their own reproductive potential by selecting larger males as partners (Miyazaki & Waas 2003). However, we would expect there to be an optimal size in female choice for larger males. Very large sizes may disadvantage individuals (e.g. through heat stress, loss of mobility for swimming), so these costs may eventually

override the benefits. Thus, females may not prefer just the largest males, but males of an 'optimally' large size. In our tests, we used only two dominant frequencies to represent large and small males (1.04 and 1.56 kHz) and found a pitch effect. However, it is likely that further reductions in pitch may eventually make the apparently large sized male less attractive to females. More tests would be necessary to determine the most 'attractive' or optimal pitch of male calls for female Little Penguins.

Other parameters of male advertising calls might also affect female responses. Larger male Little Penguins have longer exhalations as well as lower pitch in their advertising calls (Miyazaki & Waas, *in press*). Therefore, phrase duration or other call parameters may also affect female responsiveness to some degree. Our experiments, however, were designed to test only the influence of the dominant frequency on female responses. Thus, we cannot state whether any other call parameters were important during call selection, an issue that would be worth pursuing in future experiments.

## **Experiment 2**

In the first experiment, a pitch effect appears to occur when females are presented with two advertising strangers. In the second experiment, we predicted that females would be more likely to approach a low-pitched stranger call, in preference to her newly acquired mate's call, than a high-pitched stranger call. However, our results showed that, regardless of the dominant frequency of playback calls, most females approached recordings of their mates' calls rather than recordings of strangers. Acoustic signals can play an important role in mate recognition, and the ability of females to identify their partner's call has been well documented in penguins (Jouventin 1982). In Little Penguins, Winter (2000) played back bray calls to burrow-occupants and demonstrated mate recognition by recording changes in heart rates. Our experiments provide further support for identification abilities, with playback eliciting preferential approach and vocal responses to the current mate's playback (although we cannot exclude the possibility that recognition was simply based on an ability to discriminate familiar calls from unfamiliar ones). Paired females may prefer to maintain mate fidelity because of costs associated with divorcing the current mate. For example, females

who abandon mates may need to gain new information on nest site characteristics, defensive techniques of the mate and other routines that are settled by 'keeping company' with a potential mate. Intrasexual competition for the new mate, time spent further evaluating the new mate and the risk of breeding late or missing the breeding season are other important costs (Bried & Jouventin 2001). Therefore, paired females may normally avoid the costs of divorce even when there are opportunities to secure a higher quality partner.

In conclusion, the vocal responses of female Little Penguins to Low Calls might reveal their interest in the callers, but there was no obvious differences in the number of females approaching Low and High Calls. Females who already have partners approached the calls of their own mate more than those of stranger's regardless of the pitch of the stranger's call. The costs of re-forming a new relationship, even with a high quality male, may be too high by the end of the courtship period, promoting mate fidelity.

## ACKNOWLEDGEMENTS

We thank Barbara and Ray Walter on Tiritiri Matangi Island for invaluable help during this study. We are also grateful to Kim King, Ian Hogg, Laura Molles and Dai Morgan who commented on and improved the manuscript. The Penguin Fund Japan and the University of Waikato provided financial assistance. A permit to conduct research on Little Penguins was supplied by the Department of Conservation, New Zealand. The University of Waikato Animal Ethics Committee approved our experimental protocol.

## REFERENCES

- Bried, J. & Jouventin, P. 2001. Site and mate choice in seabirds: an evolutionary approach. In Schreiber, E.A. & Burger, J. (eds) *Biology of Marine Birds*: 263-305, Boca Raton, CRC Press.
- Bretagnolle, V., Genevois, F. & Mougeot, F. 1998. Intra- and intersexual functions in the call of a non-passerine bird. *Behaviour* 135: 1161-1184.

- Brooke, M. de L. 1978. Sexual differences in the voice and individual vocal recognition in the Manx Shearwater (*Puffinus puffinus*). *Anim. Behav.* 26: 622-629.
- Catchpole, C.K. 1980. Sexual selection and the evolution of complex songs among European warblers of the genus *Acrocephalus*. *Behaviour* 74: 149-166.
- Eens, M., Pinxten, R. & Verheyen, R.F. 1991. Male song as a cue for mate choice in the European Starling. *Behaviour* 116: 210-238.
- Gales, R. 1988. Sexing adult Blue Penguins by external measurements. *Notornis* 35: 71-75.
- Genevois, F. & Bretagnolle, V. 1994. Male Blue Petrels reveal their body mass when calling. *Ethol. Ecol. Evol.* 6: 377-383.
- Jouventin, P. 1982. *Visual and Vocal Signals in Penguins, Their Evolution and Adaptive Characters*. Berlin & Hamburg: Verlag Paul Parey.
- Jouventin, P. & Aubin, T. 2000. Acoustic convergence between two nocturnal burrowing seabirds: experiments with a penguin *Eudyptula minor* and a shearwater *Puffinus tenuirostris*. *Ibis* 142: 645-656.
- Márquez, R. & Bosch, J. 1997. Male advertisement call and female preference in sympatric and allopatric Midwife Toads. *Anim. Behav.* 54: 1333-1345.
- Miyazaki, M. & Waas, J.R. 2002. 'Last word' effects of male advertising calls on female preference in Little Blue Penguins. *Behaviour* 139: 1413-1423.
- Miyazaki, M. & Waas, J.R. 2003. Correlations between body size, defensive behaviour and reproductive success in male Little Blue Penguins *Eudyptula minor*: implications for female choice. *Ibis* 145: 98-105.
- Miyazaki, M. & Waas, J.R. *In press*. Acoustic properties of male advertisement and their impact on female responsiveness in Little Penguins *Eudyptula minor*. *J. Avian Biol.*
- Nelson, D.A. 1988. Feature weighting in species song recognition by the Field Sparrow (*Spizella pusilla*). *Behaviour* 106: 158-182.
- Ratcliffe L. & Weisman, R. 1992. Pitch processing strategies in birds: a comparison of laboratory and field studies. In McGregor, P.K. (ed) *Playback and Studies of Animal Communication*: 211-223. New York: Plenum Press.
- Ryan, M.J. 1980. Female mate choice in a neotropical frog. *Science* 209: 523-525.

