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**Informace o individuální identitě a predační
hrozbě v akustických signálech obratlovců**

Disertační práce

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2022

Čestné prohlášení

Prohlašuji, že jsem disertační práci na téma „Informace o individuální identitě a predanční hrozbě v akustických signálech živočichů“ vypracoval samostatně s použitím uvedené literatury.

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V Praze dne 12. 1. 2022

Podpis autora

Poděkování

Rád bych zde poděkoval těm, kteří mi byli nejvíce nápomocni při objevování fascinujícího světa komunikace zvířat. Jsou to zejména Vlastimil Hart se svou neutuchající podporou nejen při výzkumu, ale zejména jeho kamarádský přístup. Katce Benediktové a Janě Adámkové s početným doprovodem jejich čtyřnohých rodinných příslušníků, za společné dobrodružství při objevování štěkácího fenoménu. Ewě Łukaszewicz a Arturovi Kowalczykovi z Přírodovědecké univerzity ve Wroclavi a také Zenonovi Rzońcovi z Nadlesnictví Wisła za možnost studovat tajemný tok tetřeva hlušce v polských Beskydech.

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1 Úvod

Predační tlak zásadním způsobem ovlivňuje chování volně žijících zvířat. Během predační hrozby dochází k druhově specifické strategii s cílem redukovat rizika. To bývá často doprovázeno komunikací v různých časových fázích probíhajícího predačního tlaku. Mimo dobu vlastního lovu kořist získává přehled o distribuci predátorů ve společně sdíleném prostoru, kdy predátoři komunikují v různých kontextech během neloveckých aktivit. Při vlastním lovu potenciální kořist používá několik typů akustických signálů s narůstající hrozbou predace a v některých případech i v momentu vlastního uchvácení predátorem. Tyto komunikační interakce probíhají nejen mezi přímými účastníky predační události, ale zachycené signály mohou být využívány i dalšími jedinci. Varovné signály mohou obsahovat celou řadu informací, které mohou zvyšovat antipredační efekt. Jednou z nich může být i individuální identita volajícího. I když riziko stát se kořistí často převáží nad jinými komunikačními funkcemi přenášených signálů, tak za určitých podmínek je dostatečný selekční tlak na to, signalizovat vlastní identitu. Komunikace v predačním kontextu a individuální rozdíly ve vokalizaci jsou intenzivně studovány. Identita volajícího jedince zakódovaná v jeho vokalizaci byla dokumentována u řady savců a ptáků. V případě nevokálních signálů, tedy zvuků, které nemají svůj původ v hlasivkách savců či syringu ptáků, je však studium akustické individuality teprve v počátcích a u většiny typů těchto zvuků nebyl doposud dokumentován. Některé z doposud nevyřešených otázek antipredační komunikace a akustické individuality se snaží řešit tato práce.

2 Cíle práce

I. Otestovat reakce vůči hlasu vybraného predátora a ověřit možnost vlivu různého typu kořisti na variabilitu hlasu predátora.

Cílem první části výzkumu bylo otestovat antipredační reakce vůči hrozbě potenciálního predátora vyjádřenou jeho samotným hlasem, tzn. bez přítomnosti dalších komunikačních modalit (např. vizuální přítomnosti predátora). V případě potenciální rekognice hlasu hnízdního predátora zůstává otázka, zda vyvolává odlišnou antipredační odezvu v závislosti na pohlaví i u druhů s extrémně polygynním či dokonce lekovým reprodukčním systémem, ve kterém se samec nepodílí žádnou formou rodičovské péče.

Zatímco variabilita v signálech vydávaných potenciální kořistí (zejména v případě alarmů) je velmi intenzívně studována, variabilita hlasu predátora v závislosti na druhu potenciální kořisti zůstává nezodpovězena.

Policht, R., Hart, V., Goncharov, D., Surový, P., Hanzal, V., Červený, J. & H. Burda (2019) Vocal recognition of a nest-predator in black grouse. *PeerJ*, 7, e6533-e6533.

Policht, R., Matějka, O., Benediktová, K., Adámková, J. & V. Hart (2021) Hunting dogs bark differently when they encounter different animal species. *Scientific Reports*, 11, 17407.

II. Porovnat míru individuální variability u vokálních signálů s odlišnou akustickou strukturou.

Individuální variabilita ve varovných hlasech (alarmech) je intenzívně studována zejména u některých skupin savců a ptáků. Signalizace pozemních sciuridů představuje typickou modelovou skupinu ve výzkumu antipredačních aspektů komunikace. Míra individuálně

odlišné exprese v akustických signálech významně závisí i na akustické struktuře studovaných signálů. Pro studium vlivu akustické struktury na míru individuálních rozdílů se nabízí porovnání signálů, produkovaných různými druhy, ale ještě zajímavější model mohou představovat odlišně strukturované signály produkované stejným druhem a ve stejném kontextu.

Goncharov, D., **Policht, R.**, Hambálková, L., Salovarov, V. & V. Hart (2021) Individual-based acoustic variation of the alarm calls in the long-tailed ground squirrel. *Royal Society Open Science*, 8, 200147.

Hart, V., **Policht, R.**, Jandák, V., Brothánek, M. & H. Burda, (2020) Low frequencies in the display vocalization of the Western Capercaillie (*Tetrao urogallus*). *PeerJ*, 8, e9189.

Linhart, P., Osiejuk, T., Budka, M., Šálek, M., Špinka, M., **Policht, R.**, Syrová, M. & D.T. Blumstein (2019) Measuring individual identity information in animal signals: Overview and performance of available identity metrics. *Methods in Ecology and Evolution*, 10, 1558-1570.

III. Ověřit možnost kódování individuální identity u ne vokálních akustických signálů.

Míra individuálních rozdílů je velmi intenzívně studována. To se však týká hlavně signálů, které jsou produkovány hlasovým aparátem, tedy hlasivkami v případě savců a syringem, v případě ptáků. Jak u některých plazů a savců, tak ptáků, se však vyskytují i zvuky, které nemají svůj původ v hlasovém aparátu. Výzkum individuální variability u těchto ne vokálních signálů je teprve v počátcích.

Policht, R., Kowalczyk, A., Łukaszewicz, E. & V. Hart (2020) Hissing of geese: caller identity encoded in a non-vocal acoustic signal. *PeerJ*, 8, e10197.

Hambálková, L., **Policht, R.**, Horák, J. & V. Hart (2021) Acoustic individuality in the hissing calls of the male black grouse (*Lyrurus tetrix*). *PeerJ*, 9, e11837.

3 Literární přehled

3.1. Signalizace během predace

Predace představuje klíčový selekční tlak formující chování všech zúčastněných stran, jak kořisti a jejich predátorů, případně i dalších účastníků, kteří mohou těžit z ulovené kořisti (jako jiní predátoři, mesopredátoři či mrchožrouti), nebo jedinci, kteří zachytili signály doprovázející predační událost. Přirozený výběr tlačí predátory ke zvyšování efektivity nalezení a ulovení kořisti (viz Johnson & Belk 2020). V případě kořisti pak selekční tlak vede ke zvyšování schopnosti nebýt predátorem detekován a ke schopnosti predátorům uniknout (Krebs, Davies & Parr 1993). Pokud se zaměříme na akustický komunikační kanál, vlastní predace může být doprovázena varovnými signály jak potenciální kořisti (tzv. alarmy), tak hlasy samotných predátorů. Varovné hlasy mohou být také „odposlouchávány“ třetí stranou, tedy jedinci, kteří aktuálně nejsou cílem komunikace v interakci kořisti a predátora (viz Magrath *et al.* 2015). Zmiňovaná signalizace pak může probíhat jak mezi příslušníky stejné kategorie, kterou může být stejný druh, vlastní rodina, skupina, apod., tak mezi příslušníky odlišných kategorií, jako např. mezi jedinci odlišných skupin nebo příslušníky jiných druhů nebo jiných vyšších taxonomických kategorií, např. tříd (ptáci vs. savci či plazi). V prvním zmiňovaném případě označujeme tyto signály jako konspecifické a ve druhém případě jako signály heterospecifické (Magrath *et al.* 2015). Takovéto „odposlouchávání“ (eavesdropping) jiných jedinců bývá významné zejména u druhů žijících v relativně komplexních systémech, ve kterých dochází spolu s dalšími druhy ke společnému sdílení predátorů (McGregor & Dabelsteen 1996). Jedinci tak mohou získávat informace o potenciální hrozbě z okolí pomocí komunikace či odposloucháváním.

Dalším typem hlasů v predačním kontextu jsou stresové hlasy, vydávané kořistí, v momentu, kdy je jedinec uchvácen predátorem nebo v průběhu štvání predátorem těsně před jeho dostižením (viz Amaya *et al.* 2019). Do predačního kontextu lze zařadit i tzv. mobbing calls, což jsou hlasy vydávané potenciální kořistí v situaci, kdy je predátor objeven a přišel tak o možnost překvapení kořisti nebo byl lovecky neaktivní, jako noční predátor objevený v průběhu dne, kterým může být např. sova, ale také denní predátor či dokonce hnízdní predátor (viz Marler 2004; Strnad *et al.* 2012). Mobbing tak představuje důležitou formu antipredační strategie (Caro 2005). Funkcí těchto hlasů je obtěžování až odehnání objeveného predátora ale

také rekrutovat další jedince k jeho obtěžování (Altmann 1956; Dutour, Léna & Lengagne 2017). V kontextu aktuální predáční hrozby se objevují i tzv. seet calls, vydávané drobnými pěvci, které jsou sdíleny a rozpoznávány mezi více druhy (Marler 1955). Někteří autoři je pojmenovávají více synonymy, jako např. „flee alarms, warning calls, hawk nebo eagle alarms“ (viz Magrath *et al.* 2015). Výhody specifické struktury těchto signálů jsou popsány v kapitole „Akustická struktura kódování informací“.

3.2. Evoluce alarmových signálů

Varovné hlasy, tzv. alarmy“ představují specifické vokalizace produkované mnoha sociálními druhy v případě ohrožení či překvapení predátorem (Zuberbühler 2009). Tyto alarmy se mohou vyskytovat v rámci druhu nebo mezi různými druhy. Odposlech signálů ostatních druhů může poskytovat širší rozsah informací, než je tomu v případě samotných konspecifických signálů (Magrath *et al.* 2015). To vyplývá také z toho, že většina jedinců v přirozených společenstvích je obklopena heterospecifickými jedinci, proto také odposlech heterospecifických signálů může významně zvýšit celkové množství dostupných relevantních informací (Seppänen *et al.* 2007; Magrath *et al.* 2015). Používání varovných hlasů představuje významnou složku komunikace jako takové. Dopustit se omylu v tomto typu komunikace může snadno vézt ke smrti jedince. Proto přirozený výběr by měl favorizovat schopnosti umožňující tento specifický typ komunikace (Witkin & Ficken 1979). Nabízí se pak otázka, proč jedinci vůbec vydávají nápadné zvuky v přítomnosti predátora, kterého tím na sebe upozorňují. Tento fenomén se snaží vysvětlit následující hypotézy, kterým se věnuje několik autorů (viz např. Zuberbühler 2009).

Varovné signály poskytují varujícímu jedinci selektivní výhodu, pokud zvyšují pravděpodobnost přežití blízce příbuzným jedincům, což je na základě **hypotézy příbuzenského výběru**, kterou postuloval Maynard-Smith (Maynard Smith 1965). Jak uvádí Magrath *et al.* (2015), na základě této hypotézy jsou náklady volajícího, spočívající ve zvýšeném riziku predace převáženy benefity, vyplývajícími ze zvýšené pravděpodobnosti přežití jedinců, kteří tento signál přijímají. V případě blízkých příbuzných se jedná o jedince, kteří s volajícím jedincem sdílí poměrnou část svých genů. To bylo nejčastěji dokumentováno v interakci rodičů a jejich mláďat. Např. u sviště žlutobřichého (*Marmota flaviventris*) varovná volání představují přímou formu rodičovské péče matky. Jakmile odrůstající mláďata opouštějí noru, jejich matky volají častěji než jedinci jiných věkových kategorií obou pohlaví včetně samic bez mláďat (Blumstein *et al.* 1997). Pískomil velký (*Rhombomys opimus*) jako varovný

signál používá nejen volání, ale také dupání zadními nohama. Samice i samci pískají a dupají častěji v přítomnosti blízkých příbuzných, mlád'at či partnera. Dospělí varují častěji v přítomnosti mlád'at, zatímco soliterní jedinci varují zřídka (Randall, Rogovin & Shier 2000). U monogamně žijící orebice rudé (*Alectoris rufa*) bylo zjištěno, že samci nejčastěji používají varovné volání v přítomnosti partnerky, méně často volají v přítomnosti nepřibuzných samic, ale i nepřibuzných samců (Zaccaroni *et al.* 2013). Mangabej pláštíkovaný (*Lophocebus albigena*) představuje hlavní kořist orla korunkatého v (*Stephanoaetus coronatus*) v národním parku Kibale v Ugandě (Arlet & Isbell 2009). To se týká zejména dospělých samců. Všichni dospělí samci vydávají alarmy ale nejvýše postavení jedinci na orly dokonce útočí, což naznačuje jejich nejvyšší motivaci při ochraně potomků. Hypotéza příbuzenského výběru je robustně dokumentována zejména u primátů, ptáků a hlodavců. Kromě interakce rodičů a jejich potomků však existuje evidence i pro interakce mezi dalšími jedinci sdílejícími společné geny. V tomto případě se jedná o inkluzivní fitness na základě Hamiltonova pravidla (Hamilton 1963). Největší evidence byla doložena v případě hlodavců, ale celkový obrázek není tak konzistentní jako tomu je v případě interakce rodičů a jejich potomků (Zuberbühler 2009).

Pokud jedinec volá pouze za situace, kdy jsou přítomni další jedinci důležití pro jeho život, ať příslušníci vlastní skupiny nebo potenciální sexuální partneři, zatímco v jejich nepřítomnosti varovné signály nevydává, mluvíme o tzv. „audience efektu“ (viz Coppinger *et al.* 2017). Výše zmiňované případy pak představují tento fenomén. Ten se ovšem netýká jen samotných alarmů, ale i dalších kontextů. Klasický případ ukazuje Marler na příkladu tzv. food calls u kohoutů kura domácího, kterými kohout volá slepice při nálezů oblíbeného zdroje potravy. Kohouti vydávají tyto hlasy méně v případech, kdy jsou osamoceni, než v přítomnosti slesc (Marler, Dufty & Pickert 1986a; Marler, Dufty & Pickert 1986b). Stejným způsobem se kohouti chovají v přítomnosti potenciálního nebezpečí, konkrétně se jednalo o experiment, ve kterém kohoutům byl prezentován model vzdušného predátora. Kohouti v této situaci častěji produkovali alarmy v situacích, kdy byli obklopeni dalšími jedinci, než v případě kdy byli osamoceni (Karakashian, Gyger & Marler 1988).

Naproti tomu **hypotéza pohlavního výběru** (Darwin 1871) vysvětluje vydávání alarmů na základě výhod plynoucích pro volajícího, který je pak častěji preferován potenciálními sexuálními partnery (Magrath *et al.* 2015). V tomto případě se jedná o strategii zvyšující reprodukční fitness volajícího jedince. Tento fenomén byl dokumentován u řady primátů (viz review Zuberbühler 2009). Zajímavý příklad představuje dobře známé varovné bekání srnce obecného (*Capreolus capreolus*). Při vyrušení lidmi bekají častěji srnci než srny. Když však

byl produkován playback tohoto varování v teritoriu srnce, spíše než útěk, tento signál vyprovokoval opětování bekání nebo agresivní chování. Starší srnci pak reagovali častěji než mladší jedinci. Autoři této studie (Reby, Cargnelutti & Hewison 1999) předpokládají, že se tento signál původně vyvinul jako signál, který měl predátorovi signalizovat jeho odhalení a tak ho odradit od potenciálního pronásledování, druhotně pak získal funkci jako signál v rámci kompetice samců o samice. U severoamerického strakapouda osikového (*Picoides pubescens*) bylo zjištěno, že nikdy nevydává alarm pokud hledá potravu osamocený nebo když je v hejnu nepříbuzných jedinců či ostatních jedinců stejného pohlaví. V případě, že jsou přítomni příslušníci opačného pohlaví, tak varovná volání používají jak samci, tak samice (Sullivan 1985). To naznačuje využívání alarmů jako způsobu k přilákání pozornosti potenciálního sexuálního partnera. U sýkory černohlavé (*Poecile atricapillus*) se sexuální partneři drží pospolu i v zimních hejnech. Tam používají i alarmy, které se tak jeví zvyšovat přežívání obou partnerů v zimních podmínkách (Witkin & Ficken 1979).

Posuzování vlivu efektu alarmové signalizace na sexuální selekci však může být komplikovanější, než by naznačovaly výsledky korelačních studií. Hlubší vhled umožňují experimenty, které dovolují manipulovat testované signály za kontrolovaných podmínek. Modelový příklad představuje problematika používání alarmů u kura domácího (*Gallus gallus*). U kohoutů se vydávání alarmů na vzdušného predátora ukázal být nejlepší prediktor úspěšného páření a reprodukční úspěšnosti na rozdíl od kvality ornamentů, kterými byly velikost hřebínku včetně ostatních ornamentů na hlavě a plochy červeně zbarvené lysé kůže i reflektančního spektra jejich barvy (Wilson *et al.* 2008). Autoři této práce se domnívali, že preference kohoutů slepicemi na základě ornamentů hraje až druhotnou roli, zatímco primární volba probíhá na základě signálního chování a dominance. V následující studii výzkumníci testovali, jak byly slepice přitahovány alarmy kohoutů (Wilson & Evans 2010). V každém experimentu byly slepicím prezentován alarm se zvýšenou rychlostí a kontrolní alarm se sníženou rychlostí (vytvořené manipulací délky mezer mezi sekvencemi alarmů). Ve výsledku pak slepice nevykazovali zřejmou preferenci na manipulované alarmy a tak výsledky předešlé korelační práce (Wilson *et al.* 2008) vysvětlující vliv alarmů na reprodukční úspěšnost nebyly potvrzeny. Tyto výsledky byly konsistentní napříč třemi typy experimentů a ukázaly, že alarmy v případě kura domácího nejsou sexuálně selektovaným signálem.

Další hypotéza - **hypotéza individuálního výběru** - se snaží vysvětlit toto chování pomocí přímých výhod pro volajícího (viz Magrath *et al.* 2015). V tomto případě se nejedná o výše popisovaný efekt audience. Do této kategorie patří několik specifických kontextů. Jedním

z nich jsou výše zmiňované **mobbing calls**, které jsou nejznáměji produkovány ptáky při objevení predátora (Curio 1978). Mobbing calls produkované například sýkorou koňadrou (*Parus major*) mají odlišnou strukturu (rozdílná délka a počet elementů včetně intervalů mezi nimi) v závislosti na intenzitě hrozby, kterou představovala přítomnost krahujce obecného (vysoká hrozba) a puštíka obecného (nízká hrozba) (Kalb, Anger & Randler 2019). Mobbing calls zpěvných ptáků představuje silný stimul pro ostatní sousedící ptáky různých druhů k přidání se v obtěžování predátora (Forsman & Monkkonen 2001). Surikaty (*Suricata suricatta*) používají mobbing calls nejen k odehnání predátora, ale i k získávání informací o potenciální hrozbě, včetně posouzení míry nebezpečí. Variabilita mobbing calls tak může vyjadřovat různou intenzitu nebezpečí a to i konkrétními akustickými parametry (Ellis 2008). Některé druhy, například sýkora koňadra (*Parus major*) a pěnkava obecná (*Fringilla coelebs*), reagují jak na vlastní, konspicivní, tak i na mobbing calls několika severoamerických pěvců (Dutour, Léna & Lengagne 2017). Autoři citované práce to vysvětlují na základě akustické podobnosti těchto signálů, nicméně předpokládají, že se nejedná o hlavní rekogniční mechanismus, ale pravděpodobně se vyvinulo více odlišných mechanismů. Mláďata se tak učí odpovídající reakce na základě pozorování chování dospělých ptáků (Graw & Manser 2007). Jedinci sojky zlověstné (*Perisoreus infaustus*), kteří zaslechli mobbing calls, mohli uniknout, ještě než je zahlédl predátor, mláďata střízlíkovce bělobřvého (*Sericornis frontalis*) přestávají žadonit, když slyší mobbing calls rodičů a mláďata sýkory koňadry (*Parus major*), reagují odlišně v závislosti na typu hnízdního predátora (Carlson, Healy & Templeton 2018).

Hypotézu individuálního výběru podporují situace, ve kterých se snižuje riziko pro volajícího jedince v důsledku reakcí ostatních jedinců, kteří také představují potenciální kořist predátora (Zuberbühler 2009). U špačků obecných (*Sturnus vulgaris*) alarmy vyvolají rozpad hejna, což snižuje pravděpodobnost volajícího být potenciálním cílem. Tato varování jsou častější v případech zhoršené viditelnosti, jako například při pohybu na zemi ve vysoké trávě (Devereux *et al.* 2008). To naznačuje, že toto chování je spojeno s jistými náklady pro volajícího jedince.

Vydávání varovných hlasů představuje i určitou formu rodičovské péče, což v případě samců může tvořit významný podíl v míře poskytované péče vzhledem k tomu, že v porovnání se samicemi, je jejich příspěvek v péči o potomstvo zpravidla významně nižší. Podobně může být i součástí pohlavního výběru, konkrétně v případě kompetice samců o přístup k samicím.

Individuální selekce může formovat i stresové hlasy neboli „distress calls“. Jedná se o zvuky vydávané buď v momentu uchvácení kořisti predátorem nebo ještě předtím, než k tomu dojde (viz Jurisevic & Sanderson 1998). V případě mláďat, stresový hlas (distress call, capture call) často vydávají mláďata savců, ptáků a krokodýlů (Lingle *et al.* 2012). Ptáci stejného nebo jiného druhu a případně i další predátoři, bývají tímto hlasem přitahováni na poměrně značnou vzdálenost (Marler 2004). Vydání takového zvuku může predátora natolik překvapit, že se kořisti může podařit uniknout. Produkce těchto signálů ještě před momentem uchvácení kořisti může predátorovi indikovat marnost takového počínání. V tomto případě se jedná o hypotézu „perception advertisement“ (Bergstrom & Lachmann 2001). Takovéto signály musí být natolik nákladné aby silně korelovaly se schopností volajícího jedince úspěšně uniknout (Yachi 1995). Tato hypotéza je silně podpořena řadou prací zejména na primátech, například u nártouna celebeského (*Tarsius tarsier*) (Gursky 2006) či malpy hnědé (*Cebus apella*) (Wheeler 2013).

3.3. Benefity alarmů

Alarmy mohou poskytovat jedincům schopným je přijímat a dekodovat určité výhody. Selektce pak vybírá takové vlastnosti signálů, které svým nositelům zvyšují pravděpodobnost přežití (viz Charnov & Krebs 1975). Podle časového období účinku je můžeme rozdělit na benefity bezprostřední a dlouhodobé. Bezprostřední benefity se vztahují zejména k momentu možnosti být uloven nebo okamžiku který tomu předchází (Magrath *et al.* 2015). Záhy po detekci varovného signálu jedinec buď aktivně uniká z místa aktuálního nebezpečí nebo zvolí jinou antipredační strategii, jako např. zvýší svou ostražitost, aktivuje kryptické chování, nebo se potenciálně ohrožení jedinci k predátorovi aktivně přibližují či jej dokonce aktivně napadají (mobbing) (Magrath *et al.* 2015).

Dlouhodobé a nepřímé benefity podle Magratha *et al.* (2015) zahrnují: (1) zvýšenou efektivitu příjmu potravy a expanzi ekologické niky, (2) získání prostorové informace o nebezpečí, (3) učení se o predátorech. Jmenované benefity autoři popisují pro heterospecifické alarmy. V trochu menší míře je lze očekávat jejich platnost i pro konspecifické varovné signály. I když v případě heterospecifických signálů se jedná o větší variabilitu jak přenášených signálů, tak poskytovaných informací, vzhledem k různorodosti preferovaných habitatů a komunikačních modalit využívaných odlišnými druhy, zejména ve vztahu k příslušným predátorům (Magrath *et al.* 2015). Autoři této práce dále uvádí, že přítomnost jiných jedinců schopných vydávat varovné signály umožňuje ostatním přítomným snižovat čas věnovaný

ostrážitému chování a tento ušetřený čas investovat do příjmu potravy. Dále zmiňují, že naslouchání různým typům varovných signálů také umožňuje přizpůsobit využívání prostoru a načasování biologických aktivit vzhledem k aktuální míře predáčního rizika. Prostřednictvím sociálního učení tak dochází k učení se konkrétním predátorům, což umožňuje se specificky adaptovat aktuálním podmínkám.

3.4. Heterospecifické vs. konspecifické alarmy

Heterospecifická komunikace probíhá mezi příslušníky odlišných druhů či jiných kategorií. Už samotná přítomnost více jedinců zvyšuje pravděpodobnost detekce potenciální hrozby. Na základě hypotézy mnoha očí (many eyes hypothesis), lze očekávat s rostoucím počtem jedinců ve skupině, zvyšující se pravděpodobnost, že alespoň jeden jedinec včas zaregistruje blížícího se predátora (Breed 2017). V případě smíšené skupiny se nabízí možnost odposlouchávat varovné signály více druhů i těch s odlišnou biologií a senzoryckými schopnostmi (Goodale & Kotagama 2008; Goodale, Ratnayake & Kotagama 2014). Například ptáci, kteří sbírají potravu na zemi mají menší přehled o okolním prostředí než druhy, které loví poletující hmyz. Druhy sbírající potravu na substrátu potom častěji reagují na varovné signály (Goodale & Kotagama 2008). Příslušníci smíšené skupiny tak využívají odposlouchávání signálů jedinců obývajících odlišné ekologické niky. Rozšiřuje se tak spektrum nabídky signálů o signály pocházející z komunikace jiných druhů. To se může dokonce týkat i druhů, které nevydávají žádné akustické alarmy. Extrémním příkladem jsou leguáni mořští (*Amblyrhynchus cristatus*) z Galapág, kteří vůbec nevydávají žádné akustické signály ale přitom reagují na alarmy drozdce bělokrkého (*Mimus parvulus*) (Vitousek *et al.* 2007). Jak leguán, tak drozdec je často loven kání galapážskou (*Buteo galapagoensis*). Podobně i další nevokální ještěr, scink měděný (*Emoia cyanura*), rozlišuje varovný hlas bulbula šupinkového (*Pycnonotus cafer*) (Fuong *et al.* 2014). Využívání odposlechu jiných druhů může být dokonce energeticky výhodnější, jelikož tito jedinci představují menší nebo dokonce žádnou konkurenci, než je tomu v případě příslušníků stejného druhu (Seppänen *et al.* 2007).

3.5. Akustická struktura

Struktura alarmů může být vysoce specifická s ohledem na druh predátora nebo hrozby (Klump & Shalter 1984). Alarmy bývají kategorizovány mnoha způsoby, intenzitou své hrozby, behaviorální reakcí kterou vyvolávají, typem hrozby či stavem a záměrem volajícího jedince nebo svou akustickou strukturou (Magrath *et al.* 2015). Kategorie signálů definované svou strukturou mohou být buď diskrétní nebo graduální, tvořící určité kontinuum bez zřetelných hranic mezi sousedními kategoriemi (Keenan, Lemasson & Zuberbühler 2013). Pro graduální systém je typická přítomnost přechodných, intermediálních forem (Hammerschmidt & Fischer 1998; Tallet *et al.* 2013; Keenan *et al.* 2020). Právě nepřítomnost těchto přechodných forem signálů umožňuje kategorizaci do distinktních, jasně ohraničených kategorií. V případě alarmů byly dokumentovány oba systémy, jak diskrétní, tak graduální. Diskrétní kódování kategorií bylo ukázáno u řady stromových primátů, jako například u kočkodana obecného (*Chlorocebus aethiops*) (Seyfarth & Cheney 1990), kočkodana Dianina (*Cercopithecus diana*) (Zuberbühler, Noë & Seyfarth 1997) či kočkodana bělonosého (*Cercopithecus nictitans*) (Arnold & Zuberbühler 2006), zatímco graduální systém u guerézy červené (*Ptilocolobus badius*) (Marler 1970), šimpanze učenlivého (*Pan troglodytes*) i šimpanze bonobo (*Pan paniscus*) (de Waal 1988). Výsledný typ kategorizace ale může být spíše by-produktem lidské percepce nebo tendencí kategorizovat (Keenan, Lemasson & Zuberbühler 2013).

Variabilita akustické struktury vyplývá z interakce vlivů odlišných selekčních tlaků a limitů ovlivňujících produkci hlasů, jejich přenos a detekci (García-Navas & Blumstein 2016). Podle toho, jak je akustická energie rozložena napříč frekvenčním spektrem, můžeme alarmy kategorizovat jako tónické (tonální), kde akustická energie prochází napříč úzké frekvenční spektrum, v případě že tato energie je rozložena do několika frekvenčních píků, které tvoří násobky nejspodnější frekvence, pak hovoříme o harmonické struktuře. V takovém případě tato spodní frekvence je označována jako základní frekvence (F0) nebo tón a její násobky jako harmonické frekvence nebo harmonické tóny. Tónické alarmy mají nejčastěji podobu různých typů hvizdů (viz např. Schneiderova & Policht 2012; Goncharov *et al.* 2021). Pozoruhodně velkou podobnost v akustické struktuře vykazují stresové hlasy mláďat ve dvou behaviorálních kontextech, kdy se projevují jako „capture calls“ a „isolation calls“ (Lingle *et al.* 2012). Tato autorka ve své srovnávací studii ukázala, že jejich tónická a bohatě harmonická struktura je velmi podobná napříč šesti řády a devíti čeledi savců. Tato podobnost je dokonce taková, že

některé druhy mohou reagovat na stresové vokalizace i taxonomicky a ekologicky vzdálených druhů (Lingle & Riede 2014).

Tonální strukturu alarmů mají tzv. seet calls, což jsou tónické alarmany vyšších frekvencí vydávané drobnými pěvci v situaci nebezpečí vzdušného predátora, zpravidla dravce (Marler 1955). Takovéto vyšší frekvence jsou těžko zachytitelné pro dravce a sovy, navíc vyšší frekvence jsou nejdříve odfiltrovány vegetací. Délka vlny tohoto zvuku je řádové v cm, proto je snadno zachycen překážkami (Marler 1955). Struktura seet calls je velmi podobná mezidruhové a často také funguje jako mezidruhový signál. Někteří autoři tyto seet calls uvádějí pro evropské pěvce (viz Fallow & Magrath 2010), ale byly dokumentovány i u severoamerických druhů (Vanderhoff & Eason 2009).

Podobný efekt vykazují i ultrazvukové alarmany (frekvence vyšší než 20 kHz) umožňující vyhnout se slyšitelnému spektru predátorů (Wilson & Hare 2004b). Ultrazvukové signály v kontextu predáční hrozby jsou nejvíce dokumentovány u hlodavců (Wilson & Hare 2004a; Litvin, Blanchard & Blanchard 2007; Brudzynski & Fletcher 2009; Kozhevnikova *et al.* 2021). Různé formy ultrazvukových signálů byly v rámci hlodavců zaznamenány minimálně u 50 druhů (Sales 2010). V rámci savců pak také u primátů a letounů (Miard *et al.* 2019), pokud nepočítáme echolokační signály. Ultrazvukové signály těchto zvířat se potom často týkají i jiného kontextu než alarmu, ale s predáční hrozbou úzce související, jelikož komunikace ve vyšších frekvenčních spektrech umožňuje vyhnout se slyšitelnému spektru predátorů a případně i intenzivnímu hluku vyluzovaného nočním hmyzem (Ramsier *et al.* 2012).

V porovnání s tonálními či harmonickými hlasy jsou širokospektré signály (broadband, wideband), ve kterých je energie rozprostřena napříč široké frekvenční spektrum. Oproti tonálním zvukům, které mají nejčastěji podobu hvizdů a různých typů pískání, širokospektré zvuky neobsahují čisté tóny, ale mají podobu spíše chrčivou, obsahují hrubší a nepravidelné tóny, které mohou mít až podobu strukturního chaosu, ve kterém mohou být zvýrazněny některé frekvenční pásy, které mohou být situovány buď nepravidelně nebo případně i harmonicky. Tyto signály se často vyznačují velkou variabilitou a spíše graduálním charakterem, než že by tvořily jasně distinktní kategorie. Často také obsahují různé přechodné typy. Typickým příkladem jsou alarmany celé řady primátů nebo štekavé bekání některých jelenovitých, označovaných jako „barking“. Tyto signály vykazují podobnou strukturu, a dokonce do jisté míry i podobně znějí. U jelenovitých představují antipredáční signály s funkcí informovat predátora jeho odhalení (Reby, Cargnelutti & Hewison 1999). Takovéto signály jsou známy např. u srnce obecného (*Capreolus capreolus*), mundžaka sundského (*Muntiacus muntjak*),

srnčíka čínského (*Hydropotes inermis*) (Oli & Jacobson 1995; Reby, Cargnelutti & Hewison 1999; Cap *et al.* 2008). Je zajímavé, že v rámci tribu Cervini tento varovný signál vydávají všechny samice, zatímco samci daňka skvrnitého (*Dama dama*) a jelena siky (*Cervus nippon*) takový signál nevydávají, ani žádný jeho ekvivalent (Cap *et al.* 2008). V případě primátů se varovné barking vyskytuje velmi často: např. u sifaky malého (*Propithecus verreauxi*) (Fichtel & Van Schaik 2006), paviánů čakma (*Papio ursinus*) (Fischer *et al.* 2001b) či šimpanzů (*Pan troglodytes*) (Crockford & Boesch 2003).

Ještě větší podíl disharmonického chaosu v akustické struktuře mají různé typy funění, syčení či frknutí, tedy signálů, které nejsou produkovány činností hlasivek nebo syringu u ptáků, ale vznikají jakoukoli konstrikcí vzduchu kdekoli na průchodu dýchacího ústrojí (Caro *et al.* 2004; Policht *et al.* 2020). Takovéto nevokální alarmy se vyskytují u řady savců a ptáků. U některých pěvců hnízdících v dutinách se objevuje v přítomnosti nebezpečí produkce syčivých zvuků, které velmi přesvědčivě připomínají hadí syčení a mohou i efektivně odradit případného narušitele (Marler & Slabbekoorn 2004). Syčení samice sýkory modřinky (*Cyanistes caeruleus*) vykazuje akustickou podobnost se syčením tří sympatrických hadů vyskytujících se v Mediteránu: užovky iberské (*Zamenis scalaris*), užovky maurské (*Natrix maura*) a širohlavce ještěřčího (*Malpolon monspessulanus*) (Dutour *et al.* 2019). Autoři této práce ukázali, že oba typy syčení, jak hadí, tak sýkoří, vyvolává srovnatelnou míru úzkostného chování u myši západoevropské (*Mus musculus domesticus*), jako savčího modelu hnízdního predátora. Jedná se tak o akustickou variantu Batesovských mimiker, kdy neškodný druh napodobuje nebezpečný model. Syčení je však i typickou součástí antipredačního chování hus a labutí, tedy druhů nehnízdících v dutinách (Policht *et al.* 2020). Alarmy ve formě různých forem frknutí se často vyskytují i u kopytníků (Caro *et al.* 2004), např. u muflona (*Ovis gmelini musimon*), kabara pižmového (*Moschus moschiferus*), soba polárního (*Rangifer tarandus*), jihoamerických jelenců Odocoileini (Hirth & McCullough 1977; Cap *et al.* 2008), řady afrických antilop (Estes 1991; Kingdon 1997), ale i hrochů (Stuart & Stuart 1997), žiraf (Volodina *et al.* 2018) ale třeba i vyder (Leuchtenberger *et al.* 2016).

Vokalizace terestrických obratlovců zahrnují širokou škálu zvuků počínaje zvuky vznikajícími téměř periodickou vibrací hlasivek (či syringu), tedy harmonickými zvuky, až po kompletně atonální turbulentní hluk, tzv. „noisy nebo atonal sounds“ (Bradbury & Vehrencamp 2011). Mezi těmito dvěma extrémy však existují i zvuky vznikající na základě nelineární dynamiky hlasového aparátu, které představují různé formy nepravidelnosti a odchylek od pravidelných oscilací, kterými jsou subharmonické tóny, bifonace a deterministický chaos

(Riede, Wilden & Tembrock 1997; Wilden *et al.* 1998; Fitch & Hauser 2002; Tokuda *et al.* 2002), dále také tzv. „frequency jumps“ a „sidebands“ (Anikin, Pisanski & Reby 2020). Takové hlasy mají obvykle komplexní širokospektrální akustickou strukturu s některými zesílenými frekvenčními pruhy různých forem, případně doplněných harmonickými komponenty (Fitch & Hauser 2002). Bývají pak v repertoáru často popisovány jako „screams, např. u primátů včetně člověka, či prasetovitých a „barks“ taktéž u primátů a psovitých šelem (Tokuda *et al.* 2002). Ve své sluchem vnímané podobě obsahující hrubou či drsnou kvalitu (Anikin, Pisanski & Reby 2020). Nejčastěji dokumentovaným příkladem nelineárních fenoménů je deterministický chaos obsahující jak atonální (noisy) složku bez zřetelné periodicity, vytvářenou turbulentním hlukem pomocí konstriktce vokálního traktu, tak spektrální komponentu, generovanou nepravidelnou oscilací hlasivek (Fitch & Hauser 2002). Nelineární dynamika vokálního traktu byla dlouho přehlížena a začala se studovat zejména v posledních letech. Ukazuje se, že tento fenomén není jen by-produktem vokální mechaniky, ale spíše má potenciál obsahovat důležité informace o volajícím jedinci (Anikin, Pisanski & Reby 2020). Anikin *et al.* (2020) uvádí jejich korelaci s vyšší mírou vzrušení a emocí, včetně signalizace agrese. Uvádí pak i snížení vlivu habituace přítomných posluchačů. Nelineární fenomény se tak mohou přímo podílet na kódování informací nebo mohou přispívat ke zvyšování efektivity informace kódované jinými parametry. Například alarmy pišťuchy horské (*Ochotona alpina*) jsou vysoce individualizované a nelineární fenomény tyto individuální rozdíly ještě více zvyšují (Volodin *et al.* 2018).

Alarmové vokalizace mohou být produkovány jako jednotlivé signály (single calls) nebo produkovány v sériích elementů oddělených mezi sebou mezerami různé délky (viz např. Schneiderova & Policht 2012; Goncharov *et al.* 2021). Například alarmy řady palearktických sýslů rodu *Spermophilus* tvoří spíše jednotlivé alarmy, i když mohou být vydávány i v sériích, ale ty nevytvářejí vysloveně elementy (elements, notes) v rámci kontinuálního repetitivního signálu, zatímco alarmy severoamerických sýslů, např. rodu *Ammospermophilus* a *Otospermophilus* často mívají repetitivní strukturu (viz např. Bolles 1980). Konkrétně Matrosova *et al.* (2012) rozpoznává u sýslů čtyři typy alarmů na základě jejich akustické struktury: (1) jedno-elementové, slabě modulované hvizdy, (2) jedno nebo několik málo modulovaných hvizdů, (3) multi-elementové série, (4) série mnoha krátkých širokospektrálních elementů (Matrosova *et al.* 2012).

Jednotlivé komponenty akustické struktury lze vztáhnout ke specifické funkci studovaných signálů a korelovat s fenotypem či behaviorálním kontextem. Některé akustické

parametry se vztahují ke zdroji zvuku v závislosti na fyziologii a dynamice hlasivek (source related parameters), jiné parametry zase k rezonančním charakteristikám vokálního traktu (filter-related parameters), který je nezávislý na zdroji zvuku (viz Fitch & Hauser 2002). Výsledná podoba zvuku, který opouští tělo volajícího, tak zpravidla mívá jinou charakteristiku, než měl při svém vzniku v hlasivkovém aparátu, jelikož při průchodu vokálním traktem (supralaryngeal vocal tract), který se nachází nad hlasivkami, dochází ke změnám jeho charakteristik (viz Fitch & Hauser 2002; Taylor & Reby 2010). Na základě této teorie (source–filter theory), stanovující vibrace zdroje zvuku následně filtrovaných supralaryngeálním vokálním traktem, lze predikovat, že informace o velikosti těla, hmotnosti pohlaví či věku mohou být obsaženy jak ve vibračních charakteristikách hlasivek (nejčastěji charakterizovaných základní frekvencí F0), tak spektrální charakteristikou vydávané vokalizace hlasivek (nejčastěji charakterizovaných rezonancemi vokálního traktu, formantovými frekvencemi) (Taylor & Reby 2010). Predikce této teorie byly opakovaně potvrzeny jak analýzou konkrétních hlasů, tak následnými playbackovými experimenty (viz Fitch 1997; Charlton, Reby & McComb 2007; Cartei & Reby 2013; Charlton & Reby 2016; Baotic *et al.* 2018). Ačkoli tato teorie byla původně navržena jako model pro vokalizaci savců a člověka, její platnost se ukázala v některých případech i u ptáků (Fitch & Kelley 2000). V případě chřástala polního (*Crex crex*) však formanty s velikostí těla korelují jen slabě, na rozdíl od individuální specifity (Budka & Osiejuk 2013). V tomto případě se však jednalo o teritoriální hlas a ne o alarm. Pokud jde o alarmy, tak formanty se ukázaly kódovat identitu volajícího pokud jde o jeho individuální identitu, pohlaví nebo příslušnost ke skupině (Leuchtenberger *et al.* 2016).

Zajímavým případem jsou alarmy slonů afrických (*Loxodonta africana*). Přestože komunikace slonů je velmi intenzívně zkoumána, kdy zvláště po objevu jejich podprahové infrazvukové komunikace se staly modelem pro studium řady aspektů komunikace v rámci sociálních druhů, jejich alarmový systém zůstával poněkud stranou zájmu detailnějších studií. Sloni, oproti většině ostatních savců mají nepoměrně méně predátorů. Významněji je dokáží ohrozit zejména lvi (Estes 1991; Kingdon 1997) a samozřejmě člověk. Jejich alarmový systém odhalil některé unikátnosti. Playbackové experimenty ukázaly, že sloni se dávají na útěk, když slyší bzučení vyrušených včel. Přitom vydávají alarmy, kterými varují před tímto nebezpečím ostatní slony (King, Douglas-Hamilton & Vollrath 2007; King *et al.* 2010). Tyto alarmy mají podobu odlišného typu mručení (rumble), které se liší od mručení v jiných kontextech, zejména polohou svého druhého formantu (King *et al.* 2010). Jiná studie pak dokonce ukázala (Soltis *et*

al. 2014), že sloni vydávají další specifický typ alarmového mručení, když slyší hlasy pastevců kmene Samburu v severní Keni, se kterými sloni kompetují o vodní zdroje. Tyto alarmy (Samburu alarm rumbles) se vyznačují posunem prvních dvou formantů do vyšších frekvencí a podobně zvýšená je i základní frekvence. Tyto alarmy jsou jasně odlišitelné od dříve popsaných alarmů vyvolaných bzučením divokých včel (bee alarm rumbles). Tyto výsledky tak ukazují, že alarmy afrických slonů rozlišují mezi těmito dvěma typy hrozeb a reflektují i jejich urgenci (Soltis *et al.* 2014).

Strukturní charakteristiky akustických signálů, které reflektují fyziku přenosu zvuku, mohou poskytovat řadu informací o jejich funkcích (Marler 1955). Selekční tlaky formují použití alarmů do velké variability. Rizika volajícího způsobují, že toto chování je nákladné (Marler 2004). Různé formy alarmů mohou obsahovat širokou škálu informací. Alarmy často obsahují specifickou informaci o typu predátora. V tom případě mluvíme o referenční komunikaci, která zpravidla zahrnuje odlišné typy únikových strategií spojených s odlišnými typy predátorů (Fichtel & Kappeler 2002; Caro 2005). Klasický učebnicový příklad funkčně referenční komunikace představuje alarmový systém kočkodana obecného (*Chlorocebus aethiops*), vydávajícího tři odlišné typy alarmů: v případě přítomnosti hada, levharta a orla (Seyfarth, Cheney & Marler 1980). Playback těchto tří typů alarmů vyvolal odlišné typy reakcí. V případě leopardího alarmu, kočkodani utekli do stromů, při orlím alarmu vyhlíželi vzhůru a po playbacku hadího alarmu se vztyčili do bipedního postoje a shlíželi dolů. Obdobný referenční systém byl popsán a následně experimentálně potvrzen u dalších primátů čeledi Cercopithecidae a Lemuridae, ale i u surikat ale i dalších, u nich však bez experimentální demonstrace (někteří sciuridi rodu *Callosciurus*, *Spermophilu* a *Tamasciurus*) (viz review Fichtel & Kappeler 2002). V případě ptáků byla referenční signalizace nalezena ve třech řádech a pěti čeledích, kde většinu z nich tvořili pěvci (Smith 2017). Zde byly nejčastějším typem referenčního signálu vzdušný alarm (aerial alarm), následovaný terestrickým alarmem a pak také „food calls“. Citovaný autor uvádí, že všechny tyto tři typy funkčně referenčních signálů byly doposud zaznamenány u kura domácího a bělokura běloocasého (*Lagopus leucura*).

