

and the latitude inhabited by the female (Alkindi et al., 2006; Arslan et al., 1978; Bona-Gallo et al., 1980; Callard et al., 1978; Cree et al., 1992; Ganesh & Yajurvedi, 2007; Guillette et al., 1997; Licht et al., 1979; McPherson et al., 1982; Radder et al., 2001; Taylor et al., 2004). The endocrinology of the reptilian ovary is addressed in Chapter 4, this volume and only an outline will be presented here.

Both estrogens and testosterone are secreted by the vitellogenic ovarian follicle. Vitellogenic follicles are those that incorporate the yolk precursor, vitellogenin, into the yolk. Vitellogenesis itself is under estrogenic control; this aspect of estrogenic action is discussed in Chapter 4, this volume. Levels of estrogens in the blood typically become elevated during vitellogenesis, reaching a peak prior to or at the time of ovulation, and then decline as eggs remain in the uterus. In species possessing multiple egg clutches, spikes of estrogens are associated with each ovulatory event. Progesterone levels rise close to the time of ovulation and are short-lived in oviparous species, lasting longer in viviparous reptiles, and decline shortly before oviposition.

Vitellogenic follicles typically enlarge rapidly as they accumulate yolk, leading to the formation of a megalecithal egg in which virtually the entire volume of the egg is occupied by yolk. Following ovulation, the follicular wall forms a corpus luteum that persists for varying intervals. The corpora lutea of viviparous reptiles persist longer than those of oviparous forms (Callard et al., 1992). There is little doubt that the corpus luteum can synthesize and secrete P₄. Secretion in oviparous forms reaches a peak prior to ovulation whereas, in viviparous forms, with longer-lasting corpora lutea, elevated P₄ levels are observed after ovulation (Callard et al., 1992).

5.7 Oviduct

It is well-established that oviductal functions are influenced by ovarian sex steroids. The reader is referred to Botte (1974) for a review of earlier literature. Nevertheless, there are only a few studies examining specific estrogen or P₄ effects on the oviduct. Mead et al. (1981) examined oviductal histology following administration of E₂ or P₄ to ovariectomized garter snakes (*Thamnophis elegans*). Estradiol was partially effective in reversing the regressive changes in the oviduct following ovariectomy, but P₄ was not. A combination of the two hormones was no more effective than E₂ alone. Administration of E₂ to intact painted turtles (*C. picta*) in the fall stimulated the tubuloalveolar glands of the uterus to levels seen in the summer but had no effect on the uterine tube (Motz & Callard, 1991). Estradiol also stimulates myometrial contractility in this same species (Callard & Hirsch, 1976), whereas P₄ suppresses uterine contractility but has no effect on the uterine glands.

More recent studies have focused on the location of and changes in steroid hormone receptors in the reptilian oviduct. The reptilian oviduct contains receptors for T, E₂, and P₄. Both nuclear and cytoplasmic ARs are reported in the oviduct of *T. scripta*, localized in the glands of the lamina propria but absent from the epithelium lining the oviduct lumen and the myometrium (Selcer et al., 2005). More information is available for the latter two receptor types, and oviductal ERs are reported in the turtles *C. picta* (Salhanick et al., 1979) and *T. scripta* (Selcer & Leavitt, 1991) as well as the garter snake *T. s. parietalis* (Whittier et al., 1991) and the lizard *Podarcis sicula* (Paolucci et al., 1992). In the alligator, ERs have been characterized from the anterior uterus (Vonier et al., 1997). Progesterone receptors (PRs) are reported using immunocytochemistry in the luminal epithelium, glands of the lamina propria, and myometrium of the turtle *C. picta* (Giannoukos et al., 1995). A PR was demonstrated in the oviduct of the snake *Nerodia* (*Natrix* sp.) (Kleis-San Francisco & Callard, 1986), and two forms of the PR were found in the turtle *C. picta* (Reese & Callard, 1989).

Changes in the number of oviductal ERs and PRs over the reproductive cycle or in response to hormonal or surgical manipulation have been examined. Hypophysectomy reduces oviductal ER levels in the turtle *C. picta*, but replacement therapy with either E₂ or P₄ is unable to restore them to normal (Giannoukos & Callard, 1996). Hepatic ER levels in the lizard *P. sicula* rise during spring vitellogenic growth (Paolucci, 1989), while those in the oviduct are highest during winter ovarian quiescence (Paolucci & DiFiore, 1994; Paolucci et al., 1992). Long-term (14 days) administration of E₂ to ovariectomized *P. sicula* during quiescence induces an elevation in hepatic ER, but effects on oviductal ER levels are equivocal. On the other hand, in response to a single injection of E₂ to intact quiescent *P. sicula*, oviductal ER expression was elevated 12 hours later. These results suggest a rather complex regulation of ER expression in reptilian reproductive tissues and further studies will be of benefit.

More attention has been devoted to the regulation of the PR. Specific oviductal binding of P₄ in most species is elevated at the time of ovulation, well in advance of the rise in plasma P₄, and this elevation persists for varying periods of time following ovulation (Giannoukos et al., 1995; Kleis-San Francisco & Callard, 1986; Paolucci & DiFiore, 1994). Ovariectomy has little or no effect on oviductal PR levels in the lizard *P. sicula* (Paolucci & DiFiore, 1994), but the same operation increases specific P₄ binding by turtle (*C. picta*) oviducts (Giannoukos & Callard, 1996). In contrast to mammals, administration of E₂ to ovariectomized *P. sicula* has little effect on oviduct PR (Paolucci & DiFiore, 1994), whereas E₂ increases oviductal PR levels in the snake *Nerodia* (*Natrix* sp.) (Kleis-San Francisco &

Callard, 1986). Part of the conundrum may lie in the findings that two forms of the PR have been isolated from the turtle (*C. picta*) oviduct (Reese & Callard, 1989). One form (PR-A) has a low P₄ affinity (2.8×10^{-9} M) and is present throughout the reproductive cycle, being elevated at the time of and shortly following ovulation (Reese & Callard, 1989). The other form (PR-B) has a high P₄ affinity (28×10^{-9} M) and is expressed from the time of ovulation to egg laying, and then again during the autumnal period of ovarian growth (Giannoukos et al., 1995). While the role of estrogens in regulating oviductal PR remains obscure, it seems clear that P₄ is influential in regulating oviductal levels of its own receptor. Progesterone injections induce a downregulation of oviductal PR receptors in turtles (Giannoukos & Callard, 1996; Selcer & Leavitt, 1991). Such regulation may play a role in the timing of oviductal secretions.

