



Published in final edited form as:

Horm Behav. 2006 June ; 50(1): 101–106. doi:10.1016/j.yhbeh.2006.01.010.

Social regulation of plasma estradiol concentration in a female anuran

Kathleen S. Lynch^{a,*},¹ and Walter Wilczynski^{a,b,2}

^a *Institute for Neuroscience, University of Texas, 108 East Dean Keeton, Seay, Psychology Building, Austin, TX 78712, USA*

^b *Department of Psychology, University of Texas, 108 East Dean Keeton, Seay, Psychology Building, Austin, TX 78712, USA*

Abstract

The behavior of an individual within a social aggregation profoundly influences behavior and physiology of other animals within the aggregation in such a way that these social interactions can enhance reproductive success, survival and fitness. This phenomenon is particularly important during the breeding season when males and female must synchronize their reproductive efforts. We examined whether exposure to conspecific social cues can elevate sex steroid levels, specifically estradiol and androgens, in female túngara frogs (*Physalaemus pustulosus*). We compared plasma estradiol and androgen concentrations in wild-caught females before and after exposure to either natural mate choruses or random tones. After exposure to mate choruses for 10 consecutive nights, estradiol concentrations were significantly elevated whereas there was no significant elevation in estradiol concentrations in the group of females exposed to random tones for 10 nights. Plasma androgen concentrations were not significantly changed after exposure to either natural mate choruses or random tones for 10 consecutive nights. Social modulation of estradiol concentrations may be important in maintaining a female's reproductive state while males are chorusing. To our knowledge, this is the first study to demonstrate social regulation of estradiol concentration in female anurans.

Keywords

Social regulation; Estradiol; Female reproductive behavior; Anuran

Introduction

In order to maximize reproductive opportunity, males and females in many taxa must be able to predict the onset of the breeding season and synchronize their reproductive behaviors to one another. Ultimate factors, such as food availability, weather, competition or predation, favor individuals that reproduce when changes in the environment occur. Therefore, animals must prepare for changes in the environment by responding to proximate cues, which are features of the environment that actually influence physiology or behavior (Hahn et al., 1997). Proximate cues can be predictive, such as changes in photoperiod (Wingfield, 1983), or

*Corresponding author. E-mail addresses: lynchs@mail.utexas.edu, lynchs@jhu.edu (K.S. Lynch), wilczynski@psy.utexas.edu, wwilczynski@gsu.edu (W. Wilczynski).

¹Current address: Psychological and Brain Science, Johns Hopkins University, Ames Hall 3400 North Charles Street, Baltimore, MD 21218-2686, USA.

²Current address: Department of Psychology and Center for Behavioral Neuroscience, Georgia State University, P.O. Box 3966, Atlanta GA, 30302-3966, USA.

supplementary, such as changes in weather (Hahn et al., 1997). Predictive cues help regulate sex steroids production, which in turn, regulates reproductive behaviors in a variety of taxa including birds (Noble, 1973; Delville and Balthazart, 1987; Ball and Balthazart, 2002), fish (Tricas et al., 2000; Grober and Bass, 2002), mammals (Hull et al., 2002; Blaustein and Erskine, 2002), reptiles (Alderete et al., 1980; Rhen et al., 1999; 2000; Rhen and Crews, 2000; Godwin and Crews, 2002) and amphibians (Diakow and Nemiroff, 1981; Schmidt, 1984, 1985; Mendonça et al., 1985; Boyd, 1994, Wilczynski and Chu, 2001). Such environmental cues, however, are not the only means by which animals recognize the onset of the breeding season and synchronize their reproductive state. Lorenz demonstrated that the behavior of one individual within a social aggregation could influence the behavior of other animals within the aggregation, thereby demonstrating that social cues can impact an animal's behavior and therefore its survival and fitness (Lorenz, 1970). Since then, it has been shown that social cues also regulate sex steroid production (Dufty and Wingfield, 1986; Chu et al., 1998; Propper and Moore, 1991; Burmeister and Wilczynski, 2000, 2001; Chu and Wilczynski, 2001), function of gonadotropin releasing hormone neurons (Propper and Moore, 1991; Dellovade and Rissman, 1994; reviewed in Rissman, 1996; Burmeister and Wilczynski, 2005), gonadal status (Brzoska and Obert, 1980; Bentley et al., 2000; Lea et al., 2001) and survival of new neurons (Lipkind et al., 2002). These cues, whether environmental or social, are clearly a mechanism by which animals coordinate neural, physiological and behavioral responses that enhance the probability of reproductive success.

