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Responses of king penguin Aptenodytes patagonicus adults and chicks to two food-related odours

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Responses of king penguin Aptenodytes patagonicus adults and chicks to two food-related odours

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.

Keywords

fsc2017

Disciplines

Biology | Ornithology

Comments

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14 **Responses of King penguin (*Aptenodytes patagonicus*) adults and chicks to two**

15 **food-related odours**

16

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26 **Key words:**

27 King penguin, olfaction, dimethyl sulphide, foraging, Ratmanoff

28 **Running title:**

29 King penguins can detect DMS

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32   Increasing evidence suggests that penguins are sensitive to dimethyl sulphide

33   (DMS), a scented airborne compound that a variety of marine animals use to find

34   productive areas of the ocean where prey is likely to be found. Here we present

35   data showing that King penguins (*Aptenodytes patagonicus*) are also sensitive to

36   DMS. We deployed DMS on a lake near a King penguin colony at Ratmanoff beach in

37   the Kerguelen archipelago. We also presented DMS to "sleeping" adults on the

38   beach. On the lake, penguins responded to the DMS deployments by swimming

39   more, while on the beach, penguins twitched their heads and woke up more for the

40   DMS than for the control presentations. Interestingly, penguins did not respond to

41   cod liver oil deployments on the lake; mirroring at-sea studies of other penguins.

42   Although at-sea studies are needed to confirm that King penguins use DMS as a

43   surface cue that informs them of productivity under the water, this study is an

44   important first step in understanding how these birds locate prey over significant

45   distances.

46

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Introduction

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

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

70 traditionally been identified as visual hunters (Stonehouse 1960; Williams 1995),

71 they have recently begun to be tested for their responses to DMS. Original

72 observations by Culik et al. (2000) on Humboldt penguins (*Spheniscus humboldti*)

73 first suggested a role for olfaction in penguin foraging, as birds appeared to use

74 winds to find food during an El Niño event. Later, Culik (2001) confirmed that

75 captive Humboldt penguins could detect DMS. Cunningham et al. (2008) showed

76 DMS sensitivities in wild African penguins (*S. demersus*) by placing the odourant

77 along walkways in their colony on Robben Island, South Africa and with captive

78 penguins using a Y-maze. Wright et al. (2011) repeated and confirmed the colony

79 experiment on Robben Island and also found that DMS slicks deployed at-sea

80 attracted three times more penguins than control slicks. Sensitivity to DMS has also

81 been found in the Antarctic-breeding Chinstrap penguin (*Pygoscelis antarctica*; Amo

82 et al. 2013).

83　　　　The responses of King penguins (*Aptenodytes patagonicus*) to odours has yet

84 to be studied. These birds represent an intriguing species in which to study

85 olfactory foraging, because, similar to many procellariiforms, their foraging grounds

86 are extremely far from their nesting beaches. For example, Bost et al. (2002) found

87 that King penguins nesting at Kerguelen Island, where our study was conducted, had

88 a mean maximal foraging range of 267 +/- 88 km, with some individuals foraging

89 over 400 km away. In contrast, African penguins providing for chicks commonly

90 forage 11 – 28 km away from their colony (Wilson et al. 1989; Petersen et al. 2005)

91 while Humboldt penguins spend 90% of their time within 35 km of their colony

92 (Culik et al. 1998). During the austral summer, King penguins from the Kerguelen

93   and Crozet archipelagos forage primarily on two types of fish: the eel-cod

94   *Muraenolepis marmoratus* and a variety of pelagic myctophids (Cherel and Ridoux

95   1992; Cherel et al. 1993; Ridoux 1994; Bost et al. 1997; Bost et al. 2002). During

96   these months these fish are found in the southern waters of the Antarctic Polar

97   Frontal Zone (Sabourrenkov 1991; Koubbi 1993). Although the front moves from

98   year to year, its northern edge tends to be 70 km to the South of Kerguelen (Park et

99   a. 2014). Not surprisingly, King penguins from Sub-Antarctic islands around the

100   world focus their foraging efforts during these times in these waters (Jouventin et al.

101   1994; Bost et al. 1997; Rodhouse et al. 1998; Moore et al. 1999; Duhamel et al.

