Pre-screening acoustic and other natural signatures for use in noninvasive individual identification

Kimberly A. Pollard[1]*†, Daniel T. Blumstein[1] and Suzanne C. Griffin[2]

[1]*Department of Ecology and Evolutionary Biology, University of California, Los Angeles, CA, USA; and* [2]*Department of Ecosystem Conservation, University of Montana, Missoula, MT, USA*

Summary

1. Common ecological tasks, such as wildlife monitoring, adaptive management, and behavioural study, often make use of natural signatures (e.g. animal calls or visual markings) to identify individual animals noninvasively. However, there is no accepted method for pre-screening candidate natural signatures to select which signatures are the best-suited for this purpose. In this paper, we suggest a pre-screening checklist and focus on the challenge of assessing a candidate signature's individuality.

2. Individuality is critical, as the use of low-individuality natural signatures can lead to misidentification of individuals and therefore bias estimation of population parameters and population response to management actions. An information-based metric of individuality could assist researchers with selecting suitable signatures by allowing comparison among candidate signatures and providing an estimate of how many individuals may be reliably discriminated using a particular signature.

3. Before an individuality metric can be used to pre-screen natural signatures, the metric must first be calculated from preliminary sampling and must be robust to typical sampling concerns. We used field-collected animal vocalizations as well as simulations to test how robust the metric is to variation in sampling design.

4. We found that the metric is fairly robust to the number of animals sampled and the number of sessions (e.g. calling bouts) analysed, but that it is sensitive to the number of observations per session.

5. *Synthesis and applications.* Managers and researchers could save time and energy and improve the accuracy of estimates (such as abundance, survival, or population response) based on individual identification by first pre-screening candidate natural signatures for their individuality. As long as the number of observations per session is controlled, the relative values of the individuality metric can be meaningfully compared. The metric can thus be used as a tool to estimate relative individuality and so facilitates a difficult step in choosing a natural signature for noninvasive individual identification. We include instructions on how to calculate and interpret the individuality metric.

Key-words: censusing, individuality, individual discrimination, information theory, marking, signature, vocalization

Introduction

Many ecological studies and wildlife conservation and management applications require reliable identification of individual animals. Individual identification allows researchers to examine population response to management actions, acquire detailed life histories, track site fidelity and turnover, determine survivorship, conduct post-release follow-ups, estimate abundance and population size, and note individual differences in behaviour, condition, and conservation value (McGregor & Peake 1998; Galeotti & Sacchi 2001). Individual identification is valuable in direct monitoring and particularly important for calibrating indirect monitoring methods (such as counting tracks or nests, McGregor & Peake 1998).

Artificial markings such as numbered leg bands or fin tags are widely used for individual identification; however, artificial tagging is laborious, often expensive, and may disrupt the animals' behaviour, physiology, and habitat (Pennycuick & Rudnai 1970; Hare 1994; Baptista & Gaunt 1997; McGregor & Peake 1998; McGregor, Peake & Gilbert 2000; Hartwig

*Correspondence author. E-mail: kpollard@ucla.edu
†Present address: U.S. Army Research Laboratory, Human Research and Engineering Directorate Visual and Auditory Processes Branch, 520 Mulberry Point Rd., Aberdeen Proving Ground, MD 21005, USA.

2005; Cattet *et al.* 2008), so the use of naturally-occurring signatures is often desirable. Noninvasive individual identification, using natural signatures, can circumvent some costs associated with artificial tagging (Baptista & Gaunt 1997; Lubow & Ransom 2009).

There are multiple types of natural signatures. Individuals of many species, such as marine mammals or terrestrial carnivores, can be noninvasively identified with natural visual markings such as whisker spots or scars (Pennycuick & Rudnai 1970; Friday, Smith & Stevick 2000; Markowitz, Harlin & Würsig 2003; Evans & Hammond 2004; Anderson, Roth & Waterman 2007; Gallardo-Escarate, Goldstein-Vasquez & Thiel 2007; Gilkinson *et al.* 2007). However, many species are difficult to visually detect or discriminate (Saunders & Wooller 1988; Peake *et al.* 1998; Aldrich, Molleson & Nekaris 2008). Noninvasive genetic sampling has also become popular, but is expensive (Schwartz, Luikart & Waples 2007). Furthermore, genotyping error can introduce bias into estimates of population size or resighting frequency (Mills *et al.* 2000; Creel *et al.* 2003; Bonin *et al.* 2004; Nichols & Williams 2006; Schwartz, Luikart & Waples 2007; Soulsbury *et al.* 2007). Acoustic sampling is an attractive option when animals are visually cryptic, nocturnal, or living in difficult terrain (e.g. Gilbert, McGregor & Tyler 1994; Jones & Smith 1997; Peake *et al.* 1998; Galeotti & Sacchi 2001; Tripp & Otter 2006). Acoustic signatures have been used to noninvasively individually identify or census a wide variety of birds and mammals including African wild dogs (*Lycaon pictus*, Hartwig 2005), titi monkeys (*Callicebus oenanthe*, Aldrich, Molleson & Nekaris 2008), kingfishers (*Halcyon sancta,* Saunders & Wooller 1988), owls (*Otus scops*, Galeotti & Sacchi 2001; *Aegolius acadicus brooksi,* Holschuh & Otter 2005; *Megascops kennicottii,* Tripp & Otter 2006), fly-catchers (*Empidonax traillii extimus,* Fernandez-Juricic, del Nevo & Poston 2009), and corncrakes (*Crex crex*, Terry, McGregor & Peake 2001).

