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Chapter 8

Hormones and behavior of reptiles

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ABBREVIATIONS

α -MSH	α -melanocyte stimulating hormone
5-HT	serotonin
ACTH	corticotropin
AGBG	androgen-glucocorticoid-binding globulin
AH	anterior hypothalamus
ARS	alternative reproductive strategy
AVP	arginine vasopressin
AVT	arginine vacotocin
VSTm	bed nuclei of the stria terminalis
CBG	corticosterone-binding globulin
CNS	central nervous system
CORT	corticosterone
CRF	corticotropin-releasing factor
CRH	corticotropin-releasing hormone
DA	dopamine
DHT	dihydrotestosterone
E ₂	17 β -estradiol
ERE	see HRE
FSH	follicle stimulating hormone
GnRH	gonadotropin-releasing hormone
GTH	gonadotropin
HP	hypothalamus
HPA	hypothalamus-pituitary-adrenal
HPG	hypothalamus-pituitary-gonadal
HRE	hormone response element
L-DOPA	L-3,4-dihydroxyphenylalanine
LH	luteinizing hormone
NE	norepinephrine
OXY	oxytocin
P ₄	progesterone
POA	preoptic area
PRL	prolactin
PRL-R	prolactin receptor
RPS	rock-paper-scissors
SHBG	steroid hormone-binding globulin
T	testosterone
TSD	temperature-dependent sex determination

1 INTRODUCTION

The role of the endocrine system in regulating the expression of behavior in vertebrates has become the paradigm for studies at both the proximate level of analysis that includes a molecular analysis of behavior and the ultimate level of analysis that includes an understanding of selection (Cohen & Wade, 2019). This chapter reviews the modulation of reproductive behavior of reptiles by endocrine systems as shaped by natural and sexual selection. Studies of natural and sexual selection provide the structure for understanding the functional role of the various components of the endocrine system at the proximate level. We review models for the evolution of male–male interactions, ideas underlying evolution of density regulation (*r-K* selection) and viviparity, new emerging models for the evolution of sociality, and network models of such social interactions. New empirical results indicate that reptiles, and in particular lizards, exhibit a diversity of social systems (Doody et al., 2021); in particular, we describe advanced forms of sociality including both nuclear family structure in large groups (Chapple, 2003; Halliwell et al., 2017; O'Connor & Shine, 2003), and greenbeard selection (Sinervo, Chaine, et al., 2006; Sinervo & Clobert, 2003; Sinervo et al., 2007), which until recently were thought to be restricted to mammals, birds, and social insects (Doody et al., 2021).

Throughout this review, we highlight the generic control networks of the neuroendocrine system of vertebrates and then reference what is known from reptilian studies of behavior and hormones. We finish our review of hormones and behavior of reptiles by synthesizing the results in terms of regulatory networks applied to social systems and their endocrine control. Many of these social systems involve alternative social strategies for reproduction, and throughout our review, we highlight findings from species with ARS.

[†] Deceased.

We also highlight key research opportunities that have not been tapped in studies of the hormonal control of behavior in reptiles. In particular, we specifically posit that the hormones arginine vasotocin (AVT) and prolactin (PRL) and their receptors are the most likely control mechanisms for the evolution of diverse behavioral phenomena including ARS, territoriality, viviparity, dispersal, migration, sociality, and coloniality.

The order of the items we highlight in our overview of reptilian hormones and behavior roughly follows the role of hormones during development, beginning with early organizational events, proceeding to activational events, and ending with the effects of social neighbors on the expression of hormones and behavior. An understanding of the roles of hormones in affecting behavioral responses requires a consideration of two very different timescales. Over evolutionary time, behavior is shaped by natural and sexual selection to generate optimal responses in endocrine function. In contrast, over the lifespan of an organism, behavior changes in response to the environment, social context, and the endocrine system modulate many of these changes through interactions with the neuroendocrine system. Complicating this interaction of timescales are feedback loops that act between behavioral responses and the endocrine system to shape the organism in a plastic fashion, via social cues. Such plasticity can be favored by selection and generates new coping strategies under a variety of social circumstances, as the social system evolves. At the end of this review, we integrate social system interactions with the endocrine control of individual behaviors.

In this chapter, we also highlight the utility of network system analysis (Milo et al., 2002) in providing a comprehensive synthesis of these various timescales that shape the endocrine control of behavior. We compare and link social networks and endocrine networks to unravel the dimensions involved in the hormonal control of behavior. The network theory provides a framework that allows the unification of ultimate forces of selection with complex proximate sources generated by endocrine systems and temporal regulation of gene activation (gene cascades) on the expression of behaviors, their selection, and associated trade-offs.

1.1 Organization and other developmental effects of reptilian hormones on behavior

The degree to which an organism is responsive to change is also genetically programmed and dictated in a large measure by ontogenetic stages from the juvenile to adult phases. The organizational effects of hormones (Adkins-Regan, 2012; Phoenix et al., 1959), which act during

embryogenesis to pattern the basic behavioral repertoire of male vs female types, and the activational effects of hormones, which trigger the expression of male vs female behavior at maturity, accomplish these ontogenetic changes (Caro & Bateson, 1986; Schorálková et al., 2017). Juvenile behavior also arises in a physiological background where sex steroids are not yet circulating at significant levels. In this context, many aspects of juvenile behavior of reptiles are regulated by neuroendocrine systems such as the interplay between the control of behavioral thermoregulation (Seebacher, 2005), thermal plasticity, and thyroid hormones, e.g., metabolic regulation by thyroxine (Little, 2021; Sinervo & Dunlap, 1995), which we only mention briefly, but may play a critical role. Many organizational events arise during the earliest stages of development in both live-bearing or viviparous, and egg-laying or oviparous forms of reptiles.

In lizards, model systems with ARS have been investigated to specifically understand the organizational role of hormones in generating basic behaviors within a given sex. In *Urosaurus ornatus*, males with an orange dewlap (throat) are nonterritorial, and larger than blue-orange males that are territorial and have a blue patch on their orange dewlap (Thompson & Moore, 1991). Castration early in prematurational development results in a high frequency of the orange, nonterritorial morph, and supplemental testosterone (T) results in a high frequency of the blue-orange morph (Hews & Moore, 1995), suggesting a possible organizational role for T. However, subsequent experiments revealed that a perinatal surge in progesterone (P₄) of adrenal origin (higher in blue-orange males) governs the organization of the two male strategies, and exogenous P₄ mimics the effects of T on the generation of the blue-orange type (Weiss & Moore, 2004) (Fig. 1A). Despite these salient details on hormones in organizing the phenotype of male tree lizards, female tree lizards (and many other lizard species described in the following section) exhibit conspicuous color polymorphism associated with ARS (Zucker & Boecklen, 1989), and the interaction between the ARS locus of tree lizards with sex chromosomes is poorly understood (Fig. 1A).

Besides endogenous sources of hormones that govern organization, maternally provided yolk steroids may provide a developmental mechanism for altering behavior of progeny (Meylan et al., 2012). The organizational role of such early acting hormones plays a clear role, for example, in birds where maternally provided yolk steroids alter differential expression of aggression in chicks (Schwabl, 1993) and in other vertebrate groups (Mouton & Duckworth, 2021). However, limited data are available for reptiles. Noteworthy in this regard is the maternal modulation of yolk steroids observed in female side-blotched lizards, *Uta stansburiana*, which express three ARS that, as in *U. ornatus*, are coded by

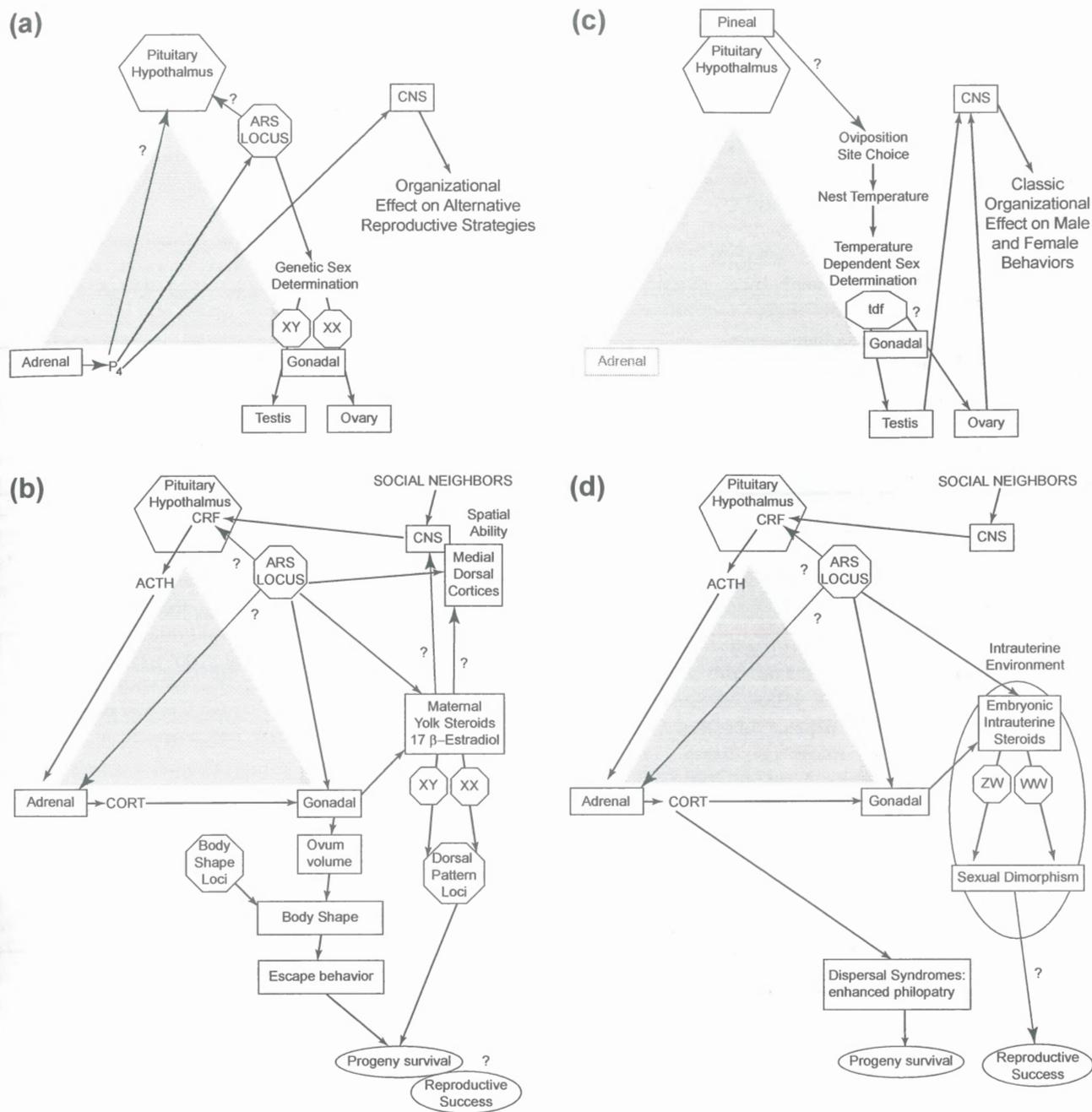


FIG. 1 Organizational effects of steroid hormones on behavior for some model reptilian systems (see Section 1 for details and refs.). (A) Progesterone (P₄) secretion by the adrenal glands organizes alternative reproductive strategies (ARS) of the ornate tree lizard, *Urosaurus ornatus*. (B) Sex differences in behavior are organized by temperature-dependent sex determination in the leopard gecko, *Eublepharis macularius*. (C) Yolk estradiol organizes dorsal patterns, an antipredator signal, in the North American side-blotched lizard, *Uta stansburiana*, and yolk volume independently organizes morphology and escape behavior. These two organizational effects, in tandem, produce highly adaptive and functionally integrated ARS in both sexes. (D) Exogenous corticosterone application to female European common lizards, *Lacerta* (=*Zootoca*) *vivipara*, organizes juvenile dispersal syndromes, but the relationship to ARS of females is unknown. Moreover, intrauterine steroids secreted by embryos (i.e., sibs) affects development of sexual dimorphism. Arrows describe positive effects of each mechanism (or inferred pathways of action) on endocrine function, and fitness related traits in the case of (C) and (D). Octagonal symbols indicate the action of key genes: ARS loci, dorsal pattern loci, sex chromosomes (XX/XY or WW/ZW systems of heterogamy) or in the case of TSD, *tdf*, a hypothetical testis determining factor that is temperature sensitive. The symbol identifies processes that are poorly understood. Abbreviations: P₄, progesterone; CRF, corticotropin-releasing factor; CNS, central nervous system; ACTH, adrenocorticotropin hormone.

throat color. Genetic crosses and gene mapping studies using a field pedigree suggests the polymorphism is due to a single gene (Sinervo et al., 2001; Sinervo, Calsbeek, et al., 2006; Sinervo, Chaine, et al., 2006). Both male and female side-blotched lizards can be yellow-, orange- or blue-throated. In males, color reflects alternative mating strategies. Orange males are territorial and aggressive, and their territories overlap with multiple females. Blue males tend to have a single female overlapping their territory and repel rival males who might attempt to copulate with the resident female (mate-guarders). This mate-guarding behavior entails cooperative behavior with other blue males (Sinervo, Chaine, et al., 2006). Finally, yellow males are sneakers that lack a territory, mimic the appearance and behavior of females, and copulate with females by stealth (Sinervo, Chaine, et al., 2006; Sinervo & Lively, 1996; Zamudio & Sinervo, 2000). In females, alternative throat colors correlate with alternative egg-laying strategies. Orange females lay larger clutches of small eggs. Yellow and blue females lay small clutches of large eggs (Sinervo, Miles, et al., 2000). Throat color and life history traits are heritable (Sinervo & Zamudio, 2001; Sinervo, Miles, et al., 2000).

In *U. stansburiana*, females deposit greater quantities of yolk 17 β -estradiol (E₂) when mated with a yellow sire, and thus, the progeny carries yellow alleles (Lancaster et al., 2007). Females also deposit increased yolk E₂ in response to crowding by orange neighbors. Thus, there are two distinct cues that alter levels of yolk E₂ – yellow alleles from the sire and crowding by orange female neighbors – and these two yolk E₂ pathways modify dorsal patterns of progeny in an interaction with the color locus (Fig. 1B). Increased yolk E₂ in combination with yellow sire alleles enhanced the barred dorsal pattern (transverse dorsal bars) phenotype of progeny, which is cryptic in meadow habitats and adaptive for yellow sneaker genotypes. Increased yolk E₂ induced by orange crowding increased the prominence of striped dorsal patterns (longitudinal dorsal stripes), but in a sex-specific manner that interacted with throat color genes. In response to yolk E₂ induced by social crowding, stripes were increased in sons destined to be orange, and daughters destined to be yellow or blue. These changes enhanced progeny survival to maturity of the induced phenotypes. The causal effects were verified with exogenous E₂ ectopically applied to eggs (Lancaster et al., 2007). Yolk E₂ could also have organizational effects on the central nervous system (CNS), akin to those described in the following section (see Section 1.3) for temperature-dependent sex determination (TSD) in geckos, and perhaps, they even play a major role in promoting the conspicuous and stereotypical female mimicking behaviors observed in yellow male morphs.

Squamate reptiles with ARSs provide potential research avenues for investigating the classic organizational effect of hormones on basic neural structures between the sexes and between the alternative male types (Miles et al., 2007). Consider the neural basis of spatial abilities, which has been identified as influenced by sex differences in the volume of the hippocampus in other vertebrates like birds and mammals (Clayton, 2001; LaDage et al., 2009). The medial and dorsal cortices are the putative reptilian homologues of the mammalian hippocampus (LaDage et al., 2009); yet, an emerging number of studies have revealed a relationship between the variation in brain regions (dorsal cortex and hippocampus) and differential spatial use strategies in reptiles (Roth et al., 2020). Recent studies indicate that spatial use strategies of male side-blotched lizards (*U. stansburiana*) are linked to the volume of the medial and dorsal cortical regions of the brain. Orange males, which defend large territories (Sinervo, Miles, et al., 2000), have larger dorsal cortical volumes compared with nonterritorial yellow males, and blue males with small territories are intermediate in volume. Future studies on alternative polymorphisms should ascertain whether this effect is due to the organizational effect of the endogenous hormone produced by the embryo (*sensu* Phoenix et al., 1959), the yolk-steroid effect and maternal programming (*sensu* Lancaster et al., 2007), activational differences between morphs that manifest at maturity (Sinervo, Miles, et al., 2000), or simply a consequence of the feedback role of the territory size, which differs among the morphs, thereby inducing increases in hippocampal volumes during ontogeny and adulthood.

The basic sex differences in the expression of male vs female behavior have been a classic question explored in model systems of reptiles that express TSD (Fig. 1C, see Singh et al., 2020). For example, incubation experiments on the leopard gecko, *Eublepharis macularius*, demonstrate the induction of female clutches at low temperature (26°C), female-biased sex ratio at 30°C, male-biased clutches at intermediate temperatures (32.5°C), and nearly all females at very high temperatures (34–35°C) (Crews & Moore, 2005). Crews et al. (Crews, 1998; Sakata & Crews, 2003) have discovered that TSD has a classic organizing effect on male vs female behaviors, similar to those first described in mammals (e.g., Phoenix et al., 1959; see also methods in Vom Saal et al., 1983). For example, males from female-biased temperatures are less likely to adopt an offensive attack posture, a stereotypical male behavior, than those raised at male-biased temperatures. Conversely, females from male-biased temperatures are more aggressive than females from female-biased temperatures, although females are less aggressive than males overall. Additional experiments with gonadectomy followed by restorative hormone treatment indicate that the expression of male vs female

stereotypical behaviors is influenced by egg rearing temperature independently from sex steroid levels (Crews & Moore, 2005).

In the turtle *Chrysemys picta* (Bowden et al., 2000), another species with TSD, the ratio in levels of maternally provided E₂ to T varies seasonally, and this variation contributes to the production of female-biased clutches late in the season. In this experiment, all eggs were incubated at 28°C, and the ratio of females shifted seasonally from 72% male to 76% female. The adaptive significance of environmental sex determination has been a subject of much debate (reviewed in Shine, 1999; Pennell et al., 2018; see also Chapter 1, this volume), but it is generally thought that females can maximize their fitness by giving the progeny an opportunity to develop into the sex that will perform best, given the prevailing environmental conditions. In the case of a mechanism coupled with yolk estrogens, it is possible for females to fine-tune both the sex ratio produced and the phenotypic expression of male vs female behaviors. Bowden et al. (2000) suggested that other aspects of offspring phenotype, such as the association between egg size and TSD (Ennen et al., 2017; Ewert et al., 1994; Roosenburg & Kelley, 1996), might be fine-tuned with the addition of a mechanism linked to egg size.

The endocrine control of egg size and ensuing effects on progeny behavior and fitness have been studied most thoroughly in *U. stansburiana*, where a battery of endocrine manipulations and normative studies have determined both the proximate mechanisms governing egg size regulation and adaptive consequences for the progeny of each sex (Fig. 1C). In yellow and blue females, but not orange females, increased egg size (which leads to increased progeny size) leads to enhanced progeny survival, reflecting an egg size × genotype interaction (Lancaster et al., 2008). One reason for this is that large vs small progeny exploit different escape behaviors, and escape behaviors exhibited by smaller progeny benefit orange progeny (Lancaster et al., 2010). The effects of maternally provided yolk steroids noted as discussed earlier are likely to play a synergistic role with egg size in altering progeny escape behavior, but experimental manipulations in which yolk is withdrawn from eggs with a syringe indicates that egg size alone alters progeny morphology and escape behavior through the modification of body size and limb allometry.

The local frequency of throat colors in the social environment affects plasticity in maternal egg size allocation strategy via impacts of social cues on the hypothalamus–pituitary–adrenal (HPA) system (discussed in Section 1.4). Specifically, orange females exhibit decreased corticosterone (CORT) in response to orange-throated neighbors in the wild (Comendant et al., 2003). Conversely, yellow and blue females exhibit increased CORT when crowded by

yellow- and blue-throated individuals (Comendant et al., 2003). The chronic elevation of CORT leads to an increase in females' average egg mass without a concomitant decrease in clutch size (Lancaster et al., 2008). A potential mechanism is the effect of CORT on increasing appetite (Jönsson, 1997) or affecting energy reserves (Hau et al., 2016; Moore & Jessop, 2003). Females experiencing chronic elevated levels of CORT are predicted to feed more during oogenesis. This mechanism allows each maternal morph to adjust offspring size in response to social cues, which can inform her of her offspring's likely genotype. Small size benefits orange hatchlings, and orange females lay small eggs. Orange females further decrease egg size via reducing plasma CORT when the local frequency of orange throat color is high (indicating that offspring will likely inherit that orange color through the sire as well as the dam). Large size benefits yellow and blue hatchlings. Yellow and blue females further increase egg size when the social environment predicts that offsprings are also likely to inherit yellow and blue throat color from the sire. Lancaster et al. (2010) performed the second experiment assigning females to controlled mating in the lab. Females mated to yellow-throated sires, but not sires of other throat colors, responded by increasing average egg size (Lancaster et al., 2010).

Adrenal-mediated allocation plasticity is an adaptive mechanism by which females respond to the trade-off among different aspects of offspring quality (Sherrif & Love, 2013). Social contexts may induce maternally derived stress (MDS), which result in modifications in offspring traits through changes in allocation to reproduction (MacLeod et al., 2021). Such maternally induced shifts in offspring traits may be adaptive if the offspring characteristics match future environments (Meylan et al., 2012). Females use social cues to predict offspring inherited traits (Alonzo & Sinervo, 2001; Alonzo & Sinervo, 2007; Weiss & Moore, 2004), and then, they use that information, via endocrine modulation, to alter progeny phenotypes and behavior. One common trade-off that is rarely considered from a life-history perspective is the trade-off among diverse aspects of the offspring quality. The traditional life history theory considers offspring quality to be synonymous with offspring size (as in the classic offspring size–number trade-off) (Smith & Fretwell, 1974). This is because offspring size is under maternal endocrine control and a direct function of her resource allocation to reproduction. However, the size–number model does not consider genetic variation among offspring, which could render maternal resource investment more or less beneficial, depending on progeny genotype. The Trivers and Willard (1973) model expanded on this early theory and explicitly treated resource allocation to each progeny sex owing to differential forces of sexual selection in sons vs natural selection in daughters

(discussed in Section 1.3). This is a form of a parent–offspring conflict, and interaction among egg size, incubation temperature, yolk steroids, and TSD is a likely evolved mechanism to ameliorate any mismatch between progeny genotypes (e.g., sex or other genotype effects, such as alternative strategies) and resource allocation (Janzen & Phillips, 2006). When considered from the offspring’s perspective, the parent–offspring conflict is known as ontogenetic conflict (Rice & Chippindale, 2001), and the most intuitive example of this is the intersexual ontogenetic conflict, in which traits that are beneficial when inherited by one sex are detrimental when inherited by the other sex (Rice & Chippindale, 2001). For instance, from reptiles, in the side-blotched lizard, genes that enhance clutch size are related to levels of gonadotropin hormones (GTH). A high level of expression of the genes for clutch size increases not only fecundity, but also female progeny survival prior to maturity (Sinervo & McAdam, 2008). These same genes, when expressed at high levels in males, not only increase sexually selected male traits at maturity (Mills et al., 2008), but also decrease male survival from birth to maturity (Sinervo & McAdam, 2008). The male progeny inherits these genes for clutch size (GTH) from the female and male parents, but the male progeny does not express clutch size, rather the “clutch size genes” serve to enhance male sexual phenotypes, such as display behaviors, plasma T, and gular color (Mills et al., 2008).

Thus, the positive selection on genes for clutch size (controlled by GTH) in females is counteracted by negative effects of such genes on the survival of the male progeny. In the context of organizational effects of gender differences, unless early acting steroids due to maternal causes are precisely targeted towards the expression of traits in one sex but not the other (e.g., in the case of yolk steroids, or intrauterine steroid effects in viviparous lizards, see Section 1.2), ontogenetic conflict due to organizational events can generate adaptive effects in one sex, but maladaptive phenotypes in the opposite sex. Selection due to such ontogenetic conflict involves a continuous refinement of organizational effects of hormones on male vs female phenotypes. Moreover, ontogenetic conflict can occur with respect to any genotype that varies among offspring, and is not limited to genotypes associated with sex, such as those found within sexes and due to the action of ARSs. Natural selection studies on the side-blotched lizard indicate that the endocrine-modulated progeny phenotypes are adaptive (e.g., antipredator behaviors due to the synergism of escape behavior and egg size (Lancaster et al., 2010) or dorsal patterns and yolk E₂ (Lancaster et al., 2008)). When we discuss the structure of the male and female endocrine systems, influenced by activational events, we will return to the kinds of natural vs sexual selection that act on the two sexes.

1.2 Endocrine effects on behavior in viviparous species

Eutherian mammals are all viviparous, and intrauterine steroids from neighboring fetuses in mammals govern the organization of salient aggressive behaviors between the sexes (Vom Saal et al., 1983). In contrast to the single origin of viviparity in mammals, viviparity has evolved over 100 times in squamate reptiles (Albergotti & Guillette, 2011; Guillette, 1993; Whittington, 2021; Zimin et al., 2022). Studies on the role of hormones in modulating progeny behavior in the viviparous European common lizard, *Lacerta* (= *Zootoca*) *vivipara*, suggest this may be a potent avenue by which a female can program progeny behavior (Meylan et al., 2012). These studies suggest roles for both fetal steroid hormones and maternal steroid hormones.

One example of maternal influence on progeny behavior is patterns of dispersal in lizards. Previous work has shown the maternal environment affects dispersal behavior in *Z. vivipara*, and the glucocorticoid hormone CORT is a key factor influencing dispersal vs philopatry (Fig. 1D). Experimental manipulation of circulating levels of CORT in females during the gestation period may be accomplished by an ectopic application of this oil-soluble hormone to the dorsal skin surface (De Fraipont et al., 2000). High levels of ectopic CORT supplied to the mother during gestation resulted in neonates that were largely philopatric and also were more attracted to the mother’s odor than were offspring from mothers with low levels of CORT during gestation (De Fraipont et al., 2000). As in the synergisms between egg size and yolk steroids or maternal steroids in oviparous species noted as discussed earlier, the effects of CORT were also modulated by juvenile nutrition, as manipulated by differences in food supplied to the mothers. Juveniles in a good condition, or born from mothers in a good condition, have also been found to disperse more (Clobert et al., 1994). Common lizards, *Z. vivipara*, exhibit three dispersal strategies (Cote et al., 2006): the first of juvenile disperses to empty habitats, the second type to low density habitats, and the last type to densely populated habitats. All types exhibit marked differences in social behavior varying from asociality to cooperation and from neophobia to neophilia. Most of the behavioral characteristics appear to be under maternal influence, although a direct genetic control cannot be excluded in some cases. These basic differences are thus under the control of maternal endocrine and nutritional environment (Meylan et al., 2012). These dispersal strategies interact with the mechanisms governing the evolution of sociality (see Section 3).

In the case of viviparity, the consequences of the maternal uterine environment need not be strictly adaptive and may arise from constraints. Uller and Olsson (2003) studied the impact of male-biased, female-biased, and equal sex

ratio in litters from the viviparous *Z. vivipara*. They found an effect of female- vs male-biased litters on a sexually dimorphic trait, head size to snout vent length, and this effect persisted after the growth period. In particular, males from female-biased litters had smaller head to snout vent length, and Uller and Olsson (2003) suggest such effects would be maladaptive in male *Z. vivipara*, which are sexually selected for the dimorphic expression of body size and other traits like head size. Thus, the evolution of viviparity imposes an adaptive constraint on organization of male and female phenotypes in the shared fetal endocrine environment of the uterus. One mechanism by which such potential negative effects could be ameliorated is with the evolution of a yolk-steroid deposition mechanism that counteracted the negative impacts of fetally-derived steroid hormones. For example, in *Z. vivipara*, the female is the heterogametic sex (Fig. 1D), and thus, when eggs are ovulated, the female determines the sex ratio by the ovulation of Z vs W follicles. If females evolved a mechanism to alter the quantity of yolk steroids (e.g., E₂ to T ratio) available to the growing follicles (which already are sex determined), the adaptive constraints of littermate sex could be counteracted. For example, the addition of yolk T to eggs fated to be sons (ZZ) or yolk E₂ to eggs fated to be daughters (ZW) would allow for egg steroids to counteract the feminizing or masculinizing effect of littermates on progeny as they complete gender development in the uterus. As noted earlier, the multiple origins of viviparity across squamates provide an unparalleled opportunity to explore such hypotheses. A recent phylogeny for *Z. vivipara* revealed the existence of a single origin of viviparity and an apparent reversal of viviparity to oviparity (Recknagel et al., 2018). The two oviparous clades of *Z. vivipara* are found in northern Spain and the Pyrenees of France and in the Slovenian and Italian Alps. The viviparous populations occur throughout the rest of the species range. The evolution of yolk steroid and fetal steroid interactions is possible to study in geographically isolated populations that exhibit different modes of reproduction (e.g., viviparity vs oviparity).

1.3 Activational events

Our understanding of the role of hormones has benefited from three very different but complementary perspectives: (1) lab and field endocrinology (Bradshaw, 2007), (2) selection analyses and studies of function (Arnold, 1983), and (3) behavioral ecology and game theory (Maynard Smith, 1982). In brief, endocrine systems are shaped by simple forms of selection and more complex forms involving competition among behavioral strategies (Fig. 2). The basic strategies of females involve density-dependent and life history allocation strategies, and the basic strategies of males include territoriality and alternatives to territoriality such as sneaker tactics, or scramble

competition. In any given population, males might express continuous variation between two strategy types or be fixed for a single strategy. In populations with ARS, males or females might express discrete types.

Whereas the organizational effects of steroids are critical for the neurodevelopment of male vs female behaviors and the organization of alternative strategies within a given sex (Crews & Moore, 2005), steroids also have activational effects that are manifested at maturity when the sexes initiate reproduction. Later, acting activational effects trigger changes in behavior and physiology in adults. Dramatic changes in behavior can be acted on by natural and sexual selection. Selection on activational events is under “direct selection” in that the survival or reproduction of males is temporally associated with behavioral changes during maturation. In contrast, direct selection on earlier-acting organizational effects is less likely as differentiation is not yet complete. However, direct selection on activational events can lead to indirect selection on organizational effects, if activational and organizational effects are governed by the same sets of genes. Thus, direct selection on activational events and indirect selection on organizational events may primarily take place during maturation when activational events are manifested. The early organizing effect of steroids is likely to occur via selection generated by ontogenetic conflict on the determination of male vs female phenotypes. Likewise, selection on the sex-limited expression of activational events will likely act via ontogenetic conflict generated by the expression of the same genes in the sexes at maturity (Sinervo & McAdam, 2008).

