<u>Honours Research Thesis</u>

Vocalisations and vocal individuality in the endangered Australasian Bittern (*Botaurus poiciloptilus*) in south-western Australia, with applications for conservation

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Abstract

The Australasian Bittern (*Botaurus poiciloptilus*) is a large, secretive member of the heron family, which has recently been listed nationally as endangered. In south-western Australia, the species has undergone significant declines, and the most recent estimates put the remaining population size at only 38-154 individuals. Conservation efforts have intensified in recent years, but many aspects of the species' biology and ecology remain poorly understood. The ability to identify individual bitterns could increase our understanding aspects of their biology and ecology, but the species' secretive nature and preference for dense habitats makes conventional techniques for individual identification challenging. Australasian Bitterns are most readily detected by their loud booming calls, made during the breeding season: vocal individuality offers a potential means for identification of individual bitterns, but has not been investigated in the species. The primary aims of this study were to determine whether Australasian Bitterns exhibit vocal individuality, whether individual differences are sufficient for effective discrimination between individuals, and to compare the performance of two methods for classification of calls. In addition, I investigated temporal patterns of calling to determine whether there might be optimal time periods for detecting calling bitterns and recording calls. The combined acoustic data are discussed as tools that can be used in the conservation of the species.

Both quadratic discriminant function and neural network approaches yielded correct classification rates from 92 % to 93 % using training and testing calls from the same calling bouts, and 63 % to 71 % using training and testing calls from different calling bouts from the same bird. These figures are significantly higher than would be expected based on chance, which confirms that Australasian Bitterns do exhibit individuality in their vocalisations. It also indicates that this variability is sufficient to reliably discriminate between individuals over short time periods. Improvements in recording and analysis techniques may further increase the proportion of calls correctly classified. However, the decline in correct classification rates when calls from different bouts were used for training and testing may indicate instability in individual characteristics of vocalisation, which may hamper longer term identification using vocalisations. Further research is required to determine whether individuality is stable enough over longer time periods to allow for repeated individual identification. Analysis techniques would also need to be adjusted to allow for an open population for most practical applications.

Bitterns were detected calling at all times through the night on occasion, but maximum calling activity was detected just prior to sunrise. A smaller peak in calling activity was also detected just after sunset. Results indicated the optimal time for surveys to detect calling birds was the period just prior to sunrise rather than the first two to three hours after sunset that has been used in most previous Australasian Bittern aural surveys.

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Introduction

Australasian Bittern

The Australasian Bittern (*Botaurus poiciloptilus*) has experienced serious declines across much of its range and has recently been listed as endangered, both nationally under the Environment Protection and Biodiversity Conservation Act 1999 and in Western Australia under the Wildlife Conservation Act 1950 (Department of Environment 2014). In south-western Australia, the Australasian Bittern population has recently been estimated at 38-154 individuals (Pickering 2013). Australasian Bitterns are large, secretive birds of the heron family, which occur primarily in south-eastern and south-western Australia, and New Zealand (Hancock and Kushlan 1984). In south-western Australia, the species typically occurs in densely vegetated wetlands with stands of the rush *Baumea articulata* interspersed with shorter sedgelands for feeding (Pickering 2013); however it does also occur in some *Typha orientalis* wetlands and may move into more open areas when not breeding (Jaensch 1982; Pickering 2013). Due largely to its cryptic habits, many aspects of the species' biology and ecology are still poorly understood, including average lifespan, breeding site fidelity, individual movements, and survival and recruitment rates (Teal 1989; Marchant and Higgins 1990). This information is critical for ongoing conservation and management of the species in south-western Australia.

Australasian Bittern vocalisations

Australasian Bitterns give deep booming calls, which are reported to carry up to one kilometre in good conditions (Marchant and Higgins 1990), and provide the easiest way to determine the presence of bitterns in a wetland. Each call (often referred to as a boom train) consists of a series of 'gasps' followed by a series of booms, each of which is preceded by another 'gasp' (Figure 2). The gasps are shorter and lower in volume than the main booms. At close range, all gasps and booms can be detected. At greater distances, the first series of gasps is no longer audible, while at the greatest distances, only the main booms are usually audible (personal observations). The number of booms per call varies; Marchant and Higgins (1990) suggest up to five but typically three, while personal observations in Western Australia indicate that three or four booms per boom train is typical, though two and five booms have also been recorded on occasion.

Australasian Bitterns call almost exclusively during the breeding season, and the calls are thought to function in advertising male location and/or quality, and in territorial defence, though these functions have not been confirmed (Marchant and Higgins 1990). Booming calls of the closely related Great Bittern (*B. stellaris*) are known to perform these functions (e.g. Polak 2006), and the seasonal timing of calling in Australasian Bitterns supports a similar explanation. Calling occurs mostly at night, with the periods around dawn and dusk traditionally regarded as the times of peak calling activity (Marchant and Higgins 1990). However, birds have been recorded calling strongly throughout the night in south-western Australia, and field observations suggest that birds often call strongly and persistently for 3-4 hours before dawn (personal observations). Birds typically call from one site on a given night; however they will occasionally move calling position, possibly in response to disturbance (Marchant and Higgins 1990; personal observations).

The relative ease of detecting Australasian Bitterns by their calls means much of the survey effort targeting the species involves listening for their calls (e.g. Pickering 2013). This has typically been performed by observers in the field, but the use of automated recording units (ARUs) is now becoming more widespread. An increased understanding of temporal calling patterns during the night would allow surveys, either by observers or ARUs, to be concentrated on times of maximum calling activity, increasing efficiency and reducing the likelihood of false negative results. However, the species' vocalisations may be able contribute more to conservation and management of Australasian Bitterns than simple presence/absence and count data. Individual differences in vocalisations could also provide a basis for identifying individual bitterns over time. This, in turn, could be used to increase our knowledge of other aspects of the species biology that are important for management, such as breeding site fidelity, and survival and recruitment rates.

Individual identification

The importance of individual recognition for conservation and management is well established. The ability of researchers to identify individual animals within a population on a repeated basis is important for determining life history characteristics such as individual survival (e.g. Gilmartin *et al*. 1993; Rudnick *et al*. 2005). Identifiable individuals can also allow us to estimate average home range requirements for a species (e.g. Hill and Grossman 1987; Stander 1991), enhance our understanding of a species' movements and site fidelity (e.g. Parra *et al*. 2006; Anderson *et al*. 2011), and allow for better estimations of population size and density (e.g. Trolle and Kery 2003; Silver *et al*. 2004). These factors in turn impact on our understanding of population size and viability, which are of critical importance for effective conservation and management (McGregor and Peake 1998).

There are a number of methods that can be used to facilitate recognition of individual animals. Traditional methods typically involve the use of visual characteristics, using either naturally-occurring or artificial marks to identify individuals. Natural markings may include distinctive patterning, scars or deformities (e.g. Wursig and Jefferson 1990; Silver *et al*. 2004), while artificial marks can be achieved through the use of tags, bands, colour marks or collars (e.g. Ogilvie 1972; Calvo and Furness 1992; Doligez *et al*. 2004; Robinson *et al*. 2005), or clipping of fur, ears or appendages (e.g. Goto 1985; Braude and Ciszek 1998**)**. More recently, technological developments have increased the utility of microchip, radio, satellite and GPS markers as methods for individual identification or analysis of individual movement patterns (e.g. Stander 1991; Elbin and Burger 1994).

All these methods have their own drawbacks. Many individuals do not have obvious natural marks suitable for individual identification, and artificial marks can be lost. The use of artificial marks, microchips and tracking devices usually requires the capture and handling of target individuals, which can result in stress, injury and, on rare occasions, death for the captured animals and in some cases may present risks to the researcher as a result of attack by the captured animal or transmission of disease (McGregor and Peake 1998). Artificial marks, microchips and radio transmitters may also alter the behaviour of the target individuals, and in some cases even cause health problems (Spray and Bayes 1992; Elcock *et al*. 2001; Barron *et al*. 2010). Capturing individuals for marking, and the use of artificial marks, can also evoke criticism from some elements of the wider community, potentially reducing community support for research being undertaken (Mellor *et al.* 2004). Finally, visual identification can be impractical for species which are secretive, live in dense habitats, or are otherwise difficult to observe visually (Gilbert *et al*. 1994).

Non-invasive methods using genetic data collected from shed fur, feather, and scats are increasing in popularity with advances in genetics and associated technology, and corresponding decreases in costs (e.g. Hedmark *et al*. 2004; Rudnick *et al*. 2005). Such methods negate the drawbacks associated with capturing and handling animals, and also do not require visual sighting of the target individuals, making it a more feasible technique for use with difficult-to-observe species. However, the use of genetic material for ongoing individual identification can still be costly in terms of time and effort, particularly in species favouring difficult-to-access habitat such as swampland, as genetic material still needs to be collected.

Vocal individuality

Vocal individuality has long been proposed as another alternative method for identifying individual animals (e.g. Beightol and Samuel 1973). Using recordings of vocalisations to identify individual animals offers similar advantages to the use of genetic material in that it does not require capture or handling of target individuals in most cases, does not require visual sighting of target individuals, and can often be achieved with minimal disturbance to the target individuals (Terry *et al*. 2005). This also reduces the likelihood of effects on fitness or behaviour from marking processes. However, the use vocal individuality also has weaknesses. Most notably, it requires that individual differences in vocalisations are stable in order for long-term re-identification to be effective (Terry *et al*. 2005). Testing for such stability requires extended periods of study and an independent means of individual identification.

Vocal individuality can arise for a variety of reasons including intra-specific recognition of kin, mates or territorial neighbours (e.g. Aubin and Jouventin 2002; Hyman and Hughes 2006; Blumenrath *et al.* 2007), an indication of individual fitness (e.g. Behr *et al*. 2006), or physical differences in individual vocal tract morphology (Fox 2008). Vocal individuality has been demonstrated across a wide range of taxa, in particular birds (e.g. Peake *et al*. 1998; Rogers and Paton 2005; Grava *et al*. 2008; Policht *et al.* 2009) and mammals (e.g. Darden *et al*. 2003; Hartwig 2005; Leliveld *et al*. 2011; Root-Gutteridge *et al*. 2014b). Studies on non-avian and non-mammalian taxa are less common, though there is a relatively extensive body of literature demonstrating individuality in the calls of several frog species (e.g. Bee *et al*. 2001; Feng *et al*. 2009), and at least one study showing individuality in the mating sounds of a fish species (Amorim and Vasconcelos 2008).

This study

The Australasian Bittern is an ideal candidate for individual identification using vocalisations. Their preference for dense swampland habitats means that Australasian Bitterns are rarely sighted, so techniques requiring individual marks or fitting of marking devices for individual identification are impractical. Capturing individuals for fitting of radio or GPS tracking equipment may be complicated by the terrain and vegetation of their habitat. Researchers in New Zealand resorted to cannon netting from helicopters to capture bitterns for radio-tagging, but only successfully tagged three individuals (Teal 1989). The strong booming calls made by Australasian Bitterns are more readily detected, and provide the easiest way to detect the presence of bitterns in a wetland. It follows that the presence of individuality within these vocalisations could also present a practical method for identifying individual Australasian Bitterns, but this has not yet been investigated in this species.

The primary aims of this research were to determine whether Australasian Bitterns exhibit individuality in their vocalisations, and whether the level of individuality demonstrated is sufficiently high to allow reliable discrimination between individuals. This represents the first steps towards determining whether vocal individuality is a feasible method for repeated identification of individual Australasian Bitterns. I expected that individuality would be found in the vocalisations, as some level of individuality has been detected in all studies I reviewed, and it has been suggested that vocal individuality may be a feature of all vocally active animals (Terry *et al*. 2005).

I also investigated patterns of calling throughout the night to determine the peak periods of calling activity, and most reliable periods for detecting the presence of calling bitterns. Surveys have traditionally focussed on the period immediately following sunset (Pickering 2013), whilst references in the literature suggest dusk and dawn as peak calling periods (e.g. Marchant and Higgins 1990). My own qualitative field observations suggest high levels of calling activity from approximately midnight through until dawn. Data on calling patterns and the more detailed descriptions of Australasian Bittern vocalisations generated by field recordings can both be used to optimise future survey effort for this species.

Materials and methods

Study sites and sample size

Surveys were conducted at 20 wetland locations across the south-west of Western Australia where Australasian Bitterns had previously been detected calling, or sites that were located in close proximity to previous records and contained potential habitat for the species (Appendix 1). The primary objective of these surveys was to locate Australasian Bitterns that were calling strongly in order to maximise the number of calls recorded in the available time. Based on previous field experience, and with this goal in mind, surveys of 20 minutes duration were used in most instances. If a bittern was detected calling, the location was noted and recordings were taken where possible. Australasian Bitterns were detected calling at nine locations, and suitable recordings were obtained from five locations: Kulunilup Swamp, swampland along the Muir Highway in Lake Muir Nature Reserve, Lake Pleasantview, a small swamp on the outskirts of Manypeaks, and Cheynes Swamp (Figure 1; Appendix 1).

