

Sex Differences in Turtle Vocalizations Reflect Social Context and Behavioural Roles

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Abstract

Acoustic communication is widespread in animals, yet its function in turtles remains poorly understood. Although turtle signaling has been considered primarily visual and chemical, many species vocalize across life stages. We investigated adult vocalizations in the Midland Painted Turtle (*Chrysemys picta marginata*) to test whether call types and acoustic traits vary across social contexts. We recorded behavior and vocalizations during 35 hours of 1-hour trials involving solitary individuals and paired interactions (male–male, female–female, and mixed-sex). We identified five distinct call types, including one produced exclusively by males. Acoustic traits varied across social contexts, and males exhibited greater within-individual trait variance than females, although some males experienced different pre-treatment conditions that may have affected vocalization patterns. While we cannot definitively identify vocalization function, theory suggests greater within-male variation may also reflect condition-dependent signaling under intrasexual selection. Additionally, vocalization rates were negatively correlated with the number of close social interactions, suggesting that vocalizations are used at a distance, while visual, tactile, or potentially chemical cues dominate at close range. Our findings push forward our understanding of vocalization types, bimodality of turtle communication, and vocalization function, identifying many exciting pathways for further investigation.

Introduction

Acoustic communication is phylogenetically widespread and serves a variety of functions that are fundamental to reproduction and survival (Jorgewich-Cohen et al. 2020; Kaplan 2014). Unlike signals that can only be sensed in close proximity, acoustic signals are multidirectional and can be sensed at large distances, in low-light conditions, and in both aquatic and terrestrial environments. Acoustic signals can encode information about the signaller's identity, location, and condition (Endler 1992), which can elicit behaviours such as the coordination of group activities (Lacroix et al. 2022), mate choice and pre-zygotic reproductive isolation (Galeotti et al. 2005a), parental care (Kostoglou et al. 2021), territoriality (Chavez-Mendoza et al. 2023), and predator deterrence (Labra & Zapata 2023). Patterns of acoustic divergence among and within populations reflect selection on acoustic traits, and in some systems, acoustic communication is a strong contributor to speciation (Wilkins et al 2013), underlining how acoustic signals both represent and contribute to biological diversity.

Acoustic communication is most widely recognized in birds, mammals, and amphibians, but it is increasingly recognized in diverse reptile groups, where acoustic communication underpins behaviour associated with parental care and mate choice. Crocodilians are well known among reptiles for their vocal behaviour, such as hatchling calls to initiate maternal care and dominance-establishing bellows (Ferrara et al. 2025; Vergne & Mathevon, 2008; Chabert et al. 2015; Dinets 2011). While our understanding of vocalization form and function in Crocodilians is rich, the discovery of acoustic communication in its sister clade, Testudines (turtles), was not appreciated until the 1950s (Wever & Vernon 1956), and only recently has there been a burst of research that aims to understand form and function of turtle vocalization at all life stages (Ferrara et al. 2014a; Zhou et al. 2022; Zhou et al. 2023a; Zhou et al. 2023b; Lacroix et al. 2022; Ferrara et al. 2017; Charrier et al. 2022; Giles et al. 2009; Jorgewich-Cohen et al. 2025).

To date, more than 50 turtle species are known to vocalize (Jorgewich-Cohen et al. 2020). While the purpose and context of turtle vocalizations are often unclear, the vocal repertoire of several turtle species has been quantified (Ferrara et al. 2014a; Zhou et al. 2022; Lacroix et al. 2022; Ferrara et al. 2017; Charrier et al. 2022; Giles et al. 2009), suggesting that these sounds likely serve social functions (Capshaw et al. 2021). Red-eared sliders (*Trachemys scripta*), for instance, produce many different vocalization types, and both sexes exhibit unique acoustic types, suggesting a potential role for acoustic signals in both inter and intra-sexual communication (Zhou et al. 2022). The Amazonian River Turtle (*Podocnemis expansa*) produces at least six different types of vocalizations, and females vocalize during nesting and during group migration, suggesting that these turtles possess a social system much more complex than previously believed (Ferrara et al. 2014a). Vocal traits in marginated tortoises (*Testudo marginata*) correlate with mating success, highlighting the importance of acoustic signals in reproductive strategies (Galeotti et al. 2005b). In sum, recent findings suggest that vocalizations in turtles and related reptiles may underpin underappreciated social relationships. To push forward our understanding of these social systems and the role of acoustic communication in turtle social behaviour requires quantifying the types of vocalizations that exist in a species, and contextualizing these vocalizations using controlled behavioural experiments.

Emydid turtles (Emydidae: pond turtles) offer an interesting opportunity to explore the emerging field of acoustic communication in chelonians. Within the Testudines, the Common Snapping Turtle (*Chelydra serpentina*) is known to exhibit intense behavioural interactions, including direct aggression (Moldowan et al. 2020a; Hawkshaw et al. 2019), mostly between male-male pairs (Keevil et al. 2017), and it is well known that aggressive communication such as territoriality and sexual coercion take its strongest form in populations with hierarchical social systems (Davis 2009; Frommen 2020). The Painted turtle, *Chrysemys picta*, is among the most widespread and common Emydids, and interesting social dynamics may underlie acoustic communication in this system. Specifically, while painted turtles have long been known for their visual courtship displays (Darwin 1871), recent discovery shows that males engage in size-specific mating tactics that are coercive (Moldowan et al. 2020a; Hawkshaw et al. 2020). Smaller male painted turtles that possess elongated foreclaws are more likely to engage in courtship behaviours through titillation which allow copulation through female mate choice (Darwin 1871; Moldowan et al. 2020b). However, larger male painted turtles often possess large bicuspid tomiodonts, which are tooth-like projections or cusps found on the upper jaw of some turtles' beaks, as well modified nuchal scutes that form a spear-like projection posterior to the males head; together these morphologies allow males to force copulation by grasping females with tomiodonts and injuring females with their sharp, serrated nuchal scute (Moldowan et al. 2020a; Hawkshaw et al. 2019). Further evidence of a social system in painted turtles arises from females that appear to engage in nesting behaviour in the presence and proximity of other nesting females (Kell et al. 2022), suggesting an ephemeral social cohesion among females that engage in nesting. Alternative male mating tactics and social cohesion among females provide opportunities for sex- and context-specific signalling to evolve. These findings raise questions about the role of acoustic signals in painted turtle social interactions. These social contexts likely involve signalling, yet the role of acoustic communication remains unexplored in painted turtles, and in most chelonians more broadly.

Here we explore the form and function of acoustic communication in the painted turtle, a widespread North American Emydid. For most chelonians, including painted turtles, our understanding of turtle vocalizations

is poorly developed, and as such our first goal was to describe the vocal repertoire of adult painted turtles. We used hydrophones and a cluster-based algorithm to extract and characterize underwater adult turtle vocalizations (Fig. 1a,b). Next, we used ex-situ experiments to gain insight into the function of vocalizations, testing the simple hypothesis that social context influences painted turtle acoustic signals. Specifically, if sex- and context-specific signalling occurs in this system, we expected that the number of vocalizations and vocalization traits would differ between inter- and intrasexual pairings of adult turtles (Wilkins et al 2013; Galeotti et al 2005c). We investigated sex- and context-specific differences in vocalizations with 5 different social treatments that manipulate the extent of inter- and intrasexual interactions: solo males (1 male), solo females, paired males (2 males), paired females, and intersexual pairs (1 male, 1 female). Finally, we used camera traps to quantify the number of social interactions (Fig. 1c, d), allowing us to test whether variation in vocalizations predicts the number of social interactions in each treatment replicate. We underscore that our study cannot definitively identify the function of painted turtle vocalizations, but rather our aim is to push forward our understanding of signalling in turtles by investigating the form of vocalizations, as the sex-specificity of vocalizations, and how vocalization function may differ among social contexts in a broadly distributed reptile.

