RESEARCH ARTICLE

The invisible cues that guide king penguin chicks home: use of magnetic and acoustic cues during orientation and short-range navigation

Anna P. Nesterova*, Jules Chiffard, Charline Couchoux and Francesco Bonadonna

Behavioural Ecology Group, CEFE–CNRS, 1919 Route de Mende, F-34293 Montpellier, Cedex 5, France

*Author for correspondence at present address: Animal Behaviour Research Group, Department of Zoology, University of Oxford, The Tinbergen Building, South Parks Road, Oxford OX1 3PS, UK (apnesterova@gmail.com)

SUMMARY

King penguins (*Aptenodytes patagonicus*) live in large and densely populated colonies, where navigation can be challenging because of the presence of many conspecifics that could obstruct locally available cues. Our previous experiments demonstrated that visual cues were important but not essential for king penguin chicks' homing. The main objective of this study was to investigate the importance of non-visual cues, such as magnetic and acoustic cues, for chicks' orientation and short-range navigation. In a series of experiments, the chicks were individually displaced from the colony to an experimental arena where they were released under different conditions. In the magnetic experiments, a strong magnet was attached to the chicks' heads. Trials were conducted in daylight and at night to test the relative importance of visual and magnetic cues. Our results showed that when the geomagnetic field around the chicks was modified, their orientation in the arena and the overall ability to home was not affected. In a low sound experiment we limited the acoustic cues available to the chicks by putting ear pads over their ears, and in a loud sound experiment we provided additional acoustic cues by broadcasting colony sounds on the opposite side of the arena to the real colony. In the low sound experiment, the behavior of the chicks was not affected by the limited sound input. In the loud sound experiment, the chicks reacted strongly to the colony sound. These results suggest that king penguin chicks may use the sound of the colony while orienting towards their home.

Key words: short-range navigation, orientation, king penguin, *Aptenodytes patagonicus*, chick, acoustic cue, magnetic cue, colony recognition.

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INTRODUCTION

Many animal species need to navigate in crowded environments, but the mechanisms that underlie navigation under such conditions are not well understood. One of the main challenges for navigation in a crowd stems from the limited availability of local cues, i.e. environmental features close to the goal. Large numbers of individuals obstruct local cues whether they be visual, acoustic, olfactory or tactile. Therefore, under crowded conditions, positional information should rather be derived from global cues, i.e. prominent features of the environment that can be perceived from farther distances.

It has been proposed that global cues are reliable indicators of a goal's approximate location and provide directional information, whereas local cues are necessary for precise positioning once an animal approaches its goal (Cheng and Spetch, 1998; Benhamou, 2001). Indeed, many species appear to rely on both global and local cues, and different sets of cues can be used in a hierarchical manner (reviewed in Shettleworth, 1998). Taking this into account, navigation by animals living in the dense colonies presents an interesting enigma: precise positioning within the colony is necessary, but local cues are limited, and the nearest global cues could be several kilometers away.

We chose king penguins (*Aptenodytes patagonicus*) as a model with which to study orientation and navigation in crowded environment for several reasons. First, they form large and very dense colonies that can stretch over several kilometers over relatively flat featureless beaches (Stonehouse, 1960; Weimerskirch et al.,

1992; Aubin and Jouventin, 1998). Second, both adults and chicks have to locate specific places in the colony. Breeding partners alternate their parental duties, and need to find each other after foraging trips. When chicks are several weeks old, they are left unattended by their parents in groups of other chicks known as 'crèches' (Stonehouse, 1960; Barrat, 1976). In order to be found and fed by their parents, chicks must remain at their specific crèche (Dobson and Jouventin, 2003). However, chicks can be displaced by the predators or adverse weather, and should be able to navigate back to their place in the crèche. Third, the colony structure is dynamic (Bried and Jouventin, 2001). King penguins do not build nests, but defend a small part of the colony. Breeding pairs incubate their egg and brood the young chick on their feet (Stonehouse, 1960). As a result, the relative position of birds in the colony can change during the season by several meters (Lengagne et al., 1999b). Consequently, birds that come back from foraging trips to relieve their partners or to feed their chicks face the shifting mosaic of the colony. Fourth, penguins cannot obtain an aerial view of the colony due to their flightless nature and are limited in the cues available to them.

In an attempt to find what types of cues are used by king penguins for orientation and navigation, we previously conducted experiments with 10-month-old chicks. Working with chicks presents many advantages for short-range navigation studies. Chicks are always present in the colony until they molt, they can be reliably found in their crèches and, most importantly, chicks are highly motivated to return immediately to their place in the colony if they have been

displaced. In addition, our previous findings suggest that results obtained with chicks can serve as a good proxy for adult navigational behaviour, as discussed below (Nesterova et al., 2009; Nesterova et al., 2010).

Earlier, we demonstrated that chicks displaced away from their crèches can orient towards the colony and return to their crèche (Nesterova et al., 2009). The results indicated that visual cues were important for homing, but not essential. Chicks displaced at night when visual cues were limited or unavailable had a lower homing success. Nevertheless, 62% of chicks were able to home at night. Some chicks homed even when neither moonlight nor starlight was present. Similarly, our experiments on adult king penguins demonstrated that breeders can walk towards their place in the colony in complete darkness (Nesterova et al., 2010). As a result, king penguins must be able to use some environmental cues, other than visual, for successful navigation under limited light conditions.

When visual cues are not available, king penguins could derive information from olfactory, magnetic or acoustic cues to navigate back to their place in the colony. At present, nothing is known regarding the olfactory abilities of king penguins, and it is hard to estimate the importance of odors for their navigation. Magnetic cues cannot be easily obstructed by conspecifics or be limited because of low light or adverse weather conditions, unlike visual, acoustic or olfactory cues. Therefore, they could be a useful source of information in crowded environments. Moreover, animal navigation based on magnetic cues has been demonstrated in a great number of species (reviewed in Wiltschko and Wiltschko, 2005).

