

1 Vocal correlates of arousal in bottlenose dolphins (*Tursiops* spp.) in human care

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## 21 **Abstract**

22 Human-controlled regimes can entrain behavioural responses and may impact animal  
23 welfare. Therefore, understanding the impact of schedules on animal behaviour can be a  
24 valuable tool to improve welfare, however information on overnight behaviour and behaviour  
25 in the absence of husbandry staff remains rare. Bottlenose dolphins (*Tursiops* spp.) are highly  
26 social marine mammals and the most common cetacean found in captivity. They  
27 communicate using frequency modulated signature whistles which are individually  
28 distinctive and used as a contact call. We investigated the vocal behaviour of ten dolphins  
29 housed in three social groups at uShaka Sea World dolphinarium to determine how acoustic  
30 behavioural patterns link to dolphinarium routines. Investigation focused on overnight  
31 behaviour, housing decisions, weekly patterns, and transitional periods between presence and  
32 absence of husbandry staff. Recordings were made from 17h00 – 07h00 over 24 nights,  
33 spanning May to August 2018. Whistle production rate decreased soon after husbandry staff  
34 left the facility, was low over night, and increased upon arrival. Results indicated elevate  
35 arousal states associated with the morning arrival and presence of husbandry staff and  
36 heightened excitement associated with feeding. Housing in pool configurations which limited  
37 visual contact between certain groups were characterised by lower vocal production rates.  
38 Production of signature whistles was greater over the weekends compared to weekdays  
39 however total whistle production did not differ between weekends and weekdays. Heightened  
40 arousal associated with staff arrival was reflected in the structural characteristics of signature  
41 whistles, particularly maximum frequency, frequency range and number of whistle loops.  
42 Overall, these results revealed a link between scheduled activity and associated behavioural  
43 responses, which can be used as a baseline for future welfare monitoring where changes in  
44 normal behaviour may reflect shifts in welfare state.

45

## 46 **Introduction**

47 Understanding and monitoring behaviour is a useful tool for welfare assessment [1] as  
48 abnormal behaviour may be indicative of poorer welfare [2]. However, the behavioural cues  
49 the animal gives must be correctly recognised and interpreted by the observer. Animals housed  
50 in captive facilities tend to have structured daily schedules of events such as food provision or  
51 routine cleaning that are highly predictable through human-driven cues [3]. Patterns of animal  
52 behaviour such as increased alertness [4] and increased vocalisation rates [3] may precede  
53 regular events [5] Conversely, reduced activity of the animals has also been documented  
54 preceding regular activities such as feeding [6] and public display [4].

55 Arousal can be classified according to its level (high or low) and its valence (positive or  
56 negative) [7,8]. Vocal expression of arousal is a common feature of communication in both  
57 humans and non-human animals [9-12] and can reflect responses to immediate experiences  
58 [13]. Understanding the vocal cues which act as markers of emotion in mammals [13-15]  
59 may be used as a non-invasive tool to monitor welfare for animals held in human care [16-  
60 18]. In captive animals, both positive and negative associations with human care givers are  
61 observed [19,20] as animals learn to associate humans with rewards and fear through both  
62 classical and operant conditioning [21]. Human presence and handling can improve welfare  
63 of captive animals, for example in weanling pigs [22] and beef calves [19]. However, in some  
64 livestock and poultry, human presence can induce fear and have a negative impact such as  
65 decreased growth rates [20,23]. Responses to human presence associated with feeding are  
66 often positive [13,24], and the combination of feeding and handling has been shown to play  
67 an important role in the development of positive human-animal interactions [25]. Feeding

68 therefore seems to be a suitable candidate to study the behavioural response of a species to a  
69 positive interaction.

70 Animals in captivity are often cared for according to fixed daily schedules where these, as  
71 well as other activities with positive outcomes, can elicit behavioural responses for example  
72 in the form of anticipatory behaviours [26]. Anticipatory behaviour is a response evoked by  
73 reward and is typically linked to elevated arousal states [24], however behaviour has been  
74 characterised as either an increase [e.g., 27,28] or decrease [e.g., 29,30] in activity prior to the  
75 reward and has been best documented in domesticated mammals [28, 31, 32]. Responses such  
76 as anticipatory behaviour can be used as an indicator of animal welfare through measuring  
77 the frequency of certain behaviours [33].

78 Vocalisations often carry prosodic cues about the arousal state of the sender [10] which  
79 makes the analysis of vocalisations a suitable tool to assess arousal states. Dolphins live in  
80 groups with many social interactions, with vocalisations being their primary method of  
81 communication with and between groups. The vocal repertoire of bottlenose dolphins  
82 consists of a range of pulsed and tonal sounds [34]. Tonal sounds include narrow-band,  
83 frequency modulated whistles to communicate during social interactions [35, 36]. Bottlenose  
84 dolphins use ‘signature whistles’ to remain in contact [37-39] and address one another [40].  
85 Signature whistles are individually distinctive whistle types that encode identity information  
86 within their frequency modulation pattern and are the most frequently emitted stereotyped  
87 whistle type produced by an individual [41]. Although the whistle contour remains stable  
88 over time, shifts in whistle production rate, as well as frequency and duration characteristics,  
89 may reflect underlying arousal of individual dolphins [38, 42, 43]. As might be expected for a  
90 cohesion call, individual production rates of signature whistles increase when motivation to  
91 maintain contact or return to the group is strong, i.e., during separation [39] and isolation  
92 events [42].

93 During the day, bottlenose dolphins housed in human care undergo multiple predictable  
94 human-controlled events such as feeding, public presentations, training sessions and medical  
95 examinations, making them good models for studying patterns in behaviour [44]. Predictable  
96 events in captive facilities can evoke behavioural patterns in bottlenose dolphins [4] such as  
97 an increase in spy-hopping frequency and surface-looking before any human-animal  
98 interaction, including receiving of toys [45]. Vocal behaviour has been reported to be highest  
99 when husbandry staff are present and during feeding/training sessions, and lowest at night  
100 when numbers of caretakers are reduced [46] with peaks in activity overnight likely  
101 associated with bouts of social activity [47]. The morning arrival of caretakers has also been  
102 associated with increased vocalisation rate in captive killer whales [48].

103 As vocal behaviour indicates underlying emotional states, the link between behavioural  
104 responses and daily events at a dolphinarium could provide insight into the animals' life in  
105 human care. Additionally, this will provide insight into emotional responses and acoustic  
106 cues of emotion in non-human animals. Therefore, the aim of this study was to investigate the  
107 overnight behaviour of bottlenose dolphins with a focus on patterns in detections and rates of  
108 whistling in response to early morning regimes including husbandry staff presence and  
109 feeding. We monitored the vocal response to scheduled events of the population as a whole,  
110 as well as at an individual level as animal personalities may vary.

111

## 112 **Methods**

113 This study took place at uShaka Sea World which was established in 2004 and is located in  
114 Durban, South Africa. The dolphinarium consists of a covered and open-air pool network  
115 with seven interconnected pools of varying size with a combined volume of 11 000 m<sup>3</sup>. At

116 the time of data collection in 2018, the dolphinarium housed ten bottlenose dolphins: three  
117 (two male and one female) common bottlenose dolphins (*T. truncatus*), one female Indo-  
118 Pacific bottlenose dolphin (*T. aduncus*) and six (four female and two male) hybrids of the two  
119 species [49]. The individuals were held in three social groups (see Table 1 for more  
120 information on the individuals and social groups) separated by gates which allowed partial  
121 visual and full acoustic contact, but not free movement between social groups. All seven  
122 pools were utilised by the dolphins during the course of the study, with the number and  
123 configuration of pools within which social groups were housed varying throughout the days  
124 and overnight. We included pool configuration as a potential predictor variable since visual  
125 contact between the different social groups may affect arousal states. Each pool configuration  
126 was defined based on which social group was housed in the outdoor presentation pool;  
127 configuration 1 – all groups housed in the inside pools with one group having access to the  
128 outer interaction pool, and none having access to the outside presentation pool; configuration  
129 2 – the female group housed in outside presentation pool while the mixed and male groups  
130 housed in the inside pools with one group having access to the outer interaction pool;  
131 configuration 3 – the male group housed in outside presentation pool while the mixed and  
132 female groups housed in the inside pools with one group having access to the outer  
133 interaction pool; and configuration 4 – the mixed group housed in outside presentation pool  
134 while the male and female groups housed in the inside pools with one group having access to  
135 the outer interaction pool. The outer interaction pool is directly linked to the outside  
136 presentation pool, allowing the dolphins in each of these pools to be in visual contact through  
137 the gate. As potential arousal-eliciting activities, we noted morning activities at the  
138 dolphinarium which include arrival of husbandry staff (hereafter referred to as ‘staff’) and  
139 food preparation at 05h00 as well as feeding and vitamin administration at 06h00. The last

140 public presentation occurs from 15h00 to 15h30 and the last trainer leaves between 17h00  
141 and 18h00, between which times all enrichment devices are removed from the pools.

142

143 **Table 1. Genetic, social grouping and individual data for the dolphins at uShaka Sea**  
 144 **World in 2018 (adapted from Gridley et al., 2018)**

<b>Social group</b>	<b>Name</b>	<b>Species</b>	<b>Sex</b>	<b>Capture date</b>	<b>Parents</b>	<b>Signature whistle ID</b>
Female group	Affrika	<i>Tt</i>	F	Captive born	P1 + <i>Tt</i>	F1
	Zulu	<i>Ta-Tt</i> Hybrid	F	Captive born	P1 + P2	F2
	Khanya	<i>Ta-Tt</i> F <sub>2</sub>	F	Captive born	M3 + hybrid	F3
	Tombi	<i>Ta-Tt</i> Hybrid	F	Captive born	P1 + P2	F4
	Khethiwe	<i>Ta-Tt</i> Hybrid	F	Captive born	P1 + P2	F5
Male group	Ingelosi	<i>Ta-Tt</i> Hybrid	M	Captive born	P1 + P2	M1/M2
	Khwezi	<i>Ta-Tt</i> Hybrid	M	Captive born	P1 + P2	M1/M2
	Kelpie	<i>Tt</i>	M	Captive born	Unknown <i>Tt</i> x <i>Tt</i>	M3
Mixed group	Gambit	<i>Tt</i>	M	08/12/1976	Unknown <i>Tt</i> x <i>Tt</i>	P1
	Frodo	<i>Ta</i>	F	26/06/1979	Unknown <i>Ta</i> x <i>Ta</i>	P2

145

## 146 **Generating a signature whistle catalogue**

147 The vocal behaviour of individuals was assessed through analysis of signature whistles. To  
 148 determine the signature whistle for each dolphin, a catalogue was compiled using data  
 149 collected during temporary isolation sessions in November 2016. Whistle contours are  
 150 characterised by their time-frequency modulation patterns and in bottlenose dolphins,  
 151 signature whistles can consist of a single contour or repeated contour (loop). A repeated  
 152 contour, or multiloop whistle, is either connected where there are no breaks in the entire  
 153 whistle contour or disconnected with a maximum inter-loop-interval of 0.25 seconds [43].



154 Acoustic data were collected using one to three High-Tec HTI-96 MIN (flat frequency  
155 response of 2 Hz – 30 kHz  $\pm$  1 dB) dipping hydrophone(s) attached to a Tascam digital  
156 recorder (model DR-680) which sampled the data at 96 000 Hz. Simultaneous vocal notes of  
157 observed behavioural data were recorded through a separate headset microphone. Time-  
158 frequency spectrograms of acoustic recordings were analysed in Adobe Audition CC v 6.0  
159 (FFT = 1024, frequency range = 0 - 60 kHz, time series window = 10 seconds, Hann window,  
160 50% overlap). To identify the signature whistle of each dolphin, they were temporarily  
161 separated with one individual being kept in solitary in one pool between 10 and 20 minutes.  
162 The signature whistle was defined as the most common whistle recorded during the  
163 temporary isolations and matched to that individual by comparing the relative amplitude of  
164 signals on the three hydrophones at different sites. Signature whistles were confirmed using  
165 the SIGID bout analysis method [41] where stereotyped calls (at least 3 out of 4) occur in a  
166 bout separated by 1 – 10 seconds are considered highly likely to be signature whistles of an  
167 individual.

