Hormones and Reproduction in Free-ranging Snakes

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7.1 INTRODUCTION: WHAT HORMONES CAN TELL US ABOUT THE REPRODUCTIVE BIOLOGY OF ANIMAL POPULATIONS

The natural history of an organism is rarely discussed without including a description of its reproductive biology. An understanding of reproduction is critical for two primary reasons. First, although reproduction is not essential for the immediate survival of an individual, it is essential for the persistence of the species and is the currency of an individual's fitness. Second, reproduction can come at a sizable energetic cost to organisms, particularly females, and these costs can represent a substantial part of annual expenditures in species where maintenance costs are relatively low (e.g., ectotherms). Therefore, reproduction typically is not a continuous endeavor, and appropriate timing of reproductive effort is critical.

Timing of reproductive events is usually influenced by both internal and external cues. Prior to reproduction, an organism must be in adequate physiological condition and, even when body condition is sufficient, reproduction must be timed to take advantages of environmentally favorable circumstances (e.g., temperature, precipitation, and energy availability). The onset of reproductive activity is signaled by relatively reliable seasonal environmental stimuli such as photoperiod, temperature, or rainfall (Perrins 1970; Wingfield 1980; Perrins and Birkhead 1983; Farner 1986). These cues, which are detected by the organism at various sensory levels, are translated by the endocrine system to hormone signals that communicate the information to the reproductive system (Wingfield 1980, 1983; Wilson and Donham 1988). Hormones also regulate morphological, physiological, and behavioral changes in anticipation of future events

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(Wingfield 1983; Jacobs and Wingfield 2000). Thus, measurements of circulating hormone concentrations are a vital component of understanding the complexity of the timing of behavioral and physiological aspects of reproduction.

Beyond being the primary interface between environmental changes and the onset of reproduction, hormones also modulate reproductive investment based on inputs that continue throughout the reproductive cycle. Changes in climatic patterns, social interactions, energy intake, and health status can all lead to adjustments in life history events including reproductive behavior and investment (Wingfield and Kitaysky 2002). Understanding hormonal responses to such events can elucidate an organism's plasticity to unpredictable situations (Jacobs and Wingfield 2000). Consequently, without an understanding of a species' reproductive endocrinology, it is essentially impossible to adequately understand the mechanisms by which cues, both internal and external, regulate reproduction and how reproductive activity influences behavior and other physiological aspects of an organism.

7.2 SNAKES AS MODELS FOR FIELD ENDOCRINOLOGY

Endocrinology is traditionally a laboratory-based science where hormone concentrations of classical laboratory animals such as mice and rats are manipulated to observe the hormone's effects on behavioral and/ or physiological parameters. While laboratory studies are valuable because they reduce or eliminate environmental variation, these studies ignore the complex interactions between animal physiology, behavior, and the environment. Such interactive effects are especially important in understanding reproductive cycles. Therefore, studies in reproductive endocrinology must use field studies to complement laboratory manipulations where conditions are more controlled and simplistic.

Studying animals in their natural habitats can illuminate the ways in which environmental factors such as temperature, photoperiod, resource availability, and social factors affect hormone concentrations and reproduction. However, such studies can be difficult to conduct in the field. For example, many animals are logistically difficult to capture in order to collect blood samples, especially when serial samples are required from the same individual. However, many species of snakes are easy to capture and monitor in the field, and they exhibit a diversity of reproductive characteristics that make them ideal model organisms for studies of field reproductive endocrinology.

As a group, snakes exhibit dramatic variation in mating systems, life history traits, and other characteristics associated with reproduction. Other chapters in this volume go into detail on this variation, but examples of the diversity of reproductive characteristics in snakes include mating tactics ranging from scramble competition to searching for widely distributed mates, oviparity and viviparity, and income and capital breeding (Seigel

and Ford 1987; Duvall *et al*. 1992). However, the roles of hormones in the diverse reproductive patterns observed in the ~3000 snake species are poorly understood and have been studied in a limited number of taxa, particularly garter snakes (*Thamnophis* spp.) and vipers from temperate regions (reviewed in Graham *et al*. 2008; Taylor and DeNardo 2010; see also Krohmer and Lutterschmidt, **Chapter 8** in this volume).

Garter snakes have been heavily studied because they are abundant, resilient to handling and experimental manipulation, and exhibit unique and interesting reproductive behaviors. Vipers have been heavily studied because they are large-bodied and therefore amenable to radiotelemetry, and often occur in high densities at predictable locations (e.g., dens). Also, many vipers are ambush predators and therefore spend substantial amounts of time above ground, which makes them readily available for blood sampling. In contrast, many other species of temperate snakes are small-bodied, fossorial, and rarely encountered; thus they are difficult subjects for field studies of reproductive endocrinology. There are numerous potentially effective study species outside of North America and Europe, but these snakes are rarely studied because scientists are much more heavily concentrated on these two continents.

Most of the studies on hormones and reproduction in free-ranging snakes are descriptive rather than experimental. Most commonly, researchers monitoring a population of snakes via mark-recapture or radiotelemetry draw periodic blood samples and quantify the circulating concentrations of target hormones using radioimmunoassay (RIA) or enzyme-linked immunosorbent assay (ELISA), and relate these results to events in the snakes' reproductive cycles. Hormone manipulations, where plasma hormone concentrations are experimentally increased or decreased, are much less common in field studies of snake reproductive endocrinology.

