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Vocal behaviour and information coding in a pelagic Arctic seabird, the little auk (*Alle alle*)

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Zachowanie wokalne i kodowanie informacji u pelagicznego ptaka arktycznego, alczyka (*Alle alle*)

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hello, hello, and are we not

of one family, in our delight of life?

You sing, I listen.

Both are necessary

if the world is to continue going around

night-heavy then light-laden, though not

everyone knows this or at least

not yet

Mary Oliver, from *Meadowlark sings and I greet him in return*

PUBLICATION LIST AND AUTHOR CONTRIBUTIONS

At the time of submission, two of the chapters of this thesis had been published in peer-reviewed journals, two chapters had been accepted for publication, and one was in preparation. I am the first and corresponding author for all these publications. Signed co-authorship statements can be found in the Appendix.

The Reader will notice that those chapters differ in formatting, referencing style, etc. This is due to the specific journals' requirements, and I trust it should not inconvenience the reading of this work as a whole.

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Abbreviations

AO – Anna N Osiecka

DK – Dorota Kidawa

EFB – Elodie Floriane Briefer

FŻ – Feliksa Żurawska

KWJ – Katarzyna Wojczulanis-Jakubas

PB – Przemysław Bryndza

SUMMARIES

Streszczenie w języku polskim

Komunikacja wokalna ma fundamentalne znaczenie dla wielu gatunków zwierząt. Wokalizacje mogą nieść informacje o tożsamości, płci, wielkości lub jakości nadawcy, ale także o kontekście behawioralnym i emocjonalnym, w którym się on znajduje. Dzięki temu, sygnały wokalne ułatwiają wiele interakcji społecznych, od identyfikacji potencjalnego partnera po sprawowanie opieki rodzicielskiej, czy też od deeskalacji agresji po unikanie drapieżników. Jest zatem zrozumiałe, że systemy komunikacji wokalnej stają się szczególnie skomplikowane u gatunków utrzymujących stabilne i złożone więzi społeczne.

W tej pracy kompleksowo przeanalizowałam właściwości bioakustyczne wokalizacji alczyka (*Alle alle*). Alczyki są długożyjącymi ptakami morskimi o wysokiej wierności zarówno wobec gniazda, jak i partnera, co wskazuje na znaczenie i stabilność ich więzi społecznych. Są również bardzo aktywne wokalnie. Jednak kolonie ptaków morskich to zatłoczone, hałaśliwe miejsca, w których komunikacja dźwiękowa może napotykać szczególne trudności. W jaki więc sposób alczyki mogą komunikować ważne społecznie informacje wokalnie?

Aby odpowiedzieć na to pytanie, wykorzystałam dostępne i nowo zebrane pasywne nagrania akustyczne alczyków, wykonane w naturalnych warunkach. Obejmowało to pasywne nagrania wokalizacji ptaków w skali kolonii, a także bezpośrednie nagrania znanych, oznaczonych do płci i zmierzonych osobników. Uzupełniłam ten materiał o nagrania ptaków w sytuacjach potencjalnie stresogennych, rejestrowane podczas standardowych procedur ornitologicznych – co objęło zarówno pisklęta, jak i dorosłe osobniki. Pozwoliło mi to uzyskać dobry ogląd na typy wokalizacji powszechnie stosowane przez ten gatunek w sezonie lęgowym, a także powiązanych z nimi kontekstów behawioralnych - a tym samym afektywnych. Wszystkie dane pozyskane do tej pracy zostały zebrane w kolonii lęgowej alczyków w Hornsundzie, południowo-zachodnim Spitsbergenie, w norweskiej części Arktyki Wysokiej.

W rezultacie, niniejsza praca odpowiada na następujące pytania: Jaki jest repertuar wokalny gatunku, tj. jakie wokalizacje produkują alczyki? W jakich kontekstach alczyki produkują te wokalizacje i czy sygnały wokalne odzwierciedlają behawioralne i emocjonalne konteksty produkcji? Jeśli tak - czy takie dynamiczne informacje są obecne także u młodych piskląt? Czy wokalizacje niosą statyczne informacje o nadawcy, takie jak jego wielkość, płeć czy tożsamość? Wreszcie, jak dobrze informacje te niosą się w ich środowisku?

Rozdział I zawiera opis repertuaru wokalnego alczyka w okresie godowym (przed złożeniem jaja) i inkubacyjnym. Korzystając z pasywnego monitoringu akustycznego kolonii w sezonach lęgowych 2019-2021, zidentyfikowałam i opisałam osiem różnych typów wokalizacji, a także związane z nimi konteksty produkcji. Wszędzie tam, gdzie było to możliwe, przypisałam do wokalizacji domniemaną walencję afektywną - tj. pozytywną/skłaniającą do zaangażowania się w bodziec, czy też negatywną/skłaniającą do unikania bodźca. Domniemana walencja kontekstualna miała znaczący wpływ na właściwości akustyczne wokalizacji: te o przypisanej walencji pozytywnej cechowały się wyższą częstotliwością podstawową i spektralnym środkiego ciężkości, a także krótszą długością dźwięku niż wokalizacje o przypisanej walencji negatywnej. Wskazuje to, że system komunikacji wokalnej alczyka może umożliwiać wyrażanie złożonych kontekstów behawioralnych i emocjonalnych.

Rozdział II powraca do kwestii wokalnej ekspresji emocji u młodych piskląt. Wykorzystując indywidualne nagrania piskląt podczas interakcji z rodzicami (kontekst pozytywny/zaangażowanie) oraz podczas standardowych procedur ornitologicznych (kontekst negatywny/unikanie), wykazałam, że ekspresja afektywna jest już obecna u tego gatunku na wczesnym etapie ontogenezy (5-8 dni po wykluciu). Wokalizacje można było wiarygodnie sklasyfikować do kontekstu ich produkcji, z dokładnością ponad 97%. Wokalizacje produkowane w czasie procedur ornitologicznych miały wyższą średnią entropię, częstotliwość podstawową, a także niższy spektralny środek ciężkości i mniej strome nachylenie spektralne w porównaniu do wokalizacji produkowanych podczas interakcji z rodzicem. Dodatkowo oceniłam zawartość informacyjną wokalizacji produkowanych w tych dwóch kontekstach, pokazując, że wokalizacje stresowe miały niższą nośność informacji niż te związane z pozytywnym kontekstem. Odkrycia te sugerują, że pisklęta alczyków mogą skutecznie wokalnie komunikować konteksty behawioralne czy afektywne, przekazując społecznie ważne informacje już na wczesnym etapie rozwoju.

Rozdział III przygląda się wokalnym wskaźnikom płci, wielkości i partnerstwa w dwóch ważnych wokalizacjach społecznych: krótkiej, jednosylabowej wokalizacji, oraz złożonej, wielosylabowej wokalizacji o wyraźnej strukturze formantowej. Podczas gdy krótkie wokalizacje zawierały informacje o wielkości ciała nadawcy, nie stwierdzono takiej zależności w przypadku złożonej wokalizacji. Żaden z rodzajów wokalizacji nie zawierał wskazówek dotyczących płci, co jest zgodne z opisami dla innych gatunków ptaków morskich. W obu typach wokalizacji, widoczne były podobieństwa wartości niektórych parametrów akustycznych pomiędzy partnerami lęgowymi. Wskazuje to, że sygnały wokalne są w najlepszym razie słabymi wskaźnikami wielkości, a więzi społeczne prawdopodobnie wpływają na strukturę wokalizacji poszczególnych ptaków.

Rozdział IV zawiera szczegółowy opis kodowania indywidualności wokalnej w obrębie i pomiędzy pięcioma typami wokalizacji alczyka. Wokalizacje można było wiarygodnie przypisać do osobnika zarówno w obrębie, jak i pomiędzy różnymi rodzajami wokalizacji, a wszystkie te rodzaje wykazywały zawartość informacyjną pozwalającą na rozróżnienie aż do około 41 osobników na podstawie samego sygnału. Zindywidualizowane cechy wokalne osobników opierały się głównie na najwyższej częstotliwości podstawowej, wartości częstotliwości na górnej granicy drugiego i pierwszego kwartyla energii, długości dźwięku i szybkości modulacji amplitudy, a także na wzorcach czasowych w obrębie wokalizacji. To silne kodowanie indywidualności prawdopodobnie odgrywa rolę w utrzymywaniu długoterminowych więzi społecznych u tego gatunku.

Wreszcie, rozdział V zawiera teoretyczny model propagacji informacji na odległość, wykorzystujący dwa powszechne typy wokalizacji społecznych alczyka, produkowane zarówno wewnątrz gniazda, jak i w locie. Obliczyłam poziomy ciśnienia akustycznego zarejestrowanych wewnątrz gniazda wokalizacji produkowanych przez znane osobniki. Następnie, przy użyciu sferycznego modelu propagacyjnego opartego na lokalnych danych meteorologicznych z lat 1983-2021, przeprowadziłam symulację propagacji tych wokalizacji aż do domniemanego progu słyszalności. Wokalizacje można było poprawnie przyporządkować do właściwych osobników niezależnie od odległości i poniżej domniemanego fizjologicznego progu słyszalności. Nośność informacyjna sygnału nie zmniejszała się wraz z propagacją. Wskazuje to, że sygnały alczyków mogą przemieszczać się na ekstremalne odległości przy minimalnej utracie informacji, co sugeruje, że ptaki te mogą rozpoznawać wokalizacje członków swoich grup społecznych, o ile są one wciąż słyszalne. Wspiera to też hipotezę, że wokalizacje alczyka mogą odgrywać rolę w komunikacji na duże odległości.

Łącznie, wszystkie razem rozdziały stanowią kompleksowy przewodnik po komunikacji wokalnej alczyka, badając ważne behawioralnie kwestie w sposób możliwie najbardziej porównywalny dla różnych gatunków. Praca zatem stanowi ważny wkład w zrozumienie nie tylko akustyki ptaków morskich, ale także ogólnych wzorców ekspresji i znaczenia komunikacji akustycznej u zwierząt.

English summary

Vocal communication is fundamental for many animal species. Vocalisations can carry information on the sender's identity, sex, size or quality, but also about the behavioural and emotional context they find themselves in. As a result, vocal signals facilitate many social interactions, from identifying a potential mate to performing parental care, or from de-escalation of aggression to predator avoidance. Not surprisingly, vocal communication systems become particularly complex in species maintaining stable and complex social bonds.

In this work, I attempted to provide a comprehensive introduction to the acoustic world of the little auk (*Alle alle*). Little auks are long-lived, monogamous seabirds of high fidelity to both nest and partner, indicating the importance and stability of their social bonds. They are also very vocally active. Yet seabird colonies are crowded, noisy places, where acoustic communication may encounter special difficulties. So how can – and do – little auks exchange socially important information vocally?

To answer this question, I used available and newly collected passive acoustic recordings of little auks in undisturbed situations, including passive monitoring at the colony scale, as well as focal recordings of known, sexed and measured individuals. I have supplemented this material with recordings of hand-held birds collected during standard ornithological procedures – this included both young chicks and adult individuals. This allowed me to have an overview of the vocalisation types commonly produced by the species over the breeding season, as well as their related behavioural – and thus affective – contexts. All the data obtained for this work were collected at the little auk breeding colony in Hornsund, SW Svalbard, Norwegian High Arctic.

As a result, this work answers the following questions: What is the vocal repertoire of the species, i.e. the vocalisations those birds produce? In what contexts do they produce those vocalisations, and do vocal signals reflect the behavioural and emotional contexts of production? If yes – is such dynamic information already present in young chicks? Do vocalisations carry static information about the sender, such as their size, sex, or identity? Finally, how well does this information travel through their environment?

Chapter I provides a description of little auk vocal repertoire over the mating and incubation periods. Using passive acoustic monitoring of the colony over breeding seasons 2019-2021, I have identified and described eight distinct call types, as well as their associated production contexts. Wherever it was possible, I assigned the calls with a putative affective valence – i.e., positive/elicited approach vs. negative/elicited avoidance. The putative contextual valence significantly affected the acoustic properties of the calls: these assigned positive valence had higher fundamental frequency and spectral centre of gravity as well as shorter sound duration than calls assigned negative valence. This indicates that the little auk's vocal communication system may allow expression of complex behavioural and emotional contexts.

Chapter II revisits the question of vocal expression of affect in young chicks. Using focal recordings of chicks during interactions with their parents (positive/approach context) and during handling for standard ornithological procedures (negative/avoidance context), I have shown that affective expression is already present in this species early in ontogeny (5-8 days after hatching). Calls could be reliably classified to their production context, with over 97% accuracy. Calls produced during handling had higher mean entropy, fundamental frequency, as well as lower spectral centre of gravity and a less steep spectral slope compared to calls produced during interactions with a parent. Additionally, I assessed the information content of the calls produced in the two contexts, showing that the distress calls had a lower carrying capacity than those uttered in a positive context. These findings suggest that seabird chicks can effectively express behavioural/affective contexts through calls, conveying socially important messages early in development.

Chapter III investigates vocal cues to sex, size, and partnership in two important social calls: a short, one-syllable call, and a complex, multi-syllable call with an apparent formant structure. While the short call carried information on the body size of the emitter, this was not true for the complex call. Neither call type carried cues to sex, which is in line with descriptions of other seabird species. In both call types, certain parameters of the calls tended to match between partners. This indicates that vocal cues are at best weak indicators of size, and that social bonds likely influence the vocal output of individual birds.

Chapter IV provides a detailed description of vocal individuality coding within and across five call types of the little auk. Calls could be reliably assigned to an individual both within and across call types, and all call types showed information content allowing to distinguish across at least 11, and up to at least 41 individuals. Vocal cues to individuality were based mostly on the peak fundamental frequency, frequency value at the upper limit of the second and first quartiles of energy, sound duration, and amplitude modulation rate, but also temporal patterning within the call. This strong individuality coding likely plays a role in maintaining long-term social bonds in the species.

Finally, chapter V provides a theoretical model of information transmission over distance, using two common social call types of the little auk, produced both inside the nest and in flight. I calculated the sound pressure levels of the vocalisations recorded inside the nest by known individuals. Then, using a spherical spreading model based on the local meteorological data from 1983-2021, I simulated the propagation of those calls up the putative hearing threshold. Calls could be correctly classified to individuals independently of the distance up to and over the putative physiological hearing threshold. The carrying capacity of the signal did not decrease with propagation. This shows that little auk signals can travel extreme distances with minimum information loss, which suggests that they can recognise calls of the members of social groups as far as those calls are actually audible, and support the hypothesis that vocalisations could play a role in long-distance communication in this species.

Together, these chapters provide a comprehensive guide to little auk vocal communication, exploring behaviourally important questions is a way intended to be as comparable as possible across species. As such, I hope this work will prove to be an important contribution to our understanding not only of seabird acoustics, but also of the overall expression patterns and the importance of acoustic communication in animals.

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General introduction

What can we learn about an animal by simply listening to them? Sound is present nearly everywhere in the animal kingdom, and vocal communication one of its many faces. For an animal, producing vocalisations can be a voluntary or involuntary act, and the produced signals themselves can carry information that the sender intended to communicate, or that are simply side products of their anatomy or emotional states. All of this information can be vital to listeners: both intended and eavesdropping members of the nearest social circle or a predator, conspecific or not. When trying to understand why and how animals communicate, it is important to know what information is conveyed by their signals, and what is the interplay between their social lives and communication patterns. My thesis adds to this topic a comprehensive description of the acoustic world of an important North Atlantic seabird, the little auk (*Alle alle*).

Vocal communication

The field of bioacoustics investigates all aspects of production, reception, and use of sound by animals. Within this field, vocal communication refers to signals produced using the vertebrate vocal apparatus, that is either larynx (in mammals), syrinx (in birds), or similar structures (in fish, amphibians, and reptiles other than birds), in connection with the nasal and oral cavities. These anatomical structures, together with lung capacity, are the first determinants of the physical output of acoustic signals. Because they reflect an individual's anatomy, these structures tend to result in stable signals carrying what we call *static information* – that is, information that is stable for an individual over time, such as their sex (e.g. Curé *et al.* 2012, Kriesell *et al.* 2018, Bowmaker-Falconer *et al.* 2022), size (e.g. Klenova *et al.* 2011, Favaro *et al.* 2017), or identity (e.g. Favaro *et al.* 2017, Charrier 2021).

This anatomical influence over an animal's vocal output can be viewed through the lens of the source-filter theory of vocal production (Fant 1960). The source-filter theory posits that sounds generated at the source (i.e. larynx or syrinx) are further resonated and modified by the filter (supra-laryngeal or supra-syringeal vocal tract), shaping the frequency spectrum of the emitted call (Fant 1960). In general, an individual's body size is negatively correlated to the frequency of their calls (Charlton *et al.* 2020). This theory holds true for many species, particularly mammals, due to the rigidity of their vocal structures (Charlton *et al.*

2020). Because formants depend on the shape and length of the vocal tract, which is in turn limited by the surrounding bone structures, they are usually strictly dependent on the body size (e.g. Charlton *et al.* 2020). However, through the use of vocal sacs or elongations of the vocal tract, an individual may sound "larger" – that is, produce vocalisations at frequencies lower than expected based on their overall size (Fitch 1999). This can be particularly useful when sounding larger results in better access to mates or more efficient intimidation of opponents or predators.

The static information carried by vocalisations can provide some of the most socially useful cues for both senders and listeners. For example, for a small Otariid pup being recognised by their mother (i.e., effectively communicating identity) will directly translate into better survival chances (Charrier 2021) – and, for their mother, being able to recognise her pup means higher offspring survival (Charrier 2021). However, the vocal output can also be modified by the vocal tract tension or fluctuating hormones, which can (but does not have to) result in changes to the *static information* and partially overriding it with more *dynamic information* (that is, short-term, often context-dependent, informing on the current state of the caller). This can include, for example, communicating one's reproductive state (Semple and McComb), but also behavioural or emotional contexts (Briefer 2020). This dynamic information is in no way less important: for example, vocalisations can bring the attention of a caretaker to a hungry or distressed offspring (Lingle *et al.* 2012), or alert one's social group of a predator present nearby (Zuberbühler 2009), increasing individual and group fitness.

From size to behavioural contexts, vocalisations can carry crucial information. All this information may be encoded differently depending on the taxon (e.g. coding information on sex in the fundamental frequency vs. the temporal pattern of a signal, e.g. Chelysheva *et al.* 2023, Bowmaker-Falconer *et al.* 2022), intended receiver (e.g. increasing or limiting the signal's active space depending on whether the information is public or private, Larsen 2020), and information type (e.g. what exactly is being conveyed, Mathevon and Aubin 2020).

Communicating in a crowd

In general, acoustic signals are a great means of information transfer. They can travel far and across obstacles, and as a result, be more useful for long-distance communication than olfactory or visual cues. Yet, in some situations, acoustic communication may encounter specific difficulties, such as dealing with noise or the need to distinguish between numerous individuals. Colonially living species face those challenges daily.

Noise is any signal interfering with the signal of interest. It can (see e.g. Shannon *et al.* 2015), but does not have to be environmental. It is not one, defined phenomenon, but instead depends on one's current focus. For example, a person in the audience talking to their neighbour during a lecture creates signals relevant to their neighbour, but noise to everyone else. Similarly, for an animal trying to communicate with a specific receiver in a colony, vocalisations of other animals constitute noise that they need to deal with. There are many ways to approach this issue. Firstly, an animal can simply try to "shout over others", that is increase the loudness of their vocal output in noisy situations (a phenomenon called the Lombard effect, see e.g. Brumm and Zollinger 2013, Luo *et al.* 2018). Alternatively, vocalisations can be timed between individuals to avoid overlap (e.g. Grafe 1999, Serrano and Terhune 2002, Araya-Salas *et al.* 2017). Finally, when the sonic environment is occupied by more than one species, they can avoid overlap by communicating at different frequencies, i.e. occupying different acoustic niches (termed Acoustic Niche Hypothesis, see Farina 2013).

A related communication issue encountered by colonial animals is the need to efficiently code information about the sender in situations with particularly high sender density. This may require increasing the information capacity of the vocal signal (that is, the amount of information a signal can carry, which can be measured in 'bits', Shannon and Weaver 1949) and developing a strong individual signature (see e.g. Wyman *et al.* 2022). Life in stable, large and complex social groups is generally thought to drive the development of strong vocal individuality through anatomy, behaviour and cognition (Pollard and Blumstein 2011, Wyman *et al.* 2022).

However, these observations may be biased due to the overrepresentation of songbirds and mammals in studies on vocal individuality, and vocal behaviour in general. This bias is understandable, since studies of the vocal behaviour of colonial animals are particularly difficult, and obtaining good quality recordings of non-overlapping vocalisations can be a challenge. Additionally, many highly colonial species inhabit places that are difficult to access. Yet, data from the underrepresented groups are necessary to fill the current gaps in knowledge.

Seabirds are an excellent candidate group for such studies. They are ecologically important (e.g. Zwolicki *et al.* 2013, Signa *et al.* 2021), often strongly colonial, and known for their complex social networks (e.g. Jones *et al.* 2018, Wojczulanis-Jakubas *et al.* 2018, Genovart *et al.* 2020). Seabirds are also very vocally active. While in the recent years they have been gaining more attention in bioacoustic research (e.g. Favaro *et al.* 2017, Thiebault *et al.* 2019, Baciadonna *et al.* 2022), most species and ecological groups still remain completely undescribed.

Little auk as a model species

This work takes on the topic of vocal communication in colonial seabirds through the perspective of an excellent model species, the little auk. Little auks are long-lived, pelagic seabirds, and the most numerous seabird of the North Atlantic (Wojczulanis-Jakubas *et al.* 2022). They breed in the High Arctic, nesting in rock crevices or rock debris on hillsides located if the vicinity of the shore (Keslinka *et al.* 2019). Those nest chambers are packed densely within the colony, with the average density of 0.2-1.99 nests per square metre (Keslinka *et al.* 2019), and the neighbouring nests are sometimes even sharing some of the corridors or open spaces. Little auk breeding colonies are outstandingly numerous, counting up to approximately 160 000 pairs per colony site in the Svalbard population (Keslinka *et al.* 2019).

Little auks show a strong fidelity to the nest, returning to the same nesting cavity over many years (Wojczulanis-Jakubas *et al.* 2022). This comes together with a strong fidelity to the partner – while extra-couple copulations occur routinely, they are rarely successful and little auks remain socially monogamous over multiple seasons (Wojczulanis-Jakubas *et al.* 2009). Breaking the bond usually occurs after the nesting pair repeatedly fails to raise a fledgling (Wojczulanis-Jakubas *et al.* 2022). Parental care in this is species is a crucial and delicate subject. A nesting pair will only have one egg per season (Wojczulanis-Jakubas *et al.* 2022). However, in the extreme Arctic conditions, with high predator pressure and immense energy expenditure on foraging flights, success requires strict cooperation between the partners (Wojczulanis-Jakubas *et al.* 2018). Little auk parents coordinate the times they spend on parental care and self-care (Wojczulanis-Jakubas *et al.* 2018), which seems to be influenced by weather conditions (Grissot *et al.* 2019). In years of particularly dire environmental conditions, females will prolong their stay at the breeding site to assist the male for longer and thus increase chick survival chances (Wojczulanis-Jakubas *et al.* 2020).

All of this indicates the stability and importance of the social bonds in the little auk. But how are those bonds maintained, e.g. how does one find their nesting partner after returning from a winter migration? Similarly, we do not know anything about the means of coordination of parental behaviours in this species. Vocal cues might be of use – in fact, auks are very vocally active. Still, there is nearly no information about their social behaviour, with the only description being a brief field note on the types of sounds seemingly observed over a couple of days in the field (Ferdinand 1969). This makes the little auk the perfect candidate for a model species: a highly colonial species with strong and stable social bonds, and a complex set of behaviours, including strong (yet little understood) vocal activity.

The little auk is also ecologically and culturally important. It is a keystone species of the High Arctic ecosystem: transporting large organic masses from the ocean to land, it enriches the soil (Zwolicki *et al.* 2013), and in result shapes the tundral communities at all levels: both floral and faunal (Wojczulanis-Jakubas *et al.* 2022), from water bears (Zawierucha *et al.* 2015) to higher vertebrates (Jakubas *et al.* 2008). The little auks are also important prey to the glaucous gull (*Larus hyperboreus*) and the Arctic fox (*Vulpes lagopus*). In Greenland, they serve as a traditional food source (*kiviaq*) to the Inughuit people. Altogether, the species is an iconic representant of the High Arctic.

Aims and outline

While some trends in vocal communication are stable throughout evolutionary lines, we cannot simply assume that they are universally true. Acoustic signals are often species-specific, and patterns observed in one group are not necessarily applicable to another. Additionally, while some questions are well studied in lab settings, we often still lack adequate data support from wild-ranging animals. Detailed investigations into communication networks and information coding strategies across different species, and particularly in understudied groups and undisturbed, wild populations, are the only way to advance our understanding of the whys and hows of animal communication.

My thesis aims to add to this understanding through a careful look at vocal communication and information coding in a socially complex seabird, the little auk. It is comprised of five chapters, each of which answers questions important from a behavioural and/or information perspective: What is the vocal repertoire of the species, i.e. the types of vocalisations that those birds produce? (Chapter I, Osiecka *et al.* 2023a); In what contexts do they produce those vocalisations, and do vocal signals reflect the behavioural and emotional contexts of production? (Chapter I, Osiecka *et al.* 2023a); If so – is such dynamic information already present early in ontogeny, that is, in young chicks? (Chapter II, Osiecka *et al.* 2024a); Do vocalisations carry static information about the sender, such as their size, sex, or identity? (Chapters III and IV, Osiecka *et al.* 2023b, 2024b); Do the social relationships of the sender influence their vocal output? (Chapter III, Osiecka et al. 2023b); Finally, how well does this information travel through the environment? (Chapter V, Osiecka *et al.* manuscript). Each of these questions is elaborated in the indicated chapters.

Together, this work provides a comprehensive guide to the acoustic world of the little auk. I invite you to discover what we can learn by eavesdropping on those important and fascinating seabirds.

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Chapter I: Vocal repertoire and expression of emotions in the little auk

Seabird's cry: repertoire and vocal expression of contextual valence in the little auk (*Alle alle*)

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Abstract

Many seabird species breed in colonies counting up to hundreds of thousands of individuals. Life in such crowded colonies might require special coding-decoding systems to reliably convey information through acoustic cues. This can include, for example, developing complex vocal repertoires and adjusting the properties of their vocal signals to communicate behavioural contexts, and thus regulate social interactions with their conspecifics.

We studied vocalisations produced by the little auk (*Alle alle*) - a highly vocal, colonial seabird - over mating and incubation periods on the SW coast of Svalbard. Using passive acoustic recordings registered in a breeding colony, we extracted eight vocalisation types: *single call, clucking, classic call, low trill, short call, short-trill, terror*, and *handling* vocalisation. Calls were grouped by production context (based on the typically associated behaviour), to which a valence (positive vs negative) was later attributed, when possible, according to fitness threats, i.e. predator or human presence (negative) and promoters, i.e. interaction with a partner (positive). The effect of the putative valence on eight selected frequency and duration variables was then investigated.

The putative contextual valence significantly affected the acoustic properties of the calls. Calls assigned positive valence had higher fundamental frequency and spectral centre of gravity as well as shorter sound duration than these assigned negative valence. These results indicate that the little auk's vocal communication system may facilitate expression of complex behavioural contexts, and seems to include vocal plasticity within vocalisation types – however, more data are necessary to better understand this effect and possible interplays of other factors.

Keywords:

affective state, alcid, dovekie, emotional valence, information coding, social behaviour, vocal communication, vocal plasticity

Introduction

Vocal communication is fundamental for many animal species[1]. Acoustic cues can carry important information about the individual, e.g., the callers' identity[2,3], sex[4], size or quality[5,6], but also about the behavioural context[7,8] and affective state[9]. Because of this, vocal signals facilitate many social interactions[10], and can become particularly complex in socially cohesive species[11].

For animals living in large aggregations, using acoustic cues may become problematic to use due to noise and density of neighbours. Therefore, life in colonies requires a species to adjust their coding-decoding system to communicate efficiently. This may lead to developing, for example, complex vocal repertoires (i.e. many different call types) or complex acoustic features (e.g. segregation of information in distinct, independent features) for more efficient communication[12,13], as well as temporal and frequency adjustments allowing to convey fine behavioural contexts[9,14,15,16]. All this can result in an increased vocal variability.

One of the drivers behind vocal complexity can be the need to accurately convey different affective contexts – this is particularly crucial for social animals, aiding in areas such as e.g. conflict de-escalation, predator avoidance, and food location. Affective states, or emotions, are short-term states elicited in response to specific stimuli of importance for the organism, and associated with neuro-physiological, behavioural and cognitive changes[17]. According to the 'two-dimensional' approach, they can be characterised by their arousal (bodily activation) and valence (positive or negative, i.e. intrinsic pleasantness or unpleasantness[18,19]). Their function is to guide adaptive behaviour to promote survival: positive states are triggered by stimuli that enhance fitness and usually result in an approach towards the stimulus, while negative states are elicited by stimuli that threaten fitness and hence often induce avoidance of the stimulus[18].

Studies of affective responses in non-human animals require taking their perspective and deciding on robust, measurable parameters[20]. One promising indicator of affective valence are vocalisations[9,21-23] – depending on the animal's state, its calls' acoustic properties may change[9], e.g. in sound duration and fundamental frequency variation[21,24]. Importantly, since vocal expressions of emotions often carry crucial information about the environment or social interactions, conspecifics can perceive them[25] and respond to them[14]. Because of the relative difficulty in objectively assessing emotional responses of non-human animals and creating controlled conditions in the wild, this topic remains relatively understudied in non-captive animals.

Most seabirds breed in large colonies, and many are known for their complex social networks[26-28] and high vocal activity. Nevertheless, vocal communication in this group is still poorly understood and notably overlooked in the scientific literature that has focused mostly on passerines. This knowledge gap is mostly due to the difficulty to study seabirds (e.g. time spent at sea, noisy environments, and difficult access), and inquiries into their acoustic behaviour is still typically limited to partial repertoire descriptions. However, recent findings show that calls of some of these species can be individually and contextually specific[7,16,29], indicating exciting new areas of seabird acoustic communication.

The little auk (*Alle alle*) is a long-lived, colonial seabird[30] with a strong nest- and partner fidelity[31,32], suggesting complex and cohesive social networks. Being the most numerous Arctic seabird species[32] living in dense, populous colonies[30], it is also relatively easy to access, making it a great model for behavioural studies[32]. Even though the little auk is a very vocal species, its acoustic behaviour remains undescribed, with only some brief observations available[34].

Here, we examine calls produced by little auks in a breeding colony in SW Svalbard. Our main objective was to provide a detailed quantitative and qualitative description of the vocal repertoire of adults, to set a reference framework for future studies. Further, we investigated whether and how affective states may affect the acoustic properties of these calls.

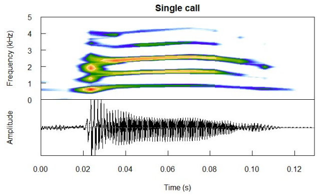
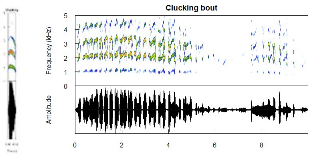
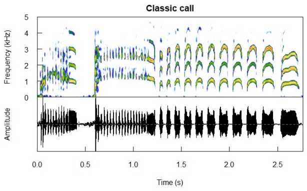
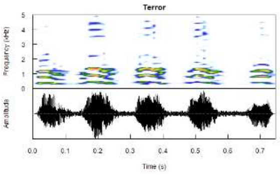
Results

Repertoire

We visually identified, based on their spectrogram and assigned production context (Table 1), eight distinct vocalisation types produced over the mating and incubation periods: *single call, clucking, low trill, short call, short-trill, single call, terror*, and *handling* (Tables 1-2). Further tests showed that these calls differed significantly in their acoustic parameters

(Permutation test: correct classification based on a discrimination function analysis = 61.7%; correct classification by chance = 12.5%; $p < 0.001$).

Table 1. Little auk vocalisations produced by adults over mating and incubation periods. Spectrograms plotted using the *seewave* package (Sueur, Aubing and Simonis 2008). If calls were described previously by Ferdinand (1969), names used therein are given in brackets. See also the summary statistics for acoustic parameters of the calls in Table 2, and *Data availability statement* for call samples.

Vocalisation type	Most common production context	Assigned valence	Example	Description
Single call (single call)	Flying alone or in flock, sitting alone in the colony	Unknown		Produced as a single vocalisation. Rarely observed.
Clucking (aggressive call/clucking call)	Vocalising and posturing with a partner, pre-, post- or without copulation, as well as during other encounters and prolonged stays in the colony with the social partner	Positive		Produced in vocalisation bouts: spectrograms show a single cluck (left) and a vocal exchange between breeding partners (right). Observed often.
Classic call (trilling call)	Flying alone or in flock, also during predator escape, in and outside nest	Unknown		The longest call of the species, composed as a single call made of a series of three syllable types. Observed often.
Terror	Flying in flock when a predator is present on the ground	Negative		Produced as a series of 2-6 identical syllables. Observed often within the

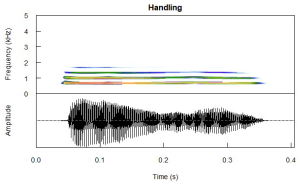
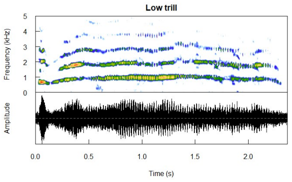
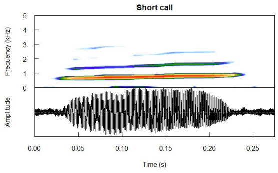
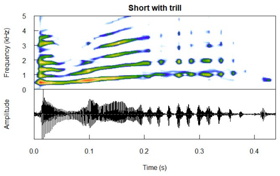
				behavioural context, often at the same time by many individuals.
Handling	Vocalisation during human handling	Negative		Produced as a single call. Observed rarely and only during the artificial context of human handling.
Low trill (snarling call)	In or in front of own nest when another bird is present	Likely negative		Observed often, sometimes in a bout with the *short* and *short with trill* calls.
Short call	In or in front of own nest when another bird is present	Likely negative		Observed often, produced as a single call or in a bout with the *low trill* and *short with trill* calls.
Short-trill	In or in front of own nest when another bird is present	Likely negative		Somewhat a combination of the *short* and *low trill* calls and often produced in a bout with these. Observed often.

Table 2. Values (mean ± SD) of acoustic parameters for the little auk call types. Positive and negative emotional valence are denoted as (+) and (-), respectively.

Acoustic	**Call type**							
parameter	**single**	**clucking (+)**	**classic**	**terror (-)**	**handling (-)**	**low trill**	**short**	**short-trill**
***f0* Min (Hz)**	657.15 ± 97.87	646.67 ± 58.99	632.45 ± 60.50	657.98 ± 73.97	685.62 ± 108.49	621.99 ± 84.97	657.49 ± 72.74	601.90 ± 63.01
***f0* Max (Hz)**	808.76 ± 177.02	809.65 ± 95.53	1209.48 ± 154.24	766.58 ± 72.57	749.48 ± 135.66	915.47 ± 133.68	815.05 ± 78.40	934.72 ± 104.98

***f0* Mean (Hz)**	759.09 ± 150.86	751.51 ± 80.18	912.61 ± 63.60	721.23 ± 65.30	717.65 ± 118.14	808.71 ± 108.99	735.81 ± 65.08	808.49 ± 77.38
Q25% (Hz)	676.43 ± 477.10	1334.59 ± 498.92	1504.70 ± 588.35	74.84 ± 271.09	377.78 ± 297.60	802.11 ± 344.17	708.10 ± 154.63	769.52 ± 286.84
Q50% (Hz)	1375.92 ± 846.78	2073.89 ± 410.24	2634.73 ± 1499.41	794.57 ± 2503.71	770.45 ± 385.00	1927.92 ± 1508.20	1390.37 ± 938.34	1589.59 ± 1303.54
Q75% (Hz)	3939.74 ± 4299.23	2718.37 ± 442.53	5278.29 ± 4903.84	1535.94 ± 4346.71	1457.16 ± 451.01	6989.95 ± 5834.44	5473.14 ± 4549.35	4417.26 ± 4727.89
Duration (s)	0.11 ± 0.05	0.09 ± 0.02	2.74 ± 0.44	0.33 ± 0.13	0.11 ± 0.10	1.08 ± 0.84	0.23 ± 0.15	0.75 ± 0.38
AM rate (s-1)	28.64 ± 10.71	26.28 ± 12.44	39.11 ± 8.08	42.21 ± 9.40	24.62 ± 11.09	47.98 ± 4.90	37.08 ± 12.17	45.40 ± 4.87
***f*M rate (s-1)**	4.78 ± 3.67	5.53 ± 1.00	5.65 ± 0.76	8.76 ± 2.56	5.13 ± 4.18	4.37 ± 2.05	2.77 ± 2.51	4.05 ± 1.72

Effects of affective valence on the acoustic parameters

MANOVA testing (with call types as a control fixed factor) of calls attributed to contexts of clear positive and negative valence revealed that the assigned affective valence had a significant effect over the call structure (F = 48.93; p < 0.001; Supplementary Table 1). Further linear models revealed that calls assigned to a positive valence (i.e. *clucking* calls exchanged between partners) had significantly higher values of acoustic parameters such as Max *f0*, Mean *f0*, *f0* Range, Q50%, *f0* Abs Slope, and *f0* var, and significantly lower values of acoustic parameters such as End *f0* as well as shorter sound duration than those assigned to a negative valence (i.e. *terror* and *handling* calls produced in response to threat; Table 3; Supplementary Figures 1-8; see Supplementary Table 2 for parameters' definitions).

