Social facilitation of reproduction and the physiological effects of vocal communication in captive boreal chorus frogs (*Pseudacris maculata*)

Jeffrey Ethier

Thesis submitted to the Department of Biology

in partial fulfillment of the requirements for the Ph.D. degree in Biology

Faculty of Science, University of Ottawa

Thèse soumise à le Département de biologie

dans le cadre des exigences du programme de doctorat en biologie

Faculté des sciences, Université d'Ottawa

Acknowledgments

I am extremely appreciative and thankful for my supervisor, Dr. Vance L. Trudeau. I don't think many supervisors would agree to my slightly non-traditional approach to a doctoral thesis. Thank you for giving me the opportunity to create something that is impactful but also my own. Your patience and guidance were essential to the completion of this degree. Thank you to my co-supervisor, Dr. Marc Mazerolle at Université Laval, for your wisdom concerning all things herpetology, ecology, and statistics. You and your students, Aurore Fayard and Jeanne Dudemaine, were indispensable for maintaining my connection with our collaborative partners in Québec. To those collaborators – Ministère de l'Environnement, de la Lutte contre les changements climatiques, de la Faune et des Parcs (formerly MFFP), SÉPAQ, and Montréal Biodôme – thank you for all your technical and financial support. I would especially like to thank the staff of Montréal Biodôme, including Odile Colin, Linda Paetow, and Dr. Emiko Wong, for sharing their animal husbandry knowledge. My frogs would have not had nearly as good of a life without your support.

I would like to acknowledge my thesis advisory committee, which included Dr. Joseph Bennett, Dr. Gabriel Blouin-Demers, and Dr. Julien Martin. It is often stated that it takes a village to raise a child. It is now clear to me that it takes a good committee to raise a graduate student. Thank you for your constructive criticism. I would also like to thank Dr. David Green for agreeing to be my external thesis reviewer. I couldn't have hoped for a better reviewer.

Thank you to the current and past members of the TeamENDO.ca lab. You made my life at the University of Ottawa a lot easier. When I was locked out, you let me into the lab. When I

was stressed, you went out for coffee and chatted with me. When I needed an extra hand with the frogs, you were always happy to help. My appreciation is boundless.

I am immensely grateful to all my friends and family, near and far, that continuously encouraged and supported me. Thank you to Bronwen and Penelope, who agreed to move from Newfoundland to Ontario during the pandemic so I could pursue my doctoral degree. Your emotional support made the seemingly impossible things possible. I would like to especially thank Shirley Ethier (née Lawson) and Robert McCormick, both of which are sadly no longer with us, for their interest in my progress. Like many other family members, they stated how important they thought my research was for the planet. However, Shirley and Robert went above and beyond to demonstrate their keen appreciation for role of amphibians in maintaining ecosystem health. I truly hope you are proud of me.

Finally, and most importantly, I would be remiss if I didn't thank the frogs. Your sacrifice allowed me to learn so much about endocrinology, physiology, and genetics – things I felt like I knew nearly nothing about before this degree – to make me a much stronger ecologist and researcher. You are proof that amazing things sometimes come in the tiniest of packages. You helped me find my place in this world. There is no greater praise than that.

Abstract

Vocalizations are an integral part of anuran breeding ecology. Acoustic signals (calls) are used to defend territories, repel competitors, attract mates, and coordinate spawning. There is a bidirectional relationship between hormonal state and reproductive behaviour, including acoustic signal production, where reception of conspecific calls can influence steroid hormone production and circulating hormone concentrations can affect calling behaviour. We examine the calling behaviour of boreal chorus frogs (*Pseudacris maculata*), a declining species in northeastern North America, and determine the effects of conspecific signals on reproductive outcomes and gonadal function. We hypothesize that conspecific acoustic signals can be used in anuran conservation to improve captive breeding outcomes as acoustic signals are a potent modulator of reproduction. First, we used autonomous recording units deployed at natural boreal chorus frog breeding locations to quantify diel and seasonal pattens in calling behaviour. We then used these acoustic recordings to produce audio playback files that were broadcasted to spawning pairs in captivity to investigate the effect of conspecific calls on the calling activity of males, reproductive output, and offspring quality in boreal chorus frogs. We found that broadcast of conspecific significantly increased the proportion of viable eggs and survival of subsequent tadpoles compared to the control. To further investigate the mechanism of reproductive enhancement, we used RNA sequencing to examine the gonadal gene expression in groups of male and female boreal chorus frogs exposed to conspecific calls. We found enhanced expression of genes and gene pathways associated with steroidogenesis, gametogenesis, and gonadal function in male boreal chorus frogs, but more limited evidence of these effects in females. Differential gene expression of male frogs exposed to conspecific calls also parallelled expression results of male frogs receiving hormone injections known to stimulate spawning,

suggesting similar stimulation of the hypothalamic-pituitary-gonadal axis in both treatment groups. Based on these results, we conclude that conspecific signals are important regulator of reproduction during the breeding season. Our research contributes to our knowledge on anuran breeding ecology as the results provide further evidence for social facilitation of reproduction in this taxon. These results also have implications for amphibian conservation, as recordings of male calling behaviour could be used a tool to enhance reproductive outcomes in captive breeding programs of declining species.

Résumé

Les vocalisations font partie intégrante de l'écologie de la reproduction des anoures. Les signaux acoustiques (chants) sont utilisés pour défendre les territoires, repousser les concurrents, attirer les partenaires et coordonner la ponte. Il existe une relation bidirectionnelle entre l'état hormonal et le comportement reproducteur, y compris la production de signaux acoustiques, où la réception d'appels de congénères peut influencer la production d'hormones stéroïdiennes, et les concentrations d'hormones circulantes peuvent affecter le comportement d'appel. Nous examinons le comportement d'appel des rainettes faux-grillon boréales (*Pseudacris maculata*), une espèce en déclin dans le nord-est de l'Amérique du Nord, et déterminons les effets des signaux conspécifiques sur la reproduction et la fonction gonadique. Nous émettons l'hypothèse que les signaux acoustiques conspécifiques peuvent être utilisés dans la conservation des anoures afin d'améliorer les résultats de la reproduction en captivité, car les signaux acoustiques sont un puissant modulateur de la reproduction. Tout d'abord, nous avons utilisé des unités d'enregistrement autonomes déployées sur des sites naturels de reproduction de la rainette faux-grillon boréale pour mesurer les motifs quotidiens et saisonniers dans le comportement d'appel. Nous avons ensuite utilisé ces enregistrements acoustiques pour produire des fichiers de lecture audio qui ont été diffusés à des couples reproducteurs en captivité, afin d'étudier l'effet des appels de congénères sur l'activité d'appel des mâles, le rendement reproductif et la qualité de la progéniture chez les rainettes faux-grillon boréales. Nous avons constaté que la diffusion d'appels de conspécifiques augmentait significativement la proportion d'œufs viables et la survie des têtards subséquents par rapport au contrôle. Pour étudier les mécanismes derrière l'amélioration de la reproduction, nous avons utilisé le séquençage de l'ARN pour examiner l'expression des gènes gonadiques dans des groupes de rainettes faux-grillon boréales mâles et

femelles exposées à des appels de conspécifiques. Nous avons constaté une augmentation de l'expression des gènes et des voies génétiques associés à la stéroïdogenèse, à la gamétogenèse et à la fonction gonadique chez les rainettes faux-grillon boréales mâles, mais des preuves plus limitées de ces effets chez les femelles. L'expression différentielle des gènes des grenouilles mâles exposées à des appels de congénères est également parallèle aux résultats d'expression des grenouilles mâles recevant des injections d'hormones connues pour stimuler le frai, ce qui suggère une stimulation similaire de l'axe hypothalamo-hypophyso-gonadique dans les deux groupes. Sur la base de ces résultats, nous concluons que les signaux conspécifiques sont des régulateurs importants de la reproduction pendant la saison de reproduction. Notre recherche contribue aux connaissances sur l'écologie de la reproduction des anoures, car les résultats fournissent des preuves supplémentaires de la facilitation sociale de la reproduction dans ce taxon. Ces résultats ont également des implications pour la conservation des amphibiens, car les enregistrements d'appels de mâles pourraient être utilisés comme outil pour améliorer les résultats de la reproduction dans les programmes d'élevage en captivité des espèces en déclin.

Table of Contents

List of Figures

List of Tables

Abbreviations

AN	anterior thalamic nucleus
AP	amphibian papilla
ART	artificial reproductive technologies
AVT	arginine vasotocin
BP	basilar papilla
CORT	cortisol
COSEWIC	Committee on the Status of Endangered Wildlife in Canada
CT	central thalamus nucleus
DA	dopamine
dBa	decibels
DEGs	differentially expressed genes
DL	dorsolateral nucleus
E2	estradiol
FSH	follicle-stimulating hormone
GnRH	gonadotropin-releasing hormone
GnRH-A	gonadotropin-releasing hormone agonist
GO	Gene Ontology
Gs	Gosner stage
HPG	hypothalamic-pituitary-gonadal
KEGG	Kyoto Encyclopedia of Genes and Genomes
LH	luteinizing hormone
P	pituitary

P4	progesterone
POA	preoptic area
RNAseq	RNA sequencing
SI	secondary isthmal nucleus
SO	superior olivary nucleus
T	testosterone
VH	ventral hypothalamus
VII n.	8th cranial nerve

Chapter 1: General Introduction

1.9 Rationale

Amphibian species over the last half-century have declined at the highest rate compared to all other vertebrates (Stuart et al. 2004; Beebee and Griffiths 2005). These steep declines within amphibian populations have prompted conservation concern for the taxon across all continents (Wake and Vredenburg 2008; Hoffmann et al. 2010). Due to multiple interacting direct threats to amphibian populations as well as continual loss of breeding habitats, captive breeding and reintroduction initiatives have been established for several species in Canada (ECCC 2015a, ECCC 2015b, ECCC 2017). Amphibians are often good candidate species for captive breeding since they tend to have higher fecundity, smaller body size, and lower associated costs for maintenance compared to other taxa (Bloxam and Tonge 1995). However, a lack of natural environmental cues and increased physiological stress associated with captivity can result in a lack of spawning activity or suboptimal reproductive outcomes (Höbel 2017). Therefore, there is increased motivation to develop new techniques to improve captive breeding protocols.

One possible solution to improve captive breeding outcomes is to incorporate broadcasts of conspecific signals (*i.e.*, acoustic playbacks). In most anuran amphibians (frogs and toads), acoustic communication is essential for reproduction (Kelley 2004). Advertisement calls produced by males during the breeding season are used to defend territories, assess the quality of competitors, attract mates, and coordinate reproduction (Wells and Schwartz 2007). The reception of conspecific signals can induce significant physiological changes via the hypothalamic-pituitary-gonadal (HPG) axis, such as stimulating the production of gonadal sex steroids (*i.e.*, androgens, estrogens, progesterone) in both sexes (Wilczynski et al. 2005; Woodley

and Leary 2024). For example, after hearing calls for several consecutive days, male frogs often have increased androgen production (Burmeister and Wilczynski 2000; Chu and Wilczynski 2001; Rodríguez et al. 2022). Conspecific calls may also have a role in maintaining gonadal function and reproductive receptivity throughout the breeding season in both sexes (Wilczynski et al. 2005; Woodley and Leary 2024). Among all these studies, the mechanisms accounting for these physiological changes are poorly explained. It is unclear if the observed increases in hormone concentration or the effects on gamete maturation have impacts on reproductive outcomes, such as fertilization rates or viability of offspring.

Given this background and the lack of data on mechanisms accounting for physiological changes following acoustic reception, we wanted to uncover the role of conspecific signals in the regulation of reproduction in anuran amphibians. We aimed to determine if broadcasts of conspecific signals stimulate gonadal processes to improve reproductive outcomes, such as increasing fertility, which then could be used to improve captive breeding protocol and bolster amphibian conservation. Additionally, we aimed to determine if reception of conspecific signals affect gonadal gene expression and how these potential effects compare to a highly effective hormonal induction technique currently utilized in captive breeding protocols. To address these questions, we used a locally declining species, the boreal chorus frog (*Pseudacris maculata*), currently being bred in captivity for the purpose of reintroduction. The hypotheses and objectives of the research are presented below, followed by a review of relevant literature that provides the foundation of the thesis.

1.10 Hypothesis

Conspecific acoustic stimulation enhances reproductive success by activating endocrine pathways that upregulate genes involved in gonadal function and fertility regulation in chorus frogs.

1.11 Objectives

The primary objective of the thesis is to describe how conspecific acoustic signals can be utilized in the captive breeding of anurans to aid in the conservation of amphibian species. Our secondary objective is to better understand the role of acoustic communication in reproduction, with an emphasis on gonadal gene activation, calling activity, and reproductive output.

Our specific research goals are:

(1) To describe the breeding ecology and conservation status of frogs in the genus *Pseudacris*, including the boreal chorus frog (*Pseudacris maculata*). This is important for determining the applicability of results for other species in the genus.

(2) To describe the daily and seasonal calling patterns of boreal chorus frogs during the breeding season within their natural environment. This is essential step to produce an accurate facsimile of natural calling behaviour which then can be replicated in the captive environment.

(3) To determine if acoustic playback of conspecific calls influence calling effort, reproductive output, and tadpole development and survival in captivity.

(4) To determine if acoustic playback of conspecific calls influence (a) gonadal gene activation and, if differences do occur, (b) compare results to known stimulators of the HPG axis (*i.e.*, hormonal induction).

1.12 Description of research

To contextualize the reproductive ecology of boreal chorus frogs (Goal 1), in Chapter 2 we provided a review of the life history traits of the 18 species of chorus frogs (genus *Pseudacris*). This genus has undergone several taxonomic reclassifications within the last 20 years, prompting us to update the species distribution and current conservation status, and to bring attention to areas of reproductive ecology research that are currently lacking. We also highlighted that the similarities in life history traits among chorus frog species to provide an opportunity for collaboration for the conservation of species within the genus and justify the applicability of results for other anuran amphibians.

To quantify the daily and seasonal calling patterns of boreal chorus frogs (Goal 2), in Chapter 3, we obtained hourly calling data across three breeding seasons (March-May). Hourly calling data was used to construct audio files and broadcast schedules to be used in subsequent experiments (Chapters 4 and 5). We were also interested in how abiotic factors influence calling behaviour. Therefore, we described the phenology of calling activity of four early spring-breeding anurans native to the Ottawa, Ontario region, including boreal chorus frogs (*Pseudacris maculata*), spring peepers (*Pseudacris crucifer*), American toads (*Anaxyrus americanus*), and wood frogs (*Boreorana sylvaticus*). We also determined the impact of a weather event where temperatures exceeded 5°C for 17 consecutive days in early March of 2024 ("false spring") on calling behaviour among the early spring-breeding species. We used audio data collected from automated recording units to model the influence of day of the year, time of day, temperature, and rainfall on the calling activity of boreal chorus frogs.

In Chapter 4, we tested whether acoustic playback of conspecific calls influence the calling effort and reproductive output of boreal chorus frogs in captivity (Goal 3). Few, if any,

captive breeding programs include vocalizations from conspecifics despite evidence that sounds from natural and anthropogenic sources can impact the rates of disease transmission and survival of captive animals (Clark and Dunn 2022). We were particularly interested in determining if broadcasts would increase the number of vocalizations produced by males and affect the number and quality of eggs produced by females, as reception of conspecific signals have been associated with stimulation the HPG axis. We also followed the development and survival of tadpoles hatched from eggs collected during breeding trials to determine if potential benefits of broadcasts extend beyond the parental individuals within the spawning period (*i.e.*, affect offspring quality).

After noting increased in egg viability within boreal chorus frogs exposed to conspecific calls in Chapter 4, we aimed to explore mechanisms that would impact reproductive function in response to acoustic stimulation. RNA sequencing is an emerging technique that allows researchers to determine the presence and quantity of all RNA within a sample and explore patterns of gene expression using pathway analysis (Wang et al. 2009). In Chapter 5, we used RNA sequencing to determine if acoustic playback of conspecific calls influence gonadal gene activation (Goal 4a) in male and female boreal chorus frogs. We compared differential gene expression patterns between sexes and determined if similar genes and pathways are affected in frogs exposed to conspecific calls compared to frogs receiving a hormone injection known to stimulate the HPG axis (Goal 4b).

In Chapter 6 we summarized the evidence that acoustic communication is an essential part of anuran ecology. We stated that our fertility and gonadal gene expression results provide some of the first evidence of the physiological changes induced by reception of conspecific signals. We described how our results support previous literature indicating that the reception of

conspecific signals is crucial for the coordination of reproduction and the sustained stimulation of the hypothalamic-pituitary-gonadal axis throughout the breeding season. We then incorporated our results in the context of male-male competition, the energetics-hormone model, and the challenge hypothesis. We concluded the chapter by stating the implications of our research for anuran conservation and by making recommendations for future studies.

1.13 Captive breeding of amphibians and assisted reproductive technologies

The two major goals of captive breeding programs are to maintain captive populations that cannot be safeguarded in their natural environment (Zippel et al. 2011) and to produce viable offspring for reintroduction into populations where species are declining or extirpated (Griffiths and Pavajeau 2008; Harding et al. 2016). Captive breeding and reintroduction programs have be initiated for a wide variety of taxa, including amphibians, fishes, reptiles, birds, and mammals but the success of these programs vary widely (Seddon et al. 2005; Farquharson et al. 2021). Common issues include high operating costs, poor captive survival, high susceptibility to disease, low genetic diversity, genetic adaptation to the captivity (*i.e.*, domestication), and loss natural behaviours (Snyder et al. 1996; Witzenberger and Hochkirch 2011). Regardless, successful initiatives where reintroduction have led to self-sustaining natural populations, such as California condor (*Gymnogyps californianus*) and black-footed ferret *(Mustela nigripes*), have led to the increased the prevalence of captive breeding and reintroduction programs in recent decades (McGowan et al. 2017).

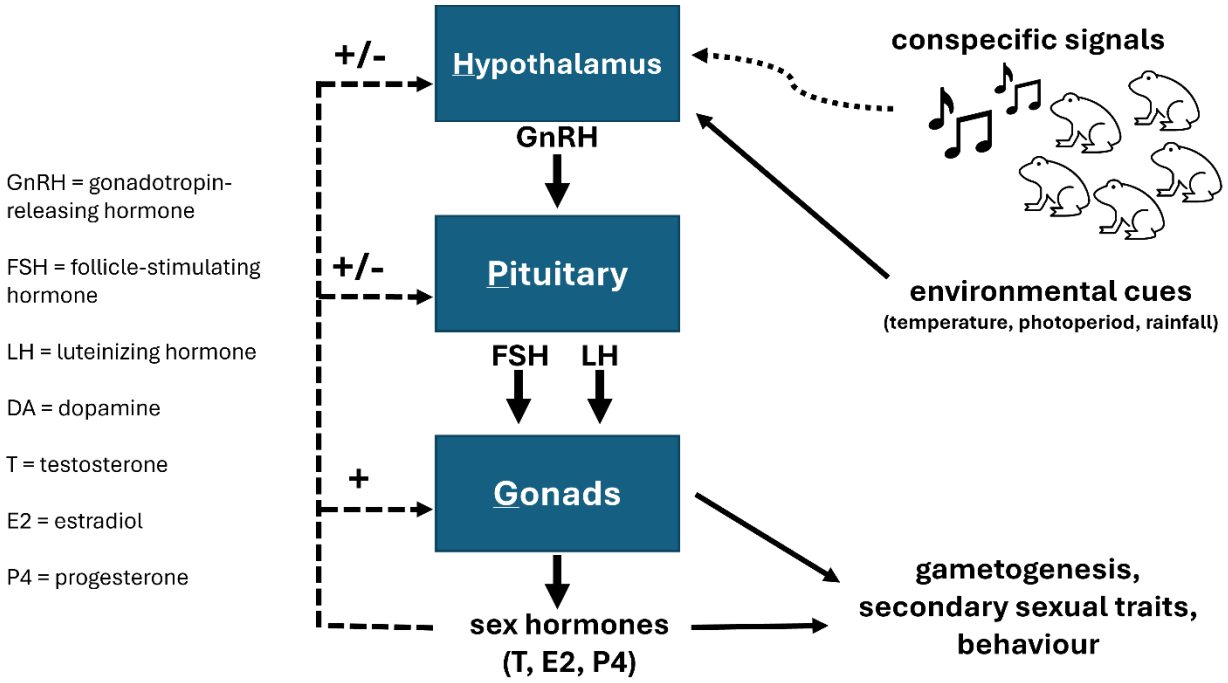

Figure 1.1: Overview of the hypothalamic-pituitary-gonadal (HPG) axis in vertebrates and the environmental and social factors initiating reproduction in amphibians (adapted from Trudeau et al. 2022). Solid lines indicate processes with well-supported evidence in amphibians or other vertebrates; dotted lines indicate an assumed process based on reproductive ecology of anuran amphibians; dashed lines indicate positive and/or negative feedback.

In theory, amphibians are good candidate species for captive breeding since they tend to have higher fecundity, smaller body size, and lower associated costs for maintenance in comparison with other taxa (Bloxam and Tonge 1995). Conversely, there are many species of frogs and toads (Order: Anura), where establishing captive colonies can be challenging, costly, and inefficient (Tapley et al. 2015; Clulow et al. 2019; Della Togna et al. 2020). Captive breeding efforts may be further complicated as many species require complex social and environmental cues to initiate breeding behaviour and therefore reproduce very poorly within a captive setting in the absence of these cues (Höbel 2017). As such, assisted reproductive technologies (ART) are often the foundation of captive breeding programs (Clulow et al. 2014; Clulow et al. 2019).

Amphibian ART encompass several techniques to increase breeding efficiency and maintain genetic diversity, including hormone therapy to induce spawning, *in vitro* fertilization, cryo-banking for the storage of gametes, and cloning (Clulow et al. 2022). Hormonal induction of spawning is one of the most common and effective tools to overcome many of the challenges faced in captive breeding (Silla et al. 2021). Hormonal induction methods primarily target the hypothalamic-pituitary-gonadal (HPG) axis to stimulate reproduction. Despite small differences among taxa, the fundamental organization and function of the HPG axis is widely conserved across all vertebrates (Figure 1.1). Gonadotropin-releasing hormone (GnRH) is a neuropeptide synthesized in specific neurons of the preoptic area and ventral hypothalamus (Rastogi et al., 1998). GnRH is then axonally transported via the infundibular hypothalamus, released into the median eminence, and then transported by the hypothalamo-hypophysial blood system to the anterior pituitary. The GnRH binds to specific GnRH receptors on gonadotropic cells to stimulate the synthesis and secretion of two glycoproteins, luteinizing hormone (LH) and follicle-stimulating hormone (FSH). These glycoproteins are transported to the gonads via the vascular system to stimulate sex steroid production (androgens, estrogens, progesterone), gametogenesis (sperm and egg production), secondary sexual traits, and reproductive behaviour (Vu and Trudeau 2016; Trudeau et al. 2022). Depending on the concentration and patterns of release, sex steroids can have either negative or positive feedback on LH and FSH release from pituitary gonadotrophs and GnRH neuron activity in the hypothalamus (Vu and Trudeau 2016; Trudeau et al. 2022).

Hormonal induction methods commonly use human chorionic gonadotrophin (hCG) and GnRH agonists, either alone or in combination, to stimulate spawning (Silla et al. 2021). Human chorionic gonadotrophin is an agonist of LH and injection of hCG simulates the actions of LH in

the gonads to stimulate ovulation and sperm release. In contrast, GnRH agonists (GnRH-A) act directly on the anterior pituitary to stimulate the production of LH and FSH. The efficacy of hCG and GnRH-A is species-dependent. Administration of GnRH-A induces spawning in most species (Silla et al. 2021), but hCG can have better results in toads (Bufonidae), Australian treefrogs (Pelodryadinae), and Australian ground frogs (Limnodynastidae) (Clulow et al. 2018; Silla and Byrne 2019). Some hormonal induction methods also incorporate an antagonist of dopamine. There is strong evidence from studies in teleost fish, as well as other vertebrates, that dopamine (DA) has an inhibitory effect on reproduction via DA-D2 type receptors in the brain and pituitary (Dufour et al. 2010; Vu and Trudeau 2016). For example, the Amphiplex method, which includes a co-injection of the DA antagonist metoclopramide in addition to GnRH-A, is an effective means of inducing spawning in leopard frogs (*Lithobates pipiens*) and three Argentinian frog species (Trudeau et al. 2010; Trudeau et al. 2013).

Challenges remain for captive breeding and amphibian conservation despite advancements over the last few decades (Della Togna et al. 2020; Tapley et al. 2022). Many captive breeding programs are initiated without proper understanding of the natural history of the species and environmental parameters required for reproduction (Michaels et al. 2014). Even with innovations in amphibian ART to induce spawning, captive breeding programs may fail to achieve high rates of fertilization or to generate significant numbers of viable offspring (Kouba et al. 2009). Captivity is associated with increased glucocorticoid production from the adrenal/interrenal gland in some species (Fischer and Romero 2019), which can inhibit sex steroid production and reduce the expression of reproductive behaviours (Carr 2024). A meta-analysis of captive breeding programs across several invertebrate and vertebrate taxa revealed

that captive-bred animals have a 42% decrease in the odds of reproducing successfully in captivity compared to wild-caught animals (Farquharson et al. 2018).

1.14 The role of acoustic signal reception and production in frog reproduction

Acoustic signal reception and reproduction in anurans are interconnected. The anuran auditory system (Figure 1.2) has direct neuronal connections to brain nuclei associated with the HPG axis, implying that conspecific signals act as important regulators of reproductive condition and expression of reproductive behaviours by modulating plasma sex hormone concentrations (Wilczynski et al. 2005; Woodley and Leary 2024). Acoustic signals are passed through and filtered by the tympanum. Differing frequencies are then detected by either the sacculus, amphibian papilla, or basilar papilla within the inner ear (Schoffelen et al. 2008). Once sorted, acoustic information is projected to the dorsolateral nucleus of the thalamus via branches of the 8th cranial nerve (or "acoustic nerve"). Projections are then relayed from the dorsolateral nucleus of the thalamus to the superior olivary nucleus and then to the midbrain torus semicircularis, the main centre of auditory information processing (Endepols et al. 2003; Arch and Narins 2009). Acoustic information via thalamic relay nuclei is then projected to and received by the preoptic area and ventral hypothalamus nuclei, leading to activation of GnRH neurons (Allison and Wilczynski 1991; Burmeister and Wilczynski 2005). Most inputs into the ventral hypothalamus are received from the central thalamic and secondary isthmal nuclei whereas inputs to the preoptic area are received primarily from the anterior thalamic and secondary isthmal nuclei (Allison and Wilczynski 1991). The relationship between the HPG axis and auditory system may be bidirectional, with gonadal hormones affecting the processing of auditory information and the reception of auditory information influencing the production and secretion of gonadal hormones. The laminar nucleus of the torus semicircularis contain cells with high concentrations of

androgen and estrogen receptors, which are connected to premotor and motor areas of the brain, providing a potential mechanism for how gonadal hormones affect behavioural and hormonal responses to acoustic signals (Arch and Narins 2009; Wilczynski and Burmeister 2016).

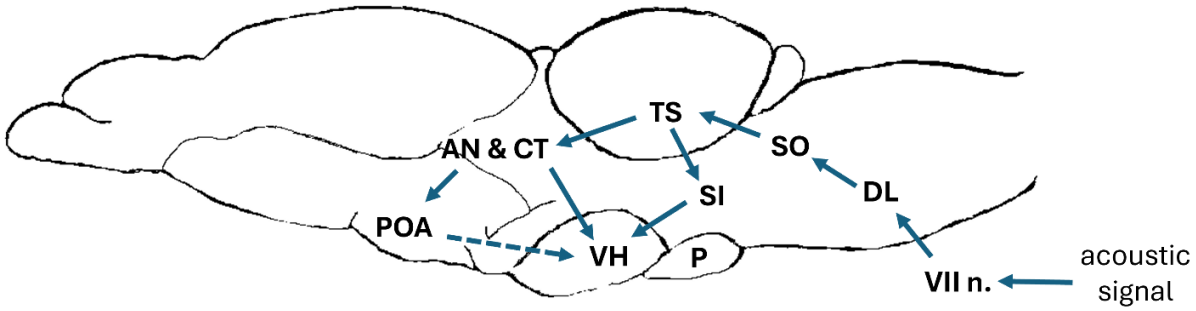

Figure 1.2: Schematic of auditory system of anurans (sagittal view). Acoustic signals travel from the tympanic membrane, where they are filtered and relayed to the inner ear organs, such as the amphibian papilla (AP) and basilar papilla (BP). Information received from the AP and BP are projected to the dorsolateral nucleus (DL) via branches of the 8th cranial nerve (VII n.). Projections are then relayed from the DL to the superior olivary nucleus (SO), then to the torus semicircularis (TS), followed by the secondary isthmal (SI) and central thalamus (CT) nuclei, which both project to the ventral hypothalamus (VH). Acoustic information is also relayed from the TS to the anterior thalamic nucleus (AN), then to the preoptic area of the hypothalamus (POA). The POA also receives projections from CT and SI, although fewer projections are received from these regions compared to the VH. The POA and VH are important neural centres for the control of reproduction in amphibians via the pituitary-gonadal axis. While anatomical data showing specific innervation of gonadotropin-releasing hormone neurons located in the POA and/or VH by AN and CT neurons, acoustic signals likely activate these circuits. GnRH neurons project via the infundibulum to the median eminence where GnRH is released to the hypothalamo-hypophsial portal blood system to the pituitary (P) to stimulate the production of luteinizing hormone and follicle stimulating hormone, which in turn regulate gonadal function. Solid lines indicate direct neuronal connections. Dotted lines indicate neuronal connections with fewer projections. Modified from Allison and Wilczynski (1991) and Burmeister and Wilczynski (2005).

Production of acoustic signals is also related to HPG axis activity. Calling, a predominately male behaviour in anurans, is significantly reduced in castrated males but can be restored with administration of androgens (Wetzel and Kelley 1983; Burmeister and Wilczynski 2001). Several studies have indicated that increased calling behaviour is associated with increasing plasma testosterone concentrations, such as in *Dryophytes cinereus* (Leary et al. 2015;

Crocker-Buta and Leary 2018) and *Lithobates grylio* (Walkowski et al. 2019). In contrast, other studies have found that androgen levels in calling males is lower (Mendonça et al. 1985) or there is no difference in calling and non-calling males (Leary et al. 2004; Leary et al. 2006). The HPG axis interacts with other hormonal pathways, such as glucocorticoids, the stress hormones of the amphibian interrenal gland, which may explain theses discrepancies (Moore and Jessop 2003; Leary 2009). Corticosterone (CORT) inhibits reproduction at many levels of the HPG axis, including moderating the production of GnRH and gonadotropins, inhibiting steroid production in the gonadal tissues, and altering the metabolism of androgens and estrogens (Carr 2024). However, positive associations between CORT and androgens can be found among calling individuals, especially in the initial stages (Woodley and Leary 2024). The energetics-hormone vocalization model (Emerson 2001) may help to explain the interplay of CORT and androgens. It proposes that calling induces androgen production while CORT synthesis and release increases to meet the energy demands, eventually surpassing a threshold that triggers an acute stress response that reduces androgen concentrations.

Another important player in moderating reproductive behaviour is the neuropeptide arginine vasotocin (AVT). AVT-containing cells are predominantly found within the preoptic area of the hypothalamus, with projections to the posterior pituitary (Rose and Moore 2002; Wilczynski et al. 2017). Injections of AVT has induced calling behaviour in non-calling males (Ten Eyck 2005) and increased calling effort by decreasing latency between calls in *Dryophytes cinerea* (Burmeister et al. 2001) and *Acris crepitans* (Chu et al. 1998). Immunoreactive AVT cells are also found within the torus semicircularis, which implies AVT has a role in auditory perception as well as call production (Boyd 1997). It was previously suggested that AVT may modulate the inhibitory effects of CORT, but this evidence is mainly garnered from studies of

clasping behaviour in roughskin newts, *Taricha granulosa* (Coddington and Moore 2003). Evidence in *Dryophytes cinerea* suggests AVT increases motivation to call rather than blocking CORT secretion, as administration of AVT increased the probability of calling but CORT did not decreased calling compared to saline-injected males (Burmeister et al. 2001).

1.8 Social facilitation of reproduction in anurans

There is empirical evidence for social facilitation of reproduction in anurans, where calling behaviour acts as a signal to initiate or maintain physiological changes associate reproduction. Male frogs rarely call in isolation but readily respond to calling conspecifics (Klump and Gerhardt 1992). Broadcasts of conspecific calls in the field increased calling rate in male *Batrachyla taeniata* frogs (Muñoz et al. 2020). Within the context of the energetics-hormone vocalization model, the challenge hypothesis (Wingfield et al. 1990) states that elevated androgen production is driven by male-male interactions and is associated with increased vocal effort. Several studies have reported rapid effects of social interactions on plasma concentrations of androgens in a variety of vertebrates, but evidence is limited in amphibians (Hirschenhauser and Oliveira 2006; Moore et al. 2020). One of the few examples of the challenge hypothesis being tested in an anuran amphibian was conducted using male *Allobates femoralis*, a territorial frog species where males attend egg masses and transport tadpoles to water after 15–20 days of development (Rodríguez et al. 2022). Consistent with the hypothesis, simulated territory intrusions significantly increased waterborne androgen concentration, a proxy significantly correlated with plasma testosterone, an hour post-challenge (Rodríguez et al. 2022).

Several studies have suggested that playbacks of conspecific calls can be used to stimulate the production of gonadal steroids in male and female frogs during the breeding season. For example, after hearing calls for several consecutive days, male American green treefrogs (*Dryophytes cinereus*) and southern leopard frogs (*Rana sphenocephala*) experienced increased plasma androgen concentration (Burmeister and Wilczynski 2000; Chu and Wilczynski 2001). Conspecific calls may also have a role in maintaining reproductive status throughout the breeding season (Wilczynski et al. 2005; Woodley and Leary 2024). Testicular regression occurs rapidly in male grass frogs (*Rana temporaria*) a few days after the end of the breeding season (Brzoska and Obert 1980). However, testis volume and size of the interstitial cells of captive male grass frogs that were exposed to recordings of synthetic conspecific calls were comparable to actively breeding males (Brzoska and Obert 1980). Male grass frogs exposed to no audio stimuli or synthetic calls with a higher spectral frequency than conspecific calls decreased total volume of testes, reduced interstitial cell size, and reduced interstitial cell number (Brzoska and Obert 1980). In female *Alytes muletensis*, eggs will continue to mature when exposed to conspecific male calls but are reabsorbed if conspecific calls cease or when exposed to heterospecific calls (Lea et al. 2001). Similarly, male sexual behaviour (*i.e.*, calling, amplexus) was found to have an important role for stimulating spawning in female *Bufo melanostictus* by increasing plasma estradiol and progesterone concentrations (Gramapurohit and Radder 2013). Take together, the data strongly implies that reception of calling behaviour is a potent regulator of reproduction and important for maintaining reproductive status during the breeding season.

1.9 Focal species: boreal chorus frog

The boreal chorus frog (*Pseudacris maculata*, Agassiz 1850) is a small-bodied (mean snout-to-vent length: 27 mm ♂, 30 mm ♀) frog species in the family Hylidae (tree frogs) that is native to Canada and the United States (Dodd 2023). Like several other species in the genus *Pseudacris*, male boreal chorus frogs produce a high-pitched trilling advertisement vocalization during the breeding season that is reminiscent of running one's fingernail across a plastic comb (Conant and Collins 1998). Vocalizations are typically < 1 second in duration and contain 14–16 notes or pulses (Bee et al. 2010). Boreal chorus frogs also produce aggressive calls, or vocalizations used to repel competitor males, that are structurally similar to advertisement calls but are longer in duration and contain more pulses (Owen 2003). Boreal chorus frogs have a wide, mostly contiguous distribution across North America (Figure 1.3) occurring from the Northwest Territories and northeastern British Columbia, southwest through portions of Montana, Idaho, Utah, Arizona, and New Mexico, across through Alberta, Saskatchewan, Manitoba and the Midwest states, to Michigan, Illinois, Indiana and Missouri in the east (Ethier et al. 2021). Tolerant of high elevations, boreal chorus frogs can be found > 3000 m above sea level in several portions of their range, including Arizona, Colorado, and Utah (Stebbins 2003). The total abundance of boreal chorus frogs is unknown but has been estimated nearly 20 years ago to be approximately 1,000,000 individuals (Hammerson 2008). The boreal chorus frog is a cold- and freeze-tolerant species (Dinsmore and Swanson 2008; Higgins and Swanson 2013), and often one of the first amphibian species to emerge from hibernation and begin spawning in late winter and early spring (Dodd 2023). The breeding season of boreal chorus frogs is approximately 6 weeks, but in some regions may be condensed to only 2–3 weeks (Desroches and Rodrigue 2004; Dodd 2023). Boreal chorus frogs utilize a variety of ephemeral wetlands for

breeding and oviposition, including temporary ponds, roadside ditches, flooded meadows, shallow bogs and marshes, buffalo wallows, furrows in plowed fields, glacial kettlepots, and vernal pools in woodlands (Dodd 2023; Ethier et al. 2021). Preferred habitat tends to be shallow (< 35 cm deep) with abundant vegetation and relatively free of predators, such as fishes and aquatic invertebrates (Ouellet et al. 2009; Shulse et al. 2010; Shulse et al. 2013).

Figure 1.3: Boreal chorus frog (*Pseudacris maculata*) distribution in North America (adapted from Lemmon et al. 2007 and Ethier et al. 2021). Image of boreal chorus frog courtesy of Chris Callaghan.

Even with a widespread distribution and relatively high abundance throughout most of it range, several boreal chorus frog populations are steeply declining. Populations in southeastern Ontario and southwestern Quebec are listed under the *Species at Risk Act* (S.C. 2002, c. 29) as "threatened" (Environmental Canada 2015), and the species is listed as "threatened" in the province of Quebec (R.S.Q, c. E-12.01). Declines are also observed in New York (Corser et al. 2012) and the species listed as "endangered" in the Vermont (Vermont Fish and Wildlife Department 2015b). Until recently, these populations in eastern North America were assigned to another species, the western chorus frog (*Pseudacris triseriata*). Mitochondrial DNA gene sequencing and behavioural experiments provide strong evidence that the frogs in these regions are more likely to be boreal chorus frogs (Lemmon et al. 2007; Rogic et al. 2015). The Government of Canada has acknowledged these findings (Bogart et al. 2015), but the species has yet to be reassigned by the Committee of the Status of Endangered Wildlife in Canada (COSEWIC) and remains listed as western chorus frog on the Species at Risk public registry (Environmental Canada 2025).

Like many other North American amphibians, the primary factor contributing to declines of boreal chorus frog populations is habitat destruction and fragmentation (Cushman 2006; Seburn et al. 2014). Other threats include the introduction of exotic species (Bucciarelli et al. 2014), disease (Rittman et al. 2003), and climate change altering hydroperiod of temporary wetlands where boreal chorus frogs breed (McMenamin et al. 2008; Amburgey et al. 2012). In the Montérégie region of southeastern Québec, boreal chorus frogs were extirpated from 90% of their historical range (Picard and Desroches 2004) and populations across the province have declined by 37% between the 1950s and 2008 (COSEWIC 2008). Suitable habitat continues to be destroyed and fragmented as land is converted for industrial and residential use (ECCC

2015b). In 2018, it was estimated that only 23% of Québec boreal chorus frog populations were viable or likely to persist if current conditions were maintained (ECCC 2021). Rapid declines have also been observed in the Ottawa Valley (Vallée d'Outaouais) region of the Ottawa River, including portions of eastern Ontario and southwestern Québec (St-Hilaire and Belleau 2005; Seburn et al. 2008; Seburn and Gunson 2011). Boreal chorus frogs were once widespread east of Ottawa, but when 184 sites of suitable habitat were surveyed in 2011 and 2012, only 5 (2.7%) had calling boreal chorus frogs, coinciding with a significant reduction of wetland cover since the 1950s (Seburn et al. 2014). This fragmentation of habitat has led to increased genetic isolation and decreased genetic variability (Rogic et al. 2019).

In response to the drastic population declines, a captive-breeding and reintroduction program was initiated in collaboration with multiple partners, including the province of Québec (Ministère de l'Environnement, de la Lutte contre les Changements climatiques, de la Faune et des Parcs), Université Laval, University of Ottawa, Société des établissements de plein air du Québec (Sépaq), and the Montréal Biodôme. Spawning of boreal chorus frog has been successfully stimulated in captivity using hormonal induction methods originally developed for other species (Trudeau et al. 2010; Trudeau et al. 2013) and protocols have been developed for rearing viable tadpoles and metamorphs (Ethier et al. 2024). However, spawning rates and egg viability are highly variable and often low, potentially reflecting suboptimal activation of the HPG axis or other reproductive constraints. This variability makes boreal chorus frogs an ideal model for investigating the role of social facilitation in reproduction in both wild and captive populations, as well as for exploring alternative strategies to enhance reproductive success in captivity.

1.10 Thesis format

Each chapter of this thesis is written as manuscripts to be, or has been, submitted for publication. As such, there is some overlap in the content presented in each chapter, though we have attempted to keep this to a minimum, where possible. Note that Chapter 2 is a reprint of a review published in *Frontiers in Zoology* with its own citation style and reference section. The references cited in all other chapters are included in the Reference section of the thesis (p. 171).

1.11 Ethics statement

All animals were collected following permits issued by the Ontario Ministry of Natural Resources. All experiments were approved by the University of Ottawa Protocol Review Committee and adhered to the guidelines of the Canadian Council on Animal Care for the use of animals in research and teaching.

Chapter 2: Life history traits and reproductive ecology of North American chorus frogs of the genus *Pseudacris* (Hylidae)

This chapter adapted from:

Ethier JP, Fayard A, Soroye P, Choi D, Mazerolle MJ, Trudeau VL. 2021. Life history traits and reproductive ecology of North American chorus frogs of the genus *Pseudacris* (Hylidae). Frontiers in Zoology 18: 40. https://doi.org/10.1186/s12983-021-00425-w

Study contributions: JPE contributed through conceptualization, investigation, methodology, data curation, and writing. AF contributed through investigation, methodology, data curation, and writing. PS contributed through investigation, methodology, and data curation. DC contributed through investigation, reviewing, and editing. MJM contributed through reviewing, editing, and supervision. VLT contributed through reviewing, editing, supervision, and funding.

2.1 Abstract

Amphibian biodiversity is declining globally, with over 40% of species being considered threatened to become extinct. In North America, chorus frogs (Genus: *Pseudacris* Fitzinger, 1843, Family: Hylidae) are common and widely distributed. While relatively abundant, several populations have experienced significant declines in the last 60 years. Captive breeding and reintroductions have been initiated to maintain wild populations while other efforts are taken to identify and mitigate the sources of these declines. Crucial to the success of these initiatives is a comprehensive understanding of life history and reproductive ecology of target species. Most data on chorus frog species were collected prior to the 2000s. Additionally, more recent genetic studies have modified classification of populations of chorus frog species to be reassigned. The combination of historical data in the face of taxonomic reclassification poses challenges to summarizing information on chorus frogs. Here we provide an overview of the *Pseudacris* genus, including breeding behaviour, reproduction, development, survival and longevity. We present an updated distribution map of the 18 species found throughout North America. We also summarize the conservation status at the national and subnational (state, provincial, and territorial) levels, in Canada, USA, and Mexico, to evaluate the relationship between life history traits and extinction risk. Results show a remarkable consistency in the life history traits of *Pseudacris* species considering their relative diversity and wide distribution in North America. However, data are lacking for several species, particularly in the Fat Frog and West Coast clades, causing some uncertainties and discrepancies in the literature. We also found that the most threatened populations of chorus frog were located in the east coast of the USA, potentially as a result of increased levels of anthropogenic disturbance. We suggest that the similarities in life

history traits among chorus frog species provides an opportunity for collaboration and united efforts for the conservation of the genus.

Key words: chorus frogs; life history; distribution; conservation; population management

2.2 Introduction

The biodiversity of wildlife is declining globally (1,2). These declines are related to several factors including habitat destruction, introduced pathogens, and climate change (1,2), and are associated with a loss of ecosystem function (3,4). Amphibian species appear to be affected disproportionately to other taxa (5,6) with over 40% of amphibians considered threatened to become extinct worldwide (6–9). North America is no exception, as amphibian species have experienced drastic declines since the 1960s (10). In the USA, 56 amphibian species are threatened to become extirpated (2), and the average rate of decline of local amphibian populations is almost 4% annually (10). In Canada, 22 amphibian species are listed as "endangered", "threatened", or of "special concern" (11). Mexico supports the greatest number of amphibian species in North America (~372 species), many of which are endemic (12,13). According to Pasquali (14), 220 amphibian species (~60%) are considered at risk of extinction in Mexico. These declines are concerning because amphibians have life stages in both aquatic and terrestrial habitats and hold an important ecological role through supporting services for primary production, decomposition, and nutrient cycling (15,16). Amphibians also act as bioindicators that provide an early warning system to degradations in ecosystem health and environmental change (7,8,17).

Conservation actions such as captive breeding and reintroductions have been initiated to maintain some wild populations, while other efforts are taken to mitigate the sources of

population declines (18,19). The Amphibian Conservation Action Plan was created in 2007 with the goal to preserve amphibian biodiversity worldwide by providing an overview on how to expand knowledge, monitor and document diversity, and respond to threats to amphibian species and their habitats (20,21). Since the Amphibian Conservation Action Plan has been established, amphibian reproduction ex situ in zoos has been prioritized in many regions (19). Specimens are collected and kept in captivity to maintain the genetic diversity of extant populations and to increase population abundance through captive breeding or translocation to new or historical habitats (22). Amphibians are often good candidates for captive breeding because they tend to have higher fecundity, smaller body size, and lower associated costs for husbandry compared to other taxa (18,23). Captive populations are currently maintained in zoos and academic institutions for several North American amphibian species, including northern leopard frogs (*Lithobates pipiens*) (24,25), dusky gopher frogs (*Lithobates sevosus*) (26,27), Wyoming toads (*Anaxyrus baxteri*) (28,29), axolotls (*Ambystoma mexicanum*) (30) and hellbenders (*Cryptobranchus alleganiensis*) (31), amongst others.

Gathering knowledge on natural population dynamics is a crucial initial step before conducting a captive breeding program, because the evaluation of success will be based on parameter values in wild populations. Although every aspect of population ecology has potential to inform recovery strategies, reintroduction success is often evaluated using indicators such as survival rates, demography, and fecundity (32). Therefore, a comprehensive understanding of the life history traits of species of interest is essential for their recovery. This information is ideally gathered when species are abundant or when population declines are first detected before species become imperilled.

Chorus frogs (genus *Pseudacris*: Hylidae) are an example of a clade that is relatively abundant but with several populations that have experienced significant declines (33–36). This species group occurs in North America and is distributed widely across Canada, the United States and Mexico. These frogs are of cultural significance, as a symbol of fertility and renewal (37) and a source of food for indigenous peoples of North Americans (38). The call of groups of male chorus frogs is a familiar sound of spring for people living in suburban areas (39). Curiously, the frog calls heard in many movies and television shows as ambient noise in nighttime scenes is that of the Pacific chorus frog (*P. regilla*) (40). Chorus frogs have also been used as flagship species, representing conservation initiatives. For example, the boreal chorus frog (*P. maculata*) is a symbol for the protection of threatened species in Québec, Canada (41). Chorus frogs play an important role in North American food webs. Larvae consume algae and adults consume insects, while chorus frogs are prey items for birds, fishes, and other animals, thus cycling nutrients between aquatic and terrestrial ecosystems (42). Despite the significance of chorus frogs, there is very limited knowledge on the physiology and ecology of several species. The majority of information for many of the chorus frog species was collected in the first half of the 20th century and requires updating. Significantly, revisions in the nomenclature and phylogenetic assignment make historical accounts confusing and challenging to interpret (43,44), prompting this review of existing information.

Our objectives are to summarize the ecology, life history strategies, and conservation status of North American chorus frogs. First, we present a map of the distribution of the 18 species using the most up to date taxonomic classifications. Second, we present a summary of the general ecology of these species, with a focus on breeding behaviour, reproduction, and development. Third, we review the life history strategies of chorus frogs. We searched databases

(Web of Science and Google Scholar) for articles pertaining to chorus frog species. We highlight the differences among species and taxonomic clades as well as gaps in current knowledge. We also compare the current conservation status of chorus frog species to explore if patterns of distribution and reproductive strategies are associated with extinction risk.

2.3 Methods

2.3.1 Taxonomic note

The taxonomy of the members in the genus *Pseudacris* is widely debated. The nomenclature and species status of many animals within this genus has changed repeatedly over the last 70–80 years (45–48). As such, it is often difficult to determine which species is being described in studies published throughout this time period. To avoid confusion, some authors group closely related species or subspecies into species complexes (*i.e.*, *Pseudacris triseriata* complex) or reinstate historical classification in separate genera (*i.e.*, *Hyliola* for *P. regilla* and *P. cadaverina*) (49–51). Others have split species concepts based on geographic distribution (48). However, recent advancements in genetic sequencing have yielded some insight into this problem with taxonomic classification. Studies from the past 20 years (43,44,52,53) indicate that there are at least 16 species within the genus, which can be separated into four clades of related species: (1) the West Coast clade containing *P. regilla* and *P. cadaverina*, (2) the Fat Frog clade containing *P. ornata*, *P. streckeri*, and *P. illinoensis*, (3) the Crucifer clade containing *P. crucifer* and *P. ocularis*, and (4) the Trilling Frog clade containing *P. brimleyi*, *P. brachyphona*, *P. clarkii*, *P. feriarum*, *P. fouquettei*, *P. kalmi*, *P. maculata*, *P. nigrita*, and *P. triseriata*. These distinctions are based on a combination of nuclear and mitochondrial DNA analyses, and morphological and behavioural data. More recent genetic studies and updates to nomenclature have listed two additional species in the West Coast clade, which are closely related to *P. regilla*; the Sierran

chorus frog (*P. sierra*) and the Baja California chorus frog (*P. hypochondriaca*) (51,54,55). However, these nomenclatural updates are still debated (44) and information regarding life history and reproductive ecology is very limited for these two species. Some authors favour the re-establishment of the genus *Hyliola* for the species within the West Coast clade based on geographic separation from the other species (51). Finally, recent genetic, acoustic, and ecological research on *P. brachyphona* by Ospina et al. (56) suggests that northern and southern populations in this species are distinct. Ospina et al. (56) propose that the southern populations be considered as a separate species, the Collinses' Mountain Chorus Frog (*P. collinsorum*). For the purpose of this review, we have chosen to retain the *Pseudacris* nomenclature, and include *P. sierra* and *P. hypochondriaca* in the West Coast clade, but not include *P. collinsorum* in the Trilling Frog clade given it is not currently recognized by the Society for the Study of Amphibians and Reptiles (57). We will discuss these 18 species (see Supplementary Table S2.1) as they are described by Barrow et al (44), and use descriptions of species' distributions to determine the likely identity of the species when considering articles published prior to 2010s.

2.3.2 Mapping distributions

To map the distributions of the 18 species of North American chorus frog, we downloaded all *Pseudacris* occurrence data recorded on the basis of preserved specimens, material samples, and human or machine observation from the Global Biodiversity Information Facility (GBIF; 61). We removed data that did not contain spatial coordinates or information on the year they were recorded. We also removed records that were not identified to species, and records where the GBIF indicated the spatial coordinate uncertainty was potentially invalid. We also cross-referenced all occurrence points for species with their known distributions according to experts (*i.e.*, Table 2.1 and Table 2.3). Recent genetic studies revealed that the distribution of

P. triseriata in Canada is largely confined to southern Ontario (43,44). Populations of chorus frogs north of Wellington County (Ontario), which were previously believed to be *P. triseriata*, are now known to be *P. maculata*. Therefore, we excluded any *P. triseriata* observations in Canada north of 44 degrees latitude. This resulted in a dataset of 72,199 species observations, collected between 1812–2021. For each species, we created concave hull polygons around the occurrence records, and created buffers around these polygons equivalent to the highest recorded measure of coordinate uncertainty for that species, to a maximum of 100 km. These final polygons represent the best-known distribution of each species according to all available occurrence data. All data were processed using R (version 3.6.1), using the packages tidyverse (59), raster (60), and rgbif (61). Mapping and visualization of spatial data was done in ArcGIS Pro (version 2.5.1).

2.3.3 Life history literature review

We compiled empirical data on the life cycle and population dynamics of the 18 *Pseudacris* species of the genus (43,44,52,62), considering all life stages, from the egg to the adult stage. We employed advanced searches with keywords in Web of Science and Google Scholar (Supplementary Table S2.2) performed between October 2020 and January 2021. We selected these reference databases as they produced more results (number of articles, books, and dissertations included), compared to BioOne, BioRxiv and Science Direct. We did not restrict results by languages or period. After reading the abstract, we retained relevant articles, books, and dissertations that dealt with the survival and the reproductive cycle of wild populations or from lab experiments of the target group. We extracted information pertaining to geographic distribution, breeding season length, fecundity (total number of eggs, number of eggs per cluster), development (time to eggs hatching, time to metamorphosis), stage-specific survival

(eggs, larvae, juveniles, adults), age of maturity, and longevity. We excluded any document that did not clearly present an estimate (*i.e.*, count, proportion, percentage) in the main body of the text, including tables and figures. When estimates of a given parameter were found in several sources, we presented the range of values. We did not distinguish between data obtained under controlled conditions (mesocosm or laboratory) and data from observational field studies, or between different methodologies of data collection.

2.3.4 Conservation status

Global status and population trends were assessed using the Red List of Threatened Species database of the International Union for Conservation of Nature (2). To determine the national and subnational (*i.e.*, provincial or state level) conservation status and distribution of the Pseudacris species, we utilized the NatureServe Explorer database (63). Status ranks are given the prefix code "N" for national status and "S" for subnational status, and a numerical suffix ordered from 1 (critically imperilled) to 5 (stable). Combinations of codes can be used to indicate uncertainty, such as S2S3 representing a status being either imperilled or vulnerable. There are also a series of unique codes (*i.e.*, SH = possibly extinct at the subnational level, SNR = unranked or not assessed at the subnational level). See supplementary materials for a full explanation of status ranks and a list of abbreviations (Supplementary Table S2.3). NatureServe databases primarily contain conservation status and distribution information in the United States of America, Canada, and Latin America. For species that are known to be extant in Mexico, we supplemented the distribution data with information from the USGS Nonindigenous Aquatic Species database (64) and AmphibiaWeb (65). Ranking was then compared to the conversation status as stated by the threatened species legislation of each North American country: Canada (Species at Risk Act, S.C. 2002, c. 29), United States of America (US Endangered Species Act,

1973, 16 U.S.C.), and Mexico (Norma Oficial Mexicana, NOM-059-ECOL-2001, Secretaría de Medio Ambiente y Recursos Naturales 2002).

2.4 Results

Our initial search of life history data using Web of Science and Google Scholar produced a total of 15,464 results, and we retained a total of 109 documents published between 1924 and 2020 for our review. Of these, over two-thirds (67.9%) were published prior to 2000, and nearly a third (31.2%) were published prior to 1970. The most widely distributed Pseudacris species were the most represented in the search; namely *P. crucifer*, *P. maculata*, *P. triseriata*, and the three species originally classified as *P. regilla*. The majority of the information was collected from regions along the coast of eastern USA (24.8% of documents; Florida, Georgia, Maryland, Pennsylvania, New York, New Jersey, North Carolina, South Carolina), in midwestern USA (18.3%; Illinois, Iowa, Michigan, Minnesota, Kansas, Wisconsin), and in California (11.0%). Only two documents (1.8%) featured data from species that occur in Mexico, and five (4.6%) from species in Canada.

2.4.1 Distribution map

Chorus frogs are found throughout Canada, USA and the Baja California peninsula of Mexico (Figure 2.1). West Coast clade frogs are distributed throughout the Pacific coast and western Canada and USA, including British Columbia, Alberta, Washington, Oregon, California, Nevada, and Arizona. Within Crucifer clade frogs, *P. crucifer* is widespread throughout eastern Canada and USA, whereas *P. ocularis* is confined to the southeastern coast of the USA, in Virginia, Georgia, North Carolina, South Carolina, and Florida. Species from the Fat Frog clade are found across central to southern USA, as far north as Illinois (*P. illinoensis*), west throughout Oklahoma, Missouri and eastern portions of Texas (*P. streckeri*), and south and eastward into

northern Florida and the southeastern seaboard. The Trilling Frog clade is the most extensively distributed. In particular, *P. maculata* are a widespread species found throughout central Canada and northeastern USA, from the Northwest Territories in the north, British Columbia, Idaho, Utah and Arizona in the west, to Quebec, Michigan, Indiana, Missouri, and Louisiana in the east. *P. nigrita* and *P. feriarum* occur in the southern and northern portions of the east coast of the USA, respectively. In the southwest of the USA, *P. clarkii* are found in Texas, Oklahoma, Kansas and Tlaxcala (Mexico), whereas *P. fouquettei* are found in Texas, Oklahoma, Alabama, Arkansas, and Mississippi. The most restricted species within the Trilling Frog clade is *P. kalmi*, which are found in small portions of Delaware, Maryland, New Jersey, Pennsylvania, and Virginia.

2.4.2 General ecology and life history traits

Morphology

Chorus frogs are small-bodied (approximately 2–4 cm, 1–5 g as adults), often heard but rarely seen (41,49). They tend to be slender with a slim waist and long limbs (Figure 2.2). Toe discs are small with minimal webbing between digits (49,66,67). Most species have a light line on the upper lip (49,66,67). Males possess a single, round vocal patch, which is yellow, grey or brown over a lighter background colour. Bellies tend to be free of pigmentation. Both sexes often have dorsal patterns with rows of dark spots, stripes, or a cross (“X”) over a brown, green, or cream body coloration. However, coloration may be highly variable, even within populations of the same species (67,68). Some species, such as *P. regilla* and *P. sierra*, may be able to change colour within a season (68–70). Albinism (lack of pigment) and erythrism (red pigmentation) have also been recorded in chorus frogs (71–73). Lemmon et al. (53) provide an excellent comparison of morphology among several species in the Trilling Frog clade.

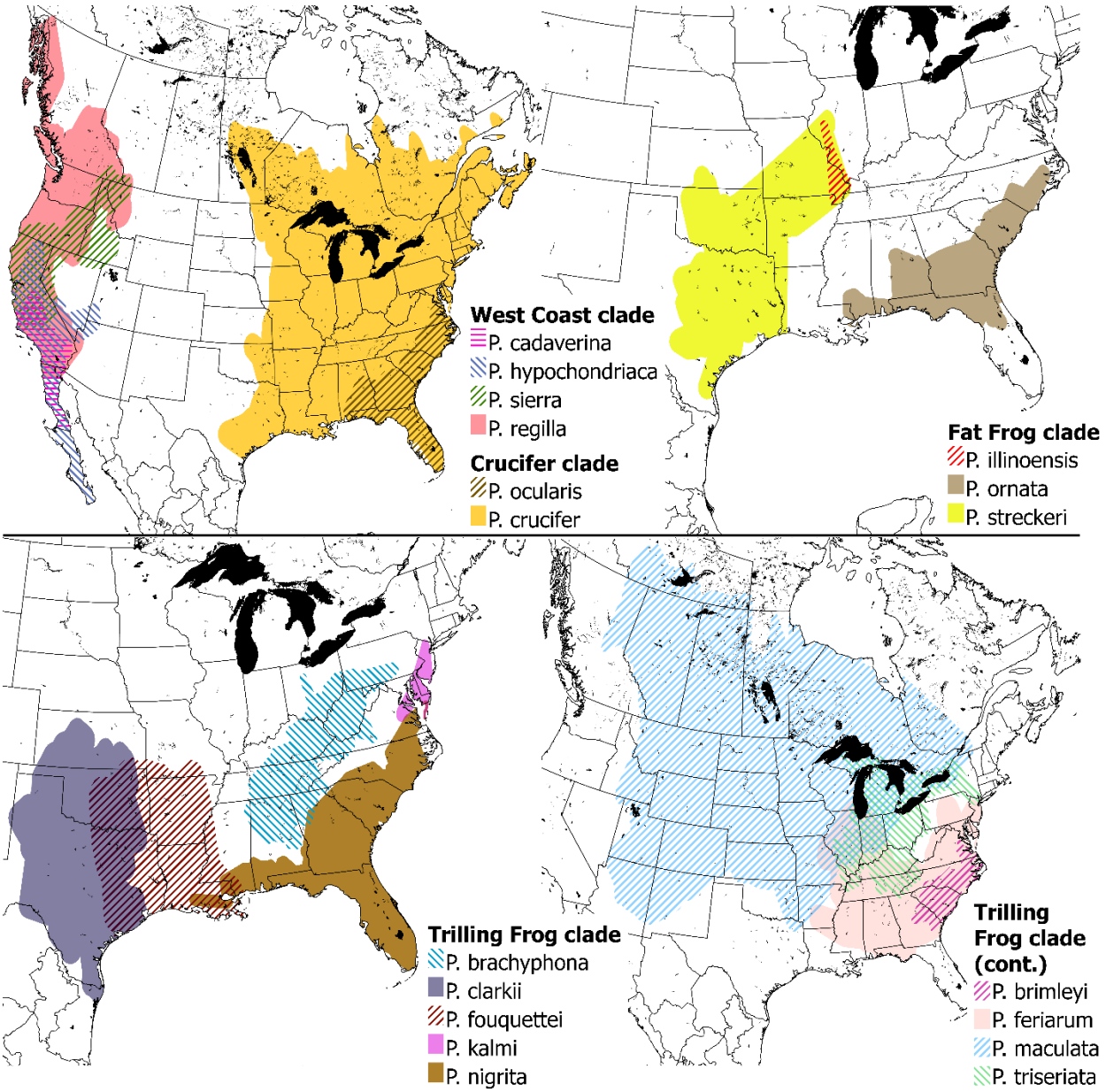

Figure 2.1: Distribution of the 18 species of North American chorus frogs (genus *Pseudacris*: Hylidae), separated by phylogenetic clade. Distribution is based on occurrence data from the Global Biodiversity Information Facility website (58).

Figure 2.2: Adult male boreal chorus frog (*Pseudacris maculata*), Great Lakes/St. Lawrence – Canadian Shield population, reared in captivity. Age = 10 months. Snout-vent length = 29.9

Table 2.1: Summary of life history traits of *Pseudacris* species in North America, separated by clade. See supplementary materials (Table S2.3) for list of abbreviations

Clade	Species	Distribution	Breeding Season	No. of eggs	No. eggs per cluster	Time to hatch	Time to metamorphose	References
West Coast	*P. cadaverina*	**US**: CA **MEX**: BCN	Feb-Oct	-	1–2 eggs	-	40–75 days	(50)
	P. hypochondriaca	**US**: AZ, CA, NV, UT **MEX**: BCN, BCS	Nov-July	400–750	9–80 eggs	2–9 days	60–75 days	(50,100,198)
	P. sierra	**US**: CA, ID, MT, NV, OR, UT	Nov-July	400–750	9–80 eggs	-	60–65 days	(50,100,199)
	P. regilla	**US**: AK, CA, MT, OR, WA **CAN**: BC	Nov-July	400–750	9–80 eggs	7–21 days	52–70 days	(66,92,100,200–204)
Fat Frog	*P. illinoensis*	**US**: AR, IL, MO	Feb-March	200–1000	8–79 eggs	-	-	(205–209)
	P. ornata	**US**: AL, FL, GA, LA, MS, NC, SC	Nov-March	10–106	20-40	~7 days	~90 days	(76,210,211)
	P. streckeri	**US**: AR, IL, KS, LA, MO, OK, TX	Nov-March	≤ 600	-	2–5 days	~60 days	(66,212,213)
Crucifer	*P. crucifer*	**US**: AL, AR, CT, DE, FL, GA, IA, IL, IN, KS, KY, LA, MA, MD, ME, MI, MN, MO, MS, NC, NH, NJ, NY, NY, OH, OK, PA, RI, SC, TN, TX, VA, VT, WI, WV **CAN**: MB, NB, NL, NS, ON, PE, QC	Nov-June	~700	2–3 eggs	6–15 days	~90 days	(66,127,214–218)
	P. ocularis	**US**: AL, FL, GA, NC, SC, VA	Jan-Sept	≤ 200	1–25 eggs	1–2 days	7–70 days	(66,88,216,219)

Note: 2-letter state/province codes for USA and Canada, and 3-letter state codes for Mexico as per ISO 3166-2 (i.e., BCN = Baja California, BCS = Baja California Sur, TLA = Tlaxcala)

Table 2.1 (continued): Summary of life history traits of *Pseudacris* species in North America, separated by clade. See supplementary materials (Table S2.3) for list of abbreviations.

Clade	Species	Distribution	Breeding Season	No. of eggs	No. eggs per cluster	Time to hatch	Time to metamorphose	References
Trilling Frog	*P. brachyphona*	**US**: KY, MD, OH, PA, TN, VA, WV	Feb-June	300–1500	4–144 eggs	7–10 days	30–64 days	(129,132,155,217,220)
	P. brimleyi	**US**: GA, NC, SC, VA	Feb-April	≤ 300	-	-	30–60 days	(221)
	P. clarkii	**US**: KS, OK, TX **MEX**: TLA	Jan-June	~1000	3–60 eggs	2–3 days	30–45 days	(66,82,153,222)
	P. feriarum	**US**: AL, DC, FL, GA, IL, KY, MD, MO, MS, NC, NJ, PA, SC, TN, VA, WV	Feb-May	≤ 1000	40–60 eggs	7–14 days	40–90 days	(217,223)
	P. fouquettei	**US**: AR, LA, MO, MS, OK, TX	Jan-May	500–1500	-	2–3 days	-	(53,67,81,224,225)
	P. kalmi	**US**: DE, MD, NJ, PA, VA	Feb-April	500–1500	6–20 eggs	7–14 days	40–90 days	(126,226)
	P. maculata	**US**: AZ, CO, IA, ID, IL, IN, KS, MI, MN, MO, MT, ND, NE, NM, NY, OK, SD, UT, VT, WI, WY **CAN**: AB, BC, MB, NT, ON, QC, SK, YK	Feb-April	137–793	5–100 eggs	10–14 days	~60 days	(67,78,227–229)
	P. nigrita	**US**: AL, FL, GA, LA, MS, NC, SC, VA	Dec-Sept	≤ 180	6–176 eggs	2–3 days	40–120 days	(66,76,89,90,125,126,131,222)
	P. triseriata	**US**: IL, IN, KY, MI, NY, OH, PA **CAN**: ON	Jan-June	440–1500	20–70 eggs	3–27 days	40–90 days	(66,71,168,230)

Note: 2-letter state/province codes for USA and Canada, and 3-letter state codes for Mexico as per ISO 3166-2 (i.e., BCN = Baja California, BCS = Baja California Sur, TLA = Tlaxcala)

Timing of breeding

Like most anurans, chorus frogs are described as polygynous, or "lekking" species (74,75). Chorus frogs are also iteroparous, although mortality is very high in the first year, so many individuals only participate in a single breeding season during their lifetime (76–78). More recent studies suggest that the proportion of chorus frog that breed more than once is greater than previously thought (79,80). Thus, long-term studies are required to evaluate the contribution of individuals to the reproductive effort across several breeding seasons. Although most chorus frogs reach sexual maturity by the end of the first summer, individuals generally do not breed during the first year (71). Species in this genus apparently capitalize on "cold weather breeding" in late winter and early spring to avoid competition with other hylid frogs (52). The timing of reproduction and calling behaviour is influenced by rainfall and temperature (81–83). However, environmental variables are not the sole determinants of reproduction and calling behaviour, as indicated by the asynchrony of timing of reproduction of sympatric species (66,71,75,76,84). Breeding seasons are highly variable (Table 2.1), generally reaching its peak in March–April in eastern regions, December–February in southern and western regions, and can occur over a prolonged period (74,75,82,85). For example, several species with southerly distributions can be observed breeding almost year-round, beginning as early as October and extending into the summer of the following year (*P. cadaverina*: (86,87); *P. ocularis*: (88); *P. ornata*: (66); *P. nigrita*: (89,90); *P. regilla*: (91,92); *P. streckeri*: (84,93,94)).

At the beginning of the breeding season, males gather in large groups shortly after emerging from hibernation, and remain within the breeding habitat for four to ten weeks (71,76,95). Conversely, females are often present in the breeding habitat for only a few nights for up to two weeks (71,76). Sex ratios on breeding grounds are generally biased towards males

(36,76,96,97). After spawning concludes, males will continue to call to attract more mates, whereas females will return to terrestrial habitat after oviposition. Both males and females are capable of mating with multiple individuals, but for the majority of species usually only one clutch of eggs will be produced per breeding season (75,98). However, multiple clutches have been observed in *P. triseriata* (99), and *P. regilla* (and potentially *P. sierra* and *P. hypochondriaca*) may produce as many as three egg clutches in a season (100). Recently, Goldberg (85) reported that chorus frogs can spawn twice in the same breeding season. Indeed, female *P. streckeri* specimens collected in Oklahoma had both mature and post-ovulatory follicles in the same ovary, indicating multiple spawning events within a single breeding season (85).

