

Social context and prey composition are associated with calling behaviour in a diving seabird

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Social cohesion and prey location in seabirds are largely enabled through visual and olfactory signals, but these behavioural aspects could potentially also be enhanced through acoustic transfer of information. Should this be the case, calling behaviour could be influenced by different social–ecological stimuli. African Penguins *Spheniscus demersus* were equipped with animal-borne video recorders to determine whether the frequency and types of calls emitted at sea were dependent on behavioural modes (commuting, sedentary and dive bout) and social status (solitary vs. group). For foraging dive bouts we assessed whether the timing and frequency of calls were significantly different in the presence of schooling prey vs. single fish. The probability of call events was significantly more likely for birds commuting early and late in the day (for solitary birds) and during dive bouts (for groups). During foraging dive bouts the frequency of calls was significantly greater for birds diving in the presence of schooling fish and birds called sooner after a catch in these foraging scenarios compared with when only single fish were encountered. Three call types were recorded, 'flat', 'modulated' and 'two-voice' calls, but there was no significant relationship detected with these call types and behavioural modes for solitary birds and birds in groups. The results of this study show that acoustic signalling by African Penguins at sea is used in a variety of behavioural contexts and that increased calling activity in the presence of more profitable prey could be of crucial importance to seabirds that benefit from group foraging.

Keywords: acoustic communication, at-sea behaviour, food-associated calls, group hunting, penguins, schooling fish.

Acoustic communication in birds has been studied extensively for songbirds (Oscines) and to a lesser extent for non-passerine species with typically more limited call repertoires (Kroodsma & Miller 1996, Bretagnolle *et al.* 1998). For seabirds, there is a significant body of research on calling behaviour at their colonies, where acoustic signalling plays a crucial role in mate and parent/offspring

recognition and in nest defence (Nelson & Baird 2001, Aubin & Jouventin 2002, Curé *et al.* 2012, Dentressangle *et al.* 2012, Jouventin & Dobson 2017). Much less is known about the calling behaviour of seabirds away from their colonies and only recently have studies shown the importance of this behaviour in the formation of groups at sea and in the coordination of foraging events (Thiebault *et al.* 2016, 2019, Choi *et al.* 2017).

Away from the colony, the marine environment poses several challenges to seabirds that search for

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favourable foraging opportunities in an often patchy and unpredictable preyscape (Hunt *et al.* 1999). Visual communication in the form of local enhancement can benefit flocking seabirds by drawing individuals' attention to patchy resources (Thiebault *et al.* 2014, Bairos-Novak *et al.* 2015). For non-flighted species, such as penguins, the benefits of local enhancement are reduced due to their visual perspective being limited to the field of view at the sea surface (Haney *et al.* 1992) and their inability to traverse large areas with the aid of flight. Despite this, penguins can effectively track the distribution of their patchy prey (Carroll *et al.* 2017) and, although this is partly achieved through olfaction based on indirect signals of prey (Culik *et al.* 2000, Culik 2001, Wright *et al.* 2011), it is likely that this ability is also facilitated through other sensory modalities.

Many penguin species associate with conspecifics at sea in a variety of behavioural contexts, including diving (both searching and foraging), commuting, preening and resting (Siegfried *et al.* 1975, Tremblay & Cherel 1999, Takahashi *et al.* 2004, Pütz & Cherel 2005, Berlincourt & Arnould 2014, Sutton *et al.* 2015). The nature of these group formations, at least in some species, is often transitory, with group size varying according to different behavioural states (Wilson & Wilson 1990, Berlincourt & Arnould 2014, McInnes *et al.* 2017). Penguins are known to use calls to communicate at sea (Stonehouse 1967, Ainley 1972, Siegfried *et al.* 1975) but the behavioural context of these vocalizations remains little explored (Choi *et al.* 2017, Thiebault *et al.* 2019).

African Penguins *Spheniscus demersus* are specialist foragers, predominantly feeding on small schooling 'bait fish' such as sardine *Sardinops sagax* and anchovy *Engraulis encrasicolus*, the most predominant mid-trophic species in the Benguela upwelling ecosystem (Wilson 1985, Cury *et al.* 2000, Crawford *et al.* 2011). Vocalizations of this species at sea have been conjectured to be associated with foraging opportunities and anti-predator behaviour, such as porpoising (Davies 1956, Siegfried *et al.* 1975). African Penguins engage in group foraging, herding schooling fish into aggregations that improve foraging efficiency (Ryan *et al.* 2012, McInnes *et al.* 2017). Fish-herding behaviour is extremely rare among birds (exceptions include American White Pelicans *Pelecanus erythrorhynchos*, McMahon & Evans 1992) but is commonly utilized as a foraging strategy by

delphinids (Leatherwood 1975, Gallo-Reynoso 1991, Similä 1997, Benoit-Bird & Au 2009). For example, Bottlenose Dolphins *Tursiops truncatus* emit food-associated calls that influence the behaviour of conspecifics (King & Janik 2015). Surface calling by foraging penguins could be used to fulfil a similar function by recruiting conspecifics in the presence of schooling prey. This is expected to be of importance to species such as African Penguins that benefit from group foraging on schooling fish.

We set out to answer three questions related to the functions of acoustic signalling in African Penguins with the aid of animal-borne video recorder (AVR) technology: (1) Does the vocal behaviour of penguins at sea significantly vary in timing and frequency depending on their behavioural (commuting, foraging or sedentary) and social contexts (presence vs. absence of conspecifics)? (2) If we identify different acoustic structures in the calls, do they relate to specific behaviours? (3) When foraging, does the vocal behaviour of penguins differ significantly depending on the type of prey (single vs. more profitable schooling prey)?

