

## Song performance improves with continued singing across the morning in a songbird

Jason P. Dinh<sup>a,\*</sup>, Susan Peters<sup>a</sup>, Stephen Nowicki<sup>a,b</sup>

<sup>a</sup> Biology Department, Duke University, Durham, NC, U.S.A.

<sup>b</sup> Neurobiology Department, Duke University Medical School, Durham, NC, U.S.A.



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Vocal performance – an animal's ability to produce physically challenging vocalizations – can reflect a signaller's overall condition and can be a reliable signal of quality. It has been suggested recently that songbirds improve vocal performance through recent practice during intense dawn singing. We tested whether recent practice improves vocal performance in swamp sparrows, *Melospiza georgiana*, a species for which the biomechanical constraints and biological implications of vocal performance are well established. We measured vocal deviation – a measure of performance – in 1527 songs recorded from 11 captive swamp sparrows, four of which were developmentally stressed as juveniles. Vocal performance improved across the morning as a function of both the cumulative number of songs previously performed and the time of day. Song types with introductory syllables showed greater improvement than more typical song types composed solely of trilled syllables, and across all song types, as song output increased, the average improvement in vocal performance also increased. However, males with high song output exhibited greater variability in vocal performance, suggesting that some individuals might experience fatigue in song production. Furthermore, birds that had been developmentally stressed as juveniles showed greater improvement over the morning than birds that were not stressed. If conspecifics attend to within-individual variation in vocal performance, then improvement in vocal performance over the course of a day may drive birds to sing early and often, although fatigue may limit the extent to which this advantage may be gained.

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Motor performance – an animal's ability to perform physically challenging displays vigorously or skilfully – reflects an individual's overall body condition and has been shown in several contexts to convey information about individual quality (Byers, Hebets, & Podos, 2010). Animals can improve motor performance through practice over both long and short timescales. Over long timescales, for example, play behaviour allows juvenile animals to practise physically demanding behaviours that are used later in functional contexts as adults (Bekoff, 1984; Caro, 1988). Furthermore, in zebra finches, *Taeniopygia guttata*, unstructured singing allows juveniles to explore vocal abilities and optimize their imitation of model songs when they are adults (Derégnaucourt, Mitra, Fehér, Pytte, & Tchernichovski, 2005). On short timescales, recent practice can improve motor performance over the course of minutes or hours. For example, during dawn choruses – periods of intense singing at

dawn – vocal performance, defined as a songbird's ability to produce physically challenging songs, has been shown to improve as an individual produces more songs (Schraft, Medina, McClure, Pereira, & Logue, 2017).

Many birds sing intensely at dawn, yet the adaptive benefit driving this behaviour remains uncertain. Several hypotheses have been proposed for the functional significance of intense dawn singing, ranging from optimizing territory defence to exploiting favourable environmental conditions for sound transmission (reviewed in Staicer, Spector, & Horn, 1996). These hypotheses are not mutually exclusive and each has received some empirical support (e.g. Henwood & Fabrick, 1979; Mace, 1986; Slagsvold, Sætre, & Dale, 1994). The 'warm-up hypothesis' suggests that individuals improve vocal performance with recent practice (Schraft et al., 2017). Accordingly, through intense dawn singing, birds may improve their vocal performance later in the day, much the same way athletes or musicians improve their performance by warming up (Amir, Amir, & Michaeli, 2005; Elliot, Sundberg, & Gramming, 1995; Fradkin, Zazryn, & Smoliga, 2010; Gish, Kunduk, Sims, & McWhorter, 2012; Shellock & Prentice, 1985). This hypothesis

\* Correspondence: J. P. Dinh, Biology Department, Duke University, Campus Box 90338, Durham, NC, 27708-0338, U.S.A.

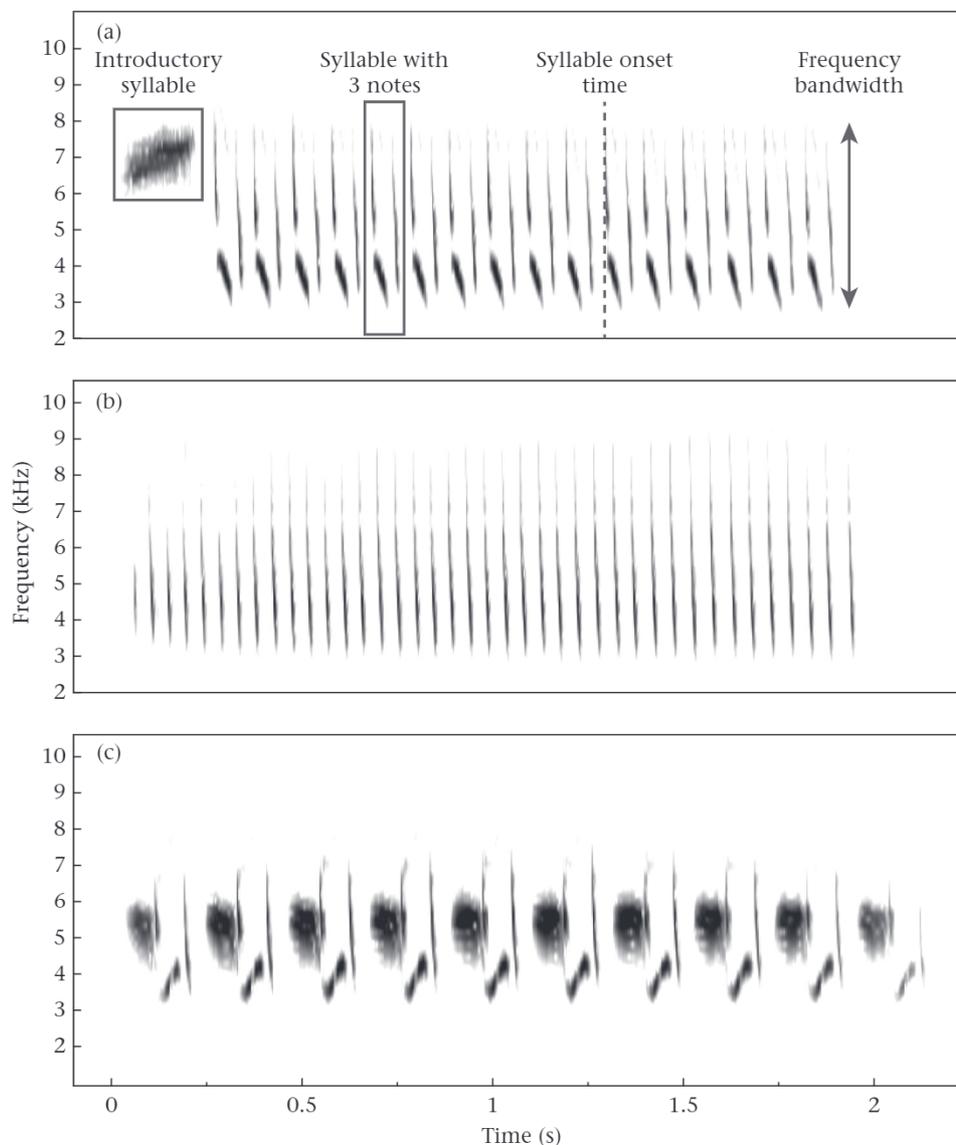
E-mail address: [jpd29@duke.edu](mailto:jpd29@duke.edu) (J. P. Dinh).

predicts that as the total number of songs an animal performs increases, vocal performance should improve. It further suggests that the greatest improvement in vocal performance should occur when song output is highest, which for many bird species occurs around dawn. If an individual's ability to perform physically challenging songs mediates fitness in some way, then the advantage of warming up could explain why many birds sing intensively at the very start of a day.