Social regulation of female reproductive physiology has been demonstrated in classic experiments by Lehrman (1965), in which he showed that the presence of a courting male enhanced the endocrine and behavioral response of the female ring dove (*Streptopelia risoria*). The effects of such stimulation are further enhanced by the presence of both visual and auditory cues provided by the male (Freidman, 1977) as well as by the female's own vocal responses to the male's courting behavior (Cheng, 1992). Further, electrophysiological studies indicate that auditory cues stimulate hypothalamic neurons (Cheng et al., 1998). Similar phenomena in which vocal signals from a sender influence the physiology and neurobiology of a receiver have also been described in anuran amphibians (Wilczynski and Chu, 2001; Wilczynski et al., 2005). This is possible because in anurans, the central auditory system sends projections into the anterior and central thalamic nuclei, which relay auditory information into the anterior preoptic area as well as the ventral hypothalamus (reviewed in Wilczynski et al., 1993). Consequently, hypothalamic neurons respond to acoustic stimulation in male (Wilczynski and Allison, 1989; Allison, 1992) and female (Hoke et al., 2005) anurans. Acoustic cues stimulate the release of gonadal hormones in male anuran amphibians (Burmeister and Wilczynski, 2000; Chu and Wilczynski, 2001). Although exposure to a conspecific mate chorus facilitates oocyte retention in female midwife toads (*Alytes mulentensis*) (Lea et al., 2001), there is no evidence as of yet that conspecific mate signals stimulate endocrine responses in female anurans. Male and female anurans do in fact have very different social interactions, in that males generally remain in choruses exposed to social signals for long periods at a time, while females enter it episodically. Their exposure to calls may be less intense and more sporadic, and it remains unknown whether the stimulatory effect of calls on gonadal hormones seen in males can be generalized to females. The objective of this study is to determine if acoustic exposure to natural, conspecific mate choruses enhances the production of reproductive steroids in a female anuran.

We examined whether conspecific mate signals stimulate the production and release of the gonadal steroid hormones, specifically estradiol and androgen, in female túngara frogs (*Physalaemus pustulosus*), a Neotropical anuran species that is subject to sporadic favorable breeding environments during the breeding season (i.e., the rainy season). Male túngara frogs gather in lek-like aggregations to form nightly mate choruses during the breeding season, as do many other species of anuran (Wells, 1977). Male choruses, however, are not constant over

the rainy season. Males do not form these choruses during heavy rainfall but the nights following a heavy rain typify the most intense mate choruses (Ryan, 1985). Females cycle through periods of high and low receptivity (Lynch et al., 2005) corresponding to cycles in gonadal steroid concentrations (Lynch and Wilczynski, 2005). Females that are gravid and ready to mate will approach these mate choruses; however, it is not clear how long the female is exposed to chorusing males before she actually mates. Therefore, it is unknown whether such acoustic social signals contribute to the regulation of gonadal hormone production in female anurans as they do in chorusing male anurans. Furthermore, the female túngara frog displays asynchronous oogenesis, in which the female túngara retains oocytes in different stages of maturity (stages I–VI, with VI being the most mature). Consequently, túngara frog females are constantly producing new eggs, while other eggs mature, allowing the female to release eggs multiple times during the season with a period of about 4 to 6 weeks (Davidson and Hough, 1969). Although the hormone profile of female túngara frogs show that they experience cyclic fluctuations in gonadal hormone concentrations (Lynch and Wilczynski, 2005; see Table 1 for summary of those results), it is possible that social cues are an additional regulator of gonadal hormone concentrations. We examined this possibility by exposing female túngara frogs to mate choruses or random tones while their gonadal steroid concentrations were at their lowest concentration (approximately midcycle). Elevation in female reproductive hormones as a consequence of social experience would indicate that females can use social cues, specifically male mate choruses, as a signal to continue cycling into the next reproductive cycle during the breeding season, whereas the absence of these social cues may contribute to the female dampening her reproductive capability, an effect that would occur at the end of the breeding season when social signals are no longer present.