5.8 Oviposition

It has been known for some time that estrogens and P₄ have antagonistic actions on the reptilian myometrium. Thus, E₂ stimulates and P₄ inhibits oviductal contractions in the turtle *C. picta* (Callard & Hirsch, 1976). Progesterone can reduce the effectiveness of arginine vasotocin (AVT), one of the reptilian neurohypophysial hormones, in stimulating uterine contractions in the same species (Callard et al., 1992) and delays parturition in the viviparous lizard *Sceloporus jarrovi* (Guillette et al., 1991a). Removal of the corpora lutea reduces the time that eggs are retained in the oviduct, and P₄ administration delays oviposition (Roth et al., 1973; Klicka & Mahmoud, 1977; Cuellar, 1979). Viviparous species, which retain eggs in the oviduct for longer times than oviparous species, maintain elevated P₄ levels for longer periods than oviparous species (Callard et al., 1992). Combined, this evidence suggest that the corpus luteum, by virtue of P₄ secretion, prevents premature oviposition.

Oviposition and its equivalent in viviparous species, parturition, are under complex neuroendocrine control. This topic has been reviewed most recently by Guillette et al. (1991b). Arginine vasotocin, a potent stimulator of oviductal contractions (Ewert & Legler, 1978; Mahmoud et al., 1988), is most effective if given late in pregnancy (viviparous forms) or during the gravid period (oviparous forms) (Guillette et al., 1992; Mahmoud et al., 1988). A similar pattern of responsiveness is seen with the prostaglandin F_{2 α} (PGF_{2 α}) in the lizard *S. jarrovi* (Guillette et al., 1992). Further, indomethacin, an inhibitor of prostaglandin synthesis, can delay parturition in this same species (Guillette et al., 1991a). Prostaglandin F_{2 α} concentrations in the blood of sea turtles (Guillette et al., 1991c), the tuatara (Guillette et al., 1990a), and the lizard *Tiliqua rugosa*

(Fergusson & Bradshaw, 1991) are all elevated at the time of oviposition. A link between AVT and prostaglandin stimulation is provided by Guillette et al. (1990a), who demonstrated in *S. jarrovi* that AVT stimulates the oviductal synthesis of PGF_{2 α} *in vitro* and increases plasma PGF_{2 α} levels *in vivo*, a response likewise blocked by indomethacin. Here too, the effectiveness of AVT in inducing uterine contractions was highest near the end of pregnancy and lowest in early pregnancy. The effectiveness of both AVT and PGF_{2 α} in stimulating oviductal contractions is greater *in vitro* than *in vivo*, leading to speculation that another factor, possibly neural, acts on the myometrium to prevent premature contractions. In the lizard *A. carolinensis*, injection of AVT will not result in oviposition unless the animal is pretreated with the β -adrenergic antagonist dichloroisoproterenol (Jones et al., 1983). Similarly, blockade of β -adrenergic receptors in the oviduct of the gecko (*Hoplodactylus maculatus*) enhances PGF_{2 α} -induced uterine contractions, but not those induced by AVT (Cree & Guillette, 1991). These data have led to the hypothesis that the neuroendocrine mechanisms influencing oviposition are directed to the uterovaginal musculature to regulate egg egress from the oviduct rather than egg expulsion as the result of myometrial activity (Guillette et al., 1991b). Thus, the uterotonic actions of AVT and PGF are inhibited by elevated P₄ levels in early pregnancy or gravidity, and are modified by the autonomic nervous system in late pregnancy or gravidity.

5.9 Unresolved questions

Fundamental aspects of oviductal function remain unknown. Coordination of deposition of eggshell protein fibers and calcification by hormones or other mechanisms have yet to be elucidated in squamates or turtles. Our knowledge of the effects of estrogens and P₄ on the oviduct are inadequate. The timing and control of expression of both ER and PR in the reptilian oviduct need further study.

6 SPERM STORAGE

Reptilian eggs are oviposited either singly or, more typically, in clusters that comprise an egg clutch. Because of the large size of reptilian eggs, multi-egg clutches and multiple egg clutches in a season pose problems with respect to fertilization because eggs may not come into contact with sufficient numbers of sperm without repeated copulations. Some reptiles have addressed this problem by the storage of sperm, either within the oviduct of the female or in the excurrent canals of the male, until ovulation occurs.

The reptilian oviduct is capable of storing sperm from matings for extended periods of time. This ability is not unique to reptiles, being common in birds, but reptiles as



FIG. 9 Sperm storage tubule in the uterine tube of the turtle *Chrysemys picta*. Bar=25 µm.

a group store sperm for longer periods than other vertebrates, up to several years, for example, in turtles (Gist & Jones, 1987). Sperm storage is common among the Reptilia, being found in all families, and is considered an integral part of the reproductive process. The locations of sperm storage within the oviduct are highly variable. In squamates, sperm may be found at the appropriate time of year in tubules formed from folds in the vaginal wall or in pouches or tubules located in the infundibulum (Sever & Hamlett, 2002). In Testudines (Fig. 9) and Crocodilidae, sperm are stored in ducts of glands located at the caudal terminations of the glandular areas of the uterine tube and uterus (DePerez & Pinilla, 2002; Gist et al., 2008; Gist & Jones, 1989). An infundibular location of sperm storage is found in the soft-shelled turtle *L. punctata* (Sarkar et al., 2003) and an anterior uterine location has been reported in another soft-shelled turtle, *Trionyx sinensis* (Han et al., 2008).

It has yet to be established whether the host glands or tubules provide sustenance for stored spermatozoa, although Han et al. (2008) reported both sperm maturation and degradation within storage tubules. In some reptiles, stored sperm are found in association with an amorphous carrier matrix (Halpert et al., 1982; Kumari et al., 1990; Sarkar et al., 2003). The epithelial cells of the tubules or glands containing sperm are generally similar to those not containing sperm, and in addition do not differ histochemically. Studies using an electron microscope reveal no contact between stored sperm and the surrounding epithelial cells (Bou-Resli et al., 1981; Gist & Fischer, 1993; Sever & Hamlett, 2002). While the possibility that the surrounding cells secrete materials that preserve or maintain sperm cannot be excluded, the available evidence suggests that sperm-storing glands or tubules provide a safe haven for

sperm and that survival of sperm is a property of the male gamete, not the female host (Gist et al., 2000, 2001).

How sperm move from the storage sites to the location of fertilization, presumably the infundibulum, is likewise unknown. In birds, sperm stored in the vaginal storage tubules evacuate the tubules at a continuous rate (Birkhead & Moller, 1992). Sperm stored in the glands and tubules of the oviduct form a reservoir that can provide sperm for upcoming ovulations. With techniques such as allozyme assay and microsatellite DNA analysis, it has become apparent that most reptilian egg clutches have multiple paternity (Davis et al., 2001; Laloi et al., 2004; Moore et al., 2008; Oppliger et al., 2007; Pearse & Avise, 2001). Thus, sperm storage sites may contain sperm from several males and the sperm within them represents an additional level of female mate choice, but at the time of fertilization. In the lizard *Ctenophorus pictus*, clutches fertilized by stored sperm are male-biased (Olsson et al., 2007) suggesting differential survival of male and female sperm within the oviduct.