Breeding habitat

Reproduction is aquatic in all species in the *Pseudacris* genus. A wide variety of shallow water habitats, both natural and artificial, are utilized for breeding (67). The majority of species use temporary or semi-permanent water bodies that are relatively free of predators and heterospecific competitors (101,102). Breeding habitats include temporary ponds, roadside ditches, flooded meadows, shallow bogs and marshes, buffalo wallows, furrows in plowed fields, glacial kettlepots, as well as ephemeral pools and vernal pools in woodlands (49,66,71,103,104). Most breeding sites are lentic freshwater systems, but Pacific chorus frogs (*P. regilla*) and California tree frogs (*P. cadavernia*) also breed in small, slow-moving streams (66,86,87).

Calling behaviour

Reproductive behaviour is initiated by males vocalizing, and long periods of calling likely have a role on circulating reproductive hormone concentrations and in maintaining sexual arousal in females (95,105). Chorus frogs get their common name from their calling behaviour

(66,106). When a sufficient number of males have gathered and are calling, a chorus of near continuously calling individuals is established (95,107). These aggregations of calling males allow females to assess the relative quality of potential mates, and for males to assess the quality and competitive abilities of other males (95). In several species (*i.e.*, *P. crucifer*, *P. regilla*, *P. triseriata*), males produce a variety of calls, including advertisement calls and courtship calls (95,108). Advertisement calls are long-range vocalizations that signal the position of a male to other males, and to attract females. Courtship calls are short-range vocalizations produced by males that are directed towards nearby females to indicate an "eagerness" to mate (95,108).

Male *Pseudacris* frogs produce either a series of repeated single notes, whistles, or a long trill (95). The advertisement call of male boreal chorus frogs (*P. maculata*) is described as a series of pulses, 750–905 ms in duration, produced at a rate of approximately 16 pulses s-1 (109). Calls are similar among species within the Trilling Frog clade, but see Cocroft and Ryan (110) for comparisons of temporal and spectral properties of calls among species within the Trilling Frog clade. The call of *P. brachyphona* is more rapid and high pitched, and described as "quack like" rather than a trill (111). Within the West Coast clade, calls consist of a one- or two-phase "rib-bit", which contains a series of pulses, approximately 232–245 ms in duration, delivered at a rate of 86–90 pulses s-1 (112). Species in the Fat Frog clade produce very short whistles (30–60 ms) repeated in quick succession (*P. ornata* and *P. streckeri*) (93).

Pulse rate and call duration are important properties for species recognition in mixed species assemblages (98,109,113). However, there is plasticity in calling behaviour, with the properties of calls influenced by environmental conditions, temperature being predominant (95,110,114). Patterns in acoustic signals are also influenced by the social context. For example, male spring peepers (*P. crucifer*) produce tone-like "peeps" when calling in a chorus

(advertisement call), but produce trill-like calls of short pulses (aggressive call) when in close proximity to a competing male (115). Males also increase the duration and intensity of their advertisement calls as the spacing between males decreases (116). Similar patterns have been noted in other *Pseudacris* species (117).

Some male individuals may adopt a non-calling strategy (118). These silent males are often referred to as satellites and associate closely with a calling male (95). Unlike in other anuran species, this behaviour is apparently not size specific nor associate with "inferior" males that cannot effectively compete (119). Individual males may switch between the calling or non-calling strategy within a single night (99,120–122). Presumably, this strategy is used to intercept females as they approach a calling male (123). However, an alternative hypothesis is that these males remain silent to conserve energy while waiting for calling territories to become available (99).

Amplexus

Consistent with other genera in the Hylidae family, *Pseudacris* species perform axillary amplexus. The male mounts the female, grasps her directly behind the forelimbs, with the male cloaca positioned above the female cloaca (75,95,124). This behaviour is initiated by female contact, indicating receptivity. Males that attempt to mount an unreceptive female are quickly dissuaded by the female moving away, although this avoidance behaviour is not always successful (99,125). Amplexus usually only occurs at night, but *P. kalmi* have been observed in amplexus during the day (126). Observations of *P. crucifer* (127) and *P. triseriata* (126) suggests that ovulation precedes amplexus. Mates remain in amplexus between a few hours up to 40 hours, as observed in *P. regilla* (128). Amplexus behaviour is concurrent with oviposition. Prior to oviposition, *P. nigrita* females perform "spasmodic" abdominal contractions (125).

Oviposition

As the *Pseudacris* female releases her eggs, she will arch her back bringing her cloaca in close proximity to the male cloaca (105,125). For the majority of species, this behavior occurs as the female straddles some form of submerged vegetation to which the eggs are attached (71,125,129). The duration of oviposition is variable and often occurs in several successive events over the course of 2–3 hours, with the female and male in amplexus moving between locations (67,95,125). Eggs are laid singly or in small clusters, depending on the species (Table 2.1). Whitaker (71) noted that egg-laying in *P. triseriata* occurred at temperatures > 10°C, and often after rainfall. Clutch size, or the full complement of eggs deposited as one to several masses, is relatively small in comparison to related taxa, such as treefrogs in the genus *Dryophytes* (=*Hyla*) that can have clutches of 2,000 to 4,000 eggs (130). For example, Southern chorus frogs (*P. nigrita*) lay ≤ 160 eggs in a series of masses of approximately 15 eggs (125,131). At the other extreme, several species of the former "*P. triseriata* complex" including *P. feriarum*, *P. kalmi*, *P. maculata*, and *P. triseriata* deposit up to 1500 eggs in masses of approximately 10–80 eggs (104). Similarly, large clutches (1479 eggs) have also been observed in *P. brachyphona* (132). Oviposition behaviour can be altered in response to predators and competition (133–135). Buxton et al. (136) found that female *P. triseriata* lay fewer eggs in experimental ponds that contained western mosquitofish (*Gambusia affinis*) than females in ponds that were fish-free. Ouellet et al. (101) observed that *P. maculata* breeding sites in Québec (Canada) were generally devoid of predatory fish. Reproductive investment and fecundity are associated with body size in several frog species, including those in the families Hylidae, Leptodactylidae, Microhylidae, Ranidae and Rhacophoridae (137–140). Duffitt and Finkler (141) found that, prior to reproduction, larger males and females of *P. crucifer* and *P. triseriata* allocate more energy to

courtship activity and gamete production, respectively, than smaller individuals. Ovarian mass is positively correlated with body size in both species, and the gonadal-somatic index is positively correlated with body size in *P. crucifer* (141).

Development

Eggs generally hatch within two weeks of being deposited, but can range from 2 to 27 days (104,142). As with many amphibian species, egg and tadpole development depends on water temperature, hydroperiod and other environmental conditions (71,143–145). At metamorphic emergence, *P. brachyphona* and *P. crucifer* have a balanced sex ratio (127,132). Larvae are generalist feeders, indiscriminately consuming a variety of items including detritus, algae, and other periphyton associated with submerged vegetation (104,142), as well as small quantities of pollen and invertebrates (146–148). The larval period is short in most species, with metamorphosis (Gosner stage 46) occurring 30 to 90 days after hatching (104). To assess the influence of hydroperiod on tadpole development, Amburgey et al. (145) collected boreal chorus frog (*P. maculata*) tadpoles from permanent and temporary ponds (Gosner stage 24–31) and then subjected tadpoles to one of three hydroperiod regimes. Whereas the hydroperiod treatment did not influence development rate, tadpoles collected from permanent ponds matured and metamorphosed faster than those collected from temporary ponds. The authors hypothesized that developmental rates are influenced by predation level as a wider variety of predators are more likely to be found in larger and more permanent water bodies (145).

Migration and hibernation

After reaching metamorphosis, juvenile frogs remain near natal ponds for several weeks and then migrate a short distance (< 500 m) into more terrestrial habitats close to water (50,67,78). Migration distance varies between populations and depends on the distribution of

suitable habitats (149,150). The majority of pond-breeding amphibians are highly philopatric (150). Since most *Pseudacris* species utilize temporary bodies of water, individuals may be philopatric to a general area rather than a specific water body and regularly switch ponds. This pattern is especially common in regions where stochastic environmental or anthropogenic conditions result in ponds regularly being created or drying up (150,151). Juvenile habitat is largely similar to adult habitat, but has not been extensively studied in any species (50).

Based on observations of *P. clarkii*, *P. crucifer*, and *P. ocularis*, adult chorus frogs are primarily terrestrial, only found in aquatic environments during breeding, and will migrate short distances away from ponds and pools after spawning (152,153). Adults generally remain within 100 m of breeding ponds during the spring and summer and rarely migrate >200 m within a single generation (*P. triseriata*: (154); Trilling Frog clade: (62)). Conversely, Green (132) observed migrations of up to 610 m within a single breeding season and up to 1219 m between breeding seasons in mountain chorus frogs (*P. brachyphona*).

Most populations of chorus frogs enter torpor and overwinter in terrestrial habitats, either underground or under logs, rocks, and leaf litter (50,155,156). Chorus frogs may migrate short distances to hibernation sites but are generally found emerging from locations close to breeding sites (71,154). Spring peepers (*P. crucifer*), Pacific tree frog (*P. regilla*), Western chorus frogs (*P. triseriata*), and boreal chorus frogs (*P. maculata*) tolerate temperatures below 0ºC. These species produce a glucose-based cryoprotectant limiting cell volume reduction and preventing intracellular freezing during sub-zero temperatures. (157–163). It is unclear whether *Pseudacris* species with southern distributions have the ability to utilize similar freeze tolerance or freeze avoidance mechanisms. Indeed, not all species are thought to hibernate. Some populations of the

ornate chorus frog (*P. ornata*) and little grass frog (*P. ocularis*) are active during the winter months and may even breed during this time (88,164,165).

2.4.3 Stage-specific survival probability

Eggs and larvae

Mean survival probability was highly variable among species and published studies (Table 2.2). The majority of data on egg and larval survival probability have been collected with species in the Trilling Frog clade, and we did not find any survival estimates on several species, including *P. cadaverina*, *P. brachyphona* and *P. brimleyi*. Development and survival probabilities in the aquatic stages depend on several abiotic and biotic factors, such as predation and competition rates, hydroperiod, and water quality (145,166). In general, survival probabilities are higher in controlled settings compared to natural conditions as eggs and larvae are able to develop without predation pressures and risks of desiccation, and with more stable environmental conditions (167). In a natural population, Whiting (78) reported a mean survival probability of only 0.05 (*P. maculata*). Most studies reviewed measured the hatching success by transferring eggs or larvae into a controlled environment (129,168–171). Even when major threats are eliminated in controlled environments, *Pseudacris* species can experience high rates of mortality between hatching and the end of the larval period. For example, survival probability of eggs was estimated to be 0.39 for *P. clarkii* (171). Survival probabilities can also be relatively high in natural settings. In *P. triseriata* reared in natural ponds, Kramer (172) reported a mean survival probability of approximately 0.62 for eggs, and Smith (173) reported a survival probability from larvae to metamorphosis between 0.25 and 0.90. Due to the lack of data, comparisons among species and clades during the aquatic stages are very limited.

Table 2.2: Summary of survival probabilities (φ) and longevity in frog species in the genus *Pseudacris*, separated by clade. To simplify the table, male and female survival parameters have been grouped.

Clade	Species	ϕ eggs	ϕ larvae	ϕ juveniles	ϕ adults	Lifespan	Age at maturity	References
West Coast	*P. cadaverina*	-	-	-	-	-	-	-
	P. hypochondriaca	0.85–0.95	-	-	0.01–0.3	-	1 year	(92,231)
	P. sierra	-	0.90–0.95	-	-	-	-	(199)
	P. regilla	-	-	-	-	1–3 years	1-3 year	(198,232,233)
Fat Frog	*P. illinoensis*	-	-	0.03–0.04	0.28	2–6 years	1 year	(36,177,185,206,209,234)
	P. ornata	-	0.94–0.97	0.32–0.85	0.52	-	-	(76,143,235)
	P. streckeri	-	-	-	-	1–3 years	-	(85,236)
Crucifer	*P. crucifer*	0.52	0.5–0.9	0.25	0.25	4 years	2 years	(67,130,170,184,226,233,237–242)
	P. ocularis	-	0.1	-	-	-	-	(219)
Trilling Frog	*P. brachyphona*	-	-	-	-	-	-	-
	P. brimleyi	-	-	-	-	-	-	-
	P. clarkii	0.39	0.22–0.84	-	-	1–2 years	-	(171,236,243,244)
	P. feriarum	0.77	0.10–0.89	-	-	-	-	(169,245)
	P. fouquettei	-	-	-	-	-	-	-
	P. kalmi	-	-	-	-	-	-	-
	P. maculata	0.4–0.9	0.3–0.9	0.09–0.13	0.14–0.49	2–7 years	1 year	(67,78–80,145,168,170,176,186,228,229,246,247)
	P. nigrita	-	-	-	0.28	1–3 years	-	(76)
	P. triseriata	0.37–0.87	0.9	0.06–0.13	0.19	1–3 years	1–2 years	(71,76,77,172,238)

Table 2.3: Summary of the national and subnational status of frog species in the genus *Pseudacris*, separated by clade.

Clade	Species	National Distribution	IUCN Status	IUCN Trend	NatureServe Subnational Status Rank (CAN & USA)
West Coast	*P. cadaverina*	USA, MEX	Least Concern	Stable	**CA**: SNR
	*P. hypochondriaca**	USA, MEX	Least Concern*	Stable*	**CA, NV**: SNR; **UT**: SU; **AZ**: S3
	*P. sierra**	USA	Least Concern*	Stable*	**CA, OR**: SNR; **UT**: SH; **MT**: S4; **ID, NV**: S5
	P. regilla	USA, CAN	Least Concern	Stable	**AK, CA**: SNR; **MT**: S4; **BC, OR, WA**: S5
Fat Frog	*P. illinoensis*†	USA	Least Concern†	Unknown†	**AR**: S1; **MO**: S2; **IL**: S2S3
	P. ornata	USA	Least Concern	Stable	**LA**: SH; **MS**: S1; **NC**: S2; **FL**: S2S3; **SC**: S3S4; **AL, GA**: S5
	P. streckeri	USA	Least Concern	Unknown	**IL, MO, OK**: SNR; **LA**: S1; **AR, KS**: S2; **TX**: S3
Crucifer	*P. crucifer*	USA, CAN	Least Concern	Stable	**FL, IN, OH, OK, SC**: SNR; **NL**: S1S2; **KS**: S3; **DC, IA, MN**: S4; **MB, NB, NS, ON, PE, QC, AL, AR, CT, DE, GA, IL, KY, LA, ME, MD, MI, MS, MO, NH, NJ, NY, NC, PA, RI, TN, TX, VT, VA, WV, WI**: S5
	P. ocularis	USA	Least Concern	Stable	**SC**: SU; **FL**: SNR; **AL**: S1; **VA**: S3; **GA**: S4S5; **NC**: S5
Trilling Frog	*P. brachyphona*	USA	Least Concern	Unknown	**OH**: SNR; **MD**: S1; **GA, NC, PA**: S2; **MS**: S3; **TN, VA, WV**: S4; **KY**: S5
	P. brimleyi	USA	Least Concern	Stable	**SC**: SNR; **GA**: S1; **NC, VA**: S4
	P. clarkii	USA, MEX	Least Concern	Stable	**OK**: SNR; **KS, TX**: S5
	P. feriarum	USA	Least Concern	Stable	**NJ**: SU; **FL**: SNR; **PA**: S1; **DC, WV**: S3; **IL**: S4; **AL, GA, KY, MD, MS, MO, NC, SC, TN, VA**: S5
	P. fouquettei	USA	Least Concern	Stable	**TX**: SU; **MS, MO**: SNR; **OK**: S3; **AR, LA**: S5
	P. kalmi	USA	Least Concern	Stable	**VA**: SNR; **PA**: S1; **NJ**: S3; **DE, MD**: S4
	P. maculata	USA, CAN	Least Concern	Stable	**ND, OK**: SNR; **MI, VT**: S1; **YT**: S1S2; **QC, IN**: S2; **NY**: S2S3; **NM**: S3; **ID, IA, ON, UT**: S4; **BC, NT**: S4S5; **AB, MB, ON, SA, AZ, CO, IL, KS, MN, MO, MT, NE, SD, WI, WY**: S5
	P. nigrita	USA	Least Concern	Stable	**FL, LA, SC**: SNR; **NC**: S2; **VA**: S3; **AL, GA, MS**: S5
	P. triseriata	USA, CAN	Least Concern	Decreasing	**QC, IL, OH**: SNR; **PA**: S1; **NY**: S2S3; **ON, IN**: S4; **KY, MI**: S5

NatureServe subnational ranks range from most at risk of extinction (critically imperilled; S1) to least at risk of extinction (stable; S5), and include ranks for species that are unrankable (SU), currently unranked (SNR), or presumed to be extirpated (SH). Multiple ranks combined (i.e., S2S3) indicate uncertainty of conservation status. See Supplementary materials (Table S2.3-S2.4) for a more detailed descriptions of ranking and a full list of abbreviations. * = species considered a subspecies of *P. regilla* by IUCN. † = species considered a subspecies of *P. streckeri* by IUCN.

Juveniles

There is considerable uncertainty in survival probabilities of juvenile chorus frogs, a pattern that is observed for many amphibians (174,175). The complexity of marking and recapturing metamorphic and juvenile anurans make estimating survival very difficult (176). For many chorus frog species, data are lacking. Studies that estimated juvenile survival probabilities in natural environments found that only a small proportion of froglets reach the adult stage. For example, in a study on *P. illoniensis*, Tucker (177) estimated a survival probability from metamorphosis to sexual maturity to be only 0.03. Whiting (78) estimated juvenile survival probability to be approximately 0.09–0.13 (*P. maculata*), whereas Smith (77) found survival probability of juveniles to adulthood was approximately 0.19 (*P. triseriata*). However, these three authors did not correct for imperfect detection probabilities, so actual survival could be very different from the reported estimates (178,179). Advancements in mark and recapture technology, such as small, light-weight visible implant elastomer tags (180,181) and alpha tags (182) offer the possibility of improved juvenile population estimations.

Adults

We found survival estimates for seven (38%) of the 18 *Pseudacris* species. For these species, the probability of survival varied between 0.01 and 0.52. Studies on the same species report conflicting adult survival rates. For example, Muths et al. (79) estimated that mean adult survival probability in *P. maculata* was approximately 0.51 (both sexes combined), whereas survival estimates from Whiting (78) ranged from 0.25 to 0.27 in males and 0.36 to 0.50 in females. These discrepancies could be due to different analytical approaches: Muths et al. (79) used a formal capture-mark-recapture model, whereas Whiting (78) used an ad hoc estimate of survival that did not account for recapture probability. Most studies on *Pseudacris* are relatively

short in duration, spanning only 2–3 years, and may not accurately capture variability of survival among years. Notable exceptions are the 30-year studies on two populations of *P. maculata* in Colorado, USA by Muths et al. (79) and Kissel, Tenan and Muths (80). Between years, Muths et al. (79) observed highly variable survival probabilities ranging from 0.19 to 0.76. Therefore, studies on adult survival probability should extend several years to capture variation in environmental conditions (hydroperiod, temperature, predation) and their impact.

2.4.4 Longevity and iteroparity

The majority of studies indicate that *Pseudacris* species have a lifespan between 1–3 years (71,76,78,92,183). However, several studies suggest longevity in chorus frogs is underestimated. Using skeletochronology, Lykens and Forester (184) estimated that *P. crucifer* could live for 4 years (n = 3 individuals, out of 43 studied). Using capture-mark-recapture methods, Tucker et al. (185) reported that some adults of *P. illinoensis* reached 6 years (mean 2–3 years). Using a similar approach, Muths et al. (79,186) recaptured tagged female *P. maculata* that were 7 years old. Together, this indicates that chorus frogs have a lifespan beyond the previously believed 1–3 years, but that individuals experience low survival between breeding seasons. Longevity estimates may be male-biased, as males are captured more easily during reproduction than females (79). Conversely, if females occur close to a breeding site during several consecutive years, it may be assumed that females attempt breeding at least twice within their lifespan (Muths E., *pers. com.*).

2.4.5 Conservation status

All 18 species in the genus of *Pseudacris* are currently classified as “least concern” by the International Union for the Conservation of Nature (2). Global population trends are considered “stable” for the majority of species (Table 2.3). However, the IUCN states that

population trends are unknown for *P. brachyphona*, *P. illinoensis*, and *P. streckeri*, and considered decreasing for *P. triseriata*. Currently, IUCN considers *P. hypochondriaca* and *P. sierra* as subspecies of *P. regilla*, and *P. illinoensis* as a subspecies of *P. streckeri*. It is possible that the rankings and population trends of these species could change if assessed separately. According to Recuero et al. (48), even if the three members of the *P. regilla* complex were considered separate species by the IUCN, they would still likely be classified as "least concern". The patterns reported by the IUCN concur with the status designated by the governments of Canada, USA, and Mexico. All three species that occur in Mexico (*P. cadaverina*, *P. hypochondriaca*, *P. clarkii*) have a status of "least concern" despite observed declines and persistent threats to populations of *P. hypochondriaca* (187). In Canada, the Great Lakes/St. Lawrence – Canadian Shield population of *P. maculata* (distributed in Ontario and Québec) is designated as threatened and is listed under the Species at Risk Act (188). Sub-nationally in Québec, *P. maculata* is listed as vulnerable (high risk of extirpation) under the Act Respecting Threatened or Vulnerable Species (R.S.Q., c. E-12.01), as the species is estimated to occupy only 10% of its historical range (189,190). The population was previously designated as *P. triseriata* (191,192), which may contribute to why the IUCN now considers the species populations to be declining. None of the species that occur in the USA are listed under the Endangered Species Act (193), but the status of *P. illinoensis* is currently under review by the US Fish and Wildlife Service (194). According to NatureServe (63) databases, all species are "secure" at the national level, with the exception of *P. illinoensis* (N3 = Vulnerable), *P. kalmi* (N4 = Apparently Secure), and *P. cadaverina* (N4 = Apparently Secure). However, several populations are considered critically imperiled, or at a very high risk of being extirpated, at the subnational level, including P. *streckeri* (in Louisiana), *P. ocularis* (in Alabama), *P. brachyphona* (in Maryland), *P. brimleyi*

(in Georgia), *P. feriarum* (in Pennsylvania), *P. kalmi* (in Pennsylvania), *P. maculata* (in Michigan and Vermont), and *P. triseriata* (in Pennsylvania). Populations of *P. illinoensis* in Illinois are classified as threatened (195) and the species has a very restricted distribution in Arkansas (183).

2.5 Conclusion

The current state of knowledge on the ecology, life history strategies and conservation status of North American chorus frogs has been reviewed. We found that there is a remarkable consistency in the life history traits of *Pseudacris* species, considering their relative diversity and wide distribution in North America. Whereas no major differences in life history traits emerge among species, the distribution of the populations appears to impact clutch size and development. Within a species, females in warmer, more southern populations tend to have smaller clutches of eggs, but the extended breeding season allows for multiple clutches within a single season. Therefore, total annual egg production amongst species is very similar. Eggs of populations in warmer climates also hatch sooner and develop more quickly than those in more temperate climates. No clear associations between conservation status and life history strategies were detected. While many populations of species within the Trilling Frog clade are considered critically imperilled at the subnational level (Table 2.3), we speculate that this is likely a result of restricted distribution and increased local threats in distal portions of the species' range. The majority of the populations in decline occur in the east coast of the USA. For example, three species (P. *feriarum*, *P. kalmi*, and *P. triseriata*) are at a high level of risk of extinction in Pennsylvania. The factors contributing to this extinction risk in Pennsylvania may be related to the spread of disease, the high human population density (9th highest of the 50 US states), and increased levels of anthropogenic disturbance (196), but this should be investigated extensively.

The most striking finding in our review is the scarcity of data on the egg, larval, and juvenile life stages. For many species there are no data available on the number of eggs laid or the length of the embryonic period, particularly in the West Coast and Fat Frog clades (see *P. cadaverina* and *P. illinoensis* in Table 2.1). Data are lacking for estimates of stage-specific survival rates and longevity for most chorus frog species (Table 2.2). More than two-thirds of data that have been collected prior to the 2000s, highlighting the need for a reassessment addressing the recent updates to phylogeny (43,44,52,53). The focus should shift to species that have been historically underrepresented or have been conflated with other species, including those recently elevated to species status (*i.e.*, *P. fouquettei*, *P. hypochondriaca*, *P. illinoensis*, *P. sierra*).

Information on life history traits is critical for understanding the ecology of chorus frogs and will improve our understanding of how environmental threats impact populations. Empirical data are also required for species conservation and mitigation efforts, to prevent further declines in regions where populations appear relatively stable or unaffected, maintaining common species common (197). We have found that there are many similarities in life history traits among species in the *Pseudacris* genus. Chorus frogs may therefore be generally susceptible to the same anthropogenic disturbance and changing climate patterns due to their characteristic cold weather breeding strategy and reliance on temporary wetlands. More promising is that the strong similarities in life histories and reproductive ecology of the 18 identified *Pseudacris* species suggests that recovery strategies we can develop for one species could be more broadly applicable. Thus, the data collected on species (or populations) that are currently stable can inform and benefit conservation efforts on species and populations declining elsewhere in North

America. There is great potential for meaningful and impactful collaboration among research and conservation groups throughout the continent.

2.6 References

1. Wake D, Vredenburg V. Are we in the midst of the sixth mass extinction? A view from the world of amphibians. PNAS. 2008;105:11466–73.
2. International union for the conservation of nature red list. Version 2020-3 [Internet]. 2020. Available from: https://www.iucnredlist.org/statistics
3. O'Connor NE, Crowe TP. Biodiversity loss and ecosystem functioning: distinguishing between number and identity of species. Ecology. 2005;86(7):1783–96.
4. Heilpern S, Weeks B, Naeem S. Predicting ecosystem vulnerability to biodiversity loss from community composition. Ecology. 2018;99:1099–107.
5. Stuart S, Chanson J, Cox N, Young B, Rodrigues A, Fischman D, et al. Status and trends of amphibian declines and extinctions worldwide. Science. 2004;306:1783–6.
6. Global assessment report on biodiversity and ecosystem services of the intergovernmental science-policy platform on biodiversity and ecosystem services. IPBES secretariat, Bonn; 2019.
7. Wake D. Declining amphibian populations. Science. 1991;253:5022.
8. Houlahan JE, Findlay CS, Schmidt BR, Meyer AH, Kuzmin SL. Quantitative evidence for global amphibian population declines. Nature. 2000;404(6779):752–5.
9. Bishop PJ, Mainguy G, Angulo A, Lewis JP, Moore RD, Rabb GB, et al. The amphibian extinction crisis - what will it take to put the action into the amphibian conservation action plan? 2012;16.
10. Grant E, Miller D, Schmidt B, Adams M, Amburgey S, Chambert T, et al. Quantitative evidence for the effects of multiple drivers on continental-scale amphibian declines. Sci Rep. 2016;6:25625.
11. Species at risk registry - species search [Internet]. Committee on the Status of Wildlife in Canada (COSEWIC). 2020 [cited 2020 Jan 20]. Available from: https://species-registry.canada.ca/index-en.html#/species?sortBy=commonNameSort&sortDirection=asc&pageSize=10
12. Campbell J. Distribution pattern of amphibians in Middle America. In: Duellman WE, Patterns of distribution of amphibians: a global perspective. Baltimore: Johns Hopkins University Press; 1999.
13. Ochoa-Ochoa LM, Rodríguez P, Mora F, Flores-Villela O, Whittaker RJ. Climate change and amphibian diversity patterns in Mexico. Biol Conserv. 2012;150(1):94–102.
14. Pasquali M. Latin America: countries with most threatened amphibian species 2019 [Internet]. Statista. 2020 [cited 2021 Feb 8]. Available from: https://www.statista.com/statistics/977935/countries-highest-number-threatened-amphibian-species-latin-america/
15. Whiles M, Lips K, Pringle C, Kilham S, Bixby R, Brenes R, et al. The effects of amphibian population declines on the structure and function of Neotropical stream ecosystems. Front Ecol Environ. 2006;4:27–34.
16. Hocking D, Babbitt K. Amphibian contributions to ecosystem services. Herpetol Conserv Biol. 2014;9:1–17.
17. Blaustein AR, Wake DB, Sousa WP. Amphibian declines: judging stability, persistence, and susceptibility of populations to local and global extinctions. Conserv Biol. 1994;8(1):60–71.
18. Bloxam QMC, Tonge SJ. Amphibians: suitable candidates for breeding-release programmes. Biodivers Conserv. 1995;4(6):636–44.
19. Harding G, Griffiths R, Pavajeau L. Developments in amphibian captive breeding and reintroduction programs. Conserv Biol. 2016;30:340–9.

20. Griffiths R, Buhlmann K, McKay J, Tuberville T. Reintroduction. In: Gascon C, Collins JP, Moore RD, Church DR, McKay J, Mendelson III, editors. Amphibian conservation action plan IUCN/SSC Amphibian Conservation Summit 2005. Gland: World Conservation Union (IUCN); 2007. p. 40–4.

21. Zippel K, Johnson K, Gagliardo R, Gibson R, McFadden M, Browne R, et al. The amphibian ark: a global community for ex situ conservation of amphibians. Herpetol Conserv Biol. 2011;6:340–52.

22. Amphibian population management guidelines. Amphibian ark amphibian population management workshop. San Diego: Amphibian ARK; 2007 p. 34.

23. Balmford A, Mace GM, Leader-Williams N. Designing the ark: setting priorities for captive breeding. Conserv Biol. 1996;10(3):719–27.

24. Trudeau V, Schueler F, Navarro-Martin L, Hamilton C, Bulaeva E, Bennett A, et al. Efficient induction of spawning of Northern leopard frogs (*Lithobates pipiens*) during and outside the natural breeding season. Reprod Biol Endocrinol. 2013;11:14.

25. Environment Canada. Recovery strategy for the northern leopard frog (*Lithobates pipiens*), Rocky Mountain population in Canada. Ottawa: Environment and Climate Change Canada; 2017 p. 71.

26. LaClaire L. Dusky gropher frog controlled propagation and reintroduction plan. Jackson: U.S. Fish and Wildlife Service; 2017 p. 20.

27. Roznik E, Reichling S. Survival, movements and habitat use of captive-bred and reintroduced dusky gopher frogs. Anim Conserv. 2021;24:51–63.

28. Browne RK, Seratt J, Vance C, Kouba A. Hormonal priming, induction of ovulation and in-vitro fertilization of the endangered Wyoming toad (*Bufo baxteri*). Reprod Biol Endocrinol. 2006;4(1):34.

29. Vincent K, Abbott T. Wyoming toad *Bufo hemiophrys baxteri* now known as *Anaxyrus baxteri* revised recovery plan 2015. Cheyenne: U.S. Fish and Wildlife Service; 2015 p. 78.

30. Voss SR, Woodcock MR, Zambrano L. A tale of two axolotls. BioScience. 2015;65(12):1134–40.

31. Browne R, Wang Z, Okada S, McGinnity D, Luo Q, Taguchi Y, et al. The sustainable management of giant salamanders (Cryptobranchoidea). Sustainability America, Belize; 2020 p. 46.

32. Ewen JG, Soorae PS, Canessa S. Reintroduction objectives, decisions and outcomes: global perspectives from the herpetofauna. Anim Conserv. 2014;17(S1):74–81.

33. Illinois Department of Natural Resources (IDNR). Illinois comprehensive wildlife conservation plan-strategy. Springfield: State of Illinois; 2005 p. 380.

34. Seburn DC, Seburn CNL, Weller WF. A localized decline in the western chorus frog, *Pseudacris triseriata*, in Eastern Ontario. Can Field Nat. 2008;122(2):158–61.

35. Seburn D, Gunson K, Schueler F. Apparent widespread decline of the boreal chorus frog (*Pseudacris maculata*) in Eastern Ottawa. Can Field Nat. 2014;128:151–7.

36. Hebenstreit L. Conservation status of the Illinois chorus frog (*Pseudacris illinoensis*) in Madison County, Illinois [MSc]. [Edwardsville]: Southern Illinois University Edwardsville; 2018.

37. Pallua J. What can the mythical frog tell us? The symbolism and role of the frog in history and modernity. Folklore. 2019;77:63–91.

38. Kuhnlein H, Humphries M. Traditional animal foods of indigenous peoples of northern North America. The contributions of wildlife diversity to the subsistence and nutrition of indigenous cultures. Montreal: Centre for Indigenous Peoples' Nutrition and Environment, McGill University; 2017.

39. Carson R. Silent spring. Boston: Houghton Mifflin; 1962.

40. Ellis M. Hollywood frogs [Internet]. KQED. 2017 [cited 2021 Feb 8]. Available from: https://www.kqed.org/perspectives/201601135544/hollywood-frogs

41. Environment Canada. Recovery strategy of the western chorus frog (*Pseudacris triseriata*), Great Lakes / St. Lawrence – Canadian Shield population, in Canada. Ottawa: Environment Canada; 2015 p. 53.

42. Pough F, Andrews R, Candle J, Crump M, Savitzky A, Wells K. Herpetology. 3rd ed. Upper Saddle River: Pearson Prentice Hall; 2004.

43. Lemmon EM, Lemmon AR, Collins JT, Lee-Yaw JA, Cannatella DC. Phylogeny-based delimitation of species boundaries and contact zones in the trilling chorus frogs (*Pseudacris*). Mol Phylogenet Evol. 2007;44(3):1068–82.

44. Barrow LN, Ralicki HF, Emme SA, Lemmon EM. Species tree estimation of North American chorus frogs (Hylidae: *Pseudacris*) with parallel tagged amplicon sequencing. Mol Phylogenet Evol. 2014;75:78–90.

45. Hedges S. An electrophoretic analysis of Holarctic hylid frog evolution. Syst Biol. 1986;35:1–21.

46. Cocroft RB. A cladistic analysis of chorus frog phylogeny (Hylidae: *Pseudacris*). Herpetologica. 1994;50(4):420–37.

47. Da Silva HR. Two character states new for hylines and the taxonomy of the genus Pseudacris. J Herpetol. 1997;31(4):609–13.

48. Recuero E, Martínez-Solano I, Parra-Olea G, García-París M. Phylogeography of *Pseudacris regilla* (Anura: Hylidae) in western North America, with a proposal for a new taxonomic rearrangement. Mol Phylogenet Evol. 2006;39(2):293–304.

49. Conant R, Collins J. Peterson field guide: reptiles and amphibians, Eastern/Central North America. Boston: Houghton Mifflin Company; 1998.

50. Lannoo M, editor. Amphibian declines: the conservation status of united states species. Berkeley: University of California Press; 2005.

51. Duellman WE, Marion AB, Hedges SB. Phylogenetics, classification, and biogeography of the treefrogs (Amphibia: Anura: Arboranae). Zootaxa. 2016;4104(1):1–109.

52. Moriarty EC, Cannatella DC. Phylogenetic relationships of the North American chorus frogs (*Pseudacris*: Hylidae). Mol Phylogenet Evol. 2004;30(2):409–20.

53. Lemmon EM, Lemmon AR, Collins JT, Cannatella DC. A new North American chorus frog species (Amphibia: Hylidae: *Pseudacris*) from the south-central United States. Zootaxa. 2008;1675(1):1.

54. Frost DR, Lemmon EM, McDiarmid RW, Mendelson JRI. Anura—Frogs. In: Crother BI, editor. Scientific and standard English names of amphibians and reptiles of North America north of Mexico, with comments regarding confidence in our understanding. 8th ed. SSAR; 2017. p. 6–21.

55. Faivovich J, Pereyra MO, Luna MC, Hertz A, Blotto BL, Vásquez-Almazán CR, et al. On the monophyly and relationships of several genera of *Hylini* (Anura: Hylidae: Hylinae), with comments on recent taxonomic changes in hylids. South Am J Herpetol. 2018;13(1):1.

56. Ospina OE, Tieu L, Apodaca JJ, Lemmon EM. Hidden diversity in the mountain chorus frog (*Pseudacris brachyphona*) and the diagnosis of a new species of chorus frog in the Southeastern United States. Copeia. 2020;108(4):778–95.

57. Crother BI, editor. Scientific and standard English names of amphibians and reptiles of North America north of Mexico, with comments regarding confidence in our understanding. SSAR Herpetol Circ. 2017;43:1–102.

58. GBIF occurrence download [Internet]. Global biodiversity information facility (GBIF.org). Available from: https://doi.org/10.15468/dl.w3hp5u

59. Wickham H. Easily install and load the "Tidyverse" [R package tidyverse version 1.3.1] [Internet]. Comprehensive R Archive Network (CRAN); 2021 [cited 2021 May 28]. Available from: https://CRAN.R-project.org/package=tidyverse

60. Hijmans RJ. Geographic data analysis and modeling [R package raster version 3.4-10] [Internet]. Comprehensive R Archive Network (CRAN); 2021 [cited 2021 May 28]. Available from: https://CRAN.R-project.org/package=raster

61. Chamberlain S, Oldoni D, Barve V, Desmet P, Geffert L, McGlinn D, et al. Interface to the global "Biodiversity" information facility API [R package rgbif version 3.5.2] [Internet]. Comprehensive R Archive Network (CRAN); 2021 [cited 2021 May 28]. Available from: https://CRAN.R-project.org/package=rgbif

62. Lemmon AR, Lemmon EM. A likelihood framework for estimating phylogeographic history on a continuous landscape. Syst Biol. 2008;57(4):544–61.

63. NatureServe. NatureServe Explorer [web application] [Internet]. NatureServe. 2021 [cited 2021 Jan 25]. Available from: https://explorer.natureserve.org/

64. Somma L. *Pseudacris hypochondriaca* (Hallowell, 1854): U.S. Geological survey, nonindigenous aquatic species database, Gainesville, FL [Internet]. 2021 [cited 2021 Jan 25]. Available from: https://nas.er.usgs.gov/queries/FactSheet.aspx?speciesID=2781

65. AmphibiaWeb. AmphibiaWeb [Internet]. 2021 [cited 2021 Jan 25]. Available from: http://amphibiaweb.org

66. Wright A, Wright A. Handbook of frogs and toads of the United States and Canada. 3rd ed. Ithaca: Comstock Publishing Company; 1995.

67. Dodd C. Frogs of the United States and Canada. Baltimore: Johns Hopkins University Press; 2013.

68. Wente WH, Phillips JB. Seasonal color change in a population of Pacific tree frogs (*Pseudacris regilla*). J Herpetol. 2005;39(1):161–5.

69. Resnick LE, Jameson DL. Color polymorphism in Pacific tree frogs. Science. 1963;142(3595):1081–3.

70. Wente W, Phillips J. Fixed green and brown color morphs and a novel color-changing morph of the Pacific tree frog *Hyla regilla*. Am Nat. 2003;162:461–73.

71. Whitaker JO. A study of the western chorus frog, *Pseudacris triseriata*, in Vigo County, Indiana. J Herpetol. 1971;5(3/4):127–50.

72. Corn PS. Genetic and developmental studies of albino chorus frogs. J Hered. 1986;77(3):164–8.

73. McAlpine D, Gilhen J. Erythrism in spring peeper (*Pseudacris crucifer*) in Maritime Canada. Can Field-Nat. 2018;132:43–5.

74. Wells K. The social behavior of anuran amphibians. Anim Behav. 1977;25:666–93.

75. Vitt L, Caldwell JP. Herpetology: an introductory biology of amphibians and reptiles. 4th ed. San Diego: Academic Press; 2014.

76. Caldwell JP. Demography and life history of two species of chorus frogs (Anura: Hylidae) in South Carolina. Copeia. 1987;1987(1):114.

77. Smith D. Adult recruitment in chorus frogs: effects of size and date at metamorphosis. Ecology. 1987;68:344–50.

78. Whiting A. Population ecology of the western chorus frog, *Pseudacris triseriata* [MSc]. [Quebec]: McGill University; 2004.

79. Muths E, Scherer RD, Amburgey SM, Corn PS. Twenty-nine years of population dynamics in a small-bodied montane amphibian. Ecosphere. 2018;9(12):e02522.

80. Kissel AM, Tenan S, Muths E. Density dependence and adult survival drive dynamics in two high elevation amphibian populations. Diversity. 2020;12(12):478.

81. Livezey RL. Some observations on *Pseudacris nigrita triseriata* (Wied) in Texas. Am Midl Nat. 1952;47(2):372–81.

82. Blair WF. Calling and spawning seasons in a mixed population of anurans. Ecology. 1961;42(1):99–110.

83. Oseen KL, Wassersug RJ. Environmental factors influencing calling in sympatric anurans. Oecologia. 2002;133(4):616–25.

84. Gray P, Stegall E. Distribution and status of Strecker's chorus frog (*Pseudacris streckeri streckeri*) in Kansas. Trans Kans Acad Sci. 1986;89(3/4):81.

85. Goldberg SR. Notes on reproduction of Strecker's chorus frog, *Pseudscris streckeri* (Anura: Hylidae), from Oklahoma. Bull Chic Herpetol Soc. 2020;3:61–3.

86. Storer T. A synopsis of the amphibia of California. Vol. 27. Berkeley: University of California Publications in Zoology; 1925.

87. Stebbins RC. Amphibians of Western North America. Berkeley, California: University of California Press; 1951.

88. Harper F. Distribution, taxonomy, nomenclature, and habits of the little tree-frog (*Hyla ocularis*). Am Midl Nat. 1939;22(1):134–49.

89. Duellman WE, Schwartz A. Amphibians and reptiles of Southern Florida. Bull Florida State Mus. 1958;3:181–324.

90. Gibbons J, Semlitsch RD. Guide to the reptiles and amphibians of the Savannah River Site. Athens: University of Georgia Press; 1991.

91. Waters D. Geographic distribution: *Pseudacris regilla* (Pacific treefrog). Herpetol Rev. 1992;23:24–5.

92. Weitzel NH, Panik. HR. Long-term fluctuations of an isolated population of the Pacific chorus frog (*Pseudacris regilla*) in northwestern Nevada. Great Basin Nat. 1993;53:379–84.

93. Blair WF, Littlejohn MJ. Stage of speciation of two allopatric populations of chorus frogs *(Pseudacris*). Evolution. 1960;14(1):82–7.

94. Brown LE. Subterranean feeding by the chorus frog *Pseudacris streckeri* (Anura: Hylidae). Herpetologica. 1978;34(2):212–6.

95. Wells K, Schwartz JJ. The ecology and behaviour of amphibians. Chicago: University of Chicago Press; 2007.

96. Matthews TC. Genetic changes in a population of boreal chorus frogs (*Pseudacris triseriata*) polymorphic for color. Am Midl Nat. 1971;85(1):208–21.

97. Tordoff W, Pettus D. Temporal stability of phenotypic frequencies in *Pseudacris triseriata* (Amphibia, Anura, Hylidae). J Herpetol. 1977;11(2):161–8.

98. Platz JE, Forester DC. Geographic variation in mating call among the four subspecies of the chorus frog: *Pseudacris triseriata* (Wied). Copeia. 1988;1988(4):1062–6.

99. Duellman WE, Trueb L. Biology of amphibians. Baltimore: Johns Hopkins University Press; 1994.

100. Perrill SA, Daniel RE. Multiple egg clutches in *Hyla regilla*, *H. cinerea* and *H. gratiosa*. Copeia. 1983;1983(2):513–6.

101. Ouellet M, Fortin C, Grimard M-J. Distribution and habitat use of the boreal chorus frog (*Pseudacris maculata*) at its extreme northeastern range limit. Herpetol Conserv Biol. 2009;4:277–84.

102. Shulse C, Semlitsch R, Trauth K, Williams A. Influences of design and landscape placement parameters on amphibian abundance in constructed wetlands. Wetlands. 2010;30:915–28.

103. Burt CE. Contributions to the herpetology of Texas I. Frogs of the genus *Pseudacris*. Am Midl Nat. 1936;17(4):770–5.

104. Moriarty E, Lannoo M. *Pseudacris triseriata* complex (including *feriarum*, *kalmi*, *triseriata*, and *maculata*) striped chorus frog. In: Lannoo MJ, editor. Amphibian declines: the conservation status of United States species. Berkeley: University of California Press; 2005. p. 485–8.

105. Wilczynski W, Lynch K. Female sexual arousal in amphibians. Horm Behav. 2011;59:630–6.

106. Conant R, Cagle FR, Goin CJ, Lowe CH, Neill WT, Netting MG, et al. Common names for North American amphibians and reptiles. Copeia. 1956;1956(3):172–85.

107. Brooke PN, Alford RA, Schwarzkopf L. Environmental and social factors influence chorusing behaviour in a tropical frog: examining various temporal and spatial scales. Behav Ecol Sociobiol. 2000;49(1):79–87.

108. Wells K. The effects of social interaction on anuran vocal behavior. In: Fritszch B, Wilczynski W, Ryan MJ, Hetherington T, Walkowiak W, editors. The evolution of the amphibian auditory system. New York: Wiley; 1988.

109. Bee MA, Cook JM, Love EK, O'Bryan LR, Pettitt BA, Schrode K, et al. Assessing acoustic signal variability and the potential for sexual selection and social recognition in boreal chorus frogs (*Pseudacris maculata*). Ethology. 2010;116(6):564–76.

110. Cocroft RB, Ryan MJ. Patterns of advertisement call evolution in toads and chorus frogs. Anim Behav. 1995;49(2):283–303.

111. Crenshaw JW, Blair WF. Relationships in the *Pseudacris nigrita* complex in Southwestern Georgia. Copeia. 1959;1959(3):215.

112. Vélez A, Guajardo A. Individual variation in two types of advertisement calls of Pacific tree frogs, *Hyliola* (=*Pseudacris*) *regilla*, and the implications for sexual selection and species recognition. Bioacoustics. 2020;2020:1–21.

113. Lemmon EM. Diversification of conspecific signals in sympatry: geographic overlap drives multidimensional reproductive character displacement in frogs. Evolution. 2009;63(5):1155–70.

114. Zimmitti SJ. Individual variation in morphological, physiological, and biochemical features associated with calling in spring peepers (*Pseudacris crucifer*). Physiol Biochem Zool. 1999;72(6):666–76.

115. Rosen M, Lemon RE. The vocal behavior of spring peepers, *Hyla crucifer*. Copeia. 1974;1974(4):940–50.

116. Schwartz JJ. Graded aggressive calls of the spring peeper, *Pseudacris crucifer*. Herpetologica. 1989;45(2):172–81.

117. Owen P. The structure, function, and evolution aggressive signals in anuran amphibians [PhD]. [Mansfield]: University of Connecticut; 2003.

118. Owen PC, Tucker JK. Courtship calls and behavior in two species of chorus frogs, genus *Pseudacris* (Anura: Hylidae). Copeia. 2006;2006(1):137–44.

119. Gerhardt HC, Huber F. Acoustic communication in insects and anurans: common problems and diverse solutions. University of Chicago Press; 2002. 552 p.

120. Perrill SA. Male mating behavior in *Hyla regilla*. Copeia. 1984;1994(3):727–32.

121. Roble SM. Observations on satellite males in *Hyla chrysoscelis*, *Hyla picta*, and *Pseudacris triseriata*. J Herpetol. 1985;19(3):432–6.

122. Lance S, Wells K. Are spring peeper satellite males physiologically inferior to calling males? Copeia. 1993;1993:1162–6.

123. Forester DC. Vocal behavior and mating tactics of the spring peeper (*Pseudacris crucifer*): a field exercise in animal behavior. In: Ploger BJ, Yasukawa K. Exploring animal behavior in laboratory and field: an hypothesis-testing approach to the development, causation, function, and evolution of animal behavior. Cambridge: Academic Press; 2003. p. 327–40.

124. Halliday T, Adler K. Firefly encyclopedia of reptiles and amphibians. Toronto: Firefly Books; 2002.

125. Martof BS, Thompson EF. Reproductive behavior of the chorus frog, *Pseudacris nigrita*. Behaviour. 1958;13(3/4):243–58.

126. Gosner KL, Rossman DA. Observations of the reproductive cycle of the swamp chorus frog, *Pseudacris nigrita*. Copeia. 1959;1959(3):263.

127. Oplinger CS. Sex ratio, reproductive cycles, and time of ovulation in *Hyla crucifer crucifer* Wied. Herpetologica. 1966;22(4):276–83.

128. Smith R. Mating and oviposition in the Pacific Coast tree toad. Science. 1940;92:379–80.

129. Green NB. The Breeding habits of *Pseudacris brachyphona* (Cope) with a description of the eggs and tadpole. Copeia. 1938;1938(2):79.

130. Trauth SE, Jr RLC, Butterfield BP, Saugey DA, Meshaka Jr WE. Reproductive phenophases and clutch characteristics of selected Arkansas amphibians. JAAS. 1990;44:107–13.

131. Brady M, Harper F. A Florida subspecies of *Pseudacris nigrita* (Hylidae). Proc Biol Soc Wash. 1935;48:107–10.

132. Green NB. A Study of the life history of *Pseudacris brachyphona* (Cope) in West Virginia with special reference to behavior and growth of marked individuals [PhD]. [Columbus]: Ohio State University; 1952.

133. Skelly D. Pond drying, predators, and the distribution of *Pseudacris* tadpoles. Copeia. 1996;1996:599–605.

134. Blaustein L. Oviposition site selection in response to risk of predation: evidence from aquatic habitats and consequences for population and community structure. In: Wasser SP, editor. Evolutionary theory and processes: modern perspectives. Dordrecht; 1999.

135. Buxton VL, Sperry JH. Reproductive decisions in anurans: A review of how predation and competition affects the deposition of eggs and tadpoles. BioScience. 2017;67(1):26–38.

136. Buxton VL, Ward MP, Sperry JH. Frog breeding pond selection in response to predators and conspecific cues. Ethology. 2017;123(5):397–404.

137. Kuramoto M. Correlations of quantitative parameters of fecundity in amphibians. Evolution. 1978;32(2):287–96.

138. Lang C. Size-fecundity relationships among stream-breeding hylid frogs. Herpetol Nat Hist. 1995;3:193–7.

139. Prado C, Haddad C. Size-fecundity relationships and reproductive investment in female frogs in the Pantanal, south-western Brazil. Herpetol J. 2005;15:181–9.

140. Nali RC, Zamudio KR, Haddad CFB, Prado CPA. Size-dependent selective mechanisms on males and females and the evolution of sexual size dimorphism in frogs. Am Nat. 2014;184(6):727–40.

141. Duffitt AD, Finkler MS. Sex-related differences in somatic stored energy reserves of *Pseudacris crucifer* and *Pseudacris triseriata* during the early breeding season. J Herpetol. 2011;45(2):224–9.

142. Harding J, Holman J. Michigan frogs, toads, and salamanders: a field guide and pocket reference. East Lansing: Michigan State University Museum; 1992.

143. Harkey GA, Semlitsch RD. Effects of temperature on growth, development, and color polymorphism in the ornate chorus frog *Pseudacris ornata*. Copeia. 1988;1988(4):1001.

144. Anderson MT, Kiesecker JM, Chivers DP, Blaustein AR. The direct and indirect effects of temperature on a predator–prey relationship. Can J Zool. 2001;79(10):1834–41.

145. Amburgey S, Funk WC, Murphy M, Muths E. Effects of hydroperiod duration on survival, developmental rate, and size at metamorphosis in boreal chorus frog tadpoles (*Pseudacris maculata*). Herpetologica. 2012;68(4):456–67.

146. Wagner W. Tadpoles and Pollen: Observations on the feeding behavior of *Hyla regilla* larvae. Copeia. 1986;1986:802–4.

147. Britson CA, Kissell RE. Effects of food type on developmental characteristics of an ephemeral pond-breeding anuran, *Pseudacris triseriata feriarum*. Herpetologica. 1996;52(3):374–82.

148. Whiles M, Gladyshev M, Sushchik N, Makhutova O, Kalachova G, Peterson S, et al. Fatty acid analyses reveal high degrees of omnivory and dietary plasticity in pond-dwelling tadpoles. Freshw Biol. 2010;55:1533–47.

149. Rittenhouse T, Semlitsch R. Distribution of amphibians in terrestrial habitat surrounding wetlands. Wetlands. 2007;27:153–61.

150. Semlitsch R. Differentiating migration and dispersal processes for pond-breeding amphibians. J Wildl Manage. 2008;72:260–7.

151. Pechmann J, Estes R, Scott D, Gibbons J. Amphibian colonization and use of ponds created for trial mitigation of wetland loss. Wetlands. 2001;21:93–111.

152. Brandt BB. The frogs and toads of Eastern North Carolina. Copeia. 1936;1936(4):215–23.

153. Bragg AN. Observations on the ecology and natural history of Anura. XVI. Life history of *Pseudacris clarkii* (Baird) in Oklahoma. Wasmann Collect. 1943;5:129–40.

154. Kramer DC. Movements of western chorus frogs *Pseudacris triseriata triseriata* tagged with Co60. J Herpetol. 1973;7(3):231–5.

155. Green NB. Postmetamorphic growth in the mountain chorus frog, *Pseudacris brachyphona* Cope. Proc West Virginia Acad Sci. 1964;36:34–8.

156. Froom B. Amphibians of Canada. Toronto: McClelland and Stewart Limited; 1982.

157. Storey K, Storey J. Freeze tolerance and intolerance as strategies of winter survival in terrestrially-hibernating amphibians. Comp Biochem Physiol A Comp Physiol. 1986;83A:613–7.

158. Storey KB, Storey JM. Persistence of freeze tolerance in terrestrially hibernating frogs after spring emergence. Copeia. 1987;1987(3):720–6.

159. Storey K, Storey JM. Freeze tolerance and freeze avoidance in ectotherms. In: Wang LCH, editor. animal adaptation to cold advances in comparative and environmental physiology. Vol. 4. Springer; 1989.

160. Churchill T, Storey K. Organ metabolism and cryoprotectant synthesis during freezing in spring peepers *Pseudacris crucifer*. Copeia. 1996;1996:517–25.

161. Layne JR, Kefauver J. Freeze tolerance and postfreeze recovery in the frog *Pseudacris crucifer*. Copeia. 1997;1997(2):260–4.

162. Croes S, Thomas R. Tolerance and cryoprotectant synthesis of the Pacific tree frog *Hyla regilla*. Copeia. 2000;2000:863–8.

163. Higgins SA, Swanson DL. Urea is not a universal cryoprotectant among hibernating anurans: evidence from the freeze-tolerant boreal chorus frog (*Pseudacris maculata*). Comp Biochem Physiol A Mol Integr Physiol. 2013;164(2):344–50.

164. Harper F. A season with Holbrook's chorus frog (*Pseudacris ornata*). Am Midl Nat. 1937;18(2):260–72.

165. Ashston, RE, Ashton, PS. Handbook of reptiles and amphibians of Florida: Part three: the amphibians. Miami: Windward Publishing; 1988.

166. Govindarajulu PP, Anholt BR. Interaction between biotic and abiotic factors determines tadpole survival rate under natural conditions. Écoscience. 2006;13(3):413–21.

167. Melvin SD, Houlahan JE. Tadpole mortality varies across experimental venues: do laboratory populations predict responses in nature? Oecologia. 2012;169(4):861–8.

168. Pettus D, Angleton GM. Comparative reproductive biology of Montane and Piedmont chorus frogs. Evolution. 1967;21(3):500–7.

169. Stanback M. Gambusia holbrooki predation on *Pseudacris feriarum* tadpoles. Herpetol Conserv Biol. 2010;5:486–9.

170. Earl JE, Cohagen KE, Semlitsch RD. Effects of leachate from tree leaves and grass litter on tadpoles. Environ Toxicol Chem. 2012;31(7):1511–7.

171. Kissner J. Amphibians of the Southern Great Plains: habitat and the impacts of temperature and pH on survival [MSc]. Texas Tech University; 2013.

172. Kramer DC. Viability of the eggs of *Pseudacris triseriata* (Amphibia, Anura, Hylidae). J Herpetol. 1978;12(1):119–20.

173. Smith D. Factors controlling tadpole populations of the chorus frog (*Pseudacris triseriata*) on Isle Royale, Michigan. Ecology. 1983;64:501–10.

174. Davis RA, Lohr CA, Roberts JD. Frog survival and population viability in an agricultural landscape with a drying climate. Popul Ecol. 2019;61(1):102–12.

175. Messerman A, Semlitsch R, Leal M. Estimating survival for elusive juvenile pond-breeding salamanders. J Wildl Manage. 2020;84:562–75.

176. Swanson J, Bailey L, Muths E, Funk WC. Factors influencing survival and mark retention in postmetamorphic boreal chorus frogs. Copeia. 2013;2013:670–5.

177. Tucker JK. Growth and survivorship in the Illinois chorus frog (*Pseudacris streckeri illinoensis*). Trans Ill State Acad Sci. 2000;93:63–8.

178. Schmidt B. Monitoring the distribution of pond-breeding amphibians when species are detected imperfectly. Aquat Conserv. 2005;15:681–92.

179. Mazerolle MJ, Bailey LL, Kendall WL, Royle JA, Converse SJ, Nichols JD. Making great leaps forward: accounting for detectability in herpetological field studies. J Herpetol. 2007;41(4):672–89.

180. Bainbridge L, Stockwell M, Valdez J, Klop-Toker K, Clulow S, Clulow J, et al. Tagging tadpoles: retention rates and impacts of visible implant elastomer (VIE) tags from the larval to adult amphibian stages. Herpetol J. 2015;25:133–1408.

181. Fouilloux C, Garcia-Costoya G, Rojas B. Visible implant elastomer (VIE) success in early larval stages of a tropical amphibian species. PeerJ. 2020;8.

182. Courtois EA, Lelong C, Calves O, Loyau A, Schmeller DS. The use of visible implant alpha tags for anuran tadpoles. Herpetol Rev. 2013;44:230–3.

183. Trauth JB, Trauth SE, Johnson RL. Best management practices and drought combine to silence the Illinois chorus frog in Arkansas. Wildl Soc Bull. 2006;34(2):514–8.

184. Lykens DV, Forester DC. Age structure in the spring peeper: do males advertise longevity? Herpetologica. 1987;43:216–23.

185. Tucker J, Chick J, Szafoni R. The Illinois chorus frog (*Pseudacris illinoensis*) and wetland mitigation: what has worked? Champaign: Division of Ecology and Conservation Science section for Field Stations and Ecosystem Science; 2008 p. 45.

186. Muths EL, Scherer RD, Amburgey SM, Matthews T, Spencer AW, Corn PS. First estimates of the probability of survival in a small-bodied, high-elevation frog (boreal chorus frog, *Pseudacris maculata*), or how historical data can be useful. Vol. 94. Can J Zool. 2016. p. 599–606.

187. Frías-Alvarez P, Zúñiga-Vega JJ, Flores-Villela O. A general assessment of the conservation status and decline trends of Mexican amphibians. Biodivers Conserv. 2010;19(13):3699–742.

188. SARA. Species at Risk Act. 2002.

189. Bonin J, Galois P. Report on the situation of the western chorus frog (*Pseudacris maculata*) in Quebec. Quebec: Wildlife and Habitats Directorate; 1996 p. 39.

190. Tardif B, Lavoie G, Lachance Y. Québec biodiversity atlas. Threatened or vulnerable species. Québec: Gouvernement du Québec, Ministère du Développement durable, de l'Environnement et des Parcs, Direction du développement durable, du patrimoine écologique et des parcs; 2005 p. 60.

191. Bogart J, Taylor E, Boles R. Western chorus frog (*Pseudacris triseriata*) and boreal chorus frog (P. maculata): clarification concerning the wildlife species listed under SARA in light of recent taxonomic interpretations. Canada: Wildlife Species Assessment Meeting (November 22- 26, 2015) of the Committee on the Status of Endangered Wildlife in Canada.; 2015 p. 5.

192. Rogic A, Tessier N, Noël S, Gendron A, Branchaud A, Lapointe F-J. A "trilling" case of mistaken identity: call playbacks and mitochondrial DNA identify chorus frogs in Southern Quebec (Canada) as *Pseudacris maculata* and not *P. triseriata*. Herpetol Rev. 2015;46:1–7.

193. US ESA. Endangered Species Act. 1983.

194. Henning B, Hinz L. Conservation guidance for species in greatest need of conservation (SGNC) – INHS technical report 2016 (09). Champaign: Illinois Department of Natural Resources, Division of Natural Heritage; 2016 p. 54.

195. Illinois Department of Natural Resources (IDNR). Endangered and threatened amphibians [Internet]. 2021 [cited 2021 Feb 5]. Available from: https://www2.illinois.gov/dnr/conservation/NaturalHeritage/Pages/Amphibians.aspx

196. Wingert HE. Amphibian declines during a 50-year period in south central Pennsylvania. J Pa Acad Sci. 2018;92(2):136–52.

197. Mitchell J, Breisch A, Buhlmann K. Habitat management guidelines for amphibians and reptiles of the Northeastern United States. Montgomery: Partners in Amphibian and Reptile Conservation; 2006.

198. Mueller CA, Bucsky J, Korito L, Manzanares S. Immediate and persistent effects of temperature on oxygen consumption and thermal tolerance in embryos and larvae of the Baja California chorus frog, *Pseudacris hypochondriaca*. Front Physiol. 2019;10:754.

199. Dimitrie D. The effects of two insecticides on California anurans (*Rana sierrae* and *Pseudacris sierra*) and the implications for declining amphibian populations. [PhD]. [Illinois]: Southern Illinois University Carbondale; 2010.

200. Brown H. Embryonic temperature adaptations of the Pacific treefrog, *Hyla regilla*. Comp Biochem Physiol. 1975;51:863–73.

201. Gardner J. Natural history notes: *Pseudacris regilla* (Pacific chorus frog) reproduction. Herpetol Rev. 1995;26:32.

202. Marnell LF. Herpetofauna of Glacier National Park. Northwest Nat. 1997;78(1):17–33.

203. Hollingsworth B, Roberts K. *Pseudacris regilla*, Pacific treefrog. San Diego natural history museum field guide [Internet]. 2001. Available from: http://www.sdnhm.org/fieldguide/herps/hyla-reg.html

204. Romansic JM, Johnson PTJ, Searle CL, Johnson JE, Tunstall TS, Han BA, et al. Individual and combined effects of multiple pathogens on Pacific treefrogs. Oecologia. 2011;166(4):1029–41.

205. Taubert D, Shetley P, Philipp D, Harrison T. Breeding biology and distribution of the Illinois chorus frog (*Pseudacris streckeri illinoensis*) in Illinois. Illinois: Illinois Dept. of Conservation: Springfield; 1982 p. 163.

206. Butterfield BP, Meshaka WE, Trauth SE. Fecundity and egg mass size of the Illinois chorus frog, *Pseudacris streckeri illinoensis* (Hylidae), from Northeastern Arkansas. Southwest Nat. 1989;34(4):556.

207. Butterfield BP. Age structure and reproductive biology of the Illinois chorus frog (*Pseudacris streckeri illin*oensis) from Northeastern Arkansas [MSc]. [Jonesboro]: Arkansas State University; 1988.

208. Tucker J, Philipp D. Population status of the Illinois chorus frog (*Pseudacris streckeri illinoensis*) in Madison County, Illinois: results of 1996 surveys. Springfield: Unpublished report to Illinois Department of Transportation; 1997.

209. Tucker JK. Fecundity in the Illinois chorus frog (*Pseudacris streckeri illinoensis*) from Madison County, Illinois. Trans Ill State Acad Sci. 1997;90:167–70.

210. Neill WT. Distributional notes on Georgia amphibians, and some corrections. Copeia. 1957;1957(1):43–7.

211. Seyle CW, Trauth SE. Life history notes: *Pseudacris ornata* (ornate chorus frog). Reproduction. Herpetol Rev. 1982;13:45.

212. Bragg AN. Observations on the ecology and natural history of Anura. X. The breeding habits of *Pseudacris streckeri* Wright and Wright in Oklahoma including a description of the eggs and tadpoles. Wasmann Collect. 1942;5:47–62.

213. Fouquette MJ, Littlejohn MJ. Patterns of oviposition in two species of hylid frogs. Southwest Nat. 1960;5(2):92.

214. Welter WA, Carr K. Amphibians and reptiles of Northeastern Kentucky. Copeia. 1939;1939(3):128–30.

215. Carr A. Dates of frog choruses in Florida. Copeia. 1940;1940:55.

216. Gosner KL, Rossman DA. Eggs and larval development of the treefrogs *Hyla crucifer* and *Hyla ocularis*. Herpetologica. 1960;16:225–32.

217. Green N, Pauley T. Amphibians and reptiles in West Virginia. Pittsburgh: University of Pittsburgh Press; 1987.

218. Martof B. Observations on the life history and ecology of the amphibians of the Athens area, Georgia. Copeia. 1955;1955(3):166–70.

219. Kehr AI. Stage-frequency and habitat selection of a cohort of *Pseudacris ocularis* tadpoles (Hylidae: Anura) in a Florida temporary pond. Herpetol J. 1997;7:103–9.

220. McClure K. Ecology of *Pseudacris brachyphona*: a second look [MSc]. [Huntington]: Marshall University; 1996.

221. Gosner KL, Black IH. Notes on the life history of Brimley's chorus frog. Herpetologica. 1958;13(4):249–54.

222. Kennedy JP. Notes on a breeding congress of *Pseudacris clarki* and *Pseudacris nigrita* in Harris County, Texas. Herpetologica. 1958;14(4):192.

223. Sias J. Natural history and distribution of the upland chorus frog, *Pseudacris feriarum* Baird, in West Virginia [MSc]. [Huntington]: Marshall University; 2006.

224. Dundee H, Rossman D. The amphibians and reptiles of Louisiana. Baton Rouge: Louisiana State University Press; 1989.

225. Dorcas M, Gibbons W. Frogs and toad of the southeast. Athens: University of Georgia Press; 2008.

226. Mitchell JC, Pague CA. Filling gaps in life-history data: clutch sizes for 21 species of North American anurans. Herpetol Conserv Biol. 2014;9:409–20.

227. Smith H. The amphibians of Kansas. Am Midl Nat. 1934;15:377–528.

228. Parmelee J, Knutson M, Lyon J. A field guide to amphibian larvae and eggs of Minnesota, Wisconsin, and Iowa. Washington: U.S. Geological Survey, Biological Resources Division; 2002 p. 48.

229. Varoux M. Optimisation des conditions d'élevage de la Rainette faux-grillon de l'Ouest (*Pseudacris triseriata*) au Biodôme de Montréal. Montréal: Diplôme Universitaire et Technologique, Génie Biologique, Université d'Avignon; 2015 p. 45.

230. Hecnar S, Hecnar D. *Pseudacris triseriata* (western chorus frog) reproduction. Herpetol Rev. 1999;30:38.

231. Luja VH, Rodriguez-Estrella R, Schaub M, Schmidt BR. Among-population variation in monthly and annual survival of the Baja California tree frog, *Pseudacris hypochondriaca curta*, in desert oases of Baja California Sur, Mexico. Herpetol Conserv Biol. 2015;10:112–22.

232. Jameson DL. Growth, dispersal and survival of the Pacific tree frog. Copeia. 1956;1956(1):25–9.

233. Jones DK, Dang TD, Urbina J, Bendis RJ, Buck JC, Cothran RD, et al. Effect of simultaneous amphibian exposure to pesticides and an emerging fungal pathogen, *Batrachochytrium dendrobatidis*. Environ Sci Technol. 2017;51(1):671–9.

234. Tucker J. Early post-transformational growth in the Illinois chorus frog (*Pseudacris streckeri illinoensis*). J Herpetol. 1995;29:314–6.

235. Blouin MS. Life history correlates of a color polymorphism in the ornate chorus frog, *Pseudacris ornata*. Copeia. 1989;1989(2):319.

236. Jameson DL. Survival of some Central Texas frogs under natural conditions. Copeia. 1956;1956(1):55–7.

237. Morin PJ. Functional redundancy, non-additive interactions, and supply-side dynamics in experimental pond communities. Ecology. 1995;76(1):133–49.

238. Skelly D. A Behavioral trade-off and its consequences for the distribution of *Pseudacris* treefrog larvae. Ecology. 1995;76:150–64.

239. Storrs SI, Kiesecker JM. Survivorship patterns of larval amphibians exposed to low concentrations of atrazine. Environ Health Perspect. 2004;112(10):1054–7.

240. Van Allen BG, Briggs VS, McCoy MW, Vonesh JR. Carry-over effects of the larval environment on post-metamorphic performance in two hylid frogs. Oecologia. 2010;164(4):891–8.

241. Stewart K, Lougheed S. Testing for intraspecific postzygotic isolation between cryptic lineages of *Pseudacris crucifer*. Ecol Evol. 2013;3:4621–30.

242. Lawrence S. Feasibility of reintroducing spring peepers (*Pseudacris crucifer*) to their historic range [MSc]. Northern Illinois University; 2018.

243. Whitehurst PH, Pierce BA. The relationship between allozyme variation and life-history traits of the spotted chorus frog, *Pseudacris clarkii*. Copeia. 1991;1991(4):1032.

244. Rosenberg EA, Pierce BA. Effect of initial mass on growth and mortality at low pH in tadpoles of *Pseudacris clarkii* and *Bufo valliceps*. J Herpetol. 1995;29(2):181–5.

245. Sours G, Petranka J. Intraguild predation and competition mediate stage-structured interactions between wood frog (*Rana sylvatica*) and upland chorus frog (*Pseudacris feriarum*) larvae. Copeia. 2007;2007:131–9.