Methods

Female túngara frogs were captured in Gamboa, Panama, while they were in the process of mating with males (i.e., amplexus). The female was permitted to release eggs and held for 15 days afterwards because our previous study (Lynch and Wilczynski, 2005) showed that gonadal hormone concentrations are depressed at this time. During the 15-day waiting period, the females were transported to the University of Texas at Austin and housed in 10-gallon aquarium with damp moss in groups of five and fed 1-week-old crickets three times per week. At exactly 15 days post-mated blood was collected via the orbital sinus using a procedure approved by the University of Texas IACUC. The blood was centrifuged, and the plasma layer was removed. The females were then individually placed in an acoustic chamber with water, moss, rocks and artificial plants. The acoustic chambers also included a speaker (RadioShack 277-1008C) and a 1.4 W light source. The hourly light cycle inside the chamber was set at 14 L/10 D, and the acoustic stimulation was set to play between 20:00 and 02:00 h. The females were kept inside the acoustic chamber for 10 consecutive nights during which time some females were exposed to natural mate choruses ($N = 15$), while others were exposed to random tones that were matched in amplitude and duration to the mate choruses ($N = 13$). The mate chorus was recorded by M.J. Ryan in Gamboa, Panama, and digitized in Sound Edit (Macromedia, Inc., San Francisco) so that four different chorus bouts were punctuated with 1 min of silence. We created the control stimulus by replacing every mate call with a pure tone between 200 Hz and 1 kHz, which are frequencies within the conspecific mate signal. The tones were not frequency or amplitude modulated. After 10 nights of acoustic exposure to either mate choruses or random tones, the females were removed and sacrificed via rapid decapitation. Blood was collected again and centrifuged to remove the plasma layer. Both the pre- and postacoustic treatment plasma samples were stored at $-20\text{ }^{\circ}\text{C}$ until assayed.

Hormone assays

Plasma volumes ranged from 5 to 20 μl . We used the plasma to first assay estradiol concentrations in chorus exposed females ($N = 15$) and females exposed to random tones ($N = 13$). The remaining plasma was then used to assay androgen concentrations ($N = 11$ in chorus exposed females and $N = 9$ in random tone exposed females). Twenty microliters of tritiated hormone (approximately 1000 cpm) was added to each plasma sample. Hormones were extracted from the plasma using 3 ml of diethyl ether. Estradiol and androgen concentrations were assayed using enzyme-linked immunosorbent (EIA) kits purchased by Caymen Chemical (Ann Arbor, MI). To validate these kits, we extracted hormone from a plasma sample and serially diluted it to be two (androgen) or three (estradiol) different concentrations. We compared the slope of the line for the serially diluted samples and the slope of the line for the area of the curve necessary to estimate the concentration of the diluted samples. These slopes were not compared statistically due to small sample sizes; however, the following slopes are clearly parallel to each other. The slope of the line for the serially diluted estradiol samples was -1.86 , and the slope of the line for the area of the estradiol standard curve in which the samples are estimated was -1.54 . The slope of the line for the serially diluted androgen samples was -3.58 , and the slope of the line for the area of the androgen standard curve in which the samples are estimated was -4.06 . Moreover, among the serial dilutions, the sample that was estimated to contain the highest concentration of estradiol was only 8% different from the sample estimated to have the lowest concentration of estradiol. There was only 3% difference between the two estimated androgen concentrations. Both estradiol and androgens were measured on two plates. The intra-assay variation was 16.8%, and inter-assay variation was 12% for estradiol. The intra-assay variation was 19.0%, and inter-assay variation was 8.4% for androgens. The estradiol EIA kits have a 0.1% cross reactivity with testosterone and 5 α -DHT, 0.07% for 17 α -estradiol, and 0.03% for progesterone and the detection limit is 8 pg/ml. The testosterone EIA kit has a 27.4% and 18.9% cross reactivity with 5 α -dihydrotestosterone and 5 β -dihydrotestosterone respectively, and therefore, we consistently refer to testosterone measurements as androgens. The detection limit for testosterone EIA kits is 6 pg/ml. Samples in which the data were extrapolated or that were not within the sensitive range of the standard curve (i.e., total percentage of bound hormone calculated under 20% or over 80%) were removed from the analysis due to unreliable estimation of hormone concentration. For these reasons, six samples were removed from the chorus-exposed group in the estradiol assay, and one sample was removed from the tone treated group. Two samples were removed from the group exposed to chorus in the androgen assay. Therefore, the final sample size in the estrogen assay was $N = 9$ in the group exposed to chorus and $N = 12$ in the group exposed to random tones, and the final sample size in the androgen assay was $N = 9$ in the group exposed to chorus and tones.

