Physiology & Behavior 152 (2015) 143–150

Contents lists available at ScienceDirect

Physiology & Behavior

journal homepage: www.elsevier.com/locate/phb

Vocalizations convey sex, seasonal phenotype, and aggression in a seasonal mammal

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HIGHLIGHTS

- We recorded vocalizations from hamsters of different sexes and photoperiods.
- Ultrasonic vocalization subtypes differentially reflect seasonal phenotype and sex.
- Broadband calls reflect seasonal phenotype and sex, and relate to aggression.
- USVs and BBCs are signals used during same-sex encounters of Siberian hamsters.

ARTICLE INFO

Article history:
Received 31 July 2015
Received in revised form 30 August 2015
Accepted 14 September 2015
Available online 18 September 2015

Keywords:
Animal communication
Vocal repertoire
Aggressive signal
Syllables
Androgens
Dwarf hamster

ABSTRACT

Seasonal variation in social behavior is often accompanied by seasonal variation in communication. In mammals, how seasonal environmental cues influence aggressive vocalizations remains underexplored. Photoperiod is the primary cue coordinating seasonal responses in most temperate zone animals, including Siberian hamsters (*Phodopus sungorus*), a species that undergoes reproductive inhibition and increased aggression in winter. During same-sex aggressive encounters, hamsters emit both broadband calls (BBCs) and ultrasonic vocalizations (USVs) that indicate aggression and the vocalizer's sex, respectively; however, it is not known whether these rodents adjust specific elements of their vocal repertoire to reflect their photoperiod-induced seasonal phenotypes. To address this, we recorded vocalizations emitted during dyadic interactions between male or female pairs of hamsters housed in long or short photoperiods and measured serum testosterone levels. USV emission rate remained stable across photoperiods, but proportional use of USV subtypes varied in novel ways: 'jump' USVs were sensitive to seasonal phenotype, but not the vocalizer's sex, whereas 'plain' USVs were sensitive only to the sex of the vocalizer. BBC emission rate varied with seasonal phenotype; short-day non-reproductive hamsters produced more BBCs and demonstrated increased aggression compared with reproductive hamsters. Testosterone, however, was not related to vocalization rates. Collectively, these findings demonstrate that changes in the vocal repertoire of Siberian hamsters reflect sex, aggression, and seasonal phenotype, suggesting that both BBCs and USVs are important signals used during same-sex social encounters.

1. Introduction

Many temperate zone species demonstrate marked seasonal variation in social behavior, including gregariousness [1], aggression [2,3], and reproductive behaviors [4]. Coupled with variation in social behavior, animals modify the use of their communicative signals on a seasonal basis. Excellent species for studying seasonal variations in communication exist across multiple vertebrate classes. For example, the substantial body of work in multiple species of songbirds has provided a detailed picture of seasonal variation in vocal behavior, particularly for vocalizations related to courtship (e.g., [5–7]). This work illustrates that animals modify acoustic signals seasonally. Vocalizations are important determinants of mate acquisition for many species, and thus, many species that exhibit seasonality in reproduction also exhibit seasonal shifts in vocal behavior with animals exhibiting increased vocalization rates during the breeding season (e.g., coyotes, frogs, humpback whales, midshipman fish, red deer, sea lions [8–13]).

Whereas courtship-related vocalizations are imperative to an animal's fitness, aggressive vocalizations can also provide fitness benefits. For example, the soft song of song sparrows (*Melospiza melodia*) is a signal of "aggressive intent," which could allow signalers to avoid potential injuries incurred during an aggressive encounter [14]. Furthermore, the production of these calls varies on a seasonal basis, with

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http://dx.doi.org/10.1016/j.physbeh.2015.09.014

proportionally more soft song being produced during the non-breeding season when territoriality dominates these sparrows' social behavioral repertoire [14]. Thus, seasonal changes in the proportional use of vocalization types may indicate differences in the information content being conveyed by animals across different seasonal contexts, or may indicate that animals have shifted between seasonal states. Here, we explicitly test the idea that vocalizations reflect shifts in seasonal phenotypes in a seasonal rodent by inducing seasonal shifts with changes in photoperiod (i.e., day length).

Photoperiod serves as the primary environmental cue used by most mammalian species to coordinate seasonally appropriate responses [15]. Temperate zone-inhabiting mammals undergo marked morphological, physiological and behavioral changes in response to changes in photoperiod. For example, animals maintained in short "winter-like" days (i.e., <12 h of light/day) undergo gonadal regression, decreases in sex steroids, and changes in critical social behaviors, such as aggression [16]. Therefore, by manipulating photoperiod within the laboratory we can gain insight into seasonal changes in vocal behavior and the relationship of this behavior to seasonal phenotypes of individuals.

Siberian hamsters (*Phodopus sungorus*) are an excellent rodent species with which to examine how the vocalizations produced during male–male or female–female social encounters are influenced by the pair's sex, seasonal phenotype, and aggression. First, both male and female Siberian hamsters exhibit gonadal regression and display *increased* territorial aggression when housed in short "winter-like" days compared with hamsters housed in long "summer-like" days [2,3]. Most vertebrate species display aggression only when reproductively active, making it difficult to dissociate the individual effects of reproductive physiology, reproductive behavior, and aggression (reviewed in: [17]). Siberian hamsters, in contrast, have elevated levels of aggression when gonads are regressed. Whereas most short-day hamsters inhibit reproductive physiology (i.e., "short-day responders"), a subset of hamsters are unresponsive to short photoperiods (i.e., "short-day non-responders") and are physiologically indistinguishable from long-day hamsters. They maintain functional reproductive physiology, brown/gray pelage, and long-day-like body mass, food intake, and thermoregulation ([18,19]; reviewed in: [20]). Thus, Siberian hamsters exhibit distinct seasonal phenotypes: a "summer" morph (long-day hamsters and short-day non-responders) and a "winter" morph (short-day responders). These natural fluctuations in seasonal phenotypes make it possible to dissociate between the relative contributions of the physiological response to photoperiod and the photoperiod cue itself to seasonal variation in vocalizations.

We have recently shown that Siberian hamsters housed in long days vocalize during same-sex aggressive encounters, emitting two vocalization classes: high frequency (>20 kHz), narrowband ultrasonic vocalizations (USVs) and lower frequency, broadband calls (BBCs) [21]. Specifically, we found that the proportional use of different vocalization types is dependent on whether the same-sex pair of hamsters is male or female. Further, BBCs, but not USVs, are related to aggression during same-sex encounters [21], demonstrating that Siberian hamster vocalizations are sensitive to social context. It is important to note that all the animals in this study were in long-day, "summer" condition; whether production of these vocalizations varies in response to changes in seasonal phenotypes, and whether environmental cues such as photoperiod play a key role in coordinating changes in vocal repertoire, remains unknown.

