Behavioural Responses of Blue Petrel Chicks (Halobaena Caerulea) to Food-Related and Novel Odours in a Simple Wind Tunnel

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Behavioural responses of blue petrel chicks (*Halobaena caerulea*) to food-related and novel odours in a simple wind tunnel

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Abstract: As a first step towards understanding the development of olfactory behaviours in Antarctic procellariiform seabirds, we recently showed that blue petrel chicks (*Halobaena caerulea*) could detect both a food-related and a novel odour while asleep. In this current study, we tested chicks in a simple wind tunnel to determine if exploratory behaviours could be initiated by olfactory stimuli as well. We compared the behavioural responses of 30 blue petrel chicks to cod liver oil (a prey-related odour) or phenyl ethyl alcohol (an unfamiliar, rosy-smelling odourant) against a control (distilled water). Six behavioural indices were measured, including head turns, body turns, bites, preening events, wing-stretches, and distance walked. In response to cod liver oil, we found that chicks increased both turning rates and distances walked whereas chicks preened more in response to phenyl ethyl alcohol. Since only cod liver oil initiated behaviours consistent with searching, our results suggest that chicks are attaching biological significance to food-related odours even before they leave the burrow to forage for the first time.

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Key words: development, foraging, Kerguelen, olfaction, procellariiform

Introduction

Petrels (sub-order: Procellariiformes; family: Procellariidae) are pelagic seabirds that forage on a range of marine organisms including krill (*Euphausia* sp.), fish, squid and a variety of crustaceans (Prince & Morgan 1987). These birds come to land to rear offspring either above ground or in burrows, depending on the species (for review see Warham 1996). Petrels have large olfactory bulbs (Bang 1966, 1971, Bang & Cobb 1968) and respond to a variety of food-related odours (Grubb 1972, Hutchison & Wenzel 1980, Lequette *et al.* 1989, Veit *et al.* 1993, Verheyden & Jouventin 1994, Nevitt 1999, Nevitt & Veit 1999), suggesting that many species hunt by smell. In burrowing species, olfaction is also used to relocate the nest site (Grubb 1973, 1974, Bonadonna & Bretagnolle 2002, Bonadonna *et al.* 2003a, 2003b) and may be involved in individual recognition (Bonadonna & Nevitt 2004, for review see Nevitt & Bonadonna 2005).

The blue petrel (*Halobaena caerulea* Gmelin) has been the subject of numerous investigations studying olfactory behaviours in burrow nestling procellariiforms. Growing experimental evidence suggests that adults rely on smell to relocate their burrows when returning from foraging trips (Bonadonna *et al.* 2001), probably recognizing burrow specific olfactory signals to help them find their own burrow amongst thousands of others at night (Bonadonna *et al.* 2004). Blue petrels are also sensitive to odours associated with food. These include fishy smelling odours (Nevitt *et al.* 2004, Nevitt & Bonadonna 2005), krill odours (Nevitt 1994) and dimethyl sulphide (Nevitt *et al.* 1995), a scented compound that is elevated where zooplankton aggregate (reviewed by Nevitt 2000).

Although extensive research has focused on better understanding olfactory-based behaviours in adult birds, little is known about how these behaviours develop. To address this, we recently investigated the responses of blue petrel and thin-billed prion (*Pachyptila belcheri* (Matthews)) chicks to a variety of odours using the Porter method (Cunningham *et al.* 2003). This method involves testing chicks’ responses to odours while they are in a ‘sleep’ state (Porter *et al.* 1999). Sleeping chicks responded to odour presentations by peeping or making head movements; these behaviours were easily scored. Using this non-invasive technique, we found that even young chicks (aged four to 17 days) were able to detect both a food-related and a novel odour, indicating that the olfactory system is well developed in some procellariiforms prior to leaving the burrow (Cunningham *et al.* 2003, see also Minguez 1997, Bonadonna *et al.* 2006). Having established that chicks can smell, we wanted to explore whether food-related odours might elicit exploratory behaviours in awake chicks once they become ambulatory.

