Conservation Biology

Conservation Methods

Using the ecological significance of animal vocalizations to improve inference in acoustic monitoring programs

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Abstract: Recent bioacoustic advances have facilitated large-scale population monitoring for acoustically active species. Animal sounds, however, can of information that is underutilized in typical approaches to passive acoustic monitoring (PAM) that treat sounds simply as detections. We developed 3 methods of extracting additional ecological detail from acoustic data that are applicable to a broad range of acoustically active species. We conducted landscape-scale passive acoustic surveys of a declining owl species and an invasive congeneric competitor in California. We then used sex-specific vocalization frequency to inform multistate occupancy models; call rates at occupied sites to characterize interactions with interspecific competitors and assess habitat quality; and a flexible multivariate approach to differentiate individuals based on vocal characteristics. The multistate occupancy models yielded novel estimates of breeding status occupancy rates that were more robust to false detections and captured known habitat associations more consistently than single-state occupancy models agnostic to sex. Call rate was related to the presence of a competitor but not habitat quality and thus could constitute a useful behavioral metric for interactions that are challenging to detect in an occupancy framework. Quantifying multivariate distance between groups of vocalizations provided a novel quantitative means of discriminating individuals with $\geq$20 vocalizations and a flexible tool for balancing type I and II errors. Therefore, it appears possible to estimate site turnover and demographic rates, rather than just occupancy metrics, in PAM programs. Our methods can be applied individually or in concert and are likely generalizable to many acoustically active species. As such, they are opportunities to improve inferences from PAM data and thus benefit conservation.

Keywords: bioacoustics, call rate, demography, multivariate distance, occupancy modeling, signal theory, vocal individuality

Uso de la Importancia Ecológica de las Vocalizaciones Animales para Mejorar la Inferencia en los Programas de Monitoreo Acústico

Resumen: Los avances bioacústicos recientes han facilitado el monitoreo a gran escala de poblaciones de especies acústicamente activas. Sin embargo, los sonidos de animales pueden transmitir cantidades sustanciales de información que queda utilizada insuficientemente en las estrategias comunes de monitoreo acústico pasivo (MAP) que tratan a los sonidos como simples detecciones. Desarrollamos tres métodos de extracción de detalles ecológicos adicionales de los datos acústicos que son aplicables a una gama amplia de especies acústicamente activas. Realizamos censos acústicos pasivos a escala de paisaje para una especie de búho en declinación y para un

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competidor congenérico invasivo en California. Después utilizamos la frecuencia de vocalizaciones específicas por sexo para orientar los modelos multiestado de ocupación; las tasas de llamados en sitios ocupados para caracterizar las interacciones con los competidores interespecíficos y evaluar la calidad de su hábitat; y una estrategia multivariada flexible para diferenciar a los individuos con base en sus características vocales. Los modelos multiestado de ocupación brindaron estimaciones novedosas para las tasas de ocupación por estado reproductivo que fueron más sólidas ante las detecciones falsas y capturaron el número de asociaciones de hábitat más sistemáticamente que los modelos de estado único agnósticos al sexo. La tasa de llamados estuvo relacionada con la presencia de un competidor pero no con la calidad del hábitat y por lo tanto podría constituir una medida conductual útil para las interacciones que son difíciles de detectar en un marco de trabajo de ocupación. La cuantificación de la distancia multivariada entre los grupos de vocalizaciones proporcionó un medio cuantitativo novedoso para discriminar a los individuos con ≥20 vocalizaciones y una herramienta flexible para balancear los errores del tipo I y del tipo II. Por lo tanto, parecer que hay posibilidad de estimar las tasas demográficas y de rotación, en lugar de sólo las medidas de ocupación, en los programas MAP. Nuestros métodos pueden aplicarse individualmente o de manera conjunta y es probable poder generalizarlas para muchas especies acústicamente activas. Dicho así, son oportunidades para mejorar las inferencias de los datos MAP y por lo tanto, beneficiar a la conservación.

Palabras Clave: bioacústica, demografía, distancia multivariada, individualidad vocal, modelado de ocupación, tasa de llamados, teoría de señales

摘要: 近年来, 生物声学的发展推动了对叫声活跃物种的大规模种群监测。然而, 动物的声音可以传递大量信息, 这些信息在将声音简单地视为检测信号的被动声学监测的经典方法中还没有得到充分利用。我们开发了三种从声学数据中提取额外生态学细节的方法, 大范围适用于叫声活跃物种。本研究在加利福尼亚对一种数量正在下降的猫头鹰和一种入侵的同类竞争者进行了景观尺度的被动声学调查。我们使用性别特定的发声频率来研究多状态占有模型、在占有位点的鸣叫率 (用于分析与种间竞争者的相互作用和评估生境质量), 以及用一种灵活的多变量方法基于声音特征来区分个体。与不能分辨性别的单一状态占有模型相比, 多状态占有模型对繁殖状态占有率做出了新的估计, 对错误检测更加稳健, 对已知栖息地关联的信息也更加一致。我们还发现, 动物的鸣叫率与竞争者的存在与否有关, 而与栖息地质量无关, 因此可以作为在占有框架中难以检测的互作行为的有效度量标准。鸣叫的组间多变量距离量化为识别超过 20 条声音记录的个体提供了一种新的定量手段, 也是平衡I类错误和II类错误的灵活工具。因此, 我们还有机会在被动声学监测估计位点周转率和种群统计参数, 而不仅仅是占有模型的指标。我们的方法可以单独使用, 也可以协同应用, 且有潜力推广到许多声学活跃的物种中, 从而为改进被动声学监测数据推断提供了机会, 因此有助于保护。**【翻译: 胡怡思; 审校: 聂永刚】**

