

NOTE

Interpreting diel activity patterns from acoustic telemetry: the need for controls

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ABSTRACT: Acoustic telemetry has emerged as a leading approach to infer diel, tidal and lunar rhythmicity in the movements of aquatic organisms in a range of taxa. Typically, studies examine the relative frequency of detections from individuals tagged with acoustic transmitters, and then infer patterns in the species' behaviour, but studies to date have not controlled for factors that may influence tag detection patterns in the absence of animal behaviour. We compared patterns in acoustic detections from tagged cuttlefish *Sepia apama* and several fixed-location control tags, and used these data to highlight the danger of misinterpreting patterns in the absence of adequate controls. Cuttlefish and control tags displayed similar detection patterns, and correcting cuttlefish-detection data for the influence of environmental factors resulted in the opposite pattern of cuttlefish activity displayed prior to correction. This study highlights the danger of using acoustic data to infer animal behaviour in the absence of adequate controls.

KEY WORDS: Acoustic telemetry · Rhythmicity · Behaviour · Diel · Activity · Temporal

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INTRODUCTION

Most animal species display diel patterns of activity, either as a function of endogenous 'circadian' rhythms or through exogenous factors such as changes in light intensity, tides, temperature or predator and prey abundance (Cole 1957, Andrews et al. 2009). Identifying those periods of greatest activity provides an understanding of the association between organisms and these cues, and how changes in exogenous factors are likely to influence organism behaviour. As a result, describing animal activity patterns has been the focus of a wide range of terrestrial and marine research (Andrews et al. 2009).

In the aquatic realm, acoustic telemetry is a prominent tool for describing diel patterns in animal behaviour, with the most common forms of analysis examining the relative frequency of acoustic detections (hereafter we refer to a 'detection' as the successful re-

solution of an ID code, which consists of a train of typically 6 or 7 acoustic pings) over various temporal scales. Spectral analyses such as Fast Fourier Transformation (FFT; e.g. Meyer et al. 2007, Yeiser et al. 2008, Afonso et al. 2009, Meyer et al. 2009, Papastamatiou et al. 2009), and non-parametric techniques such as chi-squared goodness of fit or Mann-Whitney *U*-tests are commonly used to examine patterns over 24 h (e.g. Lindholm et al. 2007, Abecasis & Erzini 2008, Carlson et al. 2008, Collins et al. 2008). With these approaches, rhythmic patterns in animal behaviour are inferred from the patterns in detection frequency (the number of successfully decoded acoustic transmissions per unit time). Since detection frequency decreases with increasing distances from acoustic receivers (Heupel et al. 2006, Simpfendorfer et al. 2008), a cyclical pattern in detection frequency may result from rhythmic movement of tagged animals away from a receiver array. However, other factors that are known to

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strongly influence detection frequency (e.g. wind speed, biological noise, current speeds; Heupel et al. 2006, Simpfendorfer et al. 2008) typically occur with diel, tidal or lunar frequency, so it is likely that one may detect rhythmic patterns in detection frequency in the complete absence of tagged-animal behaviour. Consequently, using these types of analyses to create inferences about animal behaviour is justified only if the influence of alternative factors is discounted through the employment of controls. With the increasing frequency and scale of acoustic arrays deployed worldwide, the need to accurately interpret acoustic data has never been greater. Of the myriad of acoustic telemetry studies that use the relative frequency of detections to make inferences about animal behaviour, we could find no published studies that report the use of fixed-location control tags to separate the patterns in detection frequency due to animal behaviour from those due to other (i.e. environmental) factors. Jackson et al. (2005) deployed a fixed-location reference tag to compare detection efficiency between different tag-attachment methods (also with *S. apama*), but that study did not examine patterns in detection frequency through time.

Here we examined the relationship between acoustic detections from tagged giant Australian cuttlefish *Sepia apama* and fixed-position control tags to show that patterns in detection frequency from animal tags are strongly influenced by factors other than animal behaviour.

