

Courtship Behavior and Plasma Levels of Androgens and Corticosterone in Male Marbled Salamanders, *Ambystoma opacum* (Ambystomatidae)

Lynne D. Houck,* Mary T. Mendonça,† Tracy K. Lynch,‡ and David E. Scott‡

*Department of Ecology and Evolution, University of Chicago, 940 E. 57th Street, Chicago, Illinois 60637;

†Department of Zoology and Wildlife, Auburn University, Auburn, Alabama 36849; and ‡Savannah River Ecology Laboratory, Drawer E, Aiken, South Carolina 29802

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We measured plasma levels of testosterone, dihydrotestosterone (DHT), and corticosterone for male marbled salamanders (*Ambystoma opacum*) collected during the breeding season. Our goal was to ascertain whether steroid levels changed in response to particular reproductive behaviors or laboratory confinement. Six groups of salamanders were examined: (a) MIGRATING, males migrating toward the pond basin during the breeding season; (b) LABORATORY, males kept under confined conditions in the laboratory for 10 days; (c) LAB-FIELD, laboratory males that were later released into seminatural enclosures in the field; (d) COURTING, males from male–female pairs in which the male actively courted the female (and deposited at least one spermatophore); (e) SOLO, males that were individually isolated from conspecifics; and (f) MALE-MALE, males that were placed together in pairs, and in which one male actively courted the other male. In three groups (COURTING, SOLO, and MALE-MALE), salamanders were placed in containers for observation and each male was observed for at least 2 hr prior to a plasma sample being taken. Circulating levels of testosterone, DHT, and corticosterone did not differ significantly for males in these groups. The similarity of androgen levels among the three groups indicated a lack of behaviorally evoked change under experimental conditions designed to reveal a behavior–androgen response. Male *A. opacum* differ taxonomically from other amphibians showing a behavior–androgen

response (three species of toads in the genus *Bufo*) and also lack amplexus and male–male combat during competition for mates. The effects of confinement were indicated by levels of testosterone and DHT in LABORATORY males that were significantly lower than average levels of males in the following groups: MIGRATING, LAB-FIELD, and MALE-MALE. We inferred that LAB-FIELD males, following their release to seminatural enclosures, were able to regain plasma androgen levels typical of migrating males. This increase is one of very few demonstrations for amphibians of an increase in androgen levels upon release from laboratory confinement. Levels of corticosterone did not differ significantly between males that were active in the field and males that were kept in the laboratory. The similarity of corticosterone levels among these groups differs from the typical pattern of elevated corticosterone and depressed androgen levels in captive amphibians. Maximal corticosterone levels in breeding male *A. opacum* may act differently than in other species in which chronic elevations inhibit the pituitary–gonadal axis. © 1996 Academic

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In many vertebrate species, circulating androgens usually must be elevated above some threshold level in order for reproductive behaviors to be expressed (Lofts, 1984; Licht, 1979, 1984; Moore, 1987). Above this threshold, however, androgen levels for individual

males can be highly variable. This variability in circulating androgens typically is not correlated with the intensity, frequency, or consummation of reproductive behaviors (Andreoletti *et al.* 1983; Mendonça *et al.* 1985; Wada *et al.*, 1976).

In some species, however, the expression of certain behaviors is causally linked to the subsequent elevation in levels of plasma androgens (Harding, 1981; Wingfield *et al.*, 1990). Laboratory studies have shown that mating, or even just the presence of a sexually receptive female, can elevate the levels of circulating androgens in a responsive male (see Graham and Desjardins, 1980; Harding and Follett, 1979). In some species, androgen responses also can be evoked by social stimuli other than sexual interactions. Territorial aggression between rival males has been correlated with resultant increases in plasma androgens (Harding, 1981; Wingfield and Ramenofsky, 1985; Wingfield and Moore, 1987). In a field study of territorial red-winged blackbirds, for example, plasma androgens were higher in males at the peak of aggressive encounters, compared with levels in males that had not recently been involved in aggressive interactions (Harding and Follett 1979). In white-throated sparrows, measures of the intensity and duration of territorial behavior in resident male sparrows were correlated with the level and duration of the testosterone increase in these sparrows (Wingfield *et al.*, 1987).

