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Abstract

Increasing evidence suggests that penguins are sensitive to dimethyl sulphide (DMS), a scented airborne compound that a variety of marine animals use to find productive areas of the ocean where prey is likely to be found. Here we present data showing that king penguins *Aptenodytes patagonicus* are also sensitive to DMS. We deployed DMS on a lake near a king penguin colony at Ratmanoff beach in the Kerguelen archipelago. We also presented DMS to 'sleeping' adults on the beach. On the lake, penguins responded to the DMS deployments by swimming more, while on the beach, penguins twitched their heads and woke up more for the DMS than for the control presentations. Interestingly, penguins did not respond to cod liver oil deployments on the lake; mirroring at-sea studies of other penguins. Although at-sea studies are needed to confirm that king penguins use DMS as a surface cue that informs them of productivity under the water, this study is an important first step in understanding how these birds locate prey over significant distances.

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Responses of King penguin (*Aptenodytes patagonicus*) adults and chicks to two food-related odours

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Running title:

King penguins can detect DMS

31
32 Increasing evidence suggests that penguins are sensitive to dimethyl sulphide
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45 distances.
46

47

48 **Introduction**

49 Dimethyl sulphide (DMS) has long been studied for its role in global climate
50 regulation but has only recently been studied as a signal molecule that marine
51 organisms can use to assist in foraging. In the oceans, dimethylsulphoniopropionate
52 (DMSP) is produced by phytoplankton (Keller et al. 1989; Dacey et al. 1994; Hill et
53 al. 1995; Raina et al. 2013) and its levels are increased in the water when
54 phytoplankton are grazed upon by the zooplankton that some seabirds eat (Dacey
55 and Wakeham 1986; Simo 2004). Once released, DMSP is converted to DMS which
56 then volatilizes into the air above the phytoplankton aggregation. High levels of
57 DMS exist in the air over shelf-breaks and seamounts (Berresheim et al. 1989),
58 meaning that DMS can be an indicator of high primary and secondary productivity
59 in oceanic waters (Bürgermeister et al. 1990; Andreae et al. 1994). Nevitt et al.
60 (1995) were the first to show that some procellariiform seabirds were able to detect
61 this airborne cue, likely using it as a way to locate their zooplankton prey. Since this
62 hallmark study, DMS sensitivity has been shown in a variety of other
63 procellariiforms (Nevitt and Haberman 2003; Nevitt and Bonadonna 2005;
64 Dell’Ariccia et al. 2014), seals (Harbour seal, *Phoca vitulina*, Kowalesky et al. 2006)
65 and marine invertebrates (copepod, *Temora longicornis*, Steinke et al. 2006).

66 The close evolutionary relationship of penguins to procellariiforms (Ksepka
67 et al. 2006; Hackett et al. 2008), and the fact that sensitivity to DMS is likely
68 ancestral in procellariiforms (Van Buskirk and Nevitt 2008), made this group of
69 flightless birds a logical choice for DMS sensitivity studies. Although penguins have

traditionally been identified as visual hunters (Stonehouse 1960; Williams 1995), they have recently begun to be tested for their responses to DMS. Original observations by Culik et al. (2000) on Humboldt penguins (*Spheniscus humboldti*) first suggested a role for olfaction in penguin foraging, as birds appeared to use winds to find food during an El Niño event. Later, Culik (2001) confirmed that captive Humboldt penguins could detect DMS. Cunningham et al. (2008) showed DMS sensitivities in wild African penguins (*S. demersus*) by placing the odourant along walkways in their colony on Robben Island, South Africa and with captive penguins using a Y-maze. Wright et al. (2011) repeated and confirmed the colony experiment on Robben Island and also found that DMS slicks deployed at-sea attracted three times more penguins than control slicks. Sensitivity to DMS has also been found in the Antarctic-breeding Chinstrap penguin (*Pygoscelis antarctica*; Amo et al. 2013).