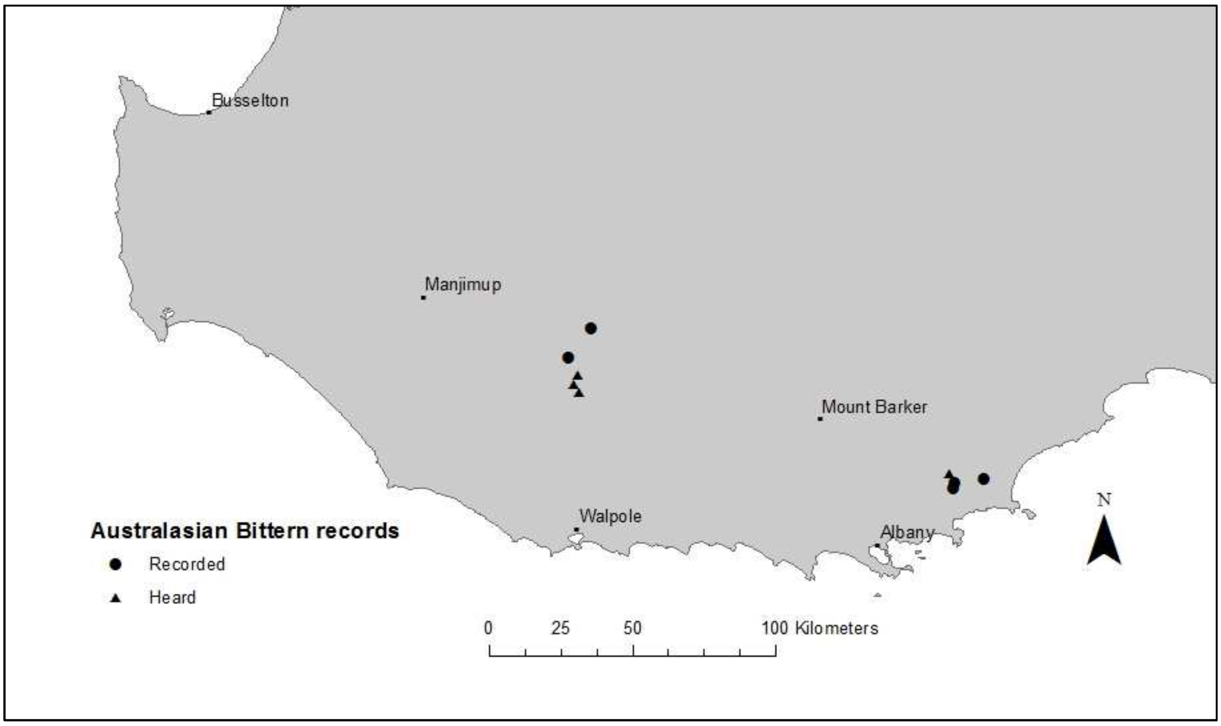

Figure 1: Locations of calling Australasian Bittern located during this project. In this context, recorded refers to a sound recording of one or more individuals being obtained at this location. For further details, refer to Appendix 1.

Identification of individuals was based on the location of calling. This introduces a level of assumption into the methodology which is regrettable; however using an independent method of individual identification is particularly challenging given the biology of this species, and impractical given the time and resources available for this study. Male Australasian Bitterns typically call from regular locations (Teal 1989; personal observations), and are territorial and aggressive towards other males (Williams 1985; Marchant and Higgins 1990), so it is highly unlikely that multiple males would be calling in very close proximity to each other. Furthermore, a similar approach was used by Gilbert *et al*. (1994) for a study on vocal individuality in the closely related Great Bittern, and calling territories have also been used as the primary means for identification of calling individuals in other vocal individuality studies (e.g. Rogers and Patton 2005). Using this technique, recordings from four of the five wetlands were attributed to a single individual, while two individuals were recorded at Lake Pleasantview (the presence of two individuals was confirmed on one occasion when both called simultaneously from their respective calling locations). The power of the individual recognition approach adopted will therefore come if birds move between locations within or between years.

Recordings

Recordings were made using two Fostex FR-2LE digital sound recorders linked to RØDE NTG-2 shotgun microphones, which have an effective, approximately flat frequency response from 20 Hz to 20 kHz (RØDE 2013), making them suitable for recording bittern calls which typically have a peak frequency in the range of 150-200 Hz (Figure 2). Recordings were made in Waveform Audio File Format (.wav files) to prevent the loss or distortion of components of the sound signal through data compression, which occurs when recordings are made in compression formats such as MP3 (Austin 2006). Recordings were made in mono with a sampling rate of 44.1 kHz. Recordings were taken from as close to the bird as was possible given water depth and other access factors, whilst also attempting to minimise the likelihood of disturbing the bird. In most cases, the estimated distance to the bird was between 50 m and 150 m, though in some instances it may have been further.

Automated recordings were also taken using SongMeter 2 automated recording units (ARUs) set up at four locations close to known calling bitterns to determine calling patterns through the night. The ARUs were programmed to record for 11 hours each night, commencing at sunset. The ARU recordings were also made in Waveform Audio File Format, but were made in stereo using a sampling rate of 16 kHz. The lower sampling rate used was necessary to allow for extended periods of recording over multiple nights given the memory available for sound files in each ARU. ARU recordings were not used in the primary vocal individuality analyses.

Analysis

Spectra of each recording were created in Audacity (Version 1.3 Beta; Audacity Development Team 2011) and examined visually to check for Australasian Bittern boom trains. All relatively clear boom trains detected were extracted, numbered and saved individually for processing. In this context, a 'clear boom train' refers to a boom train that is relatively free from other noise in a similar frequency band to the boom train. A sample of 20 booms from each recording session was then selected randomly, using random number generation, for use in analyses. In cases where fewer than 20 clear boom trains were recorded in a session, all clear boom trains were used. Recordings of each boom train were cleaned in Audacity, using the Equalisation tool to remove higher frequency noise (600 Hz and higher) and the Noise Removal tool to remove noise in the same bandwidth as bittern calls via the spectral subtraction method.

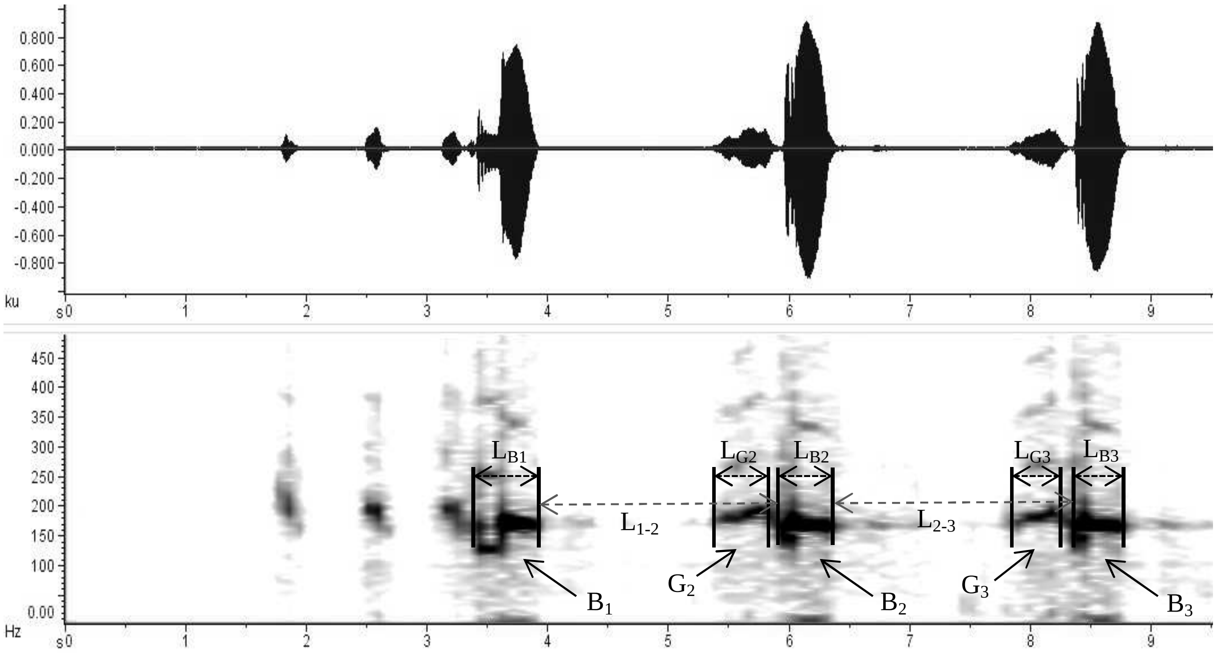

Figure 2: Waveform (upper graph) and spectrogram (lower graph) of Australasian Bittern boom train comprising three booms, recorded from Manypeaks, c. 50 km east of Albany.

Temporal and frequency measurements were then taken on cleaned boom trains using Raven Pro software (Version 1.4; Bioacoustics Research Program 2011). The program was used to create spectrograms for each cleaned boom train, using a Hann window with 5,120 samples and a discrete Fourier transform size of 65,536 samples, which gave a frequency resolution of 0.673 Hz. Sections of the call to be measured were then manually selected on spectrograms for each boom, and selected measurements for each section (Table 1) were automatically calculated by the software. The manual selection of call sections has the potential to introduce measurement errors due to relative variation in the areas selected for each call. However, testing indicated that frequency measures were relatively robust to small differences in the area selected.

Table 1: Initial variables measured for each boom train, with brief explanations for each variable

Refer to Appendix 2 for more detailed explanations of frequency measures.

Variable	Explanation
N_B	Number of booms in boom train
L_{B1}, L_{B2}, L_{B3}	Duration of 1st, 2nd and 3rd booms
L_{G2}, L_{G3}	Duration of 2nd and 3rd gasps
L_{1-2}, L_{2-3}	Duration of interval between 1st and 2nd, and 2nd and 3rd booms
PF_{B1}, PF_{B2}, PF_{B3}	Peak frequency of 1st, 2nd and 3rd booms
PF_{G2}, PF_{G3}	Peak frequency of 2nd and 3rd gasps
CF_{B1}, CF_{B2}, CF_{B3}	Centre frequency of 1st, 2nd and 3rd booms
CF_{G2}, CF_{G3}	Centre frequency of 2nd and 3rd gasps
$F5_{B1}$, $F5_{B2}$, $F5_{B3}$	5 % frequency of 1st, 2nd and 3rd booms
$F5_{G2}$, $F5_{G3}$	5 % frequency of 2nd and 3rd gasps
$F95_{B1}$, $F95_{B2}$, $F95_{B3}$	95 % frequency of 1st, 2nd and 3rd booms
$F95_{G2}$, $F95_{G3}$	95 % frequency of 2nd and 3rd gasps
$F1Q_{B1}$, $F1Q_{B2}$, $F1Q_{B3}$	1st quartile frequency of 1st, 2nd and 3rd booms
$F1Q_{G2}$, $F1Q_{G3}$	1st quartile frequency of 2nd and 3rd gasps
$F3Q_{B1}$, $F3Q_{B2}$, $F3Q_{B3}$	3rd quartile frequency of 1st, 2nd and 3rd booms
$F3Q_{G2}$, $F3Q_{G3}$	3rd quartile frequency of 2nd and 3rd gasps
$BW90_{B1}$, $BW90_{B2}$, $BW90_{B3}$	90 % bandwidth (i.e. $F95_x – F5_x$) of 1st, 2nd and 3rd booms
$BW90_{G2}$, $BW90_{G3}$	90 % bandwidth (i.e. $F95_x – F5_x$) of 2nd and 3rd gasps
$BWIQ_{B1}$, $BWIQ_{B2}$, $BWIQ_{B3}$	Inter-quartile bandwidth (i.e. $F3Q_x – F1Q_x$) of 1st, 2nd and 3rd booms
$BWIQ_{G2}$, $BWIQ_{G3}$	Inter-quartile bandwidth (i.e. $F3Q_x – F1Q_x$) of 2nd and 3rd gasps

A nested ANOVA analysis, with calling bout nested within individual bird, was conducted on each measured characteristic to determine those characteristics with relatively high inter-individual and relatively low intra-individual variation, following the procedure used in a similar study by Puglisi and Adamo (2004). Measurements were log-transformed, both to better fit the normality assumption of the ANOVA analysis and to reduce any effect of differences in magnitudes of different measurements in the final discriminant function analyses. Heteroskedasticity was an issue in measurements for all variables and was not resolved by transformation. Despite this, a nested ANOVA analysis was used because the result of most interest was the partitioning of variances rather than significance values, and there is a lack of recommended alternatives (MacDonald 2009). However, appropriate caution is recommended when considering the results from this test.

Pair-wise correlations were then calculated between those variables that were shown to have a significantly high level of inter-individual variation. Correlations between many of the frequency variables were high, so highly correlated variables were removed from the analysis. The variable chosen for use from a series of correlated variables was the variable which showed the highest proportion of inter-individual variability. Using this approach, five variables were chosen for use in the final discriminant function analyses.