The implications of sperm storage have been studied most extensively in turtles. In multiclutched painted turtles (*C. picta*) and desert tortoises (*Gopherus agassizii*), fertilization of eggs by sperm stored in the oviductal glands has been demonstrated both across subsequent egg clutches within a single year as well as from one nesting season to the next (Palmer et al., 1998; Pearse & Avise, 2001; Pearse et al., 2002). The former is in support of the conclusion of Gist and Congdon (1998) that insufficient time exists between consecutive clutches for additional matings to occur. In terms of species with only a single annual egg clutch, multiple paternity is reported to occur in the American alligator (Davis et al., 2001) and the turtle *C. serpentina* (Galbraith et al., 1993); sperm storage is also reported in these two species (Gist et al., 2008; Gist & Jones, 1989). Single-clutched species tend to have large egg clutches. Clutches of the alligator can contain up to 200 eggs (Lance, 1989) and those of the snapping turtle contain 20–40 eggs. With these large clutches of megalecithal eggs, it is unlikely that sperm residing in the oviduct lumen could fertilize more than the first few eggs descending down the oviduct of these polyautochthonic ovulators. Storage of sperm could account for the high degree of fecundity in these single-clutched species.

6.1 Unresolved questions

The role of hormones in the process of sperm storage is just beginning to be examined. Sarkar et al. (2003) have investigated the movement and storage of sperm within the oviduct of the soft-shelled turtle *L. punctata*. Sperm had reached the storage areas of the posterior uterine tube 24 hours following mating and thereafter were found in the storage tubules. Estradiol given to quiescent (non-breeding) females induced a lengthening and widening of

the sperm storage tubules. In this species, E₂ levels normally peak at or around the time of ovulation (Sarkar et al., 1995), and at that time sperm are observed exiting the sperm storage tubules. Thus, by virtue of its stimulatory action on the tubuloalveolar glands of the lamina propria, estradiol may facilitate sperm entrance and egress from storage glands as ovulation approaches. Immunological aspects of sperm storage have yet to be investigated. Further studies will examine more thoroughly the role of ovarian steroids and other hormones in regulating sperm storage.

Long-term storage of sperm in the oviducts of females or in the male epididymis poses some interesting problems. Vertebrate gametes are known to be short-lived, maintaining viability and/or fertility outside the male reproductive tract for only minutes to weeks. This contrasts with the finding that turtle sperm can retain their fertility from one year to the next stored in the female oviduct (Pearse & Avise, 2001). Whether this longevity is conferred on the sperm by the local environment (e.g., sperm storage glands, epididymis), is a property of sperm, or is due to oviductal secretions, is unknown. Results of Gist et al. (2000) showing that epididymal sperm from turtles maintain their viability in physiological saline for up to 30 days following isolation suggest that sperm survival may involve intrinsic properties of the sperm cells. Further research into the physiology of reptilian spermatozoa may reveal how male gametes of reptiles as well as non-reptilian species maintain long-term viability.

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Chapter 6

Pheromones and reproduction in reptiles

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ABBREVIATIONS

LPS	lipopolysaccharides
MHC	major histocompatibility complex
T	testosterone
TF	tongue-flick
VNO	vomeronasal organ

1 INTRODUCTION

1.1 Pheromones of vertebrates

Pheromones are classically defined as chemicals or semiochemicals produced by one individual that effect a change in the physiology (“primer” pheromone) and/or behavior (“releaser” pheromone) of conspecifics (Karlson & Lüscher, 1959). This definition originated from the study of insects, where very often just one or a pair of compounds acts as a pheromone attracting the opposite sex. Most insect species have an, often exclusive, pheromone that allows species recognition and mate attraction. In contrast, in vertebrates, pheromones are usually a mixture of many chemical compounds with different properties (Müller-Schwarze, 2006; Wyatt, 2014). These multicomponent pheromones may have multiple functions or intended receivers, but may also act together providing specific or individual “odor profiles,” also named “gestalts,” “patterns,” “mosaics,” or “signatures” (Johnston, 2005; Wyatt, 2010). Not only the mixture of chemicals but also their relative proportions or concentrations, are often needed to be biologically active as a pheromone. Moreover, the pattern of compounds in the scent of each individual may signal sex, age, social status, group, health, and condition. Thus, in vertebrates, a pheromone may be defined as a group of active compounds in a secretion that supplies information to conspecifics with the aim of changing their behavior. This has been considered different from a classical pheromone and named as “signature mixtures,” i.e., variable subsets of molecules of an animal’s chemical profile, which are learned by other animals, allowing them to distinguish individuals or groups (Wyatt, 2010, 2014).

There are also important differences between insects and vertebrates in the role of pheromones in reproduction. Chemically mediated mate choice is common in many insects, where pheromones alone can directly control reproductive physiology and behavior. However, in vertebrates, due to the multicomponent sensory nature of these animals, reproductive behavior is often mediated by a combination of sensory stimuli (visual, tactile, chemical, etc.) rather than by a single type of stimulus alone. Nevertheless, pheromones are important, and sometimes a requisite, for species and sex recognition, mate choice, and effective mating in many vertebrates, especially in fish, mammals, some amphibians, and many reptiles (for reviews see Baeckens, 2019; Houck, 2009; Mason, 1992; Mason & Parker, 2010; Müller-Schwarze, 2006; Parker & Mason, 2011; Wyatt, 2014; see also in this series Volume 1, Chapter 9; Volume 2, Chapters 5 and 8; Volume 5, Chapter 10).

1.2 Chemosensory abilities of reptiles: The vomeronasal organ

The vomeronasal organ (VNO) is a complex of different structures that forward specific chemical stimuli (including pheromones) to the central nervous system. Many reptiles have a well-developed VNO and can discriminate many different scents from prey, conspecifics or predators (Cooper, 1994; Halpern, 1992; Halpern & Martínez-Marcos, 2003; Mason, 1992; Schwenk, 1995). In many lizards and snakes, chemoreception works hierarchically, with chemicals being first received through the nares, processed by the nasal organs, and triggering tongue-flick (TF) mediated vomerolfaction (Cooper, 1994; Halpern, 1992; Schwenk, 1995). Tongue-flicking allows an individual to obtain information about conspecifics based on pheromonal cues alone (Cooper, 1994; Halpern, 1992; Mason, 1992). In fact, functional (vs sealed) VNO ducts are necessary for accurate discrimination of prey chemicals and pheromones in lizards (Cooper & Alberts, 1991; Graves & Halpern, 1990). The VNO is important for activating accessory olfactory pathways that are involved in

sexually dimorphic reproductive behaviors. For example, the VNO of male garter snakes is critically important for detection of, and response to, female sex pheromones (Huang et al., 2006). Female pheromones directly affect male, but not female, snake VNO neurons and result in the opening of ion channels, converting the pheromone signal to an electrical signal (Huang et al., 2006). Similarly, in *Liolaemus* lizards, VNO neurons exhibit chemosignal-evoked currents when stimulated with pheromone-containing gland secretions and feces (Labra et al., 2005).