MATERIALS AND METHODS

Study site, species and tagging. *Sepia apama* ($n = 7$) were collected via SCUBA from breeding grounds at Point Lowly, South Australia ($33^{\circ} 00' S$, $137^{\circ} 44' E$) during July of 2009 and tagged with Vemco acoustic transmitters (V9AP-2L, 69 kHz, 3.3 g in water, 46 mm length, mean delay 120 s, power output 147 dB) following Pecl et al. (2006). Tagged cuttlefish were typically returned to the water within 2 min of capture, and subsequently monitored via a linear array of 10 VemcoTM VR2W acoustic receivers. Range tests were conducted at a variety of locations throughout the reef prior to the study, and indicated that with prevailing wind speeds of 5 to 10 knots (the seasonal average), detection efficiency for tags was $46.6 \pm 15.3\%$ (means \pm SE) 200 m from receivers, and $19.5 \pm 14.7\%$ 300 m from receivers. Given the short mean delay between signal transmissions, we viewed the detection efficiency at 200 m as acceptable, so receivers were spaced 400 m apart in water depths ranging from 6 to 10 m. To estimate the influence of factors other than animal behaviour on detection frequency, we deployed 3 fixed-position con-

trol tags (V16-4H, 69 kHz, 11.0 g in water, 68 mm length, mean delay 600 s, power output 158 dB) at arbitrary locations throughout the area of cuttlefish breeding habitat (~60 ha). Tags were placed in small cotton bags and secured to bricks positioned on top of the substratum to mimic the typical position of cuttlefish (generally 2 to 5 m depth). Cuttlefish were monitored continuously for 22 d following release (all individuals had left the site by this time), and control tags for 65 d.

Analyses. Initially, FFTs were used to search for cyclical patterns in the frequency of detections from cuttlefish tags and the fixed-location control tags. Detections were summed in each hour of every day for all cuttlefish tags and control tags, and the FFT decomposed these data into component frequencies that were then searched for cyclical patterns. Resulting peaks in the power spectrum denote dominant frequencies at which cyclical patterns in detection frequency occur. The entire 22 d monitoring period was included in this analysis for cuttlefish tags, and the first 22 d of monitoring for the control tags. As the majority of cyclical patterns detected by FFTs in acoustic telemetry studies (e.g. Meyer et al. 2007, Afonso et al. 2009, Papastamatiou et al. 2009) occurred at a frequency of 24 h, we used a 2-tailed Pearson's correlation coefficient to test the strength of the positive relationship in the mean detection frequency per hourly bin between cuttlefish tags and control tags at a diel scale. Mean values per hourly bin were treated as individual data points, and as we found no evidence of non-linearity, we considered Pearson's coefficient appropriate (Quinn & Keough 2002).

We developed a simple method of correcting animal detection patterns to account for the influence of environmental factors on detection frequency. Comparisons of absolute values between cuttlefish tags and control tags is inappropriate given the different mean delay times (120 versus 600 s), power outputs (147 versus 158 dB), and varying distances of tags (both animal and control) from receivers, so we used an approach that quantified the magnitude of variation of each hourly bin (as standardised detection frequencies, SDFs) around the mean daily detection frequency for control tags:

$$\text{SDF}_b = \frac{B_b}{\mu} \quad (1)$$

where μ is the overall mean hourly detection frequency (the mean detection frequency across all 24 hourly bins, b), and B is the mean detection frequency in each 24 hourly bin for control tags. In this way, the differing magnitudes of variation that would result from tags being a variety of distances from a receiver, or having different power output or delay times, are standardised by their own mean. The mean detection frequency per

hourly bin for cuttlefish tags was then divided by the corresponding SDF for each bin. We used the entire 65 d monitoring period for calculating control tag SDFs, and applied these to the first 16 d of cuttlefish data, as several tagged animals had left the array subsequent to this. The first 16 d of control tag data were representative of the full 65 d period, but we used the entire control tag dataset to reduce the errors associated with calculation of SDFs. We included detections that were received by multiple receivers simultaneously for both cuttlefish tags and control tags, as this increased the maximum number of detections h^{-1} that were possible, and therefore allowed a greater expression of any temporal variation in detection frequency (excluding simultaneous detections would allow control tags, having a nominal delay of 600 s, to generally assume only 1 of 7 values h^{-1}).

To evaluate variation between control tags, SDFs were calculated for each of the 3 tags individually, for all 3 combined, and for each combination of pairs

(A+B, A+C, B+C). We also assessed the influence of varying the amount of control tag data (as a proxy of variable delay times) on corrected cuttlefish detection patterns. Standardised detection frequencies were calculated using all data from all 3 control tags combined, as well as with every sixth, every third and every second valid detection removed from analysis, and when considering only every sixth detection.

To compare detection frequency for cuttlefish at night versus day before and after corrections, mean detection frequencies per hourly bin were summed for day (07:00 to 18:00 h) and night, and compared using chi-squared goodness of fit tests.

