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EFFECTS OF EARLY CORTICOSTERONE TREATMENT ON VOCAL BABBLING IN
WILD GREEN-RUMPED PARROULETS (*FORPUS PASSERINUS*)

A Thesis

by

CELIA ROSE MCLEAN

Submitted to the Graduate College of
The University of Texas Rio Grande Valley
In partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

August 2021

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EFFECTS OF EARLY CORTICOSTERONE TREATMENT ON VOCAL BABBLING IN
WILD GREEN-RUMPED PARROULETS (*FORPUS PASSERINUS*)

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by
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August 2021

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ABSTRACT

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The stress axis of the endocrine system allows for animals to respond to environmental stressors in contextually appropriate ways. Elevated levels of the stress hormone corticosterone (CORT) in male songbirds can result in compromised song learning ability. Parrots form a sister group to songbirds, but it is unknown whether CORT affects vocal development in parrots. The goal of this study was to assess the effects of CORT supplements in green-rumped parrotlets (*Forpus passerinus*), a free-ranging parrot species in Venezuela. Bouts of vocal babbling were extracted from nest box videos and analyzed. CORT supplementation resulted in differences in babbling vocal production, including peak frequency average slope ($p < .0001$), inflection points ($p < .0001$), and syllable duration ($p < .0001$). Treatment predicted variation in repertoire size but was not significant ($p = 0.51$). Despite differences in stress axis maturation, parrots show some similar responses to early CORT supplementation as songbirds.

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CHAPTER I

INTRODUCTION

Overview

While the ability to produce vocalizations is widespread in animals, the ability to produce novel vocalizations or modify existing ones is rare (Janik & Slater, 1997; Jarvis, 2019). While humans are notably adept at this behavior, research on pygmy marmosets has shown that at least one other species of primate exhibits vocal plasticity (Takahashi et al., 2015). Aside from primates, the only animal taxa capable of vocal production learning (hereafter referred to as vocal learning) are cetaceans (King & Janik, 2013; Vergara & Barrett-Lennard, 2008), pinnipeds (Reichmuth & Casey, 2014), elephants (Poole et al., 2005; Stoeger & Manger, 2014), bats (Knörnschild, 2014), and, in birds; hummingbirds (Baptista & Schuchmann, 1990), oscine songbirds (Marler, 1970), and parrots (Berg et al., 2011, 2013; Bradbury & Balsby, 2016). Vocal learning enables animals to acquire the vocal signals necessary to maintain social cohesion, defend territories, and attract potential mates (reviewed in Farabaugh & Dooling, 1996; Janik & Slater, 1997; Nowicki & Searcy, 2004; Tyack & Clark, 2000). In order for an animal to learn new sounds, it must coordinate respiratory, phonatory, and filter systems with memorized templates (Chakraborty et al., 2015; Janik & Slater, 2000).

Altricial animals such as humans, songbirds, and parrots undergo an extended period of post-embryonic brain growth (Iwaniuk & Nelson, 2003); during this time the brain is at its most

plastic and subject to long-term, organizational changes mediated by steroid hormones (Adkins-Regan, 2005). An extended exposure to stressors can have profound and lasting effects on cognition, behavior, and physiology (Adkins-Regan, 2005; Boonstra, 2005; Kitaysky et al., 2003) that can impact future vocal production (MacDougall-Shackleton & Spencer, 2012).

Songbird vocalizations have been studied extensively as a way to understand the evolution of human language (Doupe & Kuhl, 1999; Kaplan, 2018; Marler, 1970; Nottebohm, 1972; Petkov & Jarvis, 2012), vocal culture (Fehér & Tchernichovski, 2013), and how developmental stress can affect adult phenotypes and reproductive opportunities (MacDougall-Shackleton & Spencer, 2012; Nowicki et al., 1998, 2002). While young songbirds show reduced stress responsiveness during the beginning of the nestling period (Wada et al., 2007), nestling parrots exhibit stress axis activation as young as 13 dph (Berg et al., 2019); this suggests that parrots may be more subject to the effects of developmental stress than songbirds. Parrots also differ from songbirds in several aspects of their vocal learning: both sexes of parrots are capable of learning new vocalizations throughout their lives and use vocal signals in a wide variety of contexts, traits they also share with humans (Bradbury & Balsby, 2016). The ontogeny of early vocalizations produced by humans (babbling) and songbirds (subsinging) has been researched extensively (Goldstein et al., 2003; Lipkind et al., 2013; Nathani et al., 2006; Oller, 1978; Vihman et al., 1985). Meanwhile, little is known about parrot babbling. Green-rumped parrotlets (*Forpus passerinus*) exhibit extreme hatch asynchrony (Beissinger & Waltman, 1991), which results in a developmental environment in which one nestling may be more than an order of magnitude the size of its youngest sibling, unlike the uniform ages of songbird nestlings, which tend to hatch synchronously.

Ontogeny of vocal learning

The ontogeny of vocal learning has been best studied in humans and birds (specifically the oscine-passerine suborder, songbirds). These groups have specific regions in the brain dedicated to the control of acoustic structure and possess similar brain pathways for vocal learning and production (Chakraborty et al., 2015; Jarvis, 2006). Both taxa begin their vocal development with a sensory phase in which they listen to sounds produced in their environment; this period of listening is crucial in development, as human infants and birds deprived of listening to adult vocalizations through deafening or social isolation do not develop normal vocalizations (Marler, 1970; Masataka, 2003; Oller et al., 1998). The sensory phase is followed by the sensorimotor phase, in which the individual begins to produce its own vocalizations and compare those to the adult template. Both humans and songbirds begin their vocal development with the production of amorphous signals that gradually differentiate into acoustically distinct types (syllables) that can then be combined into longer sequences (words and sentences, motifs and songs) (Lipkind et al., 2019). The highly variable vocalizations that precede human speech and songbird song is referred to as babbling and subsong, respectively.

Babbling, in both humans and birds, lacks an apparent communicative function, and develops through the addition and later selective attrition of novel sound types, guided by auditory and social feedback (Burghardt, 2005; Doupe & Kuhl, 1999; Goldstein et al., 2003; Marler & Peters, 1982). As a human infant or nestling songbird ages, the proportion of syllables it innovates decreases, and it begins to favor the sounds it imitates from its social environment (Jakobson, 1968; Marler & Peters, 1982). Whereas early researchers studying babbling in human infants disregarded the possibility of any continuity between babbling and speech, current

research supports the idea that the syllables that appear during babbling continue into true speech (Vihman et al., 1985, 2014). The same finding is supported by analysis of songbird subsong, which contains syllables later used in adult song (Marler & Peters, 1982).

Songbird vocal development generally follows the same learning pathway as human infant speech development, albeit with some species differences. Songbirds, which constitute more than half of extant bird species, belong to the order Passeriformes. While the members of the suboscine suborder demonstrate little to no vocal learning ability (Liu et al., 2013; Touchton et al., 2014), the oscine songbirds (including such diverse taxa as warblers, jays and ravens, and swallows) are well known for their song repertoires and imitative abilities. Parrots (order Psittaciformes) form a sister taxon to the Passeriformes and are another group of noted vocal performers (Hackett et al., 2008; Suh et al., 2011). In many species songbird vocal learning is confined to an early sensitive phase in the first year of life; this begins at around the time nestlings begin to fledge and adult conspecifics are most vocal (Hultsch & Todt, 2004). While female song is thought to be ancestral in songbirds, this trait has been lost in 29% of modern songbirds and research has mostly focused on species where only males sing (Odom et al., 2014; Riebel et al., 2005).

During the sensitive period juvenile birds begin to produce subsong, a series of low-amplitude and highly variable signals lacking discernable order (Hultsch & Todt, 2004; Marler, 1991; Thorpe & Pilcher, 1958). The next stage in vocal development, plastic song, is characterized by the appearance of species-specific memorized song patterns and increased stereotypy of syllables (Aronov et al., 2008; Marler, 1991). When both syntax and syllable characteristics become stereotyped, song is considered to be crystallized (Hultsch & Todt, 2004). As a bird progresses from subsong to crystallized song it discards many of the syllables it

produced during subsong, as a type of signal pruning (Marler & Peters, 1982). While this syllable attrition could be a result of neural pruning (Forssberg, 1999), findings from the experimental inactivation of song-related brain regions in zebra finches suggest that there are separate specialized pathways for the production of juvenile and adult vocalizations (Aronov et al., 2008).

The maturation of vocal production has been shown, in some species, to be guided through social interaction. Studies on human infants and juvenile songbirds have revealed that contingent interactions with social partners shape babbling production (Carouso-Peck & Goldstein, 2019; Goldstein et al., 2003; West & King, 1988). That is, juveniles are guided from producing immature forms of vocalizations to mature forms based on both vocal and visual feedback from adults, such as brief wing-strokes and “fluff-ups” (in birds) and smiling and physical touch (in humans) (Carouso-Peck & Goldstein, 2019; Goldstein et al., 2003; West & King, 1988).

The highly variable vocal behaviors during the juvenile period appear to be important for later success in adult communication. For human infants, mastering the motor skills needed to produce a bout of mature syllables is necessary to be able to produce the rapid syllable-to-syllable transitions present in speech, and by hearing its own vocal productions an infant can begin to map its “acoustic space” (Liu et al., 2009); that is, by listening to the sounds it is producing an infant can learn the acoustic consequences of its motor movements. This process has been referenced as a form of vocal play; babbling and other forms of monologue speech allow an individual to experiment with and refine sounds and syntax in a low-stakes context (Burghardt, 2005; Kuczaj, 1983; Pepperberg, 2004).

Effects of developmental stress on avian vocalization

Once an individual has mastered stereotypy of sound production it can successfully engage in social interactions with conspecifics. Animals can convey a great deal of information through their vocalizations, including identity, physiological state, sex, and motivational state (Perez et al., 2012; Seyfarth & Cheney, 2003). Birds encode such information via acoustic characteristics such as duration, pitch, fundamental frequency, and bandwidth (Aubin et al., 2007; Charrier et al., 2005; Kilner & Johnstone, 1997; Speirs & Davis, 1991). Other birds perceive and respond to this information; for example, zebra finches increase their parental investment in corticosterone- (CORT, the main stress hormone in birds) treated nestlings after hearing their stress-modified begging calls (Perez et al., 2016) and male songbirds react less aggressively to hearing the territorial song of older--and therefore less threatening--males (Zipple et al., 2020). The Developmental Stress Hypothesis, first proposed as the Nutritional Stress Hypothesis by Nowicki, Peters, and Podos in 1998 and later revised in 2002, states that adult vocalizations act as honest signals as to male quality (1998, 2002). Large song repertoire size in songbirds is correlated with higher adult survivorship, longer territory tenure, better immune function, and other physiological benefits (reviewed in MacDougall-Shackleton & Spencer, 2012). Because the costs of song production could include the cost of forming song-related brain regions (Catchpole, 1996), significant stressors during development would impede brain growth, and thus song performance (MacDougall-Shackleton & Spencer, 2012). Both experimental stressors and CORT administration during nestling development resulted in reduced High Vocal Center (HVC, a brain region critical for song production in songbirds) volume in songbird brains (MacDonald et al., 2006; Nowicki et al., 2002; Spencer et al., 2005a; Spencer et al., 2005b). Experimentally induced nutritional stress, increased brood size, and CORT administration

resulted in decreased song rate and lessened stereotypy in adult zebra finches (Zann & Cash, 2008).

Past experimentation has revealed that developmental stress produces early differences in vocal development. Nutritionally stressed swamp sparrows began subsong earlier than control birds, although both groups achieved song crystallization at the same age (Nowicki et al., 2002); this resulted in a shorter sensory and longer sensorimotor phase of song learning for the experimental group. The stressed birds also developed small brains overall, with a disproportionately reduced volume of the robust nucleus of the arcopallium (RA, another song production-related brain region); it is possible that the early onset of the sensorimotor phase of vocal development was in compensation for compromised cognitive development (Nowicki et al., 2002).

The mechanism by which stress impairs later vocal performance is unknown. The administration of exogenous CORT produces similar effects on vocal and brain development as other developmental stressors, which indicates that glucocorticoids, a main factor of the stress response, likely mediate the effects of developmental stress on vocal performance (MacDougall-Shackleton & Spencer, 2012; Wingfield, 2005).

The stress axis

The function of the stress response is to enable animals to respond to challenging situations in contextually appropriate ways. Stressors, such as a physical injury or lack of food, activate the hypothalamic-pituitary-adrenal (HPA) axis, the pathway responsible for the production and release of glucocorticoids, a class of steroid hormones (Adkins-Regan, 2005; Boonstra, 2005). The results of glucocorticoid actions include increased cardiovascular tone, the

mobilization of glucose to muscles, sharpened cognition, and suppressed reproductive behavior (Boonstra, 2005). These actions both enable an individual to respond to a current stressor and prepare for a subsequent stressor (Sapolsky et al., 2000). When the challenge has passed, negative feedback loops restore glucocorticoid levels back to baseline (Adkins-Regan, 2005). A very aversive or chronic stressor, however, can result in chronically elevated levels of glucocorticoids in circulation, which bring with them physiological costs (Adkins-Regan, 2005). Such costs include immunosuppression, gastric ulceration, and impaired brain development; the number, location, and connections between neurons can all change based on steroid presence (Adkins-Regan, 2005; Boonstra, 2005; MacDonald et al., 2006; Sapolsky et al., 2000). Even the resting state and response threshold of an adult animal's HPA axis are the result of early life experiences (Adkins-Regan, 2005). The primary glucocorticoid present in birds is CORT, for which there are membrane receptors throughout the avian brain, including in song-control regions (Adkins-Regan, 2005; Suzuki et al., 2011).

As high levels of glucocorticoids can be especially deleterious in young birds, HPA responsiveness is often delayed until young leave the nest and are capable of fending for themselves (Sims & Holberton, 2000; Wada et al., 2007). In contrast, nestling green-rumped parrotlets showed an adult-like HPA response at a much earlier stage in development, possibly as a result of competition between siblings of markedly different sizes and experience levels (Berg et al., 2019).

Responsiveness of the HPA axis is important for life in the wild, where perturbations in the environment demand quick behavioral and physiological adaptations (Wingfield, 2005). Captive birds, meanwhile, exhibit baseline and stress-induced CORT concentrations that differ markedly from free-living populations (Marra et al., 1995; Vidal et al., 2019). It is therefore

instructive to examine the stress response in wild birds, who may be more resilient to environmental challenges.

While many aspects of the developmental stress hypothesis have been examined in songbirds, it has only recently been addressed in parrots (Eggleston, 2020). Vocal learning in parrots manifests quite differently than it does in songbirds; for example, parrots are open-ended learners and exhibit a lesser degree of sexual dimorphism in vocal behavior. Researching the effects of CORT on parrot vocal development would help broaden the applicability of the developmental stress hypothesis.

