

1 **Waddling on the dark side: ambient light affects attendance behaviour of little**  
2 **penguins**

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13

14 **Running title:** The rhythmic colony attendance of little penguins

15

16 This manuscript contains: 20 pages, 3 Figures and 1 Supplementary Online Material

17 **Abstract**

18 Visible light on earth largely comes from the sun including light reflected from the  
19 moon. Predation risk is strongly determined by light conditions and some animals are  
20 nocturnal to reduce predation. Artificial lights and its consequent light pollution may  
21 disrupt this natural behaviour. Here, we used 13 years of attendance data to study the  
22 effects of sun, moon and artificial light on the attendance pattern of a nocturnal seabird,  
23 the little penguin *Eudyptula minor* at Phillip Island, Australia. The little penguin is the  
24 smallest and the only penguin species whose activity on land is strictly nocturnal.  
25 Automated monitoring systems recorded individually marked penguins every time they  
26 arrived (after sunset) at or departed (before sunrise) from two colonies under different  
27 lighting conditions: natural night skylight and artificial lights (around 3 lux) used to  
28 enhance penguin viewing for eco-tourism around sunset. Sunlight had a strong effect on  
29 attendance as penguins arrived on average around 81 mins after sunset and departed  
30 around 92 mins before sunrise. The effect of moonlight was also strong, varying  
31 according to moon phase. Fewer penguins came ashore during full moon nights. Moon  
32 phase effect was stronger on departure than arrival times. Thus, during nights between  
33 full moon and last quarter, arrival times (after sunset) were delayed, even though  
34 moonlight levels were low, while departure times (before sunrise) were earlier,  
35 coinciding with high moonlight levels. Cyclic patterns of moon effect were slightly out  
36 of phase but significantly between two colonies, which could be due to site-specific  
37 differences or presence/absence of artificial lights. Moonlight could be overridden by  
38 artificial light at our artificially lit colony, but the similar amplitude of attendance  
39 patterns between colonies suggests that artificial light did not mask the moonlight  
40 effect. Further research is indeed necessary to understand how seabirds respond to the  
41 increasing artificial night light levels.

42

43 **Keywords**

44 artificial light, attendance pattern, breeding, *Eudyptula minor*, light pollution, little  
45 penguin, moon, sun

## 46 **Introduction**

47

48 Sunlight is the main source of energy for biological systems on the surface of the  
49 Earth (Bradshaw and Holzapfel 2010). Rotation, orbital motion and tilt of the Earth  
50 cause marked daily and seasonal variation on sunlight levels. Even at night, the  
51 reflected sunlight from the moon, i.e. moonlight, causes high variation of light levels  
52 because of the moon's orbit around the Earth. Organisms on the surface of the Earth  
53 have evolved under this regime of light-dark cycles, which have remained practically  
54 invariant for long time periods, although recent proliferation of artificial lighting  
55 systems could disrupt these natural cycles (Gaston et al. 2014).

56 Animals are commonly classified as diurnal or nocturnal on the basis of the  
57 effect of sunlight on their activity patterns. For example, humans are largely diurnal as  
58 they are mainly active during daylight hours, although usage of artificial light during the  
59 last decades has extended our activity into night hours (Navara and Nelson 2007; de la  
60 Iglesia et al. 2015). Many other diurnal species have also benefited by extending their  
61 foraging times at artificially illuminated areas (Negro et al. 2000; Santos et al. 2010;  
62 Dominoni et al. 2013). Among nocturnal animals, moonlight effects have been widely  
63 studied in the context of reproduction (e.g. Harrison et al. 1984), communication (e.g.  
64 Penteriani et al. 2010), predator-prey relationships (e.g. Daly et al. 1992; Penteriani et  
65 al. 2013; Saldaña-Vázquez and Munguía-Rosas 2013) and anthropogenic-induced  
66 mortality (Gundersen and Andreassen 1998; Rodríguez and Rodríguez 2009; Mizuta  
67 2014). Artificial lights can be brighter than the moon, and thus they have the potential to  
68 mask the effect of moonlight on the physiology and behaviour of nocturnal and diurnal  
69 animals (reviewed in Kronfeld-Schor et al. 2013).

70 In marine ecosystems, the effect of light on seabirds and marine mammals is  
71 more difficult to assess due to their life cycle. Marine-adapted terrestrial breeding  
72 species have to cope with a trade-off as they live in contrasting marine and terrestrial  
73 environments. If they are well-adapted to the marine environment, they will, by  
74 comparison, under-perform on land (Griffin and Kram 2000). Many, particularly the  
75 smaller, of them show nocturnal behaviour on land (visit the breeding colonies at night)  
76 in an attempt to avoid predation. Seabirds, such as petrels and murrelets, are typical  
77 cases. Commuting behaviour, i.e. regular travels between breeding colonies and  
78 foraging locations, is strongly influenced by moon phase in petrels (e.g. Riou and  
79 Hamer 2008; Bourgeois et al. 2008; Taylor et al. 2012; Rubolini et al. 2014) often by

80 reducing visits to colonies and calling during moonlit nights (Mourgeot and Bretagnolle  
81 2000; Miles et al. 2013). Artificially increased light levels may allow visual predators to  
82 be more efficient. For example, higher predation rates have been estimated in storm-  
83 petrel colonies under the influence of artificial lights (Oro et al. 2005).

84         Among seabirds, the little penguin *Eudyptula minor* is a good model for  
85 studying the effect of light on the annual pattern of colony attendance. Even outside the  
86 breeding season, this resident species returns ashore on a regular basis and thus, adults  
87 are present at colonies throughout the year. Little penguins are diurnal foragers at sea  
88 but nocturnal on land (Stahel and Gales 1987). They arrive at the colony after sunset  
89 and depart before sunrise (Klomp and Wooller 1991; Davies and Renner 2003). During  
90 breeding, the little penguin is a diurnal central-place forager with normal foraging trips  
91 of 1 to 10 days before returning ashore at night for courtship, to incubate eggs or feed  
92 chicks (Chiaradia and Nisbet 2006; Saraux et al. 2011). During daylight hours on land,  
93 they stay in burrows, underground or under vegetation but never venture out in the  
94 open, showing a typical nocturnal behaviour on land. In this study, we assessed how  
95 sunlight and moonlight affected attendance patterns ashore of little penguins at Phillip  
96 Island, Australia, by automatically recording arrival and departure times at two colonies  
97 over 13 years. Firstly, we evaluated the effect of sunlight on the attendance behaviour of  
98 penguins annually to examine if arrivals and departures from colonies varied seasonally  
99 with respect to sunset or sunrise, respectively. Secondly, we studied the effect of  
100 moonlight on the arrival and departure times. Thirdly, we assessed the effect of  
101 moonlight on the number of penguins coming ashore at sunset and going to the sea  
102 before sunrise. Lastly, we evaluated if artificial illumination at penguin arrival time after  
103 sunset can affect the natural attendance behaviour of penguins. We compared two  
104 distinct colonies; i.e. one colony under a natural night sky and another with artificial  
105 illumination at an eco-tourism penguin viewing attraction, the Penguin Parade, on  
106 Phillip Island, Australia. We hypothesised that arrival and departure times are  
107 influenced by moonlight with penguins arriving later in the night or departing earlier in  
108 the morning during the brighter nights around the full moon. Similarly, we expected a  
109 lower number of penguins at colonies (arriving or departing) during the brighter moonlit  
110 nights. Finally, we expected penguin arrivals were independent of moon cycle at the  
111 colony lit with artificial light, as artificial light intensity can override moonlight  
112 (Kronfeld-Schor et al. 2013).