Before proceeding to our discussion of activation, we will summarize the effects of adrenal hormones and their interactions with gonadotropins (GTHs) in terms of network diagrams. Selection on most behaviors will involve selection in social networks, so the formalism of network diagrams will allow us to understand the proximate control of behavior due to endocrine networks, and the ultimate forms of selection arising from social networks; this will be outlined at the end of our review. Here, we consider the examples discussed thus far and summarize the hypothalamic–pituitary–gonadal (HPG) axis as an introduction to activational events, and how endocrine systems can be plastically modified by the HPA axis.

1.4 Endocrine network theory and the regulation of behaviors

Endocrine networks generate homeostasis and alter energy flow and metabolism among competing life history functions (Cox et al., 2016a; Lancaster & Sinervo, 2011). At maturity, once an appropriate size is reached, activational events are triggered, typically in response to photoperiod

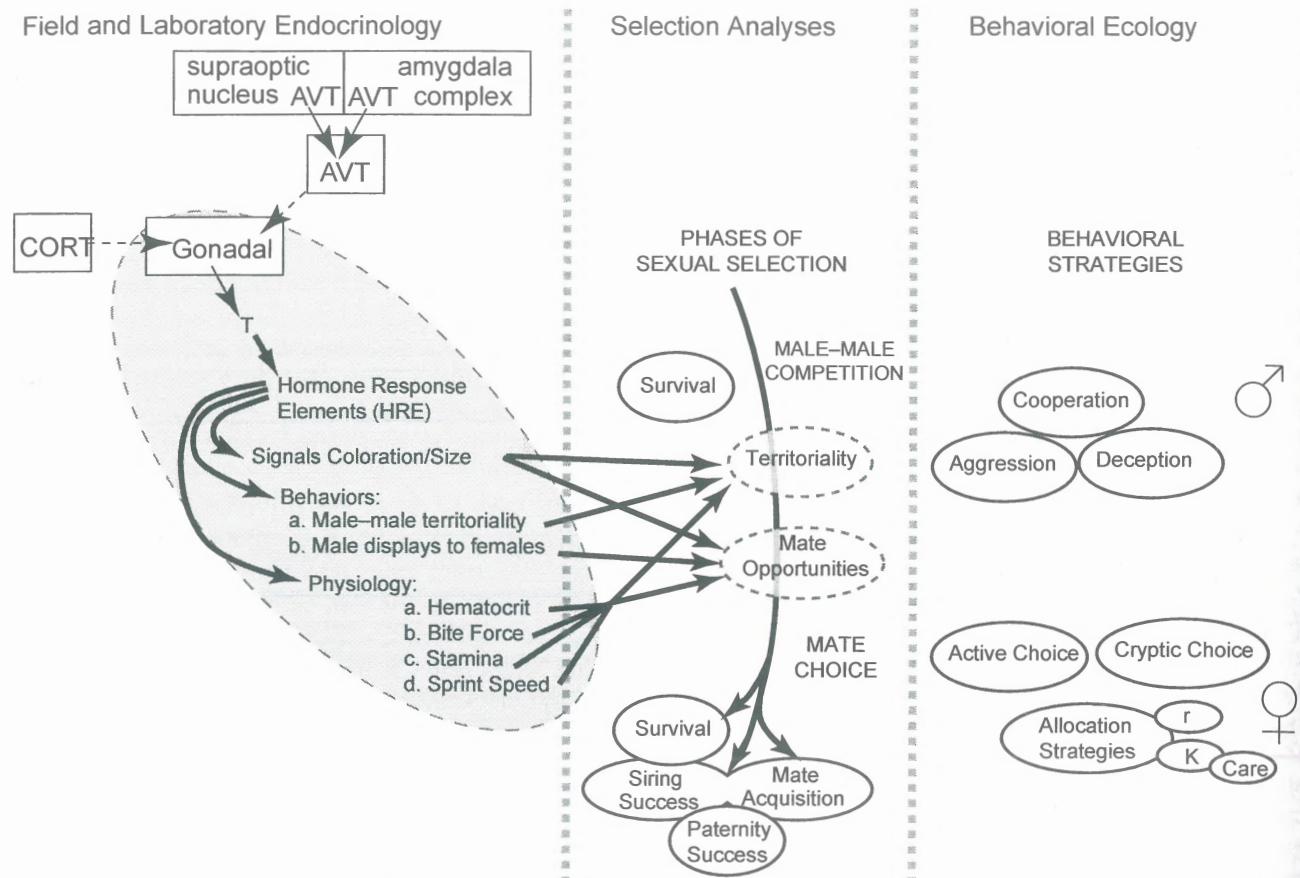


FIG. 2 The understanding of hormonal effects on behaviors has benefited from the tripartite approaches of (1) laboratory and field endocrinology of proximate mechanisms (reviewed in Crews & Moore, 2005; Bradshaw, 2007); (2) selection analyses of adaptive function (Arnold, 1983); and (3) behavioral ecology of fitness in social networks (e.g., game theory, Maynard Smith, 1982). Hormones have pleiotropic behavioral effects. A useful starting point in linking hormones to behavior is an analysis of hormones like T, which have pleiotropic effects on three classes of trait: (1) signaling traits under metabolic control (e.g., coloration controlled by carotenoids, pteridines or melatonin systems, or size controlled by growth hormones); (2) behavioral traits under neuroendocrine control (e.g., male–male territoriality, male displays to females); and (3) physiological traits that affect performance (e.g., hematocrit, stamina, speed, and bite force). The gonadal steroids T and E alter gene transcription via hormone response elements (HREs/EREs) on DNA, thereby altering gene expression in target tissues, and thus diverse traits. Testosterone generates effects in interaction with other endocrine systems such as the adrenal steroid corticosterone (CORT), the major glucocorticoid of reptiles involved in stress responses, and arginine vasotocin (AVT), a neuropeptide hormone secreted by many brain regions such as the supraoptic nucleus of the hypothalamus and the amygdala complex that innervates the hypothalamus.

cues that are monitored by the pineal gland, precipitating the release of gonadotropin releasing hormone (GnRH) by the hypothalamus. GnRH induces the release of GTHs, which are secreted by the anterior pituitary (Fig. 3) (Phillips et al., 1987). The GTHs luteinizing hormone (LH) and follicle-stimulating hormone (FSH) are essential for reproduction through their regulation of gonadal function. In mammals, LH stimulates T secretion in the testes (Habert et al., 2001; Saez, 1994) and FSH stimulates sperm production (Simoni et al., 1997). The actions of LH and FSH are modulated by the hormone prolactin (PRL), which increases the LH receptor number in Leydig cells and the FSH receptor number in Sertoli cells (Bole-Feysot et al., 1998).

Recent evidence has revealed the role of kisspeptin (K_p) and its receptor ($\text{Kiss}1r$) as an upstream mediator of reproductive behaviors in mammals and selected nonmammalian

vertebrates (Mills et al., 2018). Kisspeptin is a neuropeptide involved in the regulation of GnRH in the hypothalamus. Prior research in mammals showed that exogenous K_p activates the HPG axis in mammals and is associated with elevated levels of LH (Saito et al., 2012). Whereas most research on the role of K_p has involved mammals and fish, limited research has been done on reptiles. Prior studies verified the presence of kisspeptin/kisspeptin receptor systems in *Anolis carolinensis* (Dunham et al., 2009; Tena-Sempere et al., 2012), *Takydromus tachydromoides* (Lee et al., 2009), *U. stansburiana* (Neuman-Lee et al., 2017), and *Alligator sinensis* (Zhang et al., 2020). Despite the verification of kisspeptin receptor systems in reptiles, scant data are available regarding their effects on reproductive behavior. Previous studies identified kisspeptin-like immunoreactivity in the preoptic area (POA) of the brain of *A. carolinensis*

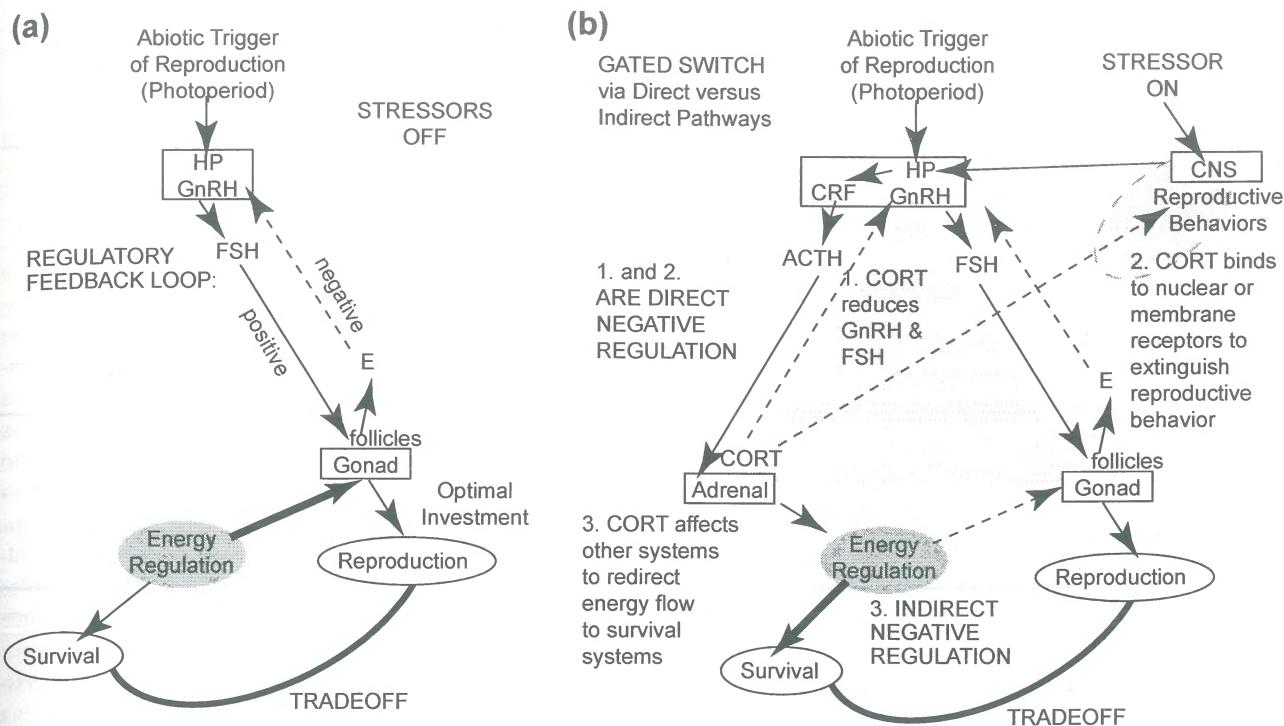


FIG. 3 The structure of endocrine networks and impacts on energy allocation. (A) A positive–negative feedback loop regulating optimal allocation to reproduction as described in Section 1.4. Solid lines denote positive effects (upregulation). Dashed lines denote negative effects (downregulation). (B) The same network modified to include effects of the adrenal glucocorticoid corticosterone (labeled CORT), which is triggered in response to a stressor that stimulates corticotropin-releasing factor (CRF) secretion, which in turn stimulates corticotropin (ACTH) secretion. The stressor can be abiotic, biotic, or social. Glucocorticoids act as a gated switch at three regulatory points to: (1) shut off gonadotropin hormone (GTH) production in the hypothalamus (HP), (2) directly bind to receptors in the central nervous system (CNS) to alter behaviors, or (3) indirectly redirect energy from reproduction to other critical metabolic functions that enhance survival. The effects of the hypothalamus–pituitary–gonadal (HPG) and hypothalamus–pituitary–adrenal (HPA) axes interact to alter behavior and fitness through effects on the trade-offs of survival and reproduction.

(Dunham et al., 2009), which is also a key site for the activation of the HPG axis. Dunham et al. (2009) suggested that kisspeptin may have a potential role in modulating reproductive behavior in lizard. In a recent study, Neuman-Lee et al. (2017) assessed the effects of kisspeptin on the HPG axis in *U. stansburiana*. They quantified the behavioral and hormonal responses to exogenous kisspeptin. Hormone assays revealed that exogenous kisspeptin increased testosterone levels in males; castration surgery eliminated testosterone release in response to exogenous kisspeptin. Males treated with kisspeptin showed an increase in some aggressive behaviors, e.g., push-up displays and head-bobs. Despite these intriguing results, additional data are needed for demonstrating the type of kiss variant present in reptiles, the seasonal expression of kisspeptin, and further investigation into how kisspeptin may regulate reproductive behaviors.

Less information is available regarding the roles of LH and FSH in reptiles, and what is known is based on heterologous GTHs from mammals. Mammalian LH increases androgen production in immature water snakes, *Nerodia sipedon* (Krohmer, 1986); yet, FSH stimulates androgen secretion

in the turtle, *Chelonia mydas* (Licht et al., 1985). In lizards, the synergism of mammalian FSH and LH increases plasma T in the side-blotched lizard *U. stansburiana* (Mills et al., 2008), but mammalian FSH and LH have distinct effects on subsets of male traits. Subcutaneous injection of LH and FSH in nonbreeding male *A. carolinensis* induced an increase in the size of the testes and testosterone levels (Kang et al., 2020).

The activational effects of gonadal steroids include changes in levels of aggression (Kelly & Wilson, 2020; Marler & Moore, 1988; Moore & Crews, 1986) and changes of morphology and physiology (Sinervo, Miles, et al., 2000; Miles et al., 2007). In females, mammalian LH positively regulates the maintenance of corpora lutea. Ovulated follicles secrete P₄, which is yet another feedback loop controlling the length of the reproductive cycle, and likely played a role in the evolution of viviparity from ancestral oviparity (Guillette, 1993).

The positive (e.g., stimulatory effect of LH and FSH on gonads) and negative endocrine regulation (e.g., negative effect of rising T and E produced by the gonads on FSH

and LH production by the HPG axis) is also subject to the action of other axes associated with the HPA that generate a “gated switch.” A gated switch is a term used in networks (Milo et al., 2002) where one network component (in this case, the HPA axis) modulates whether or not the HPG has effects on salient behaviors. Thus, the HPA axis can turn off the behavioral effects of the HPG axis or allow for behaviors to be induced by the HPG axis in a switch-like fashion. The HPG axis interacts with the HPA axis via the stress hormone CORT, the primary glucocorticoid of reptiles (Sapolsky, 1992; Summers et al., 2005). This stress system modulates the expression of behaviors in a variety of social contexts and as a result of the genetic background of an individual. For example, hormones of the HPA and HPG axes also interact in combination with the ARS locus of female lizards (Comendant et al., 2003; Svensson et al., 2002) (Figs. 1C and 3), and male lizards (discussed in Section 2.2). The HPA and HPG axes interact to determine male behaviors as evinced by the interactive effects of CORT and T on male territorial behavior (DeNardo & Licht, 1993; DeNardo & Sinervo, 1994a, 1994b) or in male–male contests (Knapp, 2003; Knapp et al., 2003). Adrenal glucocorticoids have a dual function in both metabolic regulation and the regulation of stress, including social stress (Korte et al., 2005).

In response to various abiotic and/or biotic stressors, the CNS triggers the hypothalamus to secrete corticotropin releasing factor (CRF), which in turn stimulates the release of corticotrophin (ACTH) from the pituitary. Corticotropin travels via the blood stream to stimulate release of CORT by the adrenals. CORT travels to various tissues and can be bound by CORT binding globulins (CBGs) (Jennings et al., 2000). CORT has diverse tissue targets. For example, it binds to both mineralocorticoid and glucocorticoid receptors in the hypothalamus to alter levels of FSH, and binds to components of the CNS to cause alterations in behavior in vertebrates (Korte et al., 2005). CORT is associated with color in lizards, and suppresses the effect of T on the expression of male behavior (DeNardo & Licht, 1993; DeNardo & Sinervo, 1994a, 1994b). Thus, CORT can directly switch off either the endocrine circuits that control the gonads or the behaviors that control receptivity of sexual behaviors (Fig. 3). We will use the formalism of network diagrams to summarize all of the relevant endocrine systems in reptiles and draw parallels between reptiles and other vertebrates. Each figure will include a triangle that comprises a regulatory feedback loop along one side of the triangle and a gated switch at the opposing vertex. The complete networks and selection on network structure are likely to be quite complex and involve the HPG/HPA axes, selection on reproductive function, and other systems like immune function. A brief summary of the role of life history trade-offs and plasticity in structuring endocrine systems is given in Fig. 4.

2 THE FORM OF NATURAL, SEXUAL, AND SOCIAL SELECTION

The pattern of selection on the behaviors of sexes, and on juvenile vs adult phases, is very different. The organizational events in reptiles, discussed in Section 1.1, provide a mechanism to fine-tune progeny phenotypes and ameliorate ontogenetic conflict (Sinervo et al., 2008; Sinervo & Clobert, 2003). In addition, TSD has been hypothesized to ameliorate such conflict in situations where differences in growth or maturation are linked to a developmental temperature regime (Janzen & Phillips, 2006; Singh et al., 2020). Though not universally true in vertebrates (e.g., not in the case of sex-role reversal or in the case of hermaphrodites), reptilian males are under sexual and natural selection while females tend to be under natural selection. There are few overt differences in the form of sexual selection found among reptilian groups with most exhibiting polygynous mating systems, and multiple mating is extremely common in squamate reptiles (Olsson & Madsen, 1998). Squamate reptiles provide examples of resource defense polygyny (e.g., *Uta palmeri*; Hews, 1993), lekking behaviors (e.g. *Amblyrhynchus cristatus*; Wikelski et al., 2005), scramble competition and mating balls (*Thamnophis sirtalis*; Shine et al., 2005), mate-guarding (*Vipera berus*; Luiselli (1995), *Chamaeleo chamaeleon*, Cuadrado (2001), and *Merolia spilota*, Fitzgerald and Shine (2018)), and territorial cooperation (*U. stansburiana*; Sinervo and Clobert (2003), Sinervo, Chaine, et al. (2006)).

Multiple alternative mating strategies within a single species are common in lizards and associated with color and pattern polymorphism expressed on either the ventral (e.g., *Zootoca vivipara*; Sinervo et al. (2007), Vercken et al., 2007), gular (*U. ornatus*; Carpenter, 1995); *U. stansburiana*, (Sinervo & Lively, 1996); *Podarcis muralis*; *Podarcis millesensis*, (Huyghe et al., 2007); or dorsal surfaces (*Egernia* spp.; Chapple (2003); *U. stansburiana* (Lancaster et al., 2007), *Anolis sagrei* (Calsbeek et al., 2009), Table 1). These colors have a genetic basis in several species examined: female *L. vivipara* (Vercken et al., 2007), female *A. sagrei*, male and female *U. stansburiana* (Sinervo, 2001), and male *Ctenotus decresii* (Rankin et al., 2016). As it is common in the case of color polymorphism in other vertebrates (Hoffman & Blouin, 2000), color of *U. stansburiana* has a Mendelian pattern of inheritance (linkage mapping and controlled crosses suggest a single factor control color). Males in the southern lineage of *C. decresii* exhibit a polymorphism for throat color (orange, yellow, orange + yellow, and gray) that also follows a pattern of autosomal inheritance (Rankin et al., 2016).

The cellular basis of color involves three chromatophore types: xanthophores (containing carotenoid and/or pteridines), colorless iridophores that contain light reflecting guanine crystals, and melanophores populated with melanin (McLean et al., 2017). The biochemical basis of color arises

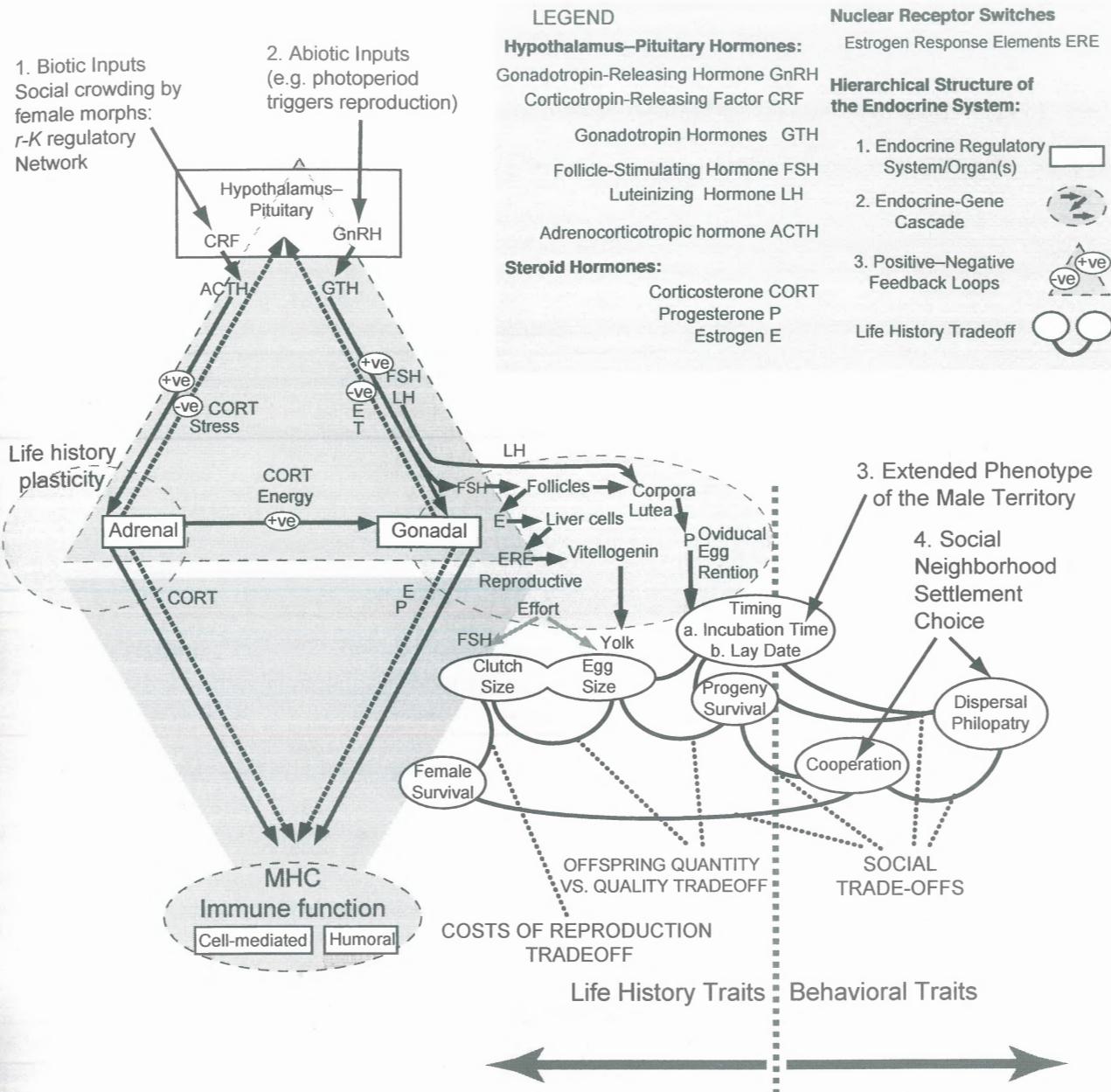


FIG. 4 The hypothalamus–pituitary–gonadal (HPG) axis, which regulates clutch size and progeny size, is associated with selection and life history trade-offs in reptiles (Sinervo, 1999; Wingfield, 2018) and other vertebrates (Oksanen et al., 2002; Vitousek & Schoenle, 2019), based on endocrine manipulations. An example of an endocrine cascade for egg size and egg number in lizards is depicted (gonadotropin-releasing hormone (GnRH) → gonadotropic hormone (GTH) → follicle proliferation → theca of follicles produce E_2 → E_2 travels to liver → initiates gene transcription via an estrogen response element (ERE) → to effectuate vitellogenin transcription and translation → vitellogenin is packaged in many small ova or a few large ova by ovaries). A single cascade regulates trade-offs in egg and clutch size. Additional endocrine cascades in this system affect levels of progesterone (P_4) via stimulation of the corpora lutea by gonadotropic hormone (GTH). However, clutch size and thus GTH determines the number of corpora lutea. Progesterone affects the degree of egg retention and incubation time in reptiles. These endocrine system interactions affect a trade-off in females: progeny survival and costs of reproduction for the female parent (Sinervo, 1999). Reproductive hormones affecting life history traits are modulated by the adrenal system, the same system that regulates territorial behavior of males. More complex theories of trade-offs implicate immune function effects on reproductive costs through effects on resource allocation and metabolic pathways (Costa & Sinervo, 2004; Lochmiller and Dabbert, 1993; Lochmiller & Deerenberg, 2000; Segner et al., 2017). For example, in the side-blotched lizard, we have observed a strong trade-off between immune function vs survival in the *r*- vs *K*-selected color morphs of females. A single gene of major effect called the *oby* locus, which is named for the colors orange, blue, and yellow, governs female throat color and reproductive strategy (*r*- vs *K*-selected). The strong negative genetic correlation (Svensson et al., 2001a, 2001b), indicative of a genetic trade-off between immune function and survival costs (Svensson et al., 2002, 2009), arises from two physiological pathways: (1) adrenal → immune system → survival, which interacts with (2) adrenal → gonadal → clutch size vs egg mass (Lancaster et al., 2007, 2008, 2010).

In summary, while the offspring size–number trade-off appears to arise from multiple effects of a single endocrine cascade, the costs of reproduction trade-offs arise from several endocrine cascades. One of these cascades is the hypothalamus–pituitary–adrenal (HPA) axis of reproductive females. In this case, the HPA axis modulates life history plasticity in the context of social stressors of female density and female morph frequency (Comendant et al., 2003). However, there are also far more complex life history trade-offs that involve egg size, oviposition date, incubation time and density regulation (Sinervo & Calsbeck, 2006; Svensson & Sinervo, 2000), and the choice of territory by the female (Calsbeck & Sinervo, 2004). In addition, male progeny experience very complex social trade-offs of altruism, mutualism, and competition that involve offspring size and offspring settlement strategies (reviewed in Sinervo et al., 2008). All of these complex behaviors are regulated by endocrine systems.

TABLE 1 Reptilian species having intraspecific polymorphisms conveying different social status.

Order	Family	Common name	Species	Type of polymorphism	References
Testudines	Emydidae	Pond slider	<i>Trachemys scripta</i>	Size and color	
Squamata					
	Gekkota	Atlas day gecko	<i>Quedensfeldia trachylepharus</i>	Color morphs	Blouin-Demers et al. (2013)
		Roze's gecko	<i>Gonatodes rozei</i>	Color morphs	Rivero-Blanco and Schargel (2012)
	Cordylidae	Augrabies flat lizard	<i>Platysaurus broadleyi</i>	Color morphs	Whiting et al. (2003)
	Lacertidae	Bocage's wall Lizard	<i>Podarcis bocagei</i>	Color morphs	Andrade et al. (2019)
		Aegean wall lizard	<i>P. erhardii</i>	Color morphs	Brock et al. (2022)
		Skyros wall lizard	<i>Podarcis gaigeae</i>	Color morphs	Runemark et al. (2010)
		Catalan wall lizard	<i>P. liolepis</i>	Color morphs	Andrade et al. (2019)
		Dalmatian wall lizard	<i>Podarcis melisellensis</i>	Color morphs	Huyghe et al. (2007)
		Common wall lizard	<i>Podarcis muralis</i>	Color morphs	Sacchi et al. (2007); J. Clober (Pers. Comm.)
		Italian wall lizard	<i>Podarcis siculus</i>	Color morphs	Andrade et al. (2019)
		Andalusian wall lizard	<i>Podarcis vaucheri</i>	Color morphs	Andrade et al. (2019)
		Sicilian wall lizard	<i>P. waglerianus</i>	Color morphs	Andrade et al. (2019)
		Algerian psammmodromus	<i>Psammmodromus algirus</i>	Color morphs	Salvador et al. (1997)
		Common lizard	<i>Lacerta (=Zootoca) vivipara</i>	Color morphs	Sinervo et al. (2007)
	Agamidae	Rainbow lizard	<i>Agama agama</i>	Color morphs	Madsen and Loman (1987)
		Lake Eyre dragon	<i>Amphibolurus (=Ctenophorus) maculosus</i>	Color morphs	Mitchell (1973)
		Tawny dragon	<i>Ctenophorus decresii</i>	Color morphs	Teasdale et al. (2013)
			<i>C. modestus</i>	Color morphs	Stuart-Fox et al. (2021)
		Painted dragon	<i>C. pictus</i>	Color morphs	Olsson et al. (2007)
	Tropiduridae	Albemarle lava lizard	<i>Microlophus albemarlensis</i>	Size morphs	Stone et al. (2003)
		Española lava lizard	<i>Microlophus delanonis</i>	Size morphs	Werner (1978)
		Striped lava lizard	<i>Tropidurus semitaeniatus</i>	Color morphs	Bruinjé et al. (2019)
	Iguanidae	Marine iguana	<i>Amblyrhynchus cristatus</i>	Behavioral morphs: Territorial, Satellite, Sneaker	Wikelski et al. (2005)

TABLE 1 Reptilian species having intraspecific polymorphisms conveying different social status—cont'd

Order	Family	Common name	Species	Type of polymorphism	References
Phrynosomatidae		Side-blotched lizard	<i>Uta stansburiana</i>	Color morphs	Sinervo and Lively (1996)
		Long-tailed brush lizard	<i>Urosaurus graciosus</i>	Color morphs	Miles (unpublished)
		Ornate tree lizard	<i>Urosaurus ornatus</i>	Color morphs	Thompson and Moore (1991, 1992)
		Mesquite lizard	<i>Sceloporus grammicus</i>	Color morphs	Bastiaans et al. (2013)
		Red-lipped plateau lizard	<i>Sceloporus undulatus erythrocheilus</i>	Color morphs	Morrison et al. (1995); Rand (1990)
		Minor lizard	<i>Sceloporus minor</i>	Color morphs	García-Rosales et al. (2019)
Liolaemidae		Fittkau's lizard	<i>Liolaemus fittkauai</i>	Color morphs	Jímenez-Robles et al. (2016)
			<i>Liolaemus sarmientoi</i>	Color morphs	Fernández et al. (2018)
Dactyloidae			<i>Anolis apletophallus</i>	Color morphs	Stapley et al. (2011)
		Green anole	<i>A. carolinensis</i>	Size morphs	Lailvaux et al. (2004), Husak et al. (2009)
		Bark anole	<i>Anolis distichus</i>	Color morphs	Schwartz (1968)
		Cuban brown anole	<i>A. sagrei</i>	Pattern morphs	Calsbeek et al. (2009)
Colubridae		Asian vine snake	<i>Ahaetulla prasina</i>	Color morphs	Amber et al. (2017)
		Red-sided garter snake	<i>Thamnophis sirtalis parietalis</i>	Behavioral morphs	Shine et al. (2005)
Viperidae		European adder	<i>Vipera berus</i>	Color morphs	Madsen et al. (2022)

from a combination of yellow and orange carotenoids acquired from food, endogenously produced pteridines (in the colors orange, blue, and yellow), and structural colors involving melanin to produce blue pigments (Morrison et al., 1995; Morrison et al., 1996; Stuart-Fox et al., 2021). Color polymorphism is not restricted to males but it is also prominently expressed in females in all of the species listed as discussed earlier. There is one example, *Sceloporus pyrocephalus*, which is unusual in that females of this species express greater color polymorphism than males (Calisi & Hews, 2007).