The responses of King penguins (*Aptenodytes patagonicus*) to odours has yet to be studied. These birds represent an intriguing species in which to study olfactory foraging, because, similar to many procellariiforms, their foraging grounds are extremely far from their nesting beaches. For example, Bost et al. (2002) found that King penguins nesting at Kerguelen Island, where our study was conducted, had a mean maximal foraging range of 267 +/- 88 km, with some individuals foraging over 400 km away. In contrast, African penguins providing for chicks commonly forage 11 – 28 km away from their colony (Wilson et al. 1989; Petersen et al. 2005) while Humboldt penguins spend 90% of their time within 35 km of their colony (Culik et al. 1998). During the austral summer, King penguins from the Kerguelen

and Crozet archipelagos forage primarily on two types of fish: the eel-cod *Muraenolepis marmoratus* and a variety of pelagic myctophids (Cherel and Ridoux 1992; Cherel et al. 1993; Ridoux 1994; Bost et al. 1997; Bost et al. 2002). During these months these fish are found in the southern waters of the Antarctic Polar Frontal Zone (Sabourrenkov 1991; Koubbi 1993). Although the front moves from year to year, its northern edge tends to be 70 km to the South of Kerguelen (Park et al. 2014). Not surprisingly, King penguins from Sub-Antarctic islands around the world focus their foraging efforts during these times in these waters (Jouventin et al. 1994; Bost et al. 1997; Rodhouse et al. 1998; Moore et al. 1999; Duhamel et al. 2000). During their commute to the foraging grounds, King penguins perform shallow dives (< 10m), and swim at speeds of up to 7 kmh⁻¹ (Kooyman et al. 1992; Jouventin et al. 1994). Once they arrive in productive waters they switch to deeper dives (100 – 300m; Kooyman et al. 1992; Jouventin et al. 1994; Bost et al. 1997; Moore et al. 1999) and begin to forage. What cues stimulate penguins to switch from the commuting style of diving and swimming into a foraging mode is unknown.

In this study, we aimed to test penguins in a controlled, aquatic environment using an experimental release of DMS, and to test individual penguins on their olfactory sensitivity to DMS using a proven methodology. Here we present evidence that implicates DMS as a cue that King penguins may use to identify productive areas where fish are likely to be encountered when diving.

Materials and Methods

Study Site

Both experiments on King penguins (*Aptenodytes patagonicus* Miller 1778) were conducted at Cape Ratmanoff, Courbet Peninsula, Kerguelen Island (70°33'13"E, 49°14'09"S) where a large colony of more than 100,000 breeding pairs plus chicks spans 1-2 km along a flat black sand beach. The experiments were carried out from 28 December 2014 – 17 January 2015 (Lake study), 27 December – 9 January (Adults, Porter method), and 27 December – 18 January (Chicks, Porter method).

A small (approx. 100 m X 116 m) lake (Fig. 1) can be found directly inland from a section of the colony. This lake is frequented by adult and chick King penguins, Giant petrels (*Macronectes sp.*), Kelp gulls (*Larus dominicanus*), Sub-Antarctic skuas (*Catharacta skua lönnbergi*), and Elephant seals (*Mirounga leonina*). Although the exact depth of the lake is unknown, it is deep enough for penguins to swim in it, but also can be traversed by a walking penguin with the water coming up to the mid-point of the bird (approximately 0.45 m). To control for any diel variation in bird activity, the experiment was carried out at the same time each day: 1000 – 1040h (local time). Wind speed (msec^{-1}), gust speed (msec^{-1}), temperature ($^{\circ}\text{C}$) and relative humidity (%) are summarized in Table 1.

The Porter method study was carried out on adult penguins found along the beach 0.5 – 1.5 km south of the main colony. We avoided testing birds closer to the colony so as to avoid extensive background scents from the colony. Due to the chick's distribution on the beach, however, it was necessary to test chicks closer to the colony (see Discussion). Data collection was carried out in the hours following

sunrise: 0430 – 0900h (local time). Wind speed (msec^{-1}), temperature ($^{\circ}\text{C}$) and relative humidity (%) are summarized in Table 1.