RESULTS

Spectral analyses revealed a strong peak at a frequency of 24 h, and secondary peaks at 12, 8 and 6 h for all cuttlefish tags combined (Fig. 1a). These same

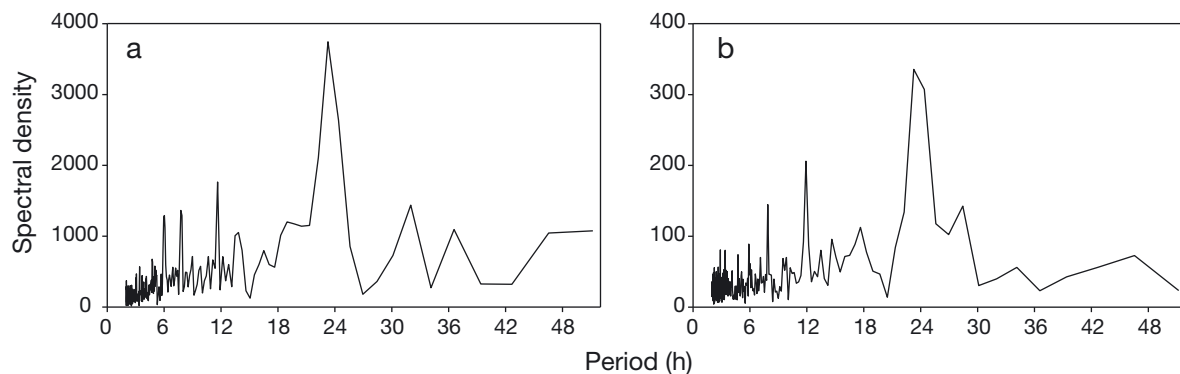


Fig. 1. *Sepia apama*. Spectral analysis of hourly detection frequency for (a) all cuttlefish tags combined, and (b) all control tags combined over a 22 d monitoring period. Note the variable scales on the y-axes

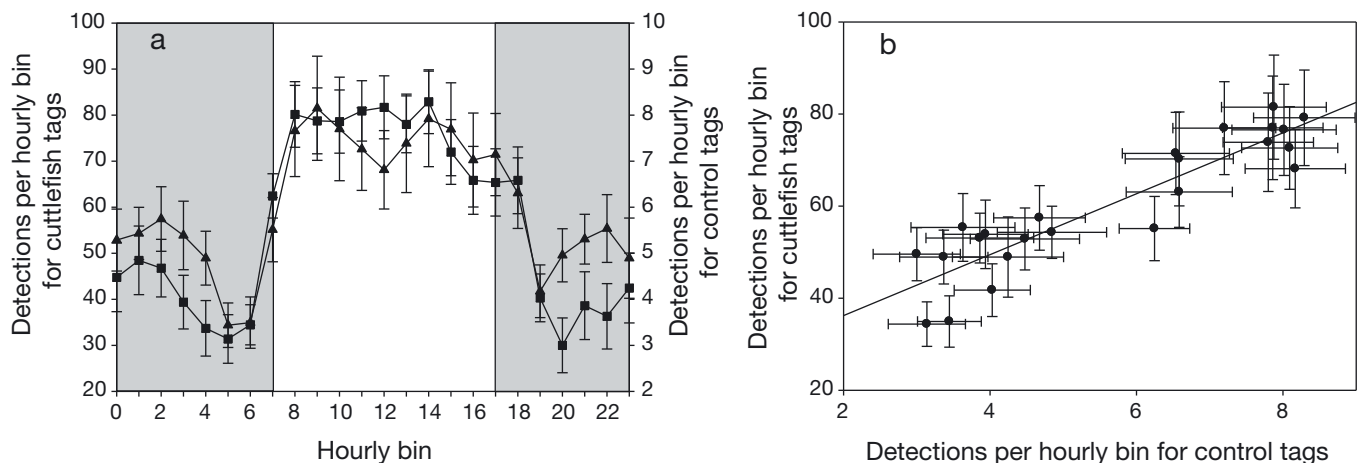


Fig. 2. *Sepia apama*. Relationship between detection frequency for cuttlefish and control tags. (a) Visual representation of the diel pattern experienced by all cuttlefish tags combined ($n = 7$, triangles) and all control tags combined ($n = 3$, squares) throughout the monitoring period (65 d for controls, 16 d for cuttlefish). Shown are mean (\pm SE) values per hourly bin, and shading indicates night time. (b) Correlation between mean (\pm SE) detection frequency per hourly bin for all cuttlefish tags combined, and the 3 control tags combined (Pearson's correlation coefficient = 0.91)

peaks were also displayed for all 3 control tags combined (although the peak at 6 h was less marked; Fig. 1b). Visual inspection of the pattern in detection frequency at a diel scale revealed a dramatic increase and subsequent decrease in mean detection frequency per hourly bin at dawn and dusk, respectively, for all cuttlefish tags and control tags (Fig. 2a), and this pattern was confirmed by Pearson's correlation coefficient ($r = 0.91$, $p < 0.01$; Fig. 2b).