METHODS

Deployment procedures

African Penguins at Stony Point, South Africa (34°22'22"S, 18°53'42"E), were fitted with AVRs during the guard phase of three breeding seasons (June to August) between 2015 and 2017. Two types of AVRs were used: Replay XD 720 (<http://www.replayxd.com>) housed in aluminium tubes (total dimensions: length × proximal diameter × distal diameter, weight: 104 × 26 × 28 mm, 100 g, i.e. <3% of the average mass of an adult penguin) for 2015 and 2016 deployments, and Replay XD 1080 Mini housed in smaller aluminium tubes (total dimensions: 94 × 28 × 23 mm, 65 g) for 2017 deployments. All observations recorded from the AVRs were limited by an angle of view of *c.* 110°. The AVR casings were all pressure-tested in a custom-built chamber to confirm their performance rating, and a few casings were tested to destruction to confirm the design safety factor. During 2017 birds were additionally fitted with depth loggers (Axydepth, TechnoSmart, Rome, Italy; dimensions: length × width × height, weight: 35 × 14 × 10 mm, 6.5 g). Devices were attached to the lower backs of African Penguins with Tesa tape 4651 (Beiersdorf AG, Hamburg, Germany)

during the late afternoon preceding a foraging trip and were removed once the birds had returned to their nests and had enough time to provision their chicks. All instrumented birds were weighed before and after deployment of the devices, and culmen and bill depth were measured to estimate the sex of each individual (Pichegru *et al.* 2013). AVRs were programmed to record 35-min bins (2015 and 2016) and 15-min bins (2017) with the initial bin set for approximately sunrise and subsequent bins programmed to initiate later in the day. Previous examination of device effects on African Penguins with the heavier AVRs during 2015 and 2016 showed no adverse effects on body condition, with the majority (83%) of equipped birds having gained weight on their return to the colony (McInnes *et al.* 2017).

Quantifying at-sea behaviours

The raw footage of the AVRs was analysed in VLC media player (VideoLAN, Paris, France), allocating time-stamps to all surface and dive events. At-sea behaviour was classified into three behavioural states: commuting, sedentary (resting or preening at the surface) and dive bouts. Estimates of dive depths of birds deployed without depth loggers during 2015 and 2016 were calculated using known descent rates of African Penguins (1.22 m/s, Ryan *et al.* 2007) following McInnes *et al.* (2017). A commuting event included more than three consecutive shallow dives less than 5 m deep with inter-dive durations less than 3 min and the direction of travel being distinctly directional (McInnes *et al.* 2017). Sedentary behaviour incorporated surface behaviour with very little horizontal movement and involved mostly preening and resting. A dive bout included a sequence of more than four searching and/or foraging dives (>3 m deep) with inter-dive durations less than the maximum bout ending criteria (BEI) calculated for all birds fitted with depth loggers. Depth loggers were only deployed during 2017 so we had to find a single threshold that could be applied to all data, including data from birds not fitted with depth loggers. The BEI was calculated using the maximum-likelihood estimation criteria method of Luque and Guinet (2007a), estimated from dive parameters quantified using the R (R Core Team 2018) package 'diveMove' (Luque & Guinet 2007b).

For each behavioural state event, the presence or absence of conspecifics was recorded. Birds

were classified as in association or solitary, the latter potentially incorporating birds within visual contact beyond the range of the AVRs, i.e. in loose associations. All prey capture events were quantified and classified as either single or schooling fish, the latter defined as fish in 'synchronized or polarized swimming groups' (Pitcher & Parrish 1993). Penguin calls were recorded directly from the AVRs recording audio data at a sampling frequency of 32 kHz at 128-kb resolution. Calls were identified and time stamped using Sonic Visualiser software (Cannam *et al.* 2010) and were cross-referenced to their associated behaviours. Calls were classified as 'call events', i.e. at least one call per surface period, and as 'sequential calls', i.e. the number of sequential calls in a 'call event'.

Acoustic structure of calls

Penguin vocalizations recorded during 2017, with improved sound quality due to a reduction in the thickness of the camera casing, were analysed using Avisoft SASLAB PRO (version 5.2.09, Avisoft Bioacoustics, Glienicke/Nordbahn, Germany). The spectrogram of each recording (Hamming function, FFT 512 points window size, 75% overlap) was visualized over a sliding window of 20 s length to identify and label all the calls. Wherever the quality of the recordings allowed, i.e. high signal-to-noise ratio, calls were measured in both temporal and frequency domains. Temporal parameters were measured on the oscillogram and included the duration of the call measured (in seconds). Spectral features were extracted from the average amplitude spectrum and included the fundamental frequency (Hz) and the frequency of maximum amplitude (Hz). When the presence of a 'two-voice' system was identified, the two fundamental frequencies were measured from the amplitude spectrum. In addition, the rate of the amplitude modulation coupled to the secondary frequency modulation of the 'modulated' calls (see Results for descriptions of call types) was measured using the 'pulse train analysis' in Avisoft SASLAB PRO software.