Parrots as model taxa for vocal learning

Research on the developmental stress hypothesis in birds has almost exclusively concentrated on male songbirds, with the perspective that birdsong evolved through sexual selection (MacDougall-Shackleton & Spencer, 2012). Not only does this disregard the many species of songbird in which female singing occurs, this hypothesis merits reexamination with the finding that the songbird ancestor possessed female song, and thus female song has been selected against, rather than having been selected for in male songbirds (Odom et al., 2014). While sexual selection undoubtedly plays a role in the evolution of song, it is just one of many natural selective pressures that have led to the wide variety of song-learning programs (Beecher & Brenowitz, 2005). Additionally, many songbirds are closed-ended vocal learners, meaning that they have only a brief sensitive phase in early development in which they can learn songs from tutors (Beecher & Brenowitz, 2005). How does the developmental stress hypothesis apply in species that can learn new vocalizations throughout life? Previous research found differences in the frequency modulation in the babbling of CORT-treated and control green-rumped parrotlets

(Eggleson, 2020). However, the individuals received CORT treatment relatively late in their nestling period (21-27 dph), during the babbling period (Eggleson, 2020). It is possible that the still-circulating CORT produced an activational effect on vocalizations, like the higher-pitched begging calls that nestling zebra finches produce after CORT treatment (Perez et al., 2016). Additionally, CORT treatment at an earlier age has the potential to have a larger organizational effect on the nestling parrotlet brain and behavior; a 21 dph parrotlet weighs 93% of its adult weight, whereas a 13 dph parrotlet, at which age its HPA axis is fully matured, weighs only 65% of its adult weight (Berg et al., 2019; Waltman & Beissinger, 1992). The gene expression and cytoarchitecture of vocal learning nuclei do not resemble those of adults until several weeks post hatch in another parrot species, the budgerigar (Hall et al., 1999; Whitney et al., 2015). At 13 dph the brain of a nestling green-rumped parrotlet is undergoing a large amount of neural organization, similar to that undergone by other altricial birds during the nestling stage (Iwaniuk & Nelson, 2003).

Bradbury and Balsby (2016) identified four vocal communication traits shared by both parrots and humans: open-ended learning, similar abilities of both males and females, large repertoires, and the use of vocalizations in a wide variety of social contexts. Like humans, young parrots are highly altricial and require extended parental care (Waltman & Beissinger, 1992). Additionally, while all three avian vocal learning lineages (parrots, songbirds, and hummingbirds) share seven analogous vocal nuclei that allow for the perception and production of learned vocalizations, the parrot vocal learning system is more elaborate, consisting of both “core” and “shell” regions that show specialized gene expression (Chakraborty et al., 2015). The parrot core region is analogous to vocal learning brain regions in oscine songbirds and hummingbirds, whereas the shell region is unique to parrots; its exact function is unknown

(Chakraborty et al., 2015). Phylogenetic comparison of shell and core sizes across parrot species revealed that the shell region is much larger in relation to the core in species capable of complex vocal communication abilities; more research needs to be done to uncover whether the shell region enables these abilities (Chakraborty et al., 2015). The body of research on developmental stress in birds has been restricted almost entirely to oscine songbirds; due to the more elaborate vocal learning brain regions and different vocal learning strategies of parrots it is possible that the effects of developmental stress manifest quite differently in parrots than in songbirds.

Summary

The developmental stress hypothesis posits that adult songbird vocalizations act as an honest indicator as to developmental condition. Parrot vocal learning strategies differ from those of songbirds, and they manifest a mature stress response at an earlier stage in development, making them potentially more vulnerable to the effects of circulating glucocorticoids on their developing brains. Earlier research on the effects of CORT on parrotlet vocalizations analyzed acoustic measurements from an age at which individuals were still receiving exogenous CORT, potentially confounding both the organizational and activational effects of CORT. Additionally, free-ranging and captive bird populations manifest different HPA axis responsiveness; to be able to extrapolate findings from research on developmental stress to the evolution of birdsong through mate choice it is essential to study these effects in an ecologically appropriate context.

CHAPTER II

HYPOTHESES

To understand how high plasma CORT levels early in development can affect later vocal learning in parrots, I analyzed various aspects of vocal babbling performance, including output, repertoire size, and spectrographic measurements from audio-video recordings of wild nestling green rumped parrotlets that received CORT treatment. Only vocalizations produced after the treatment period were incorporated in this study, as vocalizations produced during the treatment period (13-17 dph) might be altered by the activational effects of CORT. For example, zebra finch nestlings treated with exogenous CORT ten minutes earlier produced begging calls with different acoustic characteristics than control nestlings in a study by Pérez et al. (2016).

Hypothesis 1: CORT affects vocal production

In accordance with the Developmental Stress Hypothesis and previous research on both songbirds and parrots, I hypothesized that stressors early in development result in a vocal production phenotype that differs from that of birds raised under normal conditions. I predicted that prior CORT exposure would have a significant impact on nestling babbling, specifically resulting in a reduced repertoire size, lower peak frequency measurements, and reduced peak frequency slope (Eggleston, 2020; Spencer et al, 2005; 2003). I further predicted that CORT would influence the timing of vocal development, with

CORT-treated nestlings beginning to babble sooner than control nestlings, using an increased vocal learning period to compensate for reduced learning ability (Nowicki et al., 2002). These findings would support the Developmental Stress Hypothesis (MacDougall-Shackleton & Spencer, 2012; Nowicki et al., 1998, 2002): excess exposure to corticosterone during brain development results in reduced brain volume in vocal learning-related brain regions and therefore reduced song learning and production capabilities.

There is also the possibility that CORT does affect vocal production, but by resulting in an increase in repertoire size as opposed to a decrease. A study on red-winged blackbirds found an positive correlation between song repertoire size and baseline CORT (Merrill et al., 2013). However, CORT and repertoire size were measured on adult blackbirds, and within a few days of each other, so developmental condition of the males is unknown. Begging, a behavior that involves vocalization, is increased in CORT-exposed birds (Kitaysky et al., 2001). If babbling output is also increased, I could find an increased repertoire size in CORT-treated nestlings, as a higher degree of vocal exploration could lead to a larger acoustic space.

Null Hypothesis: CORT does not affect vocal production

My null hypothesis is that the parrotlet vocal learning process is somehow shielded from exposure to high levels of CORT, a necessary protection given their early HPA axis maturity (Berg et al., 2019). In this case I would predict finding no differences between CORT and control babbling output and acoustic measurements.

CHAPTER III

METHODS

Study site and species

Research is conducted at the Hato Masaguaral field site (8°33'57"N, 67°34'16"W) in Guárico, Venezuela. The site consists of a working cattle ranch surrounded by forest and tropically flooded savanna. Wild green-rumped parrotlets live year-round at the site, and nest in 120 artificial nest tubes made from polyvinyl-chloride plastic that are located in two regions on the ranch (Beissinger, 2008). The parrotlet breeding season extends from June to December and consists of up to two nesting attempts per monogamous pair (Waltman & Beissinger, 1992). In a successful nest attempt an average of 5.7 nestlings hatch over an average of 8.6 days (Beissinger & Waltman, 1991). Nestlings fledge between 28-35 dph (Beissinger & Waltman, 1991). Due to the large hatch asynchrony in this species, nestlings at a wide range of ages (and thus, size and development) are present in the nest at the same time. Nestlings are marked with symbols on the tops of their heads to aid in differentiation between individuals. Field technicians record data for each nest, including the number of eggs laid, egg mass, hatch date, nestling mass, nestling sex, fledge date, and hatch sequence. Sex is determined by the presence of sexually dimorphic plumage that is visible as early as 15 dph (Budden & Beissinger, 2004). Due to the low degree of extra-pair fertilization, siblings are assumed to be genetically related (Melland, 2000).

Twelve nests were selected for this study: six from the 2018 field season and six from the 2019 field season. Brood sizes (defined as the number of birds who fledged from a nest) consisted of four ($n=4$ nests), five ($n=2$), six ($n=4$), and seven ($n=2$) nestlings. A total of 13 nestlings from these nests died before fledging and 64 survived to fledge from the nest. 31 of these nestlings were female and 33 were male. Hatch sequence (whether a nestling is eldest, middle, and youngest within a nest) was recorded; however, sham control and CORT treatment was usually administered to the eldest two nestlings in each nest, as a way of minimizing variation due to the effects of hatch sequence on babbling production and output.

Corticosterone administration

Preparation and administration of CORT-DMSO-sesame oil solution (CORT) and the vehicle control DMSO-sesame oil solution (Oil) followed (Eggleston, 2020). Nests containing 3-8 nestlings each were selected for hormone treatment. One nestling per nest was chosen to receive the CORT treatment, one the Oil treatment, and remaining nestlings received nothing. In total, 41 nestlings received Control treatment, 12 received CORT, and 11 received Oil (one Oil nestling died before fledging at 24 dph) (Table 1). Treatment was randomly administered to the first three eldest nestlings per nest, as a way to control for hatch sequence. Subjects received 12 μ l of either CORT or Oil twice daily with a Hamilton 25- μ l syringe, starting at 13 dph and ending at 19 dph. Dosage was designed to fall within the normal range for this species, which has been previously described (Berg et al., 2019). At 13 dph a parrotlet nestling has an active HPA axis and has not yet begun to produce vocal babbling (Berg et al., 2019). Measurement of baseline and stress-induced CORT plasma concentration is previously detailed in another study (Berg et al., 2019).

Table 1: Nestling sample sizes.

| Class | <i>n</i> nestlings |
|-----------------|---------------------------|
| ♀ CORT | 4 |
| ♂ CORT | 8 |
| ♀ Oil | 4 |
| ♂ Oil | 7 |
| ♀ Control | 23 |
| ♂ Control | 18 |
| Total ♀ | 31 |
| Total ♂ | 33 |
| Total CORT | 12 |
| Total Oil | 11 |
| Total Control | 41 |
| Brood size=4 | 16 |
| Brood size=5 | 10 |
| Brood size=6 | 24 |
| Brood size=7 | 14 |
| Total nestlings | 64 |

Recording

A camcorder (Song FDR AX33) was placed at the top of each nest cavity between 7-9 am, beginning after the female parrotlet ceased incubation. Nest activity was recorded for 1-6 hours each day, until the final nestling fledged from the nest. Data were stored as Advanced Video Coding High Definition (AVCHD) files and copies stored on multiple external hard drives.

Video analysis

Adobe Audition (v13.0.3.60, 2020, Adobe Inc., San Jose, CA) was used to review audio-visual recordings. Vocal babbling bouts were defined based on the following criteria, following Eggleston (2020): (1) a series of two or more structurally distinct vocal elements, and (2) the time between elements equal to or less than four seconds long.

Bouts produced after the fourth hour of recording were not used in analysis to avoid oversampling certain days as well as certain nestlings. The audio of each bout was extracted as a Waveform Audio File (WAV) at a 16-bit resolution and a 44,100 Hz sample rate. The recorded bouts of four nestlings who died before fledging were excluded from analysis. Bouts produced before a nestling reached 20 dph were also excluded from analysis, as at 20 days and afterward all nestlings had ceased their treatments and presumably had no more exogenous CORT in circulation (oral administration of CORT results in a peak of plasma CORT levels 10 minutes later, which subsides back to baseline after 120 minutes; Spencer & Verhulst, 2007). Therefore, each nestling received equal sampling time from 20 dph until the day it fledged. These exclusions removed 9.5% (13,201 individual vocal elements) of the dataset, with the final resulting dataset consisting of 123,214 vocal elements produced by the 64 nestlings.

Spectrographic analysis

Spectrographic analysis was performed using Raven Pro (v1.6.1, 2019, Cornell Center for Conservation Bioacoustics, Cornell Lab of Ornithology, Ithaca, NY) (Figure 1). Files were first band filtered between 500 and 12,500 Hz, which encompasses the range of vocalizations produced by parrotlets (Berg et al. 2012, 2013). A Fast Fourier Transform (FFT) of 270 was used to construct spectrograms.

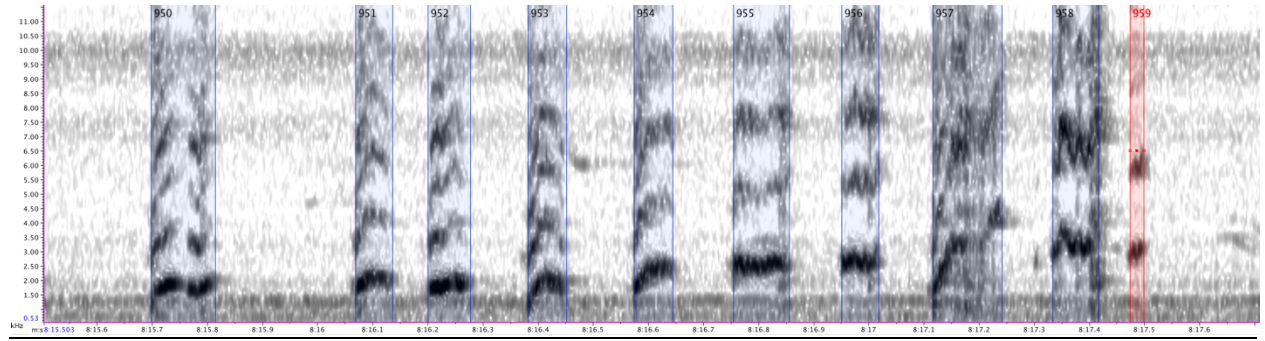


Figure 1: Example babbling sequence. The y-axis denotes frequency (Hz) and the x-axis denotes time (s).

A band limited energy detector was used to identify individual vocal elements within babbling bouts using the following detection parameters: minimum (500 Hz) and maximum (12,500 Hz) frequency, minimum (0.0122 s) and maximum (0.75 s) syllable duration, minimum inter-syllable duration (0.028 s), signal-to-noise ratio (5 dB), signal-to-noise ratio minimum occupancy (60%), noise power estimation block size (1.001 s), noise power estimation hop size (0.0459 s), and noise power estimation percentile (50). Detector selections were visually reviewed to ensure quality, as mechanical sounds and vocalizations from other species are often erroneously identified by the detector. The following spectral measurements were used in analysis: average entropy (bits) (a measure of the randomness of spectral power distribution), center frequency (Hz) and the 5% and 95% frequencies (Hz) (the frequency that divides the syllable into two sections, that contain, respectively, 50%, 5%, and 95% of the energy of the selected syllable), 95% bandwidth (Hz) (the difference between the 5% and 95% frequencies), syllable duration (s), number of inflection points of the peak frequency contour (the number of times the sign of slope of the peak frequency contour (PFC) changes), and the mean slope of the peak frequency contour (Hz/ms) (the mean of the different slope measurements that make up the

peak frequency contour) (Figure 2). Any nestling vocalizations overlapped with another sound in the frequency domain were removed from the selection table.