- Storey, A.E. 1984. Function of Manx Shearwater calls in mate attraction. *Behaviour* 89: 73-89.
- Waas, J.R. 1988. Acoustic displays facilitate courtship in Little Blue Penguins, *Eudyptula minor*. *Anim. Behav.* 36: 366-371.
- Waas, J.R. 1990. Intraspecific variation in social repertoires: evidence from cave- and burrow-dwelling Little Blue Penguins. *Behaviour* 115: 63-98.
- Waas, J.R. 1991a. Do Little Blue Penguins signal their intentions during aggressive interactions with strangers? *Anim. Behav.* 41: 375-382.
- Waas, J.R. 1991b. The risks and benefits of signalling aggressive motivation: a study of cave-dwelling Little Blue Penguins. *Behav. Ecol. Sociobiol.* 29: 139-146.
- Winter, S.J. 2000. Heart rate as a measure of individual recognition in Little Blue Penguins (*Eudyptula minor*). MSc Thesis, The University of Waikato, New Zealand.

## **Chapter 6**

# **‘Last word’ effects of male advertising calls on female preference in Little Penguins**

## ABSTRACT

Playback experiments were conducted to investigate how advertising call overlap between male Little Penguins (*Eudyptula minor*) influenced other birds along shorelines in front of breeding colonies. We used a dual-speaker design in which calls from one speaker consistently overlapped those from the other speaker. Females approached overlapping advertising calls more than overlapped calls or a silent control speaker. At a proximate level, this might have occurred because 'masking effects' obscure information in overlapped calls that females require for mate choice. Alternatively, females may simply be able to locate overlapping calls (i.e. the last call they heard) more easily. Young birds and adult males did not distinguish between calling patterns. It is possible that call overlap is associated with dominance or other quality indicators; thus, females may approach overlappers more often because they represent higher quality mates.

## INTRODUCTION

Interacting songbirds often adjust the timing of songs, perhaps to avoid acoustic overlap (Hultsch & Todt 1982, Naguib & Todt 1997). In Robins (*Erithacus rubecula*), overlapping songs elicited greater aggressive responses than alternating songs (Dabelsteen *et al.* 1997). Dabelsteen *et al.* (1997) suggested that, by overlapping the songs of rivals, males might signal a dominant status, and that an 'audience' may collect information from such contests by eavesdropping. The masking effect of call overlap reduces the possibility that information in a given call is received by perceivers, such as females attempting to select a mate (Naguib & Todt 1997). In some species of anurans, females prefer males who produce leading calls (e.g. Greenfield *et al.* 1997). In contrast, other species have important information in the middle or terminal part of male calls, so females pay more attention to overlapping calls (e.g. Hultsch & Todt 1982). In seabirds, Jouventin (1982) reported that Emperor Penguins (*Aptenodytes forsteri*) stopped calling to avoid being overlapped or having their signal jammed by playback. However, the effect of call overlap in colonial seabirds has not been explored in any detail.

Little Penguins (*Eudyptula minor*) are wholly nocturnal on land and live around the mainland and offshore islands of New Zealand and southern Australia (Reilly 1994). Their vocalisations have been thoroughly investigated (Waas 1988, 1990, 1991a,b, Jouventin & Aubin 2000, Nakagawa *et al.* 2001, Miyazaki & Waas 2003) because their courtship and agonistic behaviours have conspicuous acoustic elements. The influences of visual behaviour are limited by their nocturnal habits on land. The loud calls of solitary male penguins, given early in the breeding season, appear to be socially facilitated (Waas 1988, Waas *et al.* 2000) and may be crucial for female choice. As a result, females in large colonies will perceive the calls of many males and may be attentive to vocal interactions between males. Male Little Penguins may cluster in small clubs in cave populations or as individuals (> 3 – 4 m apart) in front of burrow populations (Waas 1988, 1990, *pers. obs.*) while advertising. They frequently overlap one another's songs (*pers. obs.*), but the significance of this behaviour in male-male or male-female interactions has never been assessed. Bill measurements can be used to sex penguins reliably in the field. Therefore, we consider penguins to be ideal subjects for examining the consequences of song overlap for both males and females in the wild.

The main objective of our research was to investigate how vocal interactions between males may influence a variety of listeners including male, female and young Little Penguins. In particular, we focused on the potential effect of overlap on female call preference. We predicted that females would be attracted to overlapping calls if overlap correlated with dominance or other quality indicators; we expected the opposite trend for males because individuals may avoid dominant rivals. We conducted playback experiments using a dual-speaker design in which songs broadcast from one speaker overlapped songs broadcast from the other speaker. We provide the first empirical study of the effect of song overlap on colonial seabird behaviour. Little information is available on the process of mate choice in seabirds in general and penguins in particular. As a result, this research also provides important comparative data to advance our general understanding of the mate choice process.