The expression of sexually dichromatic color, and alternative colors within a sex, during maturation is one of the most obvious factors influencing behaviors controlled by hormones since color is used as a signal in animals. Expression of color in females has been hypothesized to be a signal to males advertising lack of receptivity when the female is gravid (Cooper & Greenberg, 1992), or as a signal to males to advertise receptivity and stimulate male courtship (Chan et al., 2009; reviewed by Olsson et al., 2013). However,

the clear role of color in social selection among females (Comendant et al., 2003; Sinervo, Miles, et al., 2000; Sinervo, Svensson, et al., 2000; Vercken & Clobert, 2008b; Vercken et al., 2007) indicates that it also is likely to have the same role as color in males, which is used as a badge of status in lizards (Thompson & Moore, 1991). However, Olsson et al. (2013) conclude that female coloration may involve conflicts that involve interactions among mating success, physiology, and avoidance of predation. Hormonal control of female color polymorphism and effects on reproductive behavior presents a fruitful area of research.

The expression of sexually dimorphic color in vertebrates is under endocrine control, and a number of studies on reptiles indicate a positive correlation between the expression of color and steroid hormones (Calisi & Hews, 2007; Cooper & Greenberg, 1992; Cox et al., 2005b; Rankin et al., 2016; Rankin & Stuart-Fox, 2015; Yewers et al., 2017). Only a few studies have experimentally manipulated the endocrine system to alter color (Cooper et al., 1987).

Mills et al. (2008) manipulated ovine FSH and LH in a factorial design and found that blue throat color in the homozygous yellow color genotype (*yy*) of the side-blotched lizard intensified in response to an activational effect of LH, the *by* color genotype intensified in response to FSH stimulation, whereas the other color genotypes (*oo*, *bb*, *bo*) did not respond to either hormone treatment. This result is consistent with plasticity in reproductive strategy of the yellow color genotypes (*yy*, *by*). In particular, the blue color in the *by* color genotype can intensify during the breeding season as this morph transforms from a sneaker strategy and begins defending a larger territory, with increases in plasma T. Similarly, findings on the *yy* color morph suggest that the light gray melanistic stripes that provide a base to the yellow throat color can darken dramatically (Mills et al., 2008), particularly in the absence of male neighbors of the territorial color genotypes (*oo*, *bo*, *bb*, *by*). Given that plasma T is modulated by mammalian FSH and LH (Mills et al., 2008), the association of color and plasma steroids in other reptiles is likely under the control of both the GTHs and steroids. However, separate LH-like and FSH-like GTHs have not been demonstrated conclusively in squamates.

Throat color has also been manipulated in experiments investigating organization of alternative strategies discussed in Section 1.1. Early studies on the morphs of *U. ornatus* indicated that the male types did not differ in circulating levels of plasma T during the reproductive season (Moore et al., 1998) despite obvious differences in aggression (Hover, 1985), which led Moore on a search for organization effects (Crews & Moore, 2005). The polymorphism in *U. ornatus* is fixed in adults and color does not change in response to T implants (Thompson & Moore, 1992); however, manipulations of T in hatchling *U. ornatus* successfully altered throat color development (Hews & Moore, 1995), indicating that organizational events contribute to color in *U. ornatus* (Fig. 1A). We suspect similar effects are in operation in *U. stansburiana*, because while we have modulated color expression of blue in *by* genotypes, and the darker melanistic colors of *yy*, we have been unable to phenocopy the basic difference between blue (*bb*) and orange lizards (*oo*, *yo*, *bo*) with activational experiments (e.g., FSH, LH, and T have not altered the basic colors). It is likely that orange-yellow vs blue differences are organized early in development because, even though throat color is not expressed until maturity, behaviors of juveniles and selection on juveniles are markedly different among progeny genotypes (Sinervo & Zamudio, 2001).

In addition to a role for GTH and T in controlling some color expression at maturity, and P₄ and T, which act during organizational events, the hormone melatonin governs the expression of dark melanistic color. For example, in *U. ornatus*, α -melanocyte stimulating hormone, α -MSH, is the melanotropin that governs color change (Castrucci et al., 1997). The dark dorsal melanin is used as a badge of status in territorial *U. ornatus* (Zucker, 1989).

2.1 Hormone modulation of male reproductive behaviors

An organism's fitness is ultimately defined as the number of progeny produced over its lifespan. Fitness therefore depends on the ability to grow and reach adult (reproductive) body size, survival, number of reproductive episodes (clutch or litter), progeny produced per episode, and survival of young. In many reptilian species, a suite of behaviors governs reproductive success. These behaviors in turn are likely to vary with the type of the mating system. Regardless of mating systems, the two major limitations to breeding success in many male reptiles are the ability to defend a territory or repel rivals, and to court females.

Territorial status is determined by agonistic interactions among competing males (Whiting & Miles, 2019). Dominant males, as conveyed by aggressive behaviors, are more likely to have territories than less aggressive, subordinate individuals. In addition, the territory size often is tied to the number of potential mates, at least in polygynous species (e.g., *U. stansburiana*). A male with a larger territory has the potential to overlap with more females, which increases the number of progeny a male may sire (Ward-Fear et al., 2018). However, extra-pair fertilizations may reduce the number of offspring sired by a male with multiple mates or even in monogamous males. Hence, mate-guarding behaviors are likely to evolve in monogamous species as a strategy to reduce the likelihood of males sneaking copulations from a territorial male. The discovery of cooperative behavior in certain morphs within lizards (Sinervo & Clobert, 2003) suggests the importance of hormones that facilitate filiative interactions. Subordinate males without a territory may adopt other behaviors, e.g., sneaker strategy, in order to exploit furtive copulations with females. Finally, other reptilian groups, for example, snakes rarely defend territories (Shine et al., 2005). Therefore, the reproductive success of these males may entail outcompeting other rival males for access to females.

The second limitation is the ability to court and attract a female. Males may require specific signals (i.e., color pattern) or behaviors that convey dominance status to repel rivals as well as signal their attractiveness to females. Males enhance their attractiveness to females by simultaneously using phenotypic signals, such as badges, and courtship behaviors, such as push-up displays. Territorial and courtship behaviors may be distinct. Thus, agonistic and threat displays (biting, chasing, and other aggressive behaviors) may be used to chase rivals out of a territory, whereas courtship displays may entail other behaviors. The behaviors tied to dominance and mate acquisition entail two phases of sexual selection, namely, intrasexual competition (male–male interactions) and mate choice. Testosterone and other hormones modulate behaviors of males. Territorial behaviors and dominant interactions comprise one phase of sexual selection (antecedent

phase), which determines reproductive opportunities for males. Copulation and sperm transfer make up the second phase of sexual selection, mate choice. Selection operating on the HPG or HPA axis will vary according to the magnitude of male–male competition or female mate choice in affecting reproductive success.

As described earlier, each life history stage is regulated by specific endocrine cascades that influence the success of the individual in various behavioral challenges, such as territory acquisition, courtship, and parental care. Hormones are key for modulating the timing and expression of behaviors tied to fitness. Coordination via external cues synchronizes male courtship and copulatory behavior and female receptivity, timing of maturation of gametes, and territoriality. Because reptiles display a remarkable diversity of reproductive tactics, they are model organisms for investigating how hormones are involved in reproductive behavior.

In this section, we review the various reproductive behaviors influenced by T, which is the most thoroughly studied set of hormone effects. We also describe emerging details regarding the interaction between T and AVT in governing reproductive and sexual behaviors. We briefly summarize the differences between the sexes and selection on their respective endocrine systems. Moreover, we summarize the results from experiments conducted on unisexual lizards, principally *Aspidoscelis (Cnemidophorus) uniparens*, which link hormones to specific sexual behaviors.

2.2 Sexual selection, life history, and correlated selection—Hormones, behavior, and fitness

The endocrine cascades depicted in Fig. 2 reveal the complex linkages between T, CORT, and other hormones with numerous phenotypic traits and life history attributes. In the prematurational phase, T may either enhance or inhibit growth (Cox & John-Alder, 2005; Kubička et al., 2013). The action of T therefore depends on genetically determined patterns of energy allocation that may impact fitness. If body size covaries with fitness, then it may be advantageous for males to optimize growth to increase the chance of acquiring territories (through dominance interactions) and enhance mating opportunities. In contrast, T may favor reallocating energy to other competing functions, for example, musculature, which enhance physiological performance (Garland et al., 1990), or other traits that signal dominance status. By jointly influencing two trait groups, the action of T enhances the resource holding potential of a male and increases the probability of mating. The nature of the effects of T entails an interplay between traits that confer advantages for survival (avoidance of predators, foraging success), and maximizing reproductive success. Thus, T affects multiple trait complexes (Fig. 2) in interaction with other hormones, e.g., CORT and AVT (Fig. 5).

Species with alternative mating strategies offer the opportunity to dissect the role of hormones in affecting fitness (Miles et al., 2007). Morphs may be conditional or fixed (Rhen & Crews, 2002) and occur in a diversity of species in reptiles, including lizards, snakes, and turtles (Table 1). Conditional traits tend to be related to body size (e.g., marine iguanas, *A. cristatus*, Wikelski et al., 2005), age or ontogenetic polymorphisms (Lailvaux et al., 2004), or genetic polymorphisms (e.g., *U. stansburiana*; Sinervo, 2001). Most species have two alternative mating strategies, the one is a dominant male and the other is a sneaker or satellite male. In other species, there may be three morphs (e.g., *U. stansburiana*) occurring in a population. In particular, discrete morphs within a population introduce phenotypic variation that facilitates determining how hormones, physiology, behavior, and fitness are integrated (Miles et al., 2007). Comparing variation in traits among morphs generates insights into the multidimensional effects of sex steroids on sexual ornaments and behavior, the corresponding influences on other traits tied to dominance, such as physiological performance, and their role in generating life history trade-offs. Furthermore, fitness differences between morphs create the ideal system for identifying the targets of selection on hormones (Fig. 2).

2.3 Testosterone (T) and signals—Colors and badges

Substantial evidence is available supporting the importance of T in the expression of color patterns in male reptiles (Cooper & Greenberg, 1992; Cox et al., 2005a, 2005b, 2008; see also related references in Sections 2.1–2.2). Variation in color patterns among morphs also depends on androgens (Knapp, 2003; Knapp et al., 2003; Moore et al., 1998), although other hormones, e.g., P₄, may modify the expression of some morphs. As noted earlier, differences in color likely provide a signal (badge) to rival males and females about dominance status and male quality.

2.4 Testosterone, aggression, and territoriality

The connection between T and aggressive behavior, dominance status, and territoriality has received considerable attention. Rising levels of T at the beginning of the breeding season correspond with an increased frequency of agonistic displays and other forms of territorial behavior (Golinski et al., 2014; Moore & Lindzey, 1992; Tokarz et al., 2002; Wade, 2005; Watt et al., 2003). Aggressive behavior is associated with high levels of T in *Ctenophorus decressii* (Yewers et al., 2017). Male *A. sagrei* treated with exogenous T during the breeding season exhibited significantly greater dewlap extensions than castrated individuals in response to the presence of rival males (Tokarz et al., 2002).

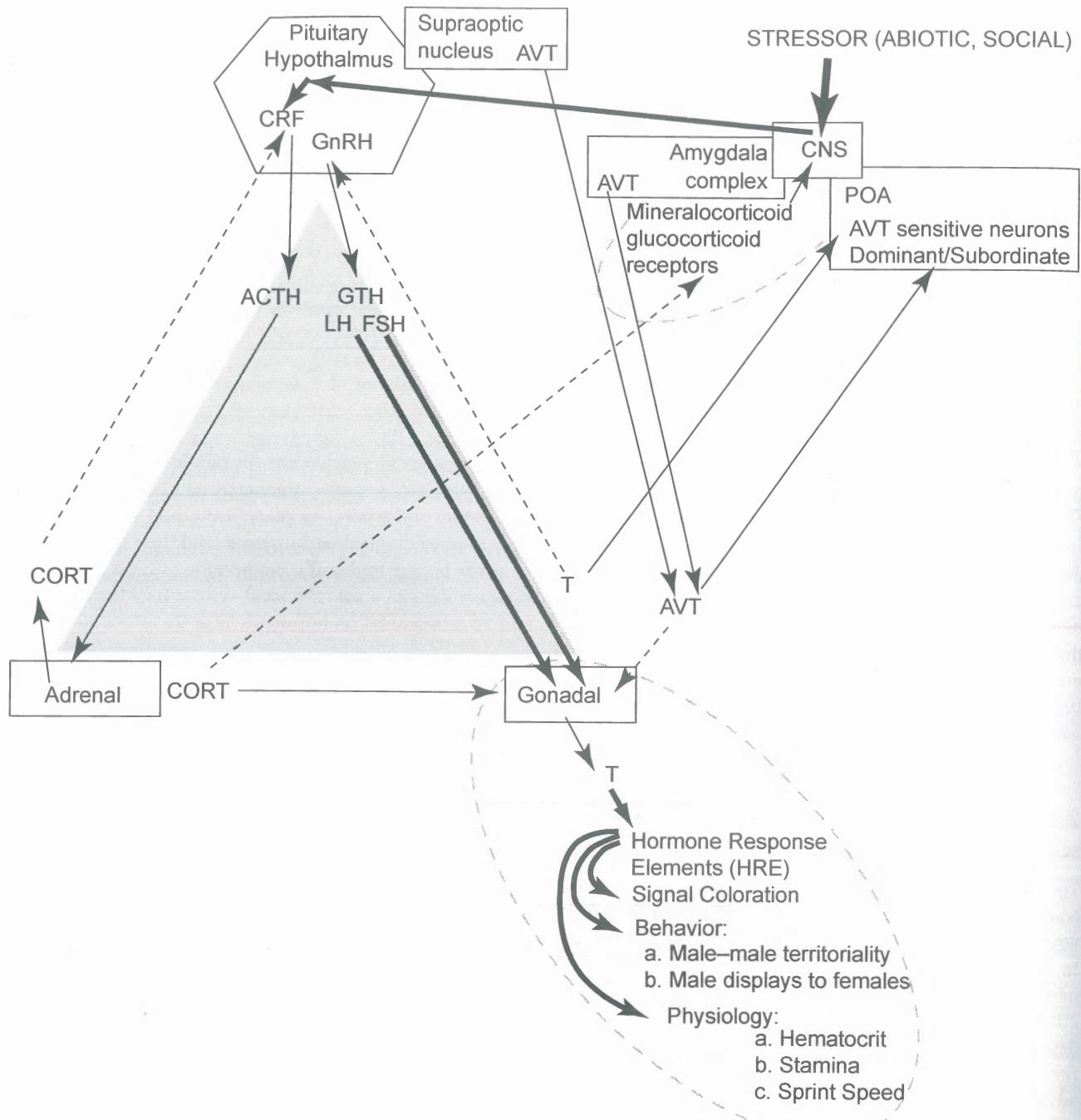


FIG. 5 The details of endocrine system regulation (e.g., negative feedback loops and gated switches, see Fig. 3) for plasma testosterone (T). Levels of plasma T secreted by the gonads are positively regulated by gonadotropin (GTH) (although the putative analogues of FSH and LH have not been definitively isolated in reptiles, injections of orthogonous mammalian FSH and LH have distinct biological effects in lizards (Mills et al., 2008)), which is in turn negatively regulated by high plasma T. The adrenal steroid corticosterone (CORT) is under positive regulation by corticotropin (ACTH), which is triggered by corticotropin-releasing factor (CRF) in the hypothalamus, in response to diverse stressors of abiotic (e.g., thermal and hydric) and biotic (e.g., social) origin. Corticosterone can also affect the neuronal activity in the central nervous system (CNS) via mineralocorticoid receptors and glucocorticoid receptors to effectuate changes in the secretion of many neuroendocrine hormones such as arginine vasotocin (AVT) and other neurotransmitters (serotonergic systems and catecholaminergic systems, not shown, see Section 2.8 for references). Arginine vasotocin can affect dominant vs subordinate behaviors of males via differences in receptor density in the anterior vs posterior preoptic area (POA), thereby adjusting social strategy.

To what extent, however, is individual variation in levels of T related to the intensity of aggression? A prior study compared aggressive displays and T levels in *U. ornatus* based on unmanipulated males and castrated males, given exogenous T (Kabelik et al., 2008). Size-matched males were paired in an arena, and the number of fullshow displays (aggressive display in *Urosaurus*) and display intensity were quantified. Fullshow displays were positively correlated with plasma T levels (Kabelik et al., 2008). To our knowledge, there is scant experimental evidence that T increases physiological characteristics associated with dominance. Identifying the link between T and physiological traits tied to dominance is key, since several studies have implicated T as a mediating factor in resource holding potential and shown to affect display capacities in male *U. stansburiana*. Mills et al. (2008) demonstrated a significant positive correlation between stamina and levels of T. Little information is available on the effects of T and aggression in snakes. T levels increased in copperheads (*Agkistrodon contortrix*) during the onset of the breeding

season (Schuett et al., 1996, 1997). However, T levels did not differ between winner and loser snakes at the end of staged agonistic encounters (Schuett et al., 1996).

Territorial status is critical for lizards to obtain copulations, and the literature is replete with examples, showing that territorial males have elevated levels of T (DeNardo & Sinervo, 1994b; Watt et al., 2003; Wikelski et al., 2005). Male *A. sagrei* treated with cyproterone exhibited fewer dewlap extensions than sham controls and spent more time in a low-quality habitat patch (Tokarz, 1995). However, in some species, the territorial status is unrelated to T. For example, T levels in older territorial male collared lizards (*Crotaphytus collaris*) did not differ from younger, nonterritorial males (Baird & Hews, 2007). Similarly, orange-blue and orange morphs in the tree lizard do not differ in T (Knapp et al., 2003), but other hormones, e.g., P₄ determine whether a male adopts a nomadic behavior or remains sedentary.

TABLE 2 Reptilian species in which T has been identified in the initiation of courtship behavior.

Order	Family	Common name	Species	References
Rhynchocephalia	Sphenodontidae	Tuatara	<i>Sphenodon punctatus</i>	Bradshaw et al. (1988)
Testudines	Emydidae	Painted turtle	<i>Chrysemys picta</i>	Licht (1982); Callard et al., (1976)
	Testudinidae	Gopher tortoise	<i>Gopherus polyphemus</i>	Ott et al. (2000)
	Cheloniidae	Loggerhead sea turtle	<i>Caretta caretta</i>	Wibbels et al. (1990)
Squamata	Gekkonidae	Ocelot gecko	<i>Paroedura picta</i>	Golinski et al. (2014)
	Scincidae	Shingleback skink	<i>Tiliqua rugosa</i>	Bourne et al. (1986)
		Blotched blue-tongued lizard	<i>Tiliqua nigrolutea</i>	Edwards and Jones (2001)
	Teiidae	Little striped whiptail	<i>Cnemidophorus (=Aspidoscelis) inornatus</i>	Lindzey and Crews (1986)
		Black and white tegu	<i>Tupinambis (=Salvator) merianae</i>	Chamut et al. (2012)
	Varanidae	White-throated monitor	<i>Varanus albicularis</i>	Phillips and Millar (1998)
	Agamidae	Central netted dragon	<i>Amphibolurus (= Ctenophorus) nuchalis</i>	Bradshaw et al. (1991)
		Ring-tailed dragon	<i>A. (=Ctenophorus) caudicinctus</i>	Bradshaw et al. (1991)
	Polychrotidae	Cuban brown anole	<i>Anolis sagrei</i>	Tokarz et al. (1998)
	Colubridae	Common garter snake	<i>Thamnophis sirtalis</i>	Clesson et al. (2002)
	Viperidae, Crotalinae	Copperhead	<i>Agkistrodon contortrix</i>	Schuett et al. (1997)
	Viperidae	Asp viper	<i>Vipera aspis</i>	Saint Girons et al. (1993)
Crocodylia	Alligatoridae	American Alligator	<i>Alligator mississippiensis</i>	Bradshaw et al. (1988)

2.5 Testosterone (T) and courtship/copulations

Considerable evidence is available showing that male courtship behaviors are induced by T (Moore & Lindzey, 1992). The onset of reproductive behaviors in the majority of reptiles coincides with a seasonal rise in androgens (Woolley et al., 2004). The association between a seasonal rise of testosterone (and 5 α -dihydrotestosterone (DHT)) and the initiation of courtship behavior in reptiles has been documented in many groups (see Table 2).

The role of T in controlling sexual behavior has been substantiated by experimental manipulations involving castration followed with exogenous T or androgen receptor blockers (e.g., flutamide) and inhibitors of androgen synthesis (e.g., cyproterone acetate), and provide additional evidence for the role of androgens in courtship and copulation behaviors (Haider & Rai, 1986). The cessation of courtship behaviors after castration has been established in *A. sagrei* (Tokarz, 1987), *A. carolinensis* (Mason & Adkins, 1976), *Sceloporus jarrovii* (Moore, 1987), *E. macularius* (Rhen et al., 1999), *Aspidoscelis inornatus* (Lindzey & Crews, 1986), *Paroedura picta* (Golinski et al., 2014), and *Goniurosaurus lichtenfelderi* (Golinski et al., 2015). For example, the musculature necessary for the dewlap display used by male anoles to attract females is responsive to levels of T and differs between the sexes (Wade, 2005). Moreover, the hemipenes increase in size in response to rising levels of T (Golinski et al., 2014; Wade, 2005). The frequency of courting behavior is also sensitive to T.

2.6 Integration—Hormones, performance, behavior, and fitness

Linking the role of natural and sexual selection on structuring hormone networks requires the quantification of individual hormone levels, the behavioral consequences of variation in hormones, and the ascertainment of the covariation between the behavioral trait and various components of fitness (for natural selection) or mating opportunities (for sexual selection) (see Bonier & Martin, 2016 for a discussion of estimating the form of association between hormone and fitness). Thus, not only it is necessary to corroborate that T influences traits that signal dominance (badges) and aggressive behaviors establishing dominance, but also that dominant males either acquire more mating opportunities or sire more offspring (Fig. 2). Disparate evidence is available supporting individual paths in the endocrine cascade, but relatively few studies have linked hormones and fitness. In particular, there are few studies that have evaluated the fitness consequences of hormone manipulations in reptiles (Bonier & Cox, 2020).

Two recent metaanalyses found striking differences in the effects of steroid hormones on fitness. Bonier and Cox (2020) found that the experimental manipulation of T resulted in a reduction in fitness across 65 studies included in their analysis. The second metaanalysis found no association between baseline or stress induced glucocorticoids and survival (Schoenle et al., 2021). In contrast, field studies have provided evidence between hormones and components of fitness. The mating system of marine iguanas (*A. cristatus*) provided an opportunity to link levels of T with potential mating success. Male marine iguanas may adopt one of the three mating strategies: dominant male holding a small display territory, satellite male, and sneaker (Wikelski et al., 2005). Territorial males have access to higher numbers of females, but the overlap with females drops when the action of T is temporarily blocked by flutamide.

The link between dominance, territory size, and fitness has been demonstrated in only a few species. The fitness consequences of dominance have been clearly established in *U. stansburiana* (Sinervo, Miles, et al., 2000; Zamudio & Sinervo, 2000). In addition, the long-term work of John-Alder and associates has linked hormones with growth, aggression, territory size, overlap with females, and number of progeny sired (Haenel et al., 2003). Males with larger territories overlap with more females (Haenel et al., 2003). Elevated T enhanced territory size and activity time (Cox et al., 2005). Interestingly, the number of clutches sired and the number of progeny sired are both associated with the territory size. However, T is unrelated to fitness; rather the paternity data show a significant, positive association with levels of CORT. A comparison of T levels and aggression in Caribbean *Anolis* lizards yielded conflicting patterns (Husak & Lovren, 2014). The study measured T in 18 species of anoles from four islands in the Greater Antilles. On three islands, the more aggressive species had lower baseline levels of T, while species on the fourth island exhibited the predicted relationship between T and aggression. A recent study on spectacled caiman, *Caiman crocodilus*, found no association between levels of T and reproductive success (Barragán-Contreras et al., 2021). Rather male size, and hence dominance, predicted number of offspring, which may be the result of an indirect effect of T.

2.7 Modulation of aggressive and courtship behavior: Arginine vasotocin (AVT)

Understanding how T can mediate sexual signals, aggressive behavior, territoriality, as well as divergent mating strategies is a fundamental goal in evolutionary

endocrinology. A growing body of evidence suggests that steroid-sensitive neuropeptides are critical for regulating social and chemosensory behaviors (Campos et al., 2020; Kabelik et al., 2008; Wilczynski et al., 2017). AVT is a neuropeptide that modulates a variety of physiological and behavioral traits (Campos & Belkasim, 2021; Goodson, 2008; Goodson & Bass, 2001; Wilczynski et al., 2017). The presence of AVT cells has been detected in lizards, snakes, and turtles (Wilczynski et al., 2017). Most information about the sexual behavioral consequence of AVT (or the mammalian analogue arginine vasopressin (AVP)) in males is based on experiments with fish, amphibians, birds, and mammals (Campos & Belkasim, 2021). The dearth of studies conducted on reptiles makes generalizations about the influence of AVT on reptilian sexual behavior difficult. Details regarding roles of AVT in social and reproductive behaviors are based on neuroendocrine studies, showing areas of the brain that have high populations of AVT-ir neurons and manipulative experiments involving exogenous AVT. The production of AVT in vertebrates occurs in neurons found in the POA and the anterior hypothalamus (AH) (Adkins-Regan, 2005). In reptiles, AVT producing neurons also occur in the supraoptic nucleus of the hypothalamus (Goodson, 2008). Despite the apparent conserved distribution of AVT associated neurons (Moore & Lowry, 1998), there is considerable diversity among vertebrates in the locations of other neurons that produce AVT, which may provide substantial diversity in patterns of aggression and sociality (Goodson, 2008). Notably, there are populations of AVT neurons associated with the medial bed nucleus of the stria terminalis (BSTm) and amygdala, two areas of the brain that mediate a suite of social behaviors (De Vries & Panzica, 2006). The sexes differ in the density of cell populations in these regions, with males having higher numbers than females in pythons (*Python regius*), pit vipers (*Bothrops jaracara*), geckos (*Gecko gecko*), anoles (*A. carolinensis*), chameleons (*Chameleon chameleon*), and turtles (*Trachemys scripta*) (De Vries & Panzica, 2006). Recent evidence suggests that these areas are also sensitive to sex steroids, suggesting a role for T or E₂ in mediating aggressive or social behaviors.

The manipulation of AVT via exogenous administration affects a variety of behaviors ranging from aggression and courtship display behavior to parental care (Dunham & Wilczynski, 2014; Goodson & Bass, 2001). T regulates mating behaviors, but recent investigations have implicated AVT in initiating courtship behaviors; other studies have suggested a role for AVT in the maintenance or continuation of sexual behavior in males in a variety of taxa (e.g., Woolley et al., 2004; Kime et al., 2007 for multiple references based on amphibian models—see also Hillsman

et al., 2007; Wilczynski et al., 2017). The comparison of the action of AVT in territorial vs colonial birds revealed contradictory responses (summarized in Goodson, 2008). AVT reduced aggression in territorial avian species, but enhanced aggressive behaviors in colonial species. In reptiles, the investigation of AVT initially focused on its physiological role as an antidiuretic hormone (Bradshaw, 2007). Individuals that are water stressed will reset their preferred body temperature to avoid water loss (Bradshaw, 2007). AVT is critical in adjusting thermoregulatory behavior in the context of water balance. Earlier evidence in reptiles suggested that the expression of AVT in the brain is affected by T (Goodson & Bass, 2001). An early manipulative study on reptiles found that injection of AVT into adult male *Podarcis siculus* inhibited completion of spermatogenesis and reduced the endocrine activity of the gonads (Ciarcia et al., 1983). Injections of AVT in green anole lizards (*A. carolinensis*) resulted in adult males performing fewer aggressive displays in size-matched contests (Dunham & Wilczynski, 2014). Courtship behavior was unaffected by exogenous AVT, yet untreated, control females responded to AVT treated males by increasing their displays. Elevated levels of AVT resulted in an increase in CORT.

A recent study by Meylan et al. (2017) found that the effects of exogenous AVT on the social behavior of male common lizards (*Z. vivipara*) was size-dependent. Small males injected with AVT avoided the odor of conspecifics compared control males; larger males exhibited no response to conspecific odors. In addition, AVT treated males had significantly lower levels of T than control males. The AVT treated males spent more time basking and enhanced locomotor performance (endurance). Size and age are correlated in *Z. vivipara*; thus, smaller males are younger and may be subordinate to larger, dominant males. The results from Meylan et al. (2017) suggest that small males also tend to be competitive subordinates, hence avoidance of conspecifics may lower the potential for interactions with dominant individuals that could lower their survival and future reproductive success.

A few studies provide evidence for a role of AVT in modulating social status and courtship behavior in species with alternative reproductive tactics (Goodson, 2008; Greenwood et al., 2008). However, the bulk of the evidence comes from studies on fish. Current data suggest that AVT mediates aggressive and territorial behavior as well as courtship behavior in fishes (Almeida & Oliveira, 2015; Goodson & Bass, 2001; Mank, 2023; Santangelo & Bass, 2006). Curiously, AVT promotes or inhibits aggression depending on the social context (Meylan et al., 2017). Hence, its action appears to vary in the context of the social/mating system. AVT promotes calling behavior of

satellite males and has a role in divergent social strategies of satellite males vs calling males in the cricket frog, *Acris crepitans* (Marier et al., 1999; Semsar et al., 1998). The comparison of mRNA expression in the brains of the cichlid fish *Astatotilapia burtoni* revealed differences between dominant and subordinate males. AVT expression in the posterior POA was higher in dominant males. In contrast, subordinate males expressed more AVT in the anterior POA (Greenwood et al., 2008). Nonterritorial, subordinate males have small testes with low amounts of sperm. Because the expression of AVT varies with male phenotype, the results of Greenwood et al. (2008) suggest that AVT may regulate alternative strategies in males via maturation of testes and hence the production of T.