113

114 **Methods**

115

116 *Study sites*

117 Phillip Island in south eastern Australia (S 38°29', E 145°15') sustains more  
118 than 1% of the global population of little penguins *Eudyptula minor* (BirdLife  
119 International 2015). Approximately 26,000-28,000 adult penguins breed on the island  
120 (Sutherland and Dann 2012). We examined the arrival and departure patterns of little  
121 penguins at the Penguin Parade and Cullen Cove on Summerlands Peninsula. The study  
122 period spanned from 1 June 2001 to 31 August 2014 (13 years) at Penguin Parade (S  
123 38°30'37'', E 145°09'01''), and from 17 July 2003 to 16 January 2014 (10 years) at  
124 Cullen Cove (S 38°31'04'', E 145°07'16''). The Penguin Parade colony is a popular  
125 ecotourism destination, receiving over half million tourists each year to watch little  
126 penguins crossing the beach in the evening (Dann and Chambers 2013). Some form of  
127 artificial lighting has been used at the Penguin Parade from the 1960s, but since 1987  
128 orange halogen lights (Linear halogen, 240V, 2600lm, Osram Sylvania, Danvers, MA,  
129 USA) of around three lux have been turned on from sunset to 1.5 hours after the arrival  
130 of the first penguins to enhance the viewing of penguins. At Penguin Parade, penguins  
131 emergence at a white sand beach, crossing a gentle slope of 15 to 110 metres of the  
132 beach depending on the tide height that oscillates by up to 1.5 metres (Laaksonen 2011).  
133 Cullen Cove is closed to the public at night and it is under a natural light regime as no  
134 artificial lights occur in the vicinity (Figure S1). At Cullen Cove, penguins cross a  
135 shorter stretch of basaltic rock beach between 20 to 35 metres at same 1.5 metre tide  
136 oscillation.

137

138 *Arrival and departure patterns*

139 Individual penguins were marked with unique passive integrated transponders  
140 (PIT). Arrival and departure patterns were continuously recorded by an automated  
141 penguin monitoring system located in Penguin Parade colony (see Chiaradia and Kerry  
142 1999), developed by the Australian Antarctic Division (Kerry et al. 1993). A simple  
143 data-logger system consisting of a transponder reader platform was set up at Cullen  
144 Cove. Both systems record time and date of attendance of PIT-marked penguins, and  
145 they were located less than 15 metres above the high tide mark and less than 10 metres  
146 from the edge of nesting sites (Figure S1).

147

148 *Variables*

149 Repeated readings from a same individual on the same night and direction (i.e.  
150 arrival or departure) were removed, only keeping the earliest arrival or the latest  
151 departure reading. To build statistical models (see below), we also removed outlier  
152 readings of birds arriving or departing after or before 5 min of sunset or sunrise  
153 respectively (less than 0.1% of the dataset). Outliers were kept in Figure 1 to provide a  
154 complete view of attendance pattern, but they were not included in the statistical  
155 analyses.

156 Apparent sunset and sunrise times were obtained from the Earth Research  
157 System Lab, NOAA (available at  
158 <http://www.esrl.noaa.gov/gmd/grad/solcalc/sunrise.html>). The fraction of moon disc  
159 illuminated per night was obtained from the U.S. Naval Observatory website  
160 (<http://aa.usno.navy.mil/data/docs/MoonFraction.php>) and used to describe the lunar  
161 phase. Fraction of the moon disc was converted into radians ( $\theta$ ) assuming a lunar cycle  
162 of 29.5 days (0 and  $2\pi$  radians correspond to a new moon, and  $\pi$  radians corresponds to  
163 a full moon). Trigonometric functions (cosine and sine) were included in the statistical  
164 models as explanatory variables to investigate possible effects of lunar phase on the  
165 departure and arrival of penguins to the colony (see deBruyn and Meeuwig 2001 for  
166 details).

167

168 *Statistical analysis*

169 To test whether time of arrival after sunset or departure before sunrise was  
170 influenced by moon phase we used general linear mixed models. Following the periodic  
171 regression approach suggested by deBruyn and Meeuwig (2001),  $\cosine(\theta)$ ,  $\sin(\theta)$ ,  
172  $\cosine(2*\theta)$  and  $\sin(2*\theta)$  transformations were included as fixed terms to model  
173 potential semilunar cycles, i.e. two peaks substantially unequal in amplitude per lunar  
174 month. A three-level factor describing the breeding phenology of penguins (Breeding:  
175 August-February; Moulting: March-April; and Interbreeding: May-July; Reilly and  
176 Cullen 1981, 1983; Salton et al. 2015) and a two-level factor for the colony (Cullen  
177 Cove or Penguin Parade) were included as fixed terms. In addition, the interaction of the  
178 trigonometric functions and the colony factor were included to test the potential  
179 different effect of moon phase between colonies. As random factors we included  
180 transponder identification (ID) of penguins and date because observations from the  
181 same individuals or nights are not independent. Response variables (time of arrival after

182 sunset and time of departure before sunrise) were log transformed. We visually checked  
183 whether the assumptions of normally distributed and homogeneous residuals were  
184 fulfilled by inspection of histograms of residuals and plots of residuals against fitted  
185 values and covariates (both indicated no obvious deviations from these assumptions).