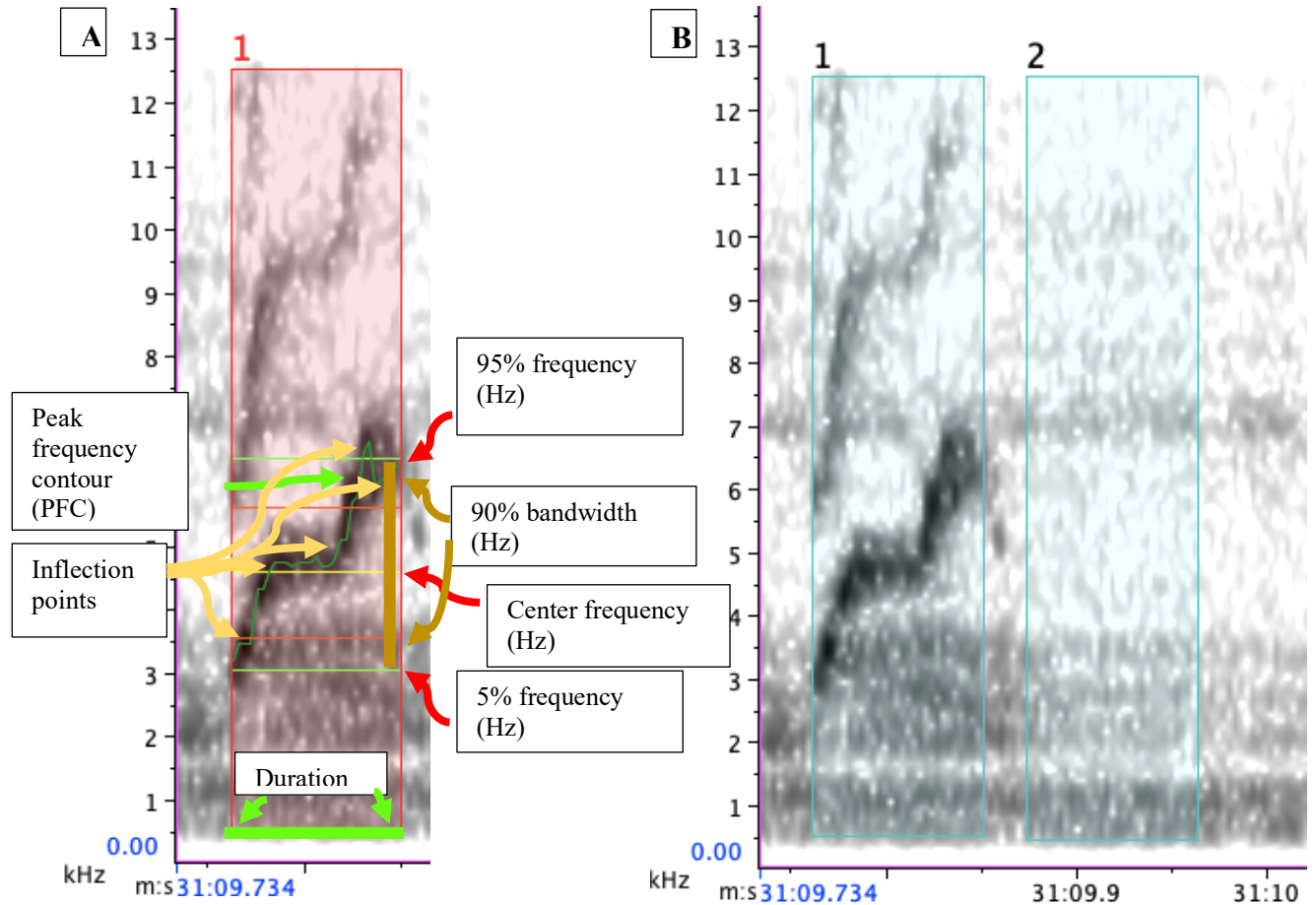


Figure 2: Visualization of spectrographic measurements. **A.** Measurements include the PFC average slope, which comes from the peak frequency contour; number of PFC inflection points; duration; 5%, center, and 95% frequency; and 90% bandwidth. **B.** Entropy measures the probability of power distribution in a spectrogram. A spectrogram with a random degree of power distribution has a high entropy value, while power concentrated in one area of the spectrogram has a low entropy score. Selection 1 has an entropy score of 3.8 while selection 2 has a score of 4.3.

Statistics

Distributions of all spectrographic measurements were visually inspected for normality, which is an assumption for later principal components, k means, and generalized linear mixed model (GLMM) analysis. GLMMs were conducted using SAS software (v.9.4, 2013, SAS Institute Inc., Cary, NC); all other analyses were conducted using JMP Pro software (v15.1.0, 2019, SAS Institute Inc., Cary, NC). The following acoustic measurements were \log_{10} transformed: center frequency, 5% frequency, 95% frequency, 95% bandwidth, and duration; while the measurements of peak frequency contour inflection points were $\log(x + 1)$ transformed. Linear regression was used to test for multicollinearity between acoustic measurements, as well as to examine change in acoustic measurements and babbling output by age. All acoustic measurements were plotted by each other: 5% and center frequency were highly correlated ($R^2=0.89$), as well as 95% frequency and 95% bandwidth ($R^2=0.94$). 95% and center frequency displayed a lower correlation ($R^2=0.82$). The 95% bandwidth and 5% frequency measurements were therefore excluded from k -means analysis, as multicollinearity among variables can result in model overfitting (Armitage & Ober, 2010). Intersyllable duration (the time between syllables in a bout), syllable output (both daily as well as total), and age of babbling onset were \log_{10} transformed.

Principal components analysis (PCA) was conducted in order to reduce the dimensionality of the dataset. Principal components were then utilized in an unsupervised k -means clustering analysis to estimate the syllable repertoire size of each nestling. Several iterations of the clustering analysis were performed, and the Cubic Clustering Criterion was used to obtain optimized number of clusters. The ensuing cluster classifications were used to calculate the Shannon-Weiner diversity index (H) of the repertoire of each individual. H is calculated as

the sum of the product of the negative proportion (p_i , the number of times a syllable type was used by an individual divided by the individual's total output) and the natural log of the proportion. A higher H score indicates a nestling with a larger repertoire size.

GLMMs were used to test for differences in vocal babbling production between nestlings who received CORT, Oil, and Control treatments. Nest ID was included as a random effect in the model, to control for the high degree of relatedness between nestlings in the same nest. Treatment (CORT, OIL, CONTROL), sex, age, and brood size (nestlings fledged from the nest) were included as fixed effects. The variables modeled were the eight previously mentioned spectrographic measurements, intersyllable duration, total syllable output, syllable repertoire size, babbling onset age, fledge age, and Shannon-Weiner diversity index, as well as the first principal component (PC1) from the principal components analysis. The corrected Akaike information criterion (AIC_C) was used to evaluate model strengths. To identify differences between groups, a p -value less than 0.05 was considered significant.

CHAPTER IV

RESULTS

Descriptive statistics

Nestlings produced an average of 1,925.2 (± 199.2 SE) vocal babbling syllables prior to fledging (Table 1). The average number of vocal babbling bouts recorded per nestling was 56.2 (± 5.38 SE) with a range of 2-1,289 syllables per bout ($\bar{x} = 34.3$ syllables ± 1.32 SE) (Table 1). The onset of babbling occurred between 12-29 dph ($\bar{x} = 19.1 \pm 0.6$ SE), and nestlings fledged between 28-37 dph ($\bar{x} = 31.7 \pm 0.2$ SE) (Table 2).

Table 2. Summary of descriptive statistics. Mean syllable output, total repertoire size, and age at babbling onset and fledging for nestling parrotlets, grouped by sex, treatment, and brood size.

| | Mean syllable output (\pm SE) | Mean repertoire size (\pm SE) | Mean onset age (dph \pm SE) | Mean fledge age (dph \pm SE) |
|--|-------------------------------------|--|----------------------------------|--------------------------------------|
| Male ($n=33$) | 2,084.6 \pm 278.67 | 34.3 \pm 0.86 | 17.7 \pm 1.13 | 31.4 \pm 0.44 |
| Female ($n=31$) | 1,755.5 \pm 286.30 | 32.4 \pm 0.88 | 20.2 \pm 1.13 | 31.8 \pm 0.44 |
| CORT ($n=12$) | 2,275.2 \pm 533.90 | 32.3 \pm 1.38 | 17.33 \pm 1.44 | 31.5 \pm 0.57 |
| Oil ($n=11$) | 1,611.6 \pm 338.17 | 34.2 \pm 1.38 | 18.5 \pm 1.48 | 30.8 \pm 0.53 |
| Control ($n=41$) | 1,906.9 \pm 255.67 | 33.4 \pm 0.79 | 19.5 \pm 1.12 | 31.8 \pm 0.43 |
| Brood size=4 ($n=16$) | 2,169.8 \pm 389.81 | 34.9 \pm 1.14 | 16.5 \pm 1.98 | 31.4 \pm 0.71 |
| Brood size=5 ($n=10$) | 1,609.9 \pm 438.14 | 29.9 \pm 1.44 | 20.1 \pm 2.73 | 30.1 \pm 0.98 |
| Brood size=6 ($n=24$) | 2,126.4 \pm 353.27 | 33.83 \pm 0.93 | 19.8 \pm 1.90 | 32.3 \pm 0.68 |
| Brood size=7 ($n=14$) | 1,526.1 \pm 420.50 | 33.14 \pm 1.22 | 20.2 \pm 2.66 | 32.1 \pm 0.95 |
| All nestlings ($n=64$) | 1,925.2 \pm 199.29 | 33.3 \pm 0.59 | 19.1 \pm 0.63 | 31.7 \pm 0.23 |

Repertoire size and diversity

The first two principal components (PCs, eigenvalues of 2.52 and 1.67, respectively) explained 69.8% of the variance in the six babbling acoustic measurements. Center frequency, 95% frequency, and average entropy were the main components of PC1, and duration and PFC inflection points were the two main components of PC2. A high PC1 score also corresponded to a high entropy, 95% frequency, and center frequency (Figures 3-5). A *k*-means analysis clustered the vocal selections into 36 syllable types (Figures 6 and 7).

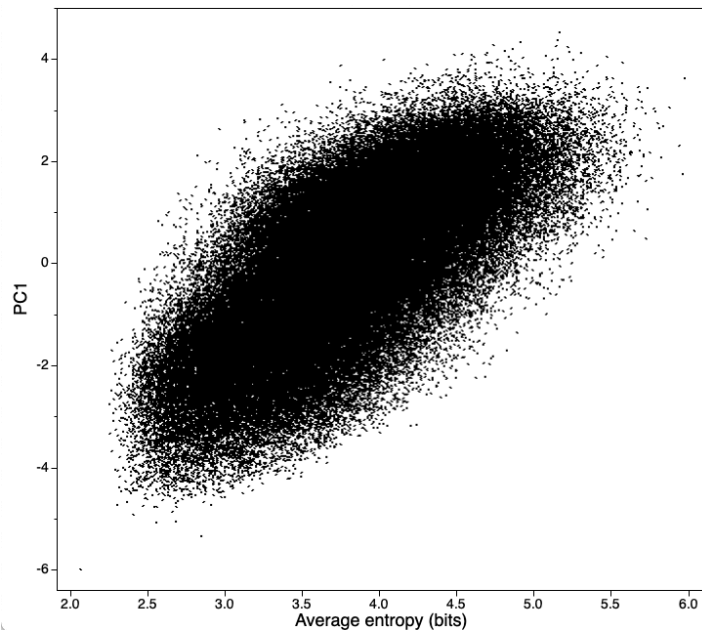


Figure 3: Average entropy by PC1. The average entropy of babbling syllables plotted by their PC1 values. A syllable with a high entropy also had a high PC1 value ($Y = -7.401 + 1.943x$, $R^2 = 0.52$).

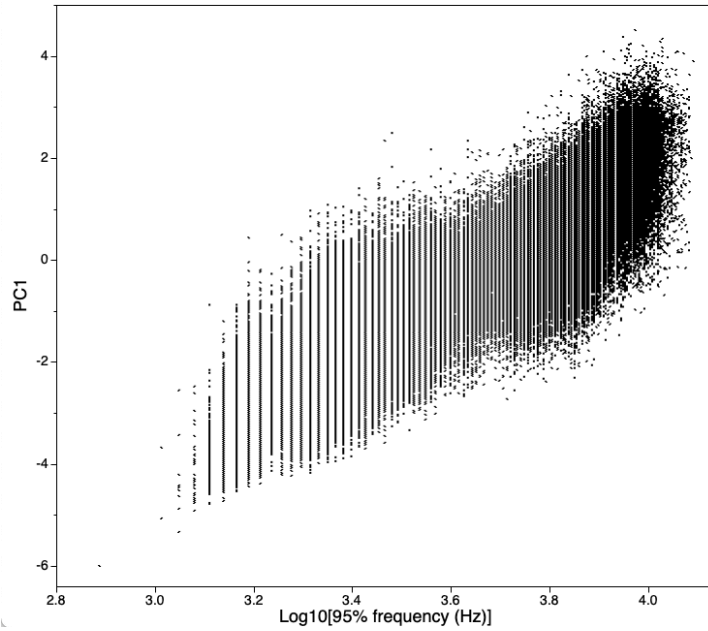


Figure 4: 95% frequency by PC1. The log-transformed 95% frequency babbling syllables plotted by their PC1 values. A high-frequency syllable also had a high PC1 value ($Y = -20.79 + 5.629x$, $R^2 = 0.77$).

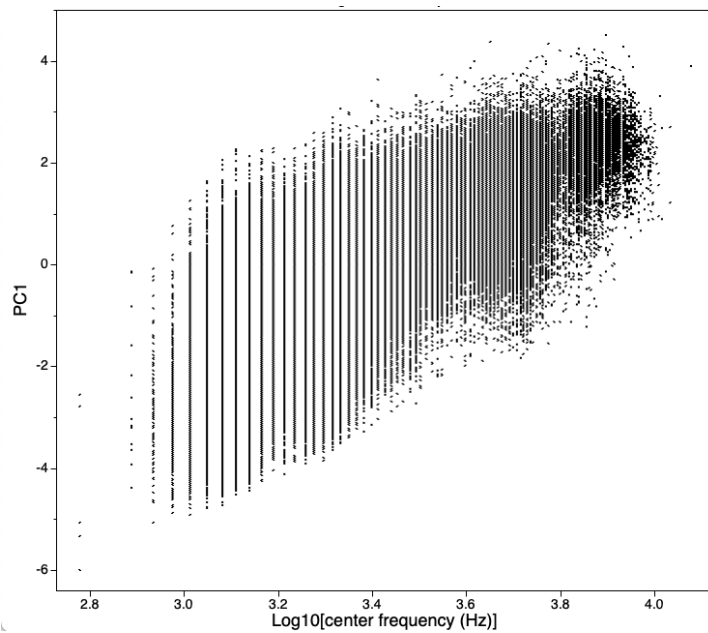


Figure 5: Center frequency by PC1. The log-transformed center frequency babbling syllables plotted by their PC1 values. A high-frequency syllable also had a high PC1 value ($Y = -18.66 + 5.452x$, $R^2 = 0.605$).