102   2000). During their commute to the foraging grounds, King penguins perform

103   shallow dives (< 10m), and swim at speeds of up to 7 kmh^{-1} (Kooyman et al. 1992;

104   Jouventin et al. 1994). Once they arrive in productive waters they switch to deeper

105   dives (100 – 300m; Kooyman et al. 1992; Jouventin et al. 1994; Bost et al. 1997;

106   Moore et al. 1999) and begin to forage. What cues stimulate penguins to switch

107   from the commuting style of diving and swimming into a foraging mode is unknown.

108   In this study, we aimed to test penguins in a controlled, aquatic environment

109   using an experimental release of DMS, and to test individual penguins on their

110   olfactory sensitivity to DMS using a proven methodology. Here we present evidence

111   that implicates DMS as a cue that King penguins may use to identify productive

112   areas where fish are likely to be encountered when diving.

113

114   **Materials and Methods**

115   *Study Site*

116   Both experiments on King penguins (*Aptenodytes patagonicus* Miller 1778)

117   were conducted at Cape Ratmanoff, Courbet Penninsula, Kerguelen Island

118   (70o33'13"E, 49o14'09"S) where a large colony of more than 100,000 breeding pairs

119   plus chicks spans 1-2 km along a flat black sand beach. The experiments were

120   carried out from 28 December 2014 – 17 January 2015 (Lake study), 27 December –

121   9 January (Adults, Porter method), and 27 December – 18 January (Chicks, Porter

122   method).

123   A small (approx. 100 m X 116 m) lake (Fig. 1) can be found directly inland

124   from a section of the colony. This lake is frequented by adult and chick King

125   penguins, Giant petrels (*Macronectes sp.*), Kelp gulls (*Larus dominicanus)*, Sub-

126   Antarctic skuas (*Catharacta skua lönnbergi*), and Elephant seals (*Mirounga leonina*).

127   Although the exact depth of the lake is unknown, it is deep enough for penguins to

128   swim in it, but also can be traversed by a walking penguin with the water coming up

129   to the mid-point of the bird (approximately 0.45 m). To control for any diel

130   variation in bird activity, the experiment was carried out at the same time each day:

131   1000 – 1040h (local time). Wind speed (msec^{-1}), gust speed (msec^{-1}), temperature

132   (oC) and relative humidity (%) are summarized in Table 1.

133   The Porter method study was carried out on adult penguins found along the

134   beach 0.5 – 1.5 km south of the main colony. We avoided testing birds closer to the

135   colony so as to avoid extensive background scents from the colony. Due to the

136   chick's distribution on the beach, however, it was necessary to test chicks closer to

137   the colony (see Discussion). Data collection was carried out in the hours following

138   sunrise: 0430 – 0900h (local time). Wind speed (msec^{-1}), temperature (^{o}C) and

139   relative humidity (%) are summarized in Table 1.

140

141   *The Lake study*

142   We followed the general methodology of Wright et al. (2011) who deployed

143   DMS and cod liver oil (CLO), a known seabird attractant (Hutchison and Wenzel

144   1980; Verheyden and Jouventin, 1994, Nevitt et al., 2004), in the ocean near an

145   African penguin colony and counted the number of birds in the area for 30 minutes.

146   For logistical reasons, however, we were unable to deploy odours at sea but instead

147   used the nearby lake where penguins commonly swam. In our study we similarly

148   (Wright et al. 2011) prepared three deployments: (1) DMS (0.2 mol l^{-1} in 1L of

149   vegetable oil, N = 6); (2) CLO (152 mL poured into 848 mL vegetable oil, N = 6); (3)

150   1L of vegetable oil alone, acting as a control, N = 6. These odours were deployed by

151   pouring the prepared solution into the lake at our site upwind of the colony. Slicks

152   deployed upon the lake were visible for up to 30 min (and often longer). Each

153   deployment was separated by at least 24 hours.