186 To investigate the potential effects of moon phase on the number of penguins  
187 crossing the beach, we ran generalized linear mixed models with negative binomial  
188 error distribution and log link function.  $\text{Cosine}(\theta)$ ,  $\text{sine}(\theta)$ ,  $\text{cosine}(2*\theta)$  and  $\text{sine}(2*\theta)$   
189 transformations were included as fixed terms to model potential semilunar cycles on the  
190 number of penguins. A two-level factor for the colony (Cullen Cove or Penguin Parade)  
191 and its interactions with trigonometric functions were included as fixed terms. We  
192 included year and month as random factors because of the huge inter-annual and  
193 seasonal variability in the number of penguins visiting the breeding colonies (see Figure  
194 S2). Overdispersion was assessed by calculating the sum of squared Pearson residuals,  
195 and divide by the sample size minus number of parameters (i.e. thirteen corresponding  
196 to ten fixed terms, two random terms, and one negative binomial dispersion parameter;  
197 Zuur et al. 2013). The overdispersion parameters of the full models ranged between  
198 0.90-0.94. Model validation included visual inspection of plots. We plotted residuals  
199 against fitted values, fixed and random terms (Zuur et al. 2013).

200 All statistical analyses were conducted in R version 3.0.3 (R Foundation for  
201 Statistical Computing, Vienna, Austria). Models were fitted using the `lmer` and `glmer.nb`  
202 functions of the R-package `lmer4` (Bates et al. 2014). P-values were obtained using the  
203 R-package `lmerTest` (Kuznetsova et al. 2014). To achieve more reliable P-values, we  
204 fitted the models using Maximum Likelihood (rather than Restricted Maximum  
205 Likelihood). The significance of the full models as compared to the null model  
206 comprising only the breeding factor and the random terms was established using  
207 likelihood ratio tests.

208

## 209 **Results**

210

211 The automated penguin monitoring system at Penguin Parade identified 885  
212 individual penguins with 295,696 readings (159,222 arrivals and 136,474 departures)  
213 over 4,830 nights. At Cullen Cove, the reading platform identified 1,849 individuals and  
214 recorded 298,027 readings (158,875 arrivals and 139,152 departures) over 2,899 nights.

215 On average, penguins arrived around 81 mins after sunset and departed around  
216 92 mins before sunrise throughout the year. Negligible numbers of penguins were  
217 recorded during daylight hours (Figure 1a). Penguin arrivals and departures varied  
218 seasonally according to sunset and sunrise times, respectively. Thus, penguin real time  
219 attendance was more than two hours earlier around winter solstice, i.e. when night  
220 duration is longer, than around summer solstice, i.e. when night duration is shorter  
221 (Figure 1a).

222 Overall, the full models explaining timing of arrival or departure as a function of  
223 moon cycle were highly significant as compared to the null models (likelihood ratio  
224 tests: Arrivals  $\chi^2 = 299.32$ ,  $df = 8$ ,  $P < 0.001$ ; Departures  $\chi^2 = 810.37$ ,  $df = 8$ ,  $P < 0.001$ ).  
225 Arrival and departure times showed semilunar cycles, i.e. terms including  $\sin(2*\theta)$  and  
226  $\cosine(2*\theta)$  were significant, but semilunar cycles were described by different terms in  
227 each colony (see Table S1). Interactions between colony and trigonometric functions  
228 reached high significance indicating that moon phase has a different effect on both  
229 colonies (Table S1). Despite of these significant differences between colonies, the  
230 modelled attendance patterns were similar at the artificially illuminated Penguin Parade  
231 and Cullen Cove, with natural light conditions. Thus, penguins arrived later and  
232 departed earlier around full moon and last quarter nights than around first quarter nights  
233 at both colonies (Figure 2). The effect of moonlight, measured as the amplitude of the  
234 cyclic pattern (i.e. maximum minus minimum value), was stronger at departure than at  
235 arrival at both two colonies. The average amplitude for arrivals was 12 minutes in  
236 comparison with the average 26 minutes for departures (Figure 2). In addition, the  
237 modelled cyclic pattern for arrivals showed similar amplitude at both colonies, despite  
238 Penguin Parade is artificially illuminated at arrivals (12.2 minutes at the artificially lit  
239 Penguin Parade and 11.8 minutes at the naturally lit Cullen Cove).

240 Overall, the full models explaining the number of penguins arriving at or  
241 departing from the colonies as a function of moon cycle were highly significant as  
242 compared to the null models (likelihood ratio tests: Arrivals  $\chi^2 = 36.44$ ,  $df = 8$ ,  $P <$   
243  $0.001$ ; Departures  $\chi^2 = 44.79$ ,  $df = 8$ ,  $P < 0.001$ ). The number of penguins arriving at or  
244 departing from the colonies showed clear lunar cycles described by  $\cosine(\theta)$  being the  
245 only transformation reaching significance at both models (Table S2). The minimum  
246 number of penguins was recorded during the full moon nights (Table S2 and Figure 3).  
247

248 **Discussion**

249

250           Despite a substantial part of the world's biodiversity being nocturnal (around  
251 30% of vertebrates and 60% of invertebrates, Hölker et al. 2010), chronobiologists have  
252 centred their attention on the effects of solar cycles (daily and annual) on biological  
253 rhythms while the effects of lunar cycles mediated by moonlight have received much  
254 less attention (Kronfeld-Schor et al. 2013). In addition, our knowledge of moonlight  
255 effects is highly skewed between terrestrial and marine ecosystems. While terrestrial  
256 ecologists have centred on predator-prey relationships, marine ecologists have focused  
257 on behaviour and reproduction (Kronfeld-Schor et al. 2013, but see for example Cruz et  
258 al. 2013). The increase in artificial lighting worldwide is an additional and growing  
259 challenge to the emerging field of night ecology. Here, we studied the effect of sunlight  
260 and moonlight on the attendance behaviour of a nocturnal seabird on land, the little  
261 penguin, over 10 years in two colonies. We examined the attendance pattern of  
262 penguins in response to the natural light of the sun and moon. Penguin arrivals and  
263 departures depended primarily on the sunlight, as they co-varied with sunset and sunrise  
264 times, respectively, throughout the annual cycle (Figure 1a; Klomp and Wooller 1991).  
265 After removing the seasonal effects by standardising arrival or departure times in  
266 relation to sunset and sunrise respectively, we found a significant effect of moon phase  
267 on the attendance timing which varied between colonies, although with similar cyclic  
268 patterns (Tables S1). Thus, penguins arrived later and departed earlier around full moon  
269 and last quarter nights than around first quarter nights at both colonies (Figure 2). The  
270 number of penguins attending both colonies was influenced by moonlight, with lower  
271 numbers around full moon nights.

