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Corticosterone and Growth in Pacific Treefrog (*Hyla regilla*) Tadpoles

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In all vertebrates, responses to environmental perturbation are regulated to at least some degree by glucocorticoid hormones (GCs). In amphibians, GCs, along with several other hormones, also play an important role in larval growth and development preceding metamorphosis. Given these two well-established functions, GCs might be expected to have a role in the well-documented effects that environmental stressors can have on larval amphibian growth and development. However, while treatment with exogenous corticosterone (the main GC in amphibians) can alter growth and development in larval anurans, few studies have addressed the question of whether corticosterone levels achieved in the treatment are representative of levels in amphibians in the field. In this study, we examined physiological levels of corticosterone in Pacific Treefrog tadpoles (*Hyla regilla*) using a confinement stress protocol in the field and then examined the effects of exogenous corticosterone on growth of treefrog tadpoles in the laboratory. We found that treefrog tadpoles in the field did respond to confinement with increasing corticosterone levels. In addition, treatment with corticosterone in the laboratory resulted in decreased growth. However, the levels of corticosterone in our laboratory treated tadpoles were outside the range of what we have observed in wild animals. Although it is possible that these levels are still in the physiological range, they would be on the high end of what is observed in the field. We suggest that to understand the impacts of environmental stressors on larval amphibians via hormonal mechanisms, we must learn more about what the natural ranges of these hormones are in amphibians developing in the field.

GLUCOCORTICOID hormones (GCs) are released from the adrenal cortex in response to activation of the hypothalamic-pituitary-adrenal (HPA) axis, and play an important role in the response of vertebrates to environmental perturbation (McEwen and Wingfield, 2003). One of their main actions, and the source of their name, is to mobilize energy stores and thereby increase the energy that is available to organisms for surviving stressful periods (Romero, 2002). In addition, with release of GCs other non-vital physiological and behavioral processes are down regulated. For instance, reproductive activities might be temporarily suppressed (e.g., Moore and Miller, 1984). In the short-term, this is obviously beneficial for survival. However, over longer periods of time and chronic exposure to the stressor, these responses can become deleterious and can result in muscle catabolism, decreased growth and development, immunosuppression in the face of disease, and decreased reproductive output (Sapolsky, 1993).

Corticosterone is the main GC in amphibians. In addition to the generalized roles described above for vertebrates, corticosterone plays an important role in amphibian metamorphosis, along with the thyroid hormones, thryoxine (T_4) and triiodothyronine(T_3), and prolactin (for review see Kikuyama et al., 1993). In metamorphosing amphibians, both thyroid-stimulating hormone (TSH), which is responsible for regulation of T_3 and T_4, and adrenocorticotropic hormone (ACTH), which stimulates corticosterone production from the adrenals, are released in response to corticotropin-releasing factor (CRF) from the hypothalamus. Given the role of corticosterone in metamorphosis, several studies have examined baseline levels of corticosterone over the course of amphibian metamorphosis in the laboratory (e.g., Krug et al., 1983; Carr and Norris, 1988; Glennemeier and Denver, 2002a). These studies have generally documented a slow rise in corticosterone, with a peak near metamorphic climax, although there appears to be some variation in the timing of the peak.

In addition, in an attempt to understand the complex interactions between thyroid hormones and corticosterone, several studies have examined the effects of experimentally treating larval amphibians with exogenous hormones (e.g., Table 1; Kobayashi, 1958; Frieden and Naile, 1995; Hayes and Wu, 1995). These studies have demonstrated that exogenous corticosterone can influence growth and development, but that the effects tend to depend upon the timing of treatment during development. Treat-

TABLE 1. STUDIES THAT HAVE TREATED LARVAL ANURANS WITH EXOGENOUS CORTICOSTERONE. Studies prior to 1955 are not included.