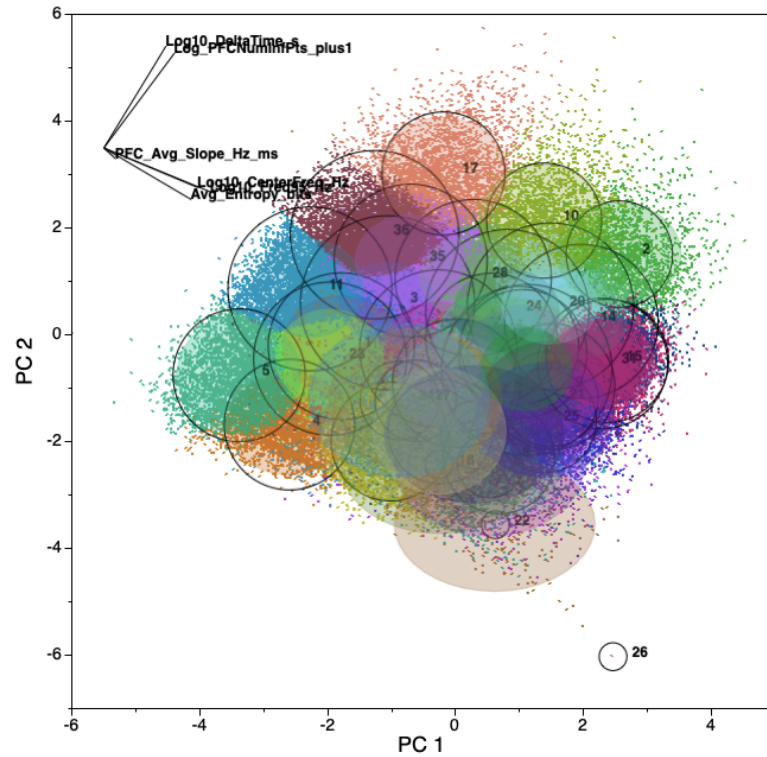
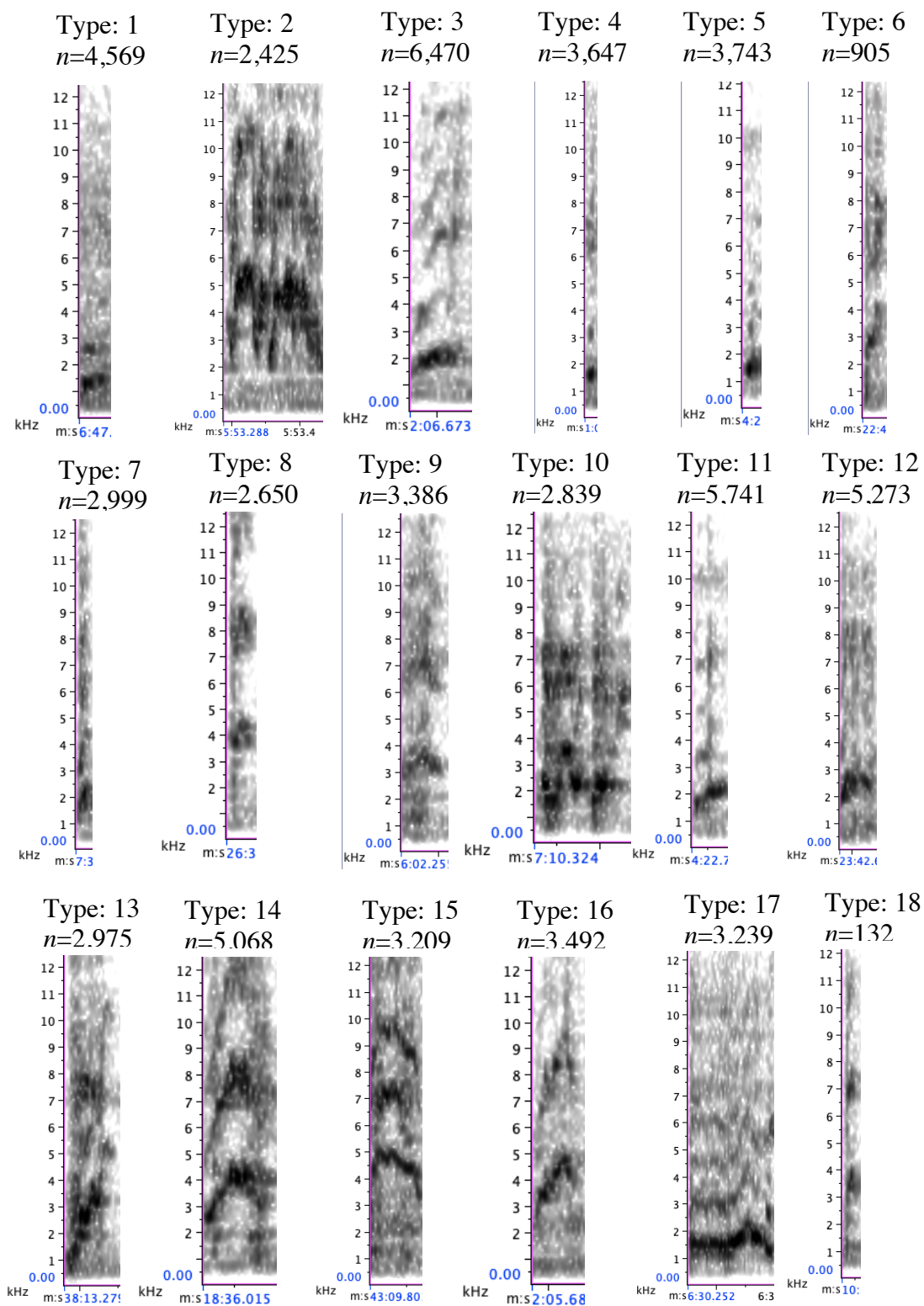


Figure 6: *k* means cluster biplot. *k*-means cluster biplot of vocal babbling syllables based on principal components analysis using the following six spectrographic measurements: average syllable entropy (bits), 95% and center (50%) frequency (Hz), duration (s), and peak frequency contour average slope (Hz/ms) and inflection points. Clustering resulted in the identification of 36 different babbling syllable types. The first two principal components (PC1 and PC2) account for 70% of the variance. Center frequency, 95% frequency, and average entropy are the highest loading for PC1 (0.49, 0.55, and 0.45, respectively) and duration and PFC inflection points are the highest for PC2 (0.64 and 0.59, respectively). PC3, which alone accounts for an additional 17% of the variance, is composed primarily of PFC average slope (0.99).



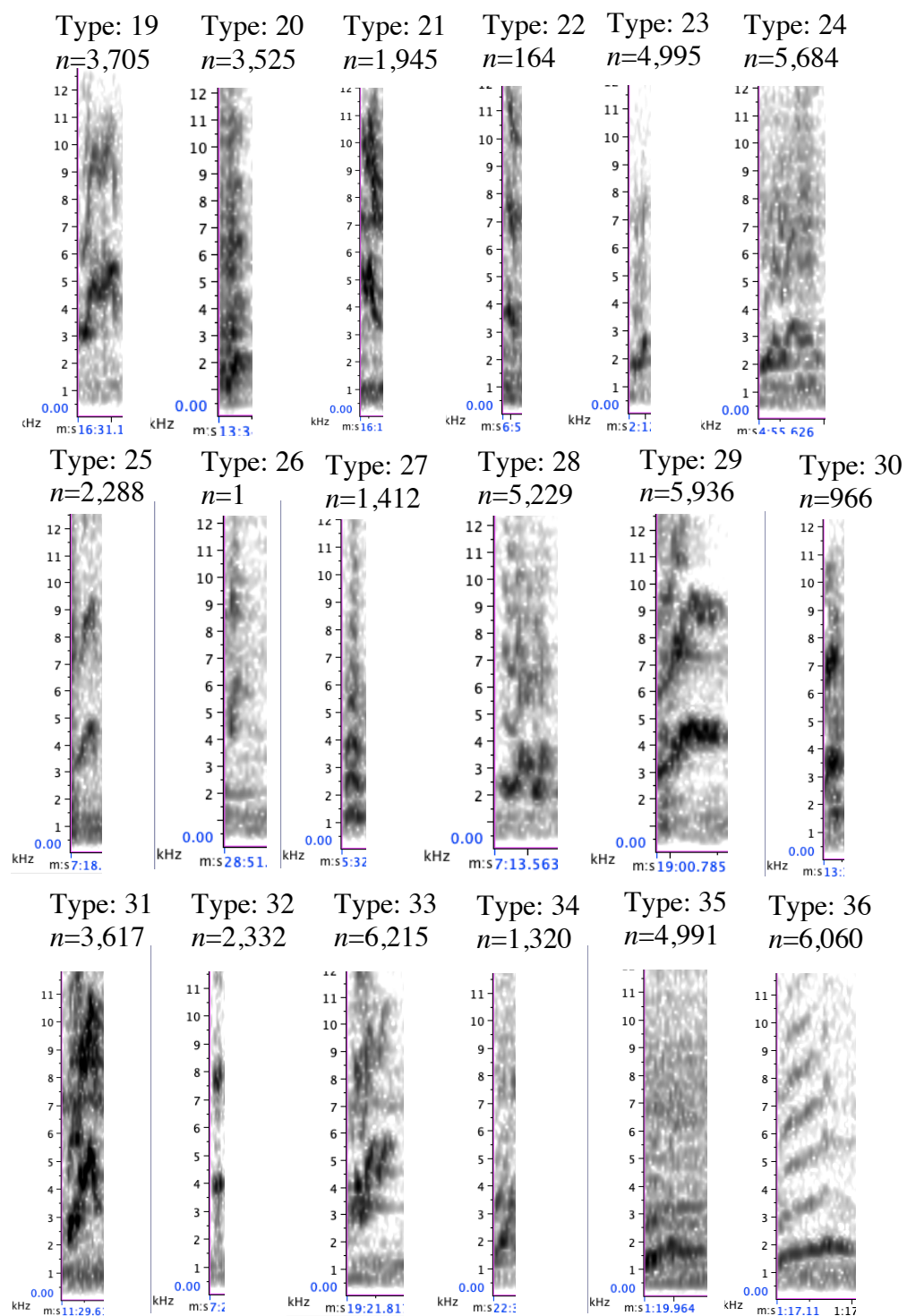


Figure 7: Syllable type exemplars. Spectrograph exemplars of the 36 syllable types, with how many times each type was recorded over the course of this study.

The top-ranked model predicting PC1 included nestling age, treatment, and sex (AIC_C $w_i=1.0$) (Table 3). Age ($F_{1,123E3}=123E3$, $p<0.0001$), treatment ($F_{2,123E3}=38.84$, $p<0.0001$), and sex ($F_{1,123E3}=246.76$, $p<0.0001$) were all significant. PC1 measurements increased with age ($Y=-4.45+0.16*\text{age}$, $R^2=0.1$), and females produced vocalizations with significantly higher PC1 values than males ($F_{123E3}=15.71$, $p<0.0001$; least squares mean PC1 values: females= 0.05 ± 0.13 SE, males= -0.10 ± 0.13 SE) (Figure 8). CONTROL, CORT, and OIL PC1 measurements all significantly differed from each other (CONTROL & CORT: $F_{123E3}=5.55$, $p<0.0001$; CONTROL & OIL: $F_{123E3}=8.11$, $p<0.0001$; $F_{123E3}=2.52$, $p<0.0001$; least squares mean PC1 values: CONTROL= 0.03 ± 0.13 , CORT= -0.04 ± 0.13 , OIL= -0.07 ± 0.13) (Figure 9).

Table 3: PC1 model comparison. Comparison of generalized linear mixed models examining the effect of early CORT treatment on principal component 1 (PC1) values of babbling syllables. Fixed factors included treatment (CORT, OIL, and CONTROL), age, sex, and brood size (4, 5, 6, and 7). Nest ID was included as a random factor. $n=41$ CONTROL, 12 CORT, and 11 OIL nestlings; $n=33$ male and 31 female; $n=16$ nestlings from 4-nestling nests, 10 from 5-nestling nests, 24 from 6-nestling nests, and 14 from 7-nestling nests.

| Model | k | AIC_C | ΔAIC_C | w_i |
|------------------------------|-----|---------|----------------|-------|
| Treatment + age + sex | 8 | 438689 | 0 | 1.00 |
| Treatment + age + brood size | 10 | 438927 | 238 | 0.00 |
| Treatment + age | 6 | 438928 | 239 | 0.00 |
| Age | 3 | 439074 | 385 | 0.00 |
| Treatment + sex | 7 | 450571 | 11882 | 0.00 |
| Sex | 4 | 450949 | 12260 | 0.00 |
| Treatment | 5 | 451824 | 13135 | 0.00 |
| Brood size | 6 | 452766 | 14077 | 0.00 |
| Random intercept | 1 | 462898 | 24209 | 0.00 |

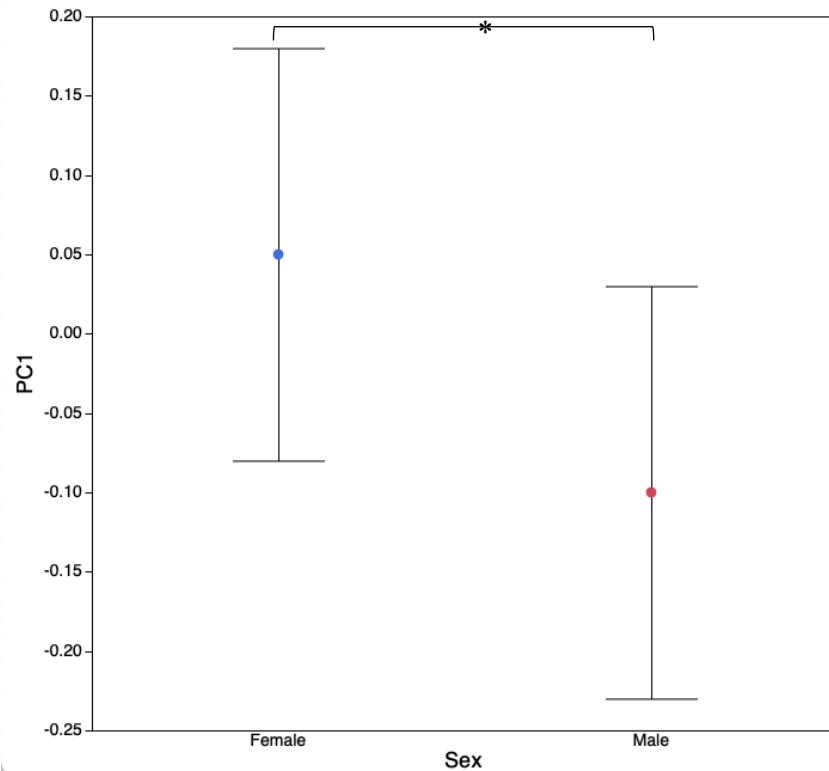


Figure 8: PC1 by sex. Least squares mean \pm SE principal component values of babbling syllables, by parrotlet sex. Female parrotlet vocalizations had higher PC1 scores than males ($F_{1,123E3}=246.76, p<0.0001$). PC1 explains 42% of the variance in the acoustic measurements recorded, and 95% frequency, center frequency, and average entropy had the highest loading for PC1 (0.54, 0.49, and 0.47 respectively). Asterisks denote significant differences between groups. $n=41$ CONTROL, 12 CORT, and 11 OIL nestlings from 12 nests.