272           Moonlight levels show a high variation due to phase and position of the moon in  
273 the sky (Austin et al. 1976; Figure S3). As expected, the effect of the moonlight on  
274 departures was stronger than on arrivals (Figure 2). It is still daylight when penguins  
275 form groups in the water before coming ashore after sunset so the sunlight has stronger  
276 influence on penguin behaviour. During full moon and last quarter nights, moonlight  
277 levels are high in the two hours before sunrise (departure). However, for the same  
278 period, moonlight levels are low in the two hours after sunset (arrival). Although the  
279 range or amplitude of arrival times was less than half of departure times, the question on  
280 why penguins arrived later during full moon and last quarter nights, i.e. when nights  
281 were dark, deserves further studies.

282 Selective pressures of penguins on arrivals and departures from colonies are  
283 different and variable throughout the annual cycle. Thus, arriving birds come back to  
284 the land after spending daylight hours foraging at sea. Penguins arriving earlier do so  
285 probably for nest-burrow defence or maintenance, courtship or thermoregulatory  
286 purposes (Davis and Renner 2003). However, departing penguins could be more  
287 interested in starting a new foraging trip as soon as practicable, especially during  
288 breeding when they must forage for nestlings and themselves. Indeed, our findings  
289 showed that penguins leave the colony earlier during breeding (Table S1).

290 But why do penguins go to sea earlier during bright nights? Light level may well  
291 determine the foraging efficiency of visual predators, especially for pursuit-diving  
292 seabirds as coefficient extinction of light is higher in the water than in the air. Depth and  
293 frequency of diving is reduced during poor light conditions in other seabirds (Wanless  
294 et al. 1999; Regular et al. 2011). Little penguins are visual predators, which forage  
295 during the day, and have never been recorded diving and foraging at night (Preston et al.  
296 2008; Pelletier et al. 2014), suggesting that light levels at night are too low for fishing  
297 (Cannell and Cullen 1998). Thus, penguins could go to the sea earlier during bright  
298 nights aiming to improve their foraging efficiency by arriving earlier at their preferred  
299 foraging areas.

300 Unlike migratory animals, little penguins are present throughout the year at their  
301 colonies, although the numbers are higher during the summer months, coinciding with  
302 the breeding season (Reilly and Cullen 1981; Salton et al. 2015), and lower during the  
303 interbreeding season or in bad years, when they have to make longer foraging trips  
304 (Chiaradia and Nisbet 2006; Kato et al. 2008; Saraux et al. 2011; Zimmer et al. 2011).  
305 Although we did not include these sources of variation, we detected significant effects  
306 of moon phase on the number of penguins coming ashore. Penguins show a more  
307 predictable time attendance pattern than other nocturnally active seabirds on land (e.g.  
308 petrels or murrelets; Nelson and Peck 1995; Warham 1996). While petrels adjust their  
309 colony arrivals at times when the moon is not in the night sky by rafting nearby (Keitt et  
310 al. 2004; Bourgeois et al. 2008; Rubolini et al. 2014), penguins come ashore almost at  
311 the same time every night (although they also congregate offshore before sunset,  
312 probably waiting for lower light levels). In this sense, the attendance behaviour of  
313 penguins is more similar to the commuting behaviour of bats; although with differences.  
314 Bats depart from colonies after sunset and arrive before sunrise.

315           It is known that weather conditions, especially fog, can delay the arrival of little  
316 penguins (Chiaradia et al. 2007; but see Klomp and Wooller 1991) as well as in other  
317 nocturnal seabirds (e.g. murrelets; Nelson and Peck 1995). In our study, we did not take  
318 into account the weather conditions because our long-term data set includes different  
319 weather conditions throughout the moon phases and it is difficult to obtain accurate  
320 weather information at the precise arrival and departure times. Tides could also affect  
321 the time of arrivals detected by the automated monitoring penguin system as during low  
322 tides penguins must traverse longer distances between the water's edge and the  
323 monitoring system (Laaksonen 2011). Thus, during nights between full moon and last  
324 quarter when the tide is low after sunset, we would expect to find delays in the arrival  
325 times (Laaksonen 2011). However, tide is also low during nights between new moon  
326 and first quarter after sunset; and delays in arrival times were not recorded. Therefore,  
327 the pattern found in our study is independent of tide levels or it is masked by other  
328 variables.

329

### 330 *Artificial illumination*

331           Moon light had a different effect on the arrival and departure timings at both  
332 colonies, which was supported by significant interactions between trigonometric  
333 functions and colony (Table S1), although timing of attendance of both colonies showed  
334 similar oscillatory patterns. The maximum and minimum values of time after sunset or  
335 before sunrise were reached at the same moon phases, i.e. shortly after full moon and  
336 after the first quarter, respectively (Figure 2).

337           Artificial lights could override the maximum luminance of the full moon, and  
338 thus high levels of light pollution could mask some of the behaviours modulated by  
339 moonlight (Kronfeld-Schor et al. 2013). In our study, artificial lights at Penguin Parade  
340 produce an illuminance of 3 lux on the beach, which is higher than the maximum  
341 moonlight illuminance (around 0.215 lux; Austin et al. 1976). Consequently, we  
342 predicted that moon light effect should be weaker in the artificially lit colony as  
343 moonlight effect would be overridden by artificial light. Contrary to our predictions, we  
344 found similar amplitude in the modelled cyclic arrival pattern at both colonies, which  
345 may indicate that artificial illumination, at least at the levels employed in our study,  
346 does not mask the moon effect on the arrival attendance behaviour.

347           Artificial illumination has been used at the Penguin Parade colony since the  
348 1980s. Given the high philopatry of penguins to breeding colonies, many of the birds

349 have been crossing a lit environment since fledging. Thus, habituation of penguins to  
350 artificial lights could explain lack of response in the predicted direction. In this sense,  
351 results of a preliminary study conducted at the Penguin Parade indicated that attendance  
352 behaviour of penguins, measured as number of penguins coming ashore, velocity, group  
353 size and timing, was not affected when artificial illumination increased to up 15 lux  
354 using the same type of lights (A.R. Unpubl. data).

355         Given that 1) site-specific differences and presence/absence of artificial  
356 illumination at both colonies are inseparable; 2) the potential complexity of the response  
357 to the interaction between artificial light and moonlight; and 3) the probable habituation  
358 of penguin to artificial lights, we cannot completely rule out an effect of artificial  
359 illumination on penguin behaviour. However, we believe that the different effect of  
360 moon on both colonies could be site-specific. The contrasting beach substrate and  
361 length of beach to be walked by penguins on the two sites could explain the different  
362 response to moon light on both colonies. If there was an effect of the artificial light, we  
363 would expect a much stronger signal as reported in other seabirds that are fatally  
364 attracted to artificial lights (Rodríguez et al. 2014). The effect of artificial lights on  
365 wildlife is an emergent field, further research is indeed necessary to understand how  
366 seabirds and other nocturnal species respond to ever increasing artificial night light  
367 levels.