Alarmy však nemusí být vždy predátor-specifické. Takové alarmy bývají podobné napříč řadou predátorů, vůči kterým jsou používány, ale např. zkracování intervalů mezi jednotlivými elementy, tedy zrychlení tempa těchto elementů za sebou v případě repetitivních signálů nebo zrychlení vydávání jednotlivých samostatných alarmů za sebou může vyjadřovat percepci potenciálního rizika (viz např. Blumstein & Armitage 1997). V takovém případě se jedná expresi aktuální míry nebezpečí, nebo-li urgenci hrozby (Yorzinski & Vehrencamp

2009). V tomto případě není specifita založená na identitě predátora, ale na specifitě vyjadřující míru nebezpečí, která je vyjádřena akustickou strukturou (Blumstein 1999; Fichtel & Kappeler 2002). Míra urgencye hrozby může být zakódována různými způsoby. Může se jednat o variabilitu akustické struktury. V případě surikat, nízkou urgenci vykazují alarmy s harmonickou strukturou, zatímco vysokou urgenci alarmy s větší mírou hlukové struktury (noisy) (Manser 2001). Častým způsobem kódování urgencye je variabilita tempa elementů jdoucích v sérii za sebou či počtu těchto elementů v repetitivních signálech. Tak tomu je u řady pozemních sciuridů, například sviště žlutobřichého (*Marmota flaviventris*), kde se snižuje interval hvizdů s vyšším predaním rizikem. Vyšší počet hvizdů v sérii vyvolává intenzivnější reakci než u jednotlivě vydávaných hvizdů (Blumstein & Armitage 1997). Urgence hrozby může být rozlišována i mezidruhově. Střízlíkovec bělobrvý (*Sericornis frontalis*) a modropláštník nádherný (*Malurus cyaneus*) navzájem rozlišují své alarmy (Fallow & Magrath 2010). Střízlíkovec komunikuje urgenci hrozby počtem elementů svého alarmu se zkracováním vzdálenosti od potencionální hrozby. Zajímavým dokladem je i rozpoznávání v rámci heterospecifické interakce mezi severoamerickou sýkorou černošedou (*Poecile atricapillus*) a brhlíkem americkým (*Sitta canadensis*). Tato sýkora má komplexní alarmový systém založený na „chick-a-dee“ alarmu, kde tento signál vykazuje největší variabilitu v počtu elementů, zejména pak koncových širokospektrých „D“ elementů (Templeton & Greene 2007). Brhlík americký reaguje na tento typ alarmu, a dokonce mnohem silněji reaguje svým mobbingem vůči alarmu směřovaného vůči menšímu dravci než tomu většímu. Menší dravec nebo sova, představuje větší hrozbu pro malé pěvce, než je tomu v případě většího dravce či sovy. Počet elementů tohoto signálu opět kóduje urgenci hrozby. Podobně mangusta liščí (*Cynictis penicillata*) používá alarmový systém založený na dvou typech alarmů pro vysokou a nízkou urgenci hrozby (Le Roux, Cherry & Manser 2009).

Další způsob vnímání urgencye hrozby může být prostřednictvím počtu volajících jedinců. Sysel Richardsonův (*Urocitellus richardsonii*) ze severních oblastí severní Ameriky, extrahuje informaci o aktuálnosti hrozby prostřednictvím počtu jedinců, kteří vydávají repetitivní alarmy, zároveň však ignorují alarmy nezkušených juvenilních jedinců (Sloan & Hare 2008). Dospělci reagují delší dobou svého ostražitého chování na druhou sérii volání odlišného jedince než stejného jedince. Dospělí jedinci tak rozlišují individuální identitu volajících. Naproti tomu mladí jedinci reagují intenzivněji na volání souseda v porovnání s voláním „nesouseda“ (Hare 1998). Jejich reakce se rychle snižuje při druhé sérii volání. Dochází tak k rychlé habituaci na opakovaná volání stejného jedince, zatímco na volání jiného dalšího jedince se opět obnoví ostražitost na úroveň před habituací. Juvenilní jedinci tohoto

sysla tedy rozlišují alarmové volání sousedů a nesousedů a reagují na ně odlišným způsobem (Hare 1998). Volání souseda tak představuje vyšší míru potenciálního nebezpečí indikující blízkého se predátora. Numerická diskriminace volajících byla dokumentována i u dalších druhů pozemních sciuridů (viz např. Robinson 1981; Blumstein, Verneyre & Daniel 2004).

Různá míra informací je obsažená i v komunikaci matky a mlád'at. Zde se v zásadě jedná o rozpoznávání na dvou úrovních, na úrovni druhu a na úrovni individuální identity. Pokud matka slyší volající mládě, může probíhat rekognice na těchto dvou úrovních, nebo jen na jedné z nich, či vlastní mlád'ata nerozpoznává, alespoň pokud se týká konkrétního signálu používaného repertoáru nebo akustických signálů vůbec. V tom případě může používat jiné komunikační modality, například pachové. Takovéto rozpoznávání je často dokumentováno u žadonění mlád'at nebo kontaktních hlasů. Ukázalo se však, že se může vyskytovat i u méně často využívaných signálů, vydávaných v jiných kontextech, například v případě aktuálního ohrožení mláděte. Stresové hlasy, tzv. „distress calls” jsou produkovány v případě, kdy je zvíře chyceno člověkem či predátorem a jsou odlišitelné od “isolation calls”, vydávaných mládětem v situaci izolace od matky a žadonění “begging calls” produkováných když je mládě hladové i u kontaktních hlasů “contact or group cohesion calls” vydávaných ve svých sociálních skupinách (Lingle *et al.* 2012). Například laně jelence ušatého (*Odocoileus hemionus*) aktivně brání kolouchy jak vlastní, tak cizí, ale dokonce i kolouchy jelence běloocasého (*Odocoileus virginianus*) (Lingle, Pellis & Wilson 2005). Naproti tomu laně jelence běloocasého brání jen vlastní kolouchy (Lingle, Rendall & Pellis 2007). Kolouši obou druhů v nebezpečí vydávají stresové hlasy (distress calls) s bohatou harmonickou strukturou (Lingle, Rendall & Pellis 2007). Stresové hlasy kolouchů obou druhů se liší jak mezidruhově, tak individuálně, proto je otázkou tak rozdílná reakce laní obou druhů. Playbackové experimenty ukázaly, že samotná akustická variabilita by pravděpodobně byla dostačující pro rychlou a spolehlivou diskriminaci obou druhů, ale nemusí být dostačující pro jednoznačné rozlišení identity vlastního koloucha (Lingle, Rendall & Pellis 2007). Naso-nasální kontakt matek s mlád'aty dříve, než umožnili kojení vlastnímu kolouchovi a následné odmítnutí cizího koloucha ukazuje nezbytnost čichu pro rozlišení identity vlastního mláděte (Lingle, Rendall & Pellis 2007).

3.6. Alarmy: vrozené vs. naučené

Rekognice predátorů je spíše vrozená a nepodléhající učení, zejména u těch druhů, jejichž mlád'ata jsou nezávislá již od svého vylíhnutí (Göth 2001; Hollen & Radford 2009), zatímco u

druhů s parentální péčí, se rekognice predátorů objevuje někdy v průběhu času (Griffin, Blumstein & Evans 2000; Griffin, Evans & Blumstein 2001; Hollen & Radford 2009). Případně se ale může jednat i o kombinaci obou variant (Haff & Magrath 2012). Reakce vůči alarmům prostřednictvím vrozených schopností či učení se nutně vzájemně nevylučují a mláďata která používají oba mechanismy mohou být úspěšnější ve vyhýbání se predaci (Haff & Magrath 2012). Expres těchto vrozených schopností může být bezprostřední, záhy po narození, nebo opožděná (Haff & Magrath 2012). Řada autorů řešila otázku, jakým způsobem se tyto schopnosti vytváří. Ukazuje se, že někteří obratlovci jsou schopni identifikovat takovéto signály díky vlastní zkušenosti, kterou získávají s věkem, zatímco jiní mohou reagovat na doposud neznámé alarmy díky podobnosti jejich akustické struktury s jejich vlastními konspecifickými signály (Fallow, Pitcher & Magrath 2013). Akustická podobnost alarmů mezi různými druhy umožňuje heterospecifické rozpoznávání bez nutnosti předchozího učení (Marler 1955; Randler 2012). Taková schopnost může být rozvinuta dokonce i mezi alopatrickými druhy, které se spolu nesetkávají, což v takových případech naznačuje, že je fylogeneticky zakonzervována (Randler 2012). Na druhou stranu by reakce na neznámý alarm mohla být důsledkem vrozeného strachu z neznámých podnětů (Magrath *et al.* 2015). Takováto neofobie vůči samotným akustickým signálům zůstává oproti vizuální neofobii nedostatečně testována (Magrath *et al.* 2015). Magrath *et al.* (2015) předpokládá, že neofobie na akustické stimuly nemusí být moc rozšířena, jelikož poplašná reakce vůči každému novému zvuku by byla nákladná redukcí času, který by jinak byl k dispozici k ostatním aktivitám. Mláďata mohou adekvátně reagovat na heterospecifické alarmy pomocí vrozené schopnosti nebo učení, či kombinací obou (Haff & Magrath 2011).

Flexibilitu alarmového komunikačního systému dokládají experimenty, při nichž byl subjektům prezentován nový, doposud neznámý typ hrozby. Když kočkodanům zeleným (*Chlorocebus sabaues*), byl prezentován letící dron, reagovali na něj alarmem, který byl jasně odlišitelný od všech ostatních alarmů tohoto primáta vydávaných na různé typy predátorů, ale velmi podobný alarmu používaného vůči vzdušným predátorům jiného kočkodana, kočkodana žlutozeleného (*Chlorocebus pygerythrus*) (Wegdell, Hammerschmidt & Fischer 2019). To indikuje konzervativnost akustické struktury alarmů. Aby autoři zjistili, jak rychle zvířata prisuzovala význam zvuku dronu, přehrávali jeho zvuk po jedné až třech expozičních. Zvířata okamžitě skenovala oblohu a utíkala se schovat do úkrytu, což ukazuje na schopnost rychlého auditivního učení. Učení se odposlouchávání alarmů umožňuje jedincům získávat ekologicky

relevantní informace, což ukazuje na fenotypovou plasticitu, která bude zvláště cenná v případech vystavení se novým druhům (Magrath & Bennett 2012).

3.7. Hlasy predátorů

U některých savců a ptáků bylo zjištěno, že reagují na hlasy predátorů, přestože tito predátoři nevokalizují v průběhu samotného lovu (Blumstein *et al.* 2008). Vokalizace během lovu se vyskytuje zcela výjimečně, například je známa u psa hyenovitého (*Lycaon pictus*) (viz Schaller 1972), ale byla zaznamenána i u pumy americké (*Puma concolor*) (Smallwood 1993) a dokonce i u tučňáků lovicích pod vodou (Thiebault *et al.* 2019). Koexistence predátorů na území sdíleném se svou kořistí bývá doprovázena signály v ostatních kontextech, například při obhajobě teritoria, reprodukčních a sociálních aktivitách (Hettena, Munoz & Blumstein 2014). Potenciální kořist tak získává informace o jejich přítomnosti, aktuálním chování či distribuci v prostoru. Kořist může mít ontogenetickou zkušenost, evoluční zkušenost nebo oba typy zkušenosti s predátory (Atkins *et al.* 2016). Zejména u dlouho žijících druhů, majících prodlouženou periodu učení, schopnost modifikace behaviorálních reakcí, jako právě antipredační chování na základě individuálních zkušeností, může být adaptivnější než fixované behaviorální strategie (Yorzinski & Ziegler 2007). Přestože kořist (los), která ztratila kontakt s predátorem na dobu 50-130 let, byla vysoce ohrožena při počátečních setkáních s navraceným predátorem (vlk), během jediné generace upravila své antipredační chování ke snížení rizika predace (Berger, Swenson & Persson 2001). V případě populací, ve kterých zmizel predátor, může dojít jak k rychlé ztrátě anti-predačního chování, tak naopak, příslušné antipredační chování může přetrvávat po mnoho generací (Blumstein, Daniel & Springett 2004; Blumstein, Ferando & Stankowich 2009; Orrock 2010; Li *et al.* 2011). Playbackové experimenty ukazují, že hlas predátora může setrvávat v paměti potenciální kořisti i v případě její dlouhodobé izolace od přirozeného habitatu (Li *et al.* 2011). Tak tomu bylo v případě jelena milu (*Elaphurus davidianus*) reagujícího na hlas tygra (Li *et al.* 2011). Rozlišování predátorů může být buď učeno pozorováním konspecifických nebo také heterospecifických jedinců v jejich reakcích vůči predátorům či prostřednictvím vlastní přímé interakce s predátorem (Hettena, Munoz & Blumstein 2014).

Hettena et al. (2014) kategorizoval čtyři typy zkušeností kořisti se svým predátorem:

- 1) Ekologická a evoluční zkušenost (eco-and-evol): kořist interagovala s daným predátorem v průběhu svého života a zároveň má koevoluční historii s tímto predátorem.
- 2) Pouze ekologická zkušenost (eco-only): kořist interagovala s daným predátorem pouze v průběhu svého života a nesdílela s ním dostatečnou dobu, aby se ustavila společná koevoluce. Například se jedná o případ nedávné introdukce predátora do oblasti výskytu kořisti.
- 3) Pouze evoluční zkušenost (evol-only): jedná se o predátor-naivní kořist, jejíž předci historicky interagovali s predátorem, ale neměla s ním žádnou interakci v průběhu vlastního života. Znamená to, že predátor v dané oblasti vymizel.
- 4) Žádná zkušenost (no-interaction): zahrnuje případy, kdy není přítomna ani ekologická, ani evoluční zkušenost s predátorem. To může například nastat, když predátor rozšíří svůj areál a tím dojde k překryvu s oblastí výskytu kořisti.

Výše citovaná rešerše zahrnuje 183 prací, věnujících se playbackovým experimentům prezentujících hlasy predátorů. Celkem bylo studováno 76 druhů, z toho 83 % se týkalo savců a v rámci savců byly nejvíce studováni primáti a hlodavci. Nejvíce prací testovalo kořist s ekologickou i evoluční zkušeností. Kořist v tomto případě na hlas predátora reagovala a byla jím ohrožována. Méně často kořist reagovala na stimuly predátora, který byl v dané oblasti vyhuben (pouze evoluční zkušenost), stejně tak tomu bylo i v případě žádné zkušenosti s predátorem, ani ekologické, ani evoluční interakce. Ve dvou případech ze tří, kde kořist měla pouze ekologickou zkušenost s introdukovaným nepůvodním predátorem, akustický podnět působil jako hrozba a kořist reagovala.

Pokud jde o ptáky, ve srovnání s rozpoznáváním alarmů, mnohem méně je známo o tom, jak ptáci rozeznávají odlišné druhy dravců (Billings, Greene & De La Lucia Jensen 2015). Výše uvedená rešerše (viz Hettena, Munoz & Blumstein 2014) ukazuje, že většina prací zkoumala reakce savců. Jako nejčastěji používané stimuly pak v playbackových experimentech byly používány hlasy dravců (55 %), kočkovitých (17 %) a psovitých šelem (rovněž 17 %). U sviště žlutobřichého (*Marmota flaviventris*) bylo testováno, zda má schopnost rozlišovat mezi vokalizacemi různých predátorů, a pokud ano, zda používaný mechanismus je výsledkem učení nebo je nezávislý na individuální zkušenosti (Blumstein *et al.* 2008). Vzhledem k tomu, že se jedná o komplexní studii, snažící se také osvětlit mechanismus rekogničních schopností, stojí za to si ji představit podrobněji. Svišťům byly prezentovány playbacky sociálních hlasů

kojota (*Canis latrans*), vlka (*Canis lupus*), orla skalního (*Aquila chrysaetos*) a rovněž vlastní konspecifické alarmy sviště žlutobřichého. Kojot a orel představoval predátory vyskytující se na zkoumané lokalitě, zatímco vlk se zde nevyskytoval od poloviny třicátých let minulého století. Autoři také kontrolovali délku vytí vlka a kojota. Kromě toho, hlas orla byl prezentován jak v přirozené formě, tak i pozpátku, aby se otestovalo, zda rozhodují obecné vlastnosti akustické struktury těchto signálů spíše než specifická asociace s orlem, kdy reverzní signál představoval pro sviště novinku. Svišti nejvíce reagovali na vlastní alarmy a nejméně na krátké vytí kojota. Střední intenzitou reagovali na orla a vlka. Kojot a orel byl pro sviště známý predátor, zatímco vlk představoval novou hrozbu. Reakce vůči vlkovi je konsistentní s hypotézou mechanismu nezávislého na zkušenosti. V experimentu testujícím jak normální, tak reverzní průběh hlasu orla svišti silněji reagovali na normální formu tohoto signálu. To ukazuje, že svišti mají nějaký druh diskriminačního/rekogničního templátu umožňujícího diskriminovat predátory od ne-predátorů. Vlastní, konspecifické alarmy svišťů vyvolávali intenzivnější reakce než sociální hlasy predátorů. Vlastní alarmy tak představovaly vyšší prediční riziko, pravděpodobně proto, že predátoři typicky nevokalizují během lovu, zatímco alarmy indikují bezprostřední hrozbu predátora.

Mezi predátory lze zařadit i hnízdní predátory, kteří většinou nejsou schopni predovat dospělce, pokud nejde vysloveně o překvapení samice sedící na hnízdě, ale zaměřují se na vejce a nevzletná mláďata v hnízdech. To se týká i některých savců, vytvářejících pro své potomky nějakou formu hnízda nebo jiného typu doupěte či úkrytu. Mláďata, která nemají doposud dostatečně vyvinutou motoriku a fyziologii, zejména pak sensorický aparát, se mohou stát objektem pozornosti hnízdních predátorů, podobně jako je tomu v případě ptáků. Vedle vajec a nevzletných mláďat ptáků se mohou stát kořistí například krkavcovitých i některá mláďata savců. Dokonce byla zaznamenána i predace mláďat lišky polární (*Alopex lagopus*) krkavcem velkým (*Corvus corax*) (Chevallier, Lai & Berteaux 2016). V případě ptáků, pozornost hnízdního predátora navíc upoutává žadonění mláďat (Husby 2018). V případě tetřívka obecného (*Lyrurus tetrix*), hnízdní predace bývá často hlavní příčinou hnízdní neúspěšnosti (Ludwig *et al.* 2010). Krkavcovití ptáci tak představují obvyklé predátory hnízd (Soderstrom, Part & Ryden 1998; Santisteban, Sieving & Avery 2002; Hayward *et al.* 2015; Husby 2018). Zejména vrány, krkavci a většina sojek patří mezi potravní generalisty, mnohdy se přiživující plněním hnízd (Winkler, Billerman & Lovette 2020).

Ačkoli interakce kořist-predátor v kontextu hnízdní predace byla intenzivně studována (viz např. Burke *et al.* 2004; Strnad *et al.* 2012), podstatně méně pozornosti bylo věnováno

reakcím na samotný hlas hnízdního predátora, tj. bez jeho vizuální přítomnosti. Playbacky hlasu hnízdních predátorů byly prezentovány převážně nidikolním ptákům. Odezva na akusticky indikovanou hrozbu zahrnovala například změny v umístění hnízda (Eggers *et al.* 2006; Emmering & Schmidt 2011), redukci snůšky (Eggers *et al.* 2006; Zanette *et al.* 2011) i snížení rodičovské péče (Zanette *et al.* 2011).

Ptáci hnízdící na zemi jsou zvláště citliví na predaci savčími a ptačími predátory (Sullivan & Dinsmore 1990; Fletcher *et al.* 2010). V případě tetřívka obecného (*Lyrurus tetrix*) bylo zjištěno, že specificky reaguje na volání krkavce velkého (*Corvus corax*) (Policht *et al.* 2019). Reagují na něj dokonce i dospělí ptáci obou pohlaví, a to i v období mimo vlastního hnízdění, ačkoli dospělci nejsou krkavcem bezprostředně ohroženi. Reakce dospělých samic mimo dobu aktivního hnízdění, a navíc i reakce dospělých kohoutů pravděpodobně ukazuje na další vliv. Autoři práce spekulují, že raná antipredační reakce mláďat by mohla setrvávat až do dospělosti, nebo by se mohlo jednat o reakci v důsledku asociace krkavců s dalšími predátory, zejména liškou obecnou (*Vulpes vulpes*). V takovém případě by volání krkavce mohlo upozorňovat na přítomnost lišky, která již pro dospělé ptáky představuje reálnou hrozbu.

V rámci interakce predátor-kořist, lze považovat i reakce zvířat vůči člověku. Vyrušování člověkem tak představuje formu predačního rizika (Frid & Dill 2001). Druhy, které jsou loveny, často využívají podobné antipredační strategie vůči lidem, podobně jako vůči svým přirozeným predátorům. Jeleni zintenzivňují své ostražitě chování v průběhu lovecké sezony, v porovnání s dobou mimo loveckou sezónu. Také vnímají rekreační aktivity jako menší hrozbu, než aktivity lovecké (Jayakody *et al.* 2008). Podobně reagují i srnci, navíc míře hrozby přizpůsobují i výběr potravního stanoviště, kdy v průběhu lovecké sezóny preferují bezpečnější ale potravně chudší stanoviště (Benhaiem *et al.* 2008). Lidská přítomnost ovlivňuje ale i druhy aktivně nelovené (viz Severcan & Yamac 2011; Wang *et al.* 2011; Li *et al.* 2016; Hume, Brunton & Burnett 2019). Vedle samotných lidí, lze zcela určitě považovat za predátory i psy, ať už jde o volně pobíhající, či toulavé jedince, nebo lovecké psy, kteří se přímo podílejí na loveckých aktivitách člověka. Štěkání psů, pak může být signálem, představujícím predační hrozbu. Playbackové experimenty skutečně ukazují zvýšení ostražitosti vůči štěkání psů (Randler 2006). Nejnovější studie pak ukázala, že štěkání loveckých psů se dokonce liší v závislosti na druhu zvířete, se kterým pes interaguje (Policht *et al.* 2021). Tato schopnost byla pozorována i u naivních psů, kteří neměli s některými druhy testovaných zvířat praktickou zkušenost. Tak tomu bylo v případě štěkání v kontaktu s prasetem divokým (*Sus scrofa*).

3.8. Individuální variabilita

Živočichové se mohou rozhodovat na základě identity jedinců se kterými interagují (Linhart *et al.* 2019). Komunikace u sociálních druhů živočichů probíhá v rámci sítě mnoha potenciálních příjemců a vysílatelů informací (McComb & Reby 2005). Za takových podmínek, identita volajícího kódovaná v akustických signálech, usnadňuje koordinaci aktivit jedinců a může tak zlepšovat celkovou kohezi skupiny (Fichtel & Manser 2010). Rozpoznávání individuální identity má také významné evoluční konsekvence, například selekční výhody plynoucími z vyhýbání se inbrední depresi (Farrow, Barati & McDonald 2019). Rozpoznávání na úrovni jedinců bývá využíváno v celé řadě kontextů. Jedinci mohou rozpoznávat sexuálního partnera, potomka, příslušníka vlastní skupiny, souseda, spřízněného jedince-kamaráda, rivala, souseda či cizince. V porovnání s jinými kontexty, volání v kontextu predáční hrozby, kdy rychlá rekognice, včetně následné relevantní reakce na alarm, zmírňuje riziko predáční hrozby se zdá převažovat nad benefity být individuálně rozpoznatelný. Nicméně v řadě případů se fixovala schopnost rozpoznávání individuální identity volajícího. Příjemci alarmu mohou rozlišovat identitu varujícího jedince a následně reagovat různým způsobem, podle toho, jak je volající jedinec spolehlivý (Pollard 2011). Řada prací tento fenomén i dokládá. Například mláďata sysla Richardsonova (*Urocitellus richardsonii*) rozlišují mezi volajícími a reagují odlišně, v závislosti na tom, zda volání pochází od souseda či nikoliv (Hare 1998). Vyšší míru ostražitosti projevují vůči signálům, znamenající potenciálně větší riziko, tedy vůči voláním sousedů, a tedy vyšší pravděpodobnosti blížícího se nebezpečí. Podobně mladí jedinci vydávající alarmy mohou být považováni za méně spolehlivé, jelikož se častěji dopouštějí chyb, kdy nesprávně identifikují konspicivní jedince jako predátory (Hollen & Radford 2009).

Akustické signály, které jsou nositeli individuální specifity musí vykazovat množství rozpoznatelných variant (Bradbury & Vehrencamp 2011). Volající jedinec je rozpoznán pomocí unikátních parametrů, které se příjemce signálu naučí rozpoznávat a používat během vzájemných interakcí (Tibbetts & Dale 2007). Takovéto inter-individuální rozdíly ve vokalizaci (vokální individualita) mohou často hrát důležitou roli v komunikaci ptáků i savců. Pro signifikanci rozdílů ve vokalizaci mezi jedinci nestačí pouze významná míra rozdílů mezi jedinci, ale záleží také na variabilitě v rámci jedince. Inter-individuální variabilita musí být větší, než variabilita intra-individuální (Falls 1982; Terry, Peake & McGregor 2005). Pokud variabilita uvnitř jedinců je vysoká, v takovém případě jedince nelze rozlišit (Puglisi & Adamo 2004). Individuálně specifická signalizace tak vyžaduje, aby variabilita hlasů vydávaných

konkrétním jedincem byla do určité míry konzervativní v porovnání s variabilitou mezi různými jedinci.

Individuální rozdíly mohou být kódovány různými typy parametrů, které lze v zásadě rozdělit zejména na frekvenční, časové a parametry měřící intenzitu, tedy hlasitost signálu. Frekvenční parametry jako parametry kvantifikující průběh základní frekvence zdroje zvuku, tedy hlasivek/syringu (fundamental frequency – F0), a případně odpovídajících harmonických tónů, tak také parametrů vyjadřujících rezonanční charakteristiky vokálního traktu nacházejícího se nad vlastním zdrojem hlasu kdekoli na průběhu dýchací trubice a navazujících dutin hlavy. Konkrétně se jedná o formanty a podobné parametry, např. LPC koeficienty, „formant like structures“ apod. Další typy parametrů mohou kvantifikovat rozložení akustické energie ve frekvenčním spektru, například kvartily, a jiné relativní vyjádření, v závislosti na použitém softwaru, viz např. programy Avisoft, Raven, PRAAT, LMA, apod.

Frekvenční parametry kvantifikující rozložení akustické energie ve frekvenčním spektru často kódují vokální individualitu u komplexních širokospektrých typů hlasů. Mezi takové signály patří například smíšená vokalizace dinga, zahrnující jak štěknutí, tak vytí tzv. bark-howl, fungující jako alarm (Déaux, Charrier & Clarke 2016). Individuální rozdíly v tomto případě byly nejvíce kódovány frekvenčními parametry. Po frekvenčních parametrech přispívala ještě délka „bark“ a „howl“ segmentu signálu. Podobný způsob nacházíme u vokalizací typu „barks“, ale velmi podobný výsledek se také ukázal u antipredačního syčení hus, kde nejdůležitějšími parametry byly frekvenční kvartily následované délkou signálu (Policht *et al.* 2020) a podobně i pšoukání tetřívka obecného (*Lyrurus tetrix*) (Hambálková *et al.* 2021), i když zde se nejedná o predační kontext. Formanty se ukázaly být důležité pro individuální rozdíly také u nevokálních alarmů typu „snort“ vydry obrovské (*Pteronura brasiliensis*) (Leuchtenberger *et al.* 2016). Formanty byly například důležité pro individuální rozdíly ve stresových hlasech novorozených mláďat antilopy džežran (*Gazella subgutturosa*) a sajgy tatarské (*Saiga tatarica*) (Volodin *et al.* 2017). V případě sajgy to byla i základní frekvence. Frekvenční parametry a délka signálu se také ukázaly být významné u čistě tónických alarmů některých evropských syslů rodu *Spermophilus* (viz Schneiderova & Policht 2010). Zajímavým příkladem jsou alarmy sysla dlouhoocasého (*Urocitellus undulatus*), který je výjimečný mezi palearktickými sysly tím, že vydává dva typy alarmů: tonální hvizd, který je podobný alarmům ostatních syslů vyskytujících se ve stejné oblasti, ale pak také širokospektrální alarmy, které často produkuje američtí sysli (Goncharov *et al.* 2021).

Individuální rozdíly byly v případě tonálních alarmů kódovány frekvenčními parametry a v případě širokospektrálního alarmu opět frekvenčními parametry a délkou signálu.

Studium individuálních rozdílů ve vokalizaci obratlovců pokračuje intenzivně zejména v posledních patnácti letech. Výzkum individuální variability ve vokalizaci ptáků a savců je rozložen napříč taxonomickým systémem velmi nerovnoměrně. Většina dosavadních znalostí o individuálně specifické vokalizaci pochází ze studia několika taxonomických skupin. Z celkového počtu 360 publikací řešících různé aspekty vokální individuality publikovaných v období let 1968 až 2016, plná třetina pochází z výzkumu primátů a pěvců, 15% prací studovala šelmy (kdy méně jak polovina prací studovala terestrické šelmy), 7% prací se zabývala letouny, sudokopytníky zkoumalo 6% prací, sovy 6%, hlodavce 6% a krátkokřídlé 4%. Mezi málo studované taxonomické skupiny patří například hrabaví (2%) či dravci (méně jak 1%) a další.

4 Metodika

Konkrétní metodické postupy řešící jednotlivé cíle disertační práce jsou detailně popsány v příslušných publikacích (včetně použité techniky, popisu lokalit, testovaných subjektů, atd.). V této části shrnuji základní použité postupy, zejména ty, které byly společné. Předložená práce využívá dva hlavní metodické přístupy: (1) playbackové experimenty a (2) akustickou analýzu.

4.1. Playbackové experimenty

Experimentální design

Metoda playbackového experimentu byla využita pro testování reakcí na hlas hnízdního predátora. Každá playbacková nahrávka obsahovala dva prezentované hlasy: (1) hlas hnízdního predátora a (2) kontrolní hlas obsahující hlas jiného, sympatricky vyskytujícího se druhu ptáka. Pro playbacky hnízdního predátora bylo použito pět různých verzí stejného typu hlasu a pro kontrolní hlas tři různé verze pěti různých druhů sympatricky vyskytujících se ptáků, které se často ozývali na zkoumaných lokalitách. Délka těchto hlasů byla standardizována na srovnatelnou délku. Stejně tak i jejich hlasitost, pomocí funkce root mean square v programu Avisoft (Avisoft Bioacoustics, R. Specht, Berlin, Germany). Každá testovací nahrávka obsahovala kontrolní hlas a hlas hnízdního predátora, které byly odděleny 2 min intervalem. Polovina nahrávek měla jako první v pořadí hlas hnízdního predátora a druhá polovina nahrávek obsahovala jako první v pořadí kontrolní hlas. Při vlastním testování v terénu byly tyto verze lišící se pořadím znáhodněny. Před začátkem prvního hlasu byl vložen 1 min interval (pre-playback) a za koncem druhého hlasu v pořadí také následoval 1 min interval (post-playback). Tento párový design (matched pair design) udržuje konstantní environmentální proměnné (viz Kroodsma 1989). Při nalezení fokálního jedince byl vlastní experiment zahájen po 5-15 min periodě habituace, kdy se jedinec vrátil ke své předešlé aktivitě.

Analýza videonahrávek

Pro analýzu bezprostřední reakce na playback byly zvoleny 15s úseky před začátkem a po konci obou testovacích hlasů (hnízdního predátora a kontroly). U každé reakce byla změřena její délka a latence (začátek reakce po startu playbackového hlasu). Video bylo analyzováno s přesností jedné sekundy.

Reakce byly kategorizovány následovně:

- (0) *No response*: žádná viditelná reakce
- (1) *Scanning*: skenování okolního prostředí (otáčení hlavou při pozorování okolí)
- (2) *Strong vigilance*: natažení krku následované skenováním nebo odletem

Pokud fokální jedinec opustil oblast zabíranou videokamerou nebo byl jakkoli vyrušen, experiment nebyl zahrnut do analýzy.

Statistická analýza

Vedle univariátních metod byla pro analýzu reakcí na playback použita analýza hlavních komponent (PCA). Výsledné jednotlivé PCA skóre z první hlavní komponenty byly použity do smíšeného lineárního modelu (GLMM) pro testování vlivu habitatu, pohlaví, počtu jedinců a vzdálenosti.

4.2. Akustické analýzy

Akustické analýzy

Pro analýzu hlasů byly vybrány hlasy, které se nepřekrývaly s jinými hlasy nebo dalšími zvuky včetně okolního hluku. V případě nahrání většího počtu hlasů bylo z důvodu vyrovnaných vzorků vstupujících do diskriminačních analýz náhodně vybrán předem určený maximální počet hlasů. Následně byl odfiltrován okolní hluk pod dolní a nad horní hranicí frekvenčního rozsahu konkrétních signálů. Nahrávky byly analyzovány použitím programů Avisoft SASLab Pro a Raven Pro. V závislosti na struktuře zkoumaných hlasů byla zvolena kombinace akustických parametrů, které kvantifikovaly průběh zejména základní frekvence, v případě tonálních hlasů a v případě širokospektrých signálů to byla kombinace parametrů kvantifikujících rozložení akustické energie ve frekvenční i časové doméně.

Statistické analýzy

Vedle standartních univariátních testů včetně univariátních GLM modelů byla použita kroková diskriminační funkční analýza (DFA) použitím softwaru IBM SPSS Statistics 24.0. Do DFA vstupovala standardizovaná data (např. Z skóre, Box-Cox transformace). Výsledky DFA byly

následně validovány buď pomocí „leave-one-out cross-validation“ a/nebo použitím permutované DFA pro „nested design“ (viz Mundry & Sommer 2007). V některém případě bylo pro testování individuální variability využito vedle DFA analýz i PIC koeficient (Potential for Individual Coding) porovnávající variační koeficienty uvnitř jedince a mezi jedinci (viz Robisson 1992).

5 Výsledky – publikované články

Cíle práce jsou naplněny souborem sedmi recenzovaných vědeckých článků publikovaných v renomovaných impaktovaných časopisech. Výsledky jsou prezentovány formou shrnujících anotací a komentářů včetně separátů publikovaných článků, které jsou pak následně interpretovány vzhledem k vytyčeným cílům v diskuzi.

I. Test reakcí vůči hlasu vybraného predátora a ověření možností vlivu různého typu kořisti na variabilitu hlasu predátora.

Vocal recognition of a nest-predator in black grouse.

Policht, R., V. Hart, D. Goncharov, P. Surový, V. Hanzal, J. Červený & H. Burda

PeerJ 7: e6533-e6533.

Studie testuje antipredační reakce vůči hrozbě potenciálního predátora vyjádřenou jeho samotným hlasem, tzn. bez přítomnosti dalších komunikačních modalit (např. vizuální přítomnosti predátora). Krkavcovití patří mezi významné predátory ptačích hnízd. Reprezentují vokální živočichy a lze očekávat, že ptáci ohrožení jejich predací, budou senzitivní na jejich hlasy, které budou rozpoznávat. Je také otázkou, jestli hlas hnízdního predátora je rozpoznáván dospělými jedinci, ačkoli dospělci nejsou přímo ohroženi a navíc, mimo dobu vlastního hnízdění. Podle dosavadních poznatků, reakce ptáků na samotný hlas krkavcovitých byl studován hlavně u altriciálních ptáků. Práce tak testuje, zda vybraný model rozpoznává a specificky reaguje na playback volání hnízdního predátora. Otázkou také zůstává, zda se reakce na hlas hnízdního predátora potenciálně liší u modelu s extrémně polygynním či dokonce lekovým reprodukčním systémem, ve kterém se samec nepodílí žádnou formou rodičovské péče.

Hunting dogs bark differently when they encounter different animal species

Policht, R., O. Matějka, K. Benedíktová, J. Adámková & V. Hart

Scientific Reports 11: 17407.

Variabilita v signálech potenciální predáční hrozby, zejména v případě alarmů, je intenzívně studována, ale případná variabilita hlasu predátora v závislosti na druhu potenciální kořisti zůstávala nezodpovězena. To pravděpodobně vyplývá z faktu, že většina predátorů během samotného aktu predace nevokalizuje. Antipredační chování zvířat srovnatelné s reakcí vůči predátorům vyvolává i samotná přítomnost člověka. Podobný efekt může vyvolat i long-distance vokalizace psů, kterou bývá nejčastěji štěkání. Psa, jako domestikovaného zástupce psovitých šelem lze tedy považovat za potenciálního predátora. V případě toulavých a zdivočelých psů tomu také skutečně je, jak dokládají páriové či australský dingo. Kromě toho, pes doprovází člověka i při aktivním lovu, ať už se jedná o organizovanou nebo jinou formu. Předešlé studie ukazují, že vokalizace psů mohou poskytovat informace svým lidským společníkům. Zatímco vokalizace psů byla intenzívně studována během poslední dekády, štěkání doprovázející lovecké aktivity zůstávalo doposud nepovšimnuto, ačkoli lze předpokládat, že štěkání u řady loveckých plemen prošlo specifickou selekcí v rámci šlechtění za účelem oznamování interakce se zvěří. Ve vzájemné interakci člověka-lovce a psa, by vokalizace psa mohla sloužit k transferu informací souvisejících s aktuální situací psa, který interaguje se zvěří mimo dosah svého majitele. Zachycení a správné dekodování případných informací ve štěkání by se mohlo ve výsledku projevovat vyšší efektivitou lovu. Selektce loveckých psů tak mohla vést ke specifickým komunikačním schopnostem, které nebyly doposud studovány. Zkušenosti lovců indikují, že majitelé psů mohou rozeznávat druh zvířete, na který psi štěkají.

II. Porovnání míry individuální variability u vokálních signálů s odlišnou akustickou strukturou.

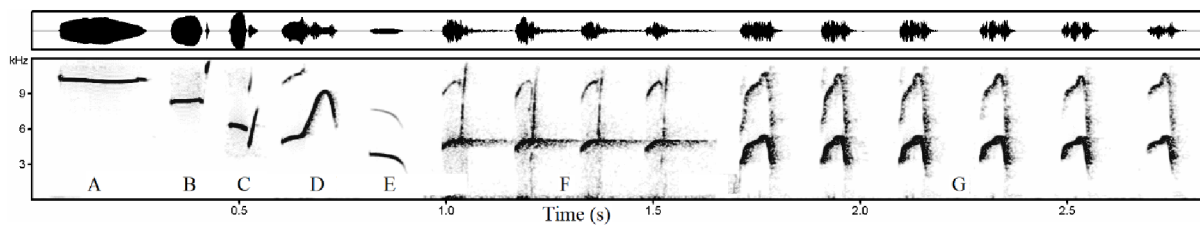
Individual-based acoustic variation of the alarm calls in the long-tailed ground squirrel.

Goncharov, D., Policht, R., Hambálková, L., Salovarov, V. & V. Hart (2021)

Royal Society Open Science, 8, 200147.

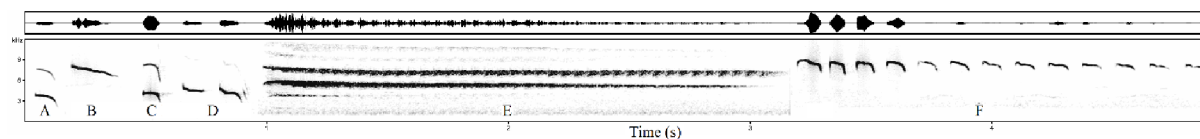
Individuální rozdíly ve vokalizaci savců jsou intenzívně studovány dlouhou dobu, zejména pak v poslední dekádě. Individuálně specifické rozdíly se ukázaly záviset na řadě faktorů. U daného druhu musí být dostatečný selekční tlak na to, aby se taková schopnost vyplatila. V některých případech může být výhodnější, jednodušší varianta rekognice, např. pamatovat si polohu hnízda, spíše než identitu každého obyvatele hnízda. Pokud jsou však hnízda ptáků či nory menších savců v těsné blízkosti, individuální rekognice se pak uplatňuje, zejména pokud mláďata začínají brzy vykazovat zvýšenou mobilitu v okolí. Míra individuálních rozdílů výrazně závisí na akustické struktuře konkrétního signálu. Tónické signály s konstantním průběhem základní frekvence mohou kódovat individuální rozdíly pomocí několika málo akustických parametrů, zatímco komplexní širokospektré signály často vyžadují kombinaci většího počtu parametrů. Oblíbeným modelem výzkumu vokální individuality jsou pozemní sciuridi. Na základě fylogenetické pozice jsou sysli nearktické oblasti nejbližší příbuzní k syslu dlouhoocasému (*Urocitellus undulates*), přestože ten se vyskytuje ve východní části palearktické oblasti. Tento druh sysla je výjimečný tím, že produkuje dva typy alarmů s kompletně odlišnou akustickou strukturou: tónické alarmy a širokospektrá volání (wideband calls). Ačkoli jsou pozemní sciuridi modelovou skupinou pro výzkum vokální individuality, individuálně specifická vokalizace doposud nebyla studována u druhu produkujícího dva takto odlišné typy alarmů. Většina druhů pozemních sciuridů produkuje buď hvízdavé nebo širokospektré alarmy. Modelový druh tak představuje unikátní model pro testování míry individuální variability v závislosti na odlišné akustické struktuře.

Prezentované publikaci předcházelo pilotní mezidruhové porovnání alarmů palearktických a nearktických syslů, které není vlastní součástí citované publikace. Cílem bylo testovat strukturální podobnost obou typů alarmů sysla dlouhoocasého se zástupci ostatních euroasijských a nearktických rodů použitím hierarchické klastrové analýzy. Spektrogramy tónických alarmů euroasijských syslů (Obr. 1) porovnávají alarm studovaného *Urocitellus undulatus* se šesti druhy rodu *Spermophilus*. Všechny tyto alarmy jsou tónické signály: jedno-elementové (A, D, E), dvou-elementové (B, C) nebo multi-elementové (F, G).

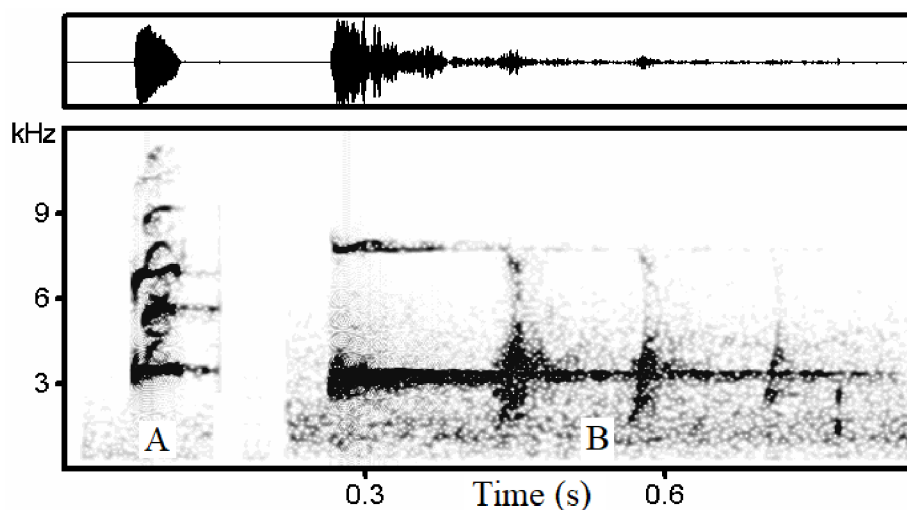


Obr. 1. Porovnání tónických alarmů euroasijských syslů: (A) *Spermophilus suslicus*, (B) *Spermophilus citellus*, (C) *Spermophilus taurinus*, (D) *Spermophilus erythrogenys*, (E) *Urocitellus undulatus*, (F) *Spermophilus pygmaeus*, G) *Spermophilus fulvus*.

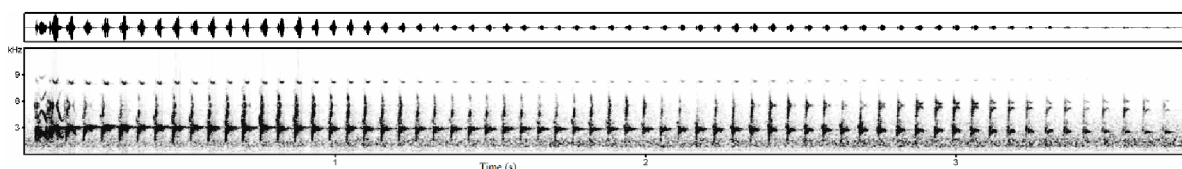
Alarmy severoamerických druhů rodu *Urocitellus* představují také tónické signály (Obr. 2-4), které mohou také být jedno-elementové (Obr. 2: A, B, C), dvou-elementové (D) nebo multi-elementové (Obr. 2: E, F). Podobně tomu je i v případě rodu *Otospermophilus* (Obr. 3) a *Ammospermophilus* (Obr. 4).



Obr. 2. Tónické alarmy severoamerických druhů rodu *Urocitellus*: (A) *Urocitellus undulatus*, (B) *U. richardsonii*, (C) *U. columbianus*, (D) *U. armatus*, (E) *U. armatus*, (F) *U. elegans*.



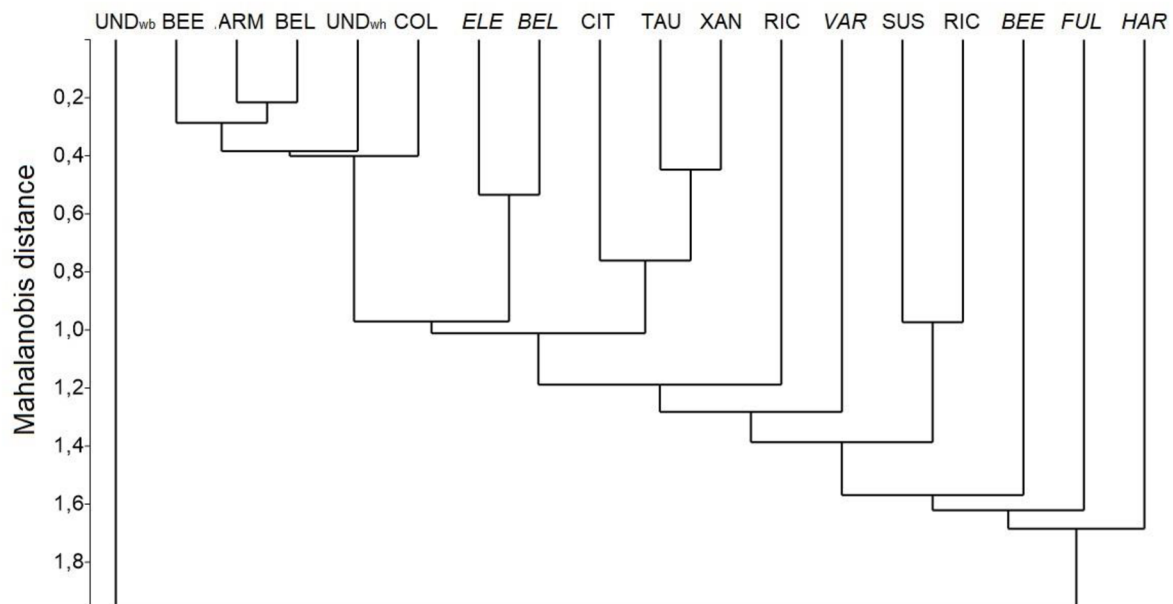
Obr. 3. Tónické alarmy zástupců rodu *Otospermophilus*: (A) *O. beecheyi*, (B) *O. variegatus*.