Calls of unknown or uncertain valence showed the largest variance for most of the measured parameters (Supplementary Figures 1-8).

Table 3. Model results: Linear Models for negative (intercept) and positive emotional valence. No. of observations = 90 for all parameters.

		Predictors		**R2/R2 adjusted**
		Negative valence (Intercept)	**Positive valence**	
Max *f0* (Hz)	*Estimates*	758.03	51.62	0.053/0.042
	95% CI	731.30-784.76	5.32-97.92	
	p	**<0.001**	**0.029**	
Mean *f0* (Hz)	*Estimates*	719.44	32.07	0.028/0.017
	95% CI	696.31-742.57	-7.99-72.12	
	p	**<0.001**	**0.115**	
Range *f0* (Hz)	*Estimates*	86.23	76.75	0.259/0.251
	95% CI	70.35-102.11	49.25-104.26	
	p	**<0.001**	**<0.001**	
Q50% (Hz)	*Estimates*	782.51	1291.38	0.149/0.139
	95% CI	404.56-1160.46	636.75-1946.01	
	p	**<0.001**	**<0.001**	
***f0* Abs Slope**	*Estimates*	1014.81	2005.70	0.566/0.561
	95% CI	799.83-12229.78	1633.35-2378.05	
	p	**<0.001**	**<0.001**	
***f0* Var (Hz/s)**	*Estimates*	764.25	1687.60	0.618/0.614
	95% CI	602.09-926.42	1406.73-1968.47	
	p	**<0.001**	**<0.001**	
End *f0* (Hz)	*Estimates*	699.09	-47.36	0.065/0.055
	95% CI	677.16-721.01	-85.34- -9.38	
	p	**<0.001**	**0.015**	
Sound Duration (s)	*Estimates*	0.22	-0.13	0.188/0.179
	95% CI	0.19-0.26	-0.19- -0.07	
	p	**<0.001**	**<0.001**	

Discussion

The complexity of vocal communication can be predicted to be high in socially cohesive[11] and colonial species[18,35], in terms of the size of a species' vocal repertoire, but also of the fine information coding (e.g. context, internal state) within call types. Here, we described eight vocalisation types with distinct acoustic structures produced by adult little auks over the mating and incubation periods at the colony site.

Additionally, we tested how these calls might be affected by the animal’s putative affective states. Based on their production context, we compared call types associated with putative positive (interactions with a social partner) and negative (response to a predator or human) valence, and found valence-specific changes. This shows that the species uses complex vocal communication, capable of conveying fine contextual information useful in social interactions.

Vocal repertoire

We observed eight acoustically distinct call types produced by the little auks in various contexts over the mating and incubation periods. Overall, little auk vocalisations are high frequency calls, with a clear harmonic structure, typical for species living in open habitats[36], such as the high-Arctic tundra. Such harmonic calls facilitate both signal transmission and location of the caller[37], which may be beneficial at a colony scale, e.g. for locating alarm calls and social partners in an acoustically crowded environment.

Little auks vocalise in different behavioural contexts, yet most calls are used in social exchanges. Half of the described call types are produced during direct interactions with conspecifics: *clucking* is most commonly uttered in a vocal dyad with a social partner, and *low trill*, *short call*, and *short-trill* are used when another bird is present and/or engages in a vocal exchange. Two call types (*terror* and *handling*) are produced only when a predator or threat (such as a human observer) is present nearby, likely serving as a warning to the colony.

While some calls are highly context-specific (e.g. *clucking* or *handling*), others may be used in various situations. The most striking example is the *classic* call – a sound any visitor to the areas inhabited by the little auk will instantly recognise as its “signature”, and which in some languages (such as Polish and Norwegian) served as the inspiration for the species’ name and/or its breeding sites. This vocalisation is used by birds returning to the breeding colonies after wintering, foraging trips, and during predator escape – in all cases, huge flocks vocalise together, resulting in a cacophony of voices. However, the *classic* call can also be heard from single birds flying or sitting outside or inside their nests. The acoustic properties of this call type are quite variable, suggesting vocal plasticity in adjusting a call type to convey a finer message.

It has previously been suggested that colonial alcids may have larger repertoire sizes than solitary-nesting species[12]. While to date no complete repertoire descriptions (i.e. covering at-sea, migration, and wintering vocalisations) exist for any of the alcid species, the little auk seems to have a larger repertoire than those described so far for most other alcids (i.e. auklets, puffins, and murres, typically with up to 5 call types described per species[12,38,39]), and much larger than the repertoire of the solitary Kittliz's murrelet *(Brachyramphus brevirostris*), with only two call types described[40]. Taking into account that this description only focuses on the vocalisations of adult birds over a selected period (mating and incubation), and other calls are likely emitted in contact with the chick and at sea, the complexity of little auk's repertoire indicates complex vocal signalling at the colony level.

Behavioural and affective context

Previous studies on vocal expression in seabirds showed that production context impact the acoustic properties of their calls[15,16]. Similarly, little auk vocalisations differ significantly across behavioural, but also assumed affective contexts. This is the first time that the impact of the putative valence on acoustic parameters has been investigated in a seabird. The acoustic variables contributing most to the observed variation between calls were related to the fundamental frequency (*f0*), modulations of which are commonly associated with affective states[9,41]. Calls produced during interactions with a partner (i.e., increasing fitness) were significantly shorter than these uttered during vocal exchanges with other birds or associated with negative contexts (e.g., handling by a human or in presence of a predator). This is in line with vocal expression patterns in previously described species[21,22]. At the same time, while positive vocalisations generally tend to have lower frequency attributes[21,22], these parameters were higher (e.g., higher fundamental frequency and frequency modulation) for little auks. While calls produced in multiple behavioural contexts (i.e. *single* and *classic* calls) seem to show a larger variance in acoustic parameters, potentially indicating vocal plasticity within a call type, the design of this study is too general to make such conclusions, but it certainly calls for a more thorough investigation in the future.

In the absence of other measures of emotions (e.g. behavioural or cognitive), the putative valence of the contexts was assessed based on whether the context promotes (positive valence) or threatens (negative valence) the fitness of the animal[17,19]. Our results are thus limited to the assumptions that affective states have indeed evolved to promote fitness (survival and reproduction), and that positive and negative states are triggered in such contexts (e.g. interactions with a partner were assigned positive valence, vocalising in the presence of a predator or during handling by a human were assigned negative valence; see Table 1. This also additionally limits the available dataset, since in this analysis we could only include calls that could be assigned to a positive or negative state with high probability, and thus calls of unknown or uncertain (i.e., *often negative*) valence had to be discarded. In addition, we did not have access to the second main dimension of emotions, which is the arousal (bodily activation) of the vocalising animals[42]. Some of the effect we found might thus be related to arousal more than valence – e.g., high arousal during vocalisation bouts related to copulation might result in increased frequencies and frequency modulations.

Consequences for social interactions

In an environment as crowded and noisy as a little auk colony, finding and communicating with one's partner or other members of one's social network may be particularly challenging. Yet little auks successfully maintain partnership over the years[32], coordinate parental care[27], and find each other not only after foraging trips, but also upon returning from annual migrations. The extent to which vocalisations facilitate these different aspects of the species' social life remains an open question, but the elaborate vocal communication system clearly suggests that acoustic cues are of importance.

While nothing is known about the social networks of the little auk outside of the breeding context, it seems possible that extra-pair or non-breeding relationships may occur, as is also suggested by the complex vocal communication within the colony. In fact, most of the described call types are social vocalisations related to a wide range of social interactions outside the breeding couple, from predator warning to, possibly, nest protection. Additionally, changes to the acoustic structure of the calls corresponding to behavioural and affective contexts can inform conspecifics about the potential risks and opportunities, increasing the fitness of the whole colony.

Some of the observed variance in acoustic parameters within call types and contexts may be due to individual differences. For example, the *classic* call, which is a long call comprised of three types of harmonic syllables, seems to hold great potential for coding information about the individual. While we were not able to establish the identity of callers in this study, it seems plausible that this call type may be used as a vocal signature, enabling the little auks to find their partners and neighbours after migrations and gull attacks, but also perhaps to coordinate other behaviours. Dedicated studies with known individuals are necessary to assess the level of individual vocal stereotypy in this species.

Finally, changes in acoustic parameters can be related to other, physiological factors, such as side-effects of amplitude and/or frequency modulation in noisy environments[43,44]. Since different factors can contribute to the overall acoustic structure of a vocalisation, further investigations controlling for their impact would help us disentangle the physiological vs. behavioural effects on the vocalisation structure.

Caveats and issues

Working on seabird bioacoustics comes with a number of challenges. While as a species the little auk is very vocal, individual animals often vocalise at unpredictable times and places. For this reason, and to ensure that we captured the whole spectrum of calls produced in the colony over a longer period, this study used passive acoustic recording of the colony, and therefore we cannot make any assumptions about how sex, age, size, and identity of the vocalising animal influence vocal behaviour of the species.

While the sample size used in this study is not very large – and in the case of valence analysis was narrowed down to calls which could be classified as *positive* or *negative* with high probability, so e.g. excluding social interactions with non-partners – it is the feasible output of long-term monitoring efforts of the study species. Further data - ideally, directly recorded vocalisations of known, positive and negative valence within one call type - would be beneficial, and enable us to make more final conclusions. Nevertheless, we believe that this unique dataset is a valuable contribution and can form a basis for future studies, indicating potential for vocal expression of emotions in an understudied but important group of birds.

Using audio (and possibly video) recording devices mounted on individuals could resolve some of these issues. However, while these prove useful on larger species[7], little auks are small and likely to be sensitive to such a burden[45]. For this reason, it is for the moment impossible to describe the vocal behaviour of the little auks during foraging trips or migrations. Nevertheless, we are confident we managed to capture the whole spectrum of vocalisations produced by the little auk at the colony over mating and incubation.

Conclusion

This study identifies and provides a quantified description of eight call types produced by adult little auks over mating and incubation, setting a framework for future studies of the vocal behaviour of this species. It also suggests emotional effects on the acoustic parameters of these calls, such as fundamental frequency, duration, and spectral centre of gravity. This is the first time that vocal expression of affective states has been studied in a seabird, shining a new light on avian behaviour. Due to the technical limitations of this study, its results should be taken with precautions, serving only as an indicator that such effects might indeed exist in this group.

Methods

Ethics and permits

Fieldwork was performed under permission from the Governor of Svalbard (17/00663-13, 20/00373-2, 20/00373-8).

Study site and recording set-up

All data were collected in the little auk colony in Hornsund, Spitsbergen (77°00' N, 15°33' E), one of Svalbard's biggest breeding aggregations of the species[30]. Recordings were made over the breeding seasons (June-August 2019-2021), during mating and incubation periods. These periods were chosen to ensure only adult birds were present in the colony.

Audio material was collected via an Olympus ME-51S stereo microphone (frequency response 100-15,000 Hz) placed right outside (mating) or inside (incubation) individual nests (n = 30) in such a way as to not disturb the birds' normal activities. Each microphone was connected to an Olympus LS-3 or LS-P4 digital voice recorder (sampling rate 48 kHz, 16 bits) placed outside of the nest and hidden under a rock.

Recording sessions took place for several hours during different stages of mating (three sessions per nest) and incubation (three sessions per nest) periods, recording vocalisations of the focal nest owners, their mutual interactions, interactions with neighbours, as well as all other vocalizations produced in the nest vicinity, i.e. neighbouring pairs and flocks of birds circling above the colony plot. All recording sessions were equal in duration, and spaced equally in time for all the monitored nests.

Additionally, recordings were made during handling the birds for standard ornithological procedures (while ringing, weighting) via a hand-held recorder (Olympus LS-12) with a built-in microphone. Although adult little auks are often silent during handling, we managed to record handling vocalisations of 21 individuals.

Sound selection

We manually processed a total of 508 hours 27 minutes of recordings, extracting all clear calls found within this set. These calls were grouped into call types based on visual inspection of the spectrograms (Hann window, FFT-length = 715) and the associated production contexts of the calls. From the extracted calls, we selected 30 high-quality (i.e. non-overlapping, untrimmed calls with the best available signal-to-noise ratio) calls per each identified call type, i.e. a total of 240 calls.

While it was not possible to assign specific calls to particular individuals, owing to the sampling design (i.e., recoding at multiple nest locations) and further sound selection routine (i.e., avoiding sampling from the same nest or vocalisation bout) we are confident that most of the selected audio samples originated from different individuals, as so could be treated as independent data points in further analyses. In the case of the *handing call*, with only 21 individuals recorded, we were forced to sample nine individuals twice – these individuals were chosen based on the signal quality, i.e. recordings with the best signal-to-noise ratio were selected.

Context and valence attribution

The context of vocal attribution was assessed based on previous behavioural observations using expert knowledge approach, matching the observed call types to their typically associated production contexts. This was based on direct long-term observations in the field (*ad libitum* observations of focal animals and colony scanning), analyses of focal video recordings from 2019-2020, and previous literature[34].

Valence is one of the main dimensions of emotions[17,19]. The valence of a context can be assumed based on threats/promoters of fitness – that means, contexts that are related to certain adverse or beneficial situations[17,19,46]. Therefore, contexts that threaten fitness and would normally be avoided were assumed to trigger negative states, while those that promote fitness and should be approached were assumed to elicit positive states[19,46,47] (Table 1). Little auks parents take care of their brood in a coordinated[27] manner where both partners contribute equally[27,32], with no parental conflict observed. Thus, we assumed that interactions with the social partner are predicted to be positive, interactions with a predator or human (i.e. handling) should be negative, interactions with other birds in the vicinity of own nest are likely negative, and other contexts are of unknown valence. For further analysis, we selected only the calls that fell into the *positive* or *negative* categories. This was done to avoid confusion in situations where the behavioural context/meaning is not always clear, such as communication with birds other than nesting partners (*likely negative*).

Sound analysis

Calls were analysed in Praat software[48] using a custom built script adjusted to the little auk[23,49-50] (Supplementary Text 1), extracting a set of 20 acoustic parameters (specified in Supplementary Table 2).

Statistical analysis

Repertoire

All analyses were performed in R environment (v. 4.1.3)[51]. Summary statistics of standard acoustic variables (Supplementary Table 2) were calculated for each call type. Principal component analysis (PCA) was performed (using *stats* package[52], function *prcomp*) on all acoustic parameters to reduce data dimensions and cross-check call types' classification. This was followed by Discriminant Function Analysis (DFA; *MASS* package[53], function *lda*) using PC scores with eigenvalues > 1. The correct classification rate obtained from the DFA with leave-one-out cross-validation was then compared to 1000 chance levels calculated by applying a randomisation procedure (permutation test), in order to investigate if call types were acoustically distinct from each other.

Vocal expression of affective valence

The first five PCA dimensions had eigenvalues > 1 (Kaiser's criterion; Supplementary Table 3). Based on the raw variables' contribution to these dimensions (i.e., over 6% contribution to all dimensions, according to the scree plot cut-off; Supplementary Table 4), we selected the following acoustic parameters for subsequent tests: Max *f0*, Range *f0*, Mean *f0*, Q50%, *f0* Abs Slope, *f0* var, End *f0*, and Sound duration (Supplementary Table 2).

Since many of the raw acoustic parameters we analysed are correlated (e.g. the different aspects of fundamental frequency changes), we have decided to use a multivariate analysis of variance based on the PC scores. To investigate variation at the level of the whole call structure, the first five PCs were entered as response variables into a MANOVA (*stats* package[52], *manova* function). The MANOVA included the assumed valence of the situation (positive or negative, Table 1) as explanatory variable and call types as a fixed factor to control for its effect.

Additionally, to understand the specific direction of changes in the eight selected parameters, their raw values were entered as outcome variables into linear models (LM; *stats* package[52], *lm* function; 8 models in total) including valence (Table 1; calls of *possibly negative* and *unknown* valence were not included in the analysis but visualized on the plots (Supplementary Figures 1-8) as a fixed factor).

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Data availability statement

Call samples with their respective spectrograms, as well as the raw data generated in this study are available at https://osf.io/83fx6/?view_only=bd9336e27dfe4f93a0e9338ccc016597

Additional information

Authors declare no competing interests.

SUPPLEMENTARY MATERIALS

Supplementary Text 1

Praat Settings

Here, we provide a detailed description of the acoustic analysis performed in Praat software using a custom-made script[46–48]. Settings used to extract the 20 acoustic parameters presented in Supplementary Table 1 are described below (Praat commands indicated in brackets; see Supplementary Table 1 for abbreviations of the parameters used).

1. **Duration**. The duration (Dur) was measured as the total duration of each wav file (s), corresponding to individual calls manually extracted from the recordings, based on the visualisation of both the oscillogram and spectrogram.

2. **Amplitude modulation**. AM Var, AM Rate, and AM Extent were calculated from the intensity contour of each individual call, using the [Sound: To Intensity] command (minimum pitch = 500 Hz, time step = 0.005 s)[52].

3. **Source-related acoustic features**. *f0* contour or each call was extracted using a cross-correlation method ([Sound: To pitch (cc)] command; time step = 0.005 s, pitch floor = 500 Hz, pitch ceiling = 2000 Hz). We included the following *f0* frequency values: *f0* at the start (*f0* Start) and at the end (*f0* End); the mean (*f0* Mean), minimum (*f0* Min) and maximum (*f0* Max); percentage of time when the maximum *f0* frequency occurs within the vocalisation (Time *f0* Max); the *f0* mean absolute slope (*f0* Abs Slope); and the *f0* range (*f0* Range). To characterise *f0* variation along the call, we measured the mean *f0* variation per second (*f0* Var) calculated as the cumulative variation in the *f0* contour in Hertz divided by call duration. Finally, we measured the number of complete cycles of *f0* modulation per second (*f*M Rate) and the mean peak-to-peak variation of each *f0* modulation (*f*M Extent)[52].

4. **Spectrum-related parameters.** Q25%, Q50%, and Q75% were measured on a spectrum applied to the whole call, and *f*Peak was measured on a cepstral-smoothed spectrum (command [Create: Cepstral smoothing]; bandwidth = 100 Hz).

5. **Noise**. Harmonicity (Harm) was measured using the [Sound: To Harmonicity (cc)] command (time step = 0.005 s, minimum pitch = 500 Hz, silence threshold = 0.2, periods per window = 1).

*f*M Extent (Hz), AM Extent (dB) and Harm (dB) could not be extracted from some of the calls. All other parameters could be measured in all calls.

Praat spectrogram settings: view range max: 8000; window length: 0.008; dynamic range: 60.

Supplementary Table 1. Model results: MANOVA for assigned emotional valence.

	Df	Pillai	Approx. F	Num Df	Den Df	Pr(>F)
valence	1	0.75	48.93	5	83	< 0.001
call type	1	0.74	47.02	5	83	< 0.001
Residuals	87					

Supplementary Table 2. Acoustic parameters extracted for each vocalisation (adapted from Briefer *et al.* 2017). Whether each parameter was selected for statistical analysis based on PCA results and in summary statistics of repertoire descriptions is indicated.

Abbreviation	Description	Analysis	Repertoire
***f*0 Mean (Hz)**	Mean fundamental frequency value across the vocalisation	+	+
***f*0 Start (Hz)**	Fundamental frequency value at the start of the vocalisation	-	-
***f*0 End (Hz)**	Fundamental frequency value at the end of the vocalisation	+	-
***f*0 Max (Hz)**	Maximum value of the fundamental frequency across the vocalisation	+	+
***f*0 Min (Hz)**	Minimum value of the fundamental frequency across the vocalisation	+	+
***f*0 Range**	Range of the fundamental frequency across the vocalisation	+	-

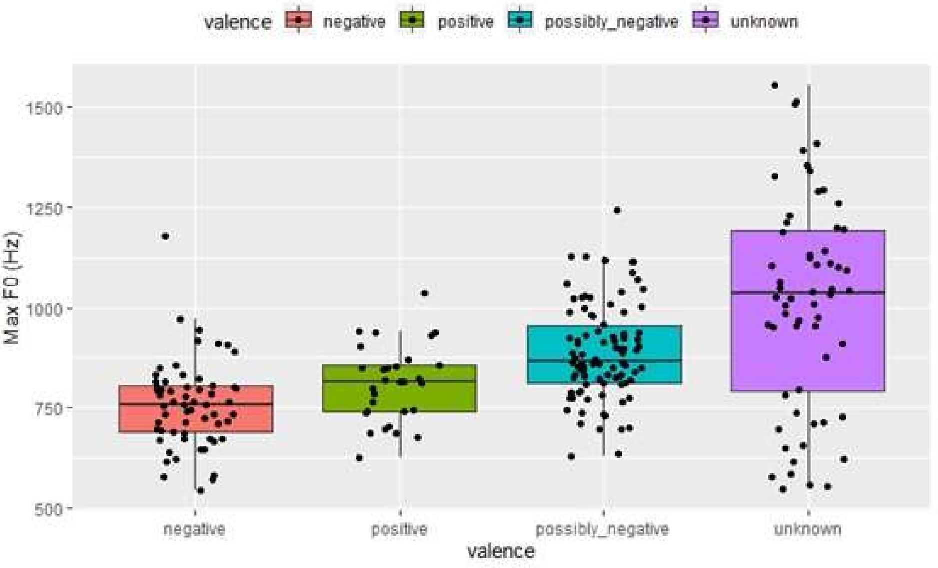
Time *f*0 Max (%)	Percentage of time when the maximum F0 frequency occurs within the vocalisation	-	-
***f*0 Abs Slope**	Absolute slope of F0 frequency	+	-
***f*0 Var (Hz/s)**	Cumulative variation in F0 frequency divided by the total vocalisation duration	+	-
***f*M Rate (s^{-1})**	Frequency modulation rate	-	+
***f*M Extent (Hz)**	Mean peak-to-peak variation of each frequency modulation	Excluded (some missing values)	Excluded (some missing values)
Q25% (Hz)	Frequency value at the upper limit of the first quartiles of energy	-	+
Q50% (Hz)	Frequency value at the upper limit of the second quartiles of energy	+	+
Q75% (Hz)	Frequency value at the upper limit of the third quartiles of energy	-	+
***f*peak (Hz)**	Peak frequency	-	-
Dur (s)	Duration of the vocalisation	+	+
AM Var (dB/s)	Cumulative variation in amplitude divided by the total vocalisation duration	-	-
AM Rate (s^{-1})	Amplitude modulation rate	-	+
AM Extent (dB)	Mean peak-to-peak variation of each amplitude modulation	Excluded (some missing values)	Excluded (some missing values)
Harm (dB)	Harmonicity (Harmonics-to-Noise Ratio)	Excluded (some missing values)	Excluded (some missing values)

Supplementary Table 3. PCA results: eigenvalues and percentage of variance for 17 dimensions.

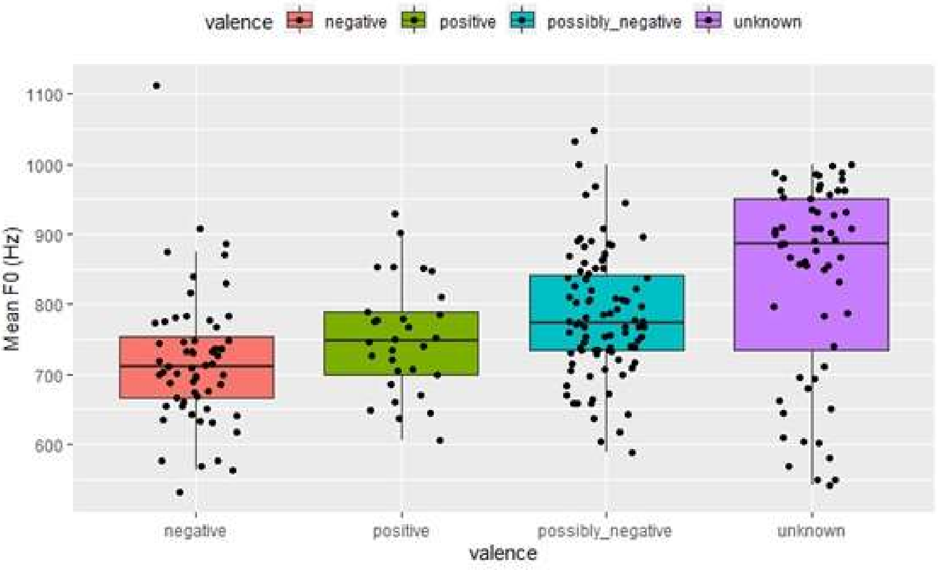
	eigenvalue	percentage of variance	cumulative percentage of variance
comp 1	4.82	28.36	28.36
comp 2	2.71	15.95	44.31
comp 3	2.46	14.47	58.78
comp 4	1.95	11.50	70.28
comp 5	1.32	7.780	78.06
comp 6	0.85	5.02	83.08
comp 7	0.74	4.36	87.43
comp 8	0.54	3.18	90.61
comp 9	0.49	2.88	93.48
comp 10	0.34	2.00	95.49
comp 11	0.32	1.85	97.34
comp 12	0.15	0.90	98.24
comp 13	0.13	0.75	98.99
comp 14	0.09	0.55	99.54
comp 15	0.06	0.32	99.86
comp 16	0.02	0.14	100.00
comp 17	0.00	0.00	100.00

Supplementary Table 4. Raw variables' contributions (%) to the first five PCA dimensions. Variables in bold were selected for MANOVA.

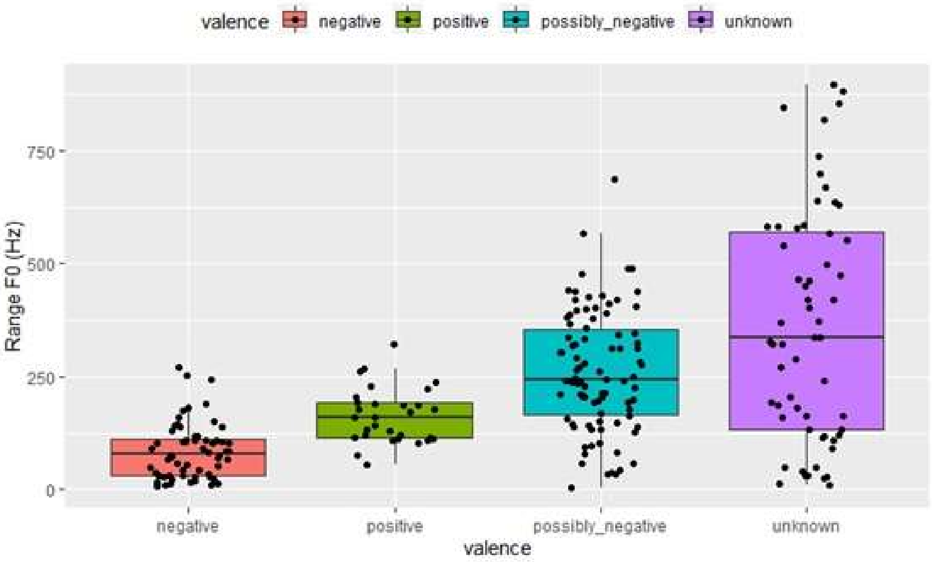
Variable	**Dim.1**	**Dim.2**	**Dim.3**	**Dim.4**	**Dim.5**
Mean *f0* (Hz)	14.41	2.27	7.01	0.03	0.10
Start *f0* (Hz)	6.55	9.16	1.32	0.63	12.97
End *f0* (Hz)	0.11	2.52	27.08	5.21	1.99
Max *f0* (Hz)	17.73	0.09	2.54	1.88	0.03
Min F0 (Hz)	0.44	14.43	11.63	6.48	7.84
Range *f0* (Hz)	15.32	1.85	0.01	6.16	1.99
Time max F0 (%)	2.05	0.43	8.00	5.08	22.28
***f0* Abs Slope**	3.48	14.73	10.50	0.02	5.27
***f0* var (Hz/s)**	5.55	12.29	8.96	0.03	5.00
*f*M Rate (s-1)	0.63	0.25	0.96	2.00	31.20
Q25% (Hz)	8.74	0.09	3.88	1.56	3.77
Q50% (Hz)	6.07	5.76	3.38	19.13	0.09
Q75% (Hz)	1.76	9.67	0.33	20.11	0.13
*f*peak (Hz)	2.64	1.84	3.85	12.46	2.66
Sound duration (s)	10.77	6.17	0.02	5.14	2.61
AM var (dB/s)	2.31	5.89	4.52	13.56	1.95
AM rate (s-1)	1.42	12.57	6.01	0.49	0.11



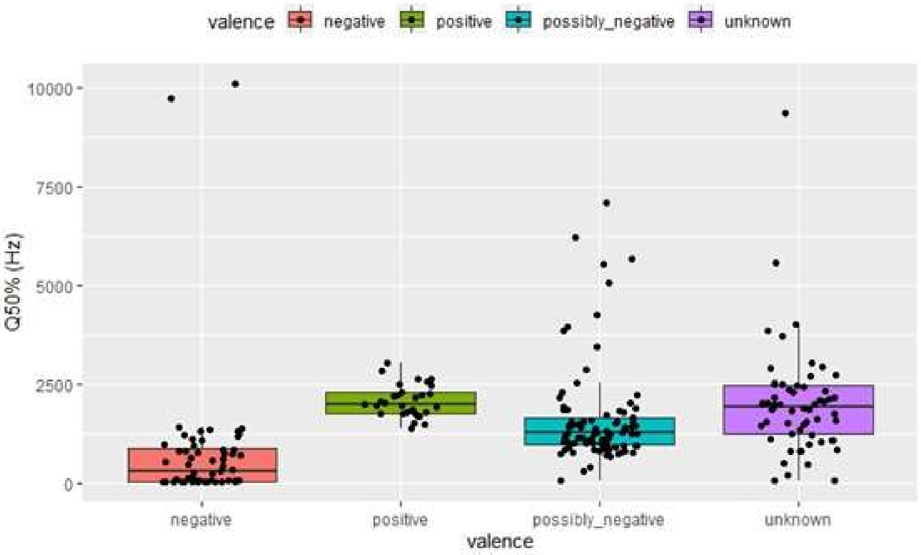
Supplementary Figure 1. Effect of the assigned emotional valence on the maximum f0. Negative and positive categories are included in the analyses, while possibly negative and unknown categories are only plotted for comparison.



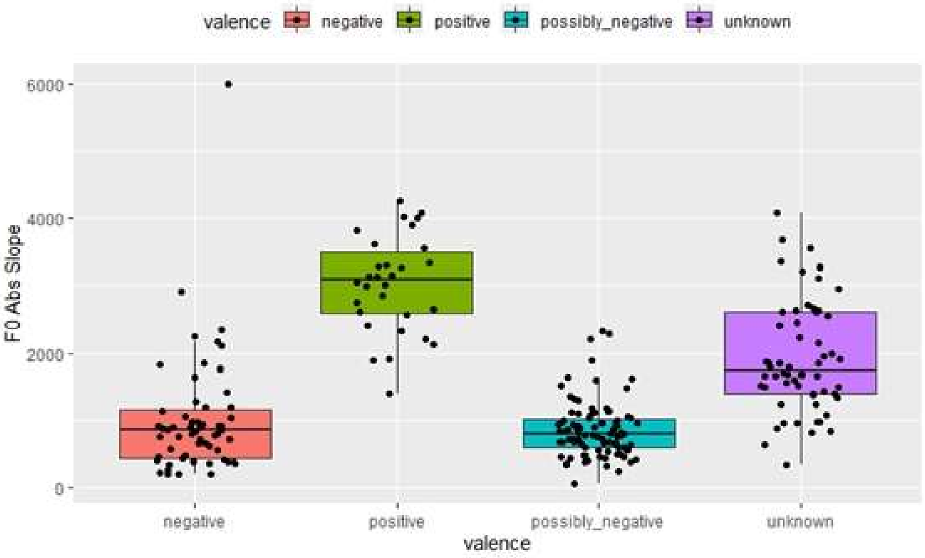
Supplementary Figure 2. Effect of the assigned emotional valence on the mean f0. Negative and positive categories are included in the analyses, while possibly negative and unknown categories are only plotted for comparison.



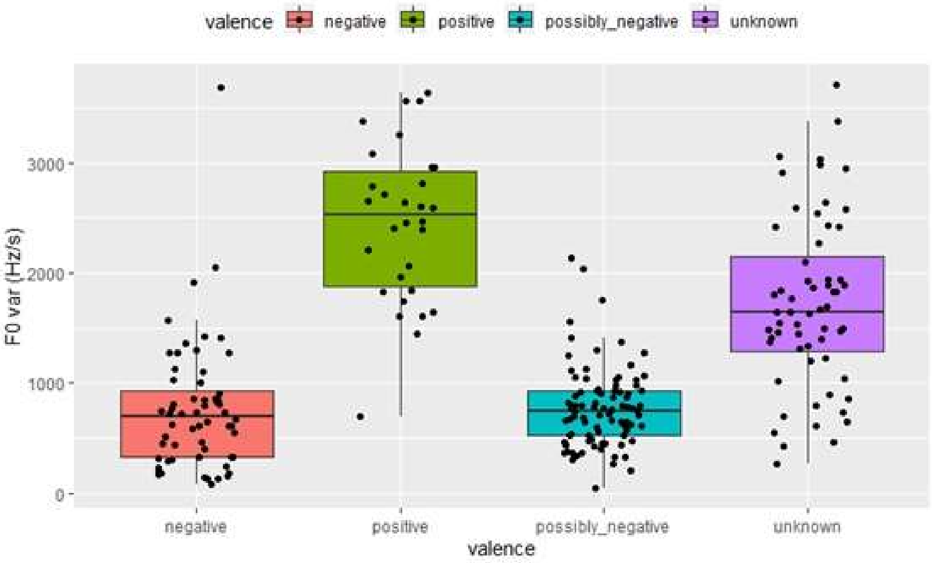
Supplementary Figure 3. Effect of the assigned emotional valence on the f0 range. Negative and positive categories are included in the analyses, while possibly negative and unknown categories are only plotted for comparison.



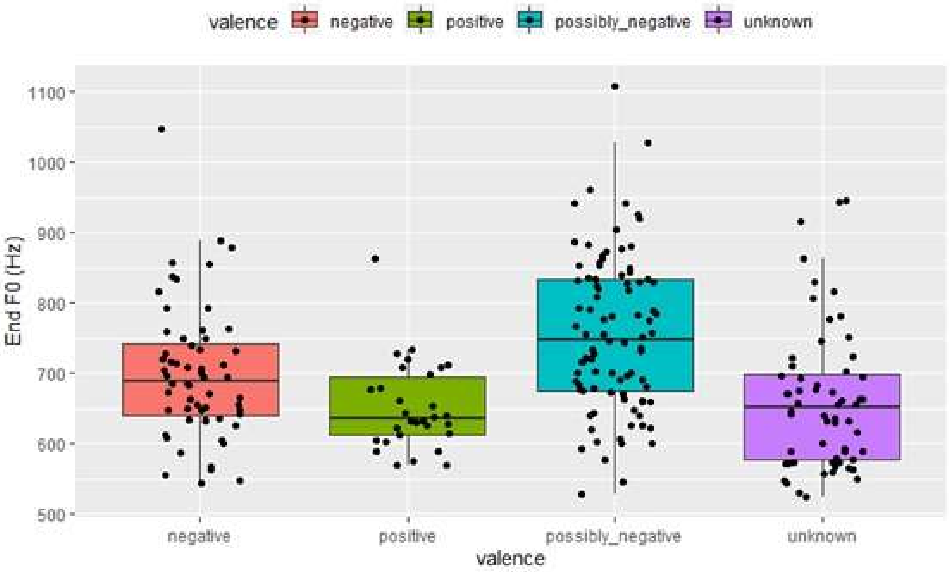
Supplementary Figure 4. Effect of the assigned emotional valence on Q50%. Negative and positive categories are included in the analyses, while possibly negative and unknown categories are only plotted for comparison.