Study	Species	Corticosterone treatment[a]	Days of exposure	Resulting mean corticosterone levels in tadpoles[b]
This study	*Hyla regilla*	0.1 μM	50	22.44 ng/g
		0.5 μM		54.50 ng/g
(Glennemeier and Denver, 2002b)	*Rana pipiens*	0.062 μM	18	0.57 ng/g
		0.125 μM		0.88 ng/g
(Glennemeier and Denver, 2002b)	*Rana pipiens*	0.062 μM	28	0.35 ng/g
		0.125 μM		0.32 ng/g
(Hayes and Wu, 1995)	*Bufo boreas*	0.55 μM	Max.27	25 ng/g
(Gray and Janssens, 1990)	*Xenopus laevis*	3.4 μM	Varied	Not reported
(Hayes et al., 1993)	*Bufo boreas*	0.275, 1.1, 4.4 μM	Varied	Not reported
(Kobayashi, 1958)	*Bufo vulgaris formosus*	1.7 μM#	Varied	Not reported
		3.4 μM#		Not reported
(Kobayashi, 1958)	*Rana japonica*	6.7 μM#	Varied	Not reported
		0.1 mg pellet implanted#		Not reported
(Frieden and Naile, 1955)	*Bufo bufo bufo, Rana hechsheri, Rana pipiens*	50 μM&	Varied	Not reported
		1–100 μM&		

a: In corticosterone treatment, all were dissolved in the water housing the amphibians except where noted (no superscript = corticosterone, # = desoxycorticosterone acetate and & = hydrocortisone).
b: Mean corticosterone levels were estimated from graphs if actual numbers were not provided in text or tables.

ment with corticosterone early during larval development tends to result in decreased growth, while corticosterone treatment later in development can actually act to speed up the rate of development (Hayes et al., 1993). Recently, Glennemeier and Denver (2002b) suggested that exposure to even very low doses of corticosterone (125 nM) can decrease growth in leopard frog (*Rana pipiens*) tadpoles.

All of these studies raise interesting questions about the impact that exposure to environmental stressors could have on amphibian metamorphosis in natural populations. However, to our knowledge, none of these studies have reported corticosterone levels in free-living tadpoles of the species studied (although Glennemeier and Denver [2002b] cite unpublished data that the levels they observed in treated tadpoles were natural and physiological, and their corticosterone treatments resulted in levels that were within the range of the endogenous increases they observed during laboratory confinement stress). Knowledge of the hormone levels in free-living animals is important for determining how close hormone treatment comes to achieving physiological levels of corticosterone. This is especially important if we are interested in understanding the potential effects that environmental stressors, acting through neuroendocrine pathways, can have on metamorphosis.

Our goals in this study were to: (1) examine physiological levels of corticosterone in Pacific Treefrog, *Hyla regilla*, tadpoles using a confinement stress protocol in the field and (2) determine the effects of exogenous corticosterone on growth of treefrog tadpoles in the laboratory.

MATERIALS AND METHODS

Confinement stress in a natural pond.—Our confinement stress experiment was completed at a natural Pacific Treefrog breeding site in the Cascade Mountains of Oregon, USA, during the summer of 2000, when tadpoles were present. Our confinement protocol involved placing single individuals in 800 ml plastic cups with mesh sides for 0, 30, or 60 minutes (N = 7 for each time point). These containers were placed in the pond so that they were filled with 4 cm depth of water and were gently shaken every 3 minutes over the course of confinement. All larvae were rapidly frozen in the field. Larvae at the 0 minute time point were netted in the field and immediately transferred to a 1.5 ml microcentrifuge tube. Tubes were then dipped into a dry ice/ethanol slurry for 1 minute, so that freezing occurred within 2 minutes of initial capture. Larvae at the other time points (30 and 60 minutes) were frozen in the same manner, with transfer to a 1.5 ml microcentrifuge tube

occurring immediately following their confinement period. Tubes were stored on dry ice until return to the laboratory, where they were stored at−70 C until the radioimmunoassay was completed.