Obr. 4. Multi-elementový alarm zástupce severoamerického rodu *Ammospermophilus*: *A. harrisii*.

Následující klastrová analýza porovnává míru podobnosti jak tónických, tak širokospektrých alarmů produkovaných palearktickými a nearktickými druhy. Cílem bylo zjistit míru podobnosti obou typů alarmu sysla dlouhoocasého (*Urocitellus undulatus*) s alarmy ostatní syslů. Bazální větev dendrogramu (Obr. 5) tvoří širokospektrý alarm *Urocitellus undulatus*, který je odlišný od alarmů všech ostatních druhů. Druhý typ alarmu tohoto sysla (tónický alarm) je lokalizován v klastru, který zahrnuje jak jedno-elementové alarmy (*Otospermophilus beecheyi*, *Urocitellus beldingi*, *Urocitellus columbianus*), tak dvou- a tři-elementové alarmy *Urocitellus armatus*. Druhý klastr zahrnuje multi-elementové alarmy *Urocitellus elegans* a *Urocitellus beldingi*. Dvou-elementové alarmy rodu *Spermophilus* tvoří jasně determinovaný klastr, kde *S. taurinus* a *S. xanthoprymnus* představují sesterské druhy. Poslední klastr zahrnuje zejména druhy produkující jak repetitivní multi-elementové alarmy (*Otospermophilus beecheyi*, *Ammospermophilus harrisii*, *Spermophilus fulvus*), tak dlouhé formy hvízdavých alarmů (*Urocitellus richardsonii*, *Spermophilus suslicus*). Alarm produkovaný od *Otospermophilus beecheyi* v tomto klastru bývá označován jako “chats” a je tvořený směsicí

struktur v rozsahu od harmonických až po hlukové (noisy) elementy (Owings & Virginia 1978). Kratší forma alarmového hvizdu produkovaného *Urocitellus richardsonii* a také *Otospermophilus variegatus*, jsou lokalizovány mezi klastrem repetitivně multi-elementových a dvou-elementových alarmů rodu *Spermophilus* (Obr. 5).



Obr. 5. Dendrogram podobnosti alarmů na základě Mahalanobisových distancí: (UNDwb) *Urocitellus undulatus* – wideband alarm, (BEE) *Otospermophilus beecheyi*, (ARM) *Urocitellus armatus*, (BEL) *Urocitellus beldingi*, (UNDwh) *Urocitellus undulatus* – whistle, (COL) *Urocitellus columbianus*, (ELE) *Urocitellus elegans*, (CIT) *Spermophilus citellus*, (TAU) *Spermophilus taurinus*, (XAN) *Spermophilus xanthopymnus*, (RIC) *Urocitellus richardsonii*, (VAR) *Otospermophilus variegatus*, (SUS) *Spermophilus suslicus*, (FUL) *Spermophilus fulvus*, (HAR) *Amмосpermophilus harrisii*. Jednotlivé alarmy (single calls - whistles, wideband alarms) písmo v normálním fontu, repetitivní multi-elementové alarmy (trills, chirps, a churrs) označeny kurzívou.

Low frequencies in the display vocalization of the Western Capercaillie

(Tetrao urogallus). Hart, V., **R. Policht**, V. Jandak, M. Brothnek & H. Burda

Peerj 8: 13.

Zatímco hlasy zvířat nad horní hranicí slyšitelnosti člověka jsou dlouho intenzivně studovány, podstatně méně toho víme o hlasech produkovaných ve frekvencích nalézajících se pod spodní hranicí slyšitelnosti. Většinu informací pak pochází od savců. Pouze u několika druhů ptáků je známa produkovat nízko-frekvenční vokalizace, které přímo nedosahují infrazvukové hladiny, ale v některých případech se k ní blíží. Nejhlubší zvuky, blíží se dolní hranici slyšitelnosti člověka, produkované ptáky byly zaznamenány u nelétavých kasuárů, jejichž hmotnost přesahuje i 50 kg. Ostatní dokumentované nízkofrekvenční hlasy ptáků dosahují podstatně vyšších frekvencí, než je tomu v případě uvedených kasuárů, což odpovídá dobře dokumentované negativní závislosti velikosti těla a frekvenčních parametrů vokalizace nejen ptáků ale i ostatních terestrických obratlovců. Nalezení vokalizace o srovnatelně nízkých frekvencích se proto jeví málo pravděpodobné, zejména pokud jde o ptáky. Podobně, o funkci nízkofrekvenčních signálů ptáků je toho známo velmi málo, proto znalost potenciálu kódovat individuální identitu u nízkofrekvenčních signálů ptáků otevírá nové perspektivy v jejich biologii.

Measuring individual identity information in animal signals: Overview and performance of available identity metrics

Linhart, P., T. Osiejuk, M. Budka, M. Šálek, M. Špinko, **R. Policht**, M. Syrová & D. T. Blumstein. *Methods in Ecology and Evolution* 2019 (10) 9: 1558-1570

Rozhodování zvířat může probíhat na základě identity jedince se kterým interagují nebo na kterého reagují. To vytváří selekční tlak jak na produkci individuálně distinktních signálů, tak na schopnost diskriminace mezi těmito signály. Kvantifikace individuální identity vyžaduje posouzení variability v jednom nebo více znacích mezi různými jedinci. Pro individuálně distinktní signály je nezbytné, aby variabilita mezi jedinci převyšovala variabilitu uvnitř jedinců. Signály identity jsou studovány více než 50 let, pozoruhodné však je, že neexistuje shoda v tom, jakým způsobem kvantifikovat individualitu v signálech živočichů. Přestože existuje celá řada různých metrik pro kvantifikaci individuality, tyto metody zůstávají

nevalidované a vztahy mezi nimi zůstávají nejasné. To činí případné porovnávání výsledků mezi různými studiemi velmi problematické.

III. Ověření možnosti kódování individuální identity u nevokálních akustických signálů.

Hissing of geese: caller identity encoded in a non-vocal acoustic signal.

Policht, R., A. Kowalczyk, E. Łukaszewicz & V. Hart (2020).

PeerJ 8: e10197.

Nevokálním signálům bylo překvapivě doposud věnováno velmi málo pozornosti, zvláště v porovnání s akustickými signály produkovanými hlasovým aparátem. Některé zvuky vydávané terestrickými obratlovci nejsou produkované hlasivkami, v případě savců nebo syringem, v případě ptáků. U některých ptáků je známo několik typů takových zvuků. Kromě instrumentálních zvuků produkovaných pomocí peří, zobáku nebo křídel, mohou být také zvuky produkované konstrikcí kdekoli na své cestě z plic do zobáku nebo nozder či ústní dutiny savců, což ve výsledku vytváří turbulentní, aerodynamické zvuky. Takovéto zvuky často připomínají hvízdání, funění nebo syčení. Ačkoli syčivé zvuky byly studovány u savců a plazů, pouze několik studií se zabývalo analýzou syčivých zvuků u ptáků. Doposud bylo studováno pouze syčení u malých pěvců v hnízdech, které slouží jako obrana proti predátorům. Syčení husy domácí reprezentuje model nepěvce hnízdícího na zemi, který často produkuje syčení i mimo hnízdo, v porovnání s pěvci, kteří produkují syčení během hnízdění v dutinách (např. sýkory). Ve srovnání s vokálně produkovanými alarmy, téměř nic není známo o tom, jaký potenciál kódovat informace o identitě volajícího jedince, mají tyto nevokální syčivé zvuky ptáků. Otázkou tak je, jestli nevokální expirace mohou kódovat individuální identitu podobně, jako zvuky produkované syringem nebo hlasivkami.

Acoustic individuality in the hissing calls of the male black grouse (*Lyrurus tetrix*)

Hambálková, L., **R. Policht**, J. Horák & V. Hart.

PeerJ 9: e11837.

Akustická individualita může hrát velkou roli během reprodukčního období u mnoha ptáků. Tetřívěk obecný (*Lyrurus tetrix*) vydává dva odlišné typy long-distance signálů během toku na tokaništích: bublání a pšoukání. První představuje nízko-frekvenční sérii bublavých zvuků a druhý představuje syčivý zvuk. Pšoukání představuje signál, který není generován syringem. V porovnání se signály produkovanými hlasovým orgánem, nevokální zvuky byly doposud studovány vzácně a tento případ, představuje teprve druhou evidenci potenciálu kódování individuální identity u ptačího syčení. Případně individuální rozdíly u tohoto nevokálního signálu, který bývá výrazně produkovan během kompetice kohoutů u druhu s lekovým reprodukčním systémem, by mohly hrát významnou roli v rámci sexuální selekce. Individualita ve vokalizaci kohoutů by mohla napomáhat slepicím při výběru sexuálního partnera, a v případě kohoutů posuzovat a rozpoznávat kondici a bojovou připravenost soupeře. Individuálně distinktní pšoukání by mohlo umožňovat jeho používání pro monitorování jedinců na tokaništích. Taková metoda by mohla překonat problémy spojené s tradičními monitorovacími metodami, kdy stejný jedinec může být započítán vícekrát, pokud během monitorovací periody přelétá na sousední tokaniště.

6 Separáty vědeckých článků

Vocal recognition of a nest-predator in black grouse

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ABSTRACT

Corvids count among the important predators of bird nests. They are vocal animals and one can expect that birds threatened by their predation, such as black grouse, are sensitive to and recognize their calls. Within the framework of field studies, we noticed that adult black grouse were alerted by raven calls during periods outside the breeding season. Since black grouse are large, extremely precocial birds, this reaction can hardly be explained by sensitization specifically to the threat of nest predation by ravens. This surprising observation prompted us to study the phenomenon more systematically. According to our knowledge, the response of birds to corvid vocalization has been studied in altricial birds only. We tested whether the black grouse distinguishes and responds specifically to playback calls of the common raven. Black grouse recognized raven calls and were alerted, displaying typical neck stretching, followed by head scanning, and eventual escape. Surprisingly, males tended to react faster and exhibited a longer duration of vigilance behavior compared to females. Although raven calls are recognized by adult black grouse out of the nesting period, they are not directly endangered by the raven. We speculate that the responsiveness of adult grouse to raven calls might be explained as a learned response in juveniles from nesting hens that is then preserved in adults, or by a known association between the raven and the red fox. In that case, calls of the raven would be rather interpreted as a warning signal of probable proximity of the red fox.

Submitted 12 December 2018

Accepted 26 January 2019

Published 15 March 2019

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Academic editor

Jose Maria Cardoso da Silva

Additional Information and
Declarations can be found on
page 8

DOI 10.7717/peerj.6533

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OPEN ACCESS

Subjects Animal Behavior, Conservation Biology, Zoology

Keywords *Tetrao*, Warning call, Corvids, Vocal recognition, Nest predator, Playback, Acoustic, Predation

INTRODUCTION

Fragmentation of extensive forests in Europe is assumed to be the key factor causing population decline of tetraonid grouse (Åhlen *et al.*, 2013; Kurki *et al.*, 2000; Storch, 2007). Changes in habitat structure and composition of predators are known to interact and affect predation pressure on birds (Fletcher *et al.*, 2010; Van der Wal & Palmer, 2008). Widespread habitat fragmentation was accompanied by an increase in local populations of mesopredators including foxes and nest predating corvids (Andren, 1992; Kurki *et al.*, 2000). Ground-nesting birds are particularly susceptible to predation both by mammalian and avian predators, and not only on incubating females, but also eggs and chicks

(Fletcher *et al.*, 2010; Newton, 1993; Sullivan & Dinsmore, 1990). Goshawk and red fox were identified as two main predators of adult black grouse (Angelstam, 1984). Fox predation plays a significant role in grouse mortality, especially during winter (Kidawa & Kowalczyk, 2011). Nest predation was recognized as the most important direct cause of nest loss in grouse (Ludwig *et al.*, 2010). Indeed, after an experimental corvid removal study, black grouse nest loss decreased (Parker, 1984). Nevertheless, compensatory nest predation by other predators has occurred in the absence of corvids. The complexity of the predator–prey relationships is evident in a study showing that the goshawk, representing the main predator of adult black grouse, might in turn provide a protective advantage for grouse, as goshawks are known to prey on corvids (Tornberg *et al.*, 2016).

By chance, within the framework of ongoing bioacoustic field experiments, we noticed that adult male black grouse (*Tetrao tetrix*), appeared to react to playback calls of the raven during periods outside the breeding season. This was unexpected and prompted us to perform further experiments under controlled conditions to study the phenomenon systematically.

Black grouse nest on the ground with only hens incubating and rearing chicks. Chicks of tetraonids are extremely precocial; they follow the mother immediately after they have dried out and they develop rapidly, and are thus able to escape predators by flight just days after birth (De Juana, 1994). Given that eggs and chicks of ground nesting birds are threatened by nest predators, their parents are expected to be able to recognize and cope with the predation risk. Indeed, mammals and birds are known to react to predator calls, even if the respective predators do not vocalize during hunting (Blumstein *et al.*, 2008).

We tested the hypothesis that black grouse recognize common ravens as a threat via their calls alone. We predicted that black grouse would exhibit greater alarm, as indicated by behavioral reactions, during playbacks of raven calls compared to playbacks of calls of nonthreatening, sympatric species.

If black grouse have a lower probability of misidentifying potentially threatening species when hearing calls of nonthreatening birds, grouse responsiveness should be similar towards calls of different control birds but different towards potentially threatening calls.

MATERIALS & METHODS

Ethics statement

The research was conducted in accordance with the guidelines of the Animal Behaviour Society for the ethical use of animals in research. The study was carried out in accordance with the recommendations in the Guide for Care and Use of Animals of the Czech University of Life Sciences Prague. The protocol was approved by the Animal Care and Use Committee of the Czech Ministry of the Environment (Permit number: 15106/ENV/14-825/630/14). We point out that every animal was provoked only once, that the disturbance did not exceed naturally occurring stimulation, and that the studied species is considered a game species in Finland. This project was carried out within the framework of the bilateral cooperation of the Czech Republic (Czech University of Life Sciences Prague) and Finland concerning game management under auspices of the Department of Natural Resources,

Ministry of Agriculture and Forestry, Finland. According to Finnish legislation in general and to the hunting legislation, this type of scientific project does not require any special permits or licenses.

Field tests

We recorded and analyzed reactions to playback calls of the common raven (a potential nest predator) and control (harmless) bird species. The study was conducted at three localities in Finland: Hollola (61°05'51.5"N 25°22'59.0"E), Kainuu (63°51'06.2"N 29°09'55.2"E), and Jäkäläkangas (63°59'30.4"N 26°53'26.6"E) during two lek seasons (2012 and 2013) in April–May. Each focal individual was tested only once (for both raven and control sound). When more individuals were present, we focused on the one individual that was most visible throughout the entire experimental session. For each session, behavior, sex, habitat (Open, Tree, Road edge) and distance from the speaker, time, weather conditions, and GPS coordinates were recorded.

Each playback session consisted of calls of control bird species and the long-distance call frequently produced during flight by the common raven (*Corvus corax*) (Bergman & Helb, 1982). For the control sound, we used calls of five sympatric bird species commonly heard in the study area: brambling (*Fringilla montifringilla*), common crane (*Grus grus*), common cuckoo (*Cuculus canorus*), the Eurasian curlew (*Numenius arquata*) and mallard (*Anas platyrhynchos*), (Fig. 1). These were selected because black grouse regularly heard these calls during the observation period and their presence does not indicate increased risk of predation, nor any threat from a possible foraging competitor. They represent long-distance signals produced in non-alarming contexts. For bramblings, we used loud, sharp nasal calls that are produced frequently by males during the breeding season (Clement & Arkhipov, 2018). For common cranes, we used duet calls which are produced in sexual, territorial, and contact context when males utter a loud trumpeting call immediately followed by a lower-pitched female call (Archibald et al., 2018; Policht & Tichácková, 2010). For common cuckoo, we used a male song, the characteristic vocalization of this species (Li et al., 2017; Payne, Christie & Kirwan, 2018). For the Eurasian curlew, we used calls that we recorded during context of meeting of both partners. For the mallard, we used one of the most familiar vocalizations, decrescendo of quacks which are frequently elicited from unmated females and from separated females (Abraham, 1974; Lorenz, 2011). We prepared five versions of the common raven playback and three playback versions of each control species. For each playback version (both for common raven and controls) calls from different individuals were selected. Stimuli used in raven playbacks were 4.1–4.5 s in duration and contained 10–12 calls, similarly brambling (4.2–4.4 s, 4 calls), common crane (6.3–6.5 s, one repetitive call), common cuckoo (4.6–4.8 s, 4 calls), the Eurasian curlew (9.8–10.0 s, one repetitive call) and mallard (4.2–4.6 s, 15–17 calls) (Fig. 1A). Instead of aligning the exact playback length, we decided not to edit inter-call intervals but rather kept their natural call length when creating the stimuli. Calls of these control bird species were obtained both from a commercial CD (all the bird songs of Britain and Europe, Roché, 1993, France) and calls recorded locally in the study area. Sound levels of all playback calls

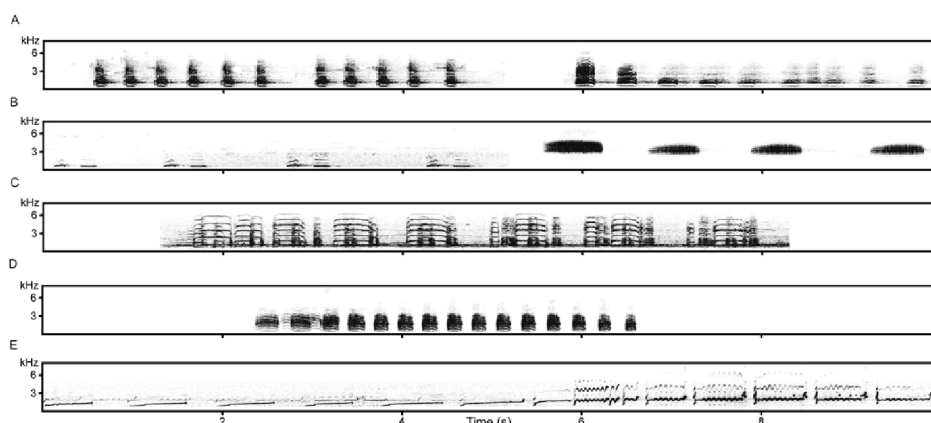


Figure 1 Spectrograms of calls used in playbacks. (A) Two playback versions of the Common raven: two series of calls (six and five calls) and one series containing ten calls. (B) Common cuckoo and Brambling. (C) Common crane. (D) Mallard. (E) The Eurasian curlew.

Full-size [DOI: 10.7717/peerj.6533/fig-1](https://doi.org/10.7717/peerj.6533/fig-1)

were standardized using root mean square in Avisoft software (Avisoft Bioacoustics, R. Specht, Berlin, Germany).

The session included one min pre-playback monitoring and one min post-playback observation which started by broadcasting the playback. The order of playbacks was randomized and the second playback followed approximately 2 min after the first playback and/or when the focal animal switched to a relaxed behavior. Such a matched pair design holds the environmental variables constant (Kroodsma, 1989). Tested sounds were played through a MIPRO MA-202 sound loudspeaker linked to Olympus PCM-11. Playbacks were played at peak sound pressure levels of about 93 dB at 1 m (measured by sound level-meter Voltcraft SL-200). Playback experiments were videotaped using a Canon digital camera (LEGRIA FS306) and the researcher remained hidden behind trees from a distance from 45 ± 16 m (mean \pm SD). When we met the birds near the road, we stopped and conducted an experiment directly from the car not to prevent any disturbance. We started the playback after a 5–15 min habituation period waiting until they returned to their previous activity.

We travelled through the area in order to find groups of grouse on leks or solitary birds. To minimize the possibility of repeated testing of the same individuals, trials were separated by at least 3 km within and between test days. Although the black grouse were not marked, high population numbers and our experimental design minimized the probability of recording the same individuals (Carrasco & Blumstein, 2012; Martínez & Zenil, 2012). We consider such design to be sufficient because this grouse is considered to be largely sedentary with limited daily movements (De Juana, 1994) and males show limited interactions with males from other leks. They are recruited on the lek locally with a high probability to remain on the same lek until the following year (Borecha, Willebrand & Nielsen, 2017).

Data analysis

We measured duration and latency of the response using frame by frame analysis of the video records 15 s before and after the onset of corvid and control sounds. Latency of

the response was measured from the beginning of playback. We categorized behavioral reactions as follows: (1) Scanning (scanning their surroundings), (2) Strong vigilance (initiated with neck stretching, followed by scanning or escaping). Only completed experiments were used in the analyses. We did not include playbacks when the focal bird disappeared from sight or when any disturbance occurred during the experiment. We used Principal Component Analysis (PCA) to express the overall response, where the individual PC factors were used as response scores of the black grouse' response to playbacks.

We used non-parametric tests: Wilcoxon Matched Pairs Tests for paired comparisons of responses to particular species-specific sounds, Mann–Whitney U Tests and Kruskal–Wallis Tests for comparing independent samples. These tests were performed with software Statistica 12.0 (StatSoft Inc., Tulsa, OK, USA). The three variables: duration, latency and intensity each describes a component of the reaction to the playback stimuli. Descriptive statistics include mean \pm SE. The Generalized Linear Mixed Model (GLMM) in IBM SPSS 23 was used for evaluation of a potential effect of habitat, sex, number of birds and distance on overall response (PC1).

RESULTS

We found that black grouse frequently exhibited vigilance shortly after hearing playback of the common raven. During the 63 trials with playback of raven calls, the majority of subjects (71%) showed an intense response, with stretched neck, scanning, or escape, Wilcoxon Matched Pairs Test: $p < 0.001$. Such a response was recorded neither during the pre-playback period nor after control calls. Black grouse were able to recognize calls of the common raven from those of control bird species. Focal birds responded to calls of the common raven faster (with shorter latency) in comparison to calls of non-corvid birds (Wilcoxon Matched Pairs Test: $p = 0.001$). Latency of the response to control stimuli lasted 7.37 ± 0.78 s (mean \pm SE), while to call of common raven 4.81 ± 0.73 s. Duration of the response to nonthreatening control stimuli was 6.83 ± 0.74 s and to the raven call was 8.98 ± 0.74 s (Fig. 2) and differed significantly (Wilcoxon Matched Pairs Test: $p = 0.001$).

Black grouse responded with increased vigilance and/or escape more frequently to raven calls in comparison to vocalization of non-predatory birds, suggesting increased vigilance to perceived predation risk. Responses of males were more intensive than those of females ($p = 0.039$; Chi-Square), were longer ($p = 0.002$, Mann–Whitney U Test) and had a shorter latency ($p = 0.024$, Mann–Whitney U Test).

To express the overall response, the individual PC factors (response scores) were used as a component measure of the black grouse' response to playbacks. The PCA revealed two principal components with an eigenvalue > 1 explaining 63% of the variation. Because both duration and latency of the response (both were Z transformed) mostly correlated with PC1 (duration: $r = -0.94$, latency: $r = 0.93$), individual PC1 factors were used for the following testing. Principal component analysis revealed highly significant response differences to the playback of raven calls compared to the calls from control species (Wilcoxon Matched Pairs Test: $p < 0.001$). Responses to different versions of control stimuli did not differ: Kruskal–Wallis Test: $H(5, n = 63) = 9.3; p = 0.097$.

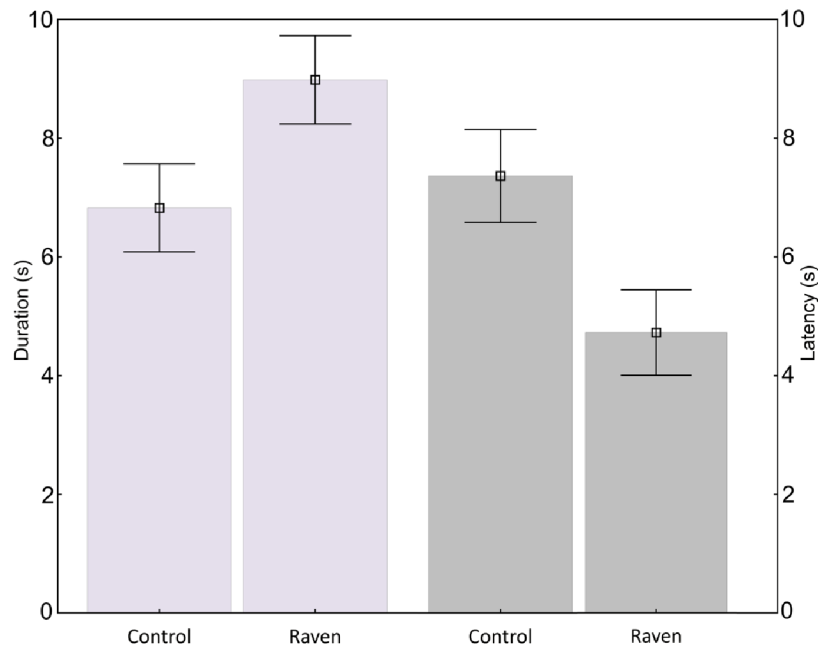


Figure 2 Duration and latency of the response. Black grouse responded to calls of the common raven in shorter latency and their reactions lasted a longer time in comparison to calls of non-corvid birds. Bars show mean with SE.

Full-size DOI: [10.7717/peerj.6533/fig-2](https://doi.org/10.7717/peerj.6533/fig-2)

Evaluation of following variables on overall response (PC1) included habitat, sex, number of birds and distance. Normal distribution of errors with Identity link function of GLMM ($F = 6.54$; $df\ 1 = 5$, $df\ 2 = 56$, $p < 0.001$) revealed only sex to have significant influence on overall responses ($F = 13.6$; $df\ 1 = 1$, $df\ 2 = 56$, $p = 0.001$), while the other variables including interactions were not significant.

DISCUSSION

Our study demonstrates that black grouse are able to distinguish calls of the common raven, a potential nest predator, from calls of non-predatory birds. Since the common raven is a vocal bird, timely reactions of black grouse to these signals, coupled with adaptive behavioral responses, such as freezing or hiding, could minimize the predation risk of the nest with eggs or chicks.

The manner and intensity with which a potential prey responds to predators depends on the ability to assess the level of predation risk and to distinguish between nonthreatening and threatening signals (Adams *et al.*, 2006). We showed that the raven is recognized by adult grouse which are actually not directly threatened by corvids. Moreover, we recorded alert responses during periods outside the breeding season, and surprisingly males, who do not provide any parental care to chicks on the nest, reacted more strongly than females. Although the females also responded outside the breeding season, the mating period represents the period immediately preceding the nesting period. Therefore, females could be theoretically motivated to the behavior that will follow soon after mating. More

robust male responses compared to that of females could reflect sexually dimorphic anti-predatory strategies. Less robust female responses could mirror the more frequent motionless camouflage response, a typical female behavioral phenotype, especially during nesting.

Potential sensitivity and specific reactivity to raven calls may be innate (analogous to responses of chicks belonging to diverse fowl species to silhouettes of raptors, cf. [Tinbergen, 1951](#)), and the responses may be preserved throughout life. The alternative (or complementary) explanation of the responses of adult black grouse to calls of ravens could be seen in the possibility that the black grouse perceive the raven call as a non-specific signal informing about danger, e.g., presence of other predators such as the red fox or humans. Particularly, the association between the common raven and the red fox is well known and a fox could follow calls of ravens to pilot to carcasses while ravens provide alarm calls after sighting a fox ([Killengreen et al., 2012](#); [Selva et al., 2003](#)). Thus, calling ravens linked with increased probability of the presence of predators may evoke adaptive anti-predator responses in adult grouse of both sexes. Similarly, ravens are scavengers, closely associated with large carnivores ([Marzluff, 2018](#)), and have been shown to be attracted by wolf howling ([Harrington, 1978](#)). Although, the impact of corvids on breeding failure has been intensively studied in many bird species, the response of birds to corvid vocalization has been a subject of relatively few studies, and focused only on altricial birds (cf. [Eggers, Griesser & Ekman, 2005](#); [Eggers et al., 2006](#)).

Predation represents a major cause of breeding failure in many bird species ([Ricklefs, 1969](#)). Corvids are generalist predators and exploit areas inhabited by grouse and altered by humans ([Manzer & Hannon, 2005](#)), particularly in fragmented landscapes ([Haegen, Schroeder & DeGraaf, 2002](#)). In the black grouse, predation has been recognized as a principal proximate cause of mortality and breeding failure, where the red fox, mustelids, and corvids represent the main predators of eggs and chicks ([Angelstam, 1984](#); [Caizergues & Ellison, 1997](#); [Parker, 1984](#); [Willebrand & Marcström, 1988](#)), whereas raptors, especially the goshawk, predate adult grouse ([Caizergues & Ellison, 2000](#); [Tornberg, 2001](#)). To our knowledge, this study is the first to show that a precocial bird species responds to calls of a nest predator. Such ability could represent an additional behavioral adaptation against nest predation. Another known strategy used by grouse species includes camouflage hen color including inactive behavior during incubation, escape by flight, grouping in flocks ([Angelstam, 1984](#)), selection of nest and brood-rearing sites with lower predator densities ([Dinkins et al., 2012](#)), and similarly, switching nest sites after nest predation ([Marjakangas, Valkeajärvi & Ijäs, 1997](#)). One possibility is that hens evaluate the potential safety of nest sites based on the acoustic landscape on the local area. Expression of this ability and recognition of calling ravens could be learned by chicks resulting in anti-predator reactions in mature adults of both sexes. To resolve these possibilities, further studies are needed on precocial birds.

CONCLUSIONS

Although predation by avian predators, such as corvids, on nests ranks among the major causes of breeding failure in birds, recognition of predator vocalizations remains poorly

studied, especially in precocial species. We tested whether the black grouse (*Tetrao tetrix*) are able to distinguish calls of the common raven, a potential nest predator, from those of sympatric and harmless bird species, representing a control group: Eurasian curlew, brambling, common crane, mallard and common cuckoo.

We show that black grouse can distinguish between calls of the common raven, a potential nest predator, and the nonthreatening birds tested. Responses of males were more pronounced compared to females. Since corvid nest predation is more likely to occur on eggs rather than precocial chicks, the study was done prior to the nesting period. Because males are not involved in brood care and adults are not directly threatened by corvids, the question raised is what is the adaptive significance of specific alert responses exhibited by male black grouse to raven calls?

Such an ability might be learned by chicks from the female, and if so, a learned reaction would then continue to mature in both sexes. We also speculate that calling signals from the common raven could inform grouse about the presence and proximity of larger predators, particularly red foxes. Such hypotheses need to be directly tested. According to our knowledge, this is the first evidence for the ability of a precocial bird species to respond to vocalizations from a nest-predator.

ACKNOWLEDGEMENTS

We very much appreciate help and support of Mr. Christian Krogell, Department of Natural Resources, Ministry of Agriculture and Forestry, Finland. We are also thankful to Mr. Ilkka Ala-Ajos for logistical support of our research in Finland. We also thank two anonymous reviewers for their comments and Michael Scott Painter for language checking.

ADDITIONAL INFORMATION AND DECLARATIONS

Funding

The research was financially supported by the grant No. QJ1220314 from Ministry of Agriculture of the Czech Republic and the Internal Grant of the Faculty of Forestry and Wood Sciences, Czech University of Life Sciences Prague, No. B06/16, B_04_18. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Grant Disclosures

The following grant information was disclosed by the authors:

Ministry of Agriculture of the Czech Republic: QJ1220314.

Internal Grant of the Faculty of Forestry and Wood Sciences, Czech University of Life Sciences Prague: No. B06/16, B_04_18.

Competing Interests

The authors declare there are no competing interests.

Author Contributions

- Richard Policht and Vlastimil Hart conceived and designed the experiments, performed the experiments, analyzed the data, prepared figures and/or tables, authored or reviewed drafts of the paper, approved the final draft.
- Denis Goncharov and Peter Surový analyzed the data, approved the final draft.
- Vladimír Hanzal contributed reagents/materials/analysis tools, approved the final draft.
- Jaroslav Červený contributed reagents/materials/analysis tools, approved the final draft, obtained the institutional support of Department of Natural Resources, Ministry of Agriculture and Forestry, Finland.
- Hynek Burda authored or reviewed drafts of the paper, approved the final draft.

Animal Ethics

The following information was supplied relating to ethical approvals (i.e., approving body and any reference numbers):

The research was conducted in accordance with the guidelines of the Animal Behaviour Society for the ethical use of animals in research. The study was carried out in accordance with the recommendations in the Guide for Care and Use of Animals of the Czech University of Life Sciences Prague. The protocol was approved by the Animal Care and Use Committee of the Czech Ministry of the Environment (Permit number: 15106/ENV/14-825/630/14). We point out that every animal was provoked only once and the disturbance was not exceeding naturally occurring stimulation and that the studied species is considered as a game species in Finland. This project was carried out within the framework of the bilateral cooperation of the Czech Republic (Czech University of Life Sciences Prague) and Finland concerning game management under auspices of the Department of Natural Resources, Ministry of Agriculture and Forestry, Finland. According to Finnish legislation in general and to hunting legislation, this type of scientific project does not need any special permits or licenses.

Data Availability

The following information was supplied regarding data availability:

The raw data are available in the [Supplemental Files](#).

Supplemental Information

Supplemental information for this article can be found online at <http://dx.doi.org/10.7717/peerj.6533#supplemental-information>.

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OPEN

Hunting dogs bark differently when they encounter different animal species

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Previous studies have shown that vocalizations of dogs can provide information to human companions. While acoustic signals of dogs have been intensively studied during the last decade, barking during hunting activities remains unstudied. The experiences of hunters indicate that owners can recognize what animal species has been found based on the bark of their dog. Such a phenomenon has never been tested before. We tested such an ability by comparing barks that were produced when dogs encountered four different animal species: wild boar, red fox, rabbit and fowl. Classification results of a discrimination analysis showed, that based on barks of dachshunds and terriers, it is possible to categorize towards which animal species barks were produced. The most distinctive barks were produced during encounters with the most dangerous of these animals, the wild boar. On the contrary, barks evoked by red fox encounters were classified similarly as those towards other smaller and non-dangerous animals like rabbits and fowl. Although the red fox represents a potentially dangerous species, the barking provoked was not classified with a much higher result than barking at animals that pose no threat. This might indicate that the key parameter could be the body size of the animal the dog meets. We further tested whether the degree of threat from the species of animal the dog encounters is reflected in the structure of the acoustic parameters based on the valence-arousal model. We found that barks produced in contact with a wild boar showed significantly lower frequency parameters and longest duration compared to other barks. According to these results, it seems that the variability of barking depending on the species of animal a dog encounters is an expression of the dog's inner state rather than functionally reference information.

Relationships between people and dogs, which represent the earliest domesticated animals, attract the attention of researchers in many ways^{1–3}. The genetic history of dogs extends into the Palaeolithic, when at least five major ancestral lineages had diversified⁴. The exact timing of the emergence of the dog lineage remains unknown⁵. Current genetic studies estimate a time of dog–wolf divergence between 25,000 and 40,000 years ago^{4,6}. Domestication of dogs caused differences from wolves in several ways. Adaptations included alterations in sequences of ritualized behaviour, and changes in motivational context for certain behaviours including changes in response thresholds^{7,8}. Dogs are better at cooperating with humans than wolves are. They are more able to recognize our facial expressions and our communication signals; therefore, they work better with humans than wolves do⁹. Dog puppies display more communicative signals to facilitate social interactions, in contrast to wolf pups^{9,10}. Thus, dogs show a unique complex of skills acquired for communication with humans¹¹. Dogs bark frequently and use them in a wider range of contexts than their close relatives the wolf^{12,13} and coyote¹², and the barks seem to have evolved from the low-frequency barks of wolves, which are mainly produced during agonistic interactions¹⁴. Barks changed quantitatively and qualitatively during the domestication process¹⁵. The complexity of the dog's vocal repertoire has been extended by using mixed sounds in barking context. Various barking forms are generated via a mix of transitions and gradations of harmonics, intermediates and noisy subunits³. Some authors suggest that the original function of barks is mobbing (alerting other pack members and calling them in to defend the territory together)¹⁶. From this, hunting barks may have also be derived, as their function is to alert humans and lead them to the prey that the dog has found. Hunting-dog breeds were originally bred to fulfill some kind of hunting work. Humans artificially selected some breeds to bark frequently^{14,17,18}. These facts indicate a strong selection force on barking performance in hunting dogs, which are also mentioned for several dog breed standards¹⁴. Additionally, sport-hunting breeds have been adapted to specific hunting work via an improved physiology, e.g. cardiac function, blood flow, and cognitive performance¹⁹. Some breeds have

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been bred for specific kinds of hunt, e.g. pointing breeds (pointers) were bred from dogs that were able to stand quietly and maintain its position in the face of the animal's scent until the human counterpart reaches the place where the animal is hiding. In contrast, other breeds were developed for multiple purposes or to be versatile and able to perform a number of tasks (e.g. hounds, retrievers, spaniels). Spaniels and retrievers will find and bring a shot animal to a hunter^{20,21}. The dachshund is considered according to Fédération Cynologique Internationale one of the most versatile hunting breeds and not just for hunting below ground. Cooperative hunting dogs keep close contact with the hunter during the hunt (e.g. retrievers) whilst non-cooperative hunting dogs perform independent work, either chasing (e.g. beagles) or attacking (e.g. terriers) the animal²². Small terriers locate and hunt smaller mammals, while larger terriers are able hunt larger animals. Selected hunting breeds were bred to follow prey while barking, and some are even capable of specialised barking; on the other hand, other breeds have to stand silently and motionless near the found animal until the arrival of the hunter^{20,23}. Specific forms of barking produced by some hunting dogs are even requested in the dog breed standards of international cynological organizations¹⁴. Recent studies have also shown that barks contain meaningful information based on context^{17,24–27}, individual identity^{24,26,27}, inner states²⁵, and emotionality^{28,29}.

We aimed to test whether hunting dogs produce barks differentially during encounters with different animal species. In order to test the barking of dogs at animals of different sizes, we needed to choose a universal dog breed. The choice of breeds for such a purpose was determined by the legislation of the Czech Republic. The Hunting Act distinguishes and defines four types of work performance. Dachshunds and terriers are the only groups of hunting dogs that can pass all four tests and be used for all types of hunting work in the Czech Republic. Although hounds are better suited to hunting wild boar, our law prohibits the use of dogs of a height of 55 cm or more for hunting ungulates. Dachshunds and terriers are no longer bred for earth-hunt work only, but are used for their independence and ability to adapt to surface work. For these reasons, dachshunds and terriers belong among the most common breeds for hunting all kinds of game in the Czech Republic. These breeds are considered to be independently working breeds that are able to work without visual contact with the hunter.

We used two different dog-breed groups: (A) dachshunds and (B) terriers. The hunting style of both breeds is as follows: looking for an animal, starting to bark, following in the footsteps of the animal, continuing to bark and chasing the animal to the hunter. In order to test potential bark differentiation, we recorded barks elicited by encounters with four different animal species. We selected animal models that would represent both (1) potentially dangerous animals (red fox, wild boar) and (2) non-dangerous animals (fowl, rabbit). Encounters with wild boars represent, for the small-bodied dog breeds used in our study, a real life-threatening situation accompanied by increased levels of emotion. Emotions with high-arousal are associated with a high sympathetic tone and a low parasympathetic tone³⁰. Emotional arousal changes the muscular actions required for vocal production (e.g. diaphragm, vocal and intercostal muscles) which affect the way air flows through the vocal system and thus alter the quality of the sounds produced³¹. Expression of emotions informs other group members about the probable behavior intentions³². Vocal responses of dogs to these animal models could show us if acoustic structure of barks allow us to make predictions about how such signals change according to emotional arousal. Review on vocal correlates of emotions revealed that vocal signals of mammals become longer with increased arousal, louder and harsher, with higher and more variable frequencies and produced at faster rates³⁰. Expression of emotions and perception of emotional states during hunting could play an important role in dogs as social species. Expression of emotions thus should benefit dogs by regulating social interactions within groups during hunting, whether it is a group of more dogs or dogs and hunters.

We have postulated the following hypotheses:

- (1) Barks produced during encounters with different animal species will have different acoustic structures. We can predict this based on previous robust literature showing that dog barks can be categorized based on various types of context^{17,24}. Barks during hunting should vary with the demands of the situation, e.g. based on urgency or a common species-specific animal response, such as running away in hares, flight in pheasants, active defence in wild boar, etc.
- (2) Barks will show a different acoustic structure depending on the arousal of the caller. We predict that barks produced during the presence of non-dangerous species might differ from those produced in the presence of potentially dangerous species. We tested whether the degree of threat from the species of animal the dog encounters (e.g. wild boar versus rabbit) is reflected in the structure of the acoustic parameters based on the valence-arousal model³³.

Results

Animal species context. We analyzed 1888 barks of 19 individual dogs belonging to two breeds—(1) dachshund and (2) terrier—which were produced in response to four different animal species (Fig. 1): wild boar, red fox, rabbit and fowl (Table 1). Figure 1 shows the bark spectrograms of the four types of barks (audio files: Additional Files 1–4).

To investigate whether acoustic parameters of barks differ in response to encountered animal species, we performed three separate stepwise discriminant function analyses (DFA) for three datasets (1) dachshunds only, (2) terriers only and (3) pooled data of both breeds.

The resulting models show a similar pattern for all three datasets independently (Table 2), including classification results (Table 3–4): the dachshund model ($N=9$, $n=810$, Wilks' lambda = 0.495, $p < 0.001$) included 8 acoustic parameters, the terrier model ($N=10$, $n=1078$, Wilks' lambda = 0.528, $p < 0.001$) included 10 parameters, and the pooled model ($N=19$, $n=1888$, Wilks' lambda = 0.549, $p < 0.001$) included 12 variables (Table 5). DFA for all three datasets showed that barks of dachshunds and terriers are recognizable based on the animal encountered with a higher probability than would be classified by chance (Table 2). The randomization procedure

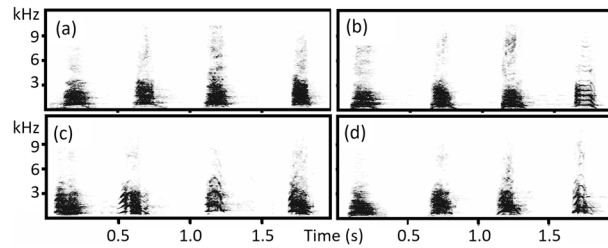


Figure 1. Spectrograms showing barking responses to wild boar, red fox, rabbit and fowl. Barks in each panel were produced by the same individual: Dachshunds Pecka (a) and Vendula (b). Fox Terriers Hard (c) and Gam (d).

Breed	Subject	Sex	Weight	Age	Wild boar	Red fox	Rabbit	Fowl
Dachshund	Hurvinek	M	7	6	30	30*	30*	30*
Dachshund	Amalka	F	5	4	0	30	30*	30*
Dachshund	Terezka	F	6	2	30	0	0*	0*
Dachshund	Nikolka	F	4	11	0*	30*	30*	30*
Dachshund	Venda-Benda	F	5	1	0	0	30*	30*
Dachshund	Vendula	F	7	10	30	30*	30*	30*
Dachshund	Kacka	F	5	4	30	30	30*	30*
Dachshund	Pecka	F	4	1	30	0	30*	30*
Dachshund	Punta	M	5	2	30	30	0*	30*
Fox Terrier	Gofi	F	7	2	0*	30*	30*	30*
Fox Terrier	Hard	M	10	1	30*	30*	30*	30*
Fox Terrier	Gam	M	9	2	30	30*	30*	30*
Fox Terrier	Cita	F	8	11	30*	30*	30*	0*
Fox Terrier	Bessy	F	8	7	30*	28*	30*	30*
Fox Terrier	Cerbis	M	10	3	30	30*	30*	30*
Fox Terrier	Nerys	M	9	9	0	30*	30*	30*
Fox Terrier	Ypsa	F	8	5	0*	30*	30*	30*
Welsh Terrier	Cit	M	7	3	30*	30*	30*	30*
Jagdterrier	Ar	M	10	2	30*	30*	30*	30*

Table 1. Tested dogs and number of analyzed barks per context. *Previous experience with tested animal model. Dachshund Nikolka was castrated, everyone else was fertile.

Result model	Classif Orig/Valid /a priori (%)		DF1 (correlation)	DF2 (correlation)
Dachshund	53.2	51.9	22.2–29.6	Duration (r = -0.49) Q3T Rel (r = -0.43)
Terrier	52.8	51.1	19.5–27.9	Duration (r = 0.78) Time 5% Rel (r = 0.57)
Pooled	50.9	49.6	20.7–27.0	Duration (r = -0.69) Q1F Rel (r = 0.58)

Table 2. The resulting discrimination function models. (Classif Orig/Valid /a priori) percentage of correct classification based on stepwise DFA, cross-validated DFA and a priori probability (classification by chance); (DF1, DF2) variable mostly correlated with the first and second discrimination function.

confirmed that these results were significant (pDFA, $p < 0.001$) for all three DFA models. Barks evoked by wild boar were classified better than those evoked by other animals (dachshund model: 60.6%; terrier model: 80.5%; pooled model: 73.3%), which is much higher than classification by chance (22.2%; 19.5% and 20.7% respectively) (Table 3). The percentages of correctly classified barks evoked by the other three animals were similar in all three models: dachshund model (43.3–52.1%), terrier model (27.8–56.2%), pooled model (35.7–49.8%). Classification outputs were significantly higher in comparison to classification by chance (dachshund model: Chi-Square = 128.7, $df = 3$, $p < 0.001$; terrier model: Chi-Square = 232.9, $df = 3$, $p < 0.001$; pooled model: Chi-Square = 170.9, $df = 3$, $p < 0.001$).