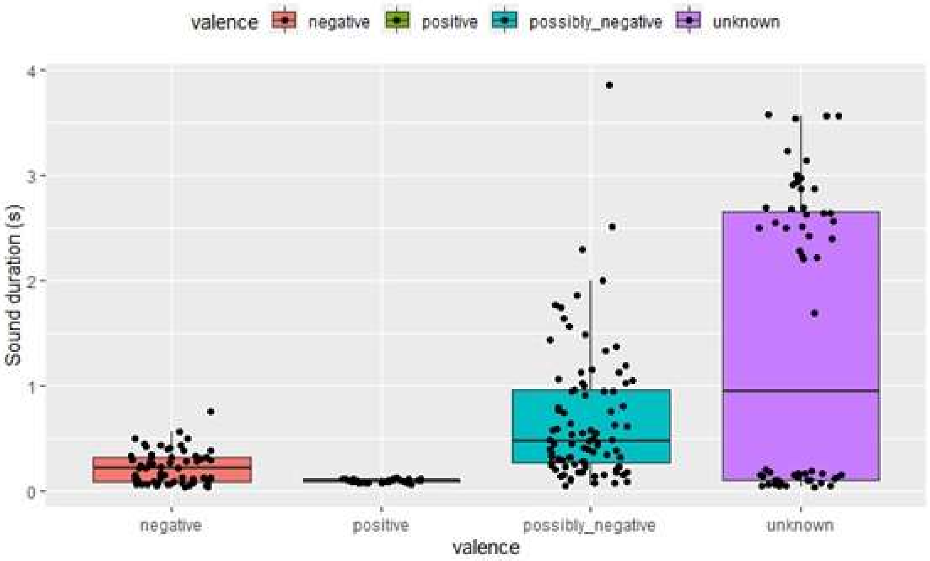
Supplementary Figure 5. Effect of the assigned emotional valence on f0 Abs Slope. Negative and positive categories are included in the analyses, while possibly negative and unknown categories are only plotted for comparison.



Supplementary Figure 6. Effect of the assigned emotional valence on f0 var. Negative and positive categories are included in the analyses, while possibly negative and unknown categories are only plotted for comparison.



Supplementary Figure 7. Effect of the assigned emotional valence on the End f0. Negative and positive categories are included in the analyses, while possibly negative and unknown categories are only plotted for comparison.



Supplementary Figure 8. Effect of the assigned emotional valence on sound duration. Negative and positive categories are included in the analyses, while possibly negative and unknown categories are only plotted for comparison.

Chapter II: Vocal expression of affect in little auk chicks

Calls of the little auk (Alle alle) chicks reflect their behavioural contexts

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ABSTRACT

Animal vocalisations can often inform conspecifics about the behavioural context of production and the underlying affective states, hence revealing whether a situation should be approached or avoided. While this is particularly important for socially complex species, little is known about affective expression in wild colonial animals, and even less to about their young. We studied vocalisations of the little auk (Alle alle) chicks in the Hornsund breeding colony, Svalbard. Little auks are highly colonial seabirds, and adults convey complex behavioural contexts through their calls. We recorded chick calls during two contexts of opposite affective valence: handing by a human, and while they interact with their parents inside the nest. Using permuted discriminant function analysis and a series of linear mixed models, we examined the effect of the production context/associated affective valence on the acoustic parameters of those calls. Calls were reliably classified to their context, with over 97% accuracy. Calls uttered during handling had higher mean entropy, fundamental frequency, as well as lower spectral centre of gravity and a less steep spectral slope compared to calls produced during interactions with a parent inside the nest. The individuality of handling calls, assessed by information content, was lower than the individuality of calls uttered in the nest. These findings suggest that seabird chicks can effectively communicate behavioural/affective contexts through calls, conveying socially important messages early in development. Our results are mostly in line with emotional expression patterns observed across taxa, supporting their evolutionary continuity.

Keywords:

affective state, seabird, distress, emotions, valence, vocal communication

Introduction

Acoustic communication plays a crucial role for many animals, and it can be especially well-developed in socially complex species[1-2]. Certain information about the environment or behavioural context can be particularly important to communicate to other group members. For example, sharing information about predator presence or food location can be key for colonial or cooperating animals.

Behavioural contexts associated with functionally important stimuli, such as food or threats, can trigger short-term responses associated with physiological, behavioural and cognitive changes, termed 'emotions' or 'affective states'[3].Affective states in non-human animals are commonly measured along two fundamental dimensions[4]: arousal (bodily activation) and valence (positive or negative)[5-6] . They are associated with neuro-physiological, behavioural and cognitive changes[3,7], serving as guides for adaptive behaviour. In other words, stimuli that promote fitness (e.g. food, caretakers, mating opportunities) are predicted to evoke positive states and typically result in approach behaviour, whereas threatening stimuli (e.g. predators, fights, perilous conditions) are predicted to elicit negative states and generally lead to avoidance[5]. It is important to note that, when discussing emotions one does not necessarily refer to complex feelings, but rather the very basic triggers of behavioural responses[3-7]. Emotional contagion, i.e. transfer of affective states to others, is a key behavioural aspect in socially living animals[8], used to alert the group about both positive and negative contexts, but also maintain social bonds[8–10]. Vocalisations are a powerful means to convey affective - and therefore behavioural - contexts to others[11–13]. Changes in acoustic signals reflecting those contexts can be perceived within[14' and even across species[15], guiding appropriate responses towards the producer of the vocalisation or the situation in which it finds itself[8,16-17]. However, dynamic information such as behavioural or emotional contexts may interfere with static information conveyed in calls, such as identity of the caller[18].

The little auk (*Alle alle*) is a highly colonial seabird that maintains long-term social bonds[19]. Little auk pairs produce one egg per year[19], so that no sibling competition occurs. Both partners contribute to and coordinate their parental efforts[20]. Adults of this species have a complex vocal repertoire (eight different call types used during mating and incubation)[13], and convey contextual and emotional information through their calls[13]. Little auk chicks are known to produce one call type during social interactions – the *begging* call[21] – used while they wait for their parent's return to the nest and during interactions with the parents. The *begging* call is highly individually specific[21] and its acoustic parameters change as the chicks grow[21]. We have also observed some chicks producing calls as they were being handled for ornithological procedures, yet these calls (from here on, the *handling* calls) were not previously described. Aside from this, little is known about the vocal behaviour of little auk chicks[21], or vocal ontogeny in this species.

In this study, we examined whether vocalisations uttered by young little auk chicks already reflect the behavioural contexts of their production - and, if so, which acoustic parameters encode this information. We also investigated whether the information content of chick calls varies between those contexts.

Methods

Ethics statement

Fieldwork was performed under permission from the Governor of Svalbard (17/00663-2, 20/00373-8), following Association for the Study of Animal Behaviour guidelines for animal studies[22]. Birds were handled by a licensed ringer (KWJ, permit no. 1095, type: C, issued by Museum Stavanger, Norway).

Site, subjects, and set-up

All data were collected in the little auk colony in Hornsund, Spitsbergen (77°00' N, 15°33' E). Recordings were made over the chick rearing period of the 2017 and 2021 breeding seasons, around the 7th day (approximately 5 to 8 days) of chicks' life.

In 2017, audio material was collected via an Olympus ME-51S stereo microphones (frequency response 100-15,000 Hz) placed inside 16 nests in such a way as to not disturb the birds' normal activities[21]. Each microphone was connected to an Olympus LS-3 or LS-P4 digital voice recorder (sampling rate 48 kHz, 16 bits) placed outside of the nest and hidden under a rock to prevent both damage to the equipment and disturbance to the animals. This was paired with video recordings to control for presence/absence of the parent. Only calls produced at times during which a parent was present in the nest were selected for this study.

In 2021, chicks were recorded during handling (weighting) via a hand-help recorder (Olympus LS-12) with a built-in microphone. Chicks were not specifically stimulated to vocalise. Therefore, all recorded calls represent 'spontaneous' vocalisations. Note, however, that not all handled chicks vocalised – and among those who did, the call production rate differed greatly (Table 1). Chick sex was unknown in either group – however, note that little auk vocalisations are not affected by the caller's sex[23].

The effect of the employed equipment (internal vs. external microphone) and recording conditions (inside a rocky burrow vs. in open air) on the acoustic parameters of the recorded calls was tested in an additional experiment (see Supplementary Material). This included broadcasting the calls in conditions mimicking the recording conditions, and re-recording them with both internal and external microphones. Results showed that while the recording set-up had a significant influence over the recorded parameters of the calls, it did not interfere with the overall classification accuracy. See Supplementary Material for a detailed description of this experiment.

We manually extracted all good quality calls found within the recordings using Raven Pro 1.6.4 (Cornell Lab of Ornithology, Ithaca, USA), assigning them to individual chicks and production contexts (during handling versus inside the nest with a parent; further on *handling* and *begging*, respectively). Both the number of successfully recorded chicks and number of vocalisations produced by those varied greatly between contexts and individuals (Table 1). Since the *begging* and *handling* calls are rather similar to the human ear and are not always obviously different upon a visual inspection of their spectrograms (Fig. 1), for the purpose of this study, we decided to treat them as a single call type emitted in two behavioural contexts.

Table 1. Number of calls per individual and production context.

Context	*begging*		*handling*	
	individual	no. calls	individual	no. calls
	N_01	224	H_01	1
	N_02	55	H_02	9
	N_03	293	H_03	4
	N_04	124	H_04	2
	N_05	26	H_05	9
	N_06	143	H_06	1
	N_07	216	H_07	2
	N_08	67	H_08	32
	N_09	81	H_09	11
			H_10	5
			H_11	69
			H_12	13
			H_13	1
			H_14	1
			H_15	7
			H_16	32
			H_17	1
			H_18	3
			H_19	1
			H_20	1
			H_21	1
Total	**9 individuals**	**1229 calls**	**21 individuals**	**206 calls**

The two behavioural contexts were assigned putative affective valence based on threats/promoters of fitness – i.e., situations that the animals should be motivated to approach or avoid[3,6,24]. Therefore, since *handling* likely represents response to a threat, it was assigned putative negative valence (avoidance). There is no sibling competition in the little auk (since broods are composed of one egg only)[19] and adults feed any chick they find in their nest chamber without adverse reaction to it[21] – therefore, *begging* calls were assigned putative positive valence (approach). Note that this approach does not correct for subtler behavioural contexts, such as e.g. frustration related to hunger or prolonged waiting, and aims to refer to the overall valence of the state as positive or negative contexts only. Also note that the use of terms “approach/avoidance” does not necessarily translate into a physical action, but rather an elicited reaction to, or internal motivation to approach/avoid the stimulus.”

Analysis

All analyses were performed in R environment (v. 4.1.3)[25]. Calls were analysed using the *analyze* function (*soundgen* package[26]) with the following settings: sampling rate = 48000, dynamic range = 60, pitch floor = 800, pitch ceiling = 3500, step = 5. The following parameters were extracted: sound duration, mean entropy, frequency value at the upper limit of the first (Q25%), second (Q50%), and third (Q75%) quartiles of energy, mean fundamental frequency (mean *f*0), and spectral slope. These were selected as standard acoustic parameters used in the studies of vocal expression of affect.

To investigate the difference in the vocal expression between the two contexts/associated putative affective states, we performed a permuted discriminant function analysis (pDFA[27]), pooling all available vocalisations from all individuals and from across the two contexts together (1435 calls in total, Table 1). The use of pDFA allowed us to test the effect of contexts/affective valence (test factor) on the extracted acoustic parameters of the calls (input variables), while controlling for repeated measures of the same individuals (control factor) and the unbalanced dataset[27]. A pDFA with nested design was conducted using the *pDFA.nested* function (R. Mundry, based on function *lda* of the *MASS* package[28]). The pDFA used all available subjects (30 individuals) to derive the discriminant function. We ran a total of 1000 permutations for the analysis.

To understand the direction of changes in call parameters, we additionally ran a series of linear mixed models (LMM; *lmer* function, *lme4* package[29]), using each parameter as a response variable (one model per parameter), context/affective valence as a fixed factor, and chick identity as a random factor to control for repeated measures of chicks within contexts (since many chicks produced several calls). Data distribution was tested using Q-Q plots (*qqnorm* function, *stats* package[30] To conform to normal distribution, mean entropy values were log-transformed as: log(*mean entropy*+1-min(*mean entropy*)). The model residuals for all other parameters did not deviate from a normal distribution. To extract the p-values of the LMMs, we used the *PBmodcop* function (*pbkrtest* package[31]), comparing models with and without context included.

To investigate whether calls associated with the two contexts carry a different individual information load, we used individuals who produced at least five calls (i.e., nine individuals per context), and randomly selected five calls for each of them. We calculated the Kaiser-Meyer-Olkin criterion (function *KMO, EFAtools* package[32]), which confirmed the data were appropriate for a principal components analysis (PCA; overall KMO score: 0.73). We then ran a PCA including all extracted acoustic parameters (function *prcomp, stats* package[30]), and used all 11 principal components to calculate Beecher's information statistic[33] (Hs; function *calcHS, IDmeasurer* package[34], which automatically provides Hs values for both all and significant variables only). Beecher's statistic provides an measure of the level of individuality coded within a signal[33] and is a robust, standard method allowing for cross-species comparisons[35]. The Hs values stand for bits of information, and can be translated as the approximate number of individuals that can theoretically be distinguished using a given signal, calculated as 2^Hs.

Results

Calls could be reliably classified to their context of production by the pDFA above chance levels (p<0.005; Table 2, Figure 1). There were significant differences between contexts in the mean entropy, mean *f*0, Q25%, Q50%, and spectral slope, but not in the call duration or Q75% (Table 3, Figure 2). Spectral slope was steeper in the *begging* calls (Table 3, Figure 2). Q25% and Q50% were both lower in the *handling* calls, and the mean *f*0 and mean entropy were higher (i.e. less tonal) in the *handling* calls compared to the *begging* calls (Table 3, Figure 2).

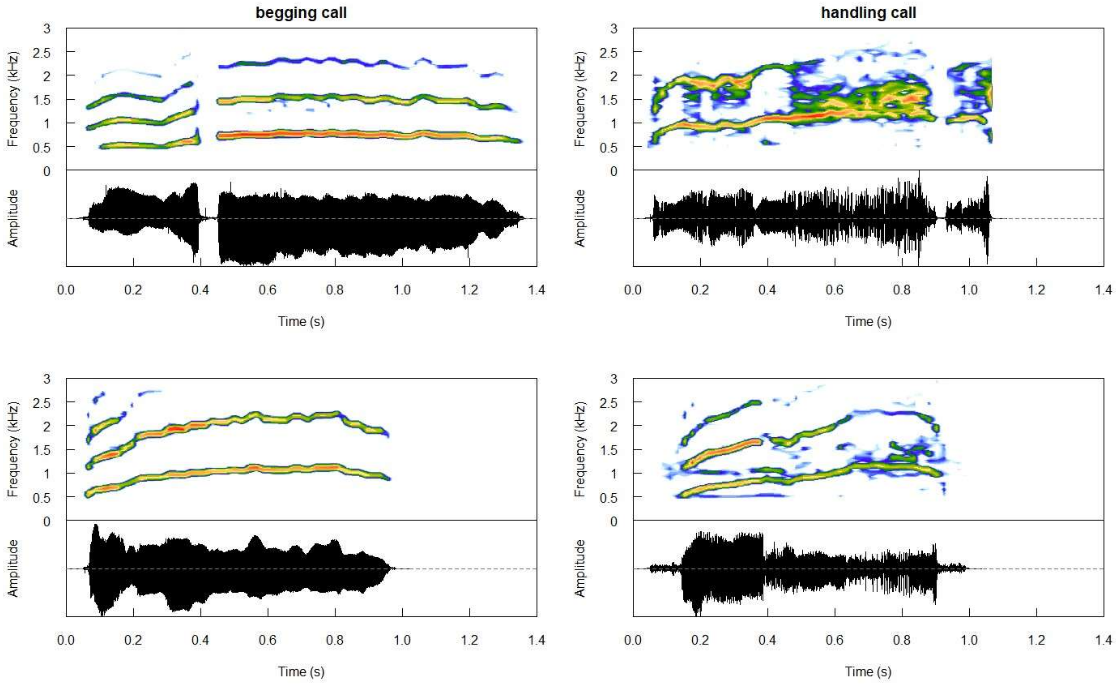

Figure 1. Spectrograms (*seewave* package[36]) of the calls produced inside the nest when interacting with a parent (left panel) and during handling (right panel). Each call was produced by a different chick.

Table 2. Results of the permuted discriminant function analysis for the *handling* and *begging* calls, using 7 raw acoustic parameters. Significance level indicated with asterisks.

Result	
No. context categories (levels of test factor)	2
No. individuals	30
Total no. calls.	1435
Correctly classified (%)	99.22
Chance level (%)	78.65
P value for classified	**0.02***
Correctly cross-classified (%)	**97.32**
Chance level for cross-classified (%)	**59.64**
Relative cross-classification level	1.63
P value for cross-classified	**0.001*****

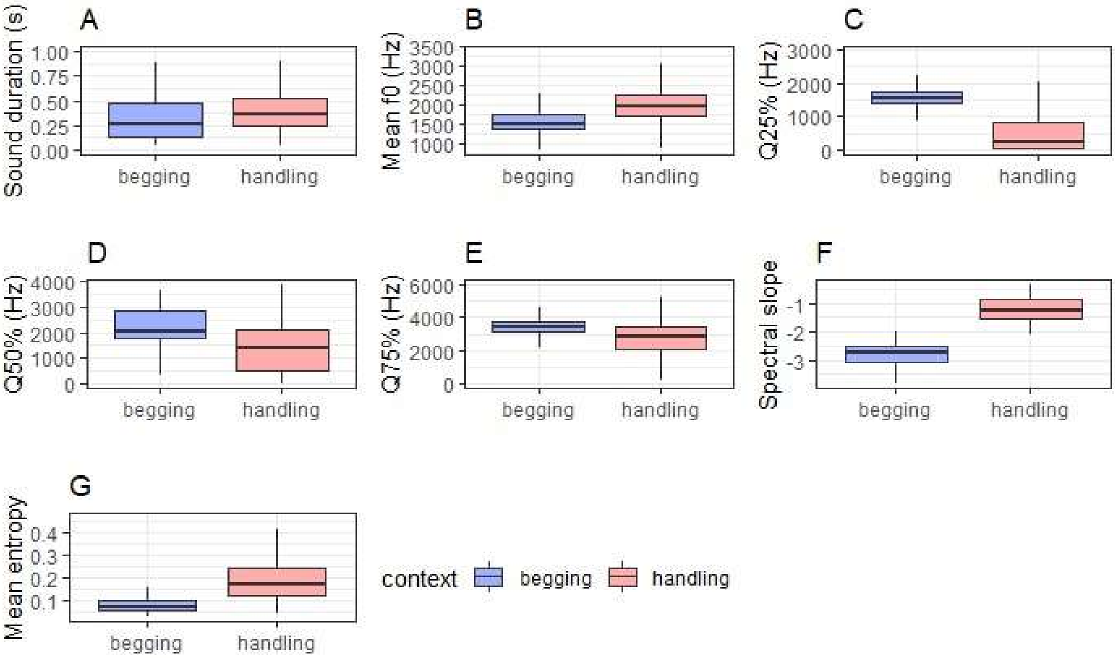

Figure 2. Effect of the behavioural context on the acoustic parameters of the calls. Plots use accessible scientific colour palettes[37-39].

Table 3. Results of the linear mixed models testing the effect of production context on raw acoustic parameters. Letters (A)-(G) refer to the respective panels in Figure 2.

		Predictors		*Scaled residuals*					**p-value**	**Interpretation**
		begging	**handling (intercept)**	**Min**	**1Q**	**Median**	**3Q**	**Max**		
Sound duration (s)	*Estimates*	-0.040	0.347	-4.77	-0.37	-0.04	0.33	8.87	>0.05	No effect (A)
	Std. Error	0.063	0.036							
	t-value	-0.639	9.509							
Mean *f0* (Hz)	*Estimates*	-317.080	1883.050	-4.27	-0.50	0.07	0.60	3.69	<0.05*	Increase during handling (B)
	Std. Error	135.73	79.930							
	t-value	-2.336	23.560							
Q25% (Hz)	*Estimates*	913.700	572.000	-3.81	-0.53	-0.06	0.56	4.87	<0.001***	Decrease during handling (C)
	Std. Error	188.400	110.700							
	t-value	4.850	5.166							
Q50% (Hz)	*Estimates*	742.200	1518.600	-4.98	-0.42	-0.03	0.40	7.47	<0.01**	Decrease during handling (D)
	Std. Error	285.700	163.100							
	t-value	2.597	9.313							
Q75% (Hz)	*Estimates*	-171.5	3535.500	-4.92	-0.40	0.02	0.43	9.31	>0.05	No effect€(E)
	Std. Error	460.100	262.00							
	t-value	-0.373	13.494							
Spectral slope	*Estimates*	-1.552	-1.240	-4.38	-0.56	0.05	0.57	5.16	<0.001***	Less steep during handling
	Std. Error	0.165	0.093							
	t-value	-9.405	-13.305							
Mean entropy	*Estimates*	-0.128	0.172	-5.42	-0.32	-0.01	0.29	7.74	<0.001***	Increase during handling, i.e. less tonal (G)
	Std. Error	0.020	0.011							
	t-value	-6.587	15.497							

Calls uttered during interaction with the parent inside the nest had a Hs = 5.26 (for both all and significant variables), while calls uttered during handling had a Hs = 0.79 for significant variables and Hs = 1.27 when including all variables (Table 4). In other words, calls uttered in during handling were less individually specific than the *begging* calls (Fig. 3).

Table 4. Beecher's information statistic of the *begging* and *handling* calls indicates, that call become much less individually specific in a situation of distress.

Context	Hs all	Hs significant	Meaning
begging	5.26	5.26	Theoretically allows distinction of at least 38 individuals
handling	1.11	0.45	Theoretically allows distinction of at least 1-2 individuals

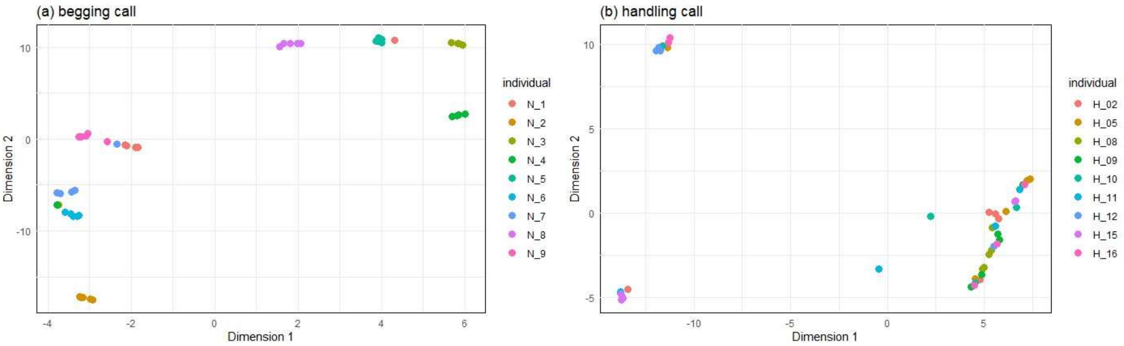

Figure 3. S-UMAP classification to individual for the a) *begging* and b) *handling* calls. While *begging* calls cluster quite well to individuals, this is not the case when the *handling* calls are considered. Plotted using *umap* function of package *uwot*[40], with five nearest neighbours, using all PCA scores of calls emitted by individuals with at least five recorded calls.

Discussion

Communicating behavioural contexts and the underlying affective information is particularly important for socially complex species. We investigated whether calls of seabird chicks carry cues to two behavioural contexts of opposite affective valence. Our results showed that within the first week after hatching, chicks of the colonial seabird, little auk, already have the potential to effectively convey in their calls, information about the context in which they find themselves, which is likely associated with a specific affective valence. Their expression patterns follow the general trends observed across taxa[6,41–47].

Vocal expression of emotional valence has previously been described in adult little auks[13]. Some of the parameters that encode behavioural or affective information in little auk chick calls align with those observed in adults[13]. Similar to adult vocalisations, chick calls associated with a positive context exhibited a steeper spectral slope and higher spectral centre of gravity compared to those associated with a negative context. However, while adult calls uttered in a positive context (interaction with the partner) show a higher mean fundamental frequency (mean *f*0) and a shorter sound duration[13] compared to negative contexts, in chick calls, we observed a lower mean *f*0 in the positive context and no context effect on sound duration. Those differences can potentially arise from the parameters being related to emotional arousal rather than valence – arousal being a dimension[4] we could not reliably measure, or might be due to differences in emotion expression between call types, as often observed in other species[44,46]. For example, while a threat presence (handling) and presence of a parent returning from a long foraging trip might well trigger emotions of opposing valence, they could both result in high arousal. At the same time, while begging calls are related to a context that should activate the pleasant-appetitive motivational system and elicit approach, they can also reflect some levels of frustration[48] and stress due to e.g. hunger[49], or prolonged waiting for the parent[50]. While handling by a human can be reliably considered a stressor[51-53], also such prolonged hunger and frustration can increase stress hormone levels[54] – and these, in turn, might reflect on the begging behaviour (e.g. rate of begging calls)[55-56]. It is therefore possible that the observed differences result from behavioural and emotional complexity not accounted for in this study. Dedicated investigations using measures of arousal (such as heart rate or corticosterone levels) and detailed behavioural observations (e.g. accounting for the time since last feeding) would be helpful to better understand affective expression patterns in the species.

Chick calls associated with a positive context had a reduced mean entropy compared to calls of negative context. This parameter was not examined in adults in our previous study[13] – to allow comparisons, we ran an additional analysis on the previously published data, the results of which showed increased entropy in calls associated with a negative valence across call types (Supplementary Material). High values of entropy reflect less tonal and therefore more chaotic/noisy calls. Therefore, the high entropy values of the calls produced during handling suggest that those calls are more noisy and less tonal than calls produced at the nest. For animals that encode their identity in the spectral parameters of their calls, like the little auk[21,57], increased noisiness of the signal will likely result in a loss of such information. That is, more chaotic calls may potentially carry less bits of information in their structure. Indeed, distress calls showed a much lower potential for individuality coding than calls associated with a positive context. This could have significant social consequences, such as impaired individual recognition, and suggest that more critically important messages are conveyed instead, e.g. presence of a threat. Some loss of individual information has previously been shown in other species, depending on the valence[58] or arousal[59-60] of the caller. If distress calls are aimed at the stressor (in this case: the human researcher handling the chick) and intended e.g. to induce release[61], losing the static individual information should not be problematic to the caller[18]. In fact, such loss can be beneficial in situations of distress, if one can catch the attention of any available rescuer, not just their caretaker's[62]. Surprisingly, cross-fostering experiments have shown that little auk parents will feed any young found in their burrow, which also suggests that individual information in chicks calls might be functionally unimportant in this species[21], or simply overridden by situationally critical information, such as communicating presence of a threat with a noisy signal.

Increased noisiness of acoustic signals is indeed often related to increased arousal or negative valence[11,41-42]. In birds, it has been shown to reflect wellbeing of commercially bred chicks[41] (*Gallus gallus domesticus*), where it has been suggested as a useful tool in welfare assessment. Being a parameter that reflects a level of disorder rather than a specific value (such as e.g. mean *f*0) that may change as an animal grows or ages, it can prove a reliable indicator of an individual's emotional state without prior knowledge of its age or weight. As such, spectral entropy is a promising parameter for social communication in large groups.

Little auk calls are highly individually specific[21,57], but also reflect the size and overall body condition of the calling chick[21] (similarly to those of the Wilson's storm petrel, *Oceanites oceanicus*[63]). Here, we did not correct for the chicks' size, yet the recordings used come from chicks of roughly the same age (5-8 days after hatching), which corresponds to comparable body sizes[21]. While we have employed methods that correct for multiple testing of the same individuals, we acknowledge that this study would have benefited from testing the same individuals in both behavioural contexts. However, this study aimed to maximise the use of already existing data to limit the disturbance caused by research activities and avoid unnecessary handling[64]. Here, we took advantage of the available recordings made inside the nests in 2017[21], supplementing them with additional recordings taken during chick handling for population monitoring in 2021. Little auk handling calls are produced in a very unpredictable manner, and few animals vocalise when handled[13] – this is true for both chicks and adults. Without external stimulation by the researcher, which we avoid, collecting recordings from both contexts for the same individuals usually proves unsuccessful, and it cannot be guaranteed that an animal recorded in one context will vocalise in another. As a result, the available recordings presented in this study are the only chick vocalisations available to investigate context-related changes in call structure. Note that we did not account for the chicks' sex in our analyses, since this information was not available. However, since adult little auks show no sex differences in their calls[23], we are confident that this factor does not significantly impact our results.

An important caveat of this study is that we only had access to two behavioural contexts (one context per putative affective valence) with their associated calls – which may represent either a gradation of one call type, or two distinct calls types. This is because the vocal activity of the young chicks in this species is rather limited in contexts – only the *begging* calls have been previously observed in the young chicks during the nesting period[21]. The data presented here enrich our knowledge of the chicks' behaviour through adding the negative contexts. They are also all that was possible to record of the young chicks' vocalisations within the logistical and ethical limitations, and as such present an important contribution. We also were not able to compare the stability of the two calls across the season (within individuals) and years (across individuals). The little auk *begging* calls change as the chicks grow[21], and it would be ideal to follow the affective expression over ontogeny – unfortunately, such data are not currently available. On the other hand, seabird calls tend to be individually stable over the years[57,65-66], and we do not expect repertoire changes to occur within small timescales. The *begging* and *handling* calls are not obviously different upon a visual inspection of their spectrograms (Fig. 1). Adult little auks use some of their calls across multiple contexts[13], and this is likely true also in case of the chicks. Nevertheless, our results may in fact reflect call type differences. In either case, the vocalisations used in this study reflect two very different behavioural contexts clearly conveyed by the sender, and we are confident that they can be safely interpreted as such. Note that by discussing conveying the behavioural or affective context, only the signal structure is meant: this study cannot assume the intended receiver of this signal (i.e., whether a vocalisation is directed at other auks or the threat itself[60]) or whether and how this intended receiver interprets it. Importantly, the expression patterns observed in this study align with both the patterns observed in the adults of the species[13], and the general trends seen in other groups taxa[6,41-47].

Inquiries into emotions of non-human animals requires taking a perspective relevant to the animal, and using robust, conservative measures[67] – ideally, physiological measures should be used, but external measures such as vocal expression patterns prove extremely helpful when such measures are impossible to take[11-12]. While affective states in birds have been studied in depth in some species under controlled, experimental conditions[43,48,68], data on non-captive animals are still scarce. Our results contribute to the growing body of research on emotions in the wild, and mostly support the general trends seen in affective signals across the vertebrate evolutionary lines[6,41-47,62].

Conclusions

Calls of the little auk chicks carry information about their production contexts early in ontogeny, i.e. within the first week after hatching. Acoustic differences between calls uttered during handling (negative context/elicited avoidance) and inside the nest during interaction with a parent (positive context/elicited approach) are mostly in line with emotional expression patterns observed across taxa, supporting its evolutionary continuity. Our findings present the first evidence of affective expression in seabird chicks, and suggest that little auk chicks might effectively convey socially important messages early in development.

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List of abbreviations

f0: fundamental frequency

LMM: linear mixed model

mean *f0*: mean fundamental frequency

pDFA: permuted discriminant function analysis

Q25%: frequency value at the upper limit of the first quartile of energy

Q50%: frequency value at the upper limit of the second quartile of energy

Q75%: frequency value at the upper limit of the third quartile of energy

Declarations

Availability of data and material

Raw data generated in this study are available at:

https://osf.io/nwpfk/

Competing interests

Authors declare no competing interests.

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SUPPLEMENTARY MATERIALS

(1) Testing for the effect of the equipment and recording conditions

To rule out that the recording equipment employed in the two field seasons and the recording conditions (inside a rocky burrow vs. in open air) had an influence the acoustic parameters of the recorded calls, and could hence explain the differences between the two contexts used in this study, we performed an additional experiment.

For each contexts, we selected one vocalisation (semi-random selection of a good quality signal) per individual (n = 9 birds) per context (vocalising inside the nest with a parent vs. vocalising during handling). These 18 vocalisations were then played back and recorded with the same equipment as used in the field, in the four following settings:

1. Recording with an external microphone, in open air
2. Recording with an external microphone, in a wooden box
3. Recording with a built-in microphone, in open air
4. Recording with a built-in microphone, in a wooden box

Each vocalisation was played back three times in each setting. The acoustic properties of the re-recorded signals were extracted using the *analyze* function of the *soundgen* package[26] (dynamic range = 60, pitch floor = 800, pitch ceiling = 3500, step = 5[13]). The following parameters were extracted (i.e. the same parameters as in the rest of the study): sound duration, mean entropy, frequency value at the upper limit of the first (Q25%), second (Q50%), and third (Q75%) quartiles of energy, mean fundamental frequency (mean *f*0), and spectral slope.

We performed two permuted discriminant function analyses (pDFA[27]; R. Mundry, based on function *lda* of the *MASS* package[28]), pooling the vocalisations from the two contexts together, using the vocalisation identification number as a control factor, and (1) recording set-up (i.e. open/closed environment and with/without external microphone) as the test factor; incomplete pDFA; and (2) production context as the test factor; nested pDFA. We ran a total of 1000 permutations per analysis.

The following results were obtained:

(1) recording set-up as the test factor: p-value for selected: 0.001; p-value for cross-classified: 0.001; expected percent correctly classified: 47.01; expected percent correctly cross-classified: 67.60; percent correctly classified: 80.60; percent correctly cross-classified: 67.60.

(2) production context as the test factor: p-value for selected: 0.001; p-value for cross-classified: 0.004; expected percent correctly classified: 72.62; expected percent correctly cross-classified: 62.05; percent correctly classified: 95.15; percent correctly cross-classified: 87.49.

Resulting accuracy shows that while the recording set-up did have a significant influence over the recorded parameters of the calls, this influence was lower than that of the production context, and vocalisations could be reliably classified to their production context independently of the equipment and recording environment.

The raw data generated in this experiment are available at:
https://osf.io/nwpfk/?view_only=a1d5577723424699b12b1c707a5369cc

(2) Entropy in negative calls of adult little auks

To allow comparisons among groups – and include more call types – we performed an additional analysis of adult calls with assigned positive and negative valence, using recordings from Osiecka *et al.* 2023[13].

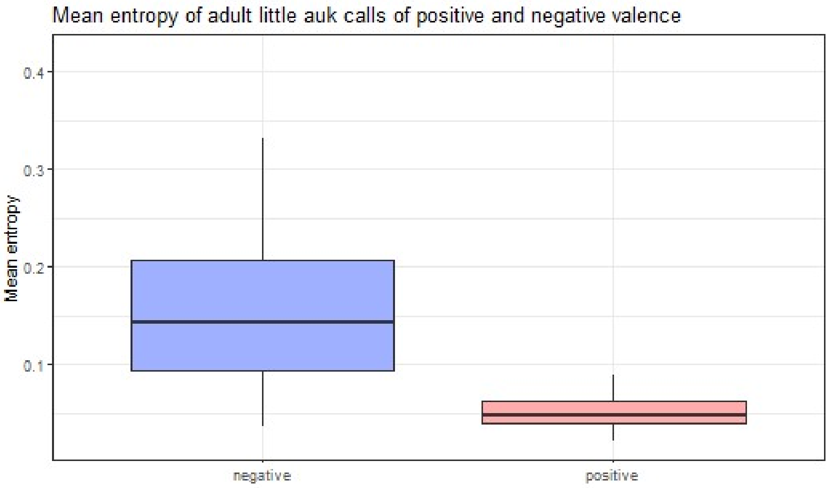
The analyses was performed in R environment (v. 4.1.3)[25]. Calls were analysed using the *analyze* function (*soundgen* package[26]) with the following settings: sampling rate = 48000, dynamic range = 60, pitch floor = 500, pitch ceiling = 2000, step = 5[13], extracting mean entropy values.

Calls were assigned positive and negative valence based on the call type/production context[13]. We ran a linear mixed model (LMM; *lmer* function, *lme4* package[29]), using mean entropy as a response variable (one model per parameter), putative valence as a fixed factor, and call type as a random factor to control for repeated measures of the same call types. Data distribution was tested using Q-Q plots (*qqnorm* function, *stats* package[30]. To extract the p-value, we used the *PBmodcop* function (*pbkrtest* package[31]), comparing models with and without valence included.

The putative affective valence had a significant effect on the mean entropy of the calls (p<0.001***; Supplementary table 1, Supplementary Figure 1). Calls assigned negative valence showed higher entropy compared to calls assigned positive valence (Supplementary Figure 1).

Supplementary Table 1. Results of a linear mixed model investigating the effect of valence on the mean entropy of adult little auks' calls.

		Predictors		*Scaled residuals*					**p-value**	**Interpretation**
		negative (intercept)	**positive**	**Min**	**1Q**	**Median**	**3Q**	**Max**		
Mean entropy	*Estimates*	0.158	-0.099	-1.64	-0.60	-0.16	0.32	3.51	<0.001***	Higher entropy in negative calls
	Std. Error	0.010	0.017							
	t-value	16.445	-5.934							



Supplementary Figure 1. Effect of the affective valence on the mean entropy of adult calls. *Positive* calls include *clucks*, and *negative* calls include *handling* and *terror* calls[13]. Plots use accessible scientific colour palettes[37–39].