Results

Sound types

In 35 hours of recordings, we detected a total of 150 individual vocalizations from adult painted turtles across 35 experimental trials. Based on audio recordings analyzed over the course of our experiment, we classified the vocalizations into five sound types, which we describe below (Table 1, Fig. 2). Overall, we found that the random forest classifier achieved 80.1% accuracy, indicating generally good agreement with manual call type assignments. However, performance varied among classes due to imbalance and limited representation of some types, with balanced accuracies of 0.79, 0.49, 0.84, 0.74, and 1.00 for call types 1–5, respectively.

Type I vocalizations (N = 96) are relatively short (mean $\pm$ SE, 0.14 ± 0.01 s) percussive click-like sounds composed of multiple short pulses produced at relatively low pulse rate (Table 1, Fig. 2, Figure S1). On average, they are composed of 4.0 ± 0.4 pulses that are 0.058 ± 0.008 s long and separated by 0.0096 ± 0.002 s intervals (Table 1). These sounds were produced at a relatively lower frequency such the minimum frequency was 0.94 ± 0.04 kHz, the maximum frequency was 3.3 ± 0.06 kHz, and the dominant frequency was 1.8 ± 0.04 kHz (Table 1). This vocalization type was the most common and was observed across all treatments (Fig. 2).

Type II vocalizations (N = 10) are relatively short (0.14 ± 0.02 s) continuous or percussive harmonic tonal sounds, similar to the sound of a zipper (Table 1, Fig. 2, Figure S1). These sounds are characterized by relatively few pulses (2.0 ± 0.4) produced at longer pulse durations (0.1 ± 0.02 s) and longer pulse intervals (0.0066 ± 0.0004 s) (Table 1). These sounds were produced at a relatively lower frequency with a longer bandwidth such the minimum frequency was 0.92 ± 0.08 kHz, the maximum frequency was 3.3 ± 0.2 kHz, and the dominant frequency was 1.9 ± 0.08 kHz (Table 1, Fig. 2, Figure S1). This vocalization type was observed across all treatments (Fig. 2).

Type III vocalizations (N = 30) are a short (0.15 ± 0.01 s) airy whistle-like sound (Table 1, Fig. 2, Figure S1). On average, they are composed of few pulses (1.6 ± 0.5) that are 0.14 ± 0.01 s long and separated by 0.00047 ± 0.0003 s intervals (Table 1). These sounds were produced at a relatively lower frequency with a shorter bandwidth such the minimum frequency was 1.6 ± 0.05 kHz, the maximum frequency was 2.7 ± 0.07 kHz, and the dominant frequency was 2.1 ± 0.02 kHz (Table 1). This vocalization type was observed in all treatments, except the Female treatment (Fig. 2).

Type IV vocalizations (N = 9) are a short (0.049 ± 0.01 s) tonal vocalization characterized by a longer bandwidth, higher minimum frequency (2.2 ± 0.3 kHz), higher maximum frequency (3.8 ± 0.2 kHz), and higher dominant frequency (3.1 ± 0.3 kHz) (Table 1, Fig. 2, Figure S1). On average, these sounds are composed of fewer pulses (1.2 ± 0.1) that are shorter in duration (0.039 ± 0.009 s) and shorter inter-pulse intervals (0.0014 ± 0.001 s) (Table 1). Only non-aggressive males were used in this treatment. This vocalization type was observed solely in the Male-Male treatment, across two trials (Fig. 2).

Type V vocalizations (N = 5) are relatively longer (0.25 ± 0.1 s) high-frequency chirps (Table 1, Fig. 2, Figure S1). They are characterized by a higher minimum frequency (2.5 ± 0.1 kHz), maximum (4.2 ± 0.3 kHz), and dominant frequency (3.4 ± 0.1 kHz) (Table 1). On average, these sounds are composed of a greater number of pulses (6.4 ± 2) that are shorter in duration (0.0045 ± 0.001 s) with shorter inter-pulse intervals (0.0021 ± 0.0006 s) (Table 1). This vocalization type was only observed in the Female-Female and Male treatments (Fig. 2).

Table 1

Descriptive statistics of the vocalization types (I-V) produced by painted turtles during behavioural trials.

Type	n	Dominant freq. (kHz) mean $\pm$ SE	Low- freq. (kHz) mean $\pm$ SE	High- freq. (kHz) mean $\pm$ SE	Duration (s) mean $\pm$ SE	No. of pulses mean $\pm$ SE	Pulse Length (s) mean $\pm$ SE	Pulse Interval Length (s) mean $\pm$ SE	No. of harmonics
I	96	1.8 ± 0.04	0.94 ± 0.04	3.3 ± 0.06	0.14 ± 0.01	4.0 ± 0.4	0.058 ± 0.008	0.0096 ± 0.002	0–4
II	10	1.9 ± 0.08	0.92 ± 0.1	3.3 ± 0.2	0.14 ± 0.02	2.0 ± 0.4	0.1 ± 0.02	0.0066 ± 0.0004	0–4
III	30	2.1 ± 0.02	1.6 ± 0.05	2.7 ± 0.07	0.15 ± 0.01	1.6 ± 0.5	0.14 ± 0.01	0.00047 ± 0.0003	0–2
IV	9	3.1 ± 0.3	2.2 ± 0.3	3.8 ± 0.2	0.049 ± 0.01	1.2 ± 0.1	0.039 ± 0.009	0.0014 ± 0.001	0–3
V	5	3.4 ± 0.1	2.5 ± 0.1	4.2 ± 0.3	0.25 ± 0.1	6.4 ± 2	0.0045 ± 0.001	0.0021 ± 0.0006	0
Total	150	2.00 ± 0.04	1.2 ± 0.05	3.2 ± 0.05	0.14 ± 0.009	3.3 ± 0.3	0.074 ± 0.006	0.0068 ± 0.001	0–4

Do vocalizations differ with social context?

We found that acoustic traits were significantly different between treatments (Table S1a). The statistical significance was largely driven by differences in treatments and not individual vocalizations, as the variance between acoustic traits covaried more with treatments (Fig. 3a) than within individual vocalizations (Fig. 3b). Significant differences were observed between MF vs MM, MF vs M, F vs MM, F vs M, and MM vs FF treatments (Table S2, Fig. 4a). NMDS plots showed that vocalization frequency (dominant and mean frequency) loaded strongly on axis 1, and vocalization duration (represented as pulse length and pulse number) loaded strongly on axis 2 (Fig. 4b). Despite some observed differences between solo (i.e., M, or F treatments) and together treatments (i.e., MM, FF, and MF), differences were not significant (Fig. 4c; Table S1). Together treatments had slightly higher variance than solo treatments, however this was not significant (Table S3, Table S4). Differences in acoustic traits were largely driven by sex, such that males had different and significantly larger trait variation than female treatments (Table S3; Table S4; Table S5), this was largely driven by MM treatments exhibiting greater variation than FF treatments (Table S2; Table S3; Table S4, Table S5). Male treatments were different than mixed treatments such that their vocalization frequency was most dissimilar (Fig. 4d). Compared to females, male turtles had larger acoustic trait variation, which was largely dissimilar in frequency and slightly dissimilar in duration (Table S4; Fig. 4d).