The five selected variables were then used to conduct two discriminant function analyses, using the MASS package (Venables and Ripley 2002) in R Stats (Version 3.0.1; R Core Team 2013). Linear discriminant function analyses have been used in most similar studies (e.g. Bee *et al*. 2001; Puglisi and Adamo 2004), though many studies do not specifically indicate the type of discriminant function analysis used (e.g. Chapman and Weary 1990; Grava *et al.* 2008). However, linear discriminant function analysis assumes constant covariance between variables. In order to avoid making any assumptions about covariance patterns, I used quadratic discriminant function analysis, a similar method which does not require constant covariances. The first of the two discriminant function analyses took training and testing calls from the same calling bouts, using a 75% training, 25% testing split; the second analysis used training and testing calls from different calling bouts. Call measurements from the training set were used to develop a series of discriminant functions, which were then used to classify the testing set.

The proportion of calls in the testing set that were classified to the correct individual was calculated for each individual, and then compared with the correct classification rate that would be expected by chance. The rationale for comparing with chance to test for individuality is that both the discriminant function analysis and the neural network approach described below use ‘nearest neighbour’ methods to assign test calls or samples to one of the individuals in the training set (which contains all individuals being tested). If vocalisations do not show any individuality, then any variation in characteristics should vary in a random manner and we would expect test calls or samples to be assigned to any individuals in the training set at random. Conversely, if the proportion of test calls or samples assigned to the correct individual is significantly higher than would be expected by chance, then we can conclude that the vocalisations vary with individual. The correct classification rate for each individual expected by chance was set at the proportion of that individual’s calls in the total training set across all birds. If characteristics of the vocalisations vary randomly, then individual calls or samples in the training set should be distributed randomly across the sample space. Hence, if an individual has more calls (or samples) in the training set, a test call (or sample), which would also be randomly placed in the sample space, is more likely to be assigned to that individual than to individuals with fewer calls in the training set.

In order to test the neural network approach, the cleaned calls used for the discriminant function analysis were grouped into a single sound file for each individual. Silence was then removed from each sound file using the Truncate Silence function in Audacity. The first 20 seconds of sound from each individual was then taken and used to train and validate a neural network in MATLAB (MathWorks 2013). 20 seconds of the remaining sound not used in the training and validation process was then used to test the trained network; the testing procedure used broke the 20 seconds of sound used for testing into 20 samples of one second each, and classified each sample to one of the individuals in the training set. The proportion of samples in the testing set that were classified to the correct individual were again calculated for each individual, and then compared with the correct classification rate that would be expected by chance, as outlined previously. The correct classification rate expected by chance for each individual was again set at the proportion of that individual’s calls in the training set.

The original uncleaned versions of the calls used were then cleaned using the same high frequency removal procedure in Audacity, but replacing the spectral subtraction noise removal method with a Weiner filtering method using MATLAB. Fox (2008) achieved higher levels of correct classification by using vocalisations cleaned using the Weiner filtering method rather than the spectral subtraction method. Any remaining areas of noise were removed manually in Audacity, then the calls were grouped into single sound files for each individual and silence was removed as previously. The training and testing procedure used previously was then repeated using the Weiner-filtered calls.

Prior to conducting the final analyses, initial testing was also conducted to determine the optimal procedure for correctly classifying bittern vocalisations. Three methods of feature extraction were compared, using either linear prediction cepstral coefficients (LPCCs), perceptual linear prediction cepstral coefficients (PLPCCs), or mel-frequency cepstral coefficients (MFCCs), in addition to determining the number of cepstral coefficients to use. For detailed explanation of these features, refer to Fox (2008) or Fox *et al.* (2008). Testing was conducted using the procedure outlined above for the final analyses, using a small subset of calls available.

In order to investigate calling patterns through the night, spectra of the full night ARU recordings were examined in Audacity, and Australasian Bittern vocalisations were counted for each hour manually through visual inspection of the spectrogram. The use of automated recognisers developed using Song Scope Bioacoustics Software (Version 4.0; Wildlife Acoustics 2011a) was tested, but the recogniser with the highest detection rate achieved only detected 50 % to 75 % of the boom trains that were detected by manual searching. The proportion of calls detected for each hour, out of the total number of calls detected for that night, was then calculated. This allowed for more comparable data for each night, as absolute counts for each night were subject to variation through differences in the total number of calls detected, and differences in the numbers of birds detected calling.

Basic statistical testing throughout was undertaken in R Stats. Where necessary, data was log-transformed prior to statistical testing to satisfy normality assumptions. If multiple comparisons were made, the family-wise error rate was controlled by using Bonferroni corrections in cases where comparisons were not independent, or more powerful Dunn-Šidák corrections in cases where comparisons were independent.

Results

Vocalisations

The majority of Australasian Bittern boom trains recorded across all study sites during field recording sessions comprised four booms, accounting for 66.2 % of the total boom trains recorded. 20.1 % of boom trains recorded comprised three booms. One bird calling along the Muir Highway was regularly recorded giving boom trains consisting of five booms; these accounted for 64.1 % of the boom trains recorded from this bird, but only 12.0 % of boom trains recorded overall. Several birds occasionally gave boom trains consisting of two booms (1.0 % of boom trains overall) and a bird along Muir Highway was recorded giving several boom trains comprising six booms. Exceptionally, a bird at Manypeaks was recorded giving a single boom train of seven booms, and a bird along Muir Highway was recorded giving a single boom train of 10 booms. No single booms were recorded during field recording sessions; however single-boom boom trains were occasionally detected on the ARU recordings.

Two types of boom were detected (Figure 3), that were distinguishable both audibly and by examination of the spectra. Poor booms sounded clipped when heard, but exhibited a more complex frequency structure (Figure 3). The peak frequency of poor booms was also marginally lower than those of typical booms; though small, this difference was statistically significant (Table 2). Poor booms comprised 9.9 % of the total number of booms analysed.

Booms were detectable over significant distances. On several occasions, bitterns were heard calling from distances up to five kilometres away, and on one occasion it is strongly suspected that a bird was heard calling from almost nine kilometres away (Table 3).

Table 2: Summary of peak frequency data, in Hz, for gasps, typical booms and poor booms

Difference in mean peak frequencies for typical booms and poor booms significant – t-test on log-transformed values assuming unequal variances, p=0.0025.

	Mean (± st. dev.)	Minimum	Maximum
Gasps (n=458)	189.0 ± 7.3	174.3	211.3
Typical booms (n=619)	160.6 ± 7.2	147.4	186.4
Poor booms (n=68)	156.6 ± 10.7	128.5	175.0

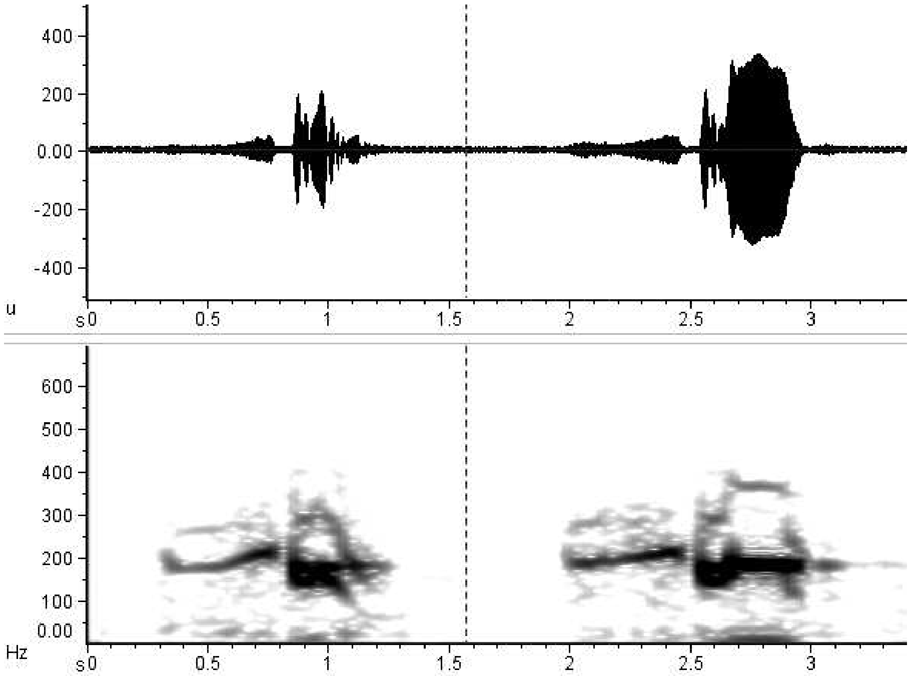

Figure 3: Comparison of waveform (upper graph) and spectrogram (lower graph) for poor boom (left) and typical boom (right), showing the more complex frequency structure of poor boom. Both booms given by same individual, recorded at Kulunilup Swamp.

Table 3: Distances between observer location and estimated location of calling bird for selected instances of long distance detections.

Location of calling birds was estimated by moving in the direction of the calls gradually, listening from multiple points for triangulation, until close to calling bird. In the instance of the Buranganup Swamp record, triangulation and assessment of intervening habitat were used to estimate the bird's location, but observers did not confirm the bird's location with certainty. Estimated distances were measured using Google Earth (Version 7.1.2; Google 2013).

Date	**Observer location**	**Estimated calling location**	**Approximate distance (km)**
28/10/2013	Unicup Hall	Kulunilup Swamp	3.5
30/10/2013	Unicup Hall	Kulunilup Swamp	3.5
04/12/2013	Geordinup Rd	Tordit-gurrup Lagoon	4.0
02/11/2013	North Sister East	Lake Pleasantview	4.7
21/11/2013	Geordinup Swamp	Poorginup Swamp	5.2
30/10/2013	Buranganup Swamp	Muir Hwy site	9.0

Discriminant function analysis

Nested ANOVA analysis indicated 20 of the measured variables exhibited significantly high levels of inter-individual variation (Table 4). Correlations between many of these variables were high; after taking correlations into account, five variables were selected that showed relatively low levels of correlation with each other (Table 4).

Table 4: Variables showing significant levels of inter-individual variability

Significance determined by comparing inter-individual p-values with the Bonferroni-corrected critical p-value of 0.00185. The five variables listed in italicised bold are those used in the final DFA analyses.

Variable	Partitioning of variance (% of total)			P-value (Inter-individual)
	Intra-bout	*Inter-bout*	*Inter-individual*	
N_{B}	38.3	16.6	45.1	0.0012
$L_{1\text{-}2}$	9.9	11.1	78.9	<0.0001
$L_{2\text{-}3}$	19.9	12.5	67.6	<0.0001
PF_{B1}	21.6	18.2	60.2	<0.001
CF_{B1}	17.3	18.6	64.1	<0.001
$F95_{B1}$	9.9	24.3	65.8	<0.001
$F3Q_{B1}$	7.9	19.7	72.4	<0.001
PF_{G2}	27.1	19.7	53.2	<0.001
CF_{G2}	20.1	22.3	57.6	<0.001
$F95_{G2}$	24.2	18.8	57.0	<0.001
$F3Q_{G2}$	18.7	21.6	59.6	<0.001
PF_{B2}	22.5	19.4	58.1	<0.001
CF_{B2}	16.4	22.4	61.2	<0.001
$F1Q_{B2}$	28.6	17.9	53.5	<0.001
$F3Q_{B2}$	9.2	26.2	64.6	<0.001
$F95_{G3}$	21.1	19.5	59.4	<0.001
$F3Q_{G3}$	25.5	21.2	53.3	0.0011
$BW90_{G3}$	33.6	11.9	54.5	<0.001
CF_{B3}	14.6	27.0	58.3	0.0016
$F1Q_{B3}$	30.3	21.2	48.5	0.0017

Quadratic discriminant function analysis conducted using all cleaned vocalisations, and using vocalisations from all calling bouts for both training and testing, produced an overall correct assignment rate of 92.6 % (50 of 54 test calls correctly assigned), with a mean correct assignment rate of 92.0 % (Table 5). This was significantly higher than the overall level of correct classification that would be expected based on chance alone (binomial test, $p < 10^{-32}$).

Correct classifications for individual birds ranged from 71.4 % to 100 % (Table 5), and correct classification levels for all individuals were significantly higher than would be expected based on chance alone (binomial tests with Dunn–Šidák correction, corrected critical value = 0.0085; $p < 0.002$ in all cases).

A second analysis using the same vocalisations, but testing with calls from different bouts to those used for training, resulted in an overall correct assignment rate of 70.4 %, with a mean correct classification rate of 63.8 %. This was significantly lower than the correct classification rates achieved with training and testing from the same bouts (Table 5), but was still significantly higher than would be expected based on chance alone (binomial test, $p < 10^{-25}$). Correct classification rates for individuals ranged from 28.6 % to 100 % (Table 5).

Table 5: Proportion of test calls assigned to the correct individual using quadratic discriminant function analysis

Training and test calls taken from the same bouts, and then from separate bouts. As the numbers of test calls per individual were not equal, the overall proportion of test calls correctly assigned is not equal to the mean proportion across individuals

Individual	**Training and test calls**	
	Same bouts	*Different bouts*
Cheynes Swamp 1	0.93 (n=15)	1.00 (n=22)
Manypeaks 1	0.88 (n=8)	0.29 (n=14)
Kulunilup Swamp 1	1.00 (n=7)	0.43 (n=7)
Muir Hwy 1	1.00 (n=10)	0.94 (n=18)
Lake Pleasantview 1	1.00 (n=7)	0.50 (n=6)
Lake Pleasantview 2	0.71 (n=7)	0.67 (n=14)
Mean (± st. dev.)	0.92* ± 0.11	0.64* ± 0.29
Overall	0.93	0.70

* Means significantly different – paired t-test on log-transformed values assuming unequal variances, p=0.039.