Chapter III: Coding information on sex, size, and partnership

Social calls of the little auk (*Alle alle*) reflect body size and possibly partnership, but not sex

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Abstract

Source-filter theory posits that an individual's size and vocal tract length are reflected in the parameters of their calls. In species that mate assortatively, this could result in vocal similarity. In the context of mate selection, this would mean that animals could listen in to find a partner that sounds – and therefore is – similar to them.

We investigated the social calls of the little auk (Alle alle), a highly vocal seabird mating assortatively, using vocalisations produced inside 15 nests by known individuals. Source- and filter-related acoustic parameters were used in linear mixed models testing the possible impact of body size. A PCA followed by a permuted discriminant function analysis tested the effect of sex. Additionally, randomisation procedures tested whether partners are more vocally similar than random birds.

There was a significant effect of size on the mean fundamental frequency of a simple call, but not on parameters of a multisyllable call with apparent formants. Neither sex nor partnership influenced the calls – there was, however, a tendency to match certain parameters between partners. This indicates that vocal cues are at best weak indicators of size, and other factors likely play a role in mate selection.

Keywords:

information coding, partner similarity, seabird, source-filter theory, vocal communication

Introduction

Finding a mate in a crowded colony can be a challenge. Acoustic signals can travel long distances and often provide cues to the caller's sex[1-3], and size[4-5], and are thus a great candidate for facilitating mate selection in dense, populous groups. The source-filter theory of vocal production postulates that sounds generated at the source (larynx or syrinx) are subsequently resonated by the filter (vocal tract), shaping the output spectrum of the call[6]. Depending on the length of the vocal tract, specific frequencies are dampened or enhanced, creating a stronger (amplified) output signal at certain frequencies, i.e. formants (resonances of the vocal tract), while others are filtered out[6]. While vocal tract elongations are used in some species to falsely indicate a larger body size[7], in general, both source- and particularly filter-related sound parameters are good indicators of body size, and are negatively correlated to it[8-10]. Although the source-filter theory was originally proposed for mammals[6], the importance of formants has been demonstrated in some bird species[10-14], including indication of size[10] and identity[10].

Body size information in vocalisations could be used in some species to achieve assortative mating, which consists in matching of certain, e.g. morphological[15-18], or physiological[17], traits between partners. In some cases, assortative mating is known to lead to certain advantages, such as improved offspring condition[19] or reproductive success[20-21]. While assortative mating tends to be somewhat overestimated[22], is not very common in birds, compared to other taxa[23]. Nevertheless, it occurs across different seabird groups: species such as the long-tailed Jaeger (*Sterocorarius longicaudus*[24]), Scopoli's Shearwater (*Calonectris diomedea*[18]), Magellanic penguin (*Spheniscus magellanicus*[25]), the masked booby (*Sula dactylara*[16]), or the little auk (*Alle alle*[17]), all select their nesting partners according to certain morphological similarities, ranging from wing length[17], to foot colour[16]. Therefore, if the vocalisations of an assortatively mating species reflect traits such as body size, it can be expected that partners will also be similar vocally.

Vocal behaviour in birds can be influenced by hormones[26], and is often sex-specific. Also call parameters can – but do not necessarily have to – depend on sex. Across species, this information can be coded differently[1], such as using temporal[2-3] or spectral[1,27] parameters. Where a significant sexual dimorphism is present, vocalisations are also likely to differ –

however, it can also assist in locating a potential mate in species with no sexual dimorphism.

Little auks are long-lived seabirds, nesting in densely populated colonies counting up to hundreds of thousands of individuals[28]. While they choose mates that are morphologically or physiologically similar to themselves[17], and usually maintain partnership over many years[29-30], nothing is known as to how these bonds are formed or are maintained over time, e.g. how potential mates are identified considering the lack of external dimorphism[30] . Little auks are very vocal, and use a variety of call types that vary significantly in their acoustic properties[31]. Most of these calls have a harmonic structure, and in case of the *classic call* we can observe formants[31] (Fig 1). Additionally, little auk calls change throughout ontogeny, with spectral parameters reflecting growth in chicks[32]. Vocal cues are thus a good candidate for coding socially important information, such as size and sex, in this species.

In this study, we investigated the information encoded in the source- (fundamental frequency, i.e. the lowest frequency of the sound, hereafter '*f*0') and filter- related parameters (formants) of the little auk social calls. We selected two commonly used social call types: the *short call* (a simple, one-syllable call with no formants; Fig. 2) and the *classic call* (a complex, multi-syllable call with clear formants; Fig. 1), both used in a range of social interactions[31]. While *short calls* are used in close-range communication in or near the nest, the *classic call* is likely a long-distance call, often uttered by birds in flight but also used from in- or outside the nest[31]. Because of the frequency of their use we selected them as socially important calls. Their very different spectral structures, on the other hand, suggests that these calls might carry different types of information. We examined whether source- (both call types) and filter-related (*classic call*) parameters could be cues to size, and whether partners' vocalisations are more similar than those of random birds. We have also tested whether sex affected the acoustic parameters of social calls.

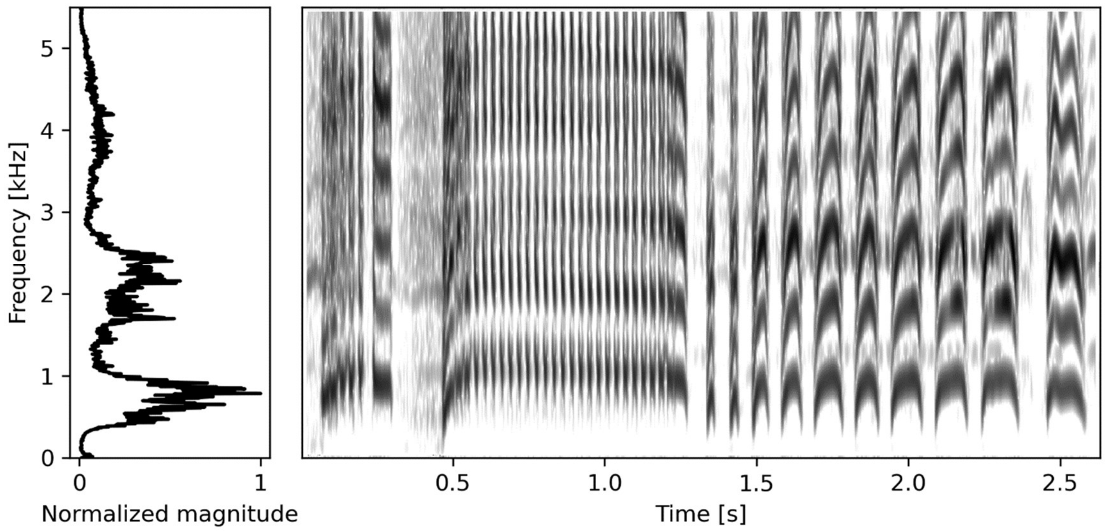

Figure 1. Spectrogram (right) and energy content at different frequencies (left) of a sample *classic call*.

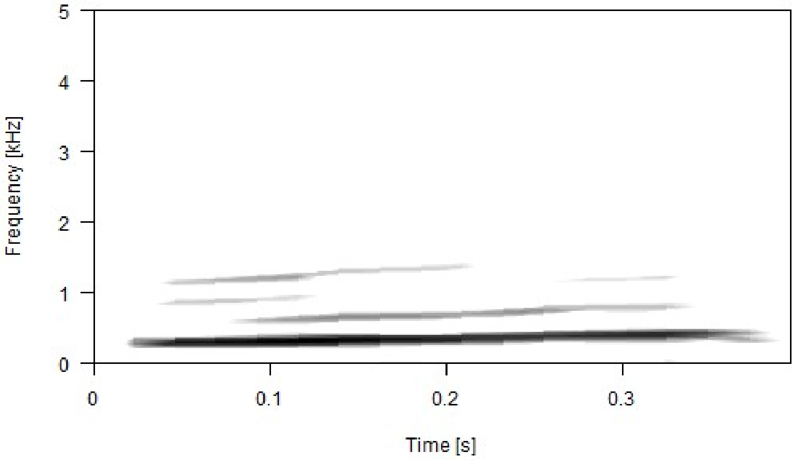

Figure 2. Spectrogram of a *short call*.

Methods

Ethics and permits

Fieldwork was performed under permission from the Governor of Svalbard (17/00663-13, 20/00373-2). The birds were handled with the greatest care, following the Animal Behavior

Society and Association for the Study of Animal Behaviour guidelines for animal treatment[33].

Choice of the size proxy

Body size is usually measured based on the individual weight, selected skeletal proxy, or a set of measures[5,7-8, 34-35]. Since the little auk's weight fluctuates heavily throughout the breeding season[36], it is not a good indicator of the overall size. Instead, we decided to use a single stable measure– the total head and beak length (THL). It is a good proxy of size in the species[37], and correlated between partners[37] (but see[17]). Moreover, containing a part of the vocal apparatus, it has the added advantage of being related to the vocal tract length.

Study site and recording set-up

All data were collected in the little auk breeding colony in Hornsund, Spitsbergen (77°00' N, 15°33' E), over two consecutive breeding seasons (2019-2020). All birds (two per nest, 18 nests in total) were handled (ringed with a unique combination of colour rings and measured) at the beginning of each field season. THL was measured using standard callipers as the distance between the back of the skull and the tip of the beak, with a 1 mm precision. The same person measured all the birds in the two seasons. If the captured individual was not yet known (i.e. had not been ringed before), aside from ringing and taking measures, its feathers were collected for molecular sexing, following a protocol adjusted to feather samples[37].

Recording little auk vocalization imposes a challenge as individual birds do not vocalize that frequently, and rather unpredictably in space and time. Moreover, vocalizing birds are often surrounded by other vocalizing individuals, creating unwanted noise in their recordings. Thus, for the purpose of this study, recording sessions were performed passively and in a continuous manner during the incubation period, with microphones inserted into the nest chamber. This way all the vocalisations produced inside the nests by focal adults (i.e. of known identity) were collected. Each nest was monitored during three different stages of incubation (early, mid and late). All sessions lasted 48 h, aiming to space them equally in time (i.e., about eight days in between sessions) for all the monitored nests.

Audio recordings were made with an Olympus ME-51S stereo microphone (frequency response 100-15,000 Hz) placed inside the nests in such a way as to not disturb the birds.

The microphones were connected to Olympus LS-3 or LS-P4 digital voice recorders (sampling rate 48 kHz, 16 bits) placed outside the nest and hidden under rocks. Synchronised video material was collected using cameras (commercial HD model of JVC, Japan; time-lapse mode: 1 frame/s) placed in front of the entrance to each nest, to control for the identity of the focal individuals.

Data selection

Video recordings were reviewed in VLC software, noting the exact time each marked individual entered or left the nest. Since the birds were equipped with an unique pattern of colour-mark rings in addition to the standard numbered rings, it was possible to know which individual exactly was observed.

Then, the time intervals at which only one individual was present inside the nest, were established using a custom-made script, and used to extract the corresponding audio fragments. This audio material was then manually reviewed in Raven Pro 1.6.4 (Cornell Lab of Ornithology, Ithaca, USA), extracting all individual vocalisations recorded inside the nest. Great care was taken to not accidentally include vocalisations coming from outside the nest (i.e. of lower amplitude and/or audible sound distortion due to the burrow’s walls), or vocalisations masked by noise. The resulting extracted vocalisations could therefore be assigned to individual of known sex, size, and breeding partner. We managed to obtain calls from 15 out of the 18 monitored nests, and both partners were successfully recorded in 11 nests (Supplementary Table 1). Because we relied on the spontaneous vocal production of wild animals in a challenging recording set-up, the final sample sizes vary between call types and individuals, ranging from 1-70 calls extracted per individual (Supplementary Table 1).

Little auks produce eight different call types[31], whose functionality is not yet well understood[31]. For this study, we selected two common social call types of a very different structure and contexts of use, i.e. the *short call* used in close-range social communication, and *classic call*, likely a long-distance call, used over a wide spectrum of contexts. This choice was made to include common calls that likely convey different types of information.

Sound analysis

To extract a standard set of 16 acoustic parameters (Supplementary Table 2), all calls were analysed in Praat software[38], using a script[39-41] adjusted to the little auk[31] (Supplementary Text 1), with the following spectrogram settings: Hann window, FFT-length = 715.

Additionally, mean values of the four first putative formants (F1-F4) were extracted from the *classic* calls using the FastTrack plug-in[42] for Praat, using the following settings: lowest analysis frequency = 500 Hz, highest analysis frequency = 7550 (Hz), number of steps = 20, number of coefficients for formant prediction = 5, number of formants = 4. The formant dispersion, i.e. the averaged difference between successive formant frequencies, was then calculated as $Fd = ((F2 - F1) + (F3 - F2) + (F4 - F3))/3$. The number of extracted formants was decided based on visual assessment of the calls' spectrograms as well as script efficiency (i.e., more than 4 formants were never extracted by the script, and the extracted values were most reliable with those settings).

Statistical analysis

All analyses were performed in R environment (v. 4.1.3[43]). The full data used in this study can be found in the Supplementary Material.

Size

We used linear mixed models (LMM; *lmer* function in *lme4* package[44]) to investigate the possible effect of size on the source- and filter-related acoustic parameters. These models included THL and sex as fixed factors (where sex was used as a control factor), and ID as a random factor to control for repeated measures. To avoid running multiple models on each parameter separately and hence avoid risks of type I error, we chose to test the effect of body size on one representative source-related parameter, the mean *f*0 value across the call (hereafter '*mean f*0'), and one representative filter-related parameter, the formant dispersion. Those parameters were chosen since they are usually reliable indicators of body size across taxa[45]. We prepared two models for the source parameter: one for the *short*, and one for the *classic* call type. For the filter parameter (formant dispersion), we prepared one model (only *classic* call type). To in the LMMs, we used the *PBmodcomp* function (*pbkrtest* package[46]), comparing models with and without THL included, i.e. providing p-values for the compared parameter.

Sex

First, we performed a Kaiser-Meyer-Olkin test on raw parameters of the *short* and *classic* calls separately (function *KMO*, *psych* package[47]). Since the overall MSA was higher than 0.5[48] for both call types (MSA$_{\text{short call =}}$ 0.75; MSA$_{\text{classic}}$ = 0.57; Supplementary Table 3), we performed a principal component analysis (PCA) was performed (function *prcomp, stats* package[49]) on the 16 extracted acoustic parameters (Supplementary Table 2) to reduce data dimensions. The scores of the PCA with eigenvalues > 1 (Kaiser's criterion) were then used as input data for the following tests (the first five PCs for the *short call,* and first six PCs for the *classic call;* Supplementary Table 4).

To investigate the influence of sex on the acoustic parameters of the calls, we analysed the data using permuted Discriminant Function Analysis (pDFA). The dataset was based on multiple sampling per individual. The use of a pDFA allowed us to test the effect of sex (test factor) on the PC scores (input variable) while controlling for repeated measures of the same individuals (included as a control factor). A pDFA with nested design was conducted using the *pDFA.nested* function (R. Mundry, based on function *lda* of the MASS package[50]). The pDFA randomly selected calls for each combination of test and control factors. This random selection was repeated 100 times, and results were averaged. The number of permutations was set at 1000 (default). This procedure was run separately for the *short* and *classic* call types.

Because temporal information can be very important in coding cues to sex in seabirds[2-3] but sound duration did not strongly contribute to the PC scores used in the pDFA (Supplementary Table 5), we additionally used linear mixed models (LMM; *lme4* package[44], *lmer* function) including sound duration as a response variable, sex and THL as fixed factors (where THL was used as a control factor), and ID as a random factor to control for repeated measures. To obtain p-values of the LMMs, we used the *Pbmodcomp* function (*pbkrtest* package[46]), comparing models with and without sex included. This was done separately for the *short* and *classic* call types.

Partner similarity

We used a correlation analysis to compare vocal similarity between nesting partners vs. randomly assigned individuals. For this, we used the mean *f*0 values and sound duration of *short* and *classic* call types, formant dispersion in the *classic* calls, as well as the scores of the first PC of each call type.

First, all parameters were averaged for each individual. Average values of partners were then compared using Spearman's correlation test (observed values; *cor* function in *stats* package[49]). To establish significance of the observed values, a randomization procedure was performed separately for each parameter, where males and females were shuffled to create random pairs. For those, correlation coefficient was calculated (randomised values; *cor* function, *stats* package[49]); the procedure was repeated 1000 times. The p-value was calculated as the proportion of randomized values that generated a correlation equal to or more extreme (in absolute terms, i.e. values equal or higher for positive correlations) than the correlation obtained from original male–female pairings, $p = 1 - \frac{sum(observed\ values \geq randomise\quad values)}{N}$. Because of the multiple testing, we used Bonferroni adjustments, so that p-values retained significance at 0.007 (i.e. 0.05/7).

Results

Size

Mean *f*0 of the *short* call decreased with size (Fig. 3; Table 1). There was no size effect on the mean *f*0 of the *classic* call (Table 1) or on the formant dispersion (Table 1).

Table 1. Model results: Linear Mixed Effect Models testing the effect of size (total head length, ‘THL’) on the source (mean *f*0) and filter (formant dispersion) parameters of the *short* and *classic* calls. Significance indicated with asterisks.

		Predictors		*Scaled residuals*					**p-value**	**Interpretation**
		Intercept	**THL**	**Min**	**1Q**	**Median**	**3Q**	**Max**		
Mean *f*0 (Hz): *short* call	*Estimates*	2064.01	-25.74	-2.78	-0.60	-0.01	0.49	3.73	0.041*	Decrease with size
	Std. Error	645.95	12.16							
	t-value	3.195	-2.117							
Mean *f*0 (Hz): *classic* call	*Estimates*	1255.38	-5.95	-2.51	-0.46	0.02	0.52	2.63	0.686	No effect
	Std. Error	676.01	12.66							
	t-value	1.857	-0.470							
Formant dispersion	*Estimates*	407.87	9.07	-1.86	-0.82	-0.03	0.76	2.42	1	No effect
	Std. Error	1175.28	21.98							
	t-value	0.35	0.41							

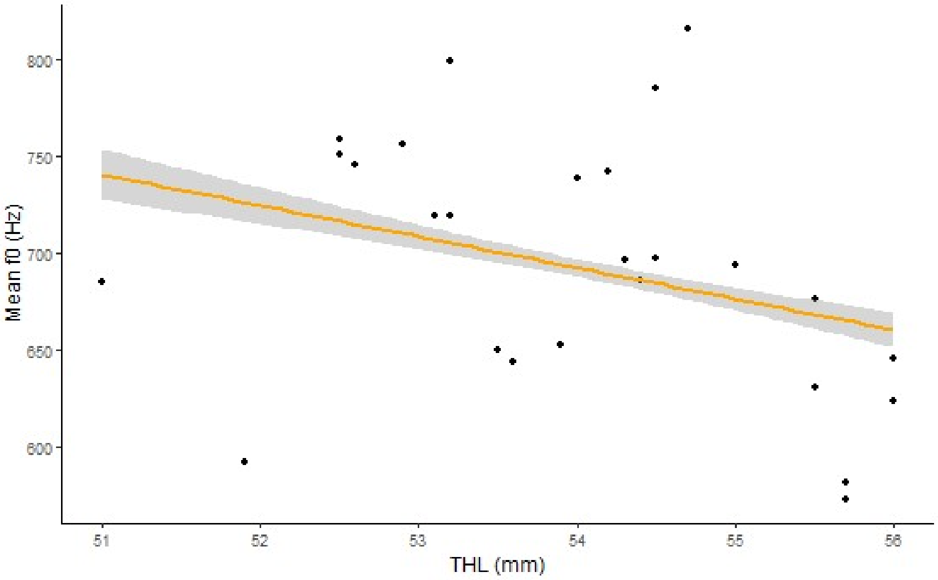

Figure 3. Relationship between size (total head length) and mean fundamental frequency of the *short* call: *f*0 decreases with size. Dots represent averaged *f*0 values for each individual, and the yellow line the best fit of the linear model.

Sex

Sex had no effect on the acoustic parameters of either call type (pDFA: p≥0.3 in both cases; Table 2), nor on the sound duration investigated separately (p>0.1 in both cases; Table 3)

Table 2. Results of the permuted discriminant function analysis for the *short* and *classic* call types, using 16 acoustic parameters in reduced dimensions. Significance indicated in bold.

Result	***short* call**	***classic* call**
No. sex categories (levels of test factor)	2	2
No. individuals	26	24
Total no. calls.	574	159
=No. calls selected	24	22
Correctly classified (%)	69.04	73.36
Chance level (%)	69.08	72.10
P value for classified	**0.51**	**0.37**
Correctly cross-classified (%)	55.41	61.84
Chance level for cross-classified (%)	55.60	55.90
Relative cross-classification level	1.00	1.11
P value for cross-classified	**0.51**	**0.26**
Interpretation	**No effect**	**No effect**

Table 3. Model results: Linear Mixed Effect Models testing the effect of sex on sound duration of the *short* and *classic* calls.

		Predictors		*Scaled residuals*					p-value	Interpretation
		Intercept	**Sex**	**Min**	**1Q**	**Median**	**3Q**	**Max**		
Sound duration (s): *short* call	*Estimates*	0.22	-0.04	-3.32	-0.44	-0.07	0.38	7.93	>0.5	No effect
	Std. Error	0.99	0.02							
	t-value	0.22	-0.71							
Sound duration (s): *classic* call	*Estimates*	-6.06	-0.33	-1.78	-0.47	-0.13	0.33	6.29	>0.1	No effect
	Std. Error	5.23	0.23							
	t-value	-1.18	-1.42							

Partner similarity

The mean *f*0 of short and classic call, as well as formant dispersion of the *classic* call tended to be more similar between partners (Table 4, Fig. 4), although the relationship remained statistically insignificant. Mean durations and scores of the first PC of both call types were not more similar between partners than between randomly assigned birds (Table 4, Fig. 4).

Table 4. Model results: correlation analysis testing similarity of different acoustic parameters between partners vs. random birds.

Parameter	*p-value*	*r-value*	*Interpretation*
Mean *f*0: *short* call	0.072	0.47	Not significant; tendency to match
Mean *f*0: *classic* call	0.036	0.64	Not significant; tendency to match
Formant dispersion: *classic* call	0.050	0.62	Not significant; tendency to match
Mean duration: *short* call	0.193	0.30	No effect
Mean duration: *classic* call	0.444	0.06	No effect
PC1: *short* call	0.163	0.37	No effect
PC1: *classic* call	0.194	0.31	No effect

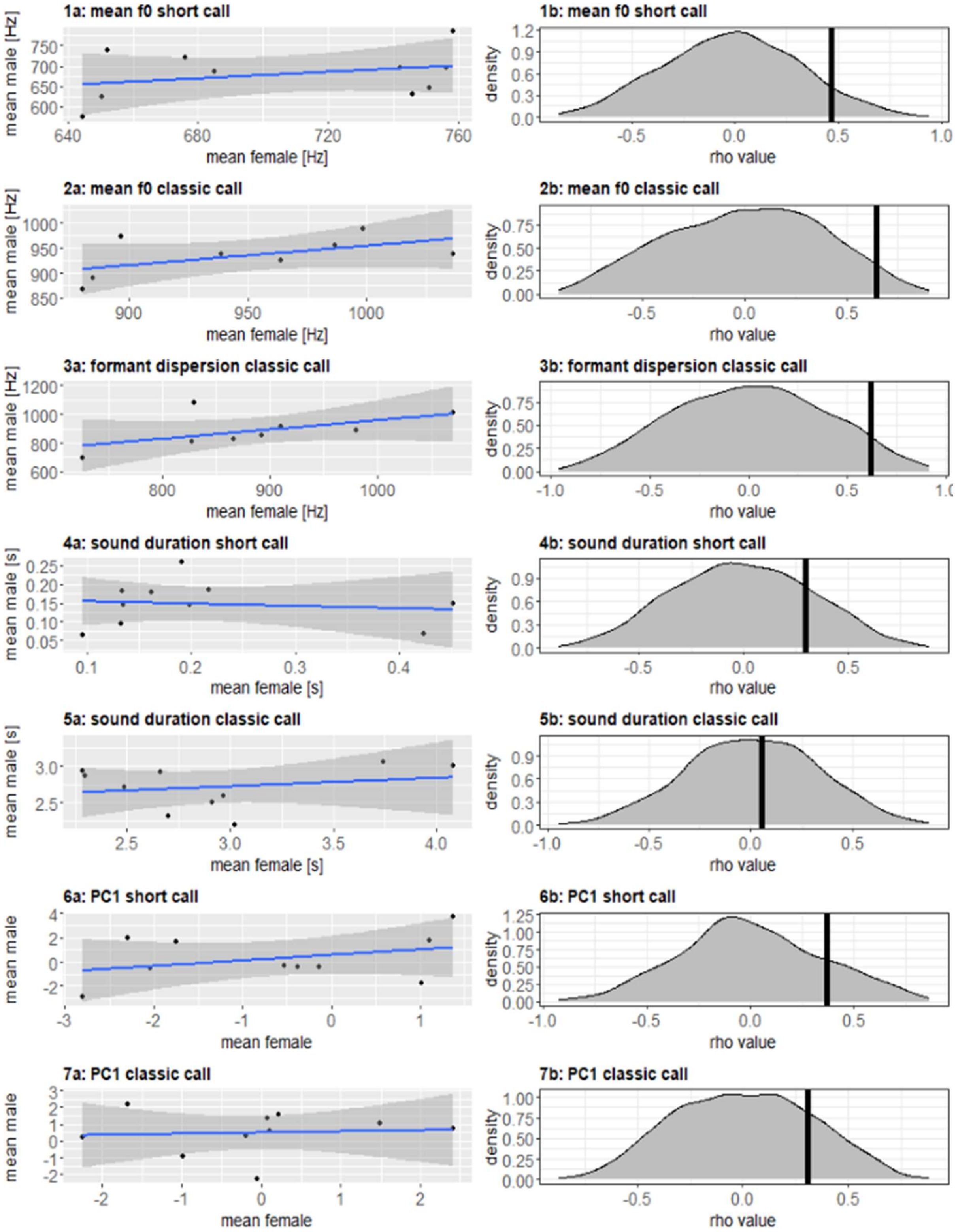

Figure 4. Observed relationship strength (a) and significance (b) of vocal similarity between partners, comparing mean *f*0 of the *short call* (1), mean *f*0 of the *classic call* (2), formant dispersion of the *classic call* (3), mean sound duration of the *short call* (4), mean sound duration of the *classic call* (5), scores of the 1st PC of the *short call* (6), and scores of the

1st PC of the *classic call*. While none of these parameters show a significant effect of partnership after Bonferroni adjustment, there is a clear tendency in the source and filter parameters of both call types to match between partners.

Discussion

We investigated the influence of size and sex on the acoustic parameters of little auk social calls, and considered partners similarity in the acoustic traits. Size had an effect on the source-related parameter (mean fundamental frequency) of one of the call types, the *short call*, with an increase in *f*0 with caller size (head length), but not on the other tested parameters. We found no influence of sex on either of the common call types. While we found no strong evidence on partners vocal similarity, there seemed to be a tendency for a matched *f*0 and formant dispersion between partners, particularly in the *classic call*.

f0 and body size

Mean fundamental frequency is a common and reliable indicator of body size across taxa[45]. Here, we found that adult body size was reflected in the *f*0 of their *short* calls – that is, larger individuals produced calls of lower fundamental frequencies. This also seems to be the case in the little auk during ontogeny; as the chicks grow, the mean *f*0 of their calls becomes lower, reflecting changes in body size[32]. Although seabirds remain quite understudied in this respect, the same negative relationship between *f*0 and body size has been observed in the African penguins[5]. Other fundamental frequency parameters were shown to correlate with the overall body condition of the great frigatebirds (*Fregata minor*[51]), and crested auklets (*Aethia cristatella*[4]). While it is unclear whether the little auks perceive this difference in vocalisations, it is possible that fundamental frequency parameters may serve as indicators of the individual's overall health, as reflected by body size or motor control of the syrinx[52] – however, dedicated studies would be necessary to understand whether this is in fact the case.

Interestingly, there was no influence of size on the *f*0 of the *classic call*. Little auk call types vary greatly[31], and likely serve very different functions. The *classic call* is a long, multi-syllable vocalisation uttered in a variety of contexts, including by birds sitting inside their nest chambers, escaping predators, flying over the colony alone or in a group. For species that depend on individual recognition to maintain crucial long-term partnerships, life in dense colonies may require extreme adjustments to signal identity[53-55]. In a social situation as complex as the little auk colony, such an elaborated vocalisation may serve as an indicator of identity, maybe at the expense of other information, such as cues to size.

While *f*0 is mainly determined by the length of the larynx in mammals[45], avian syrinx is a much more complex structure, shown to allow for production of size-independent, or even multiple *f*0 within one vocalisation[52]. Our results suggest that seabirds, or at least the little auk, are capable of both conveying honest cues to size (*short call*) and size-independent vocal modulations (*classic call*).

Formants and body size

Because the filtering process in mammals is strictly defined by the anatomical length of their vocal tract, formants are often very good indicators of body size in this group[45]. However, this relationship is neither obvious nor universal across the animal kingdom – particularly in birds, whose vocal production system is both more complex than that of mammals[52], and lacking the strict anatomical constraints by surrounding structures. Some species show modifications that distort the acoustic signal, such as tracheal prolongation[7]. As a result, the sender can not only "sound larger", which is beneficial in species with a preference for larger mates, but also produce signals of lower frequencies and an amplified output, that would propagate better through the environment[9], improving their long-distance communication.

Here, we found no indication of body size in the formant frequencies of little auk *classic* calls. This is in line with previous research on birds, where formant frequencies were shown not to[5,55] or only weakly[10] indicate body size. Unlike the *short call*, the *classic call* is produced with an extended neck (either in flight, or posturing while seated), which might suggest active modification of the output sound. Interestingly, the *classic* call of little auks is often used in situations that might require long-distance transmission: for example, signalling from within a nest chamber[31]. This might imply that this call type is fine-tuned for effective communication at a distance.

While formant frequencies might not be a honest cue to size in birds, they should nevertheless depend on, and hence reflect, the total length of the vocal tract. Here, we were only able to measure the head length, as the distance between the back of the skull and the tip of the beak, which is just a part of the filter and does not reflect the overall vocal tract length. While THL[34] and beak length and/or width[5,51] were used as body size proxies in birds in similar studies, we do recognise this is still not a standard measure, and it might render cross-species comparisons complicated. Since we studied living birds in a no-kill set-up, it was not possible to measure the total length and structure of the vocal tract of each focal individual. Further investigations into the topic might be interesting, should carcasses of naturally deceased birds become available, allowing full measurements and experiments with artificial air-flow through the excised vocal tract[56].

Sex differences

We did not find any evidence for encoding of information about the sex of the caller in the acoustic structure (defined by the 16 acoustic parameters we extracted) in two common calls of little auk calls. The negligible sexual dimorphism in this species[37] could explain the lack of information about sex in parameters that often reflect body size (e.g. fundamental frequency measures or formant dispersion). In addition, spectral properties of seabird calls do not seem to commonly indicate sex (however, see the yelkouan shearwaters (*Puffinus yelkouan*) with extremely reliable vocal differences between sexes[27]). Even species that do show sexual dimorphism in vocal tract anatomy might not encode sex in their vocalisations (as in e.g. herring gull, *Larus argentatus*[55]). However, we could have expected the temporal properties of the calls to differ between the sexes. Here, we specifically looked at the duration of little auk calls in relation to sex – still, there was no effect. In other species, some information on the caller's sex can also be conveyed by the temporal patterns of their vocalisations. For example, king penguins (*Aptenodytes patagonicus*) show a sex-specific syllable arrangement[2]. In the Cape gannet (*Morus capensis*), vocal cues to sex are encoded in the temporal rates of call displays[3]. While it seems unlikely that this is the case with little auk – *classic call* is produced as a single utterance, and the *short call* as a single vocalisation or part of a bout during vocal exchanges with neighbours – no information about calling rates is currently available for the species, and the question remains to be tested. We suggest that other means of sex recognition, such as olfactory cues[57], should be considered in future experiments.

Partner similarity

Little auks mate assortatively regarding various morphological and physiological traits[17,37]. We thus expected to find significant similarities between partners' vocalisations, at least for parameters that were expected to be related to body size. This was not the case for any of the tested parameters of either call types. Since we have also found little effect of body size on the vocal output, the absence of partner similarity could be due to the absence of size encoding in given aspects of adult vocalisations. However, while we showed no statistically significant patterns, there seems to be a tendency for little auk partners to match in their mean $f0$ and formant dispersion (Fig 4., 1-3). Little auk partners are known to match in their physiological profiles[17], namely differences between baseline and stress-induced corticosterone levels. Since vocal output can be influenced by hormones[26], it is possible that the apparent vocal similarity between little auk partners reflects physiological rather than morphological similarities.

Aside from being a result of morphological or physiological similarities, vocal similarity can be a result of vocal learning or social exposure. In some avian species, partners match their calls through a phenomenon termed 'vocal convergence'. For example, raven (*Corvus corax*) partners use similar long-distance calls to improve communication at a distance[58], but otherwise are not vocally akin. Interestingly enough, in the little auk the tendency to match was stronger in the *classic call*, which we believe is used in long-distance communication, than in the short-range *short call*. Little auks share their parenting efforts equally and coordinate their foraging trips[59,30], which likely requires behavioural adjustments between the partners. A long distance call that is easily recognisable between partners could play a role in such coordination. On the other hand, calls of the African penguins (*Spheniscus demersus*) come to be more acoustically similar to their partner's and neighbours' as the animals become more familiar[60]. It is thus possible that species maintaining long-term partnerships will show vocal convergence between partners – and this might be the case of the little auks.

Because the sample size for this analysis was rather small – we were only able to record *classic* calls of both partners in eight nests, and *short* calls in nine nests – further analyses with a larger sample size should be performed to verify these findings. Such data on seabird partners' vocalisations are very rare and challenging to acquire, making even exploratory investigations noteworthy. However, once more data becomes available, this question should be revisited with more statistical power. In particular, access to animals of known relationship history (i.e., newly mated birds vs. long-term partners) would help disentangle the potential physiological and social influences on their vocal output. While such data could be challenging to obtain from free ranging seabirds, experiments in controlled conditions or data collection from more easily accessible models would prove very useful. This could further help us understand whether the matching of certain traits is a result or driver of partnership in different assortatively mating groups[22].

Conclusions

Overall, we found that the fundamental frequency of little auk *short* calls carries information on body size. However, there seems to be no cues to sex in little auk vocalisations. While we found no strong vocal similarity between the partners, there seems to be a tendency to match source and filter parameters – yet more data would be necessary to fully investigate this question. While we do not understand yet how little auks come to form their partnerships, this study indicates that factors other than vocal cues are likely at play.

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Competing interests statement

Authors declare no competing interests.

Data accessibility statement

Raw data generated in this study are available at

https://osf.io/wp2uk/?view_only=feb0554f579c4cc08f6accc3e81af200

SUPPLEMENTARY MATERIALS

Supplementary Text 1

Praat Settings

Here, we provide a detailed description of the acoustic analysis performed in Praat software using a custom-made script (Briefer et al., 2019; Garcia et al., 2016; Reby & McComb, 2003) adjusted for little auk vocalisations (Osiecka et al. 2023). Settings used to extract the 16 acoustic parameters presented in Supplementary Table 1 are described below (Praat commands indicated in brackets; see Supplementary Table 1 for abbreviations of the parameters used).

1. **Duration**. The duration (Dur) was measured as the total duration of each wav file (s), corresponding to individual calls manually extracted from the recordings, based on the visualisation of both the oscillogram and spectrogram.

2. **Amplitude modulation**. AM Var and AM Rate were calculated from the intensity contour of each individual call, using the [Sound: To Intensity] command (minimum pitch = 500 Hz, time step = 0.005 s).

3. **Source-related acoustic features**. *f0* contour of each call was extracted using a cross-correlation method ([Sound: To pitch (cc)] command; time step = 0.005 s, pitch floor = 500 Hz, pitch ceiling = 2000 Hz). We included the following *f0* frequency values: *f0* Start, *f0* End, *f0* Mean, *f0* Min, *f0* Max, Time *f0* Max, *f0* Range. We also measured the *f0* Var and FM Rate.

4. **Spectrum-related parameters.** Q25%, Q50%, and Q75% were measured on a spectrum applied to the whole call, and *f*Peak was measured on a cepstral-smoothed spectrum (command [Create: Cepstral smoothing]; bandwidth = 100 Hz).

Praat spectrogram settings: view range max: 8000; window length: 0.008; dynamic range: 60.