## METHODS

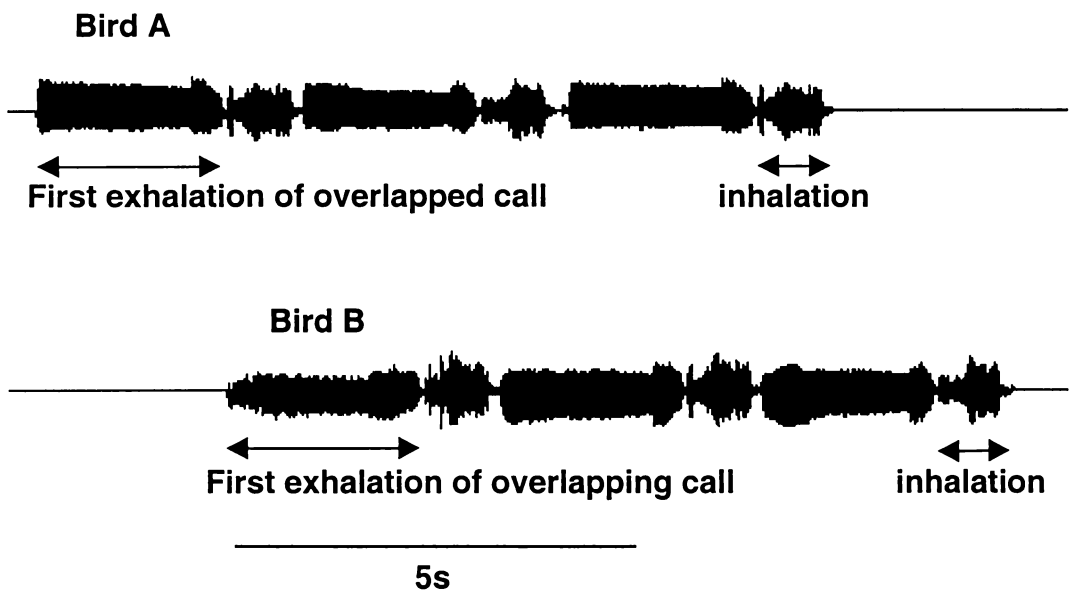
### Study area

We conducted playback experiments on Little Penguins on Motunau Island, New Zealand (43°08'S, 173°10'E). The penguins on the island start to breed in late August or September every year and are considered members of the 'white-flipped penguin' (*E. m. albosignata*) subspecies (Kinsky & Falla 1976). Advertising calls are typically used by unmated males who usually call from rocky shorelines in front of burrow areas before sunrise and after sunset during the pairing stage of the breeding season (Waas 1988).

### Analysis and structure of playback songs

The advertising songs used for playback were recorded on 31 July 1984 from the Wainui Cave on Banks Peninsula, New Zealand; the Banks Peninsula is also within the white-flipped subspecies' range. For playback, we obtained 10 high quality advertising calls from each of 10 males; calls were recorded on a Uher 4000 Report-L (frequency response 20-16,000 Hz, tape speed 9.5 cm/s) with a Nakamichi CM 300 microphone (frequency response 20-16,000 Hz  $\pm$  3.5 dB). Songs from each individual were digitised (at 44,000 Hz with 16-bit accuracy), adjusted to the same recording level and then copied using a Sony Digital Audio Tape-Coder (DAT, model TCD-D7; sampling frequency 44.1 kHz, frequency response 20-22,000 Hz  $\pm$  1 dB). To compose playback tapes, we used only full 'exhalation/inhalation' brays performed in the middle of an advertising call bout, avoiding the incomplete versions often given at the beginning of a bout. Each call we sampled consisted of three exhalation/inhalation phrases. Thus, we sampled 30 phrases from the 10 calls we obtained from each of the 10 recorded males. Jouventin (1982) suggested that the call frequencies of Little Penguins vary considerably between individuals while call duration and syllable length show less 'individuality' (Jouventin 1982). Thus, we controlled the frequency, as outlined below, to avoid possible pitch-related effects on responses to playbacks. We can not exclude the possibility that other acoustic properties contributed to responses of birds. The 10 advertising calls we recorded had a mean peak frequency of 1.10 kHz (range 0.88 – 1.25 kHz), as determined by producing sonograms using the 80

Hz high pass filter with Canary 1.2.4 software on a Macintosh computer. An ANOVA across the males we sampled indicated that there were significant differences between individuals in peak frequency ( $F_{9,20} = 6.16, P < 0.001$ ). As a result, we selected five pairs of calls that had similar peak frequencies (the difference in frequency between any two paired calls was less than 0.06 kHz, the average difference in peak frequency between paired calls was 0.04 kHz) to avoid pitch-related effects; 10 playback tapes were then created from these recordings. Each call in a given pair played the role of overlapper on one tape and the role of overlapped call on another tape. Calls were edited in stereo so that the first male's call (from one speaker) consistently overlapped the call of the second male (played by the other speaker). Each playback consisted of three exhalation/inhalation sets from each of the males. The overlap call started after the first exhalation of the overlapped call (Fig. 1). The playback tapes consisted of approximately 10 s vocal segments separated by 60 s silent periods. The total duration of each playback tape was two hours.



**Figure 1.** Example of call overlap on the playback tapes. The song of Bird B overlaps that of Bird A after the first exhalation. The short pulses between exhalations are inhalations.

## Experimental design

We carried out the two-hour playback experiments from 8 to 16 August 2000 between 1900 and 2100 h (the first experiment), and between 2130 and 2330 h (the second experiment). It was dark when the first experiments were started at this point in the season. Along the island's beaches, three circular arenas (8 m diameter), positioned side-by-side and parallel to the shoreline, were created: (1) an 'overlapping arena', (2) an 'overlapped arena' and (3) a 'control arena' (all marked with stones bound with brightly coloured tape placed along the outer edge of each arena). The natural distance between callers varies depending on numbers of birds ashore (*pers. obs.*), but the average distance at this time in the year was about 8 – 10 m (*pers. obs.*). There were no breeding nests within these shoreline experimental arenas. An amplified speaker (Sony, model SRS-77G; frequency response 80-20,000 Hz  $\pm$  1 dB) was placed centrally within each playback arena (pointing out to sea). In the control arena, a speaker was placed in the same way but no sound was broadcast from it. The observer was positioned behind obstacles such as rocks or shrubs about 10 m behind the centre of the experimental arenas. The two playback speakers were connected directly to the DAT; by using stereo playback, we broadcast the overlapped and overlapping vocalisations in the appropriate arenas. The volume of playback calls was adjusted to 85 dB at 1 m (the approximate amplitude of a natural Little Penguin advertising call) from the speaker with a sound level meter (Techcessories, model 33-2050).