Confinement stress in outdoor mesocosms.—Six *H. regilla* egg masses were collected from a natural breeding pond in the Cascadae Mountains of Oregon in May 2001 and returned to the laboratory. In the laboratory, egg masses were placed in a 38 L aquarium filled with dechlorinated tap water. Windows in the room provided a natural photoperiod and the temperature was maintained at approximately 16 C. Following hatching, tadpoles were maintained in the laboratory in the same tank and were fed ground rabbit chow *ad libitum* prior to random assignment to the field mesocosms.

Five plastic tubs (56 [L] × 36 [W] × 28 [H] cm) were set-up outdoors at the Salmon disease laboratory of Oregon State University (approximately 7 km east of Corvallis, Benton County, Oregon). Tubs were filled to a depth of 25 cm with well water, which was allowed to age for one week prior to addition of tadpoles. After one week, five tadpoles (approx. Gosner stage 27; Gosner, 1960) were randomly added to each of the mesocosms. During the experiment, temperatures in the mesocosms ranged from 8.3–30.2 C. Sixteen days after tadpoles were added, we completed a confinement stress series at 0 and 60 minutes. This was done using different confinement vessels than in the field stress series. For the mesocosms, we used opaque plastic canisters (3 cm diameter, 5 cm height) filled with 35 ml water from the appropriate mesocosm. One tadpole from each of the five tubs was frozen immediately (time = 0) and one was placed in the plastic canister and floated in the mesocosm for 60 minutes (time = 60) prior to freezing. Freezing was done as in the field stress series, using a dry ice/ethanol slurry. Frozen samples were stored at −70 C until the radioimmunoassay could be completed.

Treatment of tadpoles with exogenous corticosterone.—Twenty *H. regilla* egg masses were collected from a natural breeding pond in the Cascadae Mountains of Oregon in June 2000 and returned to the laboratory. In the laboratory, egg masses were placed in a 38 L aquarium filled with dechlorinated tap water. Windows in the room provided a natural photoperiod and the temperature was maintained at approximately 16 C. One week after hatching, we began to feed the tadpoles ground rabbit chow every other day. We began corticosterone treatments two weeks after hatching, when all tadpoles were at Gosner stage 25 (Gosner, 1960). We randomly assigned 15 tadpoles to each of 20, 4 L plastic tubs (4 treatments × 5 replicates/treatment). The four treatments were: (1) 3.5 L dechlorinated water control, (2) ethanol control (0.1 ml ETOH in 3.5 L dechlorinated tap water = 0.0028% ethanol), (3) 0.1 μM corticosterone (0.00012 g corticosterone/0.1 ml ETOH in 3.5 L dechlorinated tap water), and (4) 0.5 μM corticosterone (0.0006 g corticosterone/0.1 ml ETOH in 3.5 L dechlorinated tap water). Levels of corticosterone administered to tadpoles were based on levels utilized in previous studies of larval anurans (Table 1). Tadpoles were reared in plastic tubs and complete water changes were done every three days to maintain appropriate treatment concentrations. Tadpoles were fed ground rabbit chow *ad libitum* during the experiment. After 50 days, we collected a single tadpole from each tank for analysis of baseline corticosterone levels (N = 5 for each treatment). Larvae for this test were netted in the experimental tub and immediately transferred to a 1.5 ml microcentrifuge tube and processed as for the confinement stress protocols described above. Following tadpole collection for corticosterone analysis, the mean mass of remaining tadpoles was recorded for each of the 20 tubs (N = 5 for each treatment).

Radioimmunoassay.—Whole body levels of corticosterone were measured by RIA following the procedures of Belden et al. (2003). The samples were run in two assays. Briefly, whole body homogenates were used for each assay. Each tadpole was weighed and homogenized with a mass adjusted amount of distilled water (mass × 10 ml + 0.5 ml rinse; minimum 1.5 ml and maximum 4 ml). For individual recovery determination, each sample was equilibrated overnight with 2,000 cpm of tritiated corticosterone. Each sample was then extracted in 5 ml of dichloromethane. To break the emulsion each sample was centrifuged at 2000 rpm for 15 minutes. The organic phase was then removed and dried in a warm water bath, under a stream of nitrogen gas. The extracts were resuspended in 10% ethyl acetate in isooctane. The samples were chromatographed through individual celite columns to separate the steroid fractions and neutral lipids. The fractions were eluted using stepwise increasing proportions of ethyl acetate in isooctane. The purified eluates were dried and resuspended in buffer (phosphate buffered saline with 0.1% gelatin) for the assay. For the assay, individual sample recoveries were determined from 100 μl of the sample while 200 μl