246. Whiting A. Factors affecting larval growth and development of the boreal chorus frog Pseudacris maculata [PhD]. [Alberta]: University of Alberta; 2010.

247. Hossack B, Puglis H, Battaglin W, Anderson C, Honeycutt R, Smalling K. Widespread legacy brine contamination from oil production reduces survival of chorus frog larvae. Environ Pollut. 2017;231:742–51.

Chapter 3: Warm spring weather alters calling phenology of four sympatric early-breeding anurans

Formatted as a manuscript in preparation for submission to Integrative Zoology:

Ethier JP, Habib N, Mazerolle MJ, Trudeau VL. Warm spring weather alters calling phenology of four sympatric early-breeding anurans.

Study contributions: JPE contributed through conceptualization, investigation, methodology, data curation, and writing. NH contributed through data curation and editing. MJM contributed through methodology, reviewing, editing, and supervision. VLT contributed through reviewing, editing, supervision, and funding.

3.1 Abstract

Changes in phenological patterns in breeding behaviour are useful for determining the effect of climate change on amphibian communities, many of which are declining and at risk of extinction. However, most of the climate change research focuses on long-term warming trends while giving less consideration to the increasing frequency of extreme weather events. We determined the phenology of calling activity of four early spring-breeding anurans in eastern Ontario using autonomous recording units from 2022–2024. There were the boreal chorus frog (*Pseudacris maculata*), spring peeper (*Pseudacris crucifer*), wood frog (*Boreorana sylvatica*), and American toad (*Anaxyrus americanus*) were assessed. An unusually warm March in 2024 ("false spring") followed by freezing temperatures allowed us to assess the impact of a weather event on this amphibian community. Calling activity of all four species was associated with increasing temperature and wood frog calling activity was associated with decreasing rainfall. Coinciding with the warmer temperatures in March, the first date of calling was advanced by 11 days for American toads, 15 days for wood frog and 18 days for spring peepers and boreal chorus frogs in 2024 compared to 2022. However, the probability of calling activity was significantly reduced in 2024 for all species except spring peeper, which had the highest probability of calling activity in that year. The probability of calling in boreal chorus frogs decreased from 93% in 2022 to 54% in 2024, which may lead to negative fitness consequences for this declining species. This study exemplifies the species-specific response of spring-breeding anurans to seasonal and environmental variables are species-specific, highlighting the potential impact of the atypical weather events on calling activity. Shifts in phenological patterns can create mismatches between the timing of reproduction and the availability of resources important for the survival of offspring, thus negatively impacting recruitment and persistence of local anuran populations.

3.2 Introduction

Biotic and abiotic environmental conditions greatly influence the timing of animal behaviours (*i.e.*, their phenology) (Macphie and Phillimore 2024). Indeed, life history strategies of animals are often adapted to local weather, species composition, and physical structure of habitat (Benard and Greenwald 2023). The unprecedented rapid changes in climate caused by anthropogenic activities therefore represent potential threats to animal fitness (Mantyka-Pringle et al. 2012; Urban 2015; Soroye et al. 2020). Climate change can have negative impacts across several trophic levels (*i.e.*, individual to ecosystem-wide), leading to "phenological mismatches" between interacting species (Miller-Rushing et al. 2010; Thackeray et al. 2016). These changes contribute to population declines in insects (Scranton and Amarasekare 2017; Halsch et al. 2021), birds (Saino et al. 2011), amphibians (Daszak et al. 2005), and reptiles (Sinervo et al. 2010; Griffis-Kyle et al. 2018). Evidence in migrating birds suggests that species that have the greatest phenological mismatches, or those that fail to adjust their behaviour in response to changes in weather and climate, display the largest population declines (Saino et al. 2011; Gilroy et al. 2016). Most research on the impact of climate change on wildlife focuses on long-term changes occurring gradually over many years or decades (Shriver 2025). Importantly, climate change is also associated with increases in the frequency of extreme weather events (*e.g.*, heat waves, hurricanes, floods) which predominately have negative ecological outcomes and thus are equally important to consider (Maxwell et al. 2019).

Climate change contributes to population declines in many amphibian species as the changes are often too extreme or rare for adaptation to occur, such are increasing temperature above thermal tolerances or decreasing the hydroperiod length of breeding ponds (Pottier et al. 2025). It is widely recognized that amphibians are at the highest risk of extinction among all

vertebrate taxa (Wake and Vredenburg 2008; Munstermann et al. 2022). Amphibians may be more susceptible to the direct and indirect negative effects of climate change due to their physiology, biphasic life cycle dependent on both terrestrial and aquatic ecosystems, limited dispersal ability, and high site fidelity (Blaustein et al. 2011; López-Alcaide et al. 2011; Li et al. 2013). Changes to climatic factors could also affect survival and reproduction of amphibians by altering hydroperiods, prey-predator relationships, vegetation composition, and the spread of disease (Corn 2005; Blaustein et al. 2011; Li et al. 2013). While milder winters and earlier springs could promote population viability by increasing breeding opportunities for amphibians in temperate zones (McCaffery and Maxell 2010), it may also increase the risk of freezing and elevate physiological demands throughout hibernation (Benard 2015; Bison et al. 2021). Species in temperate zones and at higher latitudes experience broader variations in temperature and precipitation throughout the year and are more tolerant of gradual climate changes compared to tropical species (Deutsch et al. 2008; While and Uller 2014). However, the impacts of extreme weather events on amphibians, as well as potential differences among species and populations remain largely unknown (Maxwell et al. 2019).

For many anuran (frog, toad, and treefrog) species, vocalizations can be used to track the occurrence, activity patterns, and trends in populations (Dorcas et al. 2010). Males gather at breeding sites and produce vocalizations used to assess the quality of competitors, attract females, and coordinate spawning (Wells and Schwartz 2007). Calling activity occurs primarily during the breeding season, but is influenced by several abiotic and biotic factors, including habitat, hydrology, and interspecific interactions (Klaus and Lougheed 2013; Ospina et al. 2013; Davis et al. 2017). Previous studies on amphibian communities in North America have reported that winter- and spring-breeding species tend to respond similarly to changes in temperature and

precipitation, but that the magnitude of responses may be influenced by breeding strategy (*i.e.*, prolonged versus explosive) and can be species-specific (Gibbs and Breisch 2001; Todd et al. 2010; Walpole et al. 2013). Thus, estimating the relationship between abiotic factors and calling phenology is important for understanding the impacts of climate change and extreme weather on anuran communities to better inform conservation and management efforts.

In this study, our primary objective was to determine how an uncommon weather anomaly associated with climate change influenced the calling activity of early spring-breeding anuran communities. In 2024, there was an unusually warm March in eastern Ontario, during which temperatures exceeded 5°C for 17 consecutive days when temperatures are typically below freezing at this time of year in this region (2014–2021 average = -4.68°C). This weather anomaly ("false spring") provided the opportunity to evaluate the effect of a weather event on the anuran community. Because patterns in calling activity of spring-breeding anurans should be highly responsive to changes in weather conditions, we predicted that frog species would advance their calling activity in response to increasing seasonal temperature associated with the false spring. We hypothesized that responses of anuran species to the false spring would be species-specific and may be related to breeding strategy and population status. To test this, we studied four early spring-breeding anurans: boreal chorus frogs (*Pseudacris maculata*), spring peepers (*Pseudacris crucifer*), American toads (*Anaxyrus americanus*), and wood frogs (*Boreorana sylvatica*). Boreal chorus frogs are a species with a widespread distribution, but local population declines have been pronounced and rapid in recent years in Eastern Canada (Seburn et al. 2014). Boreal chorus frogs are provincially listed as "threatened" in Québec (R.S.Q., chapter E-12.01), and the Great Lakes / St. Lawrence – Canadian Shield (GLSLCS) population is "threatened" in Canada under the species at risk act (Environment Canada 2015). In comparison

to boreal chorus frogs, spring peepers, American toads, and wood frogs are widespread and their populations in eastern Canada have remained relatively stable over the last decade (Lacroix et al. 2024). The breeding strategies of the four species fall along a gradient, with spring peepers and American toads having prolonged breeding seasons (April–June), wood frogs having a short, explosive breeding season that lasts 2–3 weeks, and boreal chorus frogs being somewhere in between (Dodd 2023). Therefore, this amphibian community provides an ideal system to examine how anuran calling activity varies in response to changing weather conditions.

3.3 Methods

3.3.1 Study area

Acoustic sampling occurred at four boreal chorus frog sites in Leeds and Grenville County (approx. 1 h south of Ottawa, Ontario) during three breeding seasons (2022–2024). The "Limerick Forest" site is a small, shallow pond (approx. 5 m width x 20 m length x 0.3 m depth) in a forested area adjacent to the Wolford Bog Complex. The surrounding forest is primarily eastern white pine (*Pinus strobus*), eastern white-cedar (*Thuja occidentalis*), and American beech (*Fagus grandifolia*), with smaller components of sugar maple (*Acer saccharum*), red maple (*Acer rubrum*), eastern hemlock (*Tsuga canadensis*), northern red oak (*Quercus rubra*), black cherry (*Prunus serotina*), and yellow birch (*Betula alleghaniensis*). The "County Rd 15" site is comprised of two long roadside ditches (approx. 3 m width x 115 m length x 0.5 m depth) south of Merrickville-Wolford, ON. The ditches and verges contain a mix of grasses (*Poa* sp.) and sedges (*Carex* sp.). Swamps occur east and west of this section of County Rd 15, containing European buckthorn (*Rhamnus cathartica*), white birch (*Betula papyrifera*), trembling aspen (*Populus tremuloides*), elms (*Ulmus* sp.), and dead ashes (*Fraxinus* sp.). The "Bolton Rd" site is a flooded field (approx. 20 m width x 30 m length x 0.2 m depth) off a dirt road next to a wooded

area dominated by white spruce (*Picea glauca*), eastern white-cedar, white birch, and tamarack (*Larix laricina*). The "Hare's Hill" site is a small marsh (approx. 20 m width x 24 m length x 0.7 m depth) containing cattails (*Typha* sp.) and willow (*Salix* sp.), adjacent to a dirt road, and surrounded by European buckthorn, white spruce, eastern white-cedar, white birch, and tamarack.

3.3.2 Recording schedule

We deployed autonomous recording units (model: AudioMoth 1.2.0; Open Acoustic Devices; Hill et al. 2018) at three sites in 2022, two in 2023, and four in 2024. At each site, a single AudioMoth was placed 1.5 m above the ground and set to record 5 minutes at the beginning of each hour. AudioMoths were deployed from March 30th to May 16th in 2022 and 2023, and from March 12th to May 17th in 2024. Sampling produced a total of 5364 audio files equaling 463 hours of recordings after exclusion of corrupted files. We listened to and visually inspected each 5-min file in the Audacity software (version 3.7.0), noting the calling activity of boreal chorus frog, spring peeper, American toad, and wood frog. Visually inspecting the spectrograms of recordings was essential as large choruses of some species (particularly spring peepers) can mask the signals of other anurans. We initially scored the files using the standard three-point North American Amphibian Monitoring Program (NAAMP) index for each species (Weir and Mossman 2005): (0) absence of calling activity; (1) up to two or three distinct individuals, with mostly non-overlapping calls, (2) overlapping calls but still allowing to distinguish individuals, and (3) full chorus consisting of overlapping calls with non-distinguishable individuals. This index was then converted into a binary value (0 = no calling, 1 = calling) for each species.

3.3.3 Weather data

We obtained hourly temperature and rainfall data from the Environment and Climate Change Canada historical data website for Kemptville CS station (Climate ID: 6104027; mean distance to study sites = 16.1 km) to provide a general overview of the weather conditions in the study area in which all sites are located. We compared mean daily temperature among years for the entire three-month recording period (March–May). For comparison to years of the current study (2022–2024), we also provided the mean monthly temperature and rainfall data averaged across 2014–2021. We presented the mean daily temperature and the number of hours above 5°C for each day during the "early calling initiation period" (1 March to 10 April), during which many anuran species begin calling and often reach the peak of their calling behaviour in this region (De Solla et al. 2007; Brinley Buckley et al. 2021). We used a threshold of 5°C because air and water temperature are among the strongest predictors of anuran calling activity in this region and calling is often initiated when temperatures exceed 5°C (Oseen and Wassersug 2002; Ospina et al. 2013). The 5°C temperature threshold also closely approximates the minimum air temperature of 5.6°C (42°F) used for the first survey following the NAAMP survey protocol (Weir and Mossman 2005) and thus allows for comparisons to other North American studies on early spring calling in anuran amphibians.

3.3.4 Phenology of calling activity

We determine the first day of calling for each species, defined as the first day in the calendar year where vocalizations were detected in ≥ 4 of the 24 audio files. We calculated the relative calling activity level (CAL) as the mean proportion of hours in a day that contained at least one individual calling (*i.e.*, CAL value of 0.50 = 12 out of 24 hrs contained calls) among all sites. This metric provides a comparative measure of the date when each species begins calling

as well as the increases and decreases in calling activity throughout the breeding season. As acoustic recorders had been deployed approximately two weeks earlier in 2024 than in 2022 and 2023, we restricted the dataset for all three years to overlapping deployment dates (March 30 to May 15).

3.3.5 Statistical analyses

We evaluated the relationship between the calling activity of all four species and abiotic factors using generalized linear models and generalized linear mixed models with a binomial distribution and logit link function (Gelman and Hill 2007). For spring peepers and American toads, we modelled the relationship between the binary response variable (0 = no calling, 1 = calling) and seven predictor variables, including time of day, day of the year (linear and quadratic), year type, the interaction between day of the year and year type, air temperature (°C), and rainfall (mm) using the "glmer" function in the R package "lme4" (Bates et al. 2015). Year type was defined as either "average", reflecting the weather conditions typically observed in the region (*i.e.*, 2022 and 2023 were similar to the average of 2014–2021 weather conditions), or "false spring", reflecting weather conditions in 2024 when temperatures were warmer than usual in early March followed by freezing temperatures. For the boreal chorus frog model, we also included the calling activity (binary: 0/1) of wood frogs, American toad, and spring peeper in addition to the above predictor variables. Study site was included as a random variable in the boreal chorus frog, spring peeper, American toad models, whereas site was included as a fixed effect in the wood frog model because the species occurred only at two sites ("Hare's Hill" and "Limerick Forest"). We opted to prioritize study site being treated as a random variable as they represent a random sample of locations within the region where anurans occur. We also expect responses (*i.e.*, calling probabilities) within sites to be clustered. It is generally recommended to

have ≥ 5 levels to a random variable and often multilevel models with only 2 levels provide little to no additional information compared to classical regression model (Gelman and Hill 2007). American toads were absent from the Hare's Hill site, so data from that study site was removed from the American toad model. Additionally, wood frogs were rarely detected at the Bolton Rd and County Rd 15 sites (< 1% of audio files). We removed these study sites from the dataset and used a generalized linear model for wood frog calling activity. We used the air temperature from an internal sensor within the AudioMoth recording units, rather than the local weather station, to account for any differences in temperature among locations. Day of the year (DOY), air temperature, and rainfall were standardized to mean 0 and unit variance before analyses. Time of day was converted into four 6-hr time blocks (Morning = 06:00–11:59:59, Afternoon = 12:00–17:59:59, Evening = 18:00–23:59:59, Night = 00:00–05:59:59) to circumvent the circular nature of the 24-h clock.

3.4 Results

3.4.1 Weather patterns

The mean temperature and mean rainfall accumulation across March–May in 2024 differed from 2022 and 2023 (Figure 3.1). While rainfall was largely comparable among years, daily temperatures were warmer in 2024. Notably, there was an early thaw, or false spring, in March of 2024 (DOY 61–78). Temperatures in this region, based on local weather records from 2014–2021, are typically below freezing (average = -4.68°C, range = -8.84°C – -1.03°C) during early- to mid-March (Figure 3.1). Mean daily temperature during 2022 and 2024 were comparable to the 2014-2021 average. In contrast, temperatures in 2024 were consistently above 0°C (Figure 3.2), with at least one hour per day above 5°C for 17 days, followed by 7 days with temperatures < 0°C (Figure 3.3); a pattern not observed in the previous two years (2022 and

2023) or during 2014–2021. Temperature differences were particularly pronounced during the early calling initiation period (DOY 60–100). Mean daily temperature (± standard deviation) during this period was 0.53°C (± 5.52), 0.05°C (± 3.02), and 3.02°C (± 4.55) in 2022, 2023, and 2024, respectively. The mean number of hours above 5°C per day was 5.4 hours (± 6.5), 2.6 hours (± 4.4), and 8.5 hours (± 7.1) in 2022, 2023, and 2024, respectively.

3.4.2 Phenology of calling activity

There was considerable variation in the phenology of calling activity among species and years (Figure 3.4; Table 3.1). The breeding season of the boreal chorus frog in eastern Ontario typically extends from late March to early May, with most of the activity occurring in early April (Figure 3.4). Ponds were frozen until early April in 2022 and 2023 but were free of ice by mid-March in 2024. Boreal chorus frog calling activity began on April 1 (DOY 91) and March 31 (DOY 90) in 2022 and 2023, respectively, and ended on May 13 (DOY 133) in 2022 and 2023 (Figure 3.5). In contrast, boreal chorus frog calling activity in 2024 began on March 15 (DOY 73), more than two weeks earlier than in 2022 and 2023.

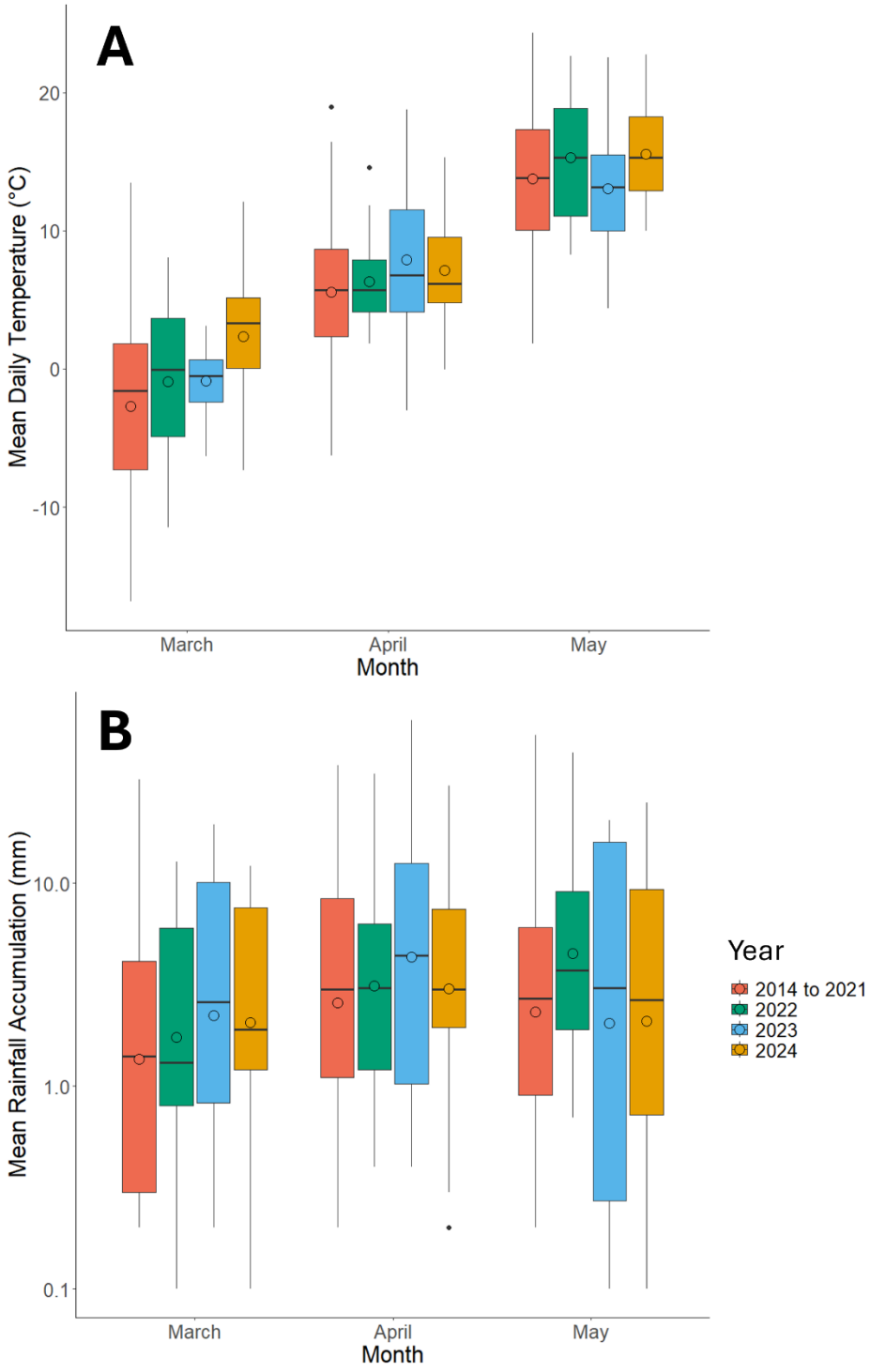

Figure 3.1: Weather conditions by month within the 2022–2024 anuran breeding seasons in Leeds and Grenville County, Ontario, Canada. Historical conditions (2014-2021) are provided for comparison. Mean daily temperature (A) and mean rainfall accumulation (B). Standard boxplots with 25th and 75th percentiles. Median values indicated by thick black line. Whiskers are minimum and maximum values with outliers indicated by black dots. Empty circles in boxplots indicate the mean values.

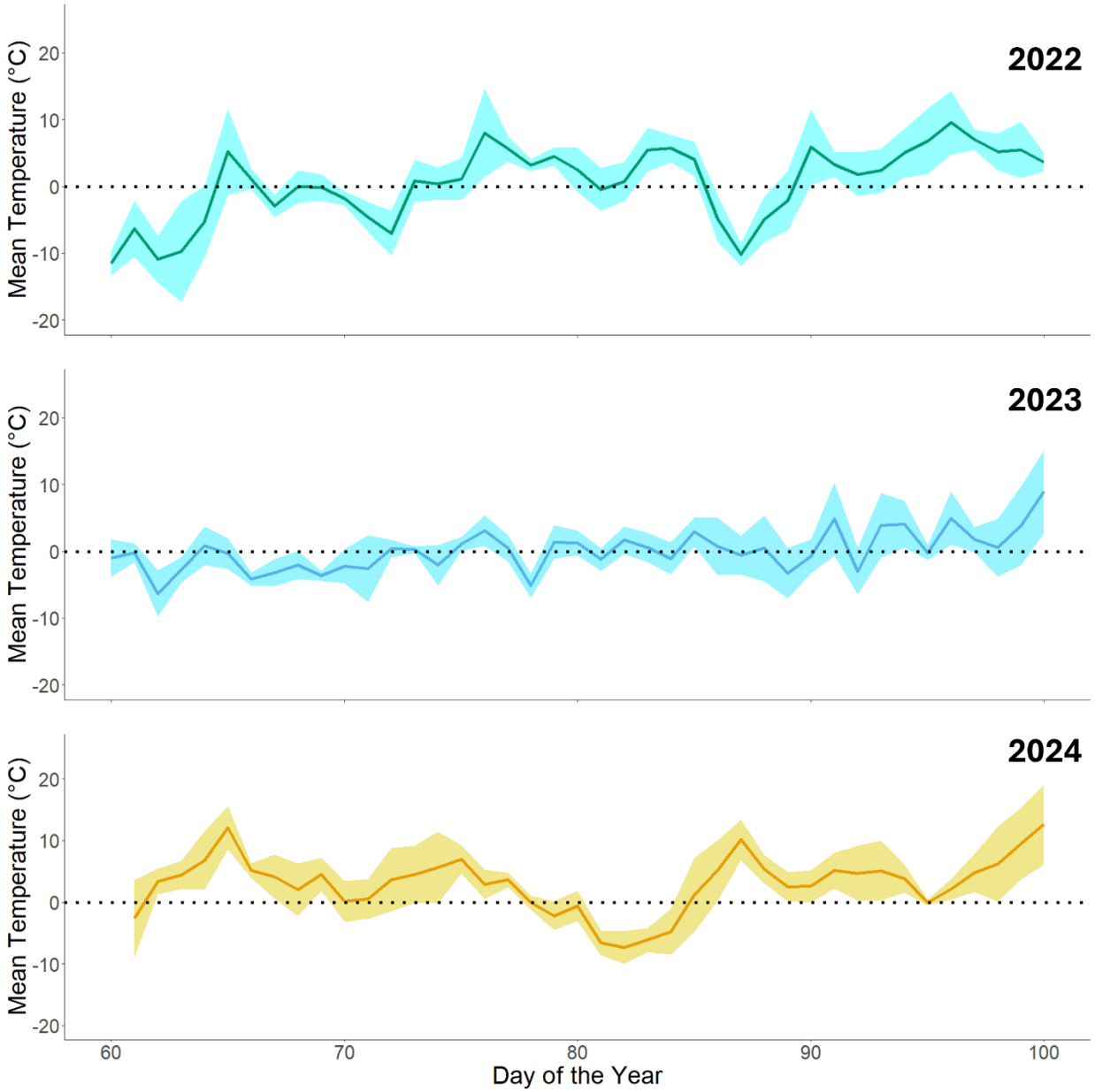

Figure 3.2: Mean daily temperature (°C) during the early calling initiation period (March 1 to April 10) within the 2022–2024 anuran breeding seasons in Leeds and Grenville County, Ontario, Canada. Solid lines are the mean daily temperature with bands denoting standard errors. The dotted line denotes 0°C. In a non-leap year, day of the year (DOY) of 60 = March 1 and DOY 100 = April 10.

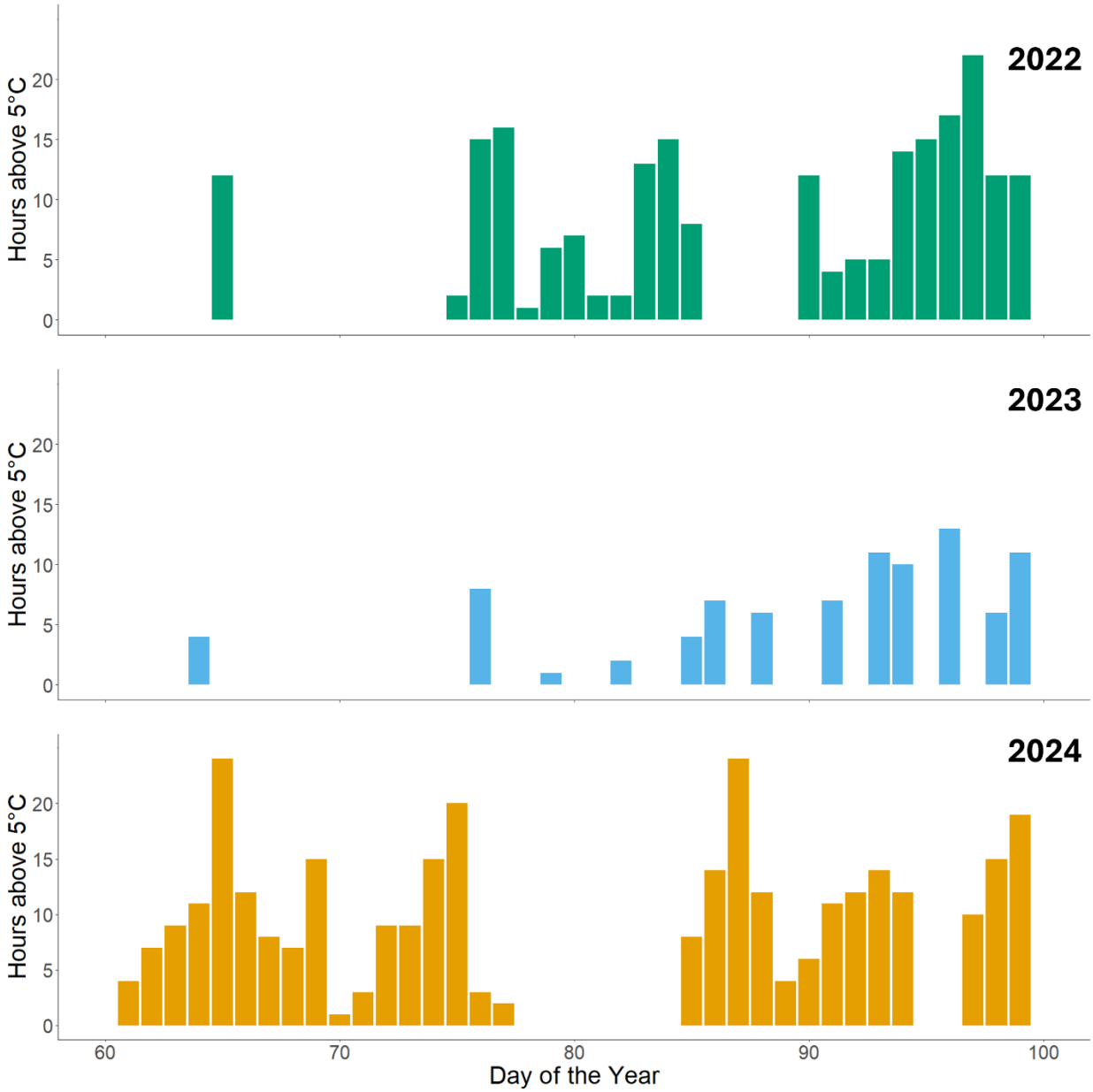

Figure 3.3: Number of hours above 5°C per day during the early calling initiation period (March 1 to April 10) within the 2022–2024 anuran breeding seasons in Leeds and Grenville County, Ontario, Canada. 5°C denotes when many early spring-breeding anuran species will begin calling. In a non-leap year, day of the year (DOY) of 60 = March 1 and DOY 100 = April 10.

Based on the same periods in the three years, mean CAL for boreal chorus frogs was ≥ 0.50 for 68.8% (33/48) of days in 2022 compared to 58.3% (28/48) and 37.5% (18/48) of days in 2023 and 2024, respectively. Calling activity in 2022 and 2023 was characterized by a rapid rise in boreal chorus frog calling activity around DOY 90, then a relatively consistent and consecutive 30-day period with CAL ≥ 0.50 (approx. DOY 100–130), followed by a rapid decline in calling activity. Conversely, boreal chorus frog calling activity in 2024 was less consistent, included several rises and falls, and had only a 10-day period where CAL was consecutively ≥ 0.50 (Figure 3.5). While the overall mean CAL differed across years, the peak of boreal chorus frog calling activity (CAL ≥ 0.95) occurred between DOY 101–104 in all three years, and there were a comparable number of days (~ 40 days) where at least one individual was calling between DOY 89–135 (Supplementary Table S3.1).

Like the boreal chorus frog, the calling onset of spring peepers, wood frogs, and American toads began several days sooner in 2024 (Figure 3.4; Table 3.1). Compared to 2022, the first day of calling in 2024 advanced by 18 days, 15 days, and 11 days for spring peepers, wood frogs, and American toads, respectively. Calling activity levels of these species throughout the breeding season were more consistent among years compared to the boreal chorus frog. CAL of spring peepers was relatively consistent throughout the breeding season with small increases generally occurring around DOY 100 and then again around DOY 120. Calling activity for wood frogs peaked between DOY 90–110 and was generally very low for the remainder of the breeding season. Calling activity for American toads was more punctuated with rapid increases in CAL occurring 2–3 times during the breeding season and was greatest between DOY 115–130. However, there was a peak in American toad calling around DOY 105–107 in 2022, and the increase in calling activity was more gradual in 2024 compared to the two previous years.

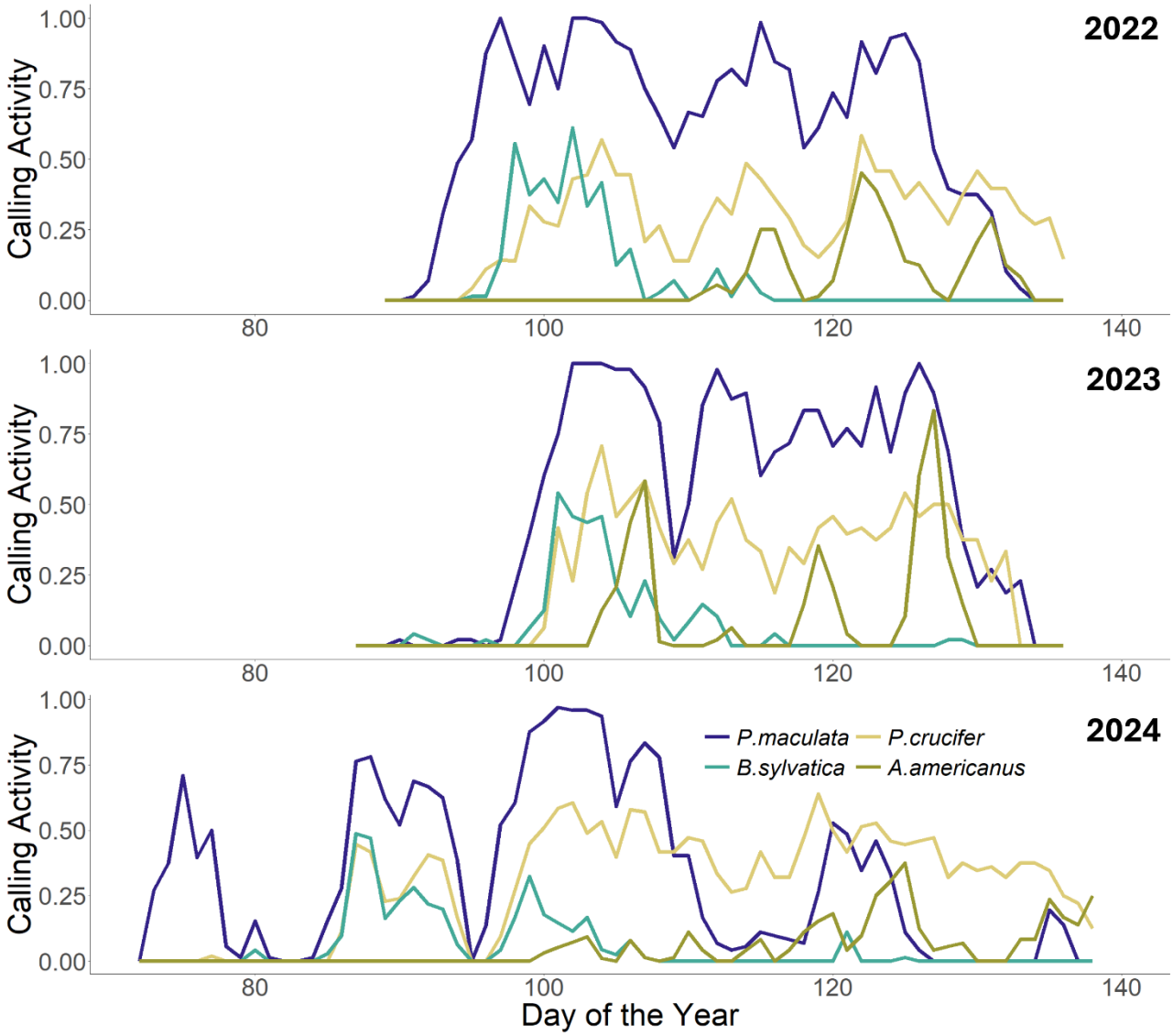

Figure 3.4: A comparison of the mean calling activity level (CAL) of boreal chorus frog (*Pseudacris maculata*), spring peeper (*Pseudacris crucifer*), wood frog (*Boreorana sylvatica*) and American toad (*Anaxyrus americanus*) during the 2022–2024 breeding seasons in Leeds and Grenville County. CAL is the mean proportion of hours for each day of the year that a species was recorded calling using AudioMoth autonomous recording units.

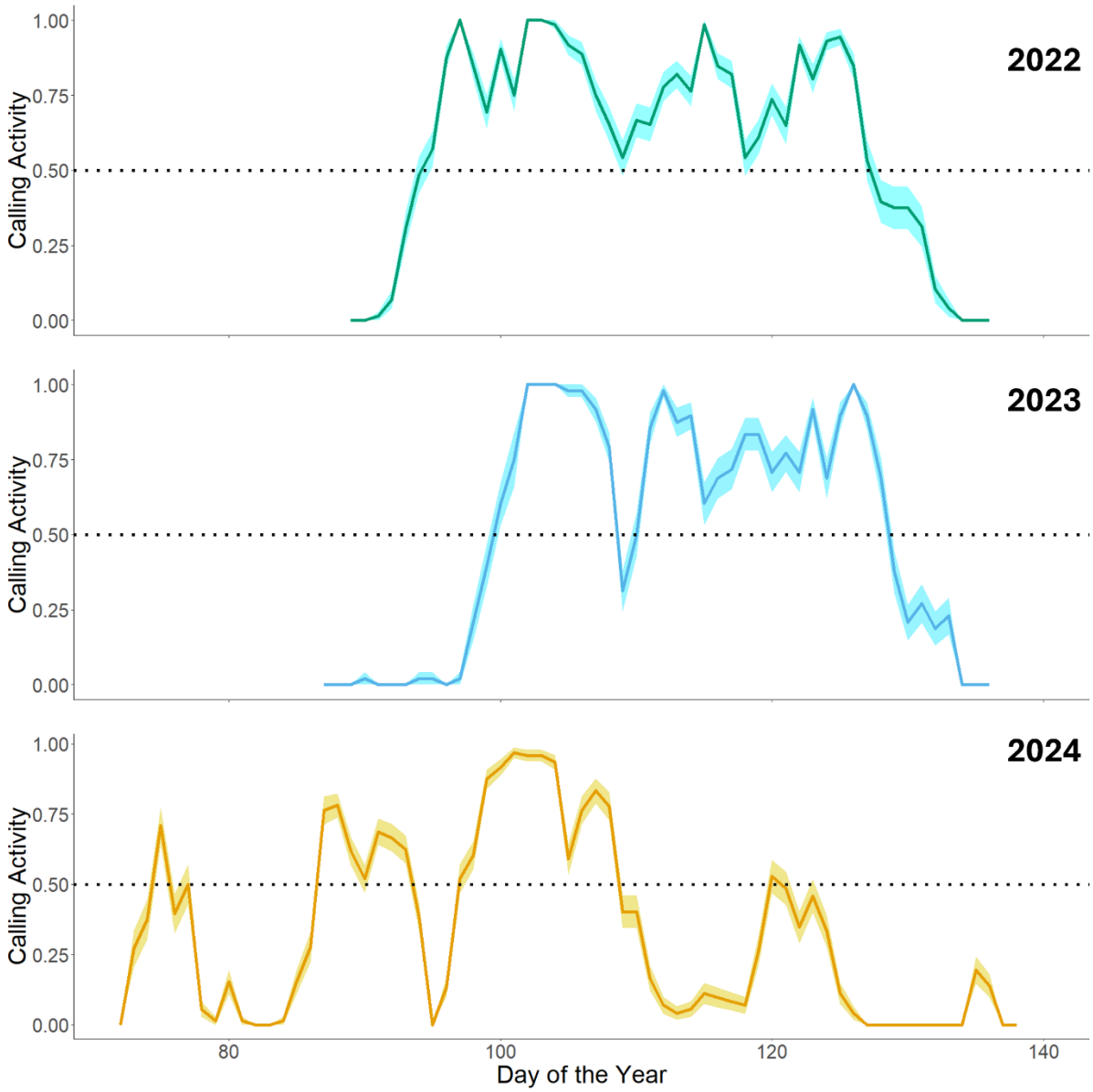

Figure 3.5: Mean calling activity level (CAL) of boreal chorus frog (*Pseudacris maculata*) during the 2022–2024 breeding seasons (March to May) in Leeds and Grenville County. CAL is the mean proportion of hours for each day of the year (± standard error band) that chorus frogs were recorded calling using AudioMoth autonomous recording units. The dotted line represents a CAL of 0.50, which signifies 12 h within a 24-h period containing calling chorus frogs. Recordings of calling activity began later in the season in 2022 and 2023 as breeding ponds were frozen.

3.4.3 Calling activity models

The probability of boreal chorus frog calling activity decreased over time (z = -13.17, p < 0.001), but these effects varied across years (Figure 3.5). Calling activity was positively correlated with increasing temperatures (z = 23.33, p < 0.001) but did not vary with rainfall (z = -0.85, p = 0.392). Although calling was observed throughout the day, calling was higher at night than at any other time (Table 3.2; Figure 3.6). Finally, calling activity of boreal chorus frogs was positively associated with the calling activity of all three sympatric species (Table 3.2).

There were several broad similarities in the effects of abiotic conditions on calling activity between boreal chorus frogs and the three sympatric species (Table 3.3–3.5). The probability of calling activity of spring peepers, wood frogs, and American toads decreased with time, but these effects varied across year types (Figure 3.7). Calling activity was significantly greater in average years than during the false spring year for wood frogs (z = 3.76, p < 0.001) and American toads (z = 4.12, p < 0.001). Conversely, probability of calling activity for spring peepers increased during the false spring (z = -4.93, p < 0.001). Probability of calling varied during the day, with the greatest calling probability occurring at night for all species. For wood frogs, the probability of calling was significantly lower in the morning (z = -9.20, p < 0.001) and the afternoon (z = 5.20, p < 0.001) but did not differ between the evening and the night (z = -0.90, p = 0.367). Calling activity increased with increasing temperature (Figure 3.8) but did not vary with rainfall.

Table 3.1: First and last date (day of the year) of calling activity of four sympatric anuran species, 2022–2024.

	2022		2023		2024	
	First	**Last**	**First**	**Last**	**First**	**Last**
Boreal chorus frog (*Pseudacris maculata*)	91	133	90	133	73	136
Wood frog (*Boreorana sylvatica*)	95	115	91	129	80	125
American toad (*Anaxyrus americanus*)	111	133	104	129	100	136
Spring peeper (*Pseudacris crucifer*)	95	136	100	132	77	136

Note: 2024 was a leap year

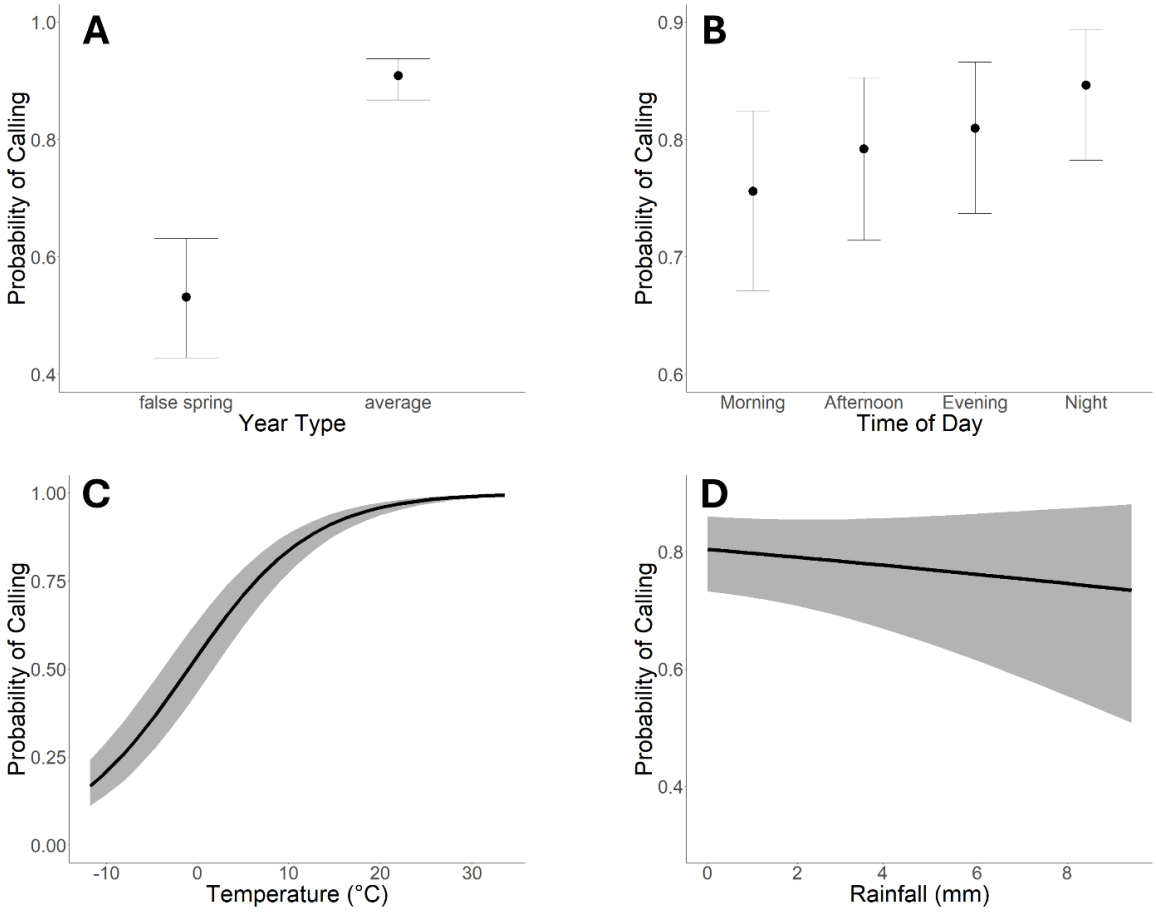

Figure 3.6: The effect of year type (A), time of day (B), mean temperature (C) and mean rainfall (D) on the predicted probability of boreal chorus frog (*Pseudacris maculata*) calling activity during the 2022–2024 breeding seasons in Leeds and Grenville County. Probabilities are based on a generalized linear mixed effects model. Error bars and bands denote 95% confidence intervals.

Table 3.2: Summary of generalized linear mixed effects model of the fixed and random effects of abiotic and biotic factors influencing boreal chorus frog (*Pseudacris maculata*) calling activity during the 2022–2024 breeding seasons in Leeds and Grenville County. DOY = day of the year; LISY = presence (1) or absence (0) of wood frog (*Boreorana sylvatica*); ANAM = presence (1) or absence (0) of American toad (*Anaxyrus americanus*); PSCR = presence (1) or absence (0) of spring peeper (*Pseudacris crucifer*). τ00 = between-subjects; ICC = intraclass correlation coefficient.

Fixed Effects		**Estimate**	**SE**	**z value**	**p**
(Intercept)		-0.13	0.23	-0.56	0.573
DOY (Linear)		-1.88	0.07	-27.14	< 0.001
DOY (Quadratic)		-0.84	0.06	-13.17	< 0.001
Year Type (=false spring)					
	Average year	2.17	0.10	22.37	< 0.001
Time of Day (=Night)					
	Morning	-0.58	0.09	-6.39	< 0.001
	Afternoon	-0.37	0.11	-3.41	0.001
	Evening	-0.26	0.09	-3.00	0.003
Temperature		1.108	0.05	23.33	< 0.001
Rainfall		-0.02	0.03	-0.85	0.393
LISY (=0)		1.71	0.17	10.09	< 0.001
ANAM (=0)		1.25	0.14	9.14	< 0.001
PSCR (=0)		1.06	0.08	13.16	< 0.001
DOY (Linear):Year Type (=false spring)					
	Average year	1.64	0.07	22.46	< 0.001
DOY (Quadratic):Year Type (=false spring)					
	Average year	-1.03	0.08	-12.62	< 0.001
Random Effects (Site)		**Value**			
σ^2		3.29			
$\tau_{00\ Site}$		0.16			
ICC		0.05			
N_{Site}		4			
Observations		9198			
Marginal R^2 / Conditional R^2		0.600/0.618			

Table 3.3: Summary of generalized linear mixed effects model of the fixed and random effects of abiotic and biotic factors influencing spring peeper (*Pseudacris crucifer*) calling activity during the 2022–2024 breeding seasons in Leeds and Grenville County. DOY = day of the year. τ00 = between-subjects; ICC = intraclass correlation coefficient.

Fixed Effects		**Estimate**	**SE**	**z value**	**p**
(Intercept)		1.78	0.38	4.66	< 0.001
DOY (Linear)		0.09	0.05	1.97	0.049
DOY (Quadratic)		-0.47	0.05	--9.54	< 0.001
Year (=false spring)					
	Average year	-0.47	0.09	-4.93	< 0.001
Time of Day (=Night)					
	Morning	-3.38	0.10	-35.57	< 0.001
	Afternoon	-4.38	0.13	-34.75	< 0.001
	Evening	-0.95	0.08	-12.54	< 0.001
Temperature		0.48	0.04	11.30	< 0.001
Rainfall		-0.01	0.03	-0.42	0.794
DOY (Linear):Year Type (=false spring)					
	Average year	0.82	0.07	11.54	< 0.001
DOY (Quadratic):Year Type (=false spring)					
	Average year	-0.59	0.07	-8.06	< 0.001
Random Effects (Site)		**Value**			
σ^2		3.29			
$\tau_{00\ Site}$		0.55			
ICC		0.14			
N_{Site}		3			
Observations		9198			
Marginal R^2 / Conditional R^2		0.521 / 0.590			

Table 3.4: Summary of generalized linear mixed effects model of the fixed abiotic and biotic factors influencing wood frog (*Boreorana sylvatica*) calling activity during the 2022–2024 breeding seasons in Leeds and Grenville County. DOY = day of the year. Pseudo R^2 = Tjur's coefficient of discrimination (Tjur 2009). Note that site was treated as a fixed effect because the species occurred on only two sites.

Fixed Effects		**Estimate**	**SE**	**z value**	**p**
(Intercept)		-3.13	0.20	-15.95	< 0.001
DOY (Linear)		-2.47	0.24	-10.43	< 0.001
DOY (Quadratic)		-0.03	0.16	-0.18	0.855
Year (=false spring)					
	Average year	0.93	0.24	3.76	< 0.001
Time of Day (=Night)					
	Morning	-1.70	0.18	-9.20	< 0.001
	Afternoon	-0.91	0.18	-5.20	< 0.001
	Evening	-0.13	0.15	-0.90	0.367
Site (=Hare's Hill)					
	Limerick	0.25	0.11	2.30	0.022
Temperature		0.98	0.08	12.37	< 0.001
Rainfall		-0.07	0.06	-1.16	0.246
DOY (Linear):Year Type (=false spring)					
	Average year	-6.16	0.65	-9.53	< 0.001
DOY (Quadratic):Year Type (=false spring)					
	Average year	-6.64	0.49	-13.57	< 0.001
Observations		5429			
pseudo R^2		0.386			

Table 3.5: Summary of generalized linear mixed effects model of the fixed and random effects of abiotic and biotic factors influencing American toad (*Anaxyrus americanus*) calling activity during the 2022–2024 breeding seasons in Leeds and Grenville County. DOY = day of the year. τ00 = between-subjects; ICC = intraclass correlation coefficient.

Fixed Effects		**Estimate**	**SE**	**z value**	**p**
(Intercept)		-1.20	0.27	-4.47	< 0.001
DOY (Linear)		1.16	0.16	7.08	< 0.001
DOY (Quadratic)		-0.86	0.14	-6.27	< 0.001
Year Type (=false spring)					
	average	0.77	0.19	4.12	< 0.001
Time of Day (=Night)					
	Morning	-3.07	0.20	-15.42	< 0.001
	Afternoon	-3.24	0.19	-17.14	< 0.001
	Evening	-1.07	0.12	-8.86	< 0.001
Temperature		1.13	0.07	15.48	< 0.001
Rainfall		0.01	0.05	0.29	0.774
DOY (Linear):Year Type (=false spring)					
	average	1.58	0.27	5.80	< 0.001
DOY (Quadratic):Year Type (=false spring)					
	average	-1.44	0.21	-6.96	< 0.001
Random Effects (Site)		**Value**			
σ^2		3.29			
$\tau_{00\ Site}$		0.16			
ICC		0.05			
N_{Site}		3			
Observations		7151			
Marginal R^2 / Conditional R^2		0.764 / 0.775			

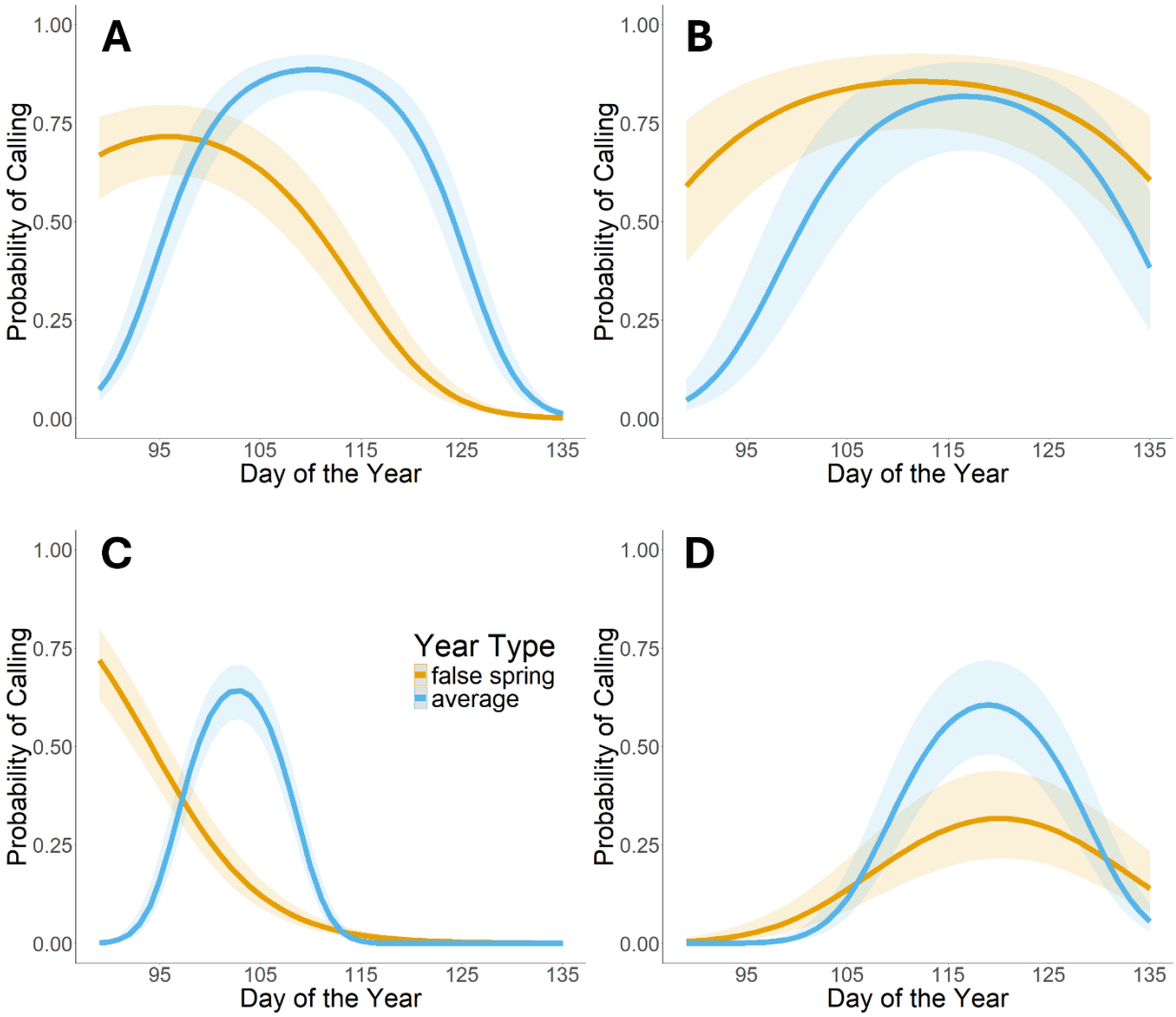

Figure 3.7: The interaction of day of year and year type on the predicted probability of anuran calling activity during the 2022–2024 breeding seasons in Leeds and Grenville County, including boreal chorus frog (A), spring peeper (B), wood frog (C), and American toad (D) Probabilities are based on generalized linear mixed effects models (A, B, D) or generalized linear model (C) which included the linear and quadratic effects of day of the year (DOY). Year type: average = typical seasonal temperatures (2022 and 2023); false spring = warm March temperatures followed by a period of freezing temperatures (2024). In a non-leap year DOY 95 = April 5 and DOY 135 = May 15. Error bands denote 95% confidence intervals.

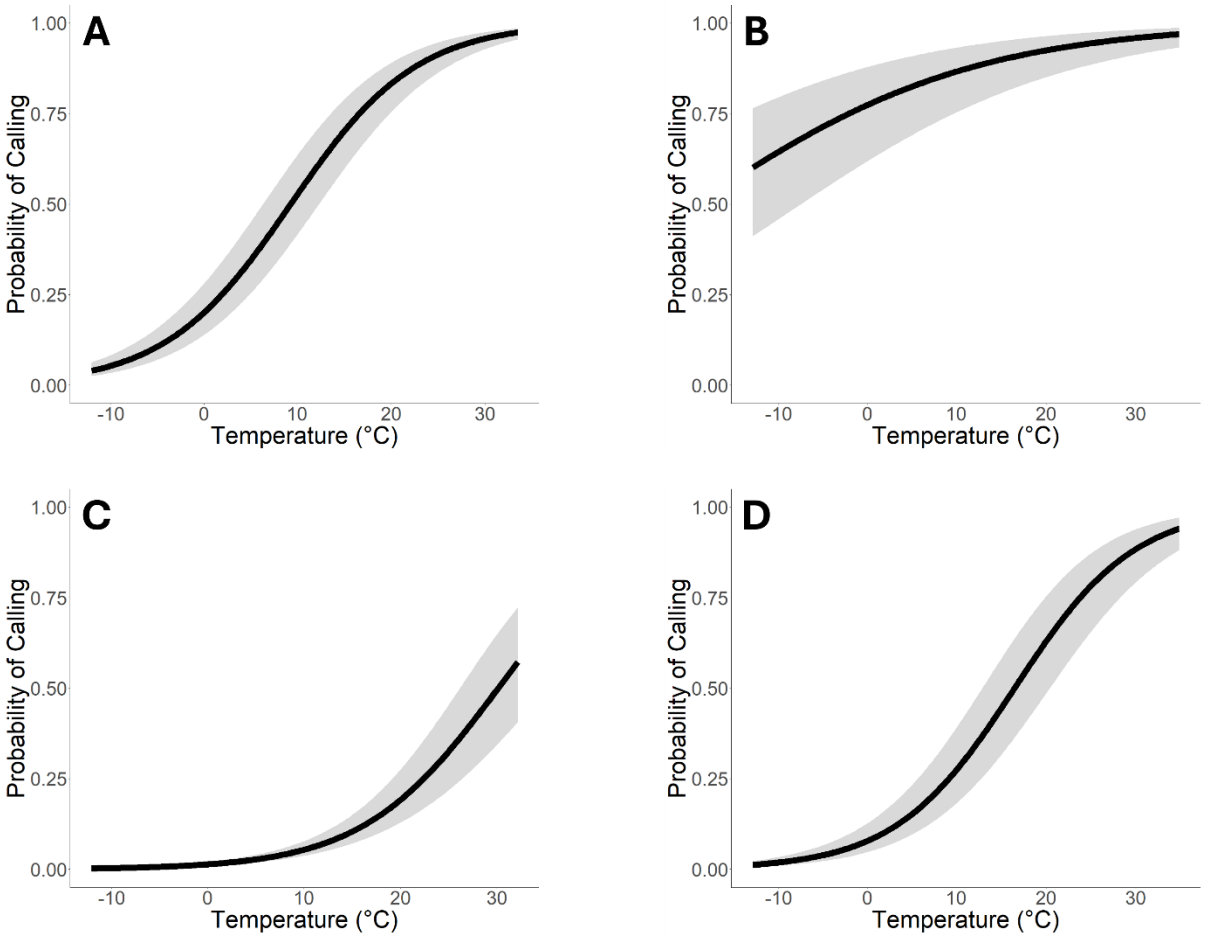

Figure 3.8: The effect of air temperature (°C) on the predicted probability of anuran calling activity during the 2022–2024 breeding seasons in Leeds and Grenville County, including boreal chorus frog (A), spring peeper (B), wood frog (C), and American toad (D) Probabilities are based on generalized linear mixed effects models (A, B, D) or generalized linear model (C). Error bands denote 95% confidence intervals.

3.5 Discussion

Using data from 2022–2024, we modeled the relationship between calling activity and abiotic factors of four spring-breeding anurans in a temperate region of North America. As predicted, calling activity was positively associated with increasing temperature for all species. In response to the false spring of 2024, frogs began calling 11–18 days earlier than the previous two years, with the greatest shift in calling phenology occurring in the two *Pseudacris* species

(*i.e.*, boreal chorus frog and spring peeper). Additionally, the impact of false spring on frogs appeared to extend beyond the two-week period of unseasonably warm temperatures. Although there was a positive association between calling activity and temperature in the models for all species, the probability of calling declined significantly in 2024 for three of the four species compared to the other years. Of the species studied, boreal chorus frogs were the most affected by the false spring. Overall calling activity level (CAL) across the 2024 breeding season was reduced and there were half as many days in 2024 that had CAL ≥ 0.50 compared to 2022. Boreal chorus frog calling activity was also more sporadic in 2024, with several sharp peaks and falls in calling, compared to the single consistently high level of calling activity from DOY 100–130 observed in previous years.

3.5.1 Comparison of responses to abiotic variables among species

Abiotic variables associated with temperature and precipitation are often the best predictors for calling activity in anurans (Ospina et al. 2013; Walpole et al. 2013). Temperature thresholds that initiate calling activity can be species- and population-specific but calling generally occurs when temperatures are ≥ 5°C in temperate regions of eastern North America (Oseen and Wassersug 2002; Saenz et al. 2006) and the United Kingdom (Scott et al. 2008). In our study, increases in temperature were associated with higher predicted probability of calling activity for all species. While precipitation may be important for emergence from hibernation (Green et al. 2016; Green 2017) and the initiating calling behaviour in many temperate anurans (Beebee 1995; Todd et al. 2010), we found that hourly rainfall was not a significant predictor of calling activity for any of the species considered in our study. Brinley Buckley et al. (2021) observed that calling was not related to daily precipitation, but boreal chorus frog calling activity was positively associated with 7-day total precipitation accumulation. Klaus and Lougheed

(2013) found calling activity in wood frogs increases with monthly rainfall. However, mean daily rainfall and total monthly precipitation accumulation in our study were similar among years (Figure 3.1; Supplementary Table S3.2), and for this reason, we did not include these variables in the models.

The magnitude of effects on phenology caused by changes in weather and climate can depend on the breeding strategy of anurans. Oseen and Wassersug (2002) and Saenz et al. (2006) observed that the calling activity of prolonged breeders was more affected by temperature and precipitation compared to explosive breeders. They argued that prolonged breeders must maintain energy stores for an extended period, whereas explosive breeders must capitalize on a short and intense breeding period. As a result, explosive breeders would be less responsive to changes in abiotic conditions once calling begins than prolonged breeders. Conversely, several studies conducted on anuran communities in North America (Walpole et al. 2012; Klaus and Lougheed 2013), South America (Schalk and Saenz 2016), and Australia (Plenderleith et al. 2018) have shown negligible differences in species responses to temperature among breeding strategies or provide evidence that prolonged breeders are less affected by abiotic conditions. In our study, the positive relationship between calling activity and air temperature was stronger for wood frogs and American toads (explosive breeders), than spring peepers (prolonged breeders).

Calling activity was strongly associated with day of the year for all species studied, but this relationship varied with year type. In response to the false spring in 2024, all species in our study advanced their first date of calling by 11–18 days compared to 2022. All species except the spring peeper experienced significant decreases in the probability of calling in the false spring year (2024) compared to the previous two years. Conversely, the probability of calling for spring peepers was greater during the false spring year than the other years. High probabilities of spring

peepers calling were predicted throughout the sampling period in the false spring year. Day of the year is strongly related to environment variables that are known to influence calling behaviour, such as temperature and rainfall (Ficetola and Maiorano 2016). Day of the year is also related to photoperiod, which may be more influential on call phenology of amphibians than previously thought (Canavero and Arim 2009). For example, day of the year was the strongest predictor of frog calling for the majority (67%) of 100 species in Australia when controlling for variation in temperature and rainfall (Thompson et al. 2022).

Time of day was a significant predictor of calling activity for all species investigated. There may be phylogenetic differences in calling activity among temperate frogs, with species in the Ranidae family (*e.g.*, wood frog) having the highest levels of calling after midnight and species in the Hylidae family (*e.g.*, spring peeper, boreal chorus frog) calling more often before midnight (Bridges and Dorcas 2000). We found that calling activity occurred mostly during the evening (18:00–23:59) or night (00:00–05:59) for spring peepers, wood frogs, and American toads. Similar effects of time of day were observed for spring peepers, wood frogs, and American toads in an anuran community in New Brunswick, Canada where calling activity for all three species was significantly reduced during the day (sunrise–sunset) compared to evening (sunset–midnight) and night (midnight–sunrise) (Oseen and Wassersug 2002). In contrast, boreal chorus frog in our study called at all times of day but had the highest calling probability at night. Most anuran species are assumed to be predominantly nocturnal (Duellman and Trueb 1994; Anderson and Wiens 2017). Diurnal calling is observed in some Ranidae species, such as *Lithobates clamitans* and *L. catesbeiana*, but calling activity during the daytime is often significantly less than at night (Bridges and Dorcas 2000). These species that call during the day often have adaptations to withstand desiccation or avoid predation (Callaghan and Rowley 2020). For

example, boreal chorus frogs are small-bodied, cryptic species that call from densely vegetated positions within breeding ponds, which reduces visual detection from potential predators.

Other factors not considered in this study likely also influenced calling activity. Social factors influence the onset of calling behaviour (Wells and Schwartz 2007) and relationships may differ depending on the scale at which it is observed. For example, Brooke et al. (2000) found that abiotic variables (*i.e.*, temperature, rainfall, moon illumination/visibility, humidity, barometric pressure) accounted for relatively little of the variation (10% at most per variable) in the number of calls by male ornate nursery-frogs (*Cophixalus ornatus*) after removing the effects of site and day of the year. These abiotic factors may shape when frogs begin calling across a population but have relatively less influence on the time an individual frog begins to call at a breeding pond. Anthropogenic noise, such as vehicle traffic, can also influence the timing and intensity of calling behaviour in anurans (Simmon and Narins 2018; Zaffaroni-Caorsi et al. 2023). In the current study, traffic volume was relatively high (2–3 vehicles/min) during the day at the County Rd 15 location potentially affecting calling activity. Anuran species that produce low frequency calls (*i.e.*, bullfrog, green frog) may reduced calling rate to avoid calling during times of heavy traffic, as vehicles produce noise in a similar frequency band that potentially mask signals of callers (Vargas-Salinas et al. 2014). Affected species may also alter the acoustic properties of their calls, such as decreasing amplitude and increasing dominant frequency, to maximize the signal-to-noise ratio and increase the likelihood of signals are received by conspecific competitors and mates (Cunnington and Fahrig 2010). In contrast, anurans species with high frequency calls (*i.e.*, American toad, grey treefrog) appear to be less affected by vehicle noise and may call independent of traffic volume (Vargas-Salinas et al. 2014).

3.5.2 Boreal chorus frogs and the response to the false spring

The calling activity of boreal chorus frog appeared to be uniquely impacted by the 2024 false spring. The warm weather in March of 2024 extended the number of days that at least one individual was calling but reduced the overall calling effort throughout the breeding season. The probability of calling decreased from 0.89 in the average years to 0.47 in 2024, when all other predictors of fixed effects were held at their mean values. Even with the full inclusion of all the days during which boreal chorus frogs were observed calling in 2024 (*i.e.*, DOY 72–135) into the model, there was a significant decrease in the probability of boreal chorus frog calling activity during the false spring compared to all other years combined (Supplementary Table S3.3). High levels of calling (CAL ≥ 0.5) of boreal chorus frogs were condensed to an approximately 30-day period in the average years (*i.e.*, 2022 and 2023). While the calling period spanned a similar number of days in each year, calling activity in 2024 was more sporadically distributed throughout March–May with several rapid increases and declines in calling. Together these results imply that there was a reduction in the number of boreal chorus frog individuals that were calling among years, caused by either a decline in the metapopulation, conflicting environmental cues associated with calling activity during the breeding season caused by the false spring, or a combination of both. It may also indicate that only a portion of the individuals within the boreal chorus frog population are shifting their calling behaviour in response to the warming temperatures, but in the absence of tracking individuals throughout the breeding season, this is impossible to definitively conclude.

Disruptions to calling phenology may have important negative consequences for populations of declining species. Boreal chorus frogs are declining in many eastern portions of its range (Corser et al. 2012; Seburn et al. 2014) and therefore are likely less resilient to any

negative impacts on reproduction and recruitment due to their shrinking population size. Boreal chorus frogs displayed a mixed response to the false spring. Despite differences in overall calling activity among years, and an advancement in the onset of calling during the false spring, the peak of boreal chorus frog calling activity (CAL ≥ 0.95) occurred at approximately the same time (early-April; DOY 101–104) in all three years. Previous evidence suggests that the breeding phenology of boreal chorus frogs is not shifting to match long-term changes in climate. Klaus and Lougheed (2013) found that there was no significant trend in the date of the first day of calling of *Pseudacris* spp. (*P. maculata* and *P. triseriata* combined) in southeastern Ontario between 1970–2010 despite an estimated increase of 2.7–2.8°C in annual spring temperature. Within the same period, wood frogs and American toads advanced their first date of calling by an average of 19.2 days (Klaus and Lougheed 2013). Although evidence is lacking in amphibians, multiple studies on migratory bird species report that species that fail to adjust their migration dates in relation to long-term climate trends and seasonal variation in weather were more likely to experience population declines (Møller et al. 2008; Saino et al. 2011; Gilroy et al. 2016).