Behavioural context of calling events and call types

The probability of a penguin calling event was modelled as a binomial response with the response

unit being the surface period between dives: 1 = calls recorded, 0 = no calls recorded. Explanatory variables included an interaction term between behavioural state and the presence or absence of conspecifics to assess the influence that group behaviour had on the likelihood of calling activity under different behavioural states. To determine whether the probability of a call event was independent of the time of day for different behavioural states, we fitted a smoother term of time (elapsed time since nautical sunrise) interacted with a categorical variable for behavioural state. Year and month were included as fixed effects to control for potential annual and seasonal variation in environmental conditions that could potentially have influenced calling behaviour. The inclusion of year also accounted for differences in the thickness of camera casings between years and the influence this may have had on call detection. A linear predictor for the duration of a surface event was included to control for the expected higher probability of a call event during longer surface periods. The potential for sex-related differences in calling propensity were accounted for by including sex as a fixed effect, and to account for pseudo-replication among individuals, bird ID was included as a random effect (fitted as a smoother term). We used generalized additive models (GAMs) using the R package 'mgcv' (Wood 2006) to incorporate the smoother terms using penalized regression splines. Initial model runs included all candidate explanatory variables with subsequent runs dropping least significant terms; the best-fitting model was selected based on Akaike's information criterion (AIC; Akaike 1973) scores.

The influence of penguin behaviour on the two most common calls identified using the 2017 sample of birds was investigated using a generalized linear mixed effect model (GLMM) with a binomial response: 1 = 'modulated' call, 0 = 'flat' call (see Results for descriptions of these call types). As with the previous model we assessed the influence of an interaction term, between behavioural state and the presence or absence of conspecifics, on the response and we included bird ID as a random effect.

Influence of prey class on number and timing of calls

Schooling prey are significantly more profitable to foraging African Penguins than single prey,

especially when schooling fish are preyed on by groups (McInnes *et al.* 2017). To test whether calling behaviour differed between these foraging scenarios (schooling fish vs. single fish encounters), for each surface event that included at least one call within a foraging dive bout, we assessed the influence of prey class on four responses: (1) the number of sequential calls; (2) the time elapsed from a call to the start of a dive that included a catch; (3) the time between surfacing from the previous dive to a call; and (4) the time elapsed from a call to the last encounter with a conspecific. Prey class was defined as either 'school' – at least one catch from schooling fish in a dive bout, or 'single' – catches only from single fish in a dive bout. These comparisons of both the frequency and the timing of calls under different foraging scenarios enabled us to examine proxies of calling arousal under different foraging stimuli. Due to the limited number of calling events in dive bouts involving solitary birds, we limited these analyses to dive bouts that included the presence of at least one conspecific. Tests of significant differences between prey classes were conducted using non-parametric Mann–Whitney tests due to the non-normality in the residuals of analyses of variance (ANOVAs). A *post hoc* Bonferroni correction was applied for four tests on the same data, corrected $\alpha = 0.0125$.

RESULTS

Footage was retrieved from 18 Penguins (eight males and ten females) which included 21.4 h of recording (mean \pm sd recorded per bird: 69.5 \pm 24.4 min). Calling events ($n = 57$) were observed for 16 of these birds (mean \pm sd events per bird: 8.5 \pm 6.7 call events). BEIs were calculated for all individuals equipped with depth loggers during 2017 ($n = 7$ birds, range, mean \pm sd: 18–75 s, 51 \pm 21 s) and the maximum BEI value, 75 s, was then used as the threshold criterion for defining dive bouts for all birds.

Behavioural context of calling events

The proportions of calling events differed significantly between behavioural categories ($\chi^2 = 27.49$, $P < 0.001$, Fig. 1) with the highest proportion (66%) of calling events recorded from solitary commuting birds and the lowest proportion (12%) from sedentary birds in groups. While associating

in groups, birds participating in dive bouts called significantly more than commuting or sedentary birds (40% of calling events; $\chi^2 = 10.49$, $P = 0.005$, Fig. 1).

The GAMs included 1059 surface events of which 130 (12%) included calling activity. Month, year and sex had no influence on the probability of calling events and were discarded from the best-fitting model (Table 1). There were significant differences between the frequency of calling events for different behaviours (Table 1). The odds of a calling event, when considering all behavioural state classes together, were about five times more likely for solitary birds than for birds in groups (Table 2). However, on average, the odds of a call event were about three times more likely for birds in dive bouts and were heavily biased toward birds in groups, as solitary birds in dive bouts called about 11 times less than the reference group (Table 2). There was a significant non-linear influence of time elapsed since nautical sunrise on the incidence of call events for commuting birds, with higher rates of calling in the early morning and late afternoon (Fig. 2). As expected, there was a strong positive correlation between calling events and the duration of surface events (Table 2).

Acoustic structure of calls

For birds recorded during 2017, 100 calls were recorded from seven individuals, although 24 calls

were unsuitable for acoustic analysis and were discarded from the following analyses. On the basis of their acoustic structure, three types of calls were identified: 'flat' calls (single voice, primary frequency modulation (FM), no secondary FM, $n = 29$ calls from six individuals), 'modulated' calls (single voice, primary and secondary FM, $n = 37$ calls from four individuals) and 'two-voice' calls (two fundamental frequencies, $n = 5$ calls from one individual) (Fig. 3). All vocalizations were characterized by a fundamental frequency of between 212 and 329 Hz (varying depending on individuals). The duration of calls varied as a function of the two most commonly recorded call types, with 'flat' calls being significantly shorter than 'modulated' calls (Table 3). There were no significant differences between average fundamental and maximum frequencies between these two call types (Table 3).