In the present study, we investigated relationships among seasonal phenotypes, aggression, and vocal production, as well as the effects of these factors on specific aspects of the vocal repertoire. We also examined the potential role of photoperiodic variation in the gonadal steroid testosterone (T) in regulating vocal production. We predicted that changes in vocal behavior would reflect photoperiodic changes in aggression, such that non-reproductive hamsters, which display more aggression, would produce more BBCs and would use proportionally more 'rattle' BBCs, which are more closely related to aggression in breeding-condition Siberian hamsters [21]. Because USVs are not related to aggression, we predicted no changes in vocalization rate for USVs across photoperiods [21]. Lastly, because T is inversely related to aggression in males of this species [2], we predicted that T would be inversely related to BBCs. By testing these predictions, we can associate known photoperiodic changes in seasonal phenotypes and aggressive behavior with changes in the composition of the vocal repertoire of a seasonal rodent, and begin to address the physiological correlates of communication in a seasonal context.

2. Materials and methods

2.1. Animal housing and photoperiodic treatment

Adult (>60 days of age) hamsters were reared in a breeding colony at Indiana University, Bloomington. Hamsters were bred and maintained under long days (light:dark, 16:8 h) and group-housed at weaning (postnatal day 18). Ambient temperature was maintained at 20 ± 2 °C, and relative humidity was maintained at 55 ± 5%. Hamsters were given ad libitum access to tap water and laboratory rodent chow (Lab Diet 5001, PMI Nutrition). All procedures were performed in accordance with the NIH Guide for the Care and Use of Laboratory Animals and were approved by the Bloomington Institutional Animal Care and Use Committee at Indiana University..

Resident hamsters were individually housed (females: $n = 40$; males: $n = 40$) and intruder hamsters were pair-housed (females; $n = 20$; males, $n = 20$) in the colony room for a one-week acclimation period. Subsequently, a random subset of hamsters was transferred to a room on a short-day light cycle (light:dark, 8:16 h), and the remaining hamsters were relocated to a new room on the same long-day light cycle as the colony room. All hamsters remained in their respective photoperiods for ten weeks.

2.2. Determination of seasonal phenotypes

Photoperiodic-induced changes in physiology and morphology indicative of seasonal phenotypes were determined based on *apriori* criteria previously established for Siberian hamsters [2,3]. Following collection of behavioral data, animals were given a lethal dose of a ketamine/xylazine cocktail, necropsies were performed, and reproductive tissues were collected to confirm functional reproductive physiology. Hamsters were deemed reproductively competent if they had functional reproductive tissue weights (i.e., paired testes mass of >0.25 g for males or the combined mass of ovaries, uterine horns, and parametrial white adipose tissue >0.1 g for females), displayed no significant changes in body mass (<10%), and maintained a brown/gray coat color (long-days; LD; females: $n = 14$; males: $n = 14$). Estrous cycles were monitored via vaginal cytology [3] to confirm cycling in reproductive females. In contrast, hamsters were deemed reproductively incompetent if they had regressed reproductive tissue masses, lost >10% of their body mass, and had a "winter" white pelage (short-day responder; SD-R; females: $n = 10$; males: $n = 15$); non-reproductive females did not demonstrate estrous cycling. As previously documented, a subset of short-day hamsters failed to respond reproductively to the short-day photoperiod treatment and remained reproductively competent (short-day non-responder, SD-NR; females: $n = 16$; males: $n = 11$) [18,19]. After examining reproductive mass, two pairs of females and two pairs of males were excluded because they were determined to be of different seasonal phenotypes.

2.3. Aggressive and vocal behavior recording and analysis

Dyadic interactions were staged between a resident hamster and a same-sex intruder hamster of the same photoperiodic and seasonal phenotype (e.g., SD-R with SD-R), of approximately the same age and mass (±5%) and from different parents. Coat color and body mass loss were used to assign pairs of SD-R and SD-NR. Same-sex social encounters were recorded and analyzed for various behaviors per previously outlined methods ([21]; electronic supplementary material, *Methods — audio/video recording*).

Trained observers used ODLog™ (Macropod Software, Eden Prairie, MN) to score both social partners for aggressive behaviors, including attacks, chases, and latency to first attack, using previously defined behaviors [2,3]. A principal components analysis on all aggression variables for males and females extracted one component that explained 74.05% of the total variance in aggression (electronic supplementary material, Table S1). All variables loaded strongly on this component (PC_{AGG}); therefore the composite aggression score was used to examine the effects of sex and seasonal phenotype on overall aggression of same-sex dyads.

USVs (>20 kHz) and BBCs (2–100 kHz) were analyzed using sound spectrograms generated by Avisoft SASlab Pro software (FFT-length of 512 and a Hamming window with 50% overlap; Avisoft Bioacoustics). The broad division of vocalizations and the subtypes within each have been described previously for both male and female Siberian hamsters in this context [21]. Briefly, USVs were classified based on the presence of frequency jumps and harmonics, resulting in four subtypes: 'Plain,' 'Jump,' 'Harmonic,' and 'Complex' USVs. BBCs were classified based on spectrotemporal structural, resulting in three subtypes: 'Squeaks,' 'Rattles,' or 'Mixed' calls ([21], see electronic supplementary material, *Methods - vocalization analysis*).

The distinct spectrotemporal structure of vocalizations allowed hamster calls to be easily distinguished from nonvocal sounds associated with movement about the cage. USVs were high frequency, narrowband vocalizations that can overlap in frequency with sounds made by hamsters' nails against the cage walls; however, cage-scratching was less intense (quieter), more broadband, and more variable in frequency structure than USVs. Broadband calls (BBCs) and noises made by rustling in the bedding have overlapping frequency ranges, but the strong harmonic signatures in the spectral structure of squeak BBCs allowed them to be easily differentiated from bed-rustling, which lacks harmonic structure. Additionally, bed-rustling was much less intense than either squeak or rattle BBCs. Both types of BBCs are audible to humans, and hamsters noticeably open their mouths when they emit BBCs. Thus, because all resident-intruder encounters were first observed with spectrographs generated in real-time, BBCs were easily distinguished from bed-rustling noises.

2.4. Testosterone quantification

Blood samples were taken 24 h prior to behavioral trials to control for daily rhythms in hormone concentration and to minimize sampling from affecting behavioral responses. Serum T was quantified using an enzyme immunoassay (EIA; Assay Design 900-065; assay sensitivity = 5.67 pg/mL) that has been validated in this species and has negligible or undetectable cross-reactivity with other steroid hormones [22]. Samples were diluted (males: 1:20, 1:40 or 1:80; females: 1:10), assayed in duplicate according to the manufacturer's recommended protocol, and were balanced across four plates of the same kit lot. Samples with C.V. >10% and maximum binding <20% or > 80% were re-analyzed. Intra-assay variability ranged from 2.49% to 8.29% and inter-assay variability was 5.67%..