168

169

## 170 **Investigating patterns of whistling behaviour**

171 Acoustic data were collected over four periods in May, July and August 2018 using a single  
172 Sound Trap 300 HF hydrophone (Ocean Instruments, New Zealand, frequency response: 20  
173 Hz – 150 kHz  $\pm$  3 dB, sensitivity: 121 dB re. 1  $\mu$ Pa) sampling the data at 576 000 Hz. The  
174 hydrophone was placed in the ‘link channel’, an area central to the pool network to which the  
175 dolphins did not have access to but was within acoustic range of dolphins held in all pools.  
176 The hydrophone was attached to a 1 kg dive weight and suspended from a rope mid water (at  
177 1.5 m depth, channel depth 2.5 m) held taut by attaching the rope to the roof with a carabiner

178 clip. Ropes tied to the pool sides and roof were used to prevent movement which could  
179 produce unnecessary noise on the hydrophone. Acoustic recording commenced in the late  
180 afternoon after the final public presentation (between 15h30 and 17h00) and continued until  
181 07h00 the following day. Recording was continuous, but files were restricted to standard 15-  
182 minute durations which constrained file sizes.

183 The first 30 minutes of each overnight deployment was discarded to obtain data unbiased by  
184 potential novelty effect as the dolphins may respond to the recorder's presence in the water.  
185 Thereafter, one 15-minute file was selected to represent each hour from 17h00 to 07h00,  
186 using the recording which spanned the start of the hour. Each of the selected files were  
187 analysed in Adobe Audition CC v 6.0 by visually locating whistle contours in the  
188 spectrogram display (FFT = 1024, frequency range = 0 – 60 kHz, time series window = 10  
189 seconds, Hann window, 50% overlap). Each whistle was documented in a database and the  
190 signal to noise ratio (SNR) was visually assessed using the following criteria: SNR – 1  
191 whistle is faint and barely visible, SNR 2 – whistle is clear and unambiguous, and SNR 3 –  
192 whistle is prominent [50]. Whistles of good quality (those graded as SNR 2 or 3) were either  
193 matched to the established signature whistle catalogue and categorised as a signature whistle  
194 from a specific individual or categorised as 'variable whistles' containing all non-signature  
195 whistles from various individuals. The category 'unclassified whistles' was used for poor  
196 quality whistles (those graded as SNR 1) which did not match to the signature whistle  
197 catalogue (Table 2).

198

199

200 **Table 2. Whistle categories included in the analyses and recording quality rating based**  
201 **on signal to noise assessment.**

Whistle type	Description	SNR
Signature whistle	A whistle type unique to an individual that matches the signature whistle catalogue	2 & 3, but can include whistles with SNR 1 if we were confident that they are signatures
Signature whistle matching	Matching of an individual's signature whistle by another individual identified through overlapping of whistle contours	2 & 3
Variable whistle	Loud, unmasked whistles which do not match the signature whistle catalogue	2 & 3
Unclassified whistle	Masked or partial whistles that were unidentifiable and faint	1

202

203 In both the wild and captive facilities, bottlenose dolphins can copy the whistles of others.  
204 Such copies can be used in addressing or matching interactions, drawing attention from, or  
205 directing information to a particular individual [40, 51, 52]. In some cases, the whistle match  
206 might not be an exact replication, but integrate features of the owner's whistle type or voice  
207 [52], however in other cases matches may be indistinguishable from the original. Whistle  
208 matching is difficult to identify unless they are overlapping in time. In general, where two

209 whistles with the same contour overlapped in time, we followed [53] in assigning the second  
210 whistle as the match and removed it from the signature whistle analyses. However, through  
211 the analysis process we identified stereotyped matching behaviour distinct from all other  
212 acoustic behaviour observed within the recordings whereby which led to the exclusion of  
213 various whistles from the analysis. We noted that the contour of one individual (P2) was  
214 emitted at various frequencies in prolonged series of matching interactions. For these  
215 stereotyped acoustic interactions, we could not confidently assign whistle production to P2,  
216 therefore we termed these whistle sequences ‘square copies’. These square copies, as well as  
217 all whistles 30 seconds before and after each event, were removed from the statistical  
218 analyses to prevent over-representation of individual P2. All other whistle matching remained  
219 in the dataset for the analyses including all whistle types.

220 To assess the production rate of whistles as a proxy of arousal, we counted the number of  
221 whistles in each category (Table 2) per 15-minute recording. We investigated how the  
222 production rate of whistles (meaning all whistle types in Table 2 or only signature whistles)  
223 was affected by several covariates using a generalised additive mixed model (GAMM)  
224 approach using the package ‘`gamm4`’ version 0.2-6 [54] in RStudio version 4.0.3 [55]. This  
225 approach allows fitting of non-linear relationships between variables as well as the inclusion  
226 of both fixed and random effects. Whistle production was investigated in terms of both  
227 presence/absence and production rate (whistles per minute). A total of four models were built  
228 (two for all whistle types in Table 2, and two for signature whistles only) and for each, a  
229 variation of the following covariates were tested: ‘hour’ (hour of the day/night), ‘pool  
230 configuration’, ‘time of week’ (week vs weekend) and ‘presence of signature whistles’.  
231 Random effects for all four models included ‘sampling day’ to account for the variability of  
232 whistles production between sampling days. The selection of covariates was based on testing  
233 the assumption that patterns of activities and housing configuration in the dolphinarium lead

234 different levels of excitement which would reflect in dolphin vocal behaviour. Of most  
235 interest were the activities of the dolphinarium which occur at scheduled times therefore  
236 ‘hour’ was of particular interest. Time of the week was of interest due to the increase of  
237 visitors to uShaka Sea World over the weekend. Codes were assigned to working days,  
238 ‘week’, (1), which consisted of Monday to Friday, and weekend days, ‘weekend’, (2), which  
239 consisted of Saturday and Sunday. The covariate ‘pool configuration’ also contained coded  
240 subcategories which were based on which social group was housed in the outdoor  
241 presentation pool overnight. All models were run using data between the hours spanning  
242 17h00 to 07h00, with one extreme outlier removed from the total whistle and signature  
243 whistle production rate models.

244 We did not incorporate signature whistle ID as a covariate in the models of the full data series  
245 because the data were zero-inflated data due to a lack of whistles emitted in most hours  
246 overnight. These models therefore investigate group, but not individual, calling behaviour for  
247 whistles. However, individual differences in vocal behaviour are of interest and relevant for  
248 animal welfare. Therefore, a separate analysis was conducted to investigate individual  
249 differences between 04h00 and 06h00 (04h00 – one hour before staff arrival and food  
250 preparation; 05h00 – staff arrival and food preparation; 06h00 – feeding time) when a  
251 sufficient number of calls for individual dolphins were available. Data were not normally  
252 distributed and had unequal variances (Shapiro-Wilks test and Levene’s test, respectively)  
253 even after transformations. Thus, Kruskal-Wallis tests were used to determine if certain  
254 individuals significantly increased their signature whistle production rate in response to staff  
255 arrival and feeding time. Multiple Dunn *post hoc* tests followed a significant Kruskal-Wallis  
256 result. We adjusted the alpha value using the Benjamini-Hochberg method to limit an  
257 increase in type I error rate, which is caused by alpha-inflation due to multiple pairwise  
258 comparisons.

259 In addition to changes in whistle production rate, the underlying arousal state may be  
260 reflected in the structural parameters of whistles such as duration and frequency  
261 characteristics [11, 42]. We investigated this for each individual by selecting five standard  
262 whistle parameters from signature whistles namely minimum and maximum frequency,  
263 frequency range, duration and number of loops, as well as production rate for each signature  
264 whistle. Measurements were taken from time-frequency spectrograms (FFT = 1024,  
265 frequency range = 0 – 40 kHz, time series window = 5 seconds, Hann window) of the  
266 fundamental frequency of 33 – 392 signature whistles from each animal in Raven Pro v1.6.1  
267 [56]. For each signature whistle, production rate data were pooled into time periods  
268 representing staff absent (20h00 – 04h00) and staff present (05h00 – 07h00). Thereafter,  
269 production rates per hour were recalculated for each time period. Times 17h00 – 19h00 were  
270 omitted from this analysis due to the uncertainty of daily staff presence that occurred at these  
271 times. Data were not normally distributed and had unequal variances (Shapiro-Wilks test and  
272 Levene’s test, respectively) therefore paired-sample Wilcoxon tests were used to investigate  
273 change in production rate between the pooled time. Additionally, all five signature whistle  
274 parameters were individually compared between the two pooled time periods for six of the  
275 ten individuals with sufficient data (at least five signature whistles for each individual for  
276 each pooled time period). Again, data were not normally distributed and had unequal  
277 variances (Shapiro-Wilks test and Levene’s test, respectively), therefore Wilcoxon tests were  
278 used to investigate change in the acoustic parameters of signature whistles between times of  
279 ‘staff absent’ and ‘staff present’.

280

## 281 **Results**

282 A total of ten signature whistles were documented for the group of ten dolphins, of which  
283 eight were assigned to individuals (see Table 1, Fig 1A). The two remaining signature  
284 whistles could not be confidently differentiated between individuals M1 and M2, which are  
285 hybrid siblings which engage in a significant amount of whistly matching. All square whistle  
286 copying sequences were removed from the analyses (Fig 1B).

287 **Fig 1. Whistle catalogues** (A) Signature whistle catalogue of all ten dolphins housed at  
288 uShaka Sea World in 2018 (F1, M3, P1 and P2 are single loop whistles; F2, F3 and F5 are  
289 connected multiloop whistles; F4, M1 and M2 are disconnected multiloop whistles). (B)  
290 Examples of (1) low, (2) middle and (3) high frequency square whistle copying, with a  
291 similar contour shape to dolphin P2.

292

293 Nocturnal whistling behaviour was investigated from acoustic recordings made over 24  
294 sampling nights (17 on weekdays and 7 on weekend days) which were conducted in four  
295 hydrophone deployment periods ranging from five to seven nights in duration. From this, 88  
296 hours of acoustic data were analysed with a total of 2640 documented whistles, 1647 of  
297 which were signature whistles (62.4%), 168 variable whistles (6.3%), 569 unclassified  
298 whistles (21.6%), 3 signature whistle matching (0.1%), 98 square whistles (3.7%) and 155  
299 square whistle copying (5.9%) (Table 2). Square whistle copying behaviour was uncommon  
300 and occurred sporadically with no particular temporal trend (S1 Fig). Furthermore, these  
301 square copying events occurred predominantly during the night when overall signature  
302 whistle production was at a minimum. In addition to P2 not confidently being assigned a  
303 whistle in these copying interactions, a closer inspection of these square whistle copying  
304 events indicated that the vocal behaviour was more involved than a simple owner-copy  
305 interaction. In the 30 seconds before and after these whistle copying events that occurred

306 throughout the data set, whistles were produced most by three of the animals (S2 Fig). After  
307 removing all whistles 30 seconds before and after each square whistle event, 2281 whistles,  
308 1561 of which were signature whistles, were included in the analyses.

309 The GAMMs show a temporal trend in presence and production rate of whistles with two  
310 clear peaks, one in the late afternoon and one in the morning (Fig 2; Table 3 – Models 1 to 4;  
311 variable ‘hour’;  $p < 0.001$ ). These peaks correspond to staff presence, particularly to the time  
312 of arrival of staff and preparation of food in the morning (05h00 – 06h00). Pool configuration  
313 also influenced overall whistle presence and production rate. Total whistle presence and  
314 signature whistle presence significantly decreased when dolphins were housed in pool  
315 configuration 2 (female group housed outside) compared to configuration 1 where all animals  
316 were housed in the indoor pools (Fig 3A; Table 3 – Model 1 and 3, variable ‘pool  
317 configuration’;  $p < 0.05$ ). Total whistle production rate decreased when dolphins were housed  
318 in pool configuration 4 (mixed group housed outside) and signature whistle production rate  
319 decreased in pool configuration 3 (male group housed outside) in comparison to  
320 configuration 1 (Fig 3B; Table 3 – Model 2 and 4, variable ‘pool configuration’;  $p < 0.05$ ).