368

### 369 **Acknowledgements**

370

371         We are grateful to Phillip Island Nature Parks: the rangers for their assistance in  
372 observational data collection and the research department for their continued support, in  
373 particularly to the late Mike Cullen, for his pioneering thinking on penguin attendance  
374 patterns at Phillip Island. We thank Ruth Martínez for her help in handling the data base  
375 and the Australian Antarctic Division for kindly donating the automated penguin  
376 monitoring system (APMS), in particularly Knowles Kerry and Kym Newbery. The  
377 APMS data collection and maintenance over the years were only possible thanks to  
378 many field assistants and electronic engineers (in chronological order since 1994): Brad  
379 Philips, Judy Clarke, Sarah Robinson-Laverick, Julija Yorke, Tom Daniel, Peter  
380 Knerborne, Marcus Salton, Julie McInnes, Rohan Long, Sandi Laaksonen, Hugh  
381 Davies, Darren Steirt, Matt Simpson, Claire Saraux, Laure Pelletier, Kean Maizels and  
382 Danielle Hedger. Two anonymous reviewers and the editor William J. Schwartz

383 provided constructive comments on early drafts. This work received grants and  
384 donations from BHP-Billiton, AAT Kings, Penguin Foundation and Australian  
385 Academy of Science. AR was supported by a Marie Curie International Outgoing  
386 Fellowship within the 7th European Community Framework Programme (330655 FP7-  
387 PEOPLE-2012-IOF).

388

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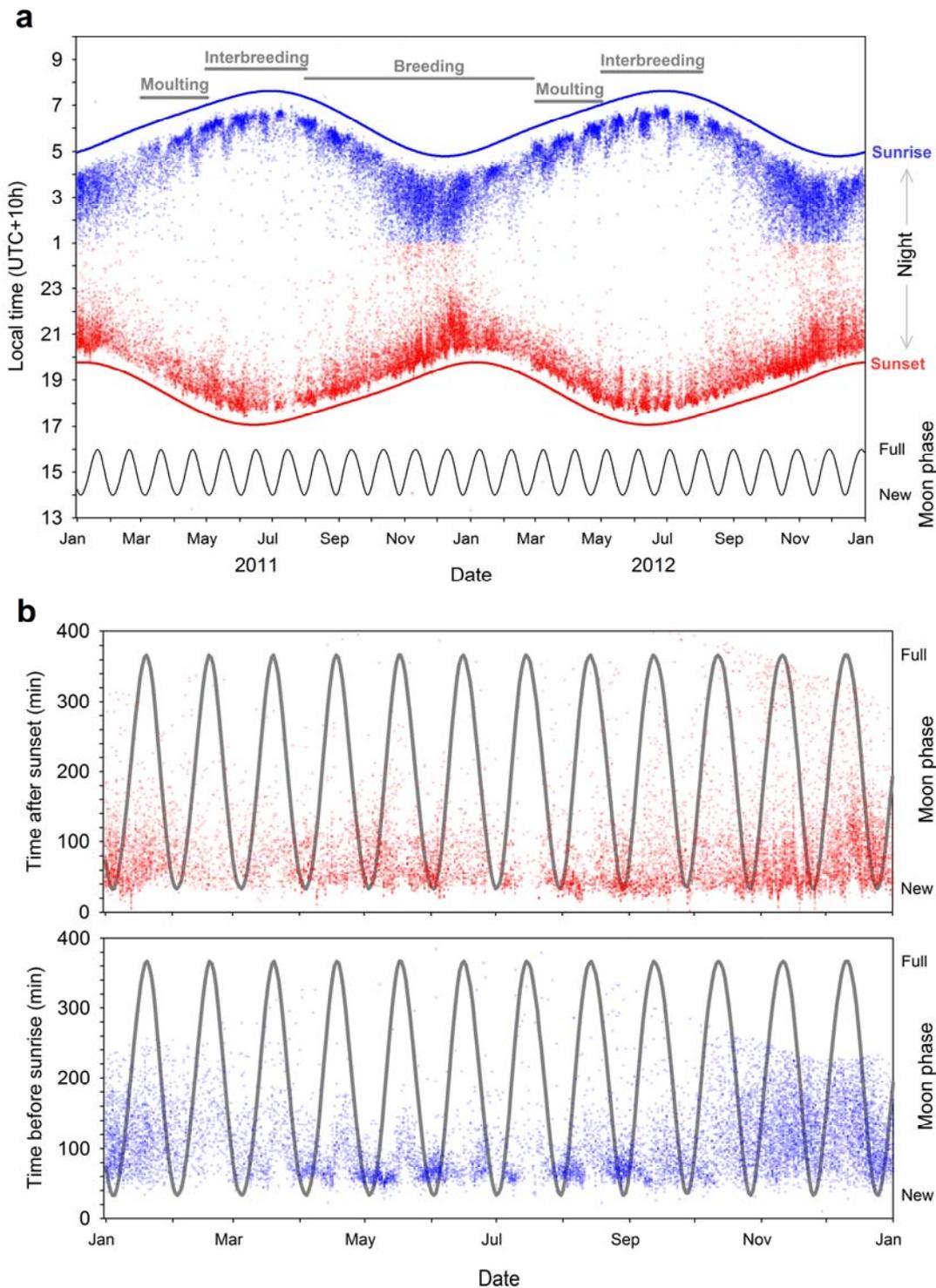
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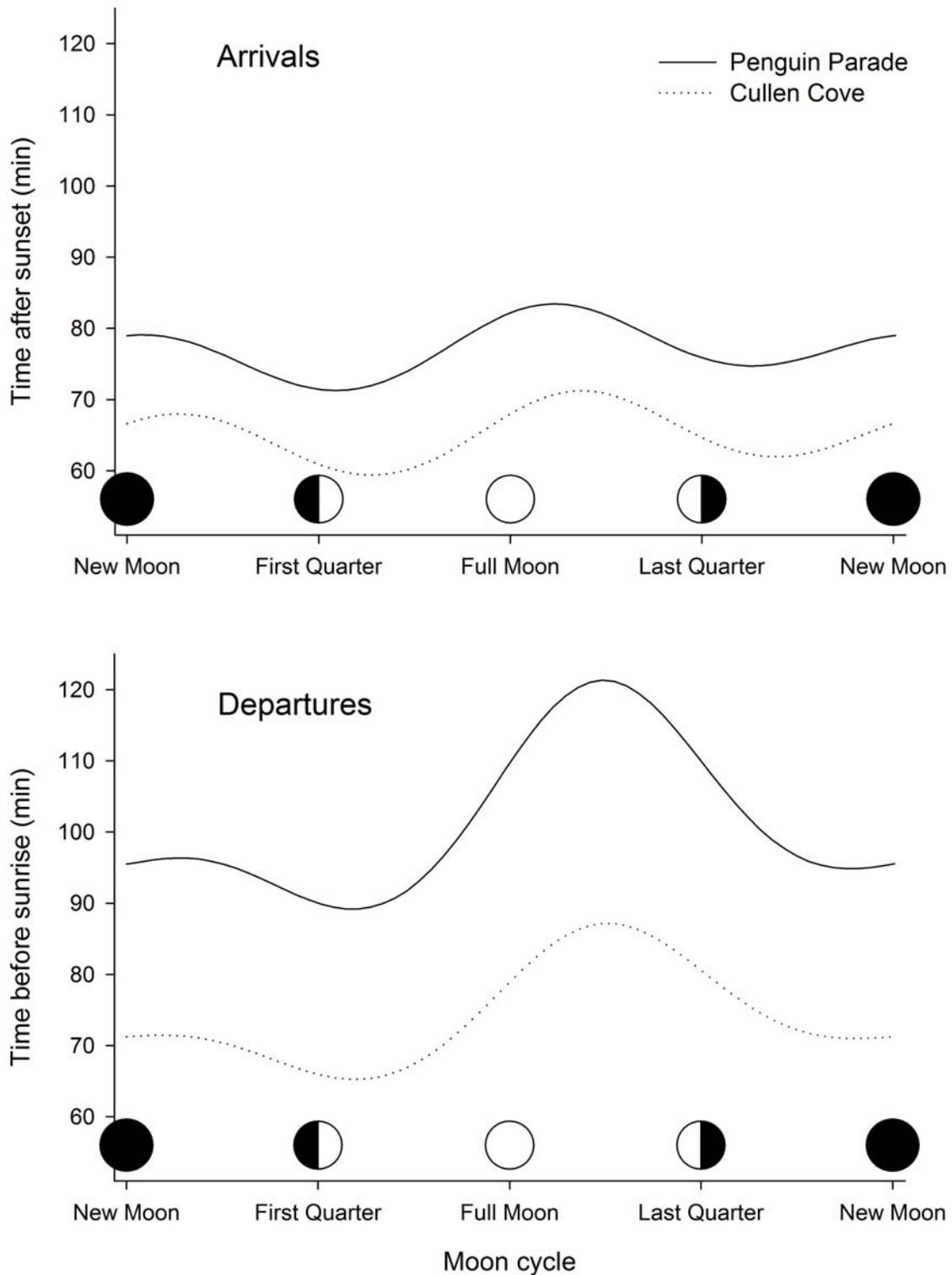
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532 **Figure 1** Times of arrival (red dots) and departures (blue dots) of little penguins at  
 533 Penguin Parade, Phillip Island, Australia. a) Seasonal sunlight effects on the arrival and  
 534 departure of penguins. Breeding phenology (moulting, interbreeding and breeding  
 535 seasons) and events of sunset (red line) and sunrise (blue line) are showed during a two-  
 536 year period (2011-2012). b) Effect of the moon phase (grey lines) on the attendance  
 537 time relative to sunset (arrivals) or sunrise (departures) during a year (2011).