The warm-up hypothesis was proposed to explain improvements in vocal performance, as measured by frequency excursion of songs in Adelaide's warblers, *Setophaga adelaidae*, a species for which this measure of vocal performance has not been shown to be physically challenging or relevant to signal receivers (Schraft et al., 2017). Swamp sparrows, *Melospiza georgiana*, by contrast, are an ideal species for testing the effects of previous song production on subsequent vocal performance because the biomechanical constraints that make songs more challenging to produce are well understood (Podos, 1996, 1997), and both male and female receivers have been shown to attend to variation in vocal performance (Ballentine, Hyman, & Nowicki, 2004; DuBois, Nowicki, &

Searcy, 2011; Moseley, Lahti, & Podos, 2013). Although swamp sparrows do not exhibit punctuated and pronounced dawn choruses as seen in other species (i.e. where most singing occurs within a brief period before sunrise), they do sing most intensely before and at dawn (Marler & Peters, 1982). Therefore, we can test whether previous singing leads to improved vocal performance in this species to an extent that might be salient to the signal's receivers.

Swamp sparrow songs are typically composed of a single syllable type, itself made up of one to five different notes, repeated for several seconds in a continuous trill (Fig. 1) (Marler & Pickert, 1984). Production of these trilled songs is constrained by the need to coordinate syringeal and respiratory activity with vocal tract motions during song, notably beak movements that modify the vocal tract's acoustic resonance properties (Nowicki, Westneat, & Hoese, 1992; Podos, 1996; Suthers & Goller, 1997). To achieve this coordination, birds must open and close their beaks in register with the acoustic frequency of the sound being produced (Hoese, Podos, Boetticher, & Nowicki, 2000; Westneat, Long, Hoese, & Nowicki, 1993), resulting in a performance trade-off between how fast a bird can repeat



**Figure 1.** Spectrograms of swamp sparrow song types collected from three different individuals. The trilled syllables are composed of (a) three, (b) one and (c) four notes. Some song types have introductory syllables that differ from the trilled syllables (a), but in the wild, song types without introductory syllables (b, c) are more common.

syllables ('trill rate') and how broad a range of frequencies the repeated syllable can encompass ('frequency bandwidth') (Podos, 1997). This physical trade-off predicts that as trill rate increases, the repeated syllable will be constrained to increasingly narrow bandwidths. In a space defined by trill rate and bandwidth, the result is an expected triangular distribution of songs (Podos, 1997), with the diagonal derived from an upper-bound regression providing an estimate of the performance limit for trill production and the orthogonal deviation from this line (referred to as 'vocal deviation') being a measure of relative vocal performance (Fig. 2) (Podos, 2001).

Vocal deviation is a measure of motor performance that has been shown to influence receiver response in both male and female swamp sparrows (Ballentine et al., 2004; DuBois et al., 2011; Moseley et al., 2013). Higher-performance songs (i.e. songs having low vocal deviation) elicit more copulation displays from females (Ballentine et al., 2004), and older and larger males sing higher-performance songs on average as compared to younger and smaller males singing the same song types (Ballentine, 2009). Also, territorial males respond more aggressively to playback of higher-performance songs as compared to lower-performance versions of the same song types sung by different males (DuBois et al., 2011). Thus, vocal deviation appears to be salient in both male–female and male–male interactions, suggesting a potential benefit to warming up and thus improving vocal performance across the morning.

Finally, because vocal performance is a display of motor performance, male song characteristics should be correlated with male quality. In swamp sparrows, nutritional limitations experienced as a nestling – hereafter called developmental stress – strongly decreases overall body growth (Searcy, Peters, Kipper, & Nowicki, 2010). In other species, developmental stress has long-term consequences on adult phenotype and fitness (Metcalfe & Monaghan, 2001). If (1) song production is physically challenging, (2) birds

that are developmentally stressed are less able to produce physically challenging songs and (3) recent practice improves an animal's ability to produce physically challenging songs, then the effects of recent practice on song performance may be different for developmentally stressed and unstressed individuals.

To test whether song production over the course of hours improves subsequent vocal performance, we exhaustively recorded 11 2-year-old laboratory-reared swamp sparrows for two to three mornings each. Four of the 11 subjects had been developmentally stressed as nestlings. From these recorded songs, we calculated vocal deviation and developed multilevel models to estimate how vocal deviation changed with time of day and the cumulative number of songs performed. To understand how song type complexity influenced the improvement in vocal performance, we also included measurements of song complexity in our models: the number of notes per syllable and the presence of an introductory syllable. Then, we compared the magnitude in the improvement of vocal deviation between developmentally stressed and unstressed birds.

## METHODS

### Study Subjects

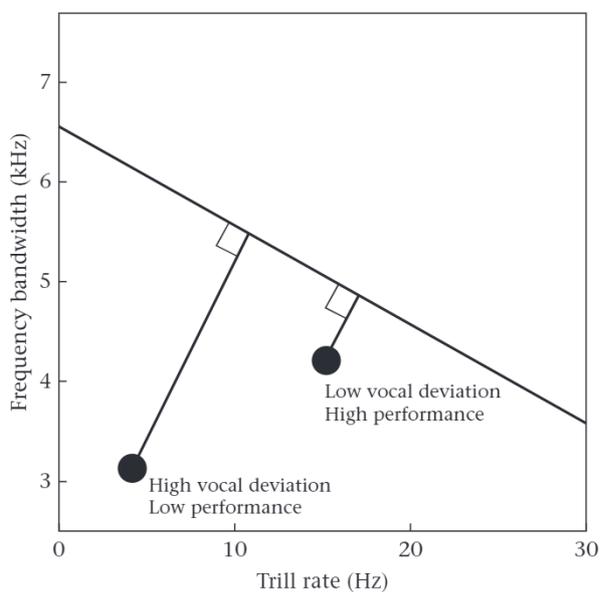
Swamp sparrows have an average of three song types in their repertoires (range 1–7), and syllable structure varies between song types (Fig. 1) (Marler & Pickert, 1984). Songs are of the same song type if they use the same syllable in their trill. Song types are functionally equivalent in the sense that there is no significant difference in male aggressiveness or female copulation solicitation to different song types in an individual's repertoire (Searcy, Searcy, & Marler, 1982). Some song types produced by the birds in our sample began with an introductory syllable that was not repeated in the trill (Fig. 1). In all cases, individuals that sang a song type with an introductory syllable always included it in their song.

We collected 11 male swamp sparrows 2–6 days posthatching (mean  $\pm$  SE =  $3.5 \pm 0.4$  days) from a population in Crawford County, Pennsylvania, U.S.A., during 13–17 June 2004. These birds were part of an experiment testing the influence of early stress on song learning. They were hand-reared in the laboratory and assigned to control (fed until sated,  $N = 7$ ) and experimental (70% of the food offered to the controls,  $N = 4$ ) groups. See Searcy et al. (2010) for more details. Birds were maintained on a normal, seasonally varying photoperiod throughout.