Noninvasive individual identification can only be effective if several conditions are met. First, the animals must possess a signature trait that is sufficiently individualistic to permit individual identification of the animals based on that trait. Additionally, the manifestations of this signature trait must be stable within the time-scale of the intended work, and the trait must be something that can be sampled multiple times (e.g. re-sighting). Practical concerns are important as well; an ideal signature trait is one that can be sampled and re-sampled frequently and easily. An ideal signature trait is thus visually, acoustically, or otherwise salient, and it should manifest frequently (e.g. a vocalization or dance) if not continually (e.g. a coat pattern). It is relatively straightforward to evaluate candidate signature traits for salience or ease of sampling. Our study therefore addresses the first and more difficult criterion: individuality. There is currently no standardized way of measuring a trait's individuality, nor a standard means of pre-screening candidate traits to see if sufficient individuality is present to permit effective noninvasive individual identification.

This is a critical problem. The use of low-individuality traits can lead to inaccurate estimates of site fidelity, survivorship, and population size (Gilbert, McGregor & Tyler 1994; Jones & Smith 1997; Holschuh & Otter 2005; Lubow & Ransom 2009). For example, individual identification has been central in mark–recapture studies that employ resighting/recapture of individuals to estimate abundance or survival rates. Mark–recapture based estimators of abundance or survival rates require the assumptions that marks are not lost or overlooked, and that individual identity is recorded correctly (Williams, Nichols & Conroy 2001); violations of these assumptions cause known biases. If more than a single animal has a specific signature mark (i.e. if signatures do not differ sufficiently between individuals), then the assumption that marks are unique is violated (e.g. Mills *et al.* 2000). The result of marks not being unique is that detection rate and apparent survival rate would be overestimated and abundance would be underestimated. Conversely, if signatures are not sufficiently consistent within individuals, then single animals may mistakenly be classified as multiple animals (e.g. Creel *et al.* 2003). This would lead to an underestimate of apparent survival rate and detection rate, and an overestimate of abundance. Inaccurate estimates of abundance and survival can lead to inappropriate adaptive management decisions, such as relaxing conservation concern when a population is still at risk or channelling limited funding into a project that is less critical (see also Caughley & Sinclair 1994). Without a reliable means of estimating the individuality in a natural signature, researchers are ill-equipped to select the most suitable natural signatures to minimize these errors.

All individual identification relies on the same statistical foundation: a signature or marker is useful for identifying individuals when the variation within individuals is low relative to the variation among individuals. Animals must be self-consistent in the focal trait, and individual animals must differ from one another in that focal trait (Pennycuick & Rudnai 1970; Pennycuick 1978; Beecher 1982, 1989; McGregor & Westby 1992; Jones & Smith 1997; Anderson, Roth & Waterman 2007). Researchers employ a variety of methods to measure this individuality. The most popular method is discriminant function analysis (DFA, McGregor, Peake & Gilbert 2000). (For examples of use, see Gilbert, McGregor & Tyler 1994; Jones & Smith 1997; Peake *et al.* 1998; Galeotti & Sacchi 2001; Hartwig 2005; Holschuh & Otter 2005; Tripp & Otter 2006;.) The DFA techniques determine the success of classifying samples within a known set and allow assessment of whether a signature trait is individually distinctive (McGregor, Peake & Gilbert 2000). The common method of interpreting discriminant function results is, however, problematic (see Mundry & Sommer 2007), and often leads to overestimating individuality in a given signature trait. Furthermore, DFA cannot answer the question of greatest practical importance to ecological researchers and managers: it cannot quantify how many individual animals may be distinguished (see also McGregor, Peake & Gilbert 2000). An alternative metric, Beecher's information statistic for individuality (Beecher 1982, 1989), can answer this question.

Beecher's information statistic quantifies individuality in terms of information theory (Shannon & Weaver 1949) and has been used to measure trait individuality in birds (Medvin,

Stoddard & Beecher 1993), bats (Wilkinson 2003), and rodents (Blumstein & Munos 2005). The information statistic equation is as follows:

$$H_i = \log_2 \sqrt{\frac{F + n - 1}{n}} \qquad \text{eqn 1}$$

where F is the F statistic from an ANOVA grouped by individual animal, n is the number of individual animals in the sample, and H_i is the individualistic information content for trait i (Beecher 1989).

