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ARTICLE

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Effects of male call pitch on female behaviour and mate fidelity in little penguins

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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) dominant frequency (low vs high) and (2) familiarity (current partner vs stranger). In the first experiment, there was no statistically significant difference in the number of approaching females between low- and high-pitch calls. In the second experiment, using calls from the current partner and a stranger, females were more likely to approach closer to their own partners' calls, regardless of the pitch of the stranger's call. We conclude that female little penguins may recognize their partners' calls (or at least familiar male calls) and appear to maintain mate fidelity even when larger, potentially more successful, strangers are on offer.

Key words Advertising call · Little penguins · Male call pitch · Mate fidelity · Playback experiments

Introduction

Acoustic features of male calls can reflect the quality of the caller and affect female choice in some species (e.g., frogs, Ryan 1980; and birds, Catchpole 1980; Eens et al. 1991; Márquez and Bosch 1997). 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, females can use male call parameters to obtain information on male body size (Genevois and Bretagnolle 1994).

Little penguins (*Eudyptula minor*) are, with few exceptions (Reilly 1974, 1994), nocturnal on land, departing and returning to/from the sea solely during darkness. They are monogamous. Mate fidelity is strong and the average divorce rate per year is approximately 17% (Reilly and Cullen 1981). Most birds start to breed by 3 years of age (Dann and Cullen 1990). Their acoustic behavior has been extensively investigated (Waas 1988, 1990, 1991a, 1991b; Jouventin and Aubin 2000; Miyazaki and Waas 2002, 2003a, 2003b). Vocalizations may play an important role in pair bonding and in the process of mate choice (Miyazaki and Waas 2002, 2003b). Male little penguins produce advertising calls to attract females, usually on rocky shorelines in front of burrow areas 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). Miyazaki and Waas (2003b) described a high degree of individual specificity in the call pitch of little penguins. The dominant frequency of male advertising calls is negatively correlated with body size (Miyazaki and Waas 2003a). Because male body size of little penguins is positively correlated with reproductive parameters (Miyazaki and Waas 2003b), 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. We examined whether acoustic signals can convey information on the quality of the caller and whether females use the signals to obtain the best mates.

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 were occurring. Next, we tested the prediction that recently paired females (that obtained their mates in the breeding season) would be more

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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.

Materials and 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 and Waas 2003b).

We first recorded the braying advertising calls of males for the playback experiments. These braying calls are the most common vocalizations 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 ± 1 dB) and an AKG shotgun microphone (head, model C460B; body, model CK68-ULS; frequency response 20–22,000 Hz ± 2 dB). Sex of the birds was identified using flipper bands that were attached during previous studies (Miyazaki and Waas 2003b).

Experiment 1: low- versus high-pitch calls

During the early pairing period, six advertising calls of six different males were recorded after sunset (1800–2100 hours) on the rocky shore of the western parts of the island. We measured the dominant frequency (kHz) of the exhalation phrase by producing sonagrams with Canary 1.2.4 software (Bioacoustics Research Program, Cornell Lab of Ornithology, Ithaca, N.Y., USA). The range of variation within this population was ±20% from the average dominant frequency (1.30 ± 0.11 kHz, range 1.03–1.55 kHz). The inter-individual coefficient of variation (CV; 10.0%) was greater than the intra-individual CV (5.8%) (see Jouventin 1982). We then changed the call pitch of both the exhalation and inhalation of the six males' calls we sampled without altering any of the other call parameters, by using Peak 2.5 software (Berkley Integrated Audio Software, Petaluma, Calif., USA). Calls that had a dominant frequency 20% lower than the average of the population were used as low-pitch calls, while calls that had a dominant frequency 20% higher than the average of the population were used as high-pitch calls. We made six low-pitch calls and six high-pitch calls from the original six calls. 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 conducted a total of 15 tests on 15 different females. We used one low-pitch call and one high-pitch call in each test. However, we did not use low- and high-pitch calls of the same male's call for each test because the same male's call broadcast from opposite ends of the enclosure may have created an "unnatural" experience for the subject. Low- and high-pitch call playback tapes were selected randomly before each test, but we never used the same tape set more than once (i.e. 15 different low- and high-pitch call tape sets were used in the 15 tests).