We used a different pair of playback tapes for the two experiments that occurred in the same night. The order in which playback tapes were broadcast and the position of each treatment arena (centre, right or left) were randomised to minimise locational biases (but the control arena was never set between overlapping playbacks and overlapped playbacks).

Before conducting experiments, we checked each experimental arena and confirmed that there were no birds present. When a bird approached a speaker within 4 m, it was regarded as 'selecting' the call or condition (i.e. the overlapper, the overlapped or the control). We entered the treatment arenas every 30 min to count the number of birds within each arena, measure their bills for sexing and to check their flipper bands. Playbacks continued throughout the counting procedure. After processing, birds were released well away from experimental areas.

According to their flipper bands and bill measurements, we did not find the same bird in any experimental arena during the same night. The second experiment conducted each evening was performed on another stretch of beach at least 24 m from the edge of the first site. We conducted 18 experiments at 18 different and widely separated sites during our nine days on the island. Differences in the number of birds found within each arena were examined using one-way factorial ANOVAs and Fisher's multiple comparisons tests.

### **Sexing of birds**

To sex the birds, bill length and depth were measured with standard calipers (Kinsky 1960). On the island, more than 13,000 Little Penguins have been banded as nestlings, measured and monitored regularly by Dr. C. N. Challies (Christchurch, New Zealand) over the past 30 years. Dr. Challies sexed the adult birds we captured using a bill measurement formula he developed that can discriminate males from females with > 95 % accuracy (see Gales 1988 and Renner & Davis 1999, for similar sexing methods developed for other Little Penguin subspecies). The bill of adult male Little Penguins is slightly bigger than the females'. However, the bills of young males (< 2 years) can have similar dimensions to adult females. To assist us in assessing the proportion of young birds on the island at the time of the experiments, one researcher captured banded birds on the shoreline around the island and checked their ages between 1900 and 2100 h each night while the other researcher conducted the experiments. This allowed us to estimate the ratio of mature females to young birds (males or females) within treatment arenas.

## **RESULTS**

### **Estimating the adult:young ratio of landing birds**

We captured a total 178 banded birds on the Island's shorelines to obtain information on their age distribution. Twenty-nine (16.3 %) of 178 birds were one year and eight months of age (defined as young birds), seventeen birds (9.6 %) were two years and eight months of age and the rest (74.1 %) were more than three years of age. Therefore, we estimated the proportion of adult birds to be

83.7 % of all landing birds during the experimental period.

### **Sexing birds in the treatment arenas**

A total of 67 birds from our treatment arenas were captured and measured during the course of playback experiments. According to bill dimensions, there were 33 (49.3 %) males, 7 (10.4 %) definite adult females, and 27 (40.3 %) adult females and/or young birds.

According to Dr. Challies' formula, 14 of the 27 unknown birds were 'probable' females (i.e. they had bill measurements that fell in the lowest range for two year old males). Thus, the number of females was assessed as being 21 (31.3 % of the total captured) and the number of young birds was thought to be 13 (19.4 %). This proportion of young birds was very similar to the estimated proportion of young birds obtained independently during the shoreline survey (16.3 %; see above).

Also, we estimated the total number of adult females in the following way. Twenty-seven of the 67 measured birds in the arenas could not be sexed with total certainty (i.e. they were either young males or adult females). If 83.7 % of the full sample of 67 were adults (as suggested by the shore survey), about 11 young occurred in the experimental arenas. This would suggest that 16 of the 27 birds we could not sex conclusively were females. Therefore, the total number of females was estimated to be 23 (16 + 7 females that could be sexed conclusively); this value is very close to value (21) derived from Dr. Challies' estimates, so we used Dr. Challies' assignments for the final analysis.

### **Distribution of birds in the playback arenas**

The mean number of birds found within the three experimental arenas during playback experiments are shown in Table 1. To compare the number of birds found in the three different treatment arenas, we excluded the data from tests where no bird of a given type (i.e. male, female or young) entered any of the treatment arenas. We recorded males in 12 of the 18 experiments. There was no statistically significant difference in the number of males found within each treatment arena ( $F_{2, 33} = 2.56$ ,  $P = 0.09$ ; Table 1). However, more females (including 'probable females'; see above) were found within the overlapping

treatment arenas than within the overlapped or control arenas during the 7 of 18 experiments they were recorded as being present ( $F_{2,18} = 4.36$ ,  $P < 0.05$ ; Table 1). There was no significant difference in the number of young birds found in each arena during the 11 of 18 tests they were recorded as being present ( $F_{2,30} = 2.16$ ,  $P = 0.13$ ; Table 1). When we analysed the data without considering young birds (i.e. we viewed all unknown birds as adult females), females tended to appear more within overlapping arenas than overlapped and control arenas during the 12 of 18 tests in which they were present, but the effect fell just short of statistical significance ( $F_{2,33} = 3.02$ ,  $P = 0.06$ ).

In 2 of the 18 tests, two birds responded vocally to playback. They called just after the playback segments ended; both were females as assessed by bill dimensions. In one test, a female approached overlapping playback with a single contact call. During another test, a female approached and responded to overlapped playback with a series of 15 contact calls over a period of 16 min.

**Table 1.** The mean  $\pm$  SD number of birds found within each treatment arena. We excluded data from tests where no bird landed on any areas ( $N$  shows the number of tests that each type of individual was represented).

