Evidence that blue petrel, *Halobaena caerulea*, fledglings can detect and orient to dimethyl sulfide

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Summary

Procellariiform seabirds (the petrels, albatrosses and shearwaters) are recognized for their acute sense of smell. These pelagic seabirds forage over thousands of miles of ocean to find patchily distributed prey resources. Over the past decade, much headway has been made in unravelling the variety of olfactory foraging strategies that Antarctic species employ, and it is becoming clearer that olfaction plays a key role in foraging, particularly for burrow nesting species. Now we are beginning to explore how these behaviours develop in chicks. Procellariiform chicks fledge and survive the open seas without aid or instruction from a parent, but how they are able to accomplish this task is unknown. Here we explore whether chicks leave the nest pre-tuned to olfactory cues necessary for foraging. In this study, we tested the hypothesis that blue petrel chicks (*Halobaena caerulea*) are able to detect and orient to a foraging cue (dimethyl sulphide, DMS) used by adults without ever having experienced this odour at sea. We first established that chicks could detect DMS at a biologically relevant concentration that they will later naturally encounter at sea (<10 pmol l⁻¹). We then performed preference tests in a Y-maze on a group of birds 1–6 days before they fledged. Sixteen out of 20 fledglings preferred DMS (e.g. DMS+propylene glycol) to a ‘control’ odour (propylene glycol alone). Our results suggest that chicks can detect and may already recognize DMS as an orientation cue even before they leave the nest to forage for the first time.

Key words: Procellariiform seabird, dimethyl sulphide, orientation, Antarctic, petrel, *Halobaena caerulea*, olfaction.

Introduction

Procellariiform seabirds – petrels, albatrosses and shearwaters – use olfaction for a variety of behaviours including foraging (reviewed by Nevitt, 2000) and partner recognition (Bonadonna and Nevitt, 2004). One of the early ideas to explain the function of the sense of smell in petrels was that these seabirds use odours as foraging cues (Grubb, 1972). For many petrel species, prey includes small crustaceans, fish and krill, and consequently early investigation mainly focused on attraction to fishy smelling compounds (reviewed in Warham, 1996).

More recently, controlled behavioural experiments have shown that different species of procellariiform seabirds are attracted to a variety of natural scented compounds associated with their prey (Nevitt et al., 2004). One such compound, dimethyl sulphide (DMS), is an odour produced by photoplankton that is associated with areas of high primary productivity where prey are likely to be found (Nevitt et al., 1995). Local emissions of marine DMS can be predictable features in the environment, reflecting bathymetric features such as shelf breaks and seamounts (Berresheim, 1987). Moreover, DMS emissions have been shown to increase during grazing by zooplankton (Dacey and Wakeham, 1986). Together, these features suggest an odour landscape that may provide birds with orientation cues for foraging at sea (Nevitt et al., 1995; Bonadonna et al., 2003). Recently, we have shown that at least one species of burrowing petrel, Antarctic prions (*Pachyptila desolata*), can detect DMS at biogenic levels, and that these birds can use DMS as an orientation cue in a non-foraging context (Nevitt and Bonadonna, 2005b). These results suggest that prions have the ability to detect DMS and potentially use a DMS odour landscape as a navigational aid at sea [harbour seals (see Kowalewsky et al., 2005)].

The blue petrel (*Halobaena caerulea*) is another sub-Antarctic burrow-species that is phylogenetically closely related to Antarctic prions (Warham, 1996; Penhallurick and Wink, 2004). Both experimental and at-sea observational data suggest that both blue petrels and prions use DMS as a foraging cue at sea (Nevitt, 2000). Since DMS is not necessarily produced by prey, birds most likely need to learn to associate DMS with foraging opportunities soon after fledging. It is
possible that this association is learned even before chicks fledge, since returning parents bring back scents on their feathers and in the regurgitations that are fed to chicks. Such early learning would be particularly advantageous for petrels since, unlike other pelagic species, parents abandon the chicks up to 10 days before they fledge, leaving them to leave their underground burrows and forage independently in a thoroughly unfamiliar environment (Warham, 1996). While it is possible that naïve fledglings find productive areas through information transfer, i.e. by way of watching or following other birds coming and going from the colony (Ward and Zahavi, 1973), it would be most efficient if chicks were able to recognize foraging opportunities on their own as soon as they leave the nest. Thus, the possibility we have been exploring is whether chicks leave their underground burrow with a sense of smell that is already finely tuned to the ocean environment (see also Cunningham et al., in press).