154   To start a trial, a site on the lake upwind of the colony was chosen. As the

155   wind's direction shifted from day to day we ended up using three different sites in

156   the northwest quadrant of the lake (see Fig. 4). The three sites were separated by

157   approximately 100 m. Odour deployment was as follows: SITE 1: 5 DMS, 3 CLO, 4

158   control deployments; SITE 2: 1 DMS, 2 CLO, 1 control deployments; SITE 3: 0 DMS, 1

159   CLO, 1 control deployments. Once the site was chosen a Sony DSC-HX400V digital

160   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.

175

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

184   our previous study we tested King penguin adults and chicks "sleeping" on the

185   beach with their beak tips tucked beneath their wings.

186   We tested 105 adult "sleeping" birds with one of three odours: (1) DMS (1

187   µmol l^{-1} dissolved in propylene glycol), N = 35; (2) Phenyl-ethyl alcohol (1 µmol l^{-1}

188   dissolved in propylene glycol), an unfamiliar rose-scented odour that has been used

189   as a positive control in a other avian olfaction studies (Cunningham et al., 2003,

190   2006; Cunningham and Nevitt, 2011), N = 35; and (3) propylene glycol, acting as a

191   control, N = 35. These concentrations are similar to what have been used in past

192   studies (Cunningham et al. 2003; Nevitt and Bonadonna 2005; Cunningham et al.

193   2008) and although higher than what birds encounter at sea, are a rough

194   approximate of the nanomolar range that birds encounter in the wild (Nevitt et al.

195   1995). Odours were deployed by pouring 5 mL of solution onto a 90 mm piece of

196   VWR filter paper taped to the end of a metal rod. Each odour had its own metal rod

197   of the same variety, size and shape. Multiple odours were tested on the same day,

198   but each bird was presented with only one odour.

199   The experiment was done blind in that the person presenting the odours and

200   scoring the responses of the birds was not the person who prepared the odour or

201   chose which odour to be tested. To decrease the likelihood of the presenter/scorer

202   accidentally smelling the scent while carrying it on the beach, the presenter placed

203   cotton balls into their nostrils during the tests.

204   To carry out the tests the presenter was handed an odour and then he

205   walked down the beach looking for "sleeping" penguins. Only penguins that had

206   their heads oriented on the up-wind side were tested. Once a penguin was

207   identified, the presenter approached the "sleeping" bird from behind, and paused

208   behind it to make sure that presenter's presence had not altered the bird's sleep and

209   to be certain that the activity of other birds in the area did not wake up the target

210   bird prematurely. The presenter then bent down and held the tip of the metal rod,

211   which held the scented filter paper, approximately 3 - 5 cm beneath the beak of the

212   bird. Birds that woke up within 2 seconds of the presentation were not included, as

213   penguins sometimes slept with their eyes partially open and we could not be sure

214   that they were not simply responding to the disturbance of the rod and filter paper.

215   The filter paper was held beneath the bird's beak for 15 seconds. The response to

216   the presentation was then noted.

217   Scores were given to the birds as follows: (0) no response; (1) a slight

218   response which could include beak clapping, twitching or head movements; (2)

219   waking up. After a bird's score was recorded it was sprayed on the back with

220   coloured Porcimark (KRUUSE, Langeskov, Denmark), a commonly used animal

221   spray for marking livestock, to prevent the bird from being tested a second time.

222   Additionally, in a similar methodology to the adults, we tested 60 chicks. Due

223   to the asynchronous breeding that King penguins undergo (Williams 1995), chicks

224   were a variety of ages. However, all chicks were likely at least 8 - 12 months old and

225   none had yet been to sea. Chicks were tested with either DMS (1 μmol l^{-1} dissolved

226   in propylene glycol), $N = 30$ or propylene glycol ($N = 30$), acting as a control. Chicks

227   were tested in their crèches along the southern edge of the main colony, or along the

228   beach.