Statistics

A Kolmogorov–Smirnov test revealed that these data were normally distributed. We therefore analyzed these data using a mixed model ANOVA with repeated measures in order to determine whether pre- and postacoustic exposure hormone concentration significantly change in females in the natural mate chorus group and the random tones group. Planned comparisons were done using paired samples t tests. All reported values are mean \pm SE.

Results

Estradiol

Estradiol concentration did significantly change within groups (Fig. 1a; $n = 12$, $df = 19$; $F = 14.1$, $P = 0.001$). Planned comparisons using paired samples t test showed that plasma concentration of estradiol were significantly increased after acoustic exposure in the group that heard mate choruses ($t = -3.26$; $df = 8$; $P = 0.01$), whereas estradiol concentrations were not

significantly increased after acoustic exposure in the group exposed to random tones ($t = -1.63$; $df = 11$; $P = 0.13$). There was a near significant interaction between the change in estradiol concentrations within groups and estradiol concentrations between the treatment groups ($F = 3.5$; $df = 19$; $P = 0.077$). There was no significant difference in estradiol concentrations between the treatment groups ($F = 0.47$; $df = 1$; $P = 0.50$).

Androgens

Androgen concentration did not significantly change within groups (Fig. 1b; $n = 9$; $df = 16$; $F = 0.034$; $P = 0.85$). There was no significant change in androgen concentrations in females that heard mate choruses ($n = 9$) or females that heard random tones ($n = 9$). There was no significant interaction between the change in androgen concentrations within groups and androgen concentrations between the treatment groups ($F = 0.84$; $df = 16$; $P = 0.37$). There was also no significant difference in androgen concentrations between the treatment groups ($F = 0.40$; $df = 1$; $P = 0.53$).

Discussion

Plasma concentrations of estradiol are significantly modified by social interactions in female túngara frogs, whereas there are no significant changes in androgen concentrations as a consequence of exposure to mate choruses or random tones. Females exposed to 10 consecutive nights of natural mate chorus had significantly elevated estradiol concentrations after acoustic treatment, whereas females exposed to random tones did not show significantly elevated estradiol concentrations after acoustic exposure. All the females tested here began their exposure to mate choruses 15 days post-egg release, approximately their midreproductive cycle. Elevated estradiol concentrations in females exposed to mate chorus at this point in their cycle suggests that continued exposure to mate choruses may serve as a cue for females that the breeding season is ongoing and to continue reproductive cycling. On the other hand, a lack of salient social signals may indicate that the conclusion of the breeding season is approaching. In this case, it is possible that the lack of social cues may cause females to show less and less fluctuation in hormone cycles. Our data do not indicate whether there is a gradual dampening of estradiol cycling, only that estradiol does not significantly elevate when social cues are absent. These data do show that estradiol rises significantly in the females exposed to the mate chorus sounds but do not in the females exposed to random tones. It is still unclear, however, whether exposure to natural mate choruses increases oocyte maturation, the most important end-point of the physiological response to salient auditory cues. This is because female túngara frogs display asynchronous oogenesis; therefore, they retain eggs at varied stages of development, making it difficult to know their exact gonadal status.

Salient acoustic inputs into central endocrine control regions does result in elevated gonadal hormone concentrations in the female túngara frog. At present, we do not know the range of signals that will induce this effect. It is still unclear as to whether this response can occur with exposure to heterospecific signals or whether alteration of the spatial and/or temporal arrangement of the conspecific signal can alter this response. For instance, forebrain auditory regions in canaries (*Serinus canaria*) and zebra finch (*Taeniopygia guttata*) show a graded immediate early gene (IEG) response to conspecific, heterospecific and tone burst, with conspecific showing the greatest IEG induction and tones showing no IEG induction (Mello et al., 1992). Furthermore, in female canaries and song sparrows (*Melospiza melodia*), follicular development and plasma luteinizing hormone concentrations are enhanced after exposure to both conspecific and heterospecific male songs (Bentley et al., 2000). This suggests that it is possible that heterospecific signals may serve as an additional cue to indicate that environmental conditions are suitable for breeding. This could be important in Neotropical frogs like the túngara that call in mixed species choruses. It is also possible for spatial and/or

temporal arrangement of the conspecific signal to alter the physiological response of the female. For instance, two distinct signals are produced by male túngara frogs and are referred to as the whine and the whine-chuck. Classic studies demonstrate that the whine is sufficient to elicit phonotaxis (i.e., species recognition), the chuck alone does not, but when placed at the end of the whine, it increases the whine's attractiveness (Rand and Ryan, 1981; Ryan, 1985; Wilczynski et al., 1995, 1999). In addition, females prefer chucks with a lower fundamental frequency (Ryan, 1980; Ryan et al., 1990). Future studies may reveal whether the female's hormonal response would reflect the known behavioral responses to these types of signals.