We conducted the first experiment by using a field-based experimental arena between 1900 and 2100 hours from June to July 2001 during the early courtship period. It was dark when our experiments were started at this point in the season. The natural distance between calling penguins varied, depending on population size and numbers of birds ashore, but averaged approximately 5–10 m on shorelines of this island (personal observation). In a bush area near the shoreline, a 5.00 × 2.50 m arena was created using temporary garden fencing (0.95 m high). There were no breeding nests within the enclosure area. An artificial nest box (33 × 33 × 90 cm) was placed in each corner of the rectangular enclosure. Two of the four boxes, diagonally opposite one another, had speakers (Sony, model SRS-77G; frequency response 80–20,000 Hz ± 1 dB) placed 50 cm inside the box entrance. No speakers were placed in the other two boxes. The position of the two playback speakers was randomized. The two silent boxes were placed to confirm that playbacks, and not some other factor, prompted females to approach boxes. 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 settling period, the 45-min tapes were broadcast to the female. Firstly, three exhalation–inhalation sets (20 s) of a low-pitch call were broadcast. After an interval of 30 s, three exhalation–inhalation sets (20 s) of a high-pitch call were played back, followed by another 30-s silent interval. Thus, low- and high-pitch calls did not overlap one another, but alternated systematically. The type of playback broadcast first (low- or high-pitch 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. Females were recorded as approaching one of the four boxes if they moved to within 1 m of the box entrance (the "approach area"). The distance between a box and the subject was further classified on a scale of 0–3 (0 = out of the approach area, 1 = between 50 cm and 1 m of the box, 2 = within 50 cm of the box, 3 = within the box). The four location scores we obtained (from the 10-min samples) were averaged for the 40-min observation period of each test; differences in the average scores in response to low- and

high-pitch call playback boxes were examined by using Wilcoxon signed rank tests. We stopped counting approaches when the subject entered a box at any time and regarded her entrance as a final choice.

Experiment 2: current partner versus stranger calls

The second experiment was conducted between 1900 and 2100 hours from September to October 2000, late in the courtship period. 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 ten bray calls of the subjects' mates we sampled had a mean dominant frequency of 0.74 ± 0.41 kHz (mean ± SD). All three low-pitched stranger calls had a dominant frequency of 0.34 kHz, while two high-pitched stranger calls both had a dominant frequency of 1.21 kHz. The ten females of the selected pairs were individually tested to determine how they reacted when presented with their partners' calls 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 tests on the ten females. Five of the ten females were tested twice (once with a lower pitched stranger call than the average pitched call, and once with a higher pitched stranger call). Consecutive tests on a given female were always at least 1 day apart. The treatments (low and high) were randomized for the females that were used twice. 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's 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

In 10 of the 15 trials, subjects approached low-pitch calls, and three of them entered the box. The remaining five females approached high-pitch calls, and two entered the boxes. No females entered boxes in corners without playback. Overall, there was no statistically significant difference in the number of approaching females between low- and high-pitch calls ($\chi^2 = 1.065$, $df = 1$, $P = 0.302$, $n = 15$). There was also no statistically significant difference in the averaged location score between low- ($1.1 \pm 1.2, n = 15$) and high-pitch calls (0.5 ± 1.0, $n = 15$, $z = -1.569$, $P = 0.117$), while the 95% confidence interval of low-pitch calls (0.44 to 1.76) was not overlapped much by that of high-pitch calls (–0.05 to 1.05). In four cases, females responded vocally to low-pitch calls with a single contact call (overlapping playback or just after playback), whereas no bird responded vocally to high-pitch calls.