2.5. Statistical analyses

All statistical analyses were performed in JMP v. 11.0.0 (SAS Institute, Inc., Cary, NC) or SPSS v. 20.0 (IBM Corp., Armonk, NY). Data were transformed to attain normality and equal variances. Welch's analysis of variance (ANOVA) was used to compare relative reproductive mass (i.e., sum of reproductive tissue mass divided by mass of the individual) within sexes and across groups. Two-way ANOVAs were used for comparisons across groups and sexes: number of attacks, PC_{AGG}, number of USVs and BBCs, duration of USVs, duration of BBCs, frequency bandwidth of USVs (see electronic supplementary material for details) and T. If a two-way ANOVA reported a statistically significant interaction between groups and sexes, Tukey's HSD post-hoc analyses were run to determine relationships. If the interaction term was not significant, but either group or sexes were, one-way ANOVAs were used followed by Tukey's HSD post-hoc analyses. Following Tukey's HSD post-hoc analyses, statistical significance was attributed at $p < 0.05$. Spearman's rank correlations were used to quantitatively assess relationships between broad vocal behavior classes and T. Reported p-values for correlations have been adjusted for multiple comparisons using the Bonferroni method [23].

To assess whether hamsters varied in the proportional use of USVs across photoperiod groups and between sexes (i.e., comparing total number of each USV subtype across groups and sexes), Fisher's exact tests of independence were used, because this test is more accurate than Pearson's chi-square test for independence when cell counts are small (<5). To assess proportional use of BBCs across groups and between sexes (i.e., comparing total number of each BBC subtype across groups and sexes), Pearson chi-square tests of independence were used. Post-hoc comparisons between proportional uses of specific vocalization subtypes among groups and between sexes were made using z-tests with standardized residuals for both Fisher's exact tests and Pearson's chi-square tests. SPSS software reports significant differences ($p < 0.05$) after correcting for multiple comparisons using the Bonferroni method.

3. Results

3.1. Photoperiodic and sex differences in reproductive physiology

Relative reproductive mass was smaller in male and female short-day responders (SD-R) compared to long-day (LD) and short-day non-responder (SD-NR) hamsters. Male, but not female levels of T were affected by photoperiod. LD males had significantly elevated levels of T compared to SD-R males, and SD-NR males had intermediate levels of T (Table *S*2).

3.2. Photoperiodic and sex differences in ultrasonic vocalizations

There was an effect of photoperiod group ($F_{2,74} = 3.38$; $p = 0.03$), but not sex ($F_{1,74} = 0.01$; $p = 0.91$), or their interaction ($F_{2,74} = 1.82$; $p = 0.17$) on number of USVs emitted by a hamster pair (Fig. 1A). Within males, the number of USVs was increased in SD-NR males ($F_{2,22} = 4.00$; $p = 0.03$) compared to LD males. SD-R males were intermediate to LD and SD-NR males. In contrast, within females, the number of USVs did not differ by photoperiod group ($p > 0.05$) (Fig. 1A). There was no effect of photoperiod group, sex, or their interaction ($p > 0.05$) on mean call duration of USVs (Table S2). In contrast, there was a significant effect of photoperiod ($F_{2,1133} = 3.81$; $p = 0.02$) and sex ($F_{2,1133} = 16.88$; $p < 0.0001$) on the frequency bandwidth of USVs (Fig. S1).

Siberian hamsters differed significantly in proportional use of USVs between seasonal phenotypes and sexes (Fisher's exact tests, $n = 1132$, $p < 0.001$; Fig. 1B–D). Proportional use of 'jump' USVs by hamsters differed depending on seasonal phenotype. LD and SD-NR hamsters produced more 'jump' USVs than those produced by SD-R hamsters (z-tests, $p < 0.05$; Fig. 1B). This difference was robust to sex, in that male and female hamsters produced the same proportion of 'jump' calls across seasonal phenotypes. Proportional use of 'complex' USVs depended on seasonal phenotype and sex. Within sexes, SD-R hamsters produced more 'complex' calls than LD and SD-NR hamsters; overall, females produced more 'complex' calls than males (z-tests, $p < 0.05$; Fig. 1C). Proportional use of 'plain' calls was constant across seasonal