321 The proportion of whistles produced during ‘staff transition’ (the afternoon period where staff  
322 were leaving and/or had just left), ‘staff absent’ and ‘staff present’ indicates that this trend in  
323 whistle production driven by pool configuration is prevalent during the overnight period  
324 where staff were absent (Fig 3A, B). The likelihood of whistle or signature whistle  
325 occurrence per hourly bin did not differ between weekdays and the weekend, however total  
326 signature whistle production rate was greater over the weekend (Fig 4; Table 3; Model 4,  
327 variable ‘Time of Week’). Although not significant, the inclusion of this variable increased  
328 model performance (R-squared from 0.178 to 0.233) which indicates that the time of the  
329 week plays a role in the production of signature whistles at the facility. Total whistle presence  
330 and production are highly dependent on the presence of signature whistles (Table 3; Model 1



331 and 2, variable ‘Signature whistle presence’). Although not significant in Model 1, this  
332 variable significantly increased overall model performance (R-squared from 0.302 to 0.649).  
333 The low-average adjusted R-squared values in all four models (Table 3; adjusted R-squared  
334 range = 0.233 – 0.649) indicate that there are other factors influencing whistle and signature  
335 whistle production rates that cannot be explained by the covariates in these models.

336 **Fig 2. GAMM summary of smoothing term ‘hour’ for all four models.**

337 **Fig 3. Total whistle and signature whistle production rate compared between pool**  
338 **configurations (A) Production rates (B) Production rates split into time periods associated**  
339 **with husbandry staff presence.**

340 **Fig 4. Signature whistle production rate compared between weekdays and weekend**  
341 **days.**

342

343 **Table 3. GAMM results for all presence/absence and production rate models (only *p*-values of significant results presented)**

Response variable	N	Explanatory variables				Adjusted R-squared
		Hour	Pool configuration	Time of week	Signature whistle presence	
<b>Model 1:</b>	347	< 0.001	0.042 (Intercept)	NA	-	0.649
<b>Whistle presence</b>			0.020 (config 2)			
<b>Model 2:</b>	346	< 0.001	0.044 (Intercept)	NA	< 0.001	0.521
<b>Whistle count</b>			0.025 (config 4)			
<b>Model 3:</b>	347	< 0.001	0.034 (Intercept)	NA	NA	0.279
<b>Signature whistle presence</b>			0.017 (config 2)			
<b>Model 4:</b>	346	< 0.001	0.001 (Intercept)	-	NA	0.233
<b>Signature whistle count</b>			0.008 (config 3)			

345 There was a large difference in signature whistle production between individuals. Of the  
346 1561 signature whistles identified ~ 60% were produced by three individuals (two female and  
347 one male, Fig 5). The three least vocal animals contributing ~ 7% to the total signature  
348 whistle production were all males (Fig 5). A comparison of individual signature whistle  
349 production rate was investigated in the morning when total whistle production was at its  
350 highest and there was sufficient data (04h00 to 06h00) to determine individual differences in  
351 response to staff arrival (05h00) and feeding time (06h00). Kruskal-Wallis tests indicated that  
352 the signature whistle production rate of five of the dolphins increased significantly only in  
353 response to feeding time (Fig 6A; no significant difference between 04h00 and 05h00,  $p <$   
354  $0.05$ ,  $n = 866$ ). The remaining five individuals did not exhibit a significant increase and P1  
355 produced no whistles during feeding time. Additionally, some of the animals were completely  
356 silent overnight when no staff were present. Investigating individual differences in signature  
357 whistle production between periods with staff absent (20h00 – 04h00) and with staff present  
358 (05h00 – 07h00), F2 was highly vocal during both of these time periods (Fig 6B) and  
359 signature whistle production from all dolphins increased with staff present (Fig 6B,  $p < 0.05$   
360 for five of them,  $n = 1413$ ). In the absence of staff, bouts of whistling occurred sporadically  
361 between sample days, driven mostly by F2 as well as four other individuals. Although  
362 signature whistle identification could not be included in the above-mentioned models as a  
363 covariate, the results strongly suggest that individual differences in signature whistle  
364 production are important.

365 **Fig 5. Total count of signature whistles produced throughout the study period.**

366 **Fig 6. Individual vocal responses to feeding and staff presence.** (A) Signature whistle  
367 production rate of each individual compared across three hours (04h00 – one hour before  
368 staff arrive, 05h00 – staff arrival and food preparation, 06h00 – feeding). Significance  
369 indicated by asterisks is consistent among individuals, with no significant shift between

370 04h00 and 05h00. (B) Whistle production rate per hour of all individuals when staff were  
371 absent and when staff were present. Significance between time periods indicated by asterisks.

372

373 Matched pairs Wilcoxon tests showed significant differences in the acoustic parameters of  
374 their signature whistles between periods of staff absence and presence (Fig 7). The three  
375 animals which exhibited the most changes in whistle characteristics all showed a significant  
376 decrease in maximum frequency and frequency range when staff were present. The two other  
377 individuals did not exhibit any significant shifts in whistle characteristics. Overall, most of  
378 the individuals showed significant decreases in maximum frequency (average: from 12.8 to  
379 11.4 kHz), half of them significant decreases in frequency range (average: from 10 to 7.9  
380 kHz) and number of loops (average: from 4.6 to 2.5 loops per whistle), while two individuals  
381 exhibited significant decreases in whistle duration (average: from 1.35 to 1.05 s) and only one  
382 individual a significant increase in minimum frequency (from 5.5 to 6.6 kHz).

383 **Fig 7. Shifts in signature whistle parameters in response to staff presence. Asterisks**  
384 **indicate significance.**

385

## 386 **Discussion**

387 The overall night-time vocal behaviour between the afternoon and morning peak of whistle  
388 production was fairly quiet, with some bouts of whistling behaviour during the night  
389 occurring randomly between recording nights. This is in accordance with [57] who found that  
390 peaks in nocturnal vocal activity of captive bottlenose dolphins may occur. Sleeping/resting  
391 occurs predominantly during the night and accounts for up to 87% of nocturnal behaviour of  
392 captive bottlenose dolphins [58]. This explains the decrease in vocal activity during this

393 night-time period, however from this study there has been no indication what might be  
394 driving the bouts of social behaviour. It has also been shown that captive bottlenose dolphins  
395 increase vocal and social interactions before their rest periods, possibly to promote  
396 synchronous swimming, a behaviour observed in wild and captive dolphins [58]. Similarly, in  
397 a study of captive bottlenose dolphins (*T. truncatus*) at facilities in Japan, vocal production  
398 rates increased during the day when their human caretakers were present [46, 58] and vocal  
399 behaviour, including whistle production rate, decreased at night when caretakers were absent  
400 [46]. These studies indicate a positive relationship between caretakers and animals in  
401 captivity may be beneficial to the welfare of the animals [59].

402 Daily arousal patterns which coincided with presence of staff and caretakers at the facility,  
403 specifically at the end of the day (last daily presentation) and early in the morning (food  
404 preparation and feeding), suggest excitement associated with these activities. Excitement is a  
405 positive arousal state [8, 60], and is less commonly studied than negative arousal. Vocal  
406 responses indicating positive and negative emotional states are hard to differentiate as they  
407 may share vocal features [61]. In the current study, signature whistle production rates  
408 increased in the morning during staff arrival and food preparation, significantly intensified  
409 during feeding and then decreased one hour after feeding and was driven by half of the  
410 individuals in the facility. At feeding time, five dolphins significantly increased signature  
411 whistle production. In wild bottlenose dolphins an increase in vocal activity is associated with  
412 foraging which may be to maintain contact or to recruit individuals [62]. Food-anticipatory  
413 activity describes increased arousal before feeding events on strict daily schedules [63] and  
414 has been observed in captive animals during scheduled feeding times [46, 64]. The emotional  
415 value of anticipatory behaviour is thought to reflect the balance of the reward system  
416 experienced by animals before and during feeding [27]. Increased vocalisations around  
417 feeding time have also been documented in captive false killer whales (*Pseudorca*

418 *crassidens*), common bottlenose dolphins (*T. truncatus*) and common dolphins (*Delphinus*  
419 *delphis*), where vocalisation rates increased upon the arrival of their caretakers and was  
420 maintained or intensified throughout feeding and decreased immediately after [48, 64]. In  
421 wild animals, detection of food may lead to an increased arousal state followed by temporary  
422 “elation” after capture of prey [8]. Other studies however observed a decrease in activity and  
423 arousal before human-animal interactions including feeding, for dolphins [4] and  
424 chimpanzees [6].

425 Of the four males in this analysis, the three younger males exhibited significant changes in  
426 most of the whistle parameters, all including a decrease in frequency range from a period  
427 with staff absent to a period with staff present. One male decreased whistle production rate  
428 when staff were present and two males exhibited significant decreases in whistle duration,  
429 one of which also decreased number of loops. The oldest male did not significantly shift any  
430 whistle parameters between these two time periods. Of the two females included in this  
431 analysis, F2 did not significantly shift any whistle parameters between these two time periods  
432 while the oldest female in the facility decreased maximum frequency and number of loops.  
433 Some dolphins may emit whistle loops faster than normal when they are excited [65]  
434 therefore duration is a function of number of loops [42]. Whistle duration has been shown to  
435 increase during isolation [42], and much like number of loops, in the context of excitement  
436 and stress, whistle duration seems to be the opposite. Whistle frequency parameters in  
437 bottlenose dolphins such as production rate and number of whistle loops are closely related to  
438 the level of arousal of an individual [42, 43]. The number of loops and whistle production  
439 rates vary by context and an increase in these parameters may indicate stress [43] or  
440 excitement [66] in bottlenose dolphins. In the current study, the whistle production rate  
441 significantly increased in six of the ten individuals, however a significant decrease in the  
442 number of loops was only exhibited by three of the six dolphins evaluated. An increase in the

443 number of loops in the context of stress [43] and a decrease in the number of loops in the  
444 context of excitement (as in the current study) suggest that loop number may be a useful tool  
445 in monitoring positive and negative arousal states in bottlenose dolphins. Although certain  
446 shifts in signature whistle parameters appear to be indicative of individual arousal,  
447 differences are not consistent across individuals [42]. The same applies to the dolphins at this  
448 facility where individuals had different combinations of whistle parameter shifts, therefore  
449 the dolphins should be individually considered and monitored.

450 In this study the dolphins were less vocal when the females were housed in the outdoor  
451 presentation pool and produced whistles less frequently when the mixed group was housed  
452 outside and produced signature whistles less frequently when the male group was housed  
453 outside and were most vocal in pool configuration 1, when they were all housed in the indoor  
454 pools (Fig 3A). This is contradictory to what you might expect from a cohesion call [39, 42]  
455 and indicates an emotive or social reaction in response to these different types of group  
456 separation. Although pool configuration affects overall whistle production rates, a vocal  
457 response to staff presence or absence is much less clear (Fig 3B) and somewhat contrary.  
458 When housed inside, human activity is in view of all dolphins, including the arrival of staff,  
459 therefore the strongest behavioural response would be expected here. Whistle production rate  
460 in response to staff presence is higher in configuration 2 for total whistles, and similar in  
461 configurations 1 and 2 for signature whistles, which is not what was expected. The social  
462 dynamics are quite different among the three groups housed at uShaka Sea World and  
463 although they are housed in separate social groups, they are always in acoustic (and mostly  
464 visual) contact with one another. This may allow inter-group social bonds to be formed,  
465 which has also been documented in wild meerkats [67] and wild bottlenose dolphins [68].  
466 Bottlenose dolphins live in complex societies and depend greatly on close social bonds [69].  
467 Because of this, they are more likely to suffer from social-related stress in both the wild and

468 captivity [70] however there is great opportunity for these animals in captivity to achieve  
469 positive welfare states due to strong social bonds within their social groups [71].

470 Whistle production rate was higher over the weekend compared to weekdays. While the  
471 number of visitors were larger on weekends than during the week, the daily schedule was the  
472 same regardless of the day of the week. Visitors to captive facilities play a role in the  
473 behavioural responses of mammals, however not all captive mammals are affected by visitor  
474 presence and noise [72]. For example, negative responses to visitors in captivity has been  
475 documented in nonhuman primates [73-76], whereas captive meerkats are behaviourally  
476 unresponsive under different intensities of visitor behaviour [77]. Bottlenose dolphins  
477 undergo activities in the form of public presentations which has been documented to elicit  
478 positive arousal rather than stress or negative arousal [4]. However, the relationship between  
479 the magnitude of arousal preceding a presentation and audience size has not yet been  
480 investigated. Increased whistle production rate over the weekend could not be directly linked  
481 to an increase in visitors as data were collected once visitors had left, however residual  
482 excitement from large crowds could have possibly been carried over into the overnight  
483 recording periods.