Snakes exhibit a diversity of reproductive cycles that are described in detail by Saint Girons (1982) and Aldridge *et al*. (2009). Briefly, snakes can be categorized into one of four types of reproductive cycles: 1) the postnuptial (dissociated) or aestival type, in which males undergo spermatogenesis during the summer, sperm are stored over the winter (in the vasa deferentia and sometimes also in female oviducts if fall mating occurs), and the principal mating season occurs in spring; 2) the prenuptial (associated) or vernal type, in which males begin spermatogenesis in the fall and complete it by the following spring or early summer, at which time mating occurs; 3) the mixed type, in which spermatogenesis begins in spring and is completed one year later with one (spring) or two (spring and fall) mating seasons; and 4) the continuous type, in which spermatogenesis and mating behavior occurs throughout the year. It is important to recognize that organisms do not always fall neatly into discrete categories assigned by biologists, and that the "type" of reproductive cycle of many species of snakes may therefore vary over time, among populations, and even among and within individuals. We therefore view the categories assigned by Saint Girons (1982) and Aldridge *et al*. (2009) simply as a

convenient framework with which to describe the relationship between hormones and reproduction in studies on free-ranging snake species, and point out situations in which species do not appear to fall discretely into the given categories.

7.3 A REVIEW OF THE STUDIES

Field studies on the reproductive endocrinology of snakes have focused on a very small number of species within a narrow taxonomic range for several reasons. First, as stated above, certain species are more amenable to field study due to their catchability, seasonal movement and aggregation patterns, and body size. Second, the vast majority of field research on snake endocrinology has been conducted in temperate regions of North America and Europe. For these reasons, our knowledge of the endocrinological mediation of the relationship between reproduction and environmental conditions in snakes is based almost entirely on studies of garter snakes and vipers from these areas. Because these snakes are viviparous, it follows that relatively few field endocrinological studies have been conducted on oviparous taxa.

The following sections separately review the studies performed on viperids, *Thamnophis*, and other snakes. We focus on field studies, although in many cases complementary laboratory studies have been performed and are cited accordingly. Table 7.1 provides a list of major field studies on the reproductive endocrinology of snake.

Table 7.1 Studies measuring hormone concentrations of free-ranging snakes. For methods, R = radiotelemetry, CR = capture/recapture, SNE = semi-natural enclosures. For hormones quantified, A = total androgen, B = corticosterone, DHT = dihydrotestosterone, E2 = estradiol, IGF-1 = insulin-like growth factor 1, M = melatonin, P = progesterone, T = testosterone, Th = thyroxine. For data series, S = serial samples on individuals, I = independent samples.

Species	Methods	Hormones quantified	Data series	Reference
Acrochordidae				
Acrochordus granulatus	CR	A,E2,P	I	Gorman *et al.* 1981
Colubridae				
Boiga irregularis	CR	B,P,T	I	Mathies *et al.* 2001
B. irregularis	CR	B,E2,P,T	I	Moore *et al.* 2005
B. irregularis	CR	B	I	Waye and Mason 2008
Cerberus rhynchops	CR	A,E2,P	I	Gorman *et al.* 1981
Natrix piscator	CR	M,T	I	Halder and Pandey 1989
Nerodia sipedon	CR	T	I	Weil and Aldridge 1981
Opheodrys aestivus	CR	A	I	Aldridge *et al.* 1990
Thamnophis elegans	CR	B	I	Robert *et al.* 2009
T. elegans	CR	IGF-1	I	Sparkman *et al.* 2009
Thamnophis sirtalis concinnus	CR	B,T	I	Moore *et al.* 2000b
T. s. concinnus	CR	B,T	I	Moore *et al.* 2001

Thamnophis sirtalis parietalis	CR	A,B	I	Krohmer *et al.* 1987
T. s. parietalis	SNE	B,E2,P,T	S	Whittier *et al.* 1987
T. s. parietalis	CR	E2	I	Mendonça and Crews 1989
T. s. parietalis	SNE	E2	I	Mendonça and Crews 1990
T. s. parietalis	SNE	B,T	I	Moore *et al.* 2000a
T. s. parietalis	SNE	B,T	I	Moore and Mason 2001
T. s. parietalis	CR	B,T	I	Moore *et al.* 2001
T. s. parietalis	SNE	A	I	Lutterschmidt *et al.* 2004
T. s. parietalis	SNE	B,T	S	Lutterschmidt and Mason 2005
T. s. parietalis	CR	A,B	I	Cease *et al.* 2007
Elapidae				
Laticauda colubrina	CR	A,E2,P	I	Gorman *et al.* 1981
Viperidae				
Agkistrodon contortrix	R,CR	P,T	S	Smith *et al.* 2010
Agkistrodon piscivorus	CR	A	I	Johnson *et al.* 1982
A. piscivorus	CR	B,T	I	Graham *et al.* 2008
A. piscivorus	CR	B,T	I	Bailey *et al.* 2009
Crotalus atrox	R,CR	B,E2,P,T	I,S	Taylor *et al.* 2004
C. atrox	CR	E2,DHT,T	I	Schuett *et al.* 2005
C. atrox	CR	E2,DHT,T	I	Schuett *et al.* 2006
Crotalus molossus	CR	E2,DHT,T	I	Schuett *et al.* 2005
Crotalus horridus	R	B,E2,T	I	Lutterschmidt *et al.* 2009
Crotalus oreganus	R,CR	B,DHT,E2,P,T	I,S	Lind *et al.* 2010
Crotalus scutulatus	CR	E2,DHT,T	I	Schuett *et al.* 2002
Vipera aspis	SNE	A,Th	S	Naulleau *et al.* 1987
V. aspis	SNE	P	S	Naulleau and Fleury 1990
V. aspis	CR	E2,DHT,P,T	I,S	Saint Girons *et al.* 1993
V. aspis	SNE	E2	S	Bonnet *et al.* 1994
V. aspis	SNE	E2,P	I,S	Bonnet *et al.* 2001
Viridovipera stejnegeri	SNE	E2,P	S	Tsai and Tu 2001

7.3.1 Viperids

Male viperids exhibit inter-specific and sometimes even intra-specific variation in the number of mating periods per year and timing of the mating season relative to gonadal activity. Several studies have shown that androgens (testosterone, T, and/or dihydrotestosterone, DHT) are elevated during the mating season(s), providing strong indication that T stimulates and possibly modulates a range of reproductive behaviors in male viperids. Many viperid species show a single annual mating season, and, where studied, plasma T concentrations are high when spermatogenesis and breeding behaviors occur. In eastern populations of Cottonmouths (*Agkistrodon piscivorus*), T peaks in late summer, at the same time that males show spermiogenic activity, hypertrophy of the sexual segment of the kidney, and breeding behavior (Johnson *et al.* 1982; Graham *et al.* 2008).