Supplementary Table 1. Sample sizes of the two call types extracted per individual. Individuals are indicated by their unique ring numbers. Grey/white segments indicate nests, i.e. mating pairs.

Ring no.	nest	sex	*classic call*	*short call*	total calls
50161	101	F	3	20	23
48567	101	M	15	40	55
55405	11_58	F	0	4	4
26937	11_58	M	2	34	36
50135	113_10	M	0	12	12
44892	113_41	F	12	40	52
46155	113_41	M	5	40	45
50259	12_16	F	1	0	1
50138	12_16	M	8	4	12
26555	D15_5	F	2	15	17
55401	D15_5	M	1	20	21
43087	D15_6	F	1	20	21
50172	D17_16	M	0	5	5
50496	K17_13	M	1	20	21
55404	K8	F	0	20	20
44931	K8	M	2	0	2
50152	W39	F	2	7	9
46028	W39	M	4	20	24
50167	W39B	F	1	8	9
50327	W39B	M	1	24	25
43817	W40	F	18	40	58
41260	W40	M	10	40	50
44955	W41J	F	7	40	47
26975	W41J	M	30	40	70
43858	W49	M	3	1	4
50099	W6	F	7	21	28
50088	W6	M	3	20	23
		total	**139**	**555**	**694**

Supplementary Table 2. Acoustic parameters extracted for each vocalisation (adapted from Briefer *et al.* 2017 and Osiecka *et al.* 2023).

Abbreviation	**Description**
***f*0 Mean (Hz)**	Mean fundamental frequency value across the vocalisation
***f*0 Start (Hz)**	Fundamental frequency value at the start of the vocalisation
***f*0 End (Hz)**	Fundamental frequency value at the end of the vocalisation
***f*0 Max (Hz)**	Maximum value of the fundamental frequency across the vocalisation
***f*0 Min (Hz)**	Minimum value of the fundamental frequency across the vocalisation
***f*0 Range**	Difference between *f*0 Max and *f*0 Min
Time *f*0 Max (%)	Percentage of the total duration when the maximum F0 frequency occurs within the vocalisation
***f*0 Var (Hz/s)**	Cumulative variation in f0 frequency divided by the total vocalisation duration
FM Rate (s^{-1})	Number of *f*0 modulations divided by the total vocalisation duration
Q25% (Hz)	Frequency value at the upper limit of the first quartiles of energy
Q50% (Hz)	Frequency value at the upper limit of the second quartiles of energy
Q75% (Hz)	Frequency value at the upper limit of the third quartiles of energy
***f*peak (Hz)**	Frequency of peak amplitude
Dur (s)	Duration of the vocalisation
AM Var (dB/s)	Cumulative variation in amplitude divided by the total vocalisation duration
AM Rate (s^{-1})	Number of amplitude modulations divided by the total vocalisation duration

Supplementary Table 3. Kaiser-Meyer-Oklin factor adequacy: results for the *short* and *classic* call types

Raw variable	*short call*	*classic call*
Mean *f0*	0.97	0.82
Start *f0*	0.89	0.79
End *f0*	0.87	0.82
Max *f0*	0.70	0.47
Min *f0*	0.61	0.36
Range *f0*	0.57	0.45
Time max *f0*	0.75	0.59
f0 var	0.64	0.80
FM rate	0.84	0.58
Q25%	0.83	0.47
Q50%	0.55	0.50
Q75%	0.56	0.60
Fpeak	0.87	0.62
AM var	0.40	0.68
AM rate	0.87	0.73
Dur (s)	0.67	0.60
Overall MSA	**0.75**	**0.57**

Supplementary Table 4. Principal Components Analysis: results for the *short* and *classic* call types

	short call			***classic call***		
	Eigenvalue	**Proportion of variance**	**Cumulative proportion**	**Eigenvalue**	**Proportion of variance**	**Cumulative proportion**
PC1	2.46	0.38	0.38	1.83	0.21	0.21
PC2	1.48	0.14	0.52	1.74	0.19	0.40
PC3	1.37	0.12	0.63	1.49	0.14	0.54
PC4	1.15	0.08	0.72	1.12	0.09	0.63
PC5	1.06	0.07	0.79	1.07	0.07	0.70
PC6	0.95	0.06	0.84	1.05	0.07	0.77
PC7	0.85	0.05	0.89	0.96	0.06	0.82
PC8	0.74	0.03	0.92	0.80	0.04	0.86
PC9	0.68	0.03	0.95	0.72	0.03	0.90
PC10	0.57	0.02	0.97	0.71	0.03	0.93
PC11	0.48	0.01	0.99	0.60	0.02	0.95
PC12	0.42	0.01	1.00	0.57	0.02	0.97
PC13	0.12	0.00	1.00	0.51	0.02	0.99
PC14	0.12	0.00	1.00	0.36	0.01	1.00
PC15	0.06	0.00	1.00	0.29	0.01	1.00

Supplementary Table 5. Principal Components Analysis: contributions of raw acoustic parameters to the first five principal components

	short call					***classic call***					
Raw variable	**PC1**	**PC2**	**PC3**	**PC4**	**PC5**	**PC1**	**PC2**	**PC3**	**PC4**	**PC5**	**PC6**
Mean *f0*	-0.94	0.23	0.14	0.01	-0.12	0.19	0.84	-0.28	-0.06	0.25	0.01
Start *f0*	-0.68	0.62	0.22	0.01	-0.16	-0.52	0.27	-0.13	-0.41	0.42	-0.01
End *f0*	-0.96	-0.14	0.10	-0.00	-0.08	-0.22	0.47	-0.49	-0.19	-0.36	-0.08
Max *f0*	-0.97	-0.12	0.09	-0.09	-0.08	0.42	0.84	-0.05	0.02	-0.08	0.10
Min *f0*	-0.73	0.59	0.22	0.13	-0.15	-0.44	0.43	-0.51	-0.48	0.02	0.01
Range *f0*	-0.65	-0.67	-0.07	-0.23	0.02	0.65	0.57	0.23	0.28	-0.08	0.09
Time max *f0*	-0.59	-0.36	0.01	0.41	-0.10	0.21	-0.27	0.21	-0.16	-0.75	-0.11
f0 var	-0.43	-0.50	-0.05	-0.60	-0.27	0.73	0.37	0.11	0.09	0.06	0.15
FM rate	0.29	0.20	0.04	0.33	-0.24	0.28	-0.02	0.17	-0.22	-0.18	0.69
Q25%	-0.55	0.33	-0.16	-0.25	0.41	-0.08	0.48	0.55	-0.13	-0.06	-0.43
Q50%	-0.16	0.25	-0.86	-0.10	0.03	-0.39	0.43	0.74	-0.11	-0.03	-0.13
Q75%	-0.15	0.27	-0.85	-0.07	0.04	-0.69	0.28	0.48	0.11	0.06	0.14
Fpeak	-0.66	0.21	0.08	-0.17	0.34	-0.30	0.05	0.56	-0.15	0.27	0.25
AM var	0.04	-0.04	0.35	-0.02	0.76	0.50	-0.23	0.28	-0.48	-0.20	-0.24
AM rate	-0.53	-0.21	-0.29	0.53	0.10	-0.72	0.09	-0.01	0.29	0.11	0.37
Dur (s)	-0.47	-0.41	-0.20	0.48	0.21	-0.28	0.21	-0.13	0.68	-1.13	-0.29

Chapter IV: Individual distinctiveness across the vocal repertoire

Strong individual distinctiveness across the vocal repertoire of a colonial seabird, the little auk, *Alle alle*

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Abstract

For species maintaining long-term social bonds, particularly in complex networks such as dense colonies, vocal cues to identity can be crucial. Here, we investigated the vocal individuality within and across five call types of the little auk: a monogamous, colonial bird with a strong nest fidelity. Calls produced inside the nest over the incubation period were recorded in 2019 and 2020 and assigned to a call type and individual. We extracted a set of 16 acoustic parameters for each vocalization, and seven additional temporal patterns for a complex, multisyllable call. Calls could be reliably assigned to an individual both within and across call types, although classification performance differed between linear and machine learning methods. All call types showed information content theoretically allowing us to distinguish at least 11 individuals, and up to at least 41 for the classic call. The raw acoustic parameters that contributed most information across the five call types (i.e. the five highest potential of individuality coding scores) were peak fundamental frequency, frequency value at the upper limit of the second and first quartiles of energy, sound duration and amplitude modulation rate. We suggest that this strong individuality across call types and parameters is selected for in the species to help maintain monogamous partnerships, and perhaps social bonds with neighbours, over the years.

Keywords

alcid, Beecher's information statistic, dovekie, individuality, information coding, information content, potential for identity coding, vocal communication

Highlights:

- Little auks are very colonial seabirds with a strong partner- and nest fidelity
- We investigated identity coding in vocalisations of 29 known individuals
- Calls could be reliably assigned to individuals within and across call types
- Their information content allowed distinction up to 41 individuals
- Parameters with the highest PIC covered domains of time, frequency, and amplitude

Introduction

Maintaining stable social bonds requires being able to recognise one's social partners – be it kin, mate, or neighbour. Signalling and individual recognition can rely on many – and often multiple – modalities, such as chemical ("What do you smell like?"), visual ("What do you look like?") or vocal ("What do you sound like?") cues (Tibbetts and Dale 2007). Acoustic signals can travel far and across obstacles, and hence can be used to signal identity at distances larger than smell or sight might allow. For this reason, they are particularly useful for colonial animals that need to locate each other in large aggregations (e.g., Klenova et al. 2012, Favaro et al. 2015, 2016, Calcari et al. 2021, Bowmaker-Falkoner et al. 2022).

Nevertheless, life in dense, noisy groups can pose some specific challenges – for example, vocal communication might need to become particularly complex to convey behaviourally (e.g. Thiebault et al. 2016, Osiecka et al. 2023a) or socially (e.g. Favaro et al. 2015, 2016) important information, such as developing particularly strong vocal stereotypy (Martin et al. 2021). As a result, it has been hypothesised that animals living in socially complex groups should show particularly strong information content in their calls (Pollard and Bulmstein 2011). Since acoustic signals are described in different domains (time, frequency and amplitude) and by different parameters within those domains (e.g. signal length, repetition rate and rhythm within the time domain, or fundamental frequency, entropy, and frequency modulation within the frequency domain), they can potentially carry very elaborate messages. For example, a seabird's vocalisation can (but does not necessarily) at the same time inform about the caller's size (e.g. Favaro et al. 2015, Osiecka et al. 2023b) and behavioural or emotional context (e.g. Thiebault et al. 2019, Osiecka et al. 2023a). However, information encoded in vocalizations, such as individuality cues, can also be lost (Charrier et al. 2001) or maintained (Favaro et al. 2015, 2016) across the vocal repertoire of a species.

Seabird colonies are a great example of complex social networks. They usually consist of animals living in dense, populous groups and maintaining strong social bonds (e.g. Jones et al. 2018, Wojczulanis-Jakubas et al. 2018b, Genovart et al. 2020). Those bonds can find reflection in various aspects of the animals' lives, e.g. how they forage (e.g. Wojczulanis-Jakubas et al. 2018b) or vocalise (Baciadonna et al. 2022). The little auk (*Alle alle*) is the most numerous seabird species of the North Atlantic (Barret et al. 2006). Over the breeding season, little auks nest in densely populated colonies counting up to hundreds of thousands of individuals (Keslinka et al. 2019), usually returning to the same nest and the same partner for many years (Wojczulanis-Jakubas et al. 2020, Wojczulanis-Jakubas et al. 2022). They also coordinate their parenting efforts, taking balanced shifts at self-maintenance and chick rearing (Wojczulanis-Jakubas et al. 2018b). How do they find their partner in the crowded colonies, and how do partners coordinate remains to be investigated. It is however likely that they use vocal cues; little auks are very vocal, and use a complex communication system with at least eight distinct call types that convey fine behavioural contexts (Osiecka et al. 2023a).

Here, we investigated individuality coding within and across five social call types of the little auk (Osiecka et al. 2023a). We examined the following problems: (1) are little auk calls individually specific? (2) can they be reliably assigned to an individual across call types? (3) what is the information content of each call type?, and finally (4) which acoustic parameters are most important for identity coding within and across call types? We expected to find strong individual distinctiveness and high information content across call types, and predicted that this information will be carried by multiple acoustic parameters.

Methods

Ethical note

Fieldwork was performed under permission from the Governor of Svalbard (17/00663-13, 20/00373-2). Little auks nest in chambers in rock crevices and rock debris (Wojczulanis et al. 2022). All animals were gently taken directly from their nesting chambers by hand, and released unharmed directly back into their nest chambers as quickly as possible after handling. Birds were handled by a licensed ringer (KWJ, permit no. 1095, type: C, issued by Museum Stavanger, Norway). All handling procedures followed approved guidelines (Buchanan et al. 2012) and were limited to the necessary minimum (i.e. only standard measurements were taken, and all collected data were subsequently used in multiple research projects of the group to avoid unnecessary repeated handling). The recording equipment was placed at a ~3m distance (cameras) or hidden under rocks (recorders) to minimise disturbance to the animals, and used external power supply and sufficiently large memory cards, to limit human presence at the site (i.e., no battery or memory card changes occurred during the recording sessions).

Study site and ornithological procedures

All data were collected in the little auk colony in Hornsund, Spitsbergen (77°00' N, 15°33' E), one of Svalbard's biggest breeding aggregations of the species (Keslinka et al. 2019). Recordings were made over the incubation period of the 2019 and 2020 breeding seasons. All birds (two per nest, 18 nests in total) were ringed with a unique combination of colour rings at the beginning of each field season.

Recording set-up

Audio material was collected via an Olympus ME-51S stereo microphone (frequency response 100-15,000 Hz) placed inside each nest in such a way as to not disturb the birds' normal activities. Each microphone was connected to an Olympus LS-3 or LS-P4 digital voice recorder (sampling rate 48 kHz, 16 bits) placed outside of the nest and hidden under a rock to prevent both damage to the equipment and disturbance to the animals. Each nest was monitored during three different stages of incubation (early, mid and late). All sessions lasted 48 h, aiming to space them equally in time (i.e., about eight days in between sessions) for all the monitored nests.

Synchronised video material was collected using cameras (commercial HD model of JVC, Japan; time-lapse mode: 1 frame/s) placed in front of the entrance to each nest, to control for the identity of the focal individuals.

Data selection

Video recordings were reviewed in VLC software, noting the exact time each marked individual entered or left the nest. Because the birds were marked with a unique pattern of coloured rings in addition to the standard numbered rings, it was possible to know which exact individual was observed. A custom-made script was used to extract the time intervals at which only one bird was present inside nest, and then extract the corresponding audio fragments. This audio material (a total of over 181 hours) was then manually reviewed in Raven Pro 1.6.4 (Cornell Lab of Ornithology, Ithaca, USA), extracting all individual vocalisations recorded inside the nest. Since not all 36 focal birds were successfully recorded (e.g. they were not the only bird present in the nest and recordings thus had to be excluded to avoid confusion, or did not vocalise during the recording sessions), the final sample size included 29 individual auks (Table 1). Great care was taken to not accidentally include vocalisations coming from outside the nest (i.e. of lower amplitude and/or audible sound distortion due to the burrow's walls), or vocalisations masked by noise. In this way, the resulting extracted vocalisations could be assigned to known individuals. Each call was also assigned to one of the eight call types produced by the species (Osiecka et al. 2023a), five of which were observed in the processed data (*classic, short, short-trill, low trill* and *single* calls; Tables 1 and 2; Figure 1).

Table 1. Number of extracted calls per individual per call type (*classic, low trill, short, short-trill, single*). Individuals are indicated by their unique ring numbers.

Ring no.	sex	*classic*	*low trill*	*short*	*short-trill*	*single*	Total (no.)	Total (%)
26975	M	30	37	40	40	37	184	10.70
41260	M	10	40	40	40	20	150	8.73
44892	F	12	40	40	35	21	148	8.61
43817	F	18	34	40	40	16	148	8.61
48567	M	15	40	40	40	6	141	8.20
44955	F	7	20	40	29	25	121	7.04
46155	M	5	18	40	40	3	106	6.17

26937	M	2	11	34	13	20	80	4.65
55401	M	1	12	20	20	20	73	4.25
50161	F	3	20	20	20	0	63	3.66
50099	F	7	0	21	24	0	52	3.03
55404	F	0	5	20	20	0	45	2.62
26555	F	2	10	15	17	0	44	2.56
50088	M	3	1	20	9	8	41	2.39
50152	F	2	15	7	8	8	40	2.33
50496	M	1	6	20	12	0	39	2.27
50327	M	1	2	24	3	6	36	2.09
46028	M	4	1	20	8	0	33	1.92
43087	F	1	4	20	0	1	26	1.51
50172	M	0	0	5	1	20	26	1.51
43858	M	3	0	1	20	2	26	1.51
50138	M	8	0	4	0	6	18	1.05
50135	M	0	3	12	2	0	17	0.99
50498	M	0	0	0	0	16	16	0.93
50167	F	1	0	8	7	0	16	0.93
50153	F	0	0	0	1	10	11	0.64
55405	F	0	1	4	4	0	9	0.52
50259	F	1	0	0	0	7	8	0.47
44931	M	2	0	0	0	0	2	0.12
	Total	139	320	555	453	252	1719	100

Table 2. Description of little auk call types observed in this study (adapted from Osiecka et al. 2023a).

Call type	**Brief description**
classic call	A complex, multi-syllable call uttered in a variety of contexts
low trill	Low frequency call with strong frequency modulation, used in or in front of own nest when another bird is present
short call	Brief, one-syllable call with a flat frequency contour, used in or in front of own nest when another bird is present
short-trill	Combination of *low trill* and *short* calls, used in or in front of own nest when another bird is present
single call	Brief, one-syllable call with a characteristic up- and down-sweep in frequency contour, used in a variety of contexts

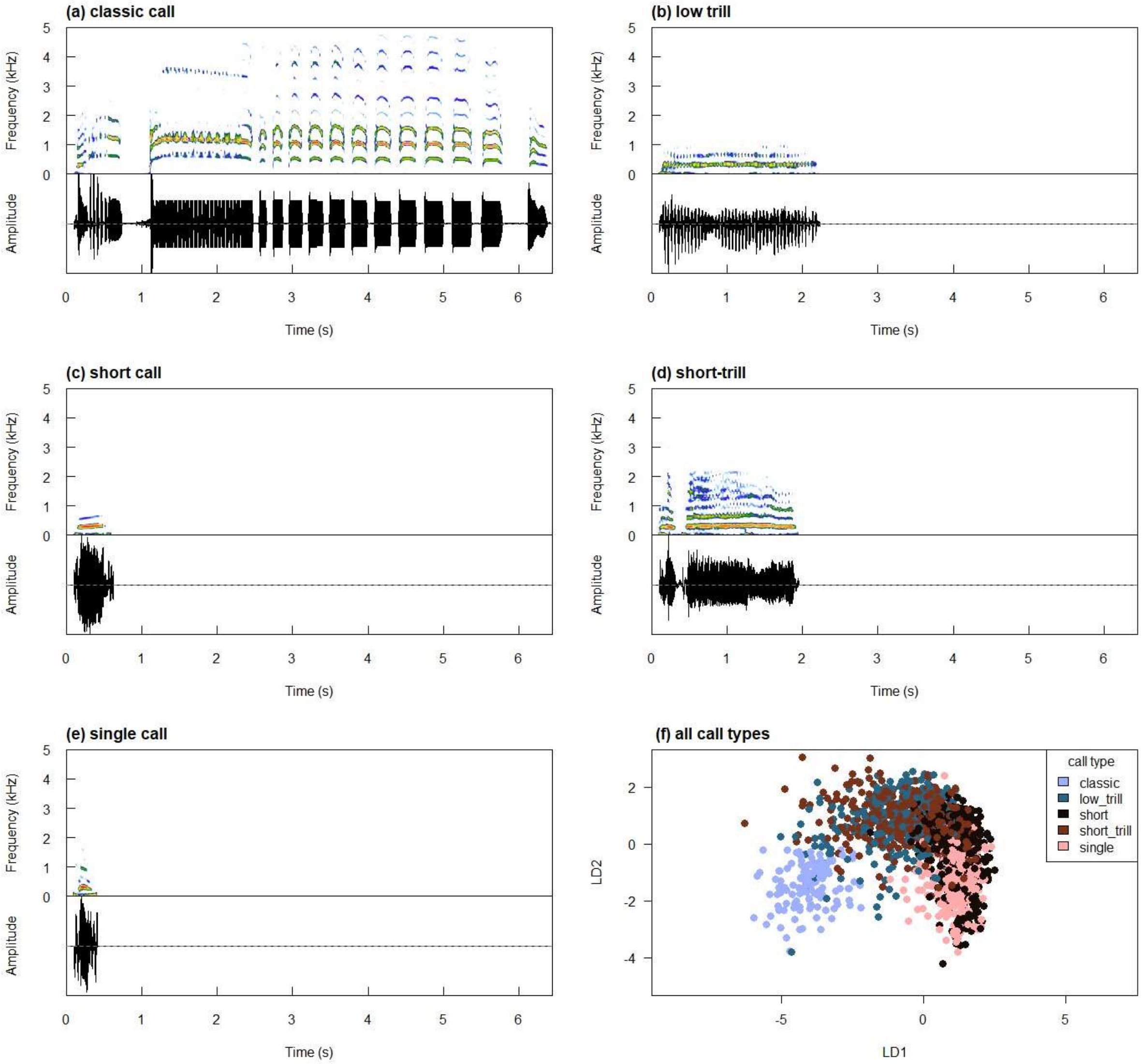

Figure 1. Example calls of the five extracted call types produced by the same individual (male, ring no. 48567), as well as separation of call types in the acoustic space (using calls of all individuals). Spectrograms were plotted using the *seewave* package (Sueur et al. 2008). LD1 represents the linear function that best separates groups (first linear discriminant), and LD2 (second linear discriminant) the uncorrelated second most important source of variation. Colours represent different call types, and each circle a specific vocalisation. Graph uses scientific colour palettes (package *khroma*; Cramieri 2018, Cramieri et al. 2020).

Sound analysis

Calls were analysed in Praat software (Boersma & Weenink, 2022) using a script (Briefer et al., 2019; Garcia et al., 2016; Reby & McComb, 2003) adjusted to the little auk (Osiecka et al. 2023a; Supplementary Text 1), extracting a set of 20 acoustic parameters (specified in Supplementary Text 1). Four parameters were excluded from further analysis because of missing or infinite values, so that 16 raw parameters were retained for further analysis (Table 3).

Table 3. Acoustic parameters extracted for each vocalisation (adapted from Briefer et al. 2017 and Osiecka et al. 2023a).

Abbreviation	**Description**
***f*0 Mean (Hz)**	Mean fundamental frequency value across the vocalisation
***f*0 Start (Hz)**	Fundamental frequency value at the start of the vocalisation
***f*0 End (Hz)**	Fundamental frequency value at the end of the vocalisation
***f*0 Max (Hz)**	Maximum value of the fundamental frequency across the vocalisation
***f*0 Min (Hz)**	Minimum value of the fundamental frequency across the vocalisation
***f*0 Range**	Range of the fundamental frequency across the vocalisation
Time *f*0 Max (%)	Percentage of time when the maximum F0 frequency occurs within the vocalisation
***f*0 Var (Hz/s)**	Cumulative variation in F0 frequency divided by the total vocalisation duration
***f*M Rate (s^{-1})**	Frequency modulation rate
Q25% (Hz)	Frequency value at the upper limit of the first quartiles of energy
Q50% (Hz)	Frequency value at the upper limit of the second quartiles of energy
Q75% (Hz)	Frequency value at the upper limit of the third quartiles of energy
***f*peak (Hz)**	Peak frequency
Dur (s)	Duration of the vocalisation
AM Var (dB/s)	Cumulative variation in amplitude divided by the total vocalisation duration
AM Rate (s^{-1})	Amplitude modulation rate

Temporal parameters

The *classic* call is the only multisyllable call type in the little auk repertoire (Osiecka et al. 2023a). To extract temporal information from these calls, we carefully reviewed all available *classic* calls in Raven Pro 1.6.4. Based on the oscillogram, we annotated the beginning and end times of the three syllable types within the call (A: mid-length beginning syllable; B: long middle syllable; and C: short syllables produced in a series in the last part of the call; Figure 1). To avoid observer and equipment bias, this was done on one day, on the same external screen by one person (ANO). Based on this information, we extracted a set of temporal parameters (Table 4) for each call using a custom-made script (see Supplementary Data). Since some calls that not suitable for spectral analysis (i.e. partially overlapping) could be used here, the obtained dataset was slightly larger, consisting of 179 calls in total.

Table 4. Temporal parameters extracted for each *classic* call.

Abbreviation	Description
Dur A (s)	Duration of the A syllable
Dur B (s)	Duration of the B syllable
Mean dur C (s)	Mean duration of the C syllables
CV C	Coefficient of variation of duration of the C syllables
No C	Number of C syllables in a call
IOI (s)	Mean inter-onset-interval, i.e. time elapsed between the beginning of each two consecutive syllables in the call
CV IOI	Coefficient of variation of IOIs in the call

Statistical analysis

All analyses were performed in R programming language (v. 4.1.3) (R Core Team, 2022) (see the Supplementary Data for the full dataset and code generated in this study).

Principal component analyses (PCA) were performed (*stats* package, function *prcomp*) on the 16 selected acoustic parameters (Table 3) to reduce redundancy. PCAs were performed for all calls pooled together, as well as for each call type and the temporal parameters separately. For each of those PCAs, scores of Principal Components (PCs) with eigenvalues > 1 (Kaiser's criterion; Supplementary Table 1) were selected, and used as input data in the subsequent tests.

Vocal individuality within call types (discriminant function analysis)

For each call type, individuals with five or more extracted calls were identified. From these, five calls were randomly selected for further analysis (for *classic*, *short*, and *low trill* call types separately). Because in the case of the *short-trill* and *single* calls, the lowest available numbers ≥ 5 were higher than five, we randomly selected seven (*short-trill*) and six (*single*) calls for further analysis. This was done to maximise the use of available data while minimising the loss of selected individuals. Because of this, the number of individuals and sample sizes differed across call types – see details in Table 5.

Table 5. Number of available individuals and randomly selected calls per call type used in the DFA (separate call types) and pDFA (all calls) analyses.

Call type	no. individuals	calls per individual	total no. calls
classic	10	5	50
short	23	5	115
short-trill	19	7	133
low trill	14	5	70
single	16	6	96
all calls	29	all available	1719

Those randomly selected calls were then used in discriminant function analyses (DFA) with leave-one-out cross-validation, performed separately for each call type and the temporal data (*MASS* package, function *lda*), with the individuals (ring no.) as a test factor, and with the scores of PCs with eigenvalues > 1 as response variables. Significance of each model was calculated using the Fisher test (*stats* package, function *fisher.test*). Little auk call parameters do not vary between sexes (Osiecka et. 2023b), so that there was no need to correct for this factor.

Vocal individuality across call types (discriminant function analysis)

To investigate the individuality of calls across the vocal repertoire, we performed a permuted discriminant function analysis (pDFA; Mundry and Sommer 2007) pooling all available vocalisations from all individuals and from across the five call types together (Table 5). The pDFA with nested design was conducted using the *pDFA.nested* function of a script provided by Roger Mundry (based on function *lda* of the *MASS* package), using individuals (ring no.) as a test factor, and call types as a restriction factor, using scores of PCs with eigenvalues > 1 as input variables. The pDFA used all available subjects (29 individuals) to derive the discriminant function. We ran a total of 1000 permutations for the analysis.

Vocal individuality within and across call types (support vector machine)

While linear methods such as DFA or pDFA are still the standard in inquiries into vocal individuality, they tend to underperform when compared to machine learning methods (Arnaud et al., 2023). To improve the reliability of this study, we performed an additional analysis employing a support vector machine (SVM) classifier. First, we reduced the data dimensions using supervised uniform manifold approximation and projection (S-UMAP; *uwot* package, *umap* function), with *n_neighbours = 5* because of the modest size of the dataset. This resulted in two-dimensional coordinates subsequently used as input for the SVM. The data were subset into 80:20 training and test datasets, and a classification task was built for each subset (*mlr* package, functions *makeClassifTask;* with individual ring number as target). A learner was then created using *makeLearner* function (*mlr* package), and corrected for individual weights due to uneven sampling of different individuals (*mlr* package, function *makeWeightedClassesWrapper*). The weighted learner was then trained (*mlr* package, function *train*) on the training task, and used to classify the test task (*mlr* package, function *predict*). Classification accuracy of the SVM was extracted using the *performance* function (*mlr* package). This was performed for each call type and the temporal data separately, as well as for all call types pooled together.

Individual information measure

To measure the level of individuality coded within little auk vocalisations, we used Beecher's information statistic, H_s (Beecher, 1989). This measure allows for cross-species comparisons, is a standard, robust method to assess the information capacity of a signal (Linhart et al. 2019), and is calculated as:

$$(1)\quad H_s = log_2\sqrt{\frac{F+n-1}{n}}$$

where H_s is the Beecher's information measure, F is the F value from a random-effects ANOVA, and n is the number of individuals. To calculate H_s, we first normalised the values of raw acoustic parameters (Beecher, 1989), and used these normalised values to perform principal components analyses (*stats* package, function *prcomp*). All PC scores were then entered into the H_s calculation (*IDmeasurer* package, function *calcHS*). We calculated H_s for each of the five call types, as well as for all the calls pooled together. For the *classic* call, we calculated two H_s values: (1) including the 16 parameters (Table 3) extracted for all calls, (2) and including the temporal parameters extracted exclusively for the *classic* calls. The H_s values stand for bits of information, and can be further used to calculate the approximate number of individuals that can be reliably distinguished using a given signal, i.e.

$$(2)\quad 2^{Hs} = number\ of\ distinguishable\ individuals.$$

Potential for individuality coding across call types

To investigate which raw acoustic variables can carry information about the callers' identity, we calculated the potential of individuality coding (PIC; Robisson et al. 1993) for each of the 16 parameters (Table 3), both for each of the five call types separately, and all call types pooled together, as well as for the seven temporal parameters (Table 4; a total of 103 sets of PIC calculations). To do this, we measured the between-individual (CV_b) and within-individual (CV_i) coefficients of variation, using the following formula adjusted for small samples (following e.g. Charrier et al. 2003, Favaro et al. 2015):

$$(3)\quad CV = 100 * \left(1 + \frac{1}{4n}\right) * \frac{SD}{MEAN}$$

where n is the number of calls. We then calculated the mean CV_i for all individuals, and finally used this mean to calculate PIC values as:

$$(4)\ PIC = \frac{CV_b}{CV_i}$$

We further calculated the mean PIC value for each acoustic parameter across call types ($PIC_{parameter}$). For this analysis, we used all available calls of individuals with ≥ 5 calls extracted to calculate CV_b and CV_i.

Results

Individuality coding across call types (discriminant function analyses)

Vocalisations could be correctly classified to an individual within each call type better than by chance (DFA: relative cross-classification level > 3.6, p≤0.002 in all cases; Table 6, Figure 2), as well as across call types (pDFA: relative cross-classification level = 2.95, p=0.001; Table 7). See Figure 3 for examples of individual stereotypy (left panel) and inter-individual variation (right panel) in the *classic call.*

Table 6. Results of the discriminant function analysis for each of the five call types, 16 acoustic parameters in reduced dimensions.

	classic	*classic (temporal)*	*short*	*short-trill*	*low trill*	*single*
No. individuals	10	10	23	20	15	16
Total no. calls	50	50	115	140	75	96
No. calls per individual	5	5	5	7	5	6
Correctly cross-classified (%)	38.00	50.91	40.80	20.71	24.00	51.04
Chance level for cross-classified (%)	10.00	9.09	4.35	5.00	6.67	6.25
Relative cross-classification level	3.80	5.60	9.40	4.14	3.60	8.17
P value for cross-classified	**<0.001*****	**<0.001*****	**<0.001*****	**0.001*****	**0.002****	**<0.001*****

Significance is indicated in bold, and significance levels with asterisks (Fisher test). The relative cross-classification level indicates the ratio between calls correctly cross-classified by the model and by chance.

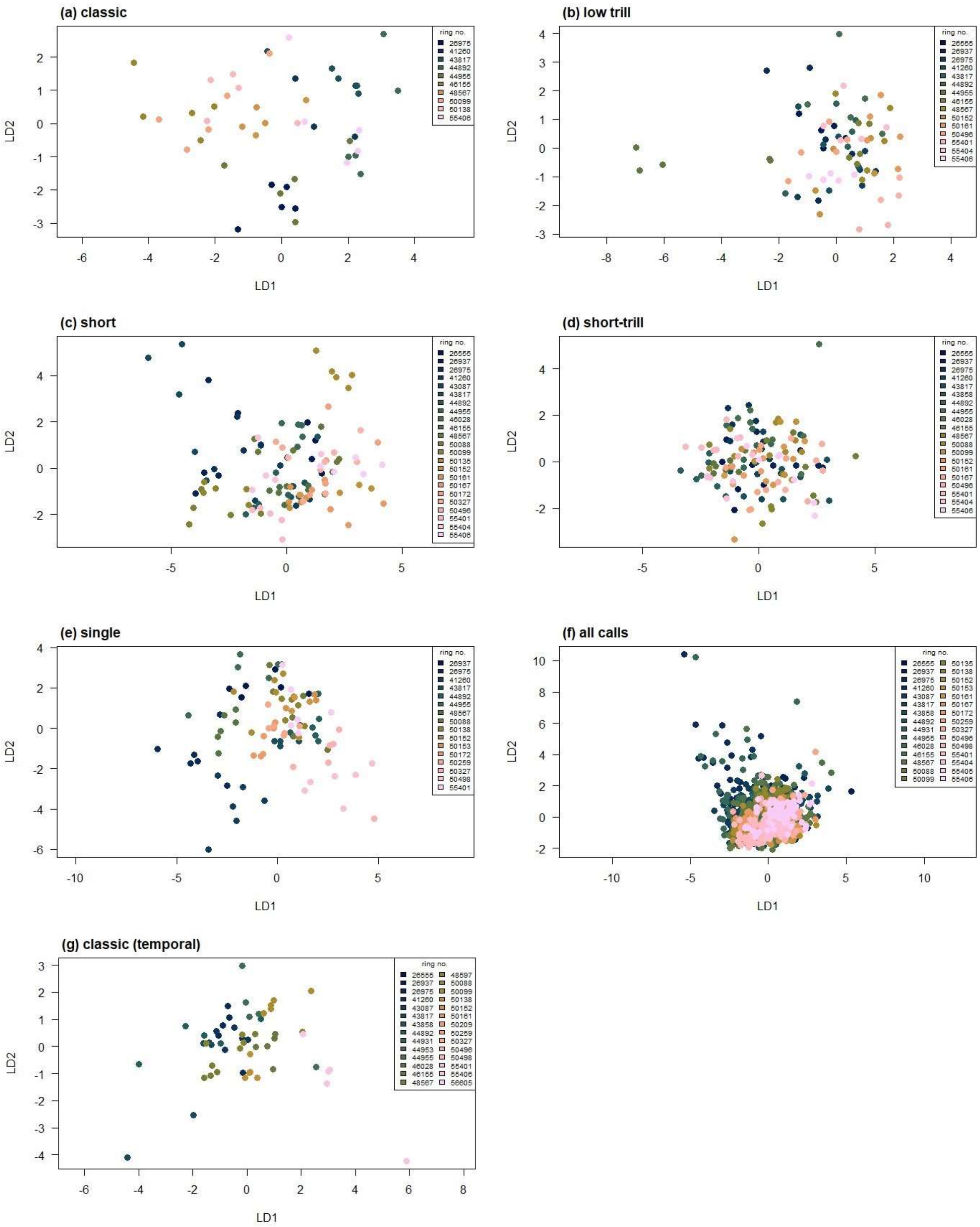

Figure 2. Discriminant function analysis scores for the different call types: a) *classic*, b) *low trill*, c) *shor*, d) *short-trill*, e) *single,* f) all calls pooled together, and g) the temporal patterns of the *classic* call.. LD1 represents the linear function that best separates groups (first linear discriminant), and LD2 (second linear discriminant) the uncorrelated second most

important source of variation. Colours represent different individuals, and each circle a specific vocalisation. Graphs use scientific colour palettes (package *khroma*; Cramieri 2018, Cramieri et al. 2020).

Table 7. Results of the permuted discriminant function analysis for the five call types pooled together, 16 acoustic parameters in reduced dimensions.

	all calls
No. individuals (levels of test factor)	29
Total no. calls	1719
No. calls selected	60
Correctly classified (%)	57.02
Chance level (%)	40.26
P value for classified	**0.005****
Correctly cross-classified (%)	10.93
Chance level for cross-classified (%)	3.71
Relative cross-classification level	2.95
P value for cross-classified	**0.001*****

Significance is indicated in bold, and significance levels with asterisks (Fisher test). The relative cross-classification level indicates the ratio between calls correctly cross-classified by the model and by chance.