229

230 *Statistical analysis*

231   For the lake study, to test for the effect of the deployment of the three

232 odours, the number of birds on the lake was modeled using a Generalised Linear

233 Mixed model, with a Poisson error distribution. As the data were overdispersed, an

234 observation level random effect was included in the model. As there were 6 trials

235 for every deployment of an odour, a random intercept for deployment number was

236 fitted in all models. Date, temperature, wind speed and the maximum number of

237 birds present on the lake during the 10 minutes before deployment were fitted as

238 fixed effects and to test for the effect of treatment over time, an interaction between

239 treatment and time was fitted (centred and scaled). We compared the change in

240 deviance after removal of a term, using a χ^2 test with the appropriate degrees of

241 freedom (Likelihood ratio test). When an interaction was tested, the corresponding

242 main effects were kept in the model. All models were run in R 3.1.2 (R Development

243 Core Team 2012) using package lme4 (Bates 2007). Temperature was correlated

244 with none of the other environmental variables (all r < 0.10, all P > 0.80), while wind

245 speed was correlated with wind gust and humidity (r = 0.97, P < 0.0001 and r = 0.49,

246 P = 0.040). Wind gust and humidity were therefore excluded from the statistical

247 analyses.

248   Since the Porter method collects categorical scores, and they were not

249 normally distributed, we used non-parametric tests to investigate differences in the

250 response to our three scents. For the adults, we first tested for overall differences

251 using a Kruskal-Wallis test. We then used a Mann-Whitney U test to compare the

252 responses to our scents against each other. For the chicks, since there was only one

253   pairwise comparison to make, we used a Mann-Whitney U test. Finally, we wanted

254   to determine whether the response of adults and chicks to DMS was similar. This

255   comparison was done with a Mann-Whitney U test. Responses of chicks and adults

256   to the control were similarly compared.

257

258   **Results**

259   *The Lake Study*

260   Once the odour was deployed we found a significant interaction between

261   treatment and the amount of time since the deployment of the stimulus (Table 2 and

262   Fig. 2). In order to interpret this interaction, we tested the effect of time since

263   deployment within each treatment and corrected for multiple comparisons using

264   the sequential Bonferroni procedure (Holm 1979). The number of birds increased

265   with time in the DMS treatment (χ^2 = 113.55, df = 1, P < 0.0001, after correction: P <

266   0.0001; Fig. 2), while it decreased with time in the control treatment (χ^2 = 27.75, df

267   = 1, P < 0.0001, after correction: P < 0.0001; Fig. 2). It did not vary with time in the

268   cod liver oil (CLO) treatment (χ^2 = 1.60, df = 1, P = 0.21, after correction: P = 0.62;

269   Fig. 2). Additionally, a higher number of birds on the lake before deployment led to a

270   higher number of birds during deployment, and as the calendar date progressed in

271   our study, fewer birds were found on the lake, regardless of the stimulus (Table 2).

272   Wind speed and temperature did not affect the number of birds after odour

273   deployment (Table 2).

274

275   *The Porter method*

276   For the adults, mean responses to DMS, PEA and control were significantly

277   different from one another (Kruskal Wallis test statistic = 8.67, d.f. = 2, P = 0.013,

278   Fig. 3).   The mean score for DMS and PEA was significantly greater than to the

279   control (Mann Whitney U test, Z = 2.38, P = 0.017 for DMS vs. control; Z = 2.67, P =

280   0.0075 for PEA vs. control). There were no significant differences in the response to

281   DMS and PEA (Z = 0.35, P = 0.73).

282   The responses of chicks to DMS were not significantly different (Z = 0.11, P =

283   0.91; Fig. 3). We also compared the responses given by chicks to the presentations

284   against those given by adults. Chicks and adults gave similar responses to control (Z

285   = 0.97, P = 0.33) and to DMS (Z = 1.17, P = 0.24).

286

287   **Discussion**

288   In this study, we show for the first time that King penguins are sensitive to an

289   olfactory stimulus. King penguins reacted to a food-related odourant, DMS, which

290   other seabirds use to forage (reviewed by Nevitt 2008), by increasing their

291   swimming in a nearby lake. Since we could not consistently differentiate between

292   adults and chicks while they were swimming we cannot be certain whether one

293   group or the other did or did not respond to our stimuli. Adults also responded to

294   DMS presentations held beneath their beak while "sleeping", though the chicks did

295   not.