The mechanism by which estradiol concentrations are elevated in females exposed to mate choruses is explained by neuroanatomical and neurophysiological studies describing the pathway by which acoustic signals may influence central endocrine control centers (reviewed in Wilczynski et al., 1993). In anurans, ascending auditory pathways project to the preoptic area (POA) and the caudal hypothalamus via the auditory midbrain nucleus (referred to as the torus semicircularis, TS) and the thalamus. Neurophysiological studies validate that the TS and thalamic projections into the POA and caudal hypothalamus do carry auditory information. The firing rate of both POA and hypothalamic neurons is altered as a consequence of auditory stimulation with conspecific calls in *Hyla cinerea* (Allison, 1992). In addition, the increase in firing rate continued past the stimulus presentation leading to the conclusion that acoustic stimulation acts as a modulator of ongoing activity within central endocrine control areas (Allison, 1992). Although neurophysiological work has not been done in túngara frogs, Hoke et al. (2004, 2005) demonstrated midbrain activation and hypothalamic sensitivity to conspecific calls in this species using immediate early gene activation. Increases in neural activity within the central endocrine regions translate into elevated gonadal steroid concentrations in male anurans (Burmeister and Wilczynski, 2000; Chu and Wilczynski, 2001). To our knowledge, this is the first study to demonstrate that estradiol, an important reproductive hormone in female anurans, is also modulated by social interactions with reproductive males; however, the exact pathway by which this arises remains unclear.

Acknowledgements

We thank the Smithsonian Tropical Research Institute for obtaining the necessary permits for this work. We also thank A.S. Rand and M.J. Ryan for their help in the field as well as Jin Yang for her help collecting plasma. This work was supported by NSF grant IBN 0078150 and NIH grant MH 05066.

References

- Alderete MR, Tokarz RR, Crews D. Luteinizing hormone-releasing hormone (LHRH) and thyrotropin releasing hormone (TRH) induction of female sexual receptivity in the lizard, *Anolis carolinensis*. *Neuroendocrinology* 1980;30:200–205. [PubMed: 6769065]
- Allison JD. Acoustic modulation of neural activity in the preoptic area and ventral hypothalamus of the green treefrog (*Hyla cinerea*). *J Comp Physiol, A* 1992;171:387–394. [PubMed: 1447726]
- Ball, G.; Balthazart, J. Neuroendocrine mechanisms regulating reproductive cycles and reproductive behavior in birds. In: Pfaff, DW.; Arnold, AP.; Etgen, AM.; Fahrbach, SE.; Rubin, RT., editors. *Hormones, Brain and Behavior*. 2. 2002. p. 649-798.
- Bentley GE, Wingfield JC, Morton ML, Ball GF. Stimulatory effects of the reproductive axis in female songbirds by conspecific and heterospecific male song. *Horm Behav* 2000;37:179–189. [PubMed: 10868481]
- Blaustein, JD.; Erskine, MS. Feminine sexual behavior: cellular integration of hormonal and afferent information in the rodent brain. In: Pfaff, DW.; Arnold, AP.; Etgen, AM.; Fahrbach, SE.; Rubin, RT., editors. *Hormones, Brain and Behavior*. 1. 2002. p. 139-215.
- Boyd SK. Arginine vasotocin facilitation of advertisement calling and call phonotaxis in bullfrogs. *Horm Behav* 1994;28:232–240. [PubMed: 7814004]