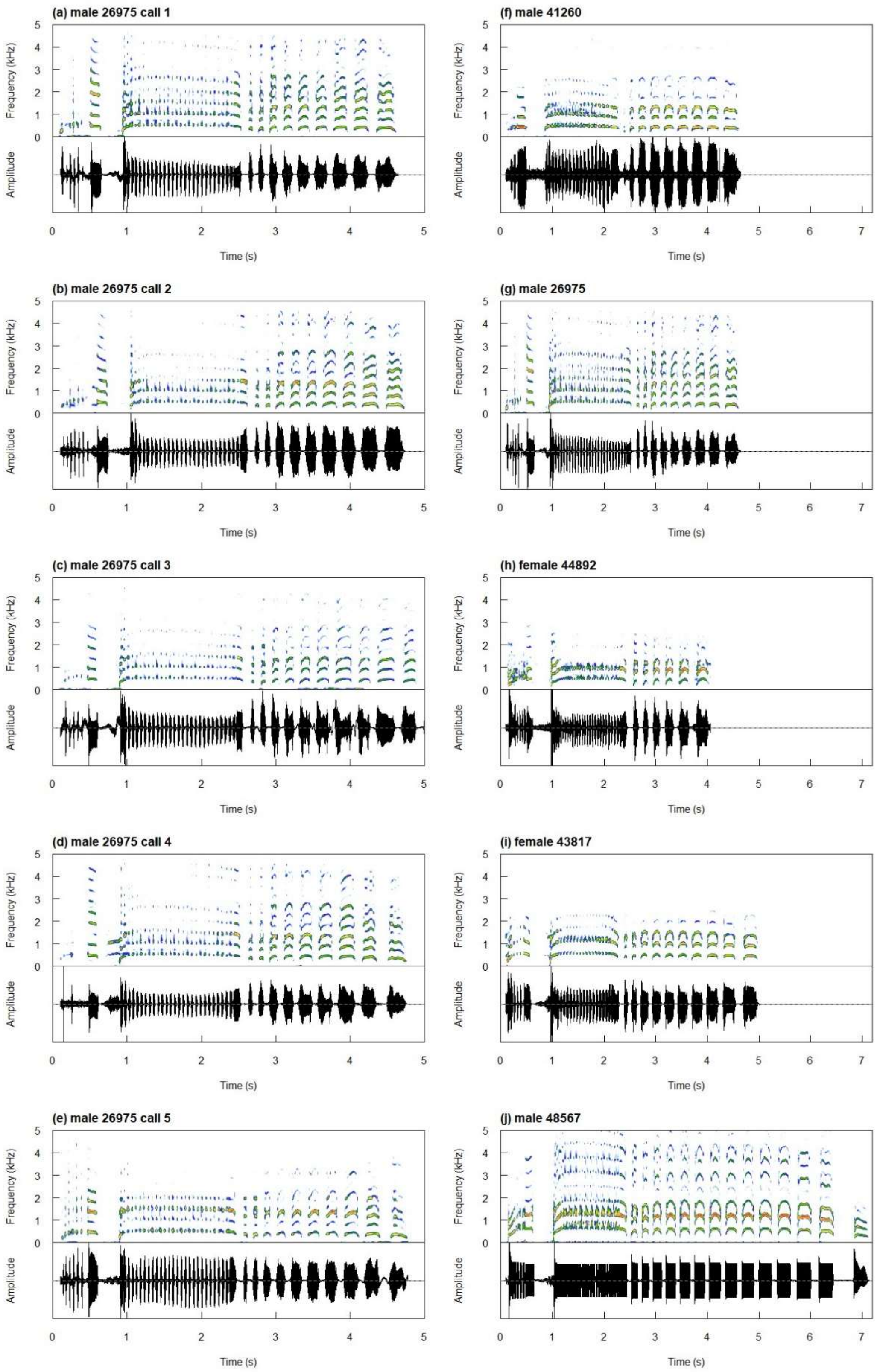

Figure 3. Example *classic calls* produced by the same individual (male, ring no. 26975; left panel) and by five different individuals (right panel). Spectrograms plotted using the *seewave* package (Sueur et al. 2008).

Individuality coding across call types (support vector machine)

Vocalisations could be correctly classified to an individual with varying accuracy, with the best performance for *short-trill, classic,* and *low trill* calls (Table 8, Figure 4).

Table 8. Accuracy of classification to individual using SVM based on S-UMAP reduced data.

Call type	accuracy (%)
classic	56
classic (temporal)	38
low trill	49
short	30
short-trill	60
single	33
all calls	10

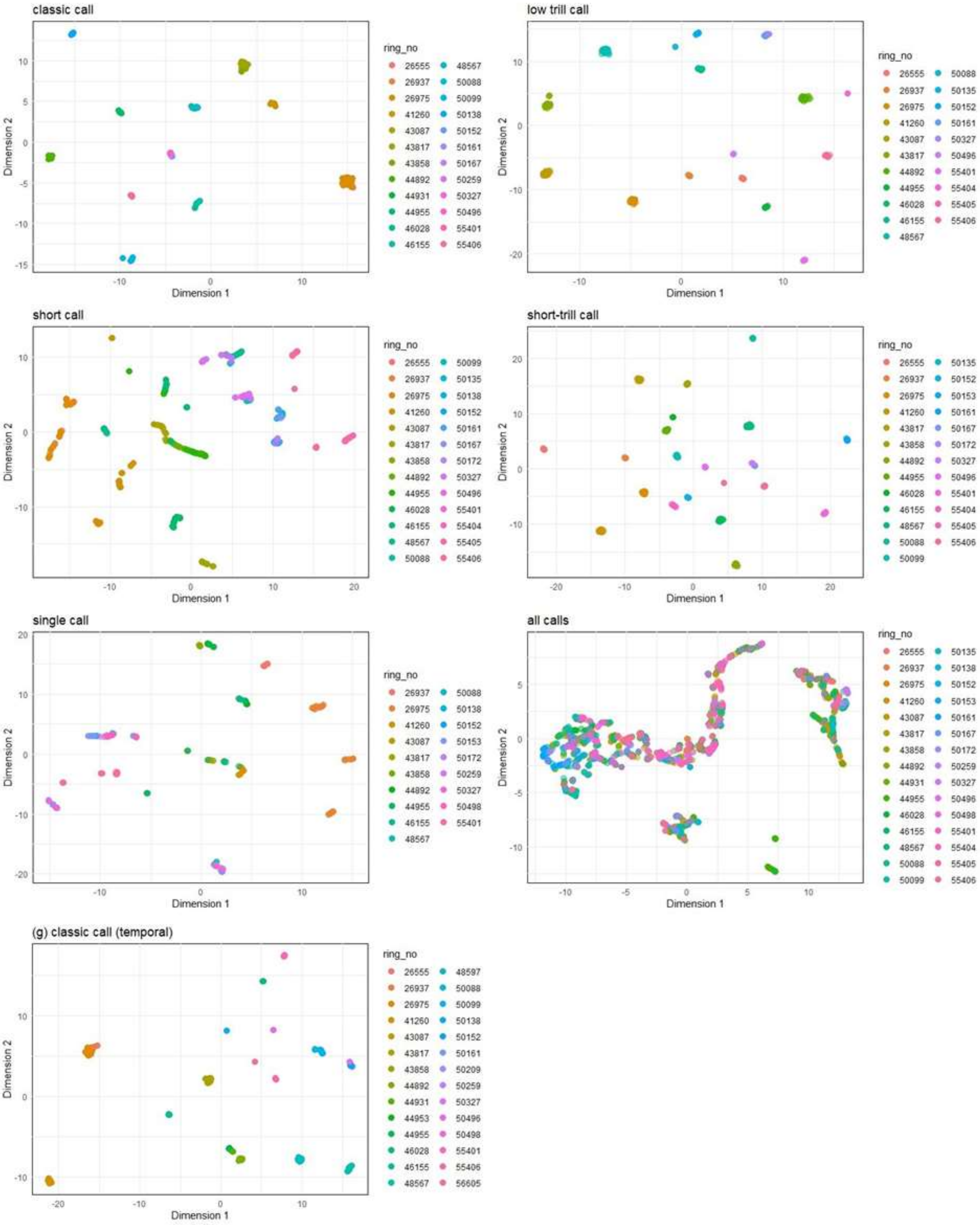

Figure 4. S-UMAP classification to individual for the different call types: a) *classic*, b) *low trill*, c) *short*, d) *short-trill*, e) *single,* f) all calls pooled together, and g) the temporal patterns of the *classic* call shows how these are represented in the acoustic space.

Individual information measure

Each of the call types contained information content that allows for distinguishing across at least 11 individuals, with the *classic* call having the highest number of bits (5.39, i.e. distinguishable across at least 41 individuals; Table 9).

Table 9. Beecher's information statistic across little auk calls, and implications for individual recognition using the significant variables only.

	$H_{s\,all}$	$H_{s\,sig}$	interpretation
all calls	4.51	4.53	allows distinction across at least 23 individuals
classic	5.53	5.39	allows distinction across at least 41 individuals
classic (temporal)	2.68	2.82	allows distinction across at least 7 individuals
short	4.41	4.33	allows distinction across at least 20 individuals
short-trill	3.56	3.58	allows distinction across at least 11 individuals
low trill	3.69	3.66	allows distinction across at least 12 individuals
single	3.99	4.19	allows distinction across at least 18 individuals

H_s *all* represents the H_s summed over all variables in the dataset, and H_s *sig* is the H_s summed over variables that differ significantly between individuals. Note that this measure indicates the information capacity of a signal, and not actual perception or recognition by the animals.

Potential for individuality coding across call types

Most raw parameters showed a considerable potential of individuality coding (PIC_r>1; Table 10) within and across the five call types. Interestingly, the CV of C syllable duration within a *classic* call had the highest PIC of all parameters (17.2; Table 10).

Table 10. Potential of individuality coding of the 16 raw acoustic parameters, calculated for the five call types, and all calls pooled together.

Parameter	**PIC**						
	all calls	*classic*	*short*	*short-trill*	*low trill*	*single*	$\mathrm{PIC}_{\mathrm{parameter}}$
f0 Mean (Hz)	1.47	2.42	1.07	1.51	1.11	1.34	1.49
f0 Start (Hz)	2.30	0.96	1.33	1.11	1.38	1.90	1.34
f0 End (Hz)	1.84	1.87	0.83	1.96	1.25	4.45	2.07
f0 Max (Hz)	1.13	1.69	0.86	2.22	1.20	2.51	1.70
f0 Min (Hz)	3.55	1.80	1.25	0.78	1.17	3.80	1.76
f0 Range	0.73	1.90	1.23	1.47	1.42	2.18	1.64
Time f0 Max (%)	2.51	1.10	0.95	1.99	1.46	2.29	1.56
f0 Var (Hz/s)	0.99	1.96	1.23	1.53	2.20	2.34	1.85
fM Rate (s-1)	0.59	1.23	0.63	1.27	0.88	0.76	0.95
Q25% (Hz)	2.65	0.87	2.81	2.04	6.01	2.84	2.91
Q50% (Hz)	4.09	1.91	7.26	8.40	2.71	4.99	5.05
Q75% (Hz)	1.64	2.58	0.97	2.69	1.33	1.28	1.77
fpeak (Hz)	4.57	1.20	2.23	12.11	6.08	5.61	5.45
Dur (s)	0.68	2.12	1.09	3.10	1.79	4.19	2.46
AM Var (dB/s)	1.74	1.65	0.88	4.28	1.32	2.30	2.09
AM Rate (s-1)	1.95	1.96	1.36	1.71	2.67	3.63	2.27
Dur A (s)	-	1.40	-	-	-	-	-
Dur B (s)	-	1.76					
Mean dur C (s)	-	2.39	-	-	-	-	-
CV C	-	17.20	-	-	-	-	-
No C	-	1.57	-	-	-	-	-
IOI (s)	-	2.16	-	-	-	-	-
CV IOI	-	1.95	-	-	-	-	-

PIC>1 indicates potential identity coding. Most parameters have a high potential of individuality coding, with peak frequency (fpeak) and frequency value at the upper limit of the second quartiles of energy (Q50%) being the most promising across call types.

Discussion

We investigated the vocal cues to individuality in a highly colonial, monogamous seabird, the little auk. Calls differed significantly between individuals, and could be correctly assigned to an individual both within and across call types above chance levels. The information content of each call type allowed distinction across at least 11, and up to at least 41 individuals, with some call types carrying more information than others. Certain acoustic parameters carried particularly strong cues to identity across all call types.

Vocal individuality across call types and parameters

All types of little auk calls carry information on the sender's identity. This identity is retained also across call types, although classification to individuals is necessarily less precise when pooling all call types together. In our analyses, the classification accuracy also depended on the used method, with machine learning likely providing the most reliable indicators. Maintaining strong individuality independently of the signal type is not obvious; it has been shown in some seabird species, such the African penguin, *Spheniscus demersus* (Favaro et al. 2015, 2016), but not others, such as the South Polar skua, *Catharacta maccormicki* (Charrier et al. 2001). Indeed, depending on the function of the call type, coding for individuality might not be needed – for example, while contact calls should inform about the sender's identity, distress calls do not necessarily need to carry such information (Charrier et al. 2001, Linn et al. 2021). In the little auk, every call type, as well as all call types pooled together, showed a considerable PIC across the raw acoustic parameters. This high vocal individuality and the extensive potential of individuality coding across acoustic parameters in little auk calls suggest that communicating one's identity is crucial across different behavioural contexts associated with those vocalisations (Osiecka et al. 2023a).

Fifteen of the 16 raw acoustic parameters showed a considerable potential of individuality coding across the five call types. Among the parameters, peak fundamental frequency, frequency value at the upper limit of the second and first quartiles of energy, sound duration, and amplitude modulation rate showed the highest PIC values across different call types, and were therefore the most promising cues to identity. The fact that these parameters cover the three different dimensions of sound: amplitude, frequency and time domains, is particularly interesting, showing that vocal individuality in the little auk does not rely on a single cue or domain. Information carried by different aspects of a vocalisation's structure is less likely to be lost in transmission – for example, should some of the frequency content be masked by noise, information on the amplitude and temporal parameters may still remain available. Sound propagation models would help understand how information content is retained over the active space of little auk calls.

Information content of the calls

While vocal individuality has been widely studied across species for decades, many publications do not use standardised indexes allowing for comparisons across taxa (Linhart et al. 2019). Here, we looked at the calls' information content using Beecher's information statistic (H_s) – the most robust and reliable of the available indices (Linhart et al. 2019). Species living in large social groups are typically expected to produce calls with a high information content (Pollard and Blumstein 2011), i.e. show high H_s values. Since the little auk lives in colonies counting up to hundreds of thousands of individuals (Keslinka et al. 2019), based on such prediction, one could expect to obtain high H_s values. This was not the case – H_s ranged from 3.58-5.39 across call types, which is comparable to mildly social sciurid rodents (H_s = 4.89-7.76; Pollard and Blumstein 2011), higher than social monk parakeets *Myiopsitta monachus* (H_s up to 2.77; Smith-Vidaurre et al. 2021), and notably lower than the somewhat extreme H_s = 13.7 of the common bottlenose dolphin, *Tursiops truncatus* (Sayigh et al. 2022). These unexpected relatively low H_s values could be a result of the employed methods. For example, Linhart et al. (2019) demonstrated that H_s values tend to be underestimated when the number of parameters used in calculations is high compared to the study population. This was indeed our case – having access to a limited number of individuals, our obtained H_s values likely underestimate the real-life information capacity of little auks signals.

It has been previously shown that vocal individuality is not driven directly by density (Blumstein et al. 2012), and may instead reflect the social complexity of a species. In this light, it may be considered that a social group of a little auk does is not made up of its entire breeding colony, but instead of the nesting partner and perhaps the neighbours. While nothing is known yet about the non-breeding interactions among the little auks, it seems plausible that neighbours of many seasons might develop some social bonds. In fact, most of the little auk's vocal repertoire consists of social calls used with non-partners (Osiecka et al. 2023a). Future studies of social interactions outside of the breeding context would be necessary to shed some light on this little understood seabird.

Consequences for social networks

Vocal parameters may result from individual anatomical differences, such as size or vocal tract length (Budka and Osiejuk 2013, Favaro et al. 2016, 2023). In the little auk, the *short* call has been found to reflect the caller's size (indicated by the total head length; Osiecka et al. 2023b), although this information was not contained in the *classic* call (Osiecka et al. 2023b). The fact that both of these call types can inform on the caller's identity, may indicate that this identity is not simply a result of size or vocal tract length. In fact, although little auks select partners that are phenotypically similar to themselves (Wojczulanis-Jakubas et al. 2018a), partners are not in overall more vocally similar than random birds (Osiecka et al. 2023b). There is, however, a trend to match the *f0* in *short* and *classic* calls, and formant dispersion of the *classic* call between little auk partners (Osiecka et al. 2023b), which together with the lack of size information in little auk calls (Osiecka et al. 2023b) may point towards the influence of vocal learning or social exposure, as in e.g. the African penguin (*Spheniscus demersus*; Baciadonna et al. 2022).

Our findings support the idea that little auk vocalisations serve different social functions (Osiecka et al. 2023a). Little auks maintain long-term partnerships (Wojczulanis-Jakubas et al. 2022) and coordinate their parenting efforts (Wojczulanis-Jakubas et al. 2018b), both of which might require the ability to recognise the partner's vocalisations. Yet not every situation might require strong individual recognition – for example, calls used in direct interactions between two individuals sitting next to each other (e.g., *short, short-trill, low trill*) might not need to convey their identity as strongly as calls used for, e.g., locating one's long-term nesting partner.

The H_s values indicate how many bits of information are carried by a signal, i.e. how many individuals can be discriminated using the information contained in it (Beecher 1989). This information content varied across little auk call types, being the highest in the *classic* call (discrimination of up to 41 individuals). The *classic* call is used across a variety of behavioural contexts (Osiecka et al. 2023a), and since unlike the *short* call it does not carry information about the caller's size, it has been suggested that it might be used as a "signature call", or one that maximises conveying identity information (Osiecka et al. 2023b).

The temporal patterning of a call could indicate individuality in some seabirds (see e.g. Cape gannets, *Morus capensis;* Bowmaker-Falkoner et al. 2022). In the case of the little auk, only the *classic* call shows a complex temporal structure. We expected to find strong cues to individuality within the temporal structure – this was indeed the case, although the temporal patterning of this call did not result in stronger individuality coding of the call type. Interestingly, one of the raw temporal parameters (CV of the C syllable duration) of this call showed a potential for individuality coding much higher than any other raw parameter. Whether this indicates usefulness to the birds or an artifact of an arbitrary parameter choice remains unclear. Dedicated playback experiments on vocal recognition in the species and complex inquiries into the rhythmic structure (see e.g. Burchardt and Knörnschild 2020, Burchardt et al. 2021, Hersh et al. 2023) of little auk vocal interactions would be necessary to assess how temporal information could be used by the species.

In real life, individual recognition is simplified through the use of contextual, visual, and spatial cues. These cues, such as knowing when and where to expect a call and visually recognising the presence of another individual, may assist animals in narrowing down their search area (Beecher 1989). One may also be better at discriminating familiar calls (e.g. Boughman and Wilkinson 1998, Lemasson et al. 2009), or even accommodate to sound similar to one's social circle (as in the African penguin; Baciadonna et al. 2022). This could be absolutely crucial in highly colonial species. In the case of the little auk, an animal and their neighbours are extremely likely to return to the same nests over many years (Wojczulanis-Jakubas et al. 2022). Once the search area – or the arena for social contacts – is limited to the immediate neighbours, vocal discrimination should be much more efficient. In other words – while discriminating between 41 individuals in a colony counting 200,000 does not sound particularly useful, it translates into being able to recognise inhabitants of the 20 neighbouring nests, or at least understanding which of the 41 birds in the neighbourhood is one's nesting partner.

Nevertheless, the fact that a signal carries some information does not necessarily mean that this information is perceived or used by the receiver. This study should be considered as an investigation of the signal structure, and not its perception. Playback experiments would be necessary to understand whether and how is such information used by the little auks in real life – for example, whether the *classic* call might be used to coordinate foraging trips or other behaviour. Data obtained from passive acoustic monitoring can only inform us about the information content of a signal (H_s, PIC) or how reliably signals can be assigned to individual callers (DFA, pDFA). The methods used in this study are a combination of the most commonly used approaches to allow cross-species comparisons – yet all those approaches come with their own strengths and weaknesses (Linhart et al. 2019). Necessarily, this will result in different indications of individuality between methods (e.g., H_s clearly indicates the *classic* call as the carrier of most individual information, whilst DFA and SVM indicate the *short* and *short-trill* calls, respectively, as those with the highest classification accuracy), and employing other tools, could further fine-tune the obtained results (see e.g. Arnaud et al 2023). Ultimately, the reality of any animals' Umwelt is likely much more complex than any study can truly acknowledge.

Caveats and issues

As is often the case with recordings of wild animals (Arnaud et al. 2023), our dataset is heavily imbalanced in terms of how the observations are distributed across individuals and call (Table 1). Yet this is the feasible output of two seasons of passive acoustic monitoring of those individuals. As a result, even though the total number of extracted calls is quite decent, it had to be limited to balance the analysis and avoid statistical issues – in some cases, this meant removing an individual from the analysis, and it other using only a subsample of the many calls available for a given individual. Note that some of the analyses do in fact use all the available calls (for individuals that produced at least five calls), as is the case with H_s and PIC calculations or the SVM classifier. However, these methods already contain a correction for weights and sample sizes within them. We are confident that following these restrictions to the sample size, the best use was made of what the available data, and trust this rare dataset will prove a valuable contribution.

In this study, we aimed to provide analyses that can be easily compared both across species, and within the little auk call types. This resulted in some necessary limitations: for example, since only the *classic* call shows a formant structure (Osiecka et al. 2023a, 2023b), we have decided to exclude formant frequencies from our analysis, even though it has the potential to indicate vocal individuality in birds (e.g. Budka and Osiejuk 2013).

Conclusions

Overall, we found strong individual distinctiveness within and across the little auk call types. The information content was particularly large for the *classic* call, suggesting it may be used as a “signature call”. Nearly all acoustic parameters showed a high potential for identity coding, also across call types – additionally, PIC values were highest parameters describing aspects of both frequency and amplitude, which indicates that vocal cues to identity are not limited to one domain. While these results do not indicate whether and to what extent is this information actually perceived by the animals, this study suggest that a particularly reliable vocal recognition system is likely present in the species.

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Competing interests

Authors declare no competing interests.

Data availability statement

Raw data and the full code generated in this study are available at

https://osf.io/q9xhd/?view_only=2b8dd1470996468ea8f961d35070d1e5

SUPPLEMENTARY MATERIALS

Supplementary Text 1

Praat Settings

Here, we provide a detailed description of the acoustic analysis performed in Praat software using a custom-made script (Briefer et al., 2019; Garcia et al., 2016; Reby & McComb, 2003; Osiecka et al. 2023a). Settings used to extract the 20 acoustic parameters presented in Table 3 are described below (Praat commands indicated in brackets; see Table 3 for abbreviations of the parameters used).

1. **Duration**. The duration (Dur) was measured as the total duration of each wav file (s), corresponding to individual calls manually extracted from the recordings, based on the visualisation of both the oscillogram and spectrogram.

2. **Amplitude modulation**. AM Var, AM Rate, and AM Extent were calculated from the intensity contour of each individual call, using the [Sound: To Intensity] command (minimum pitch = 500 Hz, time step = 0.005 s).

3. **Source-related acoustic features**. *F0* contour or each call was extracted using a cross-correlation method ([Sound: To pitch (cc)] command; time step = 0.005 s, pitch floor = 500 Hz, pitch ceiling = 2000 Hz). We included the following *f0* frequency values: *f0* at the start (*f0* Start) and at the end (*f0* End); the mean (*f0* Mean), minimum (*f0* Min) and maximum (*f0* Max); percentage of time when the maximum *f0* frequency occurs within the vocalisation (Time *f0* Max); the *f0* mean absolute slope (*f0* Abs Slope); and the *f0* range (*f0* Range). To characterise *f0* variation along the call, we measured the mean *f0* variation per second (*f0* Var) calculated as the cumulative variation in the *f0* contour in Hertz divided by call duration. Finally, we measured the number of complete cycles of *f0* modulation per second (*f*M Rate) and the mean peak-to-peak variation of each *f0* modulation (*f*M Extent) .

4. **Spectrum-related parameters.** Q25%, Q50%, and Q75% were measured on a spectrum applied to the whole call, and *f*Peak was measured on a cepstral-smoothed spectrum (command [Create: Cepstral smoothing]; bandwidth = 100 Hz).

5. **Noise**. Harmonicity (Harm) was measured using the [Sound: To Harmonicity (cc)] command (time step = 0.005 s, minimum pitch = 500 Hz, silence threshold = 0.2, periods per window = 1).

*f*M Extent (Hz), *f*0 Abs Slope (Hz), AM Extent (dB) and Harm (dB) could not be extracted from some of the calls. All other parameters could be measured in all calls.

Praat spectrogram settings: view range max: 8000; window length: 0.008; dynamic range: 60.

Supplementary Table 1. Eigenvalues (SD) and the cumulative proportion of variance (CP) explained by the principal components. PCs of eigenvalues >1 were selected as input variables for further analysis.

	classic		*short*		*short-trill*		*low trill*		*single*		*all calls*		*classic (temporal)*	
	SD	**CP**	**SD**	**CP**	**SD**	**CP**	**SD**	**CP**	**SD**	**CP**	**SD**	**CP**	**SD**	**CP**
PC1	1.80	0.20	2.46	0.38	2.09	0.27	2.02	0.26	2.32	0.34	2.18	0.30	1.49	0.32
PC2	1.70	0.38	1.48	0.52	1.67	0.45	1.74	0.44	1.67	0.52	1.58	0.45	1.27	0.55
PC3	1.60	0.54	1.37	0.63	1.59	0.61	1.49	0.58	1.35	0.63	1.50	0.60	1.19	0.75
PC4	1.27	0.65	1.15	0.72	1.37	0.72	1.33	0.69	1.18	0.72	1.28	0.70	1.00	0.89
PC5	1.06	0.72	1.06	0.79	0.96	0.78	1.01	0.76	1.10	0.79	1.04	0.76	0.74	0.97
PC6	0.99	0.78	0.95	0.84	0.89	0.83	0.94	0.81	0.95	0.85	0.90	0.82	0.35	0.99
PC7	0.85	0.82	0.85	0.89	0.79	0.87	0.81	0.85	0.86	0.90	0.85	0.86	0.30	1.00
PC8	0.82	0.86	0.74	0.92	0.76	0.91	0.76	0.89	0.76	0.93	0.78	0.90	-	-
PC9	0.75	0.90	0.68	0.95	0.65	0.93	0.67	0.92	0.63	0.96	0.66	0.93	-	-
PC10	0.68	0.93	0.57	0.97	0.54	0.95	0.62	0.94	0.52	0.97	0.61	0.95	-	-
PC11	0.60	0.95	0.48	0.99	0.54	0.97	0.58	0.96	0.45	0.99	0.50	0.96	-	-
PC12	0.56	0.97	0.42	1.00	0.49	0.98	0.46	0.98	0.33	0.99	0.48	0.98	-	-
PC13	0.49	0.99	0.17	1.00	0.37	0.99	0.40	0.99	0.29	1.00	0.43	0.99	-	-
PC14	0.36	1.00	0.12	1.00	0.33	1.00	0.37	1.00	0.16	1.00	0.38	1.00	-	-
PC15	0.30	1.00	0.06	1.00	0.20	1.00	0.30	1.00	0.05	1.00	0.20	1.00	-	-
PC16	0.00	1.00	0.00	1.00	0.00	1.00	0.00	1.00	0.00	1.00	0.00	1.00	-	-

Chapter V: Propagation of little auk social calls

Long distance calls: negligible information loss of seabird social vocalisations over propagation down to the hearing threshold

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ABSTRACT

How well does the information contained in vocal signals travel through the environment? To assess the efficiency of information transfer in little auk calls over distance, we selected two social call types with the highest potential for individuality coding among the social call types of the species. Using available recordings of known individuals, we calculated the apparent source levels, with apparent maximum peak sound pressure level (ASPL) of 63 dB re 20 μPa at 1 m for both call types. Further, we created a sound propagation model using meteorological data collected in the vicinity of the little auk colony in Hornsund, Spitsbergen. Using this model, we simulated call propagation up to the putative hearing threshold of the species, calculated to equal ASPL of signals propagated to roughly one kilometre. Those propagated calls were then used in a permuted discriminant function analysis, support vector machine models, and linear models of Beecher's information statistic, to investigate whether transmission loss will affect the retention of individual information of the signal. Calls could be correctly classified to individuals above chance level independently of the distance, down to and over the putative physiological hearing threshold. Interestingly, the information capacity of the signal did not decrease with propagation. While this study touches on signal properties purely and cannot provide evidence of the actual use by the animals, it shows that little auk signals can travel extreme distances with negligible information loss. For the animals, this could mean that they can recognize calls of the members of their social networks as far as those calls are actually audible, and support the hypothesis that vocalisations could facilitate long-distance communication in the species.

Keywords

Alle alle, active space, information coding, source level, transmission of information, vocal individuality

Introduction

The ability to recognise one's social partner – e.g. offspring, mate, or neighbour – is necessary to maintain stable social bonds. Colonial animals, such as seabirds, often rely on vocal cues to find each other in crowded aggregations (e.g., Klenova et al. 2012, Favaro et al. 2015, 2016, Calcari et al. 2021, Bowmaker-Falkoner et al. 2022). But how reliable is such communication at a distance?

While under some conditions, acoustic signals can travel over extreme distances (e.g. a blue whale's song theoretically travelling through the oceans), this is not always the case. The propagation of a soundwave, i.e. how it moves through and changes in an environment, depends on a number of factors. First of all, signals of lower amplitudes will degrade much faster due to spherical spreading, than louder sounds. Additionally, as the sound propagates, its higher frequency content will be gradually filtered out, leaving only the lower frequency components at larger distances (and finally filtering these as well). How exactly this filtering will occur, and how fast will a soundwave travel, will be impacted by the medium in which it is travelling – its density, humidity, pressure, and more. At some point, a signal's amplitude will be so low, and/or its frequency content so degraded, that it will no longer carry the information first encoded in it by the sender – and of course, as a result, the receiver will not be able to decode it.

Little auks (*Alle alle*) are highly colonial seabirds navigating complex social networks (Wojczulanis-Jakubas *et al.* 2022). Little auks are also very vocally active (Osiecka *et al.* 2023a), and their calls can carry a richness of static (Kidawa *et al.* 2023, Osiecka *et al.* 2023b, 2024a) and dynamic (Osiecka *et al.* 2023a, 2024b) information. The most complex call of the little auk repertoire, the *classic* call, is a long, compound signal with apparent formants, composed of a series of three types of syllables (Fig. 1; Osiecka *et al.* 2023a). It is produced in a range of contexts, both by animals sitting inside their rocky nest chambers, and in flight, e.g. by birds returning to the colony from the foraging grounds (Osiecka *et al.* 2023a). While it carries no information on the caller's sex or size, nesting partners tend to match certain properties of their *classic* calls (Osiecka *et al.* 2023b). This vocalisation carries reliable information on the sender's identity, mostly within its spectral centre of gravity, fundamental frequency, duration, amplitude modulation rate, and frequency variation (in this order; Osiecka *et al.* 2024a), and has a higher information capacity than any other call type of the species (Osiecka *et al.* 2024a*)*. The *classic* call likely plays a role in long-distance communication, possibly facilitating coordination of social behaviour. Therefore, it is likely to remain stable over behaviourally useful distances.

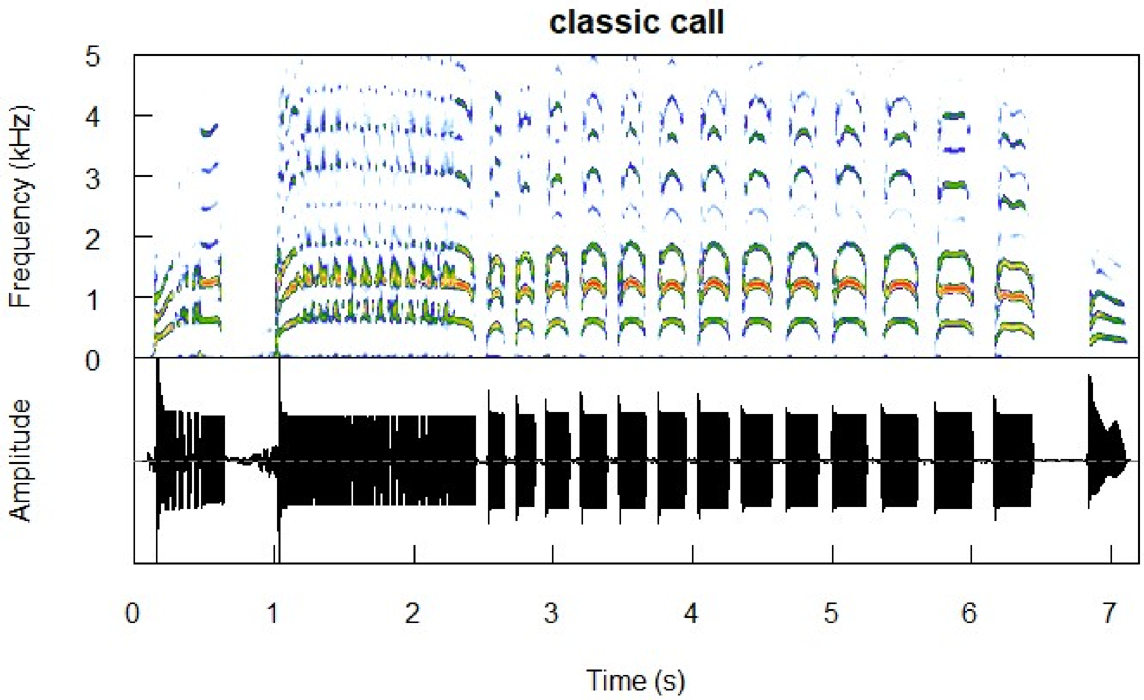


Figure 1. A sample *classic* call produced by an adult male (ring no. DA48567). Spectrogram plotted using the *seewave* package (Sueur *et al.* 2008).

Another call emitted in a range of situations, and both inside the nests and in flight, is the *single* call; a brief, one-syllable vocalisation (Fig. 2; Osiecka *et al.* 2023a). Like all little auk call types, this call is highly individually specific, and can be classified to an individual with the highest precision among all call types (Osiecka *et al.* 2024a). While the exact function of this call remains unknown, due to its short duration (less than 0.5 s; Osiecka *et al.* 2023a) and simple structure, it can be expected to serve in short-distance communication.

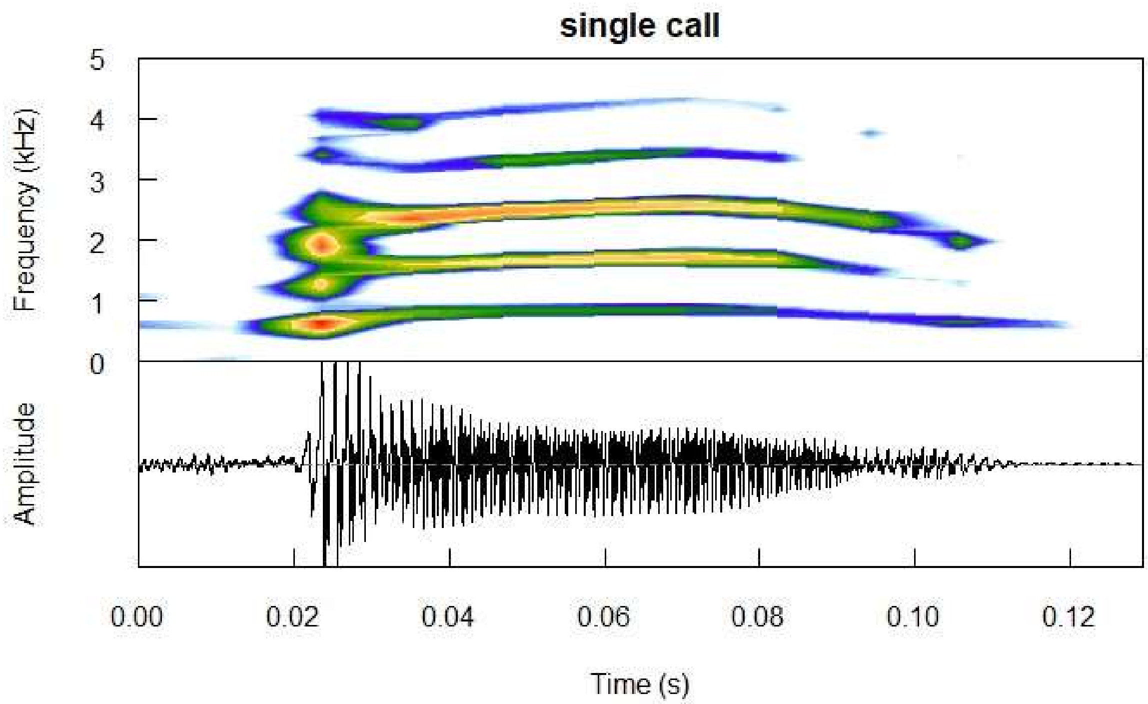


Figure 2. A sample *single* call produced by an adult male (ring no. DA48567). Spectrogram plotted using the *seewave* package (Sueur *et al.* 2008).