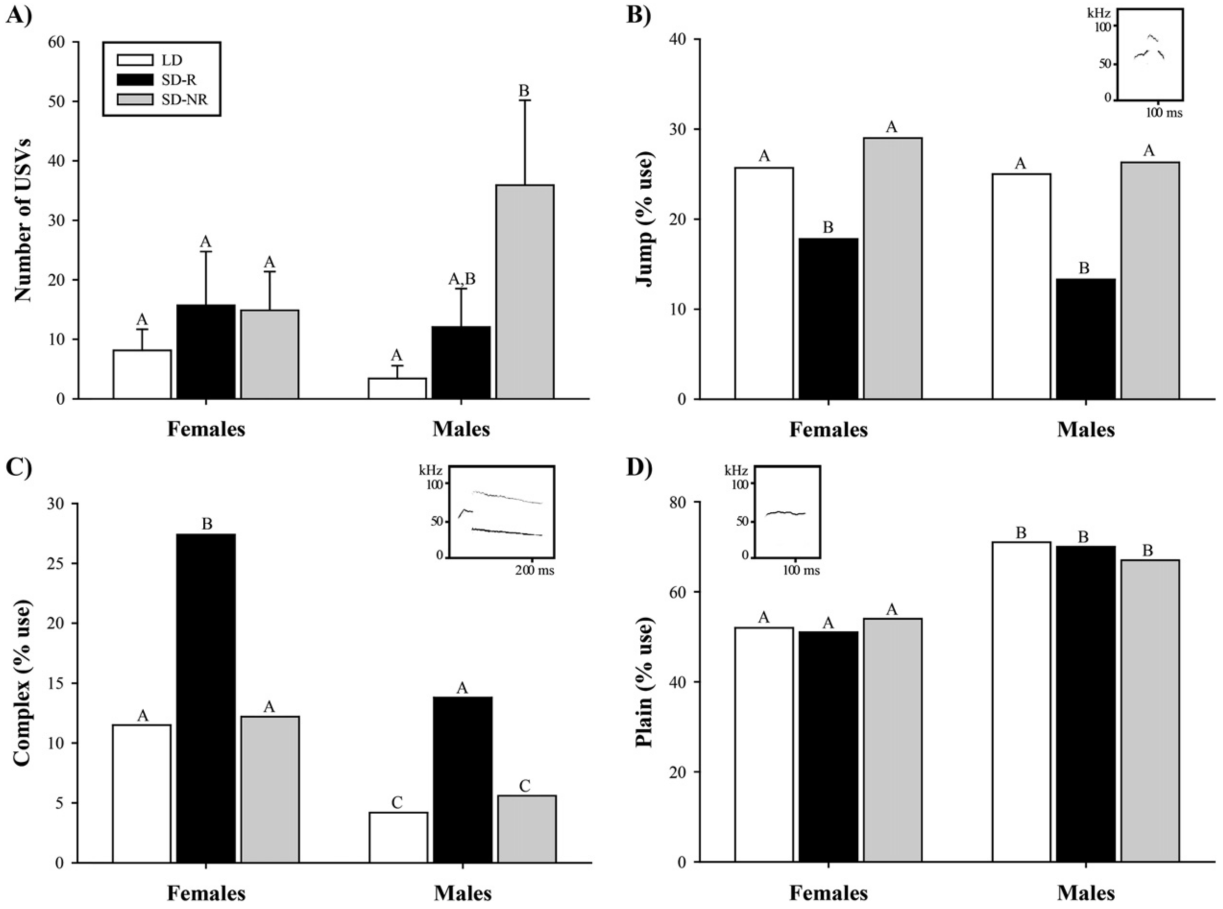

Fig. 1. Number and proportional use of USVs by hamsters of both sexes and across seasonal phenotypes. (A) Number of USVs. Bar heights represent means ± S.E.M; bars with different letters are statistically different ($p < 0.05$, Tukey's HSD). (B) Percent use of jump calls differed by seasonal phenotype. (C) Percent use of complex calls differed by both sex and seasonal phenotype. (D) Percent use of plain calls differed by sex. Harmonic calls varied based on photoperiod and sex, but are not shown, as they were a rare call type. For panels (B), (C), and (D), insets of spectrograms depict a representative call of the subtypes featured in the bar graphs. Bar heights represent proportional usage of USV subtypes; bars with different letters are statistically different ($p < 0.05$, z-tests followed by Bonferroni-corrections).

phenotypes, but differed between sexes. Male hamsters emitted proportionally more 'plain' USVs than females (z-tests, $p < 0.05$; Fig. 1D). Proportional use of 'harmonic' USVs, the rarest call-type, also varied between different groups but without distinctive patterns across photoperiods or sex (z-tests, $p < 0.05$).

3.3. Photoperiodic and sex differences in broadband calls

Photoperiod had a significant effect on the number of BBCs produced by a hamster dyad ($F_{2,74} = 2.90$; $p = 0.03$); there was no effect of sex or a photoperiod by sex interaction ($p > 0.05$) (Fig. 2A). Both male and female SD-R hamsters emitted more BBCs than their LD counterparts (males: $F_{2,22} = 2.56$; $p = 0.04$; females: $F_{2,22} = 1.67$; $p = 0.03$). For both sexes, SD-NR hamsters produced an intermediate number of BBCs compared to LD and SD-R hamsters (Fig. 2A). There was a significant effect of sex on BBC duration, with females producing longer BBCs than males ($F_{1,74} = 7.55$; $p = 0.008$). Neither photoperiod nor a photoperiod by sex interaction ($p > 0.05$) had an effect on BBC duration (Table S2).

In addition to photoperiodic effects on the number of BBCs emitted by a pair, there were also differences in proportional use of BBC subtypes across photoperiod groups (Pearson chi-square test of independence, $\chi^3 = 29.177$, $n = 4756$, $p < 0.001$) and between sexes (Pearson chi-square test of independence: $\chi^1 = 374.892$, $n = 4756$, $p < 0.001$) (Fig. 2B–D). Although the proportional use of 'rattles' by females did not vary with photoperiod, use of 'rattles' by males was influenced by photoperiod; SD-R males produced the greatest proportion of 'rattles' compared to LD and SD-NR males (z-tests, $p < 0.05$; Fig. 2B). Regardless of photoperiod group, females produced consistently more 'rattle' calls than males (z-tests, $p < 0.05$; Fig. 2B), and males produced more 'squeak' BBCs than females (z-tests, $p < 0.05$; Fig. 2C). Proportional use of 'squeaks' by females did not vary by photoperiod, whereas males varied depending on photoperiod (z-tests, $p < 0.05$; Fig. 2C). 'Mixed' BBCs were produced less frequently, and SD-R females produced more 'mixed' calls than the other groups (z-tests, $p < 0.05$; Fig. 2D).

3.4. Photoperiodic and sex differences in aggression and relationships between vocalizations and aggression

There was an overall effect of photoperiod on number of attacks for the dyad ($F_{2,74} = 12.27$; $p < 0.0001$); however, there was no effect of sex or their interaction ($p > 0.05$) (Fig. 3A). Both male and female SD-R hamsters made more attacks than LD and SD-NR hamsters (males: $F_{2,16} = 6.47$; $p = 0.006$; females: $F_{2,17} = 9.16$; $p = 0.002$; Fig. 3A). LD and SD-NR hamsters did not differ in the number of attacks. The dyad's aggression score, PC_{AGG}, was also affected by photoperiod group ($F_{2,74} = -3.87$; $p = 0.0002$); however, there was no effect of sex or their interaction ($p > 0.05$) on PC_{AGG} (Fig. 3B). Both male and female SD-R hamsters had increased aggression scores (PC_{AGG}) compared to LD hamsters, and SD-NR was intermediate to LD and SD-R hamsters (males: $F_{2,24} = 7.53$; $p = 0.003$; females: $F_{2,21} = 4.30$; $p = 0.03$; Fig. 3B). BBCs and aggression were correlated only in LD hamsters of both sexes. USVs and aggression, however, were not correlated in either sex or across photoperiod groups (Table 1).