Behavioral tests of chemosensory recognition in lizards and snakes have been often based on the relationship between chemoreception and TF, an easily observable and quantifiable characteristic behavior (Cooper & Burghardt, 1990). The TF rates can be used as a bioassay for estimating chemosensory discrimination because many lizards and snakes respond to different chemical stimuli by changing the TF rates (Cooper, 1994; Cooper & Burghardt, 1990). An elevation of TF rates in response to the presentation of a chemical stimulus above the baseline TF rates under the experimental conditions in response to an odorless control (e.g., deionized water) indicates detection of that chemical stimulus, whereas differences between TF rates in response to different scent stimuli indicates discrimination of the different stimuli (Cooper, 1994; Cooper & Burghardt, 1990). Other similar quantifiable chemosensory exploratory behaviors such as labial-licking, chin-rubbing, or gular pumping (e.g., Wilgers & Horne, 2009), or behavioral social displays (head bobbing, dewlap extensions, etc.) have also been used as indicators of chemosensory recognition, particularly in species with low or absent TF behavior. In other cases, differences in behavior and time spent in areas scent marked with different chemical stimuli are used as indicators of chemosensory discrimination and preferences for a particular scent.

1.3 Pheromones and reproduction in reptiles

Reproductive behavior of many reptiles was traditionally thought to be predominantly based on conspicuous visual signals (Cooper & Greenberg, 1992). The importance of pheromones on reproductive behavior was little considered in most studies in spite of the well-known and considerable evidence of the chemosensory abilities of most reptiles, and the widespread occurrence of multiple types of glands that secrete chemicals with the potential of being pheromones, especially during the breeding season (Halpern, 1992; Mason, 1992). Only when all this information has been considered and incorporated into new observations and experiments, the great importance of pheromones in reproduction of many reptiles has emerged. Most of the known data refer to signaling (releaser) pheromones, which can inform conspecifics on simple data such as species, sex, or individual

identity. But pheromones also may inform on individual characteristics of the signaler, such as morphological traits and health state, which can be very useful in reproductive decision-making by the receiver. Priming pheromones, which directly trigger slower endocrine or developmental processes, would be extremely rare, or not yet known, in reptiles. This is, however, a relatively new emergent field of study that will require considering new situations and new species to get a complete picture.

We will review here the sources of potential pheromones and the results of experiments showing that these chemical cues or signature mixtures of different compounds (named here pheromones for simplicity) are involved in sex and individual recognition, territoriality, intrasexual aggression between males, female mate choice, and reproductive decisions of many reptiles.

2 TESTUDINES

Many turtles have well-developed olfactory and VNO and consequently are capable of chemosensory detection (Brann & Fadool, 2006; Fadool et al., 2001; Halpern, 1992; Hatanaka & Matsuzaki, 1993). In addition, most turtles have several types of specialized secretory glands. One or more pairs of Rathke's glands, which secrete through pores on the shell bridge of the axillary or inguinal areas, are present in all turtle species except in the Testudinidae (tortoises) and some Emydidae (Ehrenfeld & Ehrenfeld, 1973; Solomon, 1984). Mental or chin glands, located in the throat area, are found in many genera of the families Emydidae, Geoemydidae, Platysternidae, and Testudinidae (Winokur & Legler, 1975). Mental glands could have appeared in an aquatic ancestor of the superfamily Testudinoidea and later expanded in other aquatic turtles and terrestrial tortoises, but mental glands have been lost and/or reduced in multiple instances independently of the macro-habitat type (Ibáñez et al., 2021). Some of these glands are quiescent in juveniles, sexually dimorphic (larger in males), and active during the breeding season only (Ibáñez et al., 2020; Rose et al., 1969), which suggests their potential role in reproduction.

Mental and Rathke's glands secrete a variety of chemicals that have been described in a few turtle species and might be potentially used in communication (e.g., Alberts, Rostal, & Lance, 1994; Ibáñez et al., 2020; Rose et al., 1969; reviewed in Weldon et al., 2008). Cloacal secretions and feces are also considered as possible sources of pheromones (Mason, 1992), but these chemicals remain undescribed. However, chemicals produced by Rathke's glands were mainly considered as predator repellents or simply products of excretion without any function in intra-specific communication (Ehrenfeld & Ehrenfeld, 1973; Eisner et al., 1977; Kool, 1981; Weldon & Tanner, 1990).

Nevertheless, secretions from the Rathke's glands of common musk turtles (*Kinosternum odoratum*) may serve a dual function, being involved in antipredator mechanisms all the year, whereas, during the mating season, female secretions may allow sex-recognition during courtship (Brann & Fadool, 2006; Eisner et al., 1977; Mahmoud, 1967). This suggests that the potential role of glandular chemicals in reproduction should be reexamined in other turtle species.

Behavioral observations suggest that chemical stimuli may play a major role in sex and species identification and that many turtle behaviors, such as foraging, orientation and homing, aggregation, aggressive interactions between males, and mating behavior seem mediated by pheromones (e.g., Alberts, Rostal, & Lance, 1994; Bulova, 1997; Poschadel et al., 2006; Quinn & Graves, 1998). Experimental elimination of olfactory function results in impaired homing ability and a large reduction in reproductive behavior in Hermann's tortoises (*Testudo hermanni*) (Chelazzi & Delfino, 1986). Evidence for the role of pheromones in reproduction of turtles is scarce. This is probably due to the paucity of studies that had considered chemical communication in turtles because recent experiments are showing the importance of pheromones in sexual behavior of different turtle species.