Do vocalizations predict the number of social interactions?

After accounting for social treatment, vocalization abundance was significantly negatively correlated with the number of interactions, such that the number of interactions decreased with increasing vocalizations (Fig. 5a, Table S6). We observed higher interactions in male treatments than female treatments (Fig. 5b, Table S6), however, this difference was not significant (Table S7). Vocalization abundance did not predict the probability of interacting with a conspecific, irrespective of sex (Table S6).

Discussion

Our study presents three major findings. First, we identified five putatively distinct types of vocalizations in painted turtles. Second, quantitative vocalization traits differed between sexes, with males producing distinct vocalizations and exhibiting greater variation in these traits than females. Third, we found that fewer vocalizations were produced during higher levels of interaction or proximity. The confluence of these findings pushes forward our understanding of the form and function of chelonian vocalization, which we discuss below.

We found that midland painted turtles exhibit at least 5 different vocalization types. We emphasize that vocalization types are a qualitative assessment of vocal diversity and are not strict categorizations. Indeed, variation of acoustic traits within and among types exist and a major goal of this field should be to understand sources and significance of this variation.

Nevertheless, it is interesting that the spectral characteristics of vocalizations uncovered in the present study show an impressive resemblance to vocalizations found in other chelonian species that are known to engage in social behaviour. For instance, we found that the most common vocalization type produced by midland painted turtles, type I, is similar in structure to vocalization type II in pig-nose turtles, *Carettochelys insculpta*, as well as type IV in the common snapping turtle, *Chelodina serpentina* (Ferrara et al. 2017; Lacroix

et al. 2022). Within our study, type II sounds are similar in harmonic structure to type A in the Chinese striped-neck turtle, *Mauremys sinensis*, of the closely related geoemydid family (Zhou et al. 2023a), and vocal types III and IV produced by *C. picta* resemble types Ia and Ib produced by Kemp's ridley sea turtle, *Lepidochelys kempii*, respectively (Ferrara et al. 2019). If these examples truly represent similarities in vocalization form, then it is interesting to consider whether these examples represent convergence or homology. Acoustic communication is likely ancestral among chelonians and tetrapods more broadly (Jorgewich-Cohen et al. 2022), but there is enormous potential for independent evolution of vocalization traits among chelonian lineages. Thus, it is paramount to uncover in chelonians the function of vocalization in general and to understand how form relates to function of chelonian vocalizations. Our experimental trials provide some limited insight into why chelonians vocalize, and under what circumstances vocalizations are used.

Evidence from our solo trials suggests that males exhibit greater variation in acoustic traits. A simple explanation for greater male variability is because many of our solo males had previously been placed in a M-F trial and had chased females. These males experienced different pre-treatment conditions than turtles other treatments, which may have affected their behaviour, even though these males had a day to recover from the failed trial. Yet, other explanations are possible. For instance, relatively greater variation is expected among traits that are sexually selected (Pomiankowski & Moller 1995; Reinhold & Engqvist, 2013; Wyman & Rowe 2014). Strong mate competition among males generates directional selection on male traits, which can favour condition-dependent expression of traits that promote male mating success (Rowe & Houle 1996), thereby generally increasing the variance in male traits subject to sexual selection. While there is little evidence of greater male variability in behaviours exhibited by both sexes (Tarka et al. 2018; Harrison et al. 2022), relative variability of vocalization traits is poorly explored as vocalizations in many non-chelonian species are generally restricted to a single-sex, such as in Aves. We speculate that greater variability in males reflects condition dependence, whereas male vocalizations reflect an honest signal of quality (Rowe & Houle 1996). Uncovering whether acoustic signalling in males at least partly reflects honest signalling of quality could involve an evaluation of associations between male vocalization traits and secondary sexual characteristics that ought to affect mating success, such as claw length, body size, and perhaps behavioural traits involved in aggression. Relatedly, the low variation in female vocalization traits may partly related to ecological selection, with signals possibly functioning in behaviours like assortative mating or as recognition cues (Wilkins et al. 2013). More broadly, an important finding in the present study is that of increased male variability in acoustic traits. While our study is not conclusive because many males experienced different pre-treatment conditions, it is possible that the greater variation in male traits arises because vocalizations are sexually-selected in males, and/or that female vocalization traits are under strong ecological selection which dampens female variance relative to males.

We observed seven instances of a unique vocalization in male-male interactions. If sexual selection is involved in increased vocal trait variation in males, then this finding suggests that it may be forces of intrasexual selection, rather than intersexual selection, that resulted in the expression of greater acoustic variation. One intriguing possibility is that male vocalizations may be involved with the formation of social hierarchies strictly among males. In many reptile groups, including turtles, there is evidence of male-male combat to compete for rank, resources, and mates (Keevil et al. 2017; Moldowan et al. 2020), where higher-

ranking individuals in each group may achieve higher mating success (Masin et al. 2020; Bush et al. 2016). Among Emydids, European Pond Turtles (*Emys orbicularis galloitalica*) form dominance hierarchies through aggression (Masin et al., 2020; Rovero et al. 1999). Similar behaviours have been documented in captive painted turtles (Ernst & Lovich 2009), as well as instances of aggression among basking individuals (Bury et al. 1979), and conspecific injuries (Keevil et al. 2017; Moldowan et al. 2020), all of which suggest the possibility that social hierarchies are formed in painted turtles. Indeed, during our experimental trials, aggression was frequently exhibited by males, including behaviours such as lunging, mouth gaping, and chasing. In extreme cases there appeared to be a threat of physical attack by a male in the tank; these trials were discontinued for ethical reasons, and males were subsequently removed from the two-turtle treatments and allocated to solo male treatments. Instances of aggression in male-male pairs would often occur within the first 5 minutes of entering the study tank. While speculative, these instances of aggression may be due to two males who had not interacted extensively in nature, outside of a manipulated environment, such that one male eventually asserted dominance over another male. Increased social interactions may help establish dominance hierarchies, which is ultimately beneficial as an established hierarchy can reduce the likelihood of aggression when searching for mates (Senar et al. 2010). We underline, however, that the hierarchy hypothesis that we discuss herein is based on circumstantial evidence, and further study is needed to understand the function of male vocalization and behaviour in painted turtles.

Vocal behaviour always occurs in an ecological context, and it often occurs in conjunction with other forms of interaction and communication (Higham & Hebets 2013), such as tactile signalling (e.g., Laird et al. 2016; Caldwell et al. 2022). We observed that vocalization abundance was negatively associated with close physical interactions, suggesting that vocalizations are used at a distance, whereas tactile or visual cues dominate close-range communication. Indeed, painted turtles are well known to engage in various tactile communication behaviours, both in sexual and aggressive contexts (Moldowan et al. 2020), and as such, tactile or visual communication may be more important in proximity. The negative correlation between close interaction and vocalizations may reflect a bimodality to signalling in painted turtles. In painted turtles, vocalizations may be used more frequently at a greater distance, while tactile or visual cues are used in closer proximity to the receiver (Uy & Safran 2013; Hebets & Papaj 2005). Painted turtle habitats, often characterized by structural complexity and murky waters, may drive the need for such signalling strategies. While turtles were likely visible to one another in the present study, such that vocalizations were likely not being used to signal location, it is notable that the aquatic environment of turtles is often characterized by low visibility. In the present study population, for instance, physical habitat features such as large sphagnum moss mats and rooted vegetation also obstruct visibility, and both organic matter and tannins render the water murky. Structural complexity and water turbidity are common features of turtle habitats. Due to a habitat with poor visibility, it would not be surprising if vocalizations play some role in conveying information about the locality of individuals at a distance beyond visual range (Giles et al. 2009). Previous work, for instance, has shown that vocalizations that provide location information often consist of short, repetitive chirps, sometimes exchanged in a call-and-response manner between two or more individuals (McCracken 1986). Brief, high-frequency, repetitive chirp sounds were also common in our trials, although whether their function is to provide location information is unknown.