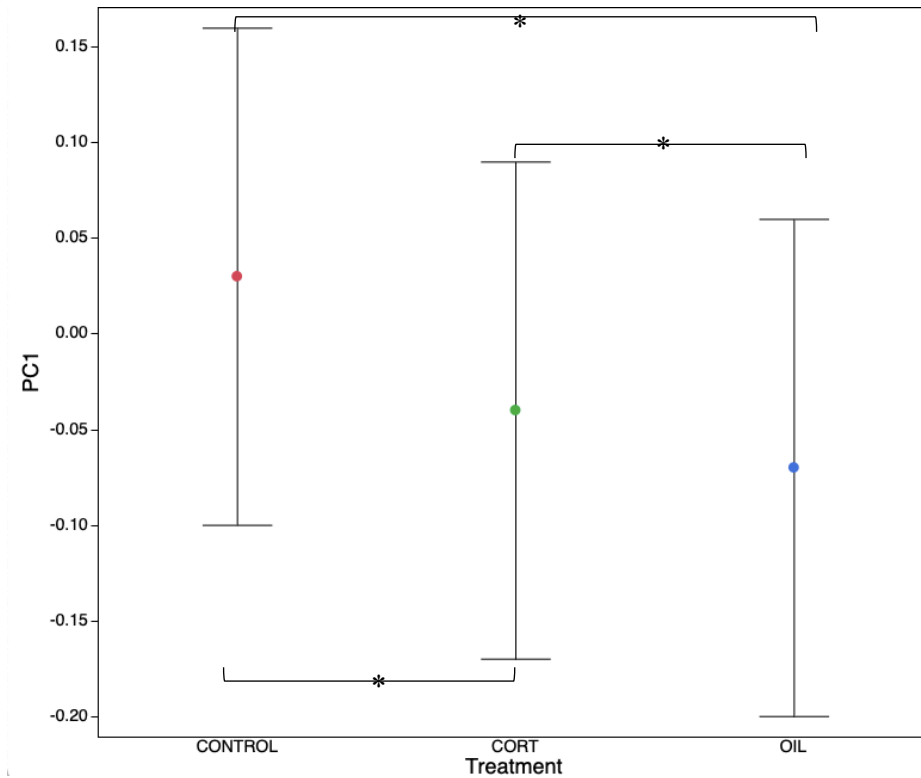


Figure 9: PC1 by treatment. Least squares mean \pm SE principal component values of babbling syllables, by treatment type. CONTROL group vocalizations had the highest PC1 values, while OIL nestlings produced vocalizations with the lowest PC1 values ($F_{2,123E3}=38.84$, $p<0.0001$). PC1 explains 42% of the variance in the acoustic measurements recorded, and 95% frequency, center frequency, and average entropy had the highest loading for PC1 (0.54, 0.49, and 0.47 respectively). Asterisks denote significant differences between groups. $n=41$ CONTROL, 12 CORT, and 11 OIL nestlings from 12 nests.

Spectrographic measurements

A model including treatment, age, and sex had the highest AIC_C weight for seven of the eight acoustic variables: average entropy (AIC_C $w_i=1.0$), 90% bandwidth (AIC_C $w_i=1.0$), center frequency (AIC_C $w_i=1.0$), duration (AIC_C $w_i=0.95$), 5% frequency (AIC_C $w_i=1.0$), 95% frequency (AIC_C $w_i=1.0$), and PFC inflection points (AIC_C $w_i=1.0$) (Tables 4-10). PFC average slope was best explained by a model including treatment, age, and brood size (AIC_C $w_i=0.82$) (Table 11).

Table 4: Average entropy model comparison. Comparison of generalized linear mixed models examining the effect of early CORT treatment on the average entropy (bits) of babbling syllables. Fixed factors included treatment (CORT, OIL, and CONTROL), age, sex, and brood size (4, 5, 6, and 7). Nest ID was included as a random factor. $n=41$ CONTROL, 12 CORT, and 11 OIL nestlings; $n=33$ male and 31 female; $n=16$ nestlings from 4-nestling nests, 10 from 5-nestling nests, 24 from 6-nestling nests, and 14 from 7-nestling nests.

| Model | k | AIC _C | Δ AIC _C | w_i |
|------------------------------|-----|------------------|---------------------------|-------|
| Treatment + age + sex | 8 | 197598 | 0 | 1.00 |
| Treatment + age + brood size | 10 | 197641 | 43 | 0.00 |
| Treatment + age | 6 | 199788 | 2190 | 0.00 |
| Age + sex | 5 | 199808 | 2210 | 0.00 |
| Age | 3 | 199887 | 2289 | 0.00 |
| Age + brood size | 7 | 199891 | 2293 | 0.00 |
| Sex | 4 | 209847 | 12249 | 0.00 |
| Treatment | 5 | 210037 | 12439 | 0.00 |
| Brood size | 6 | 210915 | 13317 | 0.00 |
| Random intercept | 1 | 218840 | 21242 | 1.00 |

Table 5: 95% bandwidth model comparison. Comparison of generalized linear mixed models examining the effect of early CORT treatment on log-transformed 90% bandwidth (Hz) of babbling syllables. Fixed factors included treatment (CORT, OIL, and CONTROL), age, sex, and brood size (4, 5, 6, and 7). Nest ID was included as a random factor. $n=41$ CONTROL, 12 CORT, and 11 OIL nestlings; $n=33$ male and 31 female; $n=16$ nestlings from 4-nestling nests, 10 from 5-nestling nests, 24 from 6-nestling nests, and 14 from 7-nestling nests.

| Model | k | AIC _C | Δ AIC _C | w_i |
|------------------------------|-----|------------------|---------------------------|-------|
| Treatment + age + sex | 8 | 96969 | 0 | 1.00 |
| Treatment + age | 6 | 97176 | 207 | 0.00 |
| Treatment + age + brood size | 10 | 97183 | 214 | 0.00 |
| Age | 3 | 97298 | 329 | 0.00 |
| Treatment + sex | 7 | 107317 | 10348 | 0.00 |
| Sex | 4 | 107729 | 10760 | 0.00 |
| Treatment | 5 | 108410 | 11441 | 0.00 |
| Treatment + brood size | 9 | 108423 | 11454 | 0.00 |
| Brood size | 6 | 109361 | 12392 | 0.00 |
| Random intercept | 1 | 118578 | 21609 | 1.00 |

Table 6: Center frequency model comparison. Comparison of generalized linear mixed models examining the effect of early CORT treatment on log-transformed center frequency (Hz) of babbling syllables. Fixed factors included treatment (CORT, OIL, and CONTROL), age, sex, and brood size (4, 5, 6, and 7). Nest ID was included as a random factor. $n=41$ CONTROL, 12 CORT, and 11 OIL nestlings; $n=33$ male and 31 female; $n=16$ nestlings from 4-nestling nests, 10 from 5-nestling nests, 24 from 6-nestling nests, and 14 from 7-nestling nests.

| Model | k | AIC _C | Δ AIC _C | w_i |
|------------------------------|-----|------------------|---------------------------|-------|
| Treatment + age + sex | 8 | -43200 | 0 | 1.00 |
| Age + sex | 5 | -43151 | 49 | 0.00 |
| Treatment + age | 6 | -42740 | 460 | 0.00 |
| Treatment + age + brood size | 10 | -42729 | 471 | 0.00 |
| Age | 3 | -42557 | 643 | 0.00 |
| Treatment + sex | 7 | -29975 | 13225 | 0.00 |
| Sex | 4 | -29517 | 13683 | 0.00 |
| Treatment | 5 | -28203 | 14997 | 0.00 |
| Brood size | 6 | -27000 | 16200 | 0.00 |
| Random intercept | 1 | -16659 | 26541 | 1.00 |

Table 7: Syllable duration model comparison. Comparison of generalized linear mixed models examining the effect of early CORT treatment on log-transformed duration (s) of babbling syllables. Fixed factors included treatment (CORT, OIL, and CONTROL), age, sex, and brood size (4, 5, 6, and 7). Nest ID was included as a random factor. $n=41$ CONTROL, 12 CORT, and 11 OIL nestlings; $n=33$ male and 31 female; $n=16$ nestlings from 4-nestling nests, 10 from 5-nestling nests, 24 from 6-nestling nests, and 14 from 7-nestling nests.

| Model | k | AIC _C | Δ AIC _C | w_i |
|------------------------------|-----|------------------|---------------------------|-------|
| Treatment + age + sex | 8 | 17132 | 0 | 0.95 |
| Treatment + age | 6 | 17138 | 6 | 0.05 |
| Treatment + age + brood size | 10 | 17151 | 19 | 0.00 |
| Treatment | 5 | 17176 | 44 | 0.00 |
| Treatment + sex | 7 | 17180 | 48 | 0.00 |
| Age + sex | 5 | 17377 | 245 | 0.00 |
| Age | 3 | 17389 | 257 | 0.00 |
| Sex | 4 | 17410 | 278 | 0.00 |
| Brood size | 6 | 17423 | 291 | 0.00 |
| Random intercept | 1 | 20575 | 3443 | 0.00 |

Table 8: 5% frequency model comparison. Comparison of generalized linear mixed models examining the effect of early CORT treatment on log-transformed 5% frequency (Hz) of babbling syllables. Fixed factors included treatment (CORT, OIL, and CONTROL), age, sex, and brood size (4, 5, 6, and 7). Nest ID was included as a random factor. $n=41$ CONTROL, 12 CORT, and 11 OIL nestlings; $n=33$ male and 31 female; $n=16$ nestlings from 4-nestling nests, 10 from 5-nestling nests, 24 from 6-nestling nests, and 14 from 7-nestling nests.

| Model | k | AIC _C | Δ AIC _C | w_i |
|------------------------------|-----|------------------|---------------------------|-------|
| Treatment + age + sex | 8 | -43266 | 0 | 1.00 |
| Treatment + age | 6 | -43112 | 154 | 0.00 |
| Age | 3 | -43111 | 155 | 0.00 |
| Treatment + age + brood size | 10 | -43102 | 164 | 0.00 |
| Sex | 4 | -37407 | 5859 | 0.00 |
| Treatment + sex | 7 | -37402 | 5864 | 0.00 |
| Treatment | 5 | -36673 | 6593 | 0.00 |
| Brood size | 6 | -36537 | 6729 | 0.00 |
| Random intercept | 1 | -28101 | 15165 | 0.00 |

Table 9: 95% frequency model comparison. Comparison of generalized linear mixed models examining the effect of early CORT treatment on log-transformed 95% frequency (Hz) of babbling syllables. Fixed factors included treatment (CORT, OIL, and CONTROL), age, sex, and brood size (4, 5, 6, and 7). Nest ID was included as a random factor. $n=41$ CONTROL, 12 CORT, and 11 OIL nestlings; $n=33$ male and 31 female; $n=16$ nestlings from 4-nestling nests, 10 from 5-nestling nests, 24 from 6-nestling nests, and 14 from 7-nestling nests.

| Model | k | AIC _C | Δ AIC _C | w_i |
|------------------------------|-----|------------------|---------------------------|-------|
| Treatment + age + sex | 8 | -23997 | 0 | 1.00 |
| Treatment + age | 6 | -23644 | 353 | 0.00 |
| Treatment + age + brood size | 10 | -23635 | 362 | 0.00 |
| Age | 3 | -23493 | 504 | 0.00 |
| Treatment + sex | 7 | -9178 | 14819 | 0.00 |
| Sex | 4 | -8683 | 15314 | 0.00 |
| Treatment | 5 | -7528 | 16469 | 0.00 |
| Brood size | 6 | -6306 | 17691 | 0.00 |
| Random intercept | 1 | 5887 | 29884 | 0.00 |

Table 10: PFC inflection points model comparison. Comparison of generalized linear mixed models examining the effect of early CORT treatment on the log-transformed number of peak frequency contour inflection points of babbling syllables. Fixed factors included treatment (CORT, OIL, and CONTROL), age, sex, and brood size (4, 5, 6, and 7). Nest ID was included as a random factor. $n=41$ CONTROL, 12 CORT, and 11 OIL nestlings; $n=33$ male and 31 female; $n=16$ nestlings from 4-nestling nests, 10 from 5-nestling nests, 24 from 6-nestling nests, and 14 from 7-nestling nests.

| Model | k | AIC _C | Δ AIC _C | w_i |
|------------------------------|-----|------------------|---------------------------|-------|
| Treatment + age + sex | 8 | 284364 | 0 | 1.00 |
| Treatment + sex | 7 | 284379 | 15 | 0.00 |
| Treatment + age | 6 | 284384 | 20 | 0.00 |
| Treatment + age + brood size | 10 | 284393 | 29 | 0.00 |
| Treatment | 5 | 284413 | 49 | 0.00 |
| Sex | 4 | 284475 | 111 | 0.00 |
| Age | 3 | 284488 | 124 | 0.00 |
| Brood size | 6 | 284522 | 158 | 0.00 |
| Random intercept | 1 | 286846 | 2482 | 0.00 |

Table 11: PFC slope model comparison. Comparison of generalized linear mixed models examining the effect of early CORT treatment on the peak frequency contour average slope (Hz/ms) of babbling syllables. Fixed factors included treatment (CORT, OIL, and CONTROL), age, sex, and brood size (4, 5, 6, and 7). Nest ID was included as a random factor. $n=41$ CONTROL, 12 CORT, and 11 OIL nestlings; $n=33$ male and 31 female; $n=16$ nestlings from 4-nestling nests, 10 from 5-nestling nests, 24 from 6-nestling nests, and 14 from 7-nestling nests.

| Model | k | AIC _C | Δ AIC _C | w_i |
|------------------------------|-----|------------------|---------------------------|-------|
| Treatment + age + brood size | 10 | 1355511 | 0 | 0.82 |
| Treatment + age + sex | 8 | 1355514 | 3 | 0.18 |
| Treatment + age | 6 | 1355531 | 20 | 0.00 |
| Treatment + sex | 7 | 1355605 | 94 | 0.00 |
| Treatment | 5 | 1355641 | 130 | 0.00 |
| Age | 3 | 1355659 | 148 | 0.00 |
| Sex | 4 | 1355742 | 231 | 0.00 |
| Brood size | 6 | 1355802 | 291 | 0.00 |
| Random intercept | 1 | 1356415 | 904 | 0.00 |

To determine which acoustic measurements were especially affected by treatment, I looked at measurements for which the treatment model ranked the highest of all single-factor

models. Treatment was the most powerful model for explaining variance in syllable duration (Table 7), PFC average slope (Table 11), and the number of PFC inflection points (Table 10).