484 Regarding the overall signature whistle production, all square copies were removed from the  
485 analysis and in doing this, there was a possibility that signature whistles of P2 were  
486 underrepresented. Square whistle copying interactions are important in terms of social  
487 interactions and bonds between certain animals and understanding this will give insight into  
488 their social vocal behaviour as a whole. Of the ten dolphins in the study, the most vocal  
489 during both overnight and morning periods was a female, the third youngest dolphin. The  
490 least vocal was a male, the oldest dolphin at the facility. Age has been documented to affect  
491 signature whistle production rate in bottlenose dolphins where higher signature whistle rates



492 are present in younger dolphins and decreases with age, more quickly in males [42, 43].  
493 Differences in whistle production in bottlenose dolphins between sexes has previously been  
494 documented due to differences in social histories of males and females [78]. Because the  
495 whistles of hybrids M1 and M2 could not be differentiated, we could not investigate the role  
496 that age and sex play together in vocal activity of these dolphins in more detail.

497 The most vocal individuals in this study were predominantly hybrids, all of which are  
498 offspring of P1 and P2. Differences in behaviour between purebred and hybrid cetaceans has  
499 been reported [79]. Hybridisation of captive bottlenose dolphins and other dolphin species  
500 has been widely documented [58, 80-82], however hybridisation between bottlenose dolphin  
501 species in captivity has seldom been seen [see 49, 83]. Although hybridisation is naturally  
502 occurring in 10% of animal species [84], in the wild there is little overlap of home ranges of  
503 *Tusriops* spp., limiting inter-species mating opportunities of bottlenose dolphins [49].

504 Hybridisation has been documented to influence behaviour in animals; for example, neonatal  
505 hiding behaviour [85] and vocalisations [86] of hybrid deer species are strongly influenced by  
506 genetic history and display intermediate characteristics of the parent species. Similarly,  
507 intermediate behaviour of free-ranging porpoise hybrids has been documented [79]. Although  
508 not included in the analyses due to small sample sizes, species may play a role in signature  
509 whistle production of bottlenose dolphins.

510 Welfare assessments in the past were heavily focussed on monitoring behaviour indicative of  
511 poorer welfare as good welfare was thought to result from lack of suffering [87].

512 Vocalisations in farm animals have been used as a measure of welfare on farms [88]. Animals  
513 that express more arousal and anticipation for events or rewards may be in poorer welfare  
514 however some level of arousal before a positive event, such as feeding, reflects positive  
515 emotions [89]. One such emotion is excitement [8], which is the emotional state that the

516 dolphins in this facility appear to be reflecting when they increase the production rate of  
517 social sounds. Additionally, some signature whistle characteristics reflect this excitement  
518 associated with the presence of staff at the facility. Undesirable behaviour may only be  
519 present in a certain group or sex, which emphasises the limitations of a unified approach to  
520 monitoring welfare and the importance to explore individual welfare states [90]. This all  
521 provides a useful index; where production rate and whistle characteristics would usually shift  
522 within this population, an abnormal change at either a group or individual level could be  
523 indicative of a shift in welfare state.

524

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529

## 530 **References**

- 531 1. Mendl M, Burman OH, Parker RM, Paul ES. Cognitive bias as an indicator of animal  
532 emotion and welfare: Emerging evidence and underlying mechanisms. *Applied*  
533 *Animal Behaviour Science*. 2009 May 1;118(3-4):161-181.
- 534 2. Gonyou HW. Why the study of animal behavior is associated with the animal welfare  
535 issue. *Journal of Animal Science*. 1994 Aug 1;72(8):2171-2177.

- 536 3. Waitt C, Buchanan-Smith HM. What time is feeding?: How delays and anticipation of  
537 feeding schedules affect stump-tailed macaque behavior. *Applied Animal Behaviour*  
538 *Science*. 2001 Dec 13;75(1):75-85.
- 539 4. Jensen AL, Delfour F, Carter T. Anticipatory behavior in captive bottlenose dolphins  
540 (*Tursiops truncatus*): a preliminary study. *Zoo Biology*. 2013 Jul;32(4):436-444.
- 541 5. Krebs BL, Torres E, Chesney C, Kantoniemi Moon V, Watters JV. Applying  
542 behavioral conditioning to identify anticipatory behaviors. *Journal of Applied Animal*  
543 *Welfare Science*. 2017 Apr 3;20(2):155-175.
- 544 6. Bloomsmith MA, Lambeth SP. Effects of predictable versus unpredictable feeding  
545 schedules on chimpanzee behavior. *Applied Animal Behaviour Science*. 1995 Aug  
546 1;44(1):65-74.
- 547 7. Morton ES. On the occurrence and significance of motivation-structural rules in some  
548 bird and mammal sounds. *The American Naturalist*. 1977 Sep 1;111(981):855-869.
- 549 8. Mendl M, Burman OH, Paul ES. An integrative and functional framework for the  
550 study of animal emotion and mood. *Proceedings of the Royal Society B: Biological*  
551 *Sciences*. 2010 Oct 7;277(1696):2895-2904.
- 552 9. Schehka S, Esser KH, Zimmermann E. Acoustical expression of arousal in conflict  
553 situations in tree shrews (*Tupaia belangeri*). *Journal of Comparative Physiology A*.  
554 2007 Aug 1;193(8):845-852.
- 555 10. Bastian A, Schmidt S. Affect cues in vocalizations of the bat, *Megaderma lyra*, during  
556 agonistic interactions. *The Journal of the Acoustical Society of America*. 2008  
557 Jul;124(1):598-608.
- 558 11. Briefer EF. Vocal expression of emotions in mammals: mechanisms of production  
559 and evidence. *Journal of Zoology*. 2012 Sep 1;288(1):1-20.

- 560 12. Filippi P, Congdon JV, Hoang J, Bowling DL, Reber SA, Pašukonis A, et al. Humans  
561 recognize emotional arousal in vocalizations across all classes of terrestrial  
562 vertebrates: evidence for acoustic universals. *Proceedings of the Royal Society B:  
563 Biological Sciences*. 2017 Jul 26;284(1859):20170990.
- 564 13. Boissy A, Manteuffel G, Jensen MB, Moe RO, Spruijt B, Keeling LJ, et al..  
565 Assessment of positive emotions in animals to improve their welfare. *Physiology &  
566 behavior*. 2007 Oct 22;92(3):375-97.
- 567 14. Soltis J, Leong K, Savage A. African elephant vocal communication II: rumble  
568 variation reflects the individual identity and emotional state of callers. *Animal  
569 Behaviour*. 2005 Sep 1;70(3):589-599.
- 570 15. Brudzynski SM. Vocalizations as indicators of emotional states in rats and cats. In:  
571 Altenmüller E, Schmidt S, Zimmermann E, editors. *Evolution of Emotional  
572 Communication: From Sounds in Nonhuman Mammals to Speech and Music in Man*.  
573 Oxford University Press; 2013 Jan 24. p 75-91.
- 574 16. Watts JM, Stookey JM. Vocal behaviour in cattle: the animal's commentary on its  
575 biological processes and welfare. *Applied Animal Behaviour Science*. 2000 Mar  
576 22;67(1-2):15-33.
- 577 17. Castellote M, Fossa F. Measuring acoustic activity as a method to evaluate welfare in  
578 captive beluga whales (*Delphinapterus leucas*). *Aquatic Mammals*. 2006 Jul  
579 1;32(3):325.
- 580 18. Leliveld LM, Döpjan S, Tuchscherer A, Puppe B. Vocal correlates of emotional  
581 reactivity within and across contexts in domestic pigs (*Sus scrofa*). *Physiology &  
582 Behavior*. 2017 Nov 1;181:117-126.

- 583 19. Boivin X, Garel JP, Mante A, Le Neindre P. Beef calves react differently to different  
584 handlers according to the test situation and their previous interactions with their  
585 caretaker. *Applied Animal Behaviour Science*. 1998 Jan 1;55(3-4):245-257.
- 586 20. Hemsworth PH. Human–animal interactions in livestock production. *Applied Animal*  
587 *Behaviour Science*. 2003 May 2;81(3):185-198.
- 588 21. Weiss E, Wilson S. The use of classical and operant conditioning in training Aldabra  
589 tortoises (*Geochelone gigantea*) for venipuncture and other husbandry issues. *Journal*  
590 *of Applied Animal Welfare Science*. 2003 Jan 3;6(1):33-38.
- 591 22. Tanida H, Miura A, Tanaka T, Yoshimoto T. Behavioral response to humans in  
592 individually handled weanling pigs. *Applied Animal Behaviour Science*. 1995 Mar  
593 1;42(4):249-259.
- 594 23. Jones RB. Fear and adaptability in poultry: insights, implications and imperatives.  
595 *World's Poultry Science Journal*. 1996 Jul 1;52(2):131-174.
- 596 24. Peters SM, Bleijenberg EH, van Dierendonck MC, van der Harst JE, Spruijt BM.  
597 Characterization of anticipatory behaviour in domesticated horses (*Equus caballus*).  
598 *Applied Animal Behaviour Science*. 2012 Apr 1;138(1-2):60-69.
- 599 25. Jago JG, Krohn CC, Matthews LR. The influence of feeding and handling on the  
600 development of the human–animal interactions in young cattle. *Applied Animal*  
601 *Behaviour Science*. 1999 Feb 15;62(2-3):137-151.
- 602 26. Watters JV. Searching for behavioral indicators of welfare in zoos: Uncovering  
603 anticipatory behavior. *Zoo Biology*. 2014 Jul;33(4):251-256.
- 604 27. Spruijt BM, van den Bos R, Pijlman FT. A concept of welfare based on reward  
605 evaluating mechanisms in the brain: anticipatory behaviour as an indicator for the  
606 state of reward systems. *Applied Animal Behaviour Science*. 2001 Apr 26;72(2):145-  
607 171.

- 608 28. van der Harst JE, Baars AM, Spruijt BM. Standard housed rats are more sensitive to  
609 rewards than enriched housed rats as reflected by their anticipatory behaviour.  
610 Behavioural Brain Research. 2003 Jun 16;142(1-2):151-156.
- 611 29. Nilsson J, Kristiansen TS, Fosseidengen JE, Stien LH, Fernö A, van den Bos R.  
612 Learning and anticipatory behaviour in a “sit-and-wait” predator: The Atlantic  
613 halibut. Behavioural Processes. 2010 Mar 1;83(3):257-266.
- 614 30. van den Bos R, Meijer MK, van Renselaar JP, van der Harst JE, Spruijt BM.  
615 Anticipation is differently expressed in rats (*Rattus norvegicus*) and domestic cats  
616 (*Felis silvestris catus*) in the same Pavlovian conditioning paradigm. Behavioural  
617 Brain Research. 2003 Apr 17;141(1):83-89.
- 618 31. van der Harst JE, Fermont PC, Bilstra AE, Spruijt BM. Access to enriched housing is  
619 rewarding to rats as reflected by their anticipatory behaviour. Animal Behaviour.  
620 2003 Sep 1;66(3):493-504.
- 621 32. Puppe B, Ernst K, Schön PC, Manteuffel G. Cognitive enrichment affects behavioural  
622 reactivity in domestic pigs. Applied Animal Behaviour Science. 2007 Jun 1;105(1-  
623 3):75-86.
- 624 33. Bassett L, Buchanan-Smith HM. Effects of predictability on the welfare of captive  
625 animals. Applied Animal Behaviour Science. 2007 Feb 1;102(3-4):223-245.
- 626 34. Herman LM. Intelligence and rational behaviour in the bottlenosed dolphin. In:  
627 Hurley S, Nudds M, editors. Rational animals? Oxford: Oxford University Press.  
628 2006. p 439-468.
- 629 35. Jones GJ, Sayigh LS. Geographic variation in rates of vocal production of  
630 free-ranging bottlenose dolphins. Marine Mammal Science. 2002 Apr;18(2):374-393.
- 631 36. Cook ML, Sayigh LS, Blum JE, Wells RS. Signature-whistle production in  
632 undisturbed free-ranging bottlenose dolphins (*Tursiops truncatus*). Proceedings of the