Acoustic communication is a cornerstone of animal behaviour in diverse taxa, as vocalization patterns often correspond to essential activities like breeding, foraging, and socializing. Uncovering the function and adaptive significance of turtle vocalizations at all life stages will represent a major step in understanding the evolution of diversity of signalling behaviours. Here we describe a vocal repertoire for a widespread turtle species, and the results of our experiments suggest that vocalizations can be sex-specific, that vocalization traits depend on social context, and that vocalizations may be part of a bimodal signalling regime in which tactile and/or visual cues are involved at closer range. We suggest that male-male dominance behaviours related to sexual selection and social hierarchies in males may be linked to vocal signalling, contributing to a broader understanding of Emydid communication systems. As our knowledge of sensory ecology and sexual selection theory is based on model systems with sensory modalities that are easy to observe in the lab or in the wild (e.g., lizards, birds, fish, frogs), less is known about species that are difficult to directly observe, such as turtles, and that spend most of their adult life in aquatic environments (Hites et al. 2013; Doody et al. 2021; Cummings and Endler 2018). While the field of chelonian communication continues to develop, carefully designed experiments can advance our knowledge of turtle behaviour and social systems (Hites et al. 2013; Doody et al. 2013; Burghardt et al. 2021). Our work provides foundational insights into the diversity of signalling behaviour in painted turtles, leading to exciting lines of inquiry in chelonian vocalization function and evolution broadly.

Methods

Study species, study area, experimental set-up

This study leverages a population of Midland Painted Turtles (*Chrysemys picta marginata*) from Algonquin Park, Ontario, Canada under long-term study (see Rollinson and Brooks 2008 for site description). In brief, the population consists of approximately 300 adults that inhabit two ponds, approximately 500m apart, and the sex ratio of the population is female-biased (3.44:1, female: male; Samson 2003). Adult painted turtles were captured from Wolf Howl Pond between July 3 to July 15, 2022; after nesting season and before autumn, when a majority of mating likely takes place (Moll 1973; Moldowan et al. 2020b). Captures were made using a canoe, dip-net, and hoop traps baited with sardines. Turtles were identified using unique numerical identifiers based on notches on the marginal scutes of the carapace. All turtles in the present study were sexually mature, and maturity and sex of turtles were determined by carapace morphology and claw length, as male foreclaw length is significantly longer than females (Cagle 1942). Each individual's unique ID was painted on their carapace with latex-based paint, using white paint for females and red paint for males, facilitating identification during experimental trials.

Experimental trials took place at the Algonquin Wildlife Research Station, about 30km east of the study site. Turtles were captured at the field site, brought back to the Wildlife Research Station, measured, and held individually in buckets (26cm diameter) overnight. Trials took place the following day, and individuals were returned to their individual buckets after the trial, and most were released the following day (see exceptions below). We set up three adjacent 170L cattle tanks in a mostly shaded area, facilitating observation during concurrent trials (Fig. 1), and filled each tank $\frac{3}{4}$ full with water from Lake Sasejewun. Trial water was not changed between trials, but typically there was only one trial per tank per day (range = 0–2), and multiple

days would often elapse between trials; thus, it is possible that chemical cues from previous trials affected vocalizations in subsequent trials. To ensure consistent and adequate thermal conditions (Edwards 2005), water temperature was measured 1 hour before every trial. We suspended a single hydrophone (Aquarian H2A hydrophone, Aquasonic Inc.) 1-inch above the bottom of each tank, as well as a single Lapelier XLR microphone (LV4-O, Movo Photo Group LLC.) 1-inch above the surface of the water (Fig. 1), to control for sounds that were external to each tank. The hydrophone and microphone for each tank were connected to a separate audio recorder (Tascam DR 40X, TEAC America Inc.). To quantify activity levels and the number of interactions, we set up a camera trap (Browning Strike Force Pro X 1080, Browning Trail Cameras) above each tank, which recorded the turtle's position in the tank every 5 seconds. Trial start time occurred between 12:00 to 17:00 hours. We monitored trials behind a mesh screen, at approximately 2 m distance. Since males can show signs of aggression in social situations and may attack and bite other turtles (Moldowan et al. 2020a), trials were discontinued for ethical reasons if we observed that males engaged in chasing behaviour, as this prevented turtle injury. Trials were discontinued on four occasions due to aggressive behaviour exhibited by males against females within male-female trials. Consequently, five males displaying repeated aggression were excluded from subsequent paired trials, but used in individual trials. The aggressive males were returned to their individual buckets in the lab, and their individual trials took place the following day. Thus, aggressive males were held for two days before being released to their natal pond. All aggressive individuals were adults that had been captured in previous years and so were not novel individuals in the study population.

Trials began by removing the turtle from its individual bucket and placing the turtle immediately in a 170L cattle tank. Treatments included 1 solo male per tank (M), 1 solo female per tank (F), 2 males per tank (MM), 2 females per tank (FF), and both 1 male and 1 female in a tank (MF). Trials were 1 hour in length and were replicated 7 times, totaling 35 trials, and 35 hours of recording. Solo trials were used because vocalizations may, for instance, be used primarily to locate other individuals in the absence of a visual cue, in which case more vocalizations might be observed in solo treatments. A total of 46 unique turtles were used in our trials: 20 males and 28 females, with each female unique to one trial. Males were used in a maximum of two trials, for two reasons. First, locating males during the study timeframe was challenging, as the sex ratio is highly female biased. Second, some males engaged in chasing behaviours when paired with another individual, and the trial had to be discontinued well before the 1-hour time mark; these trials were discarded from our analysis. All males that engaged in chasing behaviour removed from a paired treatment and later allocated to a solo M treatment. As a result, all "M" treatments involved a 1hr recording from a male that had been aggressive when paired with another turtle. These males were given a 24-hour period of 'calm down', in which, directly following an incident of aggression, they were returned to their bucket within the lab. After the 24-hour period, the 'relaxed' male was used for a solo M treatment. Other males were used once in an MM trial, and once in an MF trial. Removal of aggressive males from paired treatments, for ethical reasons, and using these males only in solo treatments weighs on our interpretation of results: a specific subset of male turtles with a particular behavioural phenotype comprise our solo male trials, and we discuss this caveat in the Discussion. Turtles were returned to their natal ponds within 24 hours of their final experimental trial.

Ethics declaration

This study, including all experimental protocols, was performed in line with the University of Toronto Animal Use Protocol #20011948, Ontario Parks research authorization for nest collection, and Wildlife Scientific Collector's Authorization no. 1100425 and 1103122. Animal collecting and use was authorized a Wildlife Scientific Collectors Authorization #1100425 issued by the Ministry of Northern Development, Mines, Natural Resources and Forestry, and an Animal Use Protocol #20011948 approved and issued by the University of Toronto Local Animal Care Committee.