关键词: 生物声学, 鸣叫率, 种群统计学, 多元距离, 占有模型, 信号理论, 声音个性

Introduction

Monitoring changes in populations and understanding the environmental and management factors affecting them is a fundamental challenge in conservation, particularly for widely distributed species. Passive acoustic monitoring (PAM) has emerged as a promising approach for efficiently surveying species at broad spatial scales that can yield high statistical power to detect population changes (Wood et al. 2019c). In many cases, researchers conducting PAM use animal sounds as indications of site occupancy (e.g., Campos-Cerqueira & Aide 2016; Wood et al. 2019a). However, for most mammals, birds, anurans, and insects, these sounds are signals that are produced deliberately, despite energetic and opportunity costs, to influence processes such as reproduction and territorial defense (Schmidt et al. 2010). Studying these sounds as signals may improve PAM programs by enabling more detailed ecological inferences.

We propose 3 methods of leveraging the ecological significance of animal vocalizations to improve PAM programs: use the frequency (i.e., pitch) of vocalizations to differentiate between sexes to implement multistate occupancy models; treat call rate as a behavioral metric to assess pair status, habitat selection, and competition; and assess vocal characteristics to discriminate between individuals. All 3 methods capture information perceived by animals that is absent from the simpler occupancy approaches (e.g., Campos-Cerqueira & Aide 2016; Wood et al. 2019a). Differences in frequency of vocalizations are a reliable method of determining the sex of birds (Volodin et al. 2015), and distinguishing sex allows assessment of pair status at occupied sites, particularly for monogamous, territorial species. Call rate (number of vocalizations per unit time) can be used to estimate abundance if multiple conspecifics are within range of a single recording unit (Pérez-Granados et al. 2019). However, most large-bodied species defend territories larger than the sampling range of a single recording unit. For these species, variation in call rate can provide insight into, for example, foraging and reproduction in taxa from raptors to anurans (Townsend & Stewart 1994; Wood et al. 2019d). Individual identity is likely communicated through vocal cues (Terry et al. 2005; Prior et al. 2018) and has been assessed for owls, small passerines, and wild dogs in small-scale studies (Hartwig 2005; Kirschel

et al. 2011; Odom et al. 2013). Discriminating between putative individuals at adjacent sites within years and putative individuals at the same site between years could substantially improve occupancy-based monitoring programs by reducing bias in population estimates and identifying turnover events. All 3 of these techniques derive additional detail from passively collected audio data. Although all 3 may not be relevant to all vocally active species, many acoustically active taxa exhibit vocal characteristics and life histories that lend themselves to at least 1 of them.

We explored how these 3 techniques can enhance the ecological inferences attainable from occupancy-based PAM programs using a focal species for which all 3 are relevant. We conducted landscape-scale passive acoustic surveys designed to yield occupancy information for the California Spotted Owl (*Strix occidentalis occidentalis*) and Barred Owl (*S. varia*) in the northern Sierra Nevada, California. Because male and female Spotted Owls can be identified easily by their vocalizations (Ganey 1990), we built acoustically informed models of Spotted Owl site occupancy: sex-specific single-state models and, for the first time with acoustic data, pair-status-based multistate models. We then compared the magnitude and precision of parameter estimates and associations between site occupancy and habitat conditions with acoustically uninformed single-state occupancy models agnostic to sex. We tested whether Spotted Owl call rate varied in response to habitat conditions, pair status, and the presence of a known competitor, the Barred Owl, to determine whether treating call rate as a behavioral metric could yield insight into important ecological processes. We also tested whether call rate varied throughout the night to help optimize the timing of sampling and data processing. Finally, we assessed the feasibility of a novel multivariate method of discriminating between individual Spotted Owls. To do this, we used vocal characteristics to determine whether repeated detections from wide-ranging individuals and site turnover events could be identified. If these techniques—used independently or in concert—can increase the information yield of occupancy-based PAM programs, they may improve the study and conservation of a broad taxonomic range of vocally active species.

Methods

Study Design

We conducted passive acoustic surveys across >6000 km^2 of the Lassen and Plumas National Forests in May–August of 2017 and 2018. Survey sites (n = 346) were 400-ha hexagonal grid cells, the approximate territory size of both species in this region (Jones et al. 2018; Wood et al. 2019a), and were randomly located noncontiguously to reduce potential nonindependence (Fig. 1a). Surveys (i.e., secondary sampling periods) consisted of 2 or 3 autonomous recording units (ARUs) (Swift Recorder, Bioacoustics Research Program, Cornell Lab of Ornithology, Ithaca, NY) deployed for 5–7 nights without knowledge of owl occupancy history in areas that were acoustically advantageous (e.g., ridges rather than gullies). The ARUs recorded at a sample rate of 32 kHz from 20:00 to 06:00 (sunset 19:50 to 20:30; sunrise 05:30 to 06:15). Sites were surveyed 1–3 times per season, and surveys were separated by ≥1 month. Survey coverage and effort were lower in 2017 (n = 167 sites, $\bar{x}$ = 1.4 surveys) than in 2018 (n = 346, $\bar{x}$ = 2.8 surveys).