The information statistic calculates how individualistic the measured signature traits are, in terms of how many binary decisions must be made to reduce the initial uncertainty about identity down to only the within-individual variation. Traits with higher values of Beecher's information statistic are more individualistic and thus may be more useful for noninvasive individual identification than traits with lower values.

The information statistic's units are expressed in bits, representing the number of these binary decisions (see also Pennycuick 1978). These multipurpose, information-based units provide Beecher's statistic with the ability to sum individuality across not only various types of traits within a signal, but also the ability to compare individuality across signal modalities, across species, and across studies. The sum of the H_i for a signal type is designated H_s.

$$H_s = \Sigma H_i \qquad \text{eqn 2}$$

Signal types (e.g. a particular type of vocalization) with higher values of H_s will be more useful for individual identification purposes than signal types with lower values. A signal type with a higher H_s value carries more individualistic information content (more individuality) than a signal type with a lower H_s value (e.g. a long-distance contact call vs. a close proximity aggressive display). Similarly, for a given signal type, a species with a higher H_s value for that signal type is more individualistic for that signal than a species with a lower H_s value.

Importantly, Beecher's information statistic can be used to estimate the number of individuals a particular signature trait can distinguish, given a certain level of acceptable error (Beecher 1989):

$$H_s = \log_2 N - \log_2 p \qquad \text{eqn 3}$$

where N is the number of individual animals distinguishable and P is the probability that a target individual's signature is also held by another individual in the group. Equation 3 has direct applicability to individual identification and monitoring tasks, as it indicates the maximum number of individuals the focal trait could discriminate. Simple rearrangement of equation 3 yields:

$$N = P \times 2^{H_s} \qquad \text{eqn 4}$$

The H_s statistic has great potential applicability for screening natural signatures for their usefulness in noninvasive individual identification; H_s estimates from candidate signatures can be compared for insight as to which signature is more individualistic. H_s estimates from different species can also be compared for insight as to which species would be most amenable to noninvasive individual identification using a given signature.

To estimate H_s, researchers must first sample the candidate signal type over multiple sessions from multiple individuals of known identity. H_s has been estimated this way in swallows (*Hirundo* spp., Medvin, Stoddard & Beecher 1993), bats (Microchiroptera, Wilkinson 2003), and marmots (*Marmota flaviventris*, Blumstein & Munos 2005). However, these studies vary greatly in their sample size and sampling design, and it is unknown how this may affect estimates of H_s. There are no standards, nor are there general recommendations, for what sorts of preliminary sampling researchers should employ to estimate individuality with H_s. Importantly, the robustness of the information statistic has not yet been assessed in light of the data structures field biologists will typically have available from which to calculate individuality. Until this is determined, the H_s method of pre-screening candidate signatures or candidate species cannot be employed to its full potential.

Using a bootstrapped sub-sampling method, we employ field-collected and simulated data to assess the effects of sampling design perturbations on Beecher's information statistic (H_s). We test whether the calculated value of H_s is influenced by: (i) the number of animals sampled, (ii) the number of sessions (bouts) sampled, and/or (iii) the number of observations per session sampled. We end by making recommendations on how to estimate H_s from preliminary sampling and how to use H_s to pre-screen candidate natural signatures or candidate species for their usefulness in noninvasive individual identification.

Materials and methods

FIELD-COLLECTED DATA

We assessed the robustness of Beecher's information statistic (H_s) by applying it to quantify individuality in natural signatures of two species of wild rodents. We used field-collected data from our study species, using what is arguably their most salient individualistic trait: alarm vocalizations. In ground-dwelling sciurid rodents, alarm vocalizations are the loudest acoustic signals produced, and these can be effectively recorded and re-recorded in the habitat. Alarm calls thus satisfy the criteria for salience and sampling, as previously discussed. We then used H_s to assess the extent to which these calls also satisfy the criterion of individuality.

Species that live in larger social groups are expected to have greater individuality in their signals than species that live in smaller social groups (Beecher 1989, 1991; Medvin, Stoddard & Beecher 1993; Mathevon, Charrier & Jouventin 2003; Wilkinson 2003; Tibbetts 2004; Blumstein & Munos 2005; Pollard 2009). We therefore used a more social species, Olympic marmots *Marmota olympus* (Merriam 1898), and a less social species, Richardson's ground squirrels *Spermophilus richardsonii* (Sabine 1822), to test Beecher's information statistic across a range of individuality (Blumstein & Armitage 1998). For each species, we selected high-quality recordings of vocal alarm calls from 10 adult females, recorded across two recording sessions (calling bouts) each, with 10 calls extracted and measured from each bout (for a total of 200 calls per species).

Calls were recorded from a free-living population of Richardson's ground squirrels at Assiniboine Park Zoo property in Winnipeg

(49°52′ N–97°14′ W) in 2006, using an Audio-Technica AT815b microphone and a Sony TCD-D8 digital audio tape recorder with a sampling rate of 48 kHz. Olympic marmot calls were recorded at Olympic National Park in Washington (at and near 47°49′ N–123°13′ W) in 2004 and 2005, using an Audix OM 3 microphone and a Sony WM-D6C audio tape recorder. For both species, researchers elicited calls by standing near the live-trapped animals (see Blumstein & Munos 2005).