Quantifying the vocal repertoire

To quantify the vocal repertoire of adult painted turtles ($n = 46$ turtles), we employed a clustering algorithm (via Kaleidoscope Pro, Wildlife Acoustics, 2021) to extract acoustic detections. We manually inspected the output of our clustering model, and refined the model parameters over multiple iterations to ensure vocalizations were not missed or split (Table S8). We set the maximum distance from the cluster centre to 2 to ensure that all audio detections within our defined acoustic window were included in the output, irrespective of their similarity to the cluster centre. C.V. manually labelled each sound detection that was extracted (i.e., visually examined the spectrogram and listened to the detection to correctly classify it) based on predefined criteria and prior knowledge of turtle acoustic signals (Table S9). This resulted in

18,809 manually labelled acoustic detections from 35 hours of audio, inclusive of both turtle vocalizations and sounds that were not vocalizations.

To help identify turtle vocalizations, and to ensure that sounds produced during turtle movement within the tanks (e.g., hitting/scratching the side of the tank) were not misrepresented as vocalizations, we replicated mechanical sounds that could be produced by turtles using a plastic toy turtle of similar body size in one of the experimental tanks. We conducted 7 different 2-minute trials to simulate movement sounds: (1) a control (no use of plastic turtle), (2) intermittent scraping on the side of the tank, (3) continuous scraping on the side of the tank, (4) intermittent scraping on the bottom of the tank, (5) continuous scraping on the bottom of the tank, (6) hitting the hydrophone, and (7) breaching the water with its body. Each detection was manually cross-referenced with suspected turtle vocalizations to discriminate between vocalizations and sounds produced during movement. If we were able to manually replicate any putative turtle vocalization using our 2-minute trial data, the sound was not labelled as a turtle vocalization. In an overwhelming majority of cases, however, sounds generated manually by researchers were different from those generated by turtles.

For each turtle vocalization detected in our experiments ($n = 150$), C.V. quantified the dominant frequency, maximum frequency, minimum frequency, the number of harmonic bands, pulse number, mean pulse length, mean pulse length interval and the duration of each vocalization, following Lacroix et al (2022), Ferrara et al. (2013), and Giles et al. (2009). We grouped vocalizations into 'types' based on qualitative acoustic similarities and common patterns in the spectral characteristics. Extracted vocalisations were re-binned by C.L. and C.V. twice to ensure consistency among vocalisation types. Accuracy among binned vocalisation types was assessed using random forest analysis, where the data was split into a 20:80 test and train data set.

Extracted vocalizations ($n = 150$) were quantified for multiple temporal and spectral traits, including the dominant frequency, maximum frequency, minimum frequency, the number of harmonic bands, pulse number, mean pulse length, mean pulse length interval and the duration of each vocalization (following Lacroix et al 2022, Ferrara et al. 2013, and Giles et al. 2009). Calls were initially grouped into vocalization types based on qualitative acoustic similarity and shared spectral patterns. They were then re-binned twice by C.L. and C.V. to ensure consistency. To evaluate the accuracy of manual categorization, we used a random forest classifier (Wright and Ziegler 2017). Acoustic traits were used to predict assigned call type, with data randomly split into training (75%) and testing (25%) sets. The model was run with 800 trees and default feature sampling. Classification performance was assessed using a confusion matrix comparing predicted and manually assigned call types.

Do vocalizations differ with social context?

As manually binned vocalization “types” represent our best qualitative categorization among a trait continuum, we chose to quantitatively investigate vocalizations among social contexts. Specifically, we investigated differences in acoustic traits among treatments using nonmetric multidimensional scaling (NMDS) ordination and permutational multivariate analysis of variance (PERMANOVA) (*vegan* package, Oksanen 2012) using a Euclidian dissimilarity matrix in R (R version 4.3.3, R Core Team 2024). Traits include dominant frequency (kHz), maximum frequency (kHz), minimum frequency (kHz), the number of harmonic bands, pulse number (s), mean pulse length (s), mean pulse length interval (s) and total duration (s) (see above). All vocalization traits were scaled and centred prior to analyses. Multicollinearity among traits were inspected using Spearman’s rank correlation, and traits exceeding correlations above 0.8 were removed. Number of pulses were removed as a result. NMDS model fit was checked by ensuring the stress value was below 0.2 and the Shepard Plot showed a high correlation between observed dissimilarity and ordination distance (*vegan* package, Oksanen et al. 2024, Figure S2). Notably, in the three treatments that featured two turtles (M-M, F-F, and M-F), we could not identify which turtles produced a given vocalization, and therefore we could not use individual-level characteristics (e.g., body size) as predictors of vocalizations in our analyses.

First, we tested whether acoustic signals were different among treatments by running a 1-factor PERMANOVA with treatment as the independent variable (M, F, MM, FF, MF) (Table S10a, S11, S12). Then, we tested whether acoustic signals were different among social and sex contexts by running a 2-factor PERMANOVA with sex (together, solo) and social (male, female, mix) context as the independent variable (Table S10b, S11, S12). Sex and social contexts were defined based on their treatment (Table S11). All PERMANOVA’s were stratified by trial ID (defined as a unique identifier for treatment, and trial number) to control for non-independence of vocalizations among trials; however, as above, we could not include individual-level information for turtles in our analysis (e.g., turtle identity as a random effect) as most treatments were paired, so we do not know which turtle in the pair emitted the sound. PERMANOVA’s with significant main effects were further investigated by running a pairwise comparison test (Arbizu 2020). Then, we tested whether variance in acoustic traits were homogenous among treatments, sex treatments, and social treatments by running an analysis of multivariate homogeneity of group dispersions (*vegan*

package, Oksanen 2012). To inspect which group pairings do not have homogenous variances, we subsequently ran a tukey test.

Finally, we explored how acoustic traits co-vary at different hierarchical units (trial vs. individual vocalizations) to test whether observed trait covariation is driven by treatment or individual vocalizations. We employed multivariate linear mixed effect models and visualized the correlation matrix among traits (Table S10c, S12) (Bates et al. 2015; Wei & Simko 2021). We fit a multivariate linear mixed-effects model using the lme4 package in R, with the standardized acoustic trait values as the response. The fixed-effects structure included the interaction between trait identity and treatment, allowing treatment effects to vary across traits. Random effects were specified as random slopes of trait nested within trial identity, and random slopes of trait within each vocalization, thereby accounting for non-independence among traits measured within the same trial and within the same vocalizations (Table S10c, S12).

Are vocalizations correlated with the number of social interactions?

To quantify the number of putative social interactions per trial, we used the instant registration procedure following methods described in Martin & Bateson (1986) and Ibañez and Vogt (2015). For each trial, we took note of each turtle's relative location every 3 minutes using camera trap footage (Fig. 1c), generating 20 observations per turtle per trial. We did not use turtle location that was captured every 5 seconds on our camera trap, as placement of turtles would be strongly autocorrelated under this approach; we instead opted for a 3 minute interval, which is likely to result in estimates of turtle position that depend less on the previous observation. For each recording session, audio recordings and camera traps were temporally synchronized. Relative position within each treatment was delimited by dividing the tank into 3 sections. As a result of an angled camera location in each trial, only two out of three sections were clearly visible in the camera footage. Thus, if a turtle was not seen in the camera, it was assumed they were in the unobservable section, as they could not be in any other location. We subsequently quantified the number of social interactions by counting the number of instances 2 turtles were in the same section of the tank. We use the number of times turtles were in the same section as a proxy for social interactions because specific social behaviours (mouth gaping, mounting, foreclaw displays, mounting or shell stacking) could not be discerned through camera trap footage. Treatments with a single turtle were excluded from this analysis.