296   On the lake birds recruited to the DMS slick, but not to the CLO slick.   Wright

297   et al. (2011), who similarly tested African penguins with scented vegetable oil slicks

298   at sea, found similar results: adults recruited to DMS scented slicks, but not to CLO

299 ones. They suggested that because penguins do not scavenge dead fish (Williams

300 1985), that they might not associate the scent of fish oil with prey. Our results here

301 support this concept. Interestingly, recent molecular research by Zhao et al. (2015)

302 suggests that some species of penguins have lost the ability to taste umami, the

303 flavour associated with the fishy taste of marine organisms. The insensitivity to

304 umami and the lack of response to fish-related odours are in line with a predator

305 that hunts underwater, and eats its prey whole, never tasting nor smelling their prey

306 directly. Finally, "sleeping" adults did not differentiate between the DMS and PEA

307 deployment. This result is consistent with a study by Cunningham et al. (2003) that

308 found that Blue petrels and Thin-billed prions (*Halobaena caerulea* and *Pachyptila*

309 *belcheri*), when tested using the Porter method, did not differentiate between DMS

310 and PEA either.

311 King penguins, which forage hundreds of kilometres from land and hundreds

312 of metres deep, must make a decision as to when to switch from their shallow

313 commuting dives to deeper dives associated with foraging. Locomotion in penguins

314 is, depending on the species, approximately 10 times slower than flying birds

315 (Meinertzhagen 1955, Wilson et al. 1989). Therefore penguins are limited in the

316 time they can spend foraging, and the area of the ocean that they can sample, and

317 must be highly selective as to where they travel to and where they dive. Dimethyl

318 sulphide is an appropriate cue for these birds to use to identify these productive

319 patches of suitable water for deep dives within the Antarctic Polar Front. Myctophid

320 fish, the primary prey item of King penguins, eat a variety of zooplankton such as

321 copepods, ostracods, euphausiids and others (Pakhomov et al. 1996). Spikes in DMS

322   in the air, associated with zooplankton foraging (Dacey and Wakeham 1986; Wolfe

323   and Steinke 1996) would alert King penguins to the presence of prey, via lower

324   trophic activity, in the waters beneath them.   Many species of fish use DMSP, a

325   precursor to DMS (Simo 2004), as a foraging cue (Nakajima et al. 1989; Nakajima et

326   al. 1990; DeBose and Nevitt 2007; DeBose et al. 2008; DeBose et al. 2010) and so

327   surface levels of DMS could inform King penguins that they have arrived in

328   productive waters where fish are located, and to start diving deep.   Although our

329   experiment did not test DMS in a foraging context, it is an important first step in

330   identifying which scents King penguins could be utilizing to target their foraging

331   efforts in productive waters.   Once these productive foraging grounds are located,

332   King penguins probably switch to visual cues to locate prey while underwater.   King

333   penguins dive deeper during the day than night, and as light levels increase at dawn,

334   dive depth proportionally increases (Kooyman et al. 1992; Bost et al. 1997; Putz et

335   al. 1998; Moore et al. 1999; Bost et al. 2002). Additionally, King penguins could use

336   temperature cues to aid in identifying the front (Guinet et al. 1997).   Clearly much

337   remains to be studied on how penguins direct themselves on these larger scales.

338   King penguin chicks did not respond to DMS held beneath their beaks.   At

339   least three possible explanations exist as to why the adults responded to DMS, but

340   the chicks did not.   First, chicks on the beach are under a high risk of predation from

341   giant petrels, during both the day and the night (Hunter and Brooke 1992; Le Bohec

342   et al. 2003).   Due to this intense predatory pressure it appears that chicks sleep

343   lightly on the beach and may wake up equally to any stimulus presented beneath

344   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 (Cunnigham 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

368   have made it more difficult for the chicks to detect the DMS presentation. Further

369   studies regarding how responses to DMS change throughout development should be

370   conducted.