Birds were trained with song recordings for 12 weeks, beginning at  $13.5 \pm 0.6$  (mean  $\pm$  SE) days of age. The peak of song learning occurs at 15–55 days of age, well within the 12 weeks of song training in our experiment (Marler & Peters, 1988). The training models were 14 song types recorded from 14 different free-living individuals in the study population. We presented songs in bouts at a rate of one song/10 s for 3 min, with 1 min of silence between each bout. We presented each song type twice per day, once in the morning and once in the afternoon, using a Marantz PMD 221 recorder and Acoustic Research 'Powered Partner' speaker. We presented song type bouts in a random order, which changed every week.

### Ethical Note

All work was conducted with the approval of the Duke University Institutional Animal Care and Use Committee (Protocols A141-02-05 and A113-05-04). We obtained all the necessary federal, state and local permission for nestling collection in 2004 (U.S. Fish and Wildlife Service MB672712P, Pennsylvania Game Commission Scientific Collecting Permit 41–2004). When collecting nestlings,



**Figure 2.** Calculation of vocal deviation. Each song was plotted in an acoustic parameter space of trill rate and frequency bandwidth. Vocal deviation was calculated as the orthogonal distance from an individual song to the upper-bound regression line, as calculated by Ballentine et al. (2004). The equation for the upper-bound regression line is  $y = 6554.70 - 99.335 \times x$ , where  $y$  is the frequency bandwidth (Hz) and  $x$  is trill rate (Hz) (Ballentine et al., 2004). Lower vocal deviation scores correspond to higher vocal performance. By convention, songs that lie above the line have negative vocal deviation.

whole nests were gently removed from the vegetation in which they were situated, wrapped in a warm cloth and transported to the laboratory within 30 min where half-hourly feeding began immediately and continued from dawn until dusk until the birds fledged (see [Searcy et al., 2010](#) for details of hand rearing). Birds began normal begging and other behaviours immediately on their arrival at the laboratory. As nestlings, birds were kept with their siblings in a natural song sparrow nest that had been sanitized through heating to eliminate potential pathogens and parasites. These nests were kept in plastic bins in a sound-attenuating room to minimize disturbance, in a facility designed and approved for songbird husbandry, with automatically monitored temperature and humidity control, and seasonally varying day length matching their natural annual cycle. As adults, birds were housed in individual cages in the same approved facility. Birds were provided with a regular rotation of enrichments, such as special food stuffs and water baths.

### *Song Recording and Analysis*

Birds were recorded exhaustively beginning early in their first spring (i.e. as 1-year-olds) and each subsequent spring for several years; for the present study, we analysed songs recorded during the first 2 weeks of May 2006, when the birds were 2 years old. When being recorded, birds were moved to individual sound isolation chambers (Industrial Acoustics AC-1, 58 × 41 × 36 cm, Industrial Acoustics, Bronx, NY, U.S.A.) and recorded using a Shure SM57 microphone, Edirol USB Audio Capture UA-1000, with Sound Analysis Pro software v.1.04 (24-bit depth, sample rate = 44.1 kHz) ([Tchernichovski & Mitra, 2004](#)). To capture all songs performed throughout the night, birds were monitored continuously for 24 h, beginning early or late afternoon on one day and continuing through late morning the following day when in the recording chamber, with songs captured using the sound-triggered recording function of Sound Analysis Pro. For this study, we only considered songs recorded between 0200 and 1200 hours. At the time of recording, lights in the recording chamber and in the holding room were turned on without a gradual increase at 0615 hours for the first week of recording and at 0630 hours for the second week of recording. Birds only experienced the lighting in their indoor holding room. Birds in the wild have been observed singing as early as 0230 hours ([Mowbray, 1997](#)), and the time span of our analysis allowed us to capture both the ramp-up and ramp-down phases of morning singing activity.

Swamp sparrows typically sing bouts of the same song type before switching to another type. We considered songs in our recorded sample to have been sung in a bout based on two criteria: (1) the song was the same song type as the preceding song and (2) the song commenced within 1 min of the preceding song. To determine whether there was short-term improvement over the course of the bout, we analysed the first and last song of each bout. The average bout length was 17 songs. We also analysed all songs that were not performed in bouts because, like in the first song in a bout, these songs would not experience within-bout warm-up effects. In total, we analysed 1527 of the 14 495 recorded songs for this 2-week period. Of these songs, 1262 songs occurred in a bout and 255 were single songs not performed in a bout.

Vocal deviation of a song was based on its trill rate and frequency bandwidth. All acoustic analyses were performed in SIGNAL 5 (Engineering Design, Berkeley, CA, U.S.A.). To calculate trill rate, we generated waveforms and measured the onset time of each syllable using an on-screen cursor (cursor resolution = 1 ms). We calculated the difference between onset times of contiguous syllables and averaged these values across the trill. After calculating the average duration between syllables for each song, we calculated the trill rate as 1/mean duration between syllables. To calculate

frequency bandwidth, we generated digital spectrograms for each song (512-point FFT, Hanning window, frequency resolution = 86.1 Hz, time resolution = 11.6 ms) and calculated the difference between the maximum and minimum frequencies measured at -36 dB relative to the peak amplitude of the song. The -36 dB cutoff maintained the frequency information in swamp sparrow songs while minimizing background noise (e.g. [Ballentine, 2006](#); [Ballentine et al., 2004](#)). In song types with introductory syllables, we did not include the introductory syllable in calculation of trill rate and frequency bandwidth. Once frequency bandwidth and trill rate were determined for each song, we calculated vocal deviation as the orthogonal distance between a song's trill rate and frequency bandwidth position and the upper-bound regression line for swamp sparrows as determined by [Ballentine et al. \(2004\)](#) ([Fig. 2](#)). By convention, points can exist above or below the regression line, and vocal deviation could be negative if the song lies above the regression line. Thus, the line is not a limit, but a reference from which to measure performance. For all acoustic analyses, the order of song analysis was randomized so that the individual measuring songs was blind to the time of day the song was performed.

Alternative methods to measure vocal performance in trilled bird songs have been proposed because (1) the methods of calculating upper-bound regression lines are subject to sampling limitations – that is, the unequal distribution of samples across bins used to calculate upper-bound regressions ([Wilson, Bitton, Podos, & Mennill, 2014](#)) – and (2) vocal deviation fails to capture within-syllable measures of complexity ([Podos et al., 2016](#)). [Wilson et al. \(2014\)](#), for example, found that after accounting for sampling limitations, the species-specific regression line for swamp sparrows no longer showed a significant slope. However, this finding is not necessarily because there is no trade-off between frequency bandwidth and trill rate in swamp sparrows, but instead could be an issue of low sample size ([Wilson et al., 2014](#)). Because older, larger males sing songs with lower vocal deviation ([Ballentine et al., 2009](#)) and swamp sparrows have been shown to attend to differences in vocal deviation calculated from the species-specific regression line ([Ballentine et al., 2004](#)), this reference point for vocal deviation likely reflects vocal performance in a biologically significant way for this species.