Similarly, Black-tailed Rattlesnakes (*Crotalus molossus*) from southeastern Arizona shows a single annual peak in T concentrations during their sole summer mating season (Schuett *et al.* 2005), and the European Adder (*Vipera berus*) shows a single peak in T at the same time as spermiogenesis and mating, although these occur in the spring rather than late summer (Naulleau *et al.* 1987). Timber Rattlesnakes (*Crotalus horridus*) show a single summer mating season but males exhibit no seasonal variation in T concentrations, although the sample size in this study was low (Lutterschmidt *et al.* 2009).

Many snake species, including some viperids, show two annual mating seasons, the first in late summer/fall and a second in spring. These mating seasons are separated by winter and summer periods of reproductive inactivity. Western Diamond-backed Rattlesnakes (*Crotalus atrox*), Northern Pacific Rattlesnakes (*Crotalus oreganus*), Mojave Rattlesnakes (*Crotalus scutulatus*), and Aspic Vipers (*Vipera aspis*) mate in both late summer/ fall and spring, and males show elevated androgens in both of these seasons (Naulleau *et al.* 1987; Saint Girons *et al.* 1993; Schuett *et al.* 2002, 2005; Taylor *et al.* 2004; Lind *et al.* 2010). Male *C. atrox*, *C. oreganus*, and *C. scutulatus* show bimodal peaks in circulating DHT that match the timing of the peaks in T (Schuett *et al.* 2002, 2005; Lind *et al.* 2010), although DHT concentrations are much lower than T concentrations. These species show the aestival or postnuptial pattern of spermatogenesis: T concentrations are high during spermatogenesis and mating in the late summer and fall, but are also high during the spring when mating is occurring in the absence of spermatogenesis. Testosterone concentrations are lowest in summer, when mating activity is absent. In the Sonoran Desert, *C. atrox* sampled while basking during the winter exhibit intermediate T concentrations between those of summer (lowest) and of the spring and fall mating seasons (Schuett *et al.* 2006). Interestingly, Copperheads (*Agkistrodon contortrix*) show a single peak in androgen (T) concentrations associated with its single summer mating season at the northern part of its range (Smith *et al.* 2010), but show two annual peaks (late summer/fall and spring) in androgen concentrations associated with its bimodal mating pattern in the southern part of its range (lab study: Schuett *et al.* 1997).

Spermatogenesis, maximal T production, and mating behaviors usually occur simultaneously in snakes with a single annual breeding season, (e.g., the "associated" reproductive pattern, Crews 1984; Whittier and Crews 1987), thereby making it difficult to assess the importance of T in each process. However, studies on species with bimodal mating seasons suggest that T is responsible for mating behaviors, since T is elevated during both mating periods regardless of whether the testes are spermatogenically active or regressed. Some exceptions are evident; for example, male *Vipera aspis* sometimes have very low androgen concentrations during the mating season (Naulleau *et al.* 1987; Saint Girons *et al.* 1993). Species with two mating seasons are intermediate in the associated-dissociated dichotomy of reproductive patterns (Crews 1984; Whittier and Crews 1987), as

they have associated reproduction in one season (late summer/fall) and dissociated reproduction in another (spring). The paradigm of associated/dissociated reproduction patterns should be viewed as describing two extremes along a continuum in which many snakes fall somewhere in the middle (Moore and Lindzey 1992; Schuett *et al.* 2006; Benner and Woodley 2007).

In general, studies of the relationship between steroid hormones and reproduction in female viperids show that plasma 17β-estradiol (E2) is elevated during vitellogenesis, reflecting its role in stimulating production of vitellogenin by the liver (Ho *et al.* 1982). Most female vipers initiate vitellogenesis in the fall, become quiescent during the winter, and complete vitellogenesis in the spring (type 2 vitellogenesis, Aldridge 1979). Female *Crotalus oreganus* follow this typical pattern and show increased E2 concentrations in the fall and spring (Lind *et al.* 2010). In contrast, female *C. atrox* in the Sonoran Desert of Arizona show a Type 1 vitellogenic pattern in which they initiate and complete vitellogenesis in the spring (Taylor and DeNardo 2005). Reproductive female *C. atrox* therefore exhibits elevated E2 concentrations from March through June, especially during peak vitellogenesis in April and May (Taylor *et al.* 2004). In non-reproductive female *C. atrox* and *C. oreganus*, plasma concentrations of E2 are low throughout the year (Taylor *et al.* 2004; Lind *et al.* 2010). Lutterschmidt *et al.* (2009) observed low concentrations of E2 in female *C horridus* throughout the year, but the sample sizes, especially of reproductive females, were low. Female *Vipera aspis* in France have the same seasonal mating patterns as *C. atrox* (spring vitellogenesis and late summer parturition), and also show elevated E2 during spring (Saint Girons *et al.* 1993). In Chinese Green Tree Vipers, *Viridovipera* (*Trimeresurus*) *stejnegeri*, E2 concentrations are slightly elevated during vitellogenesis (Tsai and Tu 2001). Almeida-Santos *et al.* (2004) found that E2 peaks during winter vitellogenesis in female Neotropical Rattlesnakes (*Crotalus durissus terrificus*) in southeastern Brazil, and is low throughout the year in non-reproductive females.