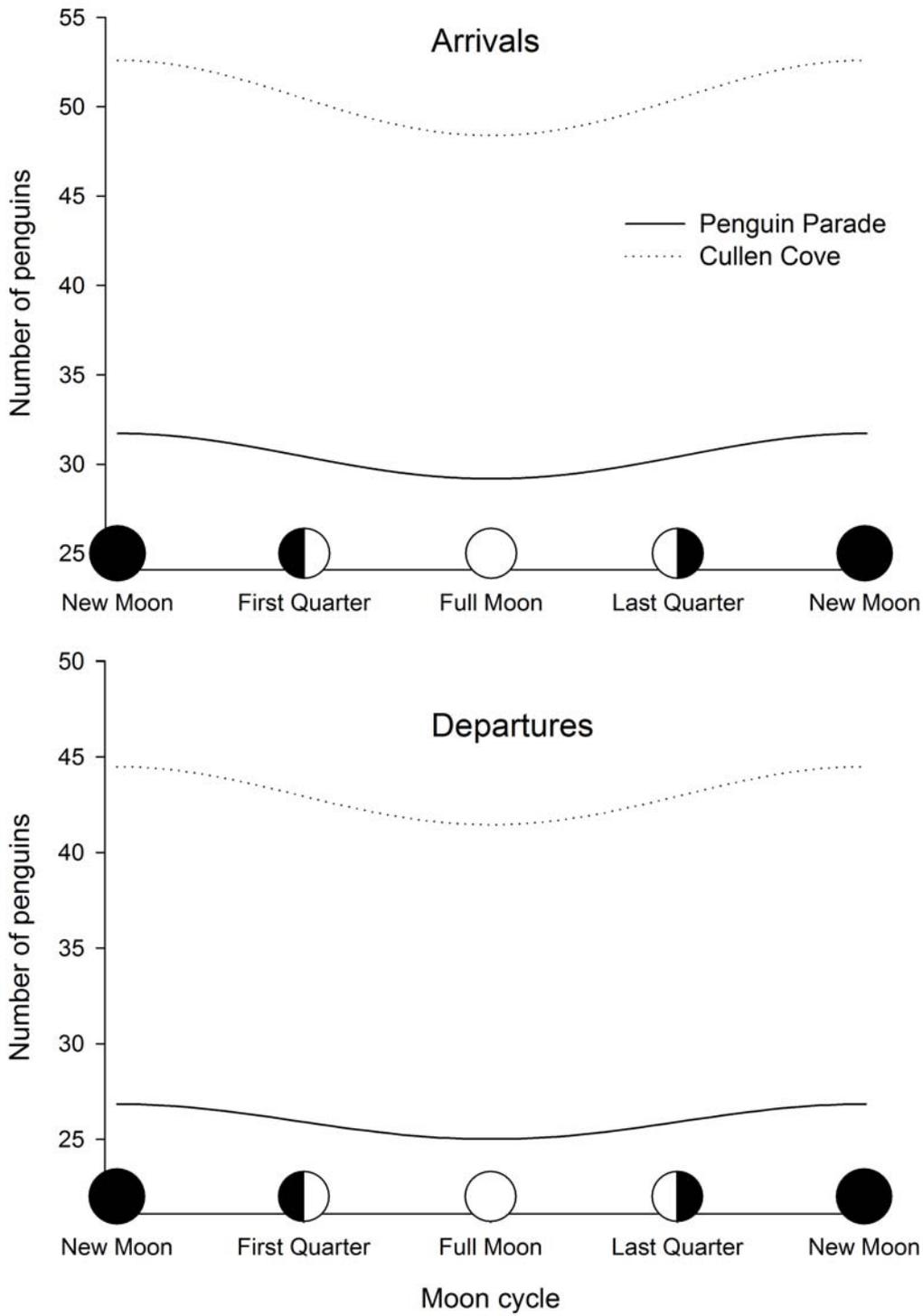
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539 **Figure 2** Arrival and departure timings of little penguins *Eudyptula minor* in relation to  
 540 moon phase at two colonies (Penguin Parade and Cullen Cove) on Phillip Island,  
 541 Australia. Cyclic effects were estimated by the inclusion of trigonometric functions of a  
 542 circular variable (moon phase) in General Linear Mixed Models (Table S1). For better  
 543 visualisation, only the effects for the level ‘breeding’ are displayed.