It would not be surprising that king penguins use acoustic cues for navigation given their extraordinary auditory abilities (Aubin and Jouventin, 1998; Aubin and Jouventin, 2002). Many different species are known to use conspecific, heterospecific, self-produced or ambient sounds for orientation and navigation. For example, male wood frogs (*Rana sylvatica*) orient towards the sound of the conspecific chorus (Bee, 2007). Palmate newts (*Lissotriton helveticus*) show positive phonotaxis towards calls of Iderian green frogs (*Rana perezi*) and European common brown frogs (*Rana temporaria*), which serve as indicators of suitable habitat (Diego-Rasilla and Luengo, 2007). White storks (*Ciconia ciconia*) move towards the mating calls of moor frogs (*Rana arvalis*), which are suggestive of the profitable foraging locations (Igaune et al., 2008). Little owls (*Athene noctua*) are attracted to those habitats where they hear the calls of their conspecifics. Scops owls (*Otus scops*) also seem to choose their breeding sites based on the calls of the little owl. Moreover, they seem to have a preference for the contact calls, and not alarm calls, of the little owl (Parejo et al., 2012). Echolocating bats use reflection of emitted sounds to reconstruct their surrounding environment (Metzner, 1991; Holland, 2007). And visually impaired humans are known to use a variety of ambient sounds while navigating in cities (Pow, 2000). These are only a few of many studies that show the importance of acoustic cues in the everyday life of animals.

The concept of a 'soundscape' was first formulated by Smith (Smith, 1994) with reference to human studies. It has been developed over the years, and the term 'soundscape orientation' defined as 'the use of sounds as cues for general orientation within a landscape' was introduced into the animal literature by Slabbekoorn and Bouton (Slabbekoorn and Bouton, 2008). Like in the case of a visual landscape, different environmental sounds can create a patchwork of 'soundmarks' that are suitable for orientation and navigation. Acoustic cues may become especially important when other types of cues, such as visual, olfactory or other cues, become unavailable or are too costly to sample. While the importance of acoustic cues appears quite strong, they have often been overlooked in favor of other sensory modalities such as visual cues.

The king penguins' highly developed acoustic abilities, 'noisy' colony lifestyle and capacity to navigate when visual cues are limited render them particularly suitable for the investigation of acoustic orientation and navigation. Individual identification in adults and chicks is based on vocal signals (Jouventin et al., 1999; Lengagne et al., 1999b). Penguins can recognize the call of their partner or chick even among thousands of calling individuals, a phenomenon known as the 'cocktail-party effect' (Aubin and Jouventin, 1998). Furthermore, king penguin colonies present an acoustically rich environment because of the highly vocal nature of these birds. The colonies can be heard several kilometers away (A.P.N., unpublished) and can potentially serve as an important soundmark.

In spite of the many studies that have been devoted to king penguin acoustic communication, there are still many unknowns. The range over which individual recognition in the colony can take place is relatively limited, around 8.8 m (Lengagne et al., 1999b). Whether king penguins use acoustic cues at larger scales of navigation remains unknown. Potentially, they use the sound of the colony while approaching it. Once inside the colony, king penguins could rely on the soundscape of the colony itself to approach a 'rendez-vous' zone where they can reliably hear and identify their partners or chicks. However, it should be taken into account that the king penguins' habitat is characterized by harsh weather conditions and prevailing strong winds that can mask or modify important sounds.

The first indirect evidence that king penguin chicks use sound for orientation comes from Nesterova et al. (Nesterova et al., 2009). Chicks placed in an experimental arena successfully oriented towards the colony in the absence of visual cues when wind came from the colony direction. If wind came from the opposite direction, the chicks showed no preference for any particular side of the arena. Because wind can alter information from several types of cues (e.g. acoustic, olfactory), the importance of acoustic cues for orientation must still be tested explicitly.

The primary objective of this study was to investigate the importance of magnetic and acoustic cues for king penguin chicks' orientation and short-range navigation. We designed a set of experiments where we manipulated separately these cues and examined the homing abilities of the chicks that were displaced from their crèches. Experiments, impairing correct geomagnetic cues perception, were conducted both during the day and at night to examine whether the use of magnetic cues depends on the availability of visual cues. To test the use of acoustic cues, we manipulated their availability to the chicks. In a low sound experiment, we displaced chicks from their crèches and examined their orientation and homing ability when their ears were covered, i.e. with limited acoustic input. In a loud sound experiment, we displaced chicks from their crèches and tested their orientation in an arena when the colony sound was broadcast adjacent to the arena, i.e. with additional acoustic input.

MATERIALS AND METHODS
Animals and study area
We conducted our experiments during austral summers at the Cape Ratmanoff king penguin (*Aptenodytes patagonicus* Miller 1778) colony situated on the Courbet Peninsula, Kerguelen Island (70°33′E, 49°42′S). We performed the magnetic day and low sound experiments during December 2008–January 2009 and the magnetic night and loud sound experiments during December 2009–January 2010. We used king penguin chicks that were 10 to 11 months old.

To estimate the age of chicks, we used their size and molting conditions (Stonehouse, 1960; Weimerskirch et al., 1992). Overall, we tested 38 (magnetic day), 34 (magnetic night), 33 (low sound) and 40 (loud sound) chicks. The acoustic experiments were performed in full daylight, between 07:00 and 19:00h. The colony was not visible from the arena. Each chick was tested only once.

This study was performed according to Institute Polaire Français – Paul-Emile Victor (IPEV) and Centre National de la Recherche Scientifique (CNRS) guidelines for the Ethical Treatment of Animals and complied with current French regulations.

Experimental arena

An arena setup similar to the one described in Nesterova et al. (Nesterova et al., 2009) was used. The circular arena (radius 5.2m) was located south of the colony on a small plateau (Fig. 1). The arena barrier (1m high) was made out of thick non-transparent fabric, to prevent chicks from seeing outside of the arena. Metal poles supported the fabric barrier during the experiments. When necessary, the barrier could be lowered down to ground level to release the chicks. The ground in the arena was divided into four quadrants by lines running north–south and east–west. Two observation posts were located 5m west and east from the arena side. Such placement minimized the observers' effect on chicks' behavior in terms of their choice of the colony (north) half or opposite (south) half of the arena. The arena was placed 140–180m away from chicks' crèches: ~180m (magnetic day), ~150m (magnetic night), ~170m (loud sound) and ~140m (low sound). The placement of the arena depended on the colony shape, location of the crèche and presence of intervening local pools of mud.

Experimental procedure

Our experiments relied on the chicks' strong motivation to return to their crèches if displaced. We captured chicks at their crèches, marked the capture location with a piece of wood and took its GPS coordinates. Immediately after capture, we covered chick's head with a cotton hood, rotated it three times, hand carried it to the arena along a non-direct path and rotated it again three times inside the arena. These manipulations were done to prevent the chicks from using internally generated cues during their return.