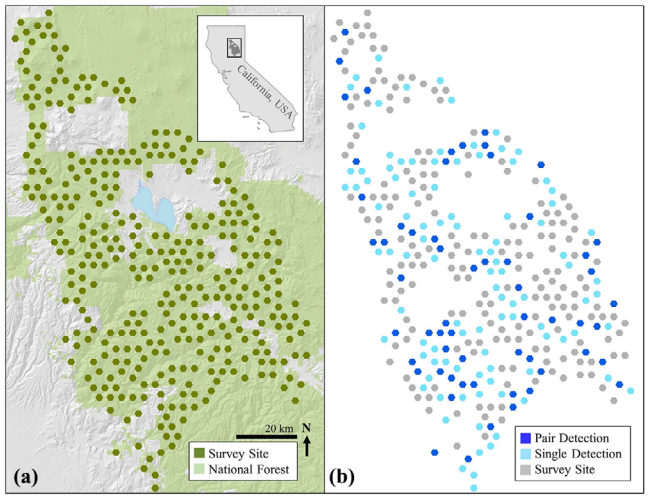

Figure 1. (a) Passive acoustic survey coverage in northern Sierra Nevada (U.S.A.) and (b) naïve site occupancy and pair status of Spotted Owls as determined by the presence of vocalizations from male, female, or both sexes (differentiated via frequency [i.e., pitch]) (Fig. 2).

We used the Template Detector feature of Raven Pro 2.0 (Bioacoustics Research Program, Cornell Lab of Ornithology) to identify territorial vocalizations in the audio data: the Spotted Owl 4-note call (Fig. 2) and the Barred Owl 2-phrased hoot. The template detector calculated the correlation between user-defined target signals—high-quality examples of the territorial vocalizations described above—and the passively collected audio data. It then identified regions of interest (ROI) in the data whose correlation with the template exceeded a user-defined threshold. We manually verified all owl vocalizations. Further bioacoustic detail is provided in Wood et al. (2019c).

To test relationships between site occupancy or call rate and environmental conditions, we derived 7 variables from remotely sensed environmental data at sites (i.e., 400-ha hexagons) and in a 250-m radius around ARU

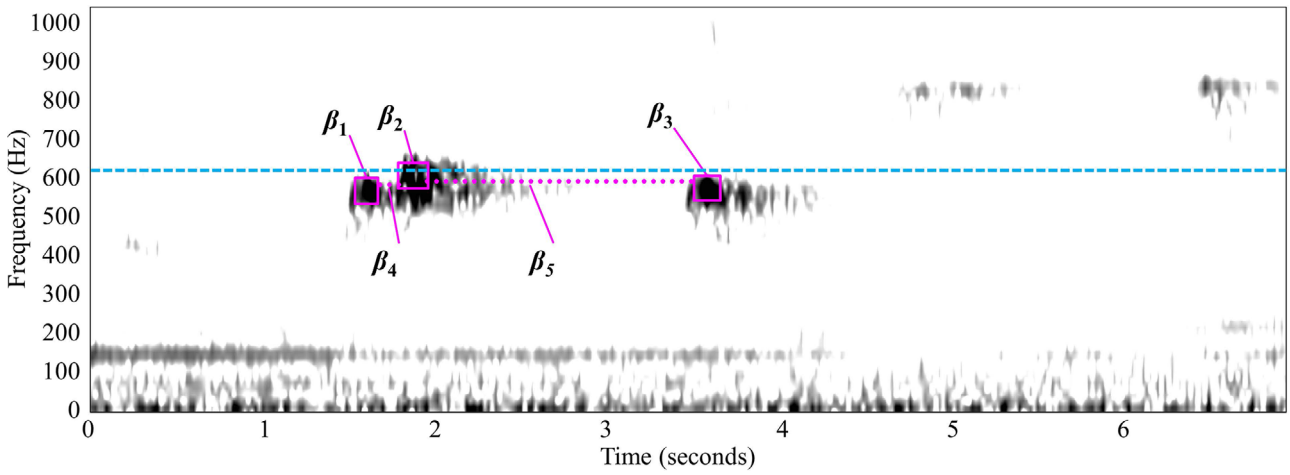

Figure 2. A spectrogram of male (left) and female (right) Spotted Owl 4-note calls. Calls with lower bounds above 631 Hz (dashed blue line) were classified as female ($\beta_{1\text{-}3}$, frequency of average of the note; $\beta_{4\text{-}5}$, call duration; variables used to calculate pairwise Mahalanobis Distance between groups of calls).

deployments because in situ testing indicated that the ARUs could record Spotted Owl calls at ~250 m with sufficient clarity to be identified by the template detector (i.e., Spotted Owl territories were approximately 20 times larger than the sampling coverage of an ARU). At both scales, we calculated average slope and elevation (5-m digital elevation model) and the amount of open forest (canopy cover [CC] < 40%), young forest (CC ≥ 40% ∩ quadratic mean diameter [QMD] < 31 cm), medium forest (CC ≥ 40% ∩ QMD 31–61 cm), and old forest (CC ≥ 40% ∩ QMD ≥ 61 cm) based on gradient nearest neighbor data (https://lemma.forestry.oregonstate.edu/data/structure-maps) and data on montane riparian forest from the National Land Cover Database (https://www.mrlc.gov/data). Open areas are unsuitable and old forest is preferred habitat (Jones et al. 2018; Blakey et al. 2019); montane riparian forest is the preferred habitat of flying squirrels (*Glaucomys sabrinus*), a major prey item.