544

545 **Figure 3** Mean number of little penguins *Eudyptula minor* coming ashore or going to  
 546 the sea in relation to moon phase at two colonies (Penguin Parade and Cullen Cove) on  
 547 Phillip Island, Australia. Cyclic effects were estimated by the inclusion of trigonometric  
 548 functions of a circular variable (moon phase) in Generalized Linear Mixed Models  
 549 (Table S2).



550

## Supplementary Online Material

### **Waddling on the dark side: ambient light affects attendance behaviour of little penguins**

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**Table S1** General linear mixed models fitted by maximum likelihood showing the effect of the moon on arrival and departure patterns (timing) of little penguins *Eudyptula minor* on Phillip Island, Australia.

<b>Explanatory variables</b>	<b>Estimate</b>	<b>SE</b>	<b>df</b>	<b>t</b>	<b>P</b>
<b>Arrivals</b>					
Intercept	4.174	0.009	1695	477.44	< <b>0.001</b>
Breeding cycle (Interbreeding) <sup>1</sup>	-0.082	0.007	4871	-11.08	< <b>0.001</b>
Breeding cycle (Moulting) <sup>1</sup>	0.053	0.009	4764	6.14	< <b>0.001</b>
Colony (Penguin Parade) <sup>2</sup>	0.170	0.012	995	14.24	< <b>0.001</b>
Sin( $\theta$ )	-0.030	0.006	6001	-5.33	< <b>0.001</b>
Cos( $\theta$ )	-0.010	0.004	6011	-2.40	<b>0.017</b>
Sin(2* $\theta$ )	0.058	0.005	5978	11.08	< <b>0.001</b>
Cos(2* $\theta$ )	0.035	0.005	6016	7.66	< <b>0.001</b>
Colony*Sin( $\theta$ )	-0.003	0.003	302400	-0.78	0.438
Colony*Cos( $\theta$ )	-0.010	0.002	302100	-4.20	< <b>0.001</b>
Colony*Sin(2* $\theta$ )	-0.032	0.003	302700	-10.29	< <b>0.001</b>
Colony*Cos(2* $\theta$ )	-0.010	0.003	301800	-3.86	< <b>0.001</b>
<b>Departures</b>					
Intercept	4.303	0.007	2408	594.00	< <b>0.001</b>
Breeding cycle (Interbreeding) <sup>1</sup>	-0.320	0.007	4979	-43.23	< <b>0.001</b>
Breeding cycle (Moulting) <sup>1</sup>	-0.255	0.009	4841	-29.42	< <b>0.001</b>
Colony (Penguin Parade) <sup>2</sup>	0.311	0.009	1070	34.34	< <b>0.001</b>
Sin( $\theta$ )	-0.100	0.005	5776	-18.26	< <b>0.001</b>
Cos( $\theta$ )	-0.051	0.004	5788	-12.58	< <b>0.001</b>
Sin(2* $\theta$ )	0.058	0.005	5759	11.25	< <b>0.001</b>
Cos(2* $\theta$ )	0.014	0.005	5788	3.10	<b>0.002</b>
Colony*Sin( $\theta$ )	0.005	0.003	272100	1.88	0.061
Colony*Cos( $\theta$ )	-0.018	0.002	272100	-8.90	< <b>0.001</b>
Colony*Sin(2* $\theta$ )	0.007	0.003	272100	2.56	<b>0.010</b>
Colony*Cos(2* $\theta$ )	0.002	0.002	272000	0.85	0.396

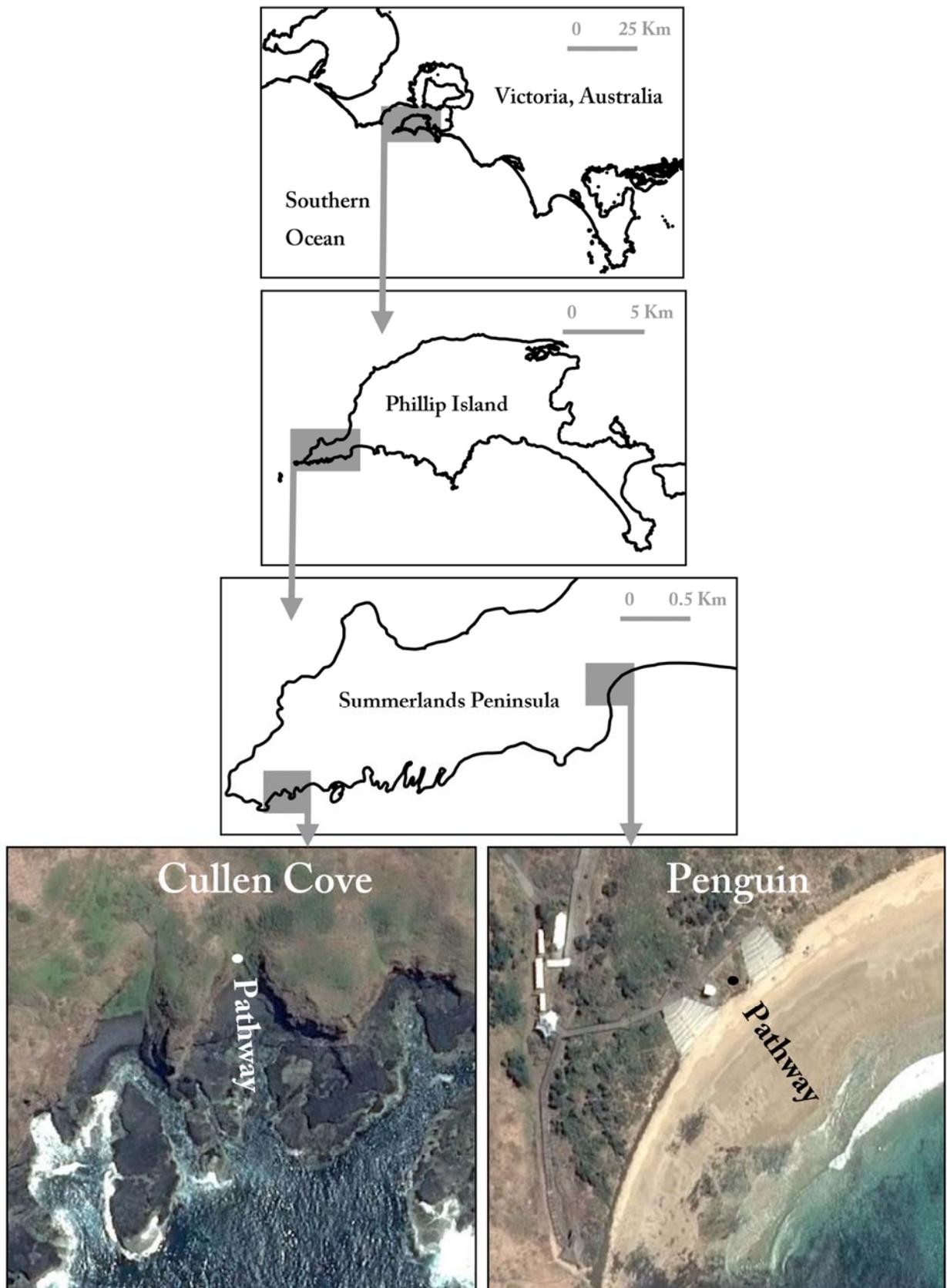
<sup>1</sup> & <sup>2</sup> 'Breeding' and 'Cullen Cove' taken as reference levels, respectively.