Behavioural context of call types

This analysis included 66 calls (29 'flat' calls and 37 'modulated' calls) from 32 call events from four individuals. Although there was a higher incidence of modulated calls for solitary birds in dive bouts (76%, Fig. 4), there were no significant differences between the proportions of the two common call types recorded for different behavioural states for both solitary birds and birds in association (between all states for both solitary and birds in association: $\chi^2 = 5.9$, $P = 0.2$; between all states

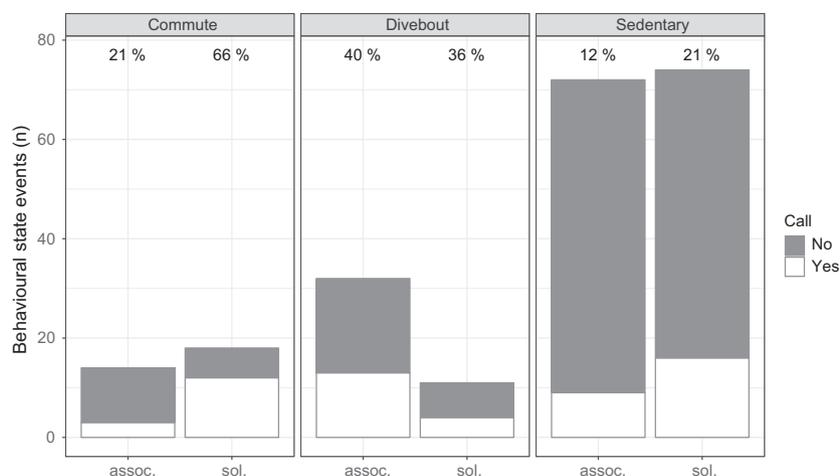


Figure 1. Counts of different behavioural state events (commuting, dive bouts and sedentary) for African Penguins, highlighting the incidence of calls for each behavioural state in the presence and absence of conspecifics, i.e. solitary birds (sol.) vs. birds in association (assoc.). Percentage values above each stacked bar represent the proportions of behavioural state events that had calling activity.

Table 1. Generalized additive model comparisons assessing binomial response of call events (CE) to different explanatory variables using Akaike's information criterion (AIC) scores to identify the best fitting models.

Model	AIC	ΔAIC
CE ~ s(TEN × BS) + BS × BM + duration + year + month + sex	694.77	1.25
CE ~ s(TEN × BS) + BS × BM + duration + month + sex	694.50	0.98
CE ~ s(TEN × BS) + BS × BM + duration + month	694.14	0.62
CE ~ s(TEN × BS) + BS × BM + duration	693.52	0

BS, behavioural state; BM, behavioural mode; TEN, time elapsed since nautical sunrise.

Table 2. Outputs for parametric and smooth term predictors of the binomial call response for the best fitting generalized additive model.

Parametric terms				
	β	se	t	P
(Intercept)	-3.28	0.54	-6.08	< 0.001
Dive bout	1.10	0.49	2.24	0.025
Sedentary	0.29	0.61	0.48	0.631
Solitary	1.67	0.55	3.05	0.002
Duration	0.003	0.001	3.46	0.001
Dive bout: solitary	-2.39	0.81	-2.95	0.003
Sedentary: solitary	-0.48	0.69	-0.69	0.493
Smooth terms				
	edf	F	P	
s(TEN): commute	2.58	3.04	15.24	0.002
s(TEN): dive bout	3.75	3.93	9.42	0.078
s(TEN): sedentary	2.62	3.13	3.05	0.355
s(ID)	11.94	15.00	50.70	< 0.001

For the parametric predictors, coefficients (β), standard errors (se), *t*-statistic values and significance estimates (*P*) are given. For the smooth terms the estimated degrees of freedom (*edf*), the *F* statistics and significance estimates (*P*) are given. Behavioural states, dive bout and sedentary are presented in relation to the reference state, commuting and the influence of solitary birds is presented in relation to birds in groups. TEN = time elapsed since nautical sunrise. Bold *P*-values denote statistically significant outcomes.

for solitary birds: $\chi^2 = 2.8$, $P = 0.3$; between all states for birds in association: $\chi^2 = 0.3$, $P = 0.6$). Approximately one-third (28%) of all call events had both call types in their call sequences, with birds in association in dive bouts being the only behavioural class that had no call events that

included both call types (Fig. 4). Results of the GLMM fit showed a negligible influence of behavioural state, the presence or absence of conspecifics and an interaction of these two variables on the incidence of the two most common call types (Table 4). 'Two-voice' calls included five calls from four call events for one individual all from the same dive bout.

Influence of prey class on number and timing of calls

There were 51 call events during dive bouts, of which 44 (86%) included catches and 45 (88%) included associations with conspecifics; only 40 call events that included both conspecifics and catches were used in the following analyses. The number of sequential calls within each surface event were significantly greater for dive bouts where fish schools were encountered compared with when only single fish were caught (Mann-Whitney test: $W = 311$, $P = 0.001$, Fig. 5a). The time elapsed from a calling event to the last catch was reduced for dive bouts involving schooling fish compared with dive bouts where only single prey were encountered (Mann-Whitney test: $W = 97$, $P = 0.03$, Fig. 5b). Although there were more incidents of birds that called sooner after surfacing in school-related dive bouts, this response was more variable than for birds that only encountered single fish prey (Fig. 5c). For dive bouts involving school encounters, birds generally called in a shorter elapsed time as their last association with conspecifics but this time difference was not statistically significant from birds that only encountered single prey (Fig. 5d).