Using Sex-Specific Vocalization Frequency to Inform Occupancy Models

We compared the parameter estimates and habitat associations derived from 3 sets of occupancy models to determine whether sexual dimorphism in vocalization frequency of Spotted Owls could be leveraged to develop sex-specific single-state occupancy models and pair-status-based multistate occupancy models (together, acoustically informed models). Male and female Spotted Owls are distinguishable because males produce lower-frequency vocalizations than females (Ganey 1990), so we compared 568 calls recorded in our study area and determined that calls whose fundamental frequency was >631 Hz were attributable to females (Fig. 2).

Wide-ranging individuals detected at multiple putatively independent sites can inflate occupancy estimates and lead to bias in habitat assessments (Berigan et al. 2019). Constructing detection histories with only female vocalizations could reduce the probability that such individuals are included in the data because females are less vocally active than males (Wood et al. 2019d). Furthermore, understanding whether sites are occupied by single owls or pairs can be important because in a single-state occupancy framework a population decline could be masked by the presence of single individuals (Tempel et al. 2014). Therefore, differentiating between sexes and determining pair status may improve on standard, acoustically uninformed models of raptor occupancy that are agnostic to sex.

We developed the 3 sets of occupancy models using 2018 data (paucity of 2017 data resulted in inestimable parameters). We considered any 4-note vocalization a detection because it is the primary territorial vocalization of this species, and estimated detection (p_a) and occupancy (ψ_a) with single-state occupancy models (MacKenzie et al. 2002). These models represented typical single-state occupancy-based applications of acoustic data that do not incorporate vocalization frequency data. We estimated detection and occupancy of females (p_f and ψ_f) with single-state occupancy models based on only detections of female 4-note calls. Finally, we used multistate models to estimate detection of single owls and of at least 1 owl of a pair (p_s and p_{op}), the probability that pairs were correctly classified as such given detection (δ), and occupancy by at least 1 owl and by pairs (ψ_{sp} and ψ_p) (Nichols et al. 2007).

We predicted that the most supported female-only model would yield lower detection (p_f) and greater sampling error in occupancy (ψ_f) than the most-supported all-detections model (p_a and ψ_a). However, significantly higher estimates of ψ_a than ψ_f could be indicative of occupancy inflation by single males when one uses all

detections given that most Spotted Owl territories are occupied by pairs (Tempel et al. 2014). Finally, we predicted that the female-only and multistate models would better reveal known associations between site occupancy and habitat covariates than the all-detections model.

For all 3 sets of models, we allowed detection to vary with open, young, medium, old, and montane riparian forest; slope; elevation (habitat variables); and presence of Barred Owls because the latter can negatively affect Spotted Owl detection (Bailey et al. 2009). We then identified the most supported p model structure while holding ψ constant by ranking models with Akaike information criterion corrected for small sample size (AIC_c). We considered models with $\Delta AIC_c < 2$ strongly supported by the data (Burnham & Anderson 2010). We allowed occupancy to vary with the 7 habitat covariates, determined the ψ structure based on the most-supported p structure, and ranked models with ΔAICc. We followed a similar procedure for the multi-state models, but constrained $p_s = p_{op}$, held δ constant, and identified the p structure, ψ_{sp}, and then ψ_p. We used R (R Core Development Team 2014) and packages xlsx (Dragulescu & Arendt 2018) and RMark (Laake 2013) for these analyses.

Evaluating Utility of Call Rate as a Behavioral Metric

PAM may provide a unique opportunity to amass unbiased behavioral data—even if those surveys are conducted for the purpose of determining site occupancy—including call rate. Stronger associations between call rates and ecological variables (e.g., habitat attributes) than relationships derived from occupancy-based analyses could indicate that call rate is an informative metric of a species' behavior, and temporal variation in call rates could influence survey design. Thus, we tested whether call rate varied in response to habitat, pair status, and interspecific competition and whether those relationships were mediated by time of night. We predicted that call rate would be higher at sites occupied by pairs, increase with the amount of older forest because it is a preferred forest type, and decrease in the presence of Barred Owls because they suppress Spotted Owl vocal activity (Bailey et al. 2009).