Several studies have also measured concentrations of circulating androgens in female vipers. Androgen concentrations of females are always lower than those of males, and are extremely low year-round in non-reproductive female *Crotalus atrox* and *C. oreganus* (Taylor *et al.* 2004; Lind *et al.* 2010). Androgen concentrations increase slightly during spring in reproductive female *C. atrox* and *C. oreganus;* this may occur because T is a precursor to E2 in steroid biosynthesis. Alternatively, the increase in androgens in reproductive females may reflect a functional role of these hormones in stimulating receptivity or other factors related to mating. For example, concentrations of DHT, a non-aromatizable androgen (i.e., cannot be converted to E2), are elevated during spring in female *C. oreganus* (Lind *et al.* 2010). Also, Saint Girons *et al.* (1993) found that female *Vipera aspis* displaying mating behaviors had significantly higher plasma DHT concentrations, but not T concentrations, than females that were not displaying mating behaviors.

Plasma progesterone (P4) is often elevated during gestation in viviparous snakes, reflecting its role in maintenance of pregnancy (Mead *et al.* 1981; reviewed in Custodia-Lora and Callard 2002). Plasma P4 concentrations are very low in non-reproductive female *Crotalus atrox*, and in reproductive females are elevated in May through August, peaking during the gestation period in June-July (Taylor *et al.* 2004; Taylor and DeNardo 2005). Reproductive *Viridovipera* (*Trimeresurus*) *stejnegeri* and *C. durissus terrificus* have dramatically elevated P4 concentrations during gestation (Tsai and Tu 2001; Almeida-Santos *et al.* 2004). Reports of plasma P4 concentrations in reproductive *Vipera aspis* are variable. Saint Girons *et al.* (1993) showed that plasma P4 was highly variable among snakes, with no clear difference between reproductive and non-reproductive females. However, in other studies, plasma P4 concentrations of reproductive female *V. aspis* were elevated in May through August (gestation), decreased in September (after parturition), and rose again during winter (Naulleau and Fleury 1990; Bonnet *et al.* 2001). Bonnet *et al.* (2001) hypothesized that the winter increase in P4, which is especially marked in post-reproductive snakes, may act to block vitellogenesis in emaciated snakes, as P4 may inhibit hepatic synthesis of vitellogenin (Callard *et al.* 1992, 1994).

While corticosterone (CORT, the major glucocorticoid in reptiles) is not typically viewed as a sex steroid hormone, it likely has important roles in reproductive physiology either directing or indirectly via its effects on energy mobilization. Taylor *et al.* (2004) measured CORT concentrations relative to reproduction in free-ranging *Crotalus atrox* and found that CORT concentrations are more variable annually than the other steroid hormones, but increase dramatically in reproductive females in July and August, during late gestation. Plasma CORT concentrations return to baseline along with P4 near the time of parturition in reproductive females. Lutterschmidt *et al.* (2009) found that both female and male *C. horridus* had lower CORT concentrations in summer than in spring or fall, but that reproductive females had higher CORT concentrations than non-reproductive females. In sum, these results demonstrate a relationship between gestation and high CORT concentrations, yet the functional role of CORT remains uncertain. CORT may be elevated in female vipers to aid in energy mobilization during reproduction, when metabolic rate is elevated (Beaupre and Duvall 1998; Beaupre 2002). Alternately, CORT may play a role in regulating development of the offspring, as is seen with glucocorticoids in mammals (Ballard and Ballard 1972). Distinguishing these roles for CORT will require manipulative experiments under controlled laboratory conditions.

In addition to potentially playing a supportive role for either the female or offspring during reproduction, CORT may have an inhibitory effect on reproduction via its role in the stress response. CORT concentrations tend to rise as a result of stressful stimuli such as handling by humans. Bailey *et al.* (2009) showed that *Agkistrodon piscivorus* subjected to 30 minutes of confinement stress in a bag exhibited higher CORT concentrations than

snakes bled immediately upon capture. The stress response did not differ between male and female snakes. Lutterschmidt *et al.* (2009) also found that CORT concentrations increased in *Crotalus horridus* subjected to one hour of confinement stress. Reproductive and post-parturient females had a greater stress-induced increase in CORT concentrations than non-reproductive females, suggesting that reproductive state modulates the hypothalamo-pituitary-adrenal axis. There was a significant relationship between baseline CORT and T concentrations in male *C. horridus*, where snakes with higher CORT concentrations had lower T concentrations. CORT and T often exhibit a reciprocal relationship because stress may inhibit reproduction (Greenberg and Wingfield 1987; Moore and Jessop 2003). Interestingly, however, confinement stress led to a slight increase in T concentrations in male *C. horridus*.

7.3.2 The Red-sided Garter Snake (*Thamnophis sirtalis parietalis*)

The Red-sided Garter Snake (*Thamnophis sirtalis parietalis*) is by far the best-studied snake species in terms of reproductive endocrinology. These snakes overwinter in communal dens at high latitudes (e.g., Manitoba, Canada), and in the spring they emerge from the dens and aggregate by the thousands to breed. Researchers are therefore able to obtain huge sample sizes for their studies. In addition, male snakes are so keen to breed that they completely ignore researchers, even when placed in experimental semi-natural enclosures in the field or returned to the laboratory, allowing easy observation of natural behaviors. These characteristics have made *T. s. parietalis* model organisms not just for reproductive endocrinology but also for studies of behavior, sexual selection, chemical ecology, and more. The literature on *T. s. parietalis* is extensive, and noteworthy review articles on their reproductive physiology include Garstka *et al.* (1982), Krohmer *et al.* (1987), Krohmer (2004), and Krohmer and Lutterschmidt (**Chapter 8** in this volume).