Although the role of AVT in modulating dominant and territorial behavior has been demonstrated in fishes (Santangelo & Bass, 2006), information on the role of AVT regulating aggression and dominance behavior in reptiles is limited. Hillsman et al. (2007) compared AVT fiber densities in the brains of *A. inornatus* (sexual species) and *A. uniparens* (asexual species). Males have higher densities of AVT in the POA than females. Furthermore, T treated males had higher AVT-immunoreactivity than sham treated males. Hillsman et al. (2007) concluded that androgens affected expression of AVT in regions that are critical for male reproductive behavior, specifically, courtship and copulatory behaviors. Dominant and subordinate *A. carolinensis* lizards differ in the number of AVT immunoreactive cells in the POA, but not in other parts of the brain (Hattori & Wilczynski, 2009). Dominant males have higher counts than subordinates. In contrast, AVT-ir cell counts for subordinate individuals were lower than dominant males, males housed singly, or paired with a female. The results of Hattori and Wilczynski (2009) provide support that the social context influences AVT. Unlike the work of Greenwood et al. (2008), the higher AVT-ir cell counts were unrelated to T levels. The lower number of AVT-ir cells in subordinate males deserves additional attention. It is unclear whether the reduced cell count is a consequence of the agonistic interactions. Unfortunately, Hattori and Wilczynski (2009) did not measure CORT levels. It is possible that the low numbers of AVT-ir cells is related to elevated CORT. Previous data have shown the effects of AVT on sexual behavior may be modified by glucocorticoids. Data from mammals suggest that vasopressin mRNA receptor binding in the hypothalamus is affected by glucocorticoids (Viau et al., 1999). Therefore, input and feedback from the HPA axis may be critical in regulating reproductive behavior as a consequence of extrinsic or intrinsic stressors (Goodson & Bass, 2001).

Data regarding differences in the AVT activity in reptiles with alternative mating strategies are limited. An earlier study by Kabelik et al. (2008) compared the association between T, aggression, and AVT in male morphs and female tree lizards

(*U. ornatus*). Males had greater densities of AVT cells in the POA, AMY, bed nucleus of the stria terminalis, and nucleus accumbens than females, which is consistent with other studies (Goodson, 2008; Goodson & Bass, 2001). Furthermore, the AVT-ir activity was lower in castrated males receiving a sham implant compared to males receiving exogenous T, supporting a role for T. However, the dominant orange-blue morph and the subordinate orange morph did not differ in AVT-ir. Testosterone affected both dominance behavior and AVT-ir in tree lizards, but AVT-ir was unrelated to aggressive behavior.

The manipulation of AVT revealed a relationship with maternal care in a viviparous snake species, the pygmy rattlesnake (*Sistrurus miliaris*). Female *S. miliaris* displays maternal attendance and defense of her offspring. A recent study blocked the vasotocin receptor, VT1a, using Manning compound, a VP1a receptor antagonist (Lind et al., 2017). Females receiving the VT1a blockade showed reduced maternal attendance of their offspring. The results supported the hypothesis that AVT mediates maternal care in pygmy rattlesnakes.

2.8 Interactions with CNS neuroendocrine hormones

Two other neuroendocrine systems have recently been linked to reproductive behavior in reptiles. The adrenergic and serotonergic systems are related to the regulation of aggressive behavior in vertebrates. The limited information on reptiles (Matter et al., 1998) indicates that male *S. jarrovii* engaged in aggressive territorial behaviors expresses higher levels of norepinephrine (NE) and dopamine (DA) and their metabolites, whereas satellite or subordinate males without territories express higher levels of serotonin (5-HT), and higher serotonergic turnover (e.g., ratio of 5-hydroxyindoleacetic acid to 5-HT). Territorial males at rest exhibited low levels of L-3,4-dihydroxyphenylalanine (L-DOPA), but diencephalic 3,4-dihydroxyphenylacetic acid, a metabolite of DA, rapidly increased after an agonistic interaction. In response to agonistic encounters, 5-HT levels of dominant males rapidly rise, but at rest, the values are low, compared to the elevated levels observed in satellite males. This finding is salient for this species, because previous studies on *S. jarrovii* did not find elevated levels of plasma T and plasma CORT after staged encounters, suggesting that the increased territorial locomotor behavior (patrolling) and agonistic displays (push-up behaviors) are driven by the monoaminergic system. Other behavioral signals, including a darkening of the eyespot in *A. carolinensis* males that win encounters, have been linked to the monoaminergic system, including elevated epinephrine and norepinephrine (Summers & Greenberg, 1994). Monoaminergic systems also regulate endocrine responsiveness (e.g., 5-HT stimulates CRF release).

2.9 Parental care—Crocodilians as model organisms

Parental care in the form of egg guarding is rare in reptiles (Shine, 1988). However, there are species that tend eggs or guard nests. Roughly, 4% of squamate reptiles (lizards and snakes) display some form of egg guarding by females (Shine, 1988). For example, female long-tailed skinks (*Eutropis longicaudata*) from Orchid Island, Taiwan, exhibit egg guarding behavior, which reduces egg loss due to snake predation (Pike et al., 2016). In contrast, 38% of crocodilians have biparental care and 62% have female only care (Murray et al., 2020; Platt & Thorbjarnarson, 2000; Reynolds et al., 2002; Shine, 1988). A key question is “do hormones permit a switch in male behavior to tend young rather than pursue additional mating opportunities to increase fitness?” The ability to address this question is hampered by the limited availability of data on the endocrine correlates of parental behavior in reptiles. Although there is evidence that T suppresses parental behavior in birds (Hau, 2007; Hunt et al., 2019; Ketterson & Nolan, 1999), the evidence is equivocal for other taxa. Oliveira et al. (2002) found little support that T reduces parental care behavior in reptiles and other ectothermic vertebrates (Oliveira, 2004). One recent study found that elevated levels of CORT may be an important mediator of parental attendance behavior in the cottonmouth pit viper (*Agkistrodon piscivorus*) whereas other hormones (e.g., P₄ or E₂) appeared to show no change (Hoss et al., 2014). CORT levels in female cottonmouths decreased after the offspring attained independence, which was defined as the first ecdysis. Other studies have shown an increase in exogenous CORT that suppresses egg guarding behavior as in the prairie lizard (*Plestiodon septentrionalis*) (Anton et al., 2018).

Increasing evidence suggests that PRL is involved in the expression of paternal care behaviors in a diverse array of vertebrates including fishes, birds, and mammals (see also Schradin & Anzenberger, 1999). However, the possible role of PRL in biparental care in reptiles, and in particular crocodilians, remains to be elucidated.

3 SOCIAL NETWORKS AND ENDOCRINE NETWORKS

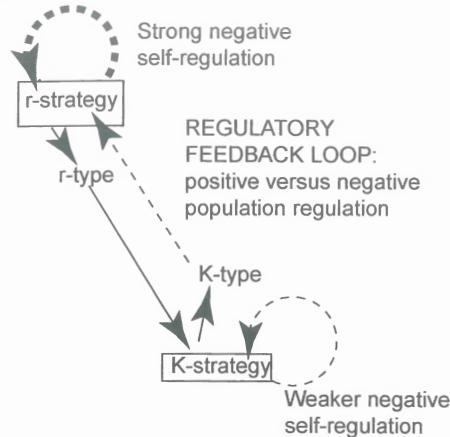
Social system networks can be coordinated with endocrine networks because social interactions commonly affect endocrine interactions within individuals and vice versa (i.e., hormones affect social behavior, and social cues affect hormones). As in endocrine networks (Figs. 3–6), social networks (Fig. 6) can be depicted as positive (solid arrows) and negative interactions (dashed arrows) that generate regulation in some aspect of system performance. Consider a population composed of an *r*-strategist, which produces large clutches of small eggs, and a *K*-strategist, which produces small clutches of large eggs (Sinervo, Svensson, et al.,

2000). Throughout this discussion, we highlight strategy types, that have a characteristic genetic/endocrine profile and gain a fitness advantage as a function of frequency or density of the alternative strategy types. Systems of *r*- and *K*-strategists can generate oscillatory density cycles, stabilize on a population density with both types present, fix on the *r*-strategist, or fix on the *K*-strategist. Under the oscillatory scenario, a strong negative regulation of *r*-strategists on self-types, when the population exceeds carrying capacity, allows the *K*-strategist to invade during the population crash. After the population has crashed, the *K*-strategist replaces the *r*-strategist. As the population recovers from low density, the *r*-strategist by virtue of a release of self-regulation can outcompete the *K*-strategist. The life history of each strategy type becomes adapted to a density cycle. The corresponding social regulatory network is depicted in Fig. 6A.

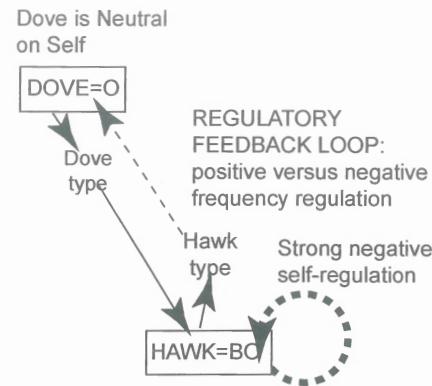
The stress hormone CORT responds to social stress and crowding differentially in *r*- vs *K*-strategists. In side-blotched lizards, the *r*-strategist with an orange throat is much more responsive to crowding, and levels of CORT (Fig. 4) are elevated in response to orange neighbors compared to the effects of either neighbor type on the yellow-throated *K*-strategy type (Comendant et al., 2003). As noted earlier, CORT has cascading effects on progeny behavior in *Z. vivipara* and *U. stansburiana*. Thus, the full regulatory network for social and endocrine effects would couple Fig. 6A with Fig. 4 at the input labeled: ‘1. Biotic inputs: Social crowding by female morphs’. Neighborhood frequency experienced by a female provides the information necessary to predict future density-dependent selection on progeny (see Section 1.4). The conserved HPG and HPA endocrine network of vertebrates (Licht et al., 1977), exemplified by *Uta*, is likely to be similar across all vertebrates.

With minor modifications, the network structure of *r*-*K* strategists can be applied to a system of aggressive and non-aggressive personality types involved in a *Hawk-Dove* dynamic (Smith, 1982) (Fig. 6B). Korte et al. (2005) reviewed the endocrine, behavioral, and metabolic networks governed by the HPA axis that regulate the expression of *Hawk*-like and *Dove*-like behavioral strategies. Besides this regulatory control over Hawk-Dove behavior by the endocrine system, *Hawks* and *Doves* also regulate one another in a social network. *Hawk* exhibits strong negative self-regulation, but the frequency of *Hawk* responds positively to *Doves*. *Dove* on the other hand is neutral to self, but decreases in frequency when subjected to more *Hawks*. This system will equilibrate with *Hawk* and *Dove* if the negative effects between *Hawks* are great enough to allow invasion of *Dove*; otherwise, it fixes on *Hawk*. The lizard species listed as discussed earlier with dimorphisms are likely to exhibit *Hawk-Dove* games (Fig. 6B), such as the two color morphs of *U. ornatus* (Table 1).

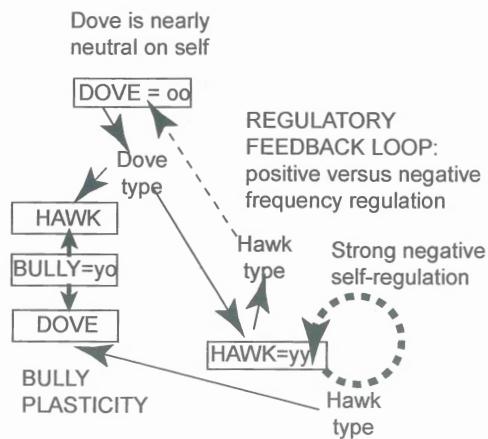
(a) POPULATION REGULATION NETWORK



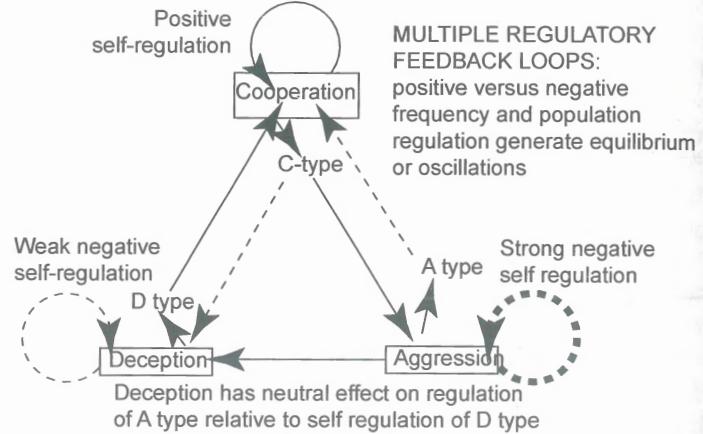
(b) HAWK–DOVE GAME NETWORK



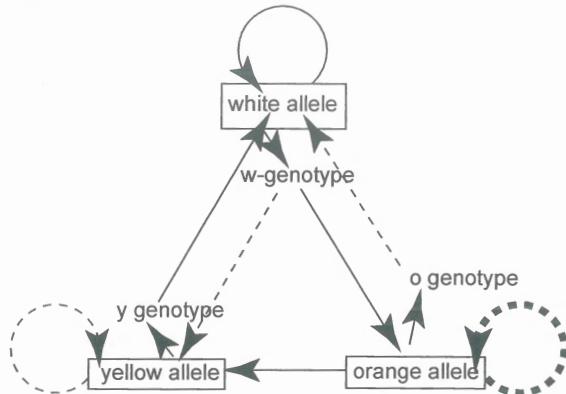
(c) HAWK–DOVE–BULLY NETWORK



(d) GENERIC SOCIAL RPS NETWORK



(e) RPS of European Common Lizard



(f) RPS in side-blotched lizards

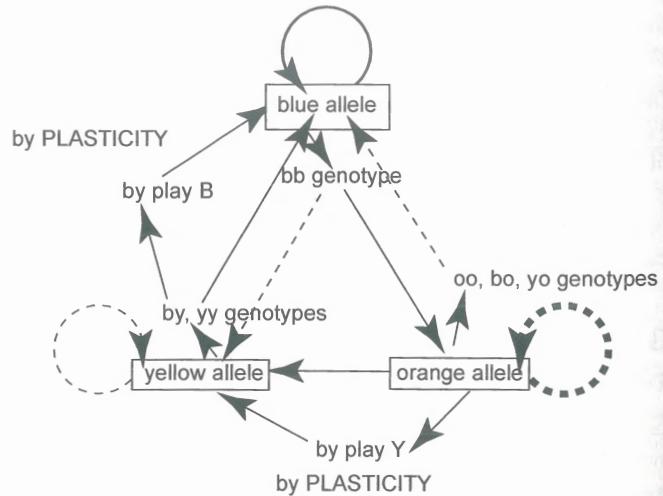


FIG. 6 Social networks of organisms can also be depicted in network diagrams with positive (solid line) and negative (dashed lines) regulation between non-self-types or between self-types (see Section 3). (A) Network diagram for a system of density genotypes, *r*- and *K*-strategists, such as the side-blotched lizard or European common lizard. (B) Network diagram for a system of *Hawks* and *Doves* in which *Hawk* is strongly self-regulating, but benefits from a high frequency of *Dove*. The *Hawk–Dove* system is likely to govern the alternative reproductive strategy (ARS) of the ornate tree lizard, *Urosaurus ornatus*. (C) Network diagram for a system of three genotypes (*oo*, *oy*, *yy*) that generate *Hawk* (*yy*), *Dove* (*oo*), and *Bully* (*yo*) phenotypes for a social system of female morphs of the European common lizard, which involve density strategies. The *Bully* strategy is heterozygous for both alleles and can plastically change phenotype depending on social situation (Vercken & Clobert, 2008a, 2008b; Vercken et al., 2007, 2008, 2010). (D) Network diagram for a system of three density types: a *cooperative* type that exhibits positive self-regulation, an *aggressive* type that exhibits negative self-regulation, and a *deceptive* type that exhibits weaker self-regulation. This system is observed in nature in (E) European common lizards, which express *o*, *w*, and *y* color alleles, and (F) side-blotched lizards, which express a parallel system of *o*, *b*, and *y* alleles. The heterozygote genotype in side-blotched lizards (*by*) reflects a *Bully* whose phenotype is plastically altered by social situation, e.g., when orange is common, *by* adopts a sneaker profile with low *T*; when orange is rare and yellow or blue is common, *by* adopts a territorial blue strategy and can even engage in cooperative interactions with blue males.

Vercken et al. (2007) describe interactions among *r*- vs *K*-strategists of female *Z. vivipara* that resembles a variant of the *Hawk-Dove* game referred to as *Hawk-Dove-Bully* (Fig. 6C), in which homozygote female *L. vivipara* *oo* and *yy* are *Hawk* and *Dove*, respectively (Vercken & Clobert, 2008a) and *Bully* is the heterozygous genotype that can play either *Hawk* or *Dove* depending on the local frequency of each. When *Hawks* are common, *Bully* plays *Dove*, but when *Dove* is common, *Bully* plays *Hawk*. This allows the heterozygous strategy to gain higher fitness and a heterozygote advantage. Although the endocrine induction of *Bully* in female *Z. vivipara* is currently unclear, it is likely related to density and frequency effects modulated by CORT based on exogenous manipulations. These observations on morphs of female *L. vivipara* are relevant for other plasticities observed in male ARSs in *U. ornatus* (Knapp, 2003; Knapp et al., 2003) and *U. stansburiana* (Sinervo, Miles, et al., 2000; Sinervo, 2001). In all these systems, social neighbors induce gated switch responses in behavior in specific genotypes; in males of both lizard species, these are the sneaker genotypes (Moore et al., 1998; Sinervo, Svensson, et al., 2000).

Hawk-Dove interactions among males involving two types of players generalize to systems with three strategies (Lancaster & Sinervo, 2011) with the addition of a *cooperative* type, which often evolves in social systems. The cooperative type generates positive self-regulation – more *cooperators* (in a group) enhance recruitment of self. *Cooperation* is vulnerable to invasion by *aggression* (i.e., *Hawk*-like type), which is self-limiting and generates negative self-regulation. *Aggression* is vulnerable to invasion by a *deceptive* (e.g., *Dove*-like strategy or *sneaker* strategy) type that is immune to or avoids *aggression*. Thus, nonaggressive *deceptive* types (e.g., *sneakers*), by virtue of lower levels of negative self-regulation, can increase in frequency when *aggressive* types are common. *Cooperative* types, which thwart *deception* via cooperation, benefit from a high frequency of *deceptive* types. A rock-paper-scissors (RPS) social system ensues from *Hawk-Dove-Cooperator* (e.g., *aggressive-deceptive-cooperative*) social interactions. RPS systems can either stabilize with all three types preserved or it can cycle in frequency of all three types (Sinervo et al., 2007). RPS networks are common in organisms (Figs. 6D–F). For example, male European common lizards (Fig. 6E) and side-blotched lizards (Fig. 6F) exhibit similar RPS frequency regulation. In general, any social organism demonstrating *cooperation* will generate similar frequency regulation and complex social trade-offs (Fig. 6D). Selection in such systems will operate on endocrine responses within and among individuals of each type. For example, *cooperation* is strongly stabilized by an endocrine response that attenuates aggression to self-types, while aggressive types benefit

from heightened agonistic behaviors induced by endocrine responses to all types (e.g., T modulated), but particularly other territorial types, and this involves the adrenal response (Knapp & Moore, 1997). *Deceptive* strategies are likely to be under facultative or plastic control, being submissive when dominants are around but aggressive when confronting self-types (Sinervo, 2001). Cooperative behavior is advantageous in interactions with *deceptive*-types, provided *aggressive* self-types are not present in local neighborhoods. *Deception* is advantageous in situations with a high frequency of *aggressive* types.

The concepts of endocrine plasticity in *Bully* genotypes discussed earlier for female *Z. vivipara* also generalize to the RPS of male *Uta* and observed endocrine plasticity in the *by* genotype in particular (Fig. 6F). When orange genotypes are common, *by* genotypes adopt a sneaker strategy, but when orange genotypes are absent and thus blue and yellow are common, *by* genotypes adopt a blue throat color (Sinervo, Miles, et al., 2000), via GTH stimulation of T (Mills et al., 2008).

Analogous behavioral plasticity occurs in the orange-throated genotype of *U. ornatus*, which switches between sedentary satellite and nomadic behavioral tactics (Moore et al., 1998). The switch between nomadic vs satellite tactic is linked to the gated switch action of P₄ and CORT (Knapp et al., 2003), which more strongly suppresses plasma T in the orange compared to blue-orange morph (Knapp & Moore, 1997). In this case, social interactions do not directly induce the switch; instead, it may be triggered by drought conditions. However, it is still possible that the drought per se generates elevated agonistic social interactions within and between morphs that result in elevated CORT in both types, and by virtue of the more resistant T levels of the blue-orange genotype, induce the orange morph to adopt an alternative tactic. The actual control mechanisms may involve steroid hormone binding globulin (SHBG) activity or levels. Jennings et al. (2000) found that *U. ornatus* possesses two SHBGs: an androgen-glucocorticoid-binding globulin (AGBG) and a typical SHBG. The capacity of AGBG is higher in the territorial orange-blue morph than in the nonterritorial orange morph and accounts for significant binding of both CORT and T. The behavior of the orange morph, due to the lower AGBG capacity, switches from satellite to nomadic type as CORT levels rise.

3.1 Social groups, monogamy, filiative behaviors, and the suppression of “aggression”

Whereas endocrine responses promoting heightened aggression are favored by sexual selection in aggressive types, cooperative types are selected for the opposite strategy and a suppression of endocrine responses for

heightened aggression (Sinervo, Chaine, et al., 2006). Quite little is known about the endocrine suppression of aggression in the context of cooperative behavior in vertebrates in general or reptiles in particular. Considerable attention has been focused on the role of adrenal glucocorticoids in the suppression of aggression in dominant-subordinate relations (Summers et al., 2005), which differs from the suppression of aggression required for advanced cooperation. Complex social systems are evident in lizards which includes transient associations between individuals, stable mating aggregations, parent-offspring associations, stable nuclear family structure, and communal aggregations consisting of overlapping generations (Chapple, 2003; Gardner et al., 2001; Halliwell et al., 2017; O'Connor & Shine, 2003; Slip & Shine, 1988). A recent review documented social grouping, defined as intergenerational social grouping, in ~8% of squamate species (95 among 1210 species) spanning 23 families (Halliwell et al., 2017). Sociability in reptiles may also be promoted by greenbeard selection, defined in the following paragraph (Sinervo, Chaine, et al., 2006; Sinervo & Clobert, 2003; Sinervo & Clobert, 2007).

The blue male genotype of the side-blotched lizard exhibits remarkable cooperative behavior in which one male will sacrifice fitness for a partner, when the cooperative dyad is attacked by an orange neighbor (Sinervo, Chaine, et al., 2006; Sinervo & Clobert, 2003). The field pedigree, which consists of 20 generations, for *U. stansburiana* reveals that neighboring blue cooperators are not related by genealogy, yet they resemble brothers in levels of gene sharing (Sinervo, Chaine, et al., 2006; Sinervo & Clobert, 2003). Genes for attraction to the blue color will be shared between blue males that are mutually attracted to blue. Other genes for self-attraction enhance the stability and fitness of pairs of cooperative blue males. Thus, the blue genotype finds other genotypes with which they share a large number of genes, via such self-attraction loci. These genes were uncovered by gene mapping behavioral traits such as settlement preference for blue neighbors or other genes that stabilize and enhance blue cooperation (Sinervo, Chaine, et al., 2006). Genes that control such social interactions are referred to as "greenbeards," and Hamilton (1964) theorized that true altruism, the expression of care between unrelated individuals, might evolve if a supergene simultaneously affected a signal and recognition of the signal, and if signal recognition elicited social acts costly to donors but beneficial to recipients. Dawkins (1976) coined Hamilton's social supergene a "greenbeard" in a hypothetical example of human altruists that sported a green beard distinct in color from other beards sported by nonaltruists. Sinervo and Clobert (2003) demonstrated that greenbeard genes and the self-attraction loci can be distributed across the genome, thereby enhancing and stabilizing cooperation (Sinervo,

Chaine, et al., 2006). Hamiltonian greenbeards also pertain to concepts of kin relatedness, but they apply directly to single genes and shared alleles, not merely genealogical sources of gene sharing.

Thus, the concepts of kin cooperation, coloniality, and nuclear family structure noted earlier are likely stabilized by suppression of aggression, as in the case of the greenbeard of *U. stansburiana*. The greenbeard cooperation of *U. stansburiana* is not unique. The lizard *L. vivipara* exhibits evidence of similar evolutionary cooperation and both species have a similar RPS social system despite 175 million years of independent evolution (Sinervo et al., 2007). A recent analysis of lizards in the family Phrynosomatidae indicates that the RPS colors are present in 22 species out of 148 species sampled. Ancestor reconstructions based on maximum likelihood suggest that RPS colors exhibited 14 independent origins (Miles, Mendez de la Cruz, & Sinervo unpublished ms.). Dimorphic systems are more common, occurring in 30 species in our sample. Both *Uta* and *Urosaurus* exhibit trimorphic RPS social systems, but the RPS social system is a more common mating system in the genus *Sceloporus*, particularly in the viviparous species of *Sceloporus*.

The *bb* genotype of *U. stansburiana* can form large clusters of adjacent territorial males of up to 12 *bb* or *by* males. These clusters resemble a primordial colony structure, even though each individual blue male retains a territory, their territories exhibit extensive overlap. In some instances, we have observed up to 4 *bb* males within a 2-m radius of one another suggesting remarkable tolerance, particularly compared to the orange male genotype, which is intolerant of territorial incursions by either orange or blue genotypes (Sinervo & Clobert, 2003). Incipient coloniality in RPS species may provide a unique, but as yet underexploited opportunity to study the suppression of the endocrine-based aggressive response. The endocrine system is likely to be controlled by the key set of genes by which suppression of aggression is achieved between cooperating and tolerant neighbors. By sharing endocrine-related alleles for suppressed aggression, for example, *bb* genotypes of *U. stansburiana* would form a much more stable territorial association. A likely place to search for such shared genes is among the factors that regulate gonadal steroid hormones like T, such as GTHs, AGBG, SHBG, or perhaps the steroidogenic enzymes that modulate metabolic conversion to T and metabolism of T (Knapp, 2003). It is unlikely that the suppression of aggression occurs via the adrenal response (Summers et al., 2005), which tends to shut down reproductive function in the context of dominance and subordinate relations.

In reptiles with more advanced social systems, both sexes are under strong social selection. Social selection includes

selection on behaviors that mediate settlement and spacing behavior, and behaviors for interactions among members of social groups. One key factor mediating the evolution of sociality in lizards may be the evolution of monogamy (Bull, 2000). The second factor favoring the evolution of sociality is the evolution of viviparity (Halliwell et al., 2017). For example, of the reptilian species that have evolved coloniality, many live in monogamous family groups (Bull, 2000; Chapple, 2003), and intriguingly most of these species are viviparous. The blue genotype of *U. stansburiana* also forms a genetic self-attraction mechanism (e.g., for neighboring blue genotypes) among males and females, owing to high levels of shared genes for self-attraction (Sinervo, Chaine, et al., 2006) and this promotes higher levels of monogamy in the blue genotype (Zamudio & Sinervo, 2000), compared to orange or yellow types, which tend to be quite promiscuous. Interestingly, color polymorphism is common in colonial species of *Egernia* (Chapple, 2003), and positive assortative mating within morphotype has been reported in some populations of *Egernia whitii* (Milton, 1987, 1990) of the form observed for the three color morphs of side-blotched lizards (Bleay & Sinervo, 2007; Sinervo, Chaine, et al., 2006).

Here, we suggest the novel hypothesis that viviparity per se predisposes lizards to an endocrine profile of suppressed aggression and the evolution of coloniality. An alternative route to protocoloniality is the suppressed aggression exhibited by cooperative RPS genotypes. We have inferential evidence that PRL is responsible for both behavioral syndromes, and here we present the arguments for the first time.

3.2 The potential role of prolactin as the master regulator of reptilian behaviors

Inferences from other vertebrate groups (birds, fishes, and mammals) suggest that prolactin (PRL) may be the key hormone related to maternal and paternal care behaviors (Schradin & Anzenberger, 1999) (Fig. 7). In viviparous species of reptiles, the association of mother and progeny would allow for natural filiative behaviors to be established by direct contact after birth, which is not as likely in oviparous species that deposit eggs in soil or cavities and progeny hatch at some time point after the female has gone through the hormonal stages in the preparation for egg-laying. The evolution of viviparity has a number of salient endocrine changes that by inference link it to PRL regulation. For example, viviparity has been linked to changes in the regulation of prostaglandins in the uterus (Gao et al., 2019; Guillette, 1993). In other vertebrates, PRL upregulates prostaglandins via phospholipase A2 prostaglandin G/H synthase 2 (Bole-Feysot et al., 1998). Prolactin also increases P₄ receptor number in the uterus and decreases

rates of P₄ metabolism in mammals. Particularly, noteworthy is the PRL's role in regulating FSH receptors and LH receptors in the gonads of other vertebrates (Schradin & Anzenberger, 1999). The broad spectrum of PRL's effects (Dobolyi et al., 2020) makes it a likely candidate in not only the evolution of reproductive strategies like viviparity, but also in the evolution of alternative mating strategies seen in RPS social systems (Schradin, 2008). The other key behavior necessary to regulate in the context of sociality is dispersal vs philopatry (e.g., sociality is enhanced when philopatry generates family groups). Dispersal and migration behaviors are also linked to PRL and thyroxine regulation in diverse vertebrates (Rankin, 1991). Whereas colonial lizard species exhibit marked philopatry (Chapple, 2003), variation in dispersal syndromes of RPS species like *Z. vivipara* and *U. stansburiana* is genetically correlated with morphs (Cote et al., 2006; Sinervo, Calsbeek, et al., 2006). The sheer number of traits genetically correlated with endocrine systems regulated by PRL strongly suggests that the PRL/PRL-R receptor (PRL-R) is the endocrine system responsible for both the RPS morphs and many instances of viviparity.

Unfortunately, there is a dearth of reptilian studies on PRL and PRL-R (Dobolyi et al., 2020). A molecular analysis of the tissues in which PRL/PRL-R are expressed in the gecko indicates mRNA for PRL in the whole brain, pituitary and oviduct, ovary, and testis, but not other tissues, and widespread presence of PRL-R mRNA in all tissues tested (Kato et al., 2005). This indicates that PRL secretion by the brain has a classic endocrine role (Kato et al., 2005), where it circulates to other tissues and effectuates changes in physiology and behavior, and potential paracrine and autocrine roles, where it can also respond in the same or adjacent tissues. This finding should allow reptilian behavioral endocrinologists to develop their own molecular probes to investigate the role of PRL in the evolution of viviparity, sociality, and ARS (e.g., RPS systems). Until now, mammalian analogues of many reptilian protein hormones have proven ineffective (e.g., receptor and hormone assays, see Knapp, 2003) including the mammalian PRL (Mills & Sinervo, unpublished data). At the same time, great progress has been made in using heterologous GTHs in eliciting behavioral and endocrine effects in reptiles (results cited in Section 1.4).