Although the magnitude of variation differed by tag, all 3 control tags showed an increased frequency of detections during daylight hours (Fig. 3a). SDFs standardised this variable magnitude of change, such that SDFs for all 3 tags individually, combined, and all combinations of pairs of tags were similar (Fig. 3b). Prior to correction, cuttlefish were detected more frequently during daylight hours than at night ($\chi^2 = 16.33$, $df = 1$, $p < 0.01$, Fig. 3c, Table 1). Following correction, however, this pattern was reversed, with cuttlefish being detected more frequently during the night when SDFs were calculated using each control tag individually, all 3 combined, and each combination of pairs (Fig. 3c, Table 1a). Although data reduction altered the magnitude of the variation in detection frequency for control tags (Fig. 3d), the resulting SDFs led to almost identical patterns in cuttlefish detection frequency when using all, five-sixths, two-thirds, one-half and one-sixth of the control tag data (Fig. 3e, Table 1b). Varying the amount of control tag data used had no influence on the significance of the chi-squared tests, with cuttlefish being detected more frequently during the night for all analyses following correction (Table 1b).

The deviation of cuttlefish detection patterns from the pattern experienced by control tags can be seen when SDFs for control tags are plotted against SDFs for cuttlefish tags (calculated in the same manner as for control tags) prior to correction (Fig. 4). If the patterns in detection frequency for cuttlefish tags were identi-