Here, we investigated how well the identity information encoded in the *classic* and *single* calls, which are both used by little auks throughout the entire breeding season, is maintained over distance, purely from a signal perspective, i.e. the transmission-related changes to carrying capacity of the vocal communication channel. To do so, we created a theoretical propagation model using local meteorological data, and, using sample calls of the two aforementioned types recorded from known individuals, we simulated call propagation down to the putative hearing threshold. We then investigated the information content of those propagated calls.

Methods

All analyses were performed in Python v. 5.11 (Rossum and Drake 1995) and R environment (v. 4.1.3), and full codes together with raw data have been provided in the supplementary materials (see Data availability statement). Visualisations use scientific colour palettes (Cramieri 2018 ; Cramieri *et al.* 2020) from package *khroma*.

Study site and subjects

This study used previously published acoustic recordings (see detailed description below; Osiecka *et al.* 2024a). These recordings were collected during fieldwork in Hornsund, Norwegian High Arctic, over the incubation period in 2019-2020, under permit from the Governor of Svalbard (20/00373-2). This included handling (e.g. colour-ringing and measuring) the birds for standard ornithological procedures by a licensed ringer (KWJ, permit no. 1095, type: C, issued by Museum Stavanger, Norway), thanks to which we were able to identify the focal individuals (see description below in the *Acoustic data* section). This study focused on 18 nesting pairs, i.e. 36 birds in total.

The study colony in Hornsund is comprised of the lower: 59-90 m a.s.l. and upper plot of the colony: 122-172 m a.s.l. Little auks maintain their flight height above their colony plots, and only descend for landing. For the purpose of this study, we selected 100 m as a representative flight height for the lower plot, i.e. the animals recorded in this study. This choice to select a flight height lower than the upper plot was made as a conservative measure to avoid accidentally increasing the modelled active space of little auk sounds (see model details below).

Acoustic data

Audio material was collected via an Olympus ME-51S stereo microphone (-40 dB sensitivity at 1 kHz, frequency response 100-15,000 Hz +/-3 dB) placed inside each nest (a rock crevice/chamber, with floor covered with pebbles, Wojczulanis-Jakubas *et al.* 2022) at approximately 10 cm from the birds inside, in such a way as to not disturb the birds' normal activities. Each microphone was connected to an Olympus LS-P4 digital voice recorder (sampling rate 48 kHz, 16 bits, high gain) placed outside of the nest chamber and hidden under a rock to prevent both disturbance of the animals and damage to the equipment. Each nest was recorded three times over the incubation period, with recording sessions lasting 48 h and spaced about equally in time (i.e., around eight days in between recording sessions).

Sound recordings were paired with video monitoring of the nest entrance, so that we could see the birds entering and exiting their nesting chambers and extract the times at which only one known (ringed with a unique colour code) individual was present inside each nest chamber. Audio recordings from those periods were manually processed, resulting in the acoustic database of vocalisations produced by known individuals inside the nest. For more details on the field procedures, refer to Osiecka *et al.* 2024a.

Apparent sound pressure level

To calculate the real-life sound pressure levels from the collected recordings, we first calibrated the equipment. First, a class II sound level meter (Volcraft SL-451) was calibrated with a class II sound level calibrator (Volcraft SLC100) following instructions provided by the producer. Then, a 1 kHz tone was played using a JBL Flip 5 loudspeaker placed at 1 m from the recorder and sound level meter, and recorded with the same equipment and set-up as used in the field recordings. The obtained recording was used in end-to-end calibration of all digital audio recordings in Raven Pro 1.6.5, following the software specifications (https://ravensoundsoftware.com/knowledge-base/calibrating-recordings-in-raven-pro/).

Back-calculated sound pressure levels are termed *apparent sound pressure levels* (hereafter 'ASPL', Møhl *et al.* 2000). ASPL (dB rms re. 20 µPa) at 10 cm of each vocalisation was extracted in Python using *numpy* package to obtain peak (i.e. the highest absolute magnitude of the signal) and root-mean-square (RMS, i.e. the RMS amplitude over signal duration, using the 95% energy threshold criterion; Madsen and Wahlberg 2007) values. The ASPL at 1 m, i.e. the Source Level (SL), was the calculated as:

$$SL_{1m} = ASPL_{10cm} - 20\ log_{10}(\frac{100cm}{10cm})$$

To estimate a global mean of the ASPL values at 1 m, we first calculated the mean ASPL value for each individual, followed by a population mean. This was done for both call types, with peak and RMS values used separately. The obtained mean values were then compared between the call types using Welch two sample t-test (function *t.test*).

Meteorological data

Long-term geosystem monitoring data are publicly available from the Polish Polar Station in Hornsund, Institute of Geophysics, Polish Academy of Sciences (https://monitoring-hornsund.igf.edu.pl). For the purpose of this study, we selected data from 1983-2021, for which full meteorological information was available (as per August 2023, when the analysis was performed), focusing on May-August, i.e. the breeding period of the little auk (Wojczulanis-Jakubas *et al.* 2022). Because those months are characterised by very different mean temperature, pressure, and relative humidity values (Fig. 3 – and therefore different sound attenuation properties – we have considered each month separately, using the 40-years average of each month in the following analyses.

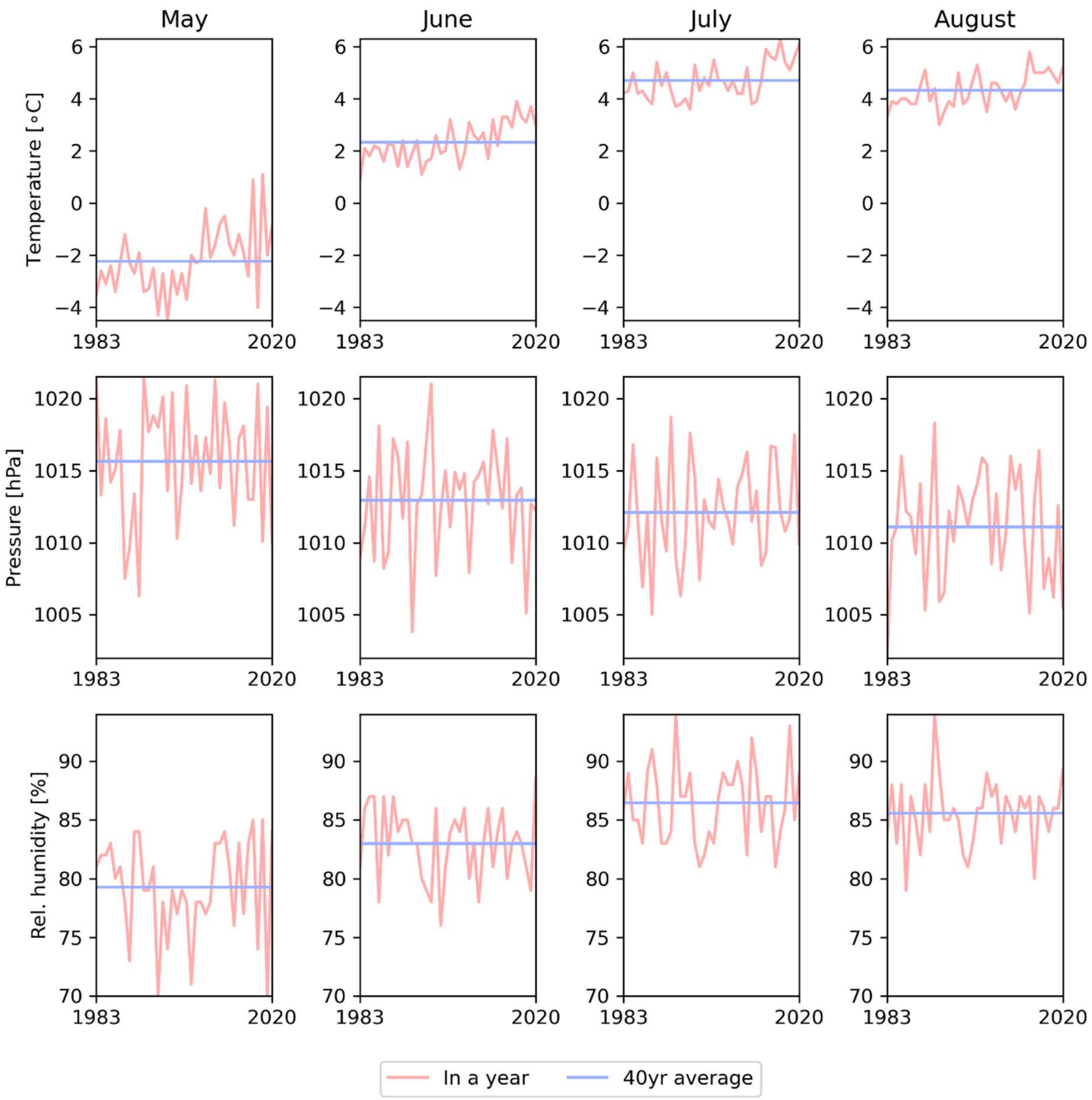


Figure 3 Annual (pink) and 40-year average (blue) meteorological data from Hornsund over the little auk's breeding period (May-August).

Propagation model

To model propagation of signals over distance, we used a spherical spreading model with the atmospheric absorption factor α based on the ISO 9613–1 standard (ISO 1993). The spherical spreading model describes how the energy of different frequency components of the signal changes over distance, working somewhat as a low-pass filter (i.e. the energy content of higher frequencies is lost earlier over propagation).

Note that this model comes with necessary simplifications: that is, it assumes simple spreading in perfect conditions, i.e. without added noise, in the absence of wind, and excluding excess attenuation. Simple spherical spreading was chosen based on the following: (1) We decided to model propagation of calls produced in flight, and not in the nest, to simplify the model. Therefore, the signal source is an individual bird in flight, that is roughly 100 m over ground. Therefore, this model is simplified to omit the impact of local topography on sound propagation (see Guibard *et al.* 2022). However, note that the calls used here were recorded inside the nest, since this is the only way we can control for the birds' identity. The implications of this are addressed in the Discussion; (2) The Hornsund ornithogenic tundra is an open habitat with a dense vegetation cover composed of species reaching a maximum of approximately 20 cm in height (Zmudczyńska *et al.* 2009), therefore expected to minimally degrade acoustic signals (Hardt and Benedict 2020); (3) The dense vegetation cover creates a soft substrate, so contribution of reflections is expected to be minimal; and (4) diel variations in meteorological conditions during the Arctic day are dictated by sea ice conditions rather than time day-night cycles (Osuch and Wawrzyniak 2017), and so reflections from different layers of the atmosphere are also expected to be minimal.

The ISO 9613-1 standard gives fitted equations for atmospheric attenuation α as a function of frequency that is dependent on temperature, pressure and relative humidity of the air. The model is valid at altitudes below 10 000 meters, and so well within our case. As described above in the Meteorological data section, we used the local mean monthly values of relevant parameters, , and subsequently α was calculated on those mean monthly values. We used the average values of the entire monitored period (1983-2021) rather than climate change-related patterns, since there was no apparent change in sound attenuation properties over the decades (Fig. 4).

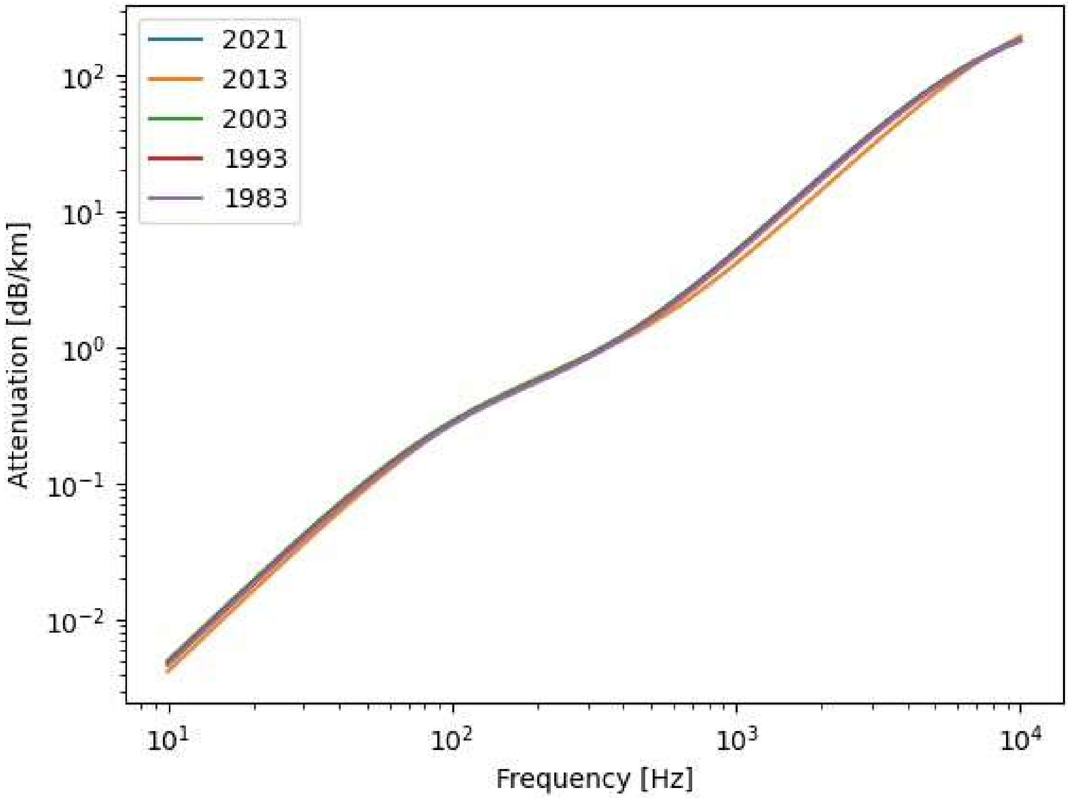


Figure 4. Sound attenuation at different frequencies, calculated from mean May conditions in Hornsund over the monitored period 1983-2021, based on the ISO 9613-1 standard. There is no apparent shift in attenuation profiles over the years.

The resulting spherical spreading model is given by the following equation:

$$ASPL(r,f) = ASPL_{1m}\frac{1m}{r^2}e^{-\alpha(\mathrm{f})\,\mathrm{r}}\ [\text{dB re 20 µPa}]$$

Where e^x is the natural exponential function, r is the distance (in metres), and α is a function of frequency as per ISO 9613-1. The full code of the propagation model is available in the Supplementary Materials.

Choice of the propagation distances

Since there is currently no information available on the hearing thresholds of the little auk, we used the in-air auditory measurements of another, related diving alcid species, the Atlantic puffin

(*Fratercula arctica*), as a reference. The average physiological hearing threshold (measured using auditory evoked potential methods) in the alcids seems relatively similar across species, namely down to 10-20 dB re 20 µPa in the 1-2.5 kHz frequency range for the Atlantic puffin (Mooney *et al.* 2020), down to 13 dB re 20 µPa in the 1-3.5 kHz range in the common murre (*Uria aalge*; Smith *et al.* 2023a), and down to 17 dB re 20 µPa in the 1-3.5 kHz range for the marbled murrelet (*Brachyramphus marmoratus*; Smith *et al.* 2023b). We chose 1000 m as the maximum propagation distance, with calculated ASPL at this distance roughly corresponding to the minimum physiological hearing threshold (i.e. the lowest SPL within the studied frequency range that still elicited brain activity during experimental procedures) of the Atlantic puffin (Mooney *et al.* 2020; Fig. 5).

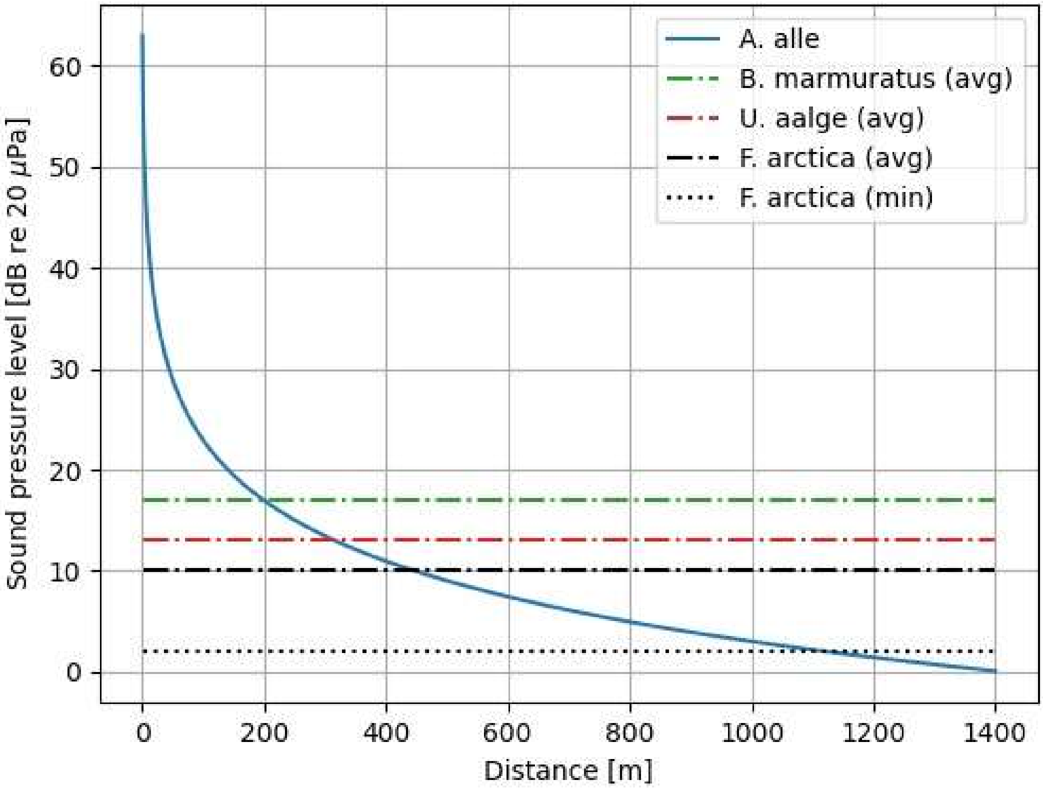


Figure 5. Average (broken lines) and minimum (dotted line) physiological hearing thresholds of three alcid species allow us to predict the expected distances at which the little auk vocalisation can still be heard (based on simple spherical spreading, i.e. -6 dB re 20 µPa per doubling of distance).

The propagation model used calibrated recordings of known individuals at 10 cm as input files. Each file was propagated (i.e., modelled in meteorological conditions for May-August separately) to 1, 2, 4, 10, 21, 46, 100, 215, 464, and 1000 m (from here on, 1-1000 m), creating a separate audio file as an output. In other words, each original call was propagated to 10 distances in mean conditions of four separate months, that is 40 times in total. Note that this does not mean performing actual propagation experiment in the air, but purely mathematical modelling resulting in selectively filtered vocalisations.

Acoustic analysis

All obtained (i.e. propagated) audio files were batch-processed in R, using the *soundgen* package (function *analyze* with settings adjusted to the little auk: dynamic range = 60 dB, pitch floor = 500 Hz, pitch ceiling = 2000 Hz, step = 5 ms) to extract a set of 15 acoustic parameters (Table 1). Both raw audio and the resulting analysed datasets can be found in the supplementary materials.

The dataset was first cleaned, i.e., entries with missing values (that is, raw acoustic parameters that could not be correctly extracted) removed. We also reduced the dataset to the individuals with at least 200 entries (i.e., at least five calls propagated four times to 10 distances). This reduced the dataset to 5521 *classic* call entries from 11 individuals, and 2640 *single* call entries from six individuals.

To reduce data dimensions for further analyses, this cleaned dataset was subsequently tested for Kaiser-Meyer-Oklin factor adequacy (function *KMO,* package *EFAtools;* Supplementary Table 1), and then used in a Principal Components Analysis (PCA; function *prcomp*, package *stats;* Supplementary Tables 2 and 3). This was done separately for each of the two call types.

Table 1. Raw acoustic parameters extracted from audio files. Variable explanations as per *soundgen* package.

Variable	**Definition**
Duration	duration from the beginning of the first non-silent short-time Fourier transform (STFT) frame to the end of the last non-silent STFT frame [s]
AM Env Dep mean	depth (0 to 1) of amplitude modulation estimated from a smoothed amplitude envelope
AM Env Freq mean	frequency [Hz] of amplitude modulation estimated from a smoothed amplitude envelope
AM Ms Freq mean	frequency of amplitude modulation
Ampl mean	root mean square of amplitude per frame
CPP mean	Cepstral Peak Prominence [dB]
Dom mean	lowest dominant frequency band [Hz]
FM Dep mean	depth of frequency modulation
Peak Freq mean	the frequency with maximum spectral power [Hz]
Pitch mean	post-processed pitch contour based on all F0 estimates [Hz]
Q25%	the 25th quantile of the spectrum of voiced frames [Hz]
Q50%	the 50th quantile of the spectrum of voiced frames [Hz]
Q75%	the 75th quantile of the spectrum of voiced frames [Hz]
Spec Centroid mean	the centre of gravity of the frame’s spectrum, first spectral moment [Hz]
Spec Slope mean	the slope of linear regression fit to the spectrum [dB/kHz]

Classification to individual over distance

To check how well can propagated calls be classified to the caller independently of the distance, we performed the following analysis. We selected the principal components with eigenvalues > 1 (Supplementary Table 2) as input variables. These PC scores of all obtained calls (i.e. calls propagated at distances 1-1000 m) for which we were able to extract the full set of acoustic parameters specified in Table 1 were used in a permuted discriminant function analysis (pDFA; Mundry and Sommer 2007), to see how well can calls be classified to the caller independently of the distance. This pDFA was conducted in a nested design, using the *pDFA.nested* function (R. Mundry, based on function *lda* of the *MASS* package), on all available calls (5521 for the *classic* call, and 2640 for the *single* call) of all the subjects (11 for the *classic* call, and six for the *single* call). Since the same calls were propagated in conditions corresponding to the four focal months (May-August), we used the file name as a control factor to correct for multiple sampling. We ran a total of 1000 permutations for the analysis. This was done separately for the two call types, for all distances pooled together and each distance separately.

Furthermore, to see how well calls propagated to different distances cluster to individuals, we performed a set of additional analyses using support vector machine (SVM) classifiers. First, to establish the approximate number of nearest neighbours to use, we used the *kNNdistplot* function of the *dbscan* package (Hahsler *et al.* 2019). We then reduced the data dimensions of the raw, cleaned datasets using supervised uniform manifold approximation and projection (S-UMAP; *uwot* package, *umap* function), with minimum distance = 0.5, n_neighbours = 500 (*classic*) or 200 (*single*), using the Euclidean metric. This gave us two-dimensional coordinates, subsequently introduced to the SVM classifiers. The data were first subset into distances, and subsequently into 8:2 training:test datasets. A classification task was built for each subset (*mlr* package, function *makeClassifTask* with individual ring number as target). A learner was then created using *makeLearner* function of the *mlr* package, and corrected for individual weights due to the uneven sampling of different individuals (*mlr* package, *makeWeightedClassesWrapper* function). The weighted learner was then trained (*mlr* package, *train* function) on the training task, and used to classify the task (*mlr* package, *predict* function). Classification accuracy of the SVM was extracted using the *performance* function of the *mlr* package. The accuracy was then compared in a simple linear model (function *lm*). This was performed for each call type and propagation distance separately.

Information loss over distance

To investigate the possible loss of information content of the signal over distance, we used Beecher's information statistic, H_s (Beecher, 1989), which informs about the information capacity of a signal. To calculate H_s, we used all PC scores into the H_s calculation (function *calcHS, IDmeasurer* package). This was performed on subsets of calls propagated at different distances (1-1000 m, 10 calculations per call type in total).

Results

Apparent sound pressure level

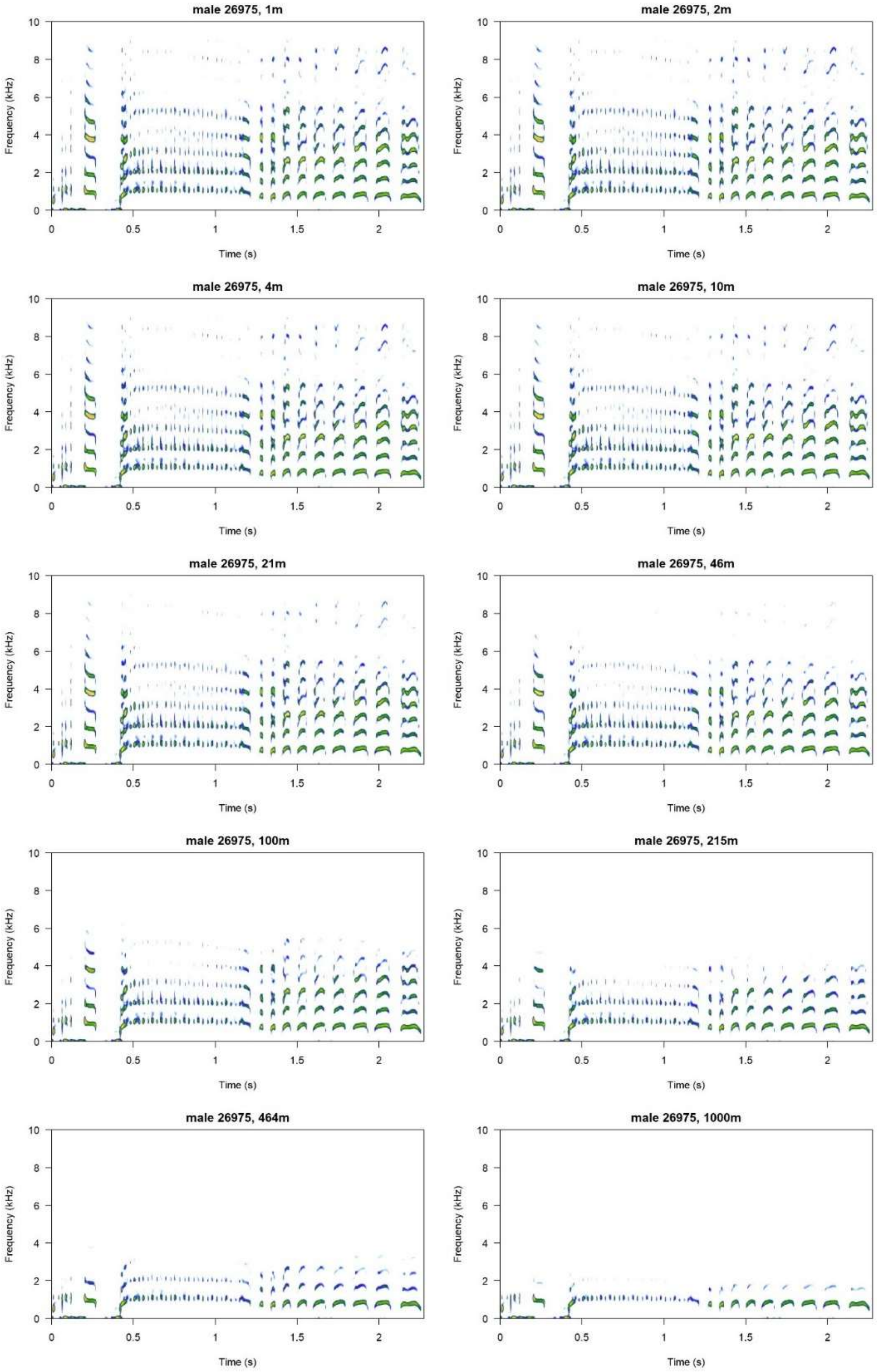
The apparent sound pressure levels, expressed as the mean peak ASPL and mean ASPL RMS, were slightly higher for the *classic* than *single* calls (Table 2). However, the maximum peak ASPL and mean ASPL RMS were similar for the two call types (Table 2).

Table 2. Maximum and mean SL values of the call types. All SL values are given in dB re 20 μPa at 1 m.

measure	*classic*	*single*	*p-value*
Mean ASPL peak	60 (SD±4)	54 (SD±7)	<0.001
Max ASPL peak	63	63	-
Mean RMS	45 (SD±4)	42 (SD±7)	<0.01
Max RMS	52	51	-

Classification to individual over distance

Call structure remained stable over large distances (Figs. 6-7), and calls could be classified to the individual independently of the distance (Tables 3-4). Clustering accuracy did not decrease with distance (Figs. 8-9, Table 5).



male 26975, 1m

male 26975, 2m

male 26975, 4m

male 26975, 10m

male 26975, 21m

male 26975, 46m

male 26975, 100m

male 26975, 215m

male 26975, 464m

male 26975, 1000m

Figure 6. A sample *classic* call propagated at 10 exponential distances in a range of 1-1000 m. Notice that the signal remains very stable across the distances, and harmonics are only lost at extreme distances, close to the putative physiological hearing threshold. Spectrograms plotted using the *seewave* package (Sueur *et al.* 2008).

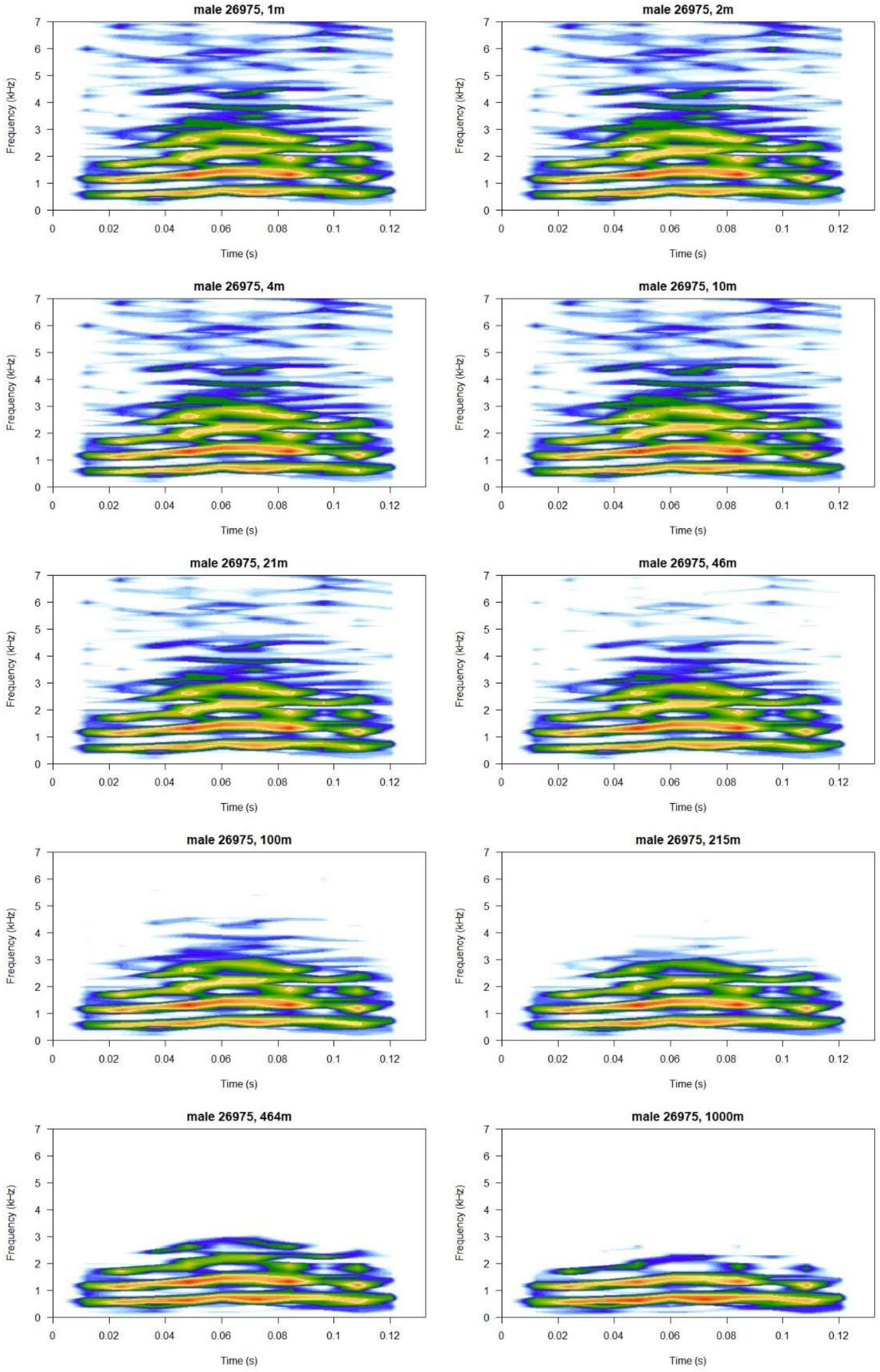


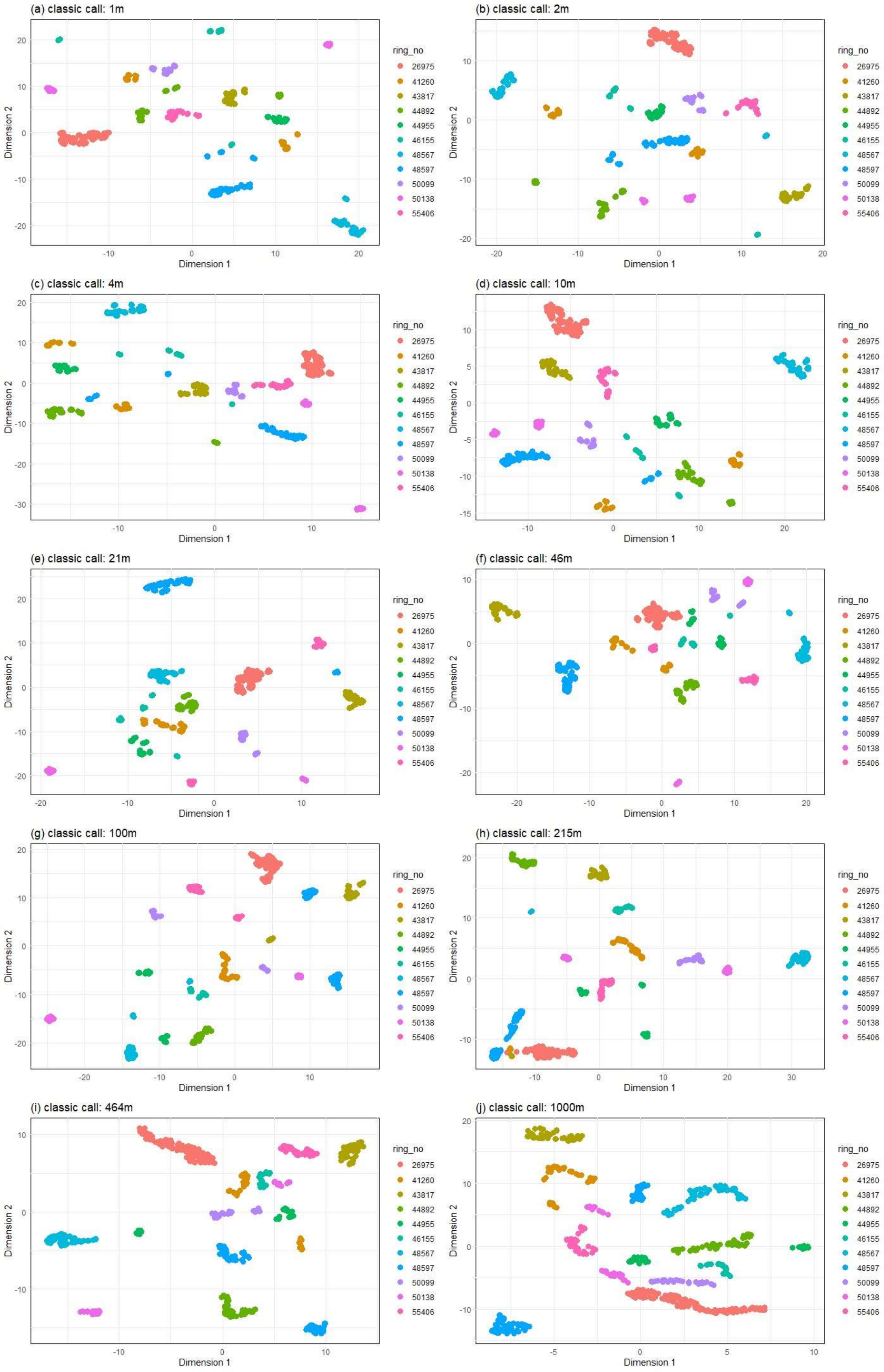
Figure 7. A sample *single* call propagated at 10 exponential distances in a range of 1-1000 m. Notice that the signal remains very stable across the distances, and harmonics are only lost at extreme distances, close to the putative physiological hearing threshold. Spectrograms plotted using the *seewave* package (Sueur *et al.* 2008).