Magnetic day and night experiments

To establish whether the use of magnetic cues for homing depends on the availability of visual cues, we displaced chicks either during the day (magnetic day experiment) or at night (magnetic night experiment) and monitored their ability to return to their crèches. For both day and night experiments the chicks were subjected to two experimental conditions: magnet or control. In the magnet condition ($N_{magnet,day}$=19, $N_{magnet,night}$=16), we attached a small cylindrical neodynium magnet (diameter 18mm, height 5mm, nominal residual induction between 1.17 and 1.22T; Calamit Trading, Milan, Italy) to each chick's head to perturb information from the geomagnetic field. This type of magnet is characterized by a very strong magnetic moment (1.2A m^2), and the field generated by it (~250–450µT at 8cm, depending on the direction with respect to the magnet axis orientation) is much stronger than the natural geomagnetic field at Kerguelen (ca. 48µT). Such magnets have been used to perturb the magnetic field around green turtles (*Chelonia mydas*) (Papi et al., 2000) and giant albatrosses (*Diomedea exulans*) (Bonadonna et al., 2005). The magnet was made mobile by suspending it on string held between two bands of Tesa tape. The first band of the tape was placed on the top of the head just behind the ears, and the second band of tape was placed 8cm
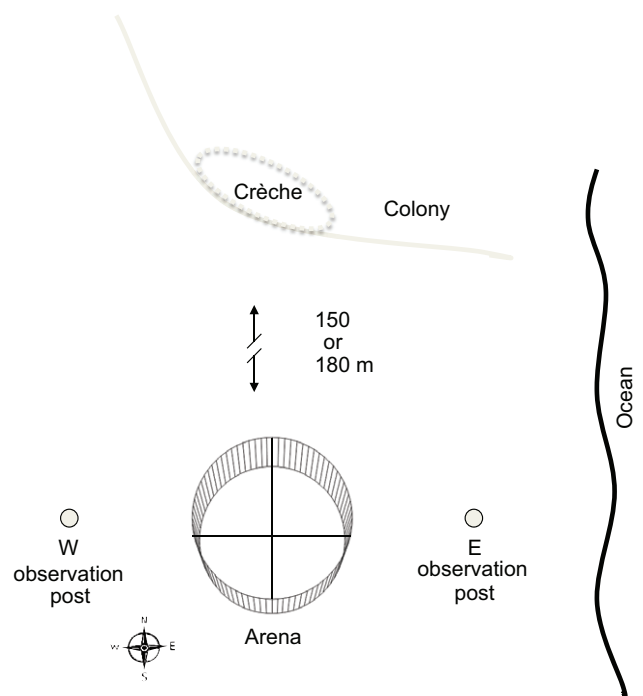

Fig. 1. Experimental arena. The diagram shows the location of the experimental arena with respect to the crèche where chicks were captured and the ocean. The drawing is not to scale.

lower on the neck. In the control condition ($N_{control,day}$=19, $N_{control,night}$=18), a piece of brass of the same mass and form as the magnet was suspended on the string.

All chicks were also equipped with a 13g miniature GPS logger (TechnoSmArt, Rome, Italy) constituting ~0.1% of the chick's mass. A small piece of reflective tape was attached to the GPS loggers to help with identification at night. GPS loggers were attached to Darvic plastic bands and fitted on the flipper for the duration of the trial. We used a 2s logging frequency acquisition rate for the GPS loggers. In addition, a band of Tesa tape was placed on the other flipper to mark animals that participated in the experiment. Tesa bands were removed at the end of the experiment after all chicks had been tested.

Equipped chicks (one at a time) were placed in the middle of the arena, the hood was removed and a plastic barrel was placed over the chick. After 1min the barrel was lifted, and two observers left the arena in the east and west directions. The timing of the trial started once a chick made a first step away from the arena center. During the first 15min we observed the chicks' orientation in the arena. An observer at each of the two posts timed the movements of the animal between the quadrants. Notes were compared at the end of the experiment for accuracy.

To test chicks' abilities to return to their crèche, the barrier was lowered completely after 15min. The chick was then free to leave arena in any direction. In the day experiment, the colony was not visible from the arena even when the barrier was down. We considered that a chick homed if it arrived within 20m of its capture location. We observed each chick for a further 10min to make sure that it remained in the area. At the end of each trial, the chick was recaptured and all equipment was removed. Chicks that did not home within 60min were brought back to their capture location. We alternated control and experimental trials to increase the likelihood

of conducting both types of trials under similar meteorological conditions.

At the beginning of each trial, we measured temperature, wind speed and direction with a Kestrel 4500 weather station (Kestrel, Boothwyn, PA, USA); luminosity level (for night experiments) with a TES 1335 luxmeter (AZ Instrument, Taichung City, Taiwan); and estimated cloud cover by eye.

Day trials were conducted between 07:00 and 19:00 h under full light conditions. Night trials started 1 h after sunset and finished at least 1 h before sunrise. Luminosity levels during night trials ranged between 0.09 and 0.18 lx (mean ± s.e.m.=0.12±0.004 lx). We monitored chick movements with binoculars. At night we used night vision binoculars. The reflective tape on the GPS loggers allowed easy tracking even at relatively long distances (up to 150 m).

Given the short removal time of the chicks from their crèches, it is highly unlikely that their feeding schedule was compromised. In our previous experiments we also observed that if a parent cannot find its chick immediately, it repeats its search later.

Low sound experiment

The experimental procedure for the low sound experiment was identical to the one described for the day magnet experiment, but instead of magnets, chicks were fitted with ear pads. The ear pads consisted of a foam layer, commonly used for human earplugs, and a cotton layer. The ear pads were secured on the head with Tesa tape for the duration of the experiment. The chicks were tested under two conditions: low sound and control. In the low sound condition (N=18), the chicks were fitted with the ear pads over the ears to reduce their hearing abilities. In the control condition (N=15), the ear pads were secured lower on the neck, keeping the ears open. The chicks were also equipped with GPS loggers (TechnoSmArt). First, the chicks were kept in the arena for 10 min to test their orientation, then the arena barrier was lowered and the chicks were free to home.

The ear pads efficiency test

To examine the efficiency of the ear pads in blocking ambient sound we tested four parent–chick pairs in the crèches at the periphery of the colony. We waited for a parent to arrive at a crèche after a foraging trip and call for its chick. We noted the distance at which the chick responded to its parent. The distance was estimated based on a grid established within the crèche. Then we captured the chick and fitted it with the ear pads. When releasing the chick, we made sure that its parent was at least 40 m away. We then observed the parent and the chick and noted the distance at which the chick again responded to the parent's call. Chick responses involved calling back and moving towards its parent. After the parent and the chick found each other for the second time, the chicks were recaptured and the ear pads were removed. We continued observations until the parent and chick were finally reunited.