To investigate whether vocalizations predict the number of social interactions among social contexts, we employed a poisson model (family = 'poisson') using the glmmTMB package in R (Brooks et al. 2023). The number of social interactions per trial was the response variable. The number of vocalizations per trial (vocalization abundance) was the dependent variable. The model was fit with sex treatment as a covariate (3 levels, M, F, or MF) and an interaction term (Table S10d, S11, S12). If the interaction term in the model was non-significant, it was removed, and the model was refit (Table S10e) (Beck and Bliwise 2014). Model assumptions were checked visually by inspecting overdispersion, model residuals with fitted values, and qq plots using the DHARMA package in R (Hartig 2022).

Declarations

Funding Declaration

This research was funded by Discovery Grants from the Natural Sciences and Engineering Research Council of Canada (NSERC) Discovery Grants to NR, and an NSERC CGS-M scholarship to CL.

Ethical note

This study was performed in line with the University of Toronto Animal Use Protocol #20011948. Ontario Parks research authorization for nest collection, and Wildlife Scientific Collector's Authorization no. 1100425 and 1103122.

Conflict of Interest

The authors declare no conflicting interests.

Author Contribution

CV conceptualized the study, collected the data, conducted the acoustic analyses, wrote the first draft of the manuscript, reviewed and edited the manuscript. NR conceptualized the study, provided supervision, reviewed and edited the manuscript. CL conceptualized the study, conducted the acoustic and statistical analyses, provided supervision, reviewed and edited the manuscript.

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Data Availability

Our data and code is archived in Dryad Digital Repository and Zenodo, respectively (DOI: 10.5061/dryad.3n5tb2rv9; reviewer link: http://datadryad.org/share/LINK_NOT_FOR_PUBLICATION/pmQ-uMyfJfUgf_CEnzJvwkGgtlUSTIyix_i-wNv8Rxg)

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Figures

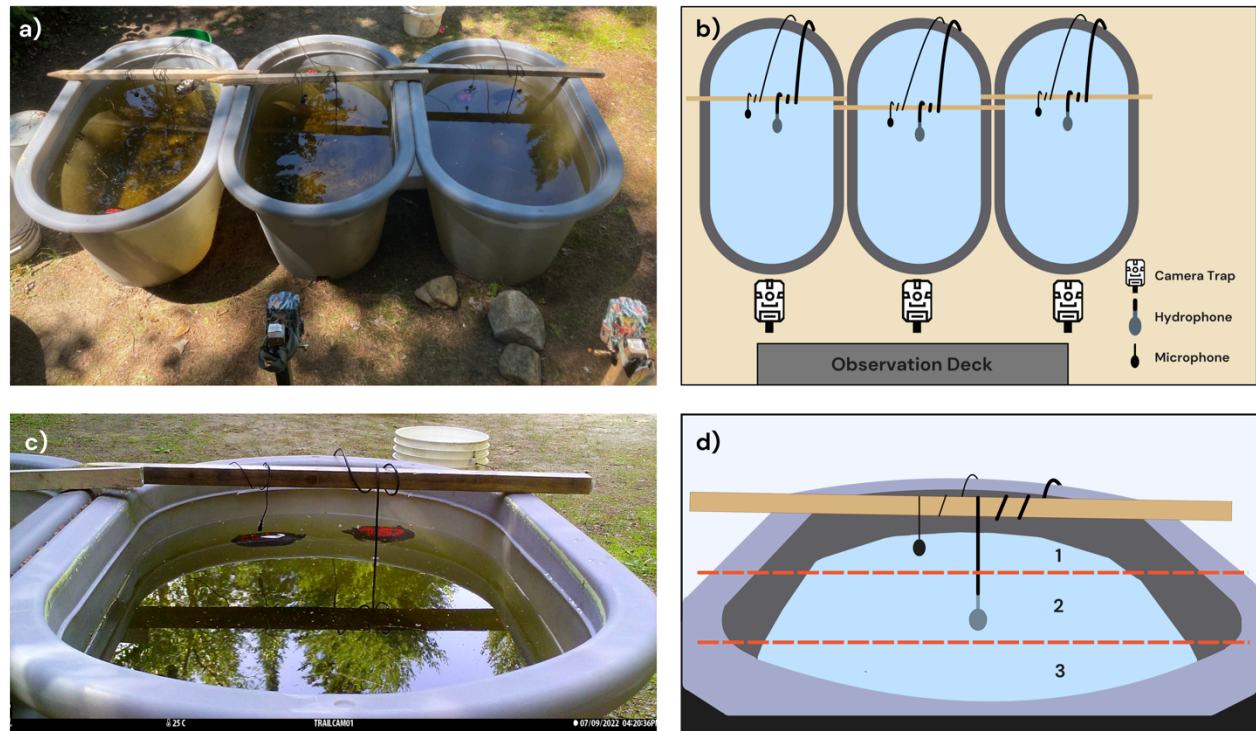


Figure 1

Photograph of the physical experimental setup (a). Schematic of experimental set-up where recording and observation took place at the Algonquin Wildlife Research Station in July 2022 (b). Image taken from camera trap timelapse footage (c). The time lapses were used to record the activity level, latency, and interaction count. The delineations were superposed onto the timelapse during analysis. Schematic of cattle tank delineating the separation of three equal sections (d). The turtle's number of interactions (when applicable) were recorded utilizing this delineation among sections.