- 633 Royal Society of London. Series B: Biological Sciences. 2004 May  
634 22;271(1543):1043-1049.
- 635 37. Sayigh LS, Tyack PL, Wells RS, Scott MD. Signature whistles of free-ranging  
636 bottlenose dolphins *Tursiops truncatus*: stability and mother-offspring comparisons.  
637 Behavioral Ecology and Sociobiology. 1990 Apr;26(4):247-260.
- 638 38. Janik VM, Todt D, Dehnhardt G. Signature whistle variations in a bottlenosed  
639 dolphin, *Tursiops truncatus*. Behavioral Ecology and Sociobiology. 1994  
640 Oct;35(4):243-248.
- 641 39. Janik VM, Slater PJ. Context-specific use suggests that bottlenose dolphin signature  
642 whistles are cohesion calls. Animal Behaviour. 1998 Oct 1;56(4):829-838.
- 643 40. King SL, Sayigh LS, Wells RS, Fellner W, Janik VM. Vocal copying of individually  
644 distinctive signature whistles in bottlenose dolphins. Proceedings of the Royal Society  
645 B: Biological Sciences. 2013 Apr 22;280(1757):20130053.
- 646 41. Janik VM, King SL, Sayigh LS, Wells RS. Identifying signature whistles from  
647 recordings of groups of unrestrained bottlenose dolphins (*Tursiops truncatus*). Marine  
648 Mammal Science. 2013 Jan;29(1):109-122.
- 649 42. Caldwell MC, Caldwell DK, Tyack PL. Review of the signature-whistle hypothesis  
650 for the Atlantic bottlenose dolphin. In: Leatherwood S, Reeves RR, editors. The  
651 Bottlenose Dolphin; 1990. P 1999-1234
- 652 43. Esch HC, Sayigh LS, Blum JE, Wells RS. Whistles as potential indicators of stress in  
653 bottlenose dolphins (*Tursiops truncatus*). Journal of Mammalogy. 2009 Jun  
654 2;90(3):638-650.
- 655 44. Clegg IL, Rödel HG, Cellier M, Vink D, Michaud I, Mercera B, et al. Schedule of  
656 human-controlled periods structures bottlenose dolphin (*Tursiops truncatus*) behavior  
657 in their free-time. Journal of Comparative Psychology. 2017 Aug;131(3):214.

- 658 45. Clegg IL, Rödel HG, Boivin X, Delfour F. Looking forward to interacting with their  
659 caretakers: dolphins' anticipatory behaviour indicates motivation to participate in  
660 specific events. *Applied Animal Behaviour Science*. 2018 May 1;202:85-93.
- 661 46. Therrien SC, Thomas JA, Therrien RE, Stacey R. Time of Day and Social Change  
662 Affect Underwater Sound Production by Bottlenose Dolphins (*Tursiops truncatus*) at  
663 the Brookfield Zoo. *Aquatic Mammals*. 2012 Jan 1;38(1).
- 664 47. Kremers D, Briseño-Jaramillo M, Böye M, Lemasson A, Hausberger M. Nocturnal  
665 vocal activity in captive bottlenose dolphins (*Tursiops truncatus*): could dolphins have  
666 presleep choruses. *Animal Behaviour and Cognition*. 2014 Nov;1:464-469.
- 667 48. Platto S, Wang D, Wang K. Variation in the emission rate of sounds in a captive  
668 group of false killer whales *Pseudorca crassidens* during feedings: possible food  
669 anticipatory vocal activity?. *Chinese Journal of Oceanology and Limnology*. 2016  
670 Nov;34(6):1218-1237.
- 671 49. Gridley T, Elwen SH, Harris G, Moore DM, Hoelzel AR, Lampen F. Hybridization in  
672 bottlenose dolphins—A case study of *Tursiops aduncus* × *T. truncatus* hybrids and  
673 successful backcross hybridization events. *PloS one*. 2018 Sep 12;13(9):e0201722.
- 674 50. Gridley T, Berggren P, Cockcroft VG, Janik VM. Whistle vocalizations of Indo-  
675 Pacific bottlenose dolphins (*Tursiops aduncus*) inhabiting the south-west Indian  
676 Ocean. *The Journal of the Acoustical Society of America*. 2012 Dec;132(6):4032-  
677 4040.
- 678 51. Janik VM. Food-related bray calls in wild bottlenose dolphins (*Tursiops truncatus*).  
679 *Proceedings of the Royal Society of London. Series B: Biological Sciences*. 2000  
680 May 7;267(1446):923-927.
- 681 52. King SL, Harley HE, Janik VM. The role of signature whistle matching in bottlenose  
682 dolphins, *Tursiops truncatus*. *Animal Behaviour*. 2014 Oct 1;96:79-86.



- 683 53. Kriesell HJ, Elwen SH, Nastasi A, Gridley T. Identification and characteristics of  
684 signature whistles in wild bottlenose dolphins (*Tursiops truncatus*) from Namibia.  
685 PloS one. 2014 Sep 9;9(9):e106317.
- 686 54. Wood S, Scheipl F. gamm4: Generalized Additive Mixed Models using 'mgcv' and  
687 'lme4'. R package version 0.2-6. <https://CRAN.R-project.org/package=gamm4>. 2020.
- 688 55. R Core Team. R: A language environment for statistical computing. R Foundation for  
689 Statistical Computing, Vienna, Austria. <https://www.R-project.org/>. 2020.
- 690 56. Center for Conservation Bioacoustics. Raven Pro: Interactive Sound Analysis  
691 Software (Version 1.6.1) [Computer software]. Ithica, NY: The Cornell Lab of  
692 Ornithology. <http://ravensoundsoftware.com/>. 2019.
- 693 57. Powell BA. Periodicity of vocal activity of captive Atlantic bottlenose dolphins:  
694 *Tursiops truncatus*. Bulletin of the Southern California Academy of Sciences.  
695 2020;65(4):237-244.
- 696 58. Sekiguchi Y, Kohshima S. Resting behaviors of captive bottlenose dolphins (*Tursiops*  
697 *truncatus*). Physiology & Behavior. 2003 Sep 1;79(4-5):643-653.
- 698 59. Waitt C, Buchanan-Smith HM, Morris K. The effects of caretaker-primate  
699 relationships on primates in the laboratory. Journal of Applied Animal Welfare  
700 Science. 2002 Oct 1;5(4):309-319.
- 701 60. Neal SJ, Caine NG. Scratching under positive and negative arousal in common  
702 marmosets (*Callithrix jacchus*). American Journal of Primatology. 2016  
703 Feb;78(2):216-226.
- 704 61. Trut L, Kharlamova A, Gogoleva S, Volodina E, Volodin I. Sign and strength of  
705 emotional arousal: vocal correlates of positive and negative attitudes to humans in  
706 silver foxes (*Vulpes vulpes*). Behaviour. 2010 Jan 1;147(13-14):1713-1736.

- 707 62. Acevedo-Gutiérrez A, Stienessen SC. Bottlenose dolphins (*Tursiops truncatus*)  
708 increase number of whistles when feeding. *Aquatic Mammals*. 2004 Dec;30(3):357-  
709 362.
- 710 63. Mistlberger RE. Circadian food-anticipatory activity: formal models and  
711 physiological mechanisms. *Neuroscience & Biobehavioral Reviews*. 1994 Jun  
712 1;18(2):171-95.
- 713 64. Moore SE, Ridgway SH. Patterns of dolphin sound production and ovulation. *Aquatic*  
714 *Mammals*. 1996;22:175-184.
- 715 65. Wood FG, Caldwell MC, Caldwell DK. Behavioral interactions between porpoises  
716 and sharks. *Marineland Research Laboratory*; 1971.
- 717 66. Scarpaci C, Bigger SW, Corkeron PJ, Nugegoda D. Bottlenose dolphins (*Tursiops*  
718 *truncatus*) increase whistling in the presence of swim-with-dolphin'tour operations.  
719 *Journal of Cetacean Research and Management*. 2000;2(3):183-5.
- 720 67. Drewe JA, Madden JR, Pearce GP. The social network structure of a wild meerkat  
721 population: 1. Inter-group interactions. *Behavioral Ecology and Sociobiology*. 2009  
722 Jul;63(9):1295-1306.
- 723 68. Connor RC. Dolphin social intelligence: complex alliance relationships in bottlenose  
724 dolphins and a consideration of selective environments for extreme brain size  
725 evolution in mammals. *Philosophical Transactions of the Royal Society B: Biological*  
726 *Sciences*. 2007 Apr 29;362(1480):587-602.
- 727 69. Wells RS. The role of long-term study in understanding the social structure of a  
728 bottlenose dolphin community. In: Pryor K, Norris KS, editors. *Dolphin societies:*  
729 *Discoveries and puzzles*. University of California Press; 1991. p 199-225.

- 730 70. Waples KA, Gales NJ. Evaluating and minimising social stress in the care of captive  
731 bottlenose dolphins (*Tursiops aduncus*). *Zoo Biology*: Published in affiliation with the  
732 American Zoo and Aquarium Association. 2002;21(1):5-26.
- 733 71. Clegg IL, Van Elk CE, Delfour F. Applying welfare science to bottlenose dolphins  
734 (*Tursiops truncatus*). *Animal Welfare*. 2017 May 1;26:165-176.
- 735 72. Quadros S, Goulart VD, Passos L, Vecci MA, Young RJ. Zoo visitor effect on  
736 mammal behaviour: Does noise matter?. *Applied Animal Behaviour Science*. 2014 Jul  
737 1;156:78-84.
- 738 73. Chamove AS, Hosey GR, Schaezel P. Visitors excite primates in zoos. *Zoo biology*.  
739 1988;7(4):359-369.
- 740 74. Simpson L. The effect of visitors on captive non-human primates. *Zoo Federation*  
741 *Research Newsletter*. 2004;5(3):6.
- 742 75. Davis N, Schaffner CM, Smith TE. Evidence that zoo visitors influence HPA activity  
743 in spider monkeys (*Ateles geoffroyii rufiventris*). *Applied Animal Behaviour Science*.  
744 2005 Feb 1;90(2):131-141.
- 745 76. Wells DL. A note on the influence of visitors on the behaviour and welfare of zoo-  
746 housed gorillas. *Applied Animal Behaviour Science*. 2005 Sep 1;93(1-2):13-17.
- 747 77. Sherwen SL, Magrath MJ, Butler KL, Phillips CJ, Hemsworth PH. A multi-enclosure  
748 study investigating the behavioural response of meerkats to zoo visitors. *Applied*  
749 *Animal Behaviour Science*. 2014 Jul 1;156:70-77.
- 750 78. Watwood SL, Tyack PL, Wells RS. Whistle sharing in paired male bottlenose  
751 dolphins, *Tursiops truncatus*. *Behavioral Ecology and Sociobiology*. 2004  
752 Apr;55(6):531-543.
- 753 79. Willis PM, Crespi BJ, Dill LM, Baird RW, Hanson MB. Natural hybridization  
754 between Dall's porpoises (*Phocoenoides dalli*) and harbour porpoises (*Phocoena*

- 755 phocoena). Canadian Journal of Zoology. 2004 May 1;82(5):828-834.
- 756 80. Zornetzer HR, Duffield DA. Captive-born bottlenose dolphin× common dolphin  
757 (Tursiops truncatus× Delphinus capensis) intergeneric hybrids. Canadian Journal of  
758 Zoology. 2003 Oct 1;81(10):1755-1762.
- 759 81. Caballero S, Baker CS. Captive-born intergeneric hybrid of a Guiana and bottlenose  
760 dolphin: Sotalia guianensis× Tursiops truncatus. Zoo Biology. 2010 Sep;29(5):647-  
761 657.
- 762 82. Zhang P, Han J, Lu Z, Chen R. Molecular Evidence of a Captive-Born Intergeneric  
763 Hybridization Between Bottlenose and Risso's Dolphins: Tursiops truncatus×  
764 Grampus griseus. Aquatic Mammals. 2014;40(1):5.
- 765 83. Lima N, Rogers T, Acevedo-Whitehouse K, Brown MV. Temporal stability and  
766 species specificity in bacteria associated with the bottlenose dolphins respiratory  
767 system. Environmental Microbiology Reports. 2012 Feb;4(1):89-96.
- 768 84. Iacolina L, Corlatti L, Buzan E, Safner T, Šprem N. Hybridisation in European  
769 ungulates: an overview of the current status, causes, and consequences. Mammal  
770 Review. 2019 Jan;49(1):45-59.
- 771 85. Endicott-Davies DR, Barrie AN, Fisher MW. Differences in the hiding behaviour of  
772 new-born red deer and hybrid 1/4 Père David's× 3/4 red deer calves. Animal Science.  
773 1996 Apr;62(2):363-367.
- 774 86. Long AM, Moore NP, Hayden TJ. Vocalizations in red deer (*Cervus elaphus*), sika  
775 deer (*Cervus nippon*), and red× sika hybrids. Journal of Zoology. 1998  
776 Jan;244(1):123-134.
- 777 87. Broom DM, Johnson KG, Broom DM. Stress and animal welfare. London: Chapman  
778 & Hall; 1993 Nov 30.