We randomly selected 30% of the 230 sites occupied in 2017 or 2018 to test for temporal patterns and ecological associations with call rates. After reviewing all ROI at selected sites, we manually reviewed the audio before and after each 4-note vocalization until >10 min had elapsed without Spotted Owl vocalizations because bouts of Spotted Owl vocalization average 10 min (Ganey 1990). Using these vocalization count data, we constructed generalized linear mixed models with the total number of vocalizations recorded and counted in up to 3 surveys at a given ARU as the response variable with a negative binomial distribution; site as a random effect, because up to 2 other ARUs could have been deployed there and recorded vocalizations; and an offset term to account for variation in the number of nights of survey effort. To that null model, we added habitat covariates separately, pair status, and competition (Barred Owl detection/nondetection at that site). This process yielded 10 models. We then created 3 sets of those models with either all vocalizations, dusk and dawn vocalizations (recorded before 22:00 or after 04:00), or nocturnal vocalizations (recorded from 22:00 to 04:00) as response variables. We ranked models with AICc. We used R and packages xlsx, glmmTMB (Magnusson et al. 2019), MuMin (Bartoń 2015), and lme4 (Bates et al. 2019) for these analyses.

Testing viability of Multivariate Vocal Individuality

The inability to distinguish individuals is a substantial weakness of occupancy-based monitoring relative to mark–recapture studies because it prevents the identification of false positive detections, which can lead to biased estimates (Berigan et al. 2019), and the identification of turnover events, which can signal declining survival in long-lived species (Sather 2002). Thus, we tested the feasibility of distinguishing male Spotted Owls by comparing groups of calls within and between individuals with multivariate distance.

We deployed ARUs at known territory centers of uniquely color-banded owls and selected ARUs included in the call rate analysis and that closely overlapped the territories of color-banded individuals (Tempel et al. 2014). The identity of individuals at or near all ARUs used was confirmed by observers. Based on extensive personal experience and the literature (Ganey 1990), we assumed that no territorial intrusions were recorded and thus that only known resident owls were recorded at each site. We recorded 1118 4-note calls in the territories of 14 pairs of color-banded Spotted Owls during 32 5- to 10-night sampling periods in 2017 and 2018. We described each call based on 5 measurements derived from feature boxes around the last 3 notes of the call (β_{1-3}, median frequency; β_{4-5}, median time; hop size, 1320 samples; Discrete Fourier Transform size, 4096 samples; grid spacing, 7.18 Hz) (Fig. 2) because Spotted Owls frequently drop the first note of the 4-note call and variation in the signal-to-noise ratio across calls precluded other more detailed measurements.

We grouped male calls ($n = 951$) by sampling period (5- to 10-night ARU deployment) and measured the Mahalanobis distance (MD), a metric of multivariate distance between groups (Manly 2005), between all pairwise combinations of groups of calls ($n = 496$). We then used logistic regression to determine if MD between groups predicted whether those groups were recorded

Table 1. Parameter estimates and precision of single- and multistate models of Spotted Owl occupancy.

		Detection[a]			*Occupancy*[a]			
Model type	*State*	*parameter*	*estimate*	*SE*	*parameter*	*estimate*	*SE*	*85% CI overlap*[b]
Single-state, all owls	any owl	p_a	0.46	0.036	ψ_a	0.53	0.041	a
Single-state, only females	female owl	p_f	0.20	0.047	ψ_f	0.39	0.092	ab
Multistate, single, or pair	true single	p_s	0.079	0.018				
	at least one owl				ψ_{sp}	not estimable		
	at least one of a pair	p_{op}	0.60	0.053				
	pair	δ	0.33	0.037	ψ_p	0.32	0.051	b

[a] *Parameters: p, detection; ψ, occupancy; δ, probability of correctly classifying a pair as such.*
[b] *Letters a and b denote numeric ranges encompassed by a parameter's 85% confidence interval.*

at the same site (i.e., were likely produced by the same individual) or at different sites. Next, we determined how a range of MD thresholds affected the type I and II error rates. An MD threshold is a value above which 2 groups of calls are considered separate clusters and thus different individuals and below which the groups are considered a single cluster and thus the products of the same individual. A type I error is the rejection of a true null hypothesis of one cluster, or concluding that 2 groups of calls were produced by 2 birds when actually they were produced by one individual (false positive). A type II error is accepting a false null hypothesis, or concluding that both groups of calls were produced by one bird when actually they were produced by 2 (false negative). We calculated error rates across the range of observed MD values and minimum group size values. We used R and packages xlsx and HDMD (McFerrin 2013) for these analyses.

We predicted MD and the probability that 2 groups were recorded at different sites (i.e., produced by different birds) would be positively related. However, the relative simplicity of the vocalization we analyzed (5 measurements of the last 3 notes of the 4-note call) led us to predict that it would be difficult to achieve an MD threshold that resulted in type I and II error rates <0.10, a criterion that is arbitrary but reflects the convention that misidentification rates of 0.01–0.05 are low (Morrison et al. 2011).