3.5.3 Climate change, false springs, and amphibian conservation

The World Meteorological Organization (WMO) reported that 2024 was the warmest year ever recorded since the 1850s. Temperatures were 1.55°C above the 1850–1900 pre-industrial global mean and are expected to continue to increase in the coming decades (WMO 2025). The impact of climate change on amphibians in Canada is expected to be “moderate”, with 34.5% (10/29) of species listed as being currently affected or projected to be affected by climate change (Woo-Durand et al. 2020). We propose that the impact of the increased frequency of extreme weather events on wildlife as well as the long-term global warming trends need to be considered. Accumulating evidence indicates that extreme weather can have long-term

consequences for wildlife beyond the initial impacts linked to the event (Harris et al. 2018; Maxwell et al. 2019). Minor weather events, such as a false spring, can impose higher daily energy expenditures (Schmidt et al. 2024), reduce immune system functioning and fecundity (Liu et al. 2018), thereby potentially exacerbating population declines. Our study provides indicative evidence that the false spring phenomenon resulted in significant advances in the first date of calling but a reduction in calling activity throughout the spring breeding season. Warmer winter and spring temperatures in temperate regions could potentially be beneficial to amphibians by reducing the risk of mortality during hibernation (Üveges et al. 2016), allowing for earlier breeding opportunities, and expediating larval development and metamorphosis (Tasker et al. 2022). However, multiple cycles of freeze-thaw likely exert greater pressures on the physiology of individuals leading to injuries compared to a single uninterrupted hibernation period (Storey and Storey 1996; Yokum et al. 2023). Lab-based hibernation experiments found that common toads (*Bufo bufo*) held at temperatures 4°C warmer than typical winter conditions had increased metabolic rates, higher daily energy demands, and reduced cold tolerance when exposed to a simulated frost (Schmidt et al. 2024). This is consistent with the false spring hypothesis that states that advancements in the phenological patterns can accrue fitness consequences for plants and animals (Yermokhin and Tabachishin 2022; Baker et al. 2024). Warm temperatures in late-winter and early-spring are often followed by a frost or a period of freezing temperatures, as we witnessed in our study. While we did not estimate mortality in the current study, earlier spawning increases the risk of adults and offspring being exposed to lethal freezing temperatures (Frisbie et al. 2000; Bison et al. 2021). Baker et al. (2024) noted that the number of days wood frogs, spring peepers, and spotted salamanders are active but exposed to freezing temperatures during the breeding season has nearly tripled in recent years (2016–2024)

compared to historical data (1900–1911). Many amphibian populations are relatively resilient to occasional mortality events (Green et al. 2003; Moss et al. 2021). However, for species or populations that are already declining, such as boreal chorus frogs in our study area, mortality in consecutive years would reduce the probability of survival for metapopulations and overall persistence in the region. While indicative, but not conclusive, reductions in calling activity may be an indicator of amphibian population declines (Dorcas et al. 2010; Lassandro et al. 2025).

Chapter 4: The effect of conspecific cues on calling activity, reproductive output, and offspring quality in the captive-bred boreal chorus frogs (*Pseudacris maculata*)

Formatted as a manuscript in preparation for submission

Ethier JP, Trudeau VL. The effect of conspecific cues on calling activity, reproductive output, and offspring quality in the captive-bred boreal chorus frogs (*Pseudacris maculata*)

Study contributions: JPE contributed through conceptualization, investigation, methodology, data curation, and writing. VLT contributed through conceptualization, methodology, reviewing, editing, supervision, and funding.

4.1 Abstract

There is considerable variation in the quantity and quality of gametes obtained, spawning rates, and fertilization success in hormone-induced captive amphibians, indicating that activation of reproductive pathways may be suboptimal. The general lack of appropriate social stimuli, such as conspecific male calls during the spawning season may, in captive settings, explain the variable success. In anurans (frogs and toads), the regions of the brain associated with acoustic reception have neural projections to the hypothalamic nuclei that regulate reproduction and calling behaviour. Therefore, incorporating acoustic stimuli is likely to be important for anurans, many of which defend territories, attract mates, and coordinate reproduction via vocalizations. In this study, we investigate the effect of playbacks of conspecific calls on the calling activity, reproductive output, and offspring quality in boreal chorus frogs (*Pseudacris maculata*) during two spawning seasons (2022–2023). Male and female frogs were injected with a mixture of a gonadotropin-releasing hormone agonist and dopamine antagonist to induce spawning and exposed to a 6h broadcast of either conspecific calls (playback group) or natural sounds (*e.g.*, wind, rain, insects and birds; control group) for 6 days. We found that playbacks of conspecific calls increased duration of calls by 186% and number of calling bouts by 145% compared to the control. Playback did not affect the number of spawning females or the number of eggs per female but significantly increased the proportion of viable eggs by 13%. Survival of tadpoles 46 days post-hatching was significant increased in the playback group but the proportion of tadpoles reaching metamorphosis was comparable to the control group. These results provide further evidence of the role of social stimulation regulating reproduction in anurans. Our findings are insightful for frogs and toads as well as any other species that use acoustic signals to announce receptivity and locate mates (*i.e.*, insects, birds).

4.2 Introduction

Captive breeding and relocation programs have been initiated in response to declining amphibian populations (Carrillo et al. 2015; Harding et al. 2016; Della Togna et al. 2020) as well as conservation programs that address direct threats to populations, such as habitat loss/degradation, disease, and overexploitation (Luedtke et al. 2023). Amphibians are good candidates for captive breeding programs (Bloxam and Tonge 1995; Griffiths and Pavajeau 2008), producing large numbers of offspring, and requiring less space and maintenance relative to other taxa (*e.g.*, mammals, birds). However, establishing captive colonies can also be challenging for many species (Clulow et al. 2019). Most anurans (frog and toad species) require complex social and environmental cues to initiate reproductive behaviour and produce viable offspring (Kouba et al. 2009). Time in captivity, even for short periods, is associated with reductions in sex steroid hormone production and reproductive performance in several taxa, including amphibians (Fischer and Romero 2019). As a result, most amphibians will not reproduce easily, or at all, within a captive setting without the application of artificial reproductive technologies (ARTs), such as hormonal induction of spermiation and ovulation (Clulow et al. 2019). Even with advancements in ARTs, the process remains imperfect and high degrees of variation in spawning and fertilization rates are observed (Silla et al. 2021).

The acoustic environment, or soundscape, can influence the health and behaviour of animals held in captivity, which may negatively impact reproductive output (Clark and Dunn 2022). Much of the research into captive soundscapes aim to measure acoustic parameters from anthropogenic sources, such as overall noise or sound pressure levels (*i.e.*, decibels or dBa) in specific frequency bands (*i.e.*, low frequency vs. high frequency sound), as anthropogenic noise often has a negative impact on captive animals (Sherwen and Hemsworth 2019). Alternatively,

sound and other sources of sensory stimulation can be used as enrichment within captivity (reviewed in (Wells 2009). Auditory enrichment techniques have shown positive effects (*e.g.*, reduced stereotypic behaviours, reduced hormone concentrations related to stress) for a variety of captive animals, including birds (Robbins and Margulis 2016), monkeys and primates (Robbins and Margulis 2014; De França Santos et al. 2022), leopards (Markowitz et al. 1995), pigs (Palermo Mendes et al. 2023), and elephants (Wells and Irwin 2008). While many of these studies compared control conditions to broadcasts anthropogenic sounds (*e.g.*, classic and rock music, human conversation), some studies have incorporated more biologically relevant sounds, such as vocalizations from prey species (Markowitz et al. 1995) and noises recorded from the species natural environment (Robbins and Margulis 2016).

In most anurans, vocal communication is essential for reproduction (Wells and Schwartz 2007). Vocalizations ("calls") are almost exclusively produced by males and are the primary method in which individuals can identify conspecifics, defend territories, assess the quality of potential competitors, and attract mates (Wells and Schwartz 2007). Furthermore, anurans possess an extraordinarily complex ear (Bell 2016) and there is building evidence that the regions of the brain associated with acoustic reception (*e.g.,* torus semicircularis) in both males and females are neurally connected to the hypothalamus that in turn regulates the pituitary-gonadal function, and thus reproduction (Burmeister and Wilczynski 2005; Wilczynski et al. 2005; Arch and Narins 2009; Maruska and Fernald 2011; Woodley and Leary 2024). Acoustic information is transduced by the preoptic area and ventral hypothalamus, which leads to activation of gonadotropin-releasing hormone (GnRH) neurons (Narins et al. 2007). The GnRH peptide is released from nerve terminals into the median eminence portal blood system and then bind to receptors on gonadotrophs in the anterior pituitary, initiating the release of luteinizing

hormone (LH) and follicle-stimulating hormone (FSH) into the blood stream. These hormones act on the gonads to stimulate oogenesis in females, spermatogenesis in males, and steroidogenesis in both sexes (Vu and Trudeau 2016; Silla and Byrne 2019, Trudeau et al. 2022). Additionally, gonadal steroids act on specific neurons to modulate sensitivity and spectral filtering of the anuran auditory system (Arch and Narins 2009).

The captive setting is associated with reduced androgens and increased corticosterone levels which may cause some species to reduce calling or cease calling all together (Burmeister et al. 2001; Fischer and Romero 2019). Calling behaviour can be induced by broadcasts of conspecific calls and playback experiments have frequently been conducted in the field and in laboratories to study competition (Reichert 2014), mate choice (Gerhardt 1991; Gerhardt 1995; Schwartz et al. 2001), and other aspects of acoustic communication in anurans (Erdtmann and Lima 2013; King 2015). Together, this suggests that broadcasts of conspecific calls are a potentially useful tool to increase natural calling behaviour and improve reproductive performance in captivity.

In our study, we evaluated the effect of broadcasts of conspecific calls on calling activity, reproductive output, and offspring survival and development of boreal chorus frogs (*Pseudacris maculata*), a declining species in eastern Ontario and southern Québec currently being bred in captivity for the conservation of the species (Environment Canada 2015). We hypothesize that the social and acoustic conditions, namely the presence of vocal cues in the form of conspecific calls, are important for the initiation and maintenance of spawning behaviour during the breeding season. The general lack of conspecific calls in captive settings may be linked to the highly variable reproductive success. We hypothesized that reception of conspecific acoustic signals stimulates increased gonadal function to improve fertility. First, we predicted that broadcasts of

conspecific calls will promote increased calling activity and reproductive output during spawning in a captive setting. Second, we predicted that broadcasts of conspecific calls will reduce the high levels of variation in fertilization by providing an additional stimulus for reproduction. Evidence in amphibians suggests that parental investment (*e.g.*, oocyte size, sperm motility), which are influenced by circulation gonadal steroids concentrations, can affect metrics of offspring quality, such as tadpole growth, development, and survival (Cheron et al. 2021; Renoirt et al. 2023). Therefore, we also monitored tadpole development and survival to determine if broadcasts of conspecific calls have an impact on the offspring produced in spawning experiments. Our study provides insight into the role of acoustic behaviour in reproductive ecology of anurans. We also present evidence for the use of acoustic enrichment not only for welfare considerations but also to improve the effectiveness of amphibian captivity breeding and reintroduction programs.

4.3 Methods

Mature boreal chorus frogs were collected from the local population from ponds and ditches in the united counties of Leeds and Grenville, Ontario, Canada (44° 50′ 0″ N, 75° 40′ 0″ W) during the first full week of April (April 03–09 in 2022 and April 02–08 in 2023). The frogs were held at 4°C for no more than 7 days prior to experiments. To determine if female frogs were gravid, we used body shape (Figure S4.1) in both years and ultrasound (Fujifilm Vevo F2 LT with UHF71x transducer probe; bandwidth: 71-30 MHz, scan depth: 10 mm) in 2023 (Figure S4.2). Additionally, if females weighed less than 0.80 g, they were not used in the experiment. All males with conspicuous yellow-to-brown colouration of the vocal sac were assumed to be sexually mature. Mean body weight (± standard deviation) for females and males was 1.37 g (± 0.32) and 1.01 g (± 0.12), respectively (Supplementary Table S4.1).

4.3.1 Experimental setup

To determine if broadcasts of conspecific calls influence male calling activity, reproductive output, and offspring survival and development, we performed a breeding trial experiment. Frogs were placed into two groups; a treatment group ("playback") where chorus frogs are exposed to a broadcast of conspecific males calling and a group ("control") where chorus frogs are exposed to a playback of ambient sound recorded at a natural breeding pond. We chose included a control recording of ambient pond sounds (*i.e.*, wind, rain, crickets, trees swaying) to ensure that the observed responses were due to the broadcast of conspecific calls rather than those elicited by the reception of any source of sound. The breeding trial experiments were conducted over four 7-day trial periods (Figure 4.1). To avoid any possibility of cross-communication, trial periods of the experiment were conducted sequentially rather than simultaneously. In 2022, the playback trial was performed before the control trial. To account for the impact of the order of trials on calling activity and reproductive variables, we reversed the order in 2023 (*e.g.*, control trial was performed before the playback trial). The playback treatment trials occurring from April 13–19 in 2022 (n = 4) and April 23–30 in 2023 (n = 5), and the control trials occurring from April 20–26 in 2022 (n = 4) and April 16–22 in 2023 (n = 5).

Previous work with several frog species, including the boreal chorus frog, has indicated that spawning in a captive setting is improved by administering a low dose of a gonadotropin-releasing hormone (GnRH) agonist 24-hrs prior to the main hormone injection (Trudeau et al. 2010; Trudeau et al. 2013; Vu and Trudeau 2016). Therefore, to induce spawning, on Day 1 frogs were given an injection of 0.04 µg/g body weight of an GnRH agonist (des-Gly10, D-Ala6, Pro-LHRH; Bachem H4070.0005; GnRHa) in a 10 µL saline vehicle with a disposable 31-gauge needle attached to a 0.3 mL syringe. Twenty-four hours later, on Day 2, an injection of a

hormone mixture (AMPHIPLEX method; Trudeau et al. 2010) containing the GnRHa (0.4 µg/g body weight) and a dopamine antagonist, metoclopramide (Sigma; 10 µg/g body weight; MET). All injections occurred between 16:00–18:00. In between Day 1 and Day 2 injections, male and female frogs were held separately in small 750 mL containers placed in a cooler with ice (4–6°C).

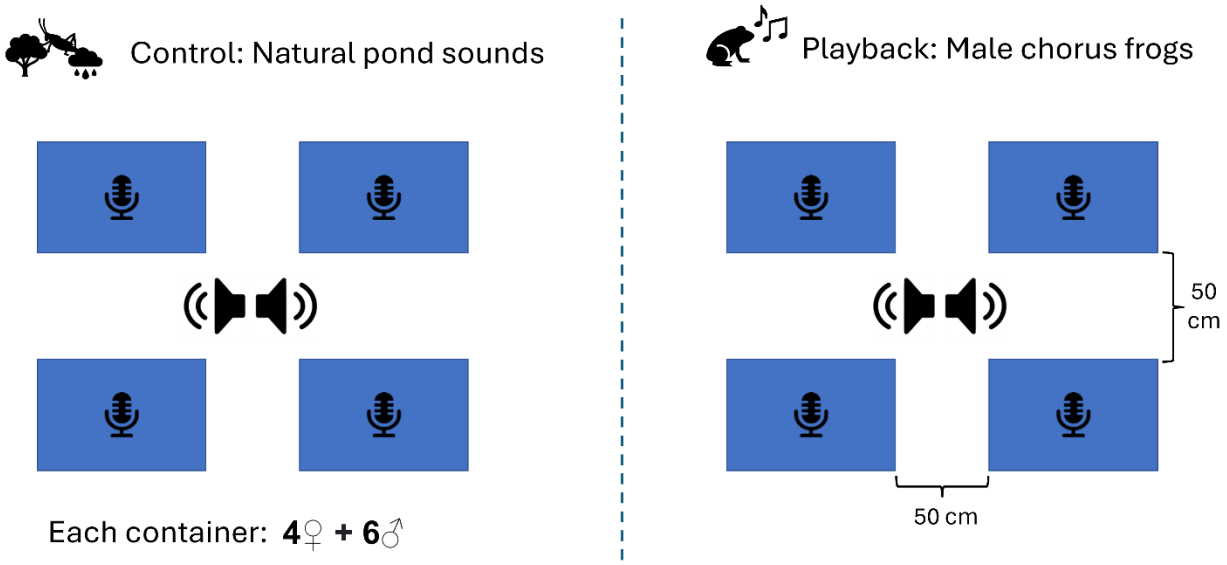

Figure 4.1: The setup of boreal chorus frog (*Pseudacris maculata*) spawning experiment. In each 120L breeding container (blue boxes), four female and six male frogs are placed with 100 L of dechlorinated water and green garden fencing to use as an oviposition substrate. Breeding containers were spaced 50 cm apart from each other. A condenser microphone (APEX 185B) was placed 15 cm above the lid of each of the breeding containers, which are connected to a central multichannel A/D converter interface device attached to a laptop to record vocalizations. In the middle of the breeding containers were two speakers, so the distance from the microphone on top of each breeding container to the speaker was 80 cm. In the control group of frogs (April 20–26, 2022 and April 23–30, 2023) were exposed for 6 hr from 18:00–24:00 each day for five days to ambient sounds from a local breeding pond just prior to chorus frog breeding season (April–May) and prior to any calling behaviour. The playback group of frogs (April 13–19, 2022 and April 23–30, 2023) were exposed to a recording of conspecific frogs calling for 6 hr from 18:00–24:00 each day for five days.

After Day 2 injections, four female and six male frogs were placed in 114-L breeding container (Sterilite; 43.8 cm H x 82.9 cm L x 50.2 cm W) filled with 100 L of dechlorinated water. Breeding containers were placed 50 cm from each other. The frogs were provided with green garden fencing weighed down with rocks as the oviposition substrate. To simulate fluctuations in temperature in natural breeding pools, we allowed water temperature to vary throughout the day (Figure S4.3). Every morning at 9:00 from Day 3–7, breeding containers were surveyed for the general health of frogs, the number of couples in amplexus, and the number of egg clusters laid.

A 6-hr composite audio file of approximately 10–12 male chorus frogs calling was used as the treatment playback. The audio file was produced using recordings of chorus frogs calling the field during the boreal chorus frog breeding season (April–May) with a direction shotgun microphone (RODE NTG4) attached to a portable multi-track audio recorder (ZOOM H6). To simulate the rising and falling in calling intensity, the audio file began with a calling rate of 43 calls/min for 1 hour, increased to 68 calls/min for 4 hours, and then decreased to 43 calls/min for 1 hour. For the control, a 6-hr audio file containing ambient pond sounds (*i.e.*, wind, rain, trees swaying) from one of the frog collection sites recorded just prior to boreal chorus frog calling activity was used. Both the playback and control audio files were normalized in Audacity (version 3.0.2) to -1.0 dB. The audio files were broadcasted at 85 dB (at 1 m) daily for five days (Day 2–6) from 18:00 until 24:00 from speakers placed an equal distance (80 cm) from each breeding container. On Day 7, frogs were removed from the breeding containers, and the eggs were collected. The eggs were transferred to rearing trays (14.1 cm H x 38.3 cm L x 24.1 cm W) filled with 6 L of dechlorinated water held at 17–19°C.

4.3.2 Calling effort

We recorded the vocalizations of male chorus frogs to estimate calling effort using a condenser microphone (APEX 185B) placed 15 cm above the center of the lid of each of the breeding containers. We connected microphones to a central multichannel A/D converter interface device (Behringer UMC404HD). As estimates of calling effort, the mean cumulative duration of calls and the mean number of calling bouts were determined in the playback and control groups. Cumulative duration was defined as the total sum of the duration in minutes of all calls produced within a breeding container within the first 12 hours. A calling bout was defined as a series of calls produced with less than 2 seconds between calls. Water and air temperature were recorded every 10 mins using waterproof temperature loggers (Onset HOBO 8K Pendant).

4.3.3 Reproductive parameters

Egg numbers were determined by photographing egg trays from above and manually counting eggs by two observers. The average number of eggs of the counts were rounded to the nearest whole integer. Eggs per female was calculated by dividing the number of number of eggs in a breeding container by the number of spawning females in a breeding container. To estimate fertilization rate, we determined the proportion of viable eggs four days post-oviposition. Egg viability was estimated by photographing the eggs in trays from above (40 cm from base of tray to camera lens) and counting viable and non-viable eggs, which can be easily differentiated from each other as tadpoles begin to develop (Figure S4.4). Photographs of eggs were divided into 32 cells of equal area, and the number of viable and non-viable eggs were counted in five of the cells selected randomly using a random number generator. The proportion of viable eggs was therefore the number of viable eggs in the five cells divided by the total number of eggs counted

in the five cells. Spawning was confirmed at the end of each experimental trial via dissection. After humane sacrifice by overdose of tricaine methanesulfonate (Syndel USA Syncaine, Catalog No. NC0872873), the spinal cord of each female was transected, and the body cavity opened and visually inspected for the presence of eggs.

4.3.4 Tadpole survival and metamorphosis

To estimate the quality of offspring produced in each treatment, we determined the mean proportion of tadpoles that survived to 46 days post-hatching, the mean proportion of tadpoles reaching metamorphosis, and the length of the larval period. The post-hatch age of tadpoles was approximate as hatching occurs 5–7 days post-oviposition and it is extremely difficult to track individuals. Two weeks after hatching, tadpoles from the control group and playback group were separated on May 11 in 2022 (Control: 5 replicates, Playback:10 replicates) and May 18 in 2023 (6 replicates per group) and reared at densities of 6–10 tadpoles/L. Gosner stage (Gs; Gosner 1960) of the tadpoles ranged from Gs 27–Gs 31 and tadpoles of different stages equally divided between groups. Every other day between 10:00h–14:00h tadpoles were fed mixture of phytoplankton, algae, boiled spinach, and frog brittle (Supplementary Table S4.2). Three times a week, excess food was removed and 80% of the water was replaced to prevent water fouling. Tadpole survival was based on the number of tadpoles survived from May 01 until June 15 in 2022 and May 05 until June 20 in 2023. Tadpole survival was expressed as a proportion to account for uneven number of tadpoles in each replicate (although reared at similar densities of 6–10 tadpoles/L). We chose mid-June (46 days post-hatching) because chorus frog tadpoles begin to reach metamorphic climax starting in early July, thus approximating survival during the larval period (Ethier et al. 2021). Mean proportion of tadpoles reaching metamorphosis was the proportion of 60 tadpoles reaching metamorphic climax (Gs 42) from May 11 in 2022 (4

replicates per group) and May 18 in 2023 (6 replicates per group) until September 01 in both years (approx. four months). Length of larval period was the mean number of days elapsed between the hatch date and data of metamorphic climax. Both environmental (*i.e.*, food availability, space) and neuroendocrinological factors affect time to metamorphosis (Wassersug 1986; Rose 2005; Denver 2013), and not all tadpoles will metamorphose. Since environmental factors such food, water quality, and space were comparable between groups and years, the proportion of tadpoles that emerge as metamorphs and the length of the larval period approximates the differences in development affected by the playback treatment.

4.3.5 Statistical analysis

All statistical analysis were performed in R (Version 4.3.3). We produced a series of models with treatment and year as independent variables. First, linear regression models were produced to compare the mean number of eggs per female, mean calling bouts, mean cumulative duration of calling, and mean length of larval period. We evaluated the assumptions of linear models via the regression diagnostic plots using the R base “plot” function. Second, we conducted a beta regression to test differences in mean proportion of egg viability, proportion of surviving tadpoles, and proportion of emerging metamorphs between years and groups using the “betareg” function within the R package “betareg” (Zeileis et al. 2024). We attempted to use a binomial regression models weighted by the total number of eggs or tadpoles to evaluate differences in the means, but variance inflation factor tests for estimating dispersion (“c_hat" function in the “AICcmodavg” package; Mazerolle 2023) indicated overdispersion and a lack-of-fit (c-hat value > 4). Beta regression is a statistical test appropriate for modelling continuous data that are distributed within the standard interval (values between 0 and 1) and is more flexible than the more traditionally used binomial regression model (Ferrari and Cribari-Neto 2004). We

refrained from applying a p-value adjustment for multiple comparisons (*e.g.*, Bonferroni correction) to balance the risks of Type I errors (false positives) against the risks of Type II errors (false negatives) and instead report effect sizes using Cohen's *d* for all significant comparisons (Cohen 1988). We define effect sizes to be low, medium, and large if Cohen's $d \geq 0.2$, 0.5, and 0.80, respectively (Cohen 1988; Nakagawa 2004). All descriptive statistics are expressed as mean values (± standard deviation) unless stated otherwise.

4.4 Results

4.4.1 Calling effort

Calling effort was highly variable in both the control and playback groups (Figure 4.2). The overall model was not significant for cumulative duration of calling ($F_{2,13} = 2.01$, $p = 0.173$, adjusted $R^2 = 0.119$) nor the number of calling bouts ($F_{2,13} = 2.46$, $p = 0.124$, adjusted $R^2 = 0.163$). However, the mean cumulative duration of calling was nearly two times (186%) longer in the playback group than the control group with frogs calling for 149.7 min (± 62.3 min) and 80.6 min (± 74.1 min), respectively, despite the difference not being significant in the model ($df = 13$, $t = 1.96$, $p = 0.072$). The mean cumulative duration of calling also did not differ between 2022 and 2023 ($df = 13$, $t = -0.43$, $p = 0.676$). There was a 145% increase in the number of calling bouts in the playback group (243.6 ± 135.4 bouts) compared to the control group (168.1 ± 143.6 bouts), but the difference was not statistically significant in the model ($df = 13$, $t = 1.18$, $p = 0.261$). The number of calling bouts also did not differ between 2022 and 2023 ($df = 13$, $t = 1.88$, $p = 0.083$).

4.4.2 Reproductive parameters

Spawning rates ranged from 50–100% (2–4 of 4 females per breeding container) in both the control and playback groups. Furthermore, an equal number of females spawned in the control and playback groups (3.2 ± 0.7 females in both groups) and there was no significant difference in the spawning rate between years (df = 15, t = -0.15, p = 0.880). The mean number of eggs was 851.7 (±478.4) and 813.3 (±246.1) in the control and playback groups, respectively, with a mean of 250.7 (±98.3) eggs per female in the control group and 261.8 (±92.7) eggs in the playback group (Figure 4.3). While the overall model was significant ($F_{2,15}$ = 9.64, p = 0.002, adjusted R^2 = 0.504) and there were significantly more eggs laid per female in 2022 compared to 2023 (df = 15, t = -4.38, p < 0.001), acoustic playback did not influence the number of eggs per female (df = 15, t = 0.36, p = 0.722). Viability was significantly higher in the playback group compared to the control group (df = 5, z = 2.13, p = 0.033). The mean proportion of viable eggs in the playback group was 0.797 (±0.129) compared to 0.663 (±0.143) in the control group (Figure 4.3), a 13.3% increase in egg viability (Cohen's d = 0.978). There was no difference in the viability of eggs between years (df = 5, z = 0.79, p = 0.427).

4.4.3 Tadpole survival and metamorphosis

Tadpole survival (Figure 4.4) was significantly correlated with treatment group (df = 5, z = 2.02, p = 0.043) and year (df = 5, z = 5.20, p < 0.001). While the mean proportion of surviving tadpoles was 0.815 (±0.119) in the playback group compared to 0.778 (±0.140) in the control group, the effect size was small (Cohen's d = 0.279). Tadpole survival was 0.896 (±0.066) in 2023 compared to 0.723 (±0.115) in 2022 (Cohen's d = 1.433). In contrast, metamorph emergence was not related to treatment group (df = 5, z = -0.64, p = 0.525) nor year (df = 5, z = -0.40, p = 0.690). The mean proportion of tadpoles emerging as metamorphs was 0.445 (±0.145)

and 0.414 (±0.133) in the control group and playback group, respectively. The overall model for mean length of the larval period was significant ($F_{2,17} = 8.69$, $p = 0.002$, adjusted $R^2 = 0.455$). The length of the larval period was comparable between groups ($df = 17$, $t = -0.31$, $p = 0.764$) with a mean length of 97.4 (±10.6) and 96.3 (±10.6) days in the control group and playback group, respectively. However, length of the larval period was significantly different between years ($df = 17$, $t = -4.22$, $p < 0.001$), lasting 105.7 (±4.4) days in 2022 compared to 91.0 (±8.9) days in 2023 (Cohen's $d = 1.978$).

4.5 Discussion

Our results provide evidence that broadcasts of conspecific calls may have positive effect on reproductive behaviours and outcomes of anurans in captivity. The playback group was associated with increases in calling behaviour and a 13% increase in the proportion of viable eggs compared to the control group. Our results provide evidence that reception of conspecific calls could enhance pituitary-gonadal function in anurans as calling behaviour in males and gamete production in both sexes are both strongly associated with gonadal sex steroid production (Wilczynski and Burmeister 2016; Woodley and Leary 2024). Tadpoles produced from eggs in the playback group had increased survival 46 days post-hatching, while the length of larval period was comparable between the playback and control groups. This is consistent with the hypothesis that effects on pituitary-gonadal function induced by conspecific signal reception may enhance fertility and leads to improved offspring quality.

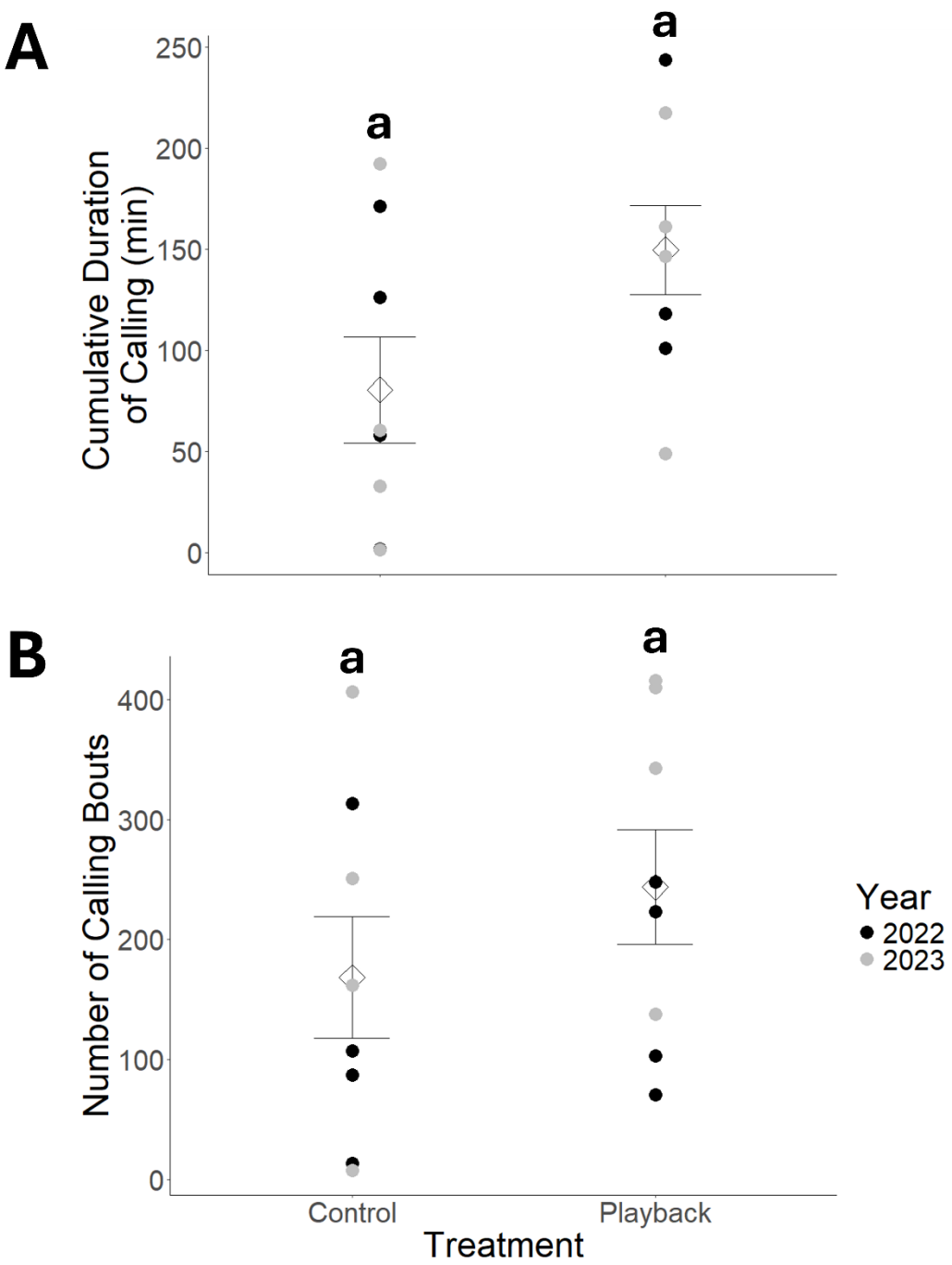

Figure 4.2: Comparison of calling behaviour of male boreal chorus frogs (*Pseudacris maculata*), including (A) mean cumulative duration and (B) mean number of calling bouts between two acoustic treatments. Control = control playback (April 20–26, 2022 and April 16–22, 2023) where the audio file contained ambient sounds (*i.e.*, wind, rain, trees swaying). Playback = acoustic playback treatment (April 13–19, 2022 and April 23–30, 2023) where the audio file contained 10–12 chorus frogs calling. Mean values are indicated by diamonds with standard error bars. Statistically significant differences ($\alpha = 0.05$) are indicated by dissimilar lower-case letters.

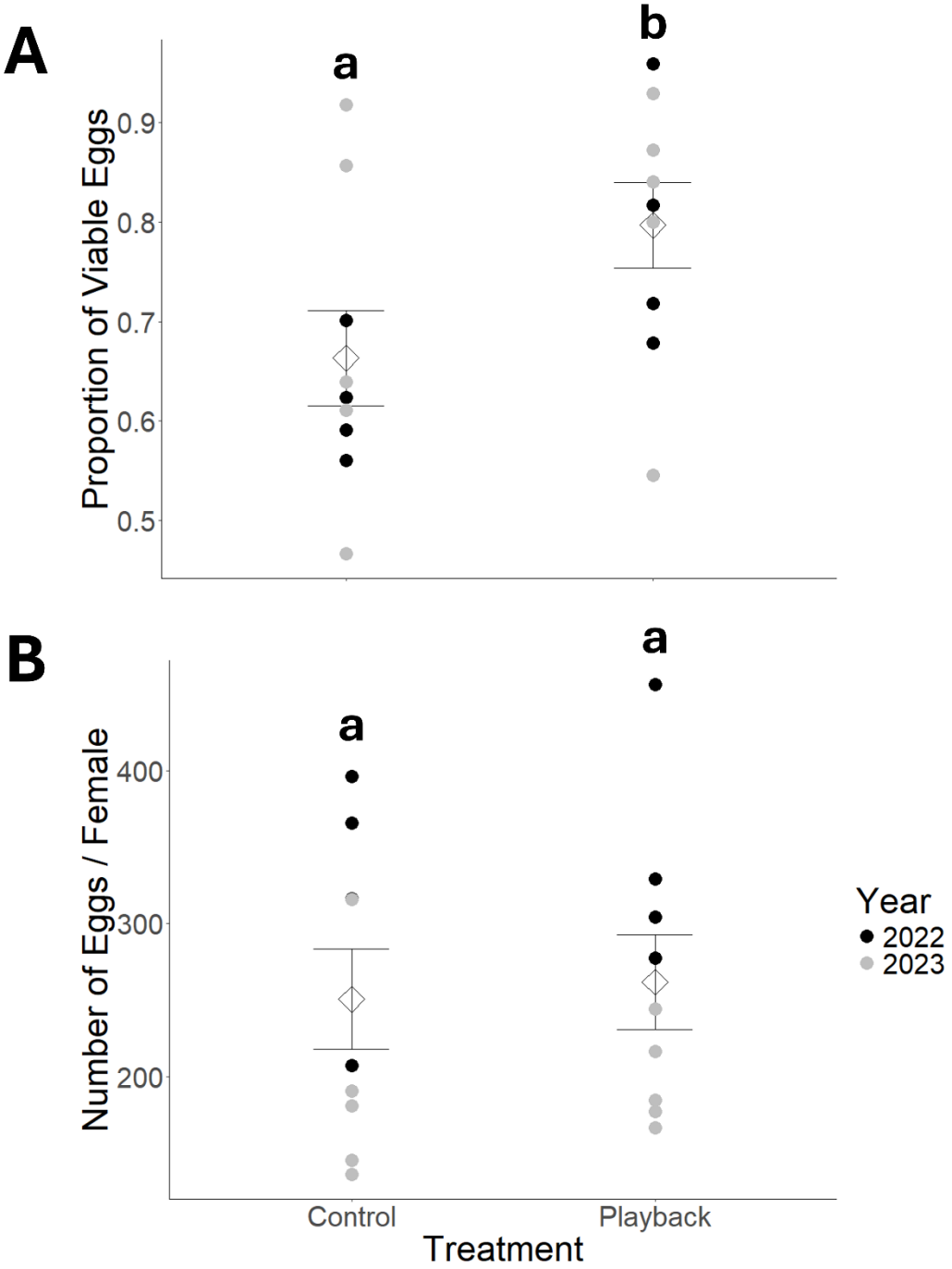

Figure 4.3: Comparison of reproductive output of spawning boreal chorus frogs (*Pseudacris maculata*) including (A) mean proportion of viable eggs and (B) mean number of eggs per female between two acoustic treatments. Control = control playback (April 20–26, 2022 and April 16–22, 2023) where the audio file contained ambient sounds (*i.e.*, wind, rain, trees swaying). Playback = acoustic playback treatment (April 13–19, 2022 and April 23–30, 2023) where the audio file contained 10–12 chorus frogs calling. Mean values are indicated by diamonds with standard error bars. Statistically significant differences ($\alpha = 0.05$) are indicated by dissimilar lower-case letters.

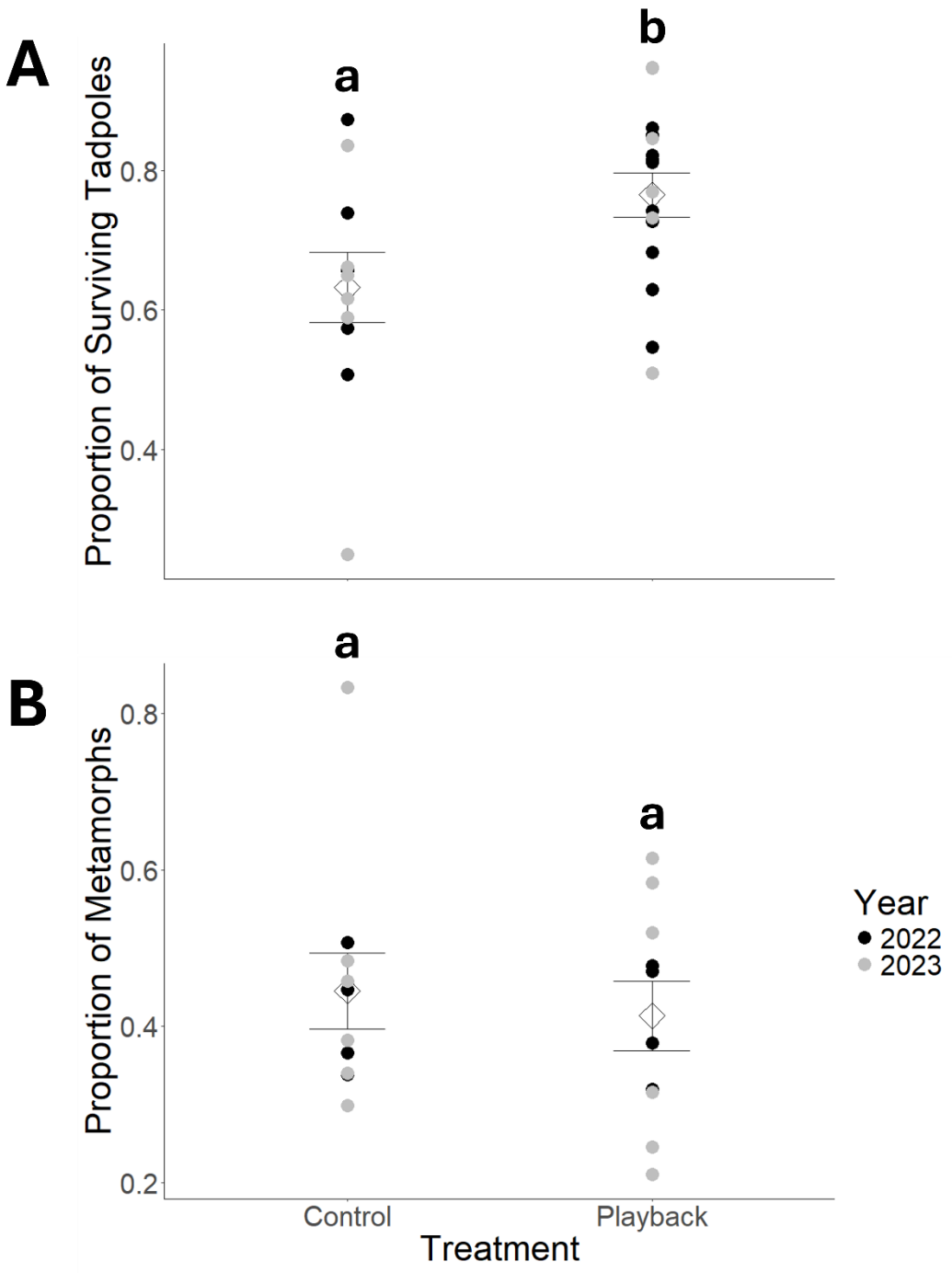

Figure 4.4: Comparison of tadpole quality of spawning boreal chorus frogs (*Pseudacris maculata*) including (A) mean proportion surviving tadpoles 46 days post-hatching and (B) mean proportion of tadpoles emerging as metamorphs (B) between two acoustic treatments. Control = control playback (April 20–26, 2022 and April 16–22, 2023) where the audio file contained ambient sounds (i.e., wind, rain, trees swaying). Playback = acoustic playback treatment (April 13–19, 2022 and April 23–30, 2023) where the audio file contained 10–12 chorus frogs calling. Mean values are indicated by diamonds with standard error bars. Statistically significant differences ($\alpha = 0.05$) are indicated by dissimilar lower-case letters.

4.5.1 Calling effort

Our observation that male chorus frogs in the playback group increased the cumulative calling duration by 186% and the number of calling bouts by 145% compared to the control group, while not statistically significant, does suggest that acoustic playbacks of conspecific calls might have increased the motivation to call in chorus frogs. This is in concordance with previous studies that have shown that anurans readily respond to playbacks of conspecific calls (Gerhardt 1991; Wells and Schwartz 2007). An increase calling behaviour in response to conspecific signals has been observed in many other anuran species, including Johnstone's whistling frog, *Eleutherodactylus johnstonei* (Tárano and Fuenmayor 2009; Tárano and Fuenmayor 2014), two species of midwife toads, *Alytes obstetricans* and *Alytes cisternasii* (Bosch and Márquez 1996), small torrent frog, *Micrixalus saxicola* (Preininger et al. 2013), Iberian parsley frog, *Pelodytes ibericus* (Márquez et al. 2001), Andean frog, *Colostethus subpunctatus* (Lüddecke 2002), and spring peeper, *Pseudacris crucifer* (Forester and Harrison 1987). We observed a large degree of variation in calling activity within treatments. Cumulative calling duration ranged from 90.5–11537.2 mins and 2931.4–14607.8 mins in the control and playback group, respectively, while number of calling bouts ranged from 8–406 bouts and 70–416 bouts in the control and playback group, respectively. Like many anurans, chorus frogs are capable of remarkable plasticity in their calling behaviour, altering their calls in response to their physical condition, the social context, and environmental conditions (Wells and Schwartz 2007; Snell-Rood 2013; Kuczynski et al. 2016; Neelon and Höbel 2019). In large choruses, males may decrease the duration or repetition rate of their calls to have the signals placed into gaps between the calls of competing conspecific males to maximize the likelihood of their call being received by a female (Klump and Gerhardt 1992; Martínez-Rivera and Gerhardt 2008). However, some studies suggest that female anurans

tend to have a behavioural sensory bias towards males that possess calls of longer duration, produced at lower fundamental frequencies (pitch), higher repetition and pulse rates, and that have a greater complexity of notes (Ryan and Keddy-Hector 1992; Gerhardt and Huber 2002).

4.5.2 Reproductive parameters

To our knowledge, our study is the first to find an increase in egg viability in an anuran in correlation with acoustic playback of conspecific calls during spawning. Most captive boreal chorus frogs do not breed without hormonal induction and those that do produce small egg clutches with low viability. Previous boreal chorus frog captive breeding trials using hormonal induction but without any playback had a mean egg viability of 67.5% (Ethier, *unpublished data*; Supplementary Table S3.4), similar to the control group in the current study. The 13% increase in egg viability to nearly 80% is a notable improvement over what can be induced by hormone injections alone. There is some evidence that broadcasts of conspecific calls can increase reproductive investment in birds. Playback of either their mate's song or recordings from conspecific colonies led to zebra finches (*Taeniopygia guttata*) laying larger clutches compared to the absence of an acoustic treatment (Waas et al. 2005). Similarly, female canaries (*Serinus canaria*) exposed to male conspecific calls produce more eggs compared to those that heard no songs and playbacks of synthetic sounds mimicking highly attractive mates resulted in larger eggs being produced (Leitner et al. 2006). Additionally, in captive brown-headed cowbirds (*Molothrus ater*) the number of eggs produced/day was significantly correlated with the number of counter-singing interactions among males (White et al. 2010). We also observed that there were no apparent negative effects on reproduction associated with the broadcast of the control treatment, which included sounds of wind, crickets, and trees swaying. Environmental noise can have impacts on the neuroendocrine system, increasing circulating hormones associated with the

stress response and leading to reduced reproduction and development (Barber et al. 2010; Kight and Swaddle 2011). Effects of environmental noise are particularly understudied in amphibians and warrant further investigation (Kight and Swaddle 2011).

There is strong evidence that the regions of the brain associated with acoustic reception have neural projections to nuclei that regulate reproduction in vertebrates (Burmeister and Wilczynski 2005; Wilczynski et al. 2005; Arch and Narins 2009; Wilczynski and Ryan 2010; Wilczynski and Burmeister 2016). For example, after hearing calls for several consecutive days, male green treefrogs (*Dryophytes* [*Hyla*] *cinerea*) exhibited a significant 25% increase in the number of immunoreactive GnRH neurons accompanied by increased plasma androgen production (Burmeister and Wilczynski 2005). Similarly, male grass frogs (*Rana temporaria*) maintain mature testes longer (*i.e.*, increased testis volume and interstitial tissue size) when exposed to a synthetic signal that resembled conspecific calls (Brzoska and Obert 1980). In female midwife toads (*Alytes muletensis*), eggs will continue to mature when exposed to conspecific male calls but are reabsorbed if conspecific calls cease or when exposed to heterospecific calls (Lea et al. 2001). The relationship between sex steroid hormone concentrations and gamete quality/quantity is well established in anuran amphibians (Iimori et al. 2005; Evaul et al. 2007; Deng et al. 2009) as well as fish (Zohar and Mylonas 2001; Cabrita et al. 2014). Therefore, increases in circulating concentrations of androgens and estrogens induced by exposure to biologically relevant social cues are likely to maintain higher quality and quantity of gametes which then increase the likelihood of successful fertilization of eggs. Optimal sperm concentrations for fertilization of eggs in artificial fertilization protocols have been established for several frog and toad species (see Kouba et al. 2009; Browne et al. 2015; Clulow et al. 2018). Our results are indicative that playback of conspecific cues affect gamete maturation but are not

conclusive. Direct experimentation establishing the relationship among exposure to playback of conspecific cues, gamete quality/quantity, and fertilization rates within the chorus frog study system would be beneficial to confirm this proposed method for increasing reproductive outcomes.

4.5.3 Tadpole survival and metamorphosis

Tadpole survival 46 days after hatching was 77.8% in the control group and 81.5% in the playback group with 44.5% and 41.4% of tadpoles emerging as metamorphs within four months in the control group and playback group, respectively. Previously, our research group conducted a rearing density experiment with boreal chorus frogs in laboratory (Ethier et al. 2024) and outdoor mesocosm settings (Fayard 2022), both without any form of acoustic playback. In the laboratory experiment, we found that tadpole survival to metamorphosis (Gs 42) was 75–92% for tadpoles reared at densities of 0.5–2 tadpoles/L in small 6-L aquaria (Ethier et al. 2024). In the current study, rearing densities that were up to 20 times higher (*i.e.*, 6–10 tadpoles/L) than Ethier et al. (2024) and we observed approximately half as many individuals underwent metamorphosis (*i.e.*, 41.4–44.5%). This suggests that the increased rearing density greatly impacted tadpole survival and development. However, in the mesocosm study, we reared tadpoles in 380-L water tanks and found that lower tadpole densities (0.1 vs. 1.0 tadpole/L) increased metamorph body size and weight but did not affect survival to metamorphosis, which was 46% across all treatments, comparable to the current study (Fayard 2022).

In general, higher conspecific densities are associated with reduced growth, longer developmental period, lower mass at metamorphosis and decreased survival during the larval period (Wilbur and Collins 1973; Weber et al. 2024). Chorus frog tadpole densities are usually between 0.01 and 5.25 tadpoles/L in natural ponds, but there are observations of up to

11.03 tadpoles/L on Isle Royale, Michigan (Smith 1990; Smith and Van Buskirk 1995). Survival of *Pseudacris* species in natural settings is highly variable and can range from <10–90% during the larval stage (Smith and Van Buskirk 1995; Ethier et al. 2021). For example, boreal chorus frog tadpoles had a mean 46-day survival of 72% in North Dakota (Hossack et al. 2017) while survival during the entire larval period was estimated to be only 3–6% in a Québec population (Whiting 2004). Survival tends to be much higher and more consistent in captive and experimental populations, with reports of ≥80% survival when reared singly in 1.5L tanks (Amburgey et al. 2012; Earl et l. 2012) or at very low densities of 0.001–0.16 tadpoles/L (Sours and Petranka 2007). However, spring peeper (*Pseudacris crucifer*) survival was only 52–63% when reared in 40-L aquariums at 0.225 tadpoles/L (Van Allen et al. 2010). This exemplifies the differences in survival rates that are often observed between field and laboratory settings (Melvin and Houlahan 2012) as well as differences that can occur among populations within the same species independent of density (Skelly 1996; Ethier et al. 2021).

We found that the length of the larval period, or time to metamorphosis, was not affected by the broadcast, with tadpoles reaching metamorphosis at 97.4 days and 96.3 days in the control and playback groups, respectively. However, larval period was almost two weeks longer in 2022 (105.7 days) compared to 2023 (91.0 days) despite temperature during the larval being comparable between years. The average larval period of boreal chorus frogs is reported to be approximately 60 days (Smith 1990; Ethier et al. 2021) but varies from as short as 43 days in an Arizona population (Sredl and Collins 1991) up to 160 days in a montane population in northern Colorado (Pettus and Angleton 1967). The drivers for differences in larval period among years in the current study are unknown. Several environmental factors have been reported to affect the length of the larval period in chorus frogs, including rearing density and food availability (Ethier

et al. 2024), air and water temperature (Whitaker 1971; Shadle et al. 2023), length of hydroperiod (Amburgey et al. 2012), and presence of predators (Skelly 1995; Lading and Wilcoxen 2021). In addition to environmental factors, reproductive success and quality of subsequent offspring is dependent on the quality of the parental male and female (Ratikainen et al. 2018). Both genetic and physiological (*i.e.*, body condition) factors influence the quality and quantity of gametes that a male and female contribute to each reproductive output (Ratikainen et al. 2018; Salles et al. 2020). While there is relatively little research linking how gamete and offspring quality affect developmental periods in anurans, some evidence in Bufonids suggests that maternal phenotype (*e.g.*, size, weight, body condition) affect clutch quality (*e.g.*, initial tadpole size, survival) which in turn affect the duration of developmental periods (Cheron et al. 2021; Renoirt et al. 2023). Furthermore, parental factors can also interact with the environmental factors affecting growth and development. In *Bombina orientalis*, initial tadpole size, a proxy for maternal investment, had a significant effect on metamorph size and length of the larval period when reared in low quality conditions where food was limited but there were no such relationships when tadpoles were fed ad libitum (Kaplan 1987; Parichy and Kaplan 1992). We maintained consistency in both the adult spawning and tadpole rearing conditions, largely controlling for the effects of environmental factors, suggesting that variations in the length of the larval period are due to either the intrinsic differences in the tadpoles produced or the conditions that the parental males and females experienced prior to collection. Therefore, the relative contributions of environmental and parental factors in chorus frogs, and how they may influence larval development, remain to be tested.

4.5.4 Conclusions

The increases in calling activity (*i.e.*, cumulative calling duration and number of bouts), reproductive output (*i.e.*, viability), and offspring survival (*i.e.*, 46-day survival) in response to the playback of conspecific calls provide preliminary evidence that broadcast of biologically relevant sounds can improve outcomes in captive breeding programs. In particular, the over 13% increase in mean egg viability is compelling and warrants further consideration and corroboration. While the proposed hormonal mechanisms leading to our results are tentative and require formal testing, our study imply that social cues have positive impacts on reproductive behaviour and spawning. This study also provides a framework to be used in the captive breeding of other anurans or any other species that use acoustic communication as a regulator of reproductive behaviour. This research is especially pertinent in amphibians as populations have declined over the last 60 years and many amphibians at high risk of extinction (Wake and Vredenburg 2008; Hoffmann et al. 2010). Further declines of amphibian species will likely necessitate the creation of more captive breeding, reintroduction, and translocation programs to conserve species (Carrillo et al. 2015; Harding et al. 2016; Della Togna et al. 2020). Therefore, investigations into the factors affecting reproduction and survival in both the captive and natural settings will be crucial for the conservation of amphibians into the future.

Chapter 5: Male to male acoustic communication modulates testicular steroidogenic and spermatogenic transcriptional networks in chorus frogs

Formatted as a manuscript:

Ethier JP, Lee H, Robinson SA, Trudeau VL. Male to male acoustic communication modulates testicular steroidogenic and spermatogenic transcriptional networks in chorus frogs.

Study contributions: JPE contributed through conceptualization, investigation, methodology, data analysis, and writing. HL contributed through investigation and methodology. SAR contributed through reviewing, editing, and funding. VLT contributed through conceptualization, methodology, reviewing, editing, supervision, and funding.

5.1 Abstract

Acoustic communication is essential for the coordination of reproduction in most anuran amphibians (toads and frogs). Several points of evidence suggest that the reception of biologically relevant acoustic signals, such as conspecific calls, can influence the gonad function and reproductive state via the hypothalamic-pituitary-gonadal axis in both sexes. In this study, we sampled testes and ovaries for RNA sequencing (RNAseq) to test for expected gene expression patterns in boreal chorus frogs (*Pseudacris maculata*) following exposure to broadcasts of one of two levels of conspecific males calling or an injection of a hormone mixture containing a gonadotropin-releasing hormone agonist and a dopamine antagonist that induces spawning. Pathway analysis of the RNAseq results indicated that several Gene Ontology (GO) terms and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways related to mitochondrial function were upregulated in male chorus frogs exposed to a broadcast of a large chorus of approximately 100 males ("High Chorus") and in males injected with the hormone mixture compared to control treatments. A systematic search of GO terms and KEGG pathways also revealed that genes associated with gonadal development, steroidogenesis, and spermatogenesis were upregulated in High Chorus and hormonal induction treatments in males. In contrast, in a parallel experiment in females subjected to the "High Chorus" or hormone injections revealed only 16 differentially expressed genes in the ovarian transcriptome. These results provide evidence for the reciprocal interaction between the endocrine control of reproduction and the acoustic reception of conspecific signals and implies that male-male acoustic communication has a role in initiating and maintaining reproductive behaviours. Our findings are consistent with the breeding ecology of anuran amphibians and have implications for the understanding of the molecular mechanisms that regulate reproduction. Practical

applications for species conservation, such as maintaining reproductive condition and inducing spawning in captive breeding programs can be enhanced by application of acoustics.

5.2 Introduction

In anuran amphibians (toads and frogs), acoustic communication plays a critical role in coordinating reproduction and spawning behaviour. For many species, males aggregate in large numbers at breeding sites and produce vocalizations to attract females and interact with potential competitors (Wells and Schwartz 2007; Wilczynski and Ryan 2010). Females orient toward sexually active calling males (*i.e.*, phonotaxis) and assess potential mates based on acoustics parameters of the calls. By aggregating in high densities, males benefit by reducing the rate of individual energy expenditure per hour, allowing longer stays at breeding sites, and increased mating chances by increasing female participation rates (Ryan et al. 1981; Grafe 2005).

Aside from sexual selection associated with female preference (Ryan and Rand 1993; Ptacek 2000), acoustic signals have a role in the regulation of hormones associated with the sexually active reproductive state (Wilczynski and Burmeister 2016; Woodley and Leary 2024). In frogs there is neural connectivity between the acoustic signal reception centres within the brain and the nuclei associated with the control of reproduction via stimulation of the hypothalamic-pituitary-gonadal (HPG) axis (Arch and Narins 2009; Hall and Kelley 2021). Environmental and social signals trigger the release of gonadotropin-releasing hormone (GnRH) from the hypothalamus, which acts on the pituitary to release gonadotropins (Trudeau et al. 2022). The gonadotropins are transported through the bloodstream to the gonads and stimulate the release of sex steroids, impacting gametogenesis, secondary sexual traits, and behaviour (Trudeau et al. 2022). Growing evidence from anurans suggests a bidirectional relationship between the endocrine control of reproduction and the acoustic reception (Burmeister and

Wilczynski 2000; Wilczynski et al. 2005; Crocker-Buta and Leary 2018). Sex steroid hormones can impact sensitivity of hearing in both sexes, and the reception of acoustic signals can affect hormone synthesis and secretion within the gonads (Wilczynski and Burmeister 2016; Woodley and Leary 2024). Many of these gonadal steroids are important for the maintenance of calling behaviour in anurans (Arch and Narins 2009; Hall and Kelley 2021). Reception of conspecific calls in many species is sufficient to stimulate other males in producing calls themselves (Wells and Schwartz 2007) implying feedback mechanisms associated with call production, acoustic reception, and reproductive state.

Underlying the relationship between reproduction and acoustic reception are physiological processes controlled by the expression of genes within cells. Gene expression varies with developmental stage, physiological condition, and external environment. Reception of conspecific calls can modulate the expression of immediate early genes within the torus semicircularis, the primary region of the brain associated with auditory processing in frogs (Burmeister et al. 2008) and several nuclei of the pallidum, which are associated with processing and integration of sensory signals (Mangiamele and Burmeister 2008). Similarly, RNA sequencing has been used to investigate whole brain gene expression of female frogs following exposure to visual and auditory cues from conspecific males, finding significant upregulation of genes and transcriptional networks associated with energy metabolism (Zhao et al. 2021). Gene expression in the ovary and testis following exposure to reproductive behaviours has yet to be studied in detail. Prolonged exposure to chorusing behaviour can increase circulating androgen concentration (Burmeister and Wilczynski 2000; Chu and Wilczynski 2001) and testes mass (Brzoska and Obert 1980) in male frogs, but there have been no attempts to understand the mechanisms underlying testicular responses.

This study reports the effect of conspecific acoustic signals on the gonadal gene expression profile in boreal chorus frogs (*Pseudacris maculata*), a diploid species in the family Hylidae. This small-bodied frog is widely distributed but declining in several portions of eastern North America (Gibbs et al. 2005; Seburn et al. 2014). We used a dynamic playback broadcast design that simulates the natural rising and falling of calling activity across a 30-hour period and sampled gonadal tissues at three timepoints (6 hrs, 24 hrs, 30 hrs). Male frogs were exposed to one of three intensities of conspecific calling: a low intensity chorus of a small group of 10–12 males, a high intensity chorus of a large group of approximately 100 males, and broadcast of naturally occurring sounds without calling of any anuran species (*i.e.*, a noise control). Although we focused the effect of chorusing activity on testicular gene expression in male frogs, we also investigated the effect of conspecific calls on ovarian gene expression in females, as well as changes in gonadal gene expression after administration of a gonadotropin-releasing hormone plus dopamine antagonist mixture for both sexes. As the genome of the boreal chorus frog has yet to be assembled (Chen et al., 2024), we described broad functional expression patterns and conducted gene pathway analyses. We hypothesized that reception of conspecific signals trigger endocrine responses that influence gonadal function and fertility. A previous study conducted with boreal chorus frogs (Ethier et al. *unpublished*) suggested that broadcasts of conspecific signals during the spawning period could increase the percentage of viable eggs and survival of tadpoles, suggesting reception of conspecific signals is important for gamete maturation and fertilization. Therefore, we predicted that differential gene expression analysis and pathway mapping would detect significant upregulation of genes and transcriptional networks associated with steroidogenesis, gonadal development, and gametogenesis in frogs exposed to conspecific signals. We also predicted that gene expression patterns should be similar among frogs injected

with an agonist of gonadotropin-releasing hormone and frogs exposed to broadcasts of conspecific signals, reflecting stimulation of the HPG axis.

5.3 Methods

5.3.1 Animal collection

Wild boreal chorus frogs were collected during the early amphibian breeding season from ponds in the United Counties of Leeds and Grenville, Ontario, Canada (44.904440, -75.831694). Males were collected between April 06–April 11 in 2023 and females were collected between March 26–April 01 in 2024. Male and female frogs were held separately in groups of 8 individuals in 1.7-L plastic terraria (18.3 x 8.5 x 10.8 cm) with damp moss and fed pinhead crickets every two days. Frogs were held at 4°C in a refrigerator for 10–14 days until the start of the experiments. All frogs were collected as per the Wildlife Scientific Collector's Authorization issued by the Ontario Ministry of Natural Resources. All the experimental procedures involving the handling and treatment of the frogs used in this study were approved by the University of Ottawa Animal Care and Veterinary Service prior to initiation of experiments.

5.3.2 Description of treatments

We investigated gonadal gene activation in response to the natural chorusing behaviour of wild male chorus frogs at three time points across a 30-hour period using a dynamic playback broadcast design. Our previous research (Chapter 2) indicated that calling activity of boreal chorus frogs fluctuates but has a predictable diel pattern (Figure 5.1; dotted line). The “Low Chorus” treatment simulated a small population of 10–12 chorus frogs (Figure 5.1; yellow line). The low chorus broadcast began at 10:00 with 2 hours of silence until 12:00, followed by 2 hours of two males calling from 12:00 to 14:00, then 7 hours of ten males calling from 14:00 to 21:00,

and then 7 hours of two males calling from 21:00 to 4:00 the next morning. From 4:00 to 10:00 there were 6 hours of silence. The “High Chorus” treatment simulated a large population of 80–100 chorus frogs (Figure 5.1; blue line). The broadcast began at 10:00 with 2 hours of two males calling until 12:00, followed by 2 hours of ten males calling from 12:00 to 14:00, then 7 hours of approximately 80–100 males calling from 14:00 to 21:00, and then 7 hours of ten males calling from 21:00 to 4:00 the next morning. From 4:00 to 10:00 there are 6 hours of two males calling. The “Wind” treatment simulated natural sounds (*i.e.*, wind, rain, insects, and trees swaying) that were recorded at breeding locations prior to the anuran breeding season and therefore do not contain calling behaviour of any frog or toad species. The 6-hour file was repeated five times to encompass the 30-hour period. Spectrograms of the different levels of calling activity can be seen in Supplementary Figure S5.1.

As a positive control, we recorded gonadal gene expression profiles in response to a hormonal treatment following a gonadotropin-releasing hormone (GnRH) priming protocol (Trudeau et al. 2010; 2013). This “Amphiplex” method has been shown to be an effective spawning induction protocol for numerous frog species (Trudeau et al. 2010; 2013). First, we intraperitoneally injected a group of frogs (8 males in 2023, 6 females in 2024) with a low priming dose of a GnRH agonist (GnRH-A; 0.04 μg/g body weight des-Gly10, D-Ala6, Pro-LHRH; Bachem H4070.0005) followed 24 h later with an injection of a mixture of GnRH-A (0.4 μg/g) and the type 2 dopamine receptor antagonist metoclopramide (10 μg/g) (Trudeau et al. 2013). In the negative control treatment (“Saline”), we injected frogs (8 males in 2023, 6 females in 2024) with a 0.7% saline solution.

5.3.3 Experimental playback procedure

Testis samples were collected in April 2023, and ovary samples were collected in April 2024. The evening before the first day of the experiment, three groups of 6–8 frogs were placed in small containers (946 mL; 14 cm H x 11 cm W) and gradually warmed overnight to 18°C in a cooler. At 8:30 the following morning, frogs were transferred to 114-L containers (Sterilite; 43.8 cm H x 82.9 cm L x 50.2 cm W) with 100 L of dechlorinated water and plastic platforms for perching. Frogs were allowed to acclimate to the conditions of the room (*i.e.*, light, temperature, disturbance from being moved) for 1.5 hours. The photoperiod reflected sunset/sunrise patterns of the region (lights on at 6:00, lights off at 20:00) and water temperature within 114-L containers ranged from 17.8 to 19.1°C across the entire experimental period. All 114-L containers were a standard distance of 1 m from the playback speaker. At 10:00, one of three treatments ("Wind", "Low Chorus", "High Chorus") was broadcasted at sound pressure level of 85 dB detected at 1 m. The first container was removed at 16:00 (Timepoint 1 = 6 hrs), the second was removed at 10:00 the next day (Timepoint 2 = 24 hrs) and the third was removed at 16:00 of the second day (Timepoint 3 = 30 hrs). At each timepoint, frogs were removed from the 114-L containers, humanely euthanized with an overdose of buffered 0.5% tricaine solution (Syndel Syncaine, Thermo Fisher Scientific, Waltham, MA, USA), and the spinal cord was cut. The testes or ovaries were removed for RNA extraction. Based on the gene expression results in 2023 with testes samples, all ovary samples were collected after 6 hours (Timepoint 1) and were restricted to the "High Chorus" and "Wind" treatments. For "Amphiplex" and "Saline" treatments, gonads were sampled for RNA extraction at Timepoint 1 only for both sexes. The order of treatments in each year was randomized and then ran consecutively to minimize time difference between treatments and thus time that the frogs were held before they were exposed.

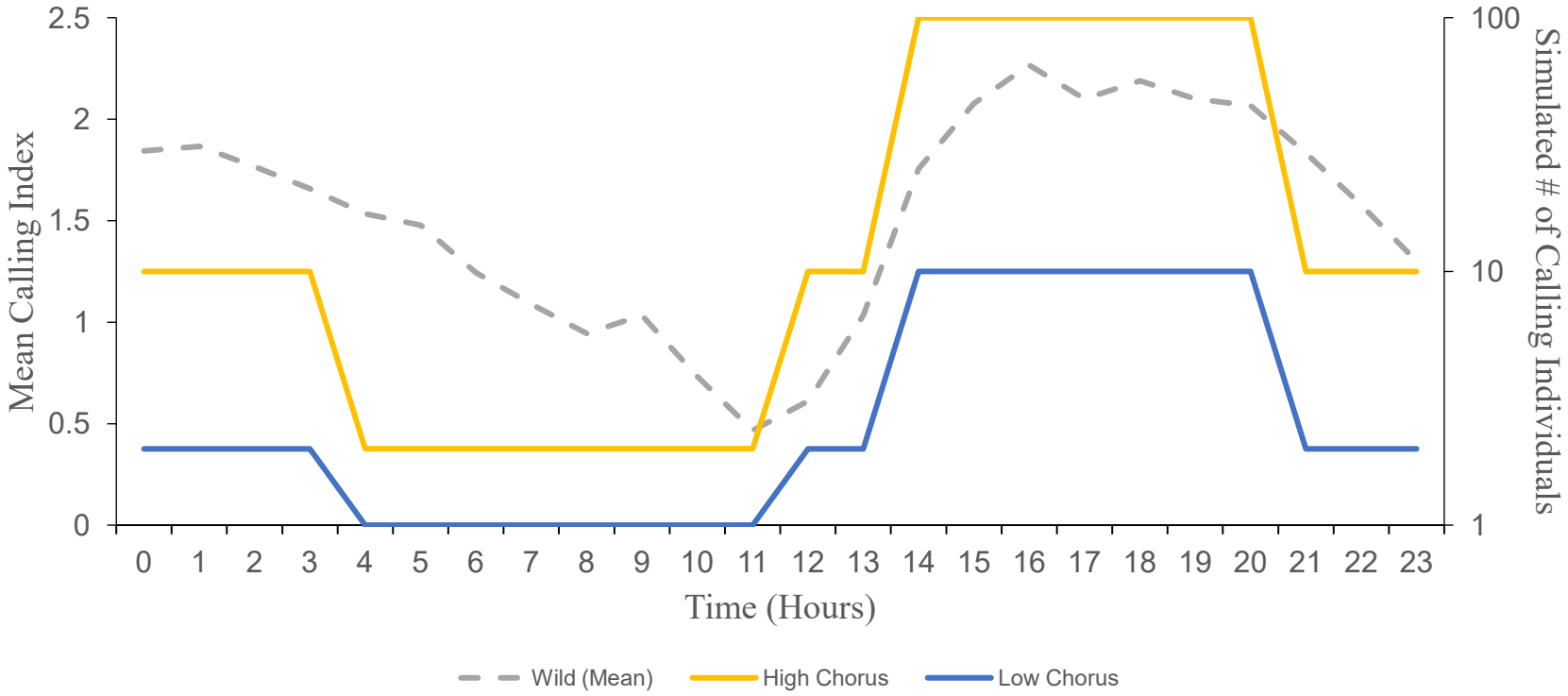

Figure 5.1: Comparison of natural calling behaviour of boreal chorus frogs (*Pseudacris maculata*) and simulated number of calling individuals. *Primary Y-axis*: Dashed grey line; the call index follows the standard call index of the North American Amphibian Monitoring Program-- (0) absence of calling activity; (1) up to two or three unique individuals, with mostly non-overlapping calls, (2) overlapping calls but still allowing to distinguish individuals, and (3) full chorus consisting of overlapping calls with non-distinguishable individuals. *Secondary Y-axis*: Solid yellow and blue lines; simulated number of calling individuals (logarithmic scale) -- (0) absence of calling activity; (2); two or three calling individuals with occasional but minimal overlap; (10) three or four individuals calling in the spectral foreground of recording with several individuals in the spectral background, approximately 10 individuals in total, with a lot of overlap of calls; (100) full chorus consisting of overlapping calls with non-distinguishable individuals, approximately 80–100 individuals in total.