According to the arousal hypothesis it is expected that frequency parameters and call duration will differ between species which differ in their life-threatening level to hunting dog. We selected four frequency-related

Result model	Rabbit	Fowl	Red fox	Wild boar	Prior probability
Dachshund					
Rabbit	51.4	32.9	2.9	12.9	25.9
Fowl	21.3	52.1	14.6	12.1	29.6
Red fox	10.6	33.9	43.3	12.2	22.2
Wild boar	13.9	23.9	1.7	60.6	22.2
Terrier					
Rabbit	46.3	19.3	27.3	7.0	27.9
Fowl	31.9	27.8	34.8	5.6	25.1
Red fox	23.6	14.8	56.2	5.4	27.6
Wild boar	10.5	1.4	7.6	80.5	19.5
Pooled					
Rabbit	49.8	24.9	16.3	9.0	27.0
Fowl	33.9	35.7	19.2	11.2	27.0
Red fox	21.0	23.9	44.9	10.3	25.3
Wild boar	9.0	11.0	6.7	73.3	20.7

Table 3. Confusion matrix for the animal species categorization task. Percentage of correct classification represents cross-validated results. A priori probability shows classification by chance (weighted by the number of analyzed barks). Bold numbers represent the percentage of correct classifications. Other values in rows show the percentages of incorrect classifications—that is, the percentage of barks wrongly classified as barks at another animal species.

Categ	No categ	Breed	N Dogs	Method	Classif	Chance	Differ	Reference
Animal	4	Dachshund	9	DFA valid	51.9	25.0	28.2	This study
Animal	4	Terriers	10	DFA valid	51.1	25.0	26.9	This study
Context	7	Mudi	8	k-nearest	55.5	14.3	41.2	⁵⁹
Context	6	Mudi		Humans listening	65–70	50	17.5	³⁵
Context	6	Mudi	14	machine learning	43	18	25	²⁷

Table 4. Classification results of bark subcategories in other studies. (Categ) classified categories, (No categ) number of classified categories, (Classif) correct classification percentage, (Chance) classification by chance, (Differ) difference between correct classification and classification by chance.

parameters (F5, Q1F, F95, Q3F) and call duration to test whether differences in the arousal state are encoded in barks.

Barks in response to a wild boar showed significantly lower F5 than in response to other animals: F5 (GLM: $F_{3,18} = 8.3$, multiple comparisons: wild boar vs. all animals: $p < 0.001$). Differences between other animals (rabbit, fowl and red fox) were not significant ($p \geq 0.52$), (Fig. 2). The lowest frequencies in the wild boar in comparison to other animals were also shown by the other three frequency parameters (Fig. 2).

Parameter F 95 showed significant differences between all animal pairs (GLM: $F_{3,18} = 12.8$, wild boar vs. all animals: $p < 0.001$, rabbit vs. red fox: $p < 0.001$, rabbit vs. fowl: $p = 0.002$, red fox vs. fowl: $p = 0.010$).

Parameter Q3F also showed the biggest differences between wild boar and all other animals (GLM: $F_{3,18} = 6.99$, $p < 0.001$). The significant difference also showed comparison of the rabbit vs. fowl ($p = 0.001$) and rabbit vs. red fox ($p < 0.001$). Pair comparison of the red fox with rabbit and wild boar showed a significant difference ($p < 0.001$), while comparison with fowl was not significant ($p = 0.073$). Model of parameter Q1F did not show a significant effect of animal species (GLM: $F_{3,18} = 1.52$, $p = 0.222$).

Temporal parameter Duration has been the longest in barks in response to a wild boar in comparison with all other animals (GLM: $F_{3,18} = 32.4$, $p < 0.001$). The comparison showed a significant difference for all other pairs ($p < 0.001$), only red fox and fowl did not differ ($p = 1.0$).

Discussion

We aimed to test for potential differences in the barking of dogs when they encounter four different animal species—wild boar, red fox, rabbit and fowl—which represent models of various size and danger level for dogs. We used two groups of dogs—dachshunds and terriers. Classification results of a discrimination analysis showed that barks of dachshunds and terriers can be categorized based on the animal species they encountered with a higher probability than would be expected if classification was random. It was revealed that the most distinctive barks were made during encounters with the most dangerous animal, the wild boar. The same pattern was shown when we pooled both datasets together. On the contrary, barks evoked by red fox encounters were classified with a similar result to the other smaller and non-dangerous animals—here, the rabbit and fowl. Although the red

Abbreviation	Name	Description	Units	DFA model
Duration	Duration	Signal duration	(s)	I,II,III
Time 5 Rel	Time 5% relative	The point in time that divides the signal into two time intervals containing 5% and 95% of the energy	(Rel)	I,II,III
Time 95 Rel	Time 95% relative	The point in time that divides the signal into two time intervals containing 95% and 5% of the energy	(Rel)	
Q1T Rel	First quartile time relative	The point in time that divides the signal into two time intervals containing 25% and 75% of the energy	(Rel)	II
Q3T Rel	Third quartile time relative	The point in time that divides the signal into two time intervals containing 75% and 25% of the energy	(s)	I,III
F5 Rel	Frequency 5% relative	The frequency that divides the signal into two frequency intervals containing 5% and 95% of the energy relative to frequency range	(Rel)	I,II
F5	Frequency 5%	The frequency that divides the signal into two frequency intervals containing 5% and 95% of the energy	(Hz)	II,III
F 95 Rel	Frequency 95% relative	The frequency that divides the signal into two frequency intervals containing 95% and 5% of the energy relative to signal duration	(Rel)	II
F 95	Frequency 95%	The frequency dividing the signal into two frequency intervals containing 95% and 5% of the energy	(Hz)	I
Q1F Rel	First quartile frequency relative	The frequency that divides the signal into two frequency intervals containing 25% and 75% of the energy relative to frequency range	(Rel)	I,II,III
Q1F	First quartile frequency	The frequency that divides the signal into two frequency intervals containing 25% and 75% of the energy	(Hz)	
Q3F	Third quartile frequency	The frequency that divides the signal into two frequency intervals containing 75% and 25% of the energy	(Hz)	III
CF	Center frequency	The frequency that divides the signal into two frequency intervals of equal energy	(Hz)	II, III
CT Rel	Center time relative	The point in time that divides a signal into two time intervals of equal energy	(Rel)	
IQRBW	Inter-quartile Range	The difference between the 1st and 3rd Quartile Frequencies	(Hz)	III
BW90	Bandwidth 90%	The difference between the 5% and 95% frequencies	(Hz)	III
Agg Entropy	Aggregate Entropy	The aggregate entropy measures the disorder (Bits) in a sound by analyzing the energy. Higher values correspond to greater disorder in the signal whereas a pure tone have zero entropy. It corresponds to the overall disorder in the sound.in a sound by analyzing the energy. Higher values correspond to greater disorder in the signal whereas a pure tone have zero entropy. It corresponds to the overall disorder in the sound	(Bits)	I,III
Avg Entropy	Average Entropy	The average entropy measures the average disorder in a sound. Describes the amount of disorder for a typical spectrum within the signal	(Bits)	II,III
Max Entropy	Maximum Entropy	This entropy is calculated by finding the entropy for each frame in the signal and then taking the maximum values	(Bits)	II,III
Min Entropy	Minimum Entropy	This entropy is calculated by finding the entropy for each frame and taking the minimum values	(Bits)	I

Table 5. Measured acoustic parameters. Measurements based on the Raven Pro manual. DFA model: (I) for dachshunds, (II) for terriers, and (III) pooled data of both breeds. The point in time that divides the signal into two specific time intervals is related to signal duration.

fox represents a potentially dangerous species for small dog breeds, the provoked barks were not classified with a much higher success rate than barks at animals that pose no threat. This would indicate that the key parameter might be the body size of the animal the dog meets. When we compare the average success in the classification of barks at different animals with the classification results of different bark-classifying methods from different contexts we can see comparable results (see Table 4); however, different classification methods with different numbers of individuals and chance levels were used in these studies. When we take into account the resulting classification by chance level using weighting classification outputs by chance level, such a comparison may give us a general overview of the classification success generated by different classification methods.

The question is why hunting dogs bark differently at different animal species; is it because of a different inner state or it is a signal directed at their human companions? The barks investigated in previous studies were collected in different distinct social scenarios such as disturbance, isolation, play, presence of a stranger, fight training, begging, walk preparation, etc.^{14,24}, and the acoustic structure of barks presumably reflects the inner states of dogs³⁴ associated with these social contexts, which can be also recognized by humans^{25,28,35}. Across these different contexts the emotional state of a dog may likely differ. We tested therefore whether the degree of threat from the species of animal the dog encounters (wild boar versus rabbit, red fox and fowl) can be reflected by the acoustic parameter structure according to the valence-arousal model³³.

Barks produced during contact with a wild boar showed significantly lower frequency parameters. Frequency parameters seems to be not such a strong reliable indicator of emotions as they may both increase and decrease with an increase in arousal³⁰, e.g. a shift in energy distribution towards higher frequencies was found in Weddel seal³⁶, silver fox³⁷, house cats³⁸, red-fronted lemur³⁹, squirrel monkey^{40,41}, while towards lower frequencies in sheep⁴² and baboon⁴³. Temporal parameters seem to be more consistent predictors of arousal. Vocalizations of mammals become longer with increase in arousal more frequently³⁰. It is consistent with our result of the longest duration of barks produced during encounters with the most dangerous of tested animals, the wild boar.

In our case, it seems that the variability of barking, which depends on the species of animal the dog encounters, is an expression of a dog's inner state rather than functionally reference information. In addition, the expression of the inner state in barking appears to depend on the size of the potential threat. Barking in the case of a great threat (wild boar) is more specific than barking in the case of a smaller threat (red fox) or no threat (rabbit,

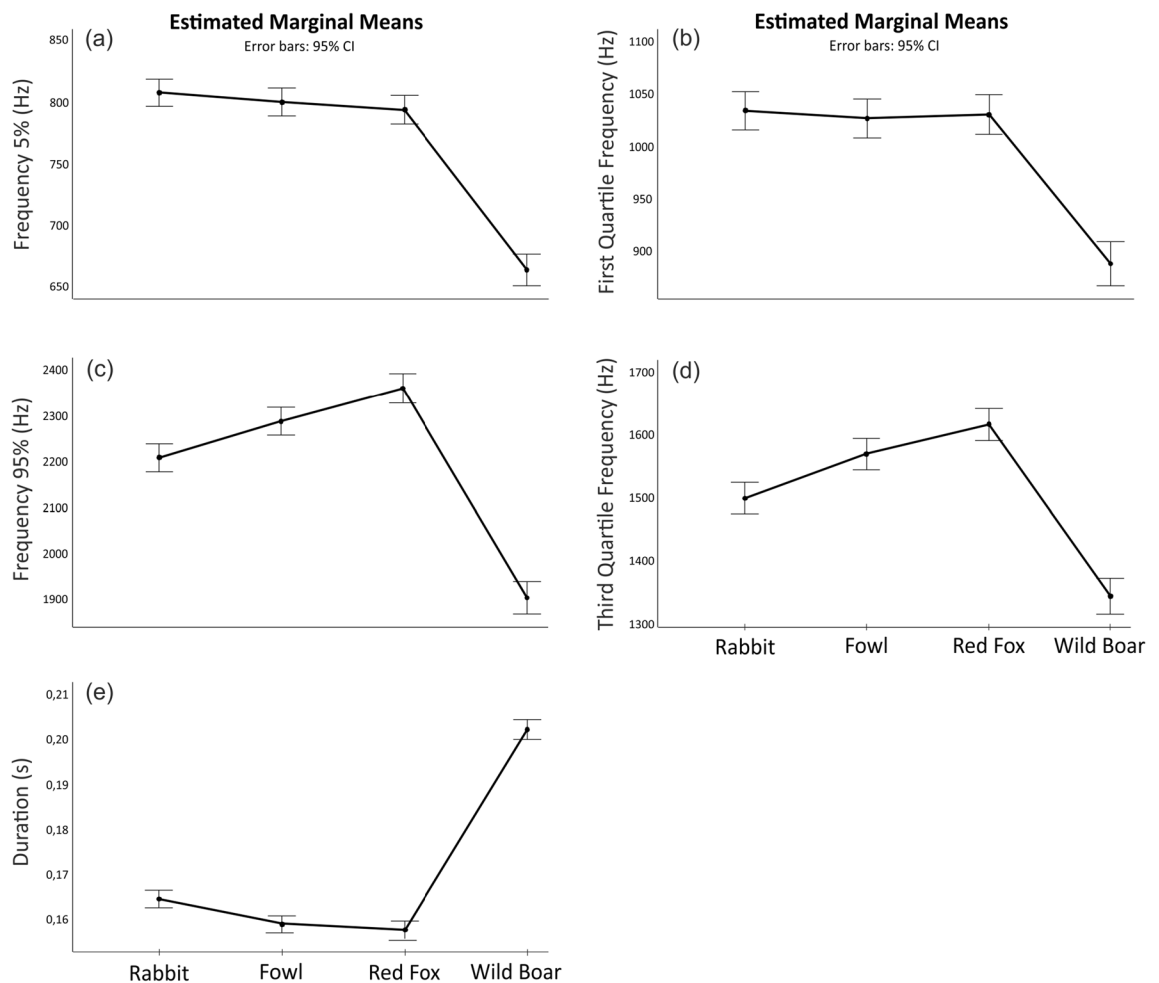


Figure 2. Univariate GLM comparison of acoustic parameters for arousal testing. Frequency parameters F5 and Q1F show the proportion of the acoustic energy in low frequencies (a–b), and F 95 and Q3F (c–d) show signal components in higher frequencies and temporal parameter Duration shows bark duration (e).

fowl). This phenomenon could then indicate an innate ability, as it has been reported in the case of naive dogs, without previous experience with wild boar.

Both dog owners and non-owners, including adults and young children, are able to categorize a dog's emotional state and barking context above the level of chance^{17,24,25,28}. Domestic dogs bark frequently in comparison to feral dogs, which produce barks relatively rarely⁴⁴. This fact could indicate that barking is at least in some way used for communication with humans²². Some authors have considered barks to be an exaggerated by-product of the domestication process that has no specific function¹². Previous studies have shown that dog barks are able to express a wider range of emotions compared to those of wolves. Such a change in the acoustic communication of dogs has resulted from their association with humans⁴⁵. Recognizing dog barks may be advantageous in inter-specific interactions since dog domestication occurred at least 30,000 years ago^{46–48}. This process was initiated by European hunter-gatherers⁴⁷. Mutualism between dog and hunter probably took place early after domestication⁴⁹ when dogs assisted in the hunting of prey⁴⁷. The ability to draw attention to different animal species complements hitherto known communicative skills like human–dog communication via eye contact⁵⁰, changes in facial expressions of dogs affected by human attentional state⁵¹, the ability of dogs to understand the communicative cues of humans⁵² or communication using eye gaze^{53,54}, and the widely known ability of dogs to understand human pointing gestures^{52,55}.

Hunting dogs were bred to follow the trail of an animal. Some of these breeds were probably selected for a specific type of barking¹⁴. Such specialization could lead to the forming of an additional ability in comparison to other breeds not selected for hunting abilities. The hearing system of canids has primarily evolved to optimize predation, especially to localize sounds produced by potential prey⁵⁶. Recognition of animal species could be favoured in reciprocal cooperation during hunting, e.g. recognition of potentially dangerous vs. non-dangerous animals could be especially favoured. Understanding the regulation mechanisms of mutual communication between humans and animals is especially important for animals such as dogs living in close contact with their human partners, depending on them for food, care and health⁵⁷. The hunting activity of dogs with humans is considered to be derived from the cooperative behavior of wolves²². In hunting dogs, we might suppose that animal-encounter-specific barking may significantly increase the effectiveness of hunting events.

Methods

Ethics statement. This is a statement to confirm that all experimental protocols were approved by a named institutional or licensing committee. The authors declare that the present study complies with the current laws of the Czech Republic. The research was carried out in accordance with recommendations in the Guide for Care and Use of Animals of the Czech University of Life Sciences, Prague. This study focused on the recording of sounds, which was not considered an invasive experimental technique by The Professional Ethics Commission of the Czech University of Life Sciences Prague (project no 14/19) and did not require a special permit.

Subjects. We recorded barks from 19 dogs (nine dachshunds—two males and seven females, eight fox terriers—four males and four females, one male Welsh terrier and one male jagdterrier) (Table 1) during December 2016 and March 2017. The age of both dachshunds and fox terriers ranged 1 to 11 years, the Welsh terrier was three years old and the jagdterrier two years old. Some dogs had previous experience with tested animal species and others were naive, with no previous experience (Table 1). The dog owners were coauthors of this study (KB and JA) and their colleagues.

Experimental procedure. Recordings were conducted under semi-controlled conditions during outdoor experiments, not during hunting events. The experimental site was chosen in isolation from other objects and potential noise. No vegetation other than low grass was present during the winter and early spring. The experiments were performed in sunny weather without rainfall and almost no wind. Each dog was tested only once per day. The interval between experimental days was longer than fourteen days. Each of 19 dogs was randomly assigned to one of the four treatments (wild boar, red fox, rabbit and fowl). Recorded barks were elicited by encounters with live four different animal species through the fence mesh. There was no direct contact between the tested animals. Only one individual dog was tested during the experiment. Each tested dog was brought to the fence, released and left alone for 5–15 min depending on the frequency of barking required. The recording microphone was placed at a distance of two metres from the fence. We used an Olympus Linear PCM LS-5 audio-recorder with a Sennheiser ME 67 microphone (frequency response 20 Hz–20 kHz) with a K6 powering module.

Acoustic analyses. We randomly selected a maximum of 30 barks per individual. These were chosen from a sample of barks of the best quality: non-overlapping barks with low background noise and a good signal-to-noise ratio. We did not obtain a full matrix as some dogs gave fewer than 30 barks. A total of 1888 barks were analyzed: 390 barks from the wild boar experiment, 508 barks from the red fox experiment, 510 barks from the rabbit experiment and 480 barks collected during the fowl experiment. We analyzed recordings using Raven Pro Sound Analysis Software (Cornell Lab of Ornithology, New York, USA) from which spectrograms were generated using the following parameters: Hann window type with a 1050 point window size, an overlap of 50%, a hop size of 11.9 ms, and grid spacing of 21 Hz. We measured 20 acoustical parameters (Table 5).

Statistical analyses. From the measured parameters we excluded those that were highly correlated ($r \geq 0.90$: F95 Rel and Avg Entropy), and the remaining variables were entered into the discriminant function analysis (DFA) (Table 3). We performed three types of analyses: (1) for dachshunds, (2) for terriers and (3) for both breeds together (pooled model). We performed a stepwise DFA in order to test whether dog barks can be classified based on the animal species that they were produced in response to. The procedure selected predictors using the Wilks' lambda criterion. We used F values as a criterion for entering or removing an acoustic parameter from a classification model (F to enter = 3.84; F to remove = 2.71). For external validation of this model we used leave-one-out cross-validation using IBM SPSS 20 (IBM Corp., Armonk, USA). Animal species were used as a group identifier and the acoustic variables were used as discriminant variables. We normalized measured variables using Z score transformation (by subtracting the mean and dividing by the variable's standard deviation), which avoids the false attribution of weights in relation to variables measured in different units (IBM Corp., Armonk, USA). We then performed a permuted DFA (pDFA) for nested designs, which serves as a randomization procedure for non-independent data⁵⁸. We calculated pDFAs using a script written in software "R" (provided by Roger Mundry) using 100 random selections and 10,000 permutations. This procedure gave a p-value which was used to determine the significance of the correct classification rate of barks to the test factor (animal species), while controlling for a single nested factor (individual). N refers to number of individuals (dogs), n refers to number of calls (barks). We used univariate general linear models (GLM) for the motivational–structural test to see if barks differ between the animal species they were produced in response to. Acoustic variables were used as dependent variables, animal species as a fixed factor, and individual dogs as a random factor. We used Bonferroni corrected post hoc multiple comparison. It was also used Chi-Square test based on the observed versus expected values to test classification outputs of DFA models.

Data availability

The datasets generated during and analyzed during the current study are available from the corresponding author on request.

Received: 18 June 2020; Accepted: 17 August 2021

Published online: 23 September 2021

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Acknowledgements

The study was funded by Internal Grants of the Faculty of Forestry and Wood Sciences, Czech University of Life Sciences Prague, reg. numbers B04_18 and A_19_13 and the "EVA 4.0" No. CZ.02.1.01/0.0/0.0/16_019/0000803 European Social Fund (Operational Programme Research, Development and Education). We also thank František Šveda who was of great help during our experiments.

Author contributions

R.P. and V.H. designed the study, R.P., K.B. and J.A. collected the data. R.P. and O.M. analyzed the data. R.P. wrote the paper. All the authors read and approved the final manuscript.

Competing interests

The authors declare no competing interests.

Additional information

Supplementary Information The online version contains supplementary material available at <https://doi.org/10.1038/s41598-021-97002-2>.

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Research



Cite this article: Goncharov D, Policht R, Hambálková L, Salovarov V, Hart V. 2021 Individual-based acoustic variation of the alarm calls in the long-tailed ground squirrel. *R. Soc. Open Sci.* **8**: 200147.
<https://doi.org/10.1098/rsos.200147>

Received: 22 June 2020
Accepted: 21 January 2021

Subject Category:
Ecology, conservation, and global change biology

Subject Areas:
behaviour

Keywords:
vocal diversity, *Urocitellus*, *Spermophilus*,
alarm call, acoustic evolution, rodent

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Electronic supplementary material is available online at <https://doi.org/10.6084/m9.figshare.c.5309820>.

Individual-based acoustic variation of the alarm calls in the long-tailed ground squirrel

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Based on their phylogenetic position, Nearctic ground squirrels are closest relatives to the long-tailed ground squirrel *Urocitellus undulates* even though it has Palearctic distribution. We aimed to investigate the variability of alarm calls of the long-tailed ground squirrel to test the individual variation in alarm calls. This species is known to produce two types of alarm calls: whistle alarms and wideband calls. Although ground squirrels are a model group for the study of vocal individuality, this phenomenon has not yet been studied in a species producing two such completely different types of alarms. Most of ground squirrel species produce either whistle or wideband alarms and this species represents a unique model for testing the degree of individual variability depending on completely different acoustic structures. We analysed 269 whistle alarms produced by 13 individuals and 591 wideband alarms from 25 individuals at the western part of Lake Baikal. A discriminant function analysis (DFA) assigned 93.5% (88.9%, cross-validated result) of whistle alarms to the correct individual and 91.4% (84%) of wideband alarms. This is the first evidence of individual variation in wideband alarms compared with whistle alarms and occurrence of vocal individuality in two warning signals of a completely different acoustic structure produced by a ground squirrel.

1. Introduction

Vocalization in both Eurasian [1–7] and North American ground squirrels [8–12] has been intensively studied, especially

during the last decade. Even though the long-tailed ground squirrel *Urocitellus undulatus* is a species with a Palaearctic distribution, its phylogenetic position (based on mitochondrial cytochrome b sequences) has been found within the Nearctic clade, and thus Nearctic ground squirrels are this species' closest relatives in comparison with other ground squirrels from the Palaearctic region [13]. The species range includes eastern Kazakhstan, southern Siberia, Transbaikalia, Yakutia (Russia), northern Mongolia and two provinces in China (Xinjiang and Heilongjiang) [14]. The long-tailed ground squirrel (*U. undulatus*) prefers short-grass steppes close to water with a thin chernozem layer and tolerates only low-density bush cover [15]. Two species (*U. undulatus* and *U. parryi*) are considered to have resulted from the more recent east–west crossings of the Bering land bridge [16]. Generic revision of the genus *Spermophilus* revealed eight morphologically distinctive genera, of which only *Spermophilus sensu stricto* is restricted to Eurasia and others have a North American distribution [14]. Of the two species of the genus *Urocitellus*, *U. parryi* has a mostly North American distribution extending to Alaska and Siberia, while *U. undulatus* inhabits the eastern Palaearctic only [14].

Variation in alarm calls of Eurasian ground squirrels has been studied in the speckled ground squirrel *Spermophilus suslicus* [6,17–20], yellow ground squirrel *S. fulvus* [6,17,19,21,22], European ground squirrel *S. citellus* [1–3,5,19,23,24], Taurus ground squirrel *S. taurensis* [1–3,23], Anatolian ground squirrel *S. xanthopyrmmus* [3,23,25], red-cheeked ground squirrel *S. erythrogegens* [5], russet ground squirrel *S. major* [26,27], Caucasian mountain *S. musicus* [25], little ground squirrel *S. pygmaeus* [25,28], *S. alaschanicus* [25], Arctic ground squirrel *Urocitellus parryii* [25], grey marmot *Marmota baibacina* [29,30] and Steppe marmot *Marmota bobak* [31]. The basic description of the alarm calls produced by ground squirrels inhabiting the Russian and Asian area included comparative study containing the following species: *Urocitellus undulatus*, *U. parryii*, *Spermophilus xanthopyrmmus*, *S. musicus*, *S. pygmaeus*, *S. alaschanicus*, *S. suslicus*, *S. relictus*, *S. citellus*, *S. erythrogegens*, *S. major*, *S. fulvus* and *S. rally* [30,32]. Results from studies up to now present various levels of distinction in alarm calls of these studied species. There is significant variation in alarm calls shown depending on species [1,2,19,23], sex [6], age [6] and between individuals [2,6,18,20,22,24]. The most striking feature in alarm vocalization of these ground squirrels is that even though their calls constitute a tonal call of very simple structure, they exhibit a high degree of individual variation, which means that each alarm call can be assigned to a specific individual with a high degree of certainty, often within the range 90–100%. The ability to recognize the calling of different individuals may increase the individual fitness of a signal recipient by adjusting its response based on the reliability of the signal. Such a recipient can save potential energetic costs by reducing its response towards unreliable signallers [33,34].

We aimed to investigate the variability of alarm calls of the long-tailed ground squirrel to test the individual variation in alarm calls. This species is known to produce two types of alarm calls: whistle alarms and wideband calls. Although ground squirrels are a model group for the study of vocal individuality, this phenomenon has not yet been studied in a species producing two such completely different types of alarms. The vast majority of ground squirrel species produce either whistle alarms or wideband alarms and this species represents a unique model for testing the degree of individual variability depending on completely different acoustic structures.

2. Methods

2.1. Study sites and animals

Alarm calls of the long-tailed ground squirrel were recorded in July 2016. In total, 14 natural colonies were found in the area of the Sarma River delta, west of Olkhon Island in the western part of Lake Baikal near to the villages of Sarma (53.1022294° N, 106.8334850° E) and Kurma (53.1799978° N, 106.9654067° E). These locations are at the foot of the Baikal Mountains in Baikal National Park in the Russian Federation. The terrain at both sites is stone and sandy. The elevation ranges from 450 to 500 m above sea level. The delta of the river creates a mosaic of water-filled meadows and dry steppes. The tree cover comprises mainly willow (*Salix* spp.), and also larch (*Larix decidua*) or pine (*Pinus* spp.). The herbaceous vegetation is dominated by grasses, accompanied by meadow blossom. Sparse settlements and free cattle grazing characterize the Sarma village; ground squirrels are not exposed to humans so often here. Kurma village is a growing tourist resort and ground squirrel encounters with humans occur on a daily basis.

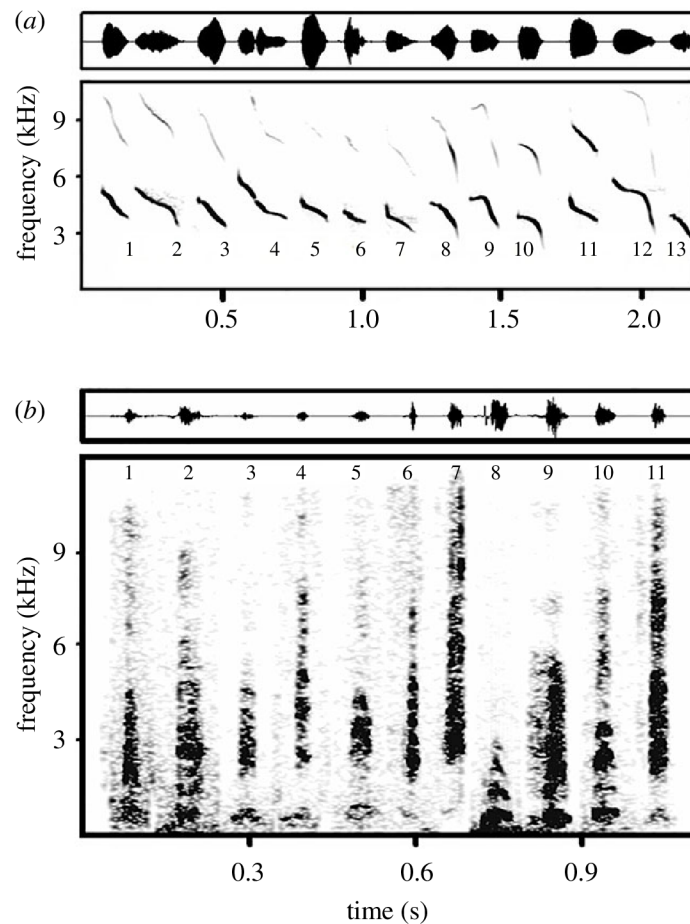


Figure 1. Spectrograms and oscilograms of (a) whistles from 13 different individuals and of (b) wideband alarms of 11 different individuals. Number of individual corresponds to the number assigned to each individual in the scatterplot. The spectrogram (a) was created with the following parameters: sampling rate 22.05 kHz, FFT 512, frame 100%, overlap 57.5%. The spectrogram (b) was created with the following parameters: sampling rate 24 kHz, FFT 1024, frame 50% and overlap 96.87%.

2.2. Recording

During pilot sampling, we tried to record alarm calls of live-trapped individuals according to methods used on other ground squirrel species [1,2] but they did not vocalize at all. Similar results were seen in attempts with live-trapped animals that were placed individually in wire-mesh hutches of $80 \times 80 \times 80$ cm [22]. We therefore decided to record free-running ground squirrels only. Each individual was recorded only once, and when we wanted to record more individuals at the same locality, each researcher selected a focal individual from within the colony and followed them closely to be sure of their identity. These focal individuals were selected from different parts of the colony to be sure with their identity. We categorized two age categories based on the body size: adults and subadults. Subadults were clearly smaller in size, an estimated half the size of adults. The vocalizations were recorded at distances from 3 to 12 m. Duration of each recording ranged from 3 to 10 min, depending on the duration of the calling behaviour of the subject. When we met an individual or a group where at least one group member was calling, we stopped and started recording in a sitting position. Each recorded individual used only one type of call, either an alarm whistle or a wideband signal. The use of the alarm type does not appear to be affected by a context that was approximately constant. We included in the final analysis only calls of those individuals in whom it was possible to observe communication behaviour when the opening of the mouth during the calling could be seen.

Alarm calls were recorded with Olympus Linear PCM LS-5 and ZOOM H5 digital audio recorders in combination with a Sennheiser ME 66 directional microphone (frequency response 20 Hz–20 kHz \pm 2.5 dB) equipped with a K6 powering module. Recordings were saved in .wav format (48 kHz sampling rate and 16-bit sample size). Long-tailed ground squirrels produce two types of alarm calls: whistle alarms (figure 1a) and wideband alarms (figure 1b).

2.3. Whistle alarms

We analysed 269 whistle alarms of the highest quality produced by 13 individuals (20.7 ± 7.4 calls, mean \pm s.d.). We included only calls with a high signal-to-noise ratio, not disturbed with wind

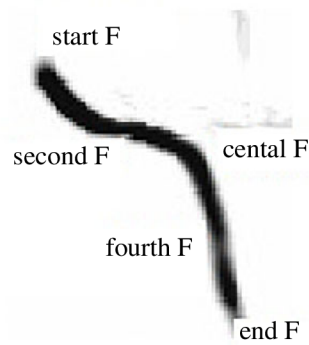


Figure 2. Measured points on the spectrograms for calculation of acoustical parameters quantifying the fundamental frequency.

and not overlapping with calls of other individuals or background noises. From these calls, we selected up to 32 calls per individual. In the case of a larger number of calls, we selected them randomly across all recorded series. The recordings were analysed using Avisoft SASLab Pro software (Avisoft Bioacoustics e.K., Schönfließer Str. 83, 16 548 Glienicke/Nordbahn, Germany). Sampling frequency was converted to 22 050 Hz and selected calls were manually labelled. We used spectrograms with the following parameters: Hamming window, FFT-length 1024, frame size 50% and overlap 96.87%. When background noise was present, it was filtered out using both high-pass (1.3–2.9 kHz) and low-pass filters (5.2–7.7 kHz). These filters show the range both for the lower- and upper-frequency limits. When surrounding noise was present, we filtered that noise which was out of the signal, e.g. when fundamental frequency reached up to 6.65 kHz, we filtered out the frequency higher than 7 kHz.

We measured 11 acoustic variables quantifying a fundamental frequency of single whistle call: duration, five frequency parameters (figure 2) using automatic measurements at five regular intervals of fundamental frequency duration of one whistle call (start frequency, second frequency, central frequency, fourth frequency, end frequency) that divided each whistle into four sections in which we measured frequency modulation as follows: first frequency modulation (start frequency minus second frequency), second frequency modulation (second frequency minus central frequency), third frequency modulation (central frequency minus fourth frequency) and fourth frequency modulation (fourth frequency minus end frequency), and frequency range (start frequency minus end frequency).

2.4. Wideband alarms

We selected 591 wideband alarms (23.7 ± 6.0 calls) of the highest quality from 25 individuals. Similarly to whistle alarms, we included only calls with a high signal-to-noise ratio, not overlapped with other calls or sounds. Signals where there was no clear lower frequency limit of the signal due to overlap with low-frequency noise were not selected. From selected calls, we randomly selected up to 34 calls per individual across all recorded series.

Sampling frequency was converted to 24 kHz and the recordings were filtered using a high-pass filter (1.6 kHz) to remove the background noise, when it was present, to increase the signal-to-noise ratio. The recordings were analysed using Raven Pro 1.5 software. Wideband alarms were manually labelled on the spectrograms set with the following parameters: Hamming window, FFT-length 512, frame size 50% and overlap 94%. Spectrogram measurements provided frequency parameters and the amplitude curve provided temporal parameters. We automatically measured 22 parameters quantifying how the acoustic energy is spread across the frequency spectrum and duration of the signal (table 1).

2.5. Statistical analyses

Individuals with a lower number of whistle alarms or wideband alarms ($n < 9$) were not included in the discriminant analysis even though they were included for descriptive statistics. Highly correlated variables were omitted ($r \geq 0.9$). The remaining 16 variables were entered into discrimination function analysis (DFA) using IBM SPSS Statistics 24.0 software (IBM Corp., Armonk, USA). In the case of whistles, all six parameters were entered into DFA. We used a leave-one-out cross-validation

Table 1. Descriptions of acoustic parameters measured in Raven Pro 1.5.

acoustic parameter name	description
call duration [duration(s)]	duration of the call.
first quartile frequency [Q1F(Hz)]	the frequency that divides the selection into two frequency intervals containing 25% and 75% of the energy in the signal.
relative first quartile frequency [Q1FRel]	the frequency that divides the selection into two frequency intervals containing 25% and 75% of the energy in the signal relative to the frequency range of the signal.
third quartile frequency [Q3F(Hz)]	the frequency that divides the selection into two frequency intervals containing 75% and 25% of the energy in the signal.
relative third quartile frequency [Q3FRel]	the frequency that divides the selection into two frequency intervals containing 75% and 25% of the energy in the signal relative to the frequency range of the signal.
aggregate entropy [AggEnt(bits)]	the aggregate entropy measures the disorder in a sound by analysing the energy distribution. Higher entropy values correspond to greater disorder in the sound whereas a pure tone with energy in only one frequency bin would have zero entropy. It corresponds to the overall disorder in the sound.
average entropy [AvgEntr(bits)]	this entropy is calculated by finding the entropy for each frame in the signal and then taking the average of these values.
max entropy [MaxEntr(bits)]	maximum entropy calculated from each frame.
bandwidth 90% [BW90%(Hz)]	the difference between the 5% and 95% frequencies.
centre frequency [CentreF(Hz)]	the frequency that divides the selection into two frequency intervals of equal energy.
centre time [CentreT(s)]	the point in time at which the selection is divided into two time intervals of equal energy.
relative centre time [CentreTRel]	the point in time at which the selection is divided into two time intervals of equal energy relative to the signal duration.
frequency 5% [F5%(Hz)]	the frequency that divides the selection into two frequency intervals containing 5% and 95%.
relative frequency 5% [F5%Rel]	the frequency that divides the selection into two frequency intervals containing 5% and 95% relative to frequency range.
frequency 95% [F95%(Hz)]	the frequency that divides the selection into two frequency intervals containing 95% and 5%.
relative frequency 95% [F95%Rel]	the frequency that divides the selection into two frequency intervals containing 95% and 5% relative to frequency range.
inter-quartile range [IQRBW(Hz)]	the difference between the first and third quartile frequencies.
peak frequency [MaxF(Hz)]	the frequency at which the maximum power occurs.
time 5% [T5%(s)]	the time that divides the signal into two time intervals containing 5% and 95%.

(Continued.)

Table 1. (Continued.)

acoustic parameter name	description
relative time 5% [T5%Rel]	the time that divides the signal into two time intervals containing 5% and 95% relative to signal duration.
time 95% [T95%(s)]	the time that divides the signal into two time intervals containing 95% and 5%.
relative time 95% [T95%Rel]	the time that divides the signal into two time intervals containing 95% and 5% relative to signal duration.

procedure (IBM SPSS Statistics 20) to validate the results of DFA. N refers to the number of individuals; n refers to the number of calls.

To avoid a potential bias in DFA results due to an unbalanced dataset, we randomly selected nine calls per each individual and such balanced datasets entered into each DFA (whistles alarms: $n = 108$ calls, $N = 12$ individuals; wideband alarms: $n = 207$ calls, $N = 23$ individuals). The descriptive statistics were calculated from the average values per each individual. All means were indicated as mean \pm s.d.

3. Results

3.1. Alarm call description

Whistle alarms represent one-syllable calls with a visible modulated fundamental frequency (figure 1*a*). Duration of whistle alarms ranged from 0.03 to 0.21 s (0.10 ± 0.03 , mean \pm s.d.). The frequency rapidly decreases and peak frequency always coincides with the fundamental frequency (f_0), sometimes with visible harmonics, and if so, these visible harmonics are always at a weaker intensity.

Only one individual showed a short, slight increase in the frequency of the initial segment followed by a rapid decrease (see individual 9; figure 1*a*). Fundamental frequency started at 4811 ± 641 Hz and rapidly decreased to 3439 ± 339 Hz (table 2).

In comparison with whistle alarms, the second alarm call type is a monosyllabic call with a wideband structure, in which acoustic energy is spread across the frequency spectrum and throughout the signal duration. Signals do not show a fundamental frequency or harmonic components but instead have several emphasized frequency bands (figure 1*b*). These alarm signals are significantly shorter than whistle alarms (duration: 0.028 ± 0.007 s, Mann–Whitney U test: $p < 0.001$). The frequency of the first quartile frequency was 2317 ± 632 Hz and the third quartile frequency 3375 ± 1119 Hz. Peak frequency was found at 2456 ± 650 Hz. Other descriptive statistics are shown in table 2.

3.2. Individual variation

3.2.1. Whistle alarms

The discrimination analyses considered 108 whistle alarms from 12 individuals. The model included five variables (fourth and second frequency, first, fourth and third frequency modulation). A discriminant function analysis assigned 93.5% (88.9% cross-validated result) of whistle alarms to the correct individual ($N = 12$, $n = 108$, Wilks' lambda = 0.001). The first four significant discriminant functions ($p < 0.001$) had eigenvalues greater than 1 and explained 97.4% of the variation. The first two functions had eigenvalues greater than 5 and explained 86.5% of the variation. The first discrimination function correlated well with the fourth frequency ($r = 0.65$), while the second discrimination function correlated with the third frequency modulation ($r = 0.48$). Five individuals reached the highest possible classification accuracy (100%), four squirrels were classified with an 80–90% result, two animals showed 78% and one individual 56% classification result. Such classification results were much higher in comparison with classification by chance (8.3%). Location of whistle alarms in the space of the first two discriminant functions is shown in figure 3*a*.

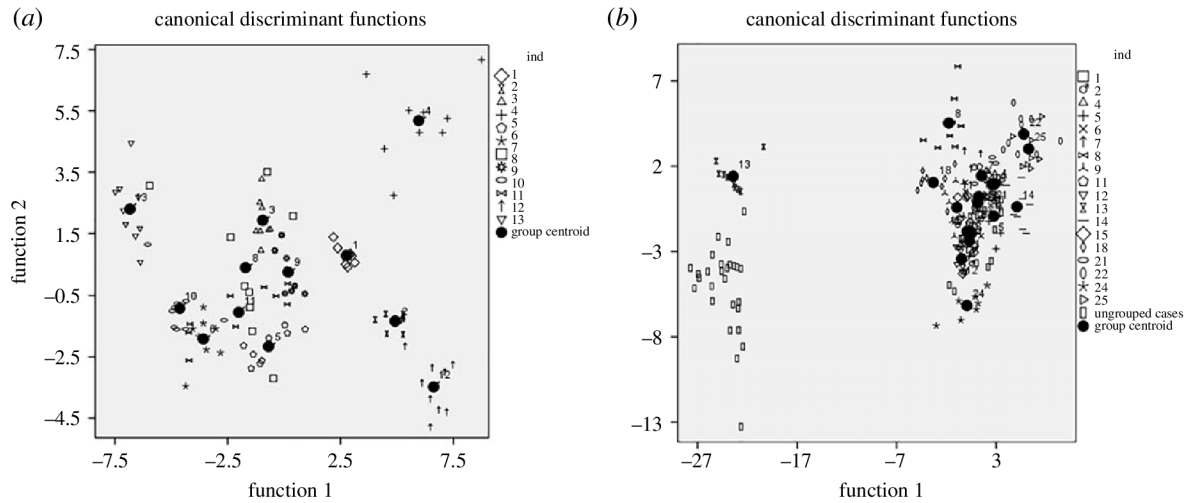


Figure 3. Scatterplot on the basis of two first discriminant functions of DFA for (a) whistle alarms from 13 individuals and for (b) wideband alarms from 25 individuals. Black circles show group centroids.

Table 2. Basic descriptive statistics.

acoustic parameter	mean	s.d.	min	max
<i>whistle alarms</i>				
duration (s)	0.10	0.03	0.03	0.21
start frequency (Hz)	4810.9	641.1	3350.0	6650.0
second frequency (Hz)	4511.0	550.3	3530.0	5980.0
centre frequency (Hz)	4245.3	498.2	3380.0	5570.0
fourth frequency (Hz)	3904.8	417.8	2900.0	4950.0
end frequency (Hz)	3438.7	339.0	2560.0	4540.0
<i>wideband alarms</i>				
duration (s)	0.028	0.007	0.014	0.048
first quartile frequency (Hz)	2316.8	631.5	281.2	3609.4
third quartile frequency (Hz)	3375.3	1118.8	421.9	7359.4
bandwidth 90% (Hz)	2984.9	1483.8	140.6	7453.1
centre frequency (Hz)	2678.8	751.6	375.0	4968.8
frequency 5% (Hz)	2105.4	650.4	46.9	3000.0
frequency 95% (Hz)	5090.3	1756.5	562.5	9796.9
peak frequency (Hz)	2455.5	649.6	328.1	4968.8

3.2.2. Wideband alarms

We entered 207 wideband alarms from 23 individuals into the following analysis. The model contained nine acoustic variables. The model assigned 91.4% (84%, cross-validated result) of wideband alarms to the correct individual ($N=23$, $n=207$, Wilks' lambda less than 0.001). The first five significant discriminant functions ($p<0.001$) had eigenvalues greater than 1 and explained 96.6% of the variation. The first two functions had eigenvalues greater than 7 and explained 82.8% of the variation. Frequency 5% Rel. mostly correlated with the first discrimination function ($r=0.88$), and duration with the second discrimination function ($r=0.63$). Wideband alarms were assigned to the correct caller with a 55–100% result. Most individuals ($N=8$) showed the highest classification accuracy (100%) and five animals were classified with a 60–89% result. Three individuals were classified with 56% success. These results were much higher than a classification by chance (5.6%). Location of wideband alarms in the space of the first two discriminant functions is shown in figure 3b.

3.2.3. Age effect

Additionally, we tested a potential distinctness between age and did not find a significant difference in any of the acoustic parameters ($0.11 < p < 0.61$; Mann–Whitney U test) in both call types.

4. Discussion

The split of Eurasian ground squirrels into two genera—*Spermophilus* and *Uroditellus* [13]—has been shown to be mirrored by vocalization pattern as well. We have described two different types of alarm calls in the long-tailed ground squirrel *Uroditellus undulatus*: (i) a whistle alarm call corresponding with whistle alarms of other ground squirrels inhabiting the Palaearctic area, but also (ii) a wideband alarm call representing a different design of alarm signal (produced both by long-tailed ground squirrel and arctic ground squirrel) has no analogue in any other Eurasian ground squirrel species. The Eurasian species of ground squirrels have wideband calls similar in structure in their vocal repertoire, but they are not used as alarm calls. In contrast with Eurasian species, which are known to produce whistle alarms only, some North American ground squirrels are known to produce both whistle and wideband alarms [19]. In our study, both call types did not differ between adults and subadults. Although young individuals differ significantly in size from adults, the fundamental frequency of alarm whistles does not differ in other studied Palearctic ground squirrels [17,35,36]. According to our results, such phenomenon seems to be valid also for wideband alarm signal. Age information could be concealed in alarm calls of Palearctic ground squirrels [36]. The two different types of alarm calls in North American species are considered as responses to aerial (whistle alarms) and terrestrial predators (wideband alarms) [37,38], but in our study, both alarm types were produced in response to terrestrial subjects as well.

Both whistle and wideband alarm calls were specific at the individual level. Based on our discrimination models, we were able to assign each whistle to the correct individual with 93.5% average success (88.9% based on cross-validated result) and wideband alarms with 91.4% average success (84%, cross-validated results). Such results are consistent with classification outputs of whistle alarms in other ground squirrels: 92% (*Spermophilus suslicus*) [18], 94% (*S. fulvus*) [21], 97% (*S. citellus*) and 95% (*S. taurensis*) [2]. This study is the first to examine individual variation in wideband alarms compared with whistle alarms and occurrence of vocal individuality in two warning signals of a completely different acoustic structure.