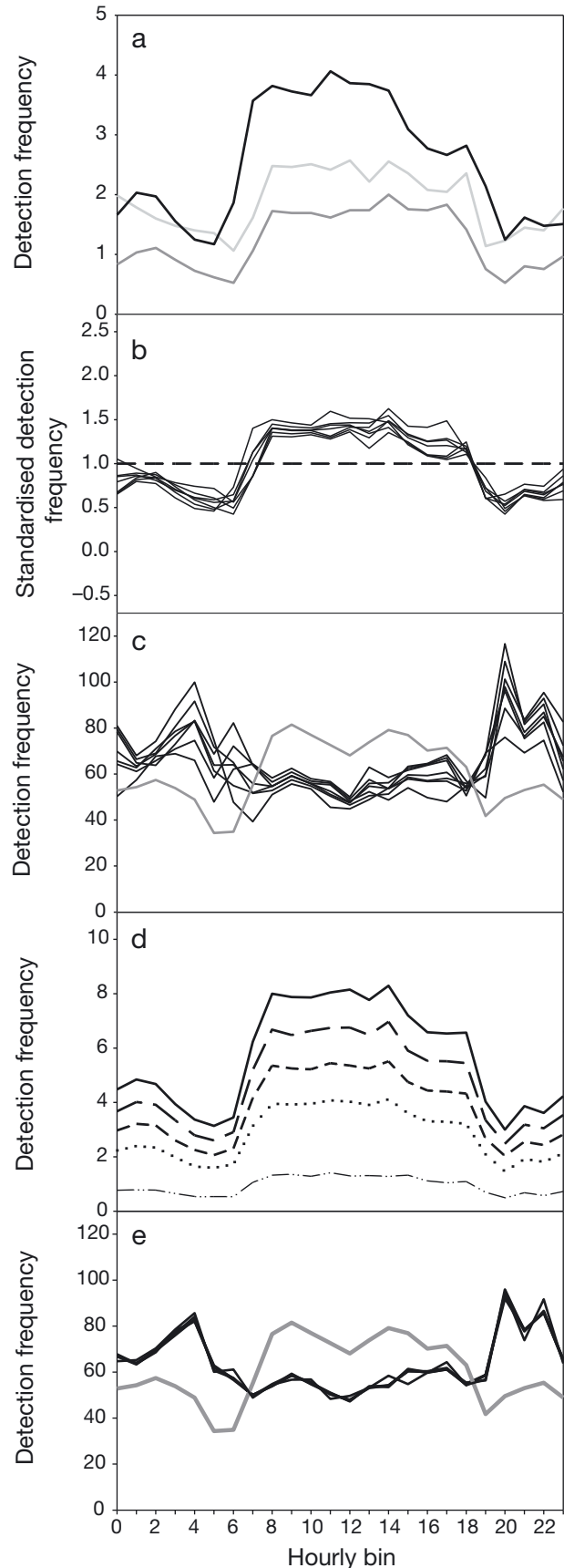


Fig. 3. *Sepia apama*. Visual representation of the process undertaken to adjust the frequency of detections per hourly bin for cuttlefish tags. (a) Mean detection frequency per hourly bin for each of the 3 control tags. (b) Standardised detection frequencies per hourly bin for each control tag individually, all 3 combined, and each combination of pairs of control tags. (c) Mean detection frequency per hourly bin for animal tags before (grey line) and after (black lines) correcting for the pattern experienced by each control tag, all 3 control tags, and each combination of pairs of control tags. (d) Mean detection frequency per hourly bin for all control tag data (solid line), and with one-sixth (long dash), one-third (short dash), one-half (dotted line), and five-sixths (dash-dot) of detections excluded from analysis. (e) Mean detection frequency per hourly bin for cuttlefish tags before (grey line) and after (solid lines) correction with all control tag data and with the reduced datasets from (d)

Table 1. *Sepia apama*. Comparison of mean number of detections during the day and at night prior to correcting for the patterns experienced by control tags and after correction, where correction factors were calculated (a) using each tag individually, each combination of pairs of tags and all 3 tags combined, and (b) with every sixth, third, second, and five-sixths of valid detection excluded from analysis

| | Detections per Day | Detections per Night | Chi-squared | df | p |
|-------------------------|--------------------|----------------------|-------------|----|-------|
| Before correction | 802.6 | 648.5 | 16.33 | 1 | <0.01 |
| After correction | | | | | |
| (a) A | 662.8 | 812.1 | 15.05 | 1 | <0.01 |
| B | 589.1 | 1003.8 | 108.11 | 1 | <0.01 |
| C | 590.2 | 988.1 | 100.38 | 1 | <0.01 |
| A+B | 626.0 | 908.0 | 51.84 | 1 | <0.01 |
| A+C | 626.5 | 900.1 | 48.81 | 1 | <0.01 |
| B+C | 589.7 | 996.0 | 103.93 | 1 | <0.01 |
| A+B+C | 614.0 | 934.7 | 66.52 | 1 | <0.01 |
| (b) Every sixth removed | 606.3 | 915.6 | 63.14 | 1 | <0.01 |
| Every third removed | 605.8 | 915.3 | 62.78 | 1 | <0.01 |
| Every second removed | 606.1 | 916.3 | 63.14 | 1 | <0.01 |
| Five-sixths removed | 605.6 | 921.3 | 64.98 | 1 | <0.01 |

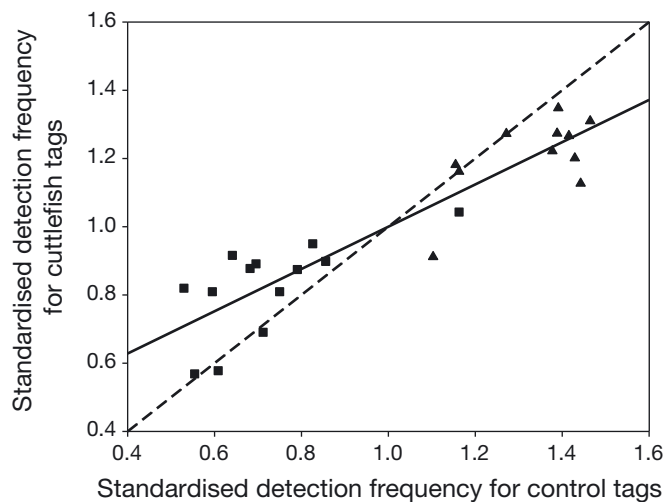


Fig. 4. *Sepia apama*. Deviation of cuttlefish tag detection frequencies from detection frequencies for control tags. Mean standardised detection frequencies were calculated for each hourly bin for cuttlefish detections (all 7 tags combined), and these were plotted against corresponding standardised detection frequencies (SDF) for control tags (all 3 combined). Dashed line represents a 1:1 ratio between the 2 variables, triangles indicate daylight hours and squares represent night time

cal to that of control tags, then the relationship between the SDFs for these 2 variables would be linear with a slope of 1. However, when above 1 (i.e. during the day), SDFs for cuttlefish tags are lower than for control tags, and this pattern is reversed when SDFs are below 1 (i.e. at night; Fig. 4). As a result, correcting cuttlefish detection frequency to account for the pattern displayed by control tags leads to lower detection frequency during the day than at night for cuttlefish (as seen in Fig. 3c,e, Table 1).