5.3.4 RNA extraction and sequencing

After dissection, testis (n = 40) and ovary (n = 22) samples were stored in RNA later® (Thermo Fisher Scientific, Waltham, MA, USA), kept at 4°C for 24 hours, and then frozen to -80°C. Upon thawing, the samples were homogenized using a VWR 4-Place Mini Bead Mill Homogenizer (VWR®, Atlanta, GA, USA), and total RNA was purified from the homogenates using NucleoZOL reagents with NucleoSpin RNA columns (Macherey-Nagel, Germany). RNA concentrations were measured with a Qubit 4 Fluorometer (Invitrogen™, Thermo Fisher Scientific, Waltham, MA, USA), and RNA integrity (RIN) was assessed using an Agilent 4150 TapeStation system (Agilent Technologies, Inc., Santa Clara, CA, USA). All samples were of high quality (average RIN: testis = 9.8; ovary = 9.4) and yield (average concentration: testis = 0.057 µg/µL; ovary = 0.973 µg/µL). rPoly(A) RNA sequencing library was performed by LC Sciences Inc. (https://lcsciences.com, Houston, TX, USA) and was prepared following Illumina's TruSeq-stranded-mRNA sample preparation protocol. Paired-ended sequencing was performed on Illumina's NovaSeq 6000 sequencing system.

Sequencing services, including a *de novo* assembly of the boreal chorus frog transcriptome, were provided by LC Sciences Inc. Cutadapt (Martin 2011) and custom Perl scripts (https://www.perl.org/about.html) were used to remove the reads that contained adaptor contamination, low quality bases, and undetermined bases. Sequence quality was then verified using FastQC (http://www.bioinformatics.babraham.ac.uk/projects/fastqc). All downstream analyses were based on these clean data of high quality. *De novo* assembly of the transcriptome was performed with Trinity 2.4.0 (Grabherr et al. 2011). Trinity groups transcripts into clusters, loosely defined as a 'gene', based on shared sequence content. Statistical significance of fold-

change of genes among comparisons were adjusted for multiple comparisons using the false discovery rate (FDR) correction (Benjamini and Hochberg 1995).

5.3.5 RNA sequencing data analysis and pathway mapping

All assembled transcripts were aligned against the National Center for Biotechnology Information non-redundant (Nr) protein database (http://www.ncbi.nlm.nih.gov), Gene ontology (GO) (http://www.geneontology.org), SwissProt (http://www.expasy.ch/sprot), Kyoto Encyclopedia of Genes and Genomes (KEGG) (http://www.kegg.jp/kegg) and eggNOG (http://eggnogdb.embl.de) databases using the DIAMOND algorithm (Buchfink et al. 2015) with a threshold of E-value < 0.00001. Different expression analysis of genes was performed using Salmon (Patro et al. 2017) based on normalized transcript per million (TPM) values (Mortazavi et al. 2008). The differentially expressed genes (DEGs) were selected as the transcripts that were annotated in at least one of the six databases with a log2 fold change of >1 or <-1 and with statistical significance (FDR p-value < 0.05) using the R package "edgeR" (Robinson et al. 2010). Enrichment analysis was performed for genes expressed at Timepoint 1 (6 hrs), mapping DEGs to GO terms (https://www.geneontology.org/docs/ontology-documentation) and KEGG pathways (https://www.genome.jp/kegg/mapper/search.html), with significance based on the hypergeometric equation:

$$P = 1 - \sum_{i=0}^{S-1} \frac{\binom{B}{i}\binom{TB-B}{TS-i}}{\binom{TB}{TS}}$$

where TB gene number = number of total genes in the comparison; TS gene number = number of differentially expressed genes in total genes in the comparison; B gene number = total number of genes in the GO term/KEGG pathway; S gene number = number of differentially expressed

genes in the GO term/KEGG pathway. GO terms/KEGG pathways with p-value < 0.05 were considered significantly enhanced. Significantly enhanced genes within terms/pathways are noted in parentheses in the Results section. Note that the naming of genes, GO terms, and KEGG pathways are largely based on previous genomic and transcriptomic research in mice and humans. In some cases, the gene, term, or pathway name may appear to have limited relevance to the organism or structure being discussed, such as the KEGG pathway "ovarian steroidogenesis" being enhanced in testis samples. When necessary, we further explain the importance of genes within terms/pathways in the Discussion section.

The top 10 GO terms and top 10 KEGG pathways were reported for pairwise comparisons (*i.e.*, Amphiplex x Saline, High Chorus x Wind) based on a combination of number of DEGs expressed within the term/pathway and level of significance (*i.e.*, adjusted p-values). To test our hypothesis that exposure to conspecific calls affects gonadal function, results of the enrichment analysis were systematically searched for significant terms/pathways associated with sex hormone steroidogenesis, gonadal development, and gametogenesis. Search terms included the following: "testis", "ovary", "gonad", "sperm", "oocyte", "hormone", "steroid", and "GNRH".

5.4 Results

5.4.1 Differential gene expression at Timepoint 1

Male

The testicular transcriptome analysis identified 25,727 transcripts that were annotated in at least one of the six databases, representing 13,988 genes. Within the Amphiplex x Saline comparison at Timepoint 1 (6 hrs), 1605 and 483 annotated transcripts were unique to the

Amphiplex and Saline treatments, respectively. Within the High Chorus x Low Chorus x Wind comparison at Timepoint 1, there were 3979, 97, and 374 annotated transcripts unique to the High Chorus, Low Chorus, and Wind treatments, respectively.

There were 4096 significant DEGs (3453 upregulated, 643 downregulated) in the Amphiplex x Saline comparison at Timepoint 1 (Figure 5.2A). When comparing Low Chorus to Wind, we observed that there were only 16 DEGs (Supplementary Table S5.1). Inspection of the z-scores among High Chorus, Low Chorus, and Wind treatments confirm that gene expression was very similar between Low Chorus and Wind treatments (Supplementary Figure S5.2). There were 3570 significant DEGs (3283 upregulated, 287 downregulated) in the High Chorus x Wind comparison at Timepoint 1 (Figure 5.2B).

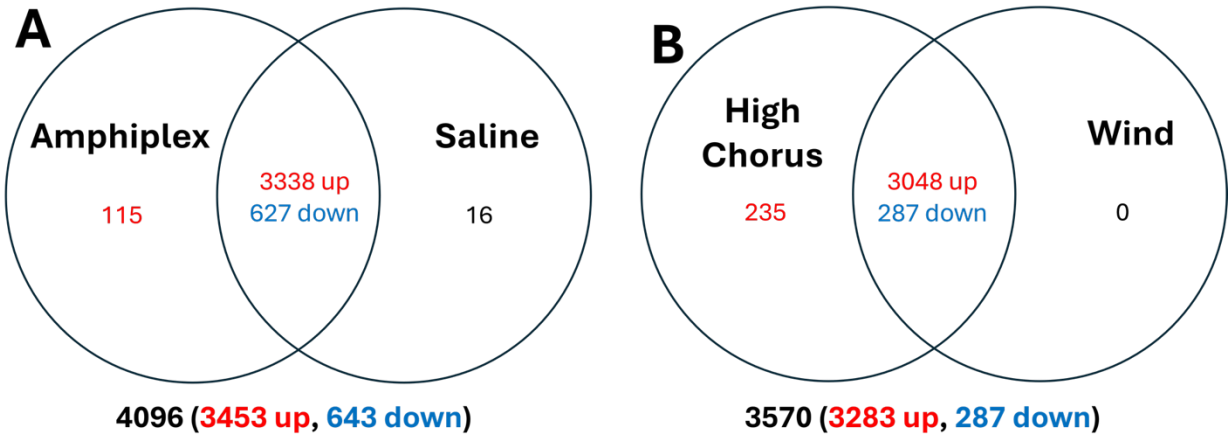

Figure 5.2: Venn diagrams of shared and unique differential expressed genes (DEGs) related to testis samples of boreal chorus frogs (*Pseudacris maculata*). In the first comparison (A) male chorus frogs were injected with a hormone mixture ("Amphiplex") or 7% saline solution ("Saline"). In the second comparison (B) male chorus frogs were exposed to 6-h broadcasts of a large chorus of 100 conspecific males calling ("High Chorus") or natural sounds recorded within the breeding environment lacking frog calls ("Wind"). Values below Venn diagrams represent the total number of DEGs upregulated (red) and down regulated (blue) in each comparison.

Female

The ovarian transcriptome analysis identified 15170 transcripts that were annotated in at least one of the six databases. Within the Amphiplex x Saline comparison at Timepoint 1, there were 14827 annotated transcripts identified, of which 441 and 317 genes were unique to the Amphiplex and Saline treatments, respectively. Within the High Chorus x Wind comparison at Timepoint 1 (6 hrs), there were 14745 genes identified, of which 181 and 222 annotated transcripts were unique to the High Chorus and Wind treatments, respectively. There were 41 significant DEGs (21 upregulated, 20 downregulated) in the Amphiplex x Saline comparison (Figure 5.3A) and there were 14 significant DEGs (6 upregulated, 8 downregulated) in the High Chorus x Wind comparison (Figure 5.3B). Two genes were upregulated in both comparisons (Figure 5.3C), *RPL21* (ribosomal protein L21) and *SPAG7* (sperm associated antigen 7).

5.4.2 GO and KEGG enrichment analyses

Male

Within the Amphiplex x Saline comparison at Timepoint 1 (6 hrs), 2178 terms and 147 pathways were enhanced in the GO (Table 5.1) and KEGG (Table 5.2) enrichment analyses, respectively. The DEGs in the Amphiplex treatment were associated with GO terms “male gonad development” (*i.e.*, *NASP*, nuclear autoantigenic sperm protein; *INSL3*; insulin like 3), “cellular response to gonadotropin-releasing hormone” (*i.e.*, *GNRHR2,* gonadotropin releasing hormone receptor-2; *ANXA5,* annexin A5), “cellular response to hormone stimulus” (*i.e.*, signal transducer and activator of transcription isoforms; transcription factor isoforms; serine/threonine-protein kinases), and “steroid hormone binding” (*i.e.*, sodium/potassium-transporting ATPase subunits).

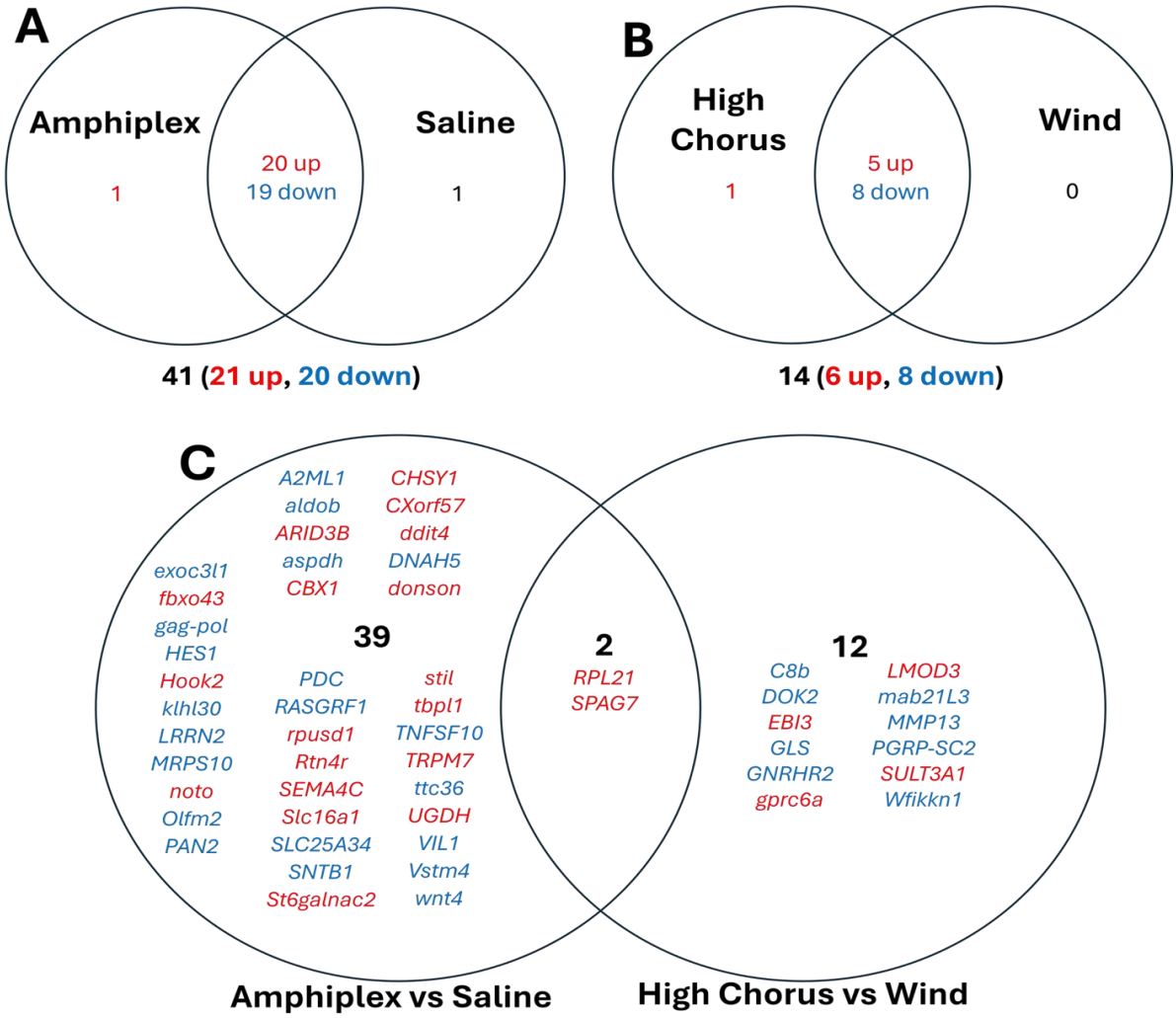

Figure 5.3: Venn diagrams of shared and unique genes related to ovary samples of boreal chorus frogs (*Pseudacris maculata*). In the first comparison (A) female chorus frogs were injected with a hormone mixture (Amphiplex) or 7% saline solution (Saline). In the second comparison (B) chorus frogs were exposed to 6-h broadcasts of a large chorus of 100 conspecific males calling ("High Chorus") or natural sounds recorded within the breeding environment lacking frog calls ("Wind"). Values below Venn diagrams A & B represent the total number of DEGs upregulated (red) and down regulated (blue) in each comparison. Gene names in the Venn diagram C are the DEGs upregulated (red) and down regulated (blue) in each comparison.

KEGG pathways significantly enriched by DEGs in the Amphiplex treatment potentially associated with reproduction and calling behaviour included “ovarian steroidogenesis” (*STAR*, steroidogenic acute regulatory protein; *PRKACA*, protein kinase cAMP-activated catalytic subunit alpha), a pathway that includes genes also important for testicular steroidogenesis and androgen signalling. Other important KEGG pathways included “estrogen signalling pathway” (heat shock protein 90 alpha family genes; *GNAS*, GNAS complex locus; *FKBP4*, FKBP prolyl isomerase 4), “prolactin signalling pathway” (prolactin isoforms; signal transducer and activator of transcription isoforms), and “oxytocin signalling pathway” (*PLCB3*, phospholipase C beta 3; *MYL6*, myosin light chain 6; *EEF2*, eukaryotic translation elongation factor 2).

Within the High Chorus x Wind comparison at Timepoint 1, 2142 terms and 174 pathways were enhanced in the GO (Table 5.3) and KEGG (Table 5.4) enrichment analyses, respectively. Notably, the High Chorus treatment and the Amphiplex treatment had several enhanced GO terms in common that were associated with spermatogenesis and response to steroid hormones (Table 5.5). The High Chorus treatment had significantly enhanced genes associated with GO terms “male gonad development” (*i.e.*, *NASP*, nuclear autoantigenic sperm protein; *INSL3*; insulin like 3), “spermatoproteasome complex” (*i.e.*, proteasome subunits), “spermatogenesis” (*i.e.*, spermatogenesis-associated proteins; testis-specific serine/threonine-protein kinases), and “follicle-stimulating hormone signaling pathway” (*i.e.*, *ADRM1*, 26S proteasome ubiquitin receptor; *ARRB2*, arrestin beta 2; *GRK2*, G protein-coupled receptor kinase 2). KEGG pathways significantly enriched by DEGs in the High Chorus treatment included “prolactin signaling pathway” (*CYP17A1*, cytochrome P450 family 17 subfamily A member 1; prolactin precursors; prolactin-like proteins), “oxytocin signaling pathway” (serine/threonine-protein phosphatases; myosin light polypeptides), “cortisol synthesis and secretion” (cAMP

responsive element binding protein 3-like proteins; cyclic AMP-dependent transcription factors), and “estrogen signaling pathway” (*MMP*, matrix metalloproteinase-2; type I keratins; heat shock proteins).

Timepoint comparisons among High Chorus and Low Chorus treatments

We described how gene expression changed between timepoints in the Low Chorus and High Chorus treatments using genes within the GO terms “steroid biosynthetic process”, “response to hormone”, and “male gonad development” to test the hypothesis that broadcasts affected testicular function in male frogs. Most of the genes within the GO terms that were upregulated in the High Chorus treatment at Timepoint 1 (6 hrs) remained significantly upregulated at Timepoint 2 (24 hrs) and at Timepoint 3 (30 hrs) when compared to the Wind Treatment at the same timepoints (Table 5.6). However, many genes that were upregulated at Timepoint 1 were significantly downregulated at Timepoint 2 when comparing within the High Chorus treatment and there were little to no significant DEGs between Timepoint 2 and Timepoint 3 (Supplementary Table S5.2). This pattern was repeatedly observed even when considering GO terms that contained several dozen or hundreds of genes. For example, for the “spermatogenesis” term, 119/163 (73%) genes were significantly upregulated ($log2FC > 1.5$, $p < 0.05$) in the High Chorus treatment compared to the Wind treatment. When comparing High Chorus at Timepoint 1 to High Chorus at Timepoint 2, we found 92/163 (56%) genes were significantly downregulated ($log2FC < -1.5$, $p < 0.05$). Only 2/163 (1.2%) genes showed differential expression when comparing Timepoint 2 to Timepoint 3, and 37/163 (23%) genes that were expressed at Timepoint 1 were not detected at Timepoint 2 or Timepoint 3 (Supplementary Table S5.3). Gene expression in the Low Chorus treatment between timepoints differed from the High Chorus treatment. Very few or no DEGs were found in the Low Chorus x

Wind at Timepoint 1 and Timepoint 3, but several genes associated with GO terms “steroid biosynthetic process”, “response to hormone”, and “male gonad development” were upregulated at Timepoint 2 (Supplementary Table S5.2). Additionally, the expression of genes in the Low Chorus x Wind comparison at Timepoint 2 was very similar to the expression of genes in the High Chorus x Wind comparison at Timepoint 1 (Table 5.7).

Female

Compared to the testicular transcriptome, fewer GO terms and KEGG pathways were enhanced in the ovarian transcriptome. There were 65 GO terms and 3 KEGG pathways enhanced in the Amphiplex x Saline comparison and 50 GO terms and 11 KEGG pathways enhanced in the High Chorus x Wind comparison. However, in most cases, only a single significant DEG characterized the enhancement. GO terms and KEGG pathways were therefore only used to identify potentially noteworthy genes contributing to ovarian function, steroidogenesis, and gametogenesis rather than considering the terms/pathways themselves significantly enhanced.

The Amphiplex treatment had significant up- and down-regulation of genes associated with GO terms “mitogen-activated protein (MAP) kinase activity” (i.e., *ALDOB*, aldolase/fructose-bisphosphate B), “embryo development” (i.e., *TBPL1*, TATA-box binding protein like 1), and “regulation of mitotic nuclear division” (i.e., *FBXO43*, F-box protein 43). Significant downregulation of KEGG pathways “apoptosis” and “necroptosis” were related to the upregulation *TRPM7* (transient receptor potential cation channel subfamily M member 7) and downregulation of *TNFSF10* (TNF superfamily member 10), respectively. Another notable KEGG pathway in the Amphiplex treatment was “glycosaminoglycan biosynthesis - chondroitin

sulfate / dermatan sulfate", which was associated with the upregulation of *CHSY1* (chondroitin sulfate synthase 1), a gene related to cell proliferation and morphogenesis.

Several GO terms were associated with the downregulation of *GNRHR2* (gonadotropin releasing hormone receptor 2) in the High Chorus treatment, including "gonadotropin-releasing hormone receptor activity", "cellular response to gonadotropin-releasing hormone", and "peptide binding". KEGG pathways enhanced in the High Chorus treatment included "il-17 signaling pathway" and "parathyroid hormone synthesis, secretion and action" (i.e., *MMP13*, matrix metallopeptidase 13). Other notable KEGG pathways included "negative regulation of DNA binding" and "negative regulation of protein binding" both of which were associated with downregulation of *WFIKKN1* (WAP, follistatin/kazal, immunoglobulin, kunitz and netrin domain containing 1).

Table 5.1: Top 10 (of 147) significantly enhanced Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways – Amphiplex x Saline testicular samples. DEG = differentially expressed gene.

Pathway Entry	Pathway Definition	Genes in pathway	DEG – total	DEG – up	DEG – down
map05200	Pathways in cancer	302	126	125	1
map05012	Parkinson disease	192	116	116	0
map04714	Thermogenesis	237	125	123	2
map05010	Alzheimer disease	218	114	114	0
map05016	Huntington disease	220	114	114	0
map04932	Non-alcoholic fatty liver disease (NAFLD)	157	84	84	0
map05418	Fluid shear stress and atherosclerosis	141	69	69	0
map05205	Proteoglycans in cancer	209	92	92	1
map04151	PI3K-Akt signaling pathway	250	116	113	3
map05165	Human papillomavirus infection	255	122	121	1

Table 5.2: Top 10 (of 2176) significantly enhanced Gene Ontology (GO) terms – Amphiplex vs Saline testicular samples. DEG = differentially expressed gene.

GO ID	GO Term	GO Function	Genes in pathway	DEG – total	DEG – up	DEG – down
GO:0043066	negative regulation of apoptotic process	Biological process	453	176	165	11
GO:0005525	GTP binding	Molecular function	568	162	151	11
GO:0042493	response to drug	Biological process	178	74	74	0
GO:0042802	identical protein binding	Molecular function	771	253	235	18
GO:0016020	membrane	Cellular component	3427	984	929	55
GO:0005615	extracellular space	Cellular component	1611	397	356	41
GO:0005515	protein binding	Molecular function	3391	1101	1045	56
GO:0005654	nucleoplasm	Cellular component	1856	416	396	20
GO:0005829	cytosol	Cellular component	2666	802	752	50
GO:0043209	myelin sheath	Cellular component	325	140	138	2

Table 5.3: Top 10 (of 174) significantly enhanced Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways – High Chorus x Wind testicular samples. DEG = differentially expressed gene.

Pathway Entry	Pathway Definition	Genes in pathway	DEG – total	DEG – up	DEG – down
map05200	Pathways in cancer	302	145	142	3
map05165	Human papillomavirus infection	255	122	122	0
map05010	Alzheimer disease	218	127	126	1
map05016	Huntington disease	220	123	122	1
map04714	Thermogenesis	237	125	122	3
map05012	Parkinson disease	192	108	106	2
map04151	PI3K-Akt signaling pathway	250	127	127	0
map05418	Fluid shear stress and atherosclerosis	141	82	81	1
map04932	Non-alcoholic fatty liver disease (NAFLD)	157	88	87	1
map05163	Human cytomegalovirus infection	151	83	83	0

Table 5.4: Top 10 (of 2141) significantly enhanced Gene Ontology (GO) terms – High Chorus x Wind testicular samples. DEG = differentially expressed gene.

GO ID	GO Term	GO Function	Genes in pathway	DEG – total	DEG – up	DEG – down
GO:0006915	apoptotic process	Biological process	327	145	139	6
GO:0015031	protein transport	Biological process	349	145	142	3
GO:0016740	transferase activity	Molecular function	516	197	192	5
GO:0007275	multicellular organism development	Biological process	482	173	161	12
GO:0007049	cell cycle	Biological process	239	117	115	2
GO:0005886	plasma membrane	Cellular component	2722	785	725	60
GO:0007283	spermatogenesis	Biological process	383	163	150	13
GO:0042995	cell projection	Cellular component	363	164	158	6
GO:0005829	cytosol	Cellular component	2666	948	915	33
GO:0070062	extracellular exosome	Cellular component	2577	675	630	45

Table 5.5: Comparison of ten enhanced Gene Ontology (GO) pathways related to spermatogenesis and hormone secretion observed in the Amphiplex x Saline and the High Chorus x Wind comparisons in testicular samples. DEG = differentially expressed gene. All reported p-values are adjusted for false discovery rate (FDR) using the Benjamini and Hochberg (1995) correction.

			Amphiplex x Saline				**High Chorus x Wind**			
GO ID	**GO Term**	**Genes in pathway**	**DEG - total**	**DEG - up**	**DEG - down**	**p-value**	**DEG - total**	**DEG - up**	**DEG - down**	**p-value**
GO:0009725	response to hormone	32	16	15	1	**< 0.01**	14	14	0	**< 0.01**
GO:0005179	hormone activity	73	22	20	2	**< 0.01**	25	22	3	**< 0.01**
GO:0071375	cellular response to peptide hormone stimulus	15	7	7	0	**< 0.01**	8	7	1	**< 0.01**
GO:0006694	steroid biosynthetic process	34	16	14	2	**< 0.01**	14	14	0	**< 0.01**
GO:0007286	spermatid development	104	9	8	1	0.98	41	34	7	**< 0.01**
GO:0007339	binding of sperm to zona pellucida	68	16	10	6	**0.04**	42	41	1	**< 0.01**
GO:0007283	spermatogenesis	383	61	55	6	0.30	163	150	13	**< 0.01**
GO:0008584	male gonad development	66	16	15	1	**0.03**	25	24	1	**< 0.01**
GO:0008202	steroid metabolic process	38	11	11	0	**0.02**	14	14	0	**0.01**
GO:0048545	response to steroid hormone	21	5	4	1	**0.01**	8	8	0	**0.04**

Table 5.6: Summary of testicular gene expression within High Chorus treatment in comparison to Wind treatment at three timepoints (6–30 hrs). Coloured nodes (red) indicate upregulation of gene based on log2 fold change (1.5 > log2FC < -1.5) and adjusted p-value (< 0.05).

steroid biosynthetic process (GO:0006694)

ID	Gene	High 6h x Wind 6h		High 24h x Wind 24h		High 30h x Wind 30h	
		log2FC	P(adj)	log2FC	P(adj)	log2FC	P(adj)
TRINITY_DN111256_c1_g3	*Dhcr24*	**2.96**	0.009	0.85	0.982	**5.17**	0.000
TRINITY_DN113871_c0_g2	*FDFT1*	**2.53**	0.038	**2.11**	0.002	**3.96**	0.000
TRINITY_DN116674_c2_g3	*Tspo*	**5.48**	0.000	**5.24**	0.000	**7.45**	0.000
TRINITY_DN131188_c0_g1	*Scp2d1*	5.36	0.475	–	–	–	–
TRINITY_DN19878_c0_g1	*Cyp17a1*	**5.65**	0.002	2.19	1.000	2.50	1.000
TRINITY_DN54102_c0_g1	*Hsd17b11*	**5.01**	0.000	**4.58**	0.012	**5.77**	0.000
TRINITY_DN65460_c2_g2	*Hsd17b12*	**4.14**	0.000	2.84	0.200	**5.16**	0.000
TRINITY_DN74172_c4_g1	*Cyb5r1*	1.54	0.435	**2.36**	0.008	**3.00**	0.000
TRINITY_DN78062_c2_g2	*Sc5d*	**3.41**	0.000	**4.13**	0.027	**4.78**	0.000
TRINITY_DN78735_c0_g1	*Scp2*	**4.11**	0.000	**2.22**	0.032	**4.06**	0.000
TRINITY_DN83670_c0_g1	*Cyb5r3*	**4.60**	0.000	**3.57**	0.029	**4.93**	0.000
TRINITY_DN90946_c0_g2	*Tecr*	**4.21**	0.000	3.03	0.084	**5.24**	0.000
TRINITY_DN91956_c2_g1	*Hmgcs1*	**3.57**	0.000	1.97	0.326	**5.72**	0.000
TRINITY_DN95069_c3_g1	*Msmo1*	**2.82**	0.025	1.98	0.242	**3.69**	0.000

response to hormone (GO:0009725)

ID	Gene	High 6h x Wind 6h		High 24h x Wind 24h		High 30h x Wind 30h	
		log2FC	P(adj)	log2FC	P(adj)	log2FC	P(adj)
TRINITY_DN110262_c3_g5	*Timp2*	2.07	0.278	2.51	0.067	**3.53**	0.005
TRINITY_DN118595_c0_g1	*Mmp14*	1.49	0.535	**2.84**	0.005	**3.90**	0.000
TRINITY_DN118956_c0_g3	*Lox*	2.26	0.235	2.87	0.152	3.53	0.505
TRINITY_DN130422_c0_g1	*Mb*	**6.49**	0.000	**5.34**	0.043	6.37	0.187
TRINITY_DN130843_c0_g1	*GPX1*	0.50	1.000	–	–	–	–
TRINITY_DN64833_c0_g2	*Cox5b*	**5.33**	0.000	3.38	0.079	**5.97**	0.000
TRINITY_DN65559_c0_g11	*Mtnd3*	**6.60**	0.000	2.14	0.876	**4.45**	0.002
TRINITY_DN68843_c3_g1	*HCLS1*	**4.61**	0.000	**2.62**	0.025	**2.65**	0.002
TRINITY_DN76207_c0_g1	*SORD*	**3.49**	0.008	1.80	0.082	**3.84**	0.000
TRINITY_DN80017_c0_g1	*NCOA4*	**4.94**	0.000	**3.60**	0.011	**6.83**	0.000
TRINITY_DN84281_c3_g2	*Timp1*	2.66	0.075	**3.48**	0.004	**4.72**	0.000
TRINITY_DN84747_c0_g1	*Me1*	**3.96**	0.000	**3.48**	0.002	**6.17**	0.000
TRINITY_DN87262_c0_g4	*Aqp1*	**6.78**	0.000	1.59	0.866	**4.06**	0.046
TRINITY_DN97922_c2_g1	*Por*	**3.97**	0.000	**2.31**	0.007	**4.15**	0.000

Table 5.6 (continued): Summary of gene expression within High Chorus treatment in comparison to Wind treatment at three timepoints (6–30 hrs). Coloured nodes (red) indicate upregulation of gene based on log2 fold change (1.5 > log2FC < -1.5) and adjusted p-value (< 0.05).

male gonad development (GO:0008584)

ID	Gene	High 6h x Wind 6h		High 24h x Wind 24h		High 30h x Wind 30h	
		log2FC	P(adj)	log2FC	P(adj)	log2FC	P(adj)
TRINITY_DN100710_c0_g3	*Insl6*	**5.53**	0.018	1.04	1.000	2.06	1.000
TRINITY_DN106049_c0_g2	*BCL2L1*	**3.01**	0.001	**3.18**	0.000	**5.06**	0.000
TRINITY_DN106744_c1_g1	*Cited2*	**3.26**	0.006	**3.84**	0.000	**6.41**	0.000
TRINITY_DN107686_c0_g1	*Ybx3*	**5.16**	0.000	1.80	0.355	**3.88**	0.000
TRINITY_DN109406_c2_g1	*Kdr*	**5.40**	0.000	1.86	0.892	**4.69**	0.017
TRINITY_DN109503_c2_g2	*Eif2s2*	**2.66**	0.032	**2.48**	0.008	**3.88**	0.000
TRINITY_DN112537_c3_g2	*Tbc1d20*	**3.56**	0.000	1.95	0.090	**2.63**	0.002
TRINITY_DN112800_c0_g1	*Wdr48*	**4.82**	0.000	2.25	0.081	**5.74**	0.000
TRINITY_DN115851_c1_g4	*PRPS1*	3.91	0.635	–	–	–	–
TRINITY_DN123522_c0_g1	*CSDE1*	**2.38**	0.007	**2.06**	0.022	**4.01**	0.000
TRINITY_DN130557_c0_g1	*Klhl10*	**21.56**	0.000	–	–	–	–
TRINITY_DN1311_c0_g1	*ANKRD7*	4.93	0.543	–	–	–	–
TRINITY_DN131297_c0_g1	*Spink2*	5.95	0.380	–	–	–	–
TRINITY_DN56774_c0_g1	*Nupr1*	1.77	0.297	2.72	0.206	**6.17**	0.000
TRINITY_DN64913_c0_g2	*Rbp4*	**7.05**	0.000	**6.08**	0.002	**5.86**	0.000
TRINITY_DN66237_c2_g2	*Insl3*	**7.19**	0.002	-0.04	1.000	1.77	1.000
TRINITY_DN69790_c0_g1	*Prdx4*	2.31	0.090	1.73	0.453	**4.07**	0.000
TRINITY_DN69790_c0_g3	*PRDX4*	5.50	0.453	–	–	–	–
TRINITY_DN73303_c4_g4	*Fdps*	2.53	0.079	**4.64**	0.001	**4.14**	0.000
TRINITY_DN74726_c4_g1	*Bax*	**2.68**	0.006	**3.71**	0.000	**4.92**	0.000
TRINITY_DN86395_c1_g2	*Hmgb2*	2.59	0.073	1.44	0.404	**4.22**	0.000
TRINITY_DN86973_c0_g1	*SFRP2*	2.46	0.158	**5.78**	0.000	2.39	0.660
TRINITY_DN94714_c3_g4	*Six4*	-0.30	0.220	-0.07	1.000	-0.12	1.000
TRINITY_DN95226_c4_g4	*CITED2*	**3.77**	0.000	**3.06**	0.000	**5.18**	0.000
TRINITY_DN96193_c2_g1	*NASP*	1.96	0.419	**2.43**	0.000	**2.29**	0.028

Table 5.7: Summary of gene expression within High Chorus and Low Chorus treatments in comparison to Wind treatment. Coloured nodes (red) indicate upregulation of gene based on log2 fold change (1.5 > log2FC < -1.5) and adjusted p-value (< 0.05).

steroid biosynthetic process (GO:0006694)

ID	Gene	Low 6h x Wind 6h		High 6h x Wind 6h		Low 24h x Wind 24h	
		log2FC	P(adj)	log2FC	P(adj)	log2FC	P(adj)
TRINITY_DN111256_c1_g3	*Dhcr24*	-0.06	0.999	**2.96**	0.009	2.82	0.182
TRINITY_DN113871_c0_g2	*FDFT1*	-1.87	0.760	**2.53**	0.038	**3.42**	0.000
TRINITY_DN116674_c2_g3	*Tspo*	-0.33	1.000	**5.48**	0.000	**6.21**	0.003
TRINITY_DN131188_c0_g1	*Scp2d1*	–	–	5.36	0.475	–	–
TRINITY_DN19878_c0_g1	*Cyp17a1*	-0.56	1.000	**5.65**	0.002	0.30	1.000
TRINITY_DN54102_c0_g1	*Hsd17b11*	-1.57	1.000	**5.01**	0.000	**6.99**	0.000
TRINITY_DN65460_c2_g2	*Hsd17b12*	-0.47	0.998	**4.14**	0.000	3.95	0.080
TRINITY_DN74172_c4_g1	*Cyb5r1*	-3.58	0.356	1.54	0.435	**4.75**	0.000
TRINITY_DN78062_c2_g2	*Sc5d*	-3.01	1.000	**3.41**	0.000	**5.81**	0.001
TRINITY_DN78735_c0_g1	*Scp2*	-0.40	0.998	**4.11**	0.000	**2.98**	0.032
TRINITY_DN83670_c0_g1	*Cyb5r3*	-1.86	0.942	**4.60**	0.000	**4.62**	0.030
TRINITY_DN90946_c0_g2	*Tecr*	-0.09	0.998	**4.21**	0.000	**4.34**	0.012
TRINITY_DN91956_c2_g1	*Hmgcs1*	-1.22	0.977	**3.57**	0.000	**3.64**	0.007
TRINITY_DN95069_c3_g1	*Msmo1*	-1.89	0.846	**2.82**	0.025	**3.62**	0.005

response to hormone (GO:0009725)

ID	Gene	Low 6h x Wind 6h		High 6h x Wind 6h		Low 24h x Wind 24h	
		log2FC	P(adj)	log2FC	P(adj)	log2FC	P(adj)
TRINITY_DN110262_c3_g5	*Timp2*	-2.32	0.854	2.07	0.278	**5.19**	0.000
TRINITY_DN118595_c0_g1	*Mmp14*	-2.23	0.769	1.49	0.535	**4.17**	0.000
TRINITY_DN118956_c0_g3	*Lox*	-4.15	0.648	2.26	0.235	**4.87**	0.032
TRINITY_DN130422_c0_g1	*Mb*	2.93	1.000	**6.49**	0.000	-0.06	1.000
TRINITY_DN130843_c0_g1	*GPX1*	–	–	0.50	1.000	1.02	1.000
TRINITY_DN64833_c0_g2	*Cox5b*	0.03	1.000	**5.33**	0.000	4.12	0.091
TRINITY_DN65559_c0_g11	*Mtnd3*	1.72	0.914	**6.60**	0.000	1.95	0.877
TRINITY_DN68843_c3_g1	*HCLS1*	-0.03	0.999	**4.61**	0.000	-0.86	0.940
TRINITY_DN76207_c0_g1	*SORD*	-1.55	0.902	**3.49**	0.008	**3.91**	0.001
TRINITY_DN80017_c0_g1	*NCOA4*	-1.05	0.993	**4.94**	0.000	**5.06**	0.008
TRINITY_DN84281_c3_g2	*Timp1*	-5.57	0.142	2.66	0.075	**5.29**	0.000
TRINITY_DN84747_c0_g1	*Me1*	-0.39	0.998	**3.96**	0.000	**4.13**	0.001
TRINITY_DN87262_c0_g4	*Aqp1*	4.04	1.000	**6.78**	0.000	-2.74	1.000
TRINITY_DN97922_c2_g1	*Por*	-0.83	0.956	**3.97**	0.000	1.81	0.125

Table 5.7 (continued): Summary of gene expression within High Chorus and Low Chorus treatments in comparison to Wind treatment. Coloured nodes (red) indicate upregulation of gene based on log2 fold change (1.5 > log2FC < -1.5) and adjusted p-value (< 0.05).

male gonad development (GO:0008584)

ID	Gene	Low 6h x Wind 6h		High 6h x Wind 6h		Low 24h x Wind 24h	
		log2FC	P(adj)	log2FC	P(adj)	log2FC	P(adj)
TRINITY_DN100710_c0_g3	*Insl6*	–	–	**5.53**	0.018	3.43	1.000
TRINITY_DN106049_c0_g2	*BCL2L1*	-2.38	0.658	**3.01**	0.001	**4.47**	0.000
TRINITY_DN106744_c1_g1	*Cited2*	-1.65	0.910	**3.26**	0.006	**4.09**	0.000
TRINITY_DN107686_c0_g1	*Ybx3*	0.66	0.995	**5.16**	0.000	2.79	0.106
TRINITY_DN109406_c2_g1	*Kdr*	-0.58	1.000	**5.40**	0.000	2.67	0.643
TRINITY_DN109503_c2_g2	*Eif2s2*	-1.38	0.925	**2.66**	0.032	**3.78**	0.000
TRINITY_DN112537_c3_g2	*Tbc1d20*	-1.69	0.799	**3.56**	0.000	**4.80**	0.000
TRINITY_DN112800_c0_g1	*Wdr48*	-2.22	1.000	**4.82**	0.000	**3.38**	0.011
TRINITY_DN115851_c1_g4	*PRPS1*	–	–	3.91	0.635	–	–
TRINITY_DN123522_c0_g1	*CSDE1*	-1.43	0.836	**2.38**	0.007	**3.16**	0.001
TRINITY_DN130557_c0_g1	*Klhl10*	–	–	**21.56**	0.000	–	–
TRINITY_DN1311_c0_g1	*ANKRD7*	–	–	4.93	0.543	–	–
TRINITY_DN131297_c0_g1	*Spink2*	–	–	5.95	0.380	–	–
TRINITY_DN56774_c0_g1	*Nupr1*	-2.27	0.885	1.77	0.297	**4.48**	0.013
TRINITY_DN64913_c0_g2	*Rbp4*	0.52		**7.05**	0.000	2.59	1.000
TRINITY_DN66237_c2_g2	*Insl3*	–	–	**7.19**	0.002	0.30	1.000
TRINITY_DN69790_c0_g1	*Prdx4*	-2.11	0.759	2.31	0.090	**3.38**	0.013
TRINITY_DN69790_c0_g3	*PRDX4*	–	–	5.50	0.453	–	–
TRINITY_DN73303_c4_g4	*Fdps*	-2.09	0.924	2.53	0.079	**6.55**	0.000
TRINITY_DN74726_c4_g1	*Bax*	-2.03	0.699	**2.68**	0.006	**5.41**	0.000
TRINITY_DN86395_c1_g2	*Hmgb2*	-2.18	0.720	**2.59**	0.073	**3.02**	0.011
TRINITY_DN86973_c0_g1	*SFRP2*	-3.09	1.000	2.46	0.158	**3.88**	0.008
TRINITY_DN94714_c3_g4	*Six4*	0.27	0.720	-0.30	0.220	-0.09	0.962
TRINITY_DN95226_c4_g4	*CITED2*	-1.09	1.000	**3.77**	0.000	**3.38**	0.001
TRINITY_DN96193_c2_g1	*NASP*	-2.31	0.708	**1.96**	0.419	**3.08**	0.009

5.5 Discussion

5.5.1 Testicular gene expression

There was considerable overlap in the groups of genes that were differentially expressed in Amphiplex x Saline and High Chorus x Wind comparisons, including several GO terms (Table 5.5), strongly indicating a shared hormonal response to the co-administration of GnRH and metoclopramine and reception of conspecific male calls. GO terms “response to hormone”, “male gonad development”, and “steroid biosynthetic process” were significantly upregulated in both Amphiplex x Saline and High Chorus x Wind comparisons. Notable genes within these GO terms that were upregulated in both comparisons included *Insl3*, *Klhl10*, *Cyp17a1*, *Tspo*, *Hsd17b11*, *Hsd17b12*, and *Aqp1*.

Insulin-like peptide 3 (INSL3) has a role in spermatogenesis and germ cell survival, stimulating the differentiation of spermatogonia in zebrafish (Assis et al. 2016). INSL3 is also a biomarker for testicular function in humans and rodents, with its concentration reflecting differentiation status and number of the Leydig cells present in the testes (Ivell et al. 2013; Facondo et al. 2020). Kelch-like family member 10 (Klhl10) is exclusively produced in testes within developing spermatids and knockout of *Klhl10* allele led to the disruption of spermiogenesis and complete male infertility in mice (Yan et al. 2004; Wu et al. 2010).

Cytochrome P450 family 17 subfamily A member 1 (CYP17A1) is an important enzyme in synthesis of glucocorticoids and sex hormones, particularly for the conversion of pregnenolone (P5) to 17-hydroxypregnenolone and progesterone (P4) to 17-hydroxyprogesterone is 17α-hydroxylase activity and production of androgens via 17,20-lyase activity (Burris-Hiday and Scott 2021). CYP17A1 also has a role in sexual behaviour and secondary sexual

characteristics in males. *Cyp17a1* knockout leads to infertility and absence of sexual behaviours in mice (Liu et al. 2005). In frogs, *Cyp17a1* is highly expressed in the gonads before and after sexual differentiation in males and likely important for testis development (Maruo et al. 2008; Roco et al. 2021).

Translocator protein (TSPO) is abundant in steroidogenic cells, including Leydig cells, and important for the transport of cholesterol into mitochondria to initiate steroid hormone synthesis (Manku and Culty 2016; Papadopoulos et al. 2018). The functions of 17 beta-hydroxysteroid dehydrogenases (*e.g.*, Hsd17b11, Hsd17b12) have been inferred for amphibians as all vertebrates appear to express some form of the enzyme (Mindnich et al. 2004). *Hsd17b12* is highly expressed in meiotic and post-meiotic germ cells in mice and rats (Culty et al. 2015) and in Leydig cells of mice (Yu et al. 2018). In mice, aquaporin (Aqp1) is mainly involved in regulation of water homeostasis in male reproductive organs (*i.e.*, testis, efferent ducts, seminal vesicles), which is essential for reproductive health by maintaining proper ionic conditions for maturation and storage of spermatozoa (Lu et al. 2008; Carrageta et al. 2020).

We observed significant upregulation of KEGG pathways “ovarian steroidogenesis” (*i.e.*, *STAR*, *PRKACA*) and “estrogen signaling pathway” (*i.e.*, heat shock protein 90 alpha family genes, *FKBP4*) in both Amphiplex x Saline and High Chorus x Wind comparisons. Despite their naming conventions, these genes are also associated with steroidogenesis, androgen signalling, sperm maturation, and sexual development in males. Steroidogenic acute regulatory protein (STAR) is essential for the regulation of steroid hormone synthesis by mediating the conversion of cholesterol into pregnenolone (Clark and Stocco 2014) and is involved in the transport of cholesterol into the mitochondrial matrix (Stocco et al. 2017; Manna 2025). Protein kinase cAMP-activated catalytic subunit alpha (PRKACA) is associated with sperm maturation and

motility in mammals, potentially modulating cAMP activity and protein stability within the sperm cell (Nolan et al. 2004; Hereng et al. 2012). Heat shock protein 90 alpha family genes (*HSP90AA1*, *HSP90AB1*, *HSP90B1*) and FKBP prolyl isomerase 4 (*FKBP4*) are genes coding for proteins that have diverse functions including interacting with androgen receptors to influence signaling within the cell via androgen receptor-mediated transcription in mammals (Cheung-Flynn et al. 2005), are associated with proper formation of genitalia (Ilaslan et al. 2020), and act as chaperones that mediate the binding of sperm to zona pellucida (Dun et al. 2012).

Broadcasts of conspecific calls stimulated spermatogenesis as well as steroidogenic activity in male frogs. Numerous groups of genes that are associated with spermatogenesis were upregulated in the High Chorus treatment. Notably, the GO term "spermatogenesis" was significantly enhanced with differential expression of 163 of 383 genes in the pathway, including nine genes encoding for spermatogenesis associated proteins (*Spata6*, *Spata9*, *Spata18*, etc.) and four genes encoding for testis-specific serine/threonine-protein kinases (*Tssk1b*, *Tssk3*, *Tssk4*, *Tssk6*). Spermatogenesis associated proteins are required for several functions in the maturation of germ cells and proper formation of sperm. For example, Spata19 appears to be related to germ cell development/differentiation and mitochondrial architecture in sperm (Nourashrafeddin et al. 2014; Mi et al. 2015) and defects in the Spata16/SPATA16 gene are associated with globozoospermia (round-headed sperm cells that lack an acrosome) in humans and spermatogenic arrest in mice (Fujihara et al. 2016). In mouse models, Tssk3 and Tssk6 are essential for male fertility. Tssk6 is required for the regulation of actin polymerization and changes in the localization of the Izumo protein, which enables the binding activity of sperm to the zona pellucida of egg cells (Sosnik et al. 2009). Mice lacking Tssk3 experience

spermatogenic arrest where spermatogenesis is blocked at the stage VII/VIII spermatids, the penultimate stage of sperm maturation, and sperm is not released into the lumen of seminiferous tubules causing the mice to be sterile (Nayyab et al. 2021).

Within the GO term “binding of sperm to zona pellucida”, two zona pellucida binding protein genes (*Zpbp*, *Zpbp2*), three zona pellucida glycoprotein genes (*ZP2*, *ZP3*, *ZP4*) and five genes coding for subunits of the chaperonin containing TCP1 complex (*Cct2*, *Cct4*, *Cct5*, *Cct7*, *Cct8*) were upregulated in the High Chorus treatment. All these genes are critical during spermatogenesis for the proper binding of sperm to oocytes and fertilization. Mice lacking ZPBP and ZPBP2 were sterile and subfertile, respectively, possessing abnormal sperm morphology, with little to no forward motility, and limited ability to penetrate the zona pellucida (Yatsenko et al. 2010). Zona pellucida (ZP) glycoproteins are found on the extracellular matrix surrounding oocytes (Gupta 2021) but *ZP3* is also expressed in spermatogonia, spermatocytes and spermatids within the testes of humans and mice (Pulawska et al. 2022). Chaperonin containing TCP1 complex subunits (CCTs) are important for mediating the binding of sperm to zona pellucida through remodelling of the sperm surface (Dun et al. 2011; Dun et al. 2012).

Effects on mitochondrial function in males

Several of the enhanced KEGG pathways in the Amphiplex x Saline and High Chorus x Wind comparisons, including “Non-alcoholic fatty liver disease (NAFLD)”, “Thermogenesis”, “Pathways in cancer”, “Alzheimer disease”, “Huntington disease” and “Parkinson disease”, are associated with upregulation of genes coding for subunits of the series of four protein complexes (CI-CIV) within the electron transport chain of the mitochondria. These enzymes and protein complexes, such as NADH:ubiquinone oxidoreductase, cytochrome c oxidase, and ubiquinol-cytochrome c reductase, are related to mitochondrial function and include processes such as

generating electrochemical potential across the mitochondrial membrane, oxidative phosphorylation, and adenosine triphosphate (ATP) production (Smeitink et al. 2001; Ludwig et al. 2001; Whitehouse et al. 2019). Energy production via electron transport chain of the mitochondria is vital for proper cellular function and disruptions can have system wide effects on the organism. For instance, deficiencies in cytochrome c oxidase are related to several mitochondrial disorders that vary widely in severity, from relatively benign affects to skeletal muscles to severe diseases such as Leigh syndrome, a condition which affects the central nervous system and often leads to fatal encephalopathy (Brischigliaro and Zeviani 2021).

Disruptions to mitochondrial function within the gonad due to abnormal gene expression can have adverse effects on several levels of reproduction, from steroid production to gamete viability (Ramalho-Santos et al. 2009; Park and Pang 2021). As an example, knockdown of *COX5B* expression, a gene coding for a subunit of cytochrome c oxidase, in the Leydig cells of mice led to an increase in apoptotic activity (Wang et al. 2024). Increases of apoptosis was related to decreased intracellular ATP levels, decreased mitochondrial membrane potential, and increased reactive oxygen species levels, thus reducing the proliferative function of Leydig cells and impacting androgen production (Wang et al. 2024). Ubiquinol-cytochrome c reductase hinge protein (UQCRH) is one of 11 subunits in the ubiquinol-cytochrome c oxidoreductase complex (CIII) and mutations within the CIII-coding genes are associated with mitochondrial disorders (Fernández-Vizarra and Zeviani 2015). For example, 2.2 kb homozygous deletion of exons 2 and 3 of *UQCRH/Uqcrh* resulted in severe cases of lactic acidosis, hyperammonaemia, and impaired glucose homeostasis in humans and mice (Vidali et al. 2021).

The upregulation of “PI3K-Akt signaling pathway” was associated with genes coding for prolactin isoforms, 14-3-3 family proteins (*i.e.*, *YWHAE*), type IV collagen alpha proteins, and

heat shock protein 90 family proteins. The PI3K/Akt/mTOR (PAM) pathway is highly conserved among species and essential for cell growth, survival, and metabolism. PAM signalling is important for many aspects of male reproduction, especially the proliferation and differentiation of spermatogonia and somatic cells during spermatogenesis (Ni et al. 2019; Deng et al. 2021). The 14-3-3 proteins (YWHA or Tyrosine 3-Monooxygenase/Tryptophan 5-Monooxygenase Activation protein) are implicated in several signalling processes related to gametogenesis and reproduction (De 2021). For example, knockout of the 14-3-3 epsilon (*YWHAE*) in male mice caused infertility related to significantly reduced phosphorylation of several signal enzymes within spermatozoa, reduced sperm count and sperm motility, and a higher percentage of abnormal spermatozoa (Eisa et al. 2021).

Changes in gene expression between timepoints in males

Initial comparison of gene expression at the first timepoint (6 hrs) among Low Chorus, High Chorus and Wind treatments indicated that there were broad similarities between Low Chorus and Wind treatments with only 16 genes differentially expressed in the Low Chorus treatment. However, gene expression comparisons between Low Chorus and Wind treatments were significantly different at the second timepoint (24 hrs) and several genes that were upregulated at 24 hrs in the Low Chorus x Wind comparison were also upregulated in the High Chorus x Wind comparison at 6 hrs (Table 5.7). These results suggest that similar pathways or groups of genes associated with steroidogenesis and spermatogenesis are being activated in the Low Chorus and High Chorus treatments, but it takes longer to occur in the Low Chorus treatment.

As the Low Chorus broadcast simulated a small, low-intensity chorus of about 10 individuals and the High Chorus broadcast simulated a large, high-intensity chorus of about 100 individuals, chorus size appears to have influenced the rate of response in testicular gene expression. After prolonged exposure to chorus activity for 7–11 nights (5–12 hours per night), male frogs have elevated androgen concentrations (Burmeister and Wilczynski 2000; Chu and Wilczynski 2001) and increased testis mass (Brzoska and Obert 1980). Acoustic reception of conspecific calls also increases the probability of calling activity and overall calling effort in male frogs (Solís and Penna 1997; Burmeister and Wilczynski 2000; Emerson 2001) and calling males tend to have higher androgen levels than non-calling males (Woodley and Leary 2024). This strongly implies that both the reception of calls and the production of calls by an individual elicit a hormonal response that maintains elevated gonadal activity for a longer period (Wilczynski et al. 2005). This also implies that some minimal chorus size, or threshold amount of calling, is necessary to initiate and sustain sufficient calling effort at breeding locations. We suspect that the Low Chorus treatment met this proposed minimal size requirement but does not far exceed it, resulting in the delayed response in gene activation in comparison to the High Chorus treatment.

5.5.2 Ovarian gene expression

Compared to the testicular transcriptome, there were far fewer DEGs in the ovarian transcriptome. Furthermore, opposed to genes being primarily upregulated in the testicular transcriptome, there was a comparable number of genes that were upregulated and downregulated in ovaries. As a result, there were fewer enhanced GO terms and KEGG pathways when performing pathway analyses. There was also very little overlap in the genes that were upregulated in both the High Chorus x Wind comparison and the Amphiplex x Saline

comparison. The only exceptions were sperm associated antigen 7 (*SPAG7*) and ribosomal protein L21 (*RPL21*). The *SPAG7* gene was initially inferred to affect sperm production and maturation (Beaton et al. 1994), and decreases in expression are associated with male infertility (Abu-Halima et al. 2023). However, this gene is expressed in every tissue and cell type in humans and mice (Noguchi et al. 2017) and is highly expressed in multiple tissues in *Xenopus* frogs (Fu et al. 2021). In primates, Wang et al. (2020) documented groups of genes that are related to each stage of oocyte development in primates and found that *SPAG7* expression was associated with oocytes transitioning from primary to secondary follicles. Knockout of *SPAG7* results in decreased energy expenditure in mice, leading to obesity and glucose intolerance in adulthood, indicating that *SPAG7* has a role in metabolism (Flaherty et al. 2024). RPL21 is a component of the 60S subunit of a ribosome and are thus important for protein synthesis. Expression of *RPL21* is implicated in cell proliferation and cell cycle arrest (Sun et al. 2017; Zhu et al. 2023) and may assist with proper ovarian development (Ma et al. 2024). Upregulation of these two genes may be additional evidence that the reception of conspecific advertisement signals has a role in oocyte development and maintenance of reproductive condition in female frogs during the breeding season (Lea et al. 2001).

It is not entirely clear why such striking evidence for effects of the playback of conspecific calls were observed within the testicular transcriptome but were largely absent within the ovarian transcriptome. In both the Amphiplex and High Chorus treatments there were relatively few DEGs compared to the respective controls (*i.e.*, Saline, Wind) despite known effects of GnRH administration on gamete maturation in frogs (Browne et al. 2006; Trudeau et al. 2013). One possible explanation is that 6 hrs post-injection is not enough time to observe changes in ovarian gene expression. The exact timing of ovulation post-injection of GnRH-

agonist is unknown, but oviposition can take up to 120 hrs in boreal chorus frogs (Ethier and Trudeau, *personal observation*), 24–72 hrs in Panamanian golden frogs, *Atelopus zeteki* (Bronson et al. 2021), approximately 24 hours in three Argentinian frog species, *Ceratophrys ornata*, *Ceratophrys cranwelli,* and *Odontophrynus americanus* (Trudeau et al. 2010), 24–96 hrs in northern leopard frogs, *Lithobates pipiens* (Trudeau et al. 2013), and up to 96 hrs in boreal toads, *Anaxyrus boreas boreas* (Calatayud et al. 2015). It is therefore plausible that multiple days are required to induce ovulation post-injection.

Effect of hormone injection in females

Downregulation of KEGG pathways "apoptosis" and "necroptosis", processes which are critical to maintaining oocyte quality by removing damaged follicles (Chaudhary et al. 2019; Stringer et al. 2023), were observed in the Amphiplex x Saline. Specifically, there was downregulation of *TNFSF10*, a gene associated with regulating cell apoptosis (Kuang et al. 2014), and upregulation of *TRPM7*, which has been implicated in oocyte and embryo development (Carvacho et al. 2016). Together this suggests that apoptosis and/or necroptosis pathways were suppressed in hormone-injected females, which may have roles in the maintenance of ovarian status or continuation of the development of oocytes.

We also observed significant upregulation of *CHSY1* in the hormone-injected females, one of several glycosyltransferases that catalyze the biosynthesis of chondroitin sulfate proteoglycans (CSPGs). Studies in mouse models suggest that CHSY1 regulates cell division and differentiation and has a role in follicle development (Li et al. 2020). We also observed upregulation of *FBXO43*, a gene associated with the regulation of oocyte meiosis, specifically maintenance of metaphase II arrest (Ma et al. 2019), and the post-meiotic process in embryos (Tischer et al. 2012). Furthermore, downregulation of fructose-1,6-bisphosphate aldolase

(*ALDOB*) and the upregulation of TATA-box binding protein like 1 (*TBPL1*) suggests altered cell proliferation and differentiation within the Amphiplex treatment, which may be related to activation of genes necessary for germ cell differentiation (Shimada et al. 2003; Zehavi et al. 2015).

Effect of chorus playback in females

Ovarian *GNRHR2* expression was downregulated in High Chorus compared to Wind. While *GNRHR2* expression has been identified in several female reproductive tissues in mammals, including the ovary, granulosa cells, uterus (Desaulniers et al. 2017), there is limited evidence of ovarian expression in amphibian species. In zebrafish, *gnrhr2* receptors are co-localized with Gnrh (*gnrh2* and *gnrh3*) within early-stage oocytes suggesting an autocrine/paracrine role in oocyte maturation (Corchuelo et al. 2017). Further supporting this, female pigs with reduced GNRHR2 levels via gene knockdown produced fewer oocytes and corpora lutea steroidogenic cells had reduced progesterone concentration which reduced ovulatory rate (Desaulniers et al. 2024). Matrix metalloproteinase 13 (*MMP13*), which is involved in the breakdown of the extracellular matrix, was upregulated in the High Chorus treatment. In chickens, *MMP13* expression tends to decline throughout follicle maturation (Wolak and Hrabia 2020) but expression increases in the granulosa layer but decreases in theca layer in response to gonadotropin injection (Wolak et al. 2021). When tracking matrix metalloproteinases changes during folliculogenesis in domestic cats, *MMP13* mRNA expression peaked in primary follicles and then steeply declined in later stages (Fujihara et al. 2016). In parallel with the enhanced *SPAG7* expression observed in the High Chorus treatment mentioned above, upregulation of *MMP13* may represent the stage in folliculogenesis where primary follicles are transitioning to secondary follicles (Wang et al. 2020). We also observed

downregulation of *WFIKKN1* in the High Chorus treatment. When expressed in the ovary, WFIKKN1 (WAP, follistatin/kazal, immunoglobulin, kunitz and netrin domain containing 1) is a multidomain protein associated with binding of mature growth factors but may have a role in folliculogenesis and ovulation (Harris et al. 2014; Monestier and Blanquet 2016). Taken together, these findings suggest that the exposure to conspecific calls affect the ovulatory cycle of female chorus frogs by potentially increasing cell proliferation and oocyte maturation.

5.5.3 Conclusion

In the current study, we provide evidence that (1) playback of conspecific calls alter testicular gene expression, particularly genes associated with spermatogenesis, and (2) there is significant overlap in differential genes expression patterns induced by playback of conspecific calls and injection of a hormone mixture (*i.e.*, Amphiplex treatment). Exposure to recordings of chorusing behaviour also affected gene pathways related to mitochondrial function and energy production, and these effects are also likely related to steroidogenesis and spermatogenesis. Playback of conspecific calls may also elicit changes in gene expression within the ovary, but the effects are less pronounced than those within the testis after a 6-hr exposure. This aspect requires additional experimentation across a longer time series to fully elucidate. Our results provide strong evidence for the reciprocal interaction between endocrine control of reproduction and acoustic reception of conspecific signals (Wilczynski and Burmeister 2016). Relationships between these regions are likely important for many anurans and possibly other taxa which utilize acoustic communication to coordinate reproductive activities during the breeding season, such as birds (Ball and Dufty 1998; Gall et al. 2021), some fishes (Maruska and Fernald 2011; Maruska and Sisneros 2015), and many mammals (Reby and McComb 2003).

Acoustic communication may also be important in coordinating reproduction for proper fertilization of gametes. In Chapter 4, we exposed groups of 10 spawning chorus frogs (6 males, 4 females) to a broadcast of conspecific calls using the same audio files as the Low Chorus treatment and received the two-dose hormonal induction as the Amphiplex treatment in the current study. After broadcasting for 6 hours/day for 7 days, we collected eggs from groups of spawning frogs and observed that the viability of eggs increased by over 13% compared to a control playback treatment. Tadpoles in the broadcast treatment also had a higher survival to 46 days post-hatch and comparable survival to metamorphosis compared to the control. Combined with the results of the current study, this indicates that reception of acoustic signals have regulatory effects on the hypothalamus-pituitary-gonadal axis, promoting optimal gonadal function leading to positive impacts on gamete maturation and fertility.

Identification of the gene expression pathways and mechanisms that initiate or maintain reproductive condition during the breeding season has ramifications for not only the ecology of amphibians but also the conservation of species. Many amphibian species are declining across the globe, which has led to an increase in the prevalence of captive breeding programs for population reinforcement and reintroduction (Wake and Vredenburg 2008; Grant et al. 2020). However, many amphibian species fail to spawn in captivity or have reduced reproductive output (Clulow et al. 2019). Compared to other vertebrates, relatively little is known in amphibians about the hormones and other physiological processes associated with the final stages of gamete maturation and the coordination of ovulation in females and spermiation in males (Trudeau et al. 2022). Further investigation, using approaches such as transcriptomics, may help address shortcomings and support in developing techniques for assisted reproduction to improve the success of population management and conservation efforts.

Chapter 6: Conclusions and recommendations

6.1 Thesis summary

Acoustic communication is an essential part of anuran ecology and important to consider at all levels of observations; from gene activation within individuals to population ecology of species (Figure 6.1). To our knowledge, our results are the first to provide evidence in an amphibian that social signals can cause rapid molecular changes in the gonads (*i.e.*, differential gene expression), similar to such changes found among teleost fishes (Maruska and Fernald 2011; Maruska et al. 2022). We found that exposure to broadcasts of conspecific chorus behaviour increased the expression of genes associated with steroidogenesis, spermatogenesis, and gonadal development in male chorus frogs within 6–24 hours (Chapter 5). We also observed increased male calling activity after prolonged exposure to playbacks observed during spawning (Chapter 4), strongly implying that there is a positive feedback relationship among reception of calls, testicular activity, and call production in boreal chorus frogs.

In captive individuals, shortly after entering the captive setting, boreal chorus frogs do not display reproductive behaviour (*i.e.*, amplexus, calling) and will not spawn without hormonal induction procedures (Ethier and Trudeau, *personal observation*). The lack of reproductive behaviour is likely related to inadequate environmental or social cues necessary to maintain neuroendocrine processes associated with spawning condition within captivity (Wilczynski et. 2005; Woodley and Leary 2024), increased glucocorticoid production which inhibits the secretion of gonadotropins and sex steroids, or a combination of both (Fischer and Romero 2019). Exposure to conspecific signals induced changes to gonadal gene expression that are broadly similar to gene expression changes produced by co-injection of an agonist of

gonadotropin-releasing hormone and the dopamine antagonist metoclopramide (Chapter 5), indicating conspecific signals induce similar physiological changes as known methods that stimulate the hypothalamic-pituitary-gonadal (HPG) axis and spawning in frogs. Furthermore, our results are the first study to provide evidence in an amphibian that reception of conspecific signals might result in positive impacts on fertility and recruitment. We found that prolonged exposure to broadcasts of chorus behaviour prior to spawning increased egg viability in spawning pairs of boreal chorus frogs and was associated with increased the survival rate of tadpoles 46 days post-hatching (Chapter 4). We provide evidence that confirms previous studies suggesting that the reception of conspecific signals maintains the production of steroidal sex hormones in both males and females throughout the breeding season and extends the period where reproduction can occur (Wilczynski et al. 2005). Our 3-year study monitoring the calling activity of four anuran species (Chapter 3), including boreal chorus frogs, provide evidence that weather events possibly associated with climate change can disrupt calling phenology. Calling activity was advanced in all species during the false spring year but resulted in a decline in overall calling activity in 3 of the 4 species monitored, which could have potential repercussions for recruitment. Our review of the genus (Chapter 2) updated the distribution of the 18 *Pseudacris* species, indicated that broad similarities in life history traits exist among species, and highlighted that population declines of greatest concern occur in eastern North America.

6.2 Evidence for the social facilitation of reproduction

We provide empirical evidence demonstrating that acoustic social interactions among boreal chorus frogs result in stimulation of the HPG-axis and increased gonadal function. This is consistent with our hypothesis that reproduction in anurans is, at least in part, mediated by reception of conspecific acoustic signals. Previous research has established that there is neural

connectivity between brain regions associated with acoustic signal reception and the HPG axis (Arch and Narins 2009) and that prolonged broadcasts of conspecific signals can induce increases in circulating sex steroids (Burmeister and Wilczynski 2000; Chu and Wilczynski 2001; Rodríguez et al. 2022). Our results corroborate these findings and provide strong evidence that reception of conspecific signals can induce rapid physiological responses (within a few hours) within the gonads of both sexes (Chapter 5), which can positively impact egg viability (Chapter 4).

Our results support current theories on the relationship between hormones and reproductive behaviour in amphibians. Calling of male boreal chorus frogs, an androgen-dependent behaviour (Woodley and Leary 2024), increased following prolonged exposure to conspecific signals (Chapter 4). We found that males responded to playbacks of conspecific calls by increasing the duration of calls by 186% and increasing the number of calling bouts by 145% compared to the control group (*i.e.*, ambient sounds recorded at a natural breeding pond). This observation is consistent with the energetics-hormone model (Emerson 2001) which states that calling behaviour is initiated by an increase in circulating androgen levels (*i.e.*, testosterone, dihydrotestosterone), which continue to increase after reception of conspecific signals. In association with the energetics-hormone model, the challenge hypothesis (Wingfield et al. 1990) states that social interactions between competing males, usually associated with aggressive or territorial behaviour, elicit rapid increases in circulating androgen levels. The energetics-hormone model and the challenge hypothesis are rooted in the positive feedback and bidirectional relationship between gonadal sex steroids and acoustic signals, whereby androgens increase reproductive behaviours (*i.e.*, calling) and reception of conspecific signals can lead to increases in androgens (Wilczynski and Burmeister 2016).