- Brzoska J, Obert HJ. Acoustic signals influence the hormone production of the testes in the grass frog. *J Comp Physiol* 1980;140:25–29.
- Burmeister SS, Wilczynski W. Social signals influence hormones independently of calling behavior in the treefrog (*Hyla cinerea*). *Horm Behav* 2000;38:201–209. [PubMed: 11104638]
- Burmeister SS, Wilczynski W. Social context influences androgenic effects on calling in the green treefrog (*Hyla cinerea*). *Horm Behav* 2001;40:550–558. [PubMed: 11716585]
- Burmeister SS, Wilczynski W. Social signals regulate gonadotropin-releasing hormone neurons in the green treefrog. *Brain Behav Evol* 2005;65:26–32. [PubMed: 15489562]
- Cheng MF. For whom does the female dove coo? A case for the role of vocal self stimulation. *Anim Behav* 1992;43:1035–1044.
- Cheng MF, Peng JP, Johnson P. Hypothalamic neurons preferentially respond to female nest coo stimulation: demonstration of direct acoustic stimulation of luteinizing hormone release. *J Neurosci* 1998;18:5477–5489. [PubMed: 9651228]
- Chu J, Wilczynski W. Social influences on androgen levels in the southern leopard frog, *Rana sphenoccephala*. *Gen Comp Endocrinol* 2001;121:66–73. [PubMed: 11161771]
- Chu J, Marler CA, Wilczynski W. The effects of arginine vasotocin on the calling behavior of male cricket frogs in changing social context. *Horm Behav* 1998;34:248–261. [PubMed: 9878274]
- Davidson EH, Hough BR. Synchronous oogenesis in *Engystomops pustulosus*, a neotropical anuran suitable for laboratory studies: localization in the embryo of RNA synthesized at the lampbrush stage. *J Exp Zool* 1969;172:25–48. [PubMed: 5372000]
- Dellovade TL, Rissman EF. Gonadotropin-releasing hormone-immunoreactivity cell number change in response to social interaction. *Endocrinology* 1994;134:2189–2197. [PubMed: 8156921]
- Delville Y, Balthazart J. Hormonal control of female sexual behavior in the Japanese Quail. *Horm Behav* 1987;21:288–309. [PubMed: 3666684]
- Diakow C, Nemiroff A. Vasotocin, prostaglandin and female reproductive behavior in the frog, *Rana pipiens*. *Horm Behav* 1981;15:86–93. [PubMed: 6971247]
- Duffy AM, Wingfield JC. The influence of social cues on the reproductive endocrinology of male brown-headed cowbirds: field and laboratory studies. *Horm Behav* 1986;20:222–234. [PubMed: 3721414]
- Freidman MB. Interactions between visual and vocal courtship stimuli in the neuroendocrine response of female doves. *J Comp Physiol* 1977;91:1408–1416.
- Godwin, J.; Crews, D. Hormones, brain and behavior in reptiles. In: Pfaff, DW.; Arnold, AP.; Etgen, AM.; Fahrbach, SE.; Rubin, RT., editors. *Hormones, Brain and Behavior*. 2. Academic Press; San Diego, CA: 2002. p. 545-586.
- Grober, M.; Bass, A. Life history, neuroendocrinology, and behavior in fish. In: Pfaff, DW.; Arnold, AP.; Etgen, AM.; Fahrbach, SE.; Rubin, RT., editors. *Hormones, Brain and Behavior*. 2. Academic Press; San Diego, CA: 2002. p. 331-348.
- Hahn, TP.; Boswell, T.; Wingfield, JC.; Ball, GF. Temporal flexibility in avian reproduction: patterns and mechanisms. In: Nolan, V., Jr; Ketterson, ED.; Thompson, CF., editors. *Current Ornithology*. 14. Plenum Press; New York: 1997. p. 39-80.
- Hoke KL, Burmeister SS, Fernald RD, Rand AS, Ryan MJ, Wilczynski W. Functional mapping of the auditory midbrain during mate call reception. *J Neurosci* 2004;24:11264–11272. [PubMed: 15601932]
- Hoke KL, Ryan MJ, Wilczynski W. Social cues shift functional connectivity in the hypothalamus. *Proc Natl Acad Sci U S A* 2005;102:10712–10717. [PubMed: 16020531]
- Hull, EM.; Meisel, RL.; Sachs, BD. Male sexual behavior. In: Pfaff, DW.; Arnold, AP.; Etgen, AM.; Fahrbach, SE.; Rubin, RT., editors. *Hormones, Brain and Behavior*. 2. Academic Press; San Diego: 2002. p. 1-139.
- Lea J, Dyson M, Halliday T. Calling by male midwife toads stimulates females to maintain reproductive condition. *Anim Behav* 2001;61:373–377.
- Lehrman, DS. Interaction between internal and external environments in the regulation of the reproductive cycle of the ring dove. In: Beach, FA., editor. *Sex and Behavior*. 1965. p. 355-380.
- Lipkind D, Nottebohm F, Rado R, Barnea A. Social change affects the survival of new neurons in the forebrain of adult songbirds. *Behav Brain Res* 2002;133:31–43. [PubMed: 12048172]