DISCUSSION

Here we show that a failure to employ adequate controls can lead to incorrect interpretations of animal behaviour. The strong positive correlations between the detection frequency for cuttlefish tags and control tags suggest that the reduction in detection frequency at night is not related to animal movement, but to some other factor or factors. Indeed, examining the relative frequency of cuttlefish detections prior to correcting for environmental effects would lead to the assertion that the dominant cyclical pattern in activity (24 h) is a result of behaviour that increases detection frequency during daytime (e.g. the cuttlefish are generally closer to receivers during the

day than at night). However, correcting for the similar pattern experienced by control tags leads to the opposite inference: cuttlefish behaviour is reducing detection frequency during the day.

Intuitive models could be proposed to explain either opposing scenario: (1) *Sepia apama* metabolic activity is lower during the night (Payne et al. 2011), so resting in or near rock crevices would block more acoustic signals than during the day. These animals cease sexual signalling outside of daylight hours and settle, camouflaged, on the seafloor during the night (Hanlon et al. 2007). It may be expected that this behaviour would result in lower detection frequency than during the day, when animals are slightly higher in the water column, and less associated with the potential interference of the reef structure. (2) There is some evidence that these cuttlefish move offshore and therefore closer to receivers during the night (at least during storm events; O'Dor et al. 2002), a behaviour that would increase the relative frequency of detections at night compared to during the day. Similarly intuitive opposing models could be proposed for many systems, and this increases the risk of acceptance of incorrect interpretations of animal behaviour. Interestingly, the diel pattern in cuttlefish detection frequency prior to correction (a dramatic increase and subsequent decrease at dawn, and dusk, respectively) is very similar to that revealed by accelerometry (Payne et al. 2011), a technique that is not influenced by detection frequency. Since correcting for external factors in the present study leads to the conclusion that increased activity is not associated with increased detection frequency, the often-made assumption that the 2 parameters covary may not always be valid.

The cause of a reduction in detections during the night was not specifically addressed in this paper; however, background noise at 69 kHz was significantly lower during the day than at night (N. Payne unpublished radio-acoustic positioning data). A possible explanation for this increased noise is nocturnally active crustaceans (e.g. shrimp, zooplankton), which are abundant in the study area and are known to reduce detection frequency (Heupel et al. 2006).

On reef systems where animals are concentrated, biological noise at 69 kHz generally increases at night and decreases during the day (D. Webber unpublished data). During windy days, this diel difference may be less marked because wind-induced noise is generally higher during daylight hours. In other systems, range may be lower during the day than at night (e.g. many lakes or marine systems where reefs are absent). Patterns in detection frequency and causes thereof are likely to vary significantly between systems, and rather than attempt to explicitly isolate the cause of reduced detection frequency here, we urge future researchers to consider the potential influence of the wide variety of external factors that may affect patterns of animal detection frequency.

Corrected cuttlefish detection patterns were generally similar regardless of which individuals or combinations of control tags were used to correct cuttlefish detection patterns, and whether all data or a reduced control tag dataset were considered. However, varying the number and combination of control tags used for correction resulted in significantly greater variation in cuttlefish detection patterns than adjusting the amount of data used (Fig. 3c versus 3e), suggesting that increasing the number of control tags deployed throughout an array may be more valuable than using a smaller number of tags with faster pinging rates. Particularly for large arrays, increasing the number of control tags used will help to account for spatial variation in acoustic conditions that are likely to occur throughout the array. Given the minimal influence of reducing the amount of data used to correct animal patterns, researchers may be wise to choose tags with larger delay times, such that battery life is extended, and code collision rates are reduced. Choice of delay times will of course depend upon the temporal resolution of interest, for example, whether comparisons are to be made between day and night or among hours within days.

Ecological experiments often offer challenges when designing appropriate controls (Hairston 1989, Underwood 1997, Quinn & Keough 2002), and acoustic telemetry has presented such a challenge for examining diel activity patterns in aquatic taxa. The significant cost of tags may be a deterrent to the use of controls in some studies, or there may be many cases where control tags are employed, but results are not

reported if environmental effects prove to be minimal. However, this study provides an example of how a lack of controls can profoundly influence interpretation of animal activity patterns, and we urge future researchers to identify, account for, and report those factors that may be acting in the absence of animal behaviour.

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