Our results provide evidence that reception of advertisement calls, a behaviour primarily produced for mate attraction, can generate similar physiological responses as antagonistic behaviours are predicted to produce by the challenge hypothesis. While we neither directly tested the challenge hypothesis nor measured circulating androgen concentrations in boreal chorus frogs, our testicular gene expression results (Chapter 5) indicate that male-male interactions elicit rapid physiological changes in males consistent with increased androgen production, such as upregulation of genes associated with steroidogenesis. Several notable genes included *STAR* (steroidogenic acute regulatory protein), *Cyp17a1* (cytochrome P450 family 17 subfamily A member 1), and the 14 upregulated genes within the Gene Ontology term "steroid biosynthetic process".

We also provide evidence that conspecific signals maintain gonadal state and receptivity. Genes within the spermatogenesis, steroidogenesis, and gonadal development pathways remained significantly upregulated at all three time points (6 hrs, 24 hrs, 30 hrs) compared to the control treatment. Our ovarian gene expression results (Chapter 5), including the upregulation of the genes *SPAG7* (sperm associated antigen 7) and *RPL21* (ribosomal protein L21), provide evidence that conspecific signals likely positively regulate oocyte development in female boreal chorus frogs. This is consistent with findings in female Majorcan midwife toads (*Alytes muletensis*), where eggs will continue to mature when exposed to conspecific male calls but are reabsorbed if conspecific calls cease or when exposed to heterospecific calls (Lea et al. 2001).

6.3 Conservation considerations and implications

A greater consideration and appreciation for the role of acoustic communication in reproduction is essential for the conservation of anuran amphibians. The first application is that broadcasts of conspecific signals may be used to attract individuals to newly created or restored

habitat. Models produced from our field recordings of the chorusing behaviour allowed us to produce audio files that accurately simulate hourly changes in calling activity of boreal chorus frog and calling phenology of four anuran species throughout the breeding season (Chapter 3). Several taxa, including amphibians, select habitat based on the presence of conspecifics (Buxton et al. 2020) and display a strong phonotactic response to conspecific signals (Wells 1977; Bee 2007). Therefore, broadcasts of chorusing behaviour could be used to attract male and female boreal chorus frogs to restored breeding habitat or locations that have yet to be occupied, as has been indicated in other anuran species (Bee 2007; Buxton et al. 2015; Pizzatto et al. 2016). Evidence from our research strongly implies that these broadcasts can also be used to induce reproductive behaviour and increase fertility (Chapters 4 and 5), especially in captive breeding scenarios where the housing of large numbers of individuals would be unfeasible or impractical and where stress associated with confinement can impair reproductive behaviour (Fischer and Romero 2019). The egg viability of boreal chorus frogs bred in captivity is usually 60–65% with hormonal induction methods (Supplementary Materials; Table S3.4). Inclusion of the conspecific signal broadcast during the breeding period, in addition to hormone injections, further increased egg viability by an average of 13% and often resulted in overall egg viability being > 75% (Chapter 4). This would represent a significant increase in tadpole numbers for a captive breeding and reintroduction program.

Our research also presents a potential conservation concern that is applicable to all declining anuran populations but is currently underappreciated. We observed an overall reduction of calling activity of boreal chorus frogs among years (Chapter 3), representing either a decline in the number of calling individuals within the population or decrease calling effort. The evidence from the study indicates that the reduced calling was related to a “false spring” weather

event. A reduction in calling activity among years is often one of the first indicators that a frog or toad population is declining. While there are limitations to using acoustic monitoring data to infer population trends (Dorcas et al. 2010; McClintock et al. 2010), several studies including our own have used audio surveys to provide evidence of population declines, local extinctions of metapopulations, and disruptions to reproduction behaviour (De Solla et al. 2007; Weir et al. 2009; Seburn et al. 2014). It is unknown if there were any negative impacts to reproduction and recruitment associated with the reduction of calling activity observed in our study. However, since production of advertisement calls during the breeding season is tied to spawning activity in anuran species (Dorcas et al. 2010; Lassandro et al. 2025), there are potential impacts to the population either directly related due to mortality or indirectly due to a reduction in recruitment.

We found that a broadcast simulating a large chorus of approximately 100 individuals caused an upregulation of genes associated with steroidogenesis and gametogenesis at 6 hours post-exposure in male chorus frogs, but a broadcast simulating a small chorus of approximately 10 individuals was ineffective in eliciting a similar gene expression response within the same period (Chapter 5). These findings may be preliminary evidence of a component Allee effect; a positive relationship between individual fitness and population density (Allee 1931; Stephens et al. 1999). As populations decline, there is an increased probability of negative effects to individual fitness accumulating due to reduced density of individuals, ultimately threatening the persistence of the species in the local ecosystem. Allee effects in amphibians are not well documented but are likely to be present (Kramer et al. 2009). Allee effects are predominantly observed in species that occur at low population densities, particularly in those that historically occurred in large densities, and in species that require group coordination of reproduction for survival (Courchamp et al. 2008). Widespread amphibian declines have led to reductions in

population densities and local extinctions in many regions where species were once highly abundant (Wake and Vredenburg 2008). The propensity of anurans to aggregate in high densities during the breeding season where reproduction is socially facilitated through pheromonal or acoustic signals make amphibians prone to Allee effects (Angulo et al. 2018; Woodley and Leary 2024). For example, locations with larger choruses of Houston toads (*Anaxyrus houstonensis*) had a greater probability of reproductive success as mean chorus size at breeding ponds was significantly higher at ponds exhibiting signs of reproduction (*i.e.*, eggs, tadpoles, post-metamorphic individuals) than those with no evidence of reproduction (Gaston et al. 2010). A correlation between calling behaviour and reproductive success may seem intuitive, but the presence of calling individuals does not necessarily equate to successful reproduction (Blaustein et al. 2004). The proportion of ponds with at least one calling male far exceeded the proportion of ponds with evidence of reproduction in the Gaston et al. (2010) study. Female attendance is correlated with chorus size, suggesting larger congregations of males have a greater mate attraction effect (Ryan et al. 1981; Schwartz 1994). The acoustic signals produced by large choruses of calling males may also have more cryptic benefits. Perceived chorus size may influence circulating steroid hormone concentrations and other fertility metrics (*i.e.*, sperm concentration and motility). Reception of conspecific signals that is above certain intensity threshold maybe necessary to optimize reproductive success. If true, broadcasts of conspecific calls used in field settings might compensate for any potential inadequate hormonal stimulation associated with small metapopulations.

6.4 Future directions in research on social facilitation of reproduction

We provide evidence that conspecific calls can enhance fertility (Chapter 4) and alter gonadal function by the expression of increasing genes associated with steroidogenesis and gametogenesis (Chapter 5). We recommend further investigation into the genes associated with the acoustic and hormonal induction groups in our gene expression study. The most promising gene targets for females are *SPAG7* and *RPL21*, as both were significantly upregulated and have previously been associated with oocyte maturation in other vertebrates (Wang et al. 2020; Zhu et al. 2023). Several gene pathways related to gametogenesis and steroidogenesis were enhanced in male chorus frogs, and we recommend that follow up studies focus on the genes within the Gene Ontology terms "steroid biosynthetic process" (GO:0006694), "male gonad development" (GO:0008584), and "spermatogenesis" (GO:0007283).

We also provided evidence that chorus size can influence these physiological responses, as a larger, high-intensity chorus evokes a change in gene expression within 6 hours compared to a small, lower-intensity chorus which required 24 hours to elicit a similar response in gene expression as the large chorus (Chapter 5). However, it is unknown how quickly these physiological responses are initiated and how long they are maintained. Additional time points (< 6 hrs and > 30 hrs) should be included in future acoustic exposure studies. Furthermore, several acoustic properties of male calls could influence female choice and likely impact reproduction in both male and female boreal chorus frogs. However, we focused on call duration and number of calling bouts because these variables are associated with female choice (Schwartz 1994; Sullivan et al. 1995) Females tend to prefer calls produced by males that are lower in frequency, longer in duration, and have a shorter latency between consecutive calls (Gerhardt and Huber 2002; Leary et al. 2021). Sound pressure level, or loudness, is also important for female mate choice, as

females display preference for louder conspecific signals even when given alternatives with more attractive qualities (*i.e.*, lower frequency, shorter latency) broadcasted at lower sound pressure levels (Sullivan et al. 1995). By using recordings of natural choruses of boreal chorus frogs to create audio playback files which were broadcasted at a constant sound pressure level, we aimed to sample a variety of individuals with differences in vocal qualities along the natural spectrum of acoustic properties. It is unknown what proportion of high quality or "attractive" males were featured in these recordings. Future studies should alter acoustic parameters (*i.e.*, frequency, duration, latency) of broadcasts to determine the effect of these metrics on gene expression in receivers.

The response of females to playback in the gene expression experiment was less pronounced than the response of males to the recordings (Chapter 5). This result suggests that the increased egg viability after prolonged exposure to chorus activity (Chapter 4) was due to physiological changes in males that enhanced their fertility. On the other hand, it is important to consider that the ovary sample comparisons in our study were restricted to the first time point (6 hrs). Changes in ovarian gene expression may require more time to be observed. This is somewhat expected as males are required to rapidly respond to other competing males within a breeding location, including adjusting their vocalizations. In contrast, females of some anuran species are known to attend breeding locations with active chorusing activity for several days before spawning activity is observed (Iwai 2018; Buxton et al. 2015). Laboratory studies using hormone injections to stimulate spawning found that males will often begin to call and produce spermic urine within 30–90 mins of receiving a hormone injection (Della Togna et al. 2017; Clulow et al. 2018; Silla et al. 2019). In some species, females oviposit 24–96 hrs post-injection (Calatayud et al. 2015; Bronson et al. 2021), providing further support that endocrine responses

in females occur over a longer time sequence compared to males. Additional timepoints would greatly benefit our understanding whether conspecific calls affect ovarian gene expression and subsequent fertility of oocytes.

Female anurans may require a visual or tactile stimulus in addition to acoustic signal reception to induce hormonal responses, increase receptivity, and trigger oocyte development, similar to observations made with urodeles (Wilczynski and Lynch 2011). Male-female pairs can remain in amplexus for several hours and up to multiple days before oviposition occurs (ref). Amplexus may have a role in synchronizing optimal gamete production and fertility. In some species, males in amplexus have higher plasma gonadotropin concentrations than solitary males (Mendonça et al. 1985; Itoh and Ishii 1990; Ishii and Itoh 1992). The hormonal responses of females appear to be relatively understudied. Female edible frog (*Pelophylax esculentus*) in amplexus had higher estradiol and lower testosterone concentrations than solitary females (Gobbetti and Zerani 1999). Increased gonadal estradiol concentration is associated with vitellogenesis, the production of egg-yolk proteins, which is important for larval development post-fertilization (Trudeau et al. 2022). Future studies should include multiple stimuli, alone and in combination, to further elucidate relationships between social signals and factors associated with gonadal function and fertility.

An alternative explanation is that the observed chorusing activity may be indicative of cooperative behaviour among male chorus frogs. This proposal combines the observations of the rapid changes in key testicular genes expressed in specific pathways (*e.g.*, steroidogenesis and fertility-related), and the relative lack of differential gene expression in ovaries after 6 hrs of exposure of the females to the male conspecific cues (Chapter 5). Prevailing anuran communication theory states that males produce advertisement calls to signal receptivity, size,

position and species identity, and that females then select males based on the attributes of these calls (Huber and Gerhardt 2002; Wells and Schwartz 2007). However, there is growing evidence that female choice and selection on acoustic parameters of male calls (*i.e.*, dominant frequency, pulse rate) is context dependent and influenced by hormonal, social, and environmental factors (Lynch et al. 2006; Cayuela et al. 2017; Caldwell et al. 2022). Additionally, if female choice is strongly linked to calling attributes, one would expect that selection would act on males that have developed methods to suppress calling behaviour of competitors or reduce the "attractiveness" of the competitors calls. Conversely, reception of conspecific calls often increases rates of calling, and has positive effects on sex steroid secretion and gonadal condition (Burmeister and Wilczynski 2000; Chu and Wilczynski 2001; Rodríguez et al. 2022). These effects on steroidogenesis and gonadal function are counterintuitive if males are competing for mates. We suspect that, rather than selection favouring individuals that inhibit calling behaviour of competitors, there is a selective advantage of males that cooperate and coordinate their calling effort to increase the probability of attracting females to breeding ponds. We hypothesize that locations with larger choruses or increased calling activity are associated with increased rates of successful reproduction, similar to what has been observed in Houston toads, *Anaxyrus houstonensis* (Gaston et al. 2010). Further research into the potential cooperation among chorus frog males and Allee effects related to calling activity would greatly benefit our understanding of social facilitation of reproduction in anurans. The relationship between calling activity and reproduction is also largely an assumption and requires empirical testing, potentially with artificial modifications to chorus size or translocation of individuals. This is particularly pertinent to small, remnant populations or locations where metapopulations are isolated, such as the boreal chorus frog metapopulations found in southern Québec.

6.5 Concluding remarks

We postulate that acoustic signals can be used as a tool, one based on the fundamental role of acoustic communication in anuran reproduction, to aid in the captive breeding of frogs and toads. We build upon the hormone and reproductive behaviour literature, providing convincing evidence that reproduction in boreal chorus frogs is mediated by social factors. Broadcasts of conspecific signals increased egg viability during captive breeding trials (Chapter 4) and upregulated genes and gene pathways related to gametogenesis, steroidogenesis, and gonad development (Chapter 5). Our gene activation and fertility results will directly apply to these *Pseudacris* species to benefit both local and national conservation programs as reproductive ecology is consistent among species in the genus (Chapter 2). Our contributions to understanding the role of acoustic communication in reproductive biology will also benefit the amphibian taxon in general as most anuran amphibians rely on acoustic signals to attract mates and coordinate reproduction (Wells and Schwartz 2007). Prompted by a conservation concern of declining amphibian populations observed at the landscape level, we provide meaningful insights into anuran reproduction garnered from gene expression that can be used to improve reproductive outcomes in captive breeding initiatives, increasing the number of individuals that can be reintroduced, and thereby enhancing native populations (Figure 6.1). Our findings also provide a starting point for further discovery in the realm of HPG axis stimulation and regulation of reproduction not just in anurans but all vertebrates that use vocalizations to coordinate breeding.

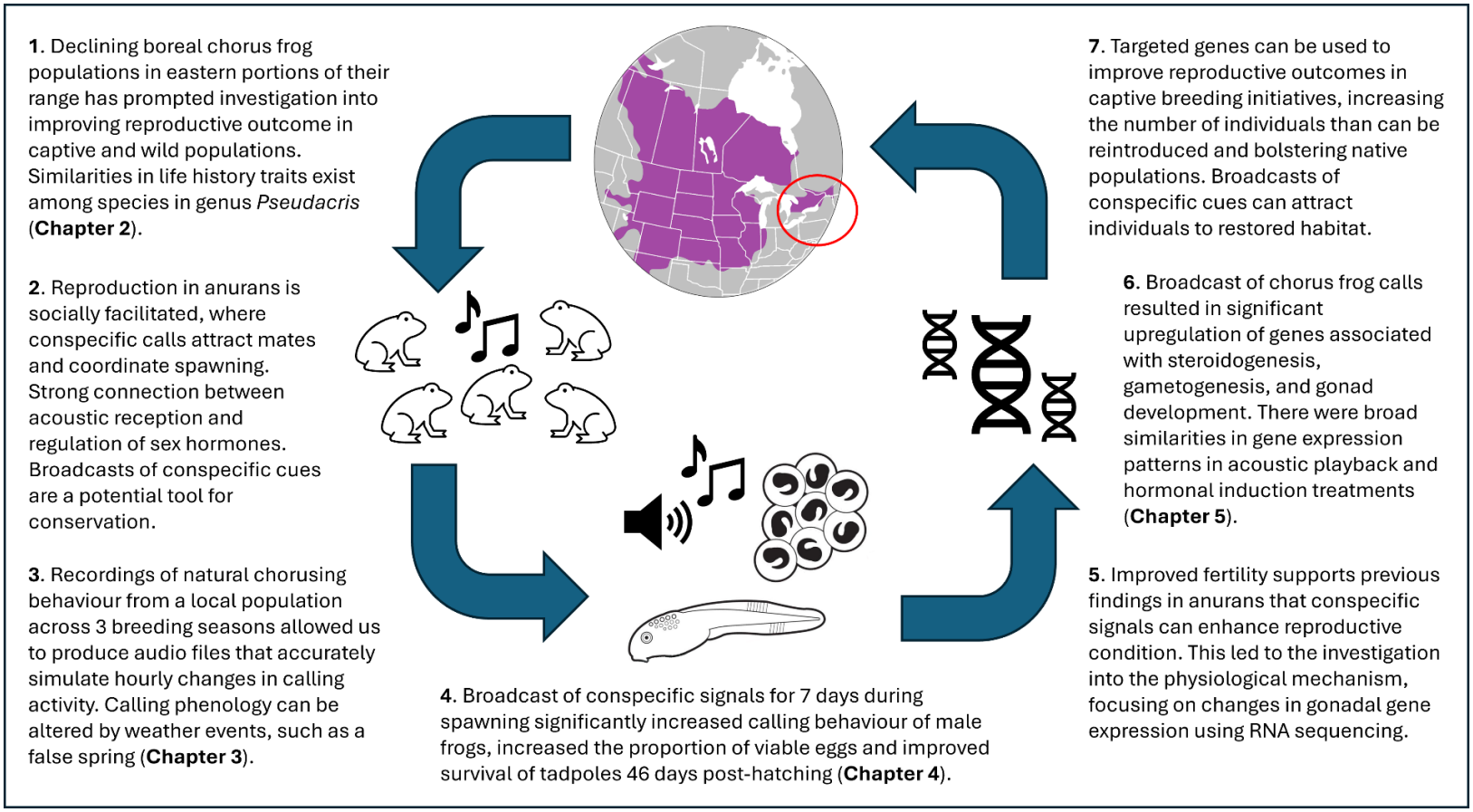

Figure 6.1: Overview of social facilitation of reproduction in captive and wild boreal chorus frogs (Pseudacris maculata). Conservation concern for boreal chorus frog populations in eastern Ontario and southern Québec led to the establishment of a captive breeding and reintroduction program. This novel research indicates that conspecific signals can be broadcasted to induce physiological responses that stimulate the hypothalamic-pituitary-gonadal axis to enhance gonadal function and improve fertility. Investigation using RNA sequencing of testicular and ovarian samples revealed that reception of conspecific signals resulted in the upregulation of genes associated steroidogenesis, gametogenesis, and gonadal development. Similar gonadal gene expression patterns were observed in male and female receiving hormone injections that are proven to induce spawning in anuran amphibians. These results can be used to guide future research in reproduction in amphibians and further improve captive breeding and reintroduction initiatives.

References

Abu-Halima M, Lea Simone Becker LS, Al Smadi MA, Kunz LS, Gröger L, Meese E. 2023. Expression of SPAG7 and its regulatory microRNAs in seminal plasma and seminal plasma-derived extracellular vesicles of patients with subfertility. Scientific Reports 13: 3645. https://doi.org/10.1038/s41598-023-30744-3.

Adams MJ, Miller DAW, Muths E, Corn PS, Grant EHC, Bailey LL, Fellers GM, et al. 2013. Trends in amphibian occupancy in the United States. PLOS ONE 8: e64347. https://doi.org/10.1371/journal.pone.0064347.

Allee WC. 1931. Animal aggregations, a study in general sociology. Chicago (IL): University of Chicago Press. https://doi.org/10.5962/bhl.title.7313.

Allison JD, Wilczynski W. 1991. Thalamic and midbrain auditory projections to the preoptic area and ventral hypothalamus in the green treefrog (*Hyla cinerea*). Brain, Behavior and Evolution 38: 322–331. https://doi.org/10.1159/000114398.

Amburgey S, Funk WC, Murphy M, Muths E. 2012. Effects of hydroperiod duration on survival, developmental rate, and size at metamorphosis in boreal chorus frog tadpoles (*Pseudacris maculata*). Herpetologica 68: 456–467. https://doi.org/10.1655/HERPETOLOGICA-D-11-00093.

Amorim MCP. 2023. The role of acoustic signals in fish reproduction. The Journal of the Acoustical Society of America 154: 2959–2973. https://doi.org/10.1121/10.0022353.

Anderson SR, Wiens JJ. 2017. Out of the dark: 350 million years of conservatism and evolution in diel activity patterns in vertebrates. Evolution 71: 1944–1959. https://doi.org/ https://doi.org/10.1111/evo.13284.

Angulo E, Luque GM, Gregory SD, Wenzel JW, Bessa-Gomes C, Berec L, Courchamp F. 2018. Review: Allee effects in social species. Journal of Animal Ecology 87: 47–58. https://doi.org/10.1111/1365-2656.12759.

Arch VS, Narins PM. 2009. Sexual hearing: the influence of sex hormones on acoustic communication in frogs. Hearing Research 252: 15–20. https://doi.org/10.1016/j.heares.2009.01.001.

Assis LHC, Crespo D, Morais RDVS, França LR, Bogerd J, Schulz RW. 2016. INSL3 stimulates spermatogonial differentiation in testis of adult zebrafish (*Danio rerio*). Cell and Tissue Research 363: 579–588. https://doi.org/10.1007/s00441-015-2213-9.

Baker PJ, Hughes R, Hughes J. 2024. Onset of early spring activity in amphibians in Ithica, New York, USA. Herpetological Conservation and Biology 19:466–474.

Ball GF, Dufty AM. 1998. Assessing hormonal responses to acoustic stimulation. In: Hopp SL, Owren MJ, Evans CS, editors. Animal acoustic communication. Berlin, Heidelberg: Springer. p. 379–398. https://doi.org/10.1007/978-3-642-76220-8_12.

Barber JR, Crooks KR, Fristrup KM. 2010. The costs of chronic noise exposure for terrestrial organisms. Trends in Ecology & Evolution 25: 180–189. https://doi.org/10.1016/j.tree.2009.08.002.

Bates D, Mächler M, Bolker B, Walker S. 2015. Fitting linear mixed-effects models using lme4. Journal of Statistical Software 67: 1–48. https://doi.org/10.18637/jss.v067.i01.

Beaton S, Cleary A, Ten HJ, Bradley MP. 1994. Cloning and characterization of a fox sperm protein FSA-1. Reproduction, Fertility and Development 6: 761–770. https://doi.org/10.1071/rd9940761.

Bee MA. 2007. Selective phonotaxis by male wood frogs (*Rana sylvatica*) to the sound of a chorus. Behavioral Ecology and Sociobiology 61: 955–966. https://doi.org/10.1007/s00265-006-0324-8.

Beebee TJC. 1995. Amphibian breeding and climate. Nature 374: 219–220. https://doi.org/10.1038/374219a0.

Beebee TJC, Griffiths RA. 2005. The amphibian decline crisis: a watershed for conservation biology? Biological Conservation 125: 271–285. https://doi.org/10.1016/j.biocon.2005.04.009.

Bell A. 2016. The remarkable frog ear: implications for vertebrate hearing. Journal of Hearing Science 6: 17–30. https://doi.org/10.17430/897793.

Benard MF, Greenwald KR. 2023. Environmental drivers of amphibian breeding phenology across multiple sites. Diversity 15: 253. https://doi.org/10.3390/d15020253.

Benard MF. 2015. Warmer winters reduce frog fecundity and shift breeding phenology, which consequently alters larval development and metamorphic timing. Global Change Biology 21: 1058–1065. https:/doi.org/10.1111/gcb.12720.

Benjamini Y, Hochberg Y. 1995. Controlling the false discovery rate: a practical and powerful approach to multiple testing. Journal of the Royal Statistical Society: Series B (Methodological) 57: 289–300. https://doi.org/10.1111/j.2517-6161.1995.tb02031.x.

Bishop CA, Pettit KE, editors. 1992. Declines in Canadian Amphibian Populations: Designing a National Monitoring Strategy: Proceedings of a Symposium Sponsored by the Canadian Wildlife Service (Ontario Region) and the Metropolitan Toronto Zoo, held in Burlington, Ontario, 5–6 October 1991; Published for the Workshop Sponsors by the Canadian Wildlife Service. Occasional Paper / Canadian Wildlife Service 76. Ottawa: Minister of Supply and Services.

Bison M, Yoccoz NG, Carlson BZ, Klein G, Laigle I, Van Reeth C, Delestrade A. 2021. Earlier snowmelt advances breeding phenology of the common frog (*Rana temporaria*) but increases the risk of frost exposure and wetland drying. Frontiers in Ecology and Evolution 9: 645585. https://doi.org/10.3389/fevo.2021.645585.

Blaustein AR, Han BA, Relyea RA, Johnson PTJ, Buck JC, Gervasi SS, Kats LB. 2011. The complexity of amphibian population declines: understanding the role of cofactors in driving amphibian losses. Annals of the New York Academy of Sciences 1223: 108–119. https://doi.org/10.1111/j.1749-6632.2010.05909.x.

Blaustein AR, Han B, Fasy B, Romansic J, Scheessele EA, Anthony RG, Marco A, Chivers DP, Belden LK, Kiesecker JM, Garcia T, Lizana M, Kats LB. 2004. Variable breeding phenology affects the exposure of amphibian embryos to ultraviolet radiation AND optical characteristics of natural waters protect amphibians from UV-B in the U.S. Pacific northwest: comment. Ecology 85: 1747–1754. https://doi.org/10.1890/03-3070.

Blaustein AR, Wake DB, Sousa WP. 1994. Amphibian declines: judging stability, persistence, and susceptibility of populations to local and global extinctions. Conservation Biology 8: 60–71. http://doi.org/10.1046/j.1523-1739.1994.08010060.x.

Blaustein AR, Romansic JM, Kiesecker JM, Hatch AC. 2003. Ultraviolet Radiation, Toxic chemicals and amphibian population declines. Diversity and Distributions 9: 123–140. https://doi.org/10.1046/j.1472-4642.2003.00015.x.

Blaustein AR, Walls SC, Bancroft BA, Lawler JJ, Searle CL, Gervasi SS. 2010. Direct and indirect effects of climate change on amphibian populations. Diversity 2: 281–313. https://doi.org/10.3390/d2020281.

Bloxam QMC, Tonge SJ. 1995. Amphibians: suitable candidates for breeding-release programmes. Biodiversity and Conservation 4: 636–644. https://doi.org/10.1007/BF00222519.

Bogart JP, Taylor EB, Boles R. 2015. Western chorus frog (*Pseudacris triseriata*) and Boreal chorus frog (P. maculata): clarification concerning the wildlife species listed under SARA in light of recent taxonomic interpretations. Species at Risk Public Registry, Environment and Climate Change Canada.

Bonin J, Galois P. 1996. Rapport sur la situation de la rainette faux-grillon (*Pseudacris triseriata*) au Québec. Ministère de l'Environnement et de la Faune, Direction de la faune et des habitats, Québec, 39 pp.

Bosch J, Márquez R. 1996. Acoustic competition in male midwife toads *Alytes obstetricans* and *Alytes cisternasii*: response to neighbor size and calling rate. implications for female choice. Ethology 102: 841–855. https://doi.org/10.1111/j.1439-0310.1996.tb01205.x.

Boyd SK. 1997. Brain vasotocin pathways and the control of sexual behaviors in the bullfrog. Brain Research Bulletin 44: 345–350. https://doi.org/10.1016/s0361-9230(97)00213-x

Bridges AS, Dorcas ME. 2000. Temporal variation in anuran calling behavior: Implications for surveys and monitoring programs. Copeia 2000: 587–592. http://doi.org/10.1643/0045-8511(2000)000[0587:TVIACB]2.0.CO;2

Brinley Buckley EM, Gottesman BL, Caven AJ, Harner MJ, Pijanowski BC. 2021. Assessing ecological and environmental influences on boreal chorus frog (*Pseudacris maculata*) spring calling phenology using multimodal passive monitoring technologies. Ecological Indicators 121: 107171. https://doi.org/10.1016/j.ecolind.2020.107171.

Brischigliaro M, Zeviani M. 2021. Cytochrome c Oxidase Deficiency. Biochimica et Biophysica Acta (BBA) - Bioenergetics 1862: 148335. https://doi.org/10.1016/j.bbabio.2020.148335.

Brodie JF, Williams S, Garner B. 2021. The Decline of Mammal Functional and Evolutionary Diversity Worldwide. Proceedings of the National Academy of Sciences 118: e1921849118. https://doi.org/10.1073/pnas.1921849118.

Bronson E, Guy EL, Murphy KJ, Barrett K, Kouba AJ, Poole V, Kouba CK. 2021. Influence of oviposition-inducing hormone on spawning and mortality in the endangered Panamanian golden frog (*Atelopus zeteki*). BMC Zoology 6: 17. https://doi.org/10.1186/s40850-021-00076-8.

Brooke PN, Alford RA, Schwarzkopf L. 2000. Environmental and social factors influence chorusing behaviour in a tropical frog: examining various temporal and spatial scales. Behavioral Ecology and Sociobiology 49: 79–87. https://doi.org/10.1007/s002650000256.

Browne RK, Kaurova SA, Uteshev VK, Shishova NV, McGinnity D, Figiel CR, Mansour N, Agnew D, Wu M, Gakhova EN, Dzyuba B, Cosson J. 2015. Sperm motility of externally fertilizing fish and amphibians. Theriogenology 83: 1–13.e8. https://doi.org/10.1016/j.theriogenology.2014.09.018.

Browne RK, Seratt J, Vance C, Kouba A. 2006. Hormonal priming, induction of ovulation and in-vitro fertilization of the endangered Wyoming toad (*Bufo baxteri*). Reproductive Biology and Endocrinology 4: 34. https://doi.org/10.1186/1477-7827-4-34.

Brzoska J, Obert H-J. 1980. Acoustic signals influencing the hormone production of the testes in the grass frog. Journal of Comparative Physiology 140: 25–29. https://doi.org/10.1007/BF00613744.

Bucciarelli GM, Blaustein AR, Garcia TS, Kats LB. 2014. Invasion complexities: the diverse impacts of nonnative species on amphibians. Copeia 2014: 611–632. https://doi.org/10.1643/OT-14-014.

Buchfink B, Xie C, Huson DH. 2015. Fast and sensitive protein alignment using DIAMOND. Nature Methods 12: 59–60. https://doi.org/10.1038/nmeth.3176.

Burmeister SS, Wilczynski W. 2000. Social signals influence hormones independently of calling behavior in the treefrog (*Hyla cinerea*). Hormones and Behavior 38: 201–209. https://doi.org/10.1006/hbeh.2000.1605.

Burmeister SS, Wilczynski W. 2005. Social signals regulate gonadotropin-releasing hormone neurons in the green treefrog. Brain, Behavior and Evolution 65: 26–32. https://doi.org/10.1159/000081108.

Burmeister SS, Somes C, Wilczynski W. 2001. Behavioral and hormonal effects of exogenous vasotocin and corticosterone in the green treefrog. General and Comparative Endocrinology 122: 189–197. https://doi.org/10.1006/gcen.2001.7625.

Burmeister SS, Mangiamele LA, Lebonville CL. 2008. Acoustic modulation of immediate early gene expression in the auditory midbrain of female túngara frogs. Brain Research 1190: 105–114. https://doi.org/10.1016/j.brainres.2007.11.008.

Burris-Hiday SD, Scott EE. 2021. Steroidogenic cytochrome P450 17A1 structure and function. Molecular and Cellular Endocrinology 528: 111261. https://doi.org/10.1016/j.mce.2021.111261.

Butchart SHM, Walpole M, Collen B, van Strien A, Scharlemann JPW, Almond REA, Baillie JEM, Bomhard B, Brown C, Bruno J, et al. 2010. Global biodiversity: indicators of recent declines. Science 328: 1164–1168. https://doi.org/10.1126/science.1187512.

Buxton VL, Enos JK, Sperry JH, Ward MP. 2020. A review of conspecific attraction for habitat selection across taxa. Ecology and Evolution 10: 12690–12699. https://doi.org/10.1002/ece3.6922.

Buxton VL, Ward MP, Sperry JH. 2015. Use of chorus sounds for location of breeding habitat in 2 species of anuran amphibians. Behavioral Ecology 26: 1111–1118. https://doi.org/10.1093/beheco/arv059.

Byrne PG, Silla AJ. 2010. Hormonal induction of gamete release, and in-vitro fertilisation, in the critically endangered southern corroboree frog, *Pseudophryne corroboree*. Reproductive Biology and Endocrinology 8: 144. https://doi.org/10.1186/1477-7827-8-144.

Cabrita E, Martínez-Páramo S, Gavaia PJ, Riesco MF, Valcarce DG, Sarasquete C, Herráez MP, Robles V. 2014. Factors enhancing fish sperm quality and emerging tools for sperm analysis. Aquaculture 432: 389–401. https://doi.org/10.1016/j.aquaculture.2014.04.034.

Calatayud NE, Langhorne CJ, Mullen AC, Williams CL, Smith T, Bullock L, Kouba AJ, Willard ST. 2015. A hormone priming regimen and hibernation affect oviposition in the boreal toad (*Anaxyrus boreas boreas*). Theriogenology 84: 600–607. https://doi.org/10.1016/j.theriogenology.2015.04.017.

Caldwell MS, Britt KA, Mischke LC, Collins HI. 2022. Beyond sound: bimodal acoustic calls used in mate-choice and aggression by red-eyed treefrogs. Journal of Experimental Biology 225: jeb244460. https://doi.org /10.1242/jeb.244460.

Callaghan CT, Rowley JJL. 2020. A continental assessment of diurnality in frog calling behaviour. Austral Ecology 46: 65–71. https://doi.org/10.1111/aec.12959.

Canadian Endangered Species Conservation Council. 2022. Wild Species 2020: The General Status of Species in Canada. National General Status Working Group: 172 pp.

Canadian Herpetological Society. 2025. Amphibian and Reptile Conservation. Accessed March 2025 from: https://canadianherpetology.ca/conservation/index.html

Canavero A, Arim M. 2009. Clues supporting photoperiod as the main determinant of seasonal variation in amphibian activity. Journal of Natural History 43: 2975–2984. https://doi.org/10.1080/00222930903377539.

Carr JA. 2024. Stress and Reproduction in Amphibians. In: DO, Lopez KH, editors. Hormones and Reproduction of Vertebrates, Volume 2 (Second Edition). New York (NY): Academic Press. p. 121–50. https://doi.org/10.1016/B978-0-443-16020-2.00002-4.

Carrageta DF, Bernardino RL, Soveral G, Calamita G, Alves MG, Oliveira PF. 2020. Aquaporins and male (in)fertility: expression and role throughout the male reproductive tract. Archives of Biochemistry and Biophysics 679: 108222. https://doi.org/10.1016/j.abb.2019.108222.

Carvacho I, Ardestani G, Lee HC, McGarvey K, Fissore RA, Lykke-Hartmann K. 2016. TRPM7-like channels are functionally expressed in oocytes and modulate post-fertilization embryo development in mouse. Scientific Reports 6: 34236. https://doi.org/10.1038/srep34236.

Cayuela H, Lengagne T, Joly P, Léna JP. 2017. Females trade off the uncertainty of breeding resource suitability with male quality during mate choice in an anuran. Animal Behaviour 123: 179–185. https://doi.org/10.1016/j.anbehav.2016.11.002.

Ceballos G, Ehrlich PR, Barnosky AD, Garía A, Pringle RM, Palmer TM. 2015. Accelerated modern human–induced species losses: Entering the sixth mass extinction. Science Advances 1: e1400253. https://doi.org/10.1126/sciadv.1400253.

Chaudhary GR, Yadav PK, Yadav AK, Tiwari M, Gupta A, Sharma A, Sahu K, Pandey AN, Pandey AK, Chaube SK. 2019. Necrosis and necroptosis in germ cell depletion from mammalian ovary. Journal of Cellular Physiology 234: 8019–8027. https://doi.org/10.1002/jcp.27562.

Chen Y, Lougheed DR, Sun Z, Ethier JP, Trudeau VL, Lougheed SC. Insights on macrosynteny, ‘rebel’ genes, and a new sex-linked region in anurans from comparative genomics and a new chromosome-level genome for the western chorus frog. [online]. BioRxiv. https://doi.org/10.1101/2024.10.27.620512.

Cheron M, Angelier F, Ribout C, Brischoux F. 2021. Clutch quality is related to embryonic development duration, hatchling body size and telomere length in the spined toad (*Bufo spinosus*). Biological Journal of the Linnean Society 133: 135–142. https://doi.org/10.1093/biolinnean/blab035.

Cheung-Flynn J, Prapapanich V, Cox MB, Riggs DL, Suarez-Quian C, Smith DF. 2005. Physiological role for the cochaperone FKBP52 in androgen receptor signaling. Molecular Endocrinology 19: 1654–1666. https://doi.org/10.1210/me.2005-0071.

Chu J, Marler CA, Wilczynski W. 1998. The effects of arginine vasotocin on the calling behavior of male cricket frogs in changing social contexts. Hormones and Behavior 34: 248–261. https://doi.org/10.1006/hbeh.1998.1479.

Chu J, Wilczynski W. 2001. Social influences on androgen levels in the southern leopard frog, *Rana sphenocephala*. General and Comparative Endocrinology 121: 66–73. https://doi.org/10.1006/gcen.2000.7563.

Clark BJ, Stocco DM. 2014. The Steroidogenic Acute Regulatory Protein (StAR). In: Clark BJ, Stocco DM, editors. Cholesterol transporters of the START domain protein family in health and disease. New York (NY): Springer. p. 15–47. https://doi.org/10.1007/978-1-4939-1112-7_2.

Clark FE, Dunn JC. 2022. From soundwave to soundscape: a guide to acoustic research in captive animal environments. Frontiers in Veterinary Science 9: 889117. https://doi.org/10.3389/fvets.2022.889117.

Clulow J, Pomering M, Herbert D, Upton R, Calatayud N, Clulow S, Mahony MJ, Trudeau VL. 2018. Differential success in obtaining gametes between male and female Australian temperate frogs by hormonal induction: a review. General and Comparative Endocrinology 265: 141–148. https://doi.org/10.1016/j.ygcen.2018.05.032.

Clulow J, Trudeau VL, Kouba AJ. 2014. Amphibian declines in the twenty-first century: why we need assisted reproductive technologies. In: Holt WV, Brown JL, Comizzoli P, editors. Reproductive Sciences in Animal Conservation. Advances in Experimental Medicine and Biology 753. New York (NY): Springer New York. p. 275–316. https://doi.org/10.1007/978-1-4939-0820-2_12.

Clulow J, Upton R, Trudeau VL, Clulow S. 2019. Amphibian Assisted Reproductive Technologies: Moving from Technology to Application. In: Comizzoli P, Brown JL, Holt WV, editors. Reproductive Sciences in Animal Conservation. Cham: Springer International Publishing. p. 413–63. https://doi.org/10.1007/978-3-030-23633-5_14.

Clulow S, Clulow J, Marcec-Greaves R, Della Togna G, Calatayud NE, Yuan Y. 2022. Common goals, different stages: the state of the arts for reptile and amphibian conservation. Reproduction, Fertility and Development 34: i–ix. https://doi.org/10.1071/RDv34n5_FO.

Coddington E, Moore FL. 2003. Neuroendocrinology of context-dependent stress responses: vasotocin alters the effect of corticosterone on amphibian behaviors. Hormones and Behavior 43: 222–228. https://doi.org/10.1016/S0018-506X(02)00015-6.

Cohen J. 1988. Statistical power analysis for the behavioral sciences (2nd ed.). New York: Academic Press.

Committee on the Status of Endangered Wildlife in Canada (COSEWIC). 2008. COSEWIC Assessment and Update Status Report on the Western Chorus Frog *Pseudacris triseriata* in Canada – Carolinian population – Great Lakes / St. Lawrence – Canadian Shield population. Committee on the Status of Endangered Wildlife in Canada. Ottawa. vii + 54 p.

Conant R, Collins JT. 1998. A Field Guide to Reptiles & Amphibians: Eastern and Central North America. Boston: Houghton Mifflin Harcourt.

Corchuelo S, Martinez ERM, Butzge AJ, Doretto LB, Ricci JMB, Valentin FN, Nakaghi LSO, Somoza GM, Nóbrega RH. 2017. Characterization of GnRH/GnIH elements in the olfacto-retinal system and ovary during zebrafish ovarian maturation. Molecular and Cellular Endocrinology 450: 1–13. https://doi.org/10.1016/j.mce.2017.04.002

Corn PS. 2005. Climate change and amphibians. Animal Biodiversity and Conservation 28: 59–67. https://doi.org/10.32800/abc.2005.28.0059.

Corser JD, Roblee KJ, Johnson G. 2012. Shifting status and distribution of range margin chorus frog (*Pseudacris*) populations in eastern Great Lakes watersheds. Journal of Great Lakes Research 38: 806–811. https://doi.org/10.1016/j.jglr.2012.09.005.

Courchamp F, Berec L, Gascoigne J. 2008. What are Allee effects? In: Courchamp F, Berec L, Gascoigne J, editors. Allee Effects in Ecology and Conservation. Oxford University Press. https://doi.org/10.1093/acprof:oso/9780198570301.003.0001.

Crocker-Buta SP, Leary CJ. 2018. Bidirectionality of hormone-behavior relationships and satellite-caller dynamics in green treefrogs. Behavioral Ecology 2: 948–957. https://doi.org/10.1093/beheco/ary047.

Crocker-Buta SP, Leary CJ. 2018. Hormonal and social correlates of courtship signal quality and behaviour in male green treefrogs. Animal Behaviour 146:13–22. https://doi.org/10.1016/j.anbehav.2018.10.001.

Culty M, Liu Y, Manku G, Chan W-Y, Papadopoulos V. 2015. Expression of steroidogenesis-related genes in murine male germ cells. Steroids 103: 105–114. https://doi.org/10.1016/j.steroids.2015.08.011.

Cunnington GM, Fahrig L. 2010. Plasticity in the vocalizations of anurans in response to traffic noise. Acta Oecologica 36: 463–470. https://doi.org/10.1016/j.actao.2010.06.002

Cushman SA. 2006. Effects of habitat loss and fragmentation on amphibians: a review and prospectus. Biological Conservation 128: 231–240. https://doi.org/10.1016/j.biocon.2005.09.031.

Daszak P, Scott DE, Kilpatrick AM, Faggioni C, Gibbons JW, Porter D. 2005. Amphibian population declines at savannah river site are linked to climate, not chytridiomycosis. Ecology 86: 3232–3237. https://doi.org/10.1890/05-0598.

Davis CL, Miller DAW, Walls SC, Barichvich WJ, Riley JW, Brown ME. 2017. Species interactions and the effects of climate variability on a wetland amphibian metacommunity. Ecological Applications 27: 285–296. https://doi.org/10.1002/eap.1442.

De França Santos M, De Menezes Galvão AC, Santos Silva F, Dos Santos Silva E, De Sousa G, Lobão-Soares B, Gonçalves Ferreira R, De Sousa MB, Galvão-Coelho NL. 2022. Welfare improvement by enrichment programs in common marmoset females under social isolation. Journal of Applied Animal Welfare Science 25: 297–309. https://doi.org/10.1080/10888705.2021.1968863.

De Solla S, Fernie K, Barrett G, Bishop C. 2007. Population trends and calling phenology of anuran populations surveyed in Ontario estimated using acoustic surveys. Biodiversity and Conservation 15: 113–129. https://doi.org/10.1007/978-1-4020-5734-2_9.

De S. 2021. The 14-3-3 (YWHA) proteins in mammalian reproduction. International Annals of Science 10: 52–59. https://doi.org/10.21467/ias.10.1.52-59.

Della Togna G, Howell LG, Clulow J, Langhorne CJ, Marcec-Greaves R, Calatayud NE. 2020. Evaluating amphibian biobanking and reproduction for captive breeding programs according to the amphibian conservation action plan objectives. Theriogenology 150: 412–431. https://doi.org/10.1016/j.theriogenology.2020.02.024.

Della Togna G, Trudeau VL, Gratwicke B, Evans M, Augustine L, Chia H, Bronikowski EJ, Murphy JB, Comizzoli P. 2017. effects of hormonal stimulation on the concentration and quality of excreted spermatozoa in the critically endangered Panamanian golden frog (*Atelopus zeteki*). Theriogenology 91: 27–35. https://doi.org/10.1016/j.theriogenology.2016.12.033.

Deng C-Y, Lv M, Luo B-H, Zhao S-Z, Mo Z-C, Xie Y-J. 2021. The role of the PI3K/AKT/mTOR signalling pathway in male reproduction. Current Molecular Medicine 21: 539–548. https://doi.org/10.2174/1566524020666201203164910.

Deng J, Carbajal L, Evaul K, Rasar M, Jamnongjit M, Hammes SR. 2009. Nongenomic steroid-triggered oocyte maturation: of mice and frogs. Steroids 74: 595–601. https://doi.org/10.1016/j.steroids.2008.11.010.

Denver RJ. 2013. Neuroendocrinology of amphibian metamorphosis. Current Topics in Developmental Biology 103: 195–227. https://doi.org/10.1016/B978-0-12-385979-2.00007-1.

Desaulniers AT, Cederberg RA, Lents CA, White BR. 2017. Expression and role of gonadotropin-releasing hormone 2 and its receptor in mammals. Frontiers in Endocrinology 8: 269. https://doi.org/10.3389/fendo.2017.00269.

Desaulniers AT, Cederberg RA, Lents CA, White BR. 2024. Knockdown of gonadotropin-releasing hormone ii receptor impairs ovulation rate, corpus luteum development, and progesterone production in gilts. Animals 14: 2350. https://doi.org/10.3390/ani14162350.

Desroches J-F, Rodrigue D. 2004. Amphibiens et reptiles du Québec et des Maritimes. Montréal (QC): Editions Michel Quintin.

Deutsch CA., Tewksbury JJ, Huey RB, Sheldon KS, Ghalambor CK, Haak DC, Martin PR. 2008. Impacts of climate warming on terrestrial ectotherms across latitude. Proceedings of the National Academy of Sciences 105: 6668–6672. https://doi.org/10.1073/pnas.0709472105.

Dinsmore SC, Swanson DL. 2008. Temporal Patterns of Tissue Glycogen, Glucose, and Glycogen Phosphorylase Activity Prior to Hibernation in Freeze-Tolerant Chorus Frogs, *Pseudacris triseriata*. Canadian Journal of Zoology 86: 1095–1100. https://doi.org/10.1139/Z08-088.

Dodd CK. 2023. Frogs of the United States and Canada. Baltimore (MD): Johns Hopkins University Press.

Dorcas ME, Price SJ, Walls SC, Barichivich WJ. 2010. Auditory monitoring of anuran populations. In: Kenneth Dodd K, editor. Amphibian ecology and conservation: a handbook of techniques. Oxford University Press, New York, NY. p. 281–298.

Duellman WE, Trueb L. 1994. Biology of Amphibians. Baltimore (MD): Johns Hopkins University Press.

Dufour S, Sebert M-E, Weltzien F-A, Rousseau K, Pasqualini C. 2010. Neuroendocrine control by dopamine of teleost reproduction. Journal of Fish Biology 76: 129–160. https://doi.org/10.1111/j.1095-8649.2009.02499.x.

Dun MD, Smith ND, Baker MA, Lin M, Aitken RJ, Nixon B. 2011. The chaperonin containing TCP1 complex (CCT/TRiC) is involved in mediating sperm-oocyte interaction. Journal of Biological Chemistry 286: 36875–36887. https://doi.org/10.1074/jbc.M110.188888.

Dun MD, Aitken RJ, Nixon B. 2012. The role of molecular chaperones in spermatogenesis and the post-testicular maturation of mammalian spermatozoa. Human Reproduction Update 18: 420–435. https://doi.org/10.1093/humupd/dms009.

Earl JE, Cohagen KE, Semlitsch RD. 2012. Effects of leachate from tree leaves and grass litter on tadpoles. Environmental Toxicology and Chemistry 31: 1511–1517. https://doi.org/10.1002/etc.1829.

Environment and Climate Change Canada (ECCC). 2013. Management Plan for the Northern Leopard Frog (*Lithobates pipiens*), Western Boreal/Prairie Populations, in Canada. Species at Risk Act Management Plan Series. Environment Canada, Ottawa. iii + 28 pp.

ECCC. 2015a. Recovery Strategy for the Oregon Spotted Frog (*Rana pretiosa*) in Canada. Species at Risk Act Recovery Strategy Series. Environment Canada, Ottawa. 21 pp. + Annex.

ECCC. 2015b. Recovery Strategy for the Western Chorus Frog (*Pseudacris triseriata*), Great Lakes / St. Lawrence – Canadian Shield population, in Canada, Species at Risk Act Recovery Strategy Series, Environment Canada, Ottawa, vi + 50 pp.

ECCC. 2017. Recovery Strategy for the Northern Leopard Frog (*Lithobates pipiens*), Rocky Mountain population in Canada. Species at Risk Act Recovery Strategy Series. Environment and Climate Change Canada, Ottawa. 2 parts, 24 pp. + 47 pp.

ECCC. 2021. Threat assessment for the Western chorus frog (*Pseudacris triseriata*), Great Lakes / St. Lawrence River – Canadian Shield population: Boisé du Tremblay metapopulation, Longueuil, Quebec.

ECCC. 2025. Species at risk public registry. [online]. Accessed March 2025 from: https://www.canada.ca/en/environment-climate-change/services/species-risk-public-registry.html.

Eisa A, Dey S, Ignatious A, Nofal W, Hess RA, Kurokawa M, Kline D, Vijayaraghavan S. 2021. The protein YWHAE (14-3-3 epsilon) in spermatozoa is essential for male fertility. Andrology 9: 312–328. https://doi.org/10.1111/andr.12865.

Emerson SB. 2001. Male advertisement calls: behavioral variation and physiological processes. In: Ryan MJ, editor. Anuran Communication. Washington (D.C.): Smithsonian Institution Press. p. 36–44.

Endepols H, Feng AS, Gerhardt HC, Schul J, Walkowiak W. 2003. Roles of the auditory midbrain and thalamus in selective phonotaxis in female gray treefrogs (*Hyla versicolor*). Behavioural Brain Research 145: 63–77. https://doi.org/10.1016/S0166-4328(03)00098-6.

Environmental Conservation Online System. 2025. FWS-Listed U.S. Species by Taxonomic Group - All Animals. [online]. Accessed May 2025 from: https://ecos.fws.gov/ecp/report/species-listings-by-tax-group?statusCategory=Listed&groupName=All%20Animals

Erdtmann LK, Lima AP. 2013. Environmental effects on anuran call design: what we know and what we need to know. Ethology, Ecology & Evolution 25: 1–11. https://doi.org/10.1080/03949370.2012.744356.

Ethier JP, Fayard A, Soroye P, Choi D, Mazerolle MJ, Trudeau VL. 2021. Life history traits and reproductive ecology of North American chorus frogs of the genus *Pseudacris* (Hylidae). Frontiers in Zoology 18: 40. https://doi.org/10.1186/s12983-021-00425-w.

Ethier JP, Worth M, Mazerolle MJ, Trudeau VL. 2024. Rearing density and food variety impact growth, development, and survival of larvae in the declining amphibian, Pseudacris maculata. Zoo Biology 43: 416–424. https://doi-org /10.1002/zoo.21848.

Evaul K, Jamnongjit M, Bhagavath B, Hammes SR. 2007. Testosterone and progesterone rapidly attenuate plasma membrane Gβγ-mediated signaling in *Xenopus laevis* oocytes by signaling through classical steroid receptors. Molecular Endocrinology 21: 186–96. https://doi.org/10.1210/me.2006-0301.

Facondo P, Delbarba A, Maffezzoni F, Cappelli C, Ferlin A. 2020. INSL3: a marker of Leydig cell function and testis-bone-skeletal muscle network. Protein and Peptide Letters 27: 1246–1252. https://doi.org/10.2174/0929866527666200925105739.

Falaschi M, Melotto A, Manenti R, Ficetola GF. 2020. Invasive species and amphibian conservation. Herpetologica 76: 216–227. https://doi.org/10.1655/0018-0831-76.2.216.

Farquharson KA, Hogg CJ, Grueber CE. 2018. A meta-analysis of birth-origin effects on reproduction in diverse captive environments. Nature Communications 9: 1055. https://doi.org/10.1038/s41467-018-03500-9.

Farquharson KA, Hogg CJ, Grueber CE. 2021. Offspring survival changes over generations of captive breeding. Nature Communications 12: 3045. https://doi.org/10.1038/s41467-021-22631-0

Fayard A. 2022. État des populations naturelles de rainette faux-grillon boréale (*Pseudacris maculata*) et stratégies de réintroduction en milieu aménagé [thesis]. [Québec City (QC)]: Université Laval. x + 110 pp.

Fernández-Vizarra E, Zeviani M. 2015. Nuclear gene mutations as the cause of mitochondrial complex III deficiency. Frontiers in Genetics 6: 134. https://doi.org/10.3389/fgene.2015.00134.

Ferrari S, Cribari-Neto F. 2004. Beta regression for modelling rates and proportions. Journal of Applied Statistics 31: 799–815. https://doi.org/10.1080/0266476042000214501.

Ficetola GF, Maiorano L. 2016. Contrasting effects of temperature and precipitation change on amphibian phenology, abundance and performance. Oecologia 181: 683–693. https://doi.org/10.1007/s00442-016-3610-9.

Fischer CP, Romero LM. 2019. Chronic Captivity Stress in Wild Animals Is Highly Species-Specific. Edited by Steven Cooke. Conservation Physiology 7: coz093. https://doi.org/10.1093/conphys/coz093.

Flaherty SE, Bezy O, LaCarubba Paulhus B, Song LJ, Piper M, Pang K, Park Y, Asano S, Lien Y-C, Griffin JD, Robertson A, Opsahl A, Hirenallur-Shanthappa D, Ahn Y, Pashos E, Simmons RA, Birnbaum MJ, Wu Z. 2024. SPAG7 deletion causes intrauterine growth restriction, resulting in adulthood obesity and metabolic dysfunction. eLife 12: RP91114. https://doi.org/10.7554/eLife.91114.

Foden WB, Butchart SHM, Stuart SN, Vié JC, Akçakaya HR, Angulo A, DeVantier LM, Gutsche A, Turak E, Cao L, Donner SD, Katariya V, Bernard R, Holland RA, Hughes AF, O'Hanlon SE, Garnett ST, Şekercioğlu ÇH, Mace GM. 2013. Identifying the world's most climate change vulnerable species: a systematic trait-based assessment of all birds, amphibians and corals. PLOS ONE 8: e65427. https://doi.org/10.1371/journal.pone.0065427.

Forester DC, Harrison WK. 1987. The significance of antiphonal vocalisation by the spring peeper, *Pseudacris crucifer* (Amphibia, Anura). Behaviour 103: 1–15.

Frisbie MP, Costanzo JP, Lee RE. 2000. Physiological and ecological aspects of low-temperature tolerance in embryos of the wood frog, *Rana sylvatica*. Canadian Journal of Zoology 78: 1032–1041. https://doi.org/10.1139/z00-022.

Fritz KA, Whiles MR. 2018. Amphibian-mediated nutrient fluxes across aquatic–terrestrial boundaries of temporary wetlands. Freshwater Biology 63: 1250–1259. https://doi.org/10.1111/fwb.13130.

Fu L, Crawford L, Tong A, Luu N, Tanizaki Y, Shi YB. 2021. Sperm associated antigen 7 is activated by T3 during Xenopus tropicalis metamorphosis via a thyroid hormone response element within the first intron. Development, Growth & Differentiation 64: 48–58. https://doi.org /10.1111/dgd.12764

Fujihara M, Yamamizu K, Wildt DE, Songsasen N. 2016. Expression pattern of matrix metalloproteinases changes during folliculogenesis in the cat ovary. Reproduction in Domestic Animals 51: 717–725. https://doi.org/10.1111/rda.12736.

Gall MD, Baugh AT, Lucas JR, Bee MA. 2021. Social communication across reproductive boundaries: hormones and the auditory periphery of songbirds and frogs. Integrative and Comparative Biology 61: 292–301. https://doi.org/10.1093/icb/icab075.

Gallant AL, Klaver RW, Casper GS, Lannoo MJ. 2007. Global rates of habitat loss and implications for amphibian conservation. Copeia 4: 967–979. https://doi.org/10.1643/0045-8511(2007)7[967:GROHLA]2.0.CO;2

Gaston MA, Fuji A, Weckerly FW, Forstner MRJ. 2010. Potential component Allee effects and their impact on wetland management in the conservation of endangered anurans. PLOS ONE 5: e10102. https://doi.org/10.1371/journal.pone.0010102.

Gelman A, Hill J. 2007. Data analysis using regression and multilevel/hierarchical models. New York (NY): Cambridge University Press.

Gerhardt HC, Huber F. 2002. Acoustic Communication in Insects and Anurans – Common Problems and Diverse Solutions. Chicago (IL): University of Chicago Press.

Gerhardt HC. 1991. Female mate choice in treefrogs: static and dynamic acoustic criteria. Animal Behaviour 42:615–635. https://doi.org/10.1016/S0003-3472(05)80245-3.

Gerhardt HC. 1995. Phonotaxis in female frogs and toads: execution and design of experiments. In: Klump GM, Dooling RJ, Fay RR, Stebbins WC, editors. Methods in Comparative Psychoacoustics. Basel, Switzerland: Birkhäuser. p. 209-220. https://doi.org/10.1007/978-3-0348-7463-2_18.

Gibbs JP, Breisch AR. 2001. Climate warming and calling phenology of frogs near Ithaca, New York, 1900–1999. Conservation Biology 15: 1175–1178. https://doi.org/10.1046/j.1523-1739.2001.0150041175.x.

Gibbs JP, Whiteleather KK, Schueler FW. 2005. Changes in frog and toad populations over 30 years in New York state. Ecological Applications 15: 1148–1157. https://doi.org/10.1890/03-5408.

Gilroy JJ, Gill JA, Butchart SHM, Jones VR, Franco AMA. 2016. Migratory diversity predicts population declines in birds. Ecology Letters 19: 308–317. https://doi.org/10.1111/ele.12569.

Gobbetti A, Zerani M. 1999. Hormonal and cellular brain mechanisms regulating the amplexus of male and female water frog (Rana esculenta). Journal of Neuroendocrinology 11: 589–596. https://doi.org/10.1046/j.1365-2826.1999.00369.x.

Goymann W, Landys MM, Wingfield JC. 2007. Distinguishing seasonal androgen responses from male–male androgen responsiveness – revisiting the challenge hypothesis. Hormones and Behavior 51: 463–476. https://doi.org/10.1016/j.yhbeh.2007.01.007.

Grabherr MG, Haas BJ, Yassour M, Levin JZ, Thompson DA, Amit I, Adiconis X, Fan L, Raychowdhury R, Zeng Q, et al. 2011. Trinity: reconstructing a full-length transcriptome without a genome from RNA-seq data. Nature Biotechnology 29: 644. https://doi.org/10.1038/nbt.1883.

Grafe TU. 2005. Anuran choruses as communication networks. In: P. K. McGregor PK, editor. Animal Communication Networks. Cambridge University Press. p. 277–99. https://doi.org/10.1017/CBO9780511610363.017.

Gramapurohit NP, Radder RS. 2013. Changes in plasma sex steroids in relation to reproductive behavior of the toad, *Bufo melanostictus* (Schn.). Copeia 2013: 332–337. https://doi-org.proxy.bib.uottawa.ca/10.1643/CP-11-100.

Grant EHC, Miller DAW, Muths E. 2020. A synthesis of evidence of drivers of amphibian declines. Herpetologica 76: 101–107. https://doi.org/10.1655/0018-0831-76.2.101

Grant EHC, Miller DAW, Schmidt BR, Adams MJ, Amburgey SM, Chambert T, Cruickshank SS, Fisher RN, Green DM, Hossack BR, et al. 2016. Quantitative evidence for the effects of multiple drivers on continental-scale amphibian declines. Scientific Reports 6: 25625. https://doi.org/10.1038/srep25625.

Green DM. 2003. The ecology of extinction: population fluctuation and decline in amphibians. Biological Conservation 111: 331–343. https://doi.org/10.1016/S0006-3207(02)00302-6.

Green DM. 2017. Amphibian breeding phenology trends under climate change: predicting the past to forecast the future. Global Change Biology 23: 646–656. https://doi.org/10.1111/gcb.13390.

Green DM, Lannoo MJ, Lesbarrères D, Muths E. 2020. Amphibian population declines: 30 years of progress in confronting a complex problem. Herpetologica 76: 97–100. https://doi.org/10.1655/0018-0831-76.2.97.

Green T, Das E, Green DM. 2016. Springtime emergence of overwintering toads, *Anaxyrus fowleri*, in relation to environmental factors. Copeia 104: 393–401. https://doi.org/10.1643/CE-15-323.

Griffis-Kyle KL, Mougey K, Vanlandeghem M, Swain S, Drake JC. 2018. Comparison of climate vulnerability among desert herpetofauna. Biological Conservation 225: 164–175. https://doi.org/10.1016/j.biocon.2018.06.009.

Griffiths RA, Pavajeau L. 2008. Captive breeding, reintroduction, and the conservation of amphibians. Conservation Biology 22: 852–861. https://doi.org/10.1111/j.1523-1739.2008.00967.x.

Gupta SK. 2021. Human zona pellucida glycoproteins: binding characteristics with human spermatozoa and induction of acrosome reaction. Frontiers in Cell and Developmental Biology 9: 619868. https://doi.org/10.3389/fcell.2021.619868.

Hall IC, Kelley DB. 2021. Endocrine modulation of acoustic communication: xenopus laevis as a model system. In: Rosenfeld CS, Hoffmann F, editors. Neuroendocrine Regulation of Animal Vocalization. New York (NY): Academic Press. p. 81–100. https://doi.org/10.1016/B978-0-12-815160-0.00009-8.

Halsch CA, Shapiro AM, Fordyce JA, Nice CC, Thorne JH, Waetjen DP, Forister ML. 2021. Insects and recent climate change. Proceedings of the National Academy of Sciences USA 118: e2002543117. https://doi.org/10.1073/pnas.2002543117.

Hammerson GA. 2008. Amphibians and reptiles in Colorado. Denver (CO): University Press of Colorado.

Harding G, Griffiths R, Pavajeau L. 2016. Developments in amphibian captive breeding and reintroduction programs. Conservation Biology 30: 340–349. https://doi.org/10.1111/cobi.12612.

Harris RMB, Beaumont LJ, Vance TR, Tozer CR, Remenyi TA, Perkins-Kirkpatrick SE, Mitchell PJ, Nicotra AB, McGregor S, Andrew NR, Letnic M, Kearney MR, Wernberg T, Hutley LB, Chambers LE, Fletcher MS, Keatley MR, Woodward CA, Williamson G, Duke NC, Bowman DMJS. 2018. Biological responses to the press and pulse of climate trends and extreme events. Nature Climate Change 8: 579–587. https://doi.org/10.1038/s41558-018-0187-9.

Harris RA, Tardif SD, Vinar T, Wildman DE, Rutherford JN, Rogers J, Worley KC, Aagaard KM. 2014. Evolutionary genetics and implications of small size and twinning in callitrichine primates. Proceedings of the National Academy of Sciences 111: 1467–1472. https://doi.org/10.1073/pnas.1316037111.

Hereng TH, Backe PH, Kahmann J, Scheich C, Bjørås M, Skålhegg BS, Rosendal KR. 2012. Structure and function of the human sperm-specific isoform of protein kinase A (PKA) catalytic subunit Cα2. Journal of Structural Biology 178: 300–310. https://doi.org/10.1016/j.jsb.2012.03.013.

Higgins SA, Swanson DL. 2013. Urea is not a universal cryoprotectant among hibernating anurans: evidence from the freeze-tolerant boreal chorus frog (*Pseudacris maculata*). Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology 164: 344–350. https://doi.org/10.1016/j.cbpa.2012.11.002.

Hill AP, Prince P, Covarrubias EP, Doncaster CP, Snaddon JL, Rogers A. 2018. AudioMoth: Evaluation of a smart open acoustic device for monitoring biodiversity and the environment. Methods in Ecology and Evolution 9:1199-1211.

Höbel G. 2015. Socially mediated plasticity of chorusing behavior in the gladiator frog *Hypsiboas rosenbergi*. Acta Ethologica 18: 145–152. https://doi.org/10.1007/s10211-014-0199-z.

Höbel G. 2017. Social facilitation is a better predictor of frog reproductive activity than environmental factors. Biotropica 49: 372–381. https://doi.org/10.1111/btp.12437.

Hocking D, Babbitt K. 2014. Amphibian contributions to ecosystem services. Herpetological Conservation and Biology 9: 1–17.

Hoffmann M, Hilton-Taylor C, Angulo A, Böhm M, Brooks TM, Butchart SHM, Carpenter KE, Chanson J, Collen B, Cox NA, et al. 2010. The impact of conservation on the status of the world's vertebrates. Science 330: 1503–1509. https://doi.org/10.1126/science.1194442.

Hossack B, Puglis H, Battaglin W, Anderson C, Honeycutt R, Smalling K. 2017. Widespread legacy brine contamination from oil production reduces survival of chorus frog larvae. Environmental Pollution 231: 742–751. https://doi.org/10.1016/j.envpol.2017.08.070.

Houlahan JE, Findlay CS, Schmidt BR, Meyer AH, Kuzmin SL. 2000. Quantitative evidence for global amphibian population declines. Nature 404: 752–55. https://doi.org/10.1038/35008052.

Iimori E, D'Occhio MJ, Lisle AT, Johnston SD. 2005. Testosterone secretion and pharmacological spermatozoal recovery in the cane toad (*Bufo marinus*). Animal Reproduction Science 90: 163–173. https://doi.org/10.1016/j.anireprosci.2005.01.010.

Ilaslan E, Markosyan R, Sproll P, Stevenson BJ, Sajek M, MP, Hayrapetyan H, Sarkisian T, Livshits L, Nef S, Jaruzelska J, Kusz-Zamelczyk K. 2020. The FKBP4 gene, encoding a regulator of the androgen receptor signaling pathway, is a novel candidate gene for androgen insensitivity syndrome. International Journal of Molecular Sciences 21: 8403. https://doi.org/10.3390/ijms21218403.

Ishii S, Itoh M. 1992. Amplexus induces surge of luteinizing hormone in male toads, *Bufo japonicus*. General and Comparative Endocrinology 86: 34–41. https://doi.org/10.1016/0016-6480(92)90123-2.

Itoh M, Ishii S. 1990. Changes in plasma levels of gonadotropins and sex steroids in the toad, *Bufo japonicus*, in association with behavior during the breeding season. General and Comparative Endocrinology 80: 451–464. https://doi.org/10.1016/0016-6480(90)90194-Q.

Ivell R, Wade JD, Anand-Ivell R. 2013. INSL3 as a biomarker of Leydig cell functionality. Biology of Reproduction 88: 147. https://doi.org/10.1095/biolreprod.113.108969.

Iwai N. 2018. Relationship between chorusing activity and number of oviposition events in the Otton frog. Animal Biology 68: 105–111. https://doi.org/10.1163/15707563-17000143.

Kaplan RH. 1987. Developmental plasticity and maternal effects of reproductive characteristics in the frog, *Bombina orientalis*. Oecologia 71: 273–279. https://doi.org/10.1007/BF00377295.

Kats LB, Ferrer RP. 2003. Alien predators and amphibian declines: review of two decades of science and the transition to conservation. Diversity and Distributions 9: 99–110. https://doi.org/10.1046/j.1472-4642.2003.00013.x.

Kelley DB. 2004. Vocal communication in frogs. Current Opinion in Neurobiology 14: 751–757. https://doi.org/10.1016/j.conb.2004.10.015

Kiesecker JM, Blaustein AR, Belden LK. 2001. Complex causes of amphibian population declines. Nature 410: 681–684. https://doi.org/10.1038/35070552.

Kight CR, Swaddle JP. 2011. How and why environmental noise impacts animals: an integrative, mechanistic review. Ecology Letters 14: 1052–1061. https://doi.org/10.1111/j.1461-0248.2011.01664.x.

King SL. 2015. You talkin' to me? interactive playback is a powerful yet underused tool in animal communication research. Biology Letters 11: 20150403. https://doi.org/10.1098/rsbl.2015.0403.

Klaus SP, Lougheed SC. 2013. Changes in breeding phenology of eastern Ontario frogs over four decades. Ecology and Evolution 3: 835–845. https://doi.org/10.1002/ece3.501.

Kleiman DG. 1980. The sociobiology of captive propagation. In: Soulé ME, Wilcox BA, editors. Conservation Biology. Sunderland (MA): Sinauer Associates, Inc. p. 243–261.

Klump GM, Gerhardt HC. 1992. Mechanisms and function of call-timing in male-male interactions in frogs. In: McGregor PK, editor. Playback and Studies of Animal Communication. Boston (MA): Springer US. p. 153–74. https://doi.org/10.1007/978-1-4757-6203-7_11.