Treatment	Overlapping	Overlapped	Control
Males ( $N=12$ )	$1.08 \pm 1.08^a$	$1.25 \pm 0.87^a$	$0.42 \pm 0.90^a$
Females ( $N=7$ )	$2.14 \pm 1.95^a$	$0.43 \pm 0.54^b$	$0.43 \pm 0.79^b$
Young ( $N=11$ )	$0.25 \pm 0.31^a$	$0.06 \pm 0.08^a$	$0.09 \pm 0.25^a$

Values in each row with the same letter in superscript were not significantly different (Fisher's multiple comparison tests;  $P < 0.05$ ).

## DISCUSSION

Our results show that female Little Penguins approached speakers broadcasting overlapping advertising calls more than those playing overlapped calls or a silent control speaker. However, young birds and adult males did not distinguish between calling patterns although they tended to enter playback arenas more often than control arenas. When we include 'probable' females in the total female sample, females also tended to approach overlapping arenas more often.

Birds may be attracted by the vocalisations or the sight of conspecifics to avoid being predated (Lack 1968); some penguins may have approached arenas because of the presence of other responding birds (to take advantage of a 'safety in numbers' effect), rather than interest in a specific type of playback treatment (but note that the only terrestrial predators of Little Penguins in New Zealand are likely to be introduced species). In our study, however, most birds that approached each experimental arena did not respond vocally (only two females responded to playback calls by using contact calls).

Overlapping experiments using territorial birds have been conducted in previous studies (e.g. Dabelsteen *et al.* 1996, 1997, Naguib & Todt 1997, Naguib 1999). These studies demonstrated that overlapping playbacks indicated more aggressive rivals and escalated territorial behaviours of the subject. For example, Dabelsteen *et al.* (1997) found that male robins responded more intensity to overlapping in comparison to alternating playbacks. Our study shows that males tended to ( $P = 0.09$ ) occur more often within playback arenas than control arenas. Male Little Penguins frequently form groups or clubs of calling males in cave populations (Waas 1990). Males may be attracted to other callers, because their chances of being predated would be lower if they called near other birds (Lack 1968). However, in our study, males that approached the playbacks did not call themselves. Perhaps males simply enhance their chances of encountering a female by positioning themselves near males that are actively advertising. They may gain the benefits of the caller's efforts while avoiding the costs (e.g. predation).

It has been suggested that overlapping calls could attract females (e.g. Naguib & Todt 1997, Otter *et al.* 1999). Naguib & Todt (1997) suggested that overlapping songs signal dominance and dominant males may constitute higher quality partners. Female great tits (*Parus major*) approached the territories of males de-escalated by playback, probably to obtain information on the callers (Otter *et al.* 1999). Otter *et al.* (1999) suggested that visiting females may have been seeking extra-pair copulations with males who had their status or quality enhanced by the playback treatments. Our results show that females might be more attracted to overlapping than overlapped advertising calls. This might be because important information near the terminal section of calls becomes unavailable for overlapped males (Naguib & Todt 1997, Naguib *et al.* 1999) or

females may simply be able to locate overlapping calls (i.e. the last call they heard) more easily. In Little Penguins, the inhalation section of male advertising calls might differ between sub-species (Banks *et al.* 2002). Variation between individuals has not been examined in detail yet but Miyazaki & Waas (2003) showed that important information on male size and quality may be encoded by the calls of Little Penguins. In our study, overlapping calls started after the first exhalation of overlapped calls. The last inhalation section of overlapping calls was not masked. If the inhalation section of male advertising calls has important information on male quality, overlapping advertising calls might be more successful than overlapped calls attracting potential mates. Future work is required to determine if overlap really provides females with information on male quality.

Young Little Penguins are defined as being less than two years old. Because some birds first attempt to breed as two or three year olds (Dann & Cullen 1990), we expected to attract many young females to the playback. There was no significant difference ( $P = 0.13$ ) in the number of young birds that approached playbacks among the three experimental arenas. However, the sample size for young birds was small. The low numbers of young recorded in our shoreline surveys (16.3 %) suggested that young birds had not yet returned to breed in the season. Because young birds are most likely to be searching for partners, we would expect more and more young females to be attracted to male advertising calls as the season progressed.

In addition to young birds, adults that 'divorced' their previous partners are likely to look for new mates at the beginning of the breeding season. Little Penguins are monogamous but the probability of divorce is approximately 18 % per year (Reilly & Cullen 1981). These divorced birds nest earlier than birds remaining with their former partners (Reilly & Cullen 1981). As our experiments were performed early in the breeding season, many of the females that appeared in the treatment arenas could have been divorced birds. Even paired females willingly accept the company of other males when they spend time on land alone (Kinsky 1960).

## ACKNOWLEDGEMENTS

We thank Chris Challies for invaluable help during this study. The manuscript was further improved by useful comments from J. T. Darby, L. Molles, T. Aubin and the anonymous referees. Our research was supported by the Penguin Fund Japan and grants from the University of Waikato. A permit to work on Motunau Island was supplied by the Department of Conservation, New Zealand. The University of Waikato Animal Ethics Committee approved our experimental protocol.

## REFERENCES

- Banks, J.C., Mitchell, A.D., Waas, J.R. & Paterson A.M. 2002. An unexpected pattern of molecular divergence within the Blue Penguin (*Eudyptula minor*) complex. *Notornis* 49: 29-38.
- Dabelsteen, T., McGregor, P.K., Shepherd, M., Whittaker, X. & Pedersen, S.B. 1996. Is the signal value of overlapping different from that of alternating during matched singing in Great Tits? *J. Avian Biol.* 27: 189-194.
- Dabelsteen, T., McGregor, P.K., Holland, J., Tobias, J.A. & Pedersen, S.B. 1997. The signal function of overlapping singing in male Robins. *Anim. Behav.* 53: 249-256.
- Dann, P. & Cullen, J.M. 1990. Survival, patterns of reproduction and lifetime reproductive output of Little Penguins *Eudyptula minor* on Phillip Island, Victoria, Australia. In Davis, L.S. & Darby, J.T. (eds) *Penguin Biology*: 63-84. San Diego: Academic Press.
- Gales, R. 1988. Sexing adult Blue Penguins by external measurements. *Notornis* 35: 71-75.
- Greenfield, M.D., Tourtellot, M.K. & Snedden W.A. 1997. Precedence effects and the evolution of chorusing. *Proc. Roy. Soc. Lond.,B* 264: 1355-1361.
- Hultsch, H. & Todt, D. 1982. Temporal performance roles during vocal interactions in Nightingales (*Luscinia megarhynchos*). *Behav. Ecol. Sociobiol.* 11: 253-260.