Neural network approach

Initial testing using this approach, using calls cleaned using spectral subtraction, produced mean correct classification percentages ranging from approximately 40 % to 75 % depending on the type of feature extracted, and the number of cepstral coefficients used. MFCCs and PLPCCs performed significantly better than the LPCCs (Table 6). Overall, the MFCCs and

PLPCCs did not perform significantly differently from each other, though their classification rates for particular individuals varied significantly in some instances (Table 6).

Using calls cleaned by Weiner filtering rather than spectral subtraction increased correct classification rates slightly; however, the differences were not statistically significant (Table 6). Increasing the number of cepstral coefficients used further increased mean correct classification rates, up to 60 cepstral coefficients (Figure 4). However, the gains achieved by increasing the number of cepstral coefficients beyond 30 were minor (Figure 4).

Table 6: Comparison of the proportion of calls correctly classified using three different features, and two different cleaning methods

A two-factor ANOVA analysis on log-transformed values indicated no significant difference between spectral subtraction and Weiner filtering (df=1, F=0.238, p=0.63), but significant differences between some features (df=2, F=3.805, p=0.034). Superscript letters indicate significant differences according to pair-wise paired t-tests on log-transformed values – significant differences indicated by p-value ≤ Bonferroni-corrected critical p-value of 0.003.

	Spectral subtraction			**Weiner filtering**		
Individual	*PLPCC*	*LPCC*	*MFCC*	*PLPCC*	*LPCC*	*MFCC*
Cheynes Swamp 1	0.65	0.30	0.50	0.90	0.00	0.85
Manypeaks 1	1.00	1.00	1.00	1.00	1.00	1.00
Kulunilup Swamp 1	0.95	0.05	0.05	0.70	0.10	0.45
Muir Hwy 1	0.10	0.05	0.90	0.15	0.00	1.00
Lake Pleasantview 1	0.80	0.75	1.00	1.00	0.95	1.00
Lake Pleasantview 2	1.00	0.40	1.00	0.95	0.65	1.00
Mean	0.75^{a}	0.40^{b}	0.74^{a}	0.78^{a}	0.45^{b}	0.88^{a}
Standard Deviation	(0.35)	(0.38)	(0.39)	(0.33)	(0.47)	(0.22)

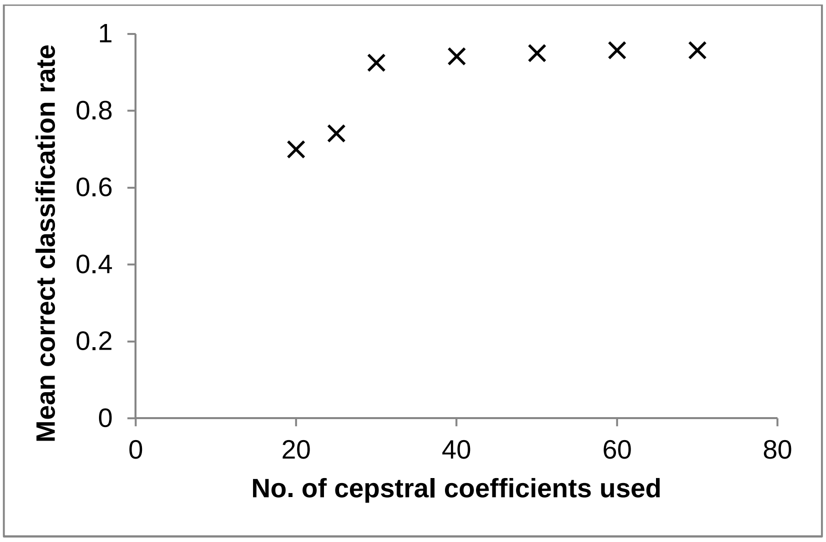

Figure 4: Variation in the proportion of calls correctly classified using the neural network approach with variation in the number of cepstral coefficients used in the training and testing procedures.

The final analysis, conducted using MFCCs, Weiner-filtered calls and 60 cepstral coefficients, produced an overall correct classification rate of 96 % when training and testing samples were taken from the same bout (Table 7). This fell to an overall rate of 71 % when using training and testing samples from different bouts. Both these classification rates were significantly higher than would be expected due to chance alone (binomial tests, $p < 10^{-80}$ for training and testing from same bouts, $p < 10^{-38}$ for training and testing calls from different bouts). Correct classification rates for individuals ranged from 75 % to 100 % when training and testing calls from the same bout were used, and 55 % to 100 % when training and testing calls from the same bout were used (Table 7). The proportions of calls correctly classified for each individual were again significantly greater than would be expected due to chance in all cases (binomial tests with Dunn–Šidák correction, corrected critical value = 0.0085; $p < 0.00002$ in all cases)

Table 7: Proportion of test samples assigned to the correct individual using neural network approach

Training and test samples taken from the same bouts, and then from separate bouts. n=20 for each individual. As 20 samples per individual were tested in these analyses, the mean proportion correctly classified is equivalent to the overall proportion correctly classified (contra the results of the discriminant function analyses; see Table 3).

Individual	**Training and test samples**	
	Same bouts	*Different bouts*
Cheynes Swamp 1	1.00	0.80
Manypeaks 1	1.00	0.60
Kulunilup Swamp 1	0.75	0.55
Muir Hwy 1	1.00	0.70
Lake Pleasantview 1	1.00	0.60
Lake Pleasantview 2	1.00	1.00
Mean^ (± st. dev.)	0.96* ± 0.10	0.71* ± 0.17

* Means significantly different – paired t-test on log-transformed values assuming unequal variances, p=0.005

Comparison of approaches

No significant differences in mean correct classification rates were found when comparing the neural network and discriminant function approaches (Table 8; Figure 5). The most notable distinction between the two methods was the increased variance in the proportion of test samples correctly classified when using the discriminant function approach.

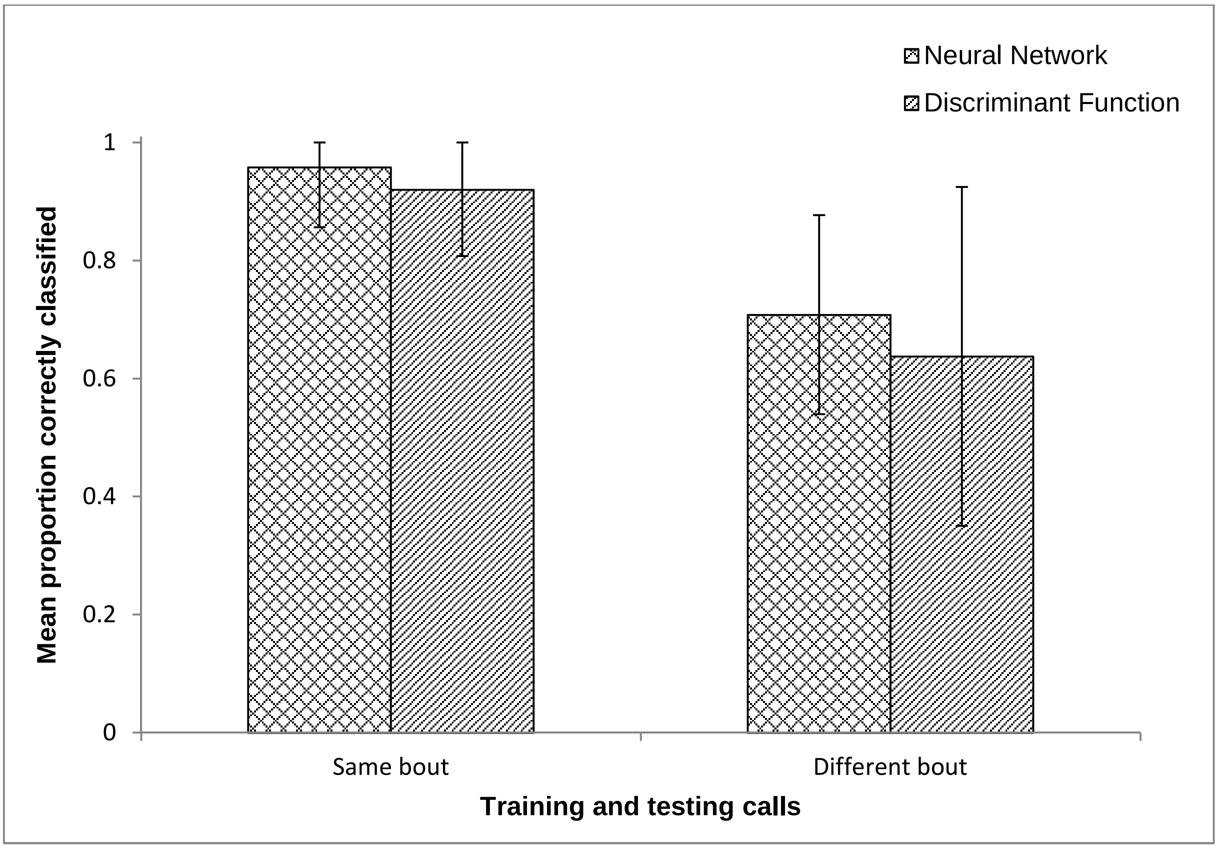

Figure 5: Comparison of mean correct classification rates for neural network and discriminant function approaches, with training and testing using calls from the same bouts, or calls from different bouts. Error bars indicate standard deviation, with upper limits constrained to 1.

Table 8: Summary of results from two-factor ANOVA on log-transformed correct classification rate data.

Asterisk (*) indicates significant effect

	DF	F-value	p-value
Method (Neural network or discriminant function)	1	0.650	0.430
Bout (Same or different)	1	12.426	0.002*
Method x Bout Interaction	1	0.121	0.732

Calling patterns

Calling patterns throughout the night showed a twin peak in calling activity, with a strong peak in calling activity close to dawn and a more minor peak in activity in the first hour after sunset (Figure 6). There were small variations in the hourly proportion of calls between the four sites; however the overall pattern is generally similar. The most notable difference was the relatively low proportion of calls given during the first hour after sunrise by the Muir Hwy birds.

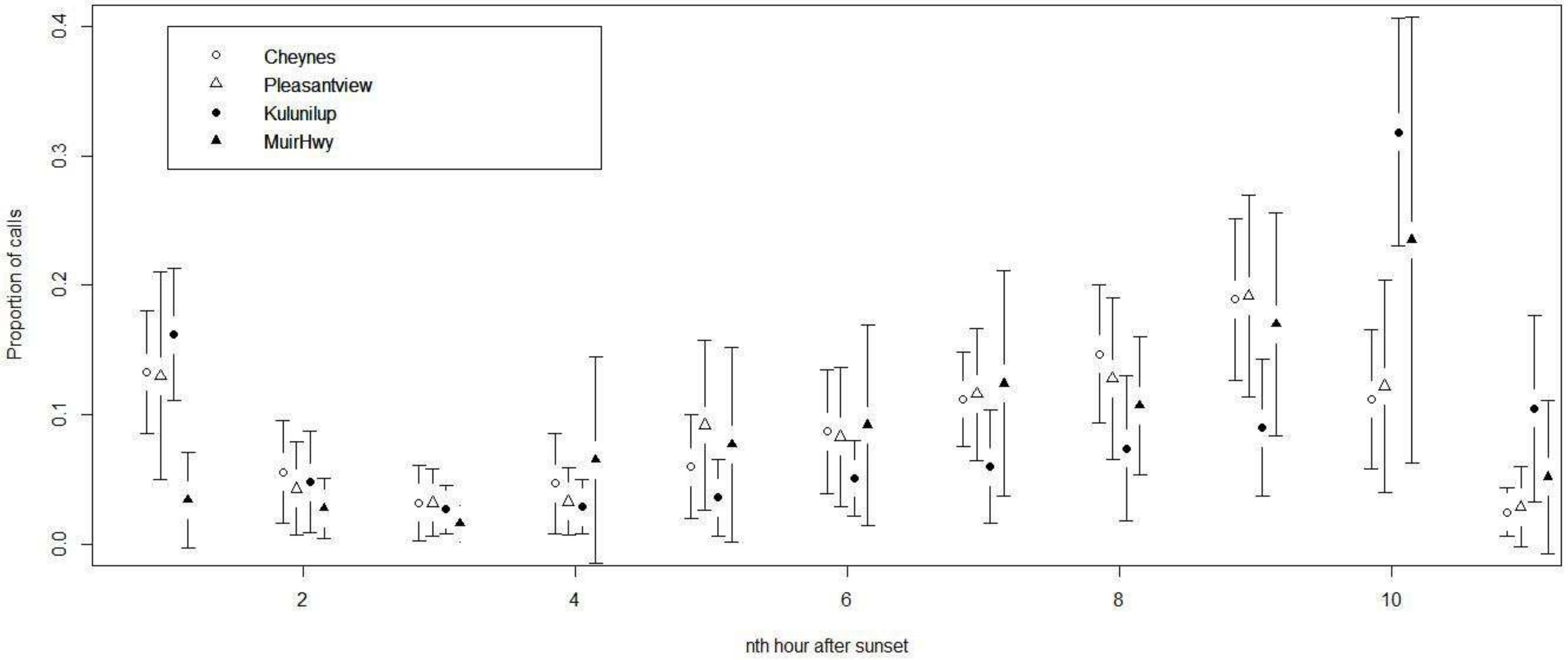

Figure 6: Mean proportion of total calls recorded during the night recorded during each of the 1st to 11th hours after sunset at four study locations. Sunrise fell from 9 hr 21 min to 10 hr 40 min after sunset during the Cheynes Swamp and Lake Pleasantview recordings, from 10 hr 33 min to 10 hr 43 min after sunset during the Kulunilup Swamp recordings, and from 10 hr 01 min to 10 hr 34 min during the Muir Highway recordings.