DISCUSSION

The differential frequency of calling events displayed by African Penguins between different behavioural states in the absence and presence of conspecifics highlights the importance of acoustic communication to these diving birds at sea. Despite African Penguins being mostly silent at sea, calling was particularly frequent for solitary birds commuting during the first and last few hours of a foraging trip and for groups of penguins involved in dive bouts. During foraging dive bouts, African Penguins called more repetitively when schooling fish were encountered and much sooner since their last catch compared with foraging bouts

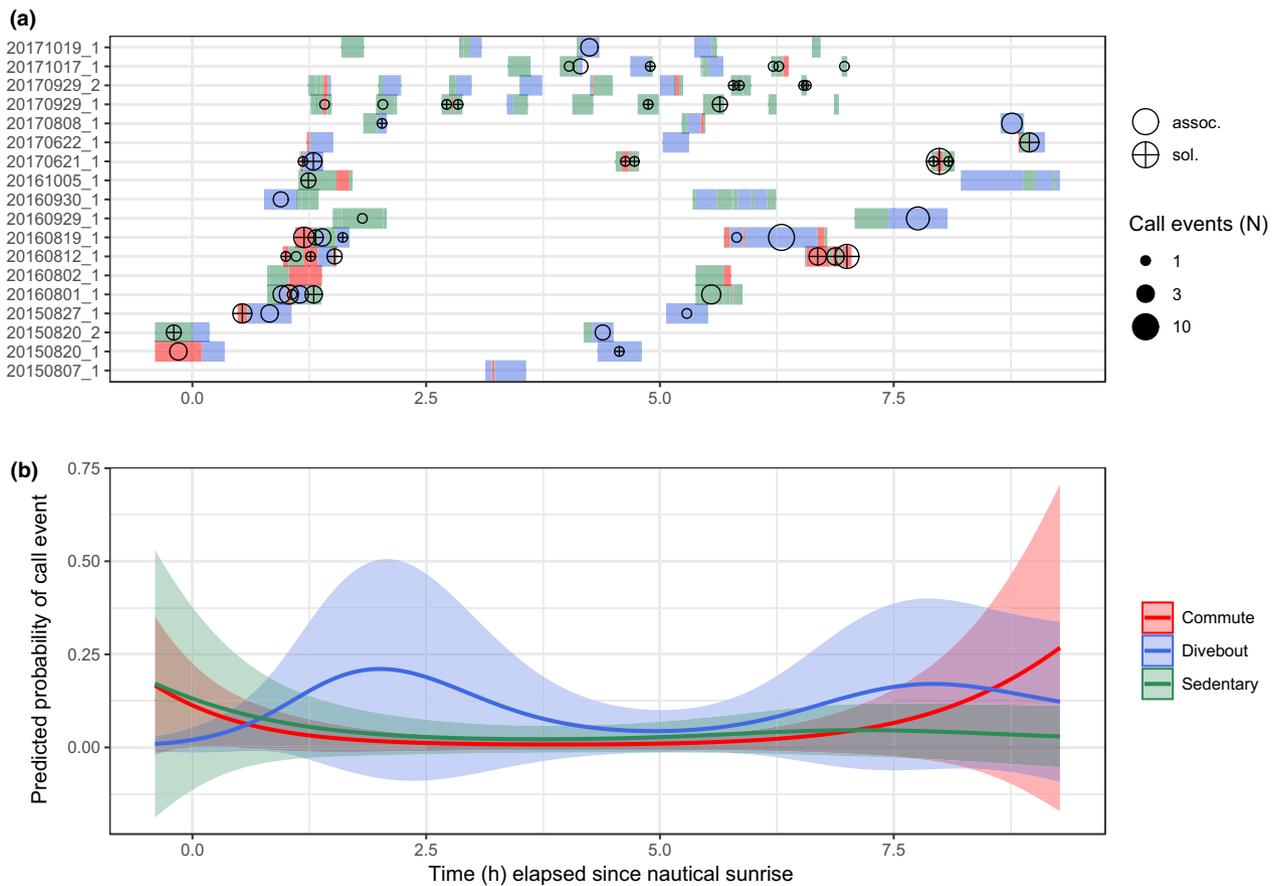


Figure 2. (a) Chronology plot of the behavioural states, commute, dive bout and sedentary (colours as in (b)), of all individual African Penguins (IDs on y-axis) deployed from Stony Point showing time elapsed since nautical sunrise. The numbers of calling events per behavioural event are superimposed as scaled circles above each bar where calls occurred and the social status of birds (assoc. – association, sol. – solitary) are denoted by different symbols. (b) Smoothed generalized additive model predictors of the probability of a call event for interactions between time elapsed since nautical sunrise and behavioural states, commuting, dive bouts and sedentary. Shaded bands denote 95% confidence intervals.

involving only single fish prey. These findings extend our understanding of the significance of acoustic communication in seabirds beyond the colony.

Despite the limited sample and relatively large confidence limits, the high incidence of solitary birds or birds in loose associations calling early in the day suggests that calling may aid group cohesion while commuting to foraging grounds. Group dispersion is likely if birds begin to dive asynchronously when they initiate prey searching behaviour, especially during exploratory dives in regions where the location of prey is less predictable, and they are therefore less likely to encounter conspecifics. The low incidence of calls during solitary dive bouts, as recorded during this

study, may increase their isolation and, if catches are minimal, they may feel the urge to re-group. The benefits of maintaining contact may also serve to stay within proximity to signals associated with the location of prey while optimizing their searching behaviour by spreading themselves out over a larger area. In an unpredictable preyscape, locating flocks of feeding birds has been demonstrated to improve foraging success of individuals, as flock cohesion is maintained by the quality of a foraging patch (Krebs 1974). Moreover, the benefits of group foraging for African Penguins extend beyond just the location of patchy resources but more directly to prey acquisition (McInnes *et al.* 2017).