Results

Acoustically Informed Occupancy Models

Detection and occupancy estimates of the most supported single-state models were higher and more precise when all detections were used to parameterize single-state occupancy models (p_a [SE] = 0.46 [0.036]; ψ_a [SE] = 0.53 [0.041]) than when only female detections were used ($p_f = 0.20$ [0.047]; $\psi_f = 0.39$ [0.092]; mean covariate values used) (Table 1). However, the 85% confidence intervals (CIs) of the 2 estimates overlapped (Table 1). The most-supported multistate occupancy model indicated that detection of true single owls was low (p_s = 0.079 [0.018]), detection of at least one owl of a pair was higher but less precise than the estimates of p_a or p_f ($p_{op} = 0.60$ [0.053]), and the likelihood of confirming occupancy by a pair given detection of one owl was relatively low ($\delta = 0.33$ [0.037]). The estimated probability of occupancy by at least 1 owl (ψ_{sp}) did not converge, likely because of the low estimate of p_s. Pair occupancy was lower than ψ_a and ψ_f ($\psi_p = 0.32$ [0.051]) (Fig. 1b), and the 85% CI overlapped with ψ_f but not ψ_a (Table 1). Barred Owl presence at a site did not affect detection in any of the 3 sets of models (Supporting Information).

Acoustically informed occupancy models appeared more sensitive to habitat types previously demonstrated to be important to Spotted Owls than acoustically uninformed models. When estimated using all detections, Spotted Owl occupancy (ψ_a) was negatively related to the amount of open forest (β = −4.37, 85% CI −6.47 to −2.26) (Supporting Information). When estimated using female detections, occupancy (ψ_f) decreased with open forest (β = −7.26, 85% CI −11.22 to −3.30, w = 0.42) and increased with slope (β = 0.072, 85% CI 0.00063 to 0.14, w = 0.16) (Supporting Information). In the multistate framework, occupancy by at least 1 owl (ψ_{sp}) increased with the amount of old forest (β = 250.24, 85% CI −65.79 to 566.27) (Supporting Information), although this point estimate did not converge. Pair occupancy (ψ_p) decreased with open forest (β = −6.2, 85% CI −9.60 to −2.83, $w = 0.31$) and increased with montane riparian forest ($\beta = 8.85$, 85% CI 1.42 to 16.28, $w = 0.14$) (Supporting Information).

Table 2. Models of Spotted Owl vocalization counts.*.

	Covariate	*β (85% CI)*	*ΔAICc*	*weight*
All data	slope	0.80 (0.25–1.35)	0.00	0.34
	pair	0.49 (0.019–0.97)	2.08	0.12
	null	NA	2.14	0.12
Dusk and dawn	pair	0.99 (0.44–1.55)	0.00	0.62
	slope	0.71 (0.032–1.39)	4.17	0.08
	null	NA	4.27	0.07
Nocturnal	Barred Owl	0.61 (0.13–1.09)	0.00	0.27
	null	NA	1.14	0.15

**Only models with a ΔAICc ≤ ΔAICcnull are shown (AIC, Akaike information criterion; NA, not applicable).*

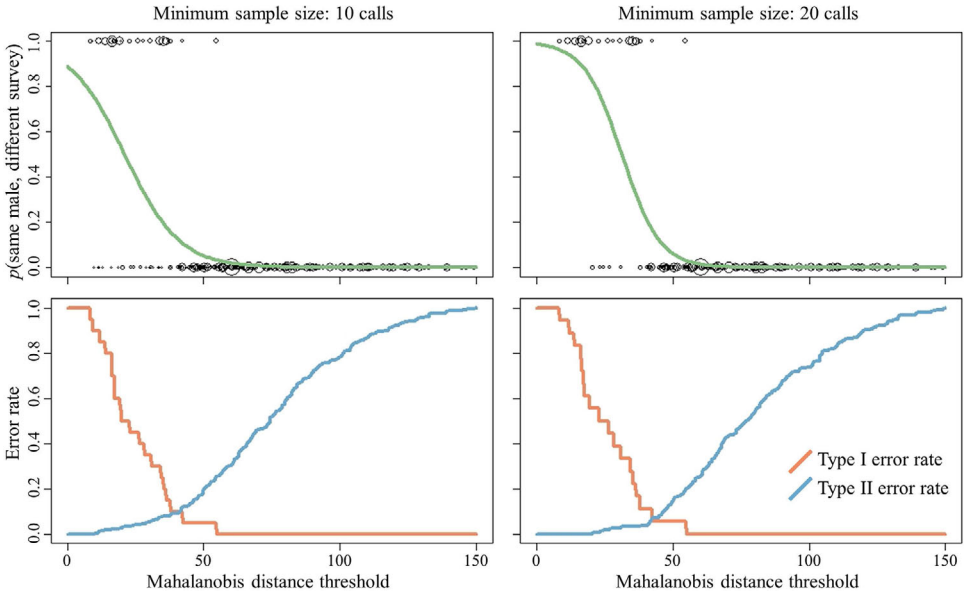

*Figure 3. The relationship (top row) between the Mahalanobis distance (MD) between groups of male Spotted Owl 4-note calls and the probability (*p*) that those groups were produced by the same individual (top row, point size scaled to sample size), and (bottom row) the type I and II error rates yielded by MD thresholds used to discriminate between individuals.*