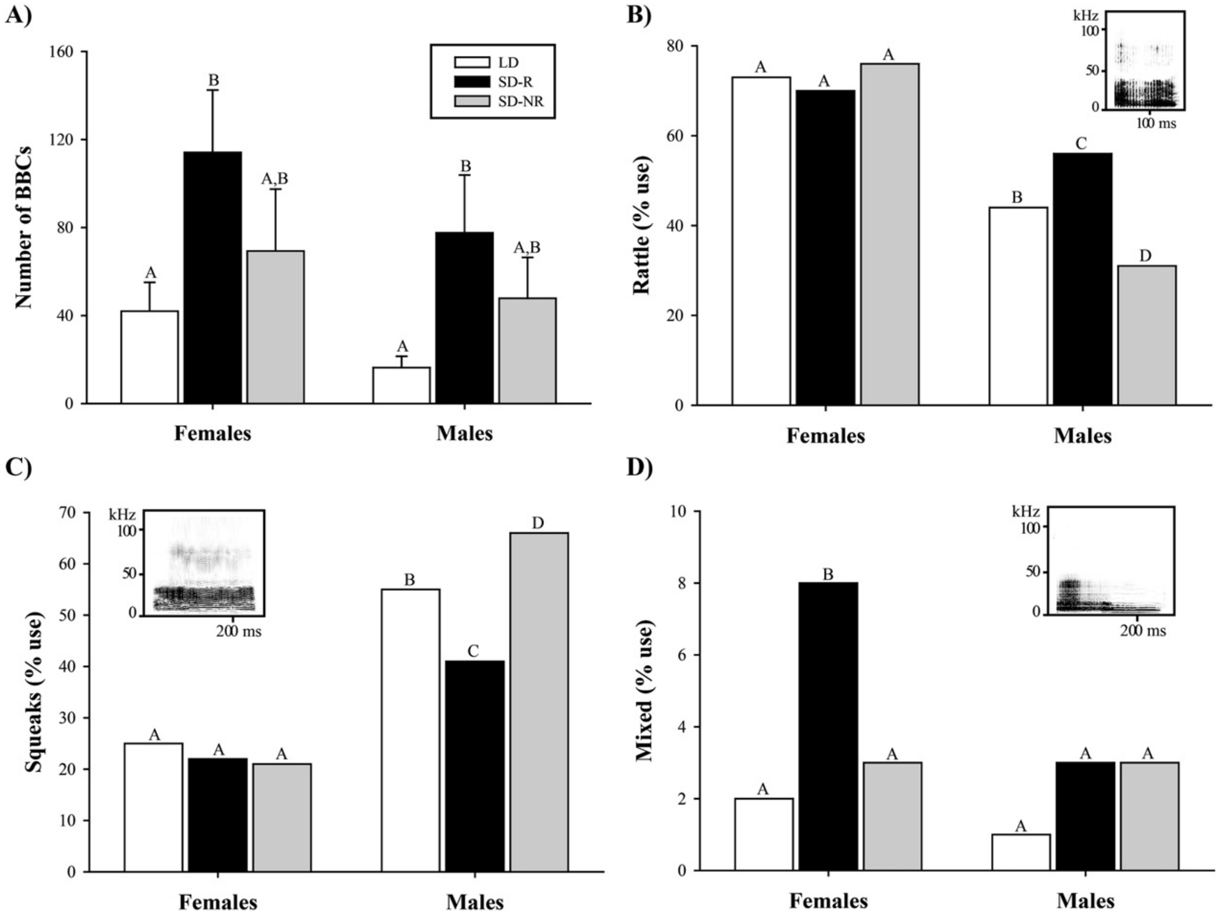

Fig. 2. Number and proportional use of BBCs by hamsters of both sexes and across seasonal phenotypes. (A) Seasonal phenotype significantly influenced emission of BBCs in both sexes. Bar heights represent means ± S.E.M.; bars with different letters are statistically different ($p < 0.05$, Tukey's HSD). (B) Percent use of rattle calls differed by sex. (C) Percent use of squeak calls differed by sex and seasonal phenotype. (D) Percent use of mixed calls differed by sex and group. For panels (B), (C), and (D), insets of spectrograms depict a representative call of the subtypes featured in the bar graphs. Bar heights represent proportional usage of BBC subtypes; bars with different letters are statistically different ($p < 0.05$, z-tests followed by Bonferroni-corrections).

3.5. Testosterone, aggression and vocalizations

Female T concentrations were unaffected by photoperiod (Table S2), thus, the T data from females across all photoperiodic groups were combined and correlated with aggression and emission rate of BBCs and USVs. In contrast, male T concentrations were significantly affected by photoperiod, so relationships were examined within each photoperiodic group. T was not related to aggression in either sex, and there was no association between either vocal class and T in either sex (Table 2).

4. Discussion

In the present study, we demonstrated that Siberian hamsters emit vocalizations that are sensitive to vocalizers' sex, as well as photoperiod-induced changes in physiology, and aggression. During same-sex aggressive encounters, male and female hamsters from all photoperiodic conditions produced two main classes of vocalizations: USVs and BBCs. These two vocalization types exhibit striking differences in their spectrotemporal structures, suggesting that USVs and BBCs also differ in function. We recently addressed this idea in a previous study of summer-condition male and female hamsters, and we found that the high frequency, tone-like USVs are not associated with aggression, whereas the harsher sounding, low frequency BBCs are positively associated with aggression [21].

Thus, we predicted that SD-R hamsters, the most aggressive seasonal phenotype, would produce BBCs at the highest rate, and our results confirm this prediction. Our results partially supported our prediction that SD-R hamsters would emit the greatest proportion of rattle BBCs, a pattern we confirmed in males but not in females. In accordance with our second main prediction, there was no effect of seasonal phenotype on emission rate of USVs, which is consistent with the idea that USVs are not directly related to aggression. In contrast, the percent use of specific subtypes of USVs was dependent on the seasonal phenotype and sex of the hamster dyad, suggesting that these calls are sensitive to some physiological feature of context. Finally, our third prediction was not confirmed, since T was not correlated with vocalization rate during these same-sex agonistic encounters. However, it is likely that some aspect of the physiological response to the change in photoperiod plays a role in regulating vocal behavior, as hamsters of the same summer phenotype (LD and SD-NR) had vocalizations that were different from hamsters of the winter phenotype (SD-R).

4.1. USV repertoire varies with sex and seasonal phenotype

A key finding in this study is that seasonal phenotype and sex affect the proportional use of USV subtypes differently. The total number of USVs emitted by same-sex hamster pairs did not vary with seasonal condition; however, proportional use of two USV subtypes, 'complex' and 'jump', differed between winter-condition (SD-R) and summer-condition (LD and SD-NR) hamsters. 'Complex' calls differed with seasonal phenotype in that SD-R hamsters produced this subtype more than the physiologically similar LD and SD-NR hamsters, whereas 'jump' calls are produced more by LD and SD-NR than SD-R hamsters. Thus, SD-R hamsters in winter condition employ greater variation in

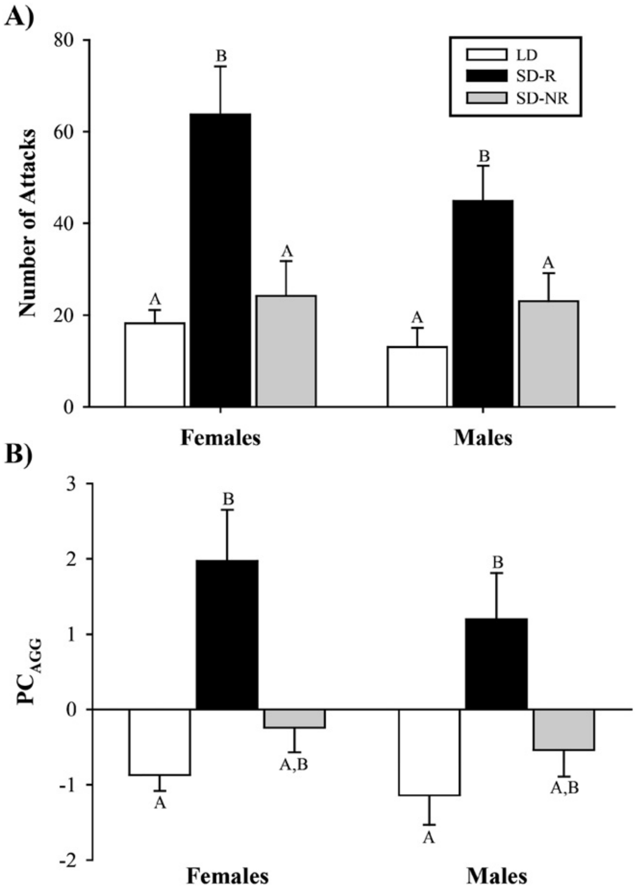

Fig. 3. Seasonal changes in aggression of hamsters. The seasonal phenotype of a hamster dyad significantly influenced (A) number of attacks and (B) aggression scores (PC_{AGG}). Bar heights represent means ± S.E.M; bars with different letters are statistically different ($p < 0.05$, Tukey's HSD).