5. Conclusion

In comparison with other Eurasian species, the long-tailed ground squirrel produces two types of alarms: (i) a whistle alarm, representing a simple signal comparable with whistles of other Eurasian species, even though it showed close similarity to simple whistles of North American species and (ii) a wideband alarm. We found that both signals are individually specific. This is the first evidence of an individually distinct structure in the wideband alarms of any ground squirrel and occurrence of vocal individuality in two warning signals of a completely different acoustic structure. We may speculate that the previous split of Eurasian ground squirrels into the two genera *Spermophilus* and *Uroditellus* based on genetics is supported by vocalization as well.

Ethics. The research was conducted in accordance with the guidelines of the Animal Behaviour Society for the ethical use of animals in research. The study was carried out in accordance with the recommendations in the Guide for Care and Use of Animals of the Czech University of Life Sciences Prague. The Animal Care and Use Committee of the Czech Ministry of the Environment approved the protocol (no.: 4/19). The Institute of Natural Resources Management of Irkutsk State Agrarian University named after A.A. Ezhevsky states that project Bioacoustic research of mammals and birds in the Irkutsk region has been considered to belong to the bilateral cooperation between our countries within the framework of wildlife biology. According to the Russian legislation, this type of scientific projects does not need any special permits or licences. In a more general way, observing, recording, catching, tagging and letting animals go back to the wild nature do not need any permission of the landowner.

Data accessibility. Data are included as electronic supplementary material.

Competing interests. We declare we have no competing interests

Funding. This work was supported by the Internal Grant Agency of the Faculty of Forestry and Wood Sciences, Czech University of Life Sciences Prague, reg. no.: B06/16.

Acknowledgements. Not applicable

Supplementary material. All data generated or analysed during this study are included in this published article or its electronic supplementary material files.

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Low frequencies in the display vocalization of the Western Capercaillie (*Tetrao urogallus*)

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ABSTRACT

Only a few bird species are known to produce low-frequency vocalizations. We analyzed the display vocalizations of Western Capercaillie males kept in breeding centers and identified harmonically structured signals with a fundamental frequency of 28.7 ± 1.2 Hz (25.6–31.6 Hz). These low-frequency components temporally overlap with the Whetting phase (96% of its duration) and they significantly contribute to the distinct vocal expression between individuals. The resulting model of discrimination analysis classified 67.6% vocalizations (63%, cross-validated result) correctly to the specific individual in comparison to the probability by chance of 12.5%. We discuss a possible function of low-frequency components that remains unclear. The occurrence of such low frequencies is surprising as this grouse is substantially smaller than cassowaries (Southern cassowary *Casuarius casuarius* and Dwarf cassowary *Casuarius bennetti*), the species that produces similarly low frequencies. Because these low frequency components temporarily overlap with the Whetting phase, they are hardly audible from a distance larger than several meters.

Subjects Animal Behavior, Zoology

Keywords Communication, Vocal individuality, Grouse, Acoustic communication, Low-frequency signals, Courtship behaviour

Submitted 12 November 2019

Accepted 23 April 2020

Published 8 July 2020

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Academic editor

Michael Wink

Additional Information and
Declarations can be found on
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DOI 10.7717/peerj.9189

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INTRODUCTION

While the uses of sound occurring in the range of human hearing have been intensively studied, and similarly studied sounds above the upper limit of human hearing (e.g., ultrasounds in bats, toothed whales, rodents, frogs and insects), very little is known about the sound below that range. Such frequencies (infrasound) were documented in whales (*Berta, Sumich & Kovacs, 2006; Mourlam & Orliac, 2017*), elephants (*Larom et al., 1997; McComb et al., 2000; McComb et al., 2003*), tigers (*Von Muggenthaler, 2000*), rhinoceroses (*Policht et al., 2008; Von Muggenthaler et al., 2003*). Only a few bird species are known to produce very low frequencies. Cassowaries produce booming calls with a minimum frequency down to 32 Hz in the Southern Cassowary (*Casuarius casuarius*) and 23 Hz in the Dwarf Cassowary (*Casuarius bennetti*) (*Mack & Jones, 2003*). Boom calls of the Great Curassows (*Crax rubra*) have frequencies of maximum amplitude between 100 and 150 Hz

(Baldo & Mennill, 2011), booms of the Houbara bustard (*Chlamydotis undulata*) with fundamental frequency ranging between 40 Hz and 54 Hz (Cornec, Hingrat & Rybak, 2014), those of the Eurasian bittern (*Botaurus stellaris*) ranging from 86.6 Hz to 248.5 Hz (Puglisi et al., 2001). Corncrakes are known to produce low-frequency soft calls which largely overlap with the frequency spectrum of the background noise (Rek, 2013; Rek & Osiejuk, 2011). Various versions of low-frequency signals also are produced by doves, e.g., the song of the tambourine dove reaches a minimum frequency of 280 Hz (Osiejuk et al., 2019) or in the spotted dove 524 ± 36 Hz (mean \pm SD) (Guo, Bonebrake & Dingle, 2016). All these calls are low-frequency calls, yet they do not fall into the real infrasound range (with frequencies below 20 Hz). The appearance of these low-frequency signals was especially observed in non-passerines of larger body size. As a matter of general bio-acoustic principle, larger animals including birds tend to produce calls of lower frequencies than smaller ones (Fletcher, 2005; Wallschläger, 1980). The validity of this rule has been found not only in songs (Azar & Bell, 2016; Brenowitz, 1982; Ryan & Brenowitz, 1985) but in calls as well (Azar & Bell, 2016; Billings, 2018; Martin et al., 2011). In addition to the influence of body size on the vocalization of grouse, other morphological adaptations can affect the resulting design of produced signals. Grouse (Tetraonidae) have an enlarged oesophagus which inflates like a balloon during mating vocalizations (Moss & Lockie, 1979) and functions as resonating chamber to amplify their signals (Ziswiler & Farner, 1972).

Previous work by Lieser, Berthold & Manley (2005) did not succeed in finding any of the low-frequency components described by Moss & Lockie (1979) based on the vocalizations of only one male. Moss & Lockie (1979) recorded signal components below 40 Hz but Lieser, Berthold & Manley (2005) did not notice any signal below 100 Hz in the vocalization of both wild and captive birds. In such fact, the existence of low-frequency components remains controversial. We therefore tested whether the Western Capercaillie with an average weight of 4–6 kg can really produce any low-frequency signals. If so, we could expect that some frequency parameters could serve as an honest signal of male body mass and/or condition, especially a negative relationship between acoustic parameters and body mass explained by correlation with the length of the trachea, size of the syrinx and vocal track resonance (Lambrechts, 1996; Wallschläger, 1980).

In that case, we could expect individual differences in vocalization among different males. We analysed recordings of vocalization of birds kept in breeding centres in the Czech Republic, Poland and Bavaria in order (1) to describe the acoustical features of potential low-frequency components, and (2) to test the hypothesis that such components have the possibility to encode individual identity.

MATERIALS & METHODS

Ethics statement

The research was conducted in accordance with the guidelines of the Animal Behaviour Society for the ethical use of animals in research. The study (recording of acoustic signals of semi-tame male capercaillies held in breeding stations) was not invasive and the animals were not handled, disturbed, or manipulated; thus it was not considered an experiment

according to the Guide for Care and Use of Animals of the Czech University of Life Sciences Prague and the laws of the Czech Republic. The breeding centre staff was responsible for all bird husbandry and care (permission nu. S/04177/BE/14).

Study objects and locations

We analysed recordings of vocalization of altogether eight adult males of the Western Capercaillie (*Tetrao urogallus*) kept in breeding centres in the Czech Republic (Šumava), Poland (Wisła Forestry District in Jaworzynka), and Germany (Bayerwald-Tierpark Lohberg). All the subjects were kept in captivity and were housed either in pairs or with several females. Their vocalizations were recorded from March 3 to May 5, 2016. This period coincided with the courting season and all these captive birds displayed courtship behaviour.

Recording and acoustic analyses

For recording, we used Olympus Linear PCM LS-5 and ZOOM H5 recorders with Sennheiser ME 62 omnidirectional microphone (frequency response 20 Hz–20 kHz) with K6 powering module and QTC50 microphone (Earthworks Inc. Milford, NH, USA) (frequency response 3 Hz–50 kHz) using sampling rate 44.1 kHz and 16 bit sample size. We were using two equipment sets because we needed to record neighbouring males synchronously in order to be sure which songs belonged to the correct individual. When we were recording successively, we were not sure of identity of the caller when neighbouring males were calling at the same time. Therefore we gave a microphone to each neighbouring male and recorded simultaneously.

Prior to analysis, we down-sampled a sampling rate at 22.05 kHz. The distance between the subjects and the microphone ranged between 0.5 and 3 m. We selected vocalizations which were clear and not overlapped by any disturbing noise including vocalizations of other males. Altogether, we analysed 108 display vocalizations (62 vocalizations of four males from the Czech Republic, 32 vocalizations of three males from Poland, and 14 vocalizations of one male from Germany). In the selected display vocalizations, we manually labelled vocal phases (Clicks, Trill, Cork and Whetting, see Fig. 1) and saved into spectrograms using Avisoft-SASLab Pro version 5.2. (Specht, 2016). We created two types of spectrograms: (1) spectrograms with high temporal resolution (FFT length 1024, frame size 100%, overlap 87.5%, Hamming window, time resolution 5.8 ms, frequency resolution 22 Hz, sampling rate 22.05 kHz); and (2) spectrograms with high-frequency resolution in which we decreased the sampling rate to 4 kHz in order to increase the frequency resolution to 4 Hz (FFT length 1024, frame size 100%, overlap 93.75%, Hamming window, time resolution 32 ms) allowing better inspection of very low-frequency signal components (Policht et al., 2008). Frequency resolution increases with decreased sampling frequency (Avisoft Bioacoustics, R. Specht, Berlin, Germany). In spectrograms with high temporal resolution, we measured temporal parameters of the whole main phases, the Whetting and the Trill, while in spectrograms with high-frequency resolution we measured parameters of the low-frequency components. Frequency parameters were measured from the amplitude spectrum (linear). We labelled these signals on spectrograms for calculation of their

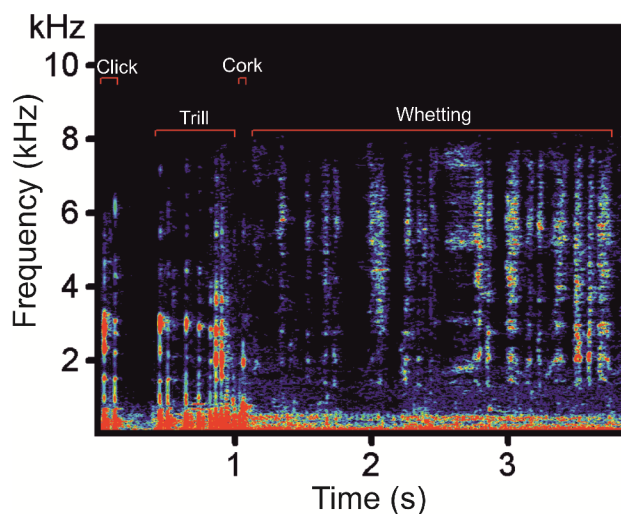


Figure 1 A spectrogram of the Western Capercaillie display vocalization showing the four typical phases.

Full-size [DOI: 10.7717/peerj.9189/fig-1](https://doi.org/10.7717/peerj.9189/fig-1)

duration and identified the fundamental frequency and upper harmonics. We measured the following acoustic parameters: song duration (Dur), fundamental frequency (F0), frequency of the highest intensity (Peak F), duration of the low-frequency component (LowF dur), duration of the Trill phase (Trill dur) and Whetting duration (Whetting dur). Additionally we computed temporal overlaps of LowF dur and Trill dur (LowF to Trill overlap) and Whetting dur (LowF to Whetting overlap).

Calibration of recording equipment

The recording sets were tested for sensitivity to low-frequency signals. We tested the following equipment combinations: Set 1 (ZOOM H5 recorder and QTC50 microphone), and Set 2 (Olympus Linear PCM LS-5 recorder and Sennheiser ME 62 omnidirectional microphone). The tests were conducted in the anechoic room at the Faculty of Electrical Engineering, Czech Technical University in Prague. The room provides an acoustic free field and sufficient signal-to-noise ratio across the whole frequency range of interest (15 Hz–20 kHz). The measurement system Brüel & Kjær Pulse type 3109 was used for the measurement and analyses of signals recorded by the equipment under tests. Pure tones were used as measurement signals. In the frequency range from 15 Hz up to 45 Hz, the fine frequency step of 2 Hz was used. In the frequency range from 45 Hz to 20 kHz, center frequencies of octave bands were used. Pure tones were played successively. For the calibration we used a high precision microphone Brüel & Kjær type 4190 as the reference one. All microphones were placed at a 1m distance from the sound source (12-inch active subwoofer with 2 way satellite loudspeaker box) producing the test signals generated by the Brüel & Kjær Pulse system. All signals were recorded and then post processed by the Brüel & Kjær Pulse system using one-twelfth octave band analyse. In general, the frequency range of equipment is defined by decrease of the frequency response of 10 dB. As the result,

it was found that the frequency response of Set 1 is flat in the frequency range of interest (decrease of 3 dB at 15 Hz), which fully corresponds to the nominal values declared by the manufacturers. The frequency range of Set 2 was determined from 20 Hz up to 20 kHz. The same frequency range is declared by the manufacturers. Both recording sets are suitable for recording the described low-frequency components of vocalizations of the Western Capercaillie males.

Statistical analysis

We used stepwise discriminant-function analysis (DFA) giving a set of discriminant functions combining the parameters of the low-frequency components per individual birds in a way that the signals are assigned to the individuals and thus give a percentage of correctly classified low-frequency signals (Cornec, Hingrat & Rybak, 2014). We excluded highly inter-correlated parameters with $r > 0.77$ (McGarigal, Cushman & Stafford, 2000). Data entered into the DFA were Z –standardized (mean subtracted from each value and divided by standard deviation).

A priori probabilities of classification were weighted by the number of the calls per individual. We used the leave-one-out cross-validation procedure to validate the results of DFA, where each call was classified by the function derived from all cases but that particular case (see IBM SPSS Statistics 20). Descriptive statistics presents the means \pm SD.

RESULTS

The typical display vocalization of the Western Capercaillie contains four phases: Clicks, Trill, Cork and Whetting (Fig. 1). A series of Clicks represent the most frequent sound produced during courting. These vocalizations are frequently produced both alone (without other remaining phase) and as an introductory part of the full display vocalization. In the latter case, the series of Clicks culminates into the Trill which is formed by a quicker repetition of Clicks. The Trill is immediately followed by the Cork, sounding like pulling the cork up of the bottle. The last phase, Whetting, is formed by syllables sounding like a scraping noise. This harsh, repetitive noisy sound is a long phase with a mean duration of 2.85 ± 0.15 s (2.37–3.28).

We found low-frequency harmonically structured signals with a fundamental frequency of 28.7 ± 1.2 Hz (Table 1) and two to five apparent harmonics (Fig. 2). The most intensive frequency was found on each of the first four harmonic tones, but most frequently on the second harmonics, as the peak frequency reached 63.5 ± 21.9 Hz and the duration of these signals was 3.13 ± 0.16 s. These low-frequency components mostly overlapped with the Whetting phase in 96% of its duration. Whetting represents the loudest phase of the display vocalization, thus masking the low frequency. Therefore it was necessary to increase frequency resolution of the lowest frequency band, i.e., the frequency range of 0–200 Hz (see Fig. 3) to reveal these components. A smaller part of low-frequency harmonics overlapped with the Trill phase (10.2%), and the Cork was always included. The Bonferroni-corrected Kruskal–Wallis test showed significant differences in all measured acoustic parameters: $16.76 > H(7, N = 108) < 64.89; p < 0.002$ (Table 1).

Table 1 Descriptive statistics and Kruskal Anova Test (SD) standard error of the mean. (Kruskal–Wallis test) Test of differences among individuals. (Bonferroni correction) P value adjusted according Bonferroni correction.

Variable correction	Mean	Min	Max	SD	Kruskal–Wallis test H (7, N = 108)	p	Bonferroni
F0 (Hz)	28.69	27.0	32.0	1.23	56.47	$p < 0.001$	$p < 0.001$
Peak F (Hz)	63.46	27.0	94.0	21.87	56.00	$p < 0.001$	$p < 0.001$
Low F duration (s)	3.13	2.07	3.52	0.16	47.74	$p < 0.001$	$p < 0.001$
Song duration (s)	6.19	3.98	18.85	2.36	38.27	$p < 0.001$	$p < 0.001$
Trill duration (s)	1.25	0.48	2.33	0.39	32.96	$p < 0.001$	$p < 0.001$
Whetting duration (s)	2.85	2.37	3.28	0.15	64.89	$p < 0.001$	$p < 0.001$
LowF-Trill overlap (%)	28.44	9.04	77.4	11.91	43.88	$p < 0.001$	$p < 0.001$
LowF-Wheet overlap (%)	96.03	87.09	116.56	3.71	16.77	$p < 0.019$	$p < 0.002$

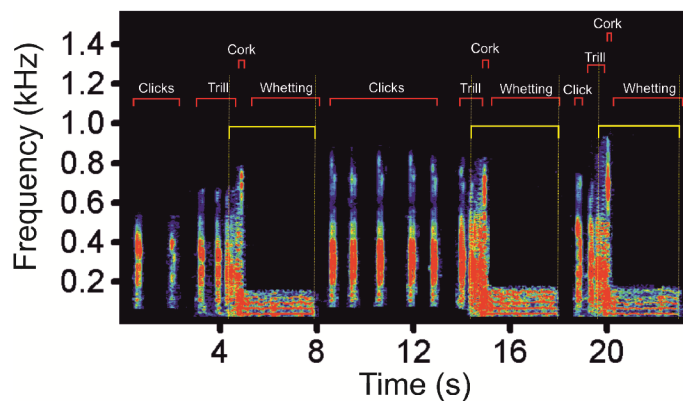


Figure 2 A spectrogram of the low-frequency part (as delimited by horizontal yellow lines) of three subsequent display vocalizations of the Western Capercaillie.

Full-size [DOI: 10.7717/peerj.9189/fig-2](https://doi.org/10.7717/peerj.9189/fig-2)

The resulting DFA model (Wilks's Lambda = 0.091) included four significant discriminating variables ($p < 0.001$): PeakF, Whetting dur, LowF to Trill overlap, LowF to Whetting overlap. This discrimination model assigns 67.6% display vocalizations (63%, cross-validated result) correctly to the correct individual in comparison to a probability of 12.5% according to assignment by chance. The first discrimination function mostly correlated with PeakF ($r = -0.56$) and Whetting dur ($r = 0.56$). With the second discrimination function, LowF correlated to Trill overlap ($r = -0.72$). The first two discrimination functions described 87.1% of variation. The amplitude spectrum of the low-frequency components shows the individually distinct pattern (Fig. 4).

DISCUSSION

The frequency at 32 Hz in the southern cassowary (*Casuarius casuarius*) and 23 Hz in the dwarf cassowary (*Casuarius bennetti*) (Mack & Jones, 2003) were reported as the lowest frequency of vocal signals in birds. The occurrence of such low frequencies in the Western

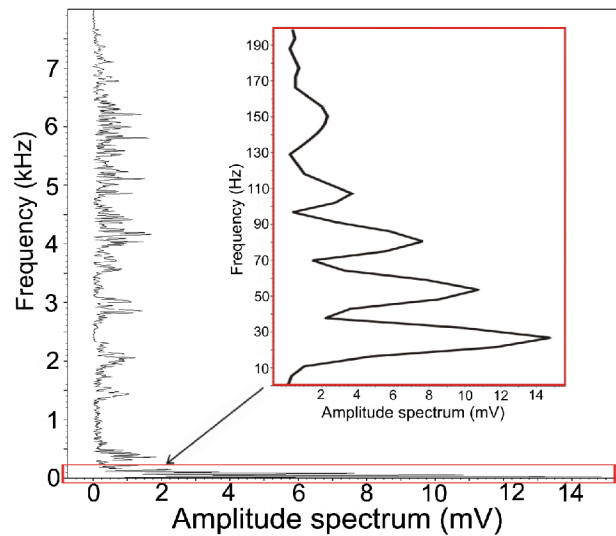


Figure 3 Amplitude spectrum of the low-frequency component. Increased frequency resolution (red rectangle) reveals a fundamental frequency of 27 Hz. This fundamental frequency also represents the most intensive frequency with three harmonic frequencies.

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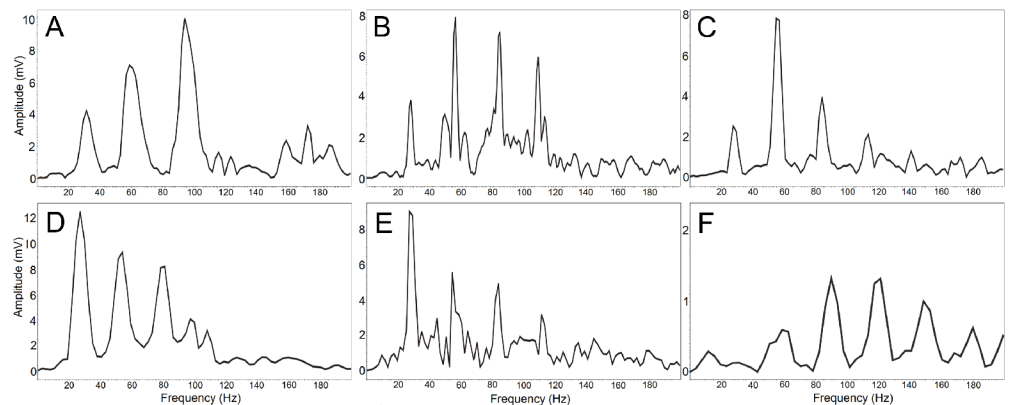


Figure 4 (A-F) The amplitude spectrum of the low-frequency components shows an individually distinct pattern in six males. The most intensive frequency may be found in each of the first four harmonic frequencies.

Full-size [DOI: 10.7717/peerj.9189/fig-4](https://doi.org/10.7717/peerj.9189/fig-4)

Capercaillie (minimum fundamental frequency 26 Hz) is therefore surprising because this grouse is substantially smaller (up to 6.5 kg) than cassowaries weighting up to 29 kg (Dwarf Cassowary) or even up to 58 kg (Southern Cassowary).

The conspicuous vocal display of the Western Capercaillie males indicates its significant function during sexual courtship, probably attracting females. Low frequencies are favourable for long-distance signalization as longer waves better propagate through vegetation (Heimann, 2003).

Another function of low-frequency components in the display vocalizations of mating males would be signalization of their size, which can indicate fighting ability (*Clutton-Brock & Albon, 1979; Clutton-Brock, Hiraiwa-Hasegawa & Robertson, 1989*). Low frequencies could thus not only be heard by hens over a long distance but also used for evaluating the male's quality during mate choice. Individually distinct pattern of these low frequency components could indicate their influence for sexual selection. It is known that most females of the Western Capercaillie select the same dominant male (*Wiley, 1974*). On the other hand, we recorded birds from a short distance and thus we do not know what is happening to the signal during propagation over longer distances, when it actually reaches the female ear from a distance of dozens meters. It has been shown that individually distinct information can change during signal propagation to a receiver (*Budka & Osiejuk, 2014*). By contrast, interactions between males and females on the lek can take at short distance. Females could be attracted to lek by well heard frequencies of males' mating calls or another long-distance signal (e.g., hissing call) from a longer distance. They move toward calling males on a lek so they usually can hear more than one male during searching mating. After approaching to calling males at close distance, females could make the choice decisions on the base of the cues reflecting the quality of male based on low-frequency components. It has been found that females in captivity preferred to mate with males with a longer calling activity (*Rosenberger et al., 2020*). The final female choice at a closer distance could reflect the tradeoff between finding a high-quality mate and predation risk including energetic costs from evaluation of several males on lek.

Although hearing in the Western Capercaillie has not been studied, the ability to hear these low frequencies is probable. Hearing of infrasound has been documented for homing pigeons (*Heffner et al., 2013; Kreithen & Quine, 1979*), the domestic chicken (*Hill et al., 2014*), and the guinea fowl (*Theurich, Langner & Scheich, 1984*). While a low frequency vocalization might be advantageous and increase the fitness of the male Western Capercaillie, it is apparent that the low-frequency phase correlates with the period of temporary deafness which is known only during the Whetting phase, which is used by hunters of this otherwise very shy bird (*Bray & Thurlow, 1942*). One of the mechanisms responsible for auditory self-masking is the contraction of the middle ear muscles (*Borg & Counter, 1989; Borg & Zakrisson, 1973*). We suggest that while it may be advantageous for communication and making an impression to vocalize in a very low frequency range, the Western Capercaillie pays with temporary deafness while singing - a deafness which made it also so popular (not only) among hunters.

Because these low-frequency components temporarily overlap with the Whetting phase, they are consequently hardly audible. It is possible to hear a growling resonating sound accompanying the whetting at a closer distance up to three meters. This fact is likely to cause a long time to escape attention, though the vocalization of the Capercaillie was studied.

Low frequencies could enable better information transfer and thus may be advantageous for long-distance signalization as longer waves propagate better through vegetation and thus enable improved information transfer.

CONCLUSIONS

This experiment is the first time that such low frequencies were unambiguously proven in vocalization of birds smaller than cassowaries. We found low-frequency harmonically structured signals with a fundamental frequency and two to five apparent harmonics. Although hearing in the Western Capercaillie has not been studied, the ability to hear these low frequencies is probable. The conspicuous vocal display of males indicates a significant function during courtship interactions. Low frequencies may be advantageous for long-distance signalization as longer waves better propagate through vegetation and thus enable better information transfer. Information gained from the fundamental frequency contour could be the key parameter used for potential information about male identity during both male–female and male-male interactions. Indeed, we found a significant distinction of low-frequency signals among males.

ACKNOWLEDGEMENTS

We thank the bird keepers and managers of breeding centres, especially to Zenon Rzońca and Vlastimil Novák for their help and support. We also thank to Martin Tharp for language corrections.

ADDITIONAL INFORMATION AND DECLARATIONS

Funding

The research was financially supported by the Internal Grant of the Faculty of Forestry and Wood Sciences, Czech University of Life Sciences Prague, reg. number B04_18. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Grant Disclosures

The following grant information was disclosed by the authors:
Faculty of Forestry and Wood Sciences.
Czech University of Life Sciences Prague: B04_18.

Competing Interests

The authors declare there are no competing interests.

Author Contributions

- Vlastimil Hart conceived and designed the experiments, analyzed the data, prepared figures and/or tables, authored or reviewed drafts of the paper, and approved the final draft.
- Richard Policht conceived and designed the experiments, performed the experiments, analyzed the data, prepared figures and/or tables, authored or reviewed drafts of the paper, and approved the final draft.
- Vojtěch Jandák and Marek Brothánek conceived and designed the experiments, analyzed the data, authored or reviewed drafts of the paper, equipment calibration, and approved the final draft.

- Hynek Burda conceived and designed the experiments, authored or reviewed drafts of the paper, and approved the final draft.

Animal Ethics

The following information was supplied relating to ethical approvals (i.e., approving body and any reference numbers):

The research was conducted in accordance with the guidelines of the Animal Behaviour Society for the ethical use of animals in research. The study (recording of acoustic signals of semi-tame male capercaillies held in breeding stations) was not invasive and the animals were not handled, disturbed, or manipulated and thus was not considered as the experiment according to the Guide for Care and Use of Animals of the Czech University of Life Sciences Prague and the laws of the Czech Republic. The breeding centre staff was responsible for all bird husbandry and care (permission nu. S/04177/BE/14).

Data Availability

The following information was supplied regarding data availability:

The measured acoustical parameters are available as a [Supplementary Files](#).

Supplemental Information

Supplemental information for this article can be found online at <http://dx.doi.org/10.7717/peerj.9189#supplemental-information>.

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RESEARCH ARTICLE

Measuring individual identity information in animal signals: Overview and performance of available identity metrics

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Funding information

Narodowe Centrum Nauki, Grant/Award Number: UMO-2015/19/P/NZ8/02507; Ministerstvo Zemědělství, Grant/Award Number: MZE-RO0718; H2020 Marie Skłodowska-Curie Actions, Grant/Award Number: 665778; Akademie Věd České republiky, Grant/Award Number: RVO 68081766; National Science Foundation; Grantová agentura České republiky, Grant/Award Number: GA14-27925S

Handling Editor: Andres Lopez-Sepulcre

Abstract

1. Identity signals have been studied for over 50 years but, and somewhat remarkably, there is no consensus as to how to quantify individuality in animal signals. While there is a variety of different metrics to quantify individuality, these methods remain un-validated and the relationships between them unclear.
2. We contrasted three univariate and four multivariate identity metrics (and their different computational variants) and evaluated their performance on simulated and empirical datasets.
3. Of the metrics examined, Beecher's information statistic (H_c) performed closest to theoretical expectations and requirements for an ideal identity metric. It could be also easily and reliably converted into the commonly used discrimination score (and vice versa). Although Beecher's information statistic is not entirely independent of study sampling, this problem can be considerably lessened by reducing the number of parameters or by increasing the number of individuals in the analysis.
4. Because it is easily calculated, has superior performance, can be used to quantify identity information in single variable or in a complete signal and because it indicates the number of individuals who can be discriminated given a set of measurements, we recommend that individuality should be quantified using Beecher's information statistic in future studies. Consistent use of Beecher's information statistic could enable meaningful comparisons and integration of results across different studies of individual identity signals.

KEYWORDS

acoustic discrimination, acoustic identification, Beecher's information statistic, discriminant analysis, identity signal, individual recognition, social behaviour, vocal individuality

1 | INTRODUCTION

The fact that individuals differ in consistent ways is a both a central attribute of life and one that underlies a number of theoretically important questions such as explaining cooperative behaviour or understanding the evolution of sociality (Bradbury & Vehrencamp, 1998; Crowley et al., 1996; Tibbetts, 2004). Such individuality can also be important in wildlife conservation as well when it is used to help census or monitor individuals based on individually distinctive traits (Blumstein et al., 2011; Terry & McGregor, 2002). And, because, animals may base their decisions on the identity of the individual with whom they interact or respond to (Godard, 1991; Wilkinson, 1984), there may be selection to both produce individually distinctive signals, and selection to discriminate among them (Tibbetts & Dale, 2007; Wiley, 2013).

Quantification of individual identity (individuality) requires the assessment of variation in one or more traits between at least two individuals. For identity signals to function properly, they should maximize the between-individual variation and minimize the within-individual variation (Beecher, 1982, 1989). A variety of identity metrics have proliferated because of recognized biases (e.g. it is more likely to find similar individuals in larger populations and, hence, it will be more difficult to discriminate individuals in large populations or studies involving more individuals). These biases make the comparison of results among studies unreliable (Beecher, 1989; Mathevon, Koralek, Weldele, Glickman, & Theunissen, 2010). Additionally, some existing metrics were considered unsuitable for a particular signal type (Searby & Jouventin, 2004). Nevertheless, new alternatives were not always thoroughly tested and were not shown to be superior to the metrics they attempted to replace. Furthermore, there are methodological problems that result from the calculation of particular identity metrics, and some studies have used different equations to calculate the same identity metric. Thus, somewhat remarkably given its importance, there is no consensus about how to properly measure identity. As a result, researchers have generally avoided quantitative comparisons between studies (Insley, Phillips, & Charrier, 2003). In a few cases, researchers tried to overcome problems with identity metrics in comparative analyses by using exactly the same methods across involved species (Beecher, Medvin, Stoddard, & Loesche, 1986; Lengagne, Lauga, & Jouventin, 1997; Pollard & Blumstein, 2011). Thus, hundreds of isolated studies have been published on individuality in animal signals but because they used different metrics there is limited prospect that we can benefit from the cumulative evidence of these studies. The lack of a commonly used identity metric is a major impediment towards understanding the evolution of identity signalling and indeed, the evolution of individuality.

Here, we review previously developed univariate (quantifying individuality within a single trait) and multivariate metrics (quantifying individuality across multiple traits) that have been used to quantify individual identity information in signals and we test their performance on simulated and empirical datasets. In particular, we

examine the following metrics: *F*-value, Potential of individual coding PIC, Beecher's information statistic H_S , Information capacity H_M , and Mutual information MI. We further evaluate the different computational variants found in the literature in case of PIC and H_S (see Table 1 and Supplement 1 for a detailed overview of metrics and their variants).

We compare the performance of metrics to hypothetical ideal identity information metric. The main principle of measuring individual identity in continuous traits is to quantify the ratio of between- and within-individual variation (Beecher, 1982, 1989; Robisson, Aubin, & Bremond, 1993; Searby & Jouventin, 2004). Thus, an ideal individual identity metric should be expressed on a ratio scale with a meaningful zero value, equivalent to the situation when there is no between-individual variation. Further, there is no expected upper limit for individuality. High between- to within-individual variation ratio indicates easy discrimination of individuals.

The datasets for the assessment of individual identity in different species vary in properties such as the number of individuals, the number of samples per individual, the number of variables measured (i.e. number of individualistic traits) and the covariance between the multiple variables measured. Hence, we further propose that an ideal identity metric should be robust or respond predictably to these dataset parameters to allow meaningful comparisons between studies. Therefore, an ideal identity metric: (a) should not be systematically biased by the sampling effort, that is there should be no systematic effects of number of individuals and number of observations per individual in a study on individuality estimate, and the sampling should ideally only impact on precision of individuality estimate; and (b) in the multivariate case, it should well capture the intrinsic multidimensionality of identity signals. In particular, it should rise with number of meaningful variables because each of the uncorrelated variables can encode another level of individual variation. In addition, it should also decrease with covariance between the variables because increasing covariance between the variables essentially decreases the number of independent variables. For our comparison, we gave the same weight to all criteria because these are very basic requirements and an ideal metric should fulfil all of them. In addition, we will list other potential pros and cons of each metric to provide a comprehensive evaluation of existing metrics.

We also wished to see if each of two commonly used metrics (Beecher's information statistic H_S and discrimination score DS) could be converted to the other metric. We focused only on H_S and DS metrics. DS has been used in the vast majority of past studies and DS has been found to correlate well with potentially unbiased H_S in a previous study (Beecher, 1989). However, the previous study only tested the relationship between H_S and DS on datasets with equal number of individuals and observations per individual, thus, ignoring the known biases associated with DS. Reliable conversion of DS into potentially unbiased H_S could facilitate comparative analyses of results reported in past and future studies.

TABLE 1 Overview of the identity metrics and their variants

Metric	Metric variant and equation	Description	Reference	IDmeasurer function
Univariate Metrics				
ANOVA F -value (F);	$F = \frac{MS_b}{MS_w}$	F from one-way ANOVA where the individual is treated as independent variable and trait as dependent variable; MS_b = between group mean squares; MS_w = within group mean squares	e.g. Miller (1978)	calcF
Potential of individual coding (PIC);	$PIC_{\text{between tot}} = \frac{CV_{\text{between tot}}}{CV_w}$	$CV_{\text{between tot}}$ = between-individual coefficient of variation calculated from all data points; CV_w = within-individual coefficient of variation	e.g. Robisson et al. (1993)	calcPICbetweentot
	$PIC_{\text{between means}} = \frac{CV_{\text{between means}}}{CV_w}$	$CV_{\text{between means}}$ = between-individual coefficient of variation calculated with means from each individual; CV_w = within-individual coefficient of variation	e.g. Lein (2008)	calcPICbetweenmeans
Beecher's information statistic (H_s)	$H_{S\text{tot}} = \log_2 \sqrt{\frac{F + n_{\text{tot}} - 1}{n_{\text{tot}}}}$	F = ANOVA F -value; n_{tot} = total sample size	Possible variant from Beecher (1989)	calcHSntot
	$H_{S\text{ngroups}} = \log_2 \sqrt{\frac{F + n_{\text{groups}} - 1}{n_{\text{groups}}}}$	F = ANOVA F -value; n_{groups} = number of groups (individuals)	possible variant from Beecher (1989); e.g. Pollard, Blumstein, & Griffin (2010)	calcHSngroups
	$H_{S\text{pergroup}} = \log_2 \sqrt{\frac{F + n_{\text{pergroup}} - 1}{n_{\text{pergroup}}}}$	F = ANOVA F -value; n_{pergroup} = number of samples in each group (observations per individual)	possible variant from Beecher (1989)	calcHSnpergroup
	$H_{S\text{varcomp}} = \log_2 \frac{\sigma_T}{\sigma_W}$	σ_T = total variance in mixed model; σ_W = residual variance associated with random factor in mixed model	Beecher (1989); Carter, et al. (2012)	calcHSvarcomp
Multivariate Metrics				
Beecher's information statistic (H_s)	$H_{S\text{pergroup}} = \log_2 \sqrt{\frac{F + n_{\text{pergroup}} - 1}{n_{\text{pergroup}}}}$	F = ANOVA F -value; n_{pergroup} = number of samples in each group (observations per individual); original variables are subjected to PCA to get uncorrelated components and H_s is calculated and summed over each independent component	Beecher (1989)	calcHSnpergroup
Information capacity (H_M)	$H_M = \log_2 \sqrt{\frac{F_M + n - 1}{n}}$ $F_M = \frac{n-1}{g-1} \times \frac{\text{dist}_t - g \times \text{dist}_w}{\text{dist}_w}$	dist_t = sum of distances of all samples from their centroid; dist_w = sum of distances of samples within individual to its centroid; n = number of observations; g = number of groups;	Searby and Jouventin (2004)	calcHM
Discrimination score (DS)	$DS = \frac{C}{N}$	C = samples correctly classified by Discriminant analysis; N = total number of samples	e.g. Hafner et al. (1979)	calcDS
Mutual information (MI)	$MI = \sum_{ij} \log_2 \frac{p(i,j)}{p(i) \times p(j)}$	$p(i)$ = probability of predicted individual; $p(j)$ = probability of actual individual; $p(i,j)$ = probability of match between predicted and actual individual	Mathevon et al. (2010)	calcMI

2 | MATERIALS AND METHODS

We used R for simulations and statistical analysis (R Core Team, 2012). Functions to calculate identity metrics, associated functions and datasets are available within an IDMEASURER package. This package is available on CRAN (<https://cran.r-project.org/web/packages/IDmeasurer/index.html>) and GitHub (<https://github.com/pygmy83/IDmeasurer>).

2.1 | Datasets

2.1.1 | Simulated datasets

Datasets were constructed to mimic typical data on individuality (Figure 1). Parameters of datasets vary among studies. In particular, there are different numbers of individuals, observations (samples) per individual, variables and different covariances among variables. Effect of these parameters was simulated along with individuality within datasets. The level of individual identity in data was modified by changing the ratio of between- and within-individual variance in accordance with theoretical assumptions of individual identity signals and previous studies (Beecher, 1989; Searby & Jouventin, 2004). We developed R scripts involving “rnorm” and MASS package (Venables & Ripley, 2002) “mvrnorm” function to generate the datasets. These functions generate random values with a given standard deviation around pre-specified mean and, in “mvrnorm”, with pre-specified covariance.

We constructed datasets with univariate and multivariate normal distributions with parameters covering a wide range of values, specifically, five values for individuality ($id = 0.01, 1, 2.5, 5$ and 10), five values for number of observations per individual ($o = 4, 8, 12, 16$ and 20), eight values for number of individuals ($i = 5, 10, 15, 20, 25, 30, 35$ and 40). Additionally, for multivariate datasets, five values for covariance among variables ($cov = 0, 0.25, 0.5, 0.75$ and 1) and five values for number of variables ($p = 2, 4, 6, 8$ and 10). Thus, 200 and 5,000 unique parameter combinations were possible in case of univariate and multivariate datasets respectively. Individuality (id) represents the ratio of standard deviations

between and within individuals ($id = SD_{between}/SD_{within}$; $SD_{between}$ was calculated from means for each individual and SD_{within} was set to be $SD_{between}/id$) (Figure 1). A single covariance (cov) value was used in the variance-covariance matrix to define covariances between all pairs of variables. For univariate datasets, we first generated individual means for a pre-defined number of individuals (normal distribution, “rnorm” function, $M = 1,000$, $SD_{between} = 1$) and then we generated a pre-defined number of random observations “o” around each individual mean (normal distribution, “rnorm” function, mean = individual mean, $SD_{within} = SD_{between}/id$). In the multivariate case, we first created a matrix representing mean individual values of variables for each of the individuals (multivariate normal distribution, “mvrnorm” function, mean for each variable = 0, variance-covariance matrix). Variances on the diagonal of the covariance matrix were set equal to 1 (hence $SD_{between} = 1$) and all covariances between variable pairs were set equal to the pre-defined covariance “cov”. Then, we generated a pre-defined number of random observations “o” around each individual and a variable mean (“rnorm” function, mean = individual mean, $SD_{within} = SD_{between}/id$).

We asked how dataset parameters (i, o, p, cov, id) influenced the value of each identity metric. To explore this, 20 randomization cycles were run for each unique combination of parameter values. For example, in the multivariate case, $20 * 5,000 = 100,000$ independent datasets were generated (datasets 1–20: $i = 5, o = 4, p = 2, cov = 0, id = 0.01$; datasets 21–40: $i = 10, o = 4, p = 2, cov = 0, id = 0.01$; ...; datasets 99,981–100,000: $i = 40, o = 20, p = 10, cov = 1, id = 10$). Identity metrics were calculated for each dataset.

2.1.2 | Empirical datasets

While the general performance of identity metrics was evaluated on simulated datasets, empirical datasets were used to evaluate the consistency of DS and H_S metrics and reliability of H_S and DS conversion on real data. We used six empirical datasets from four different species: little owls *Athene noctua* (ANmodulation, ANspec) (Linhart & Šálek, 2017), corncrake *Crex crex* (CCformants, CCspec) (Budka & Osiejuk, 2013), yellow-breasted boubous *Laniarius atrofloavus*

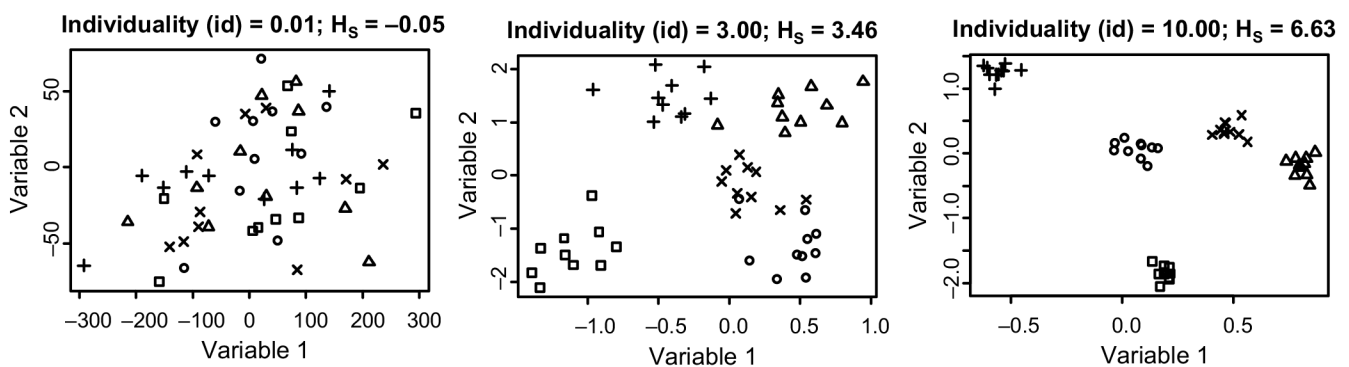


FIGURE 1 Illustration of three artificial multivariate datasets that differ only in the individuality used to generate datasets. Settings for the function generating these datasets: $i = 5, o = 10, p = 2, cov = 0, id = 0.01, 3$ and 10

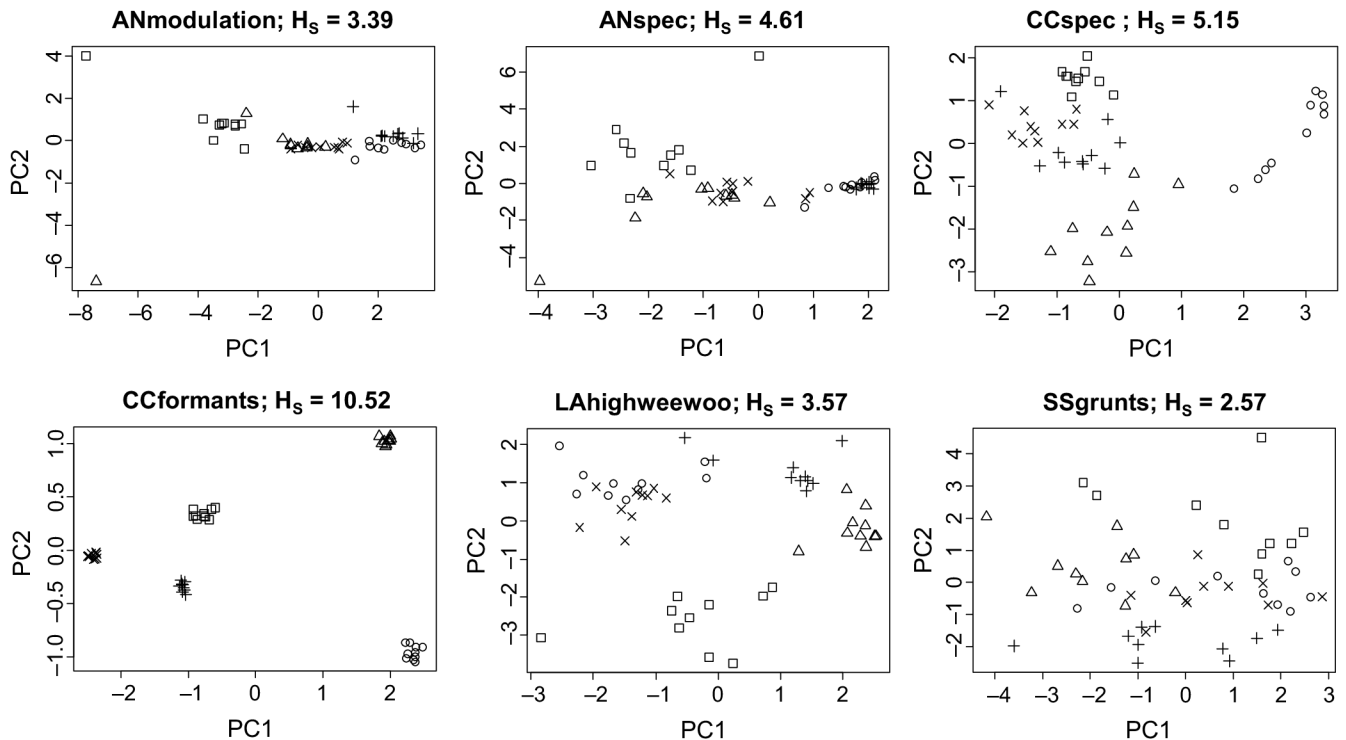


FIGURE 2 Illustration of empirical datasets. Five individuals were randomly sampled from each dataset of 33 individuals and all 10 calls per individual were selected. H_S for a full dataset is shown. Data were centred and scaled and subjected to PCA. The first two Principal Components are plotted

(LAhighweewoo) (Osiejuk et al. unpubl. data) and domestic pigs *Sus scrofa* (SSgrunts) (Syrová, Policht, Linhart, & Špinka, 2017) (Figure 2). In two species – corncrakes and little owls – calls were described by two different sets of variables. In little owls, we described calls by frequency modulation by measuring fundamental frequency at 10 measuring points evenly spread through the duration of the call (ANmodulation) or parameters describing the distribution of the frequency spectrum such as peak frequency, minimum and maximum frequencies and frequencies dividing spectrum by energy content (ANspec). In corncrakes, we used formants (CCformants) and parameters describing the distribution of the frequency spectrum (CCspec) (see the Supplement 2 for detail description of empirical datasets). Because datasets varied with respect to the number of individuals (33–100) and the number of calls per individual available (10–20), we scaled all datasets down to lowest common denominator by randomly selecting individuals and calls from bigger datasets. Eventually, each dataset had 33 individuals and 10 calls per individual. Each dataset also used different numbers of variables to describe the calls' acoustic structure (ANmodulation = 11, ANspec = 7, CCformants = 4, CCspec = 7, LAhighweewoo = 7, SSgrunts = 10). In all these empirical datasets, assumptions of multivariate normality were tested (Korkmaz, Goksuluk, & Zararsiz, 2014), but not met. We found various issues on the level of univariate variables and the whole dataset. For instance, there were issues with outliers, skewness, kurtosis and multimodal distributions (see Supplement 2 for univariate histograms and multivariate Chi-square Q-Q plots). Normality issues are common for research studies on acoustic individual identity. Authors

deal with normality issues by eliminating problematic variables (e.g. Couchoux & Dabelsteen, 2015), using nonparametric classification methods (e.g. Mielke & Zuberbuehler, 2013), or by relying on robustness of cross-validated DFA and Principle Component Analysis (PCA) towards relaxed assumptions (e.g. Mathevon et al., 2010). We used the last approach. If the assumptions of discriminant analysis that are not met the results should be less stable when using different sampling and hence our results should be viewed as conservative.