Spatiotemporal Variation in Call Rate

Relationships between call rate and habitat, pair status, and interspecific competition were mediated by the time of night (Table 2). When all 4-note vocalizations were considered regardless of time ($n = 4223$), call rate increased with slope ($w = 0.34$, $\beta = 0.80$, 85% CI 0.25 to 1.35) and if the site was occupied by a pair of Spotted Owls rather than a single owl ($w = 0.12$, $\beta = 0.49$, 85% CI 0.019 to 0.97; Table 2). When only dusk or dawn 4-note vocalizations were considered (i.e., those recorded before 22:00 or after 04:00; $n = 2148$), call rate increased if the site was occupied by a pair of Spotted Owls ($w = 0.63$, $\beta = 0.99$, 85% CI 0.44 to 1.55). There was some support for an increase in call rate with slope ($\Delta AIC_c = 4.17$; $w = 0.080$; $\beta = 0.71$, 85% CI 0.032 to 1.39) (Table 2). When only nocturnal 4-note vocalizations were considered (i.e., those recorded between 22:00 and 04:00; $n = 2075$), call rate increased when Barred Owls were present ($w = 0.27$; $\beta = 0.61$, 85% CI 0.13 to 1.09) (Table 2). The influence of Barred Owl presence on Spotted Owls was not evident in the occupancy framework.

Vocal Individuality

The MD between groups of male Spotted Owl 4-note calls recorded during different sampling periods strongly predicted whether those calls had been recorded at the same site and, on the basis of visual confirmation of color-banded individuals residing in those areas, were thus the same individual ($AIC_{MD} = 193.3$, $AIC_{null} = 244.6$). The probability that 2 groups of calls formed a single cluster, indicating that they originated from the same individual, increased as MD decreased (β_{MD} [SE] $= -0.061$ [0.010]), and this relationship grew stronger as the minimum number of calls per group increased.

Increasing group size also decreased the type I and II error rates, and an MD threshold from 40 to 45 balanced type I and II error rates (Fig. 3). For example, if comparisons were limited to groups of 20 or more calls and an MD threshold of 43 was used, groups separated by that distance or more would have a 0.16 probability of being produced by the same individual and the type I and II error rates would be 0.056 and 0.072, respectively (Fig. 3).

Discussion

Our results showed that sexual dimorphism in vocalization frequency (i.e., pitch) can be used to estimate occupancy rates for mated pairs with multistate models and to more effectively characterize occupancy–habitat associations than models agnostic to sex. We found that call rates served as a behavioral metric that was complementary to—if not more sensitive than—occupancy models regarding interspecific competition and pair status. We successfully used vocal characteristics to discriminate between individuals yet flexibly balanced error rates and thus showed our method reduces false positive detections and estimate turnover rates. Our methods can substantially enhance the ecological inferences attainable from occupancy-based PAM programs and thus improve conservation outcomes. Because PAM data can be very species rich (Wood et al. 2019b) and the acoustic traits we explored are shared by diverse taxa (e.g., anurans, small passerines, raptors, and canids), these methods could be broadly applicable within and among studies.

Acoustically Informed Occupancy Models

We implemented acoustically informed sex-specific single-state occupancy models and, for the first time, used PAM data and multi-state occupancy models to provide estimates of pair status based on the fact that male and female Spotted Owls can be distinguished by vocalization frequency. As predicted, ψ_f was lower than ψ_a, but had greater sampling error presumably resulting from low female detection probabilities owing to females' tendency to produce fewer 4-note vocalizations than males (Ganey 1990; Wood et al. 2019d). Consequently, detecting biologically meaningful changes in female occupancy rates would likely require increased sampling, such as more secondary sampling periods or sites surveyed (Wood et al. 2019c). Nonetheless, acoustically informed occupancy models may still be preferable for trend estimation because they may reduce bias in occupancy rates relative to sex-agnostic all-detections models. Occupancy was 36% greater in the all-detections model (ψ_a) than the female-only model (ψ_f) and 65% greater than pair occupancy (ψ_p), and ψ_a and ψ_p differed significantly (Table 1). These differences likely arose because ψ_a included both territorial single male owls and wide-ranging males that inflate estimates of occupancy (Tempel et al. 2014; Berigan et al. 2019). The estimate of ψ_p was more precise than ψ_f, and p_p was greater than p_a or p_f (Table 1). Coupled with the fact that this ecologically important metric is relatively insensitive to wide-ranging males, it could be used to monitor species with sexual vocal dimorphism more effectively than conventional approaches.

As predicted, acoustically informed occupancy models revealed associations between site occupancy and habitat that acoustically uninformed models did not. Only the multistate models indicated positive associations between site occupancy (ψ_{sp}) and older forest, a habitat type that is important for roosting (Blakey et al. 2019), and between pair occupancy (ψ_p) and montane riparian forest, a habitat type that is likely important for foraging (Meyer et al. 2005) (Supporting Information). Therefore, leveraging sexual dimorphism in vocalization frequency to develop multi-state occupancy models may provide a more realistic assessment of habitat associations, as well as more rigorous means of estimating population trends. This approach could be applied to any species with consistent sexual dimorphism in vocalization frequency or that produces sexually diagnostic vocalizations, making it applicable for avian or canid research (East & Hofer 1991; Volodin et al. 2015). Nevertheless, achieving sufficiently precise parameter estimates, particularly from multistate occupancy models, may require substantial data and be sensitive to sex-biased differences in detection.