USV subtypes, with greater use of harmonically structured USVs than summer-condition (LD and SD-NR) hamsters. These similarities in USV repertoires of LD and SD-NR hamsters suggest that an animal's seasonal phenotype, and not the photoperiod cue *per se*, is an important modulator of vocal repertoire in hamsters and likely other rodents.

Seasonal changes in production rate of vocalizations and/or vocal repertoire have been reported previously in other species [8–10,13,24]. For example, in song sparrows, rate of vocalizations does not vary seasonally, but proportional use of different call types ("soft" song versus "broadcast" song) are dependent on season [14]. During the winter, song sparrows in non-reproductive condition produce less stereotyped songs [25], similar to the greater variety of USVs emitted by SD-R Siberian hamsters. Additionally, vocal behavior persists during the winter in bowhead whales, with a higher signaling rate and larger repertoire of call types in winter compared with the spring [26,27]. In these studies, however, 'season' is used to refer to short segments of time within a few months (i.e., beginning versus end of the breeding season), rather than changes that occur on a yearly basis (e.g., long summer-like versus short winter-like days in the present study). Further, in the present study we isolated a single environmental cue, photoperiod; our study is the first to our knowledge to demonstrate photoperiodic changes in vocal repertoire in rodents, which may reflect seasonal changes in vocal repertoire that could occur in nature.

Table 1
Spearman's rank correlations between broad vocalization classes and aggression in male and female hamsters. Significant *p* values are shown in bold.

Group	BBCs and aggression			USVs and aggression		
	n	ρ	*p*	*n*	ρ	*p*
Female LD	14	0.52	**0.04**	14	−0.17	0.57
Female SD-R	10	0.18	0.63	10	−0.09	0.80
Female SD-NR	16	0.25	0.35	16	0.05	0.85
Male LD	14	0.81	**0.0005**	14	−0.02	0.94
Male SD-R	15	0.43	0.11	15	0.33	0.23
Male SD-NR	11	0.16	0.63	11	0.22	0.51

Table 2
Spearman's rank correlations between T, broad vocalization classes, and aggression in male and female hamsters.

Group	T and aggression			T and BBCs			T and USVs		
	n	ρ	*p*	*n*	ρ	*p*	*n*	ρ	*p*
Female LD	40	−0.12	0.45	40	0.01	0.93	40	−0.25	0.13
Female SD-R									
Female SD-NR									
Male LD	14	−0.05	0.88	14	−0.16	0.58	14	0.23	0.43
Male SD-R	15	−0.22	0.43	15	−0.43	0.10	15	0.01	0.97
Male SD-NR	11	0.41	0.21	11	0.04	0.92	11	0.03	0.93

In addition to changes in proportional call usage that reflect changes in seasonal phenotype, we demonstrated that the ultrasonic vocal repertoires of males and females also differ. 'Plain' USVs, which lack frequency jumps and harmonic features, are used proportionally more by male hamsters than female hamsters, and this difference is stable across seasonal phenotypes. Additionally, females emitted proportionally more 'complex' USVs than males, regardless of their seasonal phenotype. Thus, female Siberian hamsters have a more diverse ultrasonic vocal repertoire with more spectrographically complex vocalizations than males, which predominately use the "plain" USV subtype. This is consistent with previous findings in this and other mammalian species [21,28,29]. Furthermore, we demonstrate here that this increased variability in proportional use of USV subtypes by female hamsters is stable across seasonal phenotypes. The profile of USVs produced by Siberian hamsters is likely a stable indicator of sex across seasons, because males are much more likely to produce 'plain' USVs than females. An alternative interpretation is that the production of 'plain' calls is associated with a particular behavior expressed differentially by the sexes.

USVs in rodents are often studied in association with reproductive behavior [30]; therefore one might expect seasonal differences purely in the rate of USVs due to seasonal differences in reproductive behavior. We recorded from same-sex rather than mixed-sex pairs; it is not surprising that the rate of USVs emitted did not vary photoperiodically or with seasonal phenotype. These findings do not imply USVs are irrelevant to same-sex social encounters, however. In fact, USVs are emitted by several rodent species (e.g., gerbils, mice, rats, squirrels, and voles) across a variety of contexts, including predator sightings [31], mother-pup interactions [32], same-sex affiliative encounters [33,34], and same-sex aggressive encounters [35–37]. Furthermore, USVs are only produced by Siberian hamsters during direct encounters with a social partner, suggesting that they are immediately relevant to this context [21]. Thus, USVs may play a different role in transferring social information between Siberian hamsters, as conveyed by seasonal phenotype and sex differences in the proportional use of USV subtypes.

4.2. BBCs vary with sex and seasonal phenotype

We demonstrated not only differences across seasonal phenotypes in the emission rate of BBCs, but also differences in proportional use of BBC subtypes by hamsters of different seasonal phenotypes and sexes. BBCs, which are associated with aggression in this species [21], were produced in greater numbers by winter-condition (SD-R) hamsters, which display elevated aggression compared with summer-condition (LD and SD-NR) hamsters. BBC repertoire did not vary with seasonal phenotype for female hamsters; however, male hamsters demonstrated variation in BBC repertoire with respect to seasonal phenotype, producing more 'rattle' BBCs when in winter condition (SD-R). This is significant because 'rattles' are more closely associated with aggression in this species, and thus, male hamsters that display more aggression (SD-R) use these calls more often than less aggressive, summer-condition males. Females emitted BBCs that were longer in duration and contained proportionally more ‘rattles’ than males, regardless of seasonal phenotype. Hamster ‘rattles’ are spectrotemporally similar to the buzzes emitted by some songbirds; both ‘rattles’ and trills are rapidly modulated, pulsatile bursts of noise and the use of these calls in some songbird species is sensitive to changes in reproductive physiology [14,21,38]. However, the immediate behavioral significance of buzzes used by songbirds is unknown, although they are part of a class of vocalization related to aggression.

Another important aspect of our findings was the photoperiodic variation in seasonal phenotype and its relationship to BBCs and aggression. Specifically, number of BBCs was positively related to aggression in LD hamsters. This was not the case for SD-R or SD-NR hamsters of either sex, despite both groups displaying increased aggression and increased production of BBCs compared with LD hamsters. This could be due to a lack of exact coupling of vocal and non-vocal behavior during times of high aggression (i.e., perhaps vocalizations reach a maximum rate); alternatively, this could be due to hamsters emitting BBCs even when not immediately engaging in an aggressive act or to halt an aggressive act, which we occasionally observed. This might indicate that short-day hamsters are more sensitive to social stimuli that elicit BBCs than long-day hamsters.