Discussion

The results of this study supported the hypothesis that Australasian Bitterns show individuality in their vocalisations, as both quadratic discriminant function and neural network approaches produced correct classification levels significantly higher than would have been expected by chance alone. Very high levels of correct classification were achieved using both methods when training and test calls from the same calling bouts were used, indicating that individuality present is significant enough to allow discrimination between individuals on a given night with a high degree of certainty. Correct classification rates were lower when test calls from different bouts were used; however the overall correct classification rates were still significantly higher than predicted by chance alone, indicating individuality was still detectable. This is not surprising, as many previous studies of vocal individuality demonstrated some level of vocal individuality in a variety of species (e.g. Grava *et al*. 2008; Policht *et al.* 2009; Leliveld *et al*. 2011), including the closely related Great Bittern in Europe (Gilbert *et al.* 1994; Puglisi and Adamo 2004).

There are a number of reasons why vocal individuality may exist within a species. The success of the neural network approach indicates that in this case at least some variability is attributable to physical differences in vocal tract morphology (Fox 2008). Investigating other potential reasons for individuality, and the information about individual bitterns that may be encoded in their vocalisations, was beyond the scope of this research; however, this does provide a potential area for future research. Vocalisations may carry information about the fitness of individual Australasian Bitterns. Little is known about physical conflict over territories in the Australasian Bittern, although Williams (1985) describes males as being aggressive towards other males. Male Great Bitterns are reported to fight violently during territorial disputes (Voisin 1991), with such conflicts sometimes resulting in death for the losing combatant (Percy 1951). It would make sense for males to avoid such conflicts where possible, and as vocalisations appear to be the primary means of territorial advertisement in the species, it is conceivable that they may carry information on the strength and fitness of a particular individual. This has been shown in other species (e.g. Behr *et al*. 2006), however such information may be conveyed by differences in call rate and time spent calling (e.g. Hedrick 1985; McComb 1991) which would not have contributed to the individuality detected in this study. Any vocal individuality as a result of vocalisations reflecting male fitness would likely vary over longer time periods with variation in male condition, although whether this would preclude long-term identification of individuals by researchers would depend on the proportion of overall individuality in vocalisations that reflects individual condition.

I consider kin recognition a less likely explanation for vocal individuality in Australasian Bitterns, although it is possible that male booms could contain information to aid females in choosing non-related males for breeding. Kin recognition through vocalisations has been found more often in parent-offspring interactions (e.g. Aubin and Jouventin 2002) and within social species (e.g. Sharp *et al*. 2005). There is limited evidence of females recognising male kin through advertising calls to avoid inbreeding; however, this has been demonstrated in frog species (Waldman *et al*. 1992) and cannot be ruled out as an explanation for individuality in Australasian Bittern vocalisations. A more likely explanation may be that vocal individuality could allow neighbouring male bitterns to recognise each other, allowing for differential responses to known neighbours and unknown individuals. Several other territorial bird species have been shown to recognise the calls of their territorial neighbours, and react differently to recognised neighbours as compared to new individuals (e.g. Yasukawa *et al*. 1982; Stoddard *et al*. 1990). It is hypothesized that this allows territory holders to defend territories more efficiently by reducing the energy expended on territorial defence and allowing individuals to focus more on new individuals which pose a greater threat of territorial takeover (Temeles 1994). Such neighbour-recognition is typically found where individuals are defending breeding territories, rather than feeding territories (Temeles 1994), which is the case in Australasian Bitterns.

The proportion of calls correctly classified using both techniques was significantly lower when calls from different bouts were used for training and testing, compared to when calls from the same bouts were used. This suggests that there may be sufficient intra-individual variation in vocalisations within a season to reduce the practicality of repeated individual recognition. Similar results have been obtained in a number of other studies (e.g. Reby *et al.* 2006; Matrosova *et al*. 2009), most pertinently on Great Bitterns in Italy (Puglisi and Adamo 2004). However, there are several potential confounding factors which are also likely to have influenced between-bout variability**.** First, recordings of the “same” bird made on different dates were often subject to different recorder-to-subject distances, and different environmental conditions (e.g. wind and temperature), which could introduce variability in some characteristics through differing levels of degradation or interference with the signal (Ingard 1953; Hartwig 2005). In addition, the relatively low recording level, and low signal-to-noise ratio of the recordings used may also have reduced the success of classification; low signal-to-noise ratios, and particularly variation in signal-to-noise ratios, have been shown to decrease classification accuracy (Fox 2008).

The low number of individuals' used in this study is likely to have resulted in higher levels of correct classification than if a greater number of individuals had been tested. As the number of individuals tested increases, the probability of overlap within the sample space of each characteristic increases, in turn increasing the likelihood of incorrect classification (e.g. Fox 2008). Ideally, a greater number of individuals' calls would have been recorded and analysed; however it should be noted that this is a preliminary study, and the time and number of birds calling restricted the number of individuals that could be sampled. Nonetheless, it is important to take the effect of the small sample size into account before generalising conclusions on the effectiveness of vocal individuality for individual discrimination to larger bittern populations.

In addition to the low sample size, it is also possible that differences in recording environments between sites could have enhanced differences between individual birds. Mismatched noise between recordings of the same individual often results in reduced rates of correct classification, as previously noted (e.g. Fox 2008). However, the presence of a greater mismatch in noise between recordings of different individuals than within different recordings of the same individual could result in increased rates of correct classification, as classification may be based on similarities in background noise rather than vocalisations themselves. Similarly, differences in surrounding vegetation and terrain could affect the acoustic characteristics of the recording environment, and result in differences in recorded sound signals (Eyring 1946; Albert 2004). Any effect of mismatched additive noise should have been significantly reduced by the use of noise reduction prior to analysis, but any effects of differing acoustic environments would be less likely to be mitigated by noise reduction. The presence of two individuals in Lake Pleasantview, whose calls were still classified correctly in analyses, suggests this is not likely to have been a serious factor at that location but does not rule out minor impacts, nor preclude significant impacts at other locations.

Conversely, whilst the low sample size and differences in recording environment between locations may have contributed to artificially increased levels of correct classification in this study, improvements to the both recording and analysis methods could yield genuine increases in correct classification rates compared to those achieved in this study. While the level of background noise was successfully lowered to very low levels in most recordings used, variation in recorder-to-target distances between different recording sessions was significant in some cases, both because the distance to the target bird could only be estimated based on qualitative assessment in the field, and because in some instances the location of the recorder (and to a lesser extent the calling birds) varied between different recording sessions. Since vocal signals, and individuality encoded within them, can degrade as they travel through the atmosphere (Hartwig 2005), this variation in recording distance is likely to have increased variation in recorded vocalisations, and hence may have lowered the rate of correct classification.

The characteristics measured for the discriminant function approach were restricted to temporal and frequency characteristics, as the relatively high variability in recorder to subject distance in this study would most likely have rendered amplitude characteristics unreliable (e.g. Richards and Wiley 1980). However, the use of amplitude variables in addition to frequency variables has recently been shown to increase correct classification levels compared to correct classification rates achieved using frequency variables alone (Root-Gutteridge *et al.* 2014a). If recorder-to-target distances were more effectively controlled, this could allow the use of amplitude variables in discriminant function analyses, which may in turn result in increased levels of correct classification. The use of radio or GPS transmitters to confirm the identity of birds in a longer term study could also allow for greater control over recording distances.

A comparison of the two classification approaches used in this research indicated little difference in effectiveness with regard to mean and overall correct classification rates. However, when testing calls were taken from different calling bouts to training calls, the neural network approach produced less variation in correct classification rates between different individuals compared to the discriminant function analysis, and also correctly classified a majority of samples to the correct individual for every individual tested. Conversely, the discriminant function approach assigned a majority of test calls correctly in only four of the six study individuals. In practical terms, the neural network approach is also more efficient, but required access to MATLAB and more user training. On the basis of this project, the neural network approach is recommended for future use; however DFA is a very adequate alternative which should not produce significantly inferior results.

This study indicates that individuality does exist in the booming calls of Australasian Bitterns, and that this variation is sufficient to distinguish between individuals. However, significant further work is required before it can be determined whether vocal individuality is an effective tool for longer term individual identification. First and foremost, a longer term study utilising an independent method of individual identification is required to determine whether inter-individual variation is stable enough over time to allow longer term identification of individuals. In addition, the methods used for this study could be further refined, both when obtaining field recordings, through reduced recorder-to-subject variation and increased recording levels, and during analyses, through investigating the use of additional characteristics in DFA and possibly the use of improved noise reduction methods. Investigating any age-related variation in vocalisations may also be valuable; age-cohort based vocal characteristics have recently been demonstrated in elephants (Stoeger *et al.* 2014), and a similar result in bitterns could provide valuable data on the age profile of populations, and recruitment levels of young males into populations. Further investigation of the use of ARUs with respect to identifying individuals would also be valuable, as these provide a less labour-intensive method of obtaining recordings of bittern vocalisations.

In addition to the requirement for a longer study on the stability of individual characteristics in vocalisations, there are other potential shortcomings to using vocalisations for long-term individual identification in the Australasian Bittern. Firstly, as it is generally considered that only male Australasian Bitterns boom (Marchant and Higgins 1990), as is the case in Great Bitterns (Cramp and Simmons 1977), any life history information determined using vocal individuality for individual identification may not be applicable to females or younger individuals. This is an issue with the use of vocal individuality that needs to be considered in any species where particular subsets of individuals are more vocally active than others (Terry *et al*. 2005). In addition, Australasian Bitterns boom for only a few months each year during the breeding season, so the use of vocal individuality for tracking individuals' movements would provide information on breeding site fidelity, but not on post-breeding movements.

Given the potential challenges and shortcomings of individual identification using vocalisations that have been discussed, it is recommended that supplemental methods of individual identification also be considered. Recent trials using automated motion-sensing cameras placed in swamps have successfully photographed Australasian Bitterns on a number of occasions (A. Clarke, pers. comm.). The use of such cameras may improve the feasibility of visual techniques for individual identification; photographs taken by camera traps have been successfully used for individual identification in several other species (e.g. Trolle and Kery 2003; Hohnen *et al*. 2012), although as yet there are few examples of their use with bird species. This approach could be particularly useful if naturally-occurring plumage patterns are able to be used, avoiding the need to capture individuals for marking, although Whiteside (1989) briefly mentions being unable to distinguish individual Australasian Bitterns from photographs.

Calling patterns through the night determined from ARU recordings in this study broadly supported qualitative impressions of peak calling activity times in the field, in that calling rates were relatively high for the first hour or so after sunset, before decreasing to very low levels for a few hours, and then increasing again towards midnight. However, there was also noticeable peak in calling activity approximately corresponding to sunrise, which had not been noted during field work. Recorded calling patterns of the Great Bittern in Britain show a similar dawn peak (Gilbert *et al*. 1994), so this result is relatively unsurprising given the close relationship between the species. Marchant and Higgins (1990) also describe dawn and dusk as periods of peak calling activity for Australasian Bitterns, although there is no mention of higher calling rates during the dawn peak compared to the dusk peak.

Aural surveys conducted in south-western Australia by BirdLife Western Australia have typically been targeted at the period from sunset until as late as 10 pm (Pickering 2013). Whilst birds were detected calling at all times throughout the night, the results of this study indicate that pre-dawn surveys are likely to be more effective than evening surveys for determining the presence of calling birds in a wetland, especially if surveys of several hours duration are planned, for example to encompass multiple locations consecutively, as calling rates in the 2nd to 4th hours after sunset were the lowest of any one-hour periods during the night. However, it should also be acknowledged that pre-dawn surveys are likely to be less optimally timed for maximising volunteer involvement.

In recent years, deployment of automated recording units (ARUs) for the purpose of detecting bittern presence has increasingly been investigated as an alternative to observers listening for birds in the field. The duration of time for which an ARU records each night impacts on the total number of nights that can be recorded with a given amount of data storage space and also increases the battery use per night, reducing the number of nights that can be recorded without battery replacement (Wildlife Acoustics 2011b). Hence, if the objective is simply to determine the presence of calling bitterns in an area, maximising the likelihood of achieving this whilst minimising the recording time per night reduces battery costs and improves efficiency. The results of this study indicate that recording for the hour before sunrise is likely to be the most effective for detecting the presence of calling bitterns.