Spatiotemporal Variation in Call Rate

The intra- and interspecific interactions revealed by the call rate analyses indicated this metric can be adapted from a density and abundance estimator for small-bodied species to a behavioral metric for large-bodied species, thus providing valuable additional insight in a PAM framework. The strong positive association between dawn/dusk call rate and pair status was consistent with our predictions. Elevated call rates, then, could be a useful indication of occupancy by a pair, particularly given that the probability of identifying sites occupied by pairs was low ($\delta = 0.33$). The finding that Barred Owl presence was correlated with an increase in Spotted Owl nocturnal call rate was the opposite of our prediction. This finding could be a function of the relative novelty and lower density of Barred Owls relative to the Pacific Northwest, where vocalization suppression is occurring (Bailey et al. 2009). Call rate was sensitive to the influence of Barred Owls but occupancy models were not (Supporting Information).

Interpreting associations between call rate and habitat requires careful consideration of data structure and assumptions. Our prediction that call rate would be greater in older forest was not supported, which may be due to territorial defense (i.e., bouts of 4-note calls) occurring at the periphery of territories, rather than core areas that are positively associated with older forest habitat (Blakey et al. 2019). It may also be due to our exclusion of unoccupied sites from the analysis, because, as the occupancy models indicated, site occupancy was positively associated with older forest (Supporting Information). Finally, relationships between call rate and habitat may require further analytical effort to provide further behavioral

detail. For example, an analysis of the association between Spotted Owl vocalization count-bout duration residuals and habitat features suggested a reduction in vocalization in likely foraging habitat (Wood et al. 2019d). Overall, call rate may be an informative behavioral metric for any species whose territory size is large relative to the sampling range of an ARU and whose vocalizations are understood well enough for variation in the number of times they occur to inform plausible ecological hypotheses.

Vocal Individuality

As predicted, MD was a strong predictor of vocal individuality even for a relatively simple vocalization like a truncated 4-note call (Fig. 2). Contrary to our prediction, type I and II error rates could both be moderately low (i.e., <0.10 [Morrison et al. 2011]) with an appropriate MD threshold (43 in this case). This required that groups being compared include ≥ 20 calls, a quantity that was not uncommon even for units deployed without knowledge of owl occupancy.

Discriminating between individuals using vocal characteristics may allow for the elimination of false positive detections generated by wide-ranging individuals (Berigan et al. 2019) and potentially turnover events between years, both of which are unobservable and problematic in a conventional occupancy framework. False positive detections are likely to result in detections occurring during only one secondary sampling period, and such sites (and adjacent occupied sites) could be prioritized for assessments of vocal individuality. However, this would be hindered if such detection events entailed few vocalizations (<20 in this case). Turnover rates could be estimated from a random sample of continuously occupied sites and used as a proxy for survival because increasing turnover can be indicative of decreasing survival (Sather 2002). However, individual owl vocalizations can vary more between years than within seasons (Odom et al. 2013) so assessments of turnover would benefit from several years of vocalization measurements from multiple confirmed individuals.

Nevertheless, our results highlight the potential for multivariate assessments of vocal individuality. The MD thresholds are a novel advance in individual identification because, in contrast to probabilistic or information theoretic approaches to vocal individuality (e.g., multivariate analysis of variance or logistic regression), they afford managers an empirically based and quantitative standard with which to differentiate individuals while balancing error rates. Moreover, these analyses relied on 5 relatively simple vocalization measurements (Fig. 2), which indicates that this approach can be applied to relatively simple and highly stereotyped vocalizations. The assessment of vocal individuality could therefore be effective for many species, including many or even most birds, and many canids. The applicability of this approach will be greatly enhanced if the considerable amount of time required to manually quantify vocalization traits can be reduced by automation (e.g., Stowell et al. 2019).

Animal vocalizations and conservation

PAM is suitable for occupancy modeling (Campos-Cerqueira & Aide 2016; Wood et al. 2019a, 2019c), but animal vocalizations can be more than just cues indicating site occupancy: they are signals with ecological significance and researchers can study them as such. A diverse range of animals—from frogs to songbirds to raptors to canids to primates—display context-dependent call rates and can distinguish not just sexes but individuals via vocal characteristics (Townsend & Stewart 1994; Terry et al. 2005; Prior et al. 2018; Wood et al. 2019d). We found that animal vocalizations passively recorded for occupancy analyses can provide this information and that it can be used to improve ecological inferences in PAM programs beyond those attainable from simple detection and nondetection data. The vocal traits on which these techniques rely are widespread among vocally active species, but taking advantage of them will require assessments similar to ours for the species of interest. Their implementation, however, can increase the information yield of PAM programs, thus reducing the uncertainty that conservationists face. This can enhance the conservation of vocally active species.

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Author Contributions

All authors contributed critically to the drafts and gave final approval for publication. C.M.W., M.Z.P., S.C.S., J.J.K., and R.J.G. conceived and designed the study; C.M.W. and M.G. collected the data; C.M.W., M.G., and H.K. designed and conducted the acoustic analyses.

Conflict of Interest

The authors declare no conflicts of interest.

Supporting Information

Complete model selection tables of Spotted Owl occupancy (Appendix S1) are available online. The authors are solely responsible for the content and functionality of these materials. Queries (other than absence of the material) should be directed to the corresponding author. Data used in these analyses are available from https://zenodo.org/record/3368381#.XVQSCuhKhEY (https://doi.org/10.5281/zenodo.3368381).

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