In *Thamnophis sirtalis parietalis*, concentrations of circulating hormones have been quantified in free-living snakes, in snakes exposed to experimental conditions in semi-natural enclosures, and in the laboratory. Many of these studies have gone beyond describing seasonal hormone profiles to actually performing experimental manipulations to examine the functional role of hormones in reproductive physiology and behavior. The reproductive endocrinology of *T. s. parietalis* is discussed in detail in Krohmer (**Chapter 8**, this volume) and Taylor and DeNardo (in press), so in this chapter we will focus only on several key field studies on reproductive endocrinology in this species.

Male *Thamnophis sirtalis parietalis* emerge from communal hibernacula in spring and initiate courtship behavior that lasts several weeks (Crews and Garstka 1982). During spring mating, males utilize sperm produced the previous summer and stored through the winter in the vasa deferentia (Krohmer *et al.* 1987); they therefore exhibit the postnuptial, dissociated

reproductive tactic (Crews 1976). However, other studies show that *T. s. parietalis* also mate in the late summer and fall (Mendonça and Crews 1989; Whittier and Crews 1989). It was initially reported that plasma androgen concentrations are low in the spring when mating occurs (Camazine *et al.* 1980), again highlighting the postnuptial, dissociated pattern of reproduction in this species. However, later studies demonstrated that androgen concentrations are, in fact, elevated upon emergence from hibernation but drop rapidly after emergence (Krohmer *et al.* 1987; Moore *et al.* 2000b; Cease *et al.* 2007). The sexual segment of the kidney, which is known to be stimulated by androgens in squamate reptiles (Bishop 1959), is hypertrophied in late summer and spring but regressed during the summer when androgen concentrations are low (Krohmer *et al.* 1987). Since there is now evidence for both spring and late summer mating, and elevated T during both fall and emergence from hibernation in spring, it is clear that this species does not exhibit a strictly dissociated pattern of reproduction. However, some laboratory studies have questioned the dependence of mating behavior on T in male *T. s. parietalis* (Camazine *et al.* 1980; Crews 1984; Crews *et al.* 1984). Courtship behavior persists despite castration, adrenalectomy, or hypophysectomy, and treatment with hypothalamic hormones, gonadotropins, arginine vasotocin, or sex steroid hormones does not stimulate courtship behavior in adult males (Garstka *et al.* 1982; Crews *et al.* 1984). Nonetheless, it is possible that prior exposure to androgens organizes brain regions involved in reproduction but the onset of reproductive behavior does not occur until initiated by environmental cues at a later time (Crews 1991). Alternately, steroids may be synthesized in the brain, bypassing the influence of circulating hormone ~~levels~~ on reproductive behavior (Soma 2006). Further studies, perhaps incorporating the use of steroid receptor antagonists, are needed to more effectively evaluate the role of T in regulating reproductive behavior in *T. s. parietalis* and other snake taxa.

Several studies have examined the relationship between reproduction and the stress response in male *Thamnophis sirtalis parietalis*, as well as the closely related *T. s. concinnus* (Moore *et al.* 2000a, Moore *et al.* 2001; Lutterschmidt and Mason 2005; Cease *et al.* 2007). Baseline CORT concentrations in *T. s. parietalis* are highly variable from year to year, and are generally highest during the spring breeding season, lowest in summer, and intermediate in fall (when breeding can also occur, Moore *et al.* 2001). Male *T. s. parietalis* show variable hormonal responses to confinement stress in cloth bags. When CORT concentrations are low (e.g., in summer), males exhibited a stress-induced increase in CORT and decrease in T (Moore *et al.* 2001). During the main breeding season in spring, Moore and colleagues (2001) and Lutterschmidt and Mason (2005) observed no stress-induced change in CORT or T concentrations. If male *T. s. parietalis* suppress the stress response during their brief spring breeding season, they may avoid the negative effect that the stress response may have on their ability to reproduce. In support of this, Cease and colleagues (2007) found that

actively courting males do not show a CORT stress response, but males already dispersing from the den site do exhibit an increase in CORT in response to stress. However, unlike the studies above, Moore *et al.* (2000a) observed a stress-induced increase in CORT and decrease in T in actively courting males. The rationale behind this discrepancy among studies is unclear at this time and warrants further study.

Male *Thamnophis s. concinnus* exhibit a stress-induced increase in CORT throughout the year. This subspecies has an extended breeding season when compared to *T. s. parietalis*; thus it may not face the same pressure to suppress the stress response during breeding. During spring, T concentrations decreased with stress but during summer and fall they actually increased. Moore *et al.* (2000b) found that throughout their annual cycle, baseline CORT and T concentrations were positively correlated. The differences in the stress-induced changes in CORT and T observed in the two subspecies (Moore *et al.* 2001) highlight the fact that the traditional negative relationship between stress and reproduction (e.g., Greenberg and Wingfield 1987; Moore and Jessop 2003) is not always true. Interestingly, while males continue to display courtship behaviors even when stressed (Moore *et al.* 2000a), injection of exogenous CORT suppresses courtship behavior in a dose-dependent manner but does not affect circulating androgen concentrations (Moore and Mason 2001; Lutterschmidt *et al.* 2004). Also, treatment with exogenous melatonin suppresses courtship behavior but does not affect androgen concentrations (Lutterschmidt *et al.* 2004; Lutterschmidt and Mason 2005). This suggests that any suppression of reproductive behavior by CORT is not due to suppression of androgens, but via another mechanism.