In two of the parent–chick pairs, the chicks responded for the first time to their parents' calls when parents were 22 and 25 m away. These distances are greater than an average of 8.8 m reported previously for king penguins inside the colony (Lengagne et al., 1999a), most likely because our experiments were conducted at the periphery of the colony. After the ear pads were fitted, the chicks responded to their calling parents at 12 and 10 m away, but not at greater distances. Thus, the ear pads reduced the chicks' hearing abilities, but did not completely remove all sound input. For the other two pairs no information was obtained because the chicks called first; the parents quickly approached the chicks and called only when they were within a 5 m radius.

Loud sound experiment

We captured and brought chicks to the arena as was previously described. In the control condition (N=21), the chicks were exposed only to the natural colony sound coming from the north of the arena. In the speakers condition (N=19), the chicks were exposed to the natural sound of the colony coming from the north and recordings of the colony broadcast from the south of the arena. To simulate colony sounds, we placed two loudspeakers (MA 705 EXP, frequency response 50 Hz–20 kHz, Mipro Electronics, Taiwan) in the SE (150 deg) and SW (210 deg) directions from the arena center. The speakers were placed 3 m away from the arena barrier. With such placement we intended to mimic more closely the presence of the natural colony. The 10 min colony recording (wave sound format) was played from an iPod shuffle (Apple, Cupertino, CA, USA) in a loop during the trial. Before the start of each trial, we verified with a sound level meter (Bioblock Scientific, Illkirch, France) that the sound level of the speakers was similar to the sound levels obtained 20–30 m away from the colony. In the speaker condition, the sound coming from the speaker was always louder than the natural colony sound.

The recordings used in the experiment were collected with an omnidirectional microphone (Sennheiser Electronic Corp., Old Lyme, CT, USA) attached to a 2 m pole and connected to a recorder (Model PMP660, Marantz LLC, Mahwah, NJ, USA) in the area of the colony where the crèches of interest were located. The recordings were performed at a sampling frequency of 44.1 kHz on a non-windy day.

We observed the chicks' behavior in the arena for 15 min, then the arena barrier was lowered and the chicks were free to home. In the speaker condition, the speakers continued to broadcast the colony sound even after the barrier was lowered. We noted the direction of departure from the arena (N, NE, E, SE, S, SW, W, NW). In addition, we recorded the coordinates of each chick's location 5 min after the barrier was opened. It was not the purpose of the experiment to record complete homing paths because we could not control the acoustic environment once chicks left the arena. Afterwards, the chicks were guided to their crèches by walking 3–5 m behind them. Chicks that were too far from their crèches were recaptured and brought back.

Data analysis

The colony was north of the arena. If chicks spent more time in the colony half (north half) of the arena, it would be suggestive of their ability to orient towards the colony. Therefore, we compared the amount of time chicks spent in the colony half of the arena under control and magnet conditions with a t-test. We compared the number of chicks that preferred the colony half of the arena with a Fisher exact test. If a chick spent more than half of the arena time in the colony half, it was considered to 'prefer' the colony half.

The number of transitions that chicks made between each arena quadrant provided an estimate of chicks' overall activity and was compared between different conditions with a t-test. A chick was considered to move from one quadrant to another when it completely crossed the line separating the quadrants. Chicks that did not move within the arena (magnetic experiments: $N_{control,day}$=1, $N_{magnet,day}$=3, $N_{magnet,night}$=2; low sound experiment: $N_{low\ sound}$=3; loud sound experiment: $N_{control}$=2) were not included in the analysis of the orientation behavior.

In some experiments, we were not able to investigate the effect of wind direction on the orientation in the arena due to the low number of trails with the south (non-colony) wind direction

(magnetic day experiment: $N_{control,day}$=1, $N_{magnet,day}$=3; low sound experiment: $N_{control}$=1, $N_{low\ sound}$=5; loud sound experiment: $N_{control}$=4, $N_{speakers}$=3).

ANOVA using a general linear model (GLM) was used to test for the differences in chicks' behavior in the magnetic night experiments. We started with a saturated model including the following variables and their two-way interactions: experimental condition, wind direction, wind speed and luminosity. In a stepwise

regression approach, we subsequently dropped all the non-significant terms ($P>0.05$).

The chicks were equipped with the GPS loggers in the magnetic day, magnetic night and low sound experiments. From the GPS data, we extracted the following information: the total path length (D_t), the straight-line distance (D_s) between the arena and the final point of the chick's path, and the chick's position 10 and 30 m away from the arena. A linearity index, defined as LI=D_s/D_t, was

Table 1. Statistical tests: magnetic day and night experiments

Parameter tested	Groups tested	N	Mean ± s.e.m.	Test	Test statistic value	d.f.	P
Day experiment							
Orientation in the arena							
No. of chicks that preferred the colony half	Control vs magnet	18 16		Fisher			0.275
Time at the colony half (s)	Control vs magnet	18 16	505±80.4 478±59.7	t	0.268	32	0.79
Activity in the arena	Control vs magnet	18 16	4.0±0.9 7.0±0.9	t	−2.283	32	0.029*
Homing							
No. of chicks that homed	Control vs magnet	19 19		Fisher			0.162
Homing time (min)	Control vs magnet	15[a] 12[a]	32.4±3.3 33.6±3.8	t	−0.237	25	0.814
Path straightness	Control vs magnet	19 18	0.38±0.03 0.33±0.04	t	0.936	35	0.356
Unusual homing paths	Control vs magnet	19 19		Fisher			0.24
Orientation at 10 m	Control	19		Rayleigh	3.068		0.044*
Orientation at 10 m	Magnet	18		Rayleigh	1.333		0.267
Orientation at 30 m	Control	19		Rayleigh	7.519		<0.001*
Orientation at 30 m	Magnet	18		Rayleigh	6.727		<0.001*
Orientation at 10 m	Control vs magnet	19 18		Watson U^2	0.051		>0.5
Orientation at 30 m	Control vs magnet	19 18		Watson U^2	0.098		0.5>P>0.2
Night experiment							
Orientation in the arena							
No. of chicks that preferred the colony half	Control vs magnet	16 16		Fisher			0.099
Time at the colony half (s)	Control vs magnet	16 16	627±51.1 471±79.4	GLM – Experimental condition GLM – Wind direction	2.18 3.76	1.29 1.29	0.151 0.062
Activity in the arena	Control vs magnet	16 16	7.4±1.0 4.9±1.1	t	1.665	30	0.106
Homing							
No. of chicks that homed	Control vs magnet	18 16		Fisher			0.165
Homing time (min)	Control vs magnet	7[a] 9[a]	37±5.3 31±4.1	t	0.925	14	0.371
Path straightness (All data)	Control vs magnet	15 12	0.12±0.02 0.20±0.04	t	−2.167	25	0.040*
Path straightness (No outlying data point)	Control vs magnet	15 11	0.12±0.02 0.18±0.03	t	−1.796	24	0.085
Unusual homing paths	Control vs magnet	15 12		Not observed			
Orientation at 10 m	Control	15		Rayleigh	3.351		0.032*
Orientation at 10 m	Magnet	12		Rayleigh	2.476		0.089
Orientation at 30 m	Control	15		Rayleigh	4.138		0.013*
Orientation at 30 m	Magnet	11		Rayleigh	7.188		0.001*
Orientation at 10 m	Control vs magnet	15 12		Watson U^2	0.036		>0.5
Orientation at 30 m	Control vs magnet	15 11		Watson U^2	0.044		>0.5