- Lorenz, K. *Studies in Animal and Human Behaviour*. 1. Harvard Univ. Press; Cambridge, MA: 1970. Companions as factors in the bird's environment; p. 101-254.
- Lynch KS, Wilczynski W. Gonadal steroids vary with reproductive stage in a tropically breeding female Anuran. *Gen Comp Endocrinol* 2005;143:51–56. [PubMed: 15993104]
- Lynch KS, Rand AS, Ryan MJ, Wilczynski W. The influence of reproductive stage in producing within-individual plasticity in female mate choice. *Anim Behav* 2005;69:689–699.
- Mello CV, Vicario DS, Clayton DF. Song presentation induces gene expression in the songbird forebrain. *Proc Natl Acad Sci U S A* 1992;89:6818–6822. [PubMed: 1495970]
- Mendonça MT, Licht P, Ryan MJ, Barnes R. Changes in hormone levels in relation to breeding behavior in male bullfrogs (*Rana catesbeiana*) at the individual and population levels. *Gen Comp Endocrinol* 1985;58:270–279.
- Noble R. Hormonal control of receptivity in female quail (*Coturnix coturnix japonica*). *Horm Behav* 1973;4:61–72.
- Propper CR, Moore FL. Effects of courtship on brain gonadotropin hormone releasing hormone and plasma steroid concentrations in a female amphibian (*Taricha granulosa*). *Gen Comp Endocrinol* 1991;81:304–312. [PubMed: 2019403]
- Rand AS, Ryan MJ. The adaptive significance of a complex vocal repertoire in a neotropical frog. *Z Tierpsychol* 1981;57:209–214.
- Rhen T, Crews D. Organization and activation of sexual and agonistic behavior in the leopard gecko, *Eublepharis macularius*. *Neuroendocrinology* 2000;71:252–261. [PubMed: 10773745]
- Rhen T, Ross J, Crews D. Effects of testosterone on sexual behavior and morphology in adult female leopard geckos, *Eublepharis macularius*. *Horm Behav* 1999;36:119–128. [PubMed: 10506536]
- Rhen T, Sakata JT, Zeller M, Crews D. Sex steroid levels across the reproductive cycle of female leopard geckos, *Eublepharis macularius*, from different incubation temperatures. *Gen Comp Endocrinol* 2000;118:322–331. [PubMed: 10890571]
- Rissman EF. Behavioral regulation of gonadotropin-releasing hormone. *Biol Reprod* 1996;54:413–419. [PubMed: 8788194]
- Ryan MJ. Female mate choice in a neotropical frog. *Science* 1980;209:523–525. [PubMed: 17831371]
- Ryan, MJ. *The Túngara Frog: a Study in Sexual Selection and Communication*. University of Chicago Press; Chicago: 1985.
- Ryan MJ, Fox JH, Wilczynski W, Rand AS. Sexual selection for sensory exploitation in the frog, *Physalaemus pustulosus*. *Nature* 1990;343:66–67. [PubMed: 2296291]
- Schmidt RS. Mating call phonotaxis in the female American toad: induction by hormones. *Gen Comp Endocrinol* 1984;55:150–156. [PubMed: 6235146]
- Schmidt RS. Prostaglandin-induced mating call phonotaxis in female American toad: facilitation by progesterone and arginine vasotocin. *J Comp Physiol, A* 1985;156:823–829.
- Tricas TC, Maruska KP, Rasmussen L. Annual cycles of steroid hormone production, gonad development, and reproductive behavior in the Atlantic stingray. *Gen Comp Endocrinol* 2000;118:209–225. [PubMed: 10890563]
- Wells KD. The social behaviour of anuran amphibians. *Anim Behav* 1977;25:666–693.
- Wilczynski W, Allison JD. Acoustic modulation of neural activity in the hypothalamus of the leopard frog. *Brain, Behav Evol* 1989;33:317–324. [PubMed: 2788475]
- Wilczynski, W.; Chu, J. Acoustic communication, endocrine control, and the neurochemical systems of the brain. In: Ryan, MJ., editor. *Anuran Communication*. Smithsonian Institution Press; Washington, DC: 2001. p. 23-35.
- Wilczynski W, Allison JD, Marler CA. Sensory pathways linking social and environmental cues to endocrine control regions of amphibian forebrains. *Brain, Behav Evol* 1993;42:252–264. [PubMed: 8252377]
- Wilczynski W, Rand AS, Ryan MJ. The processing of spectral cues in the call analysis system of the túngara frog, *Physalaemus pustulosus*. *Anim Behav* 1995;49:911–929.
- Wilczynski W, Rand AS, Ryan MJ. Female preferences for temporal order of call components in the túngara frog: a Bayesian analysis. *Anim Behav* 1999;58:841–851. [PubMed: 10512658]