An initial study using the ‘Porter method’ showed that blue petrel chicks are able to detect both DMS at micromolar concentrations and a second, presumably unfamiliar, rose-scented odour, phenyl ethyl alcohol (Cunningham et al., 2003). While the DMS concentration tested was considerably higher than ambient levels that adults would encounter at sea (discussed in Nevitt and Bonadonna, 2005b), these results demonstrate that petrel chicks have a well-developed olfactory sense and may be responsive to odours. Since we know that olfactory sensitivities may be shaped by early experience in many vertebrate species, such as rabbits (Semke et al., 1995), ferrets (Vargas and Anderson, 1996), salmon (Nevitt and Dittman, 1998) and chickens (Sneddon et al., 1998), we have since hypothesized that blue petrel chicks may be able to learn biologically important odours before they leave the nest (Nevitt and Bonadonna, 2005b; Cunningham et al., in press; G. A. Nevitt, R. W. Van Buskirk, G. B. Cunningham and H. Weimerskirch, manuscript submitted for publication).

If this were the case, then chicks would be predisposed to use odours they have associated with food in the nest as foraging or orientation cues at sea. Inspired by this idea, we wanted to test whether blue petrel chicks can detect DMS at biogenic levels, and whether they are predisposed to use DMS as an orientation cue before fledging.

Materials and methods

We performed our experiments on Île Verte (49°51′S, 70°05′E, ~1 km diameter) in the gulf of Morbihan in the Kerguelen Archipelago. We chose blue petrels, Halobaena caerulea Gmelin 1789 for this investigation because at-sea studies indicate that these species naturally associate with DMS (Nevitt, 2000), suggesting that they can smell it, and potentially use it as an orientation cue. Experiments were performed between 24 December 2003 and 15 January 2004, and from 18 January and 12 March 2005. Birds were banded prior to the study to identify individuals. For each experiment, birds were used only once. Since blue petrels lay a single egg, only one chick per nest was tested.

Chick’s response to DMS

We tested the olfactory responses of 22 blue petrel Halobaena caerulea chicks using the Porter method (see Porter et al., 1999; Cunningham et al., 2003). Briefly, chicks enter a sleep-like state, and the chick’s responses are scored in response to odour stimuli. This technique is non-invasive, does not affect fledging success, and has been successfully applied to blue petrel chicks for other studies (Cunningham et al., 2003). Chicks were tested at approximately 15–20 days post hatch (24 Dec–15 Jan; wing chord, 33.4±1.7 mm; tarsus, 24.8±0.6 mm; mass, 135.3±6.6 g).

For each test, a chick was removed from its burrow and transported in a cotton bag to a well ventilated field hut (4 m × 6 m) about 0.5 km from the colony. The chick was then positioned in a freshly lined holding chamber (approximately 10 cm × 5 cm × 5 cm) opened at both ends. The chick’s head protruded from one end while the chamber walls provided contact around the bird’s body. Once in the chamber, the chick quickly (within 3 min) entered a sleep-like state in which the head drooped slightly and the eyes closed. As in earlier studies, chicks were considered to be ‘asleep’ when the eyes were closed, the head drooped, and the legs and wings relaxed. We let chicks sleep for at least 3 min before initiating a trial.

Stimuli (DMS or Control, see below) were presented by puffing odour above the tube nose using a 500 ml Nalgene® squeeze bottle. The tip of the bottle was positioned ~3 cm from the opening of the tube nose. The bottle was then squeezed 1–2 times in a 5 s period, producing brief puffs of odorant-saturated air near the bird’s nostrils. Responses to odorant presentations were recorded for 1 min and scored categorically as one (indicated by biting movements, vocalizations, distinct head or body movements) or zero (typically no reaction) for each bird (modified from Porter et al., 1999). Scoring was done blind in that the solutions were prepared and coded by one experimenter prior to the start of a trial. The second experimenter delivering the stimulus and recording the response did not know the identity or concentration of the stimulus being delivered. If the chick woke up during a test, the chick was given up to 3 min for it to return to a sleep-like state. The bird was then allowed to sleep before continuing the experiment. If the bird did not fall asleep within 3 min, it was immediately returned to its burrow.