The two types of boom detected during this study do not appear to have been widely described previously in the Australasian Bittern. In particular, there is limited reference to the poor booms, which were regularly produced by a number of individuals in this study, and have been noted in previous seasons in south-western Australia (personal observations). Marchant & Higgins (1990) mention that birds may become hoarse during the calling season, which may refer to the poor booms described here. However, poor booms were made throughout the calling season, and do not appear to become more prevalent as the season progresses (though this requires further testing); hence, I prefer the use of the term 'poor boom', following several European authors (e.g. Gilbert *et al.* 1994; Puglisi *et al*. 2001). The presence of poor booms is well-described for the Great Bittern (e.g. Gilbert *et* al. 1994; Polak 2006), and is suspected to relate to seasonal development of the oesophagus for booming (Gilbert *et al*. 1994). It is likely that the same explanation applies to poor booms in the Australasian Bittern.

It is also of interest to note the distance over which birds could be heard by observers on some occasions during fieldwork. Published sources typically describe the calls as being audible from up to one kilometre away in good conditions (e.g. Marchant and Higgins 1990), and the maximum recorded distance over which a calling bird was detected during BirdLife Western Australia surveys in the south-west is reported as 1.8 km (Pickering 2013). Observations during the fieldwork for this project indicate these figures are a significant underestimate. On several occasions during fieldwork birds, were heard calling from four to five kilometres away, and on one occasion a bird was strongly suspected to have been heard from almost nine kilometres away. Whilst these observations may seem to be an interesting aside rather than a result of real importance, the distance over which calls can be heard is an important consideration for interpreting results from aural surveys, especially as several key areas for Australasian Bitterns include multiple wetlands within relatively close proximity to each other. Underestimating the distance over which birds can be detected by observers could result in overestimates of the number of birds being detected, and lead to incorrect conclusions about the presence of bitterns in particular wetlands. Similar caution needs to be exercised when analysing recording data from ARUs, as ARUs are likely to be able to record birds over similar, if not greater, distances. A more rigorous investigation into the distances over which Australasian Bittern calls can be detected is also urgently needed given these results and the importance of aural surveys to the ongoing management of the species.

A further interesting result was the detection of two separate birds calling in both Lake Pleasantview and Cheynes Swamp. In each case, on one occasion both birds were heard concurrently to confirm the presence of two individuals (on that particular night at least). Based on identification by calling location, both individuals in Pleasantview remained present for most of the season; this was further confirmed as test calls from both individuals were consistently classified correctly by both classification techniques. However, on all other nights surveyed at these locations, only one individual was detected calling. This suggests that non-calling males may be present in wetlands, which is an important consideration if surveying populations by counting the number of individuals calling. While the second individual at Lake Pleasantview appears to have been present throughout the season, the second individual at Cheynes Swamp was only detected with certainty on one occasion, and could be an example of a ‘floater’, as described by Eriksen (1999) in the Great Bittern.

In summary, Australasian Bitterns do exhibit vocal individuality, and the level of individuality is sufficient to allow discrimination between individual Australasian Bitterns within small populations over short time periods. However, further research and development is required before vocal individuality could be used for individual identification over longer time periods. Australasian Bitterns were detected calling at all times throughout the night on occasion, but peak calling activity occurred in the hour before sunrise, suggesting that pre-dawn surveys are likely to be the most effective for detecting calling birds.

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Appendix 1 – Survey locations

Table A1: Summary of locations surveyed for calling Australasian Bitterns during this project, indicating the number of calling Australasian Bittern detected over the course of fieldwork, and whether recordings were obtained from the location. Australian Little Bitterns (*Ixobrychus* [*minutus*] *dubius*) occur in many of the same wetlands as Australasian Bitterns and records of this species are also included for information

Locations	Australasian Bittern	Australian Little Bittern	Recordings obtained
Muir-Unicup Wetlands			
Kulunilup Swamp	1	3	Yes
Muir Hwy, just E of Cowerup Rd	2	-	Yes
Cobertup Swamp	-	1 possible	-
Kodjinup Nature Reserve	-	-	-
Geordinup Swamp	-*	3+	-
Byenup Lagoon	1	-	-
Tordit-gurrup Lagoon	2^	-	-
Poorginup Swamp	1	-	-
Albany area			
Cheynes Swamp	2	-	Yes
Waychinicup turn-off swamp	-	-	-
Lake Pleasantview	2*	-	Yes
Manypeaks Swamp	1*	-	Yes
North Sister East	-*	-	-
North Sister West	1*	-	-
Pfeiffer Rd Swamp	-*	-	-
Walpole-Denmark area			
Boat Harbour Swamp	-	-	-
Esperance (Cape Le Grand NP) area			
'Big Boom Swamp'	-	2-3	-
Swamps near Cape Le Grand Rd	-	-	-
Dunns Rock swamps	-	1-2	-
Frenchmans Peak swamps	-	-	-

* Birds from other wetlands were heard from this location, in addition to the number of individuals listed as heard in the wetland

^ The bird subsequently heard in Poorginup Swamp is suspected to be one of these two individuals.

Appendix 2 – Explanation of features measured for DFA

Table A2: Further explanation of features measured for use in discriminant function analyses. For further details on how the Raven software calculates the frequency measures listed here, refer to Charif *et al*. (2010)*.

Measure	Explanation
N_B	The number of booms in a boom train
L_x	The length of time of a particular call note
$L_{x\text{-}y}$	The length of time between the end of the main note of a boom and the commencement of the initial gasp of the following boom
PF_x	The peak frequency of the selected note; that is, the frequency at which the peak amplitude of the note occurs.
CF_x	The frequency value that divides the selected note into two frequency intervals of equal energy (Charif *et al*. 2010)*
$F5_x$	The frequency value that divides the selected note into two frequency intervals containing 5 % and 95 % of the energy in the selection (Charif *et al*. 2010)*
$F95_x$	The frequency value that divides the selected note into two frequency intervals containing 95 % and 5 % of the energy in the selection (Charif *et al*. 2010)*
$F1Q_x$	The frequency value that divides the selected note into two frequency intervals containing 25 % and 75 % of the energy in the selection (Charif *et al*. 2010)*
$F3Q_x$	The frequency value that divides the selected note into two frequency intervals containing 75 % and 25 % of the energy in the selection (Charif *et al*. 2010)*
$BW90_x$	The difference between the $F95_x$ and $F5_x$ values
$BWIQ_x$	The difference between the $F3Q_x$ and $F1Q_x$ values

* Charif R. A., Waack, A. M., and Strickman L. M. (2010). ‘Raven Pro 1.4 User’s Manual’. (Cornell Lab of Ornithology: Ithaca, New York)

Appendix 3 – Research proposal

RESEARCH PROPOSAL

Variation in vocalisations and vocal individuality in the Australasian Bittern (*Botaurus poiciloptilus*) in south-western Australia, with applications for conservation

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Abstract

The Australasian Bittern (*Botaurus poiciloptilus*) is a large, secretive member of the heron family, occurring in densely vegetated wetlands across southern Australia and New Zealand. In south-western Australia, the species has undergone significant declines and is now estimated to have a population size of only 50-150 individuals. Their secretive nature and preference for dense wetland habitat means that listening for the territorial booming calls of males in spring and early summer is usually the easiest way to detect the species. The ability to identify individual male bitterns using their calls is likely to make individual identification significantly easier, and facilitate investigation of other aspects of the species' ecology, including survival, recruitment, and breeding site fidelity, which are important for ongoing conservation and management. However, individuality in the booms of Australasian Bittern has not yet been investigated. During this project, Australasian Bittern calls will be recorded at a number of locations in south-western Australia and analysed to determine whether they do exhibit individual differences, and whether these differences are sufficient for reliably discriminating between individuals. Individuality in vocalisations has been investigated in many animal species, with individual differences in voice detected in almost all cases, most notably in the Australasian Bittern's close relative, the Great Bittern (*B. stellaris*). As a result, differences are also expected to be detectable in the Australasian Bittern. If this is the case, further work to investigate the temporal stability of their vocalisations and individual identification over longer periods can be considered.

Introductory Statement

The Australasian Bittern has undergone significant declines across its range, and as a result was recently listed nationally as Endangered under the Environmental Protection and Biodiversity Conservation (EPBC) Act 1999. However, identifying individual birds is particularly challenging due to their secretive nature, so little is known about important areas of their life history, such as survival rates, recruitment, and breeding site fidelity, hindering management. The most effective method of detecting bitterns is through their deep booming calls made during the breeding season, so the individuality of an individual's calls may provide a relatively simple means of individual identification. Vocal individuality has yet to be formally investigated in this species. However, individuality in vocalisations has been demonstrated in many animal species previously studied, so I expect that this will also be the case for the Australasian Bittern.

Background

Individual identification

The importance of the identification of individual animals within a population for conservation and management is obvious. Individual identification is important for determining life history characteristics, such as individual survival rates, and individual dispersal (e.g. Gilmartin *et al*. 1993; Rudnick *et al*. 2005). Identifiable individuals can also be used to estimate the average home range requirements for the species (e.g. Hill and Grossman 1987; Stander 1991), enhance our understanding of a species' movements and site fidelity (e.g. Parra *et al*. 2006; Anderson *et al*. 2011), and allow for better estimations of population size and density (e.g. Trolle and Kery 2003; Silver *et al*. 2004). These factors in turn impact on population size and viability, which are of critical importance for effective conservation and management (McGregor and Peake 1998).

Traditionally, visual observation has been used for individual identification. This may be achieved by using natural markings already present on the animals, such as distinctive patterning, scars and deformities (e.g. Wursig and Jefferson 1990; Silver *et al*. 2004), or by artificially marking animals using a variety of methods, including the use of tags, bands, colour marks or collars (e.g. Ogilvie 1972; Calvo and Furness 1992; Doligez *et al*. 2004; Robinson *et al*. 2005), and clipping of fur, ears or appendages (e.g. Goto 1985; Braude and Ciszek 1998**)**. Such methods have their drawbacks; many individuals do not have natural marks useful for individual identification, while artificial marks can be lost and their use can impact on the health and survival of marked individuals, or alter their behaviour (Calvo and Furness 1992; Spray and Bayes 1992). The process of artificial marking usually involves the capture and handling of individuals. This can result in stress, injury, and on rare occasions death for the captured animal, and in some cases may present risks to the researcher as a result of attack by the captured animal or transmission of disease (McGregor and Peake 1998). Individuals may also be captured in a non-random way, introducing bias into a study (McGregor and Peake 1998). Capturing individuals for marking, and the use of artificial marks, can also evoke criticism from some elements of the wider community, potentially reducing community support for research being undertaken (Mellor *et al*. 2004). In addition, visual identification can be impractical and expensive in species which are challenging to observe visually (Gilbert *et al*. 1994).

Recent technological advances have increased the utility of microchipping and radio-tagging as non-visual methods of individual identification (e.g. Stander 1991; Elbin and Burger 1994). However, these techniques still require capture and handling of individuals in most cases, and recapturing individuals in many cases of microchipping, which present the same issues as capturing and handling for visual marking. There is also some evidence of deleterious effects of implanted microchips on some microchipped animals (Elcock *et al*. 2001). Additionally, the equipment required for these methods may increase the costs of a project. Genetic data collected using non-invasive sampling techniques such as the collection of shed hair or feathers, and scats is also increasingly being used as a method of individual identification (Sloane *et al*. 2000; Hedmark *et al*. 2004; Rudnick *et al*. 2005). This has a number of advantages over traditional techniques; notably, it does not require capture or handling of the target animals, avoiding associated issues, and does not require sighting of target animals, making it a more feasible method for difficult-to-observe species. However, it can still present challenges in species favouring difficult-to-access habitat, such as swampland, as genetic material still needs to be collected.

The use of individuality in vocalisations has long been recognised as a potential alternative to visual identification (e.g. Beightol and Samuel 1973). This technique is most useful in species with prominent, easily recordable, and relatively invariant vocalisations. Though its primary benefit is likely to be in species where visual recognition is challenging and resource-intensive, using vocalisations may still present a less logistically demanding and less invasive method of identification even where visual identification is practical, as it does not require capture and handling of target animals. It may also provide a useful supplemental method of identification when used in combination with other techniques.

Vocal individuality

Individuality in vocalisations can arise for a number of reasons, and serve a number of purposes for animals. Acoustic cues are often used for kin recognition, particularly in colonial breeding species where they are used by parents and offspring to recognise each other amongst other individuals within the colony; evidence of such behaviour has been detected in species of bats (Scherrer and Wilkinson 1993), penguins (Aubin and Jouventin 2002) and pinnipeds (Campbell *et al*. 2002; van Opzeeland and van Parijs 2004). Vocal individuality may also be used for recognition of mates in a similar manner (e.g. Speirs and Davis 1991; Blumenrath *et al.* 2007), and there is evidence of its use in recognition of territorial neighbours (e.g. McGregor 1993; Hyman and Hughes 2006). Territorial and advertising vocalisations may also provide indicators of an individual's fitness, which can be reflected by features within vocalisations in some cases (e.g. Behr *et al.* 2006), though most research has focussed on calling rate and time spent calling. Some individuality also results from physical differences in individual vocal tract morphology (Fox 2008a), and is likely to be present regardless of the presence of ecological or behavioural reasons for individuality. This individuality is also likely to be more stable as it is a result of individual physical characteristics.