779 88. Manteuffel G, Puppe B, Schön PC. Vocalization of farm animals as a measure of  
780 welfare. *Applied Animal Behaviour Science*. 2004 Sep 1;88(1-2):163-82.

781 89. Clegg IL, Delfour F. Cognitive judgement bias is associated with frequency of  
782 anticipatory behavior in bottlenose dolphins. *Zoo biology*. 2018 Mar;37(2):67-73.

783 90. Stoinski TS, Jaicks HF, Drayton LA. Visitor effects on the behavior of captive  
784 western lowland gorillas: The importance of individual differences in examining  
785 welfare. *Zoo Biology*. 2012 Sep;31(5):586-599.

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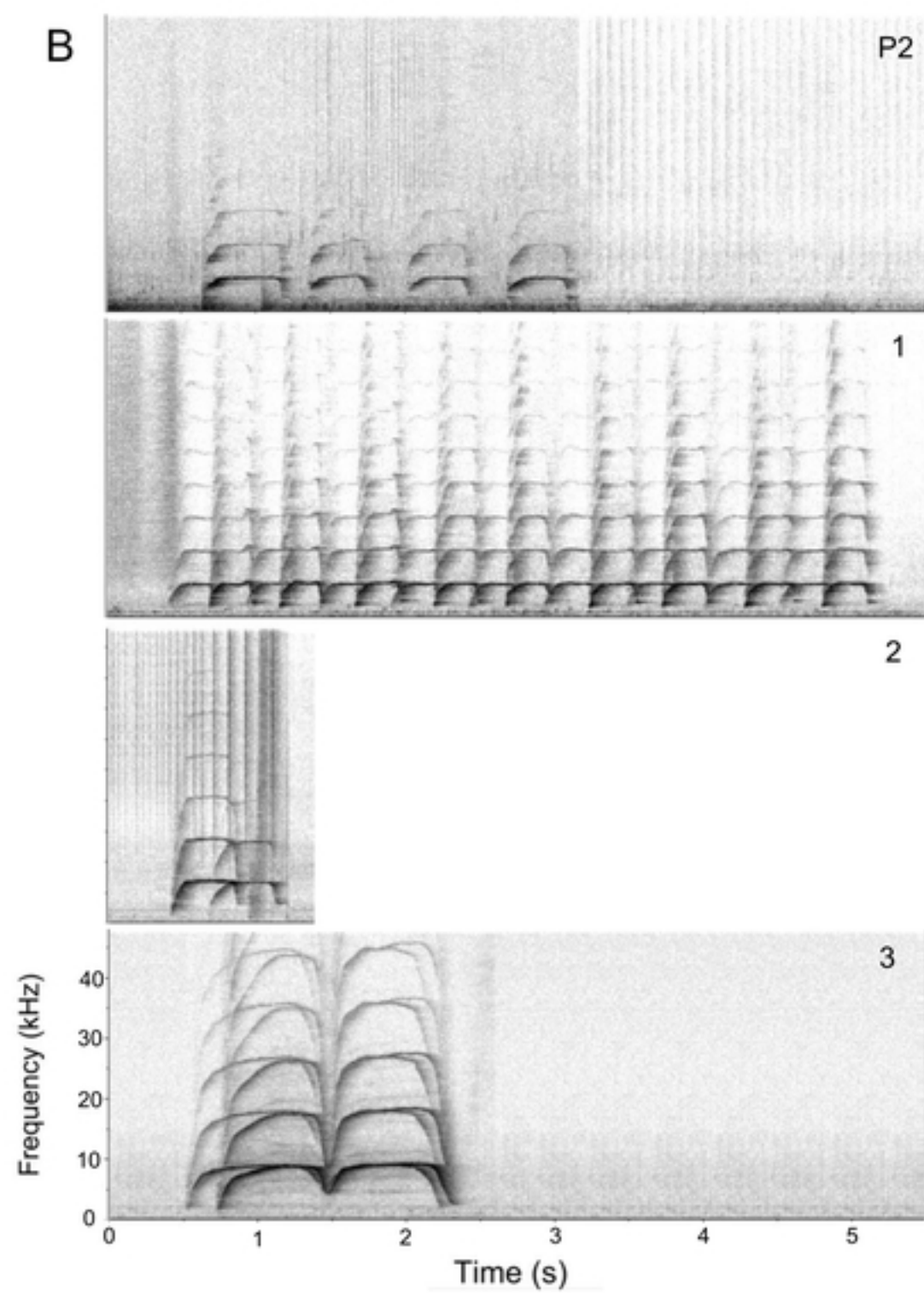
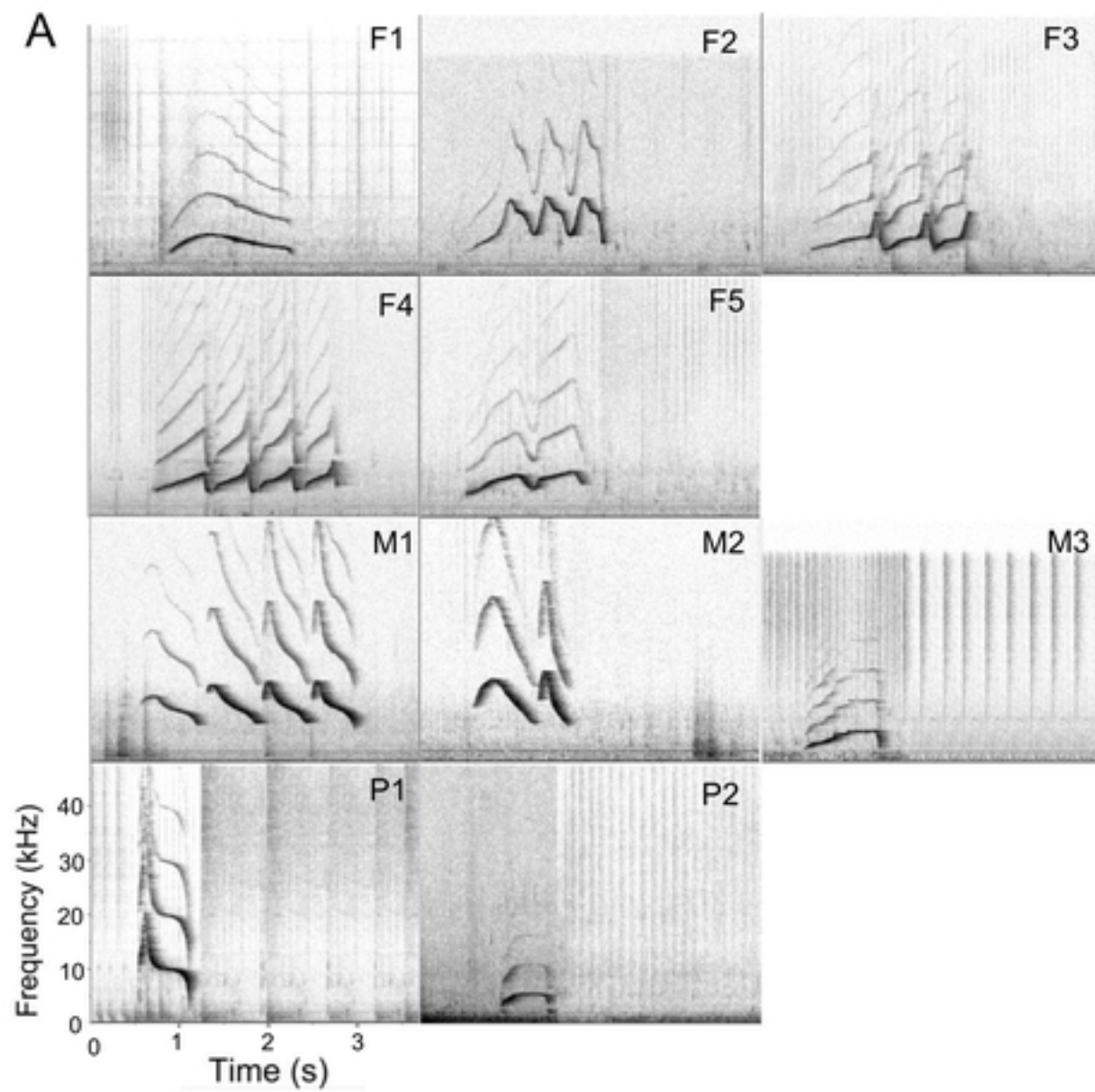
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## 790 **Supporting information**

791 **S1 Fig. Total number of square whistle copying events during each time bin throughout**  
792 **the study period.**

793 **S2 Fig. Total whistle counts, as well as P2 as the initiator of the copying events (Kriesell**  
794 **et al., 2014).**