A behaviorally induced androgen response—whether related to sexual interactions or to male–male aggression—has been found only in some vertebrate species and only under certain conditions. All territorial species do not show androgen responses, even when male–male interactions are highly intense (Wingfield *et al.*, 1990). In fact, the general case for vertebrates is that aggressive interactions trigger an adrenal corticoid response, following which luteinizing hormone (LH) and testosterone levels decrease (Bronson, 1973; Bronson *et al.*, 1973; Louch and Higgenbotham, 1967; Moore and Miller, 1984; Mendonça *et al.*, 1985). Why, then, is an androgen response found for particular species, but not for others?

This question was addressed in a synthetic review of behavior–androgen responses in field studies of avian species (Wingfield *et al.*, 1990). In analyzing these studies, Wingfield and colleagues observed that male–male aggression typically evoked a temporary increase

in androgen levels in species having a monogamous mating system and male parental care. Under these conditions, an androgen response typically was evoked only during periods when a male was actively caring for offspring, usually in response to intense male–male aggression associated with territorial defense. Periods of paternal care apparently are characterized by a relative reduction in androgen levels, compared to levels found earlier in males that were establishing territories (Hegner and Wingfield, 1986). Reduced androgen levels during paternal care presumably reduce male aggression that might be directed toward the young. Wingfield *et al.* (1990) hypothesized that a transient androgen increase in response to behavioral challenges from rivals allows the parental male to mobilize temporary physiological support on the occasions when a rival intrusion may warrant increased aggression.

The well-developed hypothesis for avian patterns of androgen response was based on field studies that combined an evaluation of mating systems (e.g., monogamy vs polygyny, levels of male parental care) with measures of reproductive behaviors and androgen levels. This type of approach is now being used to guide field studies of other vertebrate groups (e.g., Emerson *et al.*, 1993; Townsend *et al.*, 1991). In amphibians, studies of five species of anurans and one species of salamander (reviewed by Houck and Woodley, 1995) revealed a behaviorally evoked androgen response for three species of toads in the genus *Bufo* (Lupo *et al.*, 1993; Orchinick *et al.*, 1988; Siboulet, 1981). Although data available for amphibians are few, these studies demonstrate that androgen responses can occur in amphibians.

A single amphibian study has identified an androgen response and also has interpreted this response in terms of the breeding system. In studying the marine toad, *Bufo marinus*, from Hawaii, Orchinick *et al.* (1988) noted that mating occurs unpredictably during the breeding season, with bursts of activity correlated with sporadic rains. Male–male competition for access to potential mates is intense, but males do not defend territories. Males are polygynous and do not engage in parental care. Among *B. marinus* active on courtship nights, males in amplexus had significantly higher androgen levels (testosterone and dihydrotestosterone) than did other adult males not engaged in

amplexus. Furthermore, circulating androgens were positively correlated with the duration of amplexus (Orchinik *et al.*, 1988).

These results suggest that an androgen response might be favored in amphibian species characterized by competition for mates that is both intense and unpredictably sporadic. We tested this hypothesis in the marbled salamander, *Ambystoma opacum* (Family Ambystomatidae). Like *B. marinus*, *A. opacum* has explosive and highly sporadic bouts of mating behavior that are determined by unpredictable rainfall (Noble and Brady, 1933). On sporadic nights of courtship activity, *A. opacum* males typically experience intense competition for mates, and the operational sex ratio is heavily skewed toward males (Krenz and Scott, 1994).