Vocal individuality has been demonstrated across a wide range of taxa, in particular birds, including Rufous Bristlebirds (Rogers and Paton 2005), Corncrakes (Peake *et al*. 1998), two hornbill species (Policht *et al.* 2009), Common Divers (Walcott *et al.* 2006), and several owl species (Otter 1996; Tripp and Otter 2006; Grava *et al*. 2008); and mammals, including Fallow Deer (Reby *et al*. 1998), Harp Seals (van Opzeeland and van Parijs 2004), Swift Foxes (Darden *et al*. 2003), African Wild Dogs (Hartwig 2005), and several primate species (Chapman and Weary 1990; Butynski *et al*. 1992; Leliveld *et al*. 2011). Studies on non-avian and non-mammalian taxa are less common, though there is a relatively extensive body of literature demonstrating individuality in the calls of several frog species (e.g. Rogers 1999; Bee *et al*. 2001; Feng *et al*. 2009), and at least one study on a fish species (Amorim and Vasconcelos 2008). In all studies I have examined in the literature, some level of individual variation in vocalisations was reported, so it seems likely that some level of vocal individuality is present in the majority of vocal species (Terry *et al*. 2005).

Discrimination and identification

Most individuality studies test our ability to accurately discriminate between individuals' vocalisations on the basis of individual variation. This involves using recorded vocalisations from known individuals ('training calls') to develop an assignment model based on individual differences in vocalisations. This model is then used to assign additional recorded vocalisations from known individuals ('test calls') to an individual. The proportion of calls assigned to the correct individual then gives an indication of the level of vocal individuality shown, and effectiveness of the assignment technique. The ability to discriminate between individuals is generally considered to be demonstrated if vocalisations are assigned to the correct individual at a rate significantly greater than would be expected based on chance alone. Thus there is significant variation in the rates of assignment both between studies, and between individuals within studies. Mean rates for correct assignment within studies examined ranged from 67 % up to 100 % (Lengagne 2001; Hartwig 2005). It is worth pointing out however, that a large proportion of such studies use relatively small sample sizes (often $n < 10$), which is likely to reduce the possibility of overlap compared to larger sample sizes and thus increase the likelihood of correct assignment.

Whilst there are many studies demonstrating vocal individuality and the ability to discriminate individuals' vocalisations, there are fewer robust studies demonstrating long-term individual identification using vocalisations. A number of studies draw tentative conclusions based on small numbers of identifiable individuals (e.g. Butynski *et al* 1992); however there are fewer long-term individual identification studies (e.g. Puglisi and Adamo 2004). Similarly, numerous studies in the literature have discussed potential conservation applications of individual identification using vocalisations (e.g. Darden *et al*. 2003; Tripp and Otter 2006). Despite this, there are few examples of its practical use in conservation and management. There are several challenges and shortcomings that should be considered in regards to the use of individual vocal recognition, which explain at least in part the lack of long-term studies and practical use of vocal identification. These include the relative challenge of demonstrating long-term identification in some species, the stability of vocalisations over time, the effects of population size, demographic bias in calling individuals, and the quality of recording required for effective identification.

Superficially, it may appear that individual identification using vocalisations should follow from the presence of vocal individuality, and that discrimination and identification are one and the same. However, this is not the case and there are important differences between the two concepts. Discrimination only requires that individuals' vocalisations differ enough at a given point in time to be separable, but does not necessarily imply an ability to effectively identify individuals over time based on vocalisations, particularly over longer time periods. A certain level of individuality and vocal stability over time is required before vocalisations can be assigned to particular individuals with a high degree of reliability (Terry *et al*. 2005). For example, Matrosova *et al*. (2009) investigated individuality in alarm calls of the Speckled Ground Squirrel (*Spermophilus suslicus*) and found that whilst some individuality was detectable within alarm calls even across long time periods, the degree of individuality was insufficient to allow reliable identification of individuals except over very short periods of time. The ability to identify individuals repeatedly over time is more difficult to demonstrate than the ability to discriminate individuals; it requires longer study periods and, more importantly, an independent method of individual identification so the identity of recorded individuals can be determined with certainty.

The effectiveness of long-term individual recognition using vocalisations can be confounded by instability in an individual's vocalisations. The alarms calls of ground squirrels were able to be effectively identified to individuals only over very short time periods (Matrosova *et al*. 2009), which would make them unsuitable for individual identification. Similarly, Noisy Scrub-birds (*Atrichornis clamosus*) have been shown to change their song types continuously over time, with significant changes sometimes detectable within one month (Berryman 2003). Indeed, instability in an individual's vocalisations has the potential to confound analysis even in situations where vocalisations generally appear to be stable. Research into territorial calls of the Great Northern Diver (*Gavia immer*) in North America found that an individual male's calls were stable from year to year providing they remained within the same territory; however when males changed territories, they also changed their vocalisations (Walcott *et al*. 2006).

In addition, population size can affect the success of individual identification based on vocalisations. As the number of individuals within a population increases, the likelihood of overlap in vocal characteristics between individuals increases. Studies in this area are sparse, but Fox (2008b) found that identification accuracy in canaries steadily fell from 100 % to just over 70 % as the population size increased from two to 40 individuals. As a result, vocal individuality is likely to be most effective for use on relatively small populations.

When considering the use of vocalisations for individual identification, and the subsequent use of individual identifications for the investigation of other demographic factors, it is also important to be aware of bias that may be introduced by relying on vocalisations. In some species, certain subsets of individuals are more vocal and thus easier to identify by their vocalisations, which can introduce bias if life history traits also vary between the vocal and non-vocal subsets of the population. The most obvious example of this is based on sex; in a number of species, particularly bird species, males are far more vocal than females (e.g. Armstrong 1963). This is often particularly so in relation to territorial and advertising calls. If most or all identified individuals are male, then any life history information determined may not apply to female or immature individuals in a population. It is generally considered that the characteristic booming calls of Australasian Bitterns are given by males (Marchant and Higgins 1990), so the issue of bias must be considered if using vocalisations to identify individuals. This does not directly impact on the current study, but is an important consideration in the longer term aims of this research.

Whilst sex-based bias is the most obvious example, other situations can also be conceived; for example it is plausible that in some species dominant adult individuals may be more vocal and thus overrepresented in recorded vocalisations. In such a situation, information determined may not be equally applicable to subordinate or immature individuals. Such biases do not render vocal identification unusable, but it is important to be aware of their existence, or potential existence, when interpreting data or applying results.

Finally, consideration must also be given to the quality of recording required for adequate identification, and the feasibility of obtaining recordings of that quality in a field situation. Recordings with relatively low signal to noise ratios as a result of noisy environments can be very challenging to work with, and can result in decreased success in individual assignment and identification, particularly in situations where background noise is mismatched between recordings (Fox 2008b). There is also the risk that some vocal characteristics used for identification may in fact be characteristics of the background noise in a recording, resulting in recordings being classified to locations with similar sound environments rather than to individual species (Fox 2008b). These effects are minimised by recording in environments relatively free of background noise; however this is often not feasible in a field setting. Fortunately, it is often possible to use various cleaning and filtering techniques to improve the signal to noise ratio of noisy recordings, which can significantly enhance the success of discrimination (Fox 2008b).

Australasian Bittern and their vocalisations

The Australasian Bittern (*Botaurus poiciloptilus*) is a large, secretive bird of the heron family, occurring in south-eastern and south-western Australia, and New Zealand (Marchant and Higgins 1990). The species has declined across much of its range, and was recently listed federally as Endangered under the EPBC Act 1999. In south-western Australia, the species occurs in swamps in the south-west, historically ranging as far north as Moora, but now restricted to the south coast from Gingilup Swamps in the west to swamps in the vicinity of Cape Le Grand National Park in the east, with an additional population in the Muir-Unicup wetlands east of Manjimup and a few birds at scattered localities as far north as Benger Swamp (Serventy and Whittell 1976; Pickering 2013). The population in south-western Australia is currently estimated at 50 to 150 individuals (Pickering 2013). Until relatively recently, the species was regularly recorded at several locations near Perth, with Johnstone and Storr (1998) describing the species as locally common on the Swan Coastal Plain. There are still occasional records from the Perth metropolitan area; most recently a bird was recorded calling strongly at Forrestdale Lake in spring 2011 (D. James pers. comm.; personal observations). However the species appears to have largely disappeared from the Perth area (Pickering 2013).

Botaurus bitterns are largely secretive in habit, favouring densely vegetated wetland habitats (Hancock and Kushlan 1984). The Australasian Bittern is typical in this respect, occurring predominantly in freshwater wetlands with extensive areas of dense reeds or rushes (Marchant and Higgins 1990). In south-western Australia, they are largely restricted to wetlands with large stands of the rush *Baumea articulata* for nesting, and areas of finer sedges for feeding, though they do occur in some wetlands dominated by the introduced rush *Typha orientalis* (Jaensch 1982; Pickering 2013). The combination of their cryptic colouration and behaviour, and preference for dense, difficult-to-access swamp habitat means Australasian Bitterns are rarely seen in south-western Australia; their presence is most easily noted by the deep booming calls of males during the breeding season, which can carry at least one kilometre in good conditions (Marchant and Higgins 1990; personal observations).

Each call consists of a series of ‘gasps’, followed by a series of double-noted booms, the first note of which is shorter and lower in volume. At relatively close range, both the ‘gasps’ and both notes of the booms can be detected. At greater distances, the gasps are no longer audible and only the double-noted booms are detected, while at the greatest distances, only the second, louder note of each boom is usually audible (personal observations). The number of booms per call varies; Marchant and Higgins (1990) suggest up to five but typically three, while personal observations in Western Australia indicate that three or four booms per call is typical, though two and five booms have also been recorded on occasion.

Birds call almost exclusively during the breeding season, and the calls are generally thought to function as advertising and territorial defence, though this does not appear to have been confirmed (Marchant and Higgins 1990). However, booming calls of the closely related

Eurasian Bittern (*B. stellaris*) are known to perform these functions (Polak 2006), and the timing of calling in Australasian Bitterns supports a similar explanation. Calling occurs mostly at night, with the periods around dawn and dusk traditionally regarded as the times of peak calling activity (Marchant and Higgins 1990). However, birds have been recorded calling strongly throughout the night in south-western Australia, and field observations suggest that birds often call strongly and persistently for 3-4 hours before dawn (personal observations). Birds typically call from one site on a given night; however they will occasionally move calling position, possibly in response to disturbance (Marchant and Higgins 1990; personal observations). Birds often call from a similar location throughout the breeding season, and there is some evidence from observations at Cheynes Swamp near Albany that birds will use the same calling site over several years (personal observations).

This study

A number of factors make the Australasian Bittern a strong candidate for individual identification using their vocalisations, and the ability to do so is likely to have applications for the ongoing conservation and management of the species. The difficulty in observing the species in the field means that individual recognition of bitterns by sight is difficult and time-consuming. Visual identification of individuals would almost certainly also require capturing and marking individuals, which is even more time and cost intensive. Conversely, detecting calling birds during the breeding season is relatively easy, and is generally considered the most effective method for determining their presence in a wetland (e.g. Pickering 2013). Furthermore, the birds often call strongly for several hours pre-dawn, a time of day that is often conducive to obtaining relatively high quality sound recordings, and their small population size is likely to make discrimination between individuals more effective. Vocal individuality has already been investigated in the closely related Great Bittern (*B. stellaris*) with some promising results. McGregor and Byle (1992) showed that the booming calls of male Great Bitterns were individually distinctive, a result confirmed by Gilbert *et al*. (1994). However, no similar work has been carried out on the Australasian Bittern.

This project will investigate vocal individuality in the Australasian Bittern in south-western Australia. Specifically, I aim to determine the level of individuality that is detectable, and whether this can be used to effectively assign calls to a specific individual. If this study shows individual birds can be discriminated based on call over short time periods, further research can be undertaken to test for stability over time in individuals' vocalisations, and whether vocalisations can be used for long-term individual identification in the species.

Although Gilbert *et al.* (1994) found that Great Bitterns could be identified individually using their calls, they also found that in some instances, vocalisations were not stable between seasons, restricting the ability to re-identify individuals between seasons. The problem was even more evident in a study on Great Bitterns in Italy, which indicated that the vocalisations of individual males were not stable over time, making ongoing identification of individual bitterns based on their vocalisations problematic (Puglisi and Adamo 2004). Demonstration of vocal stability over time is challenging in the case of the Australasian Bittern, since the very reason that identification of individuals by vocalisation in this species is so attractive is that other methods of identification are particularly challenging. Hence, it is important to determine the feasibility of individual identification before more resources are devoted to investigating long-term stability in vocalisations.

Objectives

The primary objective of this project is to determine whether male Australasian Bitterns show individuality in their booming calls, and whether the level of individuality shown is sufficient to allow effective discrimination between individual males on the basis of their calls. Individuality in vocalisations has been shown in the majority of similar studies on bird species, including the Great Bittern in Europe (Gilbert *et al.* 1994). Hence, I expect that individuality in calls will also be shown in the Australasian Bittern, and that this individuality will be sufficient to allow effective discrimination of individuals. For the purposes of this research, I will consider correct call assignment rates of 90 % or above to allow effective discrimination of individuals. I am unlikely to be able to draw strong conclusions about the value of calls for re-identifying birds over longer time periods within this project due to time contraints. However if effective discrimination is demonstrated, it will provide justification for a longer term study on vocal stability and long-term individual identification.