2.2 | Statistical analysis

The relationship between a given identity metric and each of the parameters was assessed graphically by plotting the mean value and the 95% confidence intervals of an identity metric against all of the modelled data parameters separately. We then used a one-way ANOVA to test whether an identity metric was constant across all levels of a parameter. One-way ANOVA along with graphical evaluation of relationships between metrics and model parameters was preferred over multivariate regression because it simply, but adequately, addresses our main question (i.e. does the metric change in response to model parameter?) without the need to specify and compare many different multivariate regression models. If we found significant differences, we followed up these with post-hoc Tukey tests to identify which parameter levels differed. Due to the large number of comparisons, we only reported comparisons of neighbouring parameter levels. We used linear and nonparametric loess regression to convert H_S to DS and vice versa.

Loess regression identifies a function that best describes complex data by fitting simple models to sequential subsets of data. Its main advantage is that it does not require specifications of the function and, hence, it is suitable for modelling of complex relationships. Loess regression included the number of individuals and the number of observations per individual as additional predictors. We used Spearman correlation coefficients to quantify between-metric consistency of ranking individuality in datasets. Pearson correlations were used to assess consistency within identity metrics in full and partial datasets. We then used Friedman tests, followed by a series of Wilcoxon tests (for post-hoc comparison of differences between levels), to compare correlation coefficients obtained for each pair of the metrics.

3 | RESULTS

The comparison of available univariate and multivariate metrics to an ideal metric is shown in Table 2.

3.1 | Univariate metrics

All explored univariate metrics increased with increasing individuality in the data. However, only $PIC_{\text{betweentot}}$, $PIC_{\text{betweenmeans}}$, $H_{\text{Snpertgroup}}$ and H_{Svarcomp} estimates were independent of the number of observations and the number of individuals used to calculate the metric (Figure 3). These general patterns were qualitatively identical when all simulated data were pooled or if only one of the parameters (number of observations, number of individuals, individuality) was changed at a time and the others were kept constant at the middle value (see Supplement 3 for detailed results including ANOVA tests).

All four sampling-independent metrics ($PIC_{\text{betweentot}}$, $PIC_{\text{betweenmeans}}$, $H_{\text{Snpertgroup}}$ and H_{Svarcomp}) were highly correlated (Spearman correlation, all $r > 0.99$). $H_{\text{Snpertgroup}}$ and H_{Svarcomp} correctly converged to 0 in the case when individuality was set to be negligible ($id = 0.01$), while $PIC_{\text{betweentot}}$ and $PIC_{\text{betweenmeans}}$ converged to higher values (1.01 and 0.32 respectively). $PIC_{\text{betweentot}}$ reflects the number of potential individual signatures within a population in same way as 2^{H_S} does (Beecher, 1989), and, both, $PIC_{\text{betweentot}}$ and $2^{H_{\text{Snpertgroup}}}$ reflect the ratio of between- to within-individual variation. Hence, convergence of $PIC_{\text{betweentot}}$ to 1 could be also seen as desirable quality and meaningful value for a signal with no individuality. H_{Svarcomp} was equal to $2 * H_{\text{Snpertgroup}}$ (see Supplement 4 for details). We further considered only the $H_{\text{Snpertgroup}}$ variant in multivariate analyses.

3.2 | Multivariate metrics

The performance of multivariate identity metrics is illustrated in Figure 4. All metrics increased with increasing individuality. DS, H_S and MI increased with increasing number of variables available and decreased with increasing covariance between variables. Only H_M did not change in response to increasing the number of individuals.

H_S and H_M did not change in response to increasing the number of observations per individual. These general patterns were qualitatively identical when all simulated data were pooled or if only one dataset parameter was changed at a time and others were kept constant at the middle value (see Supplement 5 for detailed results including ANOVA tests).

Despite the different response of metrics to some of the simulated parameters, there was still moderate to high agreement among metrics about identity content in the data (Spearman correlations, mean $r \pm SD = 0.82 \pm 0.07$; minimum $r = 0.71$ for correlation between DS and MI; maximum $r = 0.95$ for correlation between DS and H_S). H_S had the greatest correlations with other metrics (average $R = 0.88$). We found no advantage to using H_M over H_S as previously suggested. Instead, H_M was equal to H_S per variable ($H_M = H_S/p$) in data with zero covariance between variables. (Supplement 6).

Thus, our simulations show that H_S matched the characteristics of the ideal metric in 6/7 cases, followed by H_M (5/7), DS (4/7) and MI (both 3/7) (Table 1).

3.3 | Potential for removing bias in H_S

We observed no significant association between H_S and the number of individuals in the univariate case, so we investigated the origin of the sampling bias in the multivariate case. This bias was only present when data were subjected to PCA. However, PCA is required to create uncorrelated components for H_S calculation.

It is possible that the more variables measured, the more individuals need to be sampled in order to reduce this bias. We therefore fixed the number of variables to 5, 10 and 20 ($p = 5, 10, 20$) and varied the ratio of the number of individuals to the number of variables "i to p ratio" from 0.5 to 5 ("i to p ratio" = 0.5, 1, 1.5, 2, 3, 5) by using different numbers of individuals in our simulations ($i = 3, 5, 8, 10, 15, 20, 25, 30, 40, 50, 60, 100$ depending on number of variables and "i to p ratio"). The number of observations per individual was set to 10. Individuality and covariance were both chosen randomly in each iteration from pre-defined intervals used in the earlier simulations (covariance range = [0, 0.25, 0.5, 0.75, 1]; individuality range = [0.01, 1, 2.5, 5, 10]). We used 100 iterations for each "i to p ratio". H_S did not rise significantly after the number of individuals reached at least the number of parameters (One-way ANOVA $F_{5,1794} = 7.68$, $p < 0.001$; no significant differences between levels if "i to p" ≥ 1 , all $p > 0.132$) (Figure 5).

3.4 | Converting DS to H_S and vice versa

We used simple linear regression and non-parametric loess regression to estimate H_S based on DS and vice versa. There was a previously suggested linear relationship that had a limit of $H_S = 8$ where the DS values were 100% correct discrimination (Beecher, 1989). Because the H_S values in our original simulated datasets far exceeded 8, we generated a new set of simulated datasets with individuality ranging between 0.1 and 2 ($id = 0.1, 0.25, 0.5, 0.75, 1, 1.33,$

TABLE 2 The comparison of available univariate and multivariate metrics to a hypothetical ideal metric and summary of their pros and cons. We summed the number of matches (points) to compare different metrics to the ideal metric

	Zero	Limit	id	cov	p	o	i	Points	Pros	Cons
Univariate metrics										
Ideal	y	n	+					5/5		
F	y	n	+					4/5		Sample dependent
$PIC_{\text{betweentot}}$	y	n	+					5/5	Intuitive and straightforward calculation; allows separate assessment of within- and between-individual variation	Not meaningful for variables with positive and negative values; cannot be summed or averaged over different variables = univariate only
$PIC_{\text{betweenmeans}}$	n	n	+					4/5		Converges to non-meaningful value for no individuality in data
H_{Sntot}	y	n	+					4/5		Sample dependent; incorrect HS variant
$H_{\text{Snpergroup}}$	y	n	+					5/5	Standard variant of HS; univariate and multivariate	
H_{Sngroups}	y	n	+					3/5		Sample dependent; incorrect HS variant
H_{Svarcomp}	y	n	+					5/5	Allows including various covariates in mixed models	Values twice as big as in case of standard $H_{\text{Snpergroup}}$
Multivariate metrics										
Ideal	y	n	+	-	+	ns	ns	7/7		
DS	n	y	+	-	+	+	-	3/7	Population and individual metric; the most commonly used metric	Sample dependent; not suitable for high individuality signals because values are limited from the top
H_{S}	y	n	+	-	+	ns	+	6/7	Univariate and multivariate; partial sample dependence is introduced by PCA but can be to large extent eliminated; biologically meaningful - provides number of unique individual signatures within population; good theoretical framework for both discrete and continuous individuality traits	Partially sample dependent
H_{M}	y	n	+	ns	ns	ns	ns	5/7	Sample independent; various types of similarity metrics can be potentially used (euclidean distances, Jaccard similarity, string edit distance, dynamic time warping, etc.)	Number of independent variables needs to be known to calculate total identity information
MI	n	y	+	-	+	-	+	3/7	Could be applied with various classification methods	Sample dependent; not suitable for high individuality signals because values are limited from the top

Abbreviations: zero, metric has a meaningful zero; limit, metric is limited from the top by an asymptote; id, change in response to increasing identity information in data; cov, response to increasing covariance between variables; p, response to increasing number of variables; o, response to increasing number of observations per individual; i, response to increasing number of individuals; y, yes; n, no; +, increase; -, decrease; ns, not significant, does not change with a parameter.

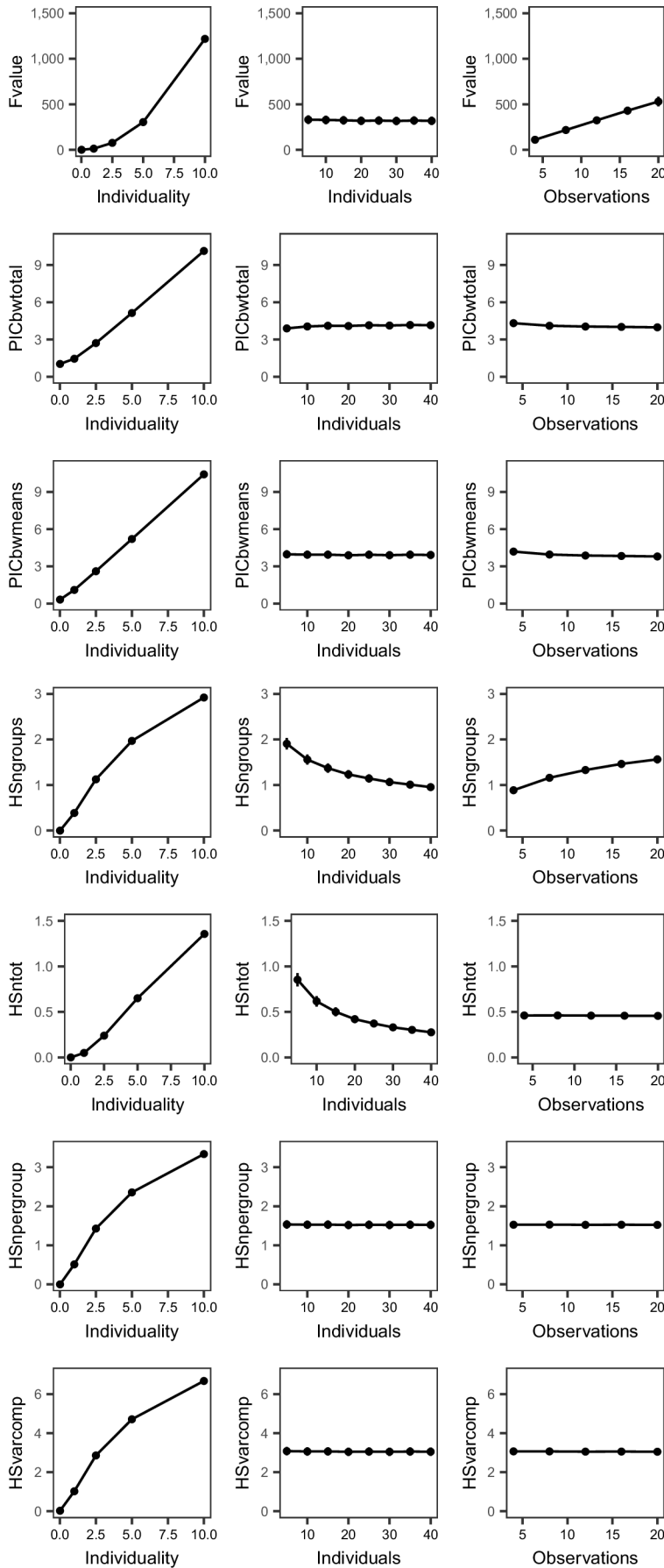


FIGURE 3 Variation in univariate identity metrics in response to simulated dataset parameters: individuality, number of observations per individual and number of individuals. Means and 95% confidence intervals are shown. Graphs were plotted using all simulated univariate data pooled together. For the graphs with only a single parameter changing at a time see Supplement 3

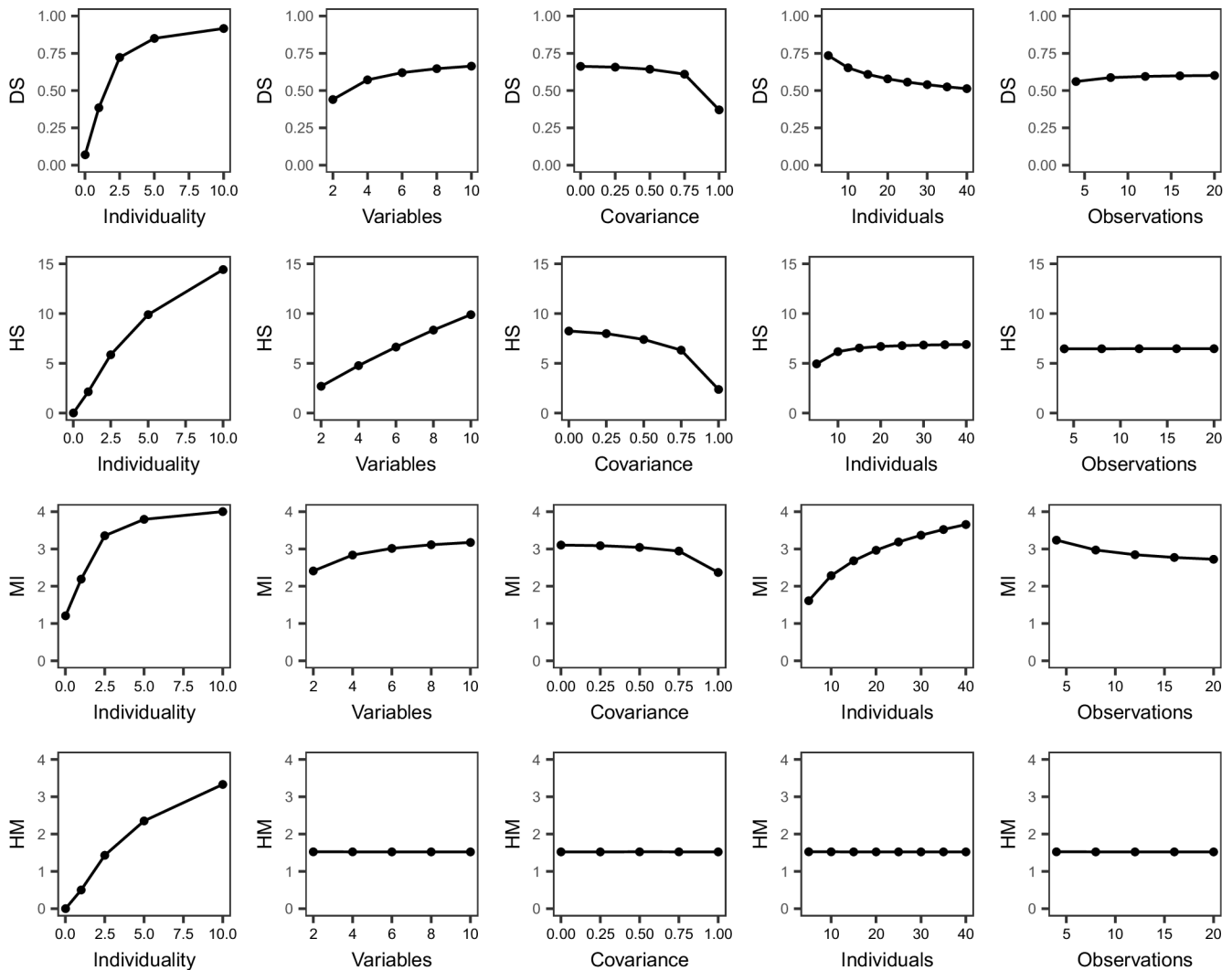


FIGURE 4 Multivariate identity metrics in response to simulated dataset parameters: individuality, covariance between variables, number of variables, number of observations per individual and number of individuals. Means and 95% confidence intervals are shown. Graphs were plotted using all simulated multivariate data pooled together. For the graphs with only a single parameter changing at a time see Supplement 4

1.66, 2), covariance set to zero ($cov = 0$), number of iterations was reduced to 10 ($it = 10$), and other parameters were set as in previous models ($p = 2, 4, 6, 8, 10$; $i = 5, 10, 15, 20, 25, 30, 35, 40$; $o = 4, 8, 12, 16, 20$). These settings led to H_S values up to about 13 for data used for model building, and H_S values up to about 14 in the case of data used for model testing. These values are much closer to 8 and also much closer to H_S values reported from nature.

Loess models took into account the number of observations per individual and the number of individuals. We compared the loess conversion and linear conversion models of DS and H_S . In general, loess estimates were closer to the ideal prediction (intercept = 0, beta = 1) and the loess model reduced the error of both DS and H_S estimates to about a half compared to linear estimates (Figure 6). Both H_S estimates were underestimated for high values of H_S . The ceiling value is clearly apparent for linear estimates of H_S . It is still visible in the case of loess estimates but loess predictions remain reasonably good up to about $H_S = 10$.

3.5 | Correlations between calculated and estimated metrics

We were further interested in how $H_{S_{est}}$ and DS_{est} might represent H_S and DS of a particular sample of individuals or $H_{S_{full}}$ and DS_{full} of the whole population. For this purpose, we first generated 50 full datasets with different identity levels representing 50 hypothetical populations of different species. Each dataset comprised 40 individuals, 20 calls per individual and 10 parameters. For these datasets, individuality was set randomly ranging between 0.2 and 2 (0.1 increments), and the covariance was set randomly ranging between 0.2 and 0.8 (0.1 increments). These settings generated datasets with $H_{S_{full}}$ values that ranged from 0.22 to 9.89 ($M \pm SD: 4.72 \pm 2.95$). Then, we repeatedly subsampled full datasets to get partial datasets which simulated different sampling of the population. We subsampled 5–40 individuals and 4–20 calls per individual per dataset in

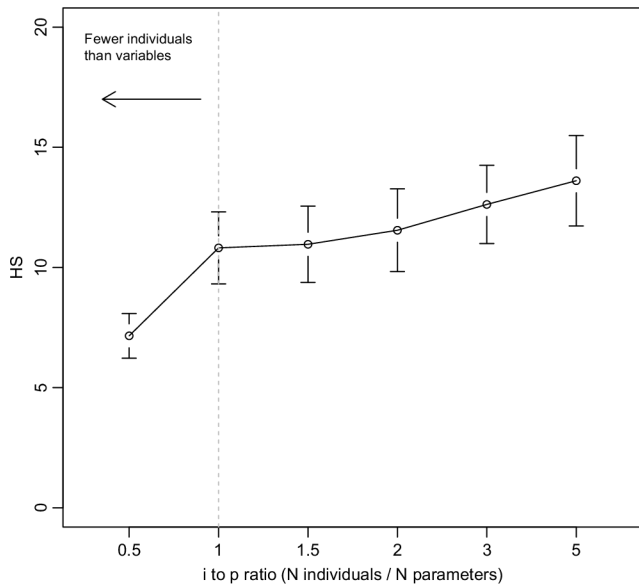


FIGURE 5 H_S and “i to p ratio” (number of individuals/ number of variables). H_S was underestimated if there were fewer individuals than variables. Means and 95% confidence intervals are shown

each of total 20 iterations. We also repeatedly subsampled our empirical datasets. We subsampled 5–33 individuals and 4–10 calls per individual per dataset in each of total 20 iterations. The number of parameters was not randomized – we always kept the original number of variables.

In simulated datasets, H_S and $H_{S_{est}}$ were correlated almost perfectly with each other and with $H_{S_{full}}$ (all average Pearson $r > 0.97$). There was no difference among the correlation coefficients from correlations between $H_{S_{full}}$, H_S and $H_{S_{est}}$ (Friedman Chi Square = 3.6,

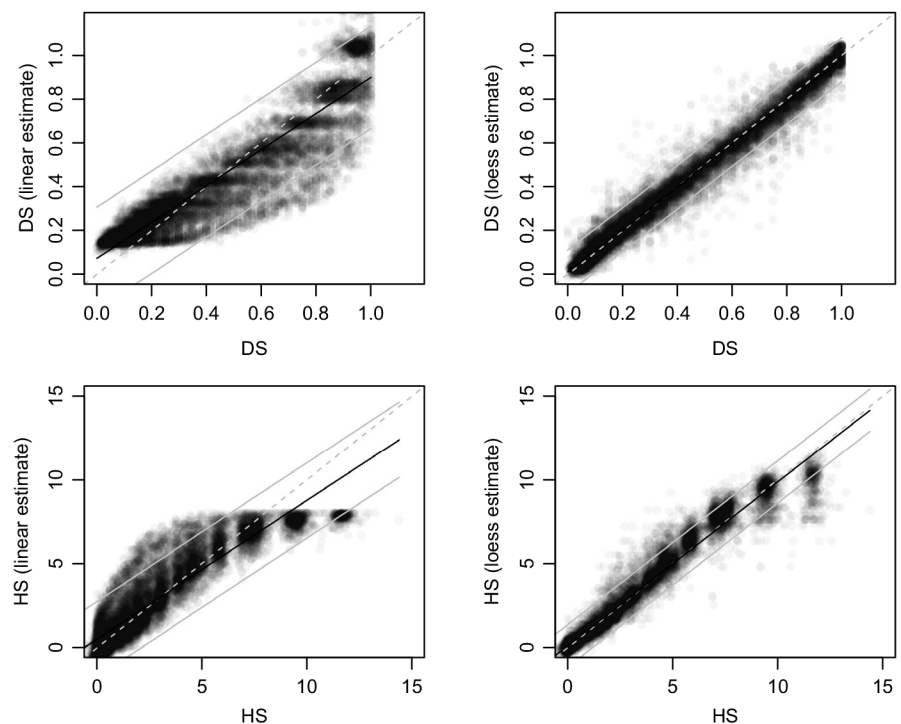
$p = 0.165$). In empirical datasets, H_S calculated on partial datasets still reflected the $H_{S_{full}}$ almost perfectly (average Pearson $r = 0.99$). While $H_{S_{est}}$ reflected H_S of partial dataset (average Pearson $r = 0.90$) and $H_{S_{full}}$ (average Pearson $r = 0.88$) slightly worse, it remained a reasonable fit. However, $H_{S_{est}}$ did not reflect $H_{S_{full}}$ as precisely as it did H_S (Friedman Chi Square = 33.6, $p < 0.001$, post-hoc test: $H_S - H_{S_{full}}$ vs. $H_{S_{est}} - H_{S_{full}}$, $p < 0.001$).

DS in simulated datasets was almost perfectly correlated with DS_{est} (average Pearson $r = 0.99$). Although the relationship between DS in full datasets (DS_{full}) and DS and DS_{est} was significantly worse (Friedman Chi Square = 40.0, $p < 0.001$; both post-hoc tests: $p < 0.005$), these associations remained strong (DS_{full} and DS: average Pearson $r = 0.95$; DS_{full} and DS_{est} : average Pearson $r = 0.96$). In empirical datasets, the correlation between DS and DS_{est} was lower than in case of artificial datasets (average Pearson $r = 0.91$). DS and DS_{est} of partial datasets had comparable correlations to DS_{full} (DS_{full} and DS: average Pearson $r = 0.88$; DS_{full} and DS_{est} : average Pearson $r = 0.86$). Thus, the performance of DS and DS_{est} to reflect each other or DS_{full} did not differ (Friedman Chi Square = 0.9, $p = 0.638$).

4 | DISCUSSION

We provided an overview of the metrics used to quantify individual identity in animal signals in order to identify the best method for reporting individuality in animal signals. Biases associated with some of the commonly used metrics, and the use of different metrics across studies, make it difficult to compare results and integrate the accumulated knowledge from the numerous published studies on individual identity in animal signals.

FIGURE 6 Estimation of H_S and DS based on linear and loess transformation of DS and H_S respectively for datasets with H_S up to 14.4. Linear DS estimation: Intercept = 0.07, Beta = 0.83, $R^2 = 0.83$, Standard Error of Estimate (SEE) = 0.12, 95% Prediction interval = predicted value ± 0.23 ; DS loess estimation: Intercept = 0.01, Beta = 0.98, $R^2 = 0.97$, Standard Error of Estimate (SEE) = 0.05, 95% Prediction interval = predicted value ± 0.10 . Linear H_S estimation: Intercept = 0.51, Beta = 0.83, $R^2 = 0.83$, Standard Error of Estimate (SEE) = 1.14, 95% Prediction interval = predicted value ± 2.24 ; H_S loess estimation: Intercept = 0.11, Beta = 0.98, $R^2 = 0.95$, Standard Error of Estimate (SEE) = 0.64, 95% Prediction interval = predicted value ± 1.26



We show that the assessment of individual identity is relatively straightforward when considering a single trait (univariate case). Both, PIC ($PIC_{\text{betweentot}}$) and H_S ($H_{S_{\text{Snpergroup}}}$) performed according to expectations. Multivariate identity metrics based on direct quantification of between- to within-individual variation ratios (H_S , H_M) performed better than the metrics derived from discrimination of individuals (DS, MI). We confirmed sampling-associated biases where they were reported previously (DS), but we found them even in metrics that had been developed to overcome these biases (H_S , MI). We also described yet unrecognized issues (the need to assess dimensionality for H_M to quantify the total individuality of a signal). We further found that some metrics created values that were so close that they could be viewed as redundant (PIC and H_S ; H_M and H_S) and using them simultaneously brings unnecessary confusion to the field.

Based on our review and systematic analysis, we suggest H_S should be routinely reported as the standard individual identity metric because it performed closest to an ideal identity metric in the univariate, as well as in the multivariate case. The partial bias in H_S caused by the number of individuals in a study could be removed by having at least the same number of individuals as the number of variables. H_S was the most consistent metric and correlated the best with DS and other identity metrics. Further, H_S could be converted reliably into DS if needed.

The robustness of H_M towards sampling bias (number of individuals, number of observations, as well as the number of variables and covariance) is an attractive feature. However, as we show, H_M quantifies identity information per variable and not the identity information of the entire signal. It is necessary to know the effective number of variables to calculate the total identity information of a signal (i.e. if there is perfect covariance between the variables, the effective number of variables is 1 no matter how many variables are used), which may be difficult to assess. On the other hand, H_M uses distances (similarity scores) of samples to calculate individuality and, hence, it could be potentially used not just with Euclidean distances (Searby & Jouventin, 2004, this study) but also together with other various methods assessing similarity (e.g. cross-correlation, dynamic time warping or string edit distances).

Mutual information (MI) is derived from a confusion matrix of discrimination analysis and we show it has similar shortcomings as discrimination scores. Our results that found systematic biases in MI are in line with previous studies that investigated measures of clustering for various machine learning purposes where potentially unbiased variants of MI are constantly searched for (e.g. Amelio & Pizzuti, 2017).

4.1 | Identity metrics in comparative analyses

We show that biases associated with DS (the most often used metric) and H_S (the best metric) are not necessarily fatal for comparisons of different published studies because H_S and DS values that are based on an entire population or subsamples from a population were well correlated in both simulated and empirical datasets.

Additionally, the conversion of sample-biased DS values into less-biased H_S values could allow better comparisons between studies. Both H_S and H_M values were previously found to correlate well with DS (Beecher, 1989; Searby & Jouventin, 2004). We extend previous findings for H_S (Beecher, 1989) to situations with unequal sampling and we show it is possible to convert between H_S and DS with an acceptable amount of error even when datasets differ in the number of individuals and observations per individual, and have important issues associated with multivariate normality (Supplement 2). Discriminant analysis (DA) and Principal component analysis (PCA) used for DS and H_S calculations both assume multivariate normality for optimal results. While using these methods with non-normal data cannot be, in general, recommended, relatively high correlations between our metrics in empirical datasets suggest that DA and PCA scores were quite robust to these normality issues. Discrimination and dimensionality reduction analytical techniques that are able to handle normal and non-normal data definitely need to be considered in future individual identity studies.

4.2 | Future individual identity metrics

We hope that our study will stimulate further discussions about how individual identity should be properly measured. Although we suggest that H_S should be generally used to quantify individuality, different metrics or more complex approaches might be required for particular interesting questions. For example, H_S can only provide a population estimate of individual identity. Researchers might be interested in whether distinctiveness of individuals increases during ontogeny (Syrová et al., 2017). In this case, discrimination scores can be reported for each individual, thus making statistical evaluation possible. Furthermore, separate assessments of within- and between-individual variations when calculating PIC might be useful to test hypotheses about which of the two has been selected for. Within-individual variation could be reduced by, for example, ritualized behaviour while between-individual variation could be increased through, for example, morphological variation in structures producing or carrying the signal (e.g. Sheehan & Nachman, 2014). The dimensionality of identity signals might be an important factor for recognition processes (Trunk, 1979) and evolution could favour low dimensional signals. Paralleling the distribution of individuals in space (territoriality, living in colonies), individual signatures within a population, too, could have random, clumped, or regular distributions depending on the mechanisms behind individual distinctiveness and the degree of plasticity of identity signals.

We evaluated the efficacy of all metrics within the acoustic modality only. It is increasingly recognized that signals employ multiple modalities (Partan, 2013; Partan & Marler, 1999). All of the identity metrics discussed here could be, in principal, used in visual or chemical domains as well. H_S has an advantage that it could be used both for discrete traits, such as colour variants, presence of particular alleles or chemicals, and for continuous traits such as size of visual patterns, duration of calls, etc. (Beecher, 1982, 1989). However, identity

information outside the acoustic domain has been rarely quantified and meaningful comparison of individual identity across modalities remains a challenge for the future.

It is likely that automatic data collection and analysis techniques will be increasingly applied for various recognition tasks, including individual recognition (Elie & Theunissen, 2018; Stowell, Petrusková, Šálek, & Linhart, 2019). While these methods will allow studying individual identity signalling on unprecedented scales and sample sizes, the resulting classification accuracy scores will be analogous to the discrimination score, with similar positives and drawbacks. However, many different feature sets, pre-defined or automatically derived from data, as well as many different classification methods could be combined to test for the robustness of identity signals and/or to mimic and test for different alternatives of possible real recognition processes (Elie & Theunissen, 2018).

5 | CONCLUSION

We suggest that, at the current state of knowledge and methodology development, H_5 should be generally reported as the “golden standard” individual identity metric to allow the best comparison of individuality in signals across different studies. Given that H_5 may not be sufficient in all cases, we encourage further research to develop new metrics to quantify identity information in signals. However, new metrics should always be appropriately assessed and their performance directly compared to the best existing metrics. We provide datasets and scripts that should help to assess individual identity information in animal signals and benchmark the future metrics.

ACKNOWLEDGEMENTS

P.L. received funding from the European Union's Horizon 2020 research and innovation programme under the Marie Skłodowska-Curie grant agreement No. 665778 administered by the National Science Centre, Poland (UMO-2015/19/P/NZ8/02507). D.T.B. is supported by the NSF. M.Šp., M.S. and R.P. were supported by the Czech Science Foundation (GA14-27925S) and the Czech Ministry of Agriculture (MZE-RO0718). M.Šá. work was supported by the research aim of the Czech Academy of Sciences (RVO 68081766).

AUTHORS' CONTRIBUTIONS

P.L. and D.T.B. conceived the ideas, designed methodology and led the writing of the manuscript; P.L., T.O., M.B., M.Šá., M.Šp., M.S. and R.P. collected the data; P.L. analysed the data; All the authors contributed critically to the drafts and gave final approval for publication.

DATA ACCESSIBILITY STATEMENT

Data and code used for this article are available within IDMEASURER R package currently available on CRAN (<https://cran.r-project.org/>

[web/packages/IDmeasurer/index.html](https://github.com/pygmy83/IDmeasurer)) and GitHub (<https://github.com/pygmy83/IDmeasurer>).

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SUPPORTING INFORMATION

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

How to cite this article: Linhart P, Osiejuk T, Budka M, et al. Measuring individual identity information in animal signals: Overview and performance of available identity metrics. *Methods Ecol Evol*. 2019;10:1558–1570. <https://doi.org/10.1111/2041-210X.13238>

Hissing of geese: caller identity encoded in a non-vocal acoustic signal

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ABSTRACT

Non-vocal, or unvoiced, signals surprisingly have received very little attention until recently especially when compared to other acoustic signals. Some sounds made by terrestrial vertebrates are produced not only by the larynx but also by the syrinx. Furthermore, some birds are known to produce several types of non-syrinx sounds. Besides mechanical sounds produced by feathers, bills and/or wings, sounds can be also produced by constriction, anywhere along the pathway from the lungs to the lips or nostrils (in mammals), or to the bill (in birds), resulting in turbulent, aerodynamic sounds. These noises often emulate whispering, snorting or hissing. Even though hissing sounds have been studied in mammals and reptiles, only a few studies have analyzed hissing sounds in birds. Presently, only the hissing of small, nesting passerines as a defense against their respective predators have been studied. We studied hissing in domestic goose. This bird represents a ground nesting non-passerine bird which frequently produces hissing out of the nest in comparison to passerines producing hissing during nesting in holes e.g., parids. Compared to vocally produced alarm calls, almost nothing is known about how non-vocal hissing sounds potentially encode information about a caller's identity. Therefore, we aimed to test whether non-vocal air expirations can encode an individual's identity similar to those sounds generated by the syrinx or the larynx. We analyzed 217 hissing sounds from 22 individual geese. We calculated the Potential for Individual Coding (PIC) comparing the coefficient of variation both within and among individuals. In addition, we conducted a series of 15 a stepwise discriminant function analysis (DFA) models. All 16 acoustic variables showed a higher coefficient of variation among individuals. Twelve DFA models revealed 51.2–54.4% classification result (cross-validated output) and all 15 models showed 60.8–68.2% classification output based on conventional DFA in comparison to a 4.5% success rate when classification by chance. This indicates the stability of the DFA results even when using different combinations of variables. Our findings showed that an individual's identity could be encoded with respect to the energy distribution at the beginning of a signal and the lowest frequencies. Body weight did not influence an individual's sound expression. Recognition of hissing mates in dangerous situations could increase the probability of their surviving via a more efficient anti-predator response.

Submitted 16 September 2019

Accepted 26 September 2020

Published 24 November 2020

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Academic editor

Lesley Rogers

Additional Information and
Declarations can be found on
page 13

DOI 10.7717/peerj.10197

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OPEN ACCESS

Subjects Agricultural Science, Animal Behavior, Zoology

Keywords Non-vocal, Communication, Vocal individuality, Non-syrinx vocalization, Recognition, Acoustic, Behavior, Anseriformes, Hiss-display, Bird

INTRODUCTION

Vocalizations in terrestrial tetrapods are usually generated by vibrations in a specialized vocal organ, the syrinx or the larynx. Sublaryngeal/subsyringeal pressure from the lungs activates laryngeal vocal folds to generate a fundamental sound, which is then filtered by the upper vocal tract (Squire, 2009). The latter comprises the trachea, the pharynx and associated nasal and buccal cavities, with the adjunction of air sacs in some species (Favaro et al., 2015; Fitch & Hauser, 2002; Fletcher, 1993). This represents most studied systems, but sounds of terrestrial vertebrates are not only produced in the larynx or syrinx. Several avian species are known to produce non-syrinx sounds, which can be produced by instrumental feathers (Murray, Zeil & Magrath, 2017), beak drumming (Budka et al., 2018; Dodenhoff, Stark & Johnson, 2001), wings beating (Garcia et al., 2012; O'Neil, Charrier & Iwaniuk, 2018), the rattling of mandibles (Eda-Fujiwara et al., 2004), step dances (Ota, Gahr & Soma, 2017) or else, by using tools (Heinsohn et al., 2017).

Besides these emissions, sounds can be produced by constriction, anywhere along the pathway from the lungs to the lips or nostrils (in mammals), or to the bill (in birds), resulting in turbulent, aerodynamic sounds (Fitch & Hauser, 2002). As of late, non-vocal, or unvoiced, signals have surprisingly received very little attention in comparison to other acoustic signals (Budka et al., 2018; Stomp et al., 2018a). The production mechanism of hissing has been studied in the domestic goose (Brackenbury, 1978). The sound is produced during a long expiration, lasts for many seconds and is preceded by a deep inspiration. Air sac pressure and flow rate reach an intermediate level, somewhere between normal breathing and vocalization. By comparing maximum pressure and flow excursions during an inspiration and expiration, it has been shown that hissing is caused by a stream of expiratory air escaping from the constricted glottis (Brackenbury, 1978). In terrestrial animals such signals are used by reptiles and mammals: snakes (Aubret & Mangin, 2014), lizards (Labra et al., 2007), crocodiles (Vergne, Pritz & Mathevon, 2009), turtles (Fitch & Hauser, 2002), rhinos (Policht et al., 2008), horses (Stomp et al., 2018a; Stomp et al., 2018b), giraffes (Volodina et al., 2018), llamas (Fitch & Hauser, 2002), both house and feral cats (Yeon et al., 2011), tigers (Rose et al., 2018), cheetahs (Smirnova et al., 2016), sloths (Fitch & Hauser, 2002), dasyurids (Dorph & McDonald, 2017) and number of invertebrates as well, e.g., cockroaches (Hunsinger et al., 2018), beetles (Lewis & Cane, 1990), sphinx caterpillars (Dookie et al., 2017), Bombycoidea caterpillars (Bura, Kawahara & Yack, 2016), honeybees (Sarma et al., 2002), mantids (Hill, 2007). Even though hissing has been studied in mammals and reptiles, only few studies have been conducted in birds, mostly in the context of parental behavior in avian species nesting in cavities or burrows. Although hissing outside the nest is common in some ground-dwelling, non-passerines, like geese and swans, we are unaware of studies that tested hissing produced by adult birds that do not nest in cavities.

Geese and swans often protect their territories and have been known to violently chase away all intruders, birds or other animals alike, including approaching humans. Both offspring and adults can easily be caught by a predator on the ground. After producing a warning hiss, they usually attack with flapping wings and biting (Lorenz, 1963; Rosiński,

1986). The production of a sudden, intense sound during such an event can deter or surprise a potential predator, but there could be other functions, e.g., to attract conspecifics for pair or collective defense, or to warn other conspecifics. In any such case, hissing sounds may contain information about social structures (e.g., group or pair membership) or an individual's identity.

Previous studies have shown the following functions of animal hissing: fear expression (Kinstler, 2009), to deter a predator (Broughton, 2005; Krams et al., 2014; Labra et al., 2007; Morley, 1953; Zub et al., 2017), acoustic mimicry (Aubret & Mangin, 2014), aggressive interactions between conspecifics (Policht et al., 2008; Rose et al., 2018; Smirnova et al., 2016) or during vigilance (Volodina et al., 2018). These sounds occur predominantly in dangerous situations, although it was also found to be a positive exhibition in horses (Stomp et al., 2018a; Stomp et al., 2018b).

Recognition of individuals during dangerous situations can be adaptive when signals provide additional important information e.g., relevancy of a threat or its urgency. For example, the calling of younger, inexperienced individuals can be less relevant than those from more experienced adults (Nakano et al., 2013; Sloan & Hare, 2008). Similarly, recognition of hissing mates in dangerous situations could increase the probability of their surviving via a more efficient anti-predator response. In comparison to vocally produced alarm calls, almost nothing is known whether non-vocal hissing sounds potentially encode information about the caller's identity. Even though individual expressions within the vocalizations of birds have been intensively studied, the majority of studies have been devoted to the sounds produced by the syrinx. Such studies of non-syrinx vocalizations remain scarce, with little being known about their sound production mechanisms or their biological functions, including within and between-individual variation (Budka et al., 2018). The extent to which an individual's variation occurs in turbulent sound like hissing has not, however, been tested. In this study, we tested the potential information contained in the hissing sounds of domestic geese, a non-passerine bird. We aimed to test the level of individual distinctiveness and the influence, if any, of sex and body weight.

MATERIALS & METHODS

Ethics statement

All applicable institutional, national and international guidelines for the care and use of animals were followed. The study has been conducted in accordance with the current laws in Poland. The university farm was responsible for goose husbandry, care and research manipulation in accordance with the regulation of Ministry of Agriculture and Rural Development (Dz. U. 2010 nr 116 poz. 778).

Birds, recording and acoustic analysis

Observations were made on a parent flock of Bilgoraj geese kept in a deep litter, under controlled environmental conditions with free access to limited catwalks. Food and water were provided ad libitum to naturally mated birds. Geese were kept in one-parent flocks consisting of 230 individuals. Males formed harem flocks with approximately four females to each group. We did not record which males mated with which females. During testing,

the specimens being tested were separated from their respective flock and were transferred to a separate room. Geese were recorded while a human approached the focal bird up to a distance of one meter. Hissing displays were defined as the action of a neck extending towards a person, stretched directly to the opponent with an opened bill, either moving or standing. We analyzed calls produced only at a close vicinity.

For recording we used a QTC50 microphone (Earthworks Inc. Milford, NH, USA, frequency response 3 Hz–50 kHz) and a ZOOM H5 digital audio recorder set to 44.1 kHz sampling rate and 16-bit sample size. After recording, each bird was weighed on an electronic balance (RADWAG WPT/R/6/15c2) with an accuracy up to 2.5 g and identified individually based on wing marks with a unique number code. After the procedure, tested individuals were returned to their flock.

We analyzed 217 hissing sounds (Fig. 1) from 22 individual geese (16 females and 6 males), produced within 5–15 min of separation from members of their respective flock. Recordings were analyzed using the Raven Pro Sound Analysis Software (Cornell Lab of Ornithology, New York, USA) from which spectrograms were generated using the following parameters: Hann window type with a 1,050 point window size, 50% overlap, 11.9 ms hop size, and 21 Hz grid spacing.

For the analysis, we visually inspected the quality of each spectrogram and selected calls with high signal-to-noise ratios that did not overlap with background noise. For the analysis we retained calls that did not differ in their root mean square amplitude (RMS). Prior to sound analysis, recordings were normalized (scaled from 0 to 1) to standardize loudness. Birds, recording and acoustic analysis Then we randomly selected the 10 best calls, or those with the highest quality, from each individual using a randomization function in IBM SPSS 20 (IBM Corp., Armonk, USA). One specimen produced only 7 calls, but was also included in the analysis.

We filtered out all background noise that was not overlapping with the frequency range of the hissing using a high pass filter on frequencies <130 Hz in Avisoft-SASLab Pro, version 5.2.13 software. We measured the following parameters (Table 1): Low frequency (LowF), Center frequency (CF), First quartile frequency (Q1F), Third quartile frequency (Q3F), First quartile time (Q1Time), Third quartile time (Q3Time), Time 95% (Time95), Time 5% (Time5), Call duration (Sample Length), Frequency 5% (F5), Frequency 95% (F95), Bandwidth 90% (BW90), Inter quartile range (IQR), Peak frequency (PF), Aggregate entropy (Agg Entropy) and Minimum entropy (Min entropy). For a more detailed description of these parameters, see *Charif, Waack & Strickman (2010)*.