We digitized alarm call recordings to 16-bit, 48 kHz AIF files, then normalized each call to 95% maximum amplitude using Sound Edit 16 (Macromedia 1995). Using Canary 1·2·4 (Charif, Mitchell & Clark 1995), with consistent settings (spectrogram: fast Fourier transformation, FFT, size = 1024 points, overlap = 99·61%, frame length = 256 points, clipping level = −80 dB, window function = Blackman, amplitude = logarithmic, display style = boxy; spectrum: FFT size = 512 points, overlap = 99·8%, frame length = 512 points, clipping level = −80 dB, window function = Blackman, amplitude = logarithmic), we measured 46 different call characteristics in Olympic marmots and 33 in Richardson's ground squirrels, including measurements in the time, frequency, and relative amplitude dimensions (see Appendix S1, Supporting Information for details). All spectrogram measurements were made while displaying only the loudest 40 dB of the call.

STATISTICAL ANALYSES

We standardized the raw data according to Beecher (1989), after which we used spss 13·0 (SPSS Inc. 2004) to calculate principal components (minimum eigenvalue = 0·01, 25 iterations, correlation method; see Appendix S2, Supporting Information). Using equations 1 and 2, we calculated Beecher's information statistic (Beecher 1982, 1989) from the principal components, using all F-values that were significant at the $\alpha = 0·05$ level.

To address the influence of sampling design on the calculated information statistic, we re-computed the statistic over a variety of sampling schemes. Using a bootstrapping method, we re-sampled our data set multiple times under pre-defined sampling regimes. We designed these regimes to mimic likely data structures collected under field constraints to identify how Beecher's information statistic (H_s) is influenced by sampling effort.

The re-sampling protocol included each combination of the following parameter values: three to ten individual animals (A), one to two observation sessions (calling bouts, B), and two to ten observations per session (calls per bout, C). For each species, each regime ($A \times B \times C$, e.g. $5 \times 1 \times 2$) was independently and randomly populated 10 times, to create 10 random iterations per regime. The one exception was the $10 \times 2 \times 10$ regime, which we iterated only once (the full dataset). Within a regime iteration, we drew individual calls at random without replacement. Across iterations and across regimes, we drew calls at random with replacement.

We wrote a Visual Basic macro to populate the regime iterations and to standardize the data within each iteration (see above). We then used spss 13·0 (SPSS Inc. 2004) and Excel SP3 (Microsoft Corporation 2001) to calculate H_s for each regime iteration. We performed multiple regressions on the H_s values to determine which sampling variations affected the calculated final value and to fit functions to describe the relationship between sampling parameters and the H_s value.

We evaluated the fit of two functions (logarithmic and inverse) to describe the relationship between calls per bout (C) and H_s. Logarithmic and inverse functions lend themselves naturally to the data structure; like H_s, both are diminishing return functions of the number of calls per bout, C, and are meaningless at $C = 0$.

SIMULATED DATA

To remove any possible effects of the particular signal type we used, and to expand our data set to a very large calls-per-bout sample size, we also ran the analyses using simulated data. For the simulated data runs, we modelled a hypothetical signature trait in which each individual animal had a unique mean trait value around which observations were normally distributed with a standard deviation of 1. The simulated data is modality-free and can be visualized as an acoustic, visual, olfactory, or other trait. We constructed a simulated species with high individuality (SimHigh), using minimally overlapping normal distributions (average distance between consecutive means = 101), and a simulated species with low individuality (SimLow), using substantially overlapping normal distributions (average distance between consecutive means = 1). For the complete data set, we drew 100 observations at random from each normal curve, using the online Simple Interactive Statistical Analysis (SISA) random number generator (Uitenbroek 1998), with MINSTD(31 bt) method, Normal II distribution, and clock as the random seed. Because analyses of field-collected data suggested little effect of either the number of animals or the number of bouts on H_s, we arbitrarily elected to use seven animals, each recorded from one bout (i.e. regimes consisted of the format $7 \times 1 \times C$, where C is the numbers of observations per bout). We varied the number of observations per bout from 2 to 100. All other analysis protocols paralleled our analysis of field-collected data. SimHigh and SimLow may be visualized as different 'species', as described above, or as different candidate signatures within a species.

Results

For both field-data species, all parameters varied in the sampling design had significant effects on H_s (Table 1). For both the number of animals and number of bouts, the effect size (partial η^2) was comparatively small. The number of animals used accounted for only 1% of the total variation in H_s in each species, while the number of bouts accounted for 18% and 3% of the variation in the Richardson's ground squirrel and Olympic marmot results, respectively. In contrast, calls per bout (observations per session) had a dramatic effect on H_s, accounting for 73–74% of the variation in both species. In both species, H_s increased with calls per bout.