The Lake study

We followed the general methodology of Wright et al. (2011) who deployed DMS and cod liver oil (CLO), a known seabird attractant (Hutchison and Wenzel 1980; Verheyden and Jouventin, 1994, Nevitt et al., 2004), in the ocean near an African penguin colony and counted the number of birds in the area for 30 minutes. For logistical reasons, however, we were unable to deploy odours at sea but instead used the nearby lake where penguins commonly swam. In our study we similarly (Wright et al. 2011) prepared three deployments: (1) DMS (0.2 mol l^{-1} in 1L of vegetable oil, $N = 6$); (2) CLO (152 mL poured into 848 mL vegetable oil, $N = 6$); (3) 1L of vegetable oil alone, acting as a control, $N = 6$. These odours were deployed by pouring the prepared solution into the lake at our site upwind of the colony. Slicks deployed upon the lake were visible for up to 30 min (and often longer). Each deployment was separated by at least 24 hours.

To start a trial, a site on the lake upwind of the colony was chosen. As the wind's direction shifted from day to day we ended up using three different sites in the northwest quadrant of the lake (see Fig. 4). The three sites were separated by approximately 100 m. Odour deployment was as follows: SITE 1: 5 DMS, 3 CLO, 4 control deployments; SITE 2: 1 DMS, 2 CLO, 1 control deployments; SITE 3: 0 DMS, 1 CLO, 1 control deployments. Once the site was chosen a Sony DSC-HX400V digital camera was set up on a tripod at a specific height (1m) with the lens pointing

161 directly downwind. A rope barrier was laid down on the grass creating a 90° angle
162 with downwind being at 45°. For 10 min before the trial started and then for 30 min
163 after deployment, we counted all birds swimming within the area outlined by the
164 projection of the rope barrier into the water every 30 sec. We elected to count only
165 swimming birds because it was not always possible to clearly determine when a
166 bird had entered the water while walking. Most birds would walk in to the lake for a
167 few metres, and then fall down and swim. Some birds, however, would walk across
168 the entire lake; these birds were never counted in our analysis. Although the
169 experiment was not done blind in that the person counting the birds on-site knew
170 the identity of the odour, the videos were blindly watched by an observer who did
171 not know the identity of the odours nor the nature of the experiment to confirm the
172 data. As some chicks in the lake were well along in the moulting process and had
173 lost most of their down feathers, adults and chicks could not be consistently
174 differentiated. Thus, they were grouped together.

176 *The Porter method*

177 To test the responses of birds to the various scents we used a modified
178 Porter method (Porter et al., 1999) where odours were presented to birds
179 “sleeping” on the beach. This technique has successfully been used to test olfactory
180 sensitivities of a variety of procellariiform chicks in a sleep-like state (for example
181 Cunningham et al., 2003). We have already confirmed that this technique works
182 with “sleeping” King penguins found on the beach, as we recently successfully tested
183 adults’ responses to social odours (Cunningham and Bonadonna, 2015). Similar to

our previous study we tested King penguin adults and chicks “sleeping” on the beach with their beak tips tucked beneath their wings.

We tested 105 adult “sleeping” birds with one of three odours: (1) DMS ($1 \mu\text{mol l}^{-1}$ dissolved in propylene glycol), $N = 35$; (2) Phenyl-ethyl alcohol ($1 \mu\text{mol l}^{-1}$ dissolved in propylene glycol), an unfamiliar rose-scented odour that has been used as a positive control in a other avian olfaction studies (Cunningham et al., 2003, 2006; Cunningham and Nevitt, 2011), $N = 35$; and (3) propylene glycol, acting as a control, $N = 35$. These concentrations are similar to what have been used in past studies (Cunningham et al. 2003; Nevitt and Bonadonna 2005; Cunningham et al. 2008) and although higher than what birds encounter at sea, are a rough approximate of the nanomolar range that birds encounter in the wild (Nevitt et al. 1995). Odours were deployed by pouring 5 mL of solution onto a 90 mm piece of VWR filter paper taped to the end of a metal rod. Each odour had its own metal rod of the same variety, size and shape. Multiple odours were tested on the same day, but each bird was presented with only one odour.