- Jouventin, P. 1982. *Visual and Vocal Signals in Penguins, Their Evolution and Adaptive Characters*. Berlin & Hamburg: Verlag Paul Parey.
- Jouventin, P. & Aubin, T. 2000. Acoustic convergence between two nocturnal burrowing seabirds: experiments with a penguin *Eudyptula minor* and a shearwater *Puffinus tenuirostris*. *Ibis* 142: 645-656.
- Kinsky, F.C. 1960. The yearly cycle of the Northern Blue Penguin (*Eudyptula minor novaehollandiae*) in the Wellington Harbour area. *Rec. Dom. Mus. Wellington* 3: 145-218.
- Kinsky, F.C. & Falla, R.A. 1976. A subspecific revision of the Australasian Blue Penguin (*Eudyptula minor*) in the New Zealand area. *Rec. Nat. Mus. NZ.* 1: 105-126.
- Lack, D. 1968. *Ecological Adaptations for Breeding in Birds*. London: Methuen.
- Miyazaki, M. & Waas, J.R. 2003. Correlations between body size, defensive behaviour and reproductive success in male Little Blue Penguins *Eudyptula minor*: implications for female choice. *Ibis* 145: 98-105.
- Naguib, M. 1999. Effects of song overlapping and alternating on nocturnally singing Nightingales. *Anim. Behav.* 58: 1061-1067.
- Naguib, M., Fichtel, C. & Todt, D. 1999. Nightingales respond more strongly to vocal leaders of simulated dyadic interactions. *Proc. Roy. Soc. Lond., B*, 266: 537-542.
- Naguib, M. & Todt, D. 1997. Effects of dyadic vocal interactions on other conspecific receivers in Nightingales. *Anim. Behav.* 54: 1535-1543.
- Nakagawa, S., Waas, J.R. & Miyazaki, M. 2001. Heart rate changes reveal that Little Blue Penguin chicks (*Eudyptula minor*) can use vocal signatures to discriminate familiar from unfamiliar chicks. *Behav. Ecol. Sociobiol.* 50: 180-188.
- Otter, K., McGregor, P.K., Terry, A.M.R., Burford, F.R.L., Peake, T.M. & Dabelsteen, T. 1999. Do female Great Tits (*Parus major*) assess males by eavesdropping? A field study using interactive song playback. *Proc. Roy. Soc. Lond., B*. 266: 1305-1309.
- Reilly P.N. & Cullen, J.M. 1981. The Little Penguin *Eudyptula minor* in Victoria, II. Breeding. *Emu* 81: 1-19.

- Reilly P.N. 1994. *Penguins of the World*. Australia: Oxford University Press.
- Renner, M. & Davis, L.S. 1999. Sexing Little Penguins (*Eudyptula minor*) from Cook Strait, New Zealand, using discriminant function analysis. *Emu* 99: 74-79.
- Waas, J.R. 1988. Acoustic displays facilitate courtship in Little Blue Penguins, *Eudyptula minor*. *Anim. Behav.* 36: 366-371.
- Waas, J.R. 1990. Intraspecific variation in social repertoires: evidence from cave- and burrow-dwelling Little Blue Penguins. *Behaviour* 115: 63-98.
- Waas, J.R. 1991a. Do Little Blue Penguins signal their intentions during aggressive interactions with strangers? *Anim. Behav.* 41: 375-382.
- Waas, J.R. 1991b. The risks and benefits of signalling aggressive motivation: a study of cave-dwelling Little Blue Penguins. *Behav. Ecol. Sociobiol.* 29: 139-146.
- Waas, J.R., Caulfield, M., Colgan, P.W. & Boag, P.T. 2000. Colony sound facilitates sex and agonistic activities in Royal Penguins. *Anim. Behav.* 60: 77-84.

# Chapter 7

## Conclusions

The aim of my Ph.D research was to investigate the influence of male size on reproductive success (Chapter 2 & 3), and relationships between male attributes and vocalisations that might affect female mating decisions in Little Penguins (Chapter 4, 5 & 6). The advantages of large body size in male-male competition (e.g. Arak 1983, Rowland 1989), parental care (e.g. Brown 1981, Côte & Hunte 1989) and feeding ability (e.g. Ryan 1980, Wiklund & Kaitala 1995) have been documented in many species. I focused my attention on how body size effects reproductive success and mate choice. Further, I compared the contribution that males make to offspring with that of females, separately, and found that male size has the most important impact on reproductive success in Little Penguins. Large males demonstrated potentially superior defensive behaviour and obtained better nest sites for breeding. However, male size did not correlate with several important parameters like fledging age and mass of offspring. (Chapter 2 & 3). Furthermore, chicks of larger males grew at a faster rate than those of smaller males (Chapter 2 & 3). Parents need to obtain enough food for their offspring and themselves during the chick rearing stage. Because large birds require more food to support their large size (Blanckenhorn 2000), they should theoretically be capable of obtaining more food than small birds (de León *et al.* 1998) (Chapter 3). However, I found no difference between larger and smaller males in the meal size delivered to offspring. The results might simply reflect a lack of statistical power, so additional research may be required with greater sample sizes to examine this possibility. Alternatively, larger males might provide better quality food to offspring than smaller ones. If diving ability varies allometrically with body size (Costa 1991), larger birds may access prey in deep water more readily and may thus obtain high quality prey. Further studies are needed to assess differences in diet between larger and smaller males (e.g. by using (1) stomach pumping techniques to obtain samples from birds arriving ashore to feed their offspring, (2) radio-tracking and satellite telemetry to monitor the foraging behaviour of large

and small males, and (3) electronic data loggers to obtain information on differences in diving behaviour). An alternative explanation for the difference in chick growth between larger and smaller males may relate to paternal genetic effects on chick growth, though female size was not related to the growth rate of offspring. Male chicks that have fathers of large size may become fathers of chicks that also grow at a faster rate. The heritability of growth rates between chicks and parents would be worth pursuing further in future research.