We examined plasma levels of both androgens and corticosterone in relation to male reproductive behaviors and to the effects of laboratory confinement in *A. opacum*. We tested the following specific hypotheses: (a) males that engaged in multiple hours of active courtship would have plasma androgen levels that were elevated relative to values for control males that were not courting, (b) androgen levels for courting males would be elevated relative to males kept under laboratory conditions, (c) corticosterone levels would be elevated in courting males, compared to levels in noncourting control males, and (d) corticosterone levels would be elevated in males kept in the laboratory.

METHODS AND MATERIALS

Study Animal and Study Site

Populations of the marbled salamander, *A. opacum*, are found in the eastern United States. In autumn, adults migrate to pond basins that are dry or that have low water levels (Noble and Brady, 1933; Jackson *et al.*, 1989). Unlike most other salamander species having aquatic larvae, courtship and sperm transfer in *A. opacum* occur on land (Noble and Brady, 1993; Krenz and Scott, 1994). Females oviposit in nests located in dry pond basins, and females brood their eggs (Nussbaum, 1985). Eggs hatch when seasonal rains begin to fill the pond basin and nests are flooded (Petranka *et al.*, 1982). The larval period is 3–6 months (Scott, 1990), followed by metamorphosis and a terrestrial

growth stage of 1 to 6 years before reproductive maturity is achieved (Scott, 1994).

This study was conducted at the U. S. Department of Energy's Savannah River Site in Aiken County, South Carolina. All salamanders were collected at night as they migrated to a breeding site (Bay 23) at the start of the breeding season in October 1993. Adult *A. opacum* (males and females) were transported to nearby Ginger's Bay (another breeding site) where behavioral observations were conducted. Both Bay 23 and Ginger's Bay are isolated, ephemeral wetlands located in mixed pine-hardwood habitat on the Upper Coastal Plain (for a detailed description see Scott, 1990).

At Ginger's Bay, animals were housed temporarily in seminatural enclosures to control for possible hormonal responses to male-female interactions that might have occurred during migration. Males and females were placed in separate enclosures. Each enclosure was a round metal tank (approx. 1.5 m in diameter) that contained moist dirt, sedge clumps, and leaves taken from the immediate area. To provide underground refugia, segments of PVC tubing (approx. 6–12 in. long) were buried in the dirt, with one end open at the surface. Salamanders in these enclosures experienced the same environmental conditions as did nonexperimental animals that had migrated to Ginger's Bay. Activity was monitored qualitatively for nonexperimental males and for males in enclosures to determine whether enclosures restricted spontaneous activity or reproductive behavior.

Behavioral Groups

Plasma samples were taken from six groups of males. The sample size for each group is given below in parentheses following the group name. Individual behavior was not observed for males in three groups (MIGRATING, LAB, and LAB-FIELD). Systematic behavioral observations were made for males in three other groups (SOLO, COURTING, MALE-MALE).

With the exception of males in the LAB group, plasma samples were taken from males that had been spontaneously active. We defined spontaneous activity as occurring when an animal had emerged from cover and was moving about at the surface. Spontaneous activity usually occurred on nights following (or during) rain. Males that spontaneously emerged from cover on rainy nights presumably were in search of

potential mates. The first of several waves of migration occurred on 10 October 1993; time limitations allowed us to collect animals on this date for five of the behavioral groups, but not for sampling the MIGRATING males.

MIGRATING ($n = 7$). Incoming males were collected shortly after dusk as they moved toward the pond basin at Bay 23 on 17 October 1993. At the time of collection, each male was not engaged in conspecific interactions. We cannot rule out the possibility of interactions prior to collection.

LAB ($n = 5$). Males captured while migrating to Bay 23 on 10 October 1993 were put in bins and transported to the Savannah River Ecology Laboratory within 4 hr of capture. Groups of approximately 20 males were housed together, each group in a plastic box (approx. $20 \times 40 \times 8$ cm) containing crumpled moist paper towels. Boxes of salamanders were kept in an environmental chamber at approximately 15° and with a photoperiod of 12D:12L. The LAB males were housed under these conditions for 10 days before plasma samples were taken on 20 October 1993.