We suggest that the regulatory role of PRL should be a new focus of behavioral endocrinology in reptiles (Fig. 7). We hypothesize that mutations in PRL, and in particular PRL-R, would be an extremely likely genetic control in the evolution of the suite of traits related to ARS, viviparity, and advanced coloniality observed in reptiles. Our hypothesis is advanced because of the suite of traits known to be genetically correlated with the color morph locus of *U. stansburiana* (Sinervo & Svensson, 2002) including

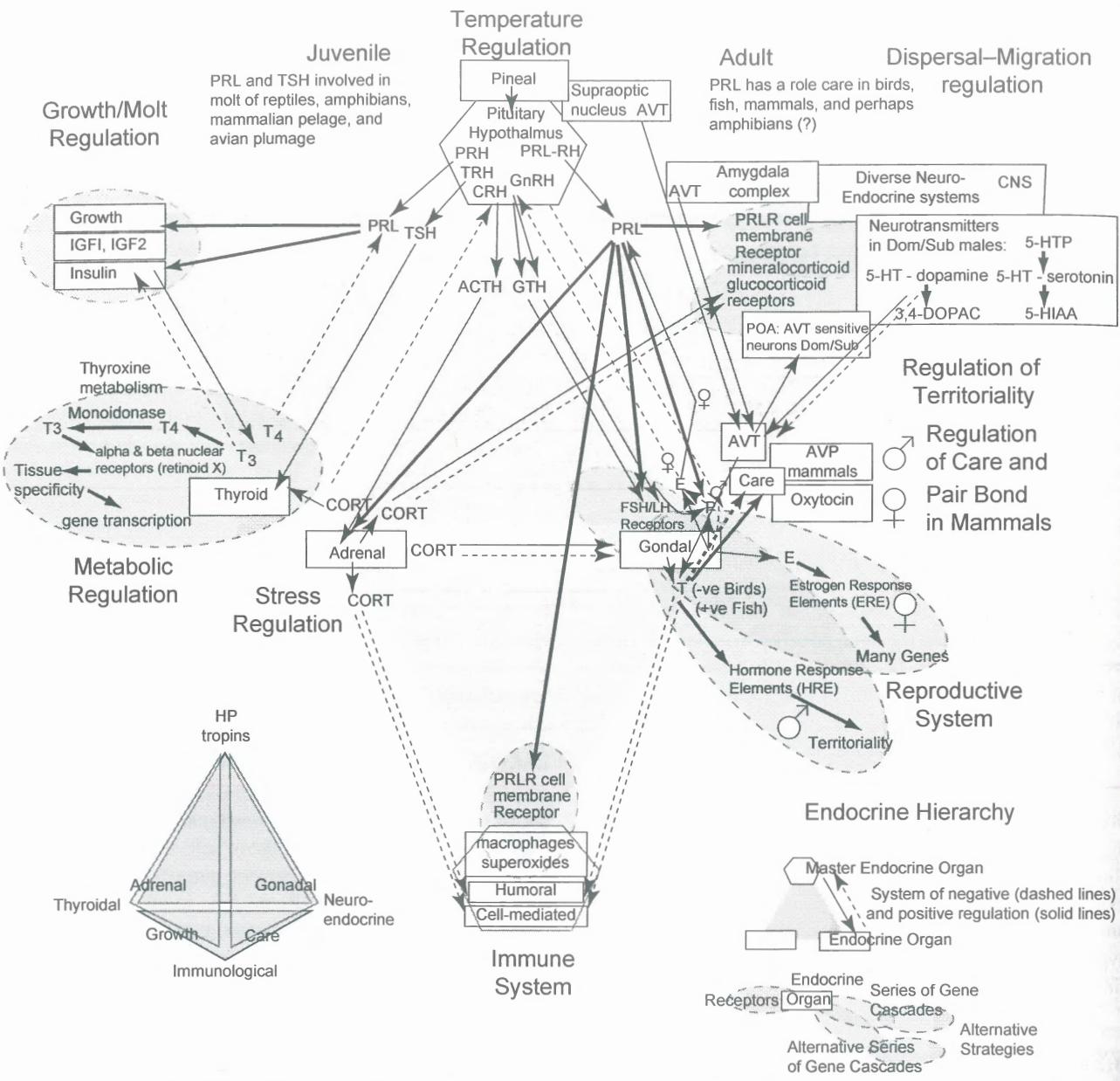


FIG. 7 The endocrine control of behavior and life history, depicted for a generic vertebrate in which resources are allocated to competing functions of growth regulation, metabolic regulation (homeostasis), immunocompetence and immune system regulation, reproduction, and life history behaviors like parental care and dispersal/migration. The topology of endocrine system network interactions that generate behaviors is multidimensional, which we depict folded as a tetrahedron (lower left). Endocrine systems at each vertex interact in positive and negative regulatory feedback loops, and the triangular “panes” forming the sides of the tetrahedron meet at adjacent vertices where regulatory feedback loops can act. Such loops can also act along the edges separating the vertex on a given triangular pane (e.g., gonadotropin hormone (GTH)–gonadal). The opposing vertex (e.g., adrenal) forms a gated switch. Real networks are more complex than the tetrahedra we use to depict network interactions, but the examples we use are holistic in that many essential functions for homeostasis are included (e.g., thermoregulatory, metabolic, immunological, and behavioral). Detailed discussion of the hierarchical structure of the endocrine system can be found in Figs. 3, 4, and 5 and in Sinervo and Calsbeek (2006) and Sinervo et al. (2008). We have clustered those hormones that largely affect juvenile life history on the left, and adult life history on the right, and the immune system, which is generic to both stages at the bottom. **Major regulatory feedback loops.** The endocrine regulators of behaviors and life history function (progeny investment, care behavior, dispersal vs philopatry, migration, territoriality, and coloniality) are found in the pituitary and hypothalamus, which produces the releasing hormones (RH) such as prolactin-releasing hormone (PRL-RH), thyrotropin-releasing hormone (TRH), corticotropin-releasing factor (CRF), and gonadotropin-releasing hormone (GnRH). In vertebrates, growth regulation (and metamorphosis if present) is achieved by the thyroid hormones, the metabolically active T_3 and its precursor T_4 , in interaction with brain prolactin (PRL) pathways and/or growth hormones. The hormone PRL is an extremely important but as yet understudied hormone from the perspective of reptilian behavior. PRL interacts with diverse endocrine systems and has multiple regulatory functions (Freeman et al., 2000). Key regulatory functions include the control of metamorphosis (Okada et al., 2019), molt cycles of reptiles, amphibians, birds, and mammals (Bole-Feysot et al., 1998; Stewart & Marshall 2022), the regulation of dispersal and/or migration behaviors (Rankin, 1991), and the expression of parental care in both sexes of several vertebrate groups (Shradin & Anzenburger, 1999). Neuroendocrine interactions. While PRL plays a major role in the regulation of parental care in both sexes in mammals (Dobolyi et al., 2020) and incubation in birds (Smiley, 2019), neuroendocrine hormones such as arginine vasopressin (AVP) play a role in generating care behaviors in males whereas the octapeptide oxytocin (OXY) plays a role in female mammals. Pair bonding in mammals arises in brain regions like the nucleus accumbens-ventral pallidum circuits (NA-VP) and involves the action of dopamine (DA) and oxytocin (OXY) (Goodson & Bass, 2001; Numan & Young, 2016). In reptiles, arginine vasotocin (AVT), the reptilian homologue of AVP, has a more ancient role in regulating territorial behavior (see Section 2.7). Arginine vasotocin, in interaction with testosterone (T), and brain regions with AVT sensitive neurons such as in the POA generate behaviors related to territoriality and sexual behavior. Dominant and subordinate lizards differ in AVT sensitive neurons in the anterior vs posterior POA, respectively (see Section 2.7). Besides AVT, DA turnover (catecholaminergic) and 5-HT turnover (serotonergic) in the dien-cephalon and telencephalon (metabolism of each neurotransmitter is shown) also differ between dominant and subordinate lizards.

clutch size (regulated by GTH), male aggression and territorial behavior (regulated by T and GTH), dispersal behavior, immune function (regulated in part by PRL), and the expression of color with the reproductive shedding of skin (regulated by PRL in reptiles). The effects of prolactin in reptiles and other vertebrates were reviewed by Bole-Feysot et al. (1998). In particular, the PRL-R is a classic membrane bound signal transduction receptor. PRL-R has been shown to be a member of the same family as the growth hormone (GH) receptor and also part of the larger class of receptors, known as the class 1 cytokine receptor superfamily (Bole-Feysot et al., 1998).

Although approaches to understanding the organizational effects of hormones on behavior in reptiles have identified several potential steroid control points, all of these are potentially regulated by PRL/PRL-R, including the production of diverse steroid hormones by the adrenal (Bole-Feysot et al., 1998), which as noted earlier have a role in organizing behaviors in embryos and juvenile mammals. The significance of the PRL in regulating behavior of reptiles is greatly understudied.

The genes directly controlling ARS in most species of vertebrates have not yet been identified, despite an exhaustive search by many research laboratories. However, several recent genomic studies have identified the genetic basis for morphs in iconic examples of ARSs. For example, two studies revealed that the alternative mating morphs in the Ruff (*Philomachus pugnax*) are determined by a supergene linked together in a chromosomal inversion (Küpper et al., 2016). An analysis of the genetic basis for morphs in the lizard *P. muralis* identified genes associated with the production of pterin and carotenoid metabolism (Andrade et al., 2019). An exciting result is that the enzyme involved in the pterin metabolism is also associated with the biosynthesis of neurotransmitters, such as dopamine, serotonin, and melatonin (Andrade et al., 2019). A recent study compared putative genes associated with dimorphism in color between two species of *Sceloporus* (Robinson et al., 2023). In one species, *S. virgatus* males and females are monochromatic and in a sister species, *S. undulatus* males and females are dichromatic. The manipulation of testosterone levels revealed the upregulation of genes associated with coloration in *S. undulatus*, whereas genes in the monomorphic species were unresponsive to exogenous testosterone. How genes associated with color variation are associated with reproduction may be explained by pleiotropic effects of genes determining color on physiology and behavior.

It is intriguing that to our knowledge, PRL/PRL-R has not yet been posited as a control system for such behavioral syndromes. The links between the evolution of viviparity and control over salient hormones (P_4 , prostaglandins)

would provide for a parsimonious pleiotropic endocrine mechanism, PRL/PRL-R, that links diverse phenomena, including the evolution of sociality, morphs, and viviparity.

Our understanding of the endocrine control of reptilian behavior has largely been focused on the agonistic behaviors that evolve under sexual selection on male display behavior. As noted earlier, the HPG axis regulates the expression of such male behavior. Less is known about the endocrine control that shapes adult female behavior despite the interesting and nuanced behaviors that evolve under social selection to generate parental care. Network visualization generalizes concepts of regulation to social forces that govern selection and life history trade-offs that shape the evolution of endocrine system control of behavior. Social trade-offs arise directly from social competition (density regulation) and cooperation that may abound in natural systems (Sinervo et al., 2007, 2008). Social networks can also provide exogenous cues of the events that control endocrine networks of organisms. In many cases, individual interactions in social networks induce gated switch effects in the internal endocrine networks of conspecifics through social stresses (e.g., aggressive types exert dominance and higher levels of CORT in neighbors).

This review highlights the growing body of evidence, demonstrating the impact of hormones on sexual behavior in reptiles. Yet, research on the influence of hormones on sexual behavior in reptiles lags that of other vertebrate groups (Kabelik & Hofmann, 2018). Clearly, sex steroids are critical in generating many patterns of sexual behaviors. However, other hormones, notably thyroid hormones, PRL, AVT, and glucocorticoids, modulate the action of sex steroids. The effects of hormones have major ramifications for individual fitness through multiple pathways, which modulate life history patterns and trade-offs between life history traits (Hau, 2007; Lancaster & Sinervo, 2011; Segner et al., 2017; Sinervo et al., 2008). Because sex steroids modulate multiple trait complexes that ultimately influence lifetime reproductive success, natural and sexual selection will operate on the complex interactions among hormone systems through the process of correlational selection by which multtrait combinations are favored (i.e., trait integration) (reviewed in Miles et al., 2007; Sinervo et al., 2008; Cox et al., 2016). The next phase in evolutionary behavioral endocrinology is to focus on how individual variation in sex steroids affects variation in behaviors and the physiological traits that are linked with fitness. This research program (as highlighted in Fig. 2) argues against sex steroids as constraints and rather highlights the potentiating (or gated response) roles of other hormone systems (adrenal, prolactin, etc.) in promoting life history variation and social system and mating system diversity.

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Chapter 9

Hormones and reproductive cycles in turtles

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ABBREVIATIONS

AVT	arginine vasotocin
CORT	corticosterone
E ₁	estrone
E ₂	estradiol
ELISA	enzyme-linked immunosorbent assay
FSH	follicle-stimulating hormone
GSI	gonadosomatic index
GTH	gonadotropins
HPA	hypothalamus-pituitary-adrenal
LH	luteinizing hormone
NP	neurophysin
P ₄	progesterone
PG E ₂	prostaglandin E ₂
PGF	prostaglandin F _{2α}
RIA	radioimmunoassay
T	testosterone
T ₃	triiodothyronine
TSD	temperature-dependent sex determination
T ₄	thyroxine
Vtg	vitellogenin

1 INTRODUCTION

After the first complete male and female reproductive cycle of a turtle (*Mauremys leprosa*) was described in the 1950s (Combescot, 1954), studies of cycles of other turtles began in earnest in the early 1970s (reviews by Licht, 1984; Moll, 1979) and have rapidly progressed (Hamann et al., 2003; Kuchling, 1999; Miller & Dinkelacker, 2008; Owens, 1997). An improved knowledge of turtle reproductive cycles is of increasing value for many reasons. First, their very highly conserved basic “life in a shell” format and their high individual longevity often combined with an “eggs for all” strategy (high egg production to account for high losses of eggs) are of particular evolutionary interest due to their

long-term success. Second, from an ecological perspective, few taxa have been as resourceful as turtles in so many different habitats, from temperate regions to tropical forests, from deserts to estuaries to oceans to lakes and streams. They actually seem to be preadapted for many of the more difficult environments of the planet. Third, a special urgency is now needed for an improved understanding of their reproduction due to the troubling declines in many turtle species around the world (Stanford et al., 2020). As their essential habitats have been destroyed or populations depleted by exploitation, a focus on reproduction in the many small remaining populations has become critical: the plowshare tortoise *Astrocelys yniphora*, for example, is now considered extinct in the wild, but, since the last edition of this book, studying the reproductive endocrinology of last wild individuals has become critical to understand requirements for captive breeding and conservation management (Currylow, Mandimbihasina, et al., 2017). Other turtle species are of distinct economic importance: certainly for food and medicine in many regions, for craft materials and artisan work in others, and worldwide as pets. Finally, as invasive species, some turtles are adding to the problems of some of their fellow chelonians. Regardless of the etiology, there are many reasons to want to improve our understanding of turtle reproductive cycles.

Despite their conservatism of living in an over 200-million-year-old box (shell) and their pervasive terrestrial egg-laying habit with embryos usually deposited at the gastrula stage, turtles have shown some very interesting and successful modifications on this basic theme. Various adaptations and flexibilities of reproductive patterns have evolved to allow them to reproduce successfully from oceans to deserts and from tropical to temperate zones throughout the world. As we will describe later, the nesting patterns of the leatherback sea turtle with vitellogenesis, ovulation, fertilization, albumin coating, shelling, and

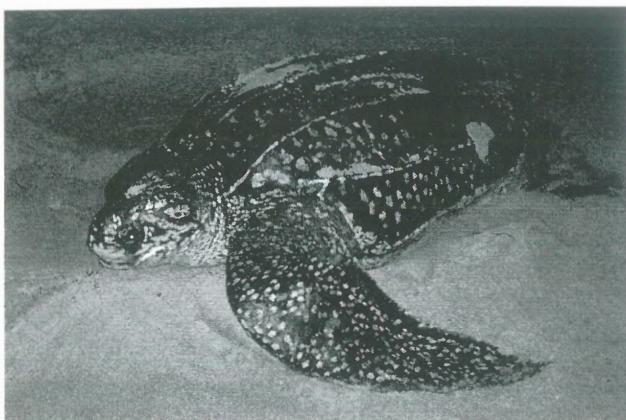


FIG. 1 Leatherback sea turtle (*Dermochelys coriacea*) covering her nest. Note the white spot on the skull that indicates location of the well-developed pineal complex.

precise oviposition of 600 or more eggs in 9–10 clutches is a *tour-de-force* of reproductive dynamics (Hirth, 1980; Tucker & Frazer, 1991) (Fig. 1). At the other extreme, some African tortoises including the world's smallest tortoise *Chersobius signatus* lay only a single egg per clutch (Loehr et al., 2004) and, depending on rainfall, may sometimes only lay one clutch per year or skip reproduction for a year (Loehr et al., 2011).

The major life-history challenge for a turtle is to survive to adulthood, which in these late-maturing animals may take several years or even decades, depending on the species (Gibbs & Amato, 2000). Once they are adults, they are typically hardy and iteroparous in their reproductive habit; i.e., they repeatedly cycle for reproduction for up to several decades. The anatomical, behavioral, endocrinological, and regulatory organization of the many forms these cycles take is both fascinating and complicated.

2 METHODOLOGIES

2.1 Gonadal cycles

The methodologies used to understand turtle reproductive cycles have improved considerably in recent decades (Kuchling, 1999). Originally, research focused mainly on gonadal cycles, often by sacrificing turtles or by using stranded or locally slaughtered animals, looking at gross morphology and histology of the gonads (e.g., Owens, 1980). Over time, the killing of animals, which is now inappropriate for protected species, and is best avoided whenever possible, was replaced by less invasive techniques including radiography, endoscopy—sometimes associated with biopsy and histology, and ultrasound scanning (Gibbons & Greene, 1979; reviews by Kuchling, 1999; Owens, 1999). Each technique has its own set of advantages and disadvantages, and

researchers are now able to choose the best methods based on their training, equipment, and the funding available, as well as the appropriateness of the technique for the question they are investigating.

Briefly, radiography represents a powerful technique used on females to determine clutch size while shelled eggs are present in the oviduct; however, this technique does not allow visualization of other structures (e.g., ovarian follicles, testes or epididymides) and concerns exist on the effects of irradiation on embryos after multiple exposures, especially when dealing with endangered species (Hinton et al., 1997; Keller, 1998; Kuchling, 1998; Zuffi et al., 2005). Endoscopy, or laparoscopy or coelioscopy, is a more invasive technique involving a surgical procedure allowing one to look directly at the gonads and therefore to obtain information on sex, maturity status, and reproductive state of the turtle through direct gonadal visualization and tissue biopsy (Fig. 2) (e.g., Blanvillain et al., 2008; Limpus, 1992; Limpus & Reed, 1985; Plotkin et al., 1996; Wibbels et al., 1990; Wood

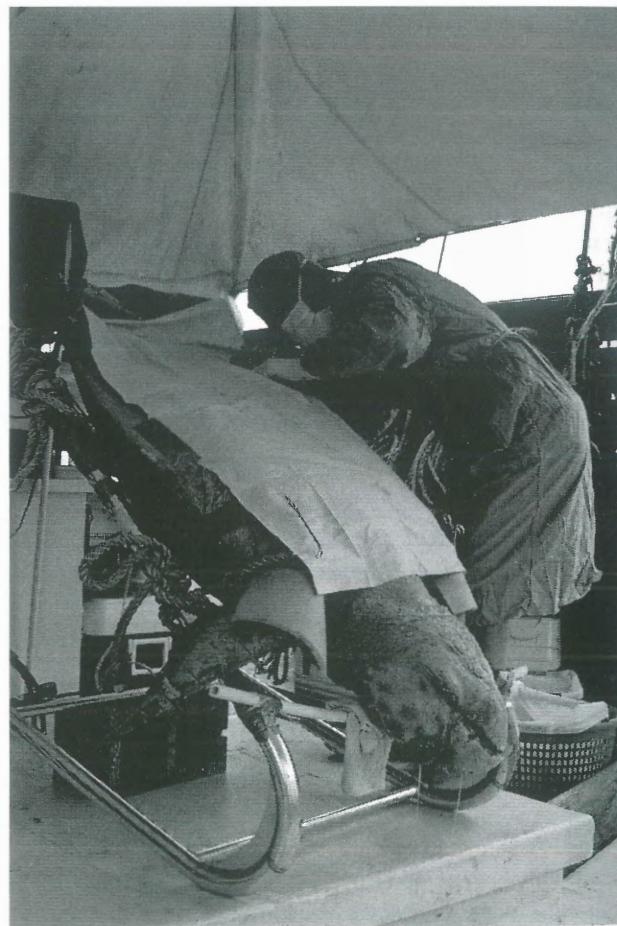


FIG. 2 Laparoscopy on an adult male loggerhead sea turtle (*Caretta caretta*) following proper surgical procedures.

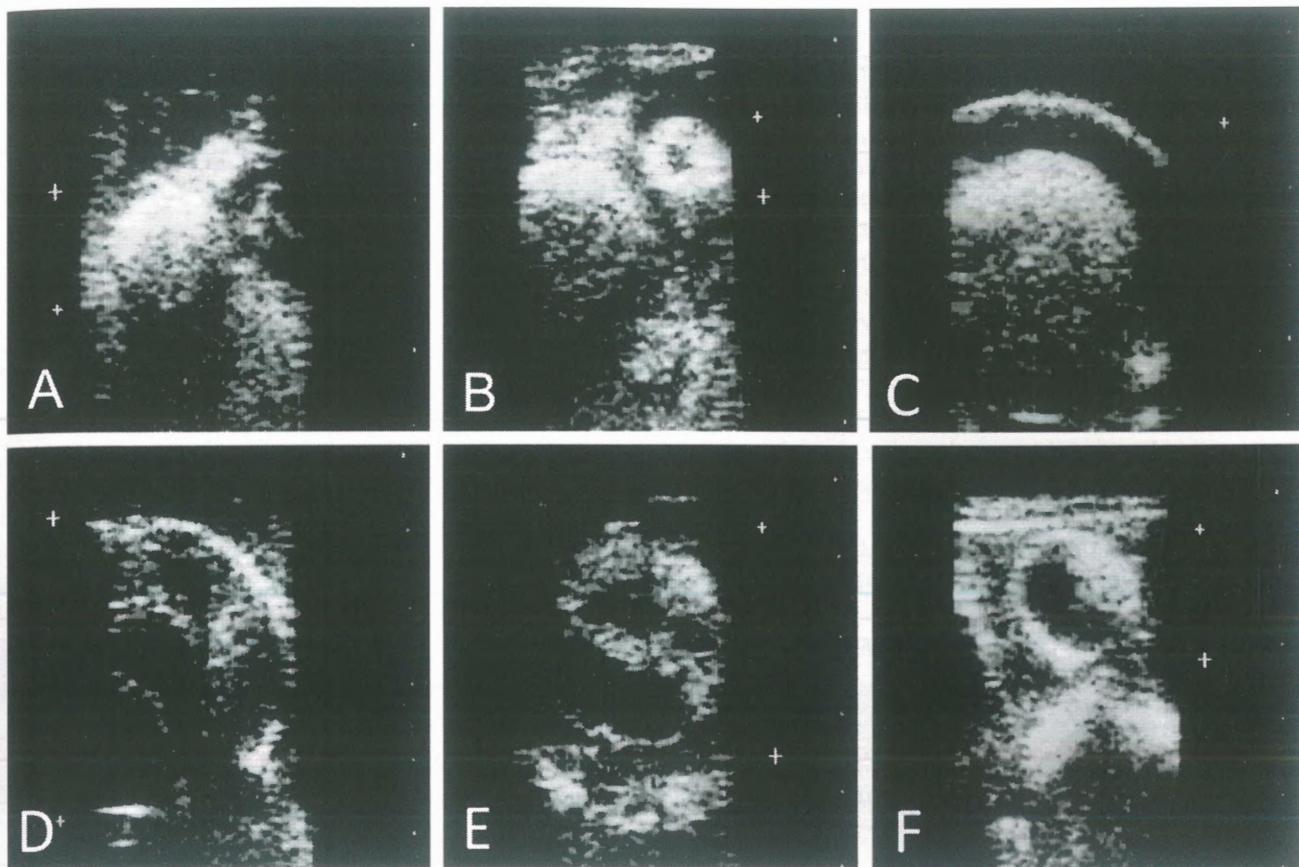


FIG. 3 Ultrasound images of reproductive organs of captive *A. yniphora* females in Ampijoroa, Madagascar. The points on the right sides of the photos indicate centimeters. (A) Vitellogenic ovarian follicles of 15 mm (center left) and 6 mm diameter (upper right), September 28, 1999. (B) Vitellogenic follicle of 22 mm (center left) and fresh corpus luteum of 10 mm diameter (upper right), January 29, 2002. (C) Soft-shelled egg in oviduct shortly after ovulation, December 10, 2002. (D) Hard-shelled egg in oviduct, February 15, 2002. (E) Preovulatory sized atretic follicle of 29 mm diameter, large anechoic caverns visible in yolk, June 9, 2001. (F) Late atretic follicle of 17 mm diameter, September 28, 1999.

et al., 1983). Laparoscopy has been used extensively over the last few decades, with the main downside being that only highly trained scientists can perform this surgery, as the death of the turtle can occur if not performed correctly (Owens, 1999). Ultrasonography can replace the techniques described earlier in many instances, as it allows researchers to visualize and measure follicles and eggs and identify follicular atresia (Fig. 3) (e.g., Kuchling, 1989a; Kuchling & Bradshaw, 1993; Kuchling & Razandrimamilafinirivo, 1999; Rostal et al., 1997, 1990; Rostal, Owens, et al., 1998; Shelby et al., 2000), as well as testes and epididymides of adult male turtles (Fig. 4) (Blanvillain et al., 2008; Lee, 2003; Pease et al., 2010). This technique has the advantage of being noninvasive; however, it usually does not allow the visualization of the corpus luteum, and therefore researchers cannot know if a female has laid a clutch of eggs in the recent past. In addition, ultrasonography cannot distinguish the inactive male gonadal structures. Finally, histology of the gonads is still performed today as it can answer basic questions on a male's reproductive status and spermatogenetic stages by



FIG. 4 Ultrasound examination of the gonads in an adult male loggerhead sea turtle (*Caretta caretta*).

looking at the cells lining the seminiferous tubules. This technique can be undertaken during endoscopy by taking a small biopsy sample (e.g., Blanvillain et al., 2008; Lee, 2003; Limpus, 1992; Plotkin et al., 1996; Wibbels et al., 1990).

2.2 Hormonal cycles

Hormonal cycles were first elucidated using direct methods to evaluate plasma hormone levels by RIA (radioimmunoassay) or ELISA (enzyme-linked immunosorbent assay). With these methods, hormonal cycles of steroids (testosterone, T; estrone, E₁; estradiol, E₂; progesterone, P₄; and corticosterone, CORT), and gonadotropins, GTHs, (follicle-stimulating hormone, FSH, and luteinizing hormone, LH) can be evaluated using a simple blood sample and relatively straightforward laboratory techniques. Nowadays, ELISA kits are available to analyze most of the sex hormones, without the downside of using radioactive chemicals. The relatively large size of some turtles including sea turtles and large tortoises has made it somewhat easier to obtain repeated blood samples (with a larger volume) compared to smaller reptiles (Jacobson et al., 1992; Owens & Ruiz, 1980). This fact has provided a distinct advantage to those interested in studying the regulatory physiology of reptiles. On the other hand, turtles can be very hard to find at times, especially during aestivation or hibernation when they understandably are less well studied.

3 DIFFERENT TYPES OF CYCLES AND TIMING OF REPRODUCTION IN TURTLES

3.1 Cyclicity

As Licht (1984) discusses most convincingly, there is strong evidence of seasonal breeding in reptiles, especially turtles. Even the tropical sea turtle species, in which nesting can occur in all months, have a clear peak of nesting during specific seasons. Most importantly, each individual has a very clear seasonal reproductive cycle.

3.1.1 Prenuptial and postnuptial reproductive cycles

One of the most useful paradigms developed for reptilian reproductive cycle descriptions is that originally described by Volsoe on *Vipera berus* (in Saint Girons, 1963) who noticed two primary patterns, which he called prenuptial and postnuptial. These two patterns are based on the timing of spermatogenesis (peak testis weight) and ovulation relative to the mating season (Fig. 5). The prenuptial pattern (type I pattern of Lance, 1998), which is more typically seen in tropical and subtropical breeding turtles, is characterized by a steady and somewhat synchronized development of the ovary and testis continuing through the winter months (or dry season) and culminating with maximal sperm production and epididymal recrudescence just prior to the spring mating period and simultaneously to the initiation of a single or multiple ovulation and nesting sequence(s) in

the female. In terms of parsimony, this would be the simpler and more basic cyclic form. One can envision the more complicated and derived condition in the postnuptial cycle (type II pattern of Lance, 1998) of more temperate regions in which testicular and gonadal growth commence full scale in the late summer (or late rainy season) and move to full gonadal development in the late fall at which time cold weather, or other extreme climatic conditions, cause a full interruption in the sequence. Instead of continuing to breed, these species then go into hibernation or aestivation for up to several months. Then, in the spring under warming or hydrating conditions, these fully developed or nearly fully recrudesced individuals emerge and soon begin their courtship and mating phase to permit nesting as early as possible in the spring. After the completion of the reproductive phase, the postnuptial cyclers begin an intense foraging period to permit nutritional accumulations for full gonadal growth in the late fall prior to the next hibernation. The critical point is that the postnuptial species must be able to store their gonadal products (sperm and ova) through the hibernation or aestivation period. Essentially, however, the regrowth phase after the breeding season starts off again in a similar way in both types of cycles.

This dichotomous categorization is very useful but many variations exist. For example, some species of reptiles have been shown to commence spermatogenesis/vitellogenesis in the late summer/fall, but complete gamete production (spermiation, preovulatory follicles) only in spring of the following year. This has been termed a "mixed" cycle or combination of prenuptial and postnuptial patterns (Saint Girons, 1963). In this case, mixed and prenuptial cycles only differ through the interruption of the cycle by winter hibernation in the first instance. One other pattern is that many species of turtles from temperate climates breed both in the fall and the spring, and their testicular cycle could be described as both prenuptial and postnuptial (Licht, 1984).