Syllable duration was significantly affected by treatment ($F_{2,123E3}=133.44, p<0.0001$), age ($F_{1,123E3}=62.97, p<0.0001$), and sex ($F_{1,123E3}=16.90, p<0.0001$). CORT-treated nestlings produced longer syllables than OIL and CONTROL nestlings (least squares means: CONTROL $\log_{10}\text{duration}=-1.31$, CORT $\log_{10}\text{duration}=-1.30$, OIL $\log_{10}\text{duration}=-1.34$) (Figure 10). The difference between CORT and OIL birds was significant ($F_{123E3}=16.03, p<0.0001$) as well as CORT and CONTROL birds ($F_{123E3}=-6.45, p<0.0001$), and CONTROL and OIL birds ($F_{2,123E3}=12.65, p<0.0001$). Furthermore, females produced shorter syllables than males ($F_{123E3}=-4.11; p<0.0001$; least squares means of \log_{10} syllable duration: males=-1.31, females=-1.32). The GLMM identified age as a significant factor in variation of syllable duration; however, the pattern over time is not generalizable as the R^2 value is low ($\log_{10}\text{duration}=-1.23-0.0015*\text{age}$, $R^2=0.000$).

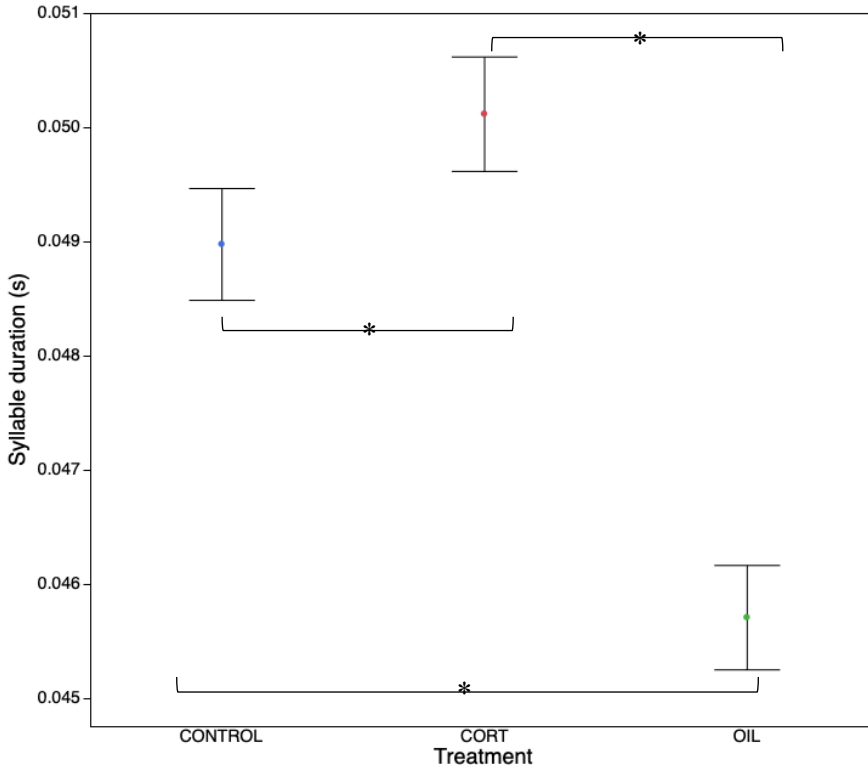


Figure 10: Syllable duration by treatment. Mean \pm SE log-transformed duration of babbling syllables, by treatment type. Means and SEs were obtained by back-transformation of the GLMM least squares means via the delta method. CORT nestlings produced longer syllables than other nestlings, while OIL nestlings produced vocalizations that were shorter than CONTROL and CORT nestlings ($F_{2,123E3}=133.44$, $p<0.0001$). Asterisks denote significant differences between groups. $n=41$ CONTROL, 12 CORT, and 11 OIL nestlings from 12 nests.

The peak frequency contour average slope of babbling elements was also significantly affected by treatment ($F_{2,123E3}=64.09$, $p<0.0001$) and age ($F_{1,123E3}=114.47$, $p<0.0001$), while brood size was marginally significant ($F_{3,123E3}=2.57$, $p=0.052$). CORT-treated birds produced less positively-inflected vocalizations than CONTROL and OIL birds (least squares mean PFC average slopes: (CONTROL= 10.18 ± 1.28 SE, CORT= 5.30 ± 1.33 SE, OIL= 11.12 ± 1.33 SE) (Figure 11). CORT PFC slopes were significantly lower than those of OIL ($F_{123E3}=-9.61$, $p<0.0001$) and CONTROL ($F_{123E3}=10.36$, $p<0.0001$), while the slopes of OIL and CONTROL

did not differ ($F_{2,123E3}=-1.80, p=0.07$). As nestlings aged, PFC average slope increased ($Y=5.125+0.47*Age, R^2=0.5$) (Figure 12).

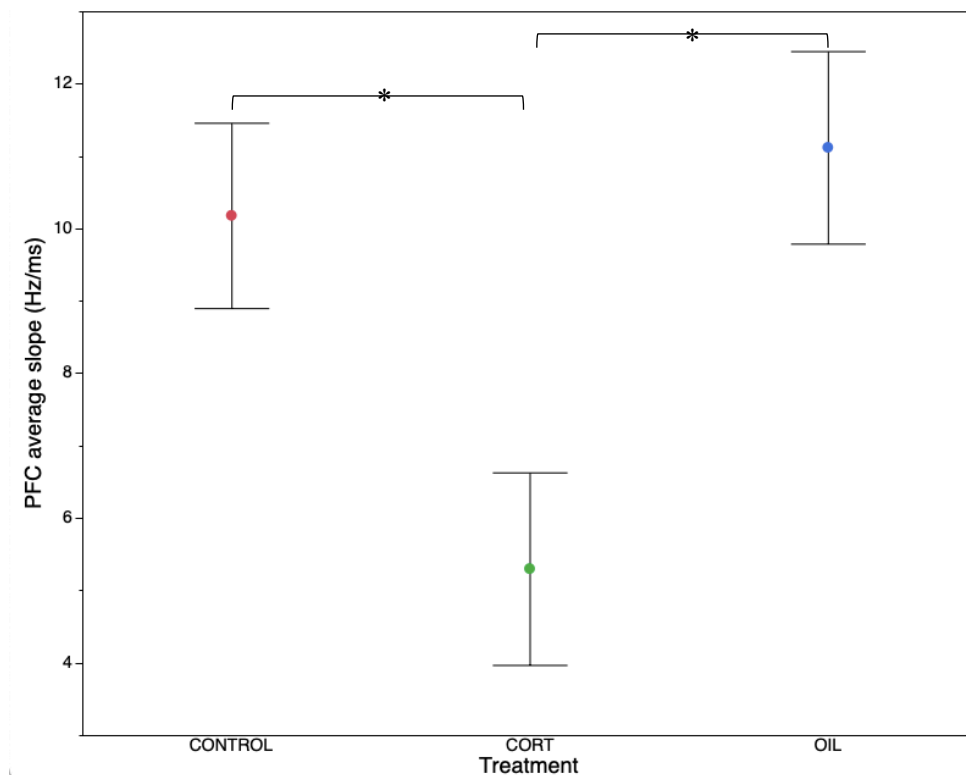


Figure 11: PFC average slope by treatment. Mean peak frequency contour average slope of babbling syllables, by treatment type. The average slope of CORT-treated nestlings was lower than that of OIL and CONTROL nestlings, while the slopes of OIL and CONTROL nestlings did not differ ($F_{2,123E3}=64.09, p<0.0001$). Asterisks denote significant differences between groups. $n=41$ CONTROL, 12 CORT, and 11 OIL nestlings from 12 nests.

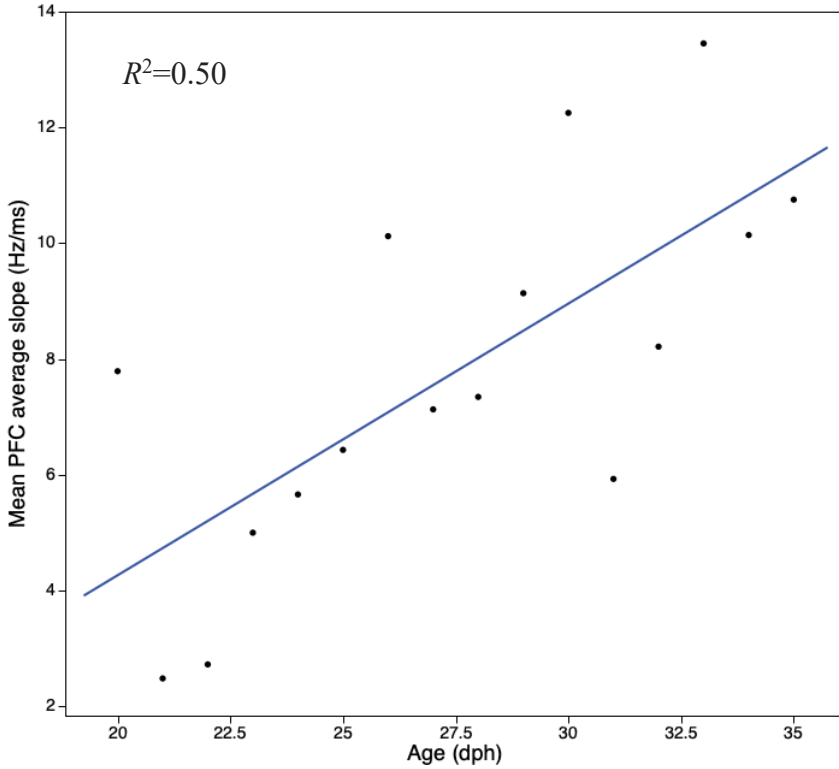


Figure 12: PFC average slope by age. Linear regression of mean peak frequency contour average slope (Hz/ms) by age.. Slope increased with age ($Y = -5.125 + 0.47 \cdot \text{Age}$, $R^2 = 0.5$), i.e., nestlings produced more positively-inflected vocalizations as they matured. $n = 64$ nestlings from 12 nests. One outlier was removed, as one nestling fledged later than others at 37 dph and was not able to be compared with other nestlings.

As with syllable duration, the number of PFC inflection points was affected by treatment ($F_{2,123E3} = 59.17$, $p < 0.0001$), age ($F_{1,123E3} = 28.13$, $p < 0.0001$), and sex ($F_{1,123E3} = 29.35$, $p < 0.0001$). CORT birds produced syllables with more inflection points than CONTROL and OIL birds (least squares means of $\log_{10}(\text{inflection points} + 1)$: CONTROL = 1.71, CORT = 1.73, OIL = 1.65), and the differences between all treatments were significant (CONTROL & CORT: $F_{123E3} = -3.02$, $p = 0.0003$; CORT & OIL: $F_{123E3} = 10.33$, $p < 0.0001$; CONTROL & OIL: $F_{2,123E3} = 9.22$, $p < 0.0001$) (Figure 13). Males produced vocalizations with fewer inflection points than females ($F_{123E3} = 5.42$, $p < 0.0001$; least squares means of $\log_{10}(\text{inflection points} + 1)$: males = 1.68, females = 1.71). While age was a significant factor in the model and regression analysis found a

negative relationship between age and inflection points, R^2 value is quite low ($Y=1.80-0.003*age$, $R^2=0.000$).

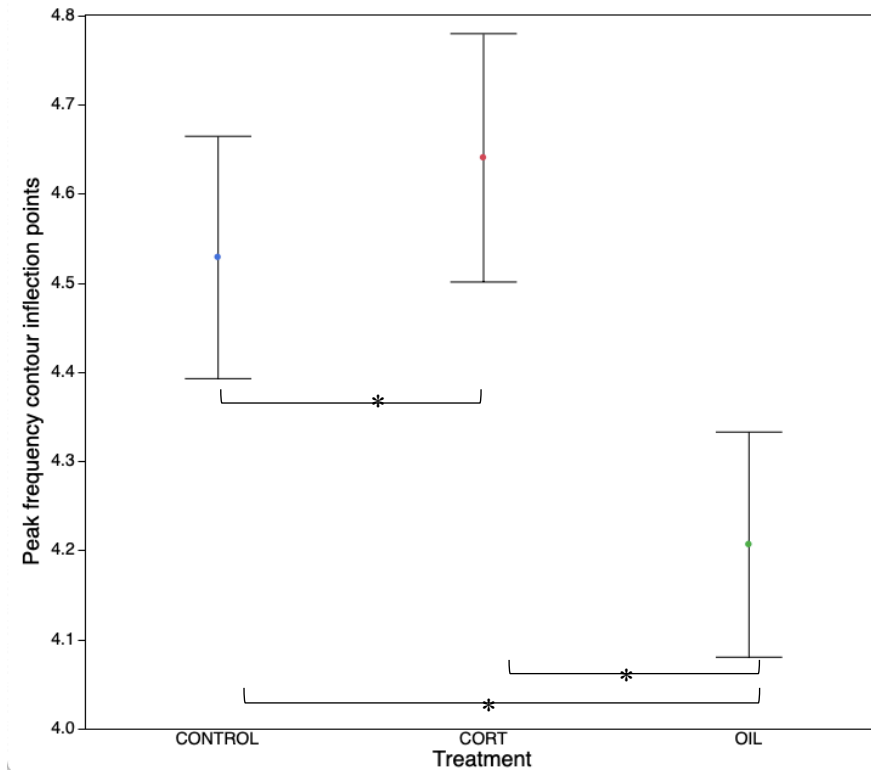


Figure 13: PFC inflection points by treatment. Mean \pm SE inflection points in babbling syllables, by treatment type. Means and SEs were obtained by back-transformation of the GLMM least square means via the delta method. CORT-treated nestlings produced vocalizations with more points of frequency inflection than other groups ($F_{2,123E3}=59.17$, $p<0.0001$). Asterisks denote significant differences between groups. $n=41$ CONTROL, 12 CORT, and 11 OIL nestlings from 12 nests.