In addition to my primary objective, I will also investigate some practical aspects of individual identification by call. This will include comparing techniques for individual recognition (discriminant function analysis and voice recognition software), comparing autonomous recording units with observer-operated units, and examining the effect of proximity to the bird and quality of recording required for recognition. As the voice recognition approach bases identification on individual differences in vocal tract morphology, which I expect to be quite stable over a season, I expect this technique to achieve greater rates of correct assignment compared to discriminant function analysis. I expect ARUs to be equally suitable for recording birds for identification in terms of recording quality. However they will most likely need to be setup when birds are calling to allow for placement sufficiently close to a calling bird. Finally, because increasing distance leads to the gradual degradation and loss of components of sounds, I expect that only high quality recordings obtained from as close as possible to the target bird will allow effective individual discrimination.

Significance and outcomes

The primary outcome of this project is expected to be an understanding of the individuality in vocalisations shown by male Australasian Bitterns in south-western Australia, and the extent to which this may be useful for individual identification within the species. If individual discrimination is shown to be feasible, further research on longer term individual identification by call can be undertaken. The ability to identify individuals by call has a variety of applications for the conservation and management of the species in south-western Australia, including refining population estimates, and investigating aspects of the species' ecology including population dynamics and breeding site fidelity, albeit largely limited to male birds. The approach is also likely to be applicable to other populations of Australasian Bittern, aiding conservation and management across the species' range.

Methodology

Study sites and sample size

Recordings of calling bitterns will be collected from as many locations in the south-west as is practical, augmented by recordings from previous seasons obtained by myself and others, and Automated Recording Unit (ARU) recordings provided by the Department of Parks and Wildlife (DPaW). The number of individuals recorded will be dependent on how many calling birds can be located, but is expected to be approximately 10 individuals based on previous surveys and known locations. Ideally, recordings of more birds would be used; however the species' small population size in south-western Australia makes this impractical and similar sample sizes have been effectively used in a number of similar studies on other species (e.g. Butynski *et al*. 1992, Hartwig 2005, Policht *et al*. 2009). A minimum of four separate calling bouts (i.e. four replicates) will be targeted for each individual, though this may not be possible at some sites with difficult access. Identification of individual will be based on location. Unfortunately, this does introduce a level of assumption into the methodology, which will need to be considered when interpreting my results. However, using an independent method of individual identification during this study is not feasible due to the challenges and cost associated with alternate identification methods for this species. As males are territorial, and typically call from a similar location during the season, calls can still be assigned to an individual with a high degree of confidence on most occasions. In addition, some similar studies have also used calling location as the primary method for individual identification (e.g. Rogers and Paton 2005)

Based on surveys conducted over the past five years, the following locations have been identified as the most reliable and accessible locations for recording: Kulunilup Swamp (Muir-Unicup Wetlands, east of Manjimup), Cheynes Swamp, Lake Pleasantview, North Sister Nature Reserve (all east of Albany), ‘Big Boom Swamp’ (Unallocated Crown Land near Cape Le Grand National Park), and Dunn Rock Nature Reserve (east of Esperance). Recording efforts will be concentrated on these locations. The following locations have also hosted bitterns in recent years and may be visited depending on conditions, available time, and access: Forrestdale Lake Nature Reserve (Perth), Benger Swamp (near Harvey), Geordinup Swamp, Tordit-Gurrup Lagoon, Byenup Lagoon, Cobertup Swamp (all in the Muir-Unicup Wetlands), Gingilup Swamps (east of Augusta), Maringup Lake (west of Walpole), Boat Harbour Swamp (between Walpole and Denmark), and swamps in Cape Le Grand National Park.

Recording

Recordings will be made using Fostex FR-2LE and Marantz PMD 660 and 670 recording units, equipped with RØDE NTG-2 shotgun microphones. Shotgun microphones are generally recommended over parabolic microphones for recording low frequency calls such as those of bitterns, as they have a flat frequency response at low frequencies (Terry *et al*. 2005). The RØDE microphones that will be used have an effective frequency range from 20 kHz down to 20 Hz (RØDE 2013), making them suitable for recording bittern calls which typically have a frequency of approximately 200 Hz. All recordings will be made in Waveform Audio File Format (.wav files) to prevent the loss or distortion of components of the sound signal through data compression, which occurs when recordings are made in compression formats such as MP3. Additional recordings are available from ARU recordings from Sarah Comer (DPaW

Albany) and, if required, from Robyn Pickering and my own recordings from previous years (2010 – 2012, seven locations).

Determining the location of a calling bird with high levels of accuracy is not feasible, particularly at night; hence it is not possible to determine exact distances at which recordings are made. A recording unit will be placed as close to a calling bird as possible to obtain the highest quality recordings possible. In some instances, additional recording units will be placed at 250 m and 500 m from the closest recording unit to obtain more distant, lower quality recordings that can be used to investigate the quality of recording required for individual recognition. Recordings of calls will be cleaned using a range of filtering techniques, such as low pass filtering, to eliminate as much background noise as possible, and ensure the signal to noise ratio of recordings is as high as possible.

Statistical analysis

Individual identification will be investigated using two quantitative approaches; the more traditional method of discriminant function analysis, and a newer approach based on techniques initially developed for human voice recognition (Fox *et al*. 2008). Discriminant function analysis (DFA) is probably the most widely used technique for investigating vocal individuality in animals; certainly the majority of studies in the literature utilise this technique (e.g. Butynski *et al*. 1992; Hartwig 2005; Grava *et al*. 2008). DFA is a multivariate technique that generates a series of functions, formed from linear combinations of the input variables, which maximise separation between the different groups in multidimensional space (Bee *et al*. 2001). The discriminant functions produced by a DFA can then be used to assign calls to particular individuals. In this case, each group will represent an individual bird's calls, and variables used for DFA will be derived from amplitude – time displays, spectrogram analyses and spectral analyses on the calls of each individual bird in the study. One of the basic assumptions of DFA is homogeneity of covariances, which is unlikely to hold for my dataset (J. Prince pers. comm.). If this assumption does not hold, quadratic DFA rather than linear DFA will be used; the use of quadratic DFA is generally recommended for situations where covariances are non-homogeneous (e.g. Huberty 1984).

Whilst DFA provides a powerful tool for testing the ability to discriminate between individuals' vocalisations, its use for conservation purposes is limited somewhat because the most widely used form is only able to classify individuals to one of several pre-defined groups (Terry *et al.* 2005). This means that all individuals, and their vocalisations, in a population must be known, and the technique cannot accommodate new individuals such as those entering the population through juvenile recruitment. However, it is possible to overcome this limitation by employing a non-parametric variation version of DFA, where classification is based on a similarity score (Terry *et al.* 2005); that is, calls that are not sufficiently similar to any of the known individuals' calls are classified as belonging to a new individual.

The voice recognition approach was developed by Fox *et al.* (2008) as a method of call-independent identification of individuals using vocalisations. This technique uses voice-recognition technology developed for use in humans and is based on the source-filter model of speech, which can be extended to many other animal vocalisations, including bird calls (Nowicki and Marler 1988; Fox 2008a). This model breaks down a vocalisation signal into two components; a source sound produced by the vocalising apparatus (vibrating vocal chords in humans; syrinx in birds) which is then modified by passing through a filter (vocal tract) to produce the final vocalisation signal (Fox 2008a). Identification is then based on individually characteristic features of the filter; in this case, the vocal tract (Fox 2008a).

The voice recognition approach was developed for use on passerine birds, whose complex vocal repertoires make individual identification using traditional techniques such as DFA more problematic, as these require comparison of the same call type to be effective (Fox 2008a). The call-independent approach has not yet been tested widely, and the few studies in the literature focus primarily on passerine songbirds (e.g. Fox *et al.* 2008; Cheng *et al.* 2010). However, this approach should theoretically also be applicable to species with less complex vocalisations such as the Australasian Bittern, and may prove valuable for long-term individual identification. Since the technique uses elements of the vocalisation that are determined by an individual's physical characteristics, it has the potential to circumvent the common problem of long-term instability in vocalisations. Such temporal variation in vocalisations has already proven a barrier to the long-term re-identification of individual Great Bitterns by call (Puglisi and Adamo 2004). However, this would only be the case where temporal changes in vocalisations are a result of changes in call structure; changes as a result of changes to an individual's 'voice' as a result of changes in vocal tract morphology would still confound analyses (Zdenek 2012).

Budget

Category	Estimated Cost ($)	Details
Travel	3700	25 days, average 200km per day at 0.65c/km plus $5/day, reserve 500km
Accomodation	1000	20 nights at $50/night
Consumables	150	Batteries, satphone calls
Equipment	In kind	On hand, provided by BWA (2x recording setups), UWA supervisor (3x recording setups), and DPaW (auto-recorders)
Data analysis	50	Matlab site license
Printing/field notepads/stationary	100	Print thesis copy, notebooks for field use
Other	80	UWA PAWES Course (Animal Ethics)
TOTAL	5080*	

* Funding provided by BirdLife Western Australia and the School of Animal Biology

Timetable

	2013								**2014**				
	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	Jan	Feb	Mar	Apr	May
Planning/ Proposal	■	■	■	■									
Proposal Seminar				■	■								
Fieldwork					■	■	■	▨					
Data Analysis							■	▨	■	■	■		
Preparation of Thesis										■	■	■	
Edit Thesis													■
Research Seminar													■

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Appendix 4 – Journal example pages

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Chatter-call harmonics in the North Island Saddleback: do they play a role in ranging?

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Abstract. Birds that counter-sing for communication and territorial maintenance need to localise the source of sound in order to promote an appropriate intraspecific response. Here, we investigate the role of harmonics in the chatter call of the North Island Saddleback (*Philesturnus rufusater*). We test whether the relative amplitude of harmonics serves as a distance cue, and whether a change of the harmonic composition of the chatter call has an effect on bird's response and its likely ability to estimate the distance of the signalling individual. North Island Saddlebacks exhibited stronger responses to playback songs with more relative energy within higher harmonics, suggesting that these are perceived as coming from a nearby individual. North Island Saddlebacks took longer to respond and counter-sang less to chatter calls with more relative energy in lower harmonics, suggesting that they were perceived as coming from a distant bird. We also found that North Island Saddlebacks responded differently to songs from which different harmonic frequencies were removed (muted). This study reveals the ability of the North Island Saddleback to differentiate between calls with different harmonic composition and proposes that harmonics are important as distance cues.

Additional keywords: bird song, *Philesturnus rufusater*, playback.

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Introduction

Acoustic signals play a significant role in animal behaviour, conveying information about signalling individuals involved in intraspecific interactions, such as repelling rivals in territorial defence or indicating the fitness of singing males to females (Krebs *et al.* 1978; Catchpole *et al.* 1984; Buchanan and Catchpole 1997; Slater 2003; Catchpole and Slater 2008). The distance of a signaller has biological significance affecting interactions within and between sexes, and transmission through the environment modifies the signal, giving receivers cues to the distance of the signaller (Catchpole and Slater 2008). For bird song, the receiver should be able to use these cues to estimate the distance of the signalling individual, a behaviour known as ranging (McGregor and Krebs 1984). Correct estimation of distance is important in determining the response of the receiver because it can lead to avoidance of unnecessary or dangerous interactions, or to the better location of a mate, or it may promote aggressive responses, to defend a territory when a rival is nearby (Richards 1981; McGregor *et al.* 1983; McGregor and Krebs 1984; Naguib 1995).

Few studies have focussed on the effect of frequency in ranging, although relative intensities of high frequencies have been used to estimate the distance of a signaller, for example in Carolina Wrens (*Thryothorus ludovicianus*) (Naguib 1995, 1997*b*). The combination of frequency-dependent attenuation and reverberation can also give information about the distance of the signaller (Naguib *et al.* 2000). Again, few studies have focussed on the role of harmonics (notes with multi-frequency bands) in ranging (e.g. Aubin and Jouventin 2002), although other aspects of the function of harmonics have been investigated. Both Zebra Finches (*Taeniopygia guttata*) and Budgerigars (*Melopsittacus undulatus*) were able to detect slight mistuning of one of the harmonics in a simulated female Zebra Finch contact call (Lohr and Dooling 1998). In Whooping Cranes (*Grus americana*) harmonics provide acoustic cues to individuality and body size (Fitch and Kelley 2000), and in Red-winged Blackbirds (*Agelaius phoeniceus*) lower frequency elements of song are essential for species recognition whereas high-frequency elements are not (Brenowitz 1982).

As the sound of a bird's vocalisation travels in the habitat, it is subject to degradation. Changes that accumulate in the songs are the result of reverberation, amplitude fluctuation and frequency-dependent attenuation (Slabbekoorn *et al.* 2002), with higher frequencies being more susceptible to degradation (Padgham 2004). Playback experiments on birds in natural conditions demonstrate that reverberated songs are judged to be further away than undegraded songs (Fotheringham *et al.* 1997). Birds approach closer towards a loudspeaker playing reverberated song or even fly beyond the loudspeaker (Wiley and Godard 1996). The relative intensities of high frequencies can also be used in avian song-ranging (Naguib 1995, 1997*a*). Combinations of reverberation and frequency-dependent attenuation may therefore serve as

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