The experiment was done blind in that the person presenting the odours and scoring the responses of the birds was not the person who prepared the odour or chose which odour to be tested. To decrease the likelihood of the presenter/scorer accidentally smelling the scent while carrying it on the beach, the presenter placed cotton balls into their nostrils during the tests.

To carry out the tests the presenter was handed an odour and then he walked down the beach looking for “sleeping” penguins. Only penguins that had their heads oriented on the up-wind side were tested. Once a penguin was

identified, the presenter approached the “sleeping” bird from behind, and paused behind it to make sure that presenter’s presence had not altered the bird’s sleep and to be certain that the activity of other birds in the area did not wake up the target bird prematurely. The presenter then bent down and held the tip of the metal rod, which held the scented filter paper, approximately 3 - 5 cm beneath the beak of the bird. Birds that woke up within 2 seconds of the presentation were not included, as penguins sometimes slept with their eyes partially open and we could not be sure that they were not simply responding to the disturbance of the rod and filter paper. The filter paper was held beneath the bird’s beak for 15 seconds. The response to the presentation was then noted.

Scores were given to the birds as follows: (0) no response; (1) a slight response which could include beak clapping, twitching or head movements; (2) waking up. After a bird’s score was recorded it was sprayed on the back with coloured PorcimarK (KRUUSE, Langeskov, Denmark), a commonly used animal spray for marking livestock, to prevent the bird from being tested a second time.

Additionally, in a similar methodology to the adults, we tested 60 chicks. Due to the asynchronous breeding that King penguins undergo (Williams 1995), chicks were a variety of ages. However, all chicks were likely at least 8 - 12 months old and none had yet been to sea. Chicks were tested with either DMS ($1\ \mu\text{mol l}^{-1}$ dissolved in propylene glycol), $N = 30$ or propylene glycol ($N = 30$), acting as a control. Chicks were tested in their crèches along the southern edge of the main colony, or along the beach.

Statistical analysis

For the lake study, to test for the effect of the deployment of the three odours, the number of birds on the lake was modeled using a Generalised Linear Mixed model, with a Poisson error distribution. As the data were overdispersed, an observation level random effect was included in the model. As there were 6 trials for every deployment of an odour, a random intercept for deployment number was fitted in all models. Date, temperature, wind speed and the maximum number of birds present on the lake during the 10 minutes before deployment were fitted as fixed effects and to test for the effect of treatment over time, an interaction between treatment and time was fitted (centred and scaled). We compared the change in deviance after removal of a term, using a χ^2 test with the appropriate degrees of freedom (Likelihood ratio test). When an interaction was tested, the corresponding main effects were kept in the model. All models were run in R 3.1.2 (R Development Core Team 2012) using package lme4 (Bates 2007). Temperature was correlated with none of the other environmental variables (all $r < 0.10$, all $P > 0.80$), while wind speed was correlated with wind gust and humidity ($r = 0.97$, $P < 0.0001$ and $r = 0.49$, $P = 0.040$). Wind gust and humidity were therefore excluded from the statistical analyses.

Since the Porter method collects categorical scores, and they were not normally distributed, we used non-parametric tests to investigate differences in the response to our three scents. For the adults, we first tested for overall differences using a Kruskal-Wallis test. We then used a Mann-Whitney U test to compare the responses to our scents against each other. For the chicks, since there was only one

pairwise comparison to make, we used a Mann-Whitney U test. Finally, we wanted to determine whether the response of adults and chicks to DMS was similar. This comparison was done with a Mann-Whitney U test. Responses of chicks and adults to the control were similarly compared.