**Fig 1**

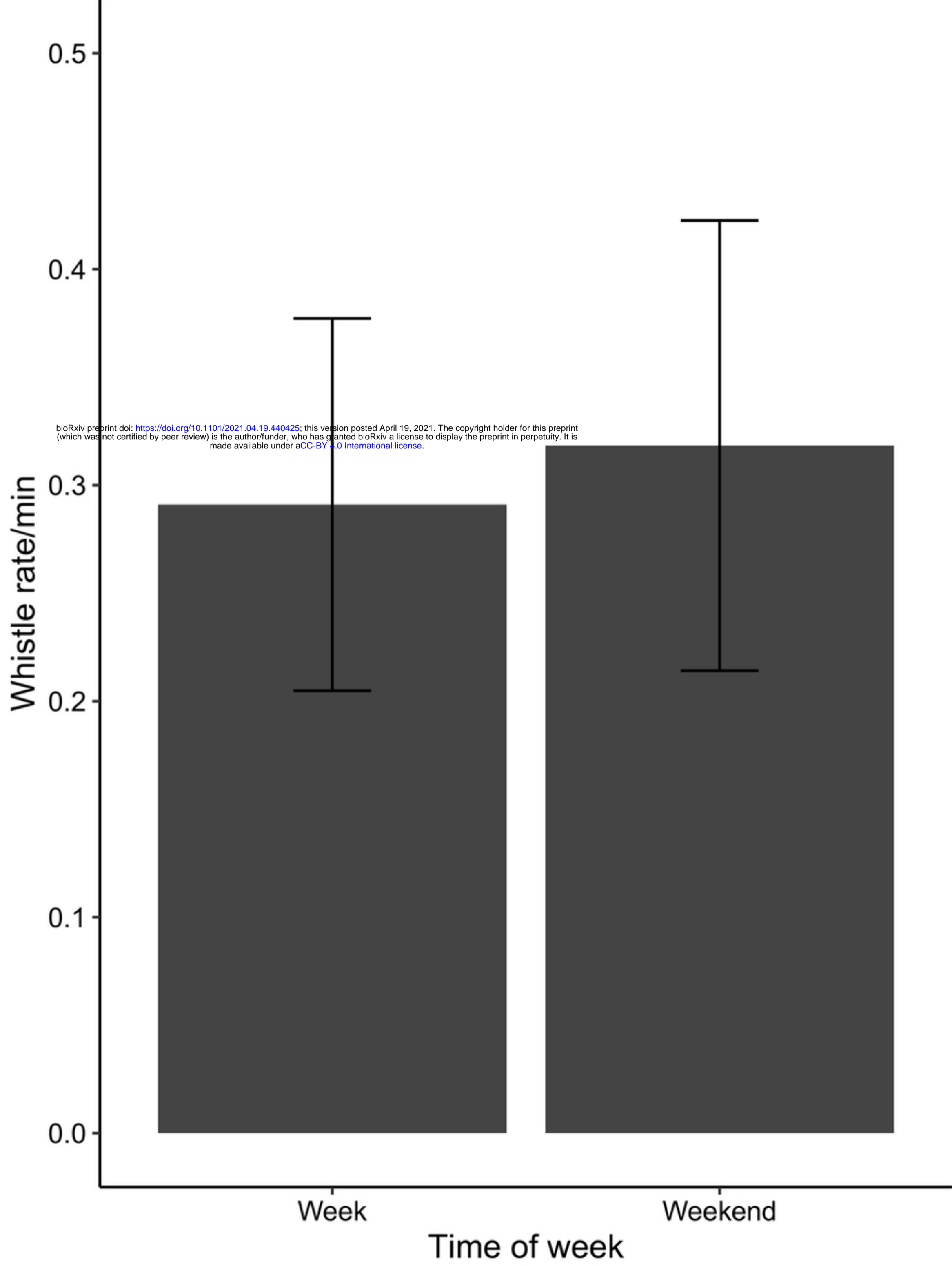


Fig 4

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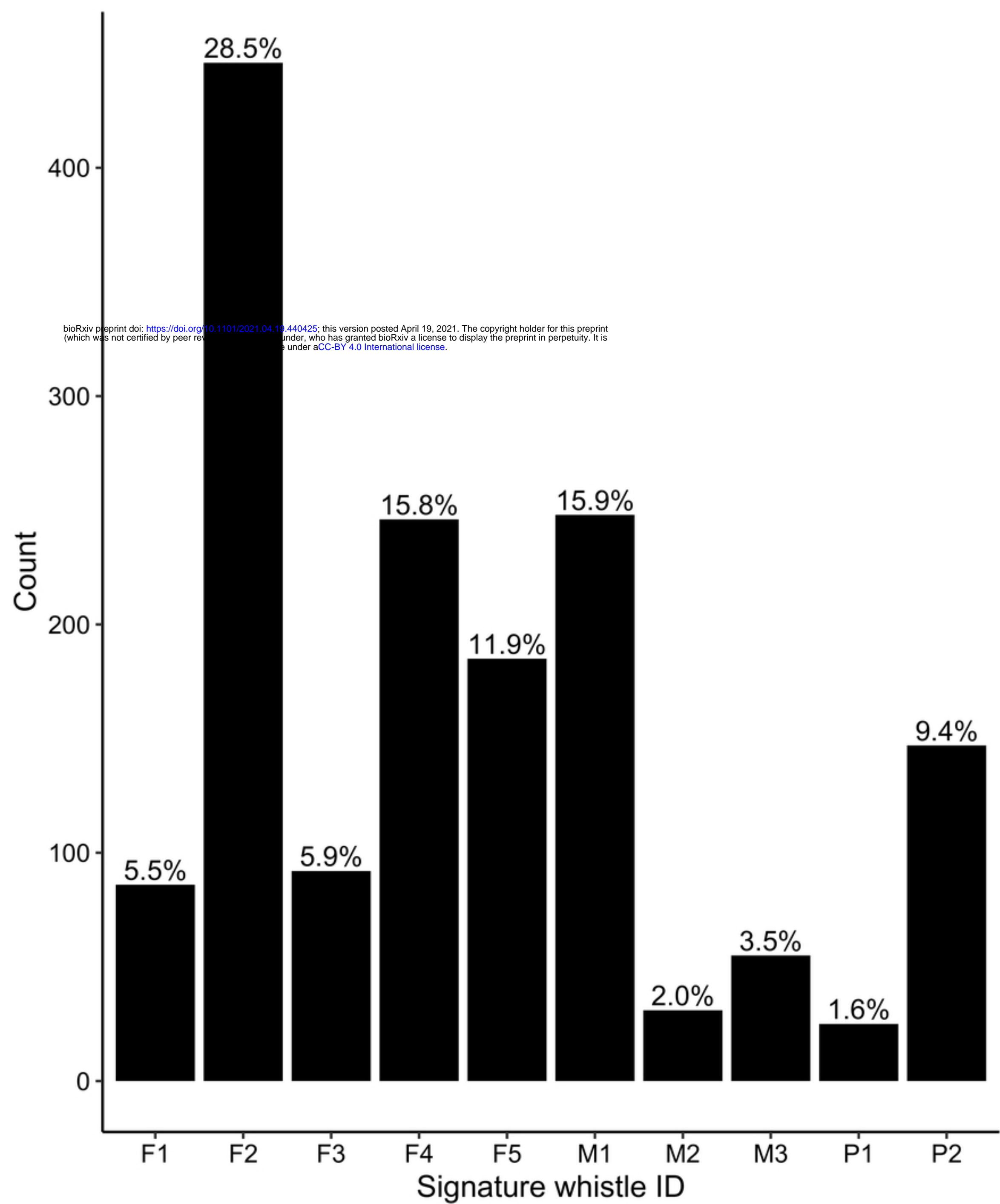


Fig 5



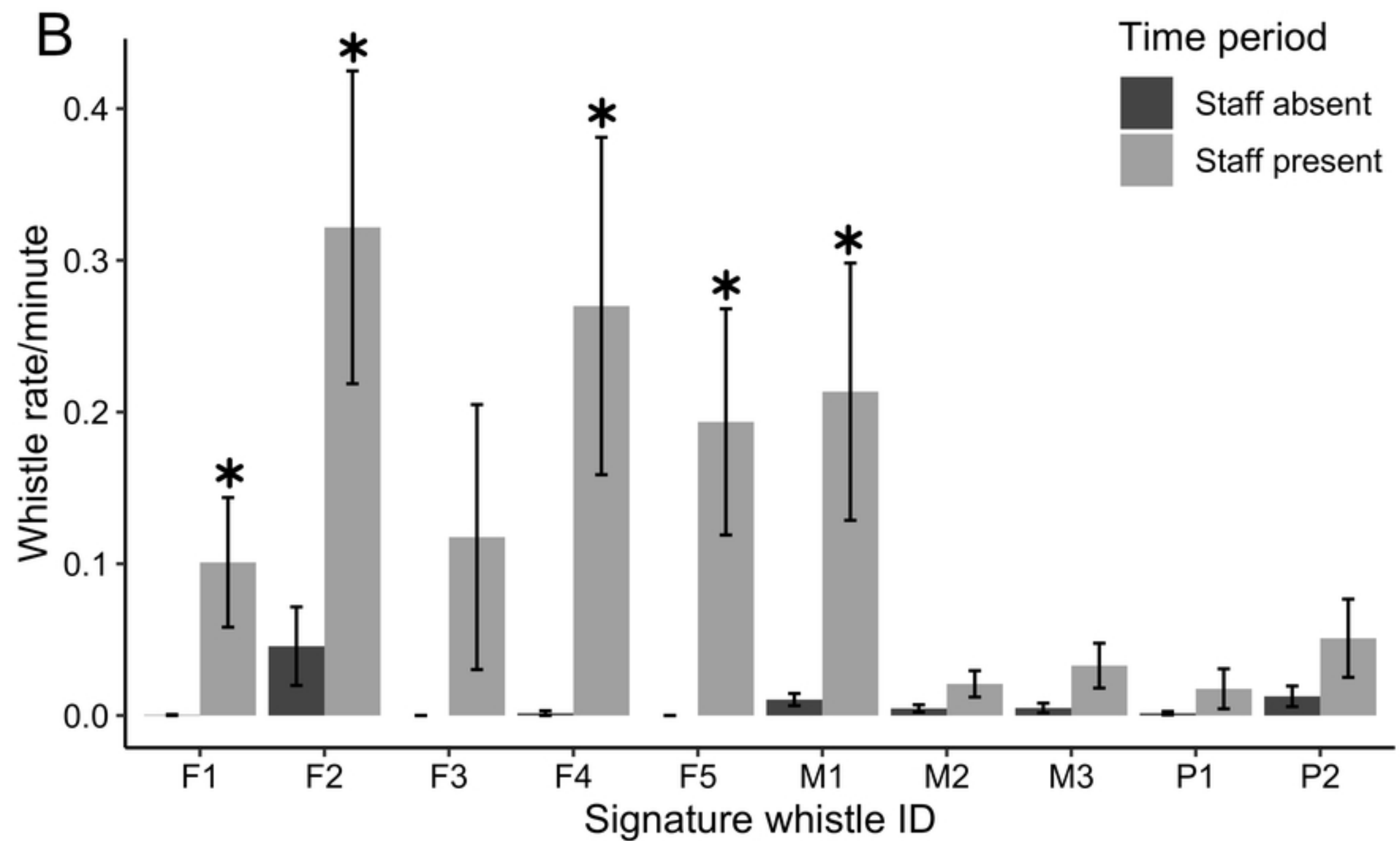
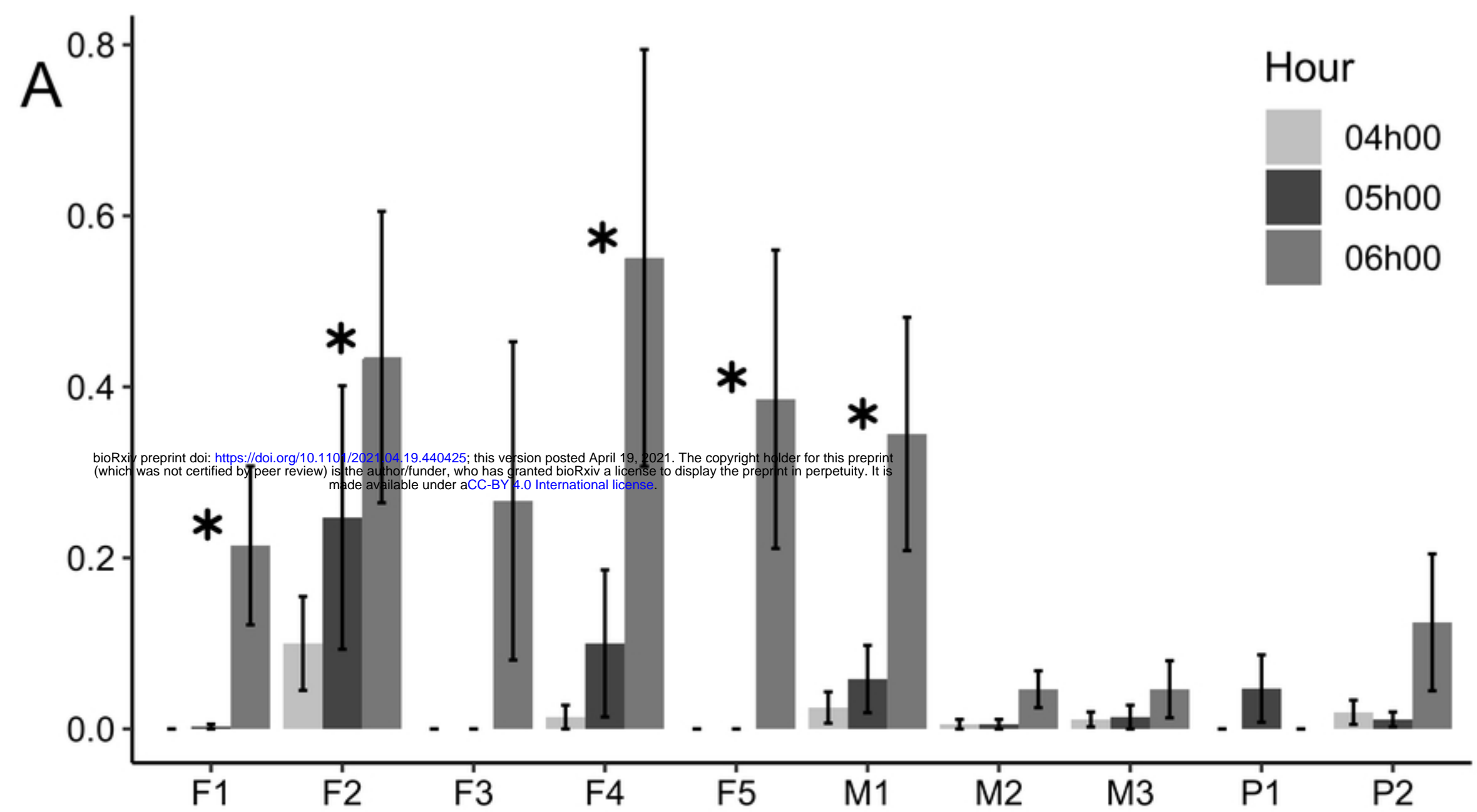