Statistical analysis

For each of the 16 sound features (Table 1), we performed Kruskal-Wallis tests with Bonferroni corrections on the non-transformed data to determine whether individual birds differed from one another. For each parameter, the Kruskal-Wallis test with Bonferroni correction showed significant differences among individuals ($p < 0.001$; Table 2).

As an index of inter-individual variation we calculated the Potential for Individual Coding (PIC), which compares the coefficient of variation both within and among individuals. The PIC ratio was computed for each acoustic parameter by dividing the

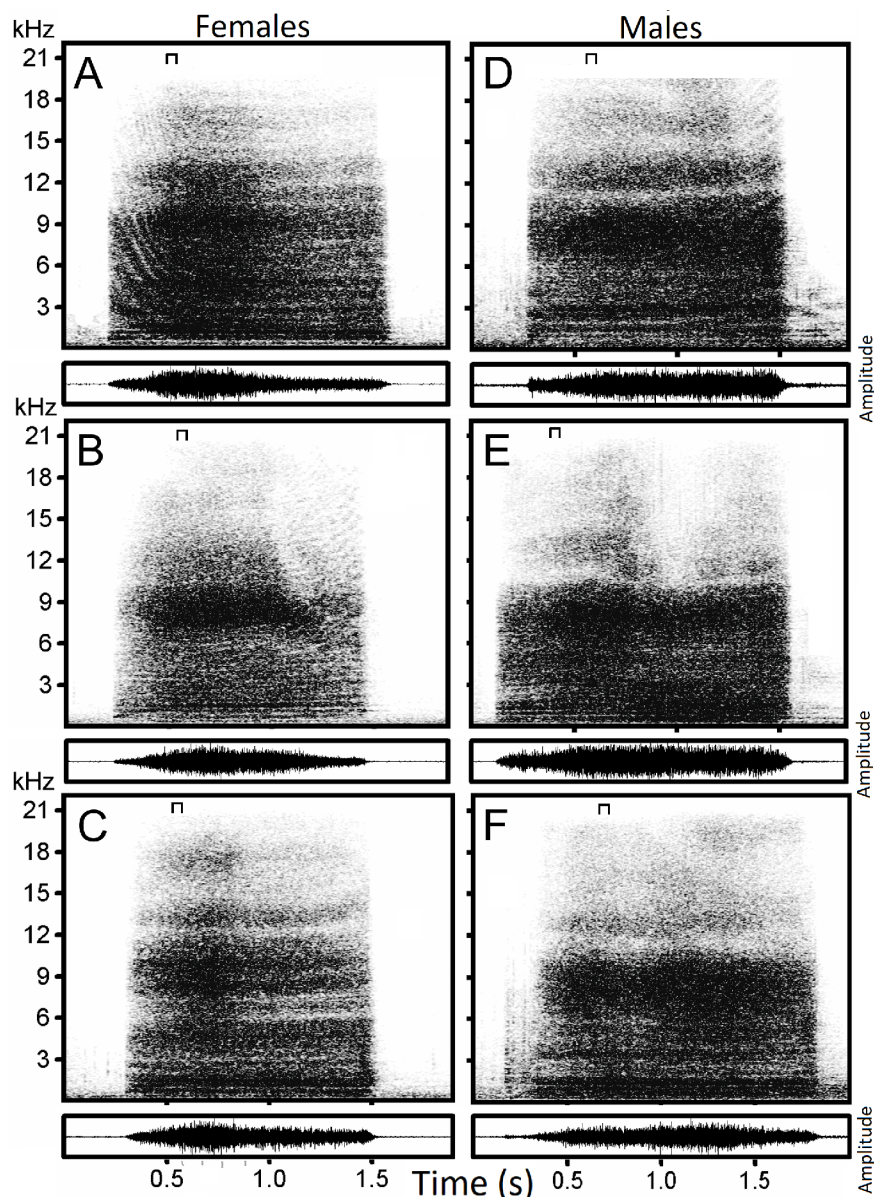


Figure 1 Examples of hissing calls recorded from six subjects. Each panel displays the spectrogram (top) and the amplitude modulation of the signal (below). Individual identity labeled according to the identity label used in DFA: (A) Female 4. (B) Female 5. (C) Female 13. (D) Male 18. (E) Male 19. (F) Male 20. The slice of sound energy representing the power spectrum is indicated by a symbol on top of each spectrogram.

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CV_{between} by the mean of the CV_{intra} values obtained from each individual (Robisson *et al.* 1993).

In order to determine whether each hissing sound could be correctly classified, we performed a stepwise procedure of discriminant function analysis (DFA). The analysis

Table 1 Measured acoustical variables. Measurements based on the Raven Pro manual (Charif, Waack & Strickman, 2010).

(LowF) Low frequency. Minimum frequency of the signal. (Hz)

(CF) Center frequency. The frequency that divides a signal into two frequency intervals of equal energy. (Hz)

(Q1F) First quartile frequency. The frequency that divides the signal into two frequency intervals containing 25% and 75% of the energy. (Seconds)

(Q3F) Third quartile frequency. The frequency that divides the selection into two frequency intervals containing 75% and 25% of the energy. (Hz)

(Q1T) First quartile time. The point in time that divides the selection into two time intervals containing 25% and 75% of the energy. (Seconds)

(Q3T) Third quartile time. The point in time that divides the selection into two time intervals containing 75% and 25% of the energy. (Seconds)

(T95) Time 95%. The point in time that divides the signal into two time intervals containing 95% and 5% of the energy. (Seconds)

(T5) Time 5%. The point in time that divides the signal into two time intervals containing 5% and 95% of the energy. (Seconds)

(Call duration) Sample length. Signal duration based on sample length. (Samples)

(F5) Frequency 5%. The frequency that divides the signal into two frequency intervals containing 5% and 95% of the energy. (Hz)

(F95) Frequency 95%. The frequency that divides the selection into two frequency intervals containing 95% and 5% of the energy in the selection. (Hz)

(BW90) Bandwidth 90%. The difference between the 5% and 95% frequencies. (Hz)

(IQR) Inter-quartile range. The difference between the 1st and 3rd quartile frequencies. (Hz).

(PeakF) Peak frequency. Frequency of the maximum amplitude. (Hz)

(AggE) Aggregate Entropy. The aggregate entropy measures the disorder in a sound by analyzing the energy. Higher values correspond to greater disorder in the signal whereas a pure tone have zero entropy. It corresponds to the overall disorder in the sound.

(MinE) Minimum Entropy. This entropy is calculated by finding the entropy for each frame in the signal and then taking the minimum values.

The entropy formula: $S = PSD(f, t) / \text{sum_over_f}(PSD(f, t)) * \log_2(PSD(f, t) / \text{sum_over_f}(PSD(f, t)))$

The units are “bits” because we use the log base 2. Since the selection may consist of multiple spectrogram slices, Raven iterates over slices and to find the minimum and maximum entropy value with the frequency bounds of the selection. Note that most signal processing applications sum over frequency and time, where Raven sums over frequency instead.

selected predictors using the Wilks' lambda criterion. F values were used as a criterion for entering or removing a parameter from a discrimination model (F-to enter = 3.84; F-to-remove = 2.71). We also used a leave-one-out cross-validation procedure for external validation using an IBM SPSS 20 (IBM Corp., Armonk, USA). The identity of the calling bird was used as a group identifier and the 16 acoustic parameters were used as discriminant variables. Normal distribution was tested (Kolmogorov–Smirnov test) and the data were transformed into the Box–Cox when necessary. Measured variables were normalized using Z score transformations (by subtracting the mean and dividing by the variable's standard deviation) which avoided the false attribution of weights in relation to acoustic parameters measured in different units (IBM Corp., Armonk, USA).

Table 2 Descriptive statistics and Potential for individual coding. (DFA) variable included in DFA model. (SE) standard error of the mean. (Krusk–Wallis) Kruskal–Wallis test after Bonferroni correction. (**) $p < 0.001$. Mean CVw (within individual comparison, $n = 22$). CVa (between individual comparison, $n = 217$).

Variable	DFA	Mean	Min	Max	SE	Krusk–Wallis	Mean CVw	CVa	PIC
Frequency 5% (Hz)	X	821.1	280.0	3359.0	29.77	**	27.14	53.41	1.97
Time 5% (ms)		263.3	41.0	687.0	8.93	**	28.87	49.98	1.73
Min Entropy	X	5.9	4.0	8.0	0.06	**	12.05	15.17	1.26
Agg Entropy		8.4	6.0	9.0	0.04	**	5.98	7.80	1.30
Low frequency (Hz)		248.0	158.0	784.0	6.82	**	19.46	40.48	2.08
First quartile frequency (Hz)	X	1920.7	560.0	7795.0	91.13	**	40.01	69.89	1.75
First quartile time (ms)	X	263.6	41.0	687.0	8.93	**	28.83	49.92	1.73
Third quartile frequency (Hz)	X	6452.9	1378.0	9281.0	130.74	**	20.88	29.85	1.43
Third quartile time (ms)		264.1	42.0	687.0	8.93	**	28.76	49.83	1.73
Bandwidth 90% (Hz)	X	8702.4	3295.0	11908.0	76.45	**	9.74	12.94	1.33
Center frequency (Hz)		3900.4	775.0	8549.0	137.37	**	38.18	51.88	1.36
Call duration (samples)	X	64124.0	29892.0	133643.0	1212.51	**	15.87	27.85	1.75
Peak frequency (Hz)		2344.2	302.0	9044.0	164.37	**	65.11	103.29	1.59
Inter-quartile range (Hz)		4532.3	302.0	7235.0	108.98	**	26.88	35.42	1.32
Time 95% (ms)		264.3	42.0	687.0	8.93	**	28.74	49.79	1.73
Frequency 95% (Hz)		9223.4	4005.0	13286.0	83.85	**	9.22	12.97	1.41

RESULTS

The hiss is a broadband and sustained sound combining a complex structure of stacked harmonics and overtones with a frequency modulation exhibiting one or more time-varying spectral peaks of energy. In some individuals, the hiss displays a sharp attack and ending (Figs. 2A, 2B, 2C) while in others, it begins or ends with a soft acoustic component (Figs. 2D, 2E, 2F). The pronounced spectral bands embedded in the call (e.g., Fig. 2B) may be formants resulting from the conformation of the vocal tract during expiration but we did not have anatomic data to support this.

Hissing calls reached a duration of 0.68–3.03 s (1.45 ± 0.03 s; mean \pm SE). Peak frequency showed 302.0–9,044.0 Hz ($2,344.2 \pm 164.4$), Inter-quartile ranged from 302.0–7,235.0 Hz ($4,532.3 \pm 109.0$) and Low frequency at 158.0–784.0 Hz (248.0 ± 6.8), (Table 2). After Bonferroni correction, the Kruskal–Wallis test for each independent acoustic variable showed significant differences among individuals ($p < 0.001$) (Table 2). The DFA model contained seven significant discriminant functions: Q1T, Call duration, F5, Q3F, BW 90, MinE and Q1F ($p < 0.001$; Table 3). The PIC value was ≥ 1.26 for all 16 acoustic variables. This model (Wilks' Lambda = 0.004; $P < 0.001$) included the first two discriminant functions, with Eigenvalues > 2 representing 59.7% of variation (Fig. 3). The first four functions had Eigenvalues > 1 and explained 87.8% of the variation (Table 3). The first discrimination function correlated with Q1T ($r = 0.736$), and the second discrimination function correlated with F5 and Q1F ($r = 0.846$ and 0.590 , respectively). Classification results revealed that any random hissing sound could be assigned to the correct individual with an accuracy rate of 67.7%. A cross-validated result showed a success rate of 54.4% in

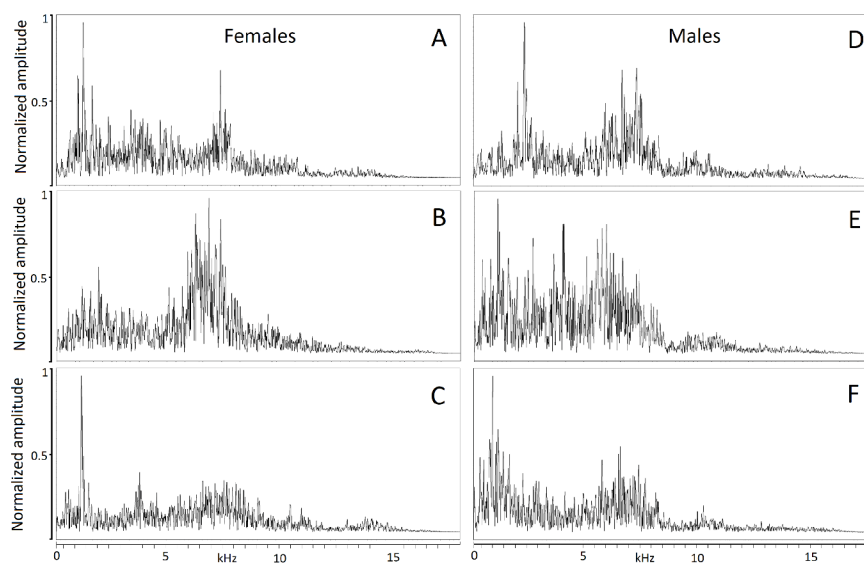


Figure 2 Powerspectrum of the hissing calls produced by six individuals showing individually distinct pattern. Individual identity labeled according to the identity label used in DFA: (A) Female 4. (B) Female 5. (C) Female 13. (D) Male 18. (E) Male 19. (F) Male 20. Power spectrum was taken from the 0.05 s interval of the first quartile time. This parameter mostly contributed to individual distinctiveness.

Full-size [DOI: 10.7717/peerj.10197/fig-2](https://doi.org/10.7717/peerj.10197/fig-2)

Table 3 Discrimination functions of the DFA model. (Funct) DFA function. (Eigenval) eigenvalue, (Cum var, %) explained cumulative variance). (Wilks' Lam) Wilks' Lambda. (Sig) Significance. (**) $p < 0.001$.

Funct	Eigenval	Cum var (%)	Wilks' Lam	Sig.	Mostly correlated variable
1	3.801	36.8	0.271	**	First quartile time (0.736)
2	2.364	59.7	0.091	**	Frequency 5% (0.846)
3	1.618	75.4	0.031	**	Call duration (0.693)
4	1.286	87.8	0.015	**	Third quartile frequency (0.798)
5	0.572	93.4	0.009	**	Bandwidth 90% (0.885)
6	0.449	97.7	0.006	**	Minimum Entropy (0.848)
7	0.234	100.0	0.004	**	First quartile frequency (0.442)

comparison to a 4.5% success rate when classification by chance. Nine individuals reached the high classification accuracy (80–100%) (Table 4). The eigenvalues of individual sounds with their respective centroid are plotted against the first two discriminant functions (Figs. 3–4). In order to see the stability of this DFA result, we conducted a series of 15 DFA models (Table 5). Twelve DFA models revealed 51.2–54.4% classification result (cross-validated output) and all 15 models showed 60.8–68.2% classification output based on conventional DFA. This indicates the stability of the DFA results when using different combinations of variables. Such results show that signaler identity is encoded in a larger set of acoustic features. The influence of body weight was inconclusive as a factor with respect to any of the acoustic parameters and did not show a significant correlation ($r < -0.34$; $p > 0.05$).

Table 4 Classification results. (ID) Individual identity. (Prior(%)) Prior probabilities of individuals. (DFA(%)) Percentage of correct classification.

ID	Sex	Nu calls	Prior(%)	DFA(%)
1	F	10	4.6	40
2	F	10	4.6	50
3	F	10	4.6	100
4	F	10	4.6	80
5	F	10	4.6	60
6	F	10	4.6	80
7	F	10	4.6	80
8	F	10	4.6	50
9	F	10	4.6	80
10	F	7	3.2	100
11	F	10	4.6	20
12	F	10	4.6	70
13	F	10	4.6	70
14	F	10	4.6	90
15	F	10	4.6	100
16	F	10	4.6	80
17	M	10	4.6	70
18	M	10	4.6	70
19	M	10	4.6	60
20	M	10	4.6	40
21	M	10	4.6	50
22	M	10	4.6	60

The sample size of males studied was too small to make an accurate assessment about any potential differences between sexes ($p > 0.25$, Mann–Whitney U Test).

DISCUSSION

The present study demonstrates that the hissing of geese, a non-vocal signal elicited in a standard experimental setup, encodes signaler identity. Our discriminant analysis found individuality embedded in specific acoustic features, which is also typical for broadband vocal signals of many animals. In the best DFA model two acoustic parameters among the sixteen that we analyzed proved to be important in separating individuals from one another: the beginning of a hiss (Q1T) and the lowest frequency (F5).

For the other 14 DFA models, in addition to Q1T, the first discrimination function mostly correlated with two other time parameters (T5 and Q3T). Three other frequency parameters (Q1F, CF and Q3F) were most often correlated with the second discrimination function. Such results show that signaler identity is encoded in a larger set of acoustic features.

In addition to F5 of the most explanatory model, three other frequency parameters were most often correlated with the second discrimination function (F25%, F50 and F75, Q1F,

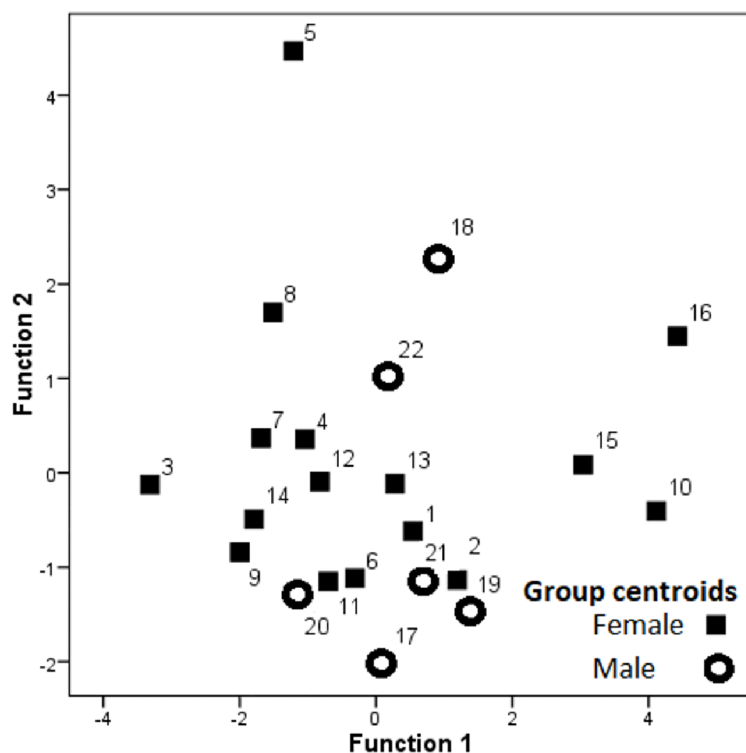


Figure 3 Dispersion of the group centroids on the two discriminant functions. Labels denote individual birds.

Full-size [DOI: 10.7717/peerj.10197/fig-3](https://doi.org/10.7717/peerj.10197/fig-3)

CF and Q3F, respectively). Such results show that identity of the signaler is encoded in a larger set of acoustic features.

Inter-individual variation of this non-vocal sound produced by constriction of the glottis during exhalation could result from differences in vocal tract anatomy and/or individual morphology (e.g., body size, body weight) in accordance with general principles of allometry. However, none of the acoustic parameters we analyzed correlated with body weight. Although we cannot exclude the influence of other factors, such as the length or the shape of the trachea, individual distinctiveness of this unvoiced call does not appear to be driven by morphological differences. The songs of some passerines have been found to be constrained by the condition of a specimen's immune system (*Gil & Gahr, 2002*), parasite load (*Garamszegi 2005*) or MHC profile (*Garamszegi et al., 2018*). Similarly, distress calls have been found influenced by an individual's health status (*Laiolo et al., 2007; Laiolo et al., 2004*).

Alternatively, the inter-individual variation found in hissing geese could result from a selective process favoring conspecific recognition. To test this hypothesis, it would be useful to collect data from wild geese living in pairs instead of domestic geese bred in flocks. This would give us an opportunity to compare within-pair and between-pair variation during performance of a joint hissing display. Subsequently, we could investigate, using playback, whether subjects are more responsive to the hissing of his/her mate relative to a stranger's.

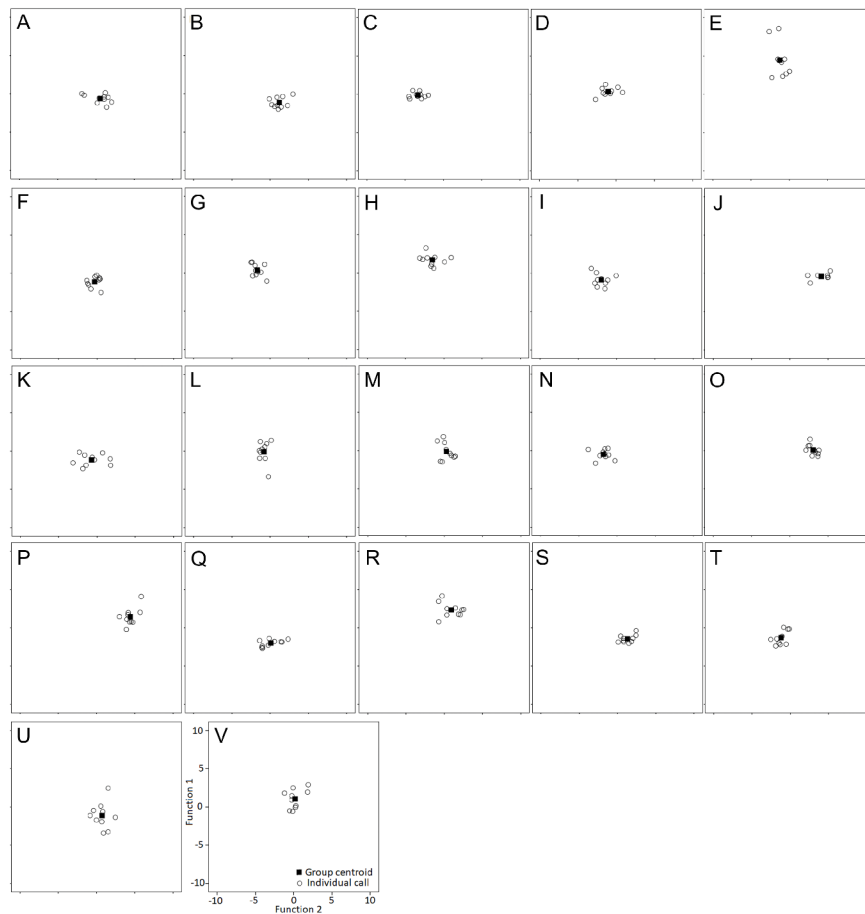


Figure 4 Individual scores are plotted with their respective centroid against the first two discriminant functions. Individual identity labeled according to the identity label used in DFA: (A–P) Female 1–16. (Q–V) Male 17–22.

Full-size  DOI: [10.7717/peerj.10197/fig-4](https://doi.org/10.7717/peerj.10197/fig-4)

The hissing display of geese could be considered as a component of parental investment. In contrast to small passerines, in which hissing is produced only by females during incubation or brood rearing, male geese often produce hissing jointly with their mate. However, male geese are not always present to warn and defend their mate against a predator (*Perrins, 1979*). Like other displays (e.g., greeting ceremony), the hissing of geese is often performed in synchrony by both mates. During the display, females may exploit the variations in expressions to test the ability of males to potentially invest in a family unit and protect it from danger.

The hissing of geese may be energetically costly as the sound is preceded by a deep inhalation and is released during a prolonged exhalation, which can last many seconds (*Brackenbury, 1978*). Its emission could be constrained by an individual's condition. Females could evaluate the fitness of their mate based on the quality of his hissing. Males in better physical condition produce longer or better quality hissing sounds although, in our study, we did not find any correlation with body weight.

Table 5 DFA models.

DFA	Nu of var	Conv class	Valid class	Variables (ordered by importance, starting with the most explanatory variable)
1	7	67.7	54.4	Q1T; Duration; F5; Q3F; BW90; Min Entr; Q1F
2	8	67.3	54.4	T5; Duration; Q3F; IQR; Q1F; BW90; Min Entr; Low F
3	6	65.0	53.0	Q3T; Duration; Q3F; Q1F; BW90; Min Entr
4	7	67.3	54.4	Q3T; Duration; Q3F; Q1F; BW90; Min Entr; Low F
5	7	67.7	53.9	T5; Duration; F5; Q3F; BW90; Min Entr; Q1F
6	8	68.2	53.9	T5; Duration; F5; IQR; Min Entr; Q3F; Q1F; BW90
7	8	66.4	52.1	T5; Duration; Q3F; IQR; Q1F; BW90; Min Entr; F95
8	7	63.6	51.2	T95; Duration; F5; IQR; Q3F; BW90; Centr F
9	7	63.6	51.2	Q3T; Duration; F5; IQR; Q3F; BW90; Centr F
10	7	66.4	51.2	T5; Duration; F5; IQR; Min Entr; Q3F; BW90
11	8	65.0	51.2	T5; Duration; Q3F; IQR; Agg Entr; Min Entr; BW90; F95
12	7	62.7	53.9	T95; Duration; Q3F; Centr F; Min Entr; Pak F; F 95
13	7	60.8	48.4	T5; Duration; Q3F; IQR; Agg Entr; Min Entr; BW 90
14	7	60.8	48.4	T95; Duration; Q3F; IQR; Agg Entr; Min Entr; BW90
15	6	62.7	47.9	T5; Duration; Q3F; BW90; F95; Min Entr

Our results provide evidence that the hissing of geese may advertise an individual's identity to a potential receiver whereas sex and body weight were inconclusive with respect to this call. Future studies could test the influence of additional individual qualities (e.g., health condition, social status, etc.). This would expand our knowledge on how non-vocal signals could potentially encode such information.

We studied hissing in a domesticated bird bred for meat production, but geese are known to retain many attributes of their wild counterparts ([Kozak, 2019](#); [Łukaszewicz et al. 2019](#)). Furthermore, hissing is common in many anserine birds ([del Hoyo et al. 1994](#)). Although there are notable behavioral differences between wild and domestic animals, many similarities still persist ([Jensen 2002](#)). Each behavior is partly under the control of genetic mechanisms, which have been adapted and designed over thousands of generations of evolution in nature and domestication has altered them only slightly ([Jensen 2002](#)).

Compared with pair-living wild geese, we do not think that the farming environment in which subjects were kept in one parent flock could selectively act against vocal individuality. Individual vocal recognition has been frequently documented in birds living in flocks. For example, colonial birds provide a robust model for research on individual acoustic recognition (e.g., penguins: [Aubin & Jouventin 2002](#); [Aubin, Jouventin & Hildebrand, 2000](#); [Jouventin, Aubin & Lengagne, 1999](#); gulls: [Aubin et al. 2007](#); [Beer 1969](#); skuas: [Charrier et al. 2001](#); swallows: [Beecher & Beecher 1983](#); [Beecher, Beecher & Hahn, 1981](#); parrots: [Berg et al. 2011](#); [Wanker & Fischer 2001](#)). Besides, wild geese also spend part of their life cycles gathering in flocks ([Cramp & Simmons, 1980](#); [Del Hoyo et al. 1994](#)). We do not think our results might indicate pair-specific rather than individual characteristics because we found significantly higher inter-individual variations in comparison to intra-individual variations. Besides, the flock of geese we studied consisted of harems with approximately four females

to one male. We would expect pair-specific characteristics to prevail in duetting birds, as has been found in cranes that have both individually specific calls (*Klenova et al. 2009b*; *Klenova et al. 2008b*) and pair specific calls produced either in unison or as a duet (*Klenova, Volodin & Volodina, 2008a*; *Klenova et al. 2009a*).

The study also raises questions about the function of this signal, i.e., what responses such hissing behavior evokes in the receivers. Playback experiments could potentially reveal whether family members or flock members respond more strongly than other individuals. However, we do have observations showing that hissing produced by an “attacked” individual attracts companions, resulting in “collective” hissing display. Recognition of hissing sounds produced by a threatened individual may trigger affective behaviors such as collective defense (pair, family, flock), which is typical for geese and swans.

CONCLUSIONS

This research is the first providing a description of the acoustic parameters of geese hissing.

We demonstrate that non-syrinx hissing sounds of geese vary between individuals more frequently than expected. Body weight proved to be inconclusive as an influential factor. Previous research of hissing sounds in birds have mainly studied hole-nesting birds in which this sound was produced by females in their respective nesting sites (*Broughton, 2005*; *Morley, 1953*). In contrast, our study deals with hissing produced by both females and males. Besides direct anti-predatory functions, hissing sounds certainly attract pairings/ mate-matching or warn other conspecifics (e.g., family or flock members). The results indicate that this non-vocal sound can encode individually specific information in a similar way as the more frequently studied vocalizations produced by the syrinx.

ACKNOWLEDGEMENTS

We thank the staff members for helping us in the study. We also thank to Jessie Perdomo Raudales for language corrections.

ADDITIONAL INFORMATION AND DECLARATIONS

Funding

The study was financially supported by the Internal Grant of the Faculty of Forestry and Wood Sciences, Czech University of Life Sciences Prague, reg. number B04_18. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Grant Disclosures

The following grant information was disclosed by the authors:
Internal Grant of the Faculty of Forestry and Wood Sciences, Czech University of Life Sciences Prague: B04_18.

Competing Interests

The authors declare there are no competing interests.

Author Contributions

- Richard Policht conceived and designed the experiments, performed the experiments, analyzed the data, prepared figures and/or tables, authored or reviewed drafts of the paper, and approved the final draft.
- Artur Kowalczyk conceived and designed the experiments, performed the experiments, prepared figures and/or tables, authored or reviewed drafts of the paper, and approved the final draft.
- Ewa Łukaszewicz and Vlastimil Hart conceived and designed the experiments, prepared figures and/or tables, authored or reviewed drafts of the paper, and approved the final draft.

Animal Ethics

The following information was supplied relating to ethical approvals (i.e., approving body and any reference numbers):

All applicable institutional, national and international guidelines for the care and use of animals were followed. The study has been conducted in accordance with the current laws in Poland. The university farm was responsible for goose husbandry, care and research manipulation in accordance with the regulation of the Ministry of Agriculture and Rural Development (Dz. U. 2010 nr 116 poz. 778).

Data Availability

The following information was supplied regarding data availability:

The raw data are available in the [Supplemental Files](#).

Supplemental Information

Supplemental information for this article can be found online at <http://dx.doi.org/10.7717/peerj.10197#supplemental-information>.

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Acoustic individuality in the hissing calls of the male black grouse (*Lyrurus tetrix*)

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ABSTRACT

Acoustic individuality may well play a big role during the mating season of many birds. Black grouse (*Lyrurus tetrix*) produces two different long-distance calls during mating on leks: rookooing and hissing calls. The first one represents low frequency series of bubbling sounds and the second one represents hissing sound. This hissing represents a signal not produced by the syrinx. We analyzed 426 hissing calls from 24 individuals in Finland and Scotland. We conducted cross-validated discrimination analyses (DFA). The discrimination model classified each call with almost 78% accuracy (conventional result) and the validated DFA revealed 71% output, that is much higher than classification by chance (4%). The most important variables were Frequency 95%, 1st Quartile Frequency, Aggregate Entropy and Duration 90%. We also tested whether between individual variation is higher than within individual variation using PIC (Potential for individual coding) and we found that all acoustic parameters had $PIC > 1$. We confirmed that hissing call of black grouse is individually distinct. In comparison to the signals produced by the syrinx, non-vocal sounds have been studied rarely and according to our knowledge, this is the second evidence of vocal individuality in avian hissing sounds which are not produced by syrinx. Individuality in the vocalization of the male black grouse may aid females in mating partner selection, and for males it may enable competitor recognition and assessment. Individually distinct hissing calls could be of possible use to monitor individuals on leks. Such a method could overcome problems during traditional monitoring methods of this species, when one individual can be counted multiple times, because catching and traditional marking is problematic in this species.

Submitted 8 October 2020
Accepted 1 July 2021
Published 9 September 2021

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Academic editor
Maria Servedio

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Declarations can be found on
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DOI 10.7717/peerj.11837

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OPEN ACCESS

Subjects Animal Behavior, Zoology

Keywords Acoustic individuality, Hissing call, *Lyrurus tetrix*, Monitoring, Phasianidae

INTRODUCTION

At the time of a decline in the black grouse (*Lyrurus tetrix*) population across its distribution range (Ciach, 2015; Jankovska et al., 2012; Kasprzykowski, 2002), determining actual number of individuals is becoming increasingly important. The causes for this decline vary: change of the environment and climate (Kurhinen et al., 2009; Kvasnes et al., 2010; Viterbi et al., 2015; White, Warren & Baines, 2013), parasite infestation (Jankovska et al., 2012), predation (Charnov, Orians & Hyatt, 1976; Korpimaki, Koivunen & Hakkarainen, 1996; Pekkola et al., 2014; Policht et al., 2019; Summers et al., 2004; Tornberg et al., 2013; Widen et al., 1987), reducing genetic diversity (Segelbacher, Hoglund &

Storch, 2003; Segelbacher et al., 2014) and human activities (*Formenti et al., 2012; Hess & Beck, 2012; Ingold, 2005; Nichter, Lipp & Gregory, 2017; Storch, 2013*). Methods and options for protection and conservation of grouse are manifold and are realized at the local, regional, and national levels (*Storch, 2013*).

An integral part of any conservation measures in general, and thus also in the case of black grouse populations in particular, is monitoring. Methods of monitoring are diverse. *Franceschi et al. (2014)* simulated two monitoring approaches: plot sampling and distance sampling. According to their study, distance sampling is a better way to monitor grouse in terms of accuracy. On the other hand, this approach is also costly, as it requires 4–5 sampling points per km² for reliable outcomes (*Franceschi et al., 2014*). The most common counting method for black grouse is to register males displaying in the spring (*Hancock et al., 1999*). Depending on the size of the area to be monitored, it is possible to perform a full-area survey or to select sample areas at random (*Hancock et al., 1999*). Monitoring based on acoustic features of bird vocalization could be a more efficient method. *Laiolo et al. (2007)* recommended combining counting based on vocalization with physical marking.

Black grouse males produce the following kinds of sounds: resonant rookooing call and hissing calls. The latter is further subdivided into tones of aggression and alarm calls (*Cramp, 1983*). This study is focused on a particular type of hissing call—crowing-hiss, described by *Cramp (1983)* as harsh and angry sound, which is produced during the display of male black grouse. This hissing sound is not produced by syrinx. Such non-vocal sounds are produced by some constriction located on the way from the lungs to the bill (*Fitch & Hauser, 2003*). Potential information encoded in non-vocal sounds of birds remains almost unstudied (*Budka et al., 2018*). Recent research of hissing sounds produced by geese confirmed encoding of individual identity during antipredator behavior (*Policht et al., 2020*). In comparison to non-vocal acoustic signals of birds, majority of bioacoustic studies focused on research of sounds produced by syrinx. A hissing sound also appears in black grouse chicks above the age of 3 weeks, but we do not suppose it is the same sound category that is the focus of our study (*Meinert & Bergmann, 1983*). This type of vocalization, along with the rookooing call, is the most prominent sound made by black grouse, which can be heard over long distances and even in closed habitats such as forests with dense undergrowth. Such calls are frequently used for population monitoring to find actually used leks and counting present males. Therefore, this type of call may play an important role in noninvasive monitoring of black grouse. The rookooing call can be characterized as a low-frequency, repetitive sound within a range of about 200 to 1,000 Hz. This is why this type of call often overlaps with background noise frequencies. Compared with this, the hissing call is found in the frequency range of 350 to 4,500 Hz and is therefore easier to filter out from background noise and to mark this type of call for measurement using acoustic software. Thanks to these characteristics, the hissing call may be more suitable for acoustic monitoring of black grouse.

In an effort to ensure quiet conditions for game wildlife, non-invasive monitoring, such as that based on vocalization, is the method of choice. This method relies on distinguishing individuals without physical marking. In our study, we analyzed the

vocalization of male black grouse to examine variation between individuals, and to find out whether vocalization characteristics could serve as a unique identifying trait.

METHODS

Study areas and recording

We recorded the hissing calls of male black grouse during their mating season. Recording took place in Finland in 2012 and 2013, and in Cairngorms National Park, Scotland in 2019. Field experiments were approved by the Department of Natural Resources, Ministry of Agriculture and Forestry, Finland and by the Game & Wildlife Conservation Trust, Scotland. According to Finnish legislation in general and to the hunting legislation, this type of scientific project does not require any special permits or licenses. All appropriate permissions were in place for the fieldwork in Scotland. The research was conducted in accordance with the guidelines of the Animal Behavior Society for the ethical use of animals in research. The study was carried out in accordance with the recommendations in the Guide for Care and Use of Animals of the Czech University of Life Sciences, Prague. The Animal Care and Use Committee of the Czech Ministry of the Environment approved the protocol (Permit number: 15106/ENV/14-825/630/14).

Vocalization of male black grouse was recorded with the audio recorder Olympus LP-100 in combination with a Sennheiser ME 66 directional microphone (frequency response 20 Hz–20 kHz \pm 2.5 dB) complemented by a K6 powering module. Recordings were saved in .wav format (48 kHz sampling rate, 16-bit sample size). We recorded all individuals in the wild during courtship at leks. Lek is an area where two or more males perform courtship displays to gain an advantage for mating with females. All leks were approached before the arrival of males, about 2 h before sunrise. Each recording session took on average 1 h and was performed from a portable hide so that the males could be observed without being disturbed. The distance of the hide from display sites was 10 m on average. During the pilot study, we only tested the variability between multiple individuals on one lek, and it turned out that the individual variability is much larger. To avoid the risk of multiple counting of the same individual, we chose the option of selecting only one, maximum of two individuals on each lek. The distance between visited display sites was at least one km and, according to [Borecha, Willebrand & Nielsen \(2017\)](#), black grouse males show strong fidelity to their lek; therefore, the risk of recording the same individual at the two display sites was low.

Acoustic analyses

Recordings were analyzed using Raven Pro 1.5 software with a 512 sample size and a Hann window. We selected good quality calls with high signal to noise ratio, non-overlapping with other hissing calls or background noise and wind. Each selected hissing call was manually bounded by the selection frame that is defined by the beginning and end of the signal and the lowest and highest frequency of the signal. Temporal and frequency variables were then measured automatically. These measurements were entered into the statistical analysis.

Statistical analyses

We analyzed 426 good-quality calls from 31 individuals (at least ten separate hissing calls per individual). We measured 29 variables (Table 1). We excluded variables with low or no variation. The remaining variables were standardized using Z-score transformation (subtracting the mean and dividing by standard deviation). In order to test individual variation, we used stepwise Discrimination Function Analysis (DFA) using IBM SPSS Statistics 24.0 software (IBM Corp., Armonk, NY, USA). We applied a leave-one-out cross-validation procedure (IBM SPSS Statistics 20) to validate the results of DFA.

To test the potential for individual variation (Potential of Individual Coding—PIC) for each parameter, we compared the coefficient of variation (CV) within and between individuals. The PIC ratio was computed for each acoustic parameter by dividing the CV_{between} by the mean of the CV_{intra} values related to each individual (Robisson, 1992). For these tested parameters, a PIC value greater than one means that an inter-individual variability is higher than intraindividual variability. We tested a significance using Kruskal–Wallis test.

RESULTS

Hissing call description

The hissing calls of black grouse represent wideband acoustic signals, in which energy is spread across a wide frequency range. The duration of such calls ranged from 0.1 to 1.21 s (0.76 ± 0.16 , mean \pm SD). This type of call can consist of one or two notes; however, the occurrence of a two-syllable form was rare ($\sim n < 1\%$)—so we did not analyze these calls.

The Low frequency ranged from 352.9 to 1,310.3 Hz (830.2 ± 195.6 Hz, mean \pm SD) and the High frequency from 1,702.4 to 4,482.8 Hz ($2,687.5 \pm 536.4$ Hz, mean \pm SD) for all individuals. Frequency range reached 729.6 to 3,241.4 Hz ($1,857.3 \pm 478.6$ Hz, mean \pm SD). The spectrograms of black grouse recorded in Finland and Scotland are shown in the figures below (Figs. 1 and 2). The spectrograms were generated in Avisoft-SASLab Pro with FFT length, 1,024 sample size, a Hamming window and 87.5% overlap. For a representative recording of a hissing call of one individual from Scotland and one individual from Finland see Audio S1 and Audio S2.

Individual variation

From selected parameters the resulting model (see Table S1) included 13 significant acoustic variables ($p < 0.001$; $r \leq 0.87$): 1st Quartile Frequency, Relative 1st Quartile Frequency, Aggregate Entropy, Average Entropy, Relative Center Time, Call Duration, Duration 90%, Frequency 5%, Relative 3rd Quartile Frequency, Frequency 95%, Inter-Quartile Range Bandwidth, Inter-Quartile Range Duration and Time 5% (Table 1). The first four discriminant functions had Eigenvalues > 1 and explained 79.7% of the variation. With the first discrimination function mostly correlated F95% (Frequency 95%) ($r = 0.767$) and Q1F (Quartile 1 Frequency) ($r = 0.707$) and the second discriminant function correlated best with AggEnt (Aggregate Entropy) ($r = 0.390$) and Dur 90% (Duration 90%) ($r = 0.387$) (Fig. 3). The Discriminant Function Analysis excluded seven

Table 1 Descriptions of acoustic parameters measured in Raven Pro 1.5 that entered statistical analysis.

Acoustic parameter name	Abbreviations (Units)	Description
*1st Quartile frequency	Q1 Freq (Hz)	The frequency that divides the signal into two frequency intervals containing 25% and 75% of the energy in the signal.
*Relative 1st quartile frequency	Q1 Freq rel,	The frequency that divides the signal into two frequency intervals containing 25% and 75% of the energy in the signal relative to the frequency range of the signal.
*3rd Quartile frequency	Q3 Freq (Hz)	The frequency that divides the signal into two frequency intervals containing 75% and 25% of the energy in the signal.
Relative 3rd quartile frequency	Q3 Freq rel,	The frequency that divides the signal into two frequency intervals containing 75% and 25% of the energy in the signal relative to the frequency range of the signal.
1st Quartile time	Q1 Time (s)	The time that divides the signal into two time intervals containing 25% and 75% of the energy in the signal.
Relative 1st quartile time	Q1 Time rel,	The time that divides the signal into two time intervals containing 25% and 75% of the energy in the signal relative to signal duration.
Relative 3rd quartile time	Q3 Time rel,	The time that divides the signal into two time intervals containing 75% and 25% of the energy in the signal relative to signal duration.
*Aggregate entropy	Agg entropy (bits)	The aggregate entropy measures the disorder in a sound by analysing the energy distribution. Higher entropy values correspond to greater disorder in the sound whereas a pure tone with energy only one frequency bin would have zero entropy. It corresponds to the overall disorder in the sound.
*Average entropy	Avg Entropy (bits)	This entropy is calculated by finding the entropy for each frame in the signal and then taking the average of these values.
Bandwidth 90%	BW 90% (Hz)	The difference between the 5% and 95% frequencies.
Center frequency	Center freq (Hz)	The frequency that divides the signal into two frequency intervals of equal energy.
Center time	Center time (s)	The point in time at which the signal is divided into two time intervals of equal energy.
*Relative center time	Center time rel,	The point in time at which the signal is divided into two time intervals of equal energy relative to the signal duration.
*Call duration	Duration (s)	The difference between begin time and end time for the signal.
*Duration 90%	Dur 90% (s)	The difference between the 5% and 95% times.
*Frequency 5%	Freq 5% (Hz)	The frequency that divides the signal into two frequency intervals containing 5% and 95%.
Relative frequency 5%	Freq 5% rel,	The frequency that divides the signal into two frequency intervals containing 5% and 95% relative to frequency range.
*Frequency 95%	Freq 95% (Hz)	The frequency that divides the signal into two frequency intervals containing 95% and 5%.
Relative Frequency 95%	Freq 95% rel,	The frequency that divides the signal into two frequency intervals containing 95% and 5% relative to frequency range.
*Inter-quartile range bandwidth	IQR BW (Hz)	The difference between the 1st and 3rd quartile frequencies.
*IQR (Inter-quartile range) duration	IQR Dur (s)	The difference between the 1st and 3rd quartile times.
Max entropy	Max entropy (bits)	Maximum entropy calculated from each frame.
Max frequency	Max freq (Hz)	The frequency at which max power occurs within the signal.
Max time	Max time (s)	The first time in the signal at which a spectrogram point with power equal to max power/peak power occurs.
Min entropy	Min entropy (bits)	The minimum entropy calculated for a spectrogram slice within the signal bounds.
Peak time	Peak time (s)	The first time in the signal at which a sample with amplitude equal to peak amplitude occurs.

(Continued)

Table 1 (continued)

Acoustic parameter name	Abbreviations (Units)	Description
*Time 5%	Time 5% (s)	The time that divides the signal into two time intervals containing 5% and 95%.
Relative time 5%	Time 5% Rel,	The time that divides the signal into two time intervals containing 5% and 95% relative to signal duration.
Relative time 95%	Time 95% Rel,	The time that divides the signal into two time intervals containing 95% and 5% relative to signal duration.

Note:

13 parameters (*) were included in resulting DFA model.