Females may select larger males to obtain benefits for their offspring. Larger males mated earlier than smaller ones, suggesting that females selected larger males first (Chapter 2); however, the relationship could simply be the result of experienced or established pairs (i.e. older pairs) occupying breeding colonies earlier in the season, and preferring sites near the sea (Chapter 3). However, the size-elevation relationship only existed for males, suggesting that my results are better explained as the result of large males obtaining mates (of any size) earlier than small males and then selecting preferred low elevation nests.

The importance of acoustic signals for female choice in birds has been thoroughly investigated (e.g. Kroodsma 1976, Eriksson & Wallin 1986, Searcy & Yasukawa 1990). However, little information is available on the role of vocalisations in female choice of seabirds (e.g. Genevois & Bretagnolle 1994), although individual recognition based on acoustic signals has been thoroughly investigated in previous studies (e.g. Jouventin 1982). In Adélie Penguins, a negative relationship between the pitch of male advertising calls and male size has been documented by Davis and Speirs (1990), but female mating decisions in relation to male calls had not yet been experimentally explored in any penguin species. Little Penguins are nocturnal on land. Because their courtship and agonistic activities occur at night, their acoustic signals, in particular, may play an important role in communication. If male calls encode information on the quality or characteristics of the caller (e.g. body size), females should be very attentive to the signals. In Chapter 4, I identified acoustic parameters of male advertisement that could reveal information on the quality of the caller. The dominant frequency in the exhalation phrase of male advertising calls was negatively related to male size, particularly body mass. I used several body size parameters in addition to body mass in this study; previous studies used only body weight as an indicator of

body size (Badyaere & Leaf 1997, Tubaro & Mahler 1998). Because body mass of seabirds is variable within individuals (Warham 1990), the call parameter may also vary to some degree within individuals. Thus, several samples of calls and weights would be needed from each caller to determine the 'average size' and 'average call pitch'.

Because the dominant frequency of male advertising calls provided reliable information on body size, females might distinguish larger from smaller males by listening to male calls. In the playback experiments reported in Chapter 4, females who occupied nests were more likely to respond vocally to medium- and lower-pitched advertising calls than to higher-pitched calls. However, more than 70 % of the females did not respond vocally to any playback. In two speaker choice tests using lower- and higher-pitched calls (Chapter 5), vocal responses were only associated with Low Calls, but there were no differences in terms of the female's approaches with respect to Low and High Calls. Females might respond to lower-pitched calls in order to obtain directional information from the caller (i.e. to facilitate a union). If a female preference for low-pitched calls occurs, this may exert directional selection for larger males.

Furthermore, as reported in Chapter 5, paired females during the late courtship period were likely to maintain mate fidelity probably even when there were opportunities to obtain a higher quality partner. If paired females change their partners, they may need to spend additional time evaluating the new mate as a breeding partner. In addition, they are exposed the risk of late breeding or missing a breeding opportunity altogether (Bried & Jouventin 2001). Therefore, paired females may remain faithful to avoid costs associated with divorcing the current mate and establishing a new relationship. It would be interesting to examine paired females' responsiveness early in the courtship period. If females have kept company with the current partner for only a couple of days, they may be more likely to abandon the mate for a more 'attractive' male.

In the study of acoustic communication, calling patterns as well as call parameters may provide important information in the context of male-male interactions and female choice. For example, the masking effects of call overlap may obscure information in overlapped calls that females require for mate choice (Naguib & Todt 1997). Females in large Little Penguin colonies would perceive

the calls of many males and may be attentive to vocal interactions between males. My two speaker choice tests investigating call overlap (Chapter 6) suggested that females approached overlapping advertising calls more than overlapped calls possibly because important information near the terminal section of the calls became unavailable for overlapped males (Naguib & Todt 1997, Naguib *et al.* 1999). Alternatively, females might simply be able to locate overlapping calls (i.e. the last call they heard) more easily. Naguib and Todt (1997) suggested that song overlappers may be signalling dominance and that dominant males might represent higher quality partners. If the 'last word' of male advertising calls has important information on the quality of the caller, overlapping advertising calls might also be a successful way of avoiding overlap by other callers (Chapter 6). If song overlap signal dominance, and dominant males make better partners, females may prefer overlappers (Naguib & Todt 1997).

My Ph.D research has revealed that male acoustic signals (e.g. dominant frequency and calling patterns), may reveal information on the quality of the caller and affect female choice in Little Penguins. In previous studies of acoustic communication in penguins, calling behaviour and acoustic characteristics in sexual calls have also been investigated (e.g. Jouventin 1982). However, the relationship between male calls and female choice has never been assessed. Thus, my research provides the first empirical data on the effects of male vocalisations on female choice in penguins. Females may prefer larger males because they represent reproductively superior males, but larger males did not fledge more young. If body size is positively correlated with age, females may simply be selecting larger (older) males on the basis of reproductive experience. However, body size as determined by PCA (performed on bill length, bill depth, head length, foot size and flipper size) was not related to age in my data (males:  $N = 19$ ,  $R = 0.18$ ,  $P = 0.44$ , range 3.3 – 8.3 years old; females:  $N = 15$ ,  $R = 0.08$ ,  $P = 0.76$ , range 1.3 – 8.4 years old; *unpublished data*). Therefore, larger males of any age may be preferred by females. By selecting larger males, females may obtain larger sized offspring that may be preferred by future mates leading to directional selection. In addition to the size-benefits I found, large body size might also reveal nutritional condition (i.e. fat stores). Good fat stores may reflect a successful forager. Large male size may also give an advantage in contests over