Here we tested the responses of blue petrel chicks to odours using a simple wind tunnel delivery system where we could record detailed behavioural responses to a standardized odour presentation. Chicks were exposed to...
either cod liver oil (CLO), a known fishy-smelling olfactory attractant for procellariiforms (Grubb 1972, Hutchison & Wenzel 1980, Lequette et al. 1989, Verheyden & Jouventin 1994, Nevitt & Haberman 2003, Nevitt et al. 2004), or phenyl ethyl alcohol (PEA), a rosy smelling novel odourant that blue petrels can detect (Cunningham et al. 2003). We predicted that these two odours would elicit different behaviours in chicks because CLO is a food-related odour whereas PEA is not.

Material and methods

Blue petrel chicks on Mayes Island (49°28'S, 69°57'E) in the Gulf of Morbihan, Iles Kerguelen, were tested in late January 2002 (21–24 January for the CLO experiment and 26–29 January for the PEA experiment). Experiments were conducted sequentially using different birds for each experiment. The mean age of the chicks at testing (± s.e.(m.)) was 32 ± 0.9 days for birds used in the CLO experiment and 36 ± 1.6 days for birds used in the PEA experiment. Hatch dates were determined by checking burrows on a daily basis around the time of hatching. All trials were conducted between 10:00 and 15:00 hours to minimize diel variation. Chicks were tested under ambient conditions. The mean air temperature was 17.2 ± 0.5°C and the mean relative humidity was 56.6 ± 0.7% during the two experiments.

Wind tunnel specifications

All experiments were performed in a simple wind tunnel (Fig. 1) situated inside a well-ventilated shelter (1.5 m x 1.5 m; 2.5 m high) located less than 500 m from a blue petrel colony. The arena where the chick was placed (0.8 m x 0.6 m; 0.3 m high) was constructed of Plexiglass®. Airflow (0.6–0.7 m sec⁻¹) was generated by a pressure box with two tubeaxial fans mounted to each side (Mechatronics model UF12A12; Preston, WA, USA; air volume: 3 m³ min⁻¹). The fans were positioned at a 90° angle to an exit port that directed air into the testing arena. Two lengths of flexible air duct (9 cm diameter) were attached to the fans and ran through vents in the hut wall to draw fresh air from outside. To reduce turbulence in the arena, air exiting the pressure box passed through egg-crate plastic lined with organdie fabric.

Olfactory stimuli

A glass dish (diameter 71 mm; height 41 mm) containing a cotton swab (1 cm x 5 cm; 0.5 cm thick) was positioned 10 cm downwind from the pressure box exit port. The cotton swab was saturated with a 1 mL solution of either 100% CLO (Squibb Pharmaceuticals, New York, NY, USA), 1 μM PEA (Sigma-Aldrich, St Louis, MO, USA), or a distilled water control. Odour or control presentations were presented sequentially and in random order according to the design shown in Table I. To control for potential order effects, half of the chicks in each group received the control stimulus first, and half received the experimental odour (CLO or PEA) first. Thirty chicks were tested for each experiment (60 chicks total). Three chicks were removed from the experiment because they either quickly walked out of the arena or showed stereotypies that suggested excessive stress. These chicks were immediately returned to their burrows and were not included in the analyses.

Experimental protocol

Chicks were tested one at a time. Prior to a trial, a chick was removed from its burrow and transported to the shelter in a cotton bag. Because chicks live in dark, underground burrows, experiments were performed in the dark and videotaped from above using infrared illumination. To start a trial, a chick was positioned inside the arena at a predefined ‘start’ point located 40 cm downwind of the glass dish (Fig. 1) which contained either an odour or a control stimulus. Chicks were positioned such that their beak faced upwind. Following a 1 min acclimation period, we removed the lid from the glass dish and recorded the chick’s behaviours for 4 min using a Sony camcorder (model DCR-TRV30) outfitted with an external infrared light emitter (Sony model HVL 1RH2). At the end of 4 min,
the chick was repositioned at the ‘start’ point, and another lidded glass dish containing the second stimulus was swapped into the testing arena. This second stimulus was then tested using the same procedure. Between trials, all chicks were handled similarly whether or not they walked during Trial One. Once Trial Two was completed, chicks were weighed, measured for wing length, tarsus length, and bill length, and returned to their burrow. These morphometric data are summarized in Table II.

A ‘blind’ observer scored videotapes in real time on a Sony Trinitron colour video monitor (model PVM-1351Q) using Jwatcher software (Blumstein, Evans & Daniel, Animal Behaviour Laboratory, Macquarie University, Australia). We identified five behaviours (see Table III): head turns, body turns, bites, preening events and wing-stretches. Body position, used to calculate the total distance that each chick walked, was noted at 15 sec intervals.