DMS solution (10 pmol l⁻¹, 100 ml) was prepared daily from stock solution (1 mmol l⁻¹; Sigma-Aldrich, St Louis, MO, USA) and bottled spring water (Evian®) using sterile glassware. Control solution contained 100 ml water only. Test solutions were transferred to clean Nalgene® squeeze bottles. Bottles were allowed to sit for at least 3 h at ambient temperature (9–13°C) to equilibrate the headspace. We calculated the headspace to be 18–21% of solution concentration using established methods ([Dacey et al., 1984], assuming an equilibrium coefficient K=15–20 at a temperature range of 10–14°C, or approximately 2 pmol l⁻¹). This concentration falls well within the biogenic concentrations that adults encounter while foraging (see Nevitt and Bonadonna, 2005b).

Tests were conducted during daylight hours within a narrow
temperature range (9–13°C) during daylight hours when parents were at sea. Chicks were transported and tested one at a time and spent approximately 15 min away from the nest. Each chick was weighed after testing. We checked burrows again prior to fledging to monitor any adverse affects to fledging success; weight gain and wing chord growth were within normal parameters (Jouventin et al., 1985) and no mortality was noted.

**Behavioural experiments**

To determine whether blue petrel chicks would orient to DMS before fledging, we presented birds with DMS in a Y-maze. We have previously established that other burrow nesting species orient to unfamiliar odours in Y-mazes. We have previously shown that adult prions prefer DMS to an unfamiliar odour (Nevitt and Bonadonna, 2005b). In this experiment, adults had previously experienced DMS at sea, and thus we assumed it was a familiar odour to them. Therefore, in the present experiment, we predicted that if chicks were also already familiar with DMS (i.e. through contact with their parents or food), then they would also orient to this compound in a Y-maze.

The Y-maze was constructed from opaque PVC wire housing and had three 60 cm symmetrical arms (for details, see Bonadonna and Nevitt, 2004; Nevitt and Bonadonna, 2005b). One arm was used as the starting point and was fitted with two trapdoors that formed a temporary holding compartment for the bird. Since chicks are negatively phototactic, odour choice arms were darkened. The end of each odour choice arm was equipped with a CPU cooling fan (Globe Fan Technology Co. Ltd., product number S05010, Taiwan) mounted on a partition to provide a low-noise, controlled airflow (9 CFM; 243 l min⁻¹).

In the compartment behind the fan, a Petri² dish (5.5 cm diameter) containing either DMS (1 μmol ml⁻¹, 4 ml) or control solution provided the stimuli. Odour stimuli were alternated between arms for each trial and frequently exchanged with fresh solutions. After each trial, the maze was washed thoroughly with methanol (70%) to remove any odour residue.

DMS solution was prepared in propylene glycol (4 ml; 1 μmol l⁻¹); control solution contained propylene glycol only (4 ml). DMS is much more soluble in propylene glycol than in water. To humans, this compound is lightly scented, suggesting that birds had to discriminate between two scented compounds rather than the presence or absence of odour. We have previously estimated the evaporation rate to be ~0.1 ml h⁻¹ or 1.7 nmol l⁻¹ min⁻¹ (Nevitt and Bonadonna, 2005b). This concentration, diluted by air flow in the maze (240 l min⁻¹), suggests that blue petrels were presented with an average stimulus concentration of <10 pmol l⁻¹ during experimental trials. This concentration is below the detection threshold for humans (Kowalewsky et al., 2005), but falls well within estimates of biogenic emissions that birds are likely to encounter at frontal zones in the Kerguelen plateau where adults are known to forage (Berresheim, 1987; Sciare et al., 1999; Nevitt, 2000).