Very few studies have been performed in the field examining the relationship between hormones and reproduction in female *Thamnophis sirtalis parietalis*. However, extensive laboratory studies have been performed on females of this species, and we refer readers to Taylor and DeNardo (2010) and Krohmer and Lutterschmidt (**Chapter 8** in this volume) for reviews of this information. The few field studies conducted have shown that female *T. sirtalis parietalis* exhibit a rather unusual relationship between reproductive events and hormones when compared to other snake species. Spring mating occurs when E2 concentrations are low (Garstka *et al.* 1982), but Mendonça and Crews (1996) have shown through ovariectomy and hormone replacement therapy that even low E2 concentrations appear to be important in making female snakes attractive and receptive to males. Additionally, the physical act of mating induces a surge in E2 in females, but plasma E2 concentrations are not necessarily elevated during vitellogenesis (Garstka *et al.* 1985; Whittier *et al.* 1987; Whittier and Crews 1989; Mendonça and Crews 1990). Like many other snakes studied, plasma T is elevated during vitellogenesis (Whittier *et al.* 1987). Interestingly, female *T. s. parietalis* do not show elevated P4 during gestation (Whittier *et al.* 1987), which is in contrast to other snakes and vertebrates, in general.

7.3.3 Other Snakes

While the vast majority of research on reproduction and hormones in free-ranging snakes has focused on temperate vipers and *Thamnophis sirtalis parietalis*, circulating hormone concentrations of several other species of free-ranging snakes have been quantified. Like the more commonly studied species, most of these species are terrestrial, temperate, North American species in which reproduction is highly seasonal. Both Northern Water Snakes (*Nerodia sipedon*) and Rough Greensnakes (*Opheodrys aestivus*) breed only in spring, but males show elevated T during the spring breeding season and during the late summer postnuptial spermatogenic period (Weil and Aldridge 1981; Aldridge *et al.* 1990). Male *T. s. concinnus* have peak T concentrations during spermatogenesis in the fall, and levels are much lower during the spring breeding season (Moore *et al.* 2000b).

A noteworthy exception to the preponderance of studies on temperate, terrestrial snakes is the study by Gorman and colleagues (1981) that describes the seasonal steroid hormone concentrations in relation to reproductive events in three marine snakes (Filesnake, *Achrochordus granulatus*; Dog-faced Water Snake, *Cerberus rhynchops*; and Banded Sea Krait, *Laticauda colubrina*) from the Philippines. These three species show varying degrees of seasonality in their reproductive patterns. *Acrochordus granulatus* is the most seasonal, with mating activity, spermatogenesis, and peak T concentrations occurring in the fall, shortly after vitellogenesis in females. In contrast, *C. rhynchops*, and especially *L. colubrina* show spermatogenesis throughout the year. Although spermatogenesis and T concentrations in *C. rhynchops* peak in the fall, there is evidence for year-round gonadal activity. In *L. colubrina*, males show no seasonal trends in spermatogenic activity or T concentrations, suggesting that they most closely follow the continuous pattern of reproduction (Saint Girons 1982). These data suggest that species inhabiting more thermally constant environments, such as the ocean and the tropics, may show year-round reproduction. However, the paucity of data on tropical snakes highlights the need for research in this area. Plasma E2 and T concentrations were elevated during vitellogenesis in the two viviparous species (*A. granulatus* and *C. rhynchops*), and in the oviparous *L. colubrina* E2 and T concentrations increased progressively through vitellogenesis and gravidity. Similarly, P4 concentrations were highest during pregnancy in the two viviparous species and increased progressively during gravidity in *L. colubrina*.

Halder and Pandey (1989) measured concentrations of T and melatonin in Indian Chequered Water Snakes (*Natrix piscator*), a tropical, aquatic snake, throughout the year to examine the relationship between pineal and testicular activity. They found that T concentrations were elevated during the fall, when maximal weights of the testes, vasa deferentia, and kidneys were observed. Plasma melatonin concentrations and pineal weight, in contrast, were lowest during the fall and peaked in the spring, suggesting that melatonin may play a role in inhibiting the reproductive axis.

Western Terrestrial Garter Snakes (*Thamnophis elegans*) exist as two ecotypes in a population near Eagle Lake, California: snakes from meadow habitats are slow-growing, long-lived, and have low annual reproductive output, whereas snakes from lakeshore habitats are fast-growing, shorter-lived, and have higher annual reproductive output. Robert *et al.* (2009) measured plasma CORT concentrations in free-living, pregnant meadow and lakeshore snakes. They found that CORT concentrations were higher in the meadow snakes, possibly because meadow snakes experienced lower food availability than lakeshore snakes. Sparkman *et al.* (2009) measured plasma concentrations of insulin-like growth factor-1 (IGF-1) in this species. They found that IGF-1 concentration was positively associated with body size in lakeshore snakes, but that the relationship between IGF-1 concentrations and size in meadow snakes depended on resource availability. Furthermore, females that gave birth to larger litters had higher concentrations of IGF-1.