The higher number of sequential calls given by African Penguins while foraging in locations with

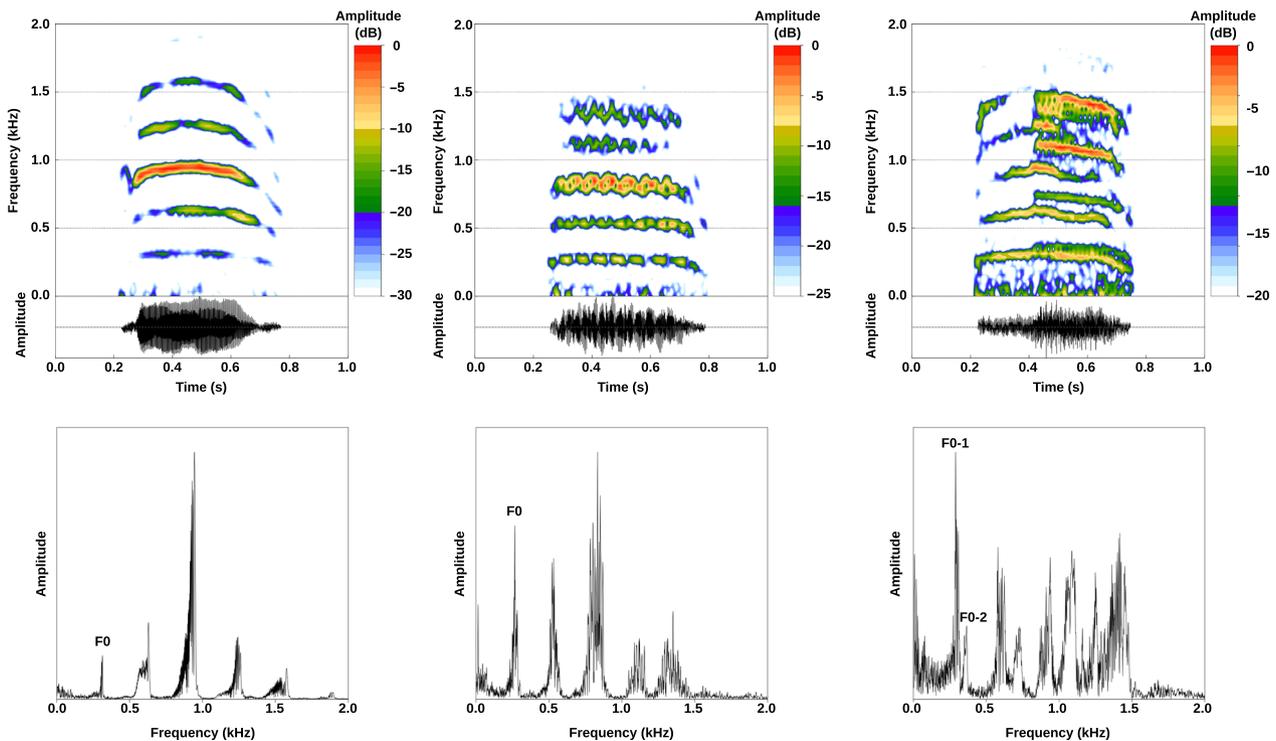


Figure 3. Vocalization types of African Penguins recorded at sea: 'flat' call (left column), 'modulated' call (middle column) and 'two-voice' call (right column): spectrograms, i.e. frequency over time (top row); oscillograms, i.e. amplitude over time (middle row); linear frequency spectra (bottom row), i.e. amplitude as a function of frequency. F0 – fundamental frequency; F0-1 and F0-2 – respectively, first and second fundamental frequencies for the 'two-voice' call. Each representation of the calls shown was recorded from different individuals. Figures generated in R software *Seewave* (Sueur *et al.* 2008).

Table 3. Acoustic analysis of African Penguin call types recorded at sea, 'flat', 'modulated' and 'two-voice' calls.

Acoustic measure	Flat			Modulated			Flat vs. Modulated		Two-voice		
	<i>n</i>	Mean/ median*	sd/ IQR*	<i>n</i>	Mean/ median*	sd/ IQR*	<i>F/W</i> *	<i>P</i>	<i>n</i>	Mean/ median*	sd/ IQR*
Duration (s)	28	0.39	0.11	36	0.45	0.08	6.6	0.01	5	0.47	0.02
F0 (Hz)	29	285	27	37	279	21	0.9	0.4	5	260	19
F0-2 (Hz)	–	–	–	–	–	–	–	–	5	334	14
F_{\max} (Hz)	29	898	668	37	810	677	536.5	1	5	554	749

Acoustic measures: duration of calls, fundamental frequency (F0), second fundamental frequency (F0-2) and the frequency of maximum amplitude (F_{\max}). Statistical comparisons are only shown for the two most common call types, 'flat' and 'modulated' calls: *F* – ANOVA test statistic, *W* – Mann–Whitney test statistic. For F_{\max} , non-parametric statistics (denoted with an asterisk) are given. Bold *P*-values denote significant differences between call types. IQR, interquartile range.