3.1.2 Sexual maturation

Turtles require years or decades to reach sexual maturity. Good foraging opportunities and an optimal temperature history will minimize the time required to reach sexual maturity. For example, in captive green sea turtles (normally herbivores) fed a high protein diet, sexual maturity was reached in 8–9 years (Wood & Wood, 1980) compared to 20–40 years in the wild (Balazs & Chaloupka, 2004; Seminoff et al., 2002). These captive conditions would appear to reflect the maximum reproductive potential for the species. Similarly, in poor foraging habitats or poor forage years, or when climatic conditions are suboptimal, like during El Niño Southern Oscillation events (associated with periods of warmer waters, therefore less productive), sea turtles will be delayed in maturation or skip a year or

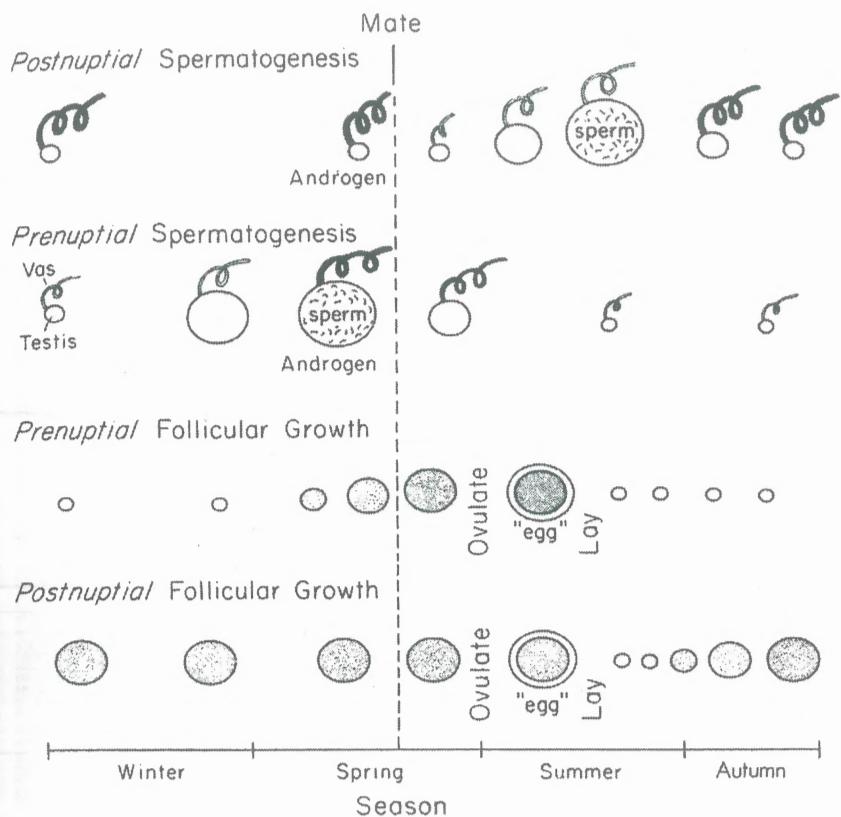


FIG. 5 Schematic description of the two types of gonadal cycles in turtles based on gametogenesis and mating. Reproduced from Licht, P. (1984). Reptiles. In G.E. Lamming (Ed.), Marshall's physiology of reproduction (pp. 206–282). Churchill Livingstone, Edinburgh, with permission.

more of reproduction as adults (Chaloupka, 2001; Limpus & Nicholls, 1988; Saba et al., 2007; Solow et al., 2002).

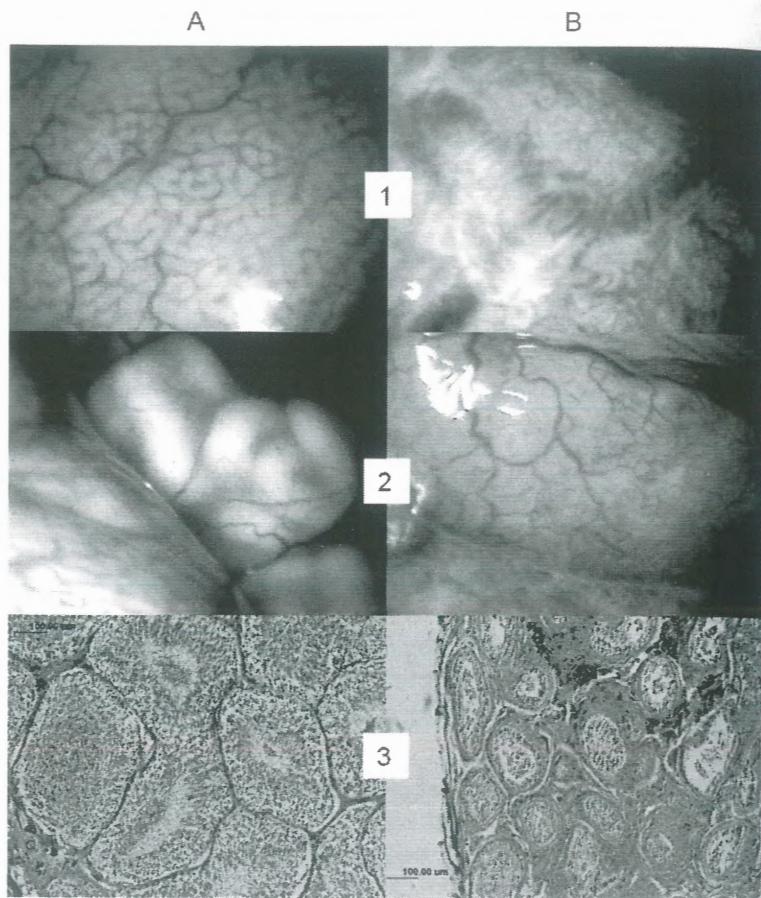
3.1.3 Nesting cyclicity

Most turtles appear capable of annual breeding when in appropriate habitats and under ideal environmental conditions. In particular, most cryptodire and pleurodire freshwater turtles (except wild *Erymnochelys madagascariensis* and *Carettochelys insculpta* in which females show biennial ovarian cycles as described later), and tortoises seem to breed annually. Interestingly, some adult females may not reproduce in a given year (see references in Kuchling, 1999; Miller & Dinkelacker, 2008). However, as Kuchling (1999) discusses, this may not reflect a multi-annual cycle, but rather the capability for the females to skip reproduction if the environmental conditions are suboptimal, or even to abort ovulation and egg production despite showing a normal vitellogenic cycle, as in the western Australian freshwater turtle, *Pseudemydura umbrina* (Kuchling & Bradshaw, 1993).

Female sea turtles of the genus *Lepidochelys* (the ridleys) often breed annually (an average of 1.8 years for the kemp's ridley) (Tripathy & Pandav, 2008; Witzell et al., 2005), whereas female sea turtles of the other genera will normally reproduce every 2–4 years. With sea turtles, as Kuchling

(1999) discusses, “capital” breeding in the sense of Drent and Daan (1980) is the basic plan: when the animal has gained enough nutritional reserves (fat probably) to allow both the energetically demanding migration and a full reproductive series, then and only then do they initiate the reproductive migration. In essence, selection must have favored an all or no reproductive effort for these migratory capital breeders. In contrast, the female giant Aldabra tortoise *Aldabrachelys gigantea* could be described as an “income” breeder (Kuchling, 1999) in that she will simply reduce the number of eggs she is producing for the season to compensate for a poor foraging year. As Kuchling further notes, these two species, the green sea turtle and the Aldabra tortoise, represent the two extremes of capital and income breeders with all other turtles showing intermediate adaptations tailored to their historic environments. Interestingly, adult male sea turtles often show annual reproductive cycles (thus much shorter than those of females) (Hays et al., 2022); however, the presence of a small number of reproductively inactive males, sometimes even remaining near the mating grounds, has been noted in a few studies (Blanvillain et al., 2008; Jessop et al., 2004; Limpus, 1993; Wibbels et al., 1987). Limpus (1993) determined the remigration interval of adult male *C. mydas* to be 2.08 years (range: 1–5 years), whereas 10%–15% of adult

FIG. 6 Laparoscopic (1-testis, 2-epididymis) and histological images (3) of reproductively active (A) and inactive (B) adult male loggerhead sea turtles (*Caretta caretta*) collected in Cape Canaveral, Florida. Reproduced from Blanvillain, G., Pease, A. P., Segars, A. L., Rostal, D. C., Richards, A. J., & Owens, D. W. (2008). Comparing methods for the assessment of reproductive activity in adult male loggerhead sea turtles *Caretta caretta* at Cape Canaveral, Florida. *Endangered Species Research*, 6, 75–85, with permission.



male *C. caretta* captured in the Cape Canaveral shipping channel were found to be inactive in 2 separate studies (Blanvillain et al., 2008; Wibbels et al., 1987). These males, captured during the mating season, showed basal T levels, regressed epididymides, and inactive testes based on laparoscopy and/or histology (Fig. 6). Moreover, it appears that inactive males do not show the soft plastra exhibited by reproductively active males of several sea turtle species during the breeding season (Fig. 7) (Blanvillain et al., 2008). In contrast to adult males *C. mydas* and *C. caretta*, the single study on the reproductive physiology of adult male *Eretmochelys imbricata* in Australia reveals that Hawksbill males might reproduce annually (Jessop et al., 2004), although this study may not have sampled sufficient numbers of males who might have remained in deeper water foraging grounds. Clearly, more studies are needed on the questions of the male's mating cyclicity.

The arribada, or synchronous nesting behavior, is well documented in the two ridley sea turtle species (Plotkin, 2007). Thousands or even hundreds of thousands of individuals have been known to nest at specific mainland beaches in brief periods of from a few hours to a few days. The most endangered sea turtle, *Lepidochelys kempii*, averages about

three nests per season, nests synchronously in the daytime, and breeds primarily in the western Gulf of Mexico (Rostal, 2007). In contrast, the sister species, the olive ridley (*Lepidochelys olivacea*), is the most abundant turtle on the planet, averages about two clutches per season, nests synchronously at night only, and breeds in the rest of the tropical oceans except for the Gulf of Mexico (Plotkin, 2007). While the overall organization of the arribada does not appear to be socially coordinated (for review see Bernardo & Plotkin, 2007), the way females seem to resist the stress of high-density interaction while the massing is occurring certainly suggests a physiological facilitation of the unusual behavior through the hypothalamus-pituitary-adrenal (HPA) axis (Valverde et al., 2007). Sea turtles typically ovulate the next clutch of follicles within hours of nesting (Owens & Morris, 1985). What is unique about the ridleys is that they will prepare the next clutch of eggs in the typical 10- to 14-day cycle as do other sea turtles and they do occasionally nest on this "short cycle"; however, most ridleys typically wait for about a month for another lunar/tidally cued arribada event (Bernardo & Plotkin, 2007). They usually hold the fully calcified eggs in the oviducts for an extra 2 weeks for the next arribada. Plotkin et al.

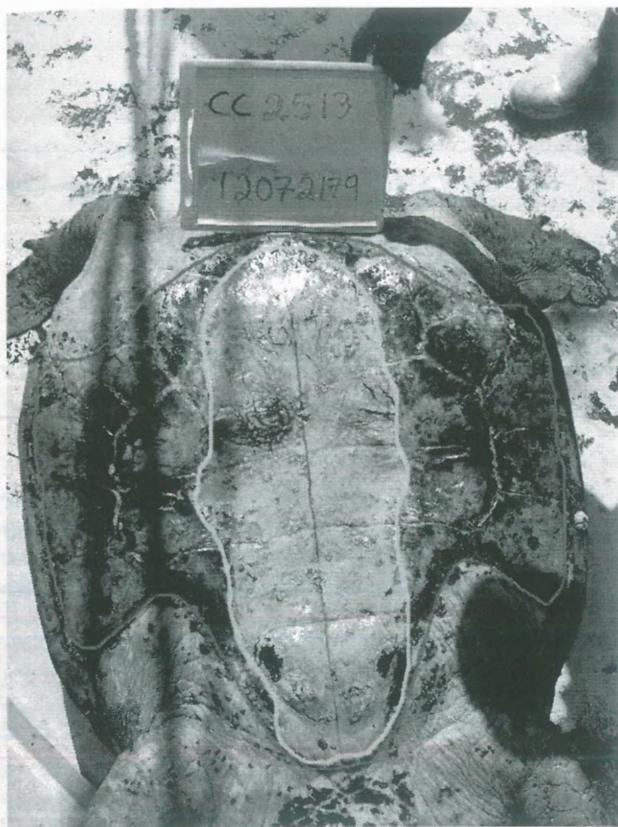


FIG. 7 Soft plastron in a reproductively active male *C. caretta* with contour lines from the PLASTron software, as described in Blanvillain et al. (2008). Reproduced from Blanvillain, G., Pease, A. P., Segars, A. L., Rostal, D. C., Richards, A. J., & Owens, D. W. (2008). Comparing methods for the assessment of reproductive activity in adult male loggerhead sea turtles *Caretta caretta* at Cape Canaveral, Florida. Endangered Species Research, 6, 75–85, with permission.

(1997) documented a surprising but apparently not unusual event when a heavy multiday rain (a possible natural stress-inducing event) occurred at Nancite in Costa Rica in which large numbers (thousands) of olive ridleys that were physiologically prepared to nest were turned away by the rains only to return after a second lunar cycle to nest synchronously and successfully after a full 60-day waiting period. The eggs were successfully retained in the oviducts for more than 50 days.

Other species of turtles are known to exhibit aggregative nesting. For example, the Amazon River turtle *P. expansa* has been shown to nest in groups of about 20 individuals, for a total of 200–500 individuals nesting on the beach in one night (Alho & Pádua, 1982). These turtles (both adult males and females) also exhibit reproductive migrations from lakes to rivers once the water level of the rivers has declined enough to expose sandy beaches on the riverbanks used as nesting grounds. This distinct aggregative behavior is not displayed by the other five species (much smaller) of *Podocnemis* from South America, and it has been

hypothesized that the unique nesting pattern of *P. expansa* could be due to their large size and density (at least in the past), resulting in few adequate nesting beaches and group nesting sites (Pritchard & Trebbau, 1984). Another explanation could be the predator swamping effect as originally described in olive ridley turtles (Bernardo & Plotkin, 2007; Eckrich & Owens, 1995). Historic records of large Asian river turtles (*Batagur baska*, *Batagur trivittata*) also report aggregative nesting events on sandbanks (Maxwell, 1904), but today these populations as well as olive ridleys of the region are extirpated or too depleted for any mass nesting to take place (Hykle et al., 2020).

3.1.4 Variation in the number of clutches

The number of clutches deposited per season varies greatly depending on the species. Some species are known to only lay one clutch, as in the common snapping turtle *Chelydra serpentina* (White & Murphy, 1973), the painted turtle *Chrysemys picta* (Gibbons, 1968), the desert and gopher tortoises *Gopherus agassizii* (Rostal et al., 1994) and *Gopherus polyphemus* (Landers et al., 1980), the Australian snapping turtles *Elseya dentata* (Kennett, 1999) and even the Amazon river turtle *P. expansa*, despite its large size (Alho & Pádua, 1982). The sea turtles, however, are all known to deposit from 2 (in *L. olivacea*) to up to a rare high of 10 clutches (in *Dermochelys coriacea*) with a renesting interval varying from 9 to 10 days (in *D. coriacea*) to a typical 30 days and a maximum of 66 days (in *L. olivacea arribada*) (Kalb, 1999; Plotkin et al., 1997; Reina et al., 2002; Rostal et al., 2001; Tripathy & Pandav, 2008). Other species of turtles are multi-clutched (e.g., *Stenotherus odoratus*, *Testudo hermanni*, *Testudo horsfieldii*, *Geochelone nigra*, discussed later); however, for many additional species, we simply don't know their nesting patterns in the wild, as it requires long-term mark-recapture or ultrasound studies, which are difficult to implement in many places.

3.2 Timing of reproduction

The timing of mating, mating receptivity, and the effectiveness of individual mounting activities are not at all well understood in turtles (Kuchling, 1999). In wild green sea turtles, Booth and Peters (1972) described a very definite “receptivity” period of female choice on Wreck Island off the Great Barrier Reef. Similarly, Crowell Comuzzie and Owens (1990), in captive green turtles, present more quantitative evidence of a distinct receptive period about 30 days prior to the first nesting, with females permitting no additional mating after this time despite the multiple clutch nesting sequence that follows. Generally speaking, spring mating, whether prenuptial or postnuptial, is well documented, with some species showing both fall and spring mating. The musk turtle (*S. odoratus*) and the desert and gopher tortoise (*G. agassizii* and *G. polyphemus*), discussed

later, seem to mate primarily in the fall (Lance & Rostal, 2002; McPherson & Marion, 1981; Mendonca & Licht, 1986; Ott et al., 2000). However, in the case of the diamondback terrapin (*Malaclemys terrapin centrata*), Estep (2005) showed a pronounced period of courtship in the spring with a much shorter and presumably less effective period in the fall. This, and similar observations of shorter fall courtship episodes, does raise the important question of the actual significance or functionality of the fall mating period, described in many turtle species. Gist et al. (1990) showed that insemination in *C. picta* occurred only during fall mating as sperm in the oviducts were recovered only during October, and electroejaculation in the males yielded sperm only during the fall. Therefore, it appears that spermatozoa can be stored in the female reproductive tract until the following spring when ovulation occurs (see Chapter 5, this volume). Finally, a few turtles have their peak mating activity in winter, e.g., *Mauremys rivulata* in Israel (Gasith & Sidis, 1985) and *Chelodina oblonga* and *Pseudemydura umbrina* in southwestern Australia (Kuchling, 1999). Since turtles have the potential to store spermatozoa in the male as well as in the female reproductive tract and, in many species, to mate at any time of the year, it would be more precise to use the time of ovulation and fertilization, rather than the time of mating and insemination, as defining parameters to differentiate between prenuptial and post-nuptial gonadal cycles.

Mating-induced ovulation has so far been described in two species: following copulations in the pre-breeding season, critically endangered female Plowshare tortoises *Astochelys yniphora* in a breeding program in Madagascar, produced several clutches of eggs over a 5- to 7-month nesting period without any additional inseminations. Ultrasound scanning demonstrated that vitellogenesis continued throughout the breeding season in both females paired with males during the mating season and in females, who were not paired with males, but no ovulations occurred in the latter and the preovulatory follicles were slowly reabsorbed through atresia (see Fig. 3). Females not paired with males showed cycles of vitellogenesis without ovulation, but concurrent atresia, over several consecutive years (Kuchling & Razandrimamafiniarivo, 1999). Ultrasound scanning of two captive female Loggerhead sea turtles *Caretta caretta* in Florida also suggested mating-induced ovulation (Manire et al., 2008). Mating-induced ovulation may still be underreported in tortoises and turtles because the effect of female exposure to males and mating per se has only rarely been studied and reported. It is nearly impossible to study this reproductive trait in wild populations in the field.

3.2.1 Nesting phenology and climate change

By far, the dominant biological and conservation questions about turtle reproduction over the past decade have involved

evaluating whether turtle species can survive the era of rapid anthropogenic climate change our planet is experiencing (Byer et al., 2020; Patrício et al., 2021). On one hand, turtles as a very diverse group of boxed-in-animals have survived splendidly innumerable environmental challenges over at least 200+ million years. On the other hand, because they are long lived, with long generation times, they may not be able to respond in evolutionary terms to the much more rapid pace of the current climate challenge. Most turtles have temperature-dependent sex determination (TSD) as their sex-determining adaptation (see Chapter 1). Warmer incubation temperatures produce females and cooler temperatures produce males. Because of global warming, the sex ratios of many populations around the world are currently being skewed toward females. For example, the largest green sea turtle nesting population in the world at Raine Island in Australia is now producing nearly all females (Jensen et al., 2018).

Many current studies have been designed to determine if turtles have sufficient behavioral and physiological plasticity to shift their reproductive cycles to cooler latitudes or cooler seasons (e.g., earlier seasonal breeding) to produce adequate males. While some adaptive flexibility has been observed in responding to temperature fluctuations (Byer et al., 2020; Livesley et al., 2022), models of migratory or phenological shifts for turtles do not support such adaptations as adequate to overcome climate change over the long-term (Laloë & Hays, 2023; Monsinjona et al., 2019). Also arguing against the ease of such adaptational shifts over the short- and long-terms in turtles is their tendency to imprint to specific breeding sites, which reduces population vagility (Levasseur et al., 2019; Roden et al., 2023). Another concern with turtles is their common use of photoperiod for seasonal cues (see Section 5.2 on the pineal gland), which may dampen the behavioral changes that might be expected from increasing environmental temperatures caused by climate change. Related to this possibility, in a recent study using female *Chrysemys picta*, Topping and Valenzuela (2023) found the lengthening photoperiod in spring to coincide with increased circulating E₂. There are two reasons why this observation could reduce the phenotypic plasticity and thus adaptability of turtles to climate change scenarios. First, increased maternal E₂ is known to increase the number of females produced in clutches (Crews et al., 1991) and second, increased nest temperatures have been shown to reduce hatchling viability at high temperatures (Booth, 2017; Fisher et al., 2014).

Despite rather dire predictions for the potential extinction of TSD turtle species due to population feminization, several studies suggest continued turtle resiliency in the face of the overall crisis. For example, in multiannual nesting species males cycle reproductively more rapidly than females and in many species males mature developmentally

earlier than females. Thus, despite an overall greater number of females, the “operational sex ratio” is not as skewed as the actual sex ratio (Hays et al., 2022). One study using paternal genotype analysis even suggested a “strong male-biased operational sex ratio” in loggerheads (Lasala et al., 2013) where female-skewed sex ratios have been observed for decades (Wibbels et al., 1991). Arendt et al. (2021) present a long-term climate-based model for loggerheads suggesting that despite increased female primary sex ratios, males should remain adequate in numbers for decades to come. Additional studies by Leivesley et al. (2022) have documented nesting biology in the snapping turtle (*Chelydra serpentina*) for 50 years during a period of significant increases in environmental temperatures at the northern limits of the species distribution. Despite these environmental challenges, their results show a resilience in the primary sex ratio for the population, which has continued to produce males. They attribute this resiliency to considerable variability in the nesting behavior of the individuals of the species (Leivesley et al., 2022).

4 HORMONAL AND GONADAL REPRODUCTIVE CYCLES

4.1 Cryptodira

Cryptodire turtles represent the majority of living turtles (10 out of 13 families) and flex their neck in the vertical plane to pull it inside the shell between the shoulder girdles.

4.1.1 Freshwater turtles

The musk, or stinkpot turtle, *S. odoratus*, has been studied extensively and represents a good example of what typical gonadal and hormonal cycles look like for both male and female freshwater turtles (McPherson et al., 1982; McPherson & Marion, 1981, 1982; Mendonca & Licht, 1986). Musk turtles hibernate during the winter months, emerging in late February to early March, depending on the geographical location (with southern populations having a reduced period of hibernation). Spermatogenesis in males begins in April, and is maximal in August until early October, at which time testis weight is also maximal, and most mating occurs (throughout fall). Epididymal duct diameters are maximal in September during spermiation and testes then regress in late October. Levels of T and FSH (measured using an antibody derived from sea turtles) are higher from August until the end of October (Mendonca & Licht, 1986). Concentrations of these hormones were correlated throughout the entire year, but LH was undetectable. The peak of FSH and T in late summer and fall occurred concomitantly with spermatogenesis and spermiation, however, the peak of FSH occurred slightly earlier (in August) than that of T (prior to October when testes started regressing) (Fig. 8). Mendonca and Licht (1986) hypothesized that FSH was the primary GTH regulating androgen secretion and gonadal growth in turtles, although differences across species may exist. However, spermatogenesis started in the spring when FSH levels were

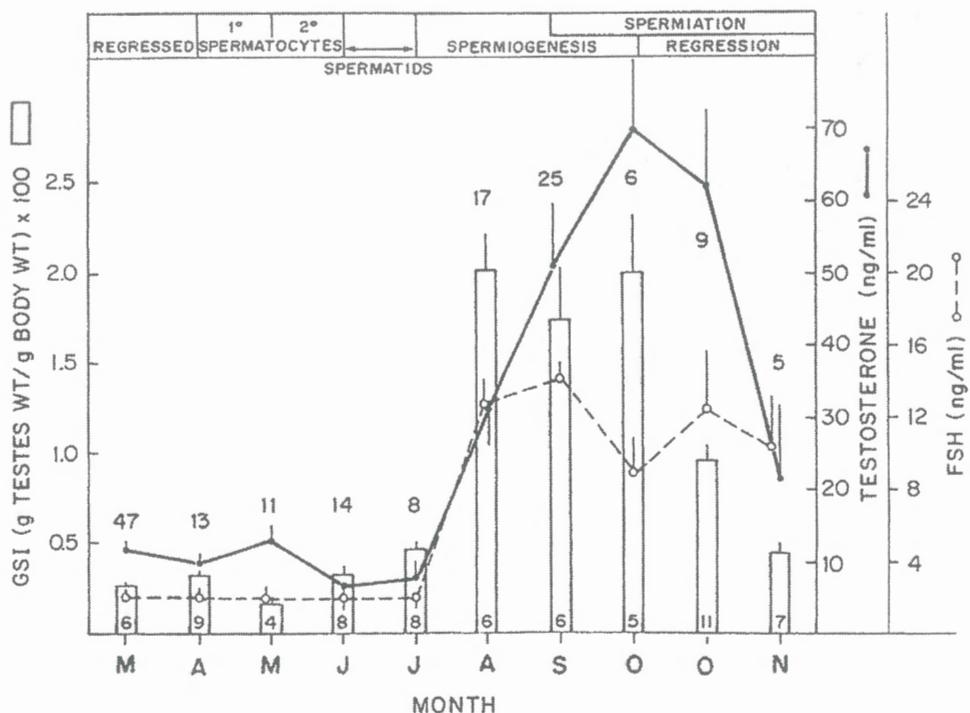


FIG. 8 Monthly mean levels of testicular gonadosomatic index (GSI) and plasma T and FSH for male musk turtles, *Sternotherus odoratus*, from South Carolina. Vertical lines are 1 standard error; numbers are sample sizes. Reproduced from Mendonca, M. T., & Licht, P. (1986). Seasonal cycles in gonadal activity and plasma GTH in the musk turtle, *Sternotherus odoratus*. General and Comparative Endocrinology, 62, 459–469.

low, suggesting that elevated FSH at lower temperatures is not necessary to stimulate spermatogenesis.

In *S. odoratus* females, LH and FSH (assayed by RIAs developed from *C. mydas*) remained undetectable throughout the year (Mendonca & Licht, 1986). Ovarian weights were high from March until July, and in November and December again, when preovulatory follicles were present. The first oviductal eggs were visible at the beginning of April until mid-July. In August and September, ovarian weight was low because females just finished laying their last clutches of eggs, and vitellogenesis started again in October–November. E₂ levels were high in the spring (and higher in females carrying eggs), during follicular growth of the next clutches of eggs, then decreased in July and remained low until November (Fig. 8). E₂ then increased in November during follicular growth for the next reproductive season (McPherson et al., 1982; Mendonca & Licht, 1986). This rise in E₂ stimulated the liver to synthesize vitellogenin (Vtg), a precursor protein of egg yolk, explaining the rise in ovarian weight at this time. Females hibernate with preovulatory follicles, which are ovulated in the next spring. T levels in females were highest in April just prior to ovulation of the first clutch of eggs and remained low thereafter, until vitellogenesis in the fall, at which time T increased again slightly (McPherson et al., 1982). One reason why T does not fluctuate with the next

nesting cycle (in this multiclutch turtle) could be that T, a known inhibitor of vitellogenesis (Ho et al., 1981), acts as a precursor for E₂, allowing vitellogenesis to proceed between clutches. P₄, on the other hand, increases during the periovulatory period and just after ovulation and then decreases quickly during the shelling of the eggs in the oviduct. This quick peak in P₄ is observed for each subsequent clutch of eggs and has been described in several other species of turtles (Fig. 9). P₄ levels also increased during the fall ovarian growth period. Ho et al. (1981) suggested that P₄ synthesized by the corpora lutea inhibits vitellogenesis, as no change in follicular size is usually observed during ovulation when P₄ levels are elevated. P₄ also may have a role in the final maturation of the follicles, the ovulation process (Callard et al., 1978), and the stimulation of enzyme production involved in follicular rupture (McPherson et al., 1982). This hormone possibly decreases myometrial contractility in the oviduct to retain the eggs so that normal shelling may occur (Callard & Hirsch, 1976; Giannoukos & Callard, 1996).

Much of this pattern of gonadal cycles remains constant for other species of freshwater turtles. Hormonal cycles, however, show considerable variations, and one notable difference is the existence of a second T peak in the spring for some male turtles, sometimes associated with mating, although the exact timing of the mating activity is often

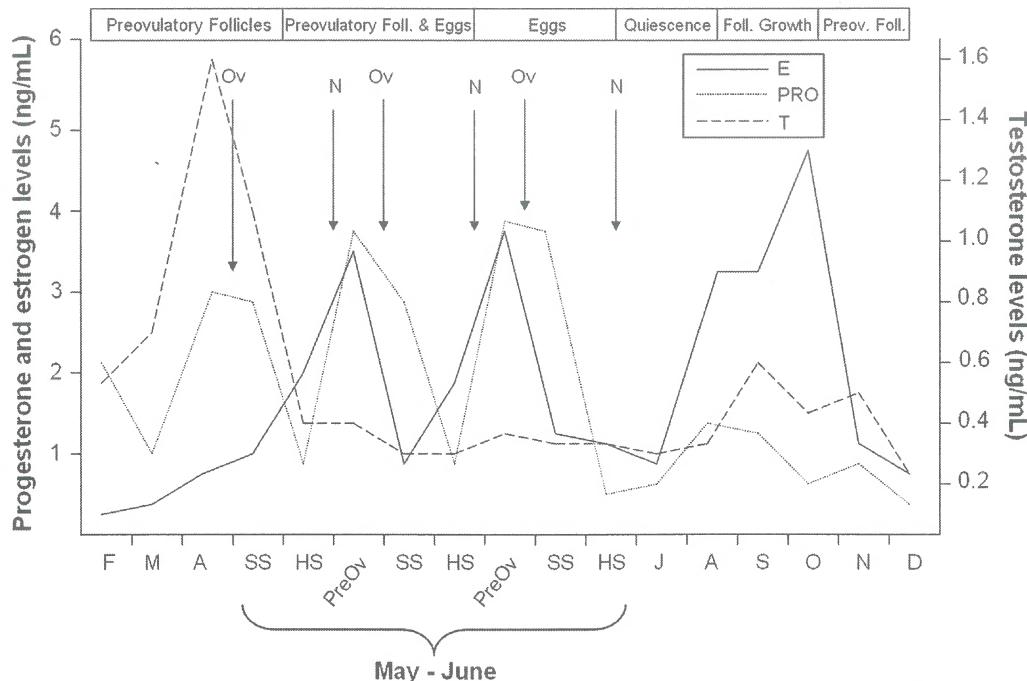


FIG. 9 Seasonal variation in sex steroid concentrations (testosterone, T; estradiol, E₂; and progesterone P₄, in ng/mL) of the female musk turtle, *Sternotherus odoratus* from Alabama. Three clutches are represented with ovulation (OV), and nesting (N) being hypothesized to occur around the time indicated by vertical arrows during the nesting season. SS = soft shelled eggs in the oviduct, HS = hard shelled eggs in oviduct, Preov = preovulatory follicles but no eggs in oviduct. Modified from McPherson, R. J., Boots, L. R., MacGregor III, R., & Marion, K. R. (1982). Steroids associated with seasonal reproductive changes in a multiclutched freshwater turtle, *Sternotherus odoratus*. General and Comparative Endocrinology, 48, 440–451.

not well known or described. This was first shown in the painted turtle, *C. picta* (Callard et al., 1976; Licht, Breitenbach, et al., 1985), and later on in the common snapping turtle, *C. serpentina* (Mahmoud et al., 1985). As in the musk turtle, FSH and T levels in *C. picta* are highly correlated and are low in animals captured just after coming out of hibernation. However, FSH and T rise quickly in April, coinciding with mating activity and a period of gonadal quiescence (Licht, Breitenbach, et al., 1985). These authors suggested that water and air temperatures might still be too low at this time to induce testicular growth and androgen secretion, even though FSH levels are elevated. As in the musk turtle, FSH and T also peak in the fall (but not as high as in the spring), just after summer testicular recrudescence is maximal. The fact that T levels are at their highest during a time of gonadal quiescence (in the spring) and after the peak of spermatogenesis (in summer) differs from *S. odoratus* (and many other turtle species) as discussed by Kuchling (1999). Spermatogenesis in *C. picta* is described as being postnuptial (spermatozoa are produced in the fall and stored over the winter until the spring mating season) with high levels of T possibly associated with mating activity. However, many species exhibit mating activity when T levels are low including *Chrysemys dorsigni* (Silva et al., 1984), *Graptemys flavimaculata* (Shelby et al., 2000), and *G. agassizii* for the spring mating period (Rostal et al., 1994, see under “terrestrial turtles”). Female painted turtles and common snapping turtles (single clutch turtles) have very similar hormonal and gonadal cycles to those of musk turtles (multiclutch turtle): high but transient levels of T, E₂ and P₄ just prior to ovulation in the spring, and smaller peaks of E₂ and T during vitellogenesis in the fall (Callard et al., 1978; Gibbons, 1968; Mahmoud & Licht, 1997). Gapp et al. (1979) also detected peaks of Vtg in the spring and the fall concurrent with elevated steroid hormones in *C. picta*. However, other species show variations in this general pattern. For example, females of *G. flavimaculata* undergo vitellogenesis in the spring when a small rise in E₂ is visible, and not in the fall (Shelby et al., 2000). In addition, T levels did not seem related to other steroid hormones throughout the study period. Similarly, a single peak in E₂ was measured in *Lissemys punctata punctata* during the final growth of ovarian follicles (late summer), in preovulatory females, during the mating season (Sarkar et al., 1996).