LAB-FIELD ($n = 6$). Males captured while migrating to Bay 23 on 10 October 1993 were kept in the laboratory for 7 days, under identical conditions described for the LAB males above. The males were transferred back to the field (Ginger's Bay) where they were kept in seminatural enclosures (described above). Males spent 4 days under field conditions before plasma samples were taken on 21 October 1993.

Behavioral Observations

Salamanders captured while migrating to Bay 23 on 10 October 1993 were placed in bins (males separated from females) and transported to seminatural enclosures at Ginger's Bay. On nights when animals were spontaneously active, males and females were collected from their respective enclosures and randomly assigned to behavioral groups. Salamanders were placed in plastic containers for behavioral observations, as described below. The containers were rinsed thoroughly before behavioral trials in order to remove possible odor cues from prior use. Containers were opaque, so that animals were visually isolated from conspecifics in other containers. Observations were conducted at Ginger's Bay, using dim lights for illumi-

nation. Scan samples were taken every 10–15 min for 1–2.5 hr. We recorded the activity (no interaction, courtship, spermatophore deposition) of each individual in each observation container. In order to control for potential differences in prior exposure to females, each male used for behavioral observations had spent at least 1 week in a field enclosure that only contained other males.

SOLO ($n = 7$). A single male was placed in each container. SOLO observations were staged on 17, 21, and 25 October 1993.

COURTING ($n = 11$). A single male and a single female were placed in a container together. The male actively courted the female for a minimum of 2 hr. During this time, each male deposited at least two spermatophores (average = 6.5, range = 2–9 spermatophores). COURTING observations were staged on 17, 21, and 25 October 1993.

MALE-MALE ($n = 5$ pairs). Two males were placed in a container together. One male actively courted the other male, which remained passive. In each case, the active male courted for a minimum of 2 hr. In two cases, the courting male deposited spermatophores ($n = 3$, $n = 6$). MALE-MALE observations were staged on 21 and 25 October.

Plasma Sampling

For each animal, a blood sample was taken by heart puncture, after which the animal was anesthetized and preserved. Blood usually was obtained within 3 min after the male was first handled (maximum = 5 min). Blood was collected in heparinized capillary tubes that were kept over ice until all samples had been obtained. Samples then were transported to the laboratory (usually within 3–5 hr after the sample was taken). Tubes were placed in a microcapillary centrifuge and spun for 10 min. Hematocrits were measured for most samples, and the plasma was frozen at -80° .

Steroid Hormone Analyses

Plasma levels of testosterone (T), dihydrotestosterone (DHT), and corticosterone (B) were analyzed without knowledge of the behavioral category for each sample. Extraction, Celite microcolumn chromatography, and radioimmunoassay followed the protocol

detailed in Wingfield *et al.* (1982). Plasma was incubated with 800–1000 cpm of the tritiated analog of the steroid to be measured for 1 hr to enable assessment of recovery efficiency. Plasma was then extracted with diethyl ether, dried under nitrogen gas, resuspended in 2% ethyl acetate in iso-octane, and chromatographed on microcolumns of a Celite:glycol mixture. Column fractions for T, DHT, and B were collected, dried under nitrogen gas, and resuspended in phosphate buffer. The sample for each fraction was divided into three aliquots. One aliquot was placed directly into a scintillation vial with scintillation fluid to determine percentage recovery. Aliquots of two different dilutions were then made for all three assays. These aliquots were incubated overnight at 4° with tritiated T, DHT, and B and their respective antibodies (T3003 for T and DHT and B21-42 antibody for B from Wein Laboratories, Succassana, NJ). After incubation, unbound steroid was removed by addition of a charcoal suspension for 15 min. Charcoal was separated by centrifugation, and the samples were then placed in scintillation vials, scintillation fluid was added, and counts were recorded by a beta counter. Control samples of stripped plasma, stripped plasma plus steroid, and buffer plus steroid also were run with each assay. Intra-assay variation averaged 8, 5, and 5% for T, DHT, and B, respectively. Inter-assay variation averaged 12.1, 12.29, and 14.3% for T, DHT, and B, respectively. Percentage recoveries for the assays averaged 65, 61, and 76% for T, DHT, and B, respectively. Sensitivity of the assays ranged from 9 to 20 pg/ml.