schooling fish may function to coordinate the diving activity of conspecifics. By foraging in groups, African Penguins facilitate the prolonged accessibility of fish in a school either as depolarized fish (i.e. escapees) from schools being herded to the surface or from fish corralled into bait-balls (McInnes *et al.* 2017). Food-related calls have been

shown to enhance prey acquisition in other bird species, such as Cliff Swallows *Hirundo pyrrhonota* that use calls to recruit conspecifics to swarms of insect prey which are tracked more efficiently in groups (Brown *et al.* 1991). In the present study, the limited sample of calls with adequate acoustic quality did not permit us to establish whether

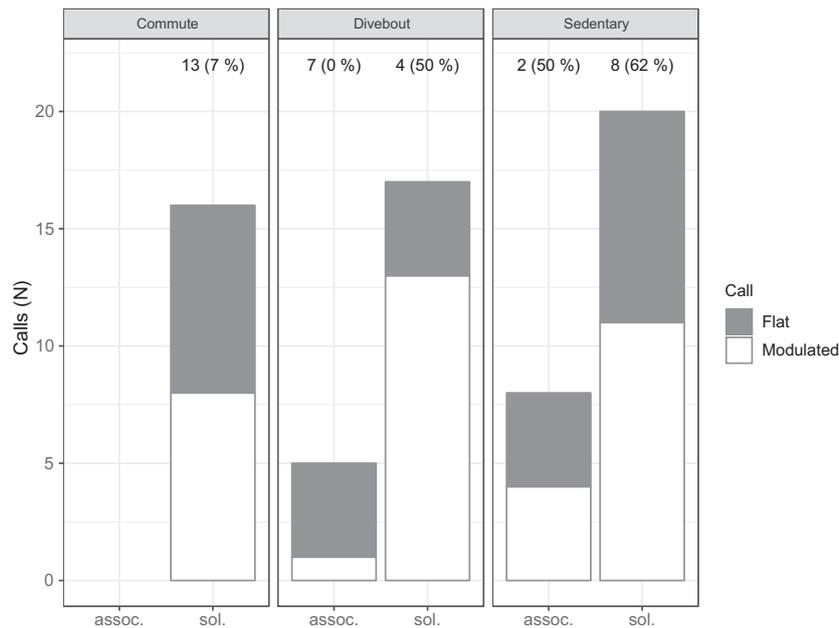


Figure 4. Frequency of call types, 'flat' and 'modulated', for different behavioural states (BS), commuting, dive bouts and sedentary, under different behavioural modes (BM), i.e. solitary birds (sol.) vs. birds in association (assoc.). Numbers above each bar represent the number of call events and the proportion of events that had both call types (in parentheses) for each behavioural category.

Table 4. Generalized linear mixed effects model outputs for parametric predictors of the binomial response for call type: 1 – modulated, 0 – flat.

Fixed effects	β	se	z	P
(Intercept)	-0.2007	0.9757	-0.206	0.837
BSdive bout	-1.1856	1.4839	-0.799	0.424
BSsedentary	0.2007	0.6723	0.298	0.765
BMsol.	0.2007	0.8379	0.24	0.811
BSdive bout:BMsol.	2.3643	1.5096	1.566	0.117

For the fixed effects, coefficients (β), standard errors (se), z-statistic values and significance estimates (P) are given. BS, behaviour state; BM, behavioural mode: sol., solitary.

food-associated calls by African Penguins were functionally referential, i.e. call types specifically associated with feeding behaviour (*sensu* Marler *et al.* 1992). During a dive bout where schooling fish were encountered, African Penguins called sooner after a catch than when only single prey were encountered. This behaviour coupled with higher numbers of sequential calls suggests a heightened sense of arousal by individuals in the vicinity of schooling prey. An elevation in acoustic signalling may be interpreted by conspecifics, within this specific social ecological context, as

being associated with the presence of schooling fish, which may then drive group cohesion. Such behaviour plays a crucial role for species, such as African Penguins, that benefit from group foraging. Specific call types can have multiple functions and can exhibit different responses by receivers of these signals depending on specific social ecological contexts and despite the lack of referential information (Owren & Rendall 2001, Seyfarth & Cheney 2003, Seyfarth *et al.* 2010). Although certain bird species exhibit functionally referential food calls, such as domestic Chickens *Gallus gallus domesticus* (Evans & Evans 1999) and Northern Raven *Corvus corax* (Bugnyar *et al.* 2001), these instances are rare and mostly associated with species that have strong social bonds (Clay *et al.* 2012). For species such as African Penguins that are more transient in their group structure, an increase in call rates during foraging behaviour is a more common phenomenon (Clay *et al.* 2012).

At-sea calling behaviour in seabirds is a relatively recent area of research with only one study that has quantified context-specific acoustic structure, that of Cape Gannets *Morus capensis* off South Africa (Thiebault *et al.* 2019). The only other study using AVRs on penguins at sea to decipher the context of calling behaviour was