### *Statistical Analysis*

To determine how vocal performance progresses throughout the morning, we built three sets of Bayesian multilevel models: one to predict trill rate, one to predict frequency bandwidth and one to predict vocal deviation ([Table 1](#)). We compared the models within these model sets to understand how trill rate, frequency bandwidth and vocal deviation changed across the morning. We included time of day, the total cumulative number of songs previously performed (herein called song number), the number of notes per syllable, the presence of an introductory syllable, and all possible interaction terms between (1) song number and time of day and (2) independent measurements of song complexity (presence of an introductory syllable and the number of notes per syllable) as fixed effects ([Table 1](#)). These fixed effects enabled us to assess (1) how vocal performance, trill rate and frequency bandwidth change throughout the morning and (2) how these changes correlate with song type complexity. To minimize the effect of multicollinearity between time and song number, we standardized these values by subtracting the mean and dividing by the standard deviation. We did not include the position of the song in the bout (i.e. whether it was the first or last song) as a fixed effect because there was not a significant difference in vocal deviation between the first and last songs in the bout (paired *t* test:  $t_{611} = 1.307$ ,  $P = 0.19$ ). This is

**Table 1**  
WAIC model weights for each model set

Model	Fixed effects	WAIC weight		
		Vocal deviation	Trill rate	Frequency bandwidth
1	Time, song number, time*song number	0.00	0.00	0.00
2	Time, song number	0.00	0.00	0.00
3	Time	0.00	0.00	0.00
4	Song number	0.00	0.00	0.00
5	Time, song number, time*song number, introductory syllable, introductory syllable*time, introductory syllable*song number, notes/syllable, notes/syllable*time, notes/syllable*song number	<b>0.14</b>	0.00	<b>0.03</b>
6	Time, song number, time*song number, introductory syllable, introductory syllable*time, introductory syllable*song number	0.00	0.00	0.00
7	Time, song number, time*song number, notes/syllable, notes/syllable*time, notes/syllable*song number	0.00	<b>0.44</b>	0.00
8	Time, song number, time*song number, introductory syllable, introductory syllable*song number, notes/syllable, notes/syllable*song number	<b>0.09</b>	0.00	0.00
9	Time, song number, time*song number, introductory syllable, introductory syllable*time, notes/syllable, notes/syllable*time	<b>0.77</b>	<b>0.56</b>	<b>0.97</b>

Higher WAIC weights indicate better fit. See [Supplementary material 1](#) for full model comparisons. Asterisks denote interaction effects. Bold numbers indicate WAIC weights greater than 0.

consistent with findings from [Schraft et al. \(2017\)](#), who found no change in frequency excursion within bouts. To control for between-song type and between-individual variation, we included random slopes for recording day within song type and bird identity (ID). Thus, our models assessed variation within song type. We implemented weakly informative priors for all model parameters (see [Supplementary material 2](#)).

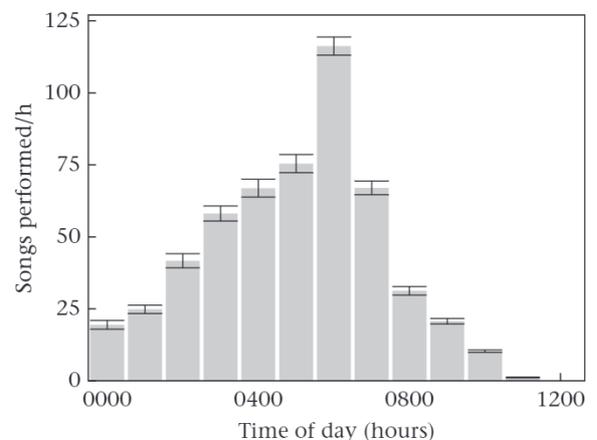
Each model set contained nine models. Within each model set, we compared Models 1–4 to confirm that time of day and song number and their interaction were all important predictors of vocal deviation as well as the two song attributes constituting vocal deviation – trill rate and frequency bandwidth. As expected, the models that included time of day, song number and their interaction were the strongest model in each model set, so we included all three fixed effects in Models 5–9. We then compared Models 5–9 to determine how measures of song complexity – the number of notes per syllable and the presence of an introductory syllable – interact with time of day and song number to influence vocal performance ([Table 1](#)).

To assess which measures of song complexity influenced the progression of vocal performance throughout the morning, we compared model fit within each model set using the widely applicable information criterion (WAIC). We used WAIC instead of Akaike information criterion (AIC) because WAIC makes no assumptions about the shape of the posterior distribution and allowed us to use weakly informative priors to regularize models ([McElreath, 2015](#)). Then, we calculated Akaike weights within each model set. Akaike weights determine relative predictive accuracy of models by rescaling WAIC values so that a total weight of 1 is distributed between all models. Higher WAIC weights indicate better fit ([McElreath, 2015](#)).

To visualize models, we generated averaged model predictions using WAIC weights as proportions. We used a model averaging approach instead of a model selection approach because it accounts for uncertainty in model fit ([McElreath, 2015](#)). We generated model predictions for songs performed between 0300 and 1100 hours because on one day of recording, four individuals were not recorded beyond 1100 hours. For each model prediction, we generated 1000 predictions that were proportioned based on Akaike weight. For instance, if a model had an Akaike weight of 0.65, we generated 650 of the 1000 predictions using that model. We generated model predictions by drawing slopes and intercepts from each of the posterior distributions in the model. In our recordings, the peak in singing was preceded by a gradual ramp up

of singing and followed by a gradual ramp down of singing, with a peak at first light (0615–0630 hours) in songs with and without introductory syllables ([Fig. 3](#)). Thus, we simulated this pattern by normally distributing songs around the mean time of song performance seen in our samples (mean  $\pm$  SD = 0633 hours  $\pm$  2.34 h). In our model predictions, we set the total number of songs produced, herein called ‘song output’, equal to the mean number of songs produced by an individual in our sample (‘average song output’, 658 songs), one standard deviation below the mean (‘low song output’, 205 songs) and one standard deviation above the mean (‘high song output’, 1110 songs). We accounted for variation in vocal performance between song types by setting all model predictions with the same intercept. Thus, in all of our model predictions, vocal performance, trill rate and frequency bandwidth at simulated sunrise were set to the same value.

We also determined whether there was a difference in the magnitude of improvement of vocal deviation between developmentally stressed and unstressed birds. For each bird, we binned songs by song type and day of performance. Then, within each of these bins, we calculated the mean vocal deviation for the first 5% of songs performed and the final 5% of songs performed. We calculated the difference in vocal deviation between the first and final 5% of songs performed and averaged the difference across song types. We compared the differences between developmentally stressed and unstressed birds using a two-sample permutation test.



**Figure 3.** Average ( $\pm$  SE) number of songs performed per individual per hour.  $N = 11$  individuals.

## RESULTS

Individuals performed different song types that varied in vocal performance, and different individuals that performed songs copied from the same song model performed those songs at varying levels of vocal performance (Fig. 4).

Within song types, vocal performance improved with both time and song number, as evidenced by decreases in vocal deviation (Fig. 5). The most substantial improvements in vocal performance occurred just after lights on in the recording chamber, when birds were singing most frequently (Fig. 5). On average, the magnitude of the improvement in vocal performance increased as total song output increased (Fig. 5).