Table 1. Multiple regression of sampling parameters on the calculation of Beecher's information statistic for individuality (H_s)

	Richardson's ground squirrels			Olympic marmots		
	B	*P*	*Partial η^2*	*B*	*P*	*Partial η^2*
Animals	−0·035	<0·001	0·014	−0·031	<0·001	0·011
Bouts	0·616	<0·001	0·175	0·244	<0·001	0·032
Calls per bout	0·421	<0·001	0·726	0·439	<0·001	0·740
Intercept	−0·011	0·902	0·000	0·629	<0·001	0·036

Using field data, the relationship between calls per bout and H_s was well-described by logarithmic and inverse functions (R^2 values of 0·713 and 0·623, respectively for Olympic marmots; and 0·672 and 0·594 for Richardson's ground squirrels; all $P < 0.001$). Simulated data, extended up to 100 observations per session, revealed a logarithmic function as the best R^2 fit in both the high-individuality and low-individuality cases (SimHigh: $R^2 = 0.900$, $P < 0.001$, $y = 0.714 \ln x + 6.818$; SimLow: $R^2 = 0.913$, $P < 0.001$, $y = 0.700 \ln x + 0.238$).

Discussion

Many ecological studies and applications (such as mark–recapture studies, population monitoring, behavioural study, or post-release follow-ups) require discrimination of individual animals, and noninvasive individual identification via natural signatures can provide important benefits over other methods. However, there is a lack of reliable means for evaluating the utility of different signatures for noninvasive individual identification. The suitability of an acoustic or other natural signature depends upon several factors, including that signature's individuality. High-individuality signatures increase the confidence of animal identification, improving the accuracy of behavioural and monitoring data and minimizing errors in mark–recapture estimates of abundance and survival. Previous studies proposed Beecher's information statistic, H_s, as a useful measure of individuality, which in turn could be utilized to evaluate the suitability of acoustic or other traits for noninvasive individual identification. Calculating the statistic requires some preliminary sampling of the focal trait, yet it is unknown how much preliminary sampling could be considered sufficient: no previous study has evaluated the statistic's sensitivity to sampling design. Using audio recordings collected from two species of wild rodents, as well as simulated data, we found that the greatest concern when estimating H_s is the number of observations per session (C), which had a substantial effect on estimates of Beecher's information statistic (H_s) in both real-world and simulated data.

The relationship between observations per session (C) and H_s had two important characteristics. First, there were diminishing returns from preliminary sampling; after approximately 10–20 observations per session, additional sampling effort contributed relatively little to H_s. A limit to the value of additional sampling is important because it indicates that preliminary field sampling can be restricted and still yield useful estimates of H_s. The 10–20 observations per session threshold may be different in different taxa, but the Richardson's ground squirrels, Olympic marmots, SimHigh, and SimLow data show this threshold to be reasonable across a wide range of individuality levels. Many species are likely to be comparable.

Secondly, for any level of observations per session (C), our data indicate that it is possible to draw inferences about relative individuality. This is because for any given C, the relative difference in individuality between two species or traits was maintained (Fig. 1). As long as observations per session sampling effort is held constant between species, populations,
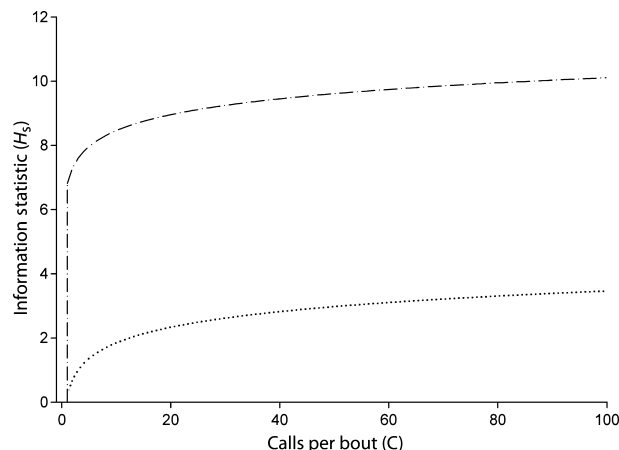

Fig. 1. Logarithmic curve fits of calls per bout (C) vs. information statistic (H_s) for simulated high-individuality data (SimHigh, dashed line) and simulated low-individuality data (SimLow, dotted line).

traits, or studies, the relative H_s – and thus potential usefulness for noninvasive individual identification – can be meaningfully compared. For example, at any level of C, our data suggest acoustic individual identification via alarm calls would be more useful and reliable in a species like Olympic marmots than in a species like Richardson's ground squirrels.

The number of animals sampled (A) and the number of observation sessions sampled (B) also had significant, though substantially smaller, effects on H_s. To compare H_s values with greatest confidence, these sampling aspects should also be controlled when comparing two candidate signatures or species. However, our data suggest that small perturbations in these sampling conditions would be of less concern than perturbations in C.

Future work could test the effect of sampling design on H_s calculation in other taxa and in other modalities. The general relationship between calls per bout and H_s in the rodent alarm calls was upheld in the modality-free, taxon-neutral simulated data, suggesting that the influence of observations per session may be of general concern when estimating H_s.