2.1 Terrestrial tortoises

Terrestrial tortoises (Fam. Testudinidae) seem to have two primary sources of pheromones, the mental or chin glands, which elicit aggression between males, and cloacal glands, which allow species and sex recognition (Mason, 1992). During the mating season, mental glands of male desert tortoises (*Gopherus* spp.) are larger and have more secretion (Alberts, Rostal, & Lance, 1994; Rose et al., 1969). Secretions are mainly composed of fatty acids, triacylglycerols, steroids, phospholipids, and also proteins, which differ between species and between sexes (Rose et al., 1969). These secretions allow discrimination between familiar and unfamiliar conspecifics in desert tortoises (*G. agassizii*) (Alberts, Rostal, & Lance, 1994), and the presence of conspecific chemical cues influences burrow choice (Bulova, 1997). Mental gland secretions, and also the fatty acids from the secretions alone, elicit intrasexual aggressive behavior in male Texas tortoises (*G. berlandieri*) (Rose, 1970). Male mental gland secretions are detected and stimulate social behaviors in both male and female gopher tortoises (*G. polyphemus*), regardless of the presence of visual stimulus (artificial tortoise models) (Kelley et al., 2021; Kelley & Mendonça, 2020). This effect is concentration-dependent, with an optimum concentration being needed to stimulate specific social behaviors (Kelley et al., 2022).

Cloacal secretions allow, at least, species and sex determination in the sympatric South American red-footed and

yellow-footed tortoises (*Geochelone carbonaria* and *G. denticulata*) (Auffenberg, 1965). Males smell the cloacal area of other tortoises, and chemical discrimination of a conspecific female induces mounting attempts, whereas heterospecific females are ignored. In the Hermann's tortoise (*T. hermanni*), both sexes discriminate between their own and related species based on scents from femoral and cloacal regions, but only males can distinguish sex and sexual maturity of potential mates (Galeotti et al., 2007).

2.2 Freshwater turtles

The olfactory anatomy and behavioral experiments suggest that some freshwater turtles can detect pheromones from conspecifics in water and that this discrimination may affect their reproductive behavior. Female painted turtles (*Chrysemys picta*) (Fam. Emydidae), but not males, discriminate and prefer to occupy water with chemical cues from home ponds over water from other ponds that contain conspecifics (Quinn & Graves, 1998). During courtship, male musk turtles (*Kinosternon* spp.) (Fam. Kinosternidae) approach females from behind and smell the cloaca, apparently to determine sex. If the turtle is identified as a female, the male moves to her side to sniff the musk (Rathke's) glands of the female, and if the female is receptive, the male attempts mounting (Mahmoud, 1967).

In laboratory experiments, freshwater turtles were tested in simultaneous binary choice trials. A focal animal was given a choice between two pools with water containing conspecific chemicals or control clean water (Ibáñez et al., 2012, 2013, 2014; Muñoz, 2004; Polo-Cavia et al., 2009; Poschadel et al., 2006). Results show inter- and intrasexual pheromonal communication in freshwater turtles. Male European Pond turtles (*Emys orbicularis*) (Fam. Emydidae) prefer water with female odor over clean water, whereas females do not show any preference, suggesting that males actively search for females using pheromones (Poschadel et al., 2006). Males prefer water with scent of larger females, probably because fecundity increases with female size. In contrast, males strongly avoid chemicals of large males, but orient toward chemicals of relatively smaller males, suggesting that pheromones are involved in the establishment of dominance hierarchies (Poschadel et al., 2006).

In similar experiments, Spanish terrapins (*Mauremys leprosa*) (Fam. Geoemydidae), which have mental and Rathke's glands, prefer water with conspecific scents and avoid water with scent from a heterospecific competitor aggressive turtle invasive species (Polo-Cavia et al., 2009) (Fig. 1). During the mating season, male *M. leprosa* select water with chemicals from conspecific females, but avoid water with chemicals from males, and prefer water from their home containers over clean water (Muñoz, 2004). Furthermore, males prefer water with chemicals of

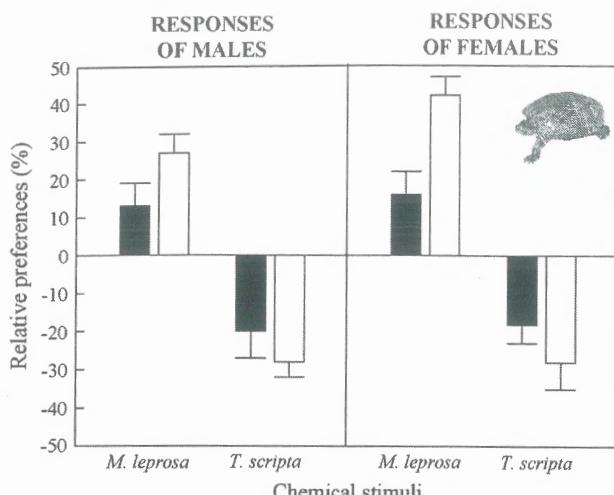


FIG. 1 Chemosensory species and sex discrimination by Spanish terrapins (*Mauremys leprosa*). Relative preferences (mean + SE) of male and female *M. leprosa* for water containing chemical stimuli of male (black boxes) or female (open boxes) conspecific or heterospecific (*Trachemys scripta*) terrapins (i.e., the difference between percent time spent on an experimental pond with a given chemical stimulus in comparison with time spent on a nearby control pond with clean water) (N. Polo-Cavia, P. López, and J. Martín, unpublished data).

healthy individual females (i.e., with higher immune responses), while females prefer water with scent of larger males (Ibáñez et al., 2012). Preferences for water used by better potential partners could increase mating opportunities with “high quality” individuals. Also, males avoid water with chemicals of relatively heavier males and prefer water with chemicals of relatively smaller males (Ibáñez et al., 2012). This chemosensory assessment of rival body size, which seems based on chemosignal concentration in water (Ibáñez et al., 2014), may allow for minimizing costly aggressive interactions with other males. Familiar discrimination also occurs, but it depends on the personality type of the responding turtle; bold individual males avoid water with chemicals of unfamiliar males, but not of familiar males, whereas shy males avoid chemicals of any male (Ibáñez et al., 2013). Secretions from the mental gland of *M. leprosa* contain mainly steroids, especially cholesterol, and also carboxylic acids, carbohydrates, alkanes, and alcohols, with intersexual differences in abundance of some compounds (Ibáñez et al., 2020). The role of specific compounds in communication remains to be examined.

Red-eared sliders (*Trachemys scripta elegans*) (Fam. Emydidae) lack inguinal and mental glands but can be trained to discriminate food chemicals (Boycott & Guillery, 1962). Sliders can also detect chemical cues of conspecifics in water and tend to prefer water with their own scent, while males avoid, more often during the mating season, water with conspecific chemicals, probably to avoid aggressive encounters (Polo-Cavia et al., 2009). However, sliders do not show a clear discrimination of male and female

conspecific chemicals in water, nor of their own scents, which suggests that sex is not discriminated, or that it does not affect their space use (Polo-Cavia et al., 2009).