Introduced Brown Tree Snakes (*Boiga irregularis*) in Guam have been the subject of several studies on reproductive and stress hormones. Moore *et al.* (2005) measured concentrations of sex steroids and CORT in free-ranging and captive *B. irregularis* throughout a two-year period. Most of the free-ranging snakes they captured were non-reproductive, had lower body condition, and had higher CORT concentrations than captive snakes, suggesting that they are in negative energy balance. Plasma T was low throughout the year in free-ranging males and exhibited a spring peak in captive males. Plasma E2 and P4 were low in free-ranging females throughout the year compared to captive females. Waye and Mason (2008) compared CORT concentrations and body condition of the snakes used in the Moore *et al.* (2005) study (collected 1991-1993) to those of snakes collected in 2003. They found that by 2003, body condition had improved and CORT concentrations had dropped, suggesting that the snakes were no longer chronically stressed by energy limitations. Waye and Mason (2008) suggest that introduction of new prey species, including frogs, may have contributed to the increased body condition and decreased CORT concentrations in *B. irregularis*. To investigate the effect of capture and confinement stress on *B. irregularis*, Mathies *et al.* (2001) compared concentrations of CORT and sex steroids in *B. irregularis* captured and held in traps for various durations with snakes bled immediately upon capture. Almost all of the snakes they captured were non-reproductive, therefore concentrations of T in males and P4 in females were low, in concordance with the results of Moore *et al.* (2005). They found that females had CORT concentrations about twice those of males. Confinement in cloth bags led to increased concentrations of CORT, similar to the studies of *Agkistrodon piscivorus* (Bailey *et al.* 2009) and *Crotalus horridus* (Lutterschmidt *et al.* 2009) described in Section 8.3.1. Snakes captured in a trap and held overnight showed increased concentrations of CORT relative to snakes bled immediately upon capture, but CORT concentrations returned to baseline in snakes held for three nights in a trap, suggesting that the snakes may acclimate to the traps.

7.4 SUGGESTED DIRECTIONS FOR FUTURE RESEARCH

To establish a comprehensive understanding of how various hormones regulate reproductive physiology and behavior among snakes in natural conditions, there is dire need for additional work. First, greater amounts of comparative data are needed. Snakes are a diverse group consisting of approximately 3,000 species in 15 genera (Integrated Taxonomic Information System 2010). Yet, only 36 field studies provide 40 species accounts of reproductive endocrinology (Table 7.1). Worse, those 40 accounts examined only 18 species in four families (two families are represented by studies on a single species). In fact, 21 of the 40 accounts (53%) are limited to two genera – *Thamnophis* and *Crotalus*.

All accounts of field endocrinology in snakes are limited to the more derived Caenophidia (Lee *et al.* 2007). While the Caenophidia contain the vast majority of extant snake species, the lack of any data from more primitive lineages prohibits any consideration of derived verses primitive patterns in reproductive endocrinology. Even within the speciose families of the Caenophidia, accounts are sparse. While the Viperidae and Elapidae each consist of over 200 species, field reports of reproductive endocrinology are limited to nine species within the Viperidae and only a single species in the Elapidae. Despite most accounts being of species within the Colubridae, this enormously diverse family (approximately 2000 species) is represented by merely seven species.

Equally concerning to the phylogenetic voids in our understanding of snake reproductive endocrinology under natural conditions is the limited coverage of the varied natural history traits among snakes. Without examining diverse snakes under many environmental conditions, we will not be able to assess the universality of findings or what phylogenetic, morphological, physiological, or environmental characteristics influence endocrinological patterns. *Thamnophis sirtalis* has been the most intensively examined species (11 of the 36 publications), and the results from these studies provide us the greatest insight into the reproductive endocrinology of snakes under natural conditions. Unfortunately, the atypical traits of this species (e.g., high latitude distribution, communal denning, formation of mating balls immediately upon female emergence) put into question the broad applicability of these results to snakes in general.

Even looking beyond the *Thamnophis sirtalis* data, there remains considerable bias in that the preponderance of studies has been conducted on temperate terrestrial species, particularly in North America. Despite snakes being widely distributed on most continents except Antarctica, there are no endocrinological field studies from South America, Africa, or Australia. Current literature is dominated by studies of viviparous species, with only four of the 18 species examined thus far being oviparous. In addition, the vast majority of field studies focus on male rather than female snakes. Males are often easier to study because they are more abundant and/or catchable in the field, but the male bias observed in studies may

also reflect a greater inherent interest in the reproductive biology of male snakes on the part of biologists. Indeed, the categories to which snakes are assigned when studying their reproductive physiology (i.e., associated/ dissociated, prenuptial/postnuptial, etc.) are often based solely on males, ignoring females. Aldridge *et al.* (2009) review studies on reproductive cycles of male and female colubrid snakes, and provide suggestions for terminology that bridges male and female reproductive patterns (i.e., pre- and post-ovulatory spermatogenesis). There is an obvious need for studies examining hormones and reproduction in both males and females of a given species so that hormonal correlates of the reproductive cycles of both sexes, and their interrelationships, can be examined.

There is a bright side. Of the 36 studies conducted, 23 (64%) of them have been published in the past ten years. Continued development of field technologies and interest in field reproductive endocrinology will hopefully lead to a continuation of the explosive growth in literature on the subject. Because of both financial and manpower limitations, study planning should be well-planned to maximize its value. While additional descriptive studies on endocrinological patterns in free-ranging snakes will make significant contributions, there would be greater benefit from studies that take an organized comparative approach. For example, great insight could come from a study that examines the reproductive endocrinology of a community of snakes that represent varied reproductive modes, activity patterns, and phylogeny. While such a study would be challenging, current technologies make it feasible. An alternate comparative approach could examine multiple viviparous and oviparous species across diverse phylogenies to ascertain the extent to which reproductive mode influences hormonal profiles. Yet another comparative approach of value would be to examine the reproductive endocrinology of multiple populations of a species that is distributed widely across habitats, since ecologically based intra-specific differences in both reproductive cycles and hormone profiles have been documented (e.g., *Agkistrodon contortrix*: Schuett *et al.* 1997, Smith *et al.* 2009; *Thamnophis elegans*: Robert *et al.* 2009). Regardless of which comparative approach is taken, studies involving comparisons of more than two species or two populations are preferable (Garland and Adolph 1994).