Babbling production

Daily syllable output was significantly affected by nestling age ($AIC_C w_i=0.62$, $F_{1,391}=58.26$, $p<0.0001$) (Table 12). As nestlings aged the number of syllables they produced increased daily ($\log_{10}[\text{daily syllable output}]=-0.2664+0.08278*Age$, $R^2=0.862$) (Figure 14).

Table 12: Daily babbling output model comparison. Comparison of generalized linear mixed models examining the effect of early CORT treatment on daily babbling syllable output. Fixed factors included treatment (CORT, OIL, and CONTROL), age, sex, and brood size (4, 5, 6, and 7). Nest ID was included as a random factor. $n=41$ CONTROL, 12 CORT, and 11 OIL nestlings; $n=33$ male and 31 female; $n=16$ nestlings from 4-nestling nests, 10 from 5-nestling nests, 24 from 6-nestling nests, and 14 from 7-nestling nests.

| Model | k | AIC _C | Δ AIC _C | w_i |
|------------------|-----|------------------|---------------------------|-------|
| Age | 3 | 917 | 0 | 0.62 |
| Treatment + age | 6 | 918 | 1 | 0.38 |
| Sex | 4 | 964 | 47 | 0.00 |
| Brood size | 6 | 966 | 49 | 0.00 |
| Treatment + sex | 7 | 969 | 52 | 0.00 |
| Random intercept | 1 | 973 | 56 | 0.00 |

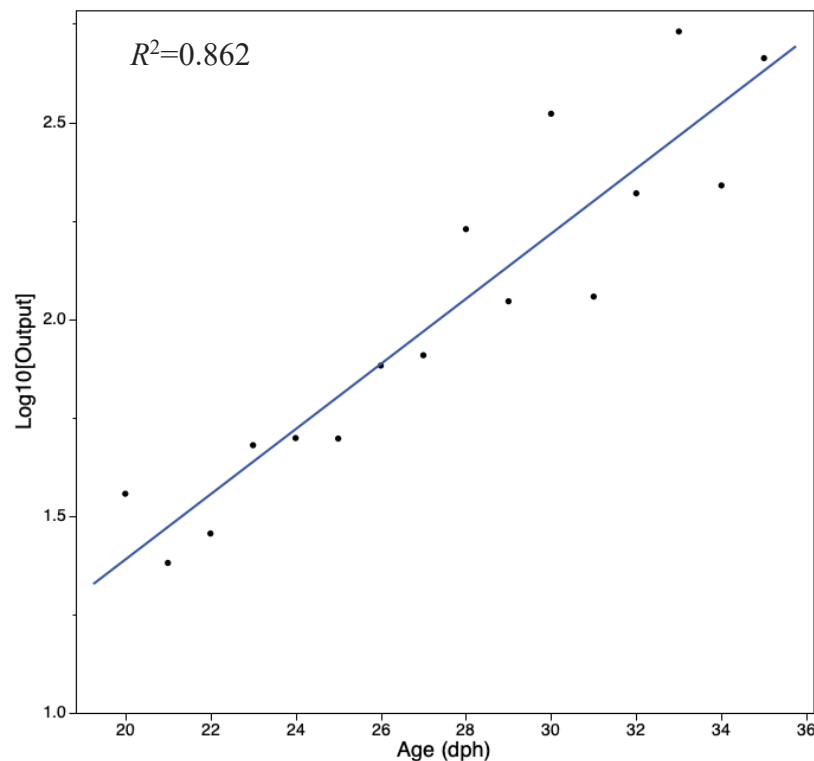


Figure 14: Output by age. Linear regression of log-transformed mean vocal output by age. Nestlings babbled more with age ($Y=-0.2664+0.08278*Age$, $R^2=0.862$). $n=64$ nestlings from 12 nests. One outlier was removed, as one nestling fledged later than others at 37 dph and was not able to be compared with other nestlings.

The model that best explained variation in daily repertoire size (the number of syllable types produced by nestlings at each day in development) included nestling age, treatment, and sex ($AIC_C w_i = 0.97$) (Table 13). Age ($F_{1,388} = 94.29, p < 0.0001$) and sex ($F_{1,388} = 4.86, p = 0.028$) were significant factors in this model, but treatment ($F_{2,388} = 0.67, p = 0.51$) was not. Male birds produced more unique syllable types on a given day than female birds (male least square mean = 22.1 ± 0.83 SE, female least square mean = 19.6 ± 1.01 SE), and nestlings steadily incorporated more syllable types in their repertoires as they aged ($Y = -11.26 + 1.201 * \text{Age}, R^2 = 0.876$) (Figure 15).

Table 13: Daily repertoire size model comparison. Comparison of models examining the effect of early CORT treatment on nestling daily repertoire size. Fixed factors included treatment (CORT, OIL, and CONTROL), age, sex, and brood size (4, 5, 6, and 7). Nest ID was included as a random factor. $n = 41$ CONTROL, 12 CORT, and 11 OIL nestlings; $n = 33$ male and 31 female; $n = 16$ nestlings from 4-nestling nests, 10 from 5-nestling nests, 24 from 6-nestling nests, and 14 from 7-nestling nests.

| Model | k | AIC_C | ΔAIC_C | w_i |
|-----------------------|-----|---------|----------------|-------|
| Treatment + age + sex | 8 | 2987 | 0 | 0.97 |
| Treatment + age | 6 | 2994 | 7 | 0.03 |
| Age | 3 | 3002 | 15 | 0.00 |
| Brood size | 6 | 3064 | 77 | 0.00 |
| Treatment + sex | 7 | 3070 | 83 | 0.00 |
| Treatment | 5 | 3073 | 86 | 0.00 |
| Random intercept | 1 | 3081 | 94 | 0.00 |
| Sex | 4 | 3975 | 988 | 0.00 |

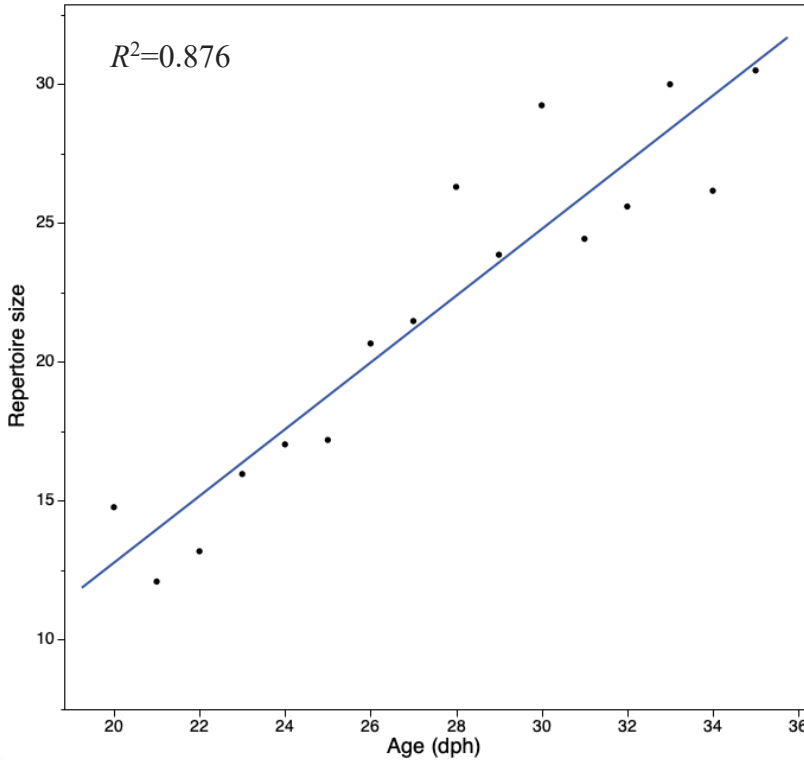


Figure 15: Repertoire size by age. Linear regression of mean nestling repertoire size by age. Repertoire size increased as nestlings grew older ($Y = -11.26 + 1.201 \cdot \text{Age}$, $R^2 = 0.876$). $n = 64$ nestlings from 12 nests. One outlier was removed, as one nestling fledged later than others at 37 dph and was not able to be compared with other nestlings.

Variation in total repertoire diversity could not be explained by treatment ($F_{2,50} = 1.12$, $p = 0.33$), sex ($F_{1,51} = 2.32$, $p = 0.13$), brood size ($F_{3,52} = 1.82$, $p = 0.16$), or combinations of these variables (treatment & sex: treatment $F_{2,49} = 1.37$, $p = 0.26$, sex $F_{1,49} = 2.78$, $p = 0.10$; treatment & brood size: treatment $F_{2,50} = 1.21$, $p = 0.31$, brood size $F_{3,50} = 1.86$, $p = 0.15$), and the model with the highest AIC_C weight contained the null intercept (AIC_C $w_i = 0.47$) (Table 14).

Table 14: Repertoire diversity model comparison. Comparison of generalized linear mixed models examining the effect of early CORT treatment on nestling repertoire diversity (H). Fixed factors included treatment (CORT, OIL, and CONTROL), sex, and brood size (4, 5, 6, and 7). Nest ID was included as a random factor. $n=41$ CONTROL, 12 CORT, and 11 OIL nestlings; $n=33$ male and 31 female; $n=16$ nestlings from 4-nestling nests, 10 from 5-nestling nests, 24 from 6-nestling nests, and 14 from 7-nestling nests.

| Model | k | AIC _C | Δ AIC _C | w_i |
|------------------------|-----|------------------|---------------------------|-------|
| Random intercept | 1 | 36 | 0 | 0.47 |
| Sex | 4 | 37 | 1 | 0.28 |
| Brood size | 6 | 39 | 3 | 0.10 |
| Treatment | 5 | 40 | 4 | 0.06 |
| Treatment + sex | 7 | 40 | 4 | 0.06 |
| Treatment + brood size | 9 | 43 | 7 | 0.01 |

The GLMM with the highest weight (AIC_C $w_i=0.98$) in evaluating intersyllable duration included nestling age, treatment, and sex (Table 15). All three factors had a significant effect on intersyllable duration. As nestlings aged, they reduced the amount of time between syllables ($F_{1,12E4}=1929.73$, $Y=-0.10-0.18*\text{Age}$, $R^2=0.80$), accelerating vocal babbling production (Figure 16). Treatment had a significant effect on intersyllable duration ($F_{2,12E4}=46.61$, $p<0.0001$), with CORT-treated birds producing syllables more rapidly than both OIL and CONTROL nestlings ($\log_{10}[\text{intersyllable duration}]$ least squares means: CORT=-0.67, CONTROL=-0.64, OIL=-0.63). CORT birds produced significantly smaller intersyllable durations than both CONTROL ($F_{12E4}=7.72$, $p<0.0001$) and OIL ($F_{12E4}=9.15$, $p<0.0001$) nestlings, and CONTROL nestlings also produced smaller intersyllable durations than OIL nestlings ($F_{12E4}=3.46$, $p=0.0005$) (Figure 17). Sex was also a significant factor ($F_{1,12E4}=17.78$, $p<0.0001$), with females producing smaller intersyllable durations than males ($\log_{10}[\text{intersyllable duration}]$ least squares means: female=-0.65, male=-0.64).

Table 15: Intersyllable duration model comparison. Comparison of generalized linear mixed models examining the effect of early CORT treatment on log-transformed intersyllable duration (s). Fixed factors included treatment (CORT, OIL, and CONTROL), sex, and brood size (4, 5, 6, and 7). Nest ID was included as a random factor. $n=41$ CONTROL, 12 CORT, and 11 OIL nestlings; $n=33$ male and 31 female; $n=16$ nestlings from 4-nestling nests, 10 from 5-nestling nests, 24 from 6-nestling nests, and 14 from 7-nestling nests.

| Model | k | AIC _C | Δ AIC _C | w_i |
|------------------------------|-----|------------------|---------------------------|-------|
| Treatment + age + sex | 8 | 149378 | 0 | 0.98 |
| Treatment + age | 6 | 149386 | 8 | 0.02 |
| Treatment + age + brood size | 10 | 149399 | 21 | 0 |
| Age | 3 | 149451 | 73 | 0 |
| Treatment + sex | 7 | 151277 | 1899 | 0 |
| Sex | 4 | 151287 | 1909 | 0 |
| Treatment | 5 | 151427 | 2049 | 0 |
| Brood size | 6 | 151456 | 2078 | 0 |
| Random intercept | 1 | 152963 | 3585 | 0 |

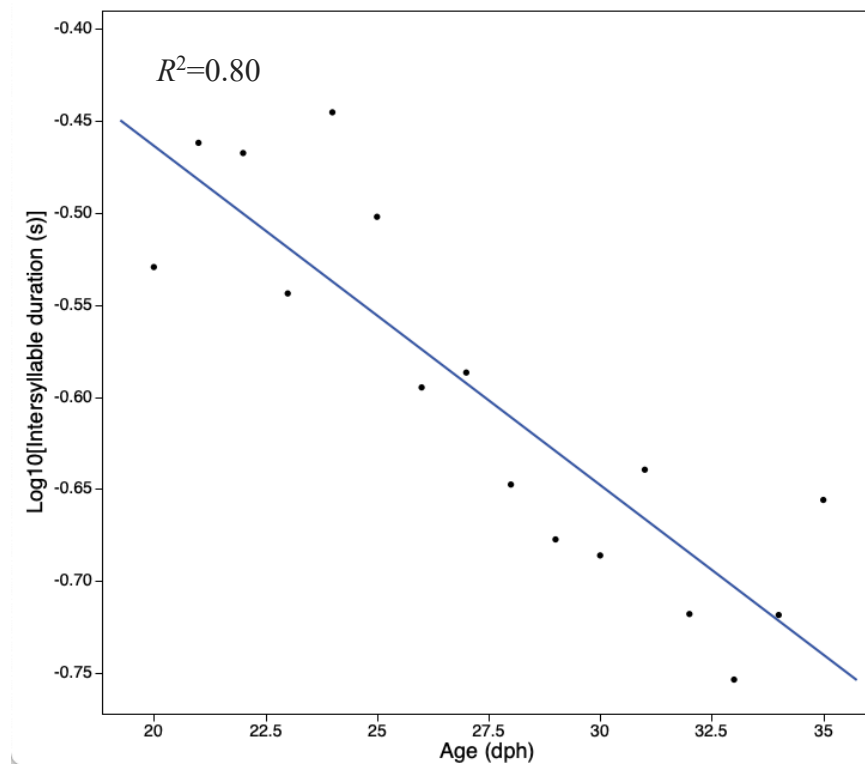


Figure 16: Intersyllable duration by age. Linear regression of mean log-transformed intersyllable duration (s) by age. The time between vocalizations decreased as nestlings grew

older ($Y=-0.10-0.18*Age$, $R^2=0.80$). $n=64$ nestlings from 12 nests. One outlier was removed, as one nestling fledged later than others at 37 dph and was not able to be compared with other nestlings.