The observed improvement of frequency bandwidth and vocal deviation differed depending on whether a song type included an introductory syllable (Table 1). In our data set, four song types had introductory syllables and 20 song types did not. The mean number of notes per trilled syllable was similar in song types with introductory syllables (2.75 notes) and without introductory syllables (3.35 notes) (two-sample permutation test:  $P = 0.34$ ). In song types with introductory syllables, frequency bandwidth increased across the morning (Fig. 6). However, frequency bandwidth decreased across the morning in song types without introductory syllables (Fig. 6). This trend was reflected in the model predictions for vocal deviation: in song types with introductory syllables, vocal deviation decreased more rapidly than in song types without introductory syllables (Fig. 5).

Song types varied in the number of notes per syllable (one note:  $N = 1$ ; two notes:  $N = 3$ ; three notes:  $N = 9$ ; four notes:  $N = 11$ ). Song types with fewer notes per syllable showed stronger increases in trill rate (Fig. 7, Table 1). However, the effect size was small and, thus, did not greatly affect how vocal deviation progressed throughout the morning (Fig. 7).

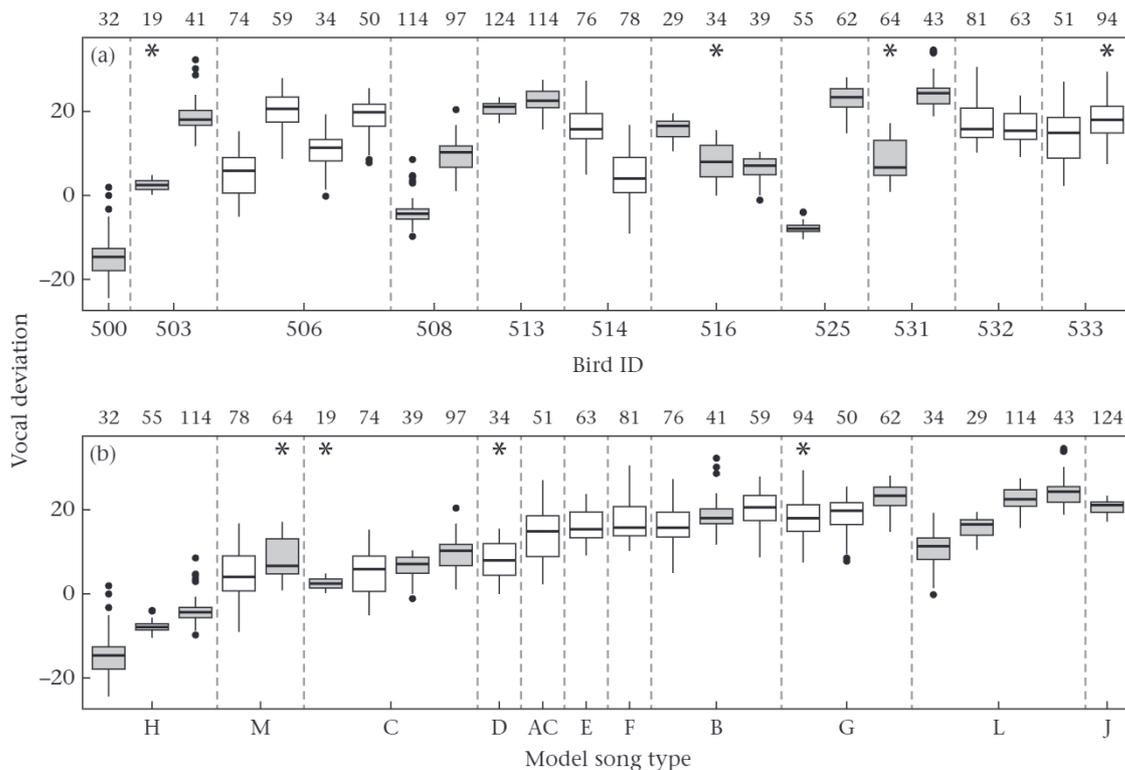
For song types with introductory syllables, as total song output increased, the confidence interval for vocal deviation narrowed (Fig. 5). In other words, as song output increased, individuals were more consistent in performing high-performance songs. However, for song types without introductory syllables, the confidence interval widened with high song output (Fig. 5). Thus, while birds performed better on average as they increased song output, they performed more inconsistently, suggesting that they might experience fatigue.

Birds that had been developmentally stressed as nestlings showed significantly stronger decreases in vocal deviation (i.e. improvements in vocal performance) compared to the control group (two-sample permutation test: mean difference in vocal deviation = 4.9,  $N = 11$ , 330 permutations,  $P = 0.04$ ) (Fig. 8).