[a]Only animals that homed were considered.
*Significant P-values ($P<0.05$).

used as a measure of the straightness (or optimality) of the path. Values close to 1 indicated a direct crèche approach. We compared LIs and homing times between different experimental conditions by means of a *t*-test. For the homing analysis we considered only chicks that homed. We analyzed and compared the distribution of the chicks' positions 10 and 30 m away from the arena with Rayleigh and Watson U^2 tests as appropriate for circular data (Batschelet, 1981). We report the length of the mean vector (*r*).

Due to technical difficulties we were not able to recover high-quality GPS data from several chicks in the magnetic experiments ($N_{magnet,day}$=1, $N_{control,night}$=2, $N_{magnet,night}$=2) and the low sound experiment ($N_{control}$=8). In the magnet night condition, two chicks never left the arena; in the control night condition, one trial was aborted due to bad weather conditions. Consequently, no paths were analyzed for these chicks.

In the loud sound experiment, we analyzed the distribution of chicks as they left the arena with Rayleigh and Watson U^2 tests. We also looked at how far the chicks were from the arena center 5 min after the barrier was opened.

We tested our linear data for normality using a Kolmagorov–Smirnov test. We report the results of the two-tailed tests, giving mean values ±1 s.e.m. The analyses were performed using SPSS 17 (IBM, Armonk, NY, USA) and Oriana (Kovach Computing Services, Pentraeth, Wales, UK) statistical packages.

RESULTS
Magnetic day experiment
Orientation in the arena
The number of animals that preferred the colony half of the arena was similar in the control day (11/18) and magnet day (10/16) conditions (Table 1). The time that chicks spent on the colony half of the arena also did not differ between the two groups (Table 1). Chicks were significantly more active in the arena under the magnet condition (Table 1).

Homing
There was no difference in the number of animals that homed in the control day (15/19) and the magnet day (12/19) conditions (Table 1). Homing times were similar between the two groups (Table 1). Also, we did not see any differences in the straightness of paths (Table 1). In general, the chicks first approached the colony, and then proceeded to their home crèche along the colony edge. The number of chicks that took unusual paths (large detours) was similar between the control (2/19) and the magnet (4/19) day conditions (Table 1).

At a distance of 10 m from the arena the control group was significantly oriented towards the crèche, but this was not the case for the magnet group. At a distance of 30 m both the control and the magnet groups were significantly oriented towards the crèche. No significant differences were observed in the distribution of homing chicks at 10 and 30 m (Table 1, Fig. 2).

Magnetic night experiment
Orientation in the arena
More animals preferred the colony half of the arena in the magnet condition (7/16) than in the control (3/16), but this difference was not significant (Table 1). The time chicks spent on the colony half of the arena was similar between the control and the magnet conditions. There was a tendency, but not significant, for chicks to spend more time on the colony half of the arena if the wind came from the north, especially in the magnet condition (control N

winds=641±61.5 s, control S winds=610±91.2 s, magnet N winds=652±87.1 s, magnet S winds=330±104.5 s; Table 1). No differences in activity levels were observed between the control and the magnet conditions (Table 1).

Homing
The number of chicks that homed was similar in the control (7/18) and magnet night (9/16) conditions, as was the homing time of the two groups (Table 1). In the magnet condition chicks tended to proceed more directly towards their crèches than under the control
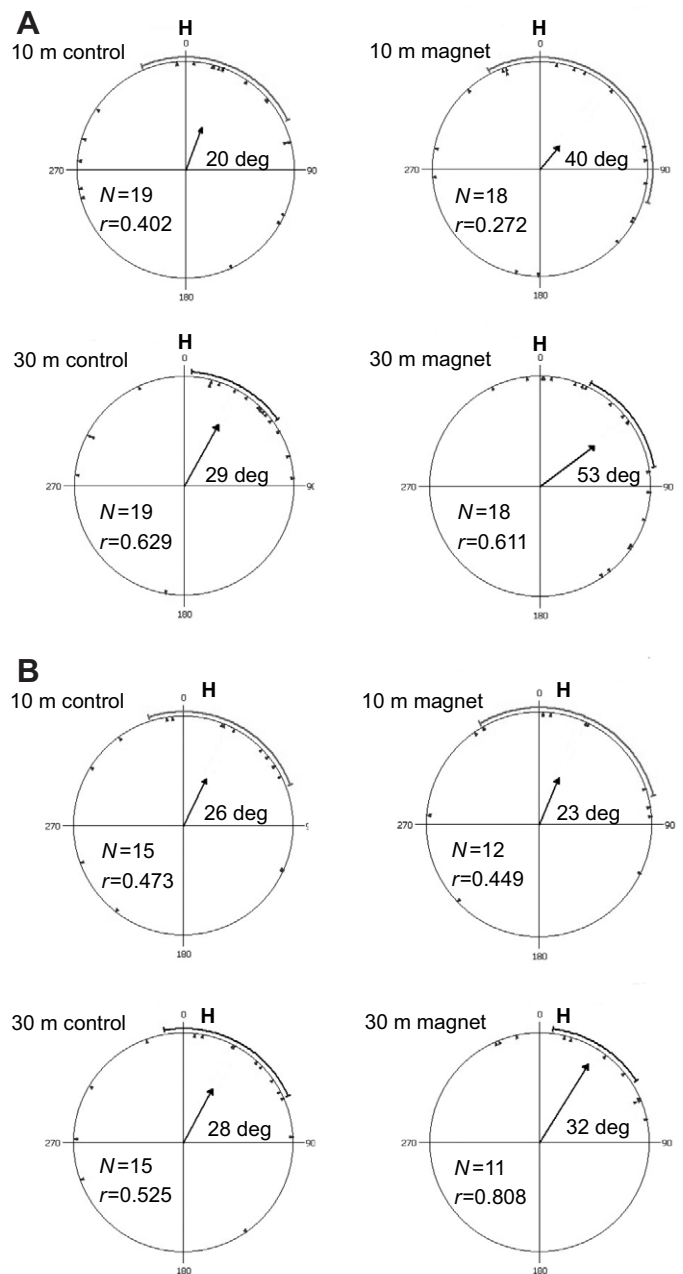