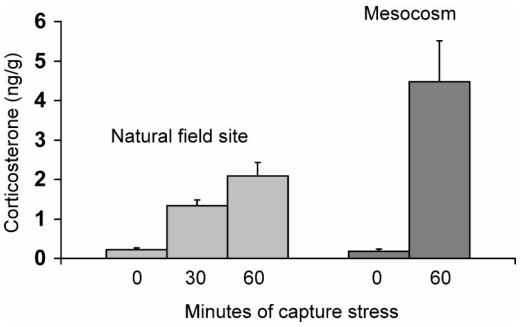

Fig. 1. Mean (±SE) whole-body corticosterone levels (ng/g) of Pacific Treefrog tadpoles in response to confinement stress in a natural pond (N = 7 for each time point) and outdoor mesocosms (N = 5 for each time point).

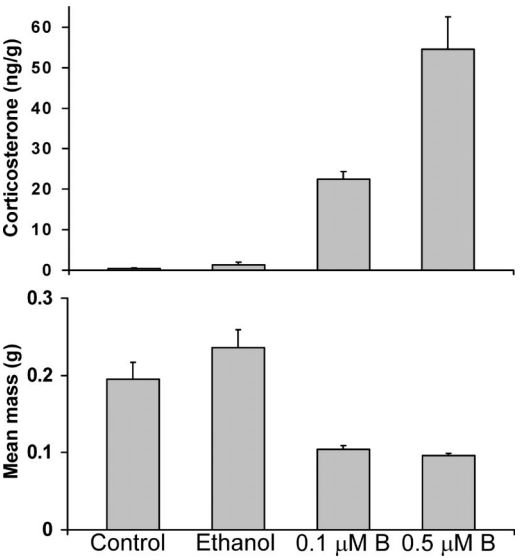

Fig. 2. Mean (±SE) whole-body corticosterone levels (ng/g) and mean (±SE) mass (g) of Pacific Treefrog tadpoles treated with exogenous corticosterone in the laboratory for 50 days (N = 5 for each treatment group).

of the sample was allocated in duplicate for the assay. Serial dilutions for the standard curves were performed in duplicate. All samples, including serial dilutions and total bound, were incubated overnight with 100 μl of antibody and 100 μl of tritiated steroid. Unbound steroid was separated using dextran-coated charcoal and the bound steroid decanted into scintillation vials. Average intraassay variation was 11.7% and interassay variation was 12.7%. Average extraction efficiency was 74%.

Results

Confinement stress in the field resulted in an increase in corticosterone levels (Fig. 1; overall ANOVA; $F = 18.06$; $P < 0.005$). The main increase appeared to occur after 30 minutes of confinement (post-hoc Tukey test 0 vs. 30 minutes, $P = 0.006$), with little additional increase at 60 minutes (post-hoc Tukey test 30 vs. 60 minutes, $P = 0.068$).

In the outdoor mesocosms, there was also a significant elevation in corticosterone following 60 minutes of confinement (Fig. 1; T-test; $P = 0.003$). The maximum level recorded during that experiment was 8.01 ng/g corticosterone, in an individual confined for 60 minutes.