Chicks were tested one at a time. Each chick was away from its nest for a maximum of 30 min and we noted no deleterious effects to fledging success. For each experimental trial, a bird was removed from its burrow, transported to the maze and then placed in the temporary holding compartment for a 1 min acclimation period. An inner trapdoor was then lifted, which allowed the bird to make a choice. We assessed the bird’s choice without disturbing the bird by the sounds it walking in the particular arm of the maze. We scored a positive choice if the bird travelled halfway down an arm and stopped for at least 1 min. Most birds stopped at the end of the arm and remained there. No-choice birds tended to sit quietly in the entryway, and were removed from the maze after 15 min. The choice time was considered to be the time that a chick took to walk halfway up each maze’s arm.

The chicks used for Y-maze experiments all had adult plumage and successfully fledged 1–6 days after testing (mean ± s.e.m.: DMS: 247±165 days) at about 44 days after hatching (43±2 days) (Jouventin et al., 1985). Fledglings were tested after parental abandonment, and thus had not been recently fed.

**Statistical analyses**

Statistical analyses were performed using SYSTAT. For Porter method studies, DMS and control scores were compared using a Wilcoxin sign-rank test for paired data. Y-maze data were analyzed using a Binomial test (Zar, 1996). Values are expressed as mean ± s.e.m.

**Results**

**Chick’s response to DMS**

During the 2003–2004 field season, we first verified that chicks could detect picomolar concentrations of DMS [10 pmol l⁻¹ solution concentration, ~2 pmol l⁻¹ headspace concentration (Dacey et al., 1984)] using the Porter method (Porter et al., 1999). Average scores were significantly higher for DMS than for the control stimulus (P<0.03, W=-63.0, Wilcoxon matched-pair sign ranks test, N=22; Fig. 1).

**Behavioural experiments**

During the 2004–2005 field season, we ran chicks in a Y-maze to test attraction to DMS under controlled conditions. Of the 24 birds tested, 20 successfully made a choice. Sixteen (mass: 167±24.8 g, mean ± s.e.m.) oriented to DMS whereas four (mass 154±10.2 g) oriented towards control (binomial test P<0.01; Fig. 2). Choices were typically made within 3–4 min (mean ± s.e.m.: DMS: 247±165 s; control, 176±183 s). Four birds (mass: 157±44.7 g) did not choose an arm but stayed in the entryway of the maze. No differences in choice time, mass, or the number of days between testing and fledging were found between birds orienting to DMS or to control (Mann–Whitney U-test: choice time, U=32, P=0.45; mass, U=30, P=0.34; number of days, U=38, P=0.74).

**Discussion**

The results presented here demonstrate that blue petrel chicks can detect and are attracted to DMS within a concentration range that they will naturally encounter while foraging as adults at sea. This concentration is less than
and is similar to the detection abilities we have calculated for Antarctic prions in Y-maze experiments (Nevitt and Bonadonna, 2005b), and at least five orders of magnitude below olfactory detection thresholds typically reported for other avian species (Roper, 1999). One of the improvements made to our experimental design in the present study is that we confirmed detection ability at picomolar concentrations using a second method, the Porter method. This method is non-invasive and allowed us to test responses of an adequate sample size of chicks to DMS under conditions in which odorant delivery and concentration were well controlled.

Although the Y-maze we used here was similar to the one we used in previous experiments that tested attraction to DMS in Antarctic prions (Nevitt and Bonadonna, 2005b), the motivation of the subjects we tested was probably different. With prions, we tested adults who had recently returned from sea and were subsequently well fed in preparation for a 10–15 day incubation shift on the nest. Because these test subjects were not motivated to look for food, we assumed that DMS in the Y-maze indicated an avenue of escape to ocean. By contrast, the blue petrel chicks that were tested in the present experiment had not been recently fed, and were within a few days of fledging. These birds had also never experienced DMS in the context of the pelagic environment. Moreover, because chicks were given a choice between two different odours (DMS in propylene glycol vs propylene glycol alone), the attraction seemed to be specific to DMS rather than to the presence of any scented cue. Based on these results, we conclude that the scent of DMS may already be associated with food before the chick leaves the nest.