Fig. 2. Chicks' homing directions at distances of 10 and 30 m away from the arena during magnetic (A) day and (B) night experiments. Circular diagrams show the heading of chicks (small triangles at the periphery). The arrow from the center of the diagram represents the mean heading vector, and the arc outside of the circle shows the 95% confidence limits of the mean. H, homing (crèche) direction; N, number of birds; r, length of mean vector.

condition. However, it should be noted that the significance of this relationship between the two groups was influenced by an outlying data point. The significant difference in the path straightness disappeared when this outlier was removed from the data set (Table 1). We did not observe any unusual paths in the control and magnet conditions.

At a distance of 10 m from the arena, the control group was significantly oriented towards the colony, but the magnet night group was not. At a distance of 30 m both control and magnet groups were significantly oriented towards the colony (Fig. 2). The distribution of the chicks did not differ between the control and the magnet conditions at 10 or 30 m (Table 1).

Low sound experiment
Orientation in the arena

Similar numbers of chicks preferred the colony half of the arena in the control (11/15) and in the low sound conditions (10/15). The time that chicks spent on the colony half of the arena was also similar between the two groups. The chicks' activity within the arena did not differ between the two conditions (Table 2).

Homing

The chicks in the low sound condition (16/18) were as successful at homing as the chicks in the control condition (14/15). The chicks' homing times were similar in the two groups tested. Examination of the homing paths revealed no difference in the path straightness of the control and the low sound groups. At 10 and 30 m from the arena chicks were oriented towards their crèches in both control and low sound conditions (Fig. 3). The distribution of chicks as a group was not different between control and low sound conditions at 10 or 30 m (Table 2).

Close examination of the paths revealed an interesting pattern. In the low sound condition, six chicks made a detour before reaching their crèche. Five chicks first headed east towards the ocean, walked around the research cabin (located close to the colony), and then turned towards the colony. One chick first headed east towards the ocean, turned west before reaching the cabin, and then turned towards the cabin (Fig. 4). In the control condition, one chick made a similar detour going around the cabin. These differences in the path shape between control and low sound groups approach significance (P=0.0650; Table 2).

Table 2. Statistical tests: low sound and loud sound experiments

Parameter tested	Groups tested	N	Mean ± s.e.m.	Test	Test statistic value	d.f.	P
Low sound experiment							
Orientation in the arena							
No. of chicks that preferred the colony half	Control vs low sound	15 15		Fisher exact			0.287
Time at the colony half (s)	Control vs low sound	15 15	397±48.3 387±48.3	t	0.148	28	0.883
Activity in the arena	Control vs low sound	15 15	4.0±0.85 3.9±0.60	t	0.064	28	0.949
No. of chicks that homed	Control vs low sound	15 18		Fisher exact			0.42
Homing							
Homing time (min)	Control vs low sound	14[a] 16[a]	22.9±3.9 22.08±3.5	t	0.021	28	0.984
Path straightness	Control vs low sound	7 18	0.55±0.09 0.48±0.05	t	0.715	23	0.482
Unusual homing paths	Control vs low sound	15 18		Fisher			0.065
Orientation at 10 m	Control	7		Rayleigh	4.011		0.012*
Orientation at 10 m	Low sound	18		Rayleigh	12.712		<0.001*
Orientation at 30 m	Control	7		Rayleigh	4.298		0.008*
Orientation at 30 m	Low sound	18		Rayleigh	11.689		<0.001*
Orientation at 10 m	Control vs low sound	7 18		Watson U^2	0.069		>0.5
Orientation at 30 m	Control vs low sound	7 18		Watson U^2	0.084		0.5>P>0.2
Loud sound experiment							
Orientation in the arena							
No. of chicks that preferred the colony half	Control vs speakers	19 19		Fisher			<0.001*
Time at the colony half (s)	Control vs speakers	19 19	562±60.5 198±47.7	t	4.73	36	<0.001*
Activity in the arena	Control vs speakers	19 19	7.0±1.2 7.0±1.5	t	−0.296	36	0.769
Initial homing							
Orientation at 0 m	Control	19		Rayleigh	3.863		0.019*
Orientation at 0 m	Speakers	17		Rayleigh	1.273		0.284
Orientation at 0 m	Control vs speakers	19 17		Watson U^2	0.063		>0.5
5 min distance (m)	Control vs speakers	19 19	57±4.3 33±6.5	t	2.984	36	0.005*

[a]Only animals that homed were considered.
*Significant P-values (P<0.05).

Loud sound experiment
Orientation in the arena

The orientation inside the arena was dramatically different between the control and the speaker conditions. In the control condition, the majority of chicks preferred the colony half of the arena (13/19). However, this preference was reversed in the speaker condition, where the majority of chicks preferred the half of the arena closest to the speakers (17/19) and not the colony. The chicks spent more time in the colony half of the arena under the control than under the speaker condition. The chicks' activity within the arena was similar under the two conditions (Table 2).

Initial homing

When the barrier was lowered and the chicks were free to leave the arena, the chicks were oriented towards the colony in the control condition but not in the speaker condition (Fig. 5). No significant differences were observed in the distribution of the two groups of chicks as they left the arena. Five minutes after the barrier was opened, the control chicks were further away from the arena than the experimental chicks (Table 2, Fig. 6).

DISCUSSION

Our experiments suggest that when the magnetic field around chicks was modified, their orientation in the arena and the overall ability to home was not compromised. Only at the beginning of the homing journey (10 m away from the arena) did chicks have difficulty in orienting towards their crèches. Limiting the sound input did not have a pronounced effect on the chicks' orientation and navigation. Chicks with covered ears oriented towards the colony and found their place in the crèche as well as chicks from the control group. However, providing additional acoustic information, such as sound of the colony, had a dramatic effect on the chicks' position within the arena. The chicks always spent more time on the side of the arena where the sound of the colony was strongest.