The diamondback terrapin *M. t. centrata* is the only truly obligatory estuarine turtle. They range widely from near tropical areas in the southern Florida Keys and Bermuda to high-temperate regions in Massachusetts (Brennessel, 2006). In general, their reproductive patterns and endocrinology are very similar to the other emydids with whom they are closely related (postnuptial) (Lee, 2003), although there is some inconsistency in the literature on the question of multiple clutches in some of the subspecies.

4.1.2 Sea turtles

The reproductive physiology of Sea turtles has been studied extensively, both in the wild and in captivity; however, most of what we have learned is based on nesting females—due to their large size and ease of sampling during nesting events. In contrast, few studies of adult males exist, especially from the wild. The only species for which hormonal cycles of adult males have been investigated in the wild are the loggerhead, *Caretta caretta*, the hawksbill, *E. imbricata*, and the green sea turtle, *C. mydas* (Blanvillain et al., 2008; Jessop et al., 2004; Wibbels et al., 1987, 1990), while Kemp’s ridley, *L. kempii*, and *C. mydas* were studied in captivity at the Cayman Turtle Farm, Grand Cayman, B.W.I. (Licht et al., 1979; Licht, Wood, et al., 1985; Rostal, Owens, et al., 1998). No data on reproductive physiology of adult male leatherbacks, *D. coriacea*, olive ridleys, *Lepidochelys olivacea*, or flatbacks, *Natator depressus*, exist, and research should be carried out to better understand their reproductive physiology.

Typically, adult males display a prenuptial rise in T levels in which T rises in the fall, coincident with spermatogenesis and testicular recrudescence, peaks in the winter, and starts decreasing around the time of the reproductive migration and at the onset of mating in the spring (Fig. 10) (Licht, Wood, et al., 1985; Rostal, Owens, et al., 1998; Wibbels et al., 1987, 1990). Generally, male T levels have returned to baseline levels again during the female’s nesting period when the males are departing on the return migration to the foraging grounds (Rostal, Owens, et al., 1998; Wibbels et al., 1990). Inactive males, however, show much lower T levels (<5 ng/mL) and no peak in winter and spring, as seen in *C. mydas* from Australia (Jessop et al., 2004), and *C. caretta* from Cape Canaveral, Florida (Blanvillain et al., 2008; Wibbels et al., 1987). Interestingly, males of *E. imbricata* (Jessop et al., 2004) did not show the same T pattern as found in the other species of sea turtles, in that T levels were significantly higher in December (Austral spring/summer) when compared to August (Austral winter). Therefore, it appears that the prenuptial rise in T is not as clear in male hawksbills; however, the authors suggested that a delayed phase shift in the T cycle might take place, as the main nesting period usually occurs later in the season in *E. imbricata* (December–March, peak in January and February) when compared to *C. mydas* (peak in December and January) (Dobbs et al., 1999, 2007; Limpus et al., 2003). Nevertheless, samples were collected at only 2 time points and more work needs to be done to clearly understand adult male hawksbill reproductive cycles.

Unlike adult males, the reproductive physiology of adult female sea turtles, in particular during the nesting period, has been studied extensively, both in the wild and in captivity, and we have a fairly good understanding of the hormonal changes happening during the reproductive cycle

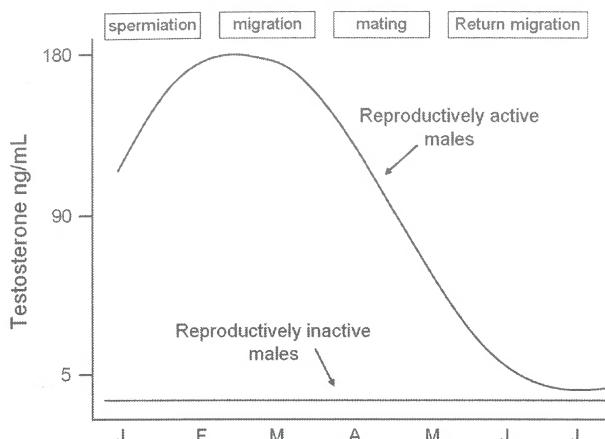


FIG. 10 Seasonal variation in T levels of reproductively active and inactive adult male loggerhead sea turtles, in relation to their reproductive behavior. Data from Wibbels, T., Owens, D. W., & Amoss, M. (1987). Seasonal changes in serum T of loggerhead sea turtles captured along the Atlantic coast of the United States. In W. Witzell (Ed.), Ecology of East Florida Sea turtles, (pp. 59–64). U.S. Dept. Commer., NOAA Tech., Rep. NMFS 53 and Blanvillain, G., Pease, A. P., Segars, A. L., Rostal, D. C., Richards, A. J., & Owens, D. W. (2008). Comparing methods for the assessment of reproductive activity in adult male loggerhead sea turtles *Caretta caretta* at Cape Canaveral, Florida. Endangered Species Research, 6, 75–85, with permission.

(Dobbs et al., 2007; Licht et al., 1982, 1979; Rostal et al., 1997, 2001, 1996; Rostal, Owens, et al., 1998; Whittier et al., 1997; Wibbels et al., 1990). Typically, T levels rise in the winter to reach a peak in March coinciding with migration and mating activity (Fig. 11). T levels then decrease slowly, following a stepwise progression, as the nesting season advances. Perhaps the granulosa cells of the follicles stop producing T every time a new set of follicles is ovulated, therefore explaining the sequential dips in T with each new ovulation (Fig. 11). E₂ levels tend to follow the same general pattern; however, the more gradual increase of this steroid hormone in the fall is correlated to long-term (several months) vitellogenesis. This was confirmed by ultrasound imaging of the growing follicles, as well as with the presence of elevated concentrations of serum calcium, indicative of vitellogenesis, from June to December. Free calcium ions readily bind to Vtg causing dissolution of bone and a rise in total circulating calcium whenever Vtg is present. Low, more constant levels of E₂ were observed during the nesting season (Rostal et al., 1997, 2001; Rostal, Owens, et al., 1998). Low levels of serum calcium during nesting also indicate that vitellogenesis might be essentially completed before females start laying their first clutch of eggs. However, other studies on *C. caretta* and *E. imbricata* have reported peak levels of E₂ only after the first or second clutches of eggs have been laid and this could suggest that vitellogenesis, at least at a lower rate, might continue into the nesting season, until all follicles are fully grown (Dobbs et al., 2007; Wibbels et al.,

1990). P₄ levels showed some contradictory results, possibly due to different sampling protocols. While *L. kempii* (Rostal et al., 1997), and *D. coriacea* (Rostal et al., 2001) showed highly variable levels of P₄ throughout the nesting period, and therefore no correlation between this hormone and the reproductive condition of the females, Whittier et al. (1997) in *C. caretta* and Rostal, Owens, et al. (1998) in *L. kempii* showed higher levels of P₄ at the beginning of the nesting season. Ikonomopoulou et al. (2014) provide the only study of nesting Australian flatbacks *N. depressus*, which they compared with a *C. mydas* colony breeding in Peninsula Malaysia. In a surprising suite of observations, they found estrone (E₁) and CORT much more dynamic and involved in (correlated to) the nesting behavior and physiology of the flatbacks than in the green turtles. Steroid profiles for the two species were readily distinguishable by discriminant analysis. E₁ has not been studied well in turtles. Ikonomopoulou et al. (2014) suggest that E₁ may have more involvement in the nesting and vitellogenesis of some species or populations than has been appreciated.

More detailed studies of hormonal changes around the time of ovulation—about 1–3 days following oviposition for the current clutch of eggs, until all clutches have been laid (Licht et al., 1982, 1979), showed a rapid surge in P₄ concentrations at the time of ovulation, rapidly returning to basal levels before shell deposition started (Licht et al., 1982; Wibbels et al., 1992). This surge was associated with similar transient high levels of LH in both studies and of T in *C. caretta* and *C. mydas* only (Fig. 11) (Wibbels et al., 1992). E₂ levels, on the other hand, were low and did not exhibit significant changes in both studies. However, in an earlier study in *C. mydas* where E₂ was measured in the middle of the internesting cycle at the Grand Cayman Turtle farm, E₂ significantly increased during this time compared to the actual time of nesting 4–6 days later (Lance et al., 1979). Interestingly, an FSH surge seen at the time of ovulation described in Wibbels et al. (1992) suggests a distinct function for this GTH in the slower and less dramatic stimulation of E₂ during the internesting period (Lance et al., 1979; Wibbels et al., 1992). As mentioned earlier, P₄, secreted by the corpora lutea after stimulation by LH from the pituitary, has been linked to the ovulation process by acting on the maturation of follicles and reducing oviductal movement during the shelling of the eggs. Owens and Morris (1985) also suggested that the P₄ surge at the time of ovulation may have an important role in oviductal albumin production as the fertilized ova are processed in the oviduct to form eggs. Indeed, the common phenomenon of “spacer” or “yolkless eggs” seen at the end of the leatherback and other sea turtle clutches is likely to be left over albumin being shelled as it follows the real clutch through the shelling region of the oviduct.

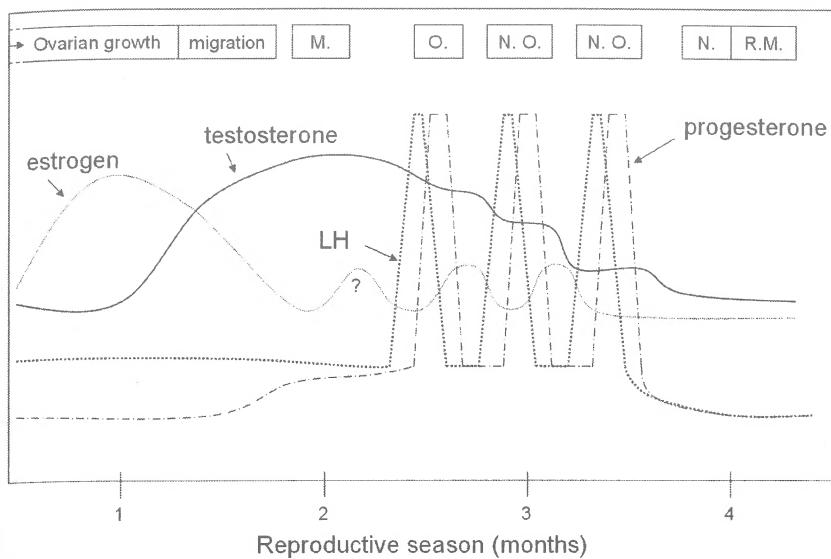


FIG. 11 The general model for endocrine fluctuations in adult female sea turtles in relation to their reproductive behavior. In this example, the female would ovulate and nest three times. M=mating, O=ovulation, N=nesting, RM=return migration. Modified from Owens, D. W. (1997). Hormones in the life history of sea turtles. In P. L. Lutz & J. A. Musick (Eds.), *The biology of sea turtles*, Vol. 1, (pp. 315–341). Boca Raton: CRC Press, with permission.

In addition, a short cycle or spike of both arginine vasotocin (AVT) and neuropephsin (NP) has been shown during the nesting period of the olive ridleys and loggerhead sea turtles by Figler et al. (1989) (Fig. 12). These hormones are secreted by the neurohypophysis and act to regulate oviductal contractions in sea turtles, and probably in other species of turtles as well. In this study, both AVT and NP increased drastically from the time of emergence from the ocean to reach a peak just prior to oviposition of the first egg. Levels of AVT then declined to low levels as the turtle returned to the water; however, NP levels stayed relatively elevated after oviposition possibly due to a longer half-life of the much larger protein. Similarly, levels of prostaglandin F (PGF) and prostaglandin E2 (PGE2) were measured at 9 different time points during nesting and oviposition in the loggerhead sea turtle (Guillette et al., 1991). Results showed an increase in both PGF and PGE₂ during nest digging reaching a peak during mid-oviposition for PGE₂ and nest covering for PGF. Concentrations returned to basal levels during body pit covering. The authors suggested that, as in other species of reptiles, AVT in turtles might induce the synthesis of PGF. However, the stimuli of release for both PGE₂ and AVT themselves are still unknown. Finally, Guillette et al. (1991) hypothesized that PGE₂ might be important for cloacal relaxation, whereas PGF, in concert with AVT, could stimulate oviductal contraction necessary for the expulsion of the eggs through the cloaca.

4.1.3 Terrestrial turtles (tortoises)

Tortoises (family Testudinidae) are found in most habitats from equatorial rainforests to hyperarid deserts to temperate

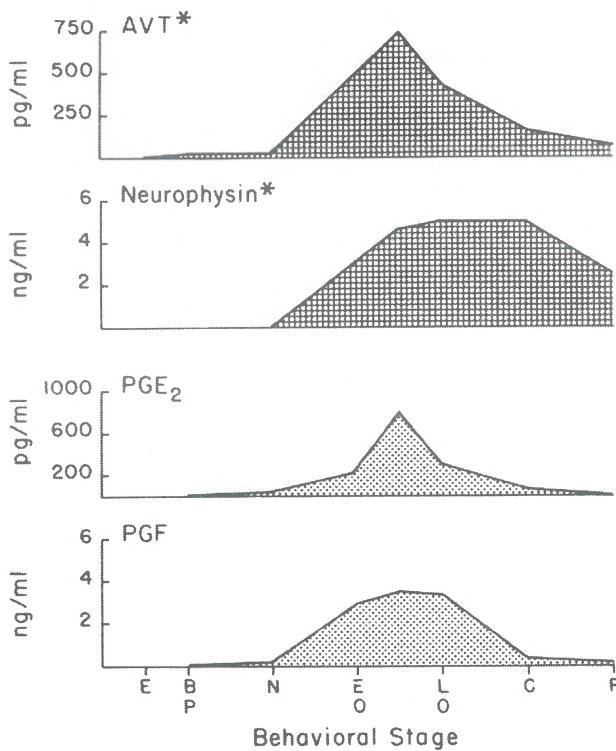


FIG. 12 Summary of relative changes in plasma arginine vasotocin (AVT), neuropephsin, prostaglandin F (PGF), and prostaglandin E₂ (PGE₂) in nesting female loggerhead sea turtles. E=emerging, BP=body pit construction, N=nest chamber construction, EO=early oviposition, LO=late oviposition, C=covering body pit, R=returning to the ocean.
* Data from Figler et al. (1989). Reproduced from Guillette, L. J. Jr., Bjorndal, K. A., Bolten, A. B., Gross, T. S., Palmer, B. D., Witherington, B. E., & Matter, J. M. (1991). Plasma E2-17 β , P4, prostaglandin F, and prostaglandin E2 concentrations during natural oviposition in the loggerhead turtle (*Caretta caretta*). General and Comparative Endocrinology, 82, 121–130.

regions and their reproductive cycles show a number of different patterns in response to the various environments and their specific challenges.

In the equatorial tropical Amazonian forest, the yellow-footed tortoise (*Chelonoidis denticulatus*) is capable of reproducing and nesting throughout the year, but egg laying still peaks (gravidity rate >50%) during the dryer season between May and September when river water levels drop, whereas the gravidity rate is <50% between October to April, which includes the high rainfall period January to April. It can thus be considered an opportunistic seasonal breeder (Mayor et al., 2023).

The equatorial Galápagos giant tortoise *Chelonoidis niger* has a well-defined nesting season and shows seasonality in their vitellogenetic cycle concerning the largest diameter of follicles, but vitellogenic follicles can be found all year round (Schramm et al., 2000). They exhibit a prenuptial cycle as presented by Rostal, Robeck, et al. (1998) for captive animals from Texas. In this scenario, T levels are high during the mating period (August–November) for both sexes, and the rise in T just prior to mating is thought to coincide with spermatogenesis in males. E₂ and calcium levels in females are highest in July, during which vitellogenesis occurs, as confirmed by ultrasonographic images of growing follicles. P₄ levels in females peaked in March and October, most likely linked to the ovulation of subsequent clutches of eggs. During nesting (November–April), T levels are low in both sexes. Schramm et al. (1999) confirmed this pattern using tortoises kept in seminatural conditions at the Charles Darwin Center in Santa Cruz, Galápagos. The mating season occurred from January through June, which corresponded to the hot and rainy season. CORT concentrations were correlated to T levels in both sexes.

Further to the south of the equator, on Aldabra island north of Madagascar, the Aldabra tortoise (*Aldabrachelys gigantea*) also shows clear seasonality in its egg production pattern: the nesting season lasts from June to September (dry season). The largest follicular diameters range from 5 to 10mm between July and November and from 25 to 46mm from January to June (Swingland & Coe, 1978), the later coinciding with the wet season and elevated plasma E₂ levels (Kummrow et al., 2020). Males show the highest plasma T concentrations during the mating season from January to April, followed by a nadir in August and September (Kummrow et al., 2020).

In the tropical subhumid climate of northwestern Madagascar, the plowshare tortoise *Astrochelys yniphora* has an extended but well-defined nesting season from mid-January to the end of May (throughout the wet season until the early dry season) during which individual females typically lay multiple clutches (Pedrono et al., 2001). In a captive colony close to its native range vitellogenic follicles are present

throughout the year, with follicles reaching preovulatory size prior to the mating season in November/January (Kuchling & Razandrimamilafiniarivo, 1999). Wild males show the highest plasma T levels from September to November. Plasma E₂ as well as P₄ in females show a slight elevation in November and reach the highest concentrations in January/February and the lowest in April. Overall plasma CORT levels do not differ between sexes and months (Currylow, Mandimbihasina, et al., 2017).

Combining the results of different investigations which only covered part of the year, *Astrochelys radiata* populations appear to show year-round vitellogenesis and egg production in the tropical arid climate of southern Madagascar with very low and often unpredictable rainfall. In a study using serial radiography to assess oviductal eggs in 12 *A. radiata* females in the far south of Madagascar from January to May 2000, gravid females could be found throughout the investigation period: seven females produced multiple clutches, one female was gravid only in January, two females were first gravid in May at the end of the study, and two females were never gravid during the January to May period (Leuteritz & Ravolanaivo, 2005). Pedrono (2008) reports nesting in southern Madagascar from late January to August. However, in an ultrasound scanning study of *A. radiata* at the Berenty Reserve in southern Madagascar in late September/early October 2001 oviductal eggs were found in 30% of *A. radiata* females while the others showed large vitellogenic and preovulatory follicles, including one which had just nested in late September. Thus, egg production and nesting can occur in September and October in southern Madagascar and, with good likelihood (based on large vitellogenic follicles in early October), also in November and December. Egg production and oviposition in *A. radiata* also occurs year-round in a captive breeding program in California (Kuchling et al., 2013). Based on the data of Leuteritz and Ravolanaivo (2005), individual females may still show discrete cycles of annual egg production (rather than continuous, acyclic egg production) that are, however, not synchronized in a population. A study of sex steroids in wild *A. radiata* from October to May found in males a single, gradual rise in T from October to January, whereas females showed a single peak of E₂ in January followed by a peak in P₄ in February and again in May. The authors concluded this pattern may have been associated with early nesting but not with peak or multiple nesting. A positive association was found between ground temperature and T concentration in males and E₂ in females, whereas ground humidity negatively affected T in males and positively P₄ concentration in females (Currylow, Rafeliarisoa, et al., 2017). The year-round egg production pattern of *A. radiata* populations appears to be based on desynchronized ovarian cycles of individual females. The year-round nesting in *A. radiata* populations could be an

adaptation to the low and unpredictable rainfall in the tropical arid climate of southern Madagascar (annual precipitation 310–460mm: Kuchling, 1989b). Much of the scarce precipitation in southern Madagascar comes in the form of unpredictable cyclonic rains which, basically, may occur at any time of the year. Spreading vitellogenesis and ovulations at the population level throughout the year may enable at least some *A. radiata* females to quickly exploit good environmental conditions, whenever they arise, through egg production and nesting.

More or less year-round egg production has also been found in the tortoise *Chersina angulata* in the arid Mediterranean climate of southwestern Africa. Females lay several, up to seven, clutches per year usually containing only single eggs. Ovulations occur from February (late summer) throughout fall and winter up to November (late spring) and individual females can have oviductal eggs for up to 11 months per year (Hofmeyr, 2004). The eggs laid during fall, winter, and early spring undergo diapause, and embryonic development beyond the gastrula stage only proceeds from late spring onward throughout summer, whereas eggs laid mid-to-late spring develop without undergoing diapause. Hatchlings emerge in early fall no matter when oviposition takes place (Kuchling & Hofmeyr, 2023). Eggs retained until early summer (December) can be oviposited with embryos advanced beyond the gastrula stage (Kuchling and Hofmeyr, 2023). In unusually hot summers or during droughts females can shift to facultative viviparity (or ovo-viviparity) by retaining the last egg clutch of the season until fall when these eggs are deposited with fully developed embryos ready to hatch (Kuchling & Hofmeyr, 2022). The hormonal regulations underlying this, for the order Testudines, novel reproductive strategy have unfortunately not yet been studied.

The smallest tortoise, *Chersobius signatus*, with a range embedded in that of the wider distributed *C. angulata*, also produces single egg clutches in extremely arid southwestern Africa, but only in spring (August–October; Loehr et al., 2004). Aridity and unpredictable rainfall determine which tortoises lay single egg clutches with taxa in these less predictable environments also producing larger eggs. *Chersobius signatus* produces the largest egg relative to the body size of any turtle, probably to enhance offspring survival in its harsh environment. These females produce rigid-shelled eggs larger than their pelvic canal and use pelvic kinesis to pass eggs at oviposition (Hofmeyr et al., 2005). *Psammobates tentorioides tentorioides* in the Karoo of southern Africa starts vitellogenesis in fall and females produce several small clutches (1–3 eggs) from spring to fall (Leuteritz & Hofmeyr, 2007).

Tortoises of the mostly Mediterranean genus *Testudo* and the North American genus *Gopherus* are also seasonal breeders, both ranging from temperate to subtropical areas

including deserts. The desert tortoise (*G. agassizii*) exhibits mating activity twice a year (in the spring and in the fall), and its cycle is described as being postnuptial, much like freshwater turtles such as *S. odoratus* (McPherson et al., 1982; Mendonca & Licht, 1986). Nesting typically occurs in the spring to early summer. In Rostal et al. (1994), the male testicular cycle was elucidated using sacrificed individuals. In May, the testes are regressed, then spermatogenesis progresses until it reaches a peak in October (prior to hibernation) when the seminiferous tubule diameters are maximal. Mature spermatozoa migrate into the epididymides prior to hibernation. Plasma T rises significantly from May to August to a peak of ~244 ng/mL, concomitantly to gonadal recrudescence, and declines prior to hibernation. Upon emergence from hibernation, T levels are relatively low (~18 ng/mL), despite the spring mating period. Female T levels rise from mid to late summer—during fall mating—to a presumed peak in April following emergence from hibernation and prior to ovulation. At this time, P₄ levels increase and then rapidly decline to baseline levels once the eggs are laid. Ultrasonography was used to investigate ovarian follicular growth in summer and fall, after nesting. Follicles had reached ovulatory size prior to hibernation, and shelled eggs were observed in the oviducts in mid-April. Nesting started in early May, after which atretic follicles were observed. E₂, lipids, and plasma calcium levels (indicative of Vtg) were high in the fall during follicular growth and started decreasing after emergence in April. Most females laid only one clutch. Similar hormonal and gonadal cycles were observed in a wild population of gopher tortoises (*G. polyphemus*) in the Southeast U.S. by Ott et al. (2000), with the only difference from *G. agassizii* being that mating occurred only during the fall, before hibernation. Oviposition typically occurred from April to July and females only laid one clutch, like most desert tortoises. In Hermann's tortoise *Testudo h. hermanni*, from Mediterranean Europe, spermatogenesis starts in April to May, just after hibernation and during the main mating season for the eastern subspecies (*Testudo h. boettgeri*) collected in the former Yugoslavia (Kuchling, 1982; Kuchling et al., 1981). Spermatogenesis continued through the summer, during which time T peaked in the eastern (August) as well as in the western (July/August) subspecies *T. h. hermanni* and declined in September and October (Huot-Daubremont et al., 2003). In the eastern subspecies, slightly elevated T was observed in April (compared to October/November), with lower levels in May and June prior to a steep rise in summer. In the western subspecies slightly elevated T was observed in March (compared to October), with lower levels in April, through June prior to a steep rise in summer. In both subspecies, the post-hibernation T increased to approximately the same levels as in September. The difference between the studies (post-hibernation spring elevation either in March or April) may reflect an earlier termination of hibernation in the west.

In both subspecies, the secondary post-hibernation spring peak does not seem to be directly related to mating: in Yugoslavia, the main mating activity in the wild occurred in May and June, with again a slight increase in September (Kuchling, 1982), whereas in France the main mating period for the western subspecies was in August/September when T levels peak. However, this mating pattern in France was observed in a semi-captive population (Huot-Daubremont & Grenot, 1997) and may have been skewed by a constant provision of water and food. During hibernation, testes are regressed, and spermatozoa are stored in the epididymides for the following mating season. T levels in females were high after hibernation, suggesting follicles are mature by April. T levels then fell to low levels in June and July during oviposition and rose again in August during renewed vitellogenesis and follicular growth. P₄ levels were highest during the periovulatory period, in April and May, and peaked prior to each nesting event (Huot-Daubremont et al., 2003).

In contrast to this somewhat general pattern, the Steppe's tortoise, *T. horsfieldii*, provides an excellent example of a tortoise that has to accomplish all of its tasks (i.e., feeding, mating, and nesting) in an extremely short amount of time (3 months) due to environmental and climatological constraints. Indeed, the Steppe's tortoise spends 9 months in dormancy underground during which spermatogenesis and vitellogenesis must occur. Lagarde et al. (2003) studied plasma T and P₄ cycles during the active period (mid-March to mid-June), and found that T levels in males were highest in March, just after hibernation and during the mating season. Brushko (1981) showed that sperm production occurs during aestivation (dormancy), in July–August,

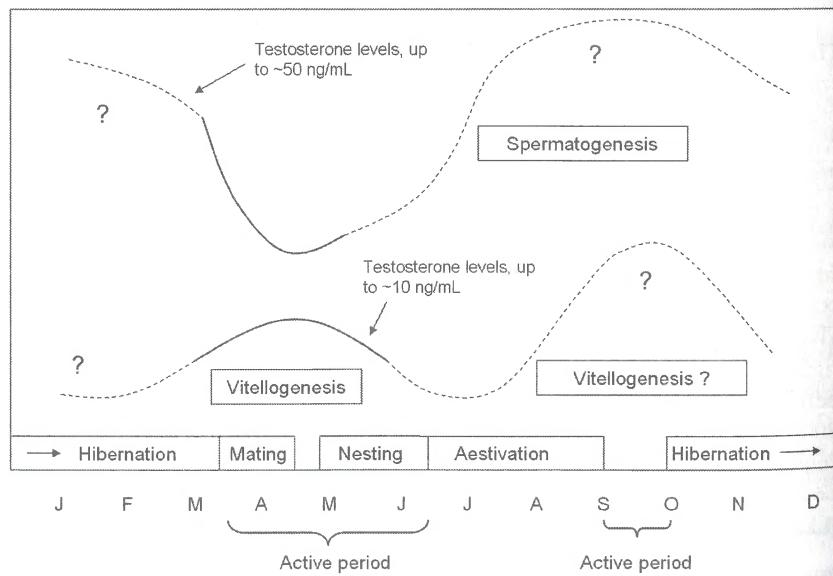
when tortoises are buried underground, and when peak levels of T might be expected. Female T and P₄ levels peaked in mid-April, prior to ovulation of the first clutches. Plasma calcium levels were higher in mid-April and May, indicative of vitellogenesis and/or egg production at this time. We suspect that vitellogenesis and ovarian growth starts during aestivation, in the summer, so that the females may have large follicles before hibernation. A summary of the hormonal pattern and hypothetical gonadal growth pattern in Steppe's tortoise is represented in Fig. 13.

In temperate climates, the North American eastern box turtle *Terrapene carolina carolina*, a terrestrial turtle of the family Emydidae, has an extended winter dormancy in its northern range and is active from April to October. During spring, females show elevated plasma T in April and May and lay a single annual clutch in late May or early June, followed by a peak in plasma Vtg and E₂ in July that correlates with the putative beginning of a new ovarian cycle. Courtship and mating occur throughout the activity period and males exhibit a dual peak of plasma T in April and August (Currylow et al., 2013), the later presumably corresponding to the period of peak spermatogenesis.

4.2 Pleurodires

Pleurodire turtles, also called “side-necked” turtles, represent an ancient group of turtles that originated in the late Triassic. They flex their neck in the horizontal plane to fold their neck and head sideways under the shell margin. Three families are extant and occur mainly in the Southern Hemisphere: the Chelidae in South America, Australia, and New Guinea, the Pelomedusidae in Africa south of the Sahara and on a

FIG. 13 Schematic representation of T levels and ovarian and testicular cycle in relation to behavior in the Steppe's tortoise *Testudo horsfieldii*. Plain lines represent T levels based on Lagarde et al. (2003), and dashed lines are hypothetical.



few islands in the Indian Ocean, and the Podocnemididae (with the former in the hyperfamily Pelomedusoides) in South America and Madagascar. Reproductive cycles of pleurodire turtles have been much less studied than those of cryptodire turtles; however, the limited data show some interesting differences compared to the cycles of a typical freshwater turtle of the Northern Hemisphere.