Table 3. Results of the permuted discriminant function analysis for *classic* calls propagated at distances from 1 to 1000 m (552 calls of 11 individuals per distance), as well as for all distances pooled together (5520 calls of 11 individuals), using the principal components of eigenvalues > 1. Calls could be reliably classified to individual independently of the distance.

	Distance (m)										
Result	**1**	**2**	**4**	**10**	**21**	**46**	**100**	**215**	**464**	**1000**	**pooled**
Correctly classified (%)	49.14	47.56	47.28	47.36	44.96	44.99	45.64	44.61	44.76	43.79	37.79
Chance level (%)	27.83	27.39	27.49	27.70	27.80	28.06	28.06	28.09	27.47	26.67	13.25
P value for classified	**0.001**	**0.001**	**0.001**	**0.001**	**0.001**	**0.003**	**0.005**	**0.002**	**0.001**	**0.001**	**0.001**
Correctly cross-classified (%)	**44.02**	**44.17**	**42.74**	**42.98**	**42.17**	**39.45**	**39.53**	**40.10**	**36.20**	**31.49**	**48.10**
Chance level for cross-classified (%)	**9.00**	**9.06**	**9.03**	**9.18**	**9.26**	**9.00**	**9.19**	**9.17**	**9.06**	**9.09**	**9.05**
Relative cross-classification level	4.89	4.88	4.73	4.68	4.55	4.38	4.30	4.37	4.00	3.46	5.31
P value for cross-classified	**0.001**	**0.001**	**0.001**	**0.001**	**0.001**	**0.001**	**0.001**	**0.001**	**0.001**	**0.001**	**0.001**

Table 4. Results of the permuted discriminant function analysis for *single* calls propagated at distances from 1 to 1000 m (264 calls of six individuals per distance), as well as for all distances pooled together (2640 calls of six individuals), using the principal components of eigenvalues > 1. Calls could be reliably classified to individual independently of the distance.

	Distance (m)										
Result	**1**	**2**	**4**	**10**	**21**	**46**	**100**	**215**	**464**	**1000**	**pooled**
Correctly classified (%)	81.17	76.95	74.43	71.13	75.87	69.27	59.33	75.23	73.43	70.30	54.63
Chance level (%)	40.59	40.75	40.15	40.06	41.12	41.46	41.79	41.77	42.22	42.11	22.40
P value for classified	**0.001**	**0.001**	**0.001**	**0.001**	**0.001**	**0.001**	**0.002**	**0.001**	**0.002**	**0.001**	**0.001**
Correctly cross-classified (%)	**64.84**	**57.53**	**57.92**	**55.26**	**58.83**	**53.14**	**51.49**	**63.62**	**62.17**	**60.96**	**51.24**
Chance level for cross-classified (%)	**25.10**	**23.34**	**25.40**	**25.45**	**25.05**	**25.69**	**24.32**	**21.00**	**25.98**	**25.62**	**19.02**
Relative cross-classification level	2.58	2.47	2.28	2.17	2.35	2.07	2.11	3.03	2.39	2.38	2.69
P value for cross-classified	**0.001**	**0.001**	**0.001**	**0.001**	**0.001**	**0.001**	**0.001**	**0.001**	**0.001**	**0.001**	**0.001**



(a) classic call: 1m

(b) classic call: 2m

(c) classic call: 4m

(d) classic call: 10m

(e) classic call: 21m

(f) classic call: 46m

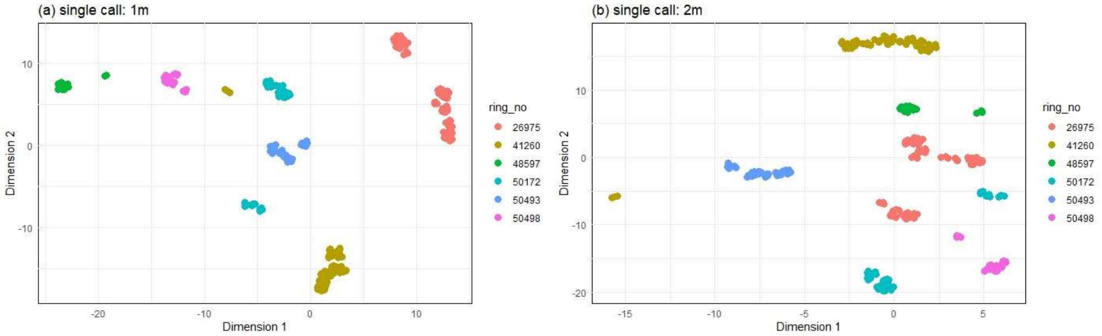
(g) classic call: 100m

(h) classic call: 215m

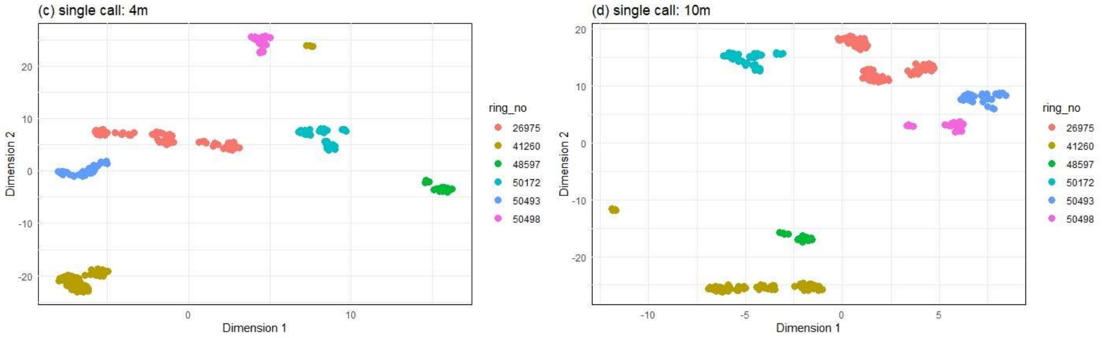
(i) classic call: 464m

(j) classic call: 1000m

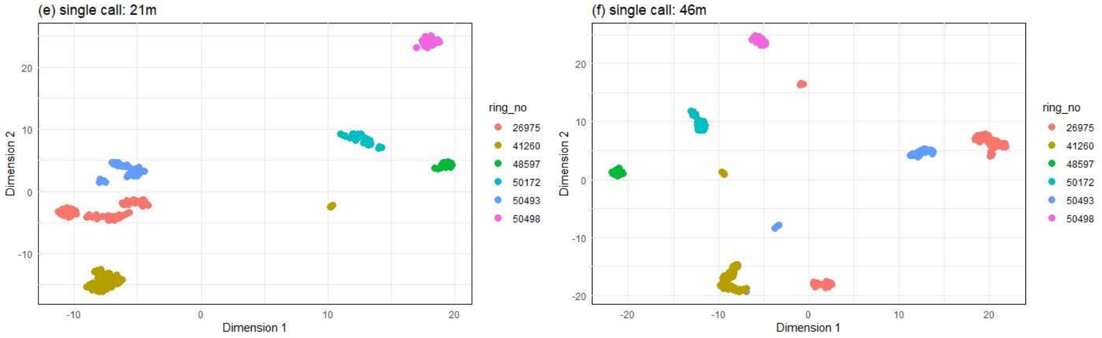
Fig 8. S-UMAP classification of the *classic* call to individual over 10 different propagation distances.


(a) single call: 1m

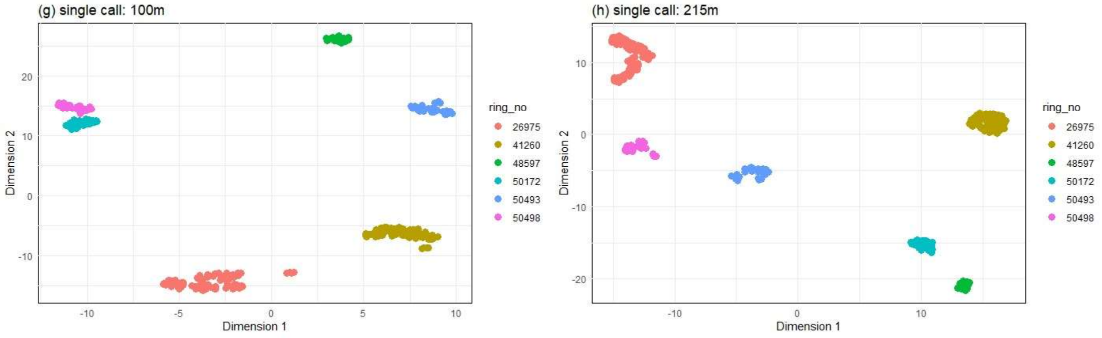
(b) single call: 2m


(c) single call: 4m

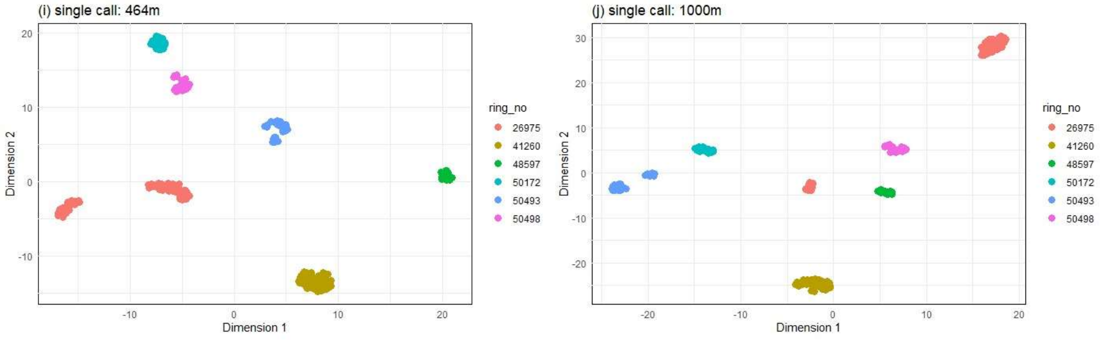
(d) single call: 10m


(e) single call: 21m

(f) single call: 46m


(g) single call: 100m

(h) single call: 215m


(i) single call: 464m

(j) single call: 1000m

Figure 9. S-UMAP classification of the *single* call to individual over different propagation distances.

Table 5. Accuracy of classification to individual using SVM based on S-UMAP reduced data.

Distance	Accuracy [%]	
	classic call	*single* call
1	58	73
2	62	72
4	61	85
10	61	69
21	59	74
46	66	78
100	56	83
215	62	77
464	57	89
1000	65	72
p-value	0.4	0.9

Information loss over distance

The information capacity of the *classic* call did not decrease with distance, theoretically allowing for a distinction of essentially constant number of individuals as at the source (Table 6). The *short* call seemed to be particularly individually specific at a very short range (1 m), and maintained roughly 50% of its original information content over propagation.

Table 6. Beecher's statistic's values in the propagated signals. Column *meaning* specifies how many individuals can be theoretically distinguished based on the signal alone.

	classic call			***single call***		
Distance [m]	**H_s significant**	**H_s all**	**meaning**	**H_s significant**	**H_s all**	**meaning**
1	2.8	2.8	7	3.5	3.6	12
2	2.8	2.8	6	2.3	2.4	5
4	2.7	2.7	6	2.2	2.2	4
10	2.7	2.7	6	1.9	2.0	3
21	2.8	2.8	7	2.4	2.4	5
46	2.9	2.9	7	3.1	3.1	8
100	2.8	2.7	6	2.5	2.6	5
215	2.8	2.8	7	3.2	3.2	9
464	2.5	2.5	5	2.7	2.7	6
1000	2.8	2.8	6	2.6	2.6	5

Discussion

We showed that, while the little auk social call is not a particularly loud signal (maximum 63 dBpeak re 20 µPa for both call types; compared to the loudest species reaching 140–150 dBpeak re 20 µPa in air; see e.g. Jakobsen *et al.* 2021), it is capable of carrying individual information over large distances. Calls could be classified to callers with very similar reliability independently of the distance, and well over the likely active space of the signal.

The *classic* call is the longest and most complex of the little auk repertoire (Osiecka *et al.* 2023a). Conspicuous signals are generally thought to have evolved for two main reasons: signalling quality and signal efficacy (Dawkins and Guilford 1997). The *classic* call certainly matches the latter description, maintaining its carrying capacity over distance. Similarly to other seabird vocalisations (e.g. Jones *et al.* 1987, Aubin *et al.* 2000, Curé *et al.* 2016, Baciadonna *et al.* 2021), little auk calls are reliable 'self-reporting signals' (Smith and Harper 1995), i.e. signals providing information about the signaller. They carry cues to identity, notably in their fundamental frequency (Osiecka *et al.* 2024a), which has been shown here to travel over 1 km. However, little auks' vocal identity can be somehow diluted when considering some parameters, since nesting partners match certain parameters of their calls, such as formant dispersion (Osiecka *et al.* 2023b). From a propagation perspective, as higher frequency formants are attenuated earlier on (see Fig. 6) and partners' vocalisations become less similar with the distance, this may result in a seemingly increasing individual information content as the *classic* call travels further and further.

Long, complex signals can be used in long-distance communication in both humans (Seifart *et al.* 2018), and non-human animals (e.g. Dawkins and Guilford 1997, Luo *et al.* 2013, Larsen 2020). One aspect of the *classic* call that we did not investigate here is individuality coding within the temporal patterning of the call's syllables – which in fact holds some of the parameters with the highest potential for individuality coding (Osiecka *et al.* 2024a). This was omitted due to the very heavy workload required to extract this information from such a large dataset. Nevertheless, the fact that strong individuality was retained even when excluding those parameters supports the notion that this call type is "designed" to facilitate efficient communication of identity. Adding the temporal information would very likely further increase the information content measured here, and improve clustering efficiency.

On the other hand, brevity often characterises short-distance communication (Luo *et al.* 2013). The *single* call is a very short, simple signal. While the classification efficiency of this call was essentially similar over distance, its information content dropped by roughly a half within the first two meters of propagation. This may suggest that even though this call type could be correctly classified over big distances, its primary role may lie more within short-range communication, i.e. to encode private information (Larsen 2020).

Of course, retaining information over long distances does not automatically translate into eliciting behavioural reactions to it. For instance, the corncrake *Crex crex,* whose calls carry cues to individuality over long distances (Ręk and Osiejuk 2011), but only result in response at behaviourally relevant distances (Ręk and Osiejuk 2010). However, the little auk's Umwelt is very different of this of a corncrake, and such efficient long-distance communication could prove particularly useful. For instance, vocalisations could facilitate important aspects of a little auk's life that might require individual recognition at long-distances, such as communication at foraging grounds, locating one's neighbours or partner after migration, or even facilitating migratory behaviours. Dedicated studies are necessary to understand whether and how sound might play a role in these behaviours.

Long distance communication in the atmosphere is more likely to occur in environments with less physical constraints for sound transmission. For example, open habitats, such as the Arctic tundra or the sea degrade acoustic signals less than closed habitats (Hardt and Benedict 2020). However, acoustic communication in the atmosphere is also constrained by a number of factors contributing to signal attenuation, such as air humidity, temperature, and pressure (Wiley and Richards 1978). In response to this, animal signals can evolve to match the optimal frequency ranges for sound communication within their environments. While the acoustic adaptation hypothesis (i.e. the notion that the vocal signal of a species will follow their habitat structure, e.g. open/closed) finds only some evidence and only in certain groups (Ey and Fischer 2009), a better match between signal properties and the environment can possibly be found at more local scales (as is e.g. the hooded crow, *Corvus cornix;* Jensen *et al.* 2008). While the fact that the Arctic tundra, as an open, humid habitat provides excellent conditions for sound propagation is not surprising, the reliability of information transmission over such distances is noteworthy.

So how far away from each other can two little auks be and still recognise the other's voice, or react to it? This remains unknown, as here, we could only show that the signals themselves can be reliably classified to a sender at least up to the putative physiological hearing threshold, i.e. over the likely effective distance. This should be considered in the frame of information *content* and *transfer*, and not *meaning* (Weaver's Levels A and B of communication problems; Shannon and Weaver 1949). That is, we cannot and do not intend to suggest to what level do little auks actually decode those transmitted signals and attribute them to individuals they know and recognise. Playback experiments in controlled conditions would be the only way to understand whether and how far away do little auks actually respond to such signals.

Caveats and issues

This study, of course, comes with a number of limitations. While we are confident that the propagation model employing spherical spreading is appropriate for the studied vocalisation (uttered at great heights in an open habitat), it is necessarily simplified and does not correct for subtle changes to air layer densities, wind speed, or topography (see Guibard *et al.* 2022 for a brilliant model of ground surface communication in mountain habitats).

It is also likely that this study underestimates the sound pressure levels of the calls: because we had to be sure of the identity of the caller, we could only use calls produces within the nest, where we can control for it. However, calls uttered in open spaces are likely to have a higher amplitude than those produced in the nest, simply because of the increased noise outside (i.e., the Lombard effect, see e.g. Brumm & Zollinger 2013). Therefore, our study likely underestimated the real-life sound pressure levels, and therefore the active space, of the vocalisations when they are produced in flight. While this is unfortunate, we feel more confident reporting under- than over-estimated values.

Perhaps the biggest issue encountered here is that the recording distance (10 cm) falls within the near field of the lower frequency components of the calls – that is, the distance at which the soundwave is not yet fully developed, and might therefore behave differently (Larsen & Wahlberg 2019). Again, this is because recording the birds inside the nest was the only feasible way of obtaining repeated recordings of known individuals in the field. While the near field should not be an issue for the higher frequency components of the little auk calls, we acknowledge that the recorded properties of the lower components might not fully reflect the actual sound properties at larger distances.

This study also provides a somewhat idealised version of signal transmission, free of environmental noise and wind that surely interfere with the signal in real life: from other birds calling to glaciers calving, there are plenty of other sounds masking the little auk signals. Sadly, we were unable to perform propagation experiments due to the great heights and distances involved, and we acknowledge the importance of the local excess attenuation that was hence unaccounted for (see e.g. Jensen *et al.* 2008 and Guibard *et al.* 2022 for theoretical propagation models confirmed experimentally). Nevertheless, taking into account that the purpose of this study was to investigate the theoretical distance those signals can travel – and not how the animals use or perceive them – we believe that this framework still provides useful insights into the acoustic world of this little understood seabird.

Conclusions

We found that the carrying capacity of the little auk social call does not decrease with distances over the likely behaviourally useful range. While these results do not indicate whether this information is actually perceived by the animals, this study suggests that vocal communication is likely used in long-distance communication, and can potentially facilitate important social interactions.

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Ethics statement

This study used previously published data in theoretical models, and did not involve direct contact with the animals. Fieldwork involved in previous data collection was performed under permit from the Governor of Svalbard (20/00373-2), following the Association for the Study of Animal Behaviour's guidelines for animal research.

Competing interests

Authors declare no competing interests.

Data availability statement

Raw data and full codes generated in this study are available at https://osf.io/esbdj/?view_only=98d5eca13d894a94b9df1c1a091661fd. Audio files obtained from Osiecka *et al.* 2024a (https://doi.org/10.1016/j.anbehav.2024.02.009), https://osf.io/q9xhd/?view_only=2b8dd1470996468ea8f961d35070d1e5

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Supplementary Materials

Supplementary Table 1. Kaiser-Meyer-Oklin factor adequacy: the overall KMO value for the dataset is middling for both call types, and data suitable for factor analysis.

Raw variable	*classic call*	*single call*
Duration	0.59	0.54
AM Env Dep mean	0.74	0.51
AM Env Freq mean	0.66	0.50
AM Ms Freq mean	0.71	0.76
Ampl mean	0.91	0.71
CPP mean	0.83	0.64
Dom mean	0.53	0.63
FM Dep mean	0.73	0.75
Peak Freq mean	0.83	0.91
Pitch mean	0.55	0.66
Q25%	0.81	0.79
Q50%	0.78	0.84
Q75%	0.76	0.72
Spec Centroid mean	0.73	0.71
Spec Slope mean	0.66	0.90
Overall KMO	**0.75**	**0.74**

Supplementary Table 2. Principal Components Analysis: eigenvalues and proportion of variance.

	classic call			*single call*		
	Eigenvalue	**Proportion of variance**	**Cumulative proportion**	**Eigenvalue**	**Proportion of variance**	**Cumulative proportion**
PC1	2.20	0.32	0.32	2.31	0.36	0.36
PC2	1.55	0.16	0.48	1.67	0.19	0.54
PC3	1.30	0.11	0.60	1.17	0.09	0.63
PC4	1.09	0.08	0.68	1.09	0.08	0.71
PC5	1.01	0.07	0.74	1.01	0.07	0.78
PC6	0.98	0.06	0.81	0.95	0.06	0.84
PC7	0.88	0.05	0.86	0.87	0.05	0.89
PC8	0.74	0.04	0.90	0.76	0.04	0.93
PC9	0.70	0.03	0.93	0.58	0.02	0.95
PC10	0.60	0.02	0.95	0.48	0.02	0.97
PC11	0.45	0.02	0.97	0.40	0.01	0.98
PC12	0.44	0.01	0.98	0.37	0.01	0.99
PC13	0.43	0.01	0.99	0.33	0.01	1.00
PC14	0.31	0.01	1.00	0.27	0.01	1.00
PC15	0.12	0.00	1.00	0.10	0.01	1.00

Supplementary Table 3. Principal Components Analysis: contributions of raw acoustic parameters to the first five principal components of both call types

	classic call				***single call***			
Raw variable	**PC1**	**PC2**	**PC3**	**PC4**	**PC1**	**PC2**	**PC3**	**PC4**
Duration	-0.10	-0.50	-0.30	-0.01	0.28	-0.72	-0.34	0.23
AM Env Dep mean	0.01	0.66	0.42	0.15	-0.26	0.65	0.62	-0.11
AM Env Freq mean	-0.31	0.19	0.42	-0.64	0.35	-0.08	-0.56	-0.52
AM Ms Freq mean	-0.05	-0.47	-0.37	0.21	-0.17	0.17	-0.56	0.06
Ampl mean	-0.04	-0.06	0.00	0.27	0.11	0.47	-0.33	0.48
CPP mean	-0.68	0.20	-0.03	0.54	0.42	0.53	-0.30	0.37
Dom mean	-0.28	0.44	-0.71	-0.23	0.24	-0.70	0.20	0.43
FM Dep mean	0.04	-0.73	0.05	-0.36	0.30	-0.30	-0.21	-0.25
Peak Freq mean	-0.87	-0.20	0.14	-0.15	0.85	-0.06	0.24	-0.19
Pitch mean	-0.31	0.42	-0.74	-0.26	0.41	-0.61	0.39	0.25
Q25%	-0.83	0.27	-0.01	0.12	0.81	-0.11	0.08	-0.29
Q50%	-0.78	0.26	0.11	-0.19	0.93	0.02	0.17	-0.15
Q75%	-0.91	-0.22	0.12	-0.00	0.88	0.24	0.05	0.11
Spec Centroid mean	-0.95	-0.15	0.08	0.09	0.95	0.22	0.04	0.09
Spec Slope mean	-0.58	-0.51	-0.03	0.07	0.79	0.47	-0.10	0.15

General discussion

Acoustic signals can travel far and across obstacles, and thus be used to facilitate communication at distances larger than smell or sight might allow. Sounds can also be very complex signals, encoding various information in the domains of time, frequency, and amplitude. Vocal communication is fundamental for many animal species, and thought to become particularly intricate in socially complex animals.

In this work, I looked at vocal communication and information coding in the acoustic signals of a highly colonial seabird, the little auk (*Alle alle*). As a result, I present a detailed guide into the vocal world of this species, from a basic description of its vocal repertoire to mathematical modelling of information transfer. A major part of this work covers coding of different types of static (e.g., size and identity of the caller) and dynamic (e.g., behavioural or affective contexts) information. I also investigated how the information content of little auk vocal signals is retained over propagation at large distances, but also across affective states.

Here, I will briefly summarise the main findings of the previous chapters, framing them in a wider perspective. I will also present some future research perspectives and directions.

Vocalisations as information carriers

The mathematical theory of communication considers vocal signals in the framework of an information transmission model (Shannon and Weaver 1949). Within this framework, we can choose to consider signals with regard to their information *content*, *transfer*, and *meaning* separately (Weaver's Levels A, B, and C of communication problems; Shannon and Weaver 1949). This work considered little auk signals through their information *content* and *transfer*.

One of the basic determinants of the vocal output's structure is anatomy. Therefore, animal vocalisations might often provide cues to their size (e.g., Klenova *et al.* 2011, Favaro *et al.* 2017), sex (e.g., Curé *et al.* 2012), or identity (e.g. Favaro *et al.* 2017). At the same time, spectral properties of seabird calls tend to lack indicators of sex even when vocal tract anatomy shows sexual dimorphism (Hardouin *et al.* 2014). Not surprisingly, in the little auk – a species lacking sexual dimorphism – vocalisations do not carry cues to the emitter's sex (Chapter III: Osiecka *et al.* 2023b). However, depending on the call type, the fundamental frequency of the calls may or may not inform on their size, with larger birds emitting lower frequency calls (Chapter III: Osiecka *et al.* 2023b).

Interestingly, little auk partners tend to match certain parameters of their calls, such as the fundamental frequency or formant dispersion (Chapter III: Osiecka *et al.* 2023b). Similar social influence over the vocal output has previously been observed in the banded penguins (Baciadonna *et al.* 2022) and long-distance calls of raven partners (*Corvus corax,* Luef *et al.* 2017). In the little auk, this frequency matching between partners was stronger in a complex call than in a simple, one-syllable call (Chapter III: Osiecka *et al.* 2023b), possibly indicating its use in long-distance communication between partners (see also Chapter V: Osiecka *et al.* manuscript).

Another pattern present in the little auk (Chapter IV: Osiecka *et al.* 2024a), and other seabird vocalisations (Kriesell *et al.* 2018, Bowmaker-Falconer *et al.* 2022), is the importance of temporal pattern in identity encoding. While the king penguins (*Aptenodytes patagonicus,* Kriesell *et al.* 2018), or Cape gannets (*Morus capensis,* Bowmaker-Falconer *et al.* 2022), mostly encode this information in the rate at which they call, the complex calls of the little auk seem to be individually specific in all their rhythmic parameters (Chapter IV: Osiecka *et al.* 2024a), and particularly so with regard to variation of syllable duration (Chapter IV: Osiecka *et al.* 2024a).

As expected for a colonial species, the individual information content of little auk calls seems to be particularly important. The information capacity of their signals (i.e., the amount of information they can carry, measured in bits) is high, theoretically allowing distinction of up to 41 individuals based on the signal alone (Chapter IV: Osiecka *et al.* 2024a). This information is encoded in nearly all temporal and spectral parameters of little auk calls, covering the three domains of sound: amplitude, time, and frequency (Chapter IV: Osiecka *et al.* 2024a). It is also retained with high precision over long-distance propagation: independently of the transmission distance, as long as they are still audible, little auk calls carry sufficient information to classify a vocalisation to its sender (Chapter V: Osiecka *et al.* preprint).

However, this identity information is not completely "bulletproof": in situations of distress, the individual information content of auk chick signals falls drastically (Chapter II: Osiecka *et al.* 2024b). A similar pattern of individual information loss depending on the valence or arousal of the caller has previously been described e.g. in Jackson's golden-backed weaver (*Ploceus jacksoni,* Reers and Jacot 2011), Holstein-Fresian cattle (*Bos taurus,* Green *et al.* 2019), and the bonobo (*Pan paniscus*, Keenan *et al.* 2020). Why does this information loss occur in distress across species? One explanation is that distress calls are not necessarily aimed at a caretaker, but trying to catch the attention of any possible rescuer (Lingle *et al.* 2012). Therefore, they tend to become less individually specific, but also more difficult to ignore (Lingle *et al.* 2012).

While individual information may be lost in situations of distress or high arousal, this does not mean that other types of information are lost as well. In fact, conveying information about the behavioural or emotional contexts in which the vocalisations are produced is one of the most crucial aspects of communication. Most little auk call types are specific to the behavioural context of their production (Chapter I: Osiecka *et al.* 2023a). Across species, calls associated with a negative valence (i.e., situations that should elicit avoidance in order to increase fitness, such as e.g. predator presence, Fridja 2010, Mendl *et al.* 2010), tend to be characterised by e.g. a higher and more variable fundamental frequency (Briefer 2020), as well as often higher entropy (Herborn *et al.* 2020), when compared to those associated with a positive valence (i.e., emitted in situation that should elicit approach to increase fitness, such as e.g. presence of food or caretakers, Fridja 2010, Mendl *et al.* 2010). With exception of a raise in the fundamental frequency of adult positive calls, vocal expression of affect in the little auk follows the same expression patterns observed throughout other groups (Chapters I-II: Osiecka *et al.* 2023a, 2024b), supporting their evolutionary continuity. These patterns are already present early in ontogeny: around five days after hatching, little auk chicks can efficiently communicate socially important messages (Chapter II: Osiecka *et al.* 2024b). At the same time, when heard by a predator, distress calls of preys might work as a dinner bell, or a notification that a tasty snack in the form of a young chick has been left unattended (see e.g. Thévenet *et al.* 2023). Because of this risk, the fact that vocal expression of affect is present suggests its importance for parent-chick communication. Nevertheless, whether and how this information is perceived and used by other members of the auk's social group (e.g., partner, offspring or neighbours) remains an open question.

Perspectives and future directions

An in-depth investigation into the vocal behaviour of a wild, colonial seabird is a rare opportunity and a major strength of this work. While it covered some of the main aspects of vocal communication in the little auk, a lot remains uncovered. Most importantly, my thesis focused purely on passive recordings of spontaneous vocalisations – as a result, while I could describe the information content of those spontaneous signals, we do not know which parameters are actually useful to the birds. In other words, going back to Shannon and Weaver's communication problems (1949), I looked at information *content* and *transfer,* but not *meaning* to the receiver. To understand this last level, we would need to "ask" the birds themselves – that is, perform a set of carefully controlled playback experiments with natural and modified or synthetic signals (see e.g. Vergne *et al.* 2012 or Anikin *et al.* 2020 for brilliant examples of such work). In addition, playback experiments directed at predators of the little auk (i.e. the glaucous gull *Larus hyperboreus* and the Arctic fox *Vulpes lagopus*) could unveil whether and how they use eavesdropping on auk calls to locate food (see e.g. Thévenet *et al.* 2023). Such studies could provide precious insights into the use of vocal cues by seabirds and their predators.

Another aspect that might require more attention is the temporal patterning of seabird calls. One of the interesting findings of this work is that the temporal patterns (expressed in different rhythmic indices) of little auk vocalisations carry cues to individuality (Chapter IV: Osiecka *et al.* 2024a). In fact, one of the temporal indices (i.e., the coefficient of variation of the *classic* call's C syllable duration) showed a Potential of Individuality Coding (Robisson *et al.* 1993) way above the values of any other temporal or spectral parameters (Chapter IV: Osiecka *et al.* 2024a). While some previous studies found temporal patterns to be individually specific (e.g. in the Cape gannet, *Morus capensis,* Bowmaker-Falconer *et al.* 2022; the elephant seal, *Mirounga leonina,* Mathevon *et al.* 2017; or the sperm whale, *Physeter macrocephalus,* Oliveira *et al.* 2016, Gero *et al.* 2016), the question of individuality encoding in rhythm remains surprisingly open. Interestingly, both fields of vocal identity (see e.g. Linhart *et al.* 2019) and acoustic rhythms in non-human animals (see e.g. Burchardt and Knörnschild 2020, Burchardt *et al.* 2021, Hersh *et al.* 2023) are currently getting more attention. I suggest that combining those two lines might shed new light even on groups that we consider really well understood, such as songbirds.

Taking the rhythmic perspective into consideration might also enrich the studies of vocal expression of emotions. Across species, vocalisations associated with elevated arousal tend to be produced at higher rates (Briefer 2020). In this work, I have shown that little auk vocalisations conform to the general affective expression patterns observed throughout taxa (Chapters I-II: Osiecka *et al.* 2023a, Osiecka *et al.* 2024b). But how might affective states influence the rhythmic structure of seabird, or other groups' calls? Maintaining acoustic rhythms may require cognitive control (Ravignani *et al.* 2019), and therefore potentially be affected by emotionally charged contexts. There is a number of important and fascinating questions that stem from this.

I am currently developing a research line combining those aspects of animal vocal behaviour I find most interesting: temporal patterns, emotional expression, and the development of these throughout both ontogeny and evolution. I hope that my current and future work will prove a useful contribution to the wider understanding of vocal communication across the animal kingdom.

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Appendix

Candidate statement

Gdańsk, 12.02.2024

Anna N. Osiecka, MSc

This is to confirm that I am the first and leading author of the following papers:

1. Osiecka, A.N., Briefer, E.F., Kidawa, D., & Wojczulanis-Jakubas, K. (2023) **Seabird's cry: repertoire and vocal expression of contextual valence in the little auk (*Alle alle*).** Scientific Reports 13, 8623. https://doi.org/10.1038/s41598-023-35857-3

2. Osiecka, A. N., Briefer, E. F., Kidawa, D., Żurawska, F., & Wojczulanis-Jakubas, K. **Calls of the little auk (*Alle alle*) chicks reflect their behavioural contexts.** Currently in press in PLoS ONE. https://www.do.org/ 10.1371/journal.pone.0299033

3. Osiecka, A. N., Briefer, E. F., Kidawa, D., & Wojczulanis-Jakubas, K. (2023). **Social calls of the little auk (*Alle alle*) reflect body size and possibly partnership, but not sex**. Royal Society Open Science, 10(9), 230845. https://doi.org/10.1098/rsos.230845

4. Osiecka, A. N., Briefer, E. F., Kidawa, D., & Wojczulanis-Jakubas, K. (2024). **Strong individual distinctiveness across the vocal repertoire of a colonial seabird, the little auk (*Alle alle*).** Currently in press in Animal Behaviour

5. Osiecka, A. N., Bryndza, P., Briefer, E. F., & Wojczulanis-Jakubas, K. **Long distance calls: negligible information loss of seabird social calls over propagation down to the hearing threshold.** Currently prepared for submission.

My role in these papers covered all major tasks, i.e. idea and design, funding, fieldwork, formal investigation, data management, analysis and visualisation, writing: first draft, review and editing. Specific standardised descriptions can also be found in the publication list at the beginning of this thesis.

Anna N. Osiecka
PhD candidate
University of Gdańsk

Co-author statements

Gdańsk, 9.01.2024

Katarzyna Wojczulanis-Jakubas, PhD

This is to confirm that I am a co-author of the following papers:

1. Osiecka, A.N., Briefer, E.F., Kidawa, D., & Wojczulanis-Jakubas, K. (2023) **Seabird's cry: repertoire and vocal expression of contextual valence in the little auk (*Alle alle*).** Scientific Reports 13, 8623. https://doi.org/10.1038/s41598-023-35857-3

My role as co-author consisted of: conceptualization, funding acquisition, investigation, methodology, supervision, writing—review and editing

2. Osiecka, A. N., Briefer, E. F., Kidawa, D., Żurawska, F., & Wojczulanis-Jakubas, K. **Calls of the little auk (*Alle alle*) chicks reflect their behavioural contexts.** Currently in review in PLoS ONE.

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My role as co-author consisted of: funding acquisition, fieldwork, supervision, writing – review and editing.

I confirm that Anna N. Osiecka was the leading author and corresponding author for all those papers. She designed the studies, participated in fund acquisition, collected data in the field, conducted formal analyses, conceived and wrote the original manuscripts, and was responsible for the review process.

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Copenhagen, January 15th, 2024

Subject: co-authorship statement for Anna Osiecka

This is to confirm that I am a co-author of the following papers:

1. Osiecka, A.N., Briefer, E.F., Kidawa, D., & Wojczulanis-Jakubas, K. (2023) **Seabird's cry: repertoire and vocal expression of contextual valence in the little auk (*Alle alle*).** Scientific Reports 13, 8623. https://doi.org/10.1038/s41598-023-35857-3
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Your sincerely,

Elodie Mandel-Briefer

Gdańsk, 9.01.2024

Dorota Kidawa, PhD

This is to confirm that I am a co-author of the following papers:

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Aachen, 9.01.2024

Przemysław Bryndza, MSc

This is to confirm that I am a co-author of the following manuscript:

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Gdańsk, 9.01.2024

Feliksa Żurawska

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I confirm that Anna N Osiecka was the leading author and corresponding author for this paper. She designed the study, took part in funds acquisition, collected the data in the field, conducted the formal analysis, conceived and wrote the original manuscript and is responsible for the reviews process.

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