Also essential to strengthening our understanding of snake reproductive endocrinology are manipulative studies that link hormonal profiles to reproductive states and behaviors, explore the functional roles of these hormones, and investigate the impact of environmental and physiological conditions on hormone profiles. Endocrine organ removal and hormone replacement therapy have been used to explore the chemical nature of physiology even before the discovery of hormones, and these manipulative techniques were vital to the emergence of the field of reproductive endocrinology in the early 20th century (Borell 1985). Not surprisingly, these techniques have been used in snakes, but relatively sporadically (e.g., Fraenkel *et al*. 1940; Bragdon 1951; Crews 1976; Camazine *et al*. 1980; Garstka *et al*. 1982; Crews *et al*. 1984, 1993; Padgoankar and Samuel

1993; Mendonça and Crews 1996). Furthermore, neither gonadectomy nor hormone treatment have been employed in long-term studies of free-ranging snakes. With the development of radiotelemetry, serially locating and processing free-ranging snakes is now relatively common and the incorporation of hormone manipulation into such studies would not only expand our understanding of reproductive endocrinology, but likely also our understanding of costs of reproduction, sex-based differences in growth and activity, and other vital ecophysiological traits.

7.5 CHAPTER SUMMARY

While there is still much to be learned about the reproductive endocrinology of snakes, especially in their natural environments, our knowledge of this field has grown considerably over the last decade. Here, we make an attempt to synthesize the existing data across taxa to identify consistencies and discrepancies within snakes in general. Additionally, since snakes have diverse reproductive cycles and reproductive modes, we can compare results from various studies to make some preliminary assessments regarding the function of hormones in reproduction despite a paucity of data from manipulative experiments.

In the vast majority of species studied, elevated plasma T concentrations in males are associated with reproductive activity, regardless of the number of mating seasons or the time of year that the mating season occurs. Species that show an associated reproductive cycle where spermatogenesis and mating occur together have a single peak in plasma T that coincides with this reproductive period (e.g., *Acrochordus granulatus*: Gorman *et al.* 1981; *Vipera berus*: Naulleau and Fleury 1987; *Agkistrodon piscivorus*: Graham *et al.* 2008). In species where breeding seems to occur year-round, plasma T remains elevated throughout the year (e.g., *Cerberus rhynchops, Laticauda colubrina*: Gorman *et al.* 1981). The most revealing descriptive data for the role of T in male snakes comes from those species where mating occurs twice a year but spermatogenesis occurs only during one of those two mating periods. In these species, plasma T is elevated during both mating seasons (e.g., *Vipera aspis*: Saint Girons *et al.* 1993; *Crotalus atrox*: Taylor *et al.* 2004; *Crotalus scutulatus*: Schuett *et al.* 2005; *C. oreganus:* Lind *et al.* 2010). Together, these data from various species that have diverse reproductive cycles strongly suggest that T is critical for reproductive activity, especially mating behavior. However, the most studied species, *Thamnophis sirtalis*, does not follow this generality. In this species, T peaks when spermatogenesis is occurring in fall. While T is initially high at spring emergence, plasma concentrations decrease through the mating season (Krohmer *et al.* 1987; Moore *et al.* 2000b; Cease *et al.* 2007). In fact, the removal of T via castration does not inhibit male courtship behavior (Garstka *et al.* 1982; Crews *et al.* 1984). The reason for this inconsistency in T cycles between *T. sirtalis* and most other species exist is uncertain, but it emphasizes the need for caution when broadly applying results derived from a single species.

In females, the general steroid profiles are similar to those of other vertebrates. Peaks in plasma E2 concentrations are associated with vitellogenesis regardless of whether vitellogenesis occurs over a single season (e.g., *Acrochordus granulatus*, *Cerberus rhynchops*: Gorman *et al.* 1981; *Viper aspis*: St. Girons *et al.* 1993; *Crotalus atrox*: Taylor *et al.* 2004) or two seasons (e.g., *C. oreganus*: Lind *et al.* 2010). Consistently, P4 peaks post-ovulation regardless of whether the species is viviparous (e.g., *A. granulatus*, *Cerberus rhynchops*: Gorman *et al.* 1981; *Viridovipera stejnegeri*: Tsai and Tu 2001; *Crotalus atrox*: Taylor *et al.* 2004) or oviparous (e.g., *Laticauda colubrina*: Gorman *et al.* 1981). However, as with the T profiles, results from female *Thamnophis sirtalis parietalis* are not consistent with those from other snakes. While mating induces a surge in E2 in females, plasma E2 concentrations are not necessarily elevated during vitellogenesis (Garstka *et al.* 1985; Mendonça and Crews 1990). Additionally, female *T. s. parietalis* do not show elevated P4 during gestation (Whittier *et al.* 1987).

Among all the steroids hormones, CORT shows the fewest consistencies in relationship to snake reproduction. Like many species, snakes typically show elevated CORT associated with stress (e.g., *Boiga irregularis*, Mathies *et al.* 2001; *Agkistrodon piscivorus*: Bailey *et al.* 2009; *Crotalus horridus*: Lutterschmidt *et al.* 2009), but this stress response is inhibited during reproduction in one subspecies of *Thamnophis sirtalis* but not another (Moore *et al.* 2001). The role of CORT in snake reproduction has simply been attributed to mobilizing energy stores during this period of high metabolic demand but low energy intake (Beaupre and Duvall 1998; Beaupre 2002). However, *C. atrox* show elevated plasma CORT concentrations associated with late gestation followed by a sudden decrease in CORT concentrations at parturition, without food intake (Taylor *et al.* 2004). This result leaves open the possibility of a direct role of CORT on embryonic development prior to parturition.

While general patterns are beginning to develop, the reproductive endocrinology of snakes essentially remains in its infancy and is in great need of further study, particularly strategically designed comparative studies intended to find causal links between hormone profiles and phylogenetic, ecologic, and physiologic characteristics. Also needed are manipulative studies to better elucidate the function of the various hormones associated with reproductive events.

7.6 ACKNOWLEDGEMENTS

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