Results

The Lake Study

Once the odour was deployed we found a significant interaction between treatment and the amount of time since the deployment of the stimulus (Table 2 and Fig. 2). In order to interpret this interaction, we tested the effect of time since deployment within each treatment and corrected for multiple comparisons using the sequential Bonferroni procedure (Holm 1979). The number of birds increased with time in the DMS treatment ($\chi^2 = 113.55$, $df = 1$, $P < 0.0001$, after correction: $P < 0.0001$; Fig. 2), while it decreased with time in the control treatment ($\chi^2 = 27.75$, $df = 1$, $P < 0.0001$, after correction: $P < 0.0001$; Fig. 2). It did not vary with time in the cod liver oil (CLO) treatment ($\chi^2 = 1.60$, $df = 1$, $P = 0.21$, after correction: $P = 0.62$; Fig. 2). Additionally, a higher number of birds on the lake before deployment led to a higher number of birds during deployment, and as the calendar date progressed in our study, fewer birds were found on the lake, regardless of the stimulus (Table 2). Wind speed and temperature did not affect the number of birds after odour deployment (Table 2).

The Porter method

For the adults, mean responses to DMS, PEA and control were significantly different from one another (Kruskal Wallis test statistic = 8.67, d.f. = 2, $P = 0.013$, Fig. 3). The mean score for DMS and PEA was significantly greater than to the control (Mann Whitney U test, $Z = 2.38$, $P = 0.017$ for DMS vs. control; $Z = 2.67$, $P = 0.0075$ for PEA vs. control). There were no significant differences in the response to DMS and PEA ($Z = 0.35$, $P = 0.73$).

The responses of chicks to DMS were not significantly different ($Z = 0.11$, $P = 0.91$; Fig. 3). We also compared the responses given by chicks to the presentations against those given by adults. Chicks and adults gave similar responses to control ($Z = 0.97$, $P = 0.33$) and to DMS ($Z = 1.17$, $P = 0.24$).

Discussion

In this study, we show for the first time that King penguins are sensitive to an olfactory stimulus. King penguins reacted to a food-related odourant, DMS, which other seabirds use to forage (reviewed by Nevitt 2008), by increasing their swimming in a nearby lake. Since we could not consistently differentiate between adults and chicks while they were swimming we cannot be certain whether one group or the other did or did not respond to our stimuli. Adults also responded to DMS presentations held beneath their beak while “sleeping”, though the chicks did not.

On the lake birds recruited to the DMS slick, but not to the CLO slick. Wright et al. (2011), who similarly tested African penguins with scented vegetable oil slicks at sea, found similar results: adults recruited to DMS scented slicks, but not to CLO

ones. They suggested that because penguins do not scavenge dead fish (Williams 1985), that they might not associate the scent of fish oil with prey. Our results here support this concept. Interestingly, recent molecular research by Zhao et al. (2015) suggests that some species of penguins have lost the ability to taste umami, the flavour associated with the fishy taste of marine organisms. The insensitivity to umami and the lack of response to fish-related odours are in line with a predator that hunts underwater, and eats its prey whole, never tasting nor smelling their prey directly. Finally, “sleeping” adults did not differentiate between the DMS and PEA deployment. This result is consistent with a study by Cunningham et al. (2003) that found that Blue petrels and Thin-billed prions (*Halobaena caerulea* and *Pachyptila belcheri*), when tested using the Porter method, did not differentiate between DMS and PEA either.