NOTE ON SIGNATURE STABILITY

Signature stability is likely to vary across species and across signal types, and this is a concern for any form of noninvasive individual identification and monitoring. An individual signature is of little practical value if it changes on short time scales. Signature stability can be assessed using traditional methods of signal resampling, following by controlled discriminant functions analysis or other comparative statistics. Importantly, the H_s statistic incorporates signature stability into its estimation of individuality. Signature stability influences H_s because it influences the amount of within-individual variation observed in signal traits (Beecher 1989). Candidate signals with high H_s values are highly individualistic over the time scale from which they were sampled, and thus could be useful in noninvasive individual identification across similar time scales.

APPLICATION OF H_S

Target species will differ in their suitability for noninvasive individual identification, as will candidate traits within a species. Suitability is influenced by practical concerns, such as trait salience, ease of resampling, etc. Noninvasive individual discrimination also requires individuality to exist in the candidate trait. The greater this individuality, the more suitable that candidate trait is for noninvasively identifying individuals (e.g. Lubow & Ransom 2009).

To calculate H_s, a number of known individual animals must first have their candidate trait sampled (with at least 10–20 observations per session). The resulting H_s then indicates how much raw individuality is available for individual identification purposes. If H_s is calculated on another species, or on a different type of signature within a species (e.g. a territorial display vs. an alarm call), the H_s values can be directly compared as long as the same sampling parameters were used. A higher H_s indicates a more suitable trait or species (in terms of individuality) for use in noninvasive individual identification. H_s thus allows researchers to pre-screen the relative usefulness of different signature types for noninvasive individual identification, and to assess which species are most amenable to noninvasive individual identification. H_s values can also be compared across modalities, allowing ecologists and mangers to evaluate the relative usefulness of, for example, acoustic calls vs. a particular visual trait.

Perhaps more intuitively, the H_s metric may be used to compare candidate traits by estimating the maximum number of individuals discriminable by those traits (eqn 4). For example, our data suggest that alarm calls would allow us to discriminate up to 34 adult female Olympic marmots and 26 adult female Richardson's ground squirrels. These numbers indicate that alarm calls may be more appropriate for individual identification in Olympic marmots than they would be in Richardson's ground squirrels. These numbers could also be compared to those calculated from alternative candidate traits (such as coat patterns or another vocalization type) to determine which trait has greater individuality.

H_S AND POPULATION ESTIMATES

Assessing the individuality of a candidate natural signature is important before embarking on any study that uses that natural signature for individual identification, including studies of abundance, habitat use, or population response for adaptive management. Any natural signature must be reliable enough to meet the assumptions that it cannot be lost or overlooked, will be recorded properly, and will be ascribed to the proper unique individual (Williams, Nichols & Conroy 2001). For population estimates based on individual identification, the signature's individuality is critical. Low-individuality signatures make resighting data ambiguous and population estimates very uncertain (Gilbert, McGregor & Tyler 1994; Jones & Smith 1997; Holschuh & Otter 2005; Lubow & Ransom 2009). If animals are not self-consistent in the signature trait, double counting becomes a concern. An animal sighted twice may appear to be two different animals. If signatures are repeated among different individuals, undercounting becomes a concern. Two different animals may be recorded as two sightings of one animal. Both over- and under-estimations of population size and population response can have negative management consequences (Caughley & Sinclair 1994). The use of highly individualistic signatures (those with high H_s values) minimizes these problems by accounting both for self-consistency within individuals and for signature repeats among individuals (Beecher 1989).

In conclusion, the use of natural signatures for noninvasive individual identification is an important tool for gauging population response to management actions, assessing abundance and habitat use, and for ecological and behavioural study of wild animals. The individuality of a natural signature is critical for the accuracy of these endeavours. Low-individuality natural signatures lead to mistakes in estimates of abundance or habitat use and hinder adaptive management efforts. Using an information metric (Beecher 1982, 1989) to calculate the individuality of candidate signatures, before embarking on a study, may save time and energy and minimize the risk of errors from using low-individuality signature traits. Our study indicated Beecher's metric is fairly robust to a range of sampling designs and hence is a good practical choice for pre-screening natural signatures for their individuality.

References

Aldrich, B.C., Molleson, L. & Nekaris, K.A.L. (2008) Vocalizations as a conservation tool: an auditory survey of the Andean titi monkey *Callicebus oenanthe* Thomas, 1924 (Mammalia : Primates : Pitheciidae) at Tarangue, Northern Peru. *Contributions to Zoology*, **77**, 1–6.

Anderson, C.J.R., Roth, J.D. & Waterman, J.M. (2007) Can whisker spot patterns be used to identify individual polar bears? *Journal of Zoology*, **273**, 333–339.

Baptista, L.F. & Gaunt, L.L. (1997) Bioacoustics as a tool in conservation studies. *Behavioral Approaches to Conservation in the Wild* (eds J.R. Clemmons & R. Buchholz), pp. 212–242. Cambridge University Press, Cambridge.