Histology

Testes and a proximal section of the epididymis from each sacrificed male were embedded for standard paraffin sectioning (Humason, 1979), sectioned at 8 μ m, and stained with hematoxylin and eosin to assess spermatogenic state and amount of sperm present in the duct.

Statistical Analyses

Comparisons of plasma hormone levels among behavioral groups were made using the Kruskal–Wallace one-way analysis of variance by ranks ($\alpha = 0.05$ for all statistical tests). This nonparametric test makes no

assumptions concerning sample variance and is 95% as efficient as the parametric *F* test when assumptions of the *F* test are met (Siegel and Castellan, 1988). Pairwise differences among groups were identified with a post hoc multiple comparison test (Tukey's range test), using rank-transformed data and a Bonferroni adjustment to account for multiple comparisons (Rice, 1989).

RESULTS

Salamanders housed in seminatural enclosures typically were active at the surface on the same nights when free-living *A. opacum* also were active at the field site. Activity levels for males in enclosures were similar to levels for free-living salamanders. The similarity in activity levels suggests that normal social activity was not disrupted for salamanders in the enclosures.

Ratio of DHT/T

Among all males sampled, the ratio of DHT to T ranged from 0.39 to 38.3 (average = 2.67; $n = 46$). Plasma values of DHT were significantly correlated with plasma T levels [$T = 2.21 + 0.31(\text{DHT})$, $r^2 = 0.62$, $P \leq 0.01$; $n = 46$]. The ratio of DHT to T was not significantly different among any of the male groups (MIG, LAB, LAB-FIELD, SOLO, COURTING, or MALE-MALE ($H = 9.68$, $P > 0.10$, $n = 46$)).

Relative Androgen Levels

Plasma levels of T and DHT are given in Fig. 1 for all six male groups. Testosterone levels ranged from 2.9 to 78.3 ng/ml (average = 26.8 ng/ml; $n = 46$). DHT levels ranged from 1.7 to 38.3 ng/ml (average = 10.6 ng/ml; $n = 46$). Plasma levels of total androgens measured (T + DHT) ranged from 5.2 to 116.7 ng/ml (average = 37.3 ng/ml, $n = 46$).

Differences among male groups in plasma T were highly significant ($H = 24.03$, $P < 0.001$, $df = 5$; $n = 46$). The highest T levels were found for MIG males and the lowest for LAB males (Fig. 1). Significant differences ($P \leq 0.05$) were found for the following pairwise com-