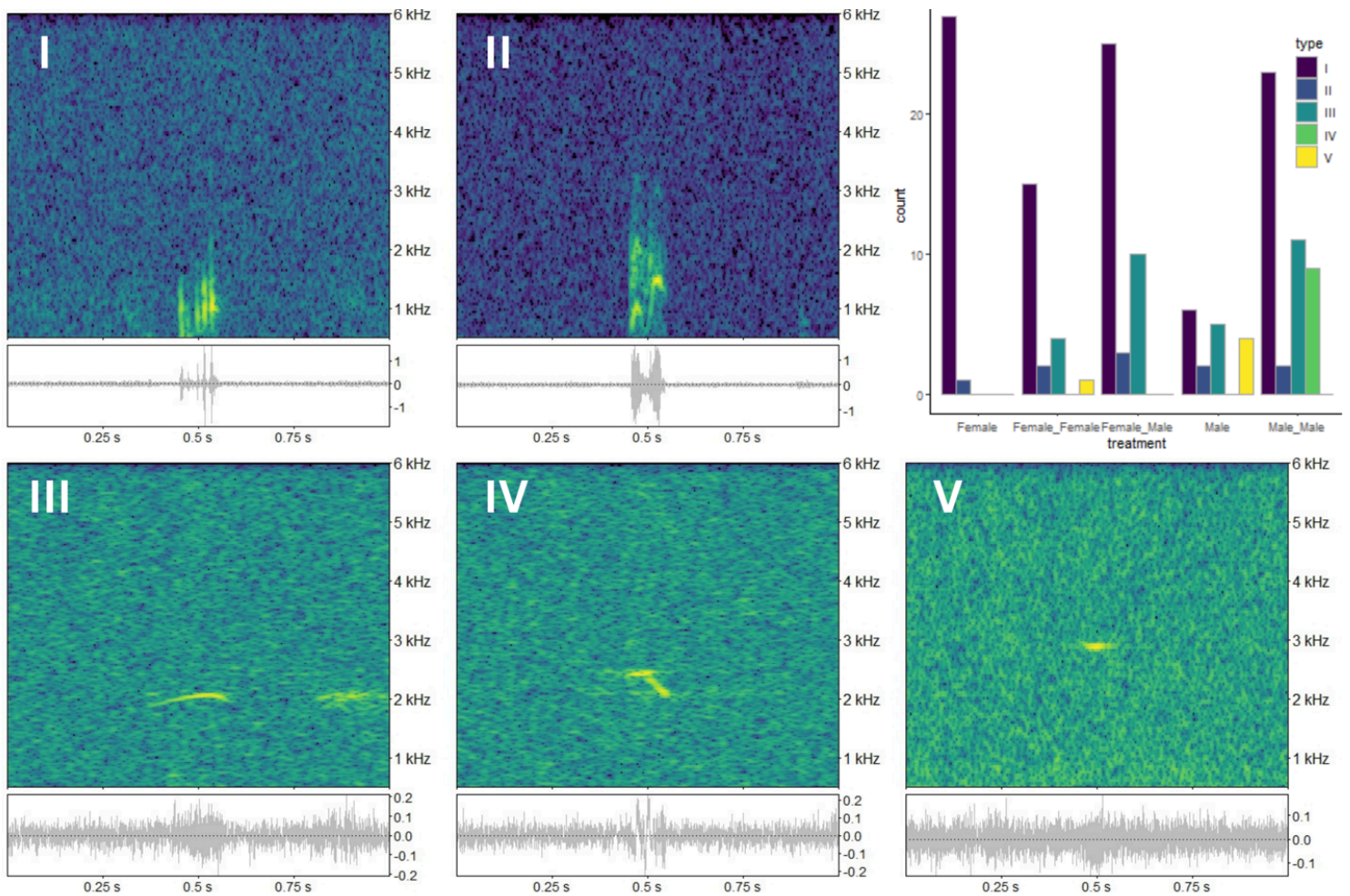


Figure 2

Spectrogram (top panel) and oscillogram (bottom panel) of all vocalization types (I-V). One representative from each vocalization type across all trials was chosen. We used a 256 sample window size with 99% overlap to generate spectrograms. The bar plot shows the relative frequency of each vocalization type with respect to each treatment.

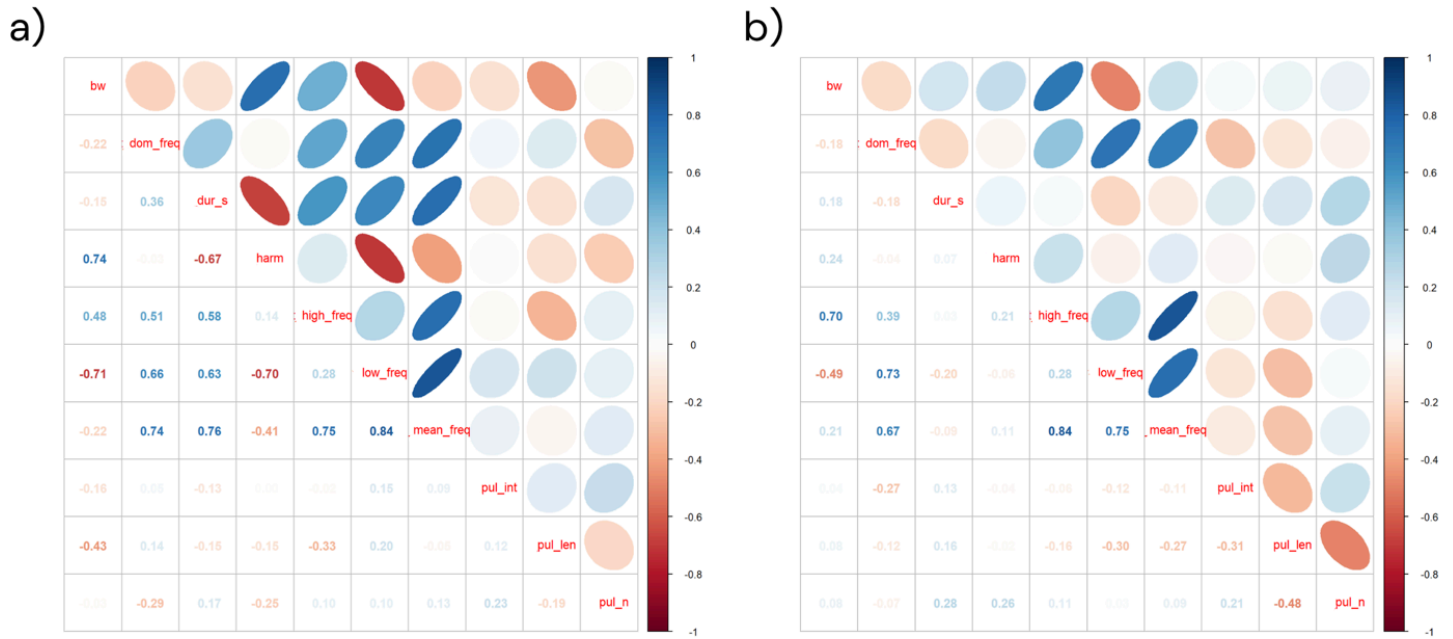


Figure 3

Covariance between all possible pairs of acoustic traits. Acoustic traits were defined as bandwidth (bw), dominant frequency (dom_freq), duration (dur_s), the number of harmonics (harm), high frequency (high_freq), low frequency (low_freq), mean frequency (mean_freq), pulse interval (pul_int), pulse length (pul_len), and pulse number (pul_n). Values indicate the relative strength of the covariance ranging from 0 to 1, where negative values indicate a negative correlation (red) and positive values indicate a positive correlation (blue). Both the degree to which shapes are not spherical and the colour gradient indicate the strength of the relationship. Variance-covariance between acoustic traits was measured for both correlations of traits across treatments (a) and within individual vocalizations (b).