Experiment 2

Current partner versus low-pitched stranger calls

There was no statistically significant difference in the number of approaching females between their partners' and low-pitched stranger call treatments (Binomial test, $P = 0.109$, $n = 8$, Fig. 1). However, females moved closer to their partners' calls (1.6 ± 1.3, $n = 8$) than to low-pitched stranger calls (0.1 ± 0.2, $n = 8$, $z = -1.980$, $P = 0.048$; 95% CI, current partner, 0.51 to 2.69; low-stranger, –0.07 to 0.27) in terms of the averaged location score. In two of the eight tests, females responded vocally to their partners' playback. They called just after the partners' playback segments ended. In one trial, the female responded to the playback of her partners' 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 partner's calls with a series of two contact calls over a period of 10 min (again, only after her partner's call was broadcast).

Current partner versus high-pitched stranger calls

Females approached their partners' calls and no birds approached the box broadcasting high-pitched stranger

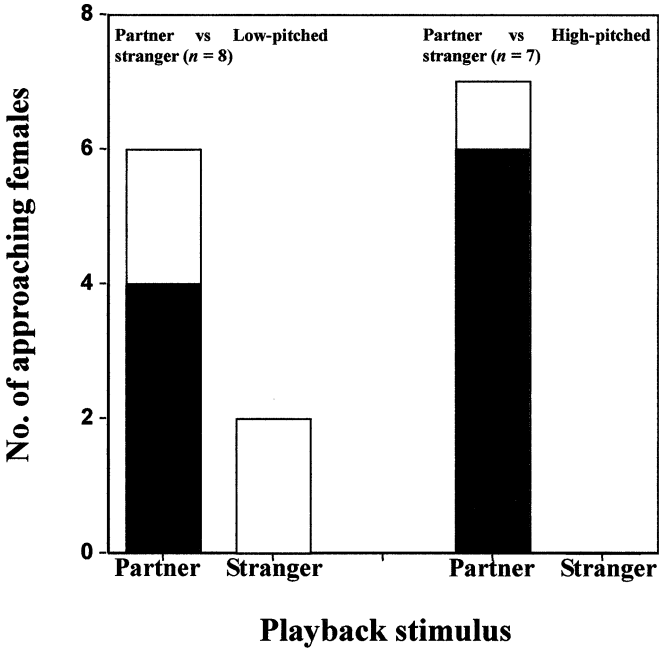

Fig. 1. The number of female little penguins (*Eudyptula minor*) approaching playback of their own partner and a stranger (*black*, entering the box; *white*, occupying the 'approach area")

calls (Binomial test, $P = 0.008$, $n = 7$; Fig. 1). In one of the seven tests, a female responded vocally to her partner's playback call. She called just after the partner's playback segments ended (two contact calls over a period of 7 min).

Discussion

Experiment 1

Songbirds can detect very small differences in call pitch (reviewed by Ratcliffe and 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). However, in this study, there were no statistically significant differences in the number of approaching birds and the averaged location score between low- and high-pitch calls. Other parameters of male advertising calls might affect female responses. Larger male little penguins have longer exhalations as well as lower pitch in their advertising calls (Miyazaki and Waas 2003a). Therefore, phrase duration or other call parameters may affect female responsiveness to some degree. However, our experiments 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. Females responded vocally only to low-pitch calls, but the sample size was small. Future research is required with greater sample sizes to examine their vocal responses.

Experiment 2

Our results showed that, regardless of the dominant frequency of playback calls, females approached closer to recordings of their partners' calls than to recordings of strangers. Females approached their partners' calls more closely in both low-pitched and high-pitched stranger experiments. However, there was no statistical difference in the number of approaching females between their partners' and low-pitched stranger calls. They may have more interest in low pitched stranger calls than in high pitched stranger calls. Future work is required to assess adequately the difference in preference between low- and high-pitched calls. Acoustic signals can play an important role in mate recognition, and the ability of a female to identify her 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 partner's playback (note, however, the sample size for vocal responses was small in our study) 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 and 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, there was no obvious difference in the number of approaching females between low- and high-pitch calls. However, in the experiment broadcasting current partner calls and stranger calls, females approached closer to the calls of their own mate than to those of strangers regardless of the pitch of the stranger's call. Females may recognize their mates' calls and prefer to maintain mate fidelity. 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.

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