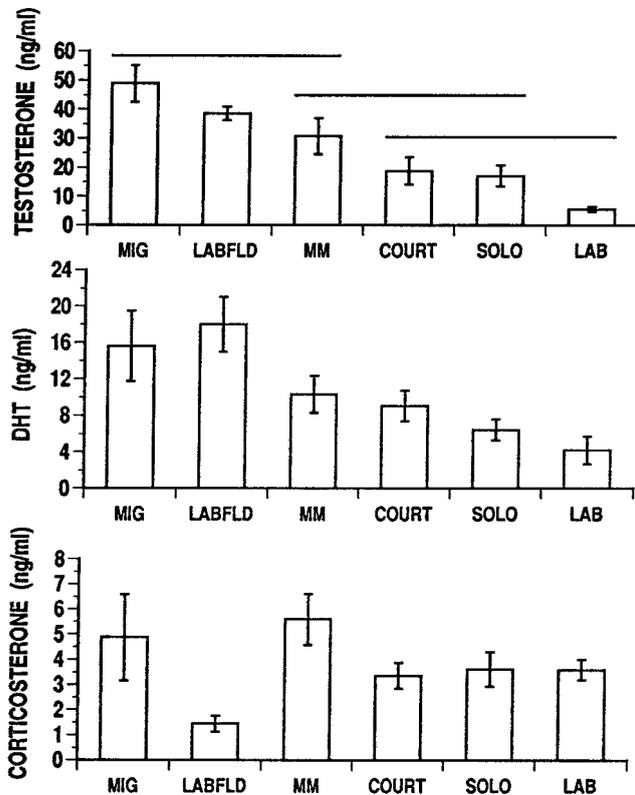


FIG. 1. Levels of testosterone (T), dihydrotestosterone (DHT), and corticosterone (B) for six groups of male *A. opacum*. MIG, migrating toward the breeding pond; LAB, kept under laboratory conditions; LAB-FIELD, originally kept in laboratory, then in field enclosure. Behavioral observations were made for males in the following categories: SOLO, alone in container (as control); MALE-MALE, male pairs in which one male courted another male that was inactive; and COURT, male that actively courted a female. Horizontal lines above T levels indicate groups that do not differ statistically. See text for further description of groups.

comparisons: LAB < MIGRATE, LAB-FIELD, and MALE-MALE; MIGRATE > LAB, SOLO, and COURT; and LAB-FIELD > LAB, SOLO, and COURT. No significant differences were found for T values among males in the three groups for which behavioral observations were made (MALE-MALE, COURT, and SOLO). Among-group differences in plasma DHT corresponded to those just described for T levels.

The highest value for total androgens (116 ng/ml) was obtained from a migrating male (on 14 October 1993). This maximal value was over 40% higher than the next highest value (82 ng/ml) for total androgens. The lowest values for total androgens (≤ 8 ng/ml) were from plasma samples taken near the end of the

courtship season (25 October 1993). These lowest values were found for a male that actively courted a female (7.64 ng/ml), a male that was alone in a container during behavioral observations (5.18 ng/ml), and a male that was courted by another male (5.86 ng/ml).

Relative Levels of Corticosterone

Plasma values of corticosterone ranged from 0.29 to 15.65 ng/ml (average = 3.89 ng/ml; $n = 46$). Levels of corticosterone differed significantly among males in the six groups ($H = 15.12$, $P \leq 0.01$, $df = 5$, $n = 46$; Fig. 1). A significant difference in circulating corticosterone was found only between the male groups having the highest (MALE-MALE) and lowest (LAB-FIELD) values ($P \leq 0.05$).

Histology

In all specimens examined, testes were regressed to the germinal layer, and seminiferous tubules were collapsed and contained no spermatozoa. All vasa examined were enlarged and contained large amounts of spermatozoa.

DISCUSSION

Our behavioral tests revealed no evidence of a behaviorally evoked androgen response in the marbled salamander, *A. opacum*. Among males that were spontaneously active, plasma androgen levels were unrelated to behavioral differences, regardless of the behavior or its intensity. Active males included those migrating to the pond basin, males that simply emerged from cover in seminatural enclosures, and emergent males placed in containers for behavioral observations. Males that courted for several hours (and deposited spermatophores) had androgen levels similar to those of control males that had been isolated from conspecific contact. Thus, under conditions designed to maximize the detection of an androgen response, *A. opacum* males showed no elevation in T or DHT in response to reproductive activities.

Differences in the nature of male-male competition

for mates may account for the findings that male marine toads, but not marbled salamanders, show a behaviorally evoked testosterone response. Males of both species were highly active for prolonged periods on nights of potential courtship activity, but marine toads exhibited intense agonistic behavior among males (Orchinik *et al.*, 1988). This behavior occurred primarily when rival males attempted to displace a male in amplexus with a female. In contrast, male *A. opacum* do not restrain or clasp the female during courtship, and interactions between males do not involve combat. Results to date, therefore, do not distinguish between the possibilities that the duration of amplexus, the intensity of male combat, or the combination of these behaviors elicit an androgen response. Resolving this issue will be accomplished when additional studies quantify male reproductive interactions (e.g., measuring the intensity of male-male aggression) in relation to the presence or absence (and duration) of amplexus. At the same time, knowledge of other reproductive variables (length of mating season, number of mating opportunities) also would help resolve the issue of whether the occurrence of an androgen response is related to variation in the nature of male mate competition.