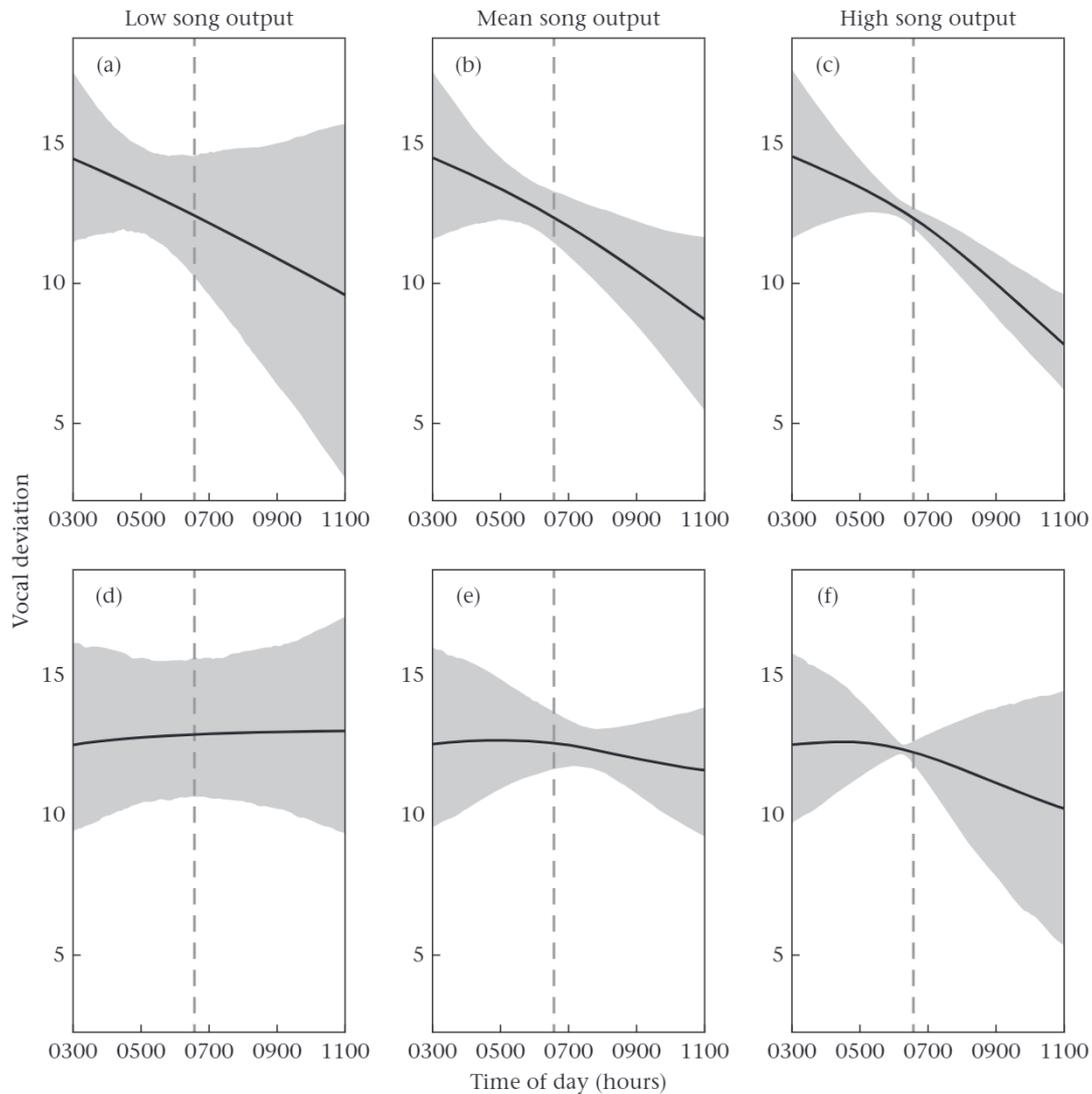
## DISCUSSION

Motor performance reflects an animal's overall condition and should therefore be relevant in assessment of mates and rivals (Byers et al., 2010). Physically challenging song production in swamp sparrows provides an excellent system to test the effects of previous song production on subsequent vocal performance because the biomechanical constraints are established (Podos, 1996, 1997) and the effects of vocal performance on receiver response are well understood in this species (Ballentine et al., 2004; DuBois et al., 2011; Moseley et al., 2013). By singing intensely early in the morning, birds can warm up to improve vocal performance later in the day (Schraft et al., 2017). We showed that in swamp sparrows, vocal performance improved with continued singing across the morning, and this effect was strongest when birds were developmentally stressed as nestlings.

The most pronounced improvements we observed in vocal performance occurred following the simulated sunrise, and the



**Figure 4.** Vocal deviation of song types sung by each bird organized by (a) individual and (b) model song type. Box plots represent quantiles from a different individual performing a given song type, and outliers are shown as dots. White box plots represent developmentally stressed birds, and grey box plots represent birds that were not developmentally stressed. Numbers above each graph show the number of songs analysed. Asterisks denote songs with introductory notes.



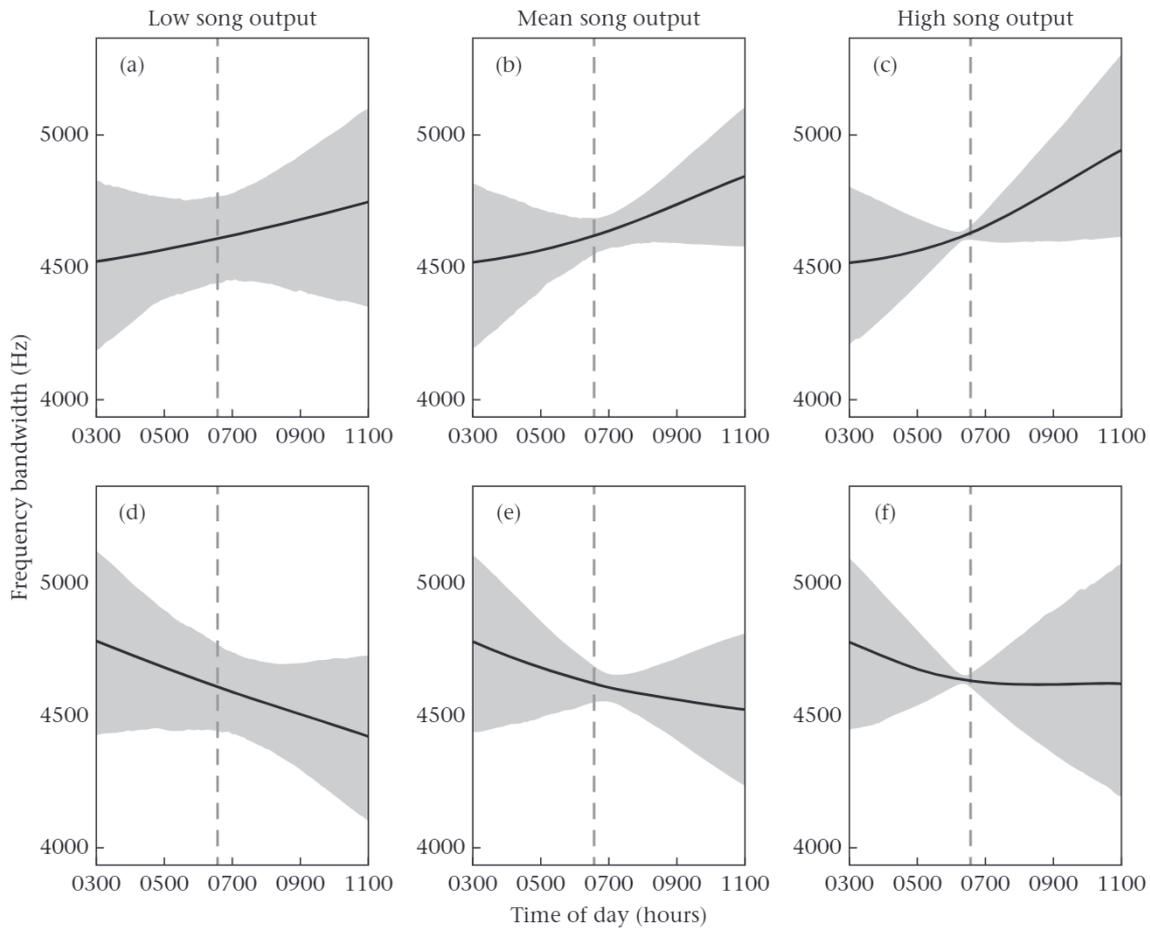
**Figure 5.** Averaged model predictions for vocal deviation in simulated song types with introductory syllables (a–c) and without introductory syllables (d–f). Each syllable consisted of three notes. The total number of songs that a bird performed was either the mean number of songs performed by our recorded individuals (658 songs) (b, e), one standard deviation less than the mean (205 songs) (a, d), or one standard deviation greater than the mean (1110 songs) (c, f). Solid lines are posterior means, shaded regions are 89th percentile intervals, and dashed lines denote first light. Model predictions were set with identical intercepts.

magnitude of the improvement in vocal performance strengthened as total song output increased (Fig. 5). After controlling for time of day, the cumulative number of songs previously performed had a substantial effect on vocal performance (Table 1). Thus, independent of time of day, swamp sparrows improved vocal performance as a result of warming up. Our findings are consistent with the warm-up hypothesis proposed by Schraft et al. (2017) because within one morning, prior song production did indeed improve subsequent vocal performance. Our results are particularly noteworthy because in swamp sparrows, we understand what makes a song more physically challenging to produce, we can reliably quantify how physically challenging a song is and we know that vocal performance is biologically relevant to conspecific receivers (Ballentine et al., 2004; DuBois et al., 2011; Podos, 1996). If this improvement across the morning in vocal performance influences receiver responses, then it could drive songbirds to sing early and often.

On average, vocal deviation of developmentally stressed birds does not differ significantly from that of unstressed birds (Searcy et al., 2010). However, in the present study, developmentally

stressed birds showed greater improvements in vocal performance compared to unstressed birds (Fig. 8). This can be explained if developmental stress reduces individual condition. If birds in worse individual condition are worse at performing physically challenging songs, then they may need to warm up in order to perform songs with high vocal performance. This would result in a greater improvement in vocal performance across the morning for developmentally stressed birds. Our current finding that developmentally stressed birds improved more in vocal performance than unstressed birds suggests that developmentally stressed birds can perform high-performance songs but they require warming up to do so.