2.3 Sea turtles

Sea turtles (Fam. Cheloniidae) have secretory Rathke's glands that produce lipids and water-soluble proteins of high molecular weight (Solomon, 1984; Weldon & Tanner, 1990; reviewed in Weldon et al., 2008), but secretions are often considered as mere excretory products. Sea turtles probably use chemoreception, together with magnetic navigation, in homing behavior, but this is not based on conspecific pheromones but on environmental chemical cues from their natal beach (Grassman, 1993). However, behavioral observations in captivity of green sea turtles (*Chelonia mydas*) show that males smell the cloacas of breeding females more often than those of nonbreeding females (Cowell Comuzzie & Owens, 1990), which suggests that pheromones might signal female receptivity, or be used by males to locate females.

3 RHYNCHOCEPHALIA

The two extant species of tuatara (*Sphenodon* spp.) have two cloacal glands that produce potential pheromones (Gabe & Saint-Girons, 1965). Cloacal secretions of adult male and female *S. punctatus* contain several triacylglycerols derived from unusual medium chain-length fatty acids (one of them named as tuataric acid), and a major glycoprotein (Flachsbarth et al., 2009). Individual tuatara shows specific long-term stable mixtures of these glycerides, which might allow individual recognition (Flachsbarth et al., 2009). However, it is still unclear whether pheromones are used in reproductive behavior because chemosensory exploration by TF is not observed during interactions between males or during courtship or mating (Gans et al., 1984). Nevertheless, tuatars frequently bite cotton balls bearing prey chemicals, suggesting that airborne chemicals may be detected via olfaction (Besson et al., 2009; Cooper et al., 2001). Also, male tuatars (*S. punctatus*) investigate faster, more often, and for longer cloacal gland secretions of conspecific males than a water control, but there is no interpopulation discrimination (Wu & Waas, 2017).

4 CROCODYLVIA

Studies of crocodiles have mainly analyzed the morphology of their glands and the chemical composition of gland secretions (Weldon et al., 2008; Weldon & Wheeler, 2000), but their behavior remains little studied, perhaps because of the practical difficulties of working with them. Crocodiles do not have a VNO, but olfaction and taste seem to be used for chemosensory detection, at least for food chemicals in

water (Halpern, 1992; Hansen, 2007; Weldon, Swenson, et al., 1990). The gular and paracloacal glands of crocodiles are thought to produce pheromones involved in nesting and mating behaviors, but their specific function is unknown (Weldon & Ferguson, 1993).

The gular glands are a pair of eversible glands, located on the ventral part of the lower jaw, which secrete lipids such as fatty acids, squalene, cholesterol, alcohols, and α -tocopherol. Gular secretions show sexual and individual variations and are thought to be used by females to scent mark the nest sites (Weldon et al., 1987; Weldon & Ferguson, 1993; Weldon & Sampson, 1988; Weldon, Scott, & Tanner, 1990; Weldon & Tanner, 1991; Weldon & Wheeler, 2000).

The paracloacal glands are a pair of glands, located in the cloaca, that secrete hydrocarbons, fatty acids, alcohols, triacylglycerols, steroids and their esters, and phospholipids (Dunn et al., 1993; García-Rubio et al., 2002; Shafagati et al., 1989; Weldon et al., 1988; Weldon, Scott, & Tanner, 1990; Weldon & Tanner, 1991; Wheeler et al., 1999). Additionally, unusual novel compounds with unknown functions are occasionally found, such as aromatic ketones and some steroidal esters, which reflects some novel biosynthetic capabilities of crocodiles (Whyte et al., 1999; Yang et al., 1999). Paracloacal gland secretions show sexual and individual variation that might be used in mating and nesting behavior (Weldon & Ferguson, 1993; Weldon & Wheeler, 2000). However, the only behavioral evidence of pheromone detection in crocodiles is that prolonged sniffing behavior has been observed in *Caiman crocodylus* in response to conspecific gland secretions (Huggins et al., 1968) and that yearling *Alligator mississippiensis* responds to airborne conspecific scents (Johnsen & Wellington, 1982).

5 SQUAMATA: AMPHISBAENIANS

Amphisbaenians are a group of reptiles morphologically and functionally adapted to a fossorial life. Amphisbaenians are genetically related to lacertid lizards, but were traditionally considered a distinct clade of squamata reptiles, and for this reason we treat them here separately. Morphological adaptations to burrowing include trunk elongation, head modification, highly reduced vision, and loss of limbs in most species (Gans, 1978). Ecology and reproductive behavior of amphisbaenians are poorly known, but chemo-reception should be especially important for these almost blind reptiles. Many amphisbaenians have several precloacal pores connected to precloacal glands that produce copious holocrine secretion, especially during the breeding season (Antoniazzi et al., 1993, 1994; Gabe & Saint-Girons, 1965; Whiting, 1967). Morphological and microscopic studies suggest that as amphisbaenians move inside tunnels, the secretion plugs are abraded against the substrate releasing a secretion trail (Jared et al., 1999). These scent marks may contain pheromones important in intraspecific communication inside tunnels (Recio et al., 2023).

Male Iberian worm lizards (*Blanus cinereus*) detect and discriminate between pheromones of males and females (Cooper et al., 1994) (Fig. 2), and between their own scent and those of other males (López et al., 1997), which might allow them to recognize their own scent marked burrows (López et al., 2000). The ability to discriminate between sexes was greater from precloacal gland secretions than from skin chemicals (Cooper et al., 1994). Chemical analyses of precloacal gland secretions of both male and female *B. cinereus* found a great amount of steroids (mainly cholesterin methyl ether and cholesterol) and minor quantities of squalene, fatty acids, waxy esters, and α -tocopherol