Fig 6

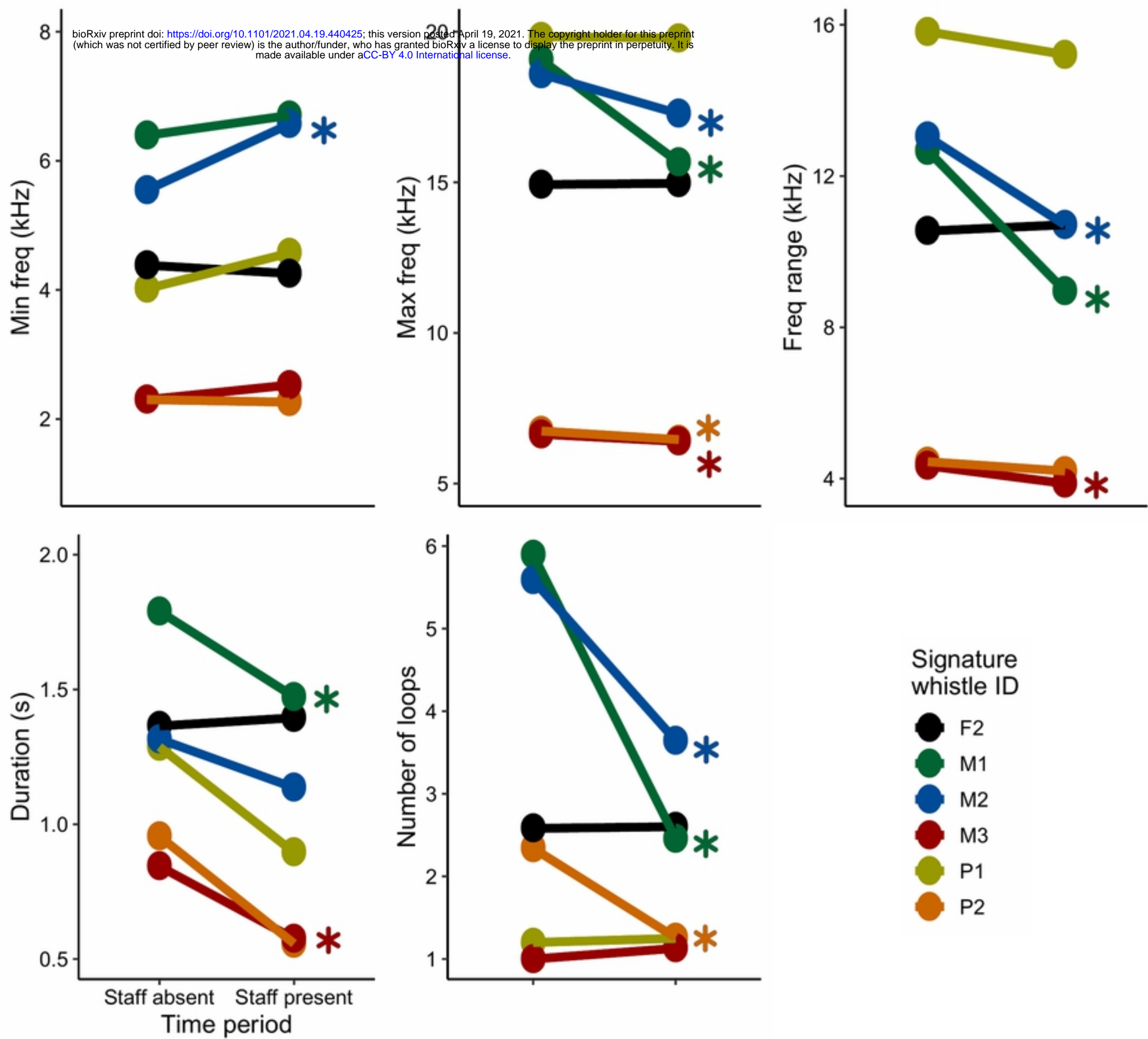


Fig 7

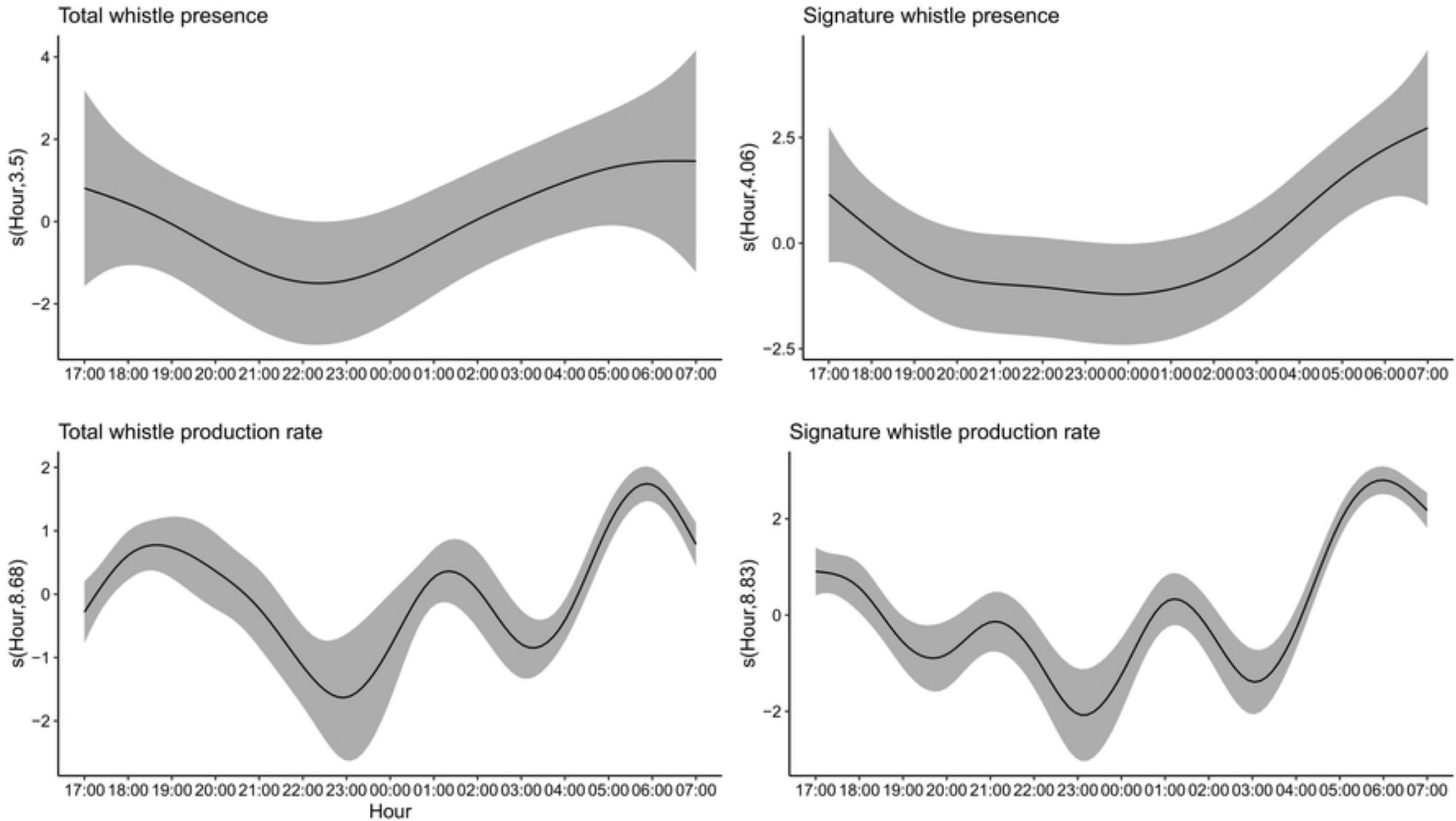


Fig 2

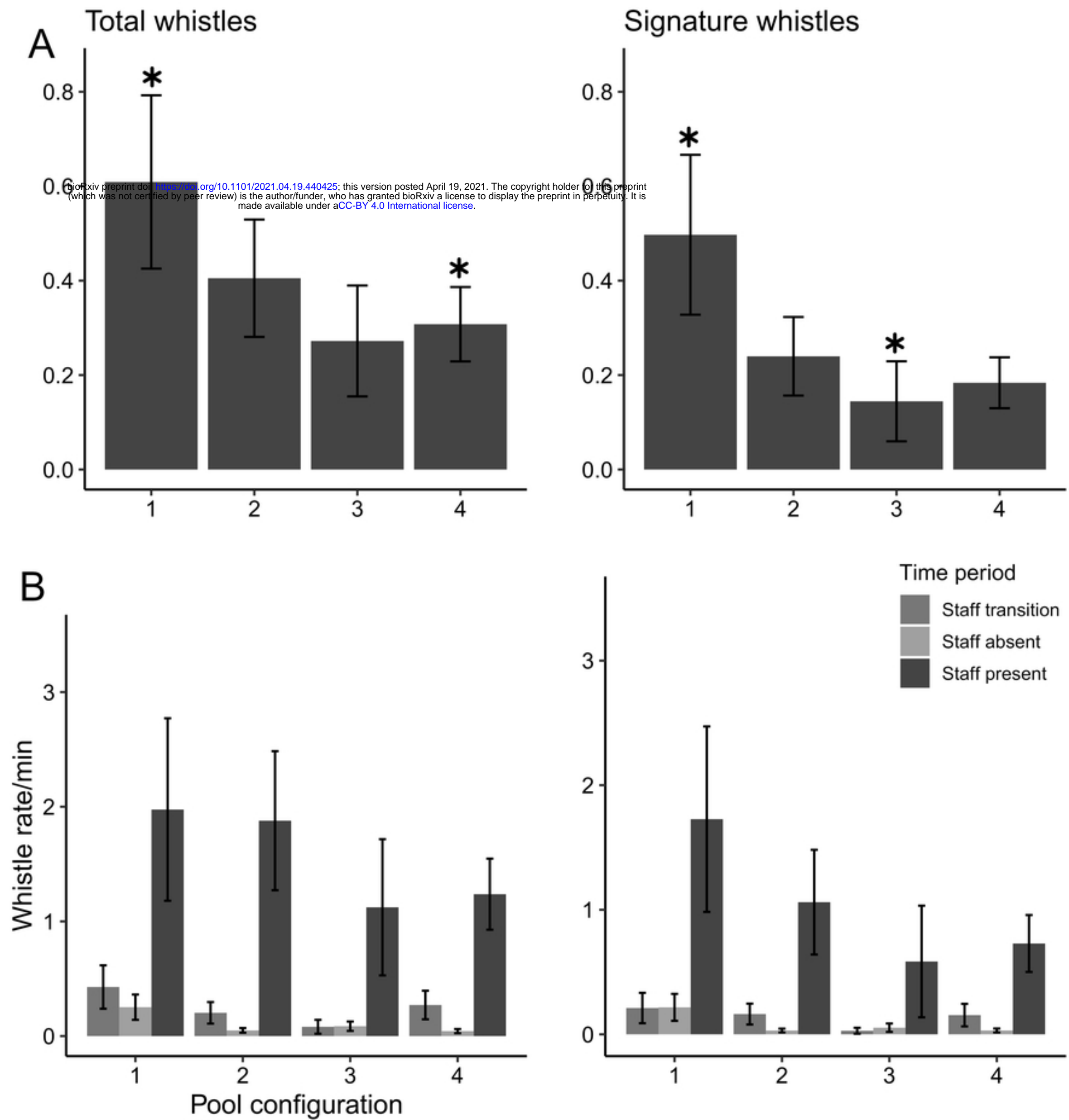


Fig 3