4.3. Vocalizations, seasonal phenotype, and T

Heightened aggression in short-day male hamsters is accompanied by relatively low levels of circulating T compared with the breeding season when circulating levels of T are maximal [2]. In contrast, circulating T in females did not vary with seasonal phenotype, despite heightened aggression in short days. Because T is inversely related to short-day aggression in males of this species [2], we predicted that T would also be inversely related to BBCs, which are positively related to aggression, and that T would not be related to USVs, which are not related to same-sex aggression. In the current study, T was not related to either BBCs or USVs, suggesting that the finer variation in vocal behavior between interactions of the same context may be more sensitive to immediate behavioral differences, such as how much aggression is displayed within a particular encounter, rather than current T concentrations. For male Syrian hamsters (*Mesocricetus auratus*), short-day photoperiods increase USVs, directed at receptive female hamsters, a contextual difference from the current study [24]. Similar to Siberian hamsters in the current study, T does not seem to regulate photoperiodic shifts in emission rate of USVs by Syrian hamsters [24]. However, T did restore female-stimulated production of USVs by castrated male Syrian hamsters in another study conducted in long-day hamsters only [39]. While inconclusive, these data suggest that seasonal shifts in T do not regulate seasonal shifts in rate of calls emitted by Siberian or Syrian hamsters [24]. This does not rule out a role for gonadal hormones in regulating vocal behavior, however, as female hamsters show seasonal variation in the sex steroid estradiol [3]. Additionally, T could modulate more subtle differences in vocal behavior, such as specific spectrotemporal characteristics, as has been demonstrated for Neotropical singing mice [40] and song sparrows [14]. Other aspects of physiology may also be associated with variation in vocalizations, such as changes in steroids or steroid receptors in brain regions associated with call production or in peripheral target tissues (e.g., syrinx muscle in birds), which are associated with changes in call production [25,41,42].

It is interesting to note that short-day responders and short-day non-responders display different levels of photoperiod-induced increases in aggression and BBC production. Specifically, SD-NR hamsters, individuals that are physiologically similar to LD hamsters despite exposure to inhibitory short days, displayed intermediate responses to LD and SD-R hamsters with respect to BBCs and aggression. This suggests that the physiological response to photoperiod, in addition to photoperiodic exposure *per se*, contributes to changes in vocal behavior in this species. Although T was not related to emission rate of BBCs in this study, there are other traits that differ across seasonal phenotypes, including thermoregulation, adiposity, and food intake, which could affect vocal behavior (reviewed in: [4,20]). However, steroid hormones remain a likely candidate mechanism for regulating seasonal shifts in emission rate of BBCs, because these hormones fluctuate seasonally (reviewed in: [4,17,20]), and have been shown to regulate vocal behavior in many rodent species (e.g., [40,43]; reviewed in: [44]). While the precise physiological mechanisms underlying the effects of seasonal phenotype on BBCs remain unknown, there likely are steroid-dependent and independent effects on vocal responses. Such steroid-dependent and steroid-independent effects on gonadotropin secretion have been well-characterized phenomena in Siberian hamsters (e.g., [45]). Regardless of mechanism, our findings demonstrate an important role for both direct and indirect actions of photoperiod on vocal and aggressive behaviors in hamsters.

5. Conclusions

We demonstrated distinct differences in vocalizations emitted by Siberian hamsters of different seasonal phenotypes during same-sex aggressive encounters. Furthermore, we show that specific aspects of vocal behavior are most closely related to an animal's seasonal phenotype, as opposed to an animal's current photoperiod. Siberian hamsters offer a novel mammalian species with which to study the interactive influences of an individual's sex, photoperiodic exposure, and seasonal phenotype on vocal communication.

Acknowledgments

The authors thank A.C. Amez for technical help in recording audio and video, and C. A. Athans and K.J. O'Malley for additional assistance in video analysis of behavior. The authors thank K.E. Sylvia for comments on an earlier version of this manuscript. The authors also thank S. Dickinson from Indiana University's Statistical Consulting Center for help in analyzing the proportional use of vocalizations dataset. This work was supported by NSF IOB 0543798 (GED), NSF GRFP (NMR) (1342962), NIH training grant T32HD049336: “Common Themes in Reproductive Diversity” (NMR), NSF REU (CA) (DBI 0851607), and Indiana University.

Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.physbeh.2015.09.014.

References

[1] J.L. Goodson, L.C. Wilson, S.E. Schrock, To flock or fight: neurochemical signatures of divergent life histories in sparrows, Proceedings of the National Academy of Sciences. 109 (2012) 10685–10692.
[2] A.M. Jasnow, K.L. Huhman, T.J. Bartness, G.E. Demas, Short-day increases in aggression are inversely related to circulating testosterone concentrations in male siberian hamsters (*Phodopus sungorus*), Hormones and Behavior. 38 (2000) 102–110.
[3] M.-A.L. Scotti, N.J. Place, G.E. Demas, Short-day increases in aggression are independent of circulating gonadal steroids in female siberian hamsters (*Phodopus sungorus*), Hormones and Behavior. 52 (2007) 183–190.
[4] B.J. Prendergast, R.J. Nelson, I. Zucker, Mammalian seasonal rhythms: behavior and neuroendocrine substrates, Hormones, Brain and Behavior. 2 (2002) 93–156.
[5] P. Marler, S. Peters, G.F. Ball, A.M. Dufty, J.C. Wingfield, The role of sex steroids in the acquisition and production of birdsong, Nature 336 (1988) 770–772.
[6] F. Nottebohm, M.E. Nottebohm, L.A. Crane, J.C. Wingfield, Seasonal changes in gonadal hormone levels of adult male canaries and their relation to song, Behavioral and Neural Biology. 47 (1987) 197–211.
[7] C.K. Thompson, G.E. Bentley, E.A. Brenowitz, Rapid seasonal-like regression of the adult avian song control system, Proceedings of the National Academy of Sciences. 104 (2007) 15520–15525.
[8] E.M. Gese, R.L. Ruff, Howling by coyotes (*Canis latrans*): variation among social classes, seasons, and pack sizes, Canadian Journal of Zoology. 76 (1998) 1037–1043.

[9] H.P. Kunc, J.B. Wolf, Seasonal changes of vocal rates and their relation to territorial status in male galápagos sea lions (*Zalophus wollebaeki*), Ethology 114 (2008) 381–388.

[10] D. Pépin, B. Cargnelutti, G. Gonzalez, J. Joachim, D. Reby, Diurnal and seasonal variations of roaring activity of farmed red deer stags, Applied Animal Behaviour Science. 74 (2001) 233–239.

[11] J.A. Sisneros, P.M. Forlano, R. Knapp, A.H. Bass, Seasonal variation of steroid hormone levels in an intertidal-nesting fish, the vocal plainfin midshipman, General and Comparative Endocrinology. 136 (2004) 101–116.

[12] K.D. Wells, The social behaviour of anuran amphibians, Animal Behaviour. 25 (1977) 666–693.

[13] A.M. Zoidis, K.S. Lomac-MacNair, A.E. Chomos-Betz, A.J. Day, A.S. McFarland, Effects of sex, seasonal period, and sea state on calf behavior in Hawaiian humpback whales (*Megaptera novaeangliae*), Aquatic Mammals. 40 (2014) 44–58.

[14] C.J. Maddison, R.C. Anderson, N.H. Prior, M.D. Taves, K.K. Soma, Soft song during aggressive interactions: seasonal changes and endocrine correlates in song sparrows, Hormones and Behavior. 62 (2012) 455–463.