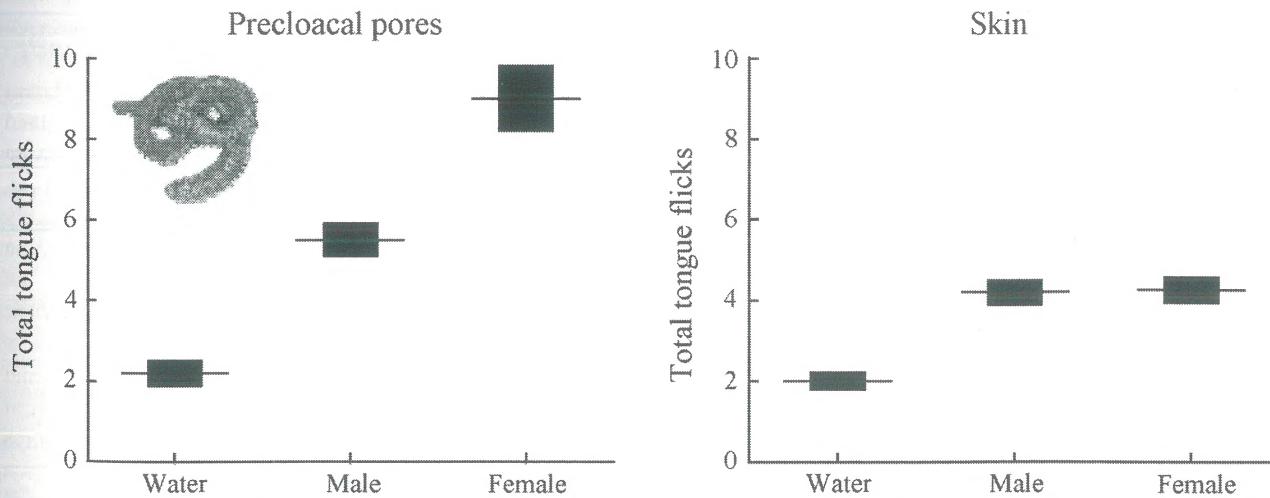


FIG. 2 Chemosensory sex discrimination by amphisbaenians. Number (mean \pm SE) of total tongue-flicks emitted in 1 min. by male amphisbaenians (*Blanus cinereus*) in response to the presentation of cotton swabs bearing deionized water (odorless control), or scent from the precloacal pores or the skin of conspecific males and females. (Redrawn from Cooper, W. E., Jr., López, P., & Salvador, A. (1994). Pheromone detection by an amphisbaenian. Animal Behaviour, 47, 1401–1411.)

(López & Martín, 2005b). The abundance of steroids and waxy esters may be used to scent mark underground tunnels, allowing orientation or conspecific trailing (Jared et al., 1999; López et al., 2000; Recio et al., 2023). There are clear intersexual differences in the presence/absence of some compounds and in relative proportions of some shared compounds (López & Martín, 2005b), which may explain pheromonal sex discrimination (Cooper et al., 1994). Squalene was much more abundant in males, and males, but not females, respond aggressively to cotton swabs bearing high concentrations of squalene (López & Martín, 2009). Thus, squalene may be part of the male sex recognition system of this amphisbaenian. Similarly, male garter snakes present more squalene in the skin, and courtship to females is partially inhibited if squalene is experimentally added to female skin (Mason et al., 1989). Furthermore, larger *B. cinereus* males have higher proportions of cholesterol and squalene, and lower proportions of cholesterol methyl ether, which might explain body-size-related responses to scent marks (Recio et al., 2023).

The checkerboard amphisbaenian (*Trogonophis wiegmanni*) does not have external precloacal pores, but at least males recognize the sex of conspecifics based on cloacal scent, probably produced by internal cloacal glands (Martín et al., 2020, 2023). Moreover, both males and females discriminate between a familiar partner and an unfamiliar individual of the same sex as the partner, and males discriminate between self and unfamiliar male scents (Martín et al., 2020). Further, adult females, but not males, can discriminate between the scents of familiar juveniles that live associated with them, likely their offspring, and unfamiliar ones (Martín et al., 2021). Most abundant lipids in feces and cloacal products are steroids (mainly cholesterol and cholestanol), alkanes, alkenes, and squalene, and there are intersexual differences (Martín et al., 2023).

6 SQUAMATA: LIZARDS

6.1 Secretory glands and potential pheromones

Lizards have several possible sources of pheromones. Chemicals with the potential function of pheromones are found in the skin and, in many lizards, secreted by large specialized external holocrine glands (femoral, preanal, or precloacal glands), or internal cloacal glands (Mason, 1992; Mayerl et al., 2015; Weldon et al., 2008). The activity of these glands depends on sex hormones, such that secretion is greater during the mating season. This strongly suggests the role of the chemicals secreted by these glands on reproduction. Morphological descriptions of these glands (Gabe & Saint-Girons, 1965) and characterization of the chemicals secreted (Weldon et al., 2008) are known for some lizard species from diverse taxonomic

groups. However, in most cases, it is not clear, or it has never been tested, whether specific compounds have activity of pheromones and their specific functions.

6.1.1 Skin

The skin of lizards contains lipids that are sequestered in the mesos layer of the epidermis. The skin of green iguanas (*Iguana iguana*) contains alcohol, steroids, fatty acids, and waxy esters (Roberts & Lillywhite, 1980). Similarly, analyses of many lizard species from most families found in the skin steroids, fatty acids, phosphatidylcholines and phosphatidylethanolamines, and hydrocarbons in some species (Weldon & Bagnall, 1987; Weldon et al., 2008). Lipids protect against water loss of the skin (Roberts & Lillywhite, 1980), but they may also allow, at least, for species and sex chemosensory recognition. Thus, there is taxonomic variation in the skin lipids, which “combinations” are characteristics of each species (Weldon & Bagnall, 1987). Moreover, in leopard geckos (*Eublepharis macularius*), there are sex differences in skin lipids (Mason & Gutzke, 1990). Fatty acids, hydrocarbons, and steroids are found in both sexes, but some steroids are only found in males, and long-chain methyl ketones are characteristic of females. Males court females, but when females are shedding the skin, males respond aggressively to females, as if they were males, suggesting that sex identification depends on skin lipids (Mason & Gutzke, 1990). Furthermore, in the striped plateau lizard (*Sceloporus virgatus*), an uncharacterized skin lipid (probably a phytosterol) is only found in receptive females, and females with larger clutches have less stearic and oleic acids in the skin (Goldberg et al., 2017), suggesting that males might predict the reproductive state and quality of females based on skin lipids.

6.1.2 Femoral, precloacal, and preanal glands

Many studies have shown pheromonal detection in different lizard species based on precloacal, preanal, or femoral gland secretions (e.g., Alberts, 1993; Aragón et al., 2001b; Labra et al., 2002; reviewed in Baeckens, 2019; Mason & Parker, 2010; Mayerl et al., 2015). All these glands are probably homologous structures that differ in their relative position on the body (Gabe & Saint-Girons, 1965). Glands are formed by an invagination of the stratum germinativum, which forms follicular units that produce copious amounts of holocrine secretion. Glands are connected to epidermal structures called femoral pores (located on the ventral surface of the thigh) (Fig. 3) or precloacal or preanal pores (located on the anterior edge of the cloacae), through which a “waxy” secretion is slowly secreted (Cole, 1966).