Within-individual differences in vocal deviation between the beginning and the end of the day were substantial (Figs 5, 8). For example, in model predictions where song types had introductory syllables and individuals performed an average number of songs, the mean difference in vocal deviation between 0300 and 1100 hours was 8.18 (Fig. 5). These differences were most pronounced in developmentally stressed birds. For example, vocal deviation of Bird 533 showed an average decrease of 10.7 and that



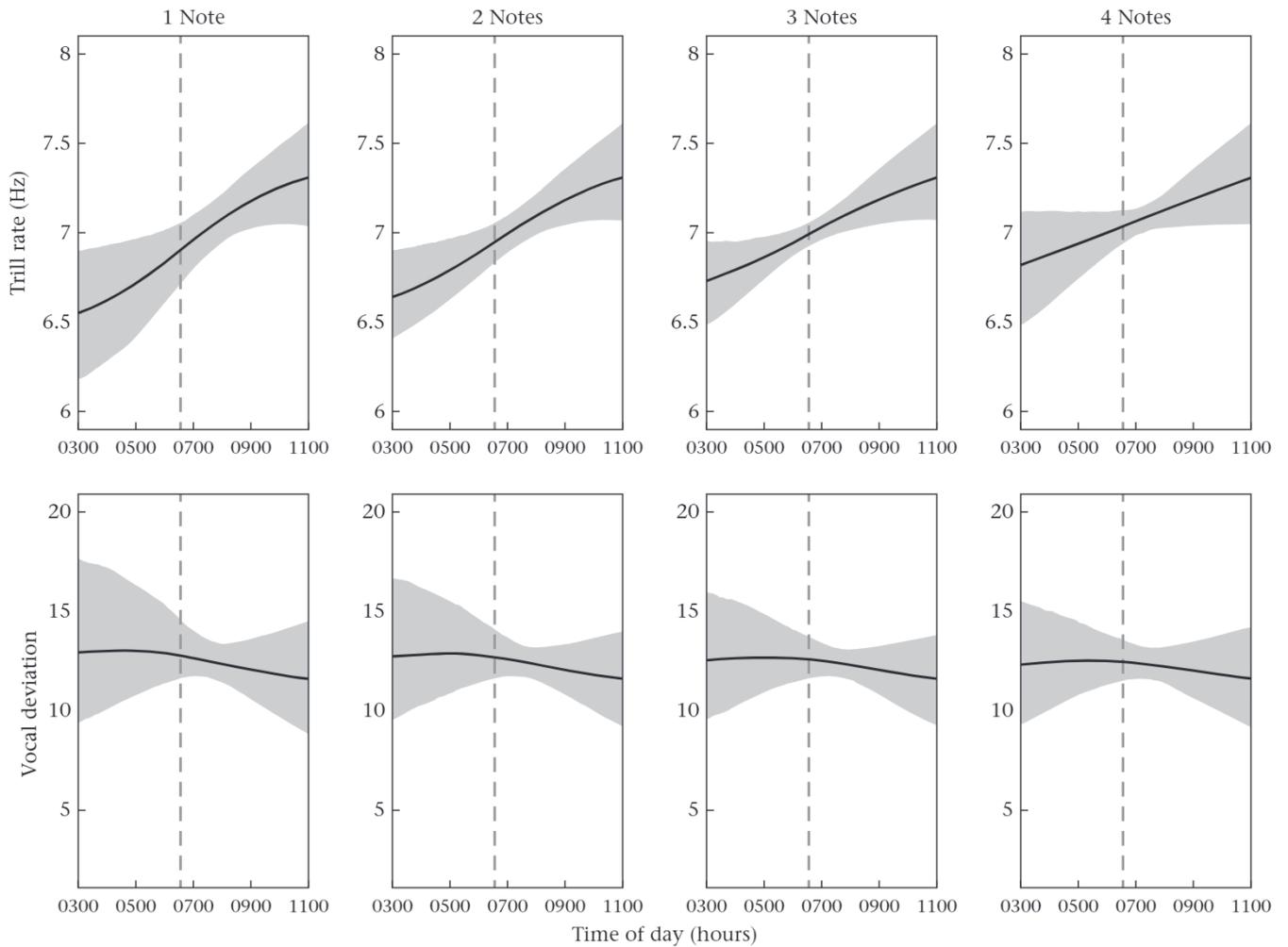
**Figure 6.** Averaged model predictions for frequency bandwidth. Simulated song types had three notes per syllable. Song types were simulated either with an introductory syllable (a–c) or without an introductory syllable (d–f). The total number of songs performed during simulations was either one standard deviation less than the mean (205 songs) (a, c), equal to the mean (658 songs) (b, e), or one standard deviation greater than the mean (1110 songs) (c, f). Solid lines are posterior means, shaded regions are 89th percentile intervals, and dotted lines represent first light. Model predictions were set with identical intercepts.

of Bird 532 showed an average decrease of 7.52 (Fig. 5). Earlier work showed that male swamp sparrows can discriminate between similar differences in vocal deviation from songs produced by different individuals (mean difference in vocal deviation = 9.9), but they do not differentiate between songs of the same type sung by the same individual (mean difference in vocal deviation = 4.19) (DuBois et al., 2011). The within-individual differences that we found are similar to those that DuBois et al. (2011) used for between-individual discrimination. Thus, after accounting for the improvement in vocal performance, within-individual differences may indeed influence the behavioural response of male conspecifics. It is not known whether within-individual differences in vocal deviation affect female response. In a previous study, females preferred high-performance songs over low-performance songs of the same song type:  $2.50 \pm 4.05$  versus  $16.83 \pm 3.50$ , respectively, or an average vocal deviation difference of about 14 (Ballentine et al., 2004). Because females are thought to be generally more discriminating than males, at least in the context of species recognition (Searcy, 1990; Searcy & Brenowitz, 1988; but see Nelson & Soha, 2004), it seems likely that they would discriminate smaller differences in vocal deviation, although this remains to be tested directly.

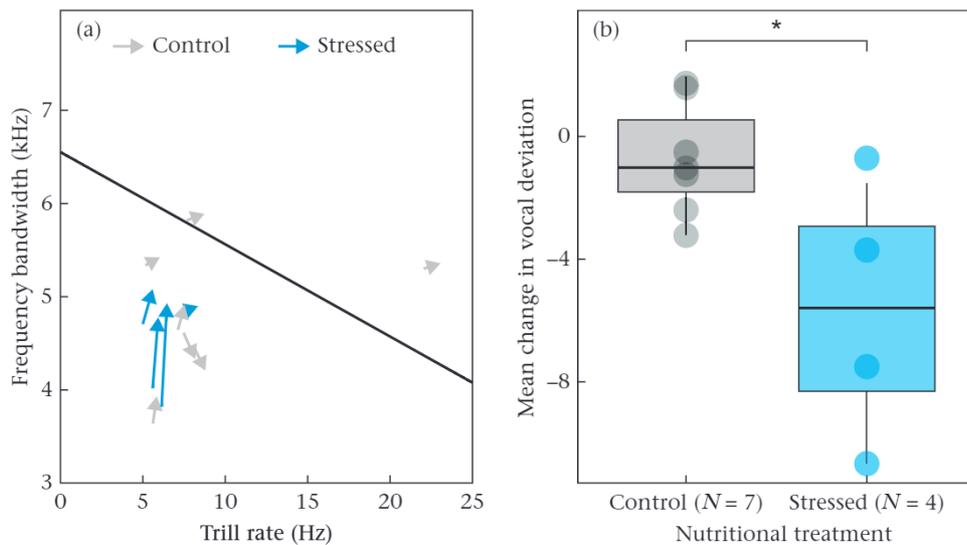
The improvement in vocal performance we observed was stronger in song types having fewer notes per syllable, although this effect was weak and likely irrelevant to receivers (Fig. 7). This trend results from song types with fewer notes per syllable