[15] F.H. Bronson, Mammalian reproduction: an ecological perspective, Biology of reproduction. 32 (1985) 1–26.

[16] B.D. Goldman, Mammalian photoperiodic system: formal properties and neuroendocrine mechanisms of photoperiodic time measurement, Journal of Biological Rhythms. 16 (2001) 283–301.

[17] K.K. Soma, N.M. Rendon, R. Boonstra, H.E. Albers, G.E. Demas, DHEA effects on brain and behavior: insights from comparative studies of aggression, The Journal of Steroid Biochemistry and Molecular Biology. 261-72 (2015).

[18] M.R. Gorman, I. Zucker, Testicular regression and recrudescence without subsequent photorefractoriness in Siberian hamsters, American Journal of Physiology-Regulatory Integrative and Comparative Physiology. 38 (1995) R800–R806.

[19] G.R. Lynch, C.B. Lynch, R.M. Kliman, Genetic analyses of photoresponsiveness in the Djungarian hamster, *Phodopus sungorus*, Journal of Comparative Physiology A. 164 (1989) 475–481.

[20] B.J. Prendergast, L.J. Kriegsfeld, R.J. Nelson, Photoperiodic polyphenisms in rodents: neuroendocrine mechanisms, costs, and functions, Quarterly Review of Biology. 293-325 (2001).

[21] S.M. Keesom, N.M. Rendon, G.E. Demas, L.M. Hurley, Vocal behaviour during aggressive encounters between Siberian hamsters, *Phodopus sungorus*, Animal Behaviour 85-93 (2015).

[22] T.A. Bedrosian, L.K. Fonken, G.E. Demas, R.J. Nelson, Photoperiod-dependent effects of neuronal nitric oxide synthase inhibition on aggression in Siberian hamsters, Hormones and Behavior. 61 (2012) 176–180.

[23] S.P. Wright, Adjusted p-values for simultaneous inference, Biometrics 1005-13 (1992).

[24] J.A. Matochik, M. Miernicki, J.B. Powers, M.L. Bergondy, Short photoperiods increase ultrasonic vocalization rates among male Syrian hamsters, Physiology & Behavior. 38 (1986) 453–458.

[25] G.T. Smith, E.A. Brenowitz, J.C. Wingfield, L.F. Baptista, Seasonal changes in song nuclei and song behavior in Gambel's white-crowned sparrows, Journal of Neurobiology. 28 (1995) 114–125.

[26] C.W. Clark, C.L. Berchok, S.B. Blackwell, D.E. Hannay, J. Jones, D. Ponirakis, et al., A year in the acoustic world of bowhead whales in the Bering, Chukchi and Beaufort Seas, Progress in Oceanography (2015).

[27] O.M. Tervo, S.E. Parks, L.A. Miller, Seasonal changes in the vocal behavior of bowhead whales (*Balaena mysticetus*) in Disko Bay, Western-Greenland, The Journal of the Acoustical Society of America. 126 (2009) 1570–1580.

[28] O.R. Floody, D.W. Pfaff, Communication among hamsters by high-frequency acoustic signals: I. Physical characteristics of hamster calls, Journal of Comparative and Physiological Psychology 91 (1977) 794–806.

[29] S. von Merten, S. Hoier, C. Pfeifle, D. Tautz, A role for ultrasonic vocalisation in social communication and divergence of natural populations of the house mouse (*Mus musculus domesticus*), PLoS ONE 9 (2014), e97244.

[30] C.V. Portfors, Types and functions of ultrasonic vocalizations in laboratory rats and mice, Journal of the American Association for Laboratory Animal Science. 46 (2007) 28–34.

[31] D.R. Wilson, J.F. Hare, Animal communication: ground squirrel uses ultrasonic alarms, Nature 430 (2004) 523.

[32] T.A. Terleph, A comparison of prairie vole audible and ultrasonic pup calls and attraction to them by adults of each sex, Behaviour 148 (2011) 1275–1294.

[33] J. Chabout, P. Serreau, E. Ey, L. Bellier, T. Aubin, T. Bourgeron, et al., Adult male mice emit context-specific ultrasonic vocalizations that are modulated by prior isolation or group rearing environment, PLoS ONE 7 (2012), e29401.

[34] A. Moles, F. Costantini, L. Garbugino, C. Zanettini, F.R. D'Amato, Ultrasonic vocalizations emitted during dyadic interactions in female mice: a possible index of sociability? Behavioural Brain Research. 182 (2007) 223–230.

[35] J. Burgdorf, R. Kroes, J. Moskal, J. Pfaus, S. Brudzynski, J. Panksepp, Ultrasonic vocalizations of rats (*Rattus norvegicus*) during mating, play, and aggression: behavioral concomitants, relationship to reward, and self-administration of playback, Journal of Comparative Psychology. 122 (2008) 357–367.

[36] B.E. Gourbal, M. Barthelemy, G. Petit, C. Gabrion, Spectrographic analysis of the ultrasonic vocalisations of adult male and female BALB/c mice, Naturwissenschaften. 91 (2004) 381–385.

[37] M. Ter-Mikaelian, W.B. Yapa, R. Rübsamen, Vocal behavior of the Mongolian gerbil in a seminatural enclosure, Behaviour 149 (2012) 461–492.

[38] R.C. Anderson, W.A. Searcy, S. Peters, S. Nowicki, Soft song in song sparrows: acoustic structure and implications for signal function, Ethology 114 (2008) 662–676.

[39] O.R. Floody, C. Walsh, M.T. Flanagan, Testosterone stimulates ultrasound production by male hamsters, Hormones and Behavior. 12 (1979) 164–171.

[40] B. Pasch, A.S. George, H.J. Hamlin, L.J. Guillette, S.M. Phelps, Androgens modulate song effort and aggression in neotropical singing mice, Hormones and Behavior. 59 (2011) 90–97.

[41] G.T. Smith, E.A. Brenowitz, M.D. Beecher, J.C. Wingfield, Seasonal changes in testosterone, neural attributes of song control nuclei, and song structure in wild songbirds, The Journal of Neuroscience. 17 (1997) 6001–6010.

[42] G.T. Smith, E.A. Brenowitz, J.C. Wingfield, Roles of photoperiod and testosterone in seasonal plasticity of the avian song control system, Journal of Neurobiology. 32 (1997) 426–442.

[43] J. Kapusta, E. Pochroń, Effect of gonadal hormones and sexual experience on vocalizations and behavior of male bank voles (*Myodes glareolus*), Canadian Journal of Zoology. 89 (2011) 1117–1127.

[44] J.G. Nyby, Auditory communication among adults, in: J.F. Willott (Ed.), Handbook of Mouse Auditory Research: From Behavior to Molecular Biology, CRC Press, Boca Raton, FL 2001, pp. 3–18.

[45] E.L. Bittman, A.E. Jetton, C. Villalba, G.J. Devries, Effects of photoperiod and androgen on pituitary function and neuropeptide staining in Siberian hamsters, American Journal of Physiology-Regulatory, Integrative and Comparative Physiology 271 (1996) R64–R72.