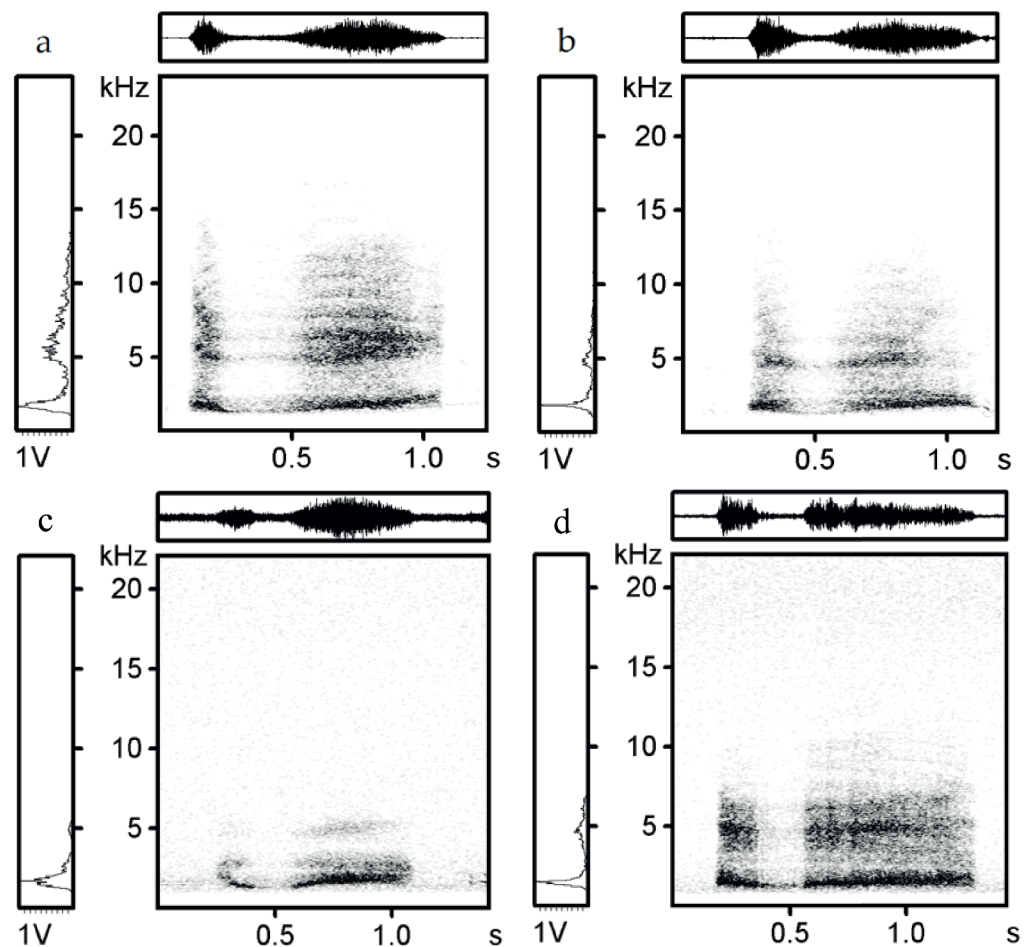


Figure 1 Spectrograms and oscilograms: Representative hissing calls of two of the black grouse from Scotland (A, B) and Finland (C, D). Each lettered panel refers to one individual bird. Spectrograms indicate observable differences between four individuals. [Full-size !\[\]\(e18234c3c79d4e9aedd7f39240ab238d_img.jpg\) DOI: 10.7717/peerj.11837/fig-1](https://doi.org/10.7717/peerj.11837/fig-1)

out of 31 individuals due to their missing or extreme values of the measured parameters. The cause could be a poorer degree of sound quality that did not pass the analysis. This selection has been made by model procedure automatically. The resulting DFA model correctly classified 77.9% (71.1%, cross-validated result) hissing calls. Six individuals showed the highest classification accuracy (80–100%), and most individuals ($N = 15$) were

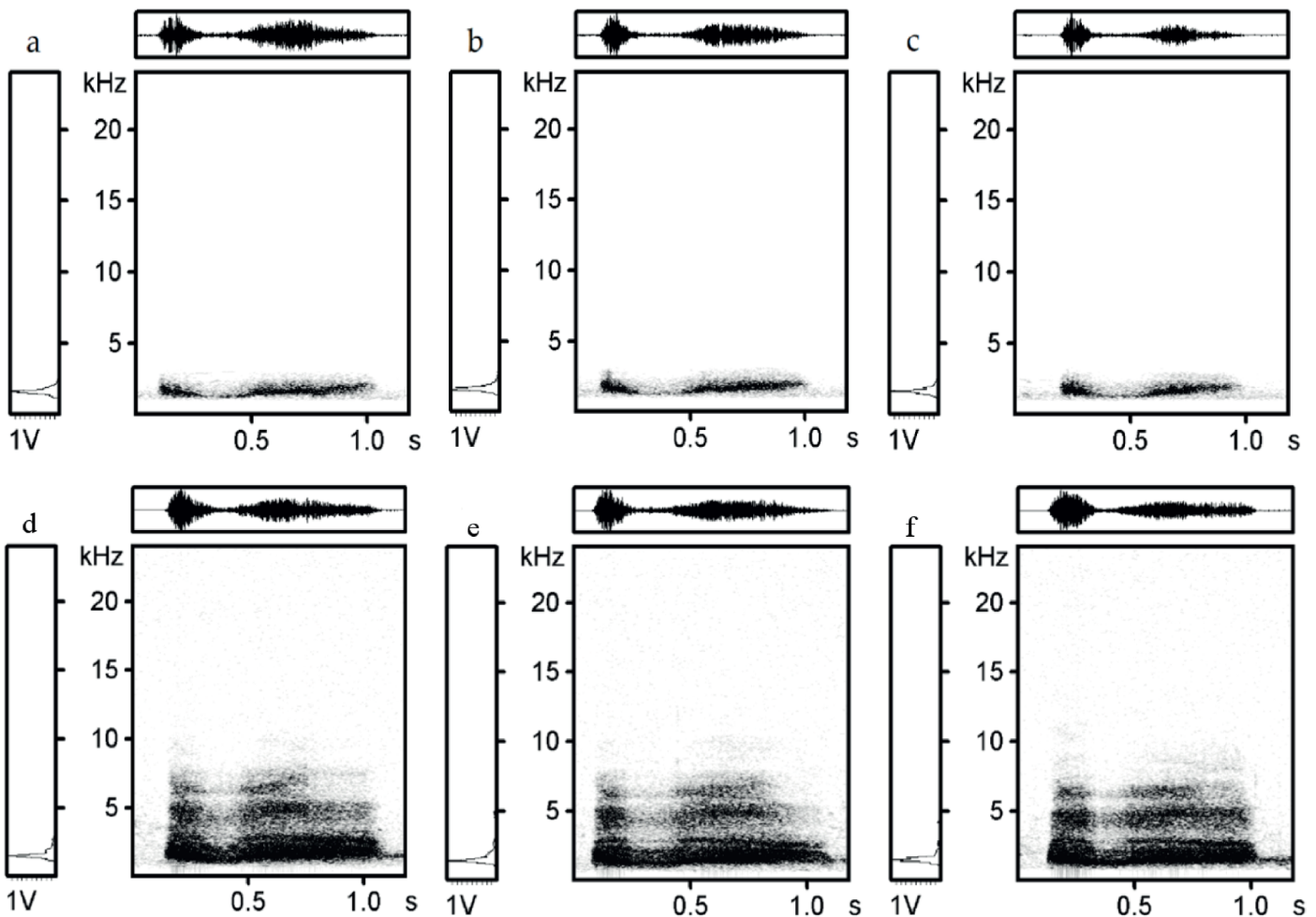


Figure 2 Spectrograms and oscilograms of three hissing calls of black grouse from one individual from Scotland (A–C) and one individual from Finland (D–F). Each lettered panel refers to one hissing call. Spectrograms in rows indicate consistent stability of individual pattern within the same individual. [Full-size !\[\]\(b345a1c4255362eec3746050dd71ccac_img.jpg\) DOI: 10.7717/peerj.11837/fig-2](https://doi.org/10.7717/peerj.11837/fig-2)

classified with 60–79% success. Only three males were classified with a lower than 59% success. These results were much higher than classification by chance (4%). The output of classification results is shown in [Table S2](#). We tested whether observed classifications differed from the expected classifications (by chance) and we found a significant difference: Chi-Square = 307.1, $df = 23$, $p < 0.001$. We also tested whether between individual variation is higher than within individual variation using PIC and we found that all acoustic parameters had $PIC > 1$ ([Table 2](#)).

DISCUSSION

Our results reveal that the wideband hissing call of male black grouse is individual specific. The discrimination model classified each call with almost 78% accuracy, and the first four discriminant functions explained nearly 80% of variation. The PIC ratio was higher than one for all parameters tested, demonstrating that the variability between individuals was higher than the variability within individuals. Therefore, the hissing call is

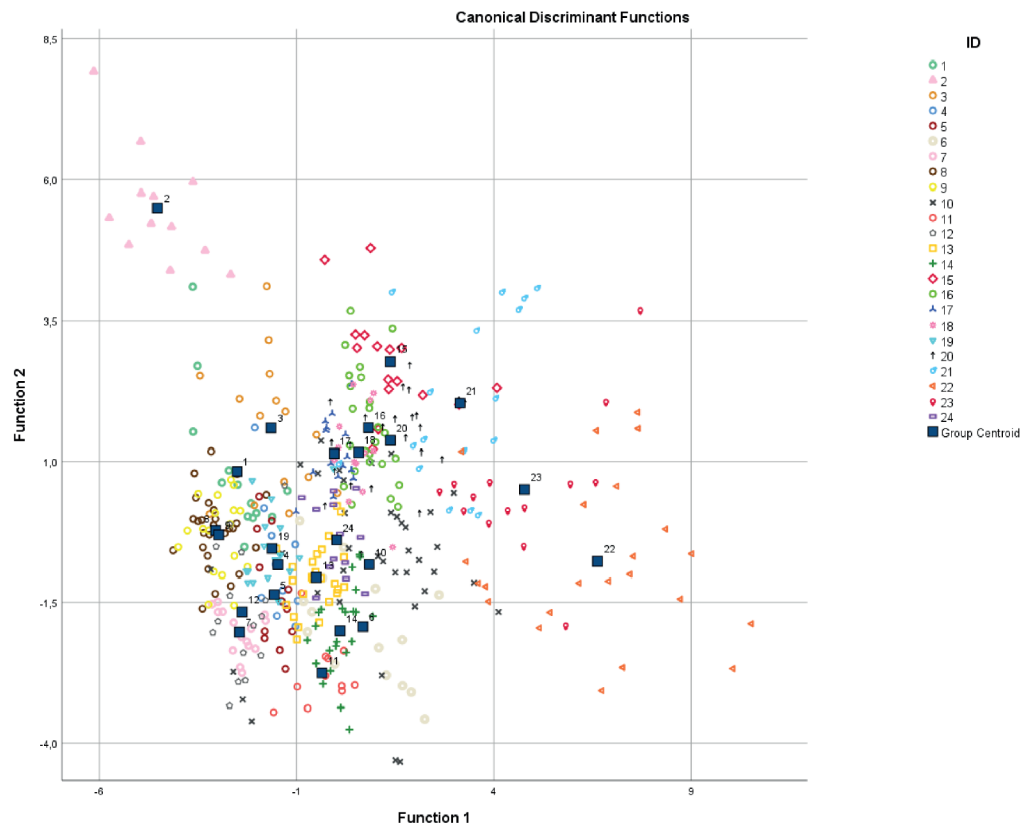


Figure 3 Scatter plot of hissing calls. Numbers refer to individuals, squares represent group centroids. Discrimination function 1 represents Frequency 95% and function 2 represents 1st Quartile Frequency.

Full-size [DOI: 10.7717/peerj.11837/fig-3](https://doi.org/10.7717/peerj.11837/fig-3)

a type of vocalization that carries information about individuality. Considering this type of call is a wideband, non-vocal sound, such a result is quite unique. There are not many studies focused on non-vocal animal sounds and even fewer of them have provided results confirming individuality in this type of vocalization; however, there are several. Individual variation was found in the male Houbara Bustard (*Chlamydotis undulata undulata*), which produces sounds called booms during courtship (Cornec, Hingrat & Rybak, 2014). Acoustic variation between individuals was also investigated in the Greater Prairie-chicken (*Tympanuchus cupido*) and the boom vocalization of this species was found to have individual characteristics (Hale, Nelson & Augustine, 2014). Thanks to temporal patterns, along with the number of drumming strokes, it is possible to discriminate individuals of the Great Spotted Woodpecker (*Dendrocopos major*) (Budka et al., 2018). According to acoustic analysis in the male Greater Sage-grouse (*Centrocercus urophasianus*), the “rustling” of wings differs between individuals (Koch, Krakauer & Patricelli, 2015). Therefore, mechanical sounds can also carry acoustic information about individuality.

Vocal individuality in some non-passerine groups has been intensively studied, such as colonial birds or nocturnal birds. On the other hand, gallinaceous species has not been

Table 2 Descriptive statistics and Potential for individual coding.

Variable	DFA	Mean	Min	Max	SE	Kruskal–Wallis	Mean CVw	CVa	PIC
1st Quartile frequency	X	1,444.3	0.9	3,000.0	271.8	*	38,503.6	73,887	1.919
Relative 1st quartile frequency	X	3.3	0.0	1500.0	62.4	*	219.9	3,896	17.714
3rd Quartile frequency	X	197.8	0.1	3,027.8	436.6	*	70,423.4	190,607	2.707
Relative 3rd quartile frequency		0.3	0.0	1.0	0.2	*	0.0	0	1.960
1st Quartile time		1,747.2	0.5	3,562.5	367.6	*	60,556.2	135,130	2.231
Relative 1st quartile time		2.4	0.1	1,687.5	56.2	*	229.5	3,159	13.761
Relative 3rd quartile time		246.2	0.2	4,3525.0	1508.0	*	216,894.9	2,274,186	10.485
Aggregate entropy	X	3.2	0.7	4.8	0.5	*	0.2	0	1.846
Average entropy	X	2.8	1.7	4.0	0.4	*	0.1	0	1.869
Bandwidth 90%		839.2	187.5	2,437.5	355.2	*	69,079.2	126,142	1.826
Center frequency		1,589.0	468.8	3,375.0	303.2	*	45,489.3	91,910	2.020
Center time		198.1	0.1	3028.1	436.6	*	70,428.0	190,611	2.706
Relative center time	X	0.5	0.1	1.0	0.2	*	0.0	0	2.470
Call duration	X	1.0	0.2	1.9	0.2	*	0.0	0	1.460
Duration 90%	X	0.7	0.1	1.6	0.2	*	0.0	0	1.657
Frequency 5%	X	1,209.8	375.0	2,250.0	249.9	*	42,818.7	62,427	1.458
Relative Frequency 5%		0.2	0.0	0.5	0.1	*	0.0	0	1.303
Frequency 95%		2,049.0	1,125.0	4,125.0	432.2	*	84,005.1	186,777	2.223
Relative frequency 95%		0.7	0.3	1.0	0.1	*	0.0	0	1.823
Inter-quartile range	X	301.8	86.1	1,218.8	188.9	*	17,712.6	35,694	2.015
IQR (Inter-quartile range) duration	X	0.4	0.0	1.2	0.1	*	0.0	0	2.301
Max entropy		3.9	2.9	4.9	0.3	*	0.0	0	2.044
Max frequency		1,572.4	468.8	3,468.8	336.3	*	58,737.8	113,076	1.925
Max time		197.9	0.1	3,027.5	436.6	*	70,409.4	190,584	2.707
Min entropy		1.6	0.1	2.9	0.4	*	0.1	0	1.685
Peak time		197.9	0.1	3,027.5	436.6	*	70,409.3	190,583	2.707
Time 5%	X	197.9	0.0	3,027.5	436.5	*	70,738.2	190,565	2.694
Relative time 5%		0.1	0.0	0.4	0.0	*	0.0	0	2.247
Relative time 95%		0.8	0.6	1.0	0.1	*	0.0	0	2.143

Note:

(DFA) 13 variables included in final DFA model (X). (SE) standard error of the mean. (Kruskal–Wallis) Kruskal–Wallis test after Bonferroni correction, (*) $p < 0.001$. (Mean CVw) within individual comparison. (CVa) between individual comparison. (PIC) Potential for Individual Coding.

studied frequently. Acoustic displays of the Japanese quail (*Coturnix coturnix japonica*) are characterized by a potential for vocal individuality in terms of temporal parameters. Spectral characteristics of the voice are then associated with the possibility of greater stability during the development of the individual, which is important in the question of long-term recognition of individuals (Sezer & Tekelioglu, 2010). Call analyses of European and Japanese quail (*Coturnix c. japonica*, *C. c. coturnix*) confirm a difference between these two subspecies based on the time structure of vocalization (Collins & Goldsmith, 1998). The hazel grouse (*Bonasa bonasia*), studied in Switzerland, exhibits 6 to

11 elements of singing during flight. These elements (individual tones or syllables) are characterized by their individual specificity (*Mulhauser & Zimmermann, 2003*). Specific parameters responsible for acoustic individuality were also found in males and females of the common quail (*Coturnix coturnix*); the results of this study also indicated that the male's inter-individuality is dependent on sexual maturation and age (*Guyomarc'h, Aupiais & Guyomarc'h, 1998*). Our study demonstrates a vocal individuality in gallinaceous species with lek mating system.

What role acoustic individuality plays in the black grouse's voice is still a question for future research. Calls of individual birds may carry information about male quality (e.g., physiological state, age) for females (*Guyomarc'h, Aupiais & Guyomarc'h, 1998*), and, at the same time, it might be a signal for other males providing information about the strength of a rival. Finally, individuality can serve to easily identify individuals among each other within a group. Its potential for scientists lies in the possibility of use for noninvasive monitoring. Taking an observation, census may be inaccurate; due to overflights of individuals within the lekking site, repeated census of the same individuals may occur and therefore the results of counting may be overestimated. Monitoring based on acoustic recognition could provide the required accuracy and assistance in areas where observation is limited by environmental conditions (e.g., the situation when males of black grouse lek individually hidden in the undergrowth).

CONCLUSION

The black grouse population is affected by many factors that contribute to its decline, and as part of its conservation, efforts are being made to develop better methods of protection, including monitoring. Vocalization recording and analysis could be a non-invasive monitoring tool, especially if there is individuality in the voice of individuals. This method could significantly reduce the risk of multiple counting of the same individual.

Surprisingly, we found this individuality in the black grouse in the non-vocal type of display. The discrimination model classified each call with high accuracy and important variables turned out to be Frequency 95% and Quartile 1 Frequency. In comparison to the signals produced by the syrinx, non-vocal sounds have been studied rarely and according to our knowledge, this is the second evidence of vocal individuality in avian hissing sounds which are not produced by syrinx. Finding specific identifiers in vocalization could lead to a more accurate determination of the number of individuals.

ACKNOWLEDGEMENTS

We very much appreciate the help and support of Mr. Christian Krogell, Department of Natural Resources, Ministry of Agriculture and Forestry, Finland. We are also thankful to Mr. Ilkka Ala-Ajos for logistical support for our research in Finland. Furthermore, we would like to thank Dr. David Parish, Marlies Nicolai and Dr. Kathy Fletcher from the Game & Wildlife Conservation Trust and the gamekeepers in Cairngorm National Park, Scotland. We would like to express our gratitude to Robert B. Davis for correction of the English language. Thanks for help in translating articles also goes to Mr. Roland Kralik.

ADDITIONAL INFORMATION AND DECLARATIONS

Funding

This research was funded by the IGA of the Faculty of Forestry and Wood Sciences, Czech University of Life Sciences, Prague. Project number: B_04_18. There was no additional external funding received for this study. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Grant Disclosures

The following grant information was disclosed by the authors:

IGA of the Faculty of Forestry and Wood Sciences, Czech University of Life Sciences, Prague: B_04_18.

Competing Interests

The authors declare that they have no competing interests.

Author Contributions

- Lucie Hambálková performed the experiments, analyzed the data, prepared figures and/or tables, and approved the final draft.
- Richard Policht conceived and designed the experiments, performed the experiments, analyzed the data, authored or reviewed drafts of the paper, and approved the final draft.
- Jiří Horák performed the experiments and approved the final draft.
- Vlastimil Hart conceived and designed the experiments, performed the experiments, authored or reviewed drafts of the paper, and approved the final draft.

Animal Ethics

The following information was supplied relating to ethical approvals (*i.e.*, approving body and any reference numbers):

The Animal Care and Use Committee of the Czech Ministry of the Environment approved the protocol (Permit number: 15106/ENV/14-825/630/14).

Field Study Permissions

The following information was supplied relating to field study approvals (*i.e.*, approving body and any reference numbers):

Field experiments were approved by the Department of Natural Resources, Ministry of Agriculture and Forestry, Finland and by the Game & Wildlife Conservation Trust, Scotland.

Data Availability

The following information was supplied regarding data availability:

The raw data are available as [Supplemental Files](#).

The resulting model of statistical analysis shows all individuals and significant variables tested. The output of classification results shows classification accuracy percentages for each individual.

Supplemental Information

Supplemental information for this article can be found online at <http://dx.doi.org/10.7717/peerj.11837#supplemental-information>.

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7 Diskuze

Reakce na hlas vybraného predátora a vliv různého typu kořisti na variabilitu hlasu predátora

U ptáků a savců je známo, že reagují na hlasy predátorů i přesto, že příslušní predátoři během vlastního lovu zpravidla nevokalizují a jedinci kořisti tak mohou získávat informace o aktivitě a prostorové distribuci ze signálů v ostatních kontextech, doprovázejících jejich reprodukci, sociální interakce, apod. (Blumstein *et al.* 2008). I když reakce zvířat vůči hlasům predátorů byly intenzivně studovány (viz Hettena *et al.* 2014), méně je známo o tom, jak je tomu v případě rekognice hlasu hnízdních predátorů zejména v případě prekociálních ptáků, zatímco reakce altriciálních ptáků byly pravidelně studovány (viz Schaeff & Mumme 2012). Neznámou zůstávala otázka, zda hlas hnízdního predátora může vyvolat antipredační odezvu v závislosti na pohlaví u druhů s extrémně polygynním či dokonce lekovým reprodukčním systémem, ve kterém samec neposkytuje žádnou formou rodičovské péče. Tato práce ukazuje, že i zástupce takového modelu, je schopen rozpoznávat hlas hnízdního predátora. Model s lekovým pářicím systémem, reaguje na hlas hnízdního predátora zvýšenou ostražitostí nebo odletem (Policht *et al.* 2019), i když hnízdní predátor zpravidla není schopen ohrozit dospělé ptáky. Proto je překvapivé, že na něj dospělí ptáci vůbec reagují, zejména mimo období vlastního hnízdění. Ve studovaném případě se jednalo o období těsně předcházející době hnízdění, takže slepice mohly být již motivovány k blížícímu se hnízdnímu chování. V tomto případě není ale jasné, proč intenzivnější reakci vykazovali kohouti, ačkoli ti se nijak nepodílí na rodičovské péči. Možným vysvětlením reakcí dospělých ptáků by mohlo být uchování raného antipredačního chování předváděného v období pobytu na hnízdě až do dospělosti. To však nevysvětluje zmiňované rozdíly mezi pohlavím. Tento rozdíl by mohl reflektovat odlišnou antipredační strategii, kde reakce slepic mohly odrážet spíše strategii nehybné kryptické pozice na hnízdě v případě nebezpečí, zatímco kohouti častěji volili odlet. Jiným možným vysvětlením by mohlo být, že hlas krkavce signalizuje přítomnost dalšího predátora, kterým by mohla být například liška obecná. Jednu z hlavních potravních strategií krkavce velkého, jako omnivorního generalisty, představuje mrchožravost, během které často asociuje s velkými predátory (Marzluff 2018). Následování volání krkavce liškou by mohlo lišce zvyšovat efektivitu nalezení případné padliny a naproti tomu krkavec by vydával varovné hlasy v případě

přibližující se lišky (viz Killengreen *et al.* 2012). V takovém případě by volání krkavce mohlo představovat adaptivní antipredační strategii dospělců tetřívka obecného vůči potenciální přítomnosti predátora, který je již schopen přímo ohrozit dospělé ptáky, navíc v průběhu celého roku, nejenom v době hnízdění.

Variabilita v signálech vydávaných potenciální kořistí byla intenzivně studována. Naproti tomu variabilita hlasu predátora v závislosti na druhu potenciální kořistí zůstává nejasnou. Jedním z hlavních důvodů pravděpodobně bude, že predátoři během samotné predace zpravidla nevokalizují (Blumstein *et al.* 2008). Za predací kontext lze považovat i reakce zvířat vůči člověku, které se také ukázaly odrážet míru potenciálního rizika. Studie ukázaly odlišné reakce v závislosti na lovecké sezóně a typu lidské aktivity, kde jejich reakce se lišili mírou ostražitosti chování nebo volbou bezpečnějšího stanoviště (viz Frid & Dill 2001; Benhaiem *et al.* 2008; Jayakody *et al.* 2008). Přítomnost lidí může být signalizována i přítomností psů, kteří je často doprovázejí. Ti také mohou vyvolávat antipredační reakci, a to jak svou přítomností, tak i samotným hlasovým projevem. Štěkání, tak může představovat signál potenciální hrozby. To se skutečně potvrdilo pomocí playbackových experimentů (Randler 2006). Především početné výzkumy ukázaly, že štěkání psů může poskytovat lidským společníkům několik různých typů informací (viz Miklosi 2007; Serpell 2016). Štěkání loveckých psů během lovu zůstávalo doposud nepovšimnuto, ačkoli zkušenosti lovců tento efekt naznačovaly. Tato práce odhaluje, že štěkání loveckých psů se při setkání se s odlišnými druhy zvířat průkazně odlišuje. Míra těchto rozdílů se zdá korelovat s mírou potenciální hrozby, kterou zvíře pro daného psa představuje. Tato práce indikuje, že míra hrozby od zvířete, se kterým se pes setká, je reflektována strukturou akustických parametrů na základě valence-arousal modelu. Štěkání produkované při setkání s nejnebezpečnějším modelem, vykazovalo parametry o nejnižších frekvencích a mělo nejdelší délku trvání, oproti štěkání na ostatní, méně nebezpečné nebo i bezpečné druhy zvířat. Štěkání se pak spíše jeví být expresí vnitřního stavu psa než funkčně referenční informací. Expresie vnitřního stavu psa během štěkání tak pravděpodobně závisí na míře potenciální hrozby. Stejný efekt byl zaznamenán i u „naivních“ psů, kteří neměli s konkrétním, potenciálně nebezpečným druhem žádnou předchozí zkušenost. To by mohlo indikovat vrozenou predispozici. V případě loveckých psů by schopnost odlišného typu štěkání na různé druhy zvířat a schopnost lidí-lovců je rozpoznávat mohlo zvyšovat efektivitu lovu. Lovečtí psi tak mohli projít silným selekčním tlakem, který by zintenzivnil tuto schopnost v porovnání s jinými plemeny psů.

Míra individuální variability u vokálních signálů s odlišnou akustickou strukturou

Míra individuálních rozdílů může významně záviset na struktuře akustické energie ve vokálních signálech savců (viz Charlton 2015). Čím jednodušší model podporuje výsledky, tím snadnější je výsledná interpretace. U hlasů s jednoduchou akustickou strukturou, kterými jsou zejména tónické či harmonické signály s konstantním průběhem základní frekvence, tedy minimální změnou frekvencí, jakými jsou například alarmy sysla perličkového (*Spermophilus suslicus*) (viz např. Matrosova *et al.* 2016), obecného (*Spermophilus citellus*) či taurského (*Spermophilus taurensis*), může být identita volajícího determinována i jen několika málo akustickými parametry. V případě některých sýslů je kombinace pouhých dvou akustických parametrů rozhodující pro rozlišení jedinců, např. u sysla obecného či taurského (Schneiderova & Policht 2010). Na druhé straně škály vyjadřující akustickou strukturu signálů jsou širokospektré signály, ve kterých je akustická energie rozprostřena napříč široké spektrum frekvencí (viz kapitola 3.5.). Tyto signály se vyznačují různým způsobem formovanou komplexitou. Takto strukturované varovné signály jsou časté např. u řady primátů (viz Fischer *et al.* 2001a; Fischer *et al.* 2001b; Crockford & Boesch 2003; Fichtel & Van Schaik 2006) a jelenovitých (Oli & Jacobson 1995; Reby, Cargnelutti & Hewison 1999; Cap *et al.* 2008). U těchto komplexních širokospektrých signálů jsou individuální rozdíly nejčastěji kódovány multiparametricky (viz Schrader & Hammerschmidt 1997; Aubin *et al.* 2007). Pro testování vlivu akustické struktury na míru individuálních rozdílů by byl ideální zejména druh, který produkuje oba dva strukturálně odlišné typy alarmů.

Varovné alarmy pozemních sciuridů jsou oblíbeným modelem výzkumu individuálně specifické vokalizace. Varovné signály pozemních sciuridů představují klasickou modelovou skupinu v těchto výzkumech. Fylogenetická poloha sysla dlouhoocasého (*Urocitellus undulates*) na základě molekulárních i kraniometrických dat vykazuje bližší příbuznost k nearktickým sýslům, přestože obývá palearktickou oblast (Harrison *et al.* 2003; Helgen *et al.* 2009). Svou schopností produkovat vedle tonálního alarmu i širokospektrálně strukturovaný alarm, tak indikuje svou příbuznost k nearktickým sýslům, kteří často širokospektré alarmy produkují. U ostatních palearktických sýslů se tento typ alarmů nevyskytuje, s jedinou výjimkou, dalšího zástupce rodu *Urocitellus*, sysla Parryova (*Urocitellus parryii*). Ten však obývá nejen východní Sibiř, ale i Aljašku a severní Kanadu (McLean 2018). I když širokospektré hlasy využívají i jiné euroasijské druhy sýslů, tak je nepoužívají jako alarmy.

Testování individuální variability alarmů sysla dlouhoocasého tak nabízí unikátní příležitost porovnat míru a způsob individuálních rozdílů u dvou zcela odlišných typů alarmů. Klastrová analýza porovnávací akustickou strukturu alarmů palearktických a nearktických syslů ukazuje bazální pozici širokospektrého alarmu sysla dlouhoocasého, který se tak odlišuje od všech ostatních druhů. Druhý typ alarmu tohoto sysla (tónický hvizd) byl lokalizován v klastru, který zahrnujícího jak jedno až tři-elementové alarmy syslů rodu *Otospermophilus* a dalších zástupců rodu *Urocitellus*. Porovnání velikosti individuální distinkce obou typů alarmů studovaného sysla dlouhoocasého pak ukázala srovnatelnou míru, konkrétně u tónického alarmu byla individuální distinkce o trochu vyšší (5%).

Některé komponenty akustického signálu mohou být méně zřetelné. To se týká zejména částí hlasů produkovaných o nižší intenzitě nebo blízko hranice slyšitelného spektra. K ještě většímu „maskování“ dojde, pokud se taková komponenta časově překrývá s jinou, intenzivněji produkovanou částí signálu. Podobná situace nastává i v případech nízkofrekvenčních komponent, které jsou často maskovány hlukem prostředí, nejčastěji větrem. Nízko-frekvenční vokalizace byla zaznamenána nejčastěji u savců, například u slonů, nosorožců, hrochů apod. (Payne, Langbauer & Thomas 1986; Barklow 2004; Policht *et al.* 2008; Herbst *et al.* 2012), ale třeba i u krokodýlů (Dinets 2013). V případě ptáků byla zaznamenána jen výjimečně. Nejhlubší produkované frekvence jsou dokumentovány u kasuárů, kde kasuár přilbový (*Casuarus casuarus*) svými „boom calls“ dosahuje až 32Hz a kasuár malý (*Casuarus bennetti*) 23Hz (Mack & Jones 2003). Podobný typ „boom calls“ vydávají i dropi, kde například základní frekvence tohoto signálu u dropa malého (*Chlamydotis undulata*) dosahuje 40 až 56 Hz (Cornec, Hingrat & Rybak 2014) a minimální frekvence u bukače velkého (*Botaurus stellaris*) dosahovala 87Hz (Puglisi *et al.* 2001). Produkci signálů o nižších frekvencích lze očekávat spíše u nepěvců s větší velikostí těla (viz Bertelli & Tubaro 2002). Případná produkce nízkých frekvencí u tetřeva hlušce (*Tetrao urogallus*) byla v minulosti diskutována, nepodařilo se však opakovaně potvrdit nález 40Hz zaznamenaný v toku jediného kohouta (viz Moss & Lockie 1979). Pozdější pokusy jak na divokých tetřevích, tak u ptáků chovaných v zajetí neodhalily ve vokalizaci frekvence nižší než 100 Hz (Lieser, Berthold & Manley 2005; Lieser, Berthold & Manley 2006). Typický tok tetřeva hlušce zahrnuje čtyři fáze: klepání, trylek, výlusk a broušení. Analýza toku tetřeva hlušce odhalila harmonicky designované signály se základní frekvencí 28.7 ± 1.2 Hz (25.6-31.6 Hz). Tyto nízko-frekvenční komponenty se časově překrývají s fází „broušení“ (96 % své délky trvání). Výskyt takto nízkých frekvencí je překvapivý, jelikož tetřev dosahuje podstatně nižší tělesné hmotnosti (do 6,5kg) v porovnání s kasuárem malým,

dorůstajícím hmotnosti 58kg (de Juana 1994; del Hoyo, Elliott & Sargatal 1994). Oba druhy přitom dosahují podobně hlubokých minimálních frekvencí (kasuár malý: 23Hz a tetřev hlušec: 26Hz). Výsledky této práce překvapivě také ukázaly individuálně specifický pattern u těchto nízko-frekvenčních komponent. Naskytá se otázka potenciální funkce těchto nízko-frekvenčních komponent v toku. Výrazný vokální projev kohoutů během toku by mohl indikovat jeho význam pro sexuální selekci, kde by mohl sloužit k přitahování pozornosti slepice v procesu samičí volby. Nižší frekvence by mohly být favorizovány jako „long-distance“ signál, jelikož delší zvukové vlny hlubších frekvencí lépe pronikají vegetací (Heimann 2003). Jiné vysvětlení by mohla představovat korelace hlubších frekvencí s větší velikostí těla. Jelikož jsou tyto nízko-frekvenční komponenty jsou v případě tetřeva hlušce překryty nejhlasitější fází toku (broušením), zdá se, že jejich zaznamenání ze vzdálenosti větší, než několika málo metrů je stěží zachytitelná. To je také asi pravděpodobný důvod proč tyto komponenty tak dlouho unikaly pozornosti. Na druhou stranu, intenzivnější interakce mezi potenciálními sexuálními partnery na ploše arény, kde se prezentují samci samicím, se koná spíše na kratší vzdálenost. Slepice by mohly být lákány k aréně z větší vzdálenosti pomocí hlasitějších složek toku. Při bližším posuzování preferovaných kohoutů z kratší vzdálenosti by pak mohly hrát roli právě tyto nízko-frekvenční komponenty. Finální rozhodnutí samice by tak mohlo reflektovat „trade-off“ mezi nalezením vysoce kvalitního samce a predačním rizikem. Navíc, posuzování mezi více samci představuje i jistou energetickou nákladnost.

Nápadný je také časový překryv výskytu nízko-frekvenčních komponent s hlasitým broušením, což je zrovna fáze toku, ve které kohouti vykazují známou „dočasnou hluchotu“, která je pak úspěšně využívána během lovu u jinak velmi plachého ptáka. Synchronní produkce hlasu ve dvou zcela odlišných frekvenčních pásmech (intenzivní broušení ve frekvenčním rozsahu cca 100 - 500Hz vs. nízko-frekvenční komponenty v pásmu 26 - 100Hz) by mohla způsobovat dočasnou hluchotu kohoutů, během této fáze toku. Mohlo by být obtížné naslouchat, a přitom produkovat hlas ve dvou zcela odlišných frekvenčních pásmech. Navíc, když se jedná o dva zcela odlišné typy hlasu. Fenomén zhoršeného slyšení během mluvení byl dokumentován i u člověka a označuje se jako „self-masking“, kde se zjistilo že za něj může kontrakce svalů středního ucha (viz Borg, Bergkvist & Gustafsson 2009; Borg *et al.* 2009).

Individuální rozdíly ve vokalizaci živočichů jsou studovány desítky let pomocí odlišných metod. Široká škála metod a použití odlišných metrik způsobuje těžkosti při porovnávání výsledků různých studií. Porovnání univariátních a multivariátních metrik testování individuální identity jak na simulovaných, tak empirických datech (Linhart *et al.*

2019) ukázalo, že Beecherova informační statistika (Beecher's information statistic: HS) byla nejbližší k teoretickým očekáváním jednotlivých modelů. Beecherova informační statistika není ale plně nezávislá na vzorkování. To je však možné řešit redukcí počtu testovaných parametrů či navýšením počtu analyzovaných jedinců. Používání Beecherovy informační statistiky by tak mohlo umožnit smysluplné porovnání výsledků napříč různými studiemi.

Kódování individuální identity u nevokálních akustických signálů

Vedle hlasů produkovaných hlasovým orgánem (hlasivkami v případě savců a syringem v případě ptáků) existuje i řada zvuků produkovaných jiným mechanismem, např. vibrací specifických per, tleskání křídly, úderý zobáku do substrátu (dřevo), klapáním zobáku, úderý nástrojem či zvuky kroků (viz Eda-Fujiwara *et al.* 2004; Garcia *et al.* 2012; Heinsohn *et al.* 2017; Murray, Zeil & Magrath 2017; Ota, Gahr & Soma 2017; Budka *et al.* 2018). Nevokálním signálům byla doposud věnována jen velmi malá pozornost v porovnání s ostatními akustickými signály (Stomp *et al.* 2018a). Zvuky mohou být také produkovány jakoukoli konstrikcí kdekoli na své cestě z plic do nozder a úst savců či zobáku ptáků, což produkuje aerodynamické, turbulentní zvuky (Fitch & Hauser 2002). Ty pak mívají podobu hvízdání, funění nebo syčení. Syčivé zvuky byly studovány zejména u savců (viz např. Policht *et al.* 2008; Stomp *et al.* 2018a; Stomp *et al.* 2018b; Volodina *et al.* 2018) a plazů (viz Labra *et al.* 2007; Vergne, Pritz & Mathevon 2009; Aubret & Mangin 2014), pouze několik studií zkoumalo syčivé zvuky ptáků. Doposud bylo ale studováno syčení pouze u malých pěvců v hnízdních dutinách používané jako obrana proti predátorům (Broughton 2005; Krams, Krama & Igaune 2006; Krams *et al.* 2014). Syčení hus, představuje model nepěvců hnízdících na zemi, produkujících syčení i mimo hnízdo v porovnání s pěvci, kteří vydávají syčení k obraně hnízdní dutiny, kterými jsou například sýkory. Potenciál ke kódování informací o individuální identitě u takovýchto nevokálních signálů nebyl doposud studován. Tato práce tak testuje, zda nevokální expirace mohou kódovat individuální identitu podobně, jako zvuky produkované syringem nebo hlasivkami (Policht *et al.* 2020). Vzájemné individuální rozpoznání syčení partnerů během nebezpečných situací by mohlo zvyšovat účinek antipredační odezvy, a tak zvyšovat pravděpodobnost přežití obou partnerů. Synchronizace partnerů během antipredačního chování pravděpodobně posiluje vzájemný svazek, podobně jako je tomu během synchronizovaného chování v jiných kontextech (např. dvoření či zdravícího ceremoniálu) (viz Ciaranca, Allin & Jones 2020). Samice by kromě toho mohla těžit z využití různých variant vyjadřujících samcovu schopnost investovat do rodiny a její ochrany v případě nebezpečí

(Ciaranca, Allin & Jones 2020). Výsledky této studie poskytují evidenci o tom, že nevkální syčení prekociálních ptáků, kromě své antipredační či agonistické funkce, může vyjadřovat individuální identitu signalizujícího jedince.

Individuální rozdíly v akustických projevech mohou hrát významnou roli i během reprodukčního chování ptáků. Tetřívka obecný vydává dva zcela odlišné long-distance signály v průběhu toku na tokaništi: bublání a pšoukání. Bublání představuje nízko-frekvenční sérii bublavých zvuků, zatímco pšoukání představuje syčivý zvuk. Vedle prvně doloženého individuálně distinktního syčení ptáků na příkladu domácích hus (Policht *et al.* 2020), tak pšoukání tetřívka obecného představuje druhou evidenci vokální individuality u tohoto typu nevkálního signálu ptáků, tentokrát u volně žijícího představitele (Hambálková *et al.* 2021).

V rámci nevkálních hlasů ptáků byly individuální rozdíly doloženy u bubnování strakapouda velkého (*Dendrocopos major*) (Budka *et al.* 2018) a mechanicky generovaných svištivých zvuků křídel tetřívka pelyňkového (*Centrocercus urophasianus*) (Koch, Krakauer & Patricelli 2015). Jakou potenciální roli akustická individualita hraje v biologii tetřívka obecného zůstává otázkou pro příští výzkumy. Pšoukání by mohlo signalizovat slepicím kvalitu kohoutů (např. fyziologický stav, věk, apod.) a přitom zároveň signalizovat informaci o kondici a síle ostatním rivalům. Nakonec by mohlo kohoutům sloužit k získávání přehledu na tokaništi, kdy ne všichni konkurenti jsou vždy v zorném poli kohouta a navíc kohouti často po tokaništi i přeletují.

Identifikace jedinců na základě akustických projevů nabízí zajímavé uplatnění jako metoda nevkálního akustického monitoringu jedinců (viz Terry, Peake & McGregor 2005; Laiolo *et al.* 2007). Během klasických metod monitoringu tetřevovitých ptáků na tokaništích pomocí sčítání tokajících kohoutů nelze s jistotou rozlišit, zda někteří jedinci nepřeletují i na sousední tokaniště a nejsou tak započítáni vícekrát. V tom případě dochází k určitému nadhodnocení výsledků sčítání. V případě nahrání hlasů kohoutů na jednotlivých tokaništích by bylo možné takovéto jedince identifikovat a verifikovat tak sčítací data. To bude efektivní zvláště na tokaništích s nižším počtem tokajících kohoutů (viz Policht *et al.* 2009). Individuálně specifický pattern signálů, který je patrný po použití diskriminační analýzy, je u některých druhů (včetně pšoukání tetřívka) rozlišitelný vizuálně na spektrogramu. To by umožnilo aplikovat metodu vizuálního posouzení spektrogramu (viz Gilbert, McGregor & Tyler 1994) například i u týmů, nepoužívajících mnohorozměrné statistické metody. Pokud by nahrávání probíhalo po určitou dobu v pravidelných intervalech, bylo by možné odhadnout velikost populace aplikováním „capture-mark-recapture“ techniky (viz Policht *et al.* 2009), podobně

jako se tato metoda používá u jinak značených jedinců, jako např. barevnými kroužky, ušními značkami, apod. (viz Lettink & Armstrong 2003). Při opakovaném nahrávání by se tak postupně měnil poměr známých (již nahraných jedinců v předchozích intervalech) a nových jedinců, doposud nenahraných. Z tohoto poměru lze pak spočítat celkovou velikost populace (viz Lettink & Armstrong 2003).

8 Závěr

Disertační práce se zaměřuje na tři hlavní cíle: (1) otestovat reakce potenciální kořisti na hlas predátora a vliv různého typu kořisti na variabilitu hlasu predátora, (2) porovnat expresi individuální variability vokálních signálů v závislosti na akustické struktuře a (3) ověřit potenciál pro kódování individuální identity u nevokálních akustických signálů. Předložená práce přináší následující nové poznatky:

- Vedle altriciálních ptáků jsou i prekociální druhy ptáků schopny rozpoznávat hlas hnízdního predátora a relevantně reagovat příslušnou antipredační strategií. Na hlas hnízdního predátora reagují i dospělí jedinci, kteří nejsou tímto predátorem bezprostředně ohroženi a jejich reakce se liší podle pohlaví. Dokumentovaný případ tak dokládá schopnost rekognice hlasu hnízdního predátora i u dospělých samců druhu s lekovým reprodukčním systémem, ve kterém samci neposkytují žádnou formu rodičovské péče.
- Štěkání loveckých psů, které také vyvolává antipredační reakce, lze kategorizovat podle druhu zvířete, se kterým se setkají. Tento specifický případ představuje pravděpodobně první příklad diferenciací hlasu predátora při setkání s odlišnými druhy kořisti. Míra odlišnosti štěkání psů v loveckém kontextu odráží potenciální míru rizik podle druhu zvířete, se kterým psi interagují. Variabilita akustické struktury doprovázejících štěkání je pak pravděpodobně vyjádřením vnitřního stavu psa, kterou lze popsat na základě valence-arousal modelu.

- V případě nepočtených druhů sciuridů, které jsou známy produkovat odlišné typy alarmů se zcela odlišnou akustickou strukturou (tonální i širokospektrý alarm), se ukázalo, že oba typy těchto signálů jsou vysoce individuálně variabilní.
- Doposud málo dokumentované nízkofrekvenční komponenty ve vokalizaci ptáků mohou přispívat k individuálně odlišné expresi vokálního projevu. Studovaný zástupce tetřevovitých ptáků dosahoval podobně nízkých frekvencí, jako mnohem větší kasuáři, přesahující svou hmotností i 50 kg.
- Dokumentované nízkofrekvenční komponenty jsou synchronně vydávány s nejhlasitější fází tetřevího toku. Produkce dvou odlišných typů hlasů ve stejný časový interval by mohla vysvětlovat dočasnou hluchotu tetřeva hlušce, která se děje právě v této fázi toku. Intenzivní vokální projev ve dvou zcela odlišných frekvenčních spektrech by mohl způsobovat „masking efekt“, kdy dochází ke zhoršené funkci slyšení během takto intenzivní vokalizace.
- Individuální rozdíly ve vokalizaci savců a ptáků jsou dlouhou dobu intenzivně studovány pomocí celé řady odlišných metod. Široká škála metod a použití odlišných metrik způsobuje těžkosti při porovnávání výsledků různých studií. Porovnání univariátních a multivariátních metrik testování individuální identity na simulovaných a empirických datech ukázalo vhodnost Beecherovy informační statistiky pro porovnávání výsledků různých studií.
- Akustické signály ptáků, které nemají svůj původ v syringu jsou doposud studovány minimálně. Mezi tyto signály patří i syčení. Tato práce poprvé dokumentuje potenciál ke kódování individuální identity ptáků produkujících tento typ nevokálního signálu na příkladu domestikovaného i volně žijícího modelu.
- Jakou potenciální roli hraje akustická individualita nevokálních hlasů a nízkofrekvenčních komponent dokumentovaných v této práci je námětem pro budoucí výzkumy. Identifikace jedinců na základě jejich hlasů také nabízí možnost neinvazivního monitoringu jedinců, kde akustická data by mohla poskytovat detailní informace o life-history jedinců neinvazivním způsobem, což by bylo zvláště výhodné pro kriticky ohrožené druhy na našem území, kterými jsou tetřev hlušec a tetřevík obecný, ale i pro řadu jiných.

9 Literatura

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