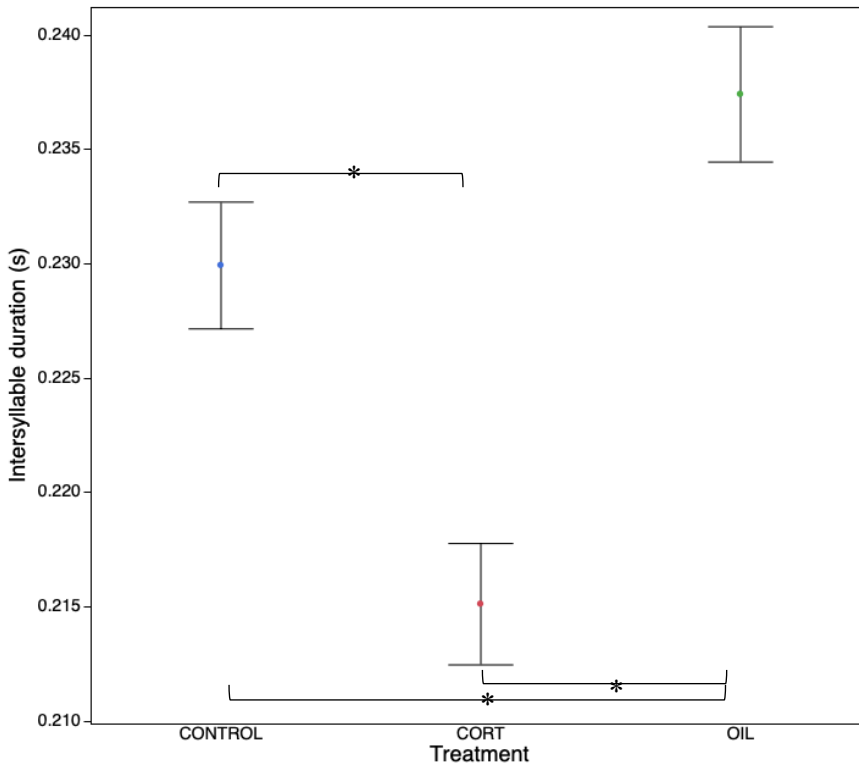


Figure 17: Intersyllable duration by treatment. Mean \pm SE duration between babbling syllables, by treatment type. Means and SEs were obtained by back-transformation of the GLMM least square means via the delta method. CORT-treated nestlings had the produced vocalizations more rapidly than other nestlings ($F_{2,12E4}=46.61$, $p<0.0001$). Asterisks denote significant differences between groups. $n=41$ CONTROL, 12 CORT, and 11 OIL nestlings from 12 nests.

Developmental milestones

Both treatment and brood size contributed to the highest-weighted model for both nestling fledge age (Table 16) and the age at babbling onset (fledge age $AIC_C w_i=0.87$, onset age $AIC_C w_i=0.95$). However, there were no significant differences between groups for either model.

Table 16: Fledge age model comparison. Comparison of generalized linear mixed models examining the effect of early CORT treatment on nestling fledge age (dph). Fixed factors included treatment (CORT, OIL, and CONTROL), sex, and brood size (4, 5, 6, and 7). Nest ID was included as a random factor. $n=41$ CONTROL, 12 CORT, and 11 OIL nestlings; $n=33$ male and 31 female; $n=16$ nestlings from 4-nestling nests, 10 from 5-nestling nests, 24 from 6-nestling nests, and 14 from 7-nestling nests.

| Model | k | AIC _C | Δ AIC _C | w_i |
|------------------------|-----|------------------|---------------------------|-------|
| Treatment + brood size | 227 | 0 | | 0.87 |
| Treatment | 232 | 5 | | 0.07 |
| Brood size | 233 | 6 | | 0.04 |
| Treatment + sex | 236 | 9 | | 0.01 |
| Sex | 241 | 14 | | 0.00 |
| Random intercept | 263 | 36 | | 0.00 |

Age at babbling onset did not differ by treatment ($F_{2,50}=1.45$, $p=0.245$) or brood size ($F_{2,50}=0.618$, $p=0.614$), and fledge age did not differ by treatment ($F_{2,50}=2.58$, $p=0.086$) or brood size ($F_{3,50}=1.17$, $p=0.332$).

CHAPTER V

DISCUSSION

This study provides evidence that corticosterone treatment affects the acoustic structure of green-rumped parrotlet vocal production, including syllable duration, inflection slope, and the number of inflection points. The first principal component, comprised primarily of frequency measurements, was also significantly affected by treatment. CORT did not only affect the acoustic characteristics of babbling syllables but output as well—birds who received the treatment produced syllables at a faster rate than the control groups. Interestingly, I also found differences in vocal production between sexes; females produced shorter, more rapid, and higher-frequency babbling syllables than males. Age was a significant factor in all aspects of vocal production; nestlings produced vocalizations with higher PC1 values (i.e., higher 95% frequency, center frequency, and average entropy) over time, and they babbled more rapidly, and produced more babbling syllables and syllable types as they aged.

Acoustic characteristics

Among the eight spectrographic measurements analyzed, treatment was the most explanatory in single-factor AIC comparisons for syllable duration, PFC average slope, and the number of PFC inflection points. CORT-treated birds produced babbling syllables with lower PFC slopes, more inflection points, and longer durations than both CONTROL and OIL nestlings (Figures 10, 11, and 13). A past study by Eggleston et al. also found that PFC average slope was significantly affected by CORT treatment (Eggleston, 2020). The contact calls of green-rumped parrotlets, used for individual recognition, have a high PFC slope over a short duration (Berg et al., 2011, 2013); the fact that CORT-treated nestlings do not produce syllables with those characteristics as commonly as control groups could indicate future communication issues with conspecifics.

CORT-treated nestlings also produced vocalizations with lower PC1 values than CONTROL nestlings (Figure 9), meaning that their vocalizations were of lower entropy, 95% frequency, and center frequency. A previous experiment found that food-restricted zebra finches also produced syllables with a lower maximum power frequency output (Zann & Cash, 2008). Fundamental frequency is directly related to how fast an animal vibrates its vocal folds, which are located in the syrinx (birds) or larynx (mammals). Corticosterone receptors are widespread in avian muscle tissues (Lattin et al., 2012); it is possible that CORT exposure during development could have an organizational effect on vocal folds, and thus impose a performance limit on frequency.

Sex differences

While there are stark differences between male and female vocal-learning related brain regions in songbirds, which are mediated by gonadal steroids, there is little research on sexual dimorphism in parrot vocal production, aside from in one species, the budgerigar. Both male and female parrots can learn new vocalizations as they age (Bradbury & Balsby, 2016); however there is some evidence that male parrots (specifically budgerigars) possess more vocal plasticity than females do. Male budgerigars learn new calls more rapidly than females (Hile & Striedter, 2000), and budgerigar vocal-learning brain regions are larger in males than in females (Brauth et al., 2005). After treatment with exogenous testosterone female budgerigars produced longer warble songs (like males) as well as exhibit other male-like social-sexual behaviors (Nespor et al., 1996).

Although it is unknown whether there are structural and size differences in male and female green-rumped parrotlet vocal-learning brain regions, there are phenotypic differences in nestling green-rumped parrotlets as early as 15 dph (Budden & Beissinger, 2004). This suggests that there are hormonal differences in males and females at that early stage (Adkins-Regan, 2005), which could contribute to differences in vocal production. This study did find differences in vocal output between sexes; male nestling parrotlets had a higher daily repertoire size than females, while females produced shorter syllables at a faster rate. Females also produced babbling syllables with higher PC1 scores than males (i.e., higher average entropy and center and 95% frequency) (Figure 8) and more peak frequency contour inflection points. As male and female parrotlets do not differ in size (Forshaw, 1989), the frequency measurements produced by females are unlikely to be simply a product of a smaller body (and thus, vocal tract) size. In future analyses I will compare body mass between sexes to be able to rule out differences in

body size causing differences in vocal production. While differences in acoustic production could be due to males tending to produce one syllable type more frequently and females another syllable type with different characteristics, this is unlikely to be the case as production of syllable types by sex did not vary greatly, although this idea does warrant further investigation.

However, hormone treatment was assigned randomly to nestlings, and there was not an even balance of sexes in the treatment groups. Twice as many males received CORT as females, and almost twice as many males received OIL as females. Female birds were overrepresented in the CONTROL group. Because treatment began before sex could be determined visually, we were unable to control which sex received treatment. In order to more definitively identify any sexual dimorphism in parrotlet vocal production, further research will compare production of non-hormone supplemented male and female nestlings from these nests, and the Control group will be pruned so as to reflect the sample size and male:female ratio present in the Oil and CORT groups.

Repertoire size and diversity

Nestling repertoire size continued to increase as they aged (Figure 11). This indicates that pruning of vocal signals occurs after fledging, as opposed to before. It is possible that juvenile parrotlets wait until they have listened to the wider variety of vocalization types produced by conspecifics before discarding some, and the template provided by the parents is not sufficient. The songbird syllable repertoire generally does not begin to decrease until many months after fledging, after extensive exposure to song mentors. The period required for a parrotlet to acquire enough auditory and production experience before they begin to cull unnecessary syllable types is likely quite long; it would be necessary to make regular recordings of parrotlet vocalizations

over a long period of time to determine when pruning occurs. Alternatively, the observed increase in repertoire size could simply be a function of the nestlings' increased vocal output; as nestlings produce more and more babbling bouts, the chance of recording lesser-used syllable types increases.

Treatment did not have a significant effect on daily nestling repertoire size. This was contrary to prediction; in previous studies on developmental stress in songbirds, CORT-administered and nutritionally stressed birds developed a smaller repertoire size (of either syllable or song types) (Buchanan et al., 2003, 2004; Spencer et al., 2003, 2005). CORT supplementation (at a similar dosage as given in this study) and nutritional stress can result in reduced HVC volume (Buchanan et al., 2004; MacDonald et al., 2006; Nowicki et al., 2002; Spencer et al., 2003, 2005), so the smaller repertoire size seen in developmentally stressed songbirds is likely due to the correlation between HVC volume and repertoire size. The lack of repertoire size difference between CORT and control nestlings could indicate a difference in how developmental stress affects songbirds and parrots. The parrot song system structure is more elaborate than that of songbirds—this structure is hypothesized to be the source of the superior vocal mimicry ability of many parrot species (Chakraborty et al., 2015). The unique song system in the parrot brain might be somehow buffered from the effects of CORT in a way that songbird brains are not, as parrots have a mature HPA axis at an earlier stage in life than songbirds.

A different method for evaluating syllable repertoire size might be more informative; while supervised and unsupervised cluster analysis is commonly used for determining repertoire size and classifying vocalization types, the measurement of “acoustic area” (the range that encompasses all possible variation in the spectral and temporal measurements of a dataset) is recommended for species with large repertoire sizes (Keen et al., 2021). Whereas my k-means

analysis used PCA to classify babbling output into discrete categories, the acoustic area method uses PCA values to calculate the volume of the convex polygon that contains the PCA variation. This would result in a more graded evaluation of vocal output. Acoustic area can serve as an accurate proxy for repertoire size, especially in cases when datasets contain over 20 vocal element types, as clustering analyses tend to be less accurate and overestimate repertoire size for datasets comprised of 20-100 unique vocal element types (Keen et al., 2021). Further analysis will compare both repertoire estimation methods and determine which one best captures the effect of CORT on vocal diversity.

Sequence production

I found that that intersyllable duration decreases with age (Figure 16). This change is unlikely to be due to maturation of the syrinx itself, which does not change over the course of vocal development, while the vocal control system inside the brain does (Maxwell et al., 2021). Intersyllable transitions are learned, just as syllables themselves are (Lipkind et al., 2013); it is likely that as nestlings become more proficient at transitioning from syllable to syllable they need less time to produce a larger number of syllables.

Intersyllable duration was lowest in the CORT treatment group. Interestingly, this finding is contrary to that of a previous study, which found lower a syllable rate (syllables per second) in stress-treated birds (Zann & Cash, 2008). The reason for this difference is unclear, and it is intriguing that while intersyllable duration was shorter in the CORT group, syllable duration was longer.

Babbling onset

Contrary to expectation, CORT-treated nestlings did not begin vocal babbling production earlier than the control groups. In a previous study nutritionally stressed swamp sparrow nestlings entered and remained in the subsong phase earlier than control nestlings (Nowicki et al., 2002); this suggests that chronic stressors may activate the pathway that signals babbling onset. Parrots are open-ended vocal learners, and can acquire new vocalizations throughout life (Bradbury & Balsby, 2016), contrary to closed-ended swamp sparrows, who can only learn songs during an early sensitive period (Marler, 1990). It is possible then that an early vocal learning onset is more crucial for songbirds than parrots, as parrots can continue acquiring new vocalizations as they age, while stressed songbirds need a longer vocal refinement period in which to practice vocal production before song crystallization occurs.

Brood size

Green-rumped parrotlet broods vary widely in size, and nestlings hatch over an extended period—up to 18 days between the first-hatched and last-hatched nestlings (Beissinger & Waltman, 1991). This hatch asynchrony is even more exacerbated in large nests. Nestling green-rumped parrotlets from larger nests exhibit higher stress-induced CORT concentrations than nestlings from smaller nests, and the same pattern was seen in earlier-hatched nestlings when compared with younger siblings (Berg et al., 2019). This high HPA response likely aids nestlings in competing with their siblings for resources. However, brood size models generally did not perform well in AIC_c analysis, and for models in which it was involved (PFC average slope, Table 10; total repertoire size, Table 18; fledge age, Table 20) it was not statistically significant. It is possible CORT upregulation in nestlings from large broods is not as large as our

experimental doses, although the dose was designed to not exceed natural ranges of acute responses.

Conclusion

This study identified specific aspects of green-rumped parrotlet vocal production that are vulnerable to the effects of developmental stress in the form of corticosterone supplementation. Evidence suggests that early CORT administration affects nestling parrotlets in a similar manner to nestling songbirds, a closely related order. Interestingly, while the parrot vocal learning program differs from songbirds in that females are observed to possess nearly equal ability, this study identified differences in male and female vocal production. Little research has been conducted on parrot neuroendocrinology in the context of developmental stress and vocal learning ability; an interesting direction for future research would be to examine sex-based differences in CORT receptors in the parrotlet brain, as well as quantifying vocal learning nuclei volume to determine whether parrots also demonstrate a correlation between song control nuclei volume and repertoire size.

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BIOGRAPHICAL SKETCH

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