Why would an ability to smell DMS be advantageous for a fledgling? At sea, DMS emissions become elevated in areas where zooplankton is grazed by phytoplankton (Dacey and Wakeham, 1986; Daly and DiTullio, 1996). Consequently, DMS characterises areas rich in zooplankton, in what is presumed to be a visually uniform environment, at least to humans. Using changes in an odour landscape would seem to be an efficient mechanism for localising patchily distributed prey (Nevitt, 2000), but how or when birds learn to associate prey-related odours with food or foraging opportunities is unclear. One possibility is that young petrels learn to associate feeding areas with DMS in their first months or years at sea. However, our results, complemented by those of other studies, indicate that chicks already may recognize DMS [or food odours (Cunningham et al., in press)] even before they leave the nest. Alternatively, it is commonly assumed that young petrels learn how to forage primarily by watching other birds [the ‘information center’ hypothesis (Ward and Zahavi, 1973)]. While monitoring the activity of conspecifics probably contributes to learning how to forage, this hypothesis ignores the critical role olfaction plays in the foraging behaviour of certain species, and particularly, burrow-nesting species (Nevitt et al., 2004).

Thus, with respect to sensitivity to DMS, two additional possibilities may be considered: (a) DMS may stimulate an innate attraction; (b) chicks reared in burrows learn odour cues before leaving the nest. It could be argued that hard-wired olfactory sensitivities usually function for highly constrained uses [i.e. pheromone attraction, for example (Shaal et al., 2003)]. However, olfactory behaviours that have evolved to contend with variability in the environment tend to be shaped by learning (reviewed by Hudson, 1999). Moreover, behaviours that can be learned are more flexible to adapt to environmental change or to differences that may exist between different populations living in different areas. For example, among the Antarctic procellariiforms, species show a great deal of variation in their foraging strategies, particularly with respect to their attraction to different scented compounds linked either to their prey or to the ecological characteristics of the species on which they forage (Nevitt, 1999; Nevitt et al., 2004; Nevitt and Bonadonna, 2005a). It follows that, in the context of foraging in a highly variable environment, an inflexible, innate sensitivity to a specific suite of prey odours is not likely to be adaptive.

Furthermore, data from a variety of systems support the hypothesis that procellariiform chicks might learn to associate relevant foraging odours to food during the rearing period. For
example, it is now well established that olfactory sensitivity is physiologically tuned after birth in a variety of animals. In some cases, this tuning has been linked to behaviour, life history and ecology. For example, salmon home to the scent of the stream of their birth (reviewed by Nevitt and Dittmann, 2002), and rabbit pups imprint on the scent of food-related odours expressed in the milk of their mother (Althacker et al., 1995; Semke et al., 1995). When we consider how and where a petrel chick develops (alone in a dark burrow), smell is probably a major sensory stimulus during the first few months of life. Odours brought back on the feathers of parents might provide chicks with the opportunity to learn scents associated with productive areas. Moreover, DMS is probably not the only odour they learn since chicks are exposed to a variety of compounds through interactions with their parents, including scented compounds in stomach oils with which they are nourished. These compounds are derived, in part, from prey species and thus are linked to foraging opportunities in the open ocean. We have recently shown that blue petrel chicks are sensitive to at least some of these compounds (see Cunningham et al., in press).

Linking these ideas together, our current hypothesis is that odour cues in the nest may condition chicks to be able to find food rapidly once they have fledged (see also G. A. Nevitt, R. W. Van Buskirk, G. B. Cunningham and H. Weimerskirch, manuscript submitted for publication). For example, in chicken chicks (*Gallus domesticus*), exposure to strawberry odour 5 days before hatching influenced the chick’s preference for this odour afterwards (Sneddon et al., 1998). Our results suggest that olfactory learning or imprinting in birds may have important consequences to foraging success. These questions should be explored further in procercaliform species that rely heavily on olfaction to forage.

To summarize, while it is commonly assumed that young fledglings follow other birds to feeding areas, or simply wander over the ocean to locate a suitable feeding zone, our results suggest instead that chicks leave the nest already tuned to potential foraging opportunities in their environment. Chicks are thus equipped to adopt the same olfactory strategy used by adults from the beginning of their life at sea.

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