**Statistical analysis**

We first examined whether the order of stimulus presentation influenced responsiveness by comparing the mean response to a stimulus obtained during Trial One against the mean response to the same stimulus obtained in Trial Two (see Table I). We used either a Student’s \( t \)-test on normal or square root transformed data, or a Mann-Whitney \( U \) test on non-normal data (Zar 1996). Mean responses to PEA and both control presentations were significantly different between trials, but the responses to CLO were not, indicating that the order of stimulus presentation influenced the behavioural response for some stimuli (see Results). Due to this order effect, we elected not to pool the results of the two trials for each stimulus (Leger & Didrichsons 1994) and carried out further analysis on each of the trials separately.

**Results**

**The order effect**

Responses to CLO-presentations were similar between trials. However, chicks were more active in Trial One
compared to Trial Two for all other stimulus presentations (PEA, both control presentations). These findings are summarized in Table IV. Since we found a significant order effect with respect to CLO-control, PEA, and PEA-control presentations, we analysed the results of Trials One and Two separately for each of the experiments.

**Behavioural responses to CLO in Trial One and Trial Two**

In Trial One, all behavioural scores were similar in response to CLO and control stimulus presentations (Fig. 2a: Student’s t-test: head turns: \( n = 28, t = -0.907, df = 26, P = 0.372 \); body turns: \( n = 14, t = 0.7, df = 12, P = 0.25 \); Mann-Whitney U test: bites: \( n = 28, U = -0.98, P = 0.32 \); preening: \( n = 28, U = 0.36, P = 0.72 \); wing-stretches: \( n = 28, U = -0.48, P = 0.63 \)). In addition, about half of the chicks walked (6/14 for CLO and 8/14 for control) but distances travelled were similar between treatments (Fig. 3a: \( n = 14, t = 0.45, df = 12, P = 0.66 \)).

A lower proportion of the chicks walked in Trial Two compared to Trial One (5/15 for both CLO and control). Chicks turned more (Fig. 2b) and walked further distances (Fig. 3b) in response to CLO, but the other scores were similar between the two stimuli (body turns: \( n = 10, t = -2.91, df = 8, P = 0.01 \); distance: \( n = 10, t = -2.31, df = 8, P = 0.05 \); head turns: \( n = 30, t = 0.84, df = 28, P = 0.41 \); bites: \( n = 30, U = -0.66, P = 0.51 \); preening: \( n = 30, U = 0.39, P = 0.70 \); wing-stretches: \( n = 30, U = -0.48, P = 0.63 \)).

**Behavioural responses to PEA in Trial One and Trial Two**

In Trial One, chicks preened more in response to PEA than to control, but other behaviours were similar (Fig. 4a: preening: \( n = 29, U = 2.69, P = 0.0071 \); head turns: \( n = 29, t = 0.71, df = 27, P = 0.48 \); body turns: \( n = 18, t = -1.37, df = 16, P = 0.09 \); bites: \( n = 29, t = 0.27, df = 27, P = 0.79 \); wing-stretches: \( n = 29, U = 1.14, P = 0.26 \)). Most chicks tended to walk more in response to PEA compared to control in Trial One (6/14 for PEA and 2/14 for control); however, this difference was not significant (\( U = 2.69, P = 0.0071 \)). In Trial Two, most chicks walked more in response to both PEA and control (8/15 for PEA and 7/15 for control), but the difference between treatments was not significant (\( U = 1.14, P = 0.26 \)).
walk in Trial One (9/15 for PEA and 9/14 for control) but distances walked were similar between treatments (Fig. 5a: \(n = 18, t = 1.20, \text{df} = 16, P = 0.25\)).

During Trial Two, chicks reacted similarly to PEA and control stimuli (Fig. 4b: head turns: \(n = 29, t = 0.76, \text{df} = 28, P = 0.45\); body turns: \(n = 10, t = -0.13, \text{df} = 8, P = 0.45\); bites: \(n = 29, U = 0.16, P = 0.88\); preening: \(n = 29, U = -1.23, P = 0.22\); wing-stretches: \(n = 29, U = 0.45, P = 0.65\)). In contrast to Trial One, fewer chicks walked (6/14 for PEA and 4/15 for control) but distances walked were similar (Fig. 5b: \(n = 10, t = -0.74, \text{df} = 8, P = 0.48\)).