Beecher, M.D. (1982) Signature systems and kin recognition. *American Zoologist*, **22**, 477–490.

Beecher, M.D. (1989) Signalling systems for individual recognition: an information theory approach. *Animal Behaviour*, **38**, 248–261.

Beecher, M.D. (1991) Successes and failures of parent–offspring recognition in animals. *Kin Recognition* (ed P.G. Hepper), pp. 94–124. Cambridge University Press, Cambridge.

Blumstein, D.T. & Armitage, K.B. (1998) Life history consequences of social complexity: a comparative study of ground-dwelling sciurids. *Behavioral Ecology*, **9**, 8–19.

Blumstein, D.T. & Munos, O. (2005) Individual, age and sex-specific information is contained in yellow-bellied marmot alarm calls. *Animal Behaviour*, **69**, 353–361.

Bonin, A., Bellemain, E., Eidesen, P.B., Pompanon, F., Brochmann, C. & Taberlet, P. (2004) How to track and assess genotyping errors in population genetics studies. *Molecular Ecology*, **13**, 3261–3273.

Cattet, M., Boulanger, J., Stenhouse, G., Powell, R.A. & Reynolds-Hogland, M.L. (2008) An evaluation of long-term capture effects in ursids: implications for wildlife welfare and research. *Journal of Mammalogy*, **89**, 973–990.

Caughley, G. & Sinclair, A.R.E. (1994) *Wildlife Ecology and Management*. Blackwell Science Ltd, Oxford.

Charif, R.A., Mitchell, S. & Clark, C.W. (1995) *Canary 1.2 User's Manual*. Cornell Laboratory of Ornithology, New York.

Creel, S., Spong, G., Sands, J.L., Rotella, J., Zeigle, J., Joe, L., Murphy, K.M. & Smith, D. (2003) Population size estimation in Yellowstone wolves with error-prone noninvasive microsatellite genotypes. *Molecular Ecology*, **12**, 2003–2009.

Evans, P.G.H. & Hammond, P.S. (2004) Monitoring cetaceans in European waters. *Mammal Review*, **34**, 131–156.

Fernandez-Juricic, E., del Nevo, A.J. & Poston, R. (2009) Identification of individual and population-level variation in vocalizations of the endangered southwestern willow flycatcher (*Empidonax traillii extimus*). *Auk*, **126**, 89–99.

Friday, N., Smith, T.D. & Stevick, P.T. (2000) Measurement of photographic quality and individual distinctiveness for the photographic identification of humpback whales, *Megaptera novaeangliae*. *Marine Mammal Science*, **16**, 355–374.

Galeotti, P. & Sacchi, R. (2001) Turnover of territorial scops owls *Otus scops* as estimated by spectrographic analyses of male hoots. *Journal of Avian Biology*, **32**, 256–262.

Gallardo-Escarate, C., Goldstein-Vasquez, J. & Thiel, M. (2007) Individual identification of decapod crustaceans I: color patterns in rock shrimp (*Rhynchocinetes typus*). *Journal of Crustacean Biology*, **27**, 393–398.

Gilbert, G., McGregor, P.K. & Tyler, G. (1994) Vocal individuality as a census tool: practical considerations illustrated by a study of two rare species. *Journal of Field Ornithology*, **65**, 335–348.

Gilkinson, A.K., Pearson, H.C., Weltz, F. & Davis, R.W. (2007) Photo-identification of sea otters using nose scars. *Journal of Wildlife Management*, **71**, 2045–2051.

Hare, J.F. (1994) Group member discrimination by Columbian ground squirrels via familiarity with substrate-borne chemical cues. *Animal Behaviour*, **47**, 803–813.

Hartwig, S. (2005) Individual acoustic identification as a non-invasive conservation tool: An approach to the conservation of the African wild dog *Lycaon pictus* (Temminck, 1820). *Bioacoustics-The International Journal of Animal Sound and Its Recording*, **15**, 35–50.

Holschuh, C.I. & Otter, K.A. (2005) Using vocal individuality to monitor Queen Charlotte saw-whet owls (*Aegolius acadicus brooksi*). *Journal of Raptor Research*, **39**, 134–141.

Jones, D.N. & Smith, G.C. (1997) Vocalisations of the marbled frogmouth II: an assessment of vocal individuality as a potential census technique. *Emu*, **97**, 296–304.

Lubow, B.C. & Ransom, J.I. (2009) Validating aerial photographic mark–recapture for naturally marked feral horses. *Journal of Wildlife Management*, **73**, 1420–1429.

Macromedia (1995) *SoundEdit 16*. Macromedia, San Francisco.

Markowitz, T.M., Harlin, A.D. & Würsig, B. (2003) Digital photography improves efficiency of individual dolphin identification. *Marine Mammal Science*, **19**, 217–223.