Corticosterone treatment in the laboratory resulted in significant increases in baseline corticosterone levels in the tadpoles after 50 days of exposure (Fig. 2; overall ANOVA; $F = 89.09$; $P < 0.005$). There was no difference in baseline corticosterone levels between control and ethanol treated groups (post-hoc Tukey test, $P = 0.281$), or between the low and high corticosterone treated groups (post-hoc Tukey test, $P = 0.121$). However, levels in both the low and high corticosterone groups were significantly higher than in either the control or ethanol groups (control vs. low, control vs. high, ethanol vs. low, ethanol vs. high, Post-hoc Tukey tests all have $P < 0.005$). The range of baseline levels in the groups was as follows: control 0.23–0.7 ng/g, ethanol 0.33–4.24 ng/g (the 4.24 ng/g individual was an outlier in the group, all others were below 1 ng/g), low corticosterone 19.22–29.91 ng/g, high corticosterone 32.23–74.71 ng/g.

Growth in the laboratory was negatively effected by corticosterone treatment (Fig. 2; overall ANOVA; $F = 91.3$; $P < 0.005$. Post-hoc Tukey tests, all groups $P \leq 0.005$, except the two corticosterone treated groups, which were not significantly different, $P = 0.87$). Mean mass of larvae treated with corticosterone was approximately half that of controls after 50 days of treatment, regardless of the concentration of corticosterone administered (Fig. 2). Our ethanol treated groups had higher mean masses than controls (control mean mass (±SD) = 0.195 ±0.02g; ethanol mean mass (±SD) = 0.236 ± 0.02g; post-hoc Tukey test, $P = 0.005$), although as stated above, baseline corticosterone levels did not differ between those two groups.

Discussion

Taken together, the results of our first two experiments suggest that larval treefrogs early in development have a functioning HPA axis and

TABLE 2. STUDIES EXAMINING THE STRESS RESPONSE OF TADPOLES.

Study	Species/ Developmental stage[a]	Mean baseline corticosterone levels[b]	ACTH injection[1] /confinement[2] mean maximum corticosterone levels[b]
WHOLE BODY LEVELS (amount of hormone per gram of tadpole)			
This study	*Hyla regilla*	0.22 ng/g[2]	2.09 ng/g[2]
	(Gosner 25+)	0.18 ng/g[2]	4.47 ng/g[2]
(Belden et al., 2003)	*Rana cascadae* (Gosner 25+)	0.42 ng/g[2]	1.21 ng/g[2]
(Glennemeier and Denver, 2002b)	*Rana pipiens*	0.3 ng/g[1]	2.2 ng/g[1]
(Glennemeier and Denver, 2002a)	*Rana pipiens*	0.25 ng/g[1]	3.6 ng/g[1]
	(Gosner 26–29)	0.16 ng/g[2]	0.64 ng/g[2]
(Glennemeier and Denver, 2002a)	*Rana pipiens*	0.16 ng/g[1]	0.83 ng/g[1]
	(Gosner 35–40)	0.2 ng/g[2]	0.5 ng/g[2]
(Glennemeier and Denver, 2002a)	*Xenopus laevis*	0.9 ng/g[1]	3.4 ng/g[1]
	Early	1.4 ng/g[2]	9.4 ng/g[2]
(Glennemeier and Denver, 2002a)	*Xenopus laevis*	0.7 ng/g[1]	9.3 ng/g[1]
	Middle	0.6 ng/g[2]	4.1 ng/g[2]
(Glennemeier and Denver, 2002a)	*Xenopus laevis*	0.8 ng/g[1]	5.1 ng/g[1]
	Late	0.8 ng/g[2]	5.8 ng/g[2]
(Glennemeier and Denver, 2001)	*Rana pipiens* (Gosner 25+)	1.5 ng/g	4.2 ng/g[1]
PLASMA LEVELS (amount of hormone per milliliter of plasma)			
(Krug et al., 1983)	*Rana catesbeiana* premetamorphosis	7 ng/ml[1]	11 ng/ml[1] (not significant increase)
(Krug et al., 1983)	*Rana catesbieana* prometamorphosis	13 ng/ml[1]	42 ng/ml[1]
(Jaffe, 1981)	*Rana catesbieana* Stages I-X T&K	0.22 μg/100ml[1]	0.58 μg/100ml[1]

a: Stage is given as the range when tadpoles were preserved for hormone analysis, or if listed with a "+" as the stage when experiment was started. Gosner = (Gosner, 1960), T&K = (Taylor and Kollros, 1946).
b: Mean corticosterone levels were estimated from graphs if actual numbers were not provided in text or tables.