**Discussion**

This study is the first to use a simple wind tunnel to explore olfactory behaviours in procellariiform chicks. By videotaping blue petrel chicks in the wind tunnel, we were successfully able to record and measure a suite of distinct behaviours that are likely to be involved with olfactory search. We found that these birds responded differently to odour and to control presentations depending on both the identity of the odour and how long chicks had been in the wind tunnel. These differences give us insight into how different types of odour stimuli may influence behaviours. For example, in response to CLO, chicks walked further distances and turned more, but only during Trial Two. In petrels, turning behaviour is linked to olfactory search both at sea (e.g. Hutchison & Wenzel 1980, Nevitt et al. 1995) and on land (Grubb 1972), and characterizes olfactory search in a variety of other animals (Willis & Arbas 1998, Leising & Franks 2002, Weissburg & Dusenbery 2002). Our results suggest that blue petrel chicks probably needed time to adjust to the testing conditions before initiating search behaviour in response to CLO. By contrast, chicks did not perform search behaviours in response to PEA, but did preen more in the presence of this stimulus during Trial One. Preening has been linked to stress in a variety of birds (Nephew et al. 2001, Olsson et al. 2002, Zimmerman et al. 2003, Meteyer et al. 2004) and is clearly not a behaviour involved with olfactory search. Our interpretation is that this preening response was associated with the stress of being placed in an arena that smelled unfamiliar.

**Potential explanations for the differences in response to stimuli**

We see a number of reasons why CLO initiated behaviours consistent with olfactory search whereas PEA did not. First, blue petrel chicks may recognize CLO as a food-related odour and associate it with parental feeding. On Mayes Island, 56.8% of the diet of breeding blue petrels consists of fish (Cherel et al. 2002), suggesting that chicks are exposed to fishy smelling compounds in the regurgitated stomach oil that they are fed. While the composition of scented compounds in various types of petrel regurgitants is not yet known, stomach oil contains squalene, which is also a fishy smelling component of commercially available cod liver oil (Clarke & Prince 1976). It is therefore likely that the chicks we tested had prior experience with at least one of the fishy smelling components of cod liver oil. This explanation suggests that chicks have already attached a biological significance to food-related odours even before they forage for the first time.

**Table IV.** Chicks tended to be more active in Trial One compared to Trial Two for both control presentations and for PEA. Only behaviours that had significant differences between Trial One and Trial Two are shown here.

<table>
<thead>
<tr>
<th>Stimulus</th>
<th>Behaviour</th>
<th>Trend</th>
<th>Statistics</th>
</tr>
</thead>
<tbody>
<tr>
<td>CLO - Control</td>
<td>Head turns</td>
<td>Trial 1 &gt; Trial 2</td>
<td>(t = 2.96, \text{df} = 27, P = 0.006)</td>
</tr>
<tr>
<td></td>
<td>Distance walked</td>
<td>Trial 1 &gt; Trial 2</td>
<td>(t = 2.18, \text{df} = 11, P = 0.05)</td>
</tr>
<tr>
<td>PEA</td>
<td>Biting</td>
<td>Trial 1 &gt; Trial 2</td>
<td>(U = 2.00, P = 0.05)</td>
</tr>
<tr>
<td></td>
<td>Preening</td>
<td>Trial 1 &gt; Trial 2</td>
<td>(U = 3.16, P = 0.002)</td>
</tr>
<tr>
<td>PEA - Control</td>
<td>Head turns</td>
<td>Trial 1 &gt; Trial 2</td>
<td>(t = 1.83, \text{df} = 28, P = 0.08)</td>
</tr>
<tr>
<td></td>
<td>Biting</td>
<td>Trial 1 &gt; Trial 2</td>
<td>(U = 1.69, P = 0.09)</td>
</tr>
</tbody>
</table>