371   Sensitivity to DMS has now been shown in four species of penguin: African

372   (Cunningham et al. 2008; Wright et al. 2011), Humboldt (Culik 2001), Chinstrap

373   (Amo et al. 2013), and King (this study). Given the close evolutionary relationship

374   between penguins and procellariiforms (Ksepka et al. 2006; Hackett et al. 2008), the

375   sensitivity to this odourant by penguins is not surprising. Although only one study

376   has tested penguins at sea (Wright et al. 2011), the emerging picture is that this

377   group of birds uses surface odour cues much in the same way as other seabirds,

378   mammals (Kowalesky et al. 2006) and marine invertebrates (Steinke et al. 2006)

379   use odour cues in their foraging behaviors. Future studies on King penguins and

380   other penguins should continue to test birds at sea, and investigate whether these

381   birds are sensitive to DMS at more biologically relevant concentrations ($pmol^{-1}$; see

382   Nevitt and Bonadonna 2005) and how these sensitivities develop as a chick ages.

383

396

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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 (^{o}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	*P*
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 ± S.E.M.
Intercept	2.94 ± 0.90
Day effect	-0.063 ± 0.020
Max number of birds before deployment	0.084 ± 0.020
Treatment: Control	0.08 ± 0.27
Treatment: Dimethyl sulphide (DMS)	0.57 ± 0.28
Time	-0.027 ± 0.039
Control*Time	-0.18 ± 0.05
DMS*Time	0.35 ± 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.
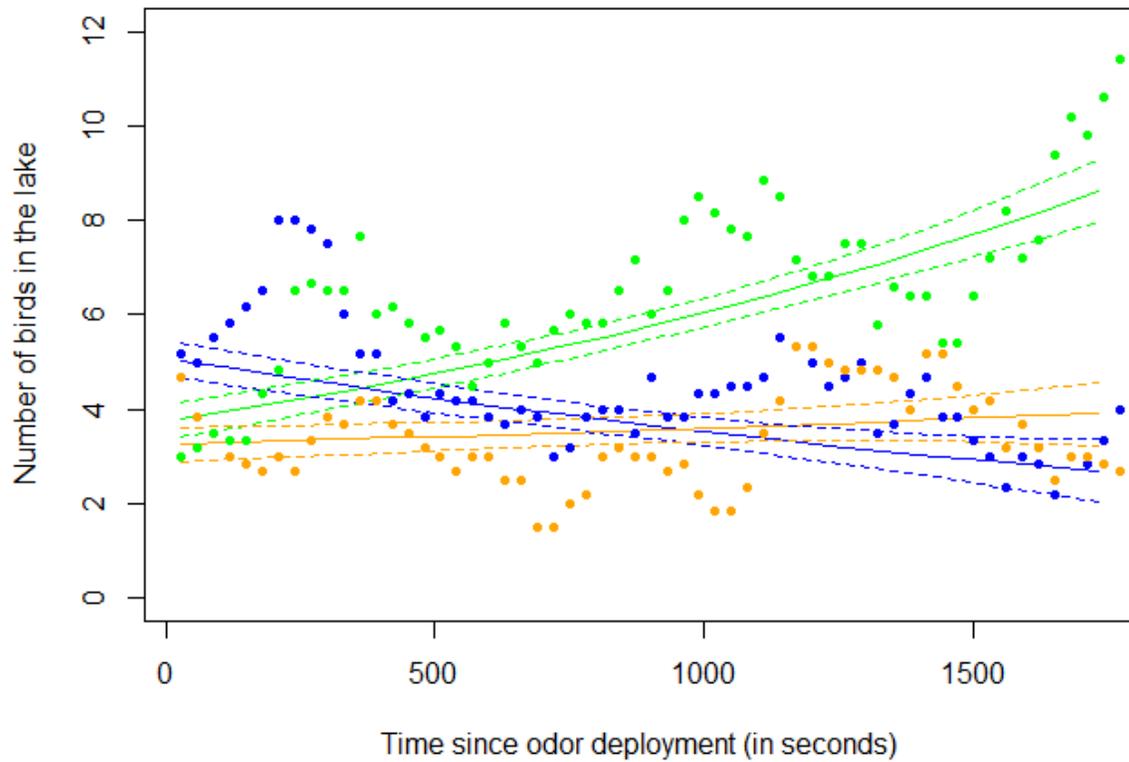
Figure 3. Mean responses of adult (N = 105) and chick (N=60) King penguins (with S.E.M.) to control (black), Dimethyl sulphide (DMS, white) and phenyl ethyl alcohol (PEA, grey) odourant presentations. For adults, significant differences were found between the DMS presentation (Mann Whitney U test, P = 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).