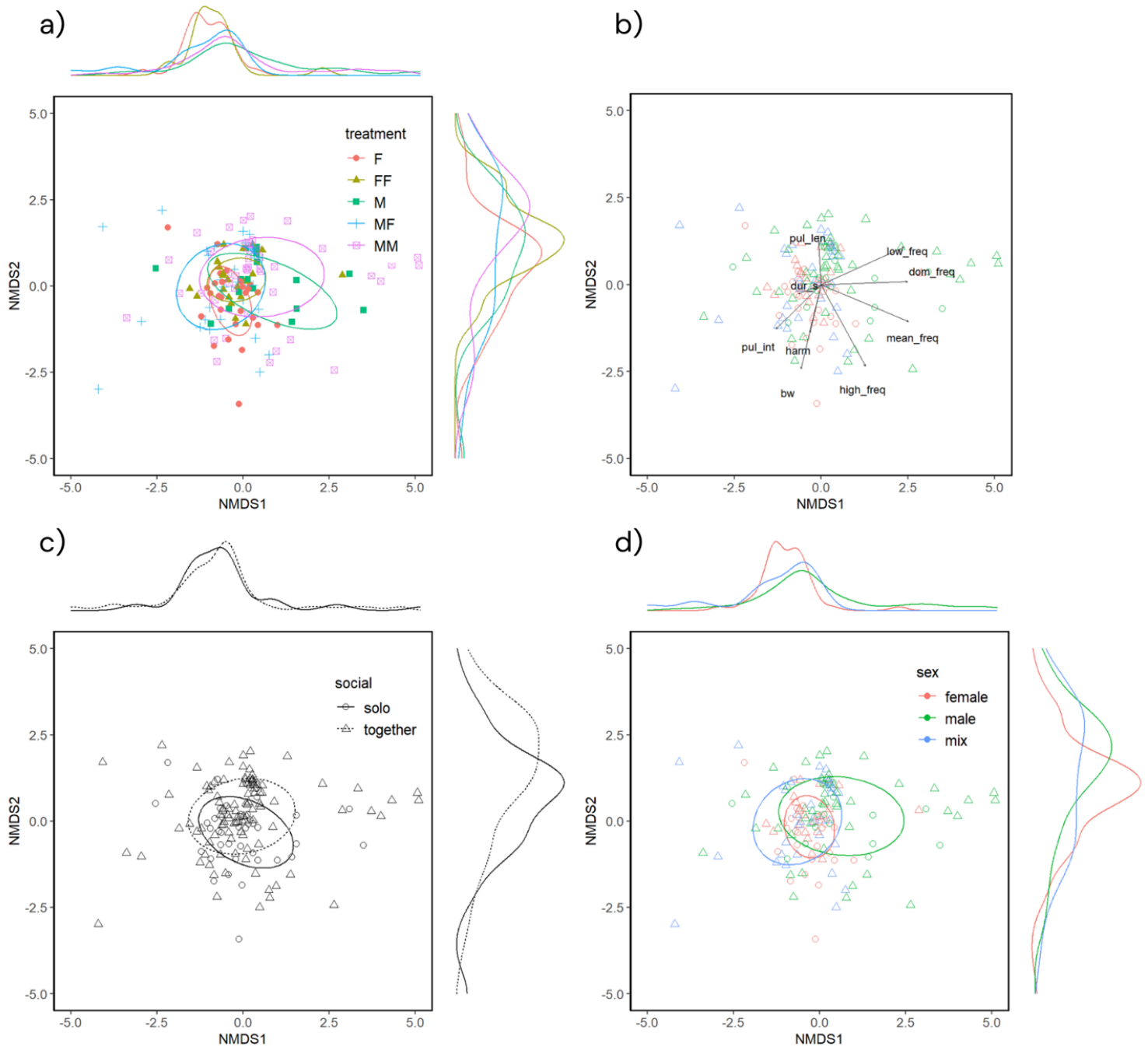


Figure 4

Non-Metric Multidimensional Scaling (NMDS) ordination plots showing differences in acoustic traits across experimental treatments. Acoustic traits were defined as bandwidth (bw), dominant frequency (dom_freq), duration (dur_s), the number of harmonics (harm), high frequency (high_freq), low frequency (low_freq), mean frequency (mean_freq), pulse interval (pul_int), and pulse length (pul_len). We investigated differences in acoustic traits between each treatment (M= 1 male, MM = 2 males, MF = 1 female and 1 male, FF = 2 females, F= 1 female) where each treatment represents a different shape and colour combination (a). Acoustic trait loadings show which traits are most represented on the NDMS axes (b). We show differences among social contexts where solo treatments (F, M) are represented as an open circle encircled by a solid line, and together treatments (MM, MF, FF) are represented as an open triangle encircled by a dotted line (c).

We show differences among the sexes where female treatments (FF, F) are represented in red, male treatments (MM, M) in green and mix treatments (MF) in blue (d). Line plots show relative density of observations along the NDMS axes.

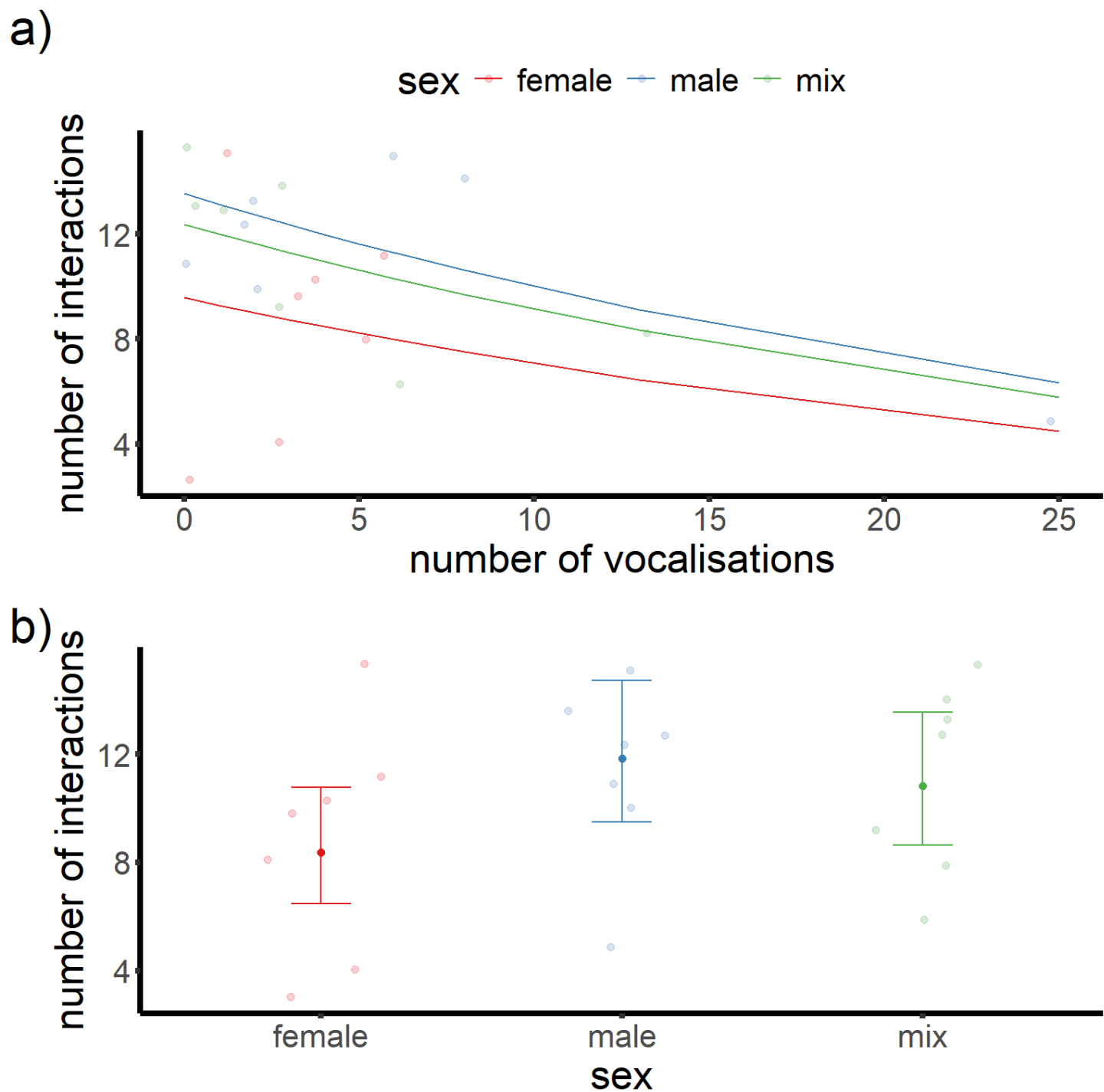


Figure 5

Line plot showing the predicted correlation between the number of vocalizations and the number of interactions (a). Model predicted number of interactions across sex treatments. Error bars show 95%

confidence intervals (b). Sex treatments were pooled as female (2 females; 1 female), Male (2 males; 1 male) and Mix (1 male and 1 female) treatments. Faded points show the raw data.

Supplementary Files

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- [Appendices.PTVocal.Mar23.2026.docx](#)