King penguins, which forage hundreds of kilometres from land and hundreds of metres deep, must make a decision as to when to switch from their shallow commuting dives to deeper dives associated with foraging. Locomotion in penguins is, depending on the species, approximately 10 times slower than flying birds (Meinertzhagen 1955, Wilson et al. 1989). Therefore penguins are limited in the time they can spend foraging, and the area of the ocean that they can sample, and must be highly selective as to where they travel to and where they dive. Dimethyl sulphide is an appropriate cue for these birds to use to identify these productive patches of suitable water for deep dives within the Antarctic Polar Front. Myctophid fish, the primary prey item of King penguins, eat a variety of zooplankton such as copepods, ostracods, euphausiids and others (Pakhomov et al. 1996). Spikes in DMS

in the air, associated with zooplankton foraging (Dacey and Wakeham 1986; Wolfe and Steinke 1996) would alert King penguins to the presence of prey, via lower trophic activity, in the waters beneath them. Many species of fish use DMSP, a precursor to DMS (Simo 2004), as a foraging cue (Nakajima et al. 1989; Nakajima et al. 1990; DeBose and Nevitt 2007; DeBose et al. 2008; DeBose et al. 2010) and so surface levels of DMS could inform King penguins that they have arrived in productive waters where fish are located, and to start diving deep. Although our experiment did not test DMS in a foraging context, it is an important first step in identifying which scents King penguins could be utilizing to target their foraging efforts in productive waters. Once these productive foraging grounds are located, King penguins probably switch to visual cues to locate prey while underwater. King penguins dive deeper during the day than night, and as light levels increase at dawn, dive depth proportionally increases (Kooyman et al. 1992; Bost et al. 1997; Putz et al. 1998; Moore et al. 1999; Bost et al. 2002). Additionally, King penguins could use temperature cues to aid in identifying the front (Guinet et al. 1997). Clearly much remains to be studied on how penguins direct themselves on these larger scales.

King penguin chicks did not respond to DMS held beneath their beaks. At least three possible explanations exist as to why the adults responded to DMS, but the chicks did not. First, chicks on the beach are under a high risk of predation from giant petrels, during both the day and the night (Hunter and Brooke 1992; Le Bohec et al. 2003). Due to this intense predatory pressure it appears that chicks sleep lightly on the beach and may wake up equally to any stimulus presented beneath their beaks. Indeed, we found that it was considerably harder to find a sleeping

345 chick on the beach than an adult, and also more difficult to approach the bird
346 without it waking up. A second explanation for chicks not responding to the DMS
347 presentation is that chicks might not recognize the significance of the odour when it
348 is placed beneath their beaks. In Blue petrels and Thin-billed prions, adults are
349 sensitive to DMS (Nevitt 2000; Nevitt et al. 1995), and the chicks respond to it while
350 asleep (Cunningham et al. 2003) and in a Y-maze (Bonadonna et al. 2006).
351 Cunningham and Nevitt (2011), testing Thin-billed prions, also found that chicks
352 exposed to novel odours as embryos showed altered behaviours towards this odour
353 after hatching. Taken together, these studies suggest that some procellariiforms
354 may be learning about odour cues while in the burrow or in the egg. Procellariiform
355 adults commonly smell of phytoplankton when returning to the burrow
356 (Cunningham and Nevitt 2011; Cunningham pers. obs.), providing the chicks with an
357 opportunity to learn about this cue before they fledge. Penguin adults foraging in
358 productive waters, however, would most likely have any DMSP or DMS washed off
359 their feathers on the return commute. Thus, a King penguin chick may never be
360 exposed to DMS until in productive waters for the first time. Since penguins are
361 social hunters that commonly leave the beach together and forage in groups at sea
362 (Stonehouse 1960; Williams 1995), chicks may learn about the significance of DMS
363 on their early foraging trips. A final explanation for the lack of response of the
364 chicks is based upon the location of the experiment. We tested adults at least 0.5 km
365 away from the colony; in this area of the beach only adults are found. Chicks,
366 however, are always found close to the colony and thus there were likely a lot of
367 odours in the air when we were testing the chicks. These background odours may

have made it more difficult for the chicks to detect the DMS presentation. Further studies regarding how responses to DMS change throughout development should be conducted.