Reproductive behaviors other than mating also may be associated with an androgen response in amphibians. The hypothesis that male parental care was related to changes in androgen levels was tested in the coqui frog (*Eleutherodactylus coqui*) from Puerto Rico. Plasma androgen levels were lower for males guarding egg clutches than for males showing territorial behavior (Townsend and Moger, 1987). This difference was consistent with the reduced androgen levels found in monogamous, territorial birds that were engaged in male parental care (Wingfield *et al.*, 1990; but see Ketterson *et al.*, 1992). Other results, however, were dissimilar from patterns found for avian species. In a second study of *E. coqui*, testosterone levels of parental males were elevated experimentally to levels of territorial males (Townsend *et al.*, 1991); this endocrine change had no effect on paternal care. The studies by Townsend and colleagues currently provide the only data on androgen levels and male care in amphibians. Studies of other amphibians that exhibit paternal care (many anurans and some salamanders; Duellmann and Trueb, 1986) would provide a more robust test of

the "paternal care-lower androgen" hypothesis that is based on results for certain avian species.

The observations of behaviorally induced changes in androgen levels do not demonstrate that these correlated changes are causally related to behavioral demands. The testosterone increases that were correlated with time spent in amplexus for several *Bufo* species (Table 1) might simply reflect a correlated response to increases in LH that accompany spermiation in anurans. This possibility was not supported by Licht *et al.* (1983), who found that spermiation in the bullfrog (*Rana catesbeiana*) was correlated with elevated levels of LH, but not with testosterone levels. Results from a single ranid species, however, may not apply to bufonids. Well-planned experiments are necessary to demonstrate causal effects of androgens (cf. Moore and Thompson, 1990). Even if the relationship between behavior and androgens is not causal, elevated androgen levels might serve as an indicator of a different neuroendocrine response to the behavior in question.

Hormonal Responses to Laboratory Conditions

Male *A. opacum* were held in the laboratory under conditions that deliberately prevented normal reproductive interactions. As we could not sample an individual male more than once, we inferred that differences between LAB males and other groups (e.g., migrating males) reflected endocrine changes resulting from laboratory captivity. Certain changes in androgen and corticosterone levels for males affected by laboratory treatments (LAB and LAB-FIELD groups, described below) provided results not reported for other amphibian species.

In captive (LAB) males, the relatively low levels of plasma androgens (T and DHT) were expected; reduced plasma testosterone commonly is reported for vertebrates brought into captivity (Licht, 1983; Zerani *et al.*, 1991; and see Houck and Woodley, 1995). Lower testosterone levels often occur in conjunction with the higher levels of corticosterone associated with the stress of captivity. The corticosterone levels for LAB males, however, were not elevated but were similar to levels reported for other male groups. The significance of this unexpected similarity is discussed below.

In a different example of hormonal response to captivity in *A. opacum*, testosterone levels for LAB-FIELD males were typical of levels reported for sponta-

TABLE 1

Relative Plasma Hormone Levels for Amphibian Males Engaged in Active Sexual Behavior (Chorusing, Amplexus, Courtship), Compared with Values for Potentially Breeding Males That Were Not Engaging in These Behaviors; Exceptions Are Data for *B. marinus*, which Reflect Whether Hormone Levels Were Positively Correlated with the Duration (0–3 hr) That Males Were in Amplexus