**Table S2** Generalized linear mixed models with negative binomial error structure and log link function and fitted by Laplace approximation showing the effect of the moon on the number of little penguins *Eudyptula minor* arriving to or departing from the two studied colonies on Phillip Island, Australia.

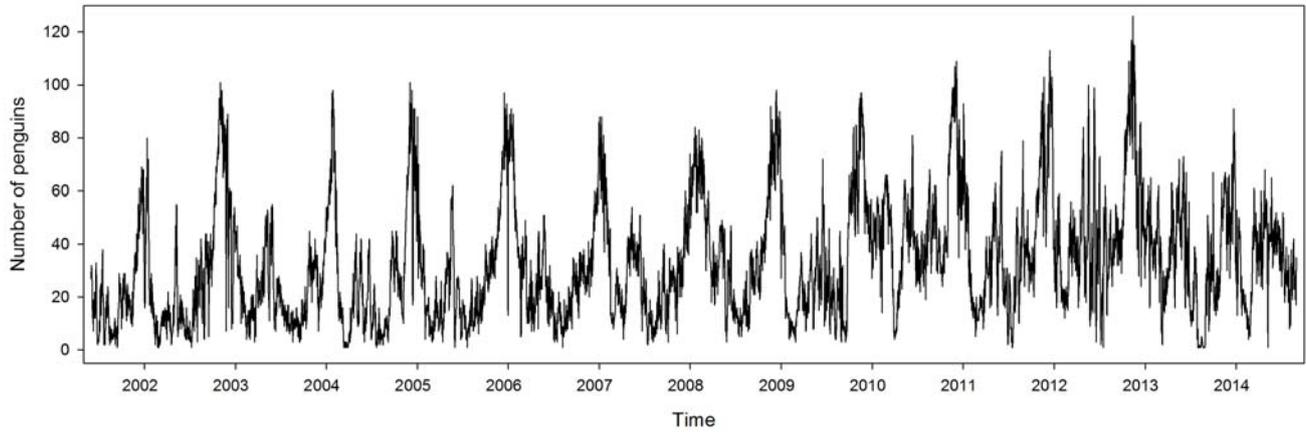
<b>Explanatory variables</b>	<b>Estimate</b>	<b>SE</b>	<b><i>t</i></b>	<b>P</b>
<b>Arrivals</b>				
Intercept	3.926	0.116	33.81	< <b>0.001</b>
Colony (Penguin Parade) <sup>1</sup>	-0.510	0.016	-32.58	< <b>0.001</b>
Sin( $\theta$ )	0.034	0.018	1.90	0.057
Cos( $\theta$ )	0.042	0.013	3.19	<b>0.001</b>
Sin(2* $\theta$ )	-0.031	0.017	-1.81	0.070
Cos(2* $\theta$ )	-0.011	0.015	-0.77	0.444
Colony*Ssin( $\theta$ )	-0.010	0.023	-0.42	0.677
Colony*Cos( $\theta$ )	-0.004	0.017	-0.25	0.803
Colony*Ssin(2* $\theta$ )	0.000	0.022	0.01	0.990
Colony*Cos(2* $\theta$ )	0.004	0.019	0.24	0.814
<b>Departures at Penguin Parade</b>				
Intercept	3.760	0.120	31.27	< <b>0.001</b>
Colony (Penguin Parade) <sup>1</sup>	-0.510	0.017	-30.23	< <b>0.001</b>
Sin( $\theta$ )	0.009	0.019	0.465	0.642
Cos( $\theta$ )	0.035	0.014	2.50	<b>0.012</b>
Sin(2* $\theta$ )	-0.016	0.018	-0.90	0.370
Cos(2* $\theta$ )	-0.011	0.016	-0.72	0.471
Colony*Ssin( $\theta$ )	-0.005	0.024	-0.22	0.824
Colony*Cos( $\theta$ )	0.030	0.018	1.66	0.097
Colony*Ssin(2* $\theta$ )	-0.008	0.023	-0.35	0.725
Colony*Cos(2* $\theta$ )	0.000	0.020	0.00	0.995

<sup>1</sup> 'Cullen Cove' taken as reference level.

**Figure S1** Location map of Summerlands Peninsula on Phillip Island, Victoria, Australia and aerial photographs of the two colonies (Cullen Cove and Penguin Parade) showing the penguin pathway and locations of transponder readers (white and black dots).



**Figure S2** Seasonal and inter-annual variation in the number of little penguins coming ashore at the Penguin Parade on Phillip Island, Australia, during the period 1 Jun 2001-31 August 2014.



**Figure S3** Estimated moon illumination for nights around the summer and winter solstices (December 2011 and June 2012). Illumination values were calculated following Austin et al. (1976). Solid, long-dashed and short-dashed black lines represent the nights of the full moon, first quarter and last quarter, respectively. Grey lines represent the remaining nights between first and last quarters.