are able to respond to stressors in the environment by increasing corticosterone levels. Increasing corticosterone levels in response to confinement or ACTH challenge have been documented now in tadpoles from four anuran families (Ranidae, Bufonidae, Pipidae, and now, Hylidae; Table 2). The whole body corticosterone levels we report for Pacific Treefrog tadpoles (range of approximately 0.2 to 8 ng/g) are similar to physiological levels (i.e., unmanipulated levels) reported for other species of larval anurans (e.g., Hayes and Wu, 1995; Denver, 1998; Glennemeier and Denver, 2001).

Our results on growth in the laboratory are also similar to those observed in other studies of larval amphibians treated with exogenous corticosterone. In general, glucocorticoid exposure early in development results in decreased growth and development, while exposure at later stages can accelerate metamorphosis, likely through interactions with the thyroid hormones. The levels of corticosterone attained in our tadpoles were much higher than Glennemeier and Denver (2002b) reported for treatment of *Rana pipiens* with a similar dose. They exposed larvae for 18 days (first basal experiment) and 28 days (ACTH injection), and in the 0.125 μM treatment, mean corticosterone levels in *Rana pipiens* were still less than 1 ng/g at both 18 and 28 days (Table 1). In this study, we exposed treefrog tadpoles for 50 days and the resultant corticosterone levels were in the range of 19.22–29.91 ng/g. One possible explanation for the dramatic differences between our results could simply be the differences in the length of exposure. However, there could also be substantial species specific differences in steroid sensitivity, metabolism, and/or excretion. Metabolism would appear to be an important component in these types of studies, as whole-body corticosterone levels, while elevated, never approach the levels in the water. Interestingly, Hayes and Licht (1993) examined metabolism of corticosterone in larval amphibians of eight species, including *Hyla regilla*, and found no evidence that any of them metabolized corticosterone.

Confinement stress protocols do not neces-

sarily indicate maximum levels of corticosterone that are possible in free-living animals. Indeed, the highest corticosterone level we have seen naturally occurring in *H. regilla* tadpoles is 11.6 ng/g (unpubl. data, from a tadpole housed in a small field enclosure for one week prior to hormone analysis), which is well above the maximum of 3.3 ng/g that we observed during the field-based confinement stress presented in this paper. However, while confinement stress may not provide a perfect representation of the possible range of endogenous hormone values, we do think that the physiological range of hormones present in wild animals should be addressed when designing studies that investigate the effects of these hormones on growth and development. Based on the range of endogenous corticosterone levels we have observed, it seems likely that treatment of treefrog tadpoles with 0.1 and 0.5 μM corticosterone resulted in levels that were pharmacological. The minimum baseline level we observed in our corticosterone treated groups was 19.22 ng/g, which is approximately twice the maximum endogenous value that we have observed.

Given the very high levels of corticosterone in our corticosterone treated tadpoles, it seems likely that the decreased growth we observed in response to corticosterone treatment may not be indicative of the response of free-living treefrog tadpoles to physiologically relevant increases in corticosterone. This is important in light of the fact that much of our knowledge about the function of corticosterone in amphibian larvae is based on manipulations of the hormone without reference to what the normal physiological range of the hormone is in free-living tadpoles. While those types of studies have been very useful in elucidating the interactions between GCs and other hormones during metamorphosis (e.g., Frieden and Naile, 1955; Kobayashi, 1958; Hayes and Wu, 1995), they are unlikely to tell us very much about the role that specific environmental stressors (and the subsequent physiological increases in corticosterone) can have in altering tadpole growth and development in the field.

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Literature Cited

Belden, L. K., I. T. Moore, R. T. Mason, J. C. Wingfield, and A. R. Blaustein. 2003. Survival, the hormonal stress response and UV-B avoidance in Cascades Frog tadpoles (*Rana cascadae*) exposed to UV-B radiation. Funct. Ecol. 17:409–416.