![Fig. 5. Mean distances walked by blue petrel chicks in response to PEA and control presentations during a. Trial One, and b. Trial Two. Responses are indicated as black (PEA) or white (control) bars. Values are given as Mean ± s.e.(m). For both trials, chicks walked the same distance in response to the two stimuli (see text).](image-url)
Alternatively, blue petrel chicks might have shown an increased turning response to CLO because they were reacting to it as a novel odour. This interpretation is supported by studies that have shown that other petrels (Antarctic prion, *Pachyptila desolata* Gmelin, Bonadonna & Nevitt 2004) and other animals can be attracted to ‘strange’ odours (reviewed by Jordan & Bruford 1998, Bernatchez & Landry 2003). These studies examined responses to individual-specific odours which were not food related, however. Given that both CLO and the regurgitants that chicks are fed contain fishy smelling compounds, the circumstances of our experiment do not support the hypothesis that chicks were responding to an unfamiliar odour. Moreover, of the two scents we tested, PEA is most likely to be unfamiliar to petrels because it is a rosy scent that is not found in the sub-Antarctic environment. Thus, if birds were responding to odours because they were unfamiliar, we would have expected the greater response to be to PEA, which we did not observe.

Lastly, chicks might have responded differently to CLO and PEA because, for technical reasons, the CLO group was tested at a slightly younger age (by four days) than the PEA group. Given that the chick rearing period is ~45 days (Jouventin et al. 1985), this difference is small, but significant (Student’s *t*-test: *t* = -2.31, df = 15, *P* = 0.04). However, since young chicks tend to be much less ambulatory than older chicks, we would have predicted that the CLO group would be less likely to show a response to an odourant. Yet these younger chicks turned more and walked further distances in response to CLO than their older counterparts did to PEA. Thus, our interpretation is that age did not contribute to the differences we saw in chicks’ responses to CLO and PEA. Alternatively, older chicks might have been more easily stressed than younger chicks, and this may partially contribute to the increase in preening we observed in response to PEA, but we have no published or anecdotal information to support this hypothesis.

The simple wind tunnel as a testing tool

In addition to providing insight into chick behaviours, this study explores the use of a simple wind tunnel as a new method for testing olfactory behaviours in the field. Using this technique, we were able to easily observe and quantify detailed behavioural responses to odour stimuli in freely moving animals, despite working under primitive field conditions. So far, such detailed observations have not been possible using alternate techniques such as Y-mazes where movements and fine scale behaviours of the subjects are hidden from the observer (see Grubb 1974, Brooke 1989, Minguez 1997, Hagelin et al. 2003, Bonadonna & Nevitt 2004, Bonadonna et al. 2004, 2006). While Y-maze testing can be used successfully to examine attraction to odours in older blue petrel chicks that are closer to fledging, we have found that it does not work well with younger chicks that have limited mobility (Nevitt, personal observation). An alternative method, the Porter method, is an easy means to assess odour detection against an unscented control, but this technique cannot be used as a discrimination tool between two scented compounds (Cunningham et al. 2003). This method also tends to fail as chicks become older because they do not stay asleep long enough to complete trials (Cunningham, personal observation).

While testing chicks in the wind tunnel offers the possibility for detailed observation and quantification of behavioural responses to odours, the methodology we used carried some restrictions. For example, our experimental design required that behaviours were scored blind to the testing conditions. In our case, this required videotaping trials that could only be properly analysed once we had returned from the field. After following this procedure, we uncovered an order effect that was not at all obvious at the time of the experimental trials. For the present experiment, this order effect gave us critical insight into the nature of the behavioural responses we observed. However, this unexpected result also suggested that blue petrel chicks might need additional acclimation time if behavioural responses to novel odours are being tested. In such cases, a better design might be to increase the acclimation period and then test chicks against a single stimulus (odour or control), rather than against sequential stimuli. This change in design, however, would double the number of individuals needed and has to be balanced against increased disturbance to the colony (for example, see Blackmer et al. 2004). In addition, other petrel species may respond differently than blue petrel species to this testing situation.

Summary and conclusions

In summary, we have shown that blue petrel chicks appear to be specifically responsive to food-related odours such as CLO, since only this odour initiated behaviours consistent with olfactory search. Furthermore, we have demonstrated that a simple wind tunnel can be successfully used in the field to test the responses of blue petrel chicks to a variety of olfactory stimuli. The emerging picture from our work suggests that chicks may be learning about odour cues that they will use while foraging as adults. Future studies will continue to investigate these ideas in greater detail (e.g. Bonadonna et al. 2006, Nevitt et al. 2006).

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R. Kelsey and K. Tooley greatly improved the quality of the manuscript as did those of the two referees. The project adhered to guidelines provided by IPEV and IACUC for the ethical treatment of animals.

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