The magnetic experiments were designed to test the overall importance of the magnetic cues for chick orientation and short-range navigation. Based on our results, it did not appear that magnetic cues are essential for successful homing in king penguin chicks. There are several explanations for our findings: chicks do not use magnetic cues for orientation and navigation around the colony; other cues compensate for the lack of information from magnetic cues (i.e. acoustic cues); or the ability to use magnetic cues develops at the later age and/or is used in a different context.

Despite the fact that the attachment of magnets did not significantly affect different parameters of the chicks' overall homing, we observed some changes in the chicks' behavior. First, the chicks were more active (made more transitions between arena quadrants) in the magnet condition than in the control condition during the day experiment, but not in the night experiment. Second, we observed differences between the control and experimental conditions in chicks' orientation when they were 10 m away from the arena both during the day and night experiments. This disorientation was transient, and by 30 m away from the arena both control and magnet groups were oriented towards the crèche.

Fig. 4. Chicks' homing paths in the low sound experiment. The figure shows the direct route to a crèche and three routes taken by homing chicks that included detours around the research cabin in the low sound condition. The thin black line shows the colony outline (only a small part of the colony is shown).
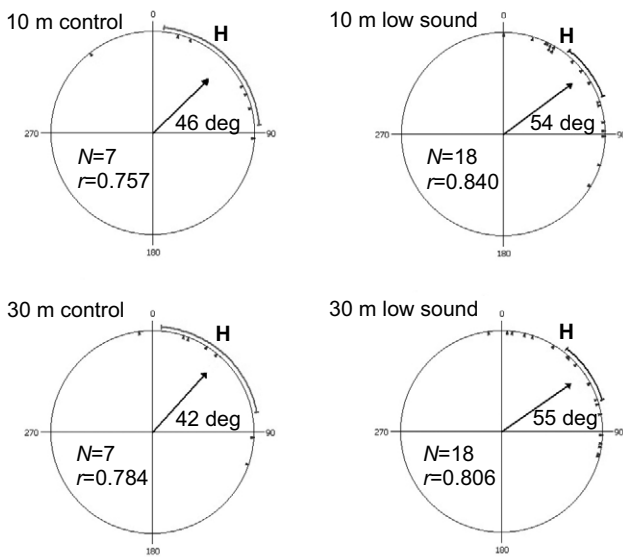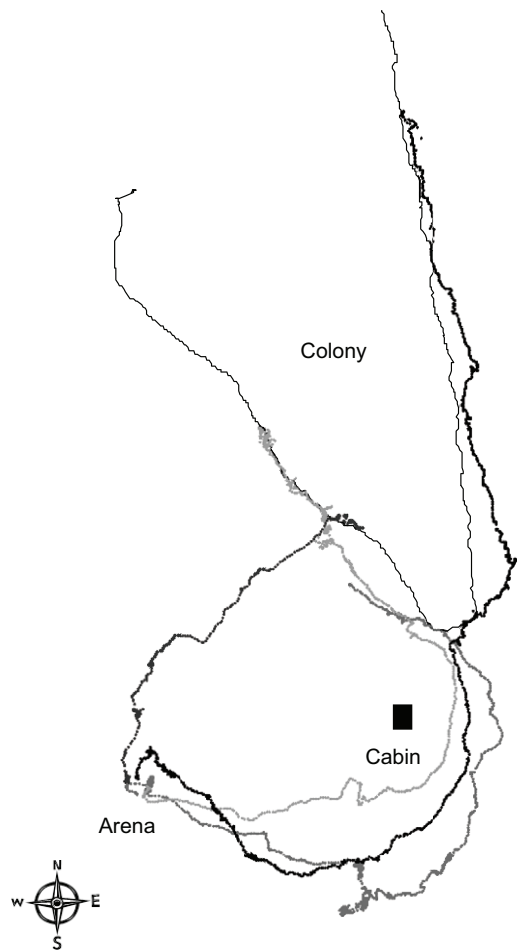

Fig. 3. Chicks' homing directions at distances of 10 and 30 m from the arena during the low sound experiment. Circular diagrams show the heading of chicks (small triangles at the periphery). The arrow from the center of the diagram represents the mean heading vector, and the arc outside of the circle shows the 95% confidence limits of the mean. H, homing (crèche) direction; N, number of birds; r, length of the mean vector.
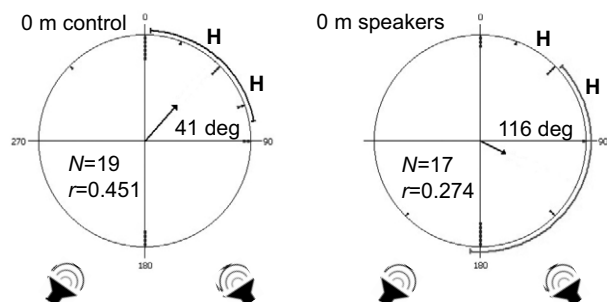
Fig. 5. Chicks' homing directions when they left the arena (0 m) in the loud sound experiment. Circular diagrams show the heading of chicks (small triangles at the periphery). The arrow from the center of the diagram represents the mean heading vector, and the arc outside of the circle shows the 95% confidence limits of the mean. H, homing (crèche) direction; *N*, number of birds; *r*, length of the mean vector. Diagrams also show the position of the speakers, which were placed 3 m from the arena barrier, SE (150 deg) and SW (210 deg) of the arena center.
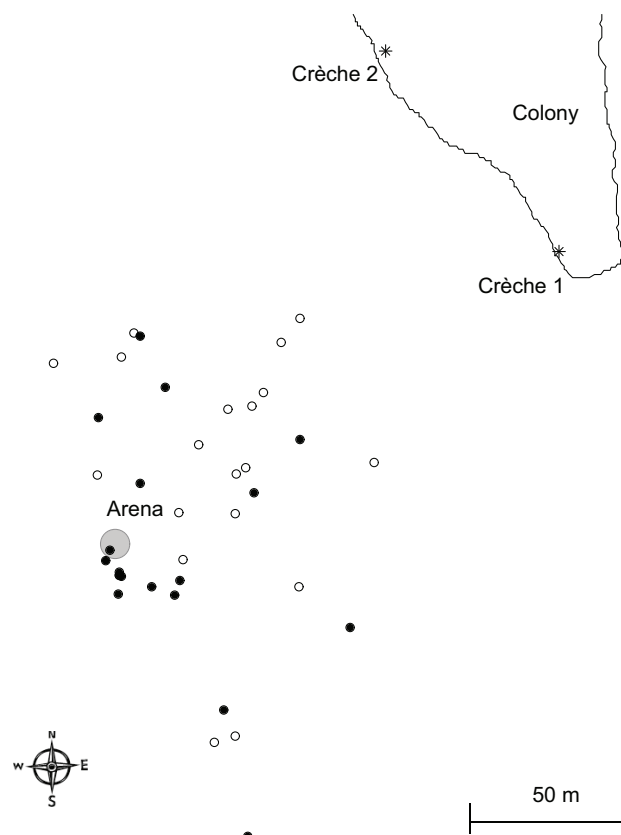