females or resources. In addition, large size may improve a male's endurance, allowing him to court and contest resources more effectively. However, I would expect there to be an optimal male size. Extremely large males may suffer disadvantages (e.g. the cost of heat stress, reduced agility, time and energy cost of supporting large size), so the costs may eventually exceed the benefits. Optimization supposes that an equivalence of costs and benefits determines the value of a trait within the range of available variation (Gadgil & Bossert 1970). It would be interesting to examine female responsiveness to male calls of various pitches by conducting playback experiments such as those reported in Chapter 5. More tests may reveal an 'optimal pitch' from the female's perspective that may reflect 'optimal male size'.

Because most size-success correlations were found to be associated with male size, sexual dimorphism in Little Penguins may be driven by female preference for larger males. As I described in Chapter 1, bill depth is one of the most sexually dimorphic physical characteristics in penguins. According to my review of previous studies listed in Marchant and Higgins (1990), variation in bill depth (on average in all subspecies) of male Little Penguins is the largest of any species of penguins. Little Penguins have six subspecies while other penguin species have 0 – 3 subspecies. Thus, the large variation on size in Little Penguins may be related to the number of subspecies (i.e. there may be differences in body size between subspecies). However, four of the six Little Penguin subspecies have larger variation in bill depth than other penguin species. Strong directional selection for large size in Little Penguins may promote variability.

## REFERENCES

- Arak, A. 1983. Sex differences in choosiness: Male-male competition and mate choice in anuran amphibians. In Bateson, P. (ed.) *Mate Choice*: 181-210. Cambridge: Cambridge University Press.
- Badyaev, A.V. & Leaf, E.S. 1997. Habitat associations of song characteristics in *Phylloscopus* and *Hippolais* warblers. *Auk* 114: 40-46.
- Blanckenhorn, W.U. 2000. The evolution of body size: what keeps organisms small? *Q. Rev. Biol.* 75: 385-407.

- Bried, J. & Jouventin, P. 2001. Site and mate choice in seabirds: an evolutionary approach. In Schreiber, E.A. & Burger, J. (eds) *Biology of Marine Birds*: 263-305. Boca Raton: CRC Press.
- Brown, J. 1981. Patterns of female choice in Mottled Sculpins (*Cottidae*, *Teleostei*). *Anim. Behav.* 29: 373-382.
- Costa, D.P. 1991. Reproductive and foraging energetics of high latitude penguins, albatrosses and pinnipeds: implications for life-history patterns. *Am. Zool.* 31: 111-130.
- Côte, I.M. & Hunte, W. 1989. Male and female mate choice in the Redlip Blenny: why bigger is better. *Anim. Behav.* 38: 78-88.
- Davis, L.S. & Speirs, E.A.H. 1990. Mate choice in penguins. In Davis, L.S. & Darby, J.T. (eds) *Penguin Biology*: 377-397. San Diego: Academic Press.
- Eriksson, D. & Wallin, L. 1986. Male bird song attracts females - a field experiment. *Behav. Ecol. Sociobiol.* 19: 297-299.
- Gadgil, M. & Bossert, W. 1970. Life history consequences of natural selection. *Am. Nat.* 104: 1-24.
- Genevois, F. & Bretagnolle, V. 1994. Male Blue Petrels reveal their body mass when calling. *Ethol. Ecol. Evol.* 6: 377-383.
- Jouventin, P. 1982. *Visual and Vocal Signals in Penguins, Their Evolution and Adaptive Characters*. Berlin & Hamburg: Verlag Paul Parey.
- Kroodsma, D.E. 1976. Reproductive development in a female songbird: differential stimulation by quality of male song. *Science* 192: 574-575.
- de León, A., Fargallo, J.A. & Moreno, J. 1998. Parental body size affects meal size in the Chinstrap Penguin (*Pygoscelis antarctica*). *Polar Biol.* 19: 358 -360.
- Marchant, S. & Higgins, P.J. 1990. *Handbook of Australian, New Zealand and Antarctic Birds. Vol.1A*. Melbourne: Oxford University Press.
- Naguib, M. & Todt, D. 1997. Effects of dyadic vocal interactions on other conspecific receivers in Nightingales. *Anim. Behav.* 54: 1535-1543.
- Naguib, M., Fichtel, C. & Todt, D. 1999. Nightingales respond more strongly to vocal leaders of simulated dyadic interactions. *Proc. Roy. Soc. Lond., B*, 266: 537-542.

- Rowland, W.J. 1989. The effects of body-size, aggression and nuptial coloration on competition for territories in male Threespine Sticklebacks, *Gasterosteus aculeatus*. *Anim. Behav.* 37: 282-289.
- Ryan, M.J. 1980. Female mate choice in a neotropical frog. *Science* 209: 523-525.
- Searcy, W.A. & Yasukawa, K. 1990. Use of song repertoire in intersexual and intrasexual contexts by male Red-winged Blackbirds. *Behav. Ecol. Sociobiol.* 27: 123-128.
- Tubaro, P.L. & Mahler, B. 1998. Acoustic frequencies and body mass in New World Doves. *Condor* 100: 54-61.
- Warham, J. 1990. *The Petrels: Their Ecology and Breeding Systems*. London: Academic Press.
- Wiklund, C. & Kaitala, A. 1995. Sexual selection for large male size in a polyandrous butterfly: the effect of body size on male versus female reproductive success in *Pieris napi*. *Behav. Ecol.* 6: 6-13.