Mathevon, N., Charrier, I. & Jouventin, P. (2003) Potential for individual recognition in acoustic signals: a comparative study of two gulls with different nesting patterns. *Comptes Rendus Biologies*, **326**, 329–337.

McGregor, P.K. & Peake, T.M. (1998) The role of individual identification in conservation biology. *Behavioral Ecology and Conservation Biology* (ed T. Caro), pp. 31–55. Oxford University Press, New York.

McGregor, P.K., Peake, T.M. & Gilbert, G. (2000) Communication behaviour and conservation. *Behaviour and Conservation* (eds L.M. Gosling & W.J. Sutherland), pp. 261–279. Cambridge University Press, Cambridge.

McGregor, P.K. & Westby, G.W.M. (1992) Discrimination of individually characteristic electric organ discharges by a weakly electric fish. *Animal Behaviour*, **43**, 977–986.

Medvin, M.B., Stoddard, P.K. & Beecher, M.D. (1993) Signals for parent–offspring recognition: a comparative analysis of the begging calls of cliff swallows and barn swallows. *Animal Behaviour*, **45**, 841–850.

Microsoft Corporation (2001) *Microsoft Excel version 2002 SP3*. Redmond, WA.

Mills, L.S., Citta, J.J., Lair, K.P., Schwartz, M.K. & Tallmon, D.A. (2000) Estimating animal abundance using noninvasive DNA sampling: promise and pitfalls. *Ecological Applications*, **10**, 283–294.

Mundry, R. & Sommer, C. (2007) Discriminant function analysis with nonindependent data: consequences and an alternative. *Animal Behaviour*, **74**, 965–976.

Nichols, J.D. & Williams, B.K. (2006) Monitoring for conservation. *Trends in Ecology & Evolution*, **21**, 668–673.

Peake, T.M., McGregor, P.K., Smith, K.W., Tyler, G., Gilbert, G. & Green, R.E. (1998) Individuality in corncrake *Crex crex* vocalizations. *Ibis*, **140**, 120–127.

Pennycuick, C.J. (1978) Identification using natural markings. *Animal Marking: Recognition Marking of Animals in Research* (ed B. Stonehouse), pp. 147–159. University Park Press, Baltimore.

Pennycuick, C.J. & Rudnai, J. (1970) A method of identifying individual lions *Panthera leo* with an analysis of the reliability of identification. *Journal of Zoology*, **160**, 497–508.

Pollard, K.A. (2009) *Causes and consequences of sociality: time allocation, individuality, and the evolution of group size in mammals*. PhD Dissertation, University of California, Los Angeles.

Saunders, D.A. & Wooller, R.D. (1988) Consistent individuality of voice in birds as a management tool. *Emu*, **88**, 25–32.

Schwartz, M.K., Luikart, G. & Waples, R.S. (2007) Genetic monitoring as a promising tool for conservation and management. *Trends in Ecology & Evolution*, **22**, 25–33.

Shannon, C.E. & Weaver, W. (1949) *The Mathematical Theory of Communication*. University of Illinois Press, Urbana.

Soulsbury, C.D., Iossa, G., Edwards, K.J., Baker, P.J. & Harris, S. (2007) Allelic dropout from a high-quality DNA source. *Conservation Genetics*, **8**, 733–738.

SPSS Inc. (2004) *SPSS version 13.0*. SPSS Inc., Chicago, IL.

Terry, A.M.R., McGregor, P.K. & Peake, T.M. (2001) A comparison of some techniques used to assess vocal individuality. *Bioacoustics – The International Journal of Animal Sound and Its Recording*, **11**, 169–188.

Tibbetts, E.A. (2004) Complex social behaviour can select for variability in visual features: a case study in *Polistes* wasps. *Proceedings of the Royal Society of London Series B-Biological Sciences*, **271**, 1955–1960.

Tripp, T.M. & Otter, K.A. (2006) Vocal individuality as a potential long-term monitoring tool for Western Screech-owls, *Megascops kennicottii*. *Canadian Journal of Zoology / Revue Canadienne de Zoologie*, **84**, 744–753.

Uitenbroek, D.G. (1998) SISA Random Number Generator, http://www.quantitativeskills.com/sisa/calculations/random.htm. Date accessed: 2/18/08.

Wilkinson, G.S. (2003) Social and vocal complexity in bats. *Animal Social Complexity: Intelligence, Culture, and Individualized Societies* (eds F.B.M. de Waal & P.L. Tyack), pp. 322–341. Harvard University Press, Cambridge.

Williams, B.K., Nichols, J.D. & Conroy, M.J. (2001) *Analysis and Management of Animal Populations*. Academic Press, San Diego.

Supporting Information

Additional Supporting Information may be found in the online version of this article.

Appendix S1. Acoustic traits measured from the animal calls.

Appendix S2. Syntax for H_s calculation.

As a service to our authors and readers, this journal provides supporting information supplied by the authors. Such materials may be re-organized for online delivery, but are not copy-edited or typeset. Technical support issues arising from supporting information (other than missing files) should be addressed to the authors.