Kouba AJ, Vance CK, Willis EL. 2009. Artificial fertilization for amphibian conservation: current knowledge and future considerations. Theriogenology 71: 214–227. https://doi.org/10.1016/j.theriogenology.2008.09.055.

Kramer AM, Dennis B, Liebhold AM, Drake JM. 2009. The evidence for Allee effects. Population Ecology 51: 341–354. https://doi.org/10.1007/s10144-009-0152-6.

Kuang H, Han D, Xie J, Yan Y, Li J, Ge P. 2014. Profiling of differentially expressed microRNAs in premature ovarian failure in an animal model. Gynecological Endocrinology 30: 57–61. https://doi.org/10.3109/09513590.2013.850659.

Kuczynski MC, Gering E, Getty T. 2016. Context and condition dependent plasticity in sexual signaling in gray treefrogs. Behavioural Processes 124: 74–79. https://doi.org/10.1016/j.beproc.2015.11.020.

Lacroix C, Schueler FW, Rollinson N. 2024. A 91% decline in a common anuran in an otherwise stable amphibian community inferred from 17 years of rapid road surveys. Animal Conservation 27:37–52. https://doi.org/10.1111/acv.12874.

Lading H, Wilcoxen TE. 2021. Western chorus frog tadpoles (*Pseudacris triseriata*) show expedited development, but no change in tail morphology, in the presence of predators and independent of diet. BIOS 92: 1–7. https://doi.org/10.1893/BIOS-D-19-00031.

Lannoo MJ. 2005. Amphibian Declines: The Conservation Status of United States Species. Berkeley (CA): University of California Press.

Lassandro M, Bolitho L, Newell D. A systematic review of passive acoustic monitoring for anuran conservation. Bioacoustics 34: 579–628. https://doi.org/10.1080/09524622.2025.2518486.

Lea J, Dyson M, Halliday T. 2001. Calling by male midwife toads stimulates females to maintain reproductive condition. Animal Behaviour 61: 373–377. https://doi.org/10.1006/anbe.2000.1604.

Leary CJ, Garcia AM, Knapp R. 2006. Elevated corticosterone levels elicit non-calling mating tactics in male toads independently of changes in circulating androgens. Hormones and Behavior 8: 425–432. https://doi.org/10.1016/j.yhbeh.2005.09.004.

Leary CJ, Crocker-Buta S, Holloway A, Kennedy JGC. 2021. Glucocorticoid-mediated changes in male green treefrog vocalizations alter attractiveness to females. Integrative and Comparative Biology 61: 283–291. https://doi.org/10.1093/icb/icab053.

Leary CJ. 2009. Hormones and acoustic communication in anuran amphibians. Integrative and Comparative Biology 49: 452–470. https://doi.org/10.1093/icb/icp027.

Leary CJ, Lippincott J, Harris S, Hawkins DL. 2015. A test of the energetics-hormone vocalization model in the green treefrog. General and Comparative Endocrinology 213: 32–39. https://doi.org/10.1016/j.ygcen.2014.12.017.

Leary CJ, Jessop TS, Garcia AM, Knapp R. 2004. Steroid hormone profiles and relative body condition of calling and satellite toads: implications for proximate regulation of behavior in anurans. Behavioral Ecology 15: 313–320. https://doi.org/10.1093/beheco/arh015.

Leitner S, Marshall RC, Leisler B, Catchpole CK. 2006. Male song quality, egg size and offspring sex in captive canaries (*Serinus canaria*). Ethology 112: 554–563. https://doi.org/10.1111/j.1439-0310.2006.01195.x.

Lemanski NJ, Williams NM, Winfree R. 2022. Greater bee diversity is needed to maintain crop pollination over time. Nature Ecology & Evolution 6: 1516–1523. https://doi.org/10.1038/s41559-022-01847-3.

Lemmon EM, Lemmon AR, Collins JT, Lee-Yaw JA, Cannatella DC. 2007. Phylogeny-based delimitation of species boundaries and contact zones in the trilling chorus frogs (*Pseudacris*). Molecular Phylogenetics and Evolution 44: 1068–1082. https://doi.org/10.1016/j.ympev.2007.04.010.

Lesbarrères D, Ashpole SL, Bishop CA, Blouin-Demers G, Brooks RJ, Echaubard P, Govindarajulu P, Green DM, Hecnar SJ, Herman T, et al. 2014. Conservation of herpetofauna in northern landscapes: threats and challenges from a Canadian perspective. Biological Conservation 170: 48–55. https://doi.org/10.1016/j.biocon.2013.12.030.

Li Y, Cohen JM, Rohr JR. 2013. Review and synthesis of the effects of climate change on amphibians. Integrative Zoology 8: 145–161. https://doi.org/10.1111/1749-4877.12001.

Li H, You L, Tian Y, Guo J, Fang X, Zhou C, Shi L, Su Y-Q. 2020. DPAGT1-mediated protein N-glycosylation is indispensable for oocyte and follicle development in mice. Advanced Science 7: 2000531. https://doi.org/10.1002/advs.202000531.

Liu Q, Piao S, Janssens IA, Fu Y, Peng S, Lian XU, Ciais P, Myneni RB, Peñuelas J, Wang T. 2018. Extension of the growing season increases vegetation exposure to frost. Nature Communications 9: 426. https://doi.org/10.1038/s41467-017-02690-y.

Liu Y, Yao Z-X, Bendavid C, Borgmeyer C, Han Z, Cavalli LR, Chan W-Y, Folmer J, Zirkin BR, Haddad BR, Gallicano GI, Papadopoulos V. 2005. Haploinsufficiency of cytochrome P450 17α-hydroxylase/17,20 lyase (CYP17) causes infertility in male mice. Molecular Endocrinology 19: 2380–2389. https://doi.org/10.1210/me.2004-0418.

López-Alcaide S, Macip-Ríos R, López-Alcaide S, Macip-Ríos R. 2011. Effects of climate change in amphibians and reptiles. In: Grillo O, editor. Biodiversity Loss in a Changing Planet. IntechOpen. https://doi.org/10.5772/24663.

Lu DY, Li Y, Bi ZW, Yu HM, Li XJ. 2008. Expression and immunohistochemical localization of aquaporin-1 in male reproductive organs of the mouse. Anatomia, Histologia, Embryologia 37: 1–8. https://doi.org/10.1111/j.1439-0264.2007.00827.x.

Lüddecke H. 2002. Male and female responses to call playback in the Andean frog *Colostethus subpunctatus*. Amphibia-Reptilia 23: 141–150. https://doi.org/10.1163/156853802760061787.

Ludwig B, Bender E, Arnold S, Hüttemann M, Lee I, Kadenbach B. 2001. Cytochrome c oxidase and the regulation of oxidative phosphorylation. ChemBioChem 2: 392–403. https://doi.org/10.1002/1439-7633(20010601)2:6<392::AID-CBIC392>3.0.CO;2-N.

Luedtke JA, Chanson J, Neam K, Hobin L, Maciel AO, Catenazzi A, Borzée A, Hamidy A, Aowphol A, Jean A, et al. 2023. Ongoing declines for the world's amphibians in the face of emerging threats. Nature 622: 308–314. https://doi.org/10.1038/s41586-023-06578-4.

Lynch AJ, Cooke SJ, Arthington AH, Baigun C, Bossenbroek L, Dickens C, Harrison I, Kimirei I, Langhans SDMurchie KJ, Olden JD, et al. 2023. People need freshwater biodiversity. WIREs Water 10: e1633. https://doi.org/10.1002/wat2.1633.

Lynch KS, Crews D, Ryan MJ, Wilczynski W. 2006. Hormonal state influences aspects of female mate choice in the Túngara Frog (*Physalaemus pustulosus*). Hormones and Behavior 49: 450–457. https://doi.org/10.1016/j.yhbeh.2005.10.001.

Ma X, Wang Y, Li W, Wang K, Zhang S. 2024. Bisphenol A disrupts ribosome function during ovarian development of mice. Toxics 12: 627. https://doi.org/10.3390/toxics12090627.

Ma Y, Xie N, Xie D, Sun L, Li S, Li P, Li Y, Li J, Dong Z, Xie X. 2019. A novel homozygous FBXO43 mutation associated with male infertility and teratozoospermia in a consanguineous Chinese family. Fertility and Sterility 111: 909–917. https://doi.org/10.1016/j.fertnstert.2019.01.007.

Macphie KH, Phillimore AB. 2024. Phenology. Current Biology 34: R183–R188. https://doi.org/10.1016/j.cub.2024.01.007.

Mangiamele LA, Burmeister SS. 2008. Acoustically evoked immediate early gene expression in the pallium of female Túngara frogs. Brain, Behavior and Evolution 72: 239–250. https://doi.org/10.1159/000171481.

Manku G, Culty M. 2016. Regulation of translocator protein 18 kDa (TSPO) expression in rat and human male germ cells. International Journal of Molecular Sciences 17: 1486. https://doi.org/10.3390/ijms17091486.

Manna PR. 2025. The multifaceted landscape of the StAR protein in steroid biosynthesis: from development to degeneration. Journal of Endocrinology 265: e240380. https://doi.org/10.1530/JOE-24-0380.

Mantyka-Pringle CS, Martin TG, Rhodes JR. 2012. Interactions between climate and habitat loss effects on biodiversity: a systematic review and meta-analysis. Global Change Biology 18: 1239–1252. https://doi.org/10.1111/j.1365-2486.2011.02593.x.

Markowitz H, Aday C, Gavazzi A. 1995. Effectiveness of acoustic 'prey': environmental enrichment for a captive African leopard (*Panthera pardus*). Zoo Biology 14: 371–379. https://doi.org/10.1002/zoo.1430140408.

Márquez R, Pargana JM, Crespo EG. 2001. Acoustic competition in male *Pelodytes ibericus* (Anura: Pelodytidae): interactive playback tests. Copeia 2001: 1142–1150. https://doi.org/10.1643/0045-8511(2001)001[1142:ACIMPI]2.0.CO;2.

Martin M. 2011. Cutadapt removes adapter sequences from high-throughput sequencing reads. EMBnet.Journal 17: 10–12. https://doi.org/10.14806/ej.17.1.200.

Martínez-Rivera CC, Gerhardt HC. 2008. Advertisement-call modification, male competition, and female preference in the bird-voiced treefrog *Hyla avivoca*. Behavioral Ecology and Sociobiology 63: 195–208. https://doi.org/10.1007/s00265-008-0650-0.

Maruo K, Suda M, Yokoyama S, Oshima Y, Nakamura M. 2008. Steroidogenic gene expression during sex determination in the frog *Rana rugosa*. General and Comparative Endocrinology 158: 87–94. https://doi.org/10.1016/j.ygcen.2008.04.019.

Maruska KP, Sisneros JA. 2015. Sex steroid-dependent modulation of acoustic communication systems in fishes. In: Ladich F, editor. Sound Communication in Fishes. Vienna: Springer. p. 207–233. https://doi.org/10.1007/978-3-7091-1846-7_7.

Maruska KP, Fernald RD. 2011. Social regulation of gene expression in the hypothalamic-pituitary-gonadal axis. Physiology 26: 412–423. https://doi.org/10.1152/physiol.00032.2011.

Maruska KP, Anselmo CM, King T, Mobley RB, Ray EJ, Wayne R. 2022. Endocrine and neuroendocrine regulation of social status in cichlid fishes. Hormones and Behavior 139: 105110. https://doi.org/10.1016/j.yhbeh.2022.105110.

Maxwell SL, Butt N, Maron M, McAlpine CA, Chapman S, Ullmann A, Segan DB, Watson JEM. 2019. Conservation implications of ecological responses to extreme weather and climate events. Biodiversity Review 25: 613–625. https://doi.org/10.1111/ddi.12878.

Mazerolle MJ. 2023. AICcmodavg: Model selection and multimodel inference based on (Q)AIC(c). R package version 2.3-3. Accessed 28 August 2024. https://cran.r-project.org/web/packages/AICcmodavg/index.html

McClintock BT, Bailey LL, Pollock KH, Simons TR. 2010. Unmodeled observation error induces bias when inferring patterns and dynamics of species occurrence via aural detections. Ecology 91: 2446–2454. https://doi.org/10.1890/09-1287.1.

McComb K, Reby D. 2009. Communication in terrestrial mammals. In: Squire LR, editor. Encyclopedia of Neuroscience Vol. 2. Oxford: Academic Press. p. 1167-1171.

McGowan PJK, Traylor-Holzer K, Leus K. 2017. IUCN guidelines for determining when and how ex situ management should be used in species conservation. Conservation Letters 10: 361–366. https://doi.org/10.1111/conl.12285.

McMenamin SK, Hadly EA, Wright CK. 2008. Climatic change and wetland desiccation cause amphibian decline in Yellowstone National Park. Proceedings of the National Academy of Sciences 105: 16988–16993. https://doi.org/10.1073/pnas.0809090105.

Melvin SD, Houlahan JE. 2012. Tadpole mortality varies across experimental venues: do laboratory populations predict responses in nature? Oecologia 169: 861–868. https://doi.org/10.1007/s00442-012-2260-9.

Mendonça MT, Licht P, Ryan MJ, Barnes R. 1985. Changes in hormone levels in relation to breeding behavior in male bullfrogs (*Rana catesbeiana*) at the individual and population levels. General and Comparative Endocrinology 58: 270–79. https://doi.org/10.1016/0016-6480(85)90343-0.

Mi Y, Shi Z, Li J. 2015. Spata19 is critical for sperm mitochondrial function and male fertility. Molecular Reproduction and Development 82: 907–913. https://doi.org/10.1002/mrd.22536.

Michaels CJ, Downie JR, Campbell-Palmer R. 2014. The importance of enrichment for advancing amphibian welfare and conservation goals: a review of a neglected topic. Amphibian & Reptile Conservation 8: 7–23.

Miller-Rushing AJ, Høye TT, Inouye DW, Post E. 2010. The effects of phenological mismatches on demography. Philosophical Transactions of the Royal Society B: Biological Sciences 365: 3177–3186. https://doi.org/10.1098/rstb.2010.0148.

Mindnich R, Möller G, Adamski J. 2004. The role of 17 beta-hydroxysteroid dehydrogenases. Molecular and Cellular Endocrinology 218: 7–20. https://doi.org/10.1016/j.mce.2003.12.006.

Møller AP, Rubolini D, Lehikoinen E. 2008. Populations of migratory bird species that did not show a phenological response to climate change are declining. Proceedings of the National Academy of Sciences 105: 16195–16200. https://doi.org/10.1073/pnas.0803825105.

Monestier O, Blanquet V. 2016. WFIKKN1 and WFIKKN2: ‘companion’ proteins regulating TGFB activity. Cytokine & Growth Factor Reviews 32: 75–84. https://doi.org/10.1016/j.cytogfr.2016.06.003.

Moore IT, Jessop TS. 2003. Stress, reproduction, and adrenocortical modulation in amphibians and reptiles. Hormones and Behavior 43: 39–47. https://doi.org/10.1016/S0018-506X(02)00038-7.

Mortazavi A, Williams BA, McCue K, Schaeffer L, Wold B. 2008. Mapping and quantifying mammalian transcriptomes by RNA-seq. Nature Methods 5: 621–628. https://doi.org/10.1038/nmeth.1226.

Moss WE, McDevitt-Galles T, Muths E, Bobzien S, Purificato J, Johnson PTJ. 2021. Resilience of native amphibian communities following catastrophic drought: Evidence from a decade of regional-scale monitoring. Biological Conservation 263: 109352. https://doi.org/10.1016/j.biocon.2021.109352.

Muñoz MI, Quispe M, Maliqueo M, \ Penna M. 2020. Biotic and abiotic sounds affect calling activity but not plasma testosterone levels in male frogs (*Batrachyla taeniata*) in the field and in captivity. Hormones and Behavior 118: 104605. https://doi.org/10.1016/j.yhbeh.2019.104605.

Munstermann MJ, Heim NA, McCauley DJ, Payne JL, Upham NS, Wang SC, Knope ML. 2022. A global ecological signal of extinction risk in terrestrial vertebrates. Conservation Biology 36: e13852. https://doi.org/10.1111/cobi.13852.

Myrberg AA, Lugli M. 2006. Reproductive behavior and acoustical interactions. In: Ladich F, Collin SP, Moller P, Kapoor BG, editors. Communication in Fishes. Enfield (NH): Science Publishers. p. 149–176.

Nakagawa S. 2004. A farewell to Bonferroni: The problems of low statistical power and publication bias. Behavioral Ecology 15: 1044–1045. https://doi.org/10.1093/beheco/arh107

Nayyab S, Gervasi MG, Tourzani DA, Caraballo DA, Jha KN, Teves ME, Cui W, Georg GI, Visconti PE, Salicioni AM. 2021. TSSK3, a novel target for male contraception, is required for spermiogenesis. Molecular Reproduction and Development 88: 718–730. https://doi.org/10.1002/mrd.23539.

Neelon DP, Höbel G. 2019. Staying ahead of the game – plasticity in chorusing behavior allows males to remain attractive in different social environments. Behavioral Ecology and Sociobiology 73: 124. https://doi.org/10.1007/s00265-019-2737-1.

Newediuk L, Ethier JP, Boyle SP, Aubin JA, Balluffi-Fry J, Dedeban E, Laforge MP, Prokopenko CM, Turner JW, Webber QMR, Vander Wal E. 2021. Sociopolitical factors drive conservation planning timelines: a Canadian case study with global implications. Biological Conservation 257: 109091. https://doi.org/10.1016/j.biocon.2021.109091.

Ni F-D, Hao S-L, Yang W-X. 2019. Multiple signaling pathways in Sertoli cells: recent findings in spermatogenesis. Cell Death & Disease 10: 541. https://doi.org/10.1038/s41419-019-1782-z.

Noguchi S, Arakawa T, Fukuda S, Furuno M, Hasegawa A, Hori F, Ishikawa-Kato S, Kaida K, Kaiho A, Kanamori-Katayama M, et al. 2017. FANTOM5 CAGE profiles of human and mouse samples. Scientific Data 4: 170112. https://doi.org/10.1038/sdata.2017.112.

Nolan MA, Babcock DF, Wennemuth G, Brown W, Burton KA, McKnight GS. 2004. Sperm-specific protein kinase a catalytic subunit Calpha2 orchestrates cAMP signaling for male fertility. Proceedings of the National Academy of Sciences of the United States of America 101: 13483–13488. https://doi.org/10.1073/pnas.0405580101.

Nourashrafeddin S, Ebrahimzadeh-Vesal R, Miryounesi M, Aarabi M, Zarghami N, Modarressi MH, Nouri M. 2014. Analysis of 19 gene expression during male germ cells development, lessons from in vivo and in vitro study. Cell Biology International Reports 21: 1–7. https://doi.org/10.1002/cbi3.10010.

Nowakowski AJ, Thompson ME, Donnelly MA, Todd BD. 2017. Amphibian sensitivity to habitat modification is associated with population trends and species traits. Global Ecology and Biogeography 26: 700-712. https://doi.org/10.1111/geb.12571.

O'Connor NE, Crowe TP. 2005. Biodiversity loss and ecosystem functioning: distinguishing between number and identity of species. Ecology 86: 1783–1796. https://doi.org/10.1890/04-1172.

Oseen KL, Wassersug RJ. 2002. Environmental factors influencing calling in sympatric anurans. Oecologia 133: 616–625. https://doi.org/10.1007/s00442-002-1067-5.

Ospina OE, Villanueva-Rivera LJ, Corrada-Bravo CJ, Aide TM. 2013. Variable response of anuran calling activity to daily precipitation and temperature: implications for climate change. Ecosphere 4: 47. https://doi.org/10.1890/ES12-00258.1.

Ouellet M, Fortin C, Grimard M-J. 2009. Distribution and habitat use of the boreal chorus frog (*Pseudacris maculata*) at its extreme northeastern range limit. Herpetological Conservation and Biology 4: 277–84.

Owen PC. 2003. The structure, function, and evolution of aggressive signals in anuran amphibians [thesis]. [Storrs (CT)]: University of Connecticut. xi + 131 pp.

Palermo Mendes J, Caldara FR, de Castro Burbarelli MF, Valentim JK, de Brito Mandú DF, Garcia RG, de Lima Almeida-Paz IC, Odakura AM, Lourenço da Silva MI. 2023. Performance and welfare of sows exposed to auditory environmental enrichment in mixed or collective housing systems. Animals 13: 1226. https://doi.org/10.3390/ani13071226.

Papadopoulos V, Fan J, Zirkin B. 2018. Translocator protein (18 kDa): an update on its function in steroidogenesis. Journal of Neuroendocrinology 30: e12500. https://doi.org/10.1111/jne.12500.

Parichy DM, Kaplan RH. 1992. Maternal effects on offspring growth and development depend on environmental quality in the frog *Bombina orientalis*. Oecologia 91: 579–586. https://doi.org/10.1007/BF00650334.

Park Y-J, Pang M-G. 2021. Mitochondrial functionality in male fertility: from spermatogenesis to fertilization. Antioxidants 10: 98. https://doi.org/10.3390/antiox10010098.

Patro R, Duggal G, Love MI, Irizarry RA, Kingsford C. 2017. Salmon provides fast and bias-aware quantification of transcript expression. Nature Methods 14: 417–420. https://doi.org/10.1038/nmeth.4197.

Pettus D, Angleton GM. 1967. Comparative reproductive biology of montane and piedmont chorus frogs. Evolution 21: 500–507. https://doi.org/10.2307/2406611.

Picard I, Desroches JF. 2004. Situation de la rainette faux-grillon de l'Ouest (*Pseudacris triseriata*) en Montérégie – Inventaire printanier 2004. Centre d'information sur l'environnement de Longueuil (CIEL), Longueuil (Quebec). 50 p.

Pizzatto L, Stockwell M, Clulow S, Clulow J, Mahony M. 2016. finding a place to live: conspecific attraction affects habitat selection in juvenile green and golden bell frogs. Acta Ethologica 19: 1–8. https://doi.org/10.1007/s10211-015-0218-8.

Plenderleith TL, Stratford D, Lollback GW, Chapple DG, Reina RD, Hero JM. 2018. Calling phenology of a diverse amphibian assemblage in response to meteorological conditions. International Journal of Biometeorology 62: 873–882. https://doi.org/10.1007/s00484-017-1490-2.

Pottier P, Kearney MR, Wu NC, Gunderson AR, Rej JE, Rivera-Villanueva AN, Pollo P, Burke S, Drobniak SM, Nakagawa S. 2025. Vulnerability of amphibians to global warming. Nature 639: 954–961. https://doi.org/10.1038/s41586-025-08665-0.

Preininger D, Boeckle M, Freudmann A, Starnberger I, Sztatecsny M, Hödl W. 2013. Multimodal signaling in the small torrent frog (*Micrixalus saxicola*) in a complex acoustic environment. Behavioral Ecology and Sociobiology 67: 1449–1456. https://doi.org/10.1007/s00265-013-1489-6.

Ptacek MB. 2000. The role of mating preferences in shaping interspecific divergence in mating signals in vertebrates. Behavioural Processes 51: 111–134. https://doi.org/10.1016/S0376-6357(00)00123-6.

Pulawska K, Ponikwicka-Tyszko D, Lebiedzinska W, Guo P, Bernaczyk P, Pilaszewicz-Puza A, Li X, Chrusciel M, Lupu O, Leskinen S et al. 2022. Novel expression of zona pellucida 3 protein in normal testis; potential functional implications. Molecular and Cellular Endocrinology 539: 111502. https://doi.org/10.1016/j.mce.2021.111502.

Ramalho-Santos J, Varum S, Amaral S, Mota PC, Sousa AP, Amaral A. 2009. Mitochondrial functionality in reproduction: from gonads and gametes to embryos and embryonic stem cells. Human Reproduction Update 15: 553–572. https://doi.org/10.1093/humupd/dmp016.

Rastogi RK, Meyer DL, Pinelli C, Fiorentino M, D'Aniello B. 1998. Comparative analysis of GnRH neuronal systems in the amphibian brain. General and Comparative Endocrinology 112: 330–345. https://doi.org/10.1006/gcen.1998.7144.

Ratikainen II, Haaland TR, Wright J. 2018. Differential allocation of parental investment and the trade-off between size and number of offspring. Proceedings of the Royal Society B: Biological Sciences 285: 20181074. https://doi.org/10.1098/rspb.2018.1074.

R Core Team. 2025. R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.

Reby D, McComb K. 2003. Vocal Communication and Reproduction in Deer. In: Slater PJB, Rosenblatt JS, Snowdon CT, Roper TJ, Naguib M, editors. Advances in the Study of Behavior, Volume 33. New York (NY): Elsevier Academic Press. p. 231–264.

Reichert MS, Gerhardt HC. 2013. Socially mediated plasticity in call timing in the gray tree frog, *Hyla versicolor*. Behavioral Ecology 24: 393–401. https://doi.org/10.1093/beheco/ars176.

Renoirt M, Angelier F, Cheron M, Brischoux F. 2023. What are the contributions of maternal and paternal traits to fecundity and offspring development? a case study in an amphibian species, the spined toad *Bufo spinosus*. Current Zoology 69: 527–534. https://doi.org/10.1093/cz/zoac072.

Rittman SE, Muths E, Green DE. 2003. *Pseudacris triseriata* (western chorus frog) and *Rana sylvatica* (wood frog) chytridiomycosis. Herpetological Review 34: 53.

Robbins L, Margulis SW. 2014. the effects of auditory enrichment on gorillas. Zoo Biology 33: 197–203. https://doi.org/10.1002/zoo.21127.

Robbins L, Margulis SW. 2016. Music for the birds: effects of auditory enrichment on captive bird species. Zoo Biology 35: 29–34. https://doi.org/10.1002/zoo.21260.

Robinson MD, McCarthy DJ, Smyth GK. 2010. edgeR: a bioconductor package for differential expression analysis of digital gene expression data. Bioinformatics 26: 139–140. https://doi.org/10.1093/bioinformatics/btp616.

Roco ÁS, Ruiz-García A, Bullejos M. 2021. Testis development and differentiation in amphibians. Genes 12: 578. https://doi.org/10.3390/genes12040578.

Rodríguez C, Fusani L, Raboisson G, Hödl W, Ringler E, Canoine V. 2022. Androgen responsiveness to simulated territorial intrusions in *Allobates femoralis* males: evidence supporting the challenge hypothesis in a territorial frog. General and Comparative Endocrinology 326: 114046. https://doi.org/10.1016/j.ygcen.2022.114046.

Rogic A, Tessier N, Lapointe F-J. 2019. Genetic characterization of imperiled boreal chorus frogs identifies populations for conservation. Journal of Herpetology 53: 89–95. https://doi.org/10.1670/17-126.

Rogic A, Tessier N, Noël S, Gendron A, Branchaud A, and Lapointe F.-J. 2015. A ‘trilling’ case of mistaken identity: call playbacks and mitochondrial DNA identify chorus frogs in southern Québec (Canada) as *Pseudacris maculata* and Not *P. triseriata*. Herpetological Review 46: 1–7.

Rose CS. 2005. Integrating ecology and developmental biology to explain the timing of frog metamorphosis. Trends in Ecology & Evolution 20: 129–135. https://doi:10.1016/j.tree.2005.01.005.

Rose JD, Moore FL. 2002. Behavioral neuroendocrinology of vasotocin and vasopressin and the sensorimotor processing hypothesis. Frontiers in Neuroendocrinology 23:317–341. https://doi.org/10.1016/S0091-3022(02)00004-3.

Ryan MJ, Rand AS. 1993. Species recognition and sexual selection as a unitary problem in animal communication. Evolution 47: 647–657. https://doi-org.proxy.bib.uottawa.ca/10.2307/2410076.

Ryan MJ, Keddy-Hector A. 1992. Directional patterns of female mate choice and the role of sensory biases. The American Naturalist 139: S4–35. https://doi.org/10.1086/285303.

Ryan MJ, Tuttle MD, Taft LK. 1981. The costs and benefits of frog chorusing behavior. Behavioral Ecology and Sociobiology 8: 273–278. https://doi.org/10.1007/BF00299526.

Saenz D, Fitzgerald LA, Baum KA, Conner RN. 2006. Abiotic correlates of anuran calling phenology: the importance of rain, temperature, and season. Herpetological Monographs 20: 64. https://doi.org/10.1655/0733-1347(2007)20[64:ACOACP]2.0.CO;2.

Saino N, Ambrosini R, Rubolini D, Von Hardenberg J, Provenzale A, Hüppop K, Hüppop O, Lehikoinen A, Lehikoinen E, Rainio K, Romano M, Sokolov L. 2011. Climate warming, ecological mismatch at arrival and population decline in migratory birds. Proceedings of the Royal Society B: Biological Sciences 278: 835–842. https://doi.org/10.1098/rspb.2010.1778.

Salles OC, Almany GR, Berumen ML, Jones GP, Saenz-Agudelo P, Srinivasan M, Thorrold SR, Pujol B, Planes S. 2020. Strong habitat and weak genetic effects shape the lifetime reproductive success in a wild clownfish population. Ecology Letters 23: 265–273. https://doi.org/10.1111/ele.13428.

Schalk CM, Saenz D. 2016. Environmental drivers of anuran calling phenology in a seasonal neotropical ecosystem. Austral Ecology 41:16–27. https://doi.org/10.1111/aec.12281.

Scheele BC, Heard GW, Cardillo M, Duncan RP, Gillespie GR, Hoskin CJ, Mahony M, Newell D, Rowley JJL, Sopniewski J. 2023. An invasive pathogen drives directional niche contractions in amphibians. Nature Ecology & Evolution 7: 1682–1692. https://doi.org/10.1038/s41559-023-02155-0.

Schiesari L, Grillitsch B, Grillitsch H. 2007. Biogeographic biases in research and their consequences for linking amphibian declines to pollution. Conservation Biology 21: 465–471. https://doi.org/10.1111/j.1523-1739.2006.00616.x.

Schlinger BA, Brenowitz EA. 2002. Neural and hormonal control of birdsong. In: Pfaff DW, Arnold AP, Fahrbach SE, Etgen AM, Rubin RT, editors. Hormones, Brain and Behavior. San Diego (CA): Academic Press. p. 799–839. https://doi.org/10.1016/B978-012532104-4/50035-4.

Schmidt R, Zummach C, Sinai N, Sabino-Pinto J, Künzel S, Dausmann KH, Ruthsatz K. 2024. Physiological responses to a changing winter climate in an early spring-breeding amphibian. Ecology and Evolution 14: e70042. https://doi.org/10.1002/ece3.70042.

Schoffelen RLM, Segenhout JM, van Dijk P. 2008. Mechanics of the exceptional anuran ear. Journal of Comparative Physiology A 194: 417–428. https://doi.org/10.1007/s00359-008-0327-1.

Schwartz JJ. 1994. Male advertisement and female choice in frogs: recent findings and new approaches to the study of communication in a dynamic acoustic environment. American Zoologist 34: 616–624. https://doi.org/10.1093/icb/34.6.616.

Scranton K, Priyanga A. 2017. Predicting phenological shifts in a changing climate. Proceedings of the National Academy of Sciences 114: 13212–13217. https://doi.org/10.1073/pnas.1711221114.

Seburn DC, Gunson K, Schueler FW. 2014. Apparent widespread decline of the boreal chorus frog (*Pseudacris maculata*) in eastern Ottawa. The Canadian Field-Naturalist 128: 151. https://doi.org/10.22621/cfn.v128i2.1579.

Seburn DC, Seburn CNL. 2000. Conservation Priorities for the Amphibians and Reptiles of Canada. Toronto (ON): World Wildlife Fund Canada and Canadian Amphibian and Reptile Conservation Network. 92 pp. http://www.carcnet.ca/francais/publications/Seburn2000.pdf

Seburn DC, Gunson K. 2011. Has the western chorus frog (*Pseudacris triseriata*) declined in Western Ottawa, Ontario? The Canadian Field-Naturalist 125: 220–226. https://doi.org/10.22621/cfn.v125i3.1224.

Seburn DC, Seburn CNL, Weller WF. 2008. A localized decline in the western chorus frog, *Pseudacris triseriata*, in Eastern Ontario. The Canadian Field-Naturalist 122: 158–161. https://doi.org/10.22621/cfn.v122i2.575.

Seddon PJ, Soorae PS, Launay F. 2005. Taxonomic bias in reintroduction projects. Animal Conservation 8: 51–58. https://doi.org/10.1017/S1367943004001799.

Shadle EJ, Hopkins WA, Belden LK, Hallmark MA, Mims MC. 2023. Experimental warming and drying act independently on developmental responses for two amphibian species. Freshwater Biology 68: 1647–1659. https://doi.org/10.1111/fwb.14156.

Sherwen SL, Hemsworth PH. 2019. The visitor effect on zoo animals: implications and opportunities for zoo animal welfare. Animals 9: 366. https://doi.org/10.3390/ani9060366.

Shimada M, Nakadai T, Tamura T. 2003. TATA-binding protein-like protein (TLP/TRF2/TLF) negatively regulates cell cycle progression and is required for the stress-mediated G2 checkpoint. Molecular and Cellular Biology 23: 4107–4120. https://doi.org/10.1128/MCB.23.12.4107-4120.2003.

Shriver RL. 2025. Quantifying how short-term environmental variation leads to long-term demographic responses to climate change. Journal of Ecology 104: 65–78. https://doi.org/10.1111/1365-2745.12490.

Shulse CD, Semlitsch RD, and Kathleen M. Community development in experimental wetlands. Journal of Applied Ecology 50: 1244–1256. https://doi.org/10.1111/1365-2664.12126.

Shulse CD, Semlitsch RD, Trauth KM, Williams A. 2010. Influences of design and landscape placement parameters on amphibian abundance in constructed wetlands. Wetlands 30: 915–928. https://doi.org/10.1007/s13157-010-0069-z.

Silla AJ, McFadden MS, Byrne PG. 2019. Hormone-induced sperm-release in the critically endangered booroolong frog (*Litoria booroolongensis*): effects of gonadotropin-releasing hormone and human chorionic gonadotropin. Conservation Physiology 7: coy080. https://doi.org/10.1093/conphys/coy080.

Silla AJ, Calatayud NE, Trudeau VL. 2021. Amphibian reproductive technologies: approaches and welfare considerations. Conservation Physiology 9: coab011. https://doi.org/10.1093/conphys/coab011.

Silla AJ, Byrne PG. 2019. The role of reproductive technologies in amphibian conservation breeding programs. Annual Review of Animal Biosciences 7: 499–519. https://doi.org/10.1146/annurev-animal-020518-115056.

Simmons AM, Narins PM. 2018. Effects of Anthropogenic Noise on Amphibians and Reptiles. In: Slabbekoom H, Dooling RJ, Popper AN, Fay RR, editors. Effects of Anthropogenic Noise on Animals. New York (NY): Springer. p. 179–208.

Sinervo B, Méndez-de-la-Cruz F, Miles DB, Heulin B, Bastiaans E, Villagrán-Santa Cruz M, Lara-Resendiz R, Martínez-Méndez N, Calderón-Espinosa ML, Meza-Lázaro RN, Gadsden H, Avila LJ, Morando M, De la Riva IJ, Sepulveda PV, Rocha CFD, Ibargüengoytía N, Puntriano CA, Massot M, Lepetz V, Oksanen TA, Chapple DG, Bauer AM, Branch WR, Clobert J, Sites JW. 2010. Erosion of lizard diversity by climate change and altered thermal niches. Science 328: 894–899. https://doi.org/10.1126/science.1184695.

Skelly D. 1995. A behavioral trade-off and its consequences for the distribution of *Pseudacris* treefrog larvae. Ecology 76: 150–164. https://doi.org/10.2307/1940638.

Skelly D. 1996. Pond drying, predators, and the distribution of *Pseudacris* tadpoles. Copeia 1996: 599–605. https://doi.org/10.2307/1447523.

Smeitink J, Sengers R, Trijbels F, van den Heuvel L. 2001. Human NADH:Ubiquinone Oxidoreductase. Journal of Bioenergetics and Biomembranes 33: 259–266. https://doi.org/10.1023/A:1010743321800.

Smith DC. 1990. Population structure and competition among kin in the chorus frog (*Pseudacris triseriata*). Evolution 44: 1529–1541. https://doi.org/10.1111/j.1558-5646.1990.tb03844.x.

Smith DC, Van Buskirk J. 1995. Phenotypic design, plasticity, and ecological performance in two tadpole species. The American Naturalist 145: 211–233. https://doi.org/10.1086/285737.

Snell-Rood EC. 2013. An overview of the evolutionary causes and consequences of behavioural plasticity. Animal Behaviour 85: 1004–1011. https://doi.org/10.1016/j.anbehav.2012.12.031.

Snyder NFR, Derrickson SR, Beissinger SR, Wiley JW, Smith TB, Toone WD, Miller B. 1996. Limitation of captive breeding in endangered species recovery. Conservation Biology 10: 338–348. https://doi.org/10.1046/j.1523-1739.1996.10020338.x.

Solís R, Penna M. 1997. Testosterone levels and evoked vocal responses in a natural population of the frog *Batrachyla taeniata*. Hormones and Behavior 31: 101–109. https://doi.org/10.1006/hbeh.1997.1366.

Soroye P, Newbold T, Kerr J. 2020. Climate change contributes to widespread declines among bumble bees across continents. Science 367: 685–688. https://doi.org/10.1126/science.aax8591.

Sosnik J, Miranda PV, Spiridonov NA, Yoon S-Y, Fissore RA, Johnson GR, Visconti PE. 2009. Tssk6 is required for izumo relocalization and gamete fusion in the mouse. Journal of Cell Science 122: 2741–2749. https://doi.org/10.1242/jcs.047225.

Sours G, Petranka J. 2007. Intraguild predation and competition mediate stage-structured interactions between wood frog (*Rana sylvatica*) and upland chorus frog (*Pseudacris feriarum*) larvae. Copeia 2007: 131–139. https://doi.org/10.1643/0045-8511(2007)7[131:IPACMS]2.0.CO;2.

Sredl MJ, Collins JP. 1991. The effect of ontogeny on interspecific interactions in larval amphibians. Ecology 72: 2232–2239. https://doi.org/10.2307/1941573.

Stebbins RC. 2003. A Field Guide to Western Reptiles and Amphibians (Third Edition). Boston (MA): Houghton Mifflin Company.

Stephens PA, Sutherland WJ, Freckleton RP. 1999. What is the Allee effect? Oikos 87: 185. https://doi.org/10.2307/3547011.

St-Hilaire D, Belleau P. 2005. Plan de protection sites et métapopulations de la rainette faux-grillon de l'Ouest (*Pseudacris triseriata*) en Outaouais. Ministère des Ressources naturelles – direction de l'aménagement de la faune, région de l'Outaouais. 45 pp.

Stocco DM, Zhao AH, Tu LN, Morohaku K, Selvaraj V. 2017. A brief history of the search for the protein(s) involved in the acute regulation of steroidogenesis. Molecular and Cellular Endocrinology, Seventeenth Conference on the Adrenal Cortex (Adrenal 2016) Boston, MA, March 28 – 31, 2016, 441: 7–16. https://doi.org/10.1016/j.mce.2016.07.036.

Stringer JM, Alesi LR, Winship AL, Hutt KJ. 2023. Beyond apoptosis: evidence of other regulated cell death pathways in the ovary throughout development and life. Human Reproduction Update 29: 434–456. https://doi.org/10.1093/humupd/dmad005

Stuart S, Chanson J, Cox N, Young B, Rodrigues A, Fischman D, Waller R. 2004. Status and trends of amphibian declines and extinctions worldwide. Science 306: 1783–1786. https://doi.org/10.1126/science.1103538.

Sullivan BK, Ryan MJ, Verrel PA. 1995. Female choice and mating system structure. In: Heatwole H, Sullivan BK, editors. Amphibian biology, volume 2: social behaviour. Chipping Norton, New South Wales, Australia: Surrey Beatty & Sons. p. 469-517.

Sun W-S, Chun J-L, Kim D-H, Ahn J-S, Kim M-K, Hwang I-S, Kwon D-J, Hwang S, ee J-W. 2017. Molecular cloning and characterization of porcine ribosomal protein L21. Journal of Veterinary Science 18: 531–540. https://doi.org/10.4142/jvs.2017.18.4.531.

Tapley B, Bradfield KS, Michaels C, Bungard M. 2015. Amphibians and conservation breeding programmes: do all threatened amphibians belong on the ark? Biodiversity and Conservation 24: 2625–2646. https://doi.org/10.1007/s10531-015-0966-9.

Tapley B, Johnson K, Michaels CJ, Bradfield K, Barber D, Randall L, Vasudevan K, Paszkowski C, Griffiths RA, Calatayud NE, Griffith E, Kacoliris F. 2022. Conservation breeding. In: Wren S, Borzée A, Marcec-Greaves R, Angulo A, editors. Amphibian conservation action plan: A status review and roadmap for global amphibian conservation. IUCN SSC Occasional Paper, No 57. Gland, Switzerland: IUCN.

Tárano Z, Fuenmayor E. 2009. Calling patterns in male responses to conspecific playbacks in the Johnstone's whistling frog *Eleutherodactylus johnstonei*. Ethology 115: 747–757. https://doi.org/10.1111/j.1439-0310.2009.01664.x.

Tárano Z, Fuenmayor E. 2014. Complex vocal responses to conspecific calls in whistling frogs *Eleutherodactylus johnstonei*: playback experiments in the field. Ethology 120: 313–323. https://doi.org/10.1111/eth.12207.

Tasker BA, Honebein KN, Erickson AM, Misslin JE, Hurst P, Cooney S, Riley S, Griffith SA, Bancroft BA. 2022. Effects of elevated temperature, reduced hydroperiod, and invasive bullfrog larvae on pacific chorus frog larvae. PLoS ONE 17: e0265345. https://doi.org/10.1371/journal.pone.0265345.

Ten Eyck GR. 2005. Arginine vasotocin activates advertisement calling and movement in the territorial Puerto Rican frog, *Eleutherodactylus coqui*. Hormones and Behavior 47: 223–229. https://doi.org/10.1016/j.yhbeh.2004.10.005.

Thackeray SJ, Henrys PA, Hemming D, Bell JR, Botham MS, Burthe S, Helaouet P, Johns DG, Jones ID, Leech DI, Mackay EB, Massimino D, Atkinson S, Bacon PJ, Brereton TM, Carvalho L, Clutton-Brock TH, Duck C, Edwards M, Elliott JM, Hall SJG, Harrington R, Pearce-Higgins JW, Høye TT, Kruuk LEB, Pemberton JM, Sparks TH, Thompson PM, White I, Winfield IJ, Sarah Wanless S. 2016. Phenological sensitivity to climate across taxa and trophic levels. Nature 535: 241–245. https://doi.org/10.1038/nature18608.

Thompson MM, Rowley JJL, Poore AGB, Callaghan CT. 2022. Citizen science reveals meteorological determinants of frog calling at a continental scale. Diversity and Distributions 28: 2375–2387. https://doi.org/10.1111/ddi.13634.

Thurman LL, Garcia TS. 2019. Asymmetric competition shapes amphibian response to rapid environmental change. Ecosphere 10: e02950. https://doi.org/10.1002/ecs2.2950

Tischer T, Hörmanseder E, Mayer TU. 2012. The APC/C Inhibitor XErp1/Emi2 is essential for xenopus early embryonic divisions. Science 338: 520–524. https://doi.org/10.1126/science.1228394.

Tjur T. 2009. Coefficients of determination in logistic regression models–a new proposal: the coefficient of discrimination. The American Statistician 63: 366–372. https://doi.org/10.1198/tast.2009.08210.

Todd BD, Scott DE, Pechmann JHK, Gibbons JW. 2010. Climate change correlates with rapid delays and advancements in reproductive timing in an amphibian community. Proceedings of the Royal Society B: Biological Sciences 278:2191–2197. https://doi.org/10.1098/rspb.2010.1768.

Trudeau VL, Raven BH, Pahuja HK, Narayan EJ. 2022. Hormonal control of amphibian reproduction. In: Silla AJ, Kouba AJ, Heatwole H, editors. Reproductive Technologies and Biobanking for the Conservation of Amphibians. Boca Raton (FL): CRC Press. p. 49–68.

Trudeau VL, Schueler FW, Navarro-Martin L, Hamilton CK, Bulaeva E, Bennett A, Fletcher W, Taylor L. 2013. Efficient induction of spawning of northern leopard frogs (*Lithobates pipiens*) during and outside the natural breeding season. Reproductive Biology and Endocrinology 11: 14. https://doi.org/10.1186/1477-7827-11-14.

Trudeau VL, Somoza GM, Natale GS, Pauli B, Wignall J, Jackman P, Doe K, Schueler FW. 2010. Hormonal induction of spawning in 4 species of frogs by coinjection with a gonadotropin-releasing hormone agonist and a dopamine antagonist. Reproductive Biology and Endocrinology 8: 36. https://doi-org.proxy.bib.uottawa.ca/10.1186/1477-7827-8-36.

Trull N, Böhm M, Carr J. 2018. Patterns and biases of climate change threats in the IUCN Red List. Conservation Biology 32: 135–147. https://doi.org/10.1111/cobi.13022.

Turvey ST, Chen S, Tapley B, Liang Z, Wei G, Yang J, Wang J, Wu M, Redbond J, Brown T, and Cunningham AA. 2021. From dirty to delicacy? Changing exploitation in China threatens the world's largest amphibians. People and Nature 3: 446–456. https://doi.org/10.1002/pan3.10185.

Urban MC. 2015. Accelerating extinction risk from climate change. Science 348: 571–573. https://doi.org/10.1126/science.aaa4984.

Üveges B, Mahr K, Szederkényi M, Bókony V, Hoi H, Hettyey A. 2016. Experimental evidence for beneficial effects of projected climate change on hibernating amphibians. Scientific Reports 6:26754. https://doi.org/10.1038/srep26754.

Valencia-Aguilar A, Cortés-Gómez AM, Ruiz-Agudelo CA. 2013. Ecosystem services provided by amphibians and reptiles in neotropical ecosystems. International Journal of Biodiversity Science, Ecosystem Services & Management 9: 257–272. https://doi.org/10.1080/21513732.2013.821168.

Van Allen BG, Briggs VS, McCoy MW, Vonesh JR. 2010. carry-over effects of the larval environment on post-metamorphic performance in two hylid frogs. Oecologia 164: 891–898. https://doi.org/10.1007/s00442-010-1728-8.

Vargas-Salinas F, Cunnington GM, Amézquita A, Fahrig L. 2024. Does traffic noise alter calling time in frogs and toads? A case study of anurans in Eastern Ontario, Canada. Urban Ecosystems 17: 945–953. https://doi-org.proxy.bib.uottawa.ca/10.1007/s11252-014-0374-z.

Vermont Fish and Wildlife Department. 2015. Vermont Wildlife Action Plan 2015. Vermont Fish and Wildlife Department. Montpelier, VT. https://vtfishandwildlife.com/node/551.

Vidali S, Gerlini R, Thompson K, Urquhart JE, Meisterknecht J, Aguilar-Pimentel JA, Amarie OV, Becker L, Breen C, Calzada-Wack J, et al. 2021. characterising a homozygous two-exon deletion in UQCRH: comparing human and mouse phenotypes. EMBO Molecular Medicine 13: e14397. https://doi.org/10.15252/emmm.202114397.

Visser ME, te Marvelde L, Lof ME. 2012. Adaptive Phenological mismatches of birds and their food in a warming world. Journal of Ornithology 153: 75–84. https://doi.org/10.1007/s10336-011-0770-6.

Vu M, Trudeau VL. 2016. Neuroendocrine control of spawning in amphibians and its practical applications. General and Comparative Endocrinology 234: 28–39. https://doi.org/10.1016/j.ygcen.2016.03.024.

Waas JR, Colgan PW, Boag PT. 2005. Playback of colony sound alters the breeding schedule and clutch size in zebra finch (*Taeniopygia guttata*) colonies. Proceedings of the Royal Society B: Biological Sciences 272: 383–388. https://doi:10.1098/rspb.2004.2949.

Wake D, Vredenburg V. 2008. Are we in the midst of the sixth mass extinction? a view from the world of amphibians. Proceedings of the National Academy of Sciences of the United States of America 105: 11466–11473. https://doi.org/10.1073/pnas.0801921105.

Wake D. 1991. Declining amphibian populations. Science 253: 5022. https://doi.org/10.1126/science.253.5022.860.

Walkowski WG, Crother BI, Valverde RA. 2019. Testosterone and corticosterone profiles and body condition of calling and non-calling *Lithobates grylio*. Copeia 107: 509–516. https://doi.org/10.1643/CP-18-134.

Walpole AA, Bowman J, Tozer DC, Badzinski DS. 2013. Community-level response to climate change: shifts in anuran calling phenology. Herpetological Conservation and Biology 7: 249–257.

Wang S, Zheng Y, Li J, Yu Y, Zhang W, Song M, Liu Z, Min Z, Hu H, Jing Y, He X, Sun L, Ma L, Rodriguez Esteban C, Chan P, Qiao J, Zhou Q, Izpisua Belmonte JC, Qu J, Tang F, Liu G-H. 2020. Single-cell transcriptomic atlas of primate ovarian aging. Cell 180: 585–600. https://doi.org/10.1016/j.cell.2020.01.009.

Wang Z, Gerstein M, Snyder M. 2009. RNA-Seq: a revolutionary tool for transcriptomics. Nature Reviews Genetics 10: 57–63. https://doi.org/10.1038/nrg2484.

Wang Z, Li T, Liu D, Li M, Liu S, Yu X, Li H, Song H, Zhao W, Liu Z, et al. 2024. The deubiquitinase cofactor UAF1 interacts with USP1 and plays an essential role in spermiogenesis. iScience 27: 109456. https://doi.org/10.1016/j.isci.2024.109456.

Wassersug R. 1986. How does a tadpole know when to metamorphose? A theory linking environmental and hormonal cues. Journal of Theoretical Biology 118: 171–181. https://doi.org/10.1016/S0022-5193(86)80132-1.

Weber T, Ozgul A, Schmidt BR. 2024. Density-dependent performance of larval and juvenile toads: implications for amphibian conservation. Basic and Applied Ecology 75: 12–17. https://doi.org/10.1016/j.baae.2024.02.001.

Weir LA, Mossman MJ. 2005. North American Amphibian Monitoring Program (NAAMP). In: Lannoo MJ, editor. Amphibian Declines: Conservation Status of United States Species. Berkeley (CA): University of California Press. p. 307–313.

Weir L, Fiske IJ, Royle JA. 2009. Trends in anuran occupancy from northeastern states of the North American amphibian monitoring program. Herpetological Conservation and Biology 4: 389–402.

Wells KD, Schwartz JJ. 2007. The Ecology and Behaviour of Amphibians. Chicago (IL): University of Chicago Press.

Wells DL. 2009. sensory stimulation as environmental enrichment for captive animals: a review. Applied Animal Behaviour Science 118: 1–11. https://doi.org/10.1016/j.applanim.2009.01.002.

Wells DL, Irwin RM. 2008. Auditory stimulation as enrichment for zoo-housed Asian elephants (*Elephas maximus*). Animal Welfare 17: 335–340. https://doi.org/10.1017/S0962728600027822.

Wells KD. 1977. The social behavior of anuran amphibians. Animal Behaviour 25: 666–693. https://doi.org/10.1016/0003-3472(77)90118-X.

Wells KD, Schwartz JJ. 2007. The behavioral ecology of anuran communication. In: Narins PM, Feng AS, Fay RR, Popper AN, editors. Hearing and sound communication in amphibians. Chicago (IL): University of Chicago Press. p. 44–86.

Wells KD. 1977. The social behavior of anuran amphibians. Animal Behaviour 25: 666–693. https://doi.org/10.1016/0003-3472(77)90118-X.

Wetzel DM, Kelley DB. 1983. Androgen and gonadotropin effects on male mate calls in South African Clawed Frogs, Xenopus laevis. Hormones and Behavior 17: 388–404.

While GM, Uller T. 2014. Quo vadis amphibia? Global warming and breeding phenology in frogs, toads and salamanders. Ecography 37: 921–929. https://doi.org/10.1111/ecog.00521.

Whitaker JO. 1971. A study of the western chorus frog, *Pseudacris triseriata*, in Vigo County, Indiana. Journal of Herpetology 5: 127. https://doi.org/10.2307/1562735.

White DJ, King AP, West MJ, Gros-Louis J, Tuttle EM. 2010. Effects of singing on copulation success and egg production in brown-headed cowbirds, *Molothrus ater*. Behavioral Ecology 21: 211–218. https://doi.org/10.1093/beheco/arp178.

Whitehouse DG, May B, Moore AL. 2019. Respiratory chain and ATP synthase. Reference Module in Biomedical Sciences. Elsevier. https://doi.org/10.1016/B978-0-12-801238-3.95732-5.

Whiting AV. 2004. Population ecology of the western chorus frog, *Pseudacris triseriata* [thesis]. [Montreal (QC)]: McGill University. x + 108 pp.

Wilbur HM, Collins JP. 1973. Ecological aspects of amphibian metamorphosis: nonnormal distributions of competitive ability reflect selection for facultative metamorphosis. Science 182: 1305–1314. https://doi.org/10.1126/science.182.4119.1305.

Wilczynski W, Lynch KS. 2011. Female sexual arousal in amphibians. Hormones and Behavior 59: 630–636. https://doi.org/10.1016/j.yhbeh.2010.08.015.

Wilczynski W, Ryan MJ. 2010. The behavioral neuroscience of anuran social signal processing. Current Opinion in Neurobiology 20: 754–763. https://doi.org/10.1016/j.conb.2010.08.021.

Wilczynski W, Burmeister SS. 2016. Effects of Steroid Hormones on Hearing and Communication in Frogs. In: Bass AH, Sisneros JA, Popper AN, Fay RR, editors. Hearing and Hormones. Cham: Springer International Publishing. p. 53–75. https://doi.org/10.1007/978-3-319-26597-1_3.

Wilczynski W, Lynch KS, O'Bryant EL. 2005. Current research in amphibians: studies integrating endocrinology, behavior, and neurobiology. Hormones and Behavior 48: 440–450. https://doi.org/10.1016/j.yhbeh.2005.06.001.

Wilczynski W, Quispe M, Muñoz MI, Penna M. 2017. Arginine vasotocin, the social neuropeptide of amphibians and reptiles. Frontiers in Endocrinology 8: 186. https://doi.org/ 10.3389/fendo.2017.00186

Wildlife Preservation Canada. 2025. Reptile & Amphibian Initiative. Accessed March 2025 from: https://wildlifepreservation.ca/reptile-amphibian-initiative/

Wingfield JC, Hegner RE, Dufty AM, Ball GF. 1990. The challenge hypothesis: theoretical implications for patterns of testosterone secretion, mating systems, and breeding strategies. The American Naturalist 136: 829–846. https://doi.org/10.1086/285134.

Witzenberger KA, Hochkirch A. 2011. Ex situ conservation genetics: A review of molecular studies on the genetic consequences of captive breeding programmes for endangered animal species. Biodiversity and Conservation 20: 1843-1861. https://doi.og/10.1007/s10531-011-0074-4.

Wolak D, Hrabia A. 2020. Tamoxifen-induced alterations in the expression of selected matrix metalloproteinases (MMP-2, -9, -10, and −13) and their tissue inhibitors (TIMP-2 and -3) in the chicken ovary. Theriogenology 148: 208–215. https://doi.org/10.1016/j.theriogenology.2019.11.008.

Wolak D, Sechman A, Hrabia A. 2021. Effect of eCG treatment on gene expression of selected matrix metalloproteinases (MMP-2, MMP-7, MMP-9, MMP-10, and MMP-13) and the tissue inhibitors of metalloproteinases (TIMP-2 and TIMP-3) in the chicken ovary. Animal Reproduction Science 224: 106666. https://doi.org/10.1016/j.anireprosci.2020.106666.

Woodley SK, Leary CJ. 2024. Hormones and reproductive behavior in amphibians. In: Norris DO, Lopez KH, editors. Hormones and Reproduction of Vertebrates. New York (NY): Academic Press. p. 179–217. https://doi.org/10.1016/B978-0-443-16020-2.00004-8.

Woo-Durand C, Matte JM, Cuddihy G, McGourdji CL, Venter O, Grant JWA. 2020. Increasing importance of climate change and other threats to at-risk species in Canada. Environmental Reviews 28: 449–456. https://doi.org/10.1139/er-2020-0032.

World Meteorological Organization (WMO). 2025. WMO confirms 2024 as warmest year on record at about 1.55°C above pre-industrial level. [online]. Accessed 31 January 2025 from: https://wmo.int/news/media-centre/wmo-confirms-2024-warmest-year-record-about-155degc-above-pre-industrial-level.

Wu Q, Song R, Yan W. 2010. SPATA3 and SPATA6 interact with KLHL10 and participate in spermatogenesis. Biology of Reproduction 83: 177. https://doi.org/10.1093/biolreprod/83.s1.177.

Yan W, Ma L, Burns KH, Matzuk MM. 2004. Haploinsufficiency of kelch-like protein homolog 10 causes infertility in male mice. Proceedings of the National Academy of Sciences 101: 7793–7798. https://doi.org/10.1073/pnas.0308025101.

Yatsenko AN, Iwamori N, Iwamori T, Matzuk MM. 2010. The power of mouse genetics to study spermatogenesis. Journal of Andrology 31: 34–44. https://doi.org/10.2164/jandrol.109.008227.

Yermokhin MV, Tabachishin VG. 2022. False spring in the spawning migrations of spadefoot toads (*Pelobates*, Anura): distribution in European Russia and the phenomenon scale in 2020. Biology Bulletin 49: 1883–1889. https://doi.org/10.1134/S1062359022100235.

Yokum EE, Wascher M, Goldstein DL, Krane CM. 2023. Repeated freeze-thaw cycles in freeze-tolerant treefrogs: novel interindividual variation of integrative biochemical, cellular, and organismal responses. American Journal of Physiology-Regulatory, Integrative and Comparative Physiology 324: R196–R206. https://doi.org/ 10.1152/ajpregu.00211.2022.

Yu J, Zhang L, Li Y, Zhu X, Xu S, Zhou X-M, Wang H, Zhang H, Liang B, Liu P. 2018. The adrenal lipid droplet is a new site for steroid hormone metabolism. PROTEOMICS 18: 1800136. https://doi.org/10.1002/pmic.201800136.

Zaffaroni-Caorsi V, Both C, Márquez R, Llusia D, Narins P, Debon M, Borges-Martins M. 2023. Effects of anthropogenic noise on anuran amphibians. Bioacoustics 32: 90–120. https://doi.org/10.1080/09524622.2022.2070543.

Zehavi Y, Kedmi A, Ideses D, Juven-Gershon T. 2015. TRF2: TRansForming the view of general transcription factors. Transcription 6: 1–6. https://doi.org/10.1080/21541264.2015.1004980.

Zeileis A, Cribari-Neto F, Grün B, Kosmidis I, Simas AB, Rocha AV. 2024. betareg: Beta regression. R package version 3.2-0. Accessed 28 August 2024. https://cran.r-project.org/web/packages/betareg/index.html

Zhao L, Wang J, Cai Y, Ran J, Brauth SE, Tang Y, Cui J. 2021. Behavioral and neurogenomic responses to acoustic and visual sexual cues are correlated in female torrent frogs. Asian Herpetological Research 12: 88–99. https://doi.org/10.16373/j.cnki.ahr.200063.

Zhu J, Long T, Gao L, Zhong Y, Wang P, Wang X, Li Z, Hu Z. 2023. RPL21 interacts with LAMP3 to promote colorectal cancer invasion and metastasis by regulating focal adhesion formation. Cellular & Molecular Biology Letters 28: 31. https://doi.org/10.1186/s11658-023-00443-y.

Zippel K, Johnson K, Gagliardo R, Gibson R, McFadden M, Browne R, Martinez C, Townsend E. 2011. The Amphibian Ark: A Global Community for Ex Situ Conservation of Amphibians. Herpetological Conservation and Biology 6: 340–352.

Zohar Y, Mylonas CC. 2001. Endocrine manipulations of spawning in cultured fish: from hormones to genes. Reproductive Biotechnology in Finfish Aquaculture 2001: 99–136. https://doi.org/10.1016/B978-0-444-50913-0.50009-6.

Appendix A: Supplementary Material

Table S2.1: List of official common names and scientific names of chorus frogs (genus *Pseudacris:* Hylidae), Society for the Study of Amphibians and Reptiles (57)

Common Name	Scientific Name
Mountain Chorus Frog	*Pseudacris brachyphona* (Cope, 1889)
Brimley's Chorus Frog	*Pseudacris brimleyi* (Brandt and Walker, 1933)
California Chorus Frog	*Pseudacris cadaverina* (Cope, 1866)
Spotted Chorus Frog	*Pseudacris clarkii* (Baird, 1854)
Collinses' Mountain Chorus Frog	*Pseudacris collinsorum* (Ospina, Tieu, Apodaca, and Lemmon, 2020)
Spring Peeper	*Pseudacris crucifer* (Wied-Neuwied, 1838)
Upland Chorus Frog	*Pseudacris feriarum* (Baird, 1854)
Cajun Chorus Frog	*Pseudacris fouquettei* (Lemmon, Lemmon, Collins, and Cannatella, 2008)
Baja California Treefrog	*Pseudacris hypochondriaca* (Hallowell, 1854)
Illinois Chorus Frog	*Pseudacris illinoensis* (Smith, 1951)
New Jersey Chorus Frog	*Pseudacris kalmi* (Harper, 1955)
Boreal Chorus Frog	*Pseudacris maculate* (Agassiz, 1850)
Southern Chorus Frog	*Pseudacris nigrita* (LeConte, 1825)
Little Grass Frog	*Pseudacris ocularis* (Holbrook, 1838)
Ornate Chorus Frog	*Pseudacris ornate* (Holbrook, 1836)
Northern Pacific Treefrog	*Pseudacris regilla* (Baird and Girard, 1852)
Sierran Treefrog	*Pseudacris sierra* (Jameson, Mackey, and Richmond, 1966)
Strecker's Chorus Frog	*Pseudacris streckeri* (Wright and Wright, 1933)
Western Chorus Frog	*Pseudacris triseriata* (Wied-Neuwied, 1838)

Table S2.2: List of key words used in Google Scholar and Web of Science databases to retrieve articles on life history traits, survival rates, and longevity of *Pseudacris* species in North America. For Web of Science searches, "TS" is used to search terms within article topics and "TI" is used to search for terms in article titles.

Database	Acronym Used
Web of Science	TS=(chorus frog* OR Pseudacris OR Trilling frog*) AND TS=(survival OR survivorship OR fertility OR demography OR longevity OR lifespan OR dynamic OR growth OR reproductive success OR life history) TI=(chorus frog* OR Pseudacris OR Trilling frog*) AND TS= (survival OR survivorship OR recruitment* OR population size OR demography OR longevity OR lifespan OR population dynamic OR growth rate OR reproductive success OR life history) TI=(chorus frog* OR Pseudacris OR Trilling frog*) AND TI=(survival OR survivorship OR fertility OR demography OR longevity OR lifespan OR dynamic OR growth OR reproductive success OR life history) TI=(chorus frog* OR Pseudacris maculata OR boreal chorus frog OR Pseudacris triseriata OR Pseudacris crucifer) AND TI =(survival OR survivorship OR fertility OR demography OR longevity OR lifespan OR dynamic OR growth OR reproductive success) TS=(chorus frog* OR Pseudacris maculata OR boreal chorus frog OR Pseudacris triseriata OR Pseudacris crucifer) AND TI =(survival OR survivorship OR fertility OR demography OR longevity OR lifespan OR dynamic OR growth OR reproductive success) TI=(chorus frog* OR Pseudacris maculata OR boreal chorus frog OR Pseudacris triseriata OR Pseudacris crucifer) AND TI=(survival OR survivorship OR fertility OR demography OR longevity OR lifespan OR dynamic OR growth OR reproductive success OR life history) TS=(egg OR larvae OR tadpole OR clutch OR life history) AND TS=(chorus frog OR Pseudacris) TS=(eggs OR larvae OR tadpole OR fertility OR oviposition OR life-history) AND TI=(chorus frog OR Pseudacris)
Google Scholar	Survival OR survivorship OR fertility OR demography OR longevity OR lifespan OR dynamic OR growth OR reproductive OR success OR life history "chorus frog* OR Pseudacris OR Trilling frog*" (chorus frog* OR Pseudacris maculata OR boreal chorus frog OR Pseudacris triseriata OR Pseudacris crucifer) AND (survival OR survivorship OR fertility OR demography OR longevity OR lifespan OR dynamic OR growth OR reproductive success OR life history) (chorus frog* OR Pseudacris maculata OR boreal chorus frog OR Pseudacris triseriata OR Pseudacris crucifer) AND (survival OR survivorship OR fertility OR demography OR longevity OR lifespan OR dynamic OR growth OR reproductive success) (egg* OR larva* OR tadpole OR clutch OR reproductive OR life history) AND ("chorus frog*" OR Pseudacris OR "Trilling frog*") Pseudacris eggs OR larva OR tadpole OR fertility OR oviposition OR "life history" "chorus frog" ("Hyla cadaverina") AND (clutch OR "life history" OR reproduction OR oviposition OR development) ("Hyla californiae") AND (clutch OR "life history" OR reproduction OR oviposition OR development) ("Hyla crucifer") AND (clutch OR "life history" OR reproduction OR oviposition OR development) ("Hyla ocularis") AND (clutch OR "life history" OR reproduction OR oviposition OR development) ("Hyla regilla") AND (clutch OR "life history" OR reproduction OR oviposition OR development) ("Hyliola cadaverina") AND (clutch OR "life history" OR reproduction OR oviposition OR development) ("Hyliola regilla") AND (clutch OR "life history" OR reproduction OR oviposition OR development)

Table S2.3: List of abbreviations of the countries, and their provinces, states and territories, of North America using the 2-letter state/province/territory codes for USA and Canada and the 3-letter state codes for Mexico (ISO 3166-2)

Country	State/Province/Territory	Code
Canada (CAN)	Alberta	AB
	British Columbia	BC
	Manitoba	MB
	New Brunswick	NB
	Newfoundland and Labrador	NL
	Northwest Territories	NT
	Nova Scotia	NS
	Nunavut	NU
	Ontario	ON
	Prince Edward Island	PE
	Quebec	QC
	Saskatchewan	SK
	Yukon	YT
United States of America (USA)	Alabama	AL
	Alaska	AK
	Arizona	AZ
	Arkansas	AR
	California	CA
	Colorado	CO
	Connecticut	CT
	Delaware	DE
	District of Columbia	DC
	Florida	FL
	Georgia	GA
	Hawaii	HI
	Idaho	ID
	Illinois	IL
	Indiana	IN
	Iowa	IA
	Kansas	KS
	Kentucky	KY
	Louisiana	LA
	Maine	ME
	Maryland	MD
	Massachusetts	MA
	Michigan	MI
	Minnesota	MN
	Mississippi	MS
	Missouri	MO

Table S2.3 (continued): List of abbreviations of the countries, and their provinces, states and territories, of North America using the 2-letter state/province/territory codes for USA and Canada and the 3-letter state codes for Mexico (ISO 3166-2)

Country	State/Province/Territory	Code
United States of America (USA)	Montana	MT
	Nebraska	NE
	Nevada	NV
	New Hampshire	NH
	New Jersey	NJ
	New Mexico	NM
	New York	NY
	North Carolina	NC
	North Dakota	ND
	Ohio	OH
	Oklahoma	OK
	Oregon	OR
	Pennsylvania	PA
	Rhode Island	RI
	South Carolina	SC
	South Dakota	SD
	Tennessee	TN
	Texas	TX
	Utah	UT
	Vermont	VT
	Virginia	VA
	Washington	WA
	West Virginia	WV
	Wisconsin	WI
	Wyoming	WY
Mexico (MEX)	Aguascalientes	AGU
	Baja California	BCN
	Baja California Sur	BCS
	Campeche	CAM
	Chiapas	CHP
	Chihuahua	CHH
	Coahuila	COA
	Colima	COL
	Mexico City	CMX
	Durango	DUR
	Guanajuato	GUA
	Guerrero	GRO
	Hidalgo	HID
	Jalisco	JAL
	México	MEX

Table S2.3 (continued): List of abbreviations of the countries, and their provinces, states and territories, of North America using the 2-letter state/province/territory codes for USA and Canada and the 3-letter state codes for Mexico (ISO 3166-2)

Country	State/Province/Territory	Code
Mexico (MEX)	Michoacán	MIC
	Morelos	MOR
	Nayarit	NAY
	Nuevo León	NLE
	Oaxaca	OAX
	Puebla	PUE
	Querétaro	QUE
	Quintana Roo	ROO
	San Luis Potosí	SLP
	Sinaloa	SIN
	Sonora	SON
	Tabasco	TAB
	Tamaulipas	TAM
	Tlaxcala	TLA
	Veracruz	VER
	Yucatán	YUC
	Zacatecas	ZAC

Table S2.4: Explanation of the NatureServe status rank codes. NatureServes status ranks are based on species rarity, severity of threats, and population trends. These three broad categories are individually scaled and weighted based on their overall impact on the risk of extirpation. The scores for each category are combined to give an overall ranking, which is reviewed and then accepted (NatureServe 2021). As an example, we use the subnational (S) prefix.