showing stronger increases in trill rate. Trill rate depends on syllable type, and it is easier to increase absolute trill rate for songs with fewer syllables (Fig. 7). For example, for a bird to increase the trill rate of a song type with one note per syllable by one syllable per second, it must add one note per second, but to increase the trill rate for a song type with four notes per syllable by one syllable per second, it must add four notes per second. Despite this effect, however, trill rate increased consistently across the morning regardless of the number of notes per syllable in a given song type (Fig. 7).

The influence of the introductory syllable on the improvement in vocal performance was stronger than the influence of the number of notes per syllable. Song types with introductory syllables showed stronger and more rapid improvements in vocal performance than song types without introductory syllables (Fig. 5). This was mainly because song types with introductory syllables increased frequency bandwidth more strongly than song types without introductory syllables (Fig. 6). This additional transition from the introductory syllable to the trilled syllable may make it difficult to perform variants of these song types with high frequency bandwidth. The structure of the introductory syllable differs substantially from the trilled syllable and requires a different vocal tract configuration. It may be physically challenging to reconfigure the vocal tract from the position used to produce the introductory syllable to the position used to produce the trilled syllable. If this is the case, then it may be difficult to establish a vocal



**Figure 7.** Model predictions for trill rate (upper panels) and vocal deviation (lower panels) for song types with one to four notes per syllable. Model predictions were set with identical intercepts. Simulated song types had a total number of songs performed equal to the mean number of songs performed in our recorded birds (658 songs). Solid lines are posterior means, shaded regions are 89th percentile intervals, and dashed lines are represent first light.



**Figure 8.** (a) Average trill rate and frequency bandwidth (denoted by arrows) from the first 5% of songs performed to the final 5% of songs performed for each individual. Each arrow represents the average position of all songs across one individual's repertoire. (b) Points represent the change in vocal deviation shown by the arrows in (a). Box plots denote the quantiles for each nutritional treatment group. \* $P < 0.05$  (permutation test).

tract configuration to produce high frequency bandwidth trills after an introductory syllable. Warming up may improve an individual's ability to transition between vocal configurations of different syllables, although the biomechanical underpinnings of this constraint remain unclear.

Interestingly, for song types without introductory syllables, frequency bandwidth was highest at the beginning of the day and decreased slightly across the morning (Fig. 6). Furthermore, the variability in frequency bandwidth (i.e. width of the confidence interval) increased later in the morning (Figs 5, 6). The downward trend and increased variability in frequency bandwidth suggests that it may be difficult for songbirds to achieve high-performance songs with high frequency bandwidth consistently over the course of the day.

Why would frequency bandwidth decline but trill rate show consistent improvements? Perhaps fatigue – a decreasing ability for vocal musculature to generate forces – reduces an animal's ability to generate wide beak gapes and thus high frequency bandwidths. However, reduced force in the muscles may not influence the rate at which beak gapes are modulated. Instead, this may depend on the rate at which forces can be applied. Thus, to compensate for decreased beak gape, animals may improve trill rate to maintain vocal performance. Furthermore, increasing frequency bandwidth could be disadvantageous if it alters note structure and phonology. Phonology is stable and stereotyped within populations of swamp sparrows (Lachlan, Ratmann, & Nowicki, 2018; Marler & Pickert, 1984). Female swamp sparrows perform more copulatory displays and males respond more aggressively to songs more typical of the population (Lachlan, Anderson, Peters, Searcy, & Nowicki, 2014). While individuals must modify note structure to increase frequency bandwidth, they can increase trill rate by shortening only the silent space between notes. Thus, to maintain song typicality (sensu Lachlan et al., 2014), an individual can compensate for decreased ability to perform high frequency bandwidth songs by maintaining a steady frequency bandwidth while increasing trill rate.

The exact mechanism underlying the improvement in vocal performance in singing birds is not known. In human exercise, warming up prevents sports-related injuries and improves muscle performance (Fradkin, 2010; Shellock & Prentice, 1985). In human singing, vocalists report that warming up improves vocal flexibility, increases vocal range and decreases vocal fatigue (Elliot et al., 1995; Gish et al., 2012; but see; Milbrath & Solomon, 2003; Motel, Fisher, & Leydon, 2003). Additionally, vocal warm-up in humans reduces frequency perturbation and amplitude perturbation while improving formant intensity and noise-to-harmonic ratios (Amir et al., 2005). The benefits of warming up in human exercise and singing are likely associated with temperature-dependent physiological effects such as increased blood flow, enhanced nervous impulse speeds, increased oxygen dissociation from haemoglobin, decreased muscle viscosity and lowered activation rates in metabolic reactions (Law & Herbert, 2007; Shellock & Prentice, 1985). Warming up may also affect resonant properties of the vocal tract by altering hydration levels and thus the viscosity and stiffness of laryngeal muscles and vocal folds (Motel et al., 2003). It is unclear whether warm-up physiology is directly comparable between humans and birds given the differences in their vocal tract anatomy, but the general benefit of warming up muscles could influence vocal performance in avian singers.

Across animals, practice can improve an animal's motor performance in the future. On long timescales, for example, juvenile zebra finches perform unstructured songs immediately after waking (Derégnaucourt et al., 2005). It has been proposed that this less structured singing allows juveniles to explore vocal abilities and optimize their imitation of the model song (Derégnaucourt

et al., 2005). While these findings suggest that on a long-term timescale, practice as a juvenile can improve motor ability as an adult, our findings show that on a short-term timescale, practice can improve motor performance in displays occurring later in the day.

In conclusion, we found that swamp sparrows improve vocal performance across the morning both as a function of time and the cumulative number of songs previously performed. The greatest improvements occurred in song types that had introductory syllables and fewer notes per syllable. Furthermore, animals that were developmentally stressed exhibited greater improvements across the morning. These findings are biologically relevant because, in swamp sparrows, we understand the biomechanics constraining vocal performance, we can quantify differences in vocal performance and individuals can improve mate attraction and territory defence by singing higher-performance songs. If the improvement in vocal performance influences mate attraction and territory defence, then it might be beneficial for songbirds to sing early and often. Even if other individuals also benefit from recent practice, an individual that does not sing often at dawn will not benefit from an increase in vocal performance whereas other individuals will. More broadly, these findings demonstrate that previous display production can improve motor performance on a short, within-day, timescale.

#### Conflict of Interest

None.

#### Acknowledgments

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#### Supplementary Material

Supplementary material associated with this article is available, in the online version, at <https://doi.org/10.1016/j.anbehav.2020.06.018>.

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