4.2.1 Chelidae

Gonadal and hormonal cycles of several species of side-necked turtles belonging to the family Chelidae have been studied in Australia, and in many cases they resemble closely those of cryptodire turtles as previously discussed. Legler (1985) defined a tropical reproductive pattern with nesting occurring during the cool dry season (fall and winter, generic groups of *Chelodina* [subgenus *Chelydera*] and *Elseya* [*dentata*]) and a temperate pattern with nesting during spring and summer (generic groups of *Chelodina* [subgenus *Chelodina*], *Myuchelys* and *Emydura*).

Male *Chelodina (Chelodina) steindachneri* and *Chelodina (Macrochelodina) oblonga*, two species of western Australian long-necked turtles, exhibit similar gonadal cycles (Kuchling, 1988a). Spermatogenesis begins in the Austral spring (September–October)—when T levels are low—and continues through the summer until June (late fall), at which time T slowly increases. Testes reach their maximal size in January and February for *C. steindachneri* (occurs mainly in temporary streams in an arid subtropical climate with unpredictable rainfall) and in April for *C. oblonga* (inhabits more permanent wetlands in Mediterranean and temperate climate) (Kuchling, 1988a). T levels in *C. oblonga* reach elevated levels in April and remain so until mid-July (Fig. 14) (Kuchling, 1999). Epididymides are maximally engorged with spermatozoa from May to November, and most mating occurs in the winter when T is low. In females of *C. oblonga*, follicular growth starts in late summer, when temperatures are declining, and follicles reach preovulatory size at the beginning of winter (May). Because this turtle does not hibernate during winter, follicles continue to grow and oviductal eggs, along with different-sized follicles, are found in the oviduct as early as September (in the spring). Several clutches of eggs are laid during spring and summer. In *C. steindachneri*, follicular growth starts in fall and progresses until spring. If water is available in early spring (September) ovulation occurs, but under dry conditions when turtles have to stay in aestivation, no further follicular development seems to take place until summer (Kuchling, 1988a). Rainfall and the availability of water are unpredictable, and ovulation and nesting may still take place after summer rains (mainly connected to cyclones) at least until January. On the other hand, if this turtle has to

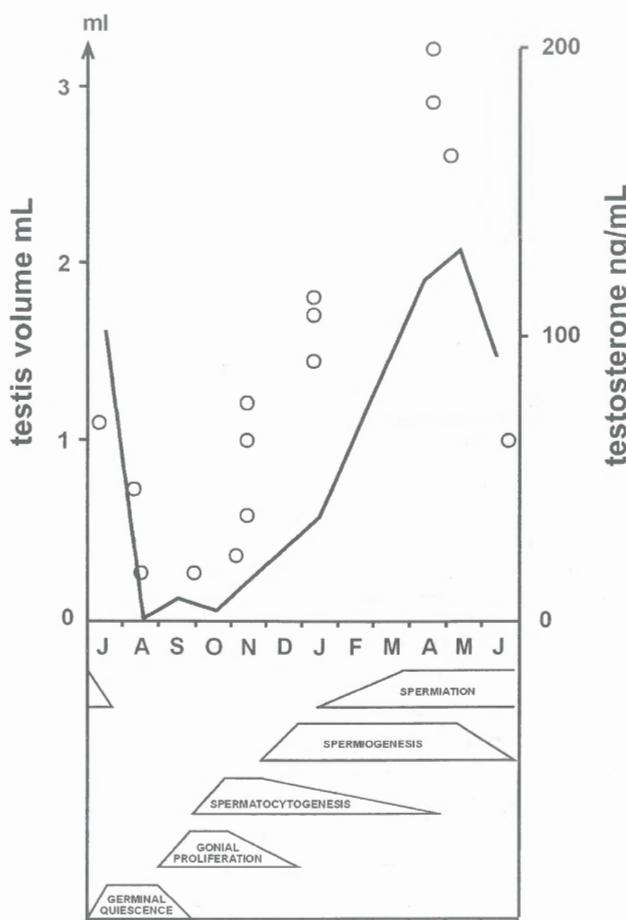


FIG. 14 Annual cycle of testis volume (open circles), spermatogenetic activity (after Kuchling, 1988a) and mean plasma T concentration (solid line, after Kuchling, 1999) in *Chelodina oblonga*. X-axis indicates the months of the year from July to June. Data from Kuchling, G. (1988a). *Gonadal cycles of the western Australian long-necked turtles Chelodina oblonga and Chelodina steindachneri (Chelonia: Chelidae)*. Records of the Western Australian Museum, 14, 189–198; Kuchling, G. (1999). *The reproductive biology of the Chelonia*. Zoophysiology, Vol. 38, Berlin Heidelberg New York: Springer-Verlag, with permission.

undergo aestivation due to the lack of rainfall while gravid, the oviductal period can be extended to several months until rainfall again allows nesting to occur (Kuchling, 1999). T levels rise in females during May and reach a peak in mid-July just prior to ovulation. Curiously, E₂ levels are low throughout the year and do not show the common periovulatory peak as seen in all other turtle species studied to date (Kuchling, 1999). This could be a problem relative to the laboratory technique employed to detect this hormone, or due to the possibility that very low levels are sufficient to stimulate vitellogenesis in this species. Moreover, the somewhat atypical ovarian cycle of *C. steindachneri*, with the possibility of a stable vitellogenic phase without follicular growth in late spring and summer, seems to be unique in the turtle world, along with an extremely reduced

period of ovarian regression. This might be the result of unpredictable environmental conditions pushing females to aestivate during dry periods and to have preovulatory follicles ready for long periods until water availability allows ovulation and nesting. The potentially long periods of aestivation along with the small size of this turtle might also explain why only one clutch of eggs is typically laid each year.

Female western swamp tortoises (*Pseudemydura umbrina*) from western Australia were studied by Kuchling and Bradshaw (1993) in an attempt to better understand their ovarian cycle and egg production. The ultimate goal was to improve captive breeding techniques to reintroduce this critically endangered species. Using both captive and wild caught tortoises, ultrasonography was effectively used to evaluate follicular growth and oviductal eggs over a period of approximately 4 years. Like many other chelonians, including other Australian chelids like *Emydura krefftii* (Georges, 1983), *C. oblonga* and *C. steindachneri* (Kuchling, 1988a) as discussed earlier, vitellogenesis starts in the summer, during the long aestivation period, and follicles continue to grow in the fall and winter, while the animals are active and feeding, until they reach a preovulatory size in spring. Interestingly, in this study, maturing females were shown to start vitellogenic cycles several years before mature follicles were finally ovulated. Another peculiarity in *P. umbrina* is that, despite being a single-clutch tortoise, different sized-follicles grow in one reproductive season, with the smaller ones always becoming atretic after the nesting season. The evolutionary significance of this observation is not well understood and may suggest that the single-clutch pattern of this tortoise

might be derived from multiple clutch capabilities. This theory was supported by the observation that animals held in captivity occasionally double clutch under optimal conditions.

4.2.2 Podocnemididae

The Podocnemididae, primarily large river turtles inhabiting tropical South America and Madagascar, are less well studied and deserve more attention, especially the *Podocnemis* and *Peltocephalus* genera from South America. Interestingly, *Erymnochelys madagascariensis*, a large turtle endemic to Madagascar, can lay several clutches of eggs per season, and nesting occurs over an extended period of time, from the first rains in the late dry season (or spring, October) to the late wet season (or fall, May) (Kuchling, 1988b; Kuchling & Garcia, 2003). In addition, wild *E. madagascariensis* seems to have a biennial ovarian cycle, based on a number of assumptions after carefully evaluating ovaries and oviducts of a dozen freshly slaughtered carcasses (Kuchling, 1993), and on ultrasound scanning and endoscopic data (Table 1). Most females lay several clutches of eggs from the late dry season to the mid-wet season, but typically only every second year. In captivity, however, a female is known to lay one clutch every year (P. Praschag, personal communication), suggesting the biennial pattern of wild females is related to nutritional limitations of the largely herbivorous wild females. The only other freshwater turtle for which a biennial ovarian cycle has been described is the large cryptodire river turtle *Carettochelys insculpta* in tropical northern Australia, which lays two clutches every second year and is also herbivorous (Doody et al., 2003). Spermatogenesis in *E. madagascariensis*,

TABLE 1 A number of breeding and nonbreeding females of *Erymnochelys madagascariensis* at Ankarafantsika National Park were assessed by ultrasound scanning and in some cases by endoscopy during the breeding season (September–May).

	Sample size (n)	Vitellogenic follicles >10 mm and/or oviducal eggs		Corpora lutea/C. albicanitia >2 mm	
		Present	Absent	Present	Absent
October 1999	1	0	1	—	—
April/May 2000	10	4	6	4	6
October 2001	4	4	0	—	—
Jan/Feb 2002	8	4	4	4	4
December 2002	2	1	1	—	1
All years combined	25	13	12	8	11
Total breeding females:	<i>n</i> =13				
Total of nonbreeding females:	<i>n</i> =12				

appears to start at the end of the nesting season, in April, with spermatogenesis and spermiation taking place in the cool dry season until it comes to an end in September, therefore showing a prenuptial pattern (Kuchling & Garcia, 2003). *E. madagascariensis* shows several reproductive patterns that are similar to those of sea turtles, rather than to other better-documented cryptodire freshwater turtles.

Females assumed to be breeding had vitellogenic follicles $>10\text{ mm}$ and/or oviductal eggs and corpora lutea/albicantia $>2\text{ mm}$. Females lacking these features were assumed to be nonbreeding.

4.2.3 Pelomedusidae

The pelomedusid turtles *Pelusios castanoides* and *Pelomedusa subrufa*, which occur sympatrically with *Erymnochelys* in Madagascar, show annual ovarian cycles, as well as postnuptial spermatogenesis, which take place during the hot wet season (Kuchling et al., 2022; Fig. 15), like most cryptodire freshwater turtles. The first clutches are laid in September (late dry season). One interesting finding in females of *P. castanoides* and *P. subrufa*, is that, despite laying several clutches of eggs within each season (up to February in the mid-wet season), follicles are not present in different size classes at one time as is usually the case in other multiclutch turtles. Instead, it appears that follicles for the next nesting event only start growing once the previous clutch of eggs has been laid. From February/March onward, vitellogenesis commences again for the first clutch of the next breeding season in September. The genus *Pelomedusa* is widely distributed in drier areas of sub-Saharan Africa, from wet-dry tropical areas to the Mediterranean climate in South Africa where its reproduction also has been studied in the western Cape in the southernmost distribution of the species *P. galeata* (Van Wyk et al., 2005). Vitellogenic recrudescence reportedly began in summer (December) and continued through winter with ovulation

occurring in spring (September–October). Reportedly, females lay only one clutch of eggs, but nesting occurred from September to January and early vitellogenic follicles were also noted during November. These observations and incongruous interpretations suggest that Van Wyk et al. (2005) may have misinterpreted their data and overlooked multiple clutching due to the fact that this species does not show concurrent different size classes of follicles as, based on the commonly accepted wisdom, the authors may have expected for a multiclutch turtle. Plasma E₂ and Vtg peaked during summer and plasma T in females varied throughout the year. P₄ was elevated prior to oviposition in summer (December). Unfortunately, the interpretation of these steroid data suffered from the probably misinterpreted ovarian cycle. As in Madagascar, spermatogenesis and spermiation took place in summer and fall and plasma T peaked once from February to March.

5 OTHER HORMONAL CYCLES

5.1 Metabolic and stress hormones

5.1.1 Thyroid hormones and possible role in reproduction

Thyroid hormones (thyroxine T₄, and triiodothyronine T₃) are very conserved within vertebrates, and their function has been linked to the regulation of many energy-demanding activities, including development, growth, reproduction, and metabolism (Eales, 1979; Norris, 2007). It has been suggested that thyroid hormones therefore should exhibit distinct annual cycles in temperate-zone animals, including turtles, during periods of metabolic activity (Kohel et al., 2001).

Levels of triiodothyronine (T₃) are much lower than those of thyroxine (T₄) in a number of turtle species and are often undetectable. Seasonal changes in hormone levels were

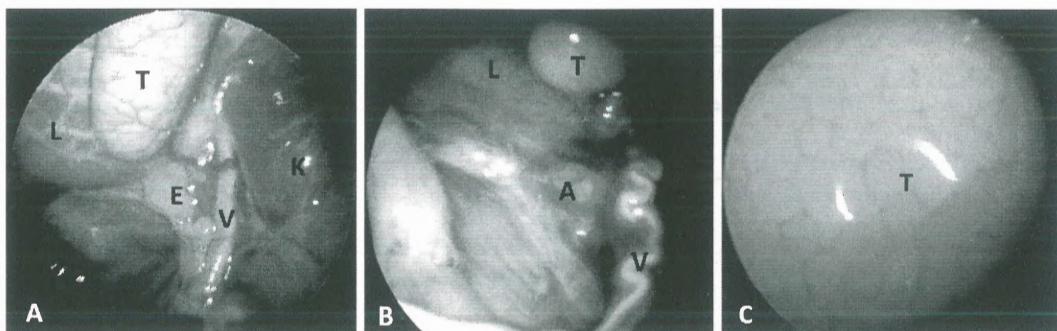


FIG. 15 Endoscopic images of testes and accessory ducts of sympatric *Erymnochelys madagascariensis* (A), *Pelusios castanoides* (B) and *Pelomedusa subrufa* (C: close up of testis) in Ankarafantsika, northwestern Madagascar, 13–15 February 2002 (height of the hot rainy season, late nesting season for all three species). Testis of *E. madagascariensis* small and regressive with no sperm in efferent ducts (prenuptial spermatogenesis during the dry season), while testes of *P. castanoides* and *P. subrufa* enlarged and undergoing spermatogenesis, with commencement of spermiation (sperm discharge into efferent ducts: postnuptial spermatogenesis). Testis (T), epididymis (E), vas deference (V), kidney (K), lung (L).

investigated over the course of 2 years in the desert tortoise (Kohel et al., 2001; Lance & Rostal, 2002). T₃ was undetectable; however, T₄ exhibited distinct cycles for both male and female *G. agassizii*. Higher levels of thyroid hormones were associated with increased activity and mating behavior. For both males and females, T₄ levels were at their lowest during hibernation (0.3 ng/mL) and rose to reach a peak in early spring (2.8 ng/mL), coinciding with feeding and mating behavior. When tortoises were fasted for 2 weeks, T₄ levels decreased and were significantly lower than in control tortoises fed a normal diet. Once tortoises were fed again, T₄ levels increased significantly, suggesting that thyroxine is directly linked to feeding activity. In reproductively active males only, T₄ concentrations exhibited a second peak in the summer, coinciding with high T levels and a period of intense aggression between males. This second peak was not observed in juvenile and subadult males although they showed the same feeding behavior as reproductively active males at this time of year. Thus, the peaks of T₄ were correlated to behavioral activity patterns including increased movement, feeding, and mating.

These results are somewhat in contrast to earlier work from Licht, Breitenbach, et al. (1985) showing a peak in plasma T₄ in male painted turtles in the summer when the T level was at its nadir. In further contrast, plasma T₄ remained fairly constant throughout the year in male green sea turtles held captive (and constantly well fed) at the Cayman Turtle Farm (Licht, Wood, et al., 1985), as well as in wild adult green turtles from Australia captured during winter and spring (Moon et al., 1998). However, wild male *L. kempii* exhibited a rise in T₄ at the onset of mating, in February–March (Rostal, Owens, et al., 1998), whereas Moon et al. (1998) did not see any trend in captive adult male *L. kempii* from the Sea Arama Marineworld (Galveston, TX). Both studies Moon et al. (1998) and Rostal, Owens, et al. (1998) found elevated T₄ levels in adult female *L. kempii* during the winter, coinciding with vitellogenesis, and in the spring, at the onset of mating. In addition, Wibbels et al. (1986) detected higher T₄ levels in *C. caretta* during the prebreeding season. In wild immature sea turtles from Australia, T₄ levels were low but slightly increased with increased water temperature in green turtles; however, levels remained low at all times in loggerheads (Moon et al., 1998). Finally, Moon et al. (1998) noted that T₄ levels were higher in captive turtles (up to 13 ng/mL in *L. kempii*) than in wild ones (up to 3 ng/mL in wild immature *C. mydas*), possibly related to the higher nutritional content of their diet. However, Rostal, Owens, et al. (1998) reported T₄ levels in wild adult female *L. kempii* as high as 11 ng/mL.

These results suggest that the thyroid system might be activated by various factors including temperature, reproductive state, and nutritional status, but it is not well understood how the changes in environmental stimuli and

endogenous conditions affect thyroid activity, and which factors, if any, might be most important in influencing reproductive activities.

5.1.2 Corticosterone (CORT)

The role of CORT during the reproductive cycle of reptiles is not well understood but results from various studies suggest that glucocorticoids are secreted during periods of increased activity and metabolism in reptiles. Lance et al. (2001) measured CORT and T levels in male and female *G. agassizii* throughout the reproductive period, finding that males had higher levels of CORT than females and that the levels peaked in late summer and fall, corresponding to peaks of T, intense male–male combat, and mating. A female CORT cycle was less clear, with a peak in May during mating and nesting, but no association with E₂ levels later in the season. Similarly, CORT (and T) levels were higher in male and female *G. nigra* during the mating season (Schramm et al., 1999). However, in *G. polyphemus*, no seasonal variation was observed for either sex, and no correlation was found with sex steroids (Ott et al., 2000).

CORT levels are commonly thought to reflect stress levels in reptiles, including turtles, especially related to capture or long-term captivity (Ott et al., 2000; Chapter 7, this volume). Stress in captivity has been suggested to result in drastic sex steroid decreases, in particular for T. For example, Mendonca and Licht (1986) noted a 35%–60% decrease in T levels in male musk turtles within 24 h of capture, which might have been correlated with increased levels of CORT (unfortunately not measured). Given these results, it is not surprising that T levels are often much lower in captive animals when compared to wild populations, although seasonal cycles are often exhibited nonetheless (Huot-Daubremont et al., 2003; Mahmoud & Licht, 1997).

In wild plowshare tortoises (*Astrochelys yniphora*) males exhibit two CORT peaks that align with the time of ovulation in females, suggesting that competition for mates is stressful for males. Since competition may determine mating rights, aggressive males with higher T would generally be more successful in combat. However, T-linked aggressive behavior could attenuate successful mating if directed at females. For courtship to take place following combat with other males, *A. yniphora* males may show a reduction in T concentrations (Fig. 16). T was generally lower in males in a captive plowshare tortoise population in Madagascar than in wild males, but captive males which sired nests had overall higher basal CORT and T concentrations than those which did not. In this species, male–male competition in captivity appears to be stressful by requiring aggression/dominance throughout the year (Currylow, Mandimbihasina, et al., 2017).

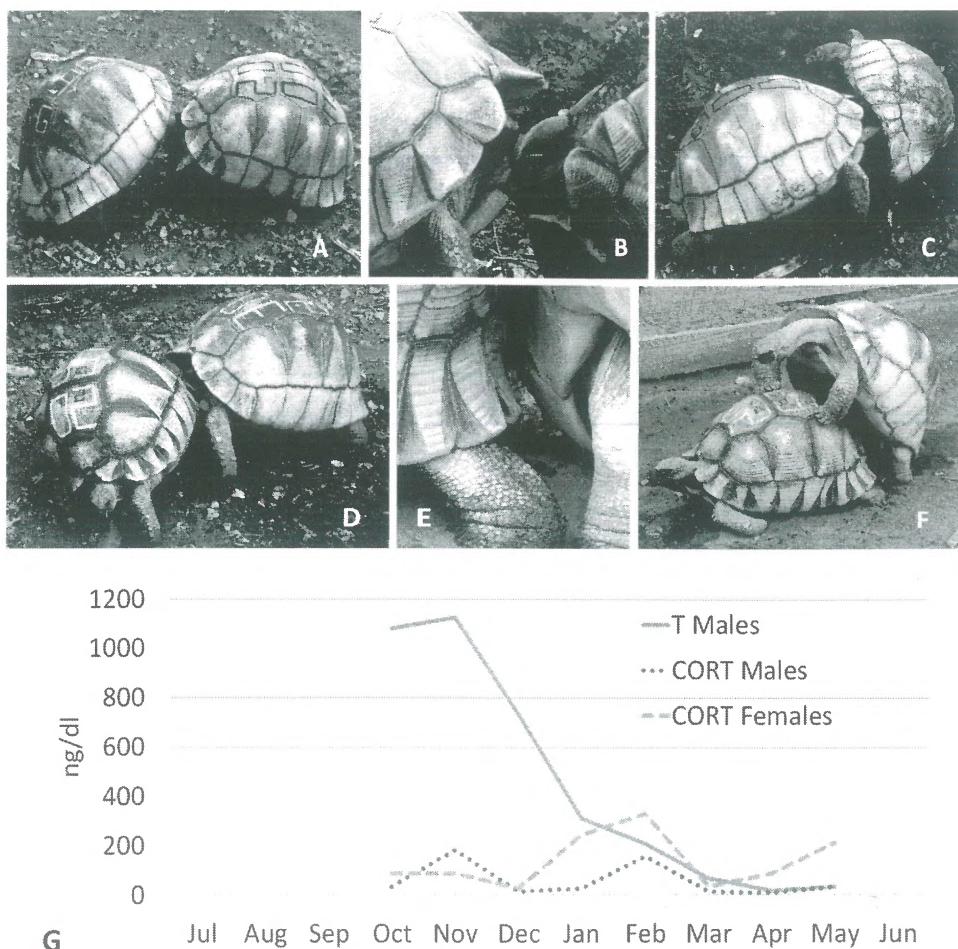


FIG. 16 Male combat (A, B, C) and courtship (D, E, F) of the plowshare tortoise *Astrochelys yniphora* (captive specimens in Ampijoroa, Madagascar) and (G) seasonal plasma levels of T in wild males and CORT in wild males and females. The main mating period lasts from December to February (the height of the wet season), preceded by a period of male combat in the late dry season/start of the wet season (October/November). Nesting occurs from mid-January to May. Combatting males use their plow-shaped gular projections to try turning each other over (A, B, C), while during mating they use the elongated gular to induce females to elevate the back of the carapace (D) to allow alignment of the cloacal vents (E) and copulation (F). See text for further details regarding behavior and circulating steroids. (G) based on graphs presented by Currylow, Mandimbahasina, et al. (2017).

CORT fluctuations during the nesting period have been fairly well studied in female sea turtles. CORT measured in female leatherback sea turtles during the nesting period showed no significant variation over time. In addition, there was no correlation between CORT and T (Rostal et al., 2001). These results are in agreement with other studies showing, for example, no significant elevation in CORT levels in female *L. olivacea* nesting in an arribada (high density) nesting aggregation in Costa Rica or green turtles on an exceptionally high-density nesting beach in Australia, when compared to a low-density nesting population (Jessop et al., 1999; Valverde et al., 1999). Furthermore, green sea turtles exposed to high daytime heat for several hours while nesting on the beach did not exhibit the expected high levels in CORT due to this potentially lethal environmental stressor. In fact, they exhibited lower CORT levels when compared to levels in nonbreeding females held captive

for the same amount of time in the shade (Jessop et al., 2000). These authors proposed that the low adrenocortical response to various stressors while nesting on these unusual but very remote beaches might be part of a generalized nesting adaptation to prevent stress from compromising reproduction along with its high investment. This inhibited stress response may be adaptive in female sea turtles, and this theory was further confirmed with the study of Jessop (2001) in which female *C. mydas* and *E. imbricata* in different reproductive states (nonbreeding, pre-breeding, and breeding) showed different levels of CORT, with breeding females exhibiting the lowest concentrations. CORT levels increased in all three categories as time after capture increased. Adult males in the three reproductive conditions did not show the pattern observed in females. Other studies in adult female sea turtles also failed to show stereotypical stress responses due to capture (Gregory et al., 1996;

Valverde et al., 1999), corroborating the studies from Jessop et al. (1999, 2000) and Jessop (2001). Although not sampling turtles at other times in their life history, in a study that looked at CORT in nesting Australian flatback sea turtles, Ikonomopoulou et al. (2014) found significantly higher levels of CORT compared to what they observed in nesting green turtles from Malaysia in the same study.

5.1.3 Catecholamines

Catecholamines, particularly epinephrine and norepinephrine, have been studied very little in turtles and showed no particular trend or association with glucose levels in nesting female green sea turtles (Jessop & Hamann, 2004). In the soft-shelled turtle, *L. p. punctata*, both epinephrine and norepinephrine plasma levels were elevated following administration of exogenous FSH (although no mention was made of its source) and high doses of E₂, and it is plausible that an annual hormonal cycle exists based on the secretion of the sex hormones Ray et al. (2002).

5.1.4 Ghrelin and leptin

Goldberg et al. (2013) described an important study of the nesting physiology of the hawksbill sea turtle in Brazil. This species nests one to five or more times in a season and usually skips at least a year between long-distance nesting migrations. Blood sampling 41 tagged females up to 5 times each as they returned to the beach for subsequent nesting events, they used RIAs to document a steady drop in levels of ghrelin while leptin showed a steady increase in the same females over several weeks. Since sea turtles (and some other turtles) fast for many weeks during the mating and nesting season, these metabolic hormones clearly could facilitate the very difficult migratory, courtship, and nesting cycles in sea turtles. Additional studies are needed in other species.

5.2 Pineal hormones and annual cycles

Turtles have a large secretory pineal gland, which is the primary source of the hormone melatonin (Owens et al., 1980; Owens & Ralph, 1978). In addition, many turtles exhibit a melatonin diurnal cycle with a nocturnal high and distinct responsiveness to both light (i.e., a dosimeter) and temperature (Fig. 17) (Owens & Gern, 1985; Owens & Morris, 1985; Vivien-Roels et al., 1988). These authors suggested that the very active pineal system of turtles could serve as a photoperiod-based annual clock, which would function as a day length measurer to coordinate the annual cycles. Thus when both the nutritional and temperature history of the individual are good, melatonin would facilitate a coordinated seasonal gonadal maturation. For a more complete discussion, see Kuchling (1999). A recent review by Ubuka et al. (2008) proposes

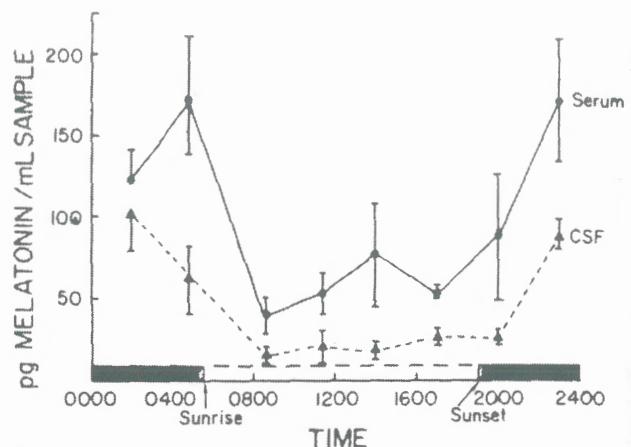


FIG. 17 Diurnal rhythm in cerebrospinal fluid and serum melatonin in green sea turtles. Each turtle was sampled simultaneously for blood and cerebrospinal fluid at only one of the 8 times. Four turtles were sampled at each time. From Owens, D. W., Gern, W. A., & Ralph, C. L. (1980). *Melatonin in the blood and cerebrospinal fluid of the green sea turtle*. General and Comparative Endocrinology, 40, 180–187.

a mechanism wherein melatonin may act centrally in the hypothalamus to in turn regulate GTH-releasing and release-inhibiting factors and thus control the release of pituitary GTHs. A curious and potentially relevant observation in the temperate-ranging leatherback turtle is a striking white spot on top of the skull just above the brain where the pineal is located (Fig. 1).

6 CONCLUSIONS

Several researchers have observed that a full understanding of endocrine regulation in turtles has been limited because of the old habit of taking too few and infrequent blood samples in turtles (Kuchling, 1999; Licht, 1984). This problem recently has been addressed in a few species; however, many gaps exist, particularly with males, hibernating or aestivating turtles, and the endangered and threatened species. Dessauer (1970) described reptilian physiology as “studies of physiology in slow motion.” Whereas this may be true in situations where temperatures are suboptimal, it is definitely not true all of the time especially not during peak reproductive periods. Indeed, some of the most striking examples of very rapid physiological changes are seen with the steroid hormone P₄. Although the baseline circulating P₄ level is what is regularly observed (e.g., Ott et al., 2000), many studies have shown that during ovulation and the albumin deposition phase of the nesting cycle, which may only last from 10 to 48 h, a tremendous surge in P₄ will occur to facilitate the rapid movement of follicles into the oviducts and the concurrent secretion of large volumes of albumin around each fertilized ovum. Just as this rapid surge facilitates nearly simultaneous ovulations of one to one hundred or more

follicles, a similar sudden drop in this steroid hormone is required to terminate the albumin deposition phase of egg formation. Since it is important for efficiency and speed in the oviduct for the entire clutch to be at the same stage of egg production, selection would favor a rapid rise and fall in P₄ secretion. The dynamics of CORT is rather unclear in some studies where infrequent sampling protocols have been used. Moreover, many early studies were done while animals were held in captivity, with blood samples being taken several hours or even days after capture. Many studies have shown that stress of capture in wild turtles, or stress from long times in captivity, suppresses hormone levels (Callard & Hirsch, 1976; Huot-Daubremont et al., 2003; Kuchling et al., 1981; Licht, Breitenbach, & Congdon, 1985; Mahmoud & Licht, 1997; Mendonca & Licht, 1986), and studies of hormonal cycles based on captive animals should be evaluated with caution (see Chapter 7, this volume).

Studying the reproductive cycles of turtles can lead to improved knowledge of the reproductive potential of the species, namely how many eggs are being produced in each clutch, how many clutches are laid per year, and what is the periodicity of the reproductive activity. As described earlier, some turtles reproduce every year, while others—mainly the sea turtles, but other species as well—have been found to only reproduce every 2–4 years or more. For example, Limpus et al. (1994) reported that green sea turtles of Eastern Australia have an average remigration interval of about 5.8 years, with many females nesting after an 8–9 year interval. Adult male sea turtles cycle faster than females do; however, resident breeding males may not be as successful in the long-term as migratory males with longer cycles. More work needs to be done to answer questions on cyclicity and turtle reproductive potential, as well as on the developmental onset of cycling and reproduction. Also, how long during their long lives can turtles reproduce? We know nothing of this in the wild. An improved understanding of turtle reproduction is critical to developing better life history and population dynamics models. In turn, such models are urgently needed for improving conservation strategies, especially when applied to threatened or endangered species. Indeed, a good understanding of the reproductive potential of a species, and how it cycles over time, can also be used in captive breeding programs for the reintroduction of endangered species (e.g., Currylow, Mandimbihasina, et al., 2017). There are too many turtle species on the verge of extinction in many countries around the globe for which nothing is known about their reproductive biology. Finally, because of the well-documented importance of ambient temperature in all phases of turtle reproduction including sex determination (see Chapter 1, this volume), the current global warming crisis adds special urgency to future research on the reproductive cycles of turtles.

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