SX	Presumed Extirpated – no evidence of species being extant despite extensive searches and very low likelihood of the species being rediscovered.
SH	Possibly Extirpated – historical records exist but has not been recently documented (20-40 years) but there is uncertainty about this assessment as thorough searches have not been performed.
S1	Critically Imperiled – very high risk of extirpation due to restricted range, low abundance, recent and widespread declines, threats, or other factors.
S2	Imperiled – high risk of extirpation due to restricted range, low abundance, recent and widespread declines, threats, or other factors.
S3	Vulnerable – moderate risk of extirpation due to restricted range, low abundance, recent and widespread declines, threats, or other factors.
S4	Apparently Secure – relatively low risk of extirpation but certain populations may be a risk of local declines, threats, or other factors.
S5	Secure – very low or no risk of extirpation.
SNR	Unranked – status not yet assessed.
SU	Unrankable – unable to rank due to a lack of information or due to conflicting information.
S#S#	Range Rank – used to indicate uncertainty

Table S3.1: Number of days between March 30th–May 15th (DOY 89–135) with at least one individual calling during the 2022–2024 anuran breeding seasons in Leeds and Grenville County tallied by species. DOY = day of the year.

Year	*Pseudacris maculata*	*Boreorana sylvatica*	*Anaxyrus americanus*	*Pseudacris crucifer*
2022	43	19	21	41
2023	40	20	16	33
2024	38	19	28	45

Table S3.2: Total monthly rain accumulation (mm) and mean daily accumulation per month by year (2022– 2024). Values in brackets are the standard deviation. Precipitation data obtained from Environment and Climate Change Canada – Meteorological Service Canada.

		2022	**2023**	**2024**
Total Monthly Accumulation	March	70.7	76.3	62.2
	April	87.5	138.3	127.8
	May	134.5	63.8	80.8
	Total	292.7	278.4	270.8
Mean daily Accumulation	March	2.3 (3.6)	2.5 (4.8)	2.0 (3.4)
	April	2.9 (6.7)	4.6 (12.6)	4.3 (6.4)
	May	4.3 (10.5)	2.1 (5.4)	2.6 (5.4)
	Total	3.2 (7.5)	3.0 (8.4)	2.9 (5.3)

Table S3.3: Summary of generalized linear mixed effects model of the fixed and random effects of abiotic and biotic factors influencing boreal chorus frog (*Pseudacris maculata*) calling activity which includes the days of the year sampled in 2024 that were not sampled in 2022 and 2023 (*i.e.*, DOY 72–88). This demonstrates that the inclusion of the additional 16 days does not impact the conclusions regarding fixed effects (Table 3.2) DOY = day of the year; LISY = presence (1) or absence (0) of wood frog (*Boreorana sylvatica*); ANAM = presence (1) or absence (0) of American toad (*Anaxyrus americanus*); PSCR = presence (1) or absence (0) of spring peeper (*Pseudacris crucifer*); τ00 = between-subjects; ICC = intraclass correlation coefficient.

Fixed Effects		**Estimate**	**SE**	**z value**	**p**
(Intercept)		0.00	0.23	0.02	0.988
DOY (Linear)		-1.78	0.06	-27.35	< 0.001
DOY (Quadratic)		-1.01	0.04	-23.00	< 0.001
Year (=false spring)					
	Average year	2.04	0.09	23.29	< 0.001
Time of Day (=Night)					
	Morning	-0.64	0.09	-7.53	< 0.001
	Afternoon	-0.43	0.10	-4.30	<0.001
	Evening	-0.26	0.08	-3.26	0.001
Temperature		1.22	0.05	27.14	< 0.001
Rainfall (=0)		-0.01	0.03	-0.31	0.760
LISY (=0)		1.58	0.15	10.65	< 0.001
ANAM (=0)		1.19	0.13	8.90	< 0.001
PSCR (=0)		1.08	0.08	13.91	< 0.001
DOY (Linear):Year Type					
	DOY:Year Type (average)	2.30	0.08	28.49	< 0.001
DOY (Quadratic):Year Type					
	DOY:Year Type (average)	-1.78	0.08	-20.98	< 0.001
Random Effects (Site)		**Value**			
σ^2		3.29			
$\tau_{00\ Site}$		0.17			
ICC		0.05			
N_{Site}		4			
Observations		10558			
Marginal R^2 / Conditional R^2		0.609 / 0.628			

Table S3.4: Comparison of boreal chorus frog (*Pseudacris maculata*) reproductive output and animal husbandry metrics between Montréal Biodôme and University of Ottawa (uOttawa) facilities, 2021–2023.

		Biodôme 2021	Biodôme 2022	Biodôme 2023	uOttawa 2021	uOttawa 2022	uOttawa 2023
# Eggs		3076	3557	—	988	9283	8870
# Tadpoles Hatched		2397	2152	4081	632	6540	5195
% Tadpoles Hatched		77.90%	60.50%	—	64.00%	70.50%	58.60%*
Hatch Date	**Min**	02-Apr	20-Apr	21-Apr	13-Apr	25-Apr	28-Apr
	Max	17-Apr	28-Apr	30-Apr	25-Apr	03-May	05-May
Length of Larval Stage (days)	**Min**	64	—	—	35	74	27
	Max	130	—	—	125	119	123
	Mean	89.9	—	—	77.5	100.4	86.9
# Metamorphs Emerged		395/1207	103/293	748/1013	210/632	277/865	281/676
% Metamorphs Emerged		32.70%	35.15%	73.84%	33.20%	32.00%	41.60%
Date of Emergence	**Min**	11-Jun	—	—	28-May	13-Jul	01-Jun
	Max	16-Aug	—	—	16-Aug	28-Aug	05-Sep
Emergence Rate (#/day)	**Mean**	5.9	—	—	2.6	5.9	2.9
	Median	2	—	—	2	6	2
	Max	30	—	—	15	24	14

***NOTE**: 7 of 39 females were larger and presumed to be declining in reproductive condition as the eggs from these females had a markedly lower viability (mean = 23.3%, range = 21.4–25.1%) compared to the eggs from the other 32 females (mean = 75.0%, range = 46.9–93.1%).

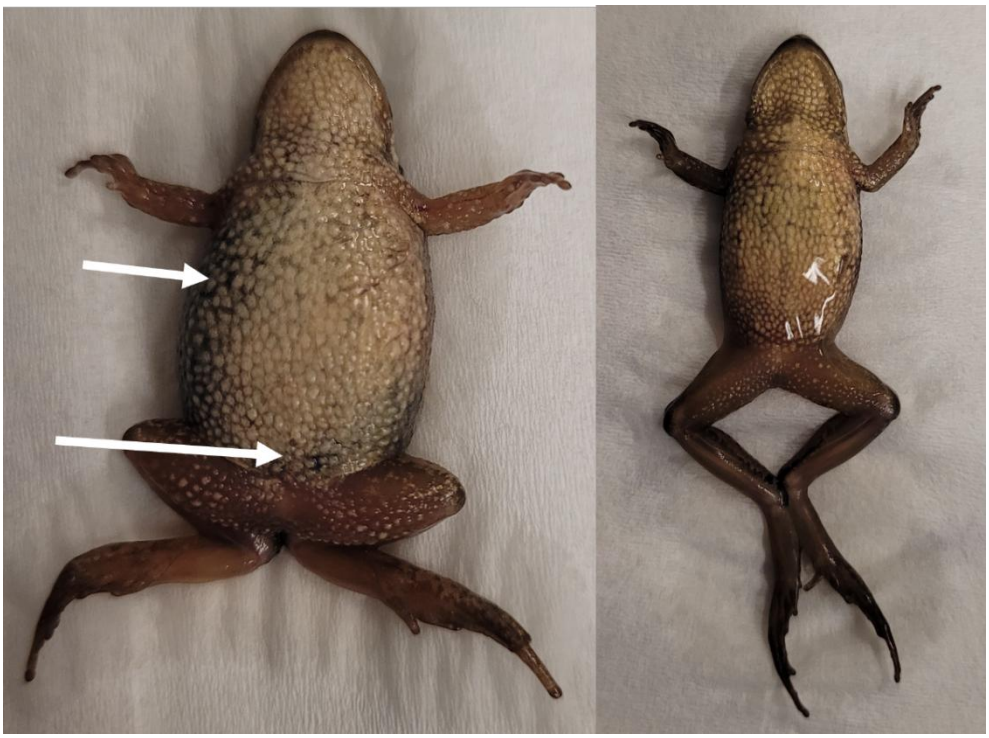

Figure S4.1: Ventral view of the body shape of a gravid (left) and a non-gravid (right) female boreal chorus frog (*Pseudacris maculata*). Gravid females are considerably rounder in body shape (both in dorsal-ventral and lateral view) with the width of the body greater than the width of the head. Note the eggs (black dots indicated by arrows) which are visible through the stretched skin of the gravid female. In non-gravid females, the width of body is equal to or less than the width of the head.

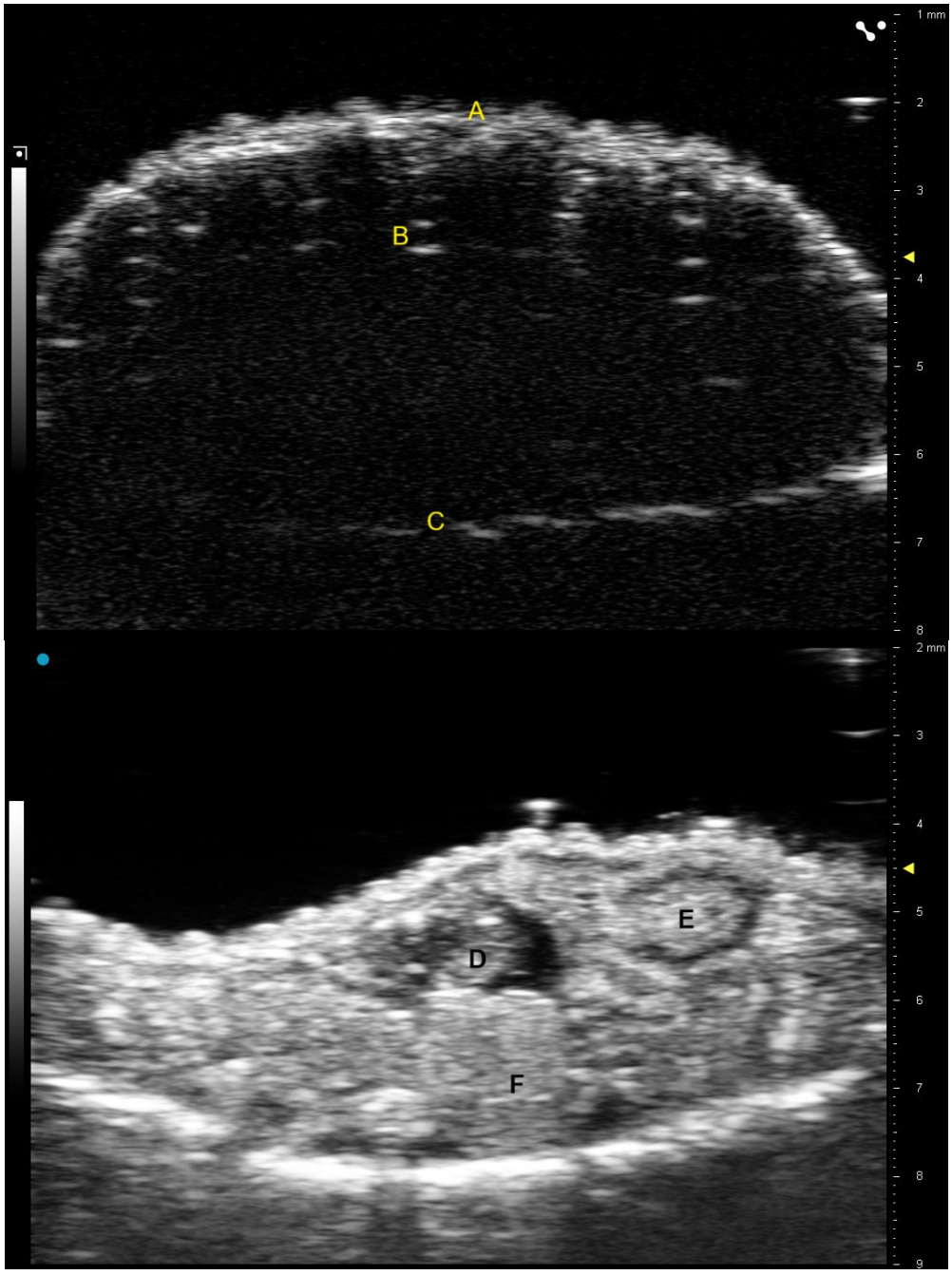

Figure S4.2: Ultrasound imaging of a gravid (top) and non-gravid (bottom) female boreal chorus frog (*Pseudacris maculata*). In ultrasound images, black areas indicate fluids while grey to white areas indicate tissues and bones. Note the ventral surface (A) of the frog where the texture of the skin is visible. Eggs are primary imaged as dark areas within the body cavity since eggs are mostly aqueous, with the upper and lower surface of eggs occasionally being visible as curved grey lines (B). The dorsal surface (C) of the frog is often visible as well but tends to be less apparent when imagining gravid females. Bottom image is a non-gravid female for comparison, including a cross-section of the heart (D) and a portion of liver (E). The ventral and dorsal surfaces are more visible. Without eggs, contents of body cavity (F) tend to be imagined as light to dark grey.

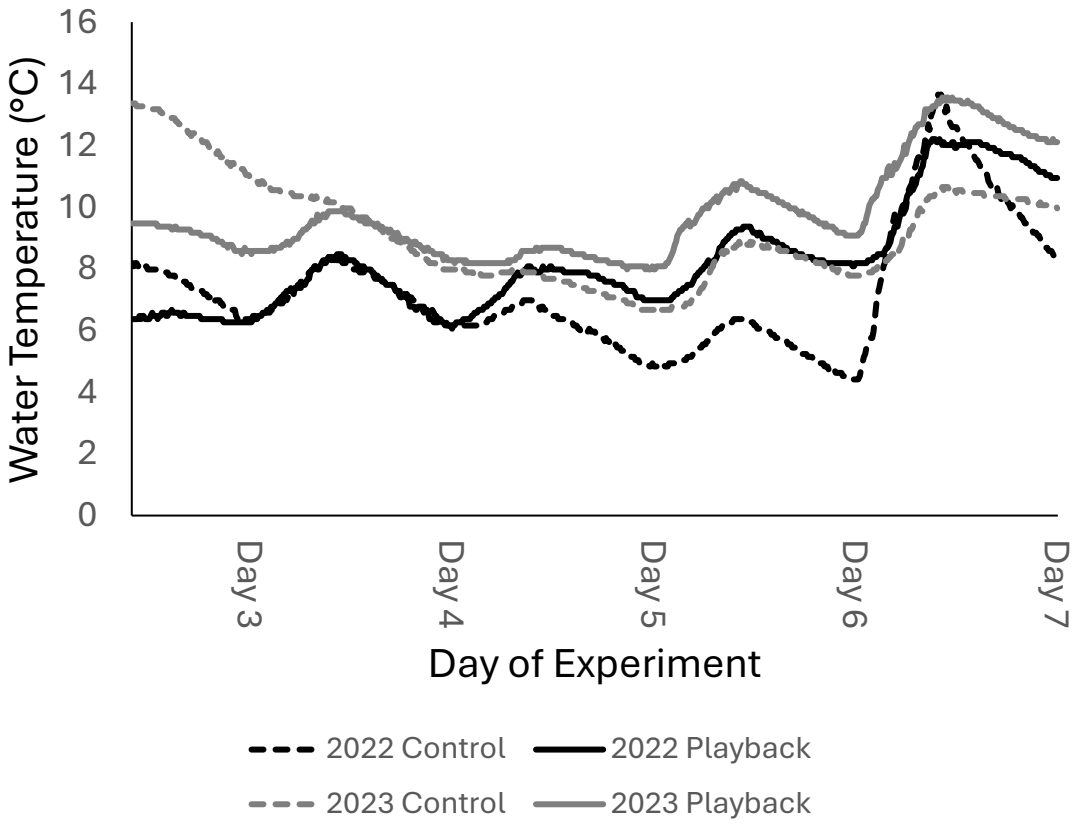

Figure S4.3: Water temperature (°C) of breeding containers during boreal chorus frog (*Pseudacris maculata*) spawning experiment. In the control group (April 20–26, 2022 and April 23–30, 2023) male and female frogs were exposed for 6 hr from 18:00–24:00 each day to ambient sounds from a local breeding pond recorded just prior to chorus frog breeding season (April–May) and prior to any calling behaviour of any frog species. In the playback group (April 13–19, 2022 and April 23–30, 2023) male and female frogs were exposed to a recording of a chorus of conspecific frogs calling for 6 hr from 18:00–24:00 each day. Day 1 frogs were injected with a priming dose of GnRHa (0.04 μg/g body weight) and then held at 4°C in a cooler. On Day 2 frogs were injected with a dose of Amphiplex hormone mixture (0.4 μg/g body weight GnRHa and 10 0.4 μg/g body weight MET) and placed into the breeding containers. Broadcasts of control or playback audio occurred during the evenings of Day 2 to Day 6. Frogs were removed from breeding containers on morning of Day 7.

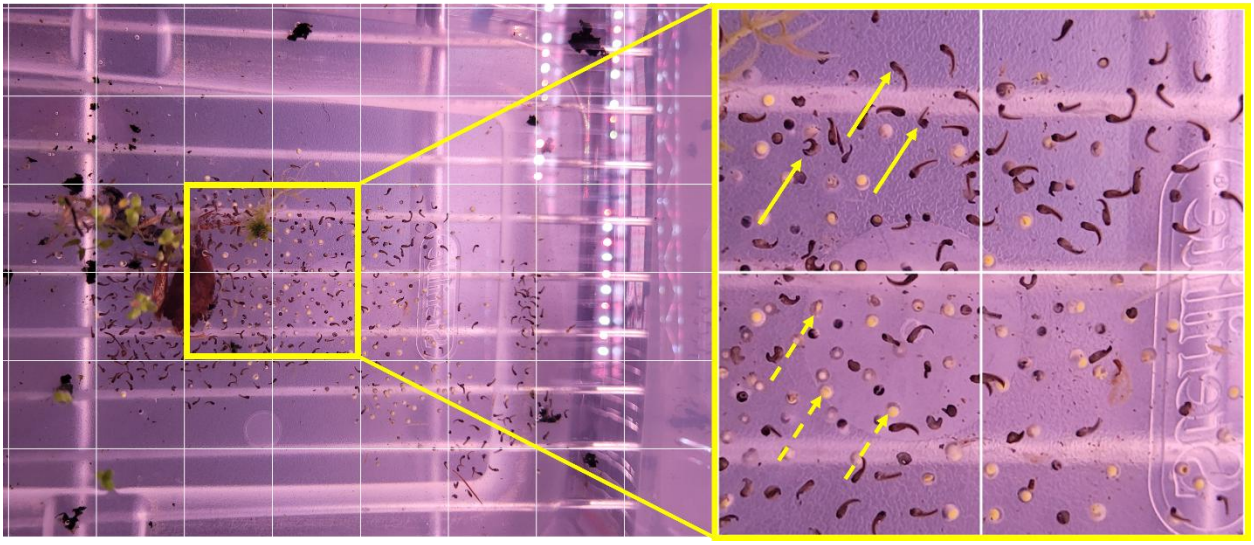

Figure S4.4: Example of the image analysis to determine the viability of boreal chorus frog (*Pseudacris maculata*) eggs in an acoustic playback experiment. Egg trays were photographed four days after being laid from a fixed distance of 40 cm. Images were then overlayed with a 32-cell grid. All eggs and developing tadpoles were counted in five randomly selected cells. Viable eggs/embryos (solid arrows) black, irregular shaped, and begun to develop, having often reached Gosner stage 15–19. Non-viable eggs (dashed arrows) are circular and white-to-yellowish in appearance with no obvious development.

Table S4.1: Mean body weights (g) of male and female boreal chorus frogs (*Pseudacris maculata*) in an acoustic playback experiment. In brackets are the standard deviation of weights.

Breeding container	Female Weight (g)	Male Weight (g)	Treatment	Year
01	1.80 (0.47)	1.24 (0.30)	Playback	2022
02	1.41 (0.26)	1.21 (0.24)	Playback	2022
03	1.67 (0.21)	1.10 (0.19)	Playback	2022
04	1.65 (0.20)	1.13 (0.22)	Playback	2022
05	1.64 (0.20)	0.98 (0.11)	Control	2022
06	1.87 (0.28	1.02 (0.17)	Control	2022
07	1.67 (0.13)	1.02 (0.14)	Control	2022
08	1.42 (0.13)	1.08 (0.27)	Control	2022
09	1.58 (0.18)	1.06 (0.07)	Control	2023
10	1.27 (0.03)	1.02 (0.06)	Control	2023
11	1.15 (0.09)	0.93 (0.04)	Control	2023
12	1.05 (0.06)	0.90 (0.04)	Control	2023
13	0.97 (0.05)	0.86 (0.07)	Control	2023
14	1.55 (0.16)	1.06 (0.07)	Playback	2023
15	1.14 (0.10)	0.93 (0.06)	Playback	2023
16	0.96 (0.09)	0.93 (0.05)	Playback	2023
17	0.92 (0.07)	0.86 (0.05)	Playback	2023
18	0.90 (0.03)	0.81 (0.03)	Playback	2023

Table S4.2: Food type, volumes, and nutritional information of the food fed to boreal chorus frog (*Pseudacris maculata*) tadpoles in experiment to determine the effect of broadcast of conspecific calls on the reproductive output and offspring quality.

<table>
<tr><td colspan="2">Every 2 days
• 90 g of Sera Micron Nature
• 4 mL of Seachem Reef Phytoplankton
• 20 mL of rotifers (supplied by uOttawa ACVS facility)
• 6 g of ground Nasco Adult Frog Brittle

Every 7 days
• 0.6 g of boiled spinach</td></tr>
<tr><td>Sera Micron Nature
(SKU: 00720)</td><td><u>Ingredients:</u>
spirulina (51%), krill (18%), brine shrimps, fish meal, wheat flour, stinging nettle, herbs, alfalfa, brewers yeast, parsley, sea algae, paprika, Ca-caseinate, gammarus, cod-liver oil (containing 34% omega fatty acids), spinach, carrots, mannan oligosaccharides, Haematococcus algae, green-lipped mussel, garlic.
<u>Guaranteed Analysis:</u>
Protein (min): 55.6%
Crude Fat (min): 6.2%
Crude Fiber (max): 11.4%
Moisture (max): 7.0%
Ash (max): 11.3%
<u>Additives:</u>
Vitamins and provitamins: Vit. A 2,800 IU/lb., Vit. D3 135 IU/lb., Vit. E (D, L-α-tocopheryl acetate) 9 IU/lb., Vit. B1 2.7 mg/lb., Vit. B2 6.8 mg/lb., Stabilized Vit. C (L-ascorbyl monophosphate) 41 mg/lb.</td></tr>
<tr><td>Seachem Reef Phytoplankton
(SKU: 20343)</td><td><u>Ingredients:</u>
Water, Sodium chloride, Thalassiosira weissflogii, Acetic Acid, Isochrysis sp, Ascorbic Acid, Citric Acid, Nannochloropsis, Astaxanthin
<u>Guaranteed Analysis:</u>
Protein (min): 0.40%
Crude Fat (min): 0.15%
Crude Fiber (max): 0.5%
Moisture (max): 96.8%
Ash (max): 3.0%</td></tr>
</table>

Table S4.2 (continued): Food type, volumes, and nutritional information of the food fed to boreal chorus frog (*Pseudacris maculata*) tadpoles in experiment to determine the effect of broadcast of conspecific calls on the reproductive output and offspring quality.

Nasco Adult Frog Brittle (SKU: SA05961[LM])	Ingredients: Fish meal, porcine meat and bone meal, dehulled soybean meal, ground corn, wheat flour, brewers dried yeast, dried egg product, glyceryl monostearate, corn distillers dried grains with solubles, whey, wheat germ, salt, choline chloride, pyridoxine hydrochloride, l-ascorbyl-2-polyphosphate (stabilized vitamin C), dl-alpha tocopheryl acetate (form of vitamin E), biotin, cholecalciferol (form of vitamin D3), vitamin A acetate, calcium carbonate, calcium pantothenate, menadione sodium bisulfite complex (source of vitamin K), ethoxyquin (a preservative), thiamine mononitrate, folic acid, riboflavin supplement, nicotinic acid, vitamin B12 supplement, manganous oxide, zinc oxide, ferrous carbonate, copper sulfate, zinc sulfate, calcium iodate, cobalt carbonate, sodium selenite. Guaranteed Analysis: Protein (min): 44.0% Crude Fat (min): 6.0% Crude Fiber (max): 5.0% Moisture (max): 12.0% Ash (max): 15%

Table S5.1: Differentially expressed genes in the Low Chorus x Wind comparison at Timepoint 1 (6 hrs) of testicular samples. Gene symbol and name based on description within the Swiss-Prot database (https://www.sib.swiss/swiss-prot).

Gene Symbol	**Name (species)**	**log2FC**	**P(adj)**
Tnnt3	troponin T3 (*Mus musculus*)	7.14	< 0.0001
METTL11B	methyltransferase 1B (*Xenopus tropicalis*)	-5.25	0.0027
ccnf	cyclin-F (*Nanorana parkeri*)	5.25	0.0027
Rab3il1	guanine nucleotide exchange factor for Rab-3A isoform X4 (*Nanorana parkeri*)	-5.17	0.0028
Pde4dip	phosphodiesterase 4D interacting protein (*Xenopus tropicalis*)	5.17	0.0028
AOX2	aldehyde oxidase-like (*Nanorana parkeri*)	-5.04	0.0047
RRNAD1	ribosomal RNA adenine dimethylase domain containing 1 (*Xenopus tropicalis*)	-4.76	0.0105
TMEM8C	transmembrane protein 8C (*Xenopus laevis*)	4.72	0.0109
RGS19	regulator of G-protein signaling 19 S homeolog isoform X2 (*Xenopus laevis*)	4.64	0.0137
cat	catalase (*Nanorana parkeri*)	-4.55	0.0153
CRYAB	Alpha-crystallin B chain (*Lithobates catesbeiana*)	-4.53	0.0160
ADGRF3	adhesion G-protein coupled receptor F3 (*Terrapene mexicana triunguis*)	-4.45	0.0205
Spp1	osteopontin isoform 2 (*Mus musculus*)	-4.36	0.0296
Abcc8	ATP-binding cassette sub-family C member 8-like (*Xenopus laevis*)	4.33	0.0328
SLC34A2	solute carrier family 34 member 2 (*Rana catesbeiana*)	-4.29	0.0385
CMAS	N-acylneuraminate cytidylyltransferase (*Nanorana parkeri*)	4.21	0.0487
ZCCHC11	zinc finger CCHC-type containing 11 (*Nanorana parkeri*)	4.20	0.0494

Table S5.2: Summary of testicular gene expression at 6, 24, and 30 hrs within High Chorus and Low Chorus treatments. For each treatment the differential gene expression in comparison to the Wind treatment (6 hrs) is provided as reference. Colour indicates upregulation (red) or downregulation (blue) of gene based on log2 fold change (1.5 > log2FC < -1.5) and adjusted p-value (< 0.05).

steroid biosynthetic process (GO:0006694)

ID	Gene	High 6h vs Wind 6h		High 24h vs High 6h		High 30h vs High 24h		Low 6h vs Wind 6h		Low 24h vs Low 6h		Low 30h vs Low 24h	
		log2FC	P(adj)	log2FC	P(adj)	log2FC	P(adj)	log2FC	P(adj)	log2FC	P(adj)	log2FC	P(adj)
TRINITY_DN111256_c1_g3	*Dhcr24*	**2.96**	0.009	**-2.87**	0.000	1.56	0.393	-0.06	0.999	2.07	0.398	**-2.62**	0.041
TRINITY_DN113871_c0_g2	*FDFT1*	**2.53**	0.038	**-2.74**	0.000	1.86	0.146	-1.87	0.760	**2.95**	0.003	-1.56	0.221
TRINITY_DN116674_c2_g3	*Tspo*	**5.48**	0.000	**-2.98**	0.000	1.16	0.609	-0.33	1.000	3.78	0.054	**-4.67**	0.041
TRINITY_DN131188_c0_g1	*Scp2d1*	5.36	0.475	-5.45	0.280	–	–	–	–	–	–	–	–
TRINITY_DN19878_c0_g1	*Cyp17a1*	**5.65**	0.002	**-3.50**	0.024	0.34	1.000	-0.56	1.000	0.80	1.000	-0.73	1.000
TRINITY_DN54102_c0_g1	*Hsd17b11*	**5.01**	0.000	**-3.68**	0.000	1.59	0.468	-1.57	1.000	**5.28**	0.007	**-4.83**	0.030
TRINITY_DN65460_c2_g2	*Hsd17b12*	**4.14**	0.000	**-2.32**	0.000	0.92	0.678	-0.47	0.998	3.38	0.118	-3.72	0.111
TRINITY_DN74172_c4_g1	*Cyb5r1*	1.54	0.435	**-2.00**	0.000	**1.54**	0.024	-3.58	0.356	**5.45**	0.000	**-2.85**	0.043
TRINITY_DN78062_c2_g2	*Sc5d*	**3.41**	0.000	**-2.81**	0.000	1.02	0.796	-3.01	1.000	**5.20**	0.003	**-4.08**	0.029
TRINITY_DN78735_c0_g1	*Scp2*	**4.11**	0.000	**-2.33**	0.000	0.81	0.681	-0.40	0.998	**2.90**	0.028	**-3.00**	0.012
TRINITY_DN83670_c0_g1	*Cyb5r3*	**4.60**	0.000	**-2.17**	0.001	0.66	0.904	-1.86	0.942	**5.33**	0.029	-3.98	0.061
TRINITY_DN90946_c0_g2	*Tecr*	**4.21**	0.000	**-2.67**	0.000	1.02	0.658	-0.09	0.998	2.91	0.190	**-4.19**	0.018
TRINITY_DN91956_c2_g1	*Hmgcs1*	**3.57**	0.000	**-3.29**	0.000	2.02	0.174	-1.22	0.977	3.14	0.051	**-2.59**	0.043
TRINITY_DN95069_c3_g1	*Msmo1*	**2.82**	0.025	**-3.10**	0.000	1.16	0.451	-1.89	0.846	**3.22**	0.008	-2.13	0.060

male gonad development (GO:0008584)

ID	Gene	High 6h vs Wind 6h		High 24h vs High 6h		High 30h vs High 24h		Low 6h vs Wind 6h		Low 24h vs Low 6h		Low 30h vs Low 24h	
		log2FC	P(adj)	log2FC	P(adj)	log2FC	P(adj)	log2FC	P(adj)	log2FC	P(adj)	log2FC	P(adj)
TRINITY_DN100710_c0_g3	*Insl6*	**5.53**	0.018	**-4.53**	0.013	1.43	1.000	–	–	3.56	1.000	-3.49	1.000
TRINITY_DN106049_c0_g2	*BCL2L1*	**3.01**	0.001	**-2.10**	0.000	**1.73**	0.020	-2.38	0.658	**4.54**	0.000	**-2.92**	0.009
TRINITY_DN106744_c1_g1	*Cited2*	**3.26**	0.006	-1.29	0.009	**1.99**	0.021	-1.65	0.910	**3.81**	0.000	**-2.85**	0.004
TRINITY_DN107686_c0_g1	*Ybx3*	**5.16**	0.000	**-3.54**	0.000	1.45	0.099	0.66	0.995	1.92	0.289	-1.85	0.237
TRINITY_DN109406_c2_g1	*Kdr*	**5.40**	0.000	**-3.49**	0.000	1.43	0.854	-0.58	1.000	3.25	0.395	-3.80	1.000
TRINITY_DN109503_c2_g2	*Eif2s2*	**2.66**	0.032	**-2.13**	0.000	1.25	0.221	-1.38	0.925	**3.19**	0.004	-1.95	0.174
TRINITY_DN112537_c3_g2	*Tbc1d20*	**3.56**	0.000	**-3.76**	0.000	1.02	0.390	-1.69	0.799	**4.33**	0.000	**-3.10**	0.035
TRINITY_DN112800_c0_g1	*Wdr48*	**4.82**	0.000	**-3.86**	0.000	1.34	0.439	-2.22	1.000	**4.24**	0.007	**-3.76**	0.017
TRINITY_DN115851_c1_g4	*PRPS1*	3.91	0.635	-3.99	1.000	–	–	–	–	–	–	–	–
TRINITY_DN123522_c0_g1	*CSDE1*	**2.38**	0.007	**-2.33**	0.000	1.48	0.092	-1.43	0.836	**2.55**	0.019	-1.79	0.175
TRINITY_DN130557_c0_g1	*Klhl10*	**21.56**	0.000	**-22.41**	0.000	0.34	1.000	–	–	–	–	–	–

TRINITY_DN1311_c0_g1	*ANKRD7*	4.93	0.543	-5.02	0.337	–	–	–	–	–	–	–	–
TRINITY_DN131297_c0_g1	*Spink2*	5.95	0.380	-6.03	0.211	0.34	1.000	–	–	–	–	–	–
TRINITY_DN56774_c0_g1	*Nupr1*	1.77	0.297	**-2.14**	0.000	1.52	0.185	-2.27	0.885	3.61	0.062	-2.50	0.371
TRINITY_DN64913_c0_g2	*Rbp4*	**7.05**	0.000	-1.01	0.542	0.18	0.995	0.52		2.01	1.000	-2.65	1.000
TRINITY_DN66237_c2_g2	*Insl3*	**7.19**	0.002	**-6.92**	0.000	1.46	1.000	–	–	0.80	1.000	-0.73	1.000
TRINITY_DN69790_c0_g1	*Prdx4*	2.31	0.090	**-2.16**	0.000	1.06	0.619	-2.11	0.759	**3.90**	0.000	-1.67	0.358
TRINITY_DN69790_c0_g3	*PRDX4*	5.50	0.453	-5.58	0.264	0.00	1.000	–	–	–	–	–	–
TRINITY_DN73303_c4_g4	*Fdps*	2.53	0.079	**-2.74**	0.000	1.13	0.554	-2.09	0.924	**3.75**	0.045	-2.66	0.302
TRINITY_DN74726_c4_g1	*Bax*	**2.68**	0.006	**-1.95**	0.000	0.98	0.328	-2.03	0.699	**4.44**	0.000	**-3.53**	0.009
TRINITY_DN86395_c1_g2	*Hmgb2*	**2.59**	0.073	**-3.72**	0.000	1.74	0.109	-2.18	0.720	**2.61**	0.044	-1.19	0.566
TRINITY_DN86973_c0_g1	*SFRP2*	2.46	0.158	1.34	0.060	-2.26	0.125	-3.09	1.000	**5.20**	0.000	-1.93	0.298
TRINITY_DN94714_c3_g4	*Six4*	-0.30	0.220	0.47	0.006	-0.01	0.996	0.27	0.720	-0.14	0.733	0.36	0.084
TRINITY_DN95226_c4_g4	*CITED2*	**3.77**	0.000	**-1.73**	0.000	**2.01**	0.020	-1.09	1.000	**3.42**	0.001	**-2.44**	0.030
TRINITY_DN96193_c2_g1	*NASP*	**1.96**	0.419	**-3.06**	0.000	0.09	0.991	-2.31	0.708	**2.58**	0.031	-1.06	0.738

response to hormone (GO:0009725)

ID	Gene	**High 6h vs Wind 6h**		**High 24h vs High 6h**		**High 30h vs High 24h**		**Low 6h vs Wind 6h**		**Low 24h vs Low 6h**		**Low 30h vs Low 24h**	
		log2FC	P(adj)	log2FC	P(adj)	log2FC	P(adj)	log2FC	P(adj)	log2FC	P(adj)	log2FC	P(adj)
TRINITY_DN110262_c3_g5	*Timp2*	2.07	0.278	**-2.48**	0.000	0.95	0.785	-2.32	0.854	**4.56**	0.006	**-3.21**	0.022
TRINITY_DN118595_c0_g1	*Mmp14*	1.49	0.535	-0.71	0.400	1.25	0.339	-2.23	0.769	**4.30**	0.001	-2.16	0.162
TRINITY_DN118956_c0_g3	*Lox*	2.26	0.235	**-2.78**	0.000	1.64	0.572	-4.15	0.648	**5.58**	0.005	-2.62	0.413
TRINITY_DN130422_c0_g1	*Mb*	**6.49**	0.000	-0.83	0.786	1.42	0.957	2.93	1.000	-2.69	1.000	–	–
TRINITY_DN130843_c0_g1	*GPX1*	0.50	1.000	-0.58	1.000	–	–	–	–	1.16	1.000	-1.09	1.000
TRINITY_DN64833_c0_g2	*Cox5b*	**5.33**	0.000	**-2.68**	0.000	1.05	0.661	0.03	1.000	3.32	0.114	-3.56	0.093
TRINITY_DN65559_c0_g11	*Mtnd3*	**6.60**	0.000	**-3.39**	0.000	1.19	0.722	1.72	0.914	1.24	0.702	-2.19	0.419
TRINITY_DN68843_c3_g1	*HCLS1*	**4.61**	0.000	**-2.03**	0.000	0.93	0.369	-0.03	0.999	-0.79	0.761	-0.30	0.948
TRINITY_DN76207_c0_g1	*SORD*	**3.49**	0.008	**-3.70**	0.000	1.27	0.316	-1.55	0.902	**3.42**	0.003	**-2.94**	0.022
TRINITY_DN80017_c0_g1	*NCOA4*	**4.94**	0.000	**-3.18**	0.000	1.14	0.621	-1.05	0.993	4.26	0.134	**-4.55**	0.031
TRINITY_DN84281_c3_g2	*Timp1*	2.66	0.075	**-2.71**	0.001	1.39	0.585	-5.57	0.142	**7.30**	0.000	-2.99	0.319
TRINITY_DN84747_c0_g1	*Me1*	**3.96**	0.000	**-2.66**	0.000	0.93	0.697	-0.39	0.998	2.33	0.176	-2.21	0.054
TRINITY_DN87262_c0_g4	*Aqp1*	**6.78**	0.000	**-2.25**	0.004	-0.02	0.998	4.04	1.000	-3.45	1.000	2.48	1.000
TRINITY_DN97922_c2_g1	*Por*	**3.97**	0.000	**-2.74**	0.000	1.29	0.074	-0.83	0.956	1.54	0.159	-1.16	0.353

Table S5.3: Expression of genes of the GO term "Spermatogenesis" (GO:0007283) within the High Chorus treatment. Differential gene expression in comparison to the Wind treatment (6 hrs) is provided as reference. Colour of node indicates upregulation (red) or downregulation (blue) of gene based on log2 fold change (1.5 > log2FC < -1.5) and adjusted p-value (< 0.05).

Spermatogenesis (GO:0007283)		High 6h vs Wind 6h		High 24h vs High 6h		High 30h vs High 24h	
Gene ID	Name	log2FC	p(adj)	log2FC	p(adj)	log2FC	p(adj)
TRINITY_DN100070_c0_g2	*Mea1*	**6.28**	0.000	**-4.48**	0.000	1.13	0.637
TRINITY_DN100710_c0_g3	*Insl6*	**5.53**	0.001	**-4.53**	0.013	1.43	1.000
TRINITY_DN101798_c1_g3	*YY1*	**1.51**	0.000	**-1.72**	0.000	0.78	0.395
TRINITY_DN102286_c1_g2	*Odf3*	5.41	0.069	-5.50	0.274	0.34	1.000
TRINITY_DN10399_c0_g1	*Spata24*	**6.02**	0.000	**-5.22**	0.004	0.71	1.000
TRINITY_DN104982_c0_g2	*Dnaja1*	**2.48**	0.001	**-2.29**	0.000	0.99	0.376
TRINITY_DN105007_c0_g1	*rpl39*	**5.61**	0.000	**-3.86**	0.000	1.92	0.055
TRINITY_DN105632_c0_g1	*SPATA6*	-1.26	0.000	0.94	0.011	-0.62	0.698
TRINITY_DN106049_c0_g2	*BCL2L1*	**3.01**	0.000	**-2.10**	0.000	**1.73**	0.020
TRINITY_DN107686_c0_g1	*Ybx3*	**5.16**	0.000	**-3.54**	0.000	1.45	0.099
TRINITY_DN109872_c0_g5	*Spata19*	**21.34**	0.000	**-22.21**	0.000	–	–
TRINITY_DN110262_c3_g5	*Timp2*	**2.07**	0.026	**-2.48**	0.000	0.95	0.785
TRINITY_DN111123_c2_g5	*Txndc8*	4.55	0.128	-4.63	0.390	–	–
TRINITY_DN112537_c3_g2	*Tbc1d20*	**3.56**	0.000	**-3.76**	0.000	1.02	0.390
TRINITY_DN112800_c0_g1	*Wdr48*	**4.82**	0.000	**-3.86**	0.000	1.34	0.439
TRINITY_DN115500_c2_g2	*JAG2*	-0.14	0.315	-0.03	0.953	0.39	0.311
TRINITY_DN116138_c3_g2	*Sgpl1*	**4.37**	0.000	**-3.22**	0.000	0.91	0.641
TRINITY_DN116402_c3_g1	*SMAD4*	-0.27	0.036	-0.08	0.820	0.13	0.871
TRINITY_DN116438_c0_g2	*ATRX*	-0.23	0.000	0.49	0.000	0.01	0.996
TRINITY_DN117068_c2_g1	*SPO11*	-0.18	0.478	0.49	0.249	0.35	0.859
TRINITY_DN118530_c0_g2	*Znf541*	3.75	0.210	-3.84	1.000	–	–
TRINITY_DN11994_c0_g1	*Slc22a16*	5.71	0.055	-5.80	0.238	–	–
TRINITY_DN120183_c1_g1	*BAG6*	**2.26**	0.010	**-2.53**	0.000	1.02	0.491
TRINITY_DN120581_c0_g1	*Meioc*	-0.66	0.001	0.93	0.001	0.12	0.977
TRINITY_DN120678_c2_g2	*CCNI*	**2.31**	0.001	**-2.52**	0.000	1.29	0.332
TRINITY_DN120821_c1_g4	*Acox1*	**4.31**	0.000	**-1.62**	0.019	0.05	0.997
TRINITY_DN121814_c0_g5	*Spem1*	**21.98**	0.000	-7.99	0.061	-0.38	1.000
TRINITY_DN122450_c1_g4	*USP42*	-0.24	0.060	0.05	0.886	-0.03	0.993
TRINITY_DN122595_c2_g2	*RAD23B*	**2.43**	0.001	**-2.35**	0.000	1.15	0.265
TRINITY_DN123430_c0_g1	*Tdrd9*	-0.25	0.307	1.13	0.000	0.26	0.884
TRINITY_DN123576_c2_g1	*Sept7*	**2.71**	0.001	**-2.25**	0.000	1.42	0.108
TRINITY_DN124093_c1_g1	*Prm3*	4.09	0.171	-4.18	1.000	–	–
TRINITY_DN124834_c1_g5	*Cdk16*	**2.49**	0.000	**-2.47**	0.000	1.00	0.558
TRINITY_DN125420_c0_g2	*BRD2*	**1.74**	0.005	**-1.81**	0.000	1.56	0.233
TRINITY_DN126114_c0_g2	*ODF2*	-0.88	0.000	0.82	0.008	-0.41	0.778
TRINITY_DN126421_c1_g4	*Tsga10*	**6.08**	0.000	**-4.17**	0.013	1.41	1.000
TRINITY_DN126526_c1_g1	*H2AFX*	**3.21**	0.000	**-2.54**	0.000	1.03	0.601
TRINITY_DN128811_c0_g1	*SPEF2*	-0.57	0.015	0.69	0.013	-0.20	0.945
TRINITY_DN1304_c0_g1	*Micalcl*	**5.51**	0.006	**-5.59**	0.042	0.34	1.000

Spermatogenesis (GO:0007283)		High 6h vs Wind 6h		High 24h vs High 6h		High 30h vs High 24h	
Gene ID	Name	log2FC	p(adj)	log2FC	p(adj)	log2FC	p(adj)
TRINITY_DN130555_c0_g1	*Tsnaxip1*	**6.35**	0.033	-6.07	0.206	-0.02	1.000
TRINITY_DN130684_c0_g1	*Odf1*	**21.99**	0.000	**-24.00**	0.000	–	–
TRINITY_DN131008_c0_g1	*Spata9*	4.79	0.108	-4.51	0.406	-0.38	1.000
TRINITY_DN131201_c0_g1	*Pdilt*	5.72	0.055	-5.80	0.237	–	–
TRINITY_DN131297_c0_g1	*Spink2*	**5.95**	0.046	-6.03	0.211	0.34	1.000
TRINITY_DN131836_c0_g1	*Spata25*	5.14	0.085	-5.23	0.309	–	–
TRINITY_DN131913_c0_g1	*Tex40*	**6.40**	0.032	-6.49	0.166	–	–
TRINITY_DN1320_c0_g1	*Ddx4*	**21.72**	0.000	**-22.56**	0.000	1.06	1.000
TRINITY_DN13913_c0_g1	*Calr3*	**4.64**	0.047	-4.73	0.200	1.25	1.000
TRINITY_DN16161_c0_g1	*Smrp1*	**6.03**	0.030	-5.39	0.286	-0.74	1.000
TRINITY_DN16875_c0_g1	*Spata16*	**21.37**	0.000	**-22.22**	0.000	–	–
TRINITY_DN17174_c0_g1	*Tssk6*	**6.35**	0.002	**-6.74**	0.009	0.70	1.000
TRINITY_DN1794_c0_g1	*Rnf151*	**21.16**	0.000	**-21.64**	0.000	0.34	1.000
TRINITY_DN1801_c0_g1	*Mns1*	**5.33**	0.000	**-4.51**	0.003	1.33	1.000
TRINITY_DN18943_c0_g1	*Tbc1d21*	5.21	0.081	-5.29	0.300	–	–
TRINITY_DN23173_c0_g1	*Tssk4*	5.10	0.059	-5.18	0.228	–	–
TRINITY_DN2322_c0_g1	*Shcbp1l*	**6.23**	0.020	-6.32	0.182	0.34	1.000
TRINITY_DN23842_c0_g1	*Tesmin*	**5.78**	0.003	**-5.87**	0.021	0.34	1.000
TRINITY_DN24967_c0_g1	*Spaca1*	**5.86**	0.004	**-4.86**	0.028	-1.10	1.000
TRINITY_DN2710_c0_g1	*H1fnt*	**21.74**	0.000	**-22.60**	0.000	0.34	1.000
TRINITY_DN28905_c0_g1	*Nme5*	4.60	0.123	-2.99	0.451	0.02	1.000
TRINITY_DN3085_c0_g1	*Mael*	**23.11**	0.000	**-9.15**	0.023	-0.38	1.000
TRINITY_DN3339_c0_g1	*Mroh2b*	**22.33**	0.000	**-23.15**	0.000	–	–
TRINITY_DN35039_c0_g1	*BCAP31*	5.80	0.052	-5.88	0.228	0.00	1.000
TRINITY_DN37815_c0_g1	-	5.06	0.090	-5.15	0.319	–	–
TRINITY_DN41114_c0_g1	*Brdt*	5.46	0.067	-5.55	0.268	–	–
TRINITY_DN42210_c0_g1	*Nlrp14*	5.24	0.079	-5.32	0.296	–	–
TRINITY_DN43261_c1_g1	*Txnrd3*	**5.99**	0.000	**-4.33**	0.004	0.71	1.000
TRINITY_DN43426_c0_g1	*Adad1*	**6.26**	0.036	-6.34	0.179	–	–
TRINITY_DN44656_c0_g1	*Sept12*	**6.12**	0.024	-6.21	0.193	0.34	1.000
TRINITY_DN46398_c0_g1	*Katnal1*	**4.34**	0.000	-1.81	0.238	-0.69	1.000
TRINITY_DN5146_c0_g1	*Sox30*	**5.95**	0.046	-6.03	0.211	–	–
TRINITY_DN53274_c0_g1	*Ccdc63*	**6.07**	0.025	-5.80	0.091	0.34	1.000
TRINITY_DN54555_c0_g1	*Oaz3*	**9.46**	0.000	**-24.26**	0.000	0.70	1.000
TRINITY_DN55194_c0_g1	*Creb3l4*	**5.35**	0.001	**-3.86**	0.026	-1.01	1.000
TRINITY_DN56009_c0_g1	*Odf4*	**5.86**	0.049	-5.95	0.221	0.34	1.000
TRINITY_DN56377_c0_g1	*Hook1*	**7.33**	0.000	**-6.33**	0.000	0.40	1.000
TRINITY_DN56398_c0_g1	*Spata6*	**5.69**	0.000	**-4.65**	0.000	1.45	0.356
TRINITY_DN56402_c0_g1	*Adam25*	4.26	0.154	-4.34	1.000	–	–
TRINITY_DN56545_c0_g1	*Mycbpap*	**5.47**	0.002	**-5.56**	0.018	0.34	1.000
TRINITY_DN57186_c0_g2	*Gtsf1*	**6.03**	0.043	-6.11	0.203	–	–
TRINITY_DN58227_c0_g2	*Tnp2*	**24.37**	0.000	**-25.13**	0.000	0.34	1.000
TRINITY_DN58246_c0_g2	*Rimbp3*	4.48	0.134	-4.56	0.400	0.34	1.000

Spermatogenesis (GO:0007283)		High 6h vs Wind 6h		High 24h vs High 6h		High 30h vs High 24h	
Gene ID	Name	log2FC	p(adj)	log2FC	p(adj)	log2FC	p(adj)
TRINITY_DN58246_c0_g3	*Rimbp3*	**21.53**	0.000	-7.24	0.103	0.34	1.000
TRINITY_DN58834_c0_g3	*Tcp11*	**24.03**	0.000	**-24.82**	0.000	1.06	1.000
TRINITY_DN58935_c0_g2	*Tnp1*	**23.76**	0.000	**-24.60**	0.000	0.34	1.000
TRINITY_DN59116_c0_g1	*H2afb1*	5.09	0.088	-5.18	0.316	–	–
TRINITY_DN59116_c0_g2	*H2afb1*	5.03	0.092	-5.11	0.324	–	–
TRINITY_DN59232_c0_g2	*B4galnt1*	**5.66**	0.000	**-3.63**	0.000	1.13	0.554
TRINITY_DN60042_c0_g3	*Cabs1*	**21.93**	0.000	**-22.76**	0.000	–	–
TRINITY_DN60766_c0_g1	*Spag4*	**5.90**	0.048	-5.62	0.186	0.34	1.000
TRINITY_DN61056_c0_g1	*Tssk1b*	**22.56**	0.000	**-22.72**	0.000	0.70	1.000
TRINITY_DN61149_c0_g1	*Hils1*	**21.94**	0.000	**-22.78**	0.000	–	–
TRINITY_DN61465_c0_g2	*Tssk3*	3.55	0.108	-4.95	0.188	0.72	1.000
TRINITY_DN61797_c0_g2	*Tbata*	**6.12**	0.040	-6.20	0.193	–	–
TRINITY_DN61868_c0_g2	*S100a11*	**6.31**	0.000	**-2.90**	0.000	1.13	0.695
TRINITY_DN62296_c0_g1	*Ccin*	**5.95**	0.046	-6.03	0.211	–	–
TRINITY_DN63069_c0_g2	*Ace*	**8.64**	0.000	**-4.83**	0.000	1.55	0.750
TRINITY_DN63104_c1_g1	*Ccdc136*	**5.97**	0.000	**-5.63**	0.000	-0.14	1.000
TRINITY_DN63193_c0_g1	*Adam25*	3.68	0.220	-3.76	1.000	–	–
TRINITY_DN64054_c0_g2	*Dzip1*	**5.21**	0.000	-2.47	0.070	-0.10	1.000
TRINITY_DN64359_c0_g2	*Bcap31*	**4.41**	0.000	**-2.46**	0.000	0.83	0.755
TRINITY_DN64713_c3_g1	*TCFL5*	**3.16**	0.039	**-4.73**	0.030	2.02	1.000
TRINITY_DN64774_c0_g4	*DNM2*	**3.13**	0.000	**-2.27**	0.000	1.07	0.301
TRINITY_DN64913_c0_g2	*Rbp4*	**7.05**	0.000	-1.01	0.542	0.18	0.995
TRINITY_DN65015_c1_g3	*Nphp1*	**5.67**	0.000	**-3.92**	0.000	0.93	0.695
TRINITY_DN65322_c0_g2	*Rad23b*	**5.57**	0.000	**-2.46**	0.002	0.36	0.975
TRINITY_DN65674_c0_g1	*Mif*	**4.79**	0.000	**-2.73**	0.000	1.59	0.087
TRINITY_DN65743_c0_g2	*Nme8*	**6.12**	0.040	-6.21	0.193	–	–
TRINITY_DN66042_c1_g1	*Piwil1*	5.18	0.083	-5.26	0.304	0.34	1.000
TRINITY_DN66161_c0_g3	*Tdrd6*	4.50	0.090	-4.58	0.300	–	–
TRINITY_DN66161_c0_g5	*Tdrd6*	**6.64**	0.001	**-6.73**	0.011	2.30	1.000
TRINITY_DN66519_c0_g3	*Ddx25*	**5.92**	0.047	-6.58	0.157	1.05	1.000
TRINITY_DN66662_c4_g2	*Tmbim6*	**5.71**	0.000	**-3.20**	0.000	1.21	0.549
TRINITY_DN67679_c0_g1	*Cib1*	**5.54**	0.000	**-3.18**	0.000	1.09	0.773
TRINITY_DN67804_c0_g1	*HSF2*	**1.78**	0.017	**-2.17**	0.001	1.17	0.705
TRINITY_DN67942_c0_g1	*Clgn*	**5.84**	0.031	**-22.76**	0.000	2.44	1.000
TRINITY_DN68550_c2_g4	*Theg*	5.63	0.059	-5.72	0.247	–	–
TRINITY_DN68873_c2_g1	-	5.70	0.056	-5.79	0.239	0.00	1.000
TRINITY_DN68988_c0_g3	*Spata20*	**23.29**	0.000	**-23.95**	0.000	0.70	1.000
TRINITY_DN69074_c0_g2	*Sycp3*	4.83	0.106	-4.91	0.351	–	–
TRINITY_DN69338_c0_g1	*CREM*	**8.04**	0.000	**-4.71**	0.000	1.37	1.000
TRINITY_DN69431_c3_g3	*Spata18*	**21.26**	0.000	**-22.67**	0.000	0.34	1.000
TRINITY_DN69790_c0_g1	*Prdx4*	**2.31**	0.005	**-2.16**	0.000	1.06	0.619
TRINITY_DN69790_c0_g3	*PRDX4*	5.50	0.065	-5.58	0.264	0.00	1.000
TRINITY_DN7062_c0_g1	*Tspan8*	**7.23**	0.000	-2.46	0.203	1.20	0.935

Spermatogenesis (GO:0007283)		High 6h vs Wind 6h		High 24h vs High 6h		High 30h vs High 24h	
Gene ID	Name	log2FC	p(adj)	log2FC	p(adj)	log2FC	p(adj)
TRINITY_DN70736_c3_g1	*Acsbg2*	3.75	0.210	-3.84	1.000	–	–
TRINITY_DN70736_c3_g2	*Acsbg2*	**6.22**	0.037	-6.30	0.183	–	–
TRINITY_DN72363_c2_g2	*Hspa2*	**4.48**	0.000	**-4.52**	0.000	0.45	1.000
TRINITY_DN72629_c0_g1	*Lamp1*	**4.21**	0.000	**-3.28**	0.000	1.20	0.432
TRINITY_DN73303_c4_g4	*Fdps*	**2.53**	0.004	**-2.74**	0.000	1.13	0.554
TRINITY_DN73435_c2_g1	*Gsr*	**4.56**	0.000	**-3.11**	0.000	**1.56**	0.012
TRINITY_DN73905_c2_g1	*Txndc2*	**21.50**	0.000	**-22.32**	0.000	–	–
TRINITY_DN74726_c4_g1	*Bax*	**2.68**	0.000	**-1.95**	0.000	0.98	0.328
TRINITY_DN75274_c0_g2	*Siah1a*	**2.25**	0.001	**-2.39**	0.000	1.35	0.726
TRINITY_DN75324_c0_g2	*Rgs2*	**5.90**	0.000	**-3.89**	0.000	1.51	0.418
TRINITY_DN7542_c0_g1	*Ggnbp1*	**7.22**	0.000	**-6.58**	0.001	1.46	1.000
TRINITY_DN77540_c0_g3	*Rai14*	**5.25**	0.000	**-3.98**	0.000	0.99	0.624
TRINITY_DN77946_c0_g1	*Sod1*	**6.00**	0.000	**-3.00**	0.000	1.28	0.335
TRINITY_DN78903_c0_g1	*TRIM27*	**2.04**	0.031	**-2.33**	0.000	0.66	0.552
TRINITY_DN80698_c4_g1	*D1Pas1*	**5.11**	0.000	**-2.47**	0.003	0.48	0.958
TRINITY_DN81516_c0_g2	*Ctsh*	**5.88**	0.000	**-3.33**	0.000	1.16	0.752
TRINITY_DN84317_c0_g1	*IGF2R*	1.43	0.077	**-1.59**	0.002	0.69	0.682
TRINITY_DN84926_c1_g4	*Catsper2*	**2.89**	0.000	**-2.16**	0.014	0.60	0.716
TRINITY_DN85246_c0_g1	*ODF2*	**4.82**	0.000	**-5.02**	0.000	0.76	0.603
TRINITY_DN86395_c1_g2	*Hmgb2*	**2.59**	0.004	**-3.72**	0.000	1.74	0.109
TRINITY_DN86806_c2_g1	*LMNA*	**2.72**	0.000	-1.26	0.001	0.80	0.632
TRINITY_DN86948_c1_g2	*PAIP2*	**2.45**	0.001	**-2.46**	0.000	1.25	0.313
TRINITY_DN89354_c0_g5	*Galntl5*	**21.83**	0.000	**-22.67**	0.000	–	–
TRINITY_DN90251_c0_g2	*Ggn*	**6.90**	0.000	**-6.98**	0.002	1.88	1.000
TRINITY_DN91648_c0_g3	*Spata32*	4.46	0.135	-4.54	0.403	–	–
TRINITY_DN91659_c2_g1	*Bag6*	**2.29**	0.010	**-3.01**	0.000	1.38	0.180
TRINITY_DN91719_c2_g7	*Cdyl*	**2.80**	0.010	**-2.97**	0.001	1.51	0.471
TRINITY_DN91828_c3_g1	*SIRT1*	-0.12	0.216	0.13	0.526	-0.05	0.977
TRINITY_DN92532_c0_g1	*Axl*	**3.16**	0.000	**-2.11**	0.000	1.84	0.393
TRINITY_DN92710_c0_g4	*Hspa1l*	**5.95**	0.011	-5.67	0.252	-0.38	1.000
TRINITY_DN93463_c2_g7	*Hspa1l*	**5.87**	0.009	-5.95	0.054	–	–
TRINITY_DN96575_c3_g1	*CRTAP*	1.12	0.124	-1.47	0.015	-0.04	0.997
TRINITY_DN96687_c0_g4	*Rfx2*	**5.45**	0.001	**-3.59**	0.017	-0.01	1.000
TRINITY_DN97606_c2_g1	*FAM50A*	1.24	0.113	-0.96	0.167	0.31	0.974
TRINITY_DN99333_c1_g1	*Zfp37*	**4.99**	0.003	**-4.35**	0.028	0.98	1.000
TRINITY_DN99357_c0_g8	*Chd5*	**5.52**	0.046	-3.96	0.287	-0.93	1.000
TRINITY_DN99460_c0_g1	*TDRD15*	-0.49	0.076	1.26	0.000	0.54	0.577
TRINITY_DN99980_c0_g1	*Pafah1b2*	**5.04**	0.000	**-2.98**	0.000	1.21	0.386

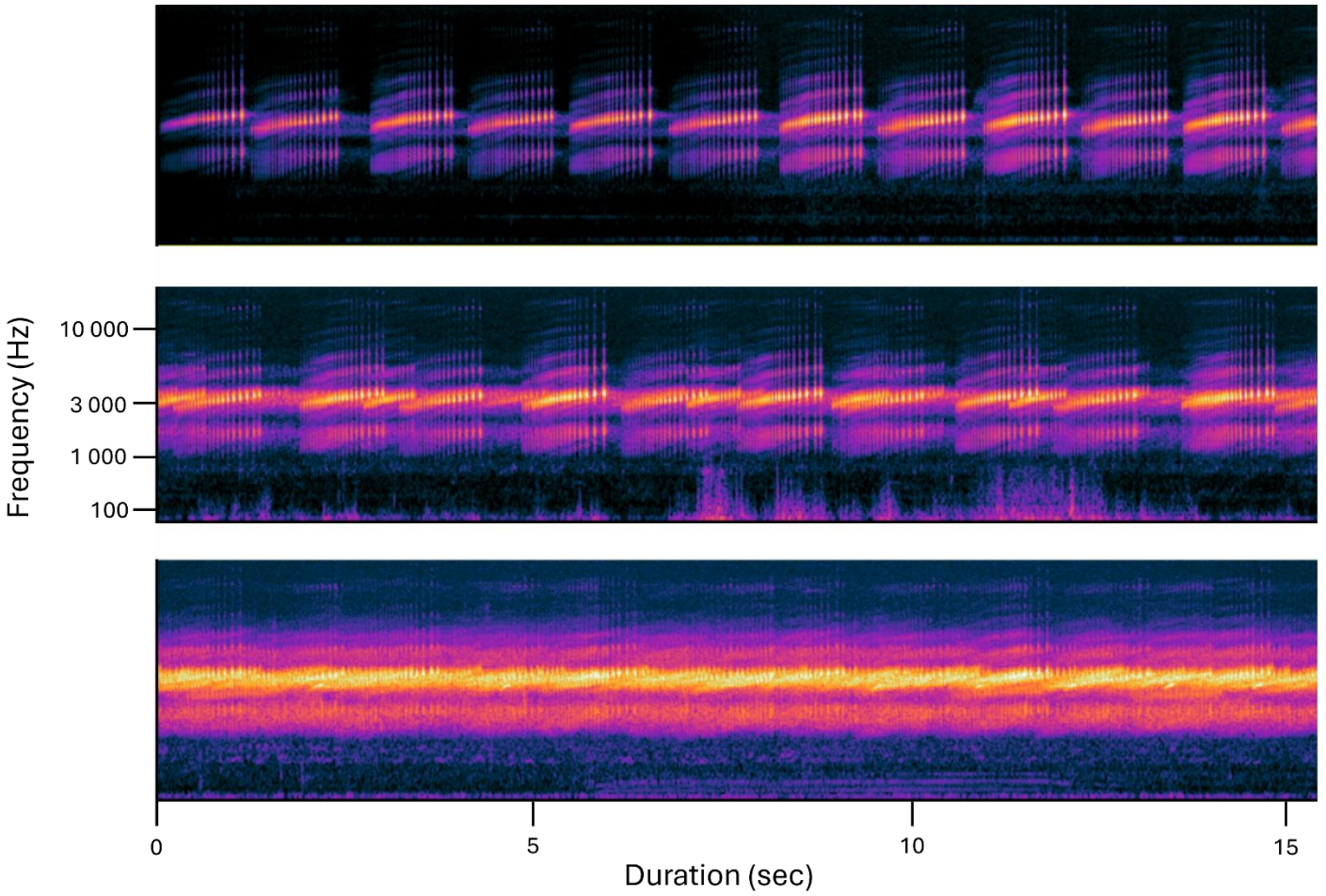

Figure S5.1: Spectrograms of simulated calling intensity levels used in playback treatments. (Top) two or three calling individuals with occasional but minimal overlap; (Middle) three or four individuals calling in the spectral foreground of recording with several individuals in the spectral background, approximately 10 individuals in total, with considerable overlap of calls; (Bottom) full chorus consisting of overlapping calls with non-distinguishable individuals, approximately 80-100 individuals in total.

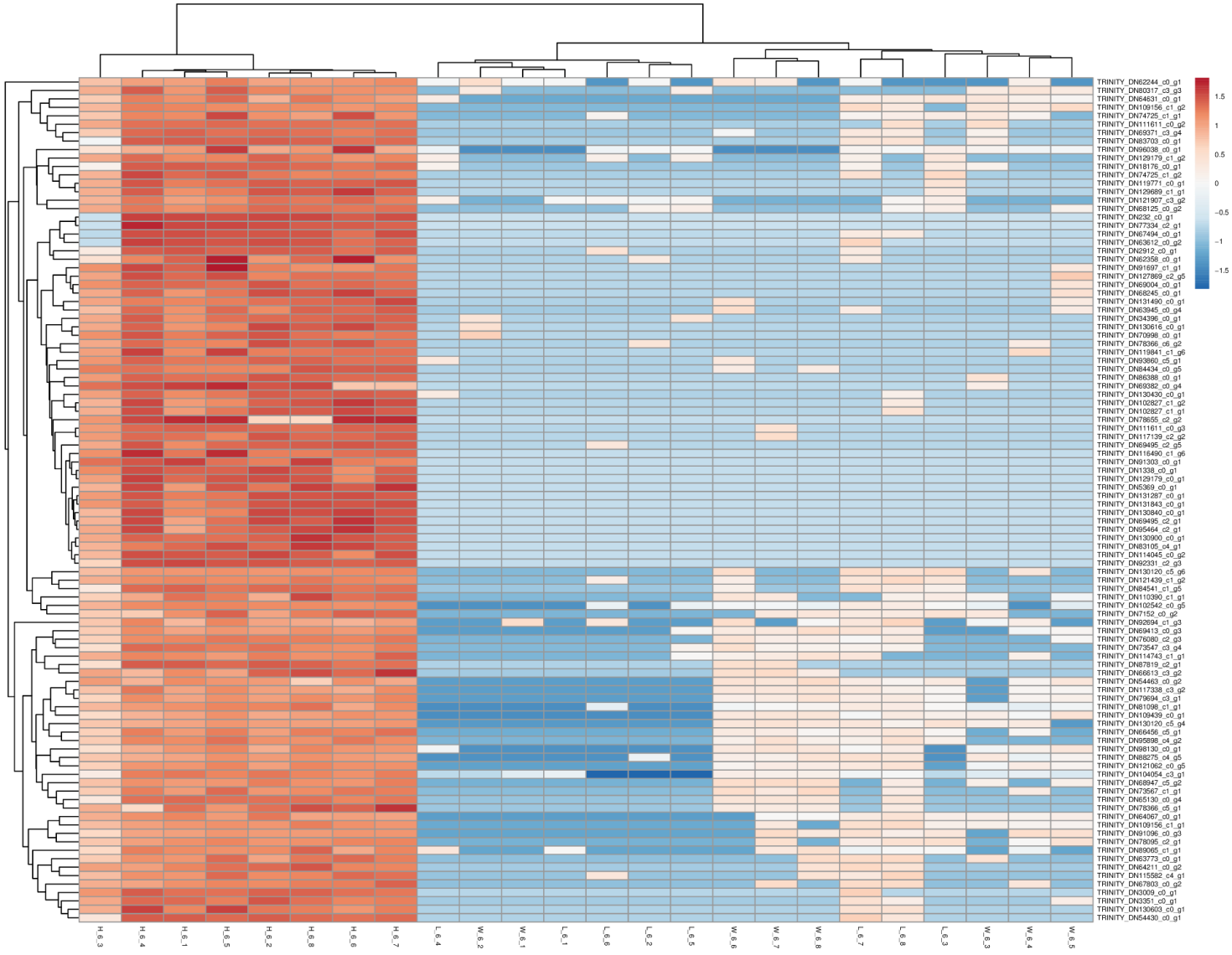

Figure S1.2: Heatmap of 100 genes in three broadcast treatments, High Chorus (H), Low Chorus (L) and Wind (W). Colour of node indicates upregulation (red) or downregulation (blue) of gene based on z-score. X-axis labels indicate the treatment, timepoint and replicate number (*i.e.*, "H_6_1," represents a sample in the High Chorus treatment, at the 6 hours time point, replicate #1).

Appendix B: Additional Contributions

Chen Y, Lougheed DR, Sun Z, **Ethier JP**, Trudeau VL, Lougheed SC. 2024. Insights on macrosynteny, 'rebel' genes, and a new sex-linked region in anurans from comparative genomics and a new chromosome-level genome for the western chorus frog. *Manuscript in preparation for publication*. bioRxiv. https://doi.org/10.1101/2024.10.27.620512.

Empey MA, Reyes YM, **Ethier JP**, Rosa CGT, Trudeau VL. 2025 Toxicity of *Bacillus thuringiensis israelensis* and deltamethrin in three anuran species. Environmental Pollution 382: 126702. https://doi.org/10.1016/j.envpol.2025.126702.

Ethier JP, Worth M, Mazerolle MJ, Trudeau VL. 2024. Rearing density and food variety impact growth, development, and survival of larvae in the declining amphibian, *Pseudacris maculata*. Zoo Biology 43: 416–424. https://doi.org/10.1002/zoo.21848.

Trudeau VL, **Ethier JP**. 2025. Amphibians and their impact on human reproduction research. F&S Reports 6: 3–6. https://doi.org/10.1016/j.xfre.2025.01.005.