- Wilczynski W, Lynch KS, O'Bryant EL. Current research in amphibians: studies integrating endocrinology, behavior, and neurobiology. *Horm Behav* 2005;48:440–450. [PubMed: 16023646]
- Wingfield, JC. Environmental and endocrine control of reproduction: an ecological approach. In: Mikami, S.; Homma, K.; Wada, M., editors. *Avian Endocrinology: Environmental and Ecological Perspective*. Springer-Verlag; Berlin: 1983. p. 265-288.

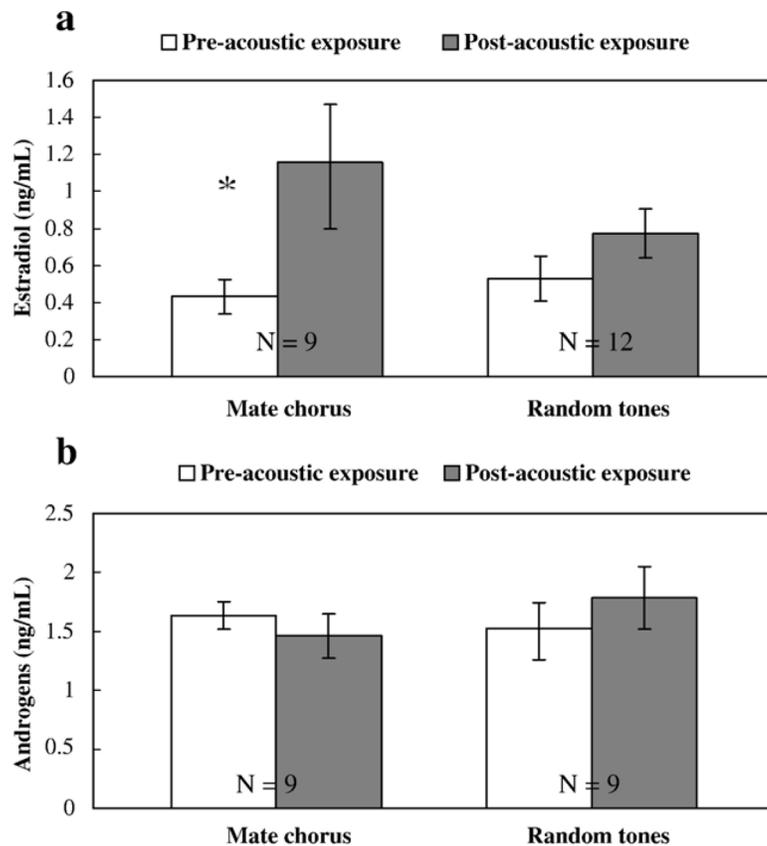


Fig. 1. (a) Repeated measurements of mean (\pm SE) plasma estradiol concentrations in females both before acoustic exposure and again after ten consecutive nights of acoustic exposure with either mate choruses or random tones. *N* equals the number of subjects tested in each treatment. A mixed ANOVA with repeated measures showed a significant within group effect ($P = 0.001$). Planned comparisons with paired samples *t* test revealed that only the group exposed to natural mate choruses showed a significant elevation in estradiol concentrations after treatment ($P = 0.01$). (b) Repeated measurements of mean (\pm SE) plasma androgen concentrations in females both before acoustic exposure and again after ten consecutive nights of acoustic exposure with either mate choruses or random tones. *N* equals the number of subjects tested in each treatment.

Table 1

Summary of results in Lynch and Wilczynski (2005)

Reproductive Stage	Hormones		
	Estradiol	Progesterone	Androgen
Unamplexed	↓	↓	↑
Amplexed	↑	↑	↓
Post-mated	↓	↓	↓

Female reproductive hormones, specifically estradiol, progesterone and androgen concentrations, fluctuate over three different reproductive stages. These reproductive stages mark the transition through a single reproductive cycle, which occurs approximately every 4–6 weeks in the female túngara frog. The unamplexed stage is while the female is alone at the breeding pond and has not mated yet. The amplexed stage is while the female is in the process of mating and the post-mated stage is 10 days after the female has laid her eggs.