Fig. 6. Chicks' locations 5 min after the arena barrier was opened in the loud sound experiment. Open circles represent the locations of chicks from the control group and filled circles represent the locations of chicks from the loud sound group. The thin black line shows the colony outline (only a small part of the colony is shown). Asterisks indicate centers of two crèches from where chicks were captured.

The observed effects of magnets in king penguin chicks could be due to stress-related factors. Potentially, non-static perturbation (due to the mobile magnet on the head) of the geomagnetic field caused stress to the chicks in our experiments, and this in turn affected their activity (magnetic day experiment) and initial orientation (magnetic day and night experiment). Luschi et al. demonstrated that the transportation of pigeons in an oscillating magnetic field affected their emotional state (stress levels) and resulted in an increased scatter and a decrease in initial homeward orientation (Luschi et al., 1996). When birds were injected with promazine, which is known to have tranquilizing effect, they behaved like the control group that was not subjected to the oscillating magnetic field, and the initial 'disorientation effect' disappeared.

When testing acoustic cues, in the low sound experiment, the chicks with covered ears behaved for the most part in a similar way to the chicks in the control group. It is likely that they were able to perceive enough acoustic information for successful orientation and homing. In addition, after the barrier was open, the chicks could have also used visual cues to compensate for the limited acoustic input. We observed an interesting pattern with respect to the homing paths. All but one chick in the control group went more or less directly towards the crèche. However, in the group with plugged ears, several animals first departed towards the ocean, and only then turned towards the colony. The colony was not visible immediately around the arena, even when the barrier was down, but the ocean was in clear view, and could have served as an important landmark. Potentially, chicks that were disturbed by the low sound input relied on the sight of the ocean for their orientation. In addition to the unusual homing paths, our data on the initial orientation show an eastward bias in both the low and loud sound experiments. This trend was also observed during magnetic experiments, and is probably due to the presence of the ocean, which potentially can provide visual, acoustic and/or olfactory information.

Broadcasting colony sounds had a strong effect on the chicks' position within the arena. In fact, the orientation preference within the arena could be completely reversed depending on where the colony sounds were strongest. When the arena barrier was lowered and the chicks were free to leave, the control group appeared to orient towards the colony. However, in the experimental condition the colony sounds coming from the south

(non-colony side) disoriented the animals. On several occasions chicks walked towards the speakers and vocalized repeatedly while standing between the speakers, which continued to broadcast colony sounds. In addition, 5 min after opening the barrier, the control group was further away from the arena than the experimental group.

Given the extraordinary auditory abilities of king penguins, it is not surprising that chicks could use acoustic cues to orient towards the colony. Individual recognition in this species is based on vocal communication where individuals can identify their partners or chicks as they move through a noisy crowd of conspecifics (Aubin, 2004). Moreover, because of the highly vocal nature of these birds, king penguin colonies are a prominent source of sound, especially during breeding season. The use of conspecific or heterospecific sounds as guiding cues has been widely demonstrated before. Migrant songbirds tend to use acoustic cues for habitat selection, especially when visual cues are limited, such as during twilight. For example, the songs of Eurasian reed warblers (*Acrocephalys scirpaceus*) and sedge warblers (*Acrocephalys shoenobaenus*) attract conspecifics and other species that are specialists in wetland habitats (Mukhin et al., 2008).

Whether acoustic cues alone are sufficient to guide chicks towards the colony remains questionable. Our previous work demonstrated that the homing success of king penguin chicks drops at night when visual cues are limited, but the colony is still

audible (Nesterova et al., 2009). However, king penguin adults seem to be able to enter the colony and proceed toward their attachment places even in complete darkness (Nesterova et al., 2010). This ability to rely solely on acoustic cues (or other non-visual cues) may develop at a later age, but this remains to be tested in the future.

King penguins present a very interesting system for testing further the idea of soundscape orientation. These birds have to find a very specific place in the colony and often under conditions with limited visual cues. It has been demonstrated that at the last stages of navigation in the colony king penguins rely on vocal signals, but the range of individual vocal recognition is quite limited, 8.8 m on average in the colony (Lengagne et al., 1999b). The question is whether vocal cues are used when an animal is more distant from its goal. Given the highly vocal nature of this species, the colony could be perceived as a patchwork of different sound neighborhoods that are used as soundmarks. At first glance this seems a daunting task because the colony is dynamic. Partners take shifts during incubation and later when feeding their chicks. As a result, each neighborhood might sound slightly different at different times. However, king penguins might be capable of deciphering particular neighborhood soundscapes, considering that they are adapted to recognize specific calls in spite of the low signal-to-noise ratio in the colony (Aubin and Jouventin, 1998). Additional support for neighborhood recognition comes from studies on royal penguins (*Eudyptes schlegeli*) (Waas et al., 2000). By means of a colony playback system, the authors exaggerated the number of calls heard in the colony. Interestingly, the perceivers reacted differently to the calls of neighbors than to individuals from a different colony. Playback from their own colony facilitated more sexual and aggressive interactions than playbacks from a different colony, suggesting that royal penguins can recognize their immediate acoustic neighborhood.

This study is only a first step towards understanding the importance of magnetic and acoustic cues for king penguin orientation and navigation. Together with our previous work, it suggests that acoustic and visual, but not magnetic, cues are important for chick orientation towards the colony.

AUTHOR CONTRIBUTIONS

A.P.N. conceived and designed the study, conducted experiments in the field, interpreted the findings, and wrote the manuscript. F.B. conceived the study, participated in the designing of the experiments and interpretation of the findings, provided logistical support with the expedition organization, and revised the manuscript. C.C. assisted in conducting field experiments and commented on the manuscript. J.C. assisted in conducting field experiments.

COMPETING INTERESTS

No competing interests declared.

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