Carr, J. A., and D. O. Norris. 1988. Interrenal activity during metamorphosis of the Tiger Salamander, *Ambystoma tigrinum*. Gen. Comp. Endocrinol. 71:63–69.

Denver, R. J. 1998. Hormonal correlates of environmentally induced metamorphosis in the Western Spadefoot Toad, *Scaphiopus hammondii*. *Ibid.* 110: 326–336.

Frieden, E., and B. Naile. 1955. Biochemistry of amphibian metamorphosis: I. Enhancement of induced metamorphosis by gluco-corticoids. Science. 121:37–39.

Glennemeier, K. A., and R. J. Denver. 2001. Sublethal effects of chronic exposure to an organochlorine compound on Northern Leopard Frog (*Rana pipiens*) tadpoles. Environ. Toxicol. 16:287–297.

———, and ———. 2002a. Developmental changes in interrenal responsiveness in anuran amphibians. Integrat. Comp. Biol. 42:565–573.

———, and ———. 2002b. Small changes in whole-body corticosterone content affect larval *Rana pipiens* fitness components. Gen. Comp. Endocrinol. 127:16–25.

Gosner, K. L. 1960. A simplified table for staging anuran embryos and larvae with notes on identification. Herpetologica. 16:183–190.

Gray, K. M., and P. A. Janssens. 1990. Gonadal hormones inhibit the induction of metamorphosis by thyroid hormones in *Xenopus laevis* tadpoles *in vivo*, but not *in vitro*. Gen. Comp. Endocrinol. 77:202–211.

Hayes, T., R. Chan, and P. Licht. 1993. Interactions of temperature and steroids on larval growth, development, and metamorphosis of a toad (*Bufo boreas*). J. Exp. Zool. 232:206–215.

———, and P. Licht. 1993. Metabolism of exogenous steroids by anuran larvae. Gen. Comp. Endocrinol. 91:250–258.

———, and T. H. Wu. 1995. Interdependence of corticosterone and thyroid hormones in toad larvae (*Bufo boreas*). II. Regulation of corticosterone and thyroid hormones. J. Exp. Zool. 271:103–111.

Jaffe, R. C. 1981. Plasma concentration of corticosterone during *Rana catesbeiana* tadpole metamorphosis. Gen. Comp. Endocrinol. 44:314–318.

Kikuyama, S., K. Kawamura, S. Tanaka, and K. Yamamoto. 1993. Aspects of amphibian metamorphosis: hormonal control. Int. Rev. Cytol. 145:105–148.

Kobayashi, H. 1958. Effect of desoxycorticosterone

acetate on metamorphosis induced by thyroxine in anuran tadpoles. Endocrinology 62:371–377.

KRUG, E. C., K. V. HONN, J. BATTISTA, AND C. S. NICOLL. 1983. Corticosteroids in serum of *Rana catesbeiana* during development and metamorphosis. Gen. Comp. Endocrinol. 52:232–241.

MCEWEN, B. S., AND J. C. WINGFIELD. 2003. The concept of allostasis in biology and biomedicine. Horm. Behav. 43:2–15.

MOORE, F. L., AND L. J. MILLER. 1984. Stress-induced inhibition of sexual behavior: corticosterone inhibits courtship behaviors of a male amphibian (*Taricha granulosa*). *Ibid.* 18:400–410.

ROMERO, L. M. 2002. Seasonal changes in plasma glucocorticoid concentrations in free-living vertebrates. Gen. Comp. Endocrinol. 128:1–24.

SAPOLSKY, R. M. 1993. Neuroendocrinology of the stress-response, p. 287–324. *In:* Behavioral Endocrinology. J. B. Becker, S. M. Breedlove, and D. Crews (eds.). MIT press, Cambridge, Massachusetts.

TAYLOR, A. C., AND J. J. KOLLROS. 1946. Stages in the normal development of *Rana pipiens* larvae. Anatomical Record. 94:7–23.

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