Sensitivity to DMS has now been shown in four species of penguin: African (Cunningham et al. 2008; Wright et al. 2011), Humboldt (Culik 2001), Chinstrap (Amo et al. 2013), and King (this study). Given the close evolutionary relationship between penguins and procellariiforms (Ksepka et al. 2006; Hackett et al. 2008), the sensitivity to this odourant by penguins is not surprising. Although only one study has tested penguins at sea (Wright et al. 2011), the emerging picture is that this group of birds uses surface odour cues much in the same way as other seabirds, mammals (Kowalesky et al. 2006) and marine invertebrates (Steinke et al. 2006) use odour cues in their foraging behaviors. Future studies on King penguins and other penguins should continue to test birds at sea, and investigate whether these birds are sensitive to DMS at more biologically relevant concentrations (pmol^{-1} ; see Nevitt and Bonadonna 2005) and how these sensitivities develop as a chick ages.

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Table 1. Environmental data for the two experiments.

	Average Wind Speed (msec ⁻¹)	Average Gust speed (msec ⁻¹)	Average Air Temperature (°C)	Average Relative Humidity (%)
Lake Study: Dimethyl sulphide (DMS)	6.4 +/- 1.0	8.0 +/- 1.2	8.8 +/- 0.5	66.5 +/- 4.2
Lake Study: Cod liver oil (CLO)	6.5 +/- 0.9	7.8 +/- 1.1	10.3 +/- 1.5	71.1 +/- 5.7
Lake Study: Control	6.5 +/- 1.0	8.5 +/- 1.2	9.7 +/- 0.6	76.5 +/- 4.4
Porter Method	3.8 +/- 0.1		6.7 +/- 0.2	77.6 +/- 1.4

Table 2. General linear mixed model testing the factors affecting the number of birds after odor deployment. Terms retained in the selected model are highlighted in bold.

Parameter	χ^2	df	<i>P</i>
Wind speed	0.57	1	0.45
Temperature	0.16	1	0.69
Day	7.69	1	0.0056
Max number of birds before deployment	11.98	1	0.00054
Treatment*Time	115.55	2	< 2.2 e-16

Selected model	Estimated coefficient $\pm$ S.E.M.
Intercept	2.94 $\pm$ 0.90
Day effect	-0.063 $\pm$ 0.020
Max number of birds before deployment	0.084 $\pm$ 0.020
Treatment: Control	0.08 $\pm$ 0.27
Treatment: Dimethyl sulphide (DMS)	0.57 $\pm$ 0.28
Time	-0.027 $\pm$ 0.039
Control*Time	-0.18 $\pm$ 0.05
DMS*Time	0.35 $\pm$ 0.05

Figure 1. A small lake is found directly inland from the main colony at Ratmanoff. Adults and chicks commonly swim in this lake, as do other species of birds and mammals. We deployed our odours at three sites (1, 2, 3), based upon wind direction. Odour release sites were always chosen so that the odour was released directly upwind of the colony. The Porter method experiments were done South of the cabin (*) along the beach.

Figure 2. Mean (of six trials) number of birds in the lake after Dimethyl sulphide (DMS, green), cod liver oil (CLO, yellow) or control (blue) deployment. Lines show GLM prediction for an average maximum number of birds before deployment and an average day and 95% confidence bands.

Figure 3. Mean responses of adult (N = 105) and chick (N=60) King penguins (with S.E.M.) to control (black), Dimethyl sulphide (DMS, white) and phenyl ethyl alcohol (PEA, grey) odourant presentations. For adults, significant differences were found between the DMS presentation (Mann Whitney U test, $P = 0.017$) and the PEA presentation ($P = 0.0075$) than to the control presentations. Mean responses of the chicks to the two deployments were not significantly different ($P = 0.91$).