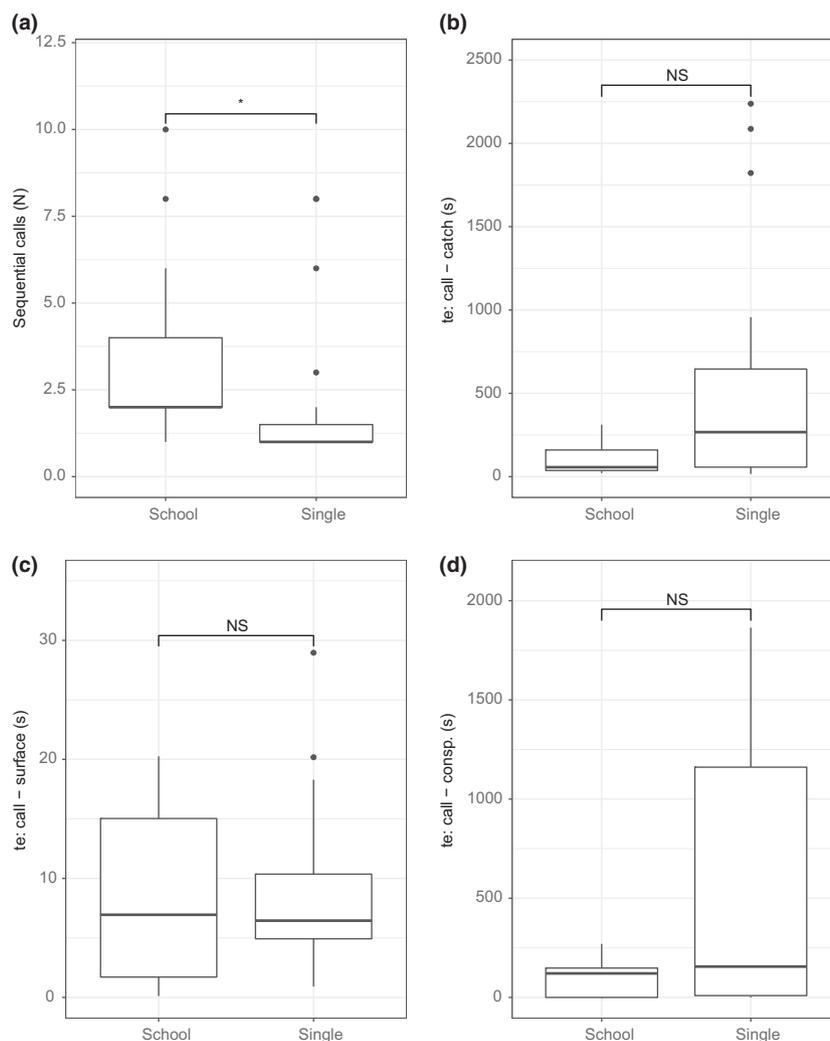


Figure 5. Influence of prey class (school vs. single fish for each dive bout) on the number of sequential calls per surface event (a), the time elapsed from the onset of each call event to the last catch (b), the time elapsed from the first call of a call event to the end of the previous dive (c) and the time elapsed from each call event to the last encounter with a conspecific (d). Significance levels are shown above each pair of boxplots representing Mann–Whitney statistics: * $P < 0.0125$; NS, not significant.

recently conducted on Gentoo Penguins *Pygoscelis papua* (Choi *et al.* 2017). Although significant recruitment events were associated with calls in this species, there was no evidence for food-associated calls. It is not known whether Gentoo Penguins benefit from group foraging but the evidence to date suggests that they are generalist, solitary foragers (Handley *et al.* 2017, 2018). The function of calling behaviour at sea may differ between penguin species according to their degree of foraging specialization and the types of dominant prey targeted. The results of this study further highlight the importance of social behaviour to the

survival of African Penguins and the need to be cognisant of potential Allee effects (Allee 1938, Courchamp *et al.* 1999) that may already be operating on this threatened species (Ryan *et al.* 2012, McInnes *et al.* 2017).

The acoustic structure of African Penguin vocalizations recorded at sea closely resembles the relatively short duration 'contact' (for 'flat' calls) and 'agonistic' calls (for 'modulated' calls) recorded by Favaro *et al.* (2014) for captive birds. These authors provided evidence for the behavioural context of these call types, with 'contact' calls mostly being associated with isolated birds and 'agonistic'

calls being associated with both aggressive encounters and feeding events. The function of 'contact' calls at sea may provide similar functions to those at the colony, primarily coordinating group cohesion. Stimuli such as the presence of schooling fish may induce heightened levels of arousal, which may have some bearing on the incidence of 'modulated' calls. Future research with bolstered sample sizes of these call types under different social-ecological contexts will hopefully elucidate this hypothesis. The 'two-voice' call emitted by one individual during this study is intriguing in that this phenomenon has only been previously documented for display calls of Emperor Penguins *Aptenodytes forsteri* and King Penguins *Aptenodytes patagonicus* emitted at the colony, where they function in individual recognition (Robisson 1992, Aubin *et al.* 2000). Unfortunately, we only recorded this call type from one individual during a single dive bout, so the behavioural relevance of this call type remains unclear. The discovery of different at-sea call types of African Penguins warrants further research into the functions of these different acoustic signals and an assessment of whether at-sea acoustic repertoires are more complex in seabird species that benefit from social cohesion. A bolstered sample of AVR data may also elucidate individual differences in calling behaviour under different socio-ecological stimuli and how acoustic signatures vary between individuals in different states.

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DATA AVAILABILITY STATEMENT

Datasets used in all analyses in this manuscript have been archived in figshare digital repository: <https://doi.org/10.6084/m9.figshare.7212173>.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Table S1. Generalized linear mixed effects model outputs for parametric predictors of the binomial response for call type: 1 – modulated, 0 – flat. For the fixed effects, coefficients (β), standard errors (*se*), z-statistic values and significance estimates (*P*) are given. BS, behaviour state; BM, behavioural mode; sol., solitary; assoc., association.