	Hormone levels		References
Chorusing males			
<i>Rana catesbeiana</i>	T	Same	Licht <i>et al.</i> (1983)
	B	Same	
<i>R. catesbeiana</i>	T	Lower	Mendonça <i>et al.</i> (1985)
	B	Higher	
Males in amplexus			
<i>Bufo bufo</i>	T	Same	Lupo <i>et al.</i> (1993)
	DHT	Higher	
	BPcap	Lower	
<i>B. marinus</i>	T	Higher	Orchinik <i>et al.</i> (1988)
	B	Higher	
<i>B. mauritanicus</i>	T	Higher	Siboulet (1981)
<i>B. japonicus</i>	T	Same	Itoh and Ishii (1990)
	LH	Higher	
	FSH	Higher	
Males courting (no amplexus)			
<i>Triturus carnifex</i>	T	Lower	Zerani and Gobetti (1993)
	B	Higher	
<i>Ambystoma opacum</i>	T	Same	This study
	DHT	Same	
	B	Same	
Duration of amplexus			
<i>B. marinus</i>			
Experimental	T	Correlated	Orchinik <i>et al.</i> (1988)
Experimental	B	Not correlated	

Note. Values are for free-living (unmanipulated) males, except for *B. marinus*, listed as experimental. T, testosterone; DHT, dihydrotestosterone; B, corticosterone; LH, luteinizing hormone; BPcap, relative capacity (i.e., availability) of T binding proteins in the brain (adapted from Houck and Woodley, 1995).

neously active males that had never been in the laboratory. The LAB-FIELD males were sampled within 4 days of their release from laboratory conditions. We assumed that these males, transferred to seminatural field enclosures after 7 days of laboratory confinement, initially had the relatively reduced testosterone levels typical of captive amphibians (cf. Zerani *et al.*, 1991). Given this assumption, testosterone levels subse-

quently were increased (within 4 days) in these salamanders. This is one of the first demonstrations for amphibians that plasma androgens can rise to precapture levels following release from laboratory captivity.

Levels of Corticosterone

Androgen levels typically are suppressed and corticosterone levels are elevated for vertebrates that are stressed by laboratory captivity (Greenberg *et al.*, 1984; Licht *et al.*, 1983; Nock and Leshner, 1976; Moore and Deviche, 1988; Moore and Miller, 1984; Nowell, 1980; Tokarz, 1987; Wingfield, 1985; Zerani *et al.*, 1991). In contrast, this negative correlation between corticosterone and testosterone was not found for most male *A. opacum*. Instead, plasma levels of both corticosterone and testosterone were high in all males that were spontaneously active (and never kept in the laboratory). These results may indicate a physiological dissociation between corticosterone and androgen levels. This conclusion is supported by the observation that LAB males had relatively low testosterone levels but corticosterone levels that were similar to those of males in groups never housed in the laboratory. The absence of elevated corticosterone levels (relative to levels found for males not experiencing laboratory conditions) suggests that reproductively active *A. opacum* males normally experience maximal corticosterone levels during the breeding season (cf. Smith *et al.*, 1994). A similar dissociation was found in the field study of *B. marinus* by Orchinik *et al.* (1988), who suggested that the intense competitive interactions typical of explosive breeders might stimulate the release of corticosterone. These authors also speculated that an acute rise in corticosterone concentration (a) might not inhibit behavior already in progress (e.g., amplexus), and (b) could act differently from the chronic elevations of corticosterone that inhibit the pituitary-gonadal axis. As only four amphibian studies provide data concerning levels of testosterone and corticosterone in relation to reproductive behavior in the field (Table 1), the frequency and pattern of testosterone-corticosterone dissociations remain to be demonstrated.

In conclusion, our focus on marbled salamanders provides a test of behavior-endocrine relationships in an understudied vertebrate group. Our results for

A. opacum indicate no behaviorally evoked androgen response, although such responses have been demonstrated for a few anuran species. In general, studies of vertebrates have revealed that male sexual and aggressive interactions can result in rapid and profound elevations in plasma levels of testosterone. This dramatic hormonal response to particular social stimuli has been found in certain species, but is conspicuously absent in others. Our understanding of evolutionary patterns of male behavior-androgen responses will profit from continued comparisons of different vertebrate species.

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