The secretory activity of the femoral and precloacal glands is greatest in the breeding season and in males, being usually

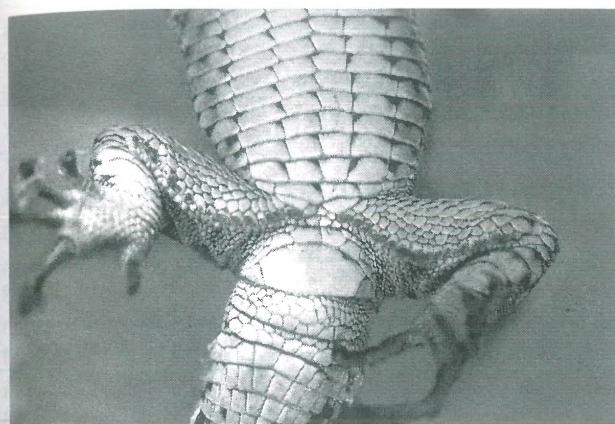


FIG. 3 Femoral glands of male rock lizards (*Iberolacerta cyreni*) (shown as a row of femoral pores with “waxy” secretion on the ventral surface of the thighs). (Photograph by J. Martín.)

scarcer or absent in females, which indicates that gland secretions are under direct androgenic control and vary seasonally with androgen production (Alberts, 1993; Cole, 1966; Van Wyk, 1990). For example, productivity and chemical composition of femoral secretions of green iguanas (*I. iguana*) vary seasonally in relationship with plasma testosterone (T) levels (Alberts, Pratt, & Phillips, 1992; Alberts, Sharp, et al., 1992). Also, in other lizards, castration of males causes femoral glands to atrophy, whereas supplementation of T increased secretion rates (e.g., Baeckens, Huyghe, et al., 2017; Chauhan, 1987; Fergusson et al., 1985; see review in Mason, 1992). Thus, the amount of secretion in scent marks may reflect the physiological condition and reproductive state of individual lizards (Martins et al., 2006). The ventral location of femoral and precloacal pores allows chemical secretions to be passively deposited on substrates as lizards move through their home ranges, which may serve to scent mark territories. Some lizards also show rubbing behavior of the cloaca and pores against substrates, suggesting active scent marking. Seasonal increases in gland production may allow lizards to mark more sites rather than influence the quality of the signal on a single site (Martins et al., 2006).

The number of precloacal or femoral glands/pores varies, within each species, within a limited characteristic range (often used in taxonomy). Interspecific variation could be directly related to the rate of secretion needed for effective scent marking, compensating for different environmental conditions (Alberts, 1992b), or may reflect the relative importance of chemical communication for a given species. Thus, in South American *Liolaemus* lizards (Fam. Liolaemidae), species with more precloacal pores inhabit more humid and cold environments, suggesting that lizards produce more secretions under harsh conditions (Escobar et al., 2001; Jara et al., 2018). However, in lacertid lizards, interspecific differences in the number of femoral pores do

not depend on climate or latitude, although shrub-climbing species tend to have fewer pores than species using other substrates (Baeckens et al., 2015). There is also a strong phylogenetic inertia component in the number of glands/pores across species (García-Roa, Jara, Baeckens, et al., 2017; Pincheira-Donoso et al., 2008). Nevertheless, despite this phylogenetic signal, the number of glands has extensively diversified over time and across lineages, with a basal ancestor that likely lacked these glands, from which glands emerged and disappeared repeatedly and independently during the evolution (García-Roa, Jara, Baeckens, et al., 2017).

Chemical composition of femoral or precloacal gland secretions has been studied in some lizard species (reviewed in Martín & López, 2014; Mayerl et al., 2015; Weldon et al., 2008) showing that secretions are composed of both lipids and proteins. Proteins may be the major components in secretions (Alberts, 1990, 1991; Alberts et al., 1993; Mangiacotti, Pezzi, et al., 2019). The patterns of protein composition of different species depend on phylogenetic affinities, but also on environmental characteristics (Alberts, 1991). Inter- and intraspecific variations in proteins of secretions are consistent over time and can be responsible for species, self and individual recognition (Alberts, 1991; Alberts & Werner, 1993; Mangiacotti et al., 2020, 2021; Mangiacotti, Gaggiani, et al., 2019), but lipids may be the main compounds involved in signaling condition (Mason, 1992; Martín & López, 2015; see below). Lipids are more volatile and have a high degree of molecular diversity, which increases the potential information content of a pheromone. Moreover, lipids are regulated by the general metabolism of each individual, such that they can vary between individuals and over time, being directly related with the current characteristics, condition, and health state of each individual signaler (Martín & López, 2015). Nevertheless, both lipids and proteins may be needed for the functionality of lizard chemical signals, and in lacertid lizards, the composition and complexity of the lipid and protein fractions are strongly correlated (Mangiacotti et al., 2023). Lipids might not simply be held in the proteinaceous matrix, but also be protected by specific proteins with binding, regulatory, anti-oxidant, anti-bacterial or immune functions (Ibáñez et al., 2022; Mangiacotti et al., 2023; Tellkamp et al., 2020).

Iguanian lizards were considered mainly visually oriented lizards, but many have secretory glands and are capable of chemosensory conspecific recognition. The lipophilic fraction of femoral gland secretions of male green iguanas (*I. iguana*) (Fam. Iguanidae) contains fatty acids and their esters, and several steroids (Alberts, Pratt, & Phillips, 1992; Weldon, Dunn, et al., 1990), which do not differ qualitatively between adults and juveniles (Weldon, Dunn, et al., 1990). However, seasonal variations in T levels in plasma are related to changes in lipid composition of secretions,

with proportions of unsaturated fatty acids increasing during the mating season, which may enhance volatility and detectability of secretions (Alberts, Pratt, & Phillips, 1992; Alberts, Sharp, et al., 1992). The amounts of steroids and fatty acids in secretions vary among individuals. Some steroids are phytosterols, which have to be obtained from the diet (Alberts, Pratt, & Phillips, 1992) and, thus, might potentially inform on the ability of an individual to obtain food or on the quality of his territory. Proteins in femoral secretions have been analyzed in desert iguanas (*Dipsosaurus dorsalis*) and green iguanas (*I. iguana*) (Alberts, 1990, 1991; Alberts et al., 1993). Intraspecific variations in protein composition may allow sex and individual recognition (Alberts & Werner, 1993). Lipids might attract conspecifics from long distances but individual identification might be based on nonvolatile proteins in substrate scent marks.

Marine iguanas from the Galápagos Islands (*Amblyrhynchus cristatus*) (Fam. Iguanidae) have femoral secretions with few lipids, mostly fatty acids and steroids (mainly cholesterol) (Ibáñez et al., 2017) and many proteins (Tellkamp et al., 2020). Lipids vary greatly among populations (islands), but are not linked to genetic differences (Ibáñez et al., 2017). Galapagos land iguanas (*Conolophus spp.*) (Fam. Iguanidae) have femoral secretions with a high variety of compounds, mainly saturated and unsaturated fatty acids, steroids, and aldehydes (which are totally absent in marine iguanas) (Colosimo et al., 2020; Ibáñez et al., 2017).

In lizards of the genus *Liolaemus* spp. (Fam. Liolaemidae), precloacal gland secretion allows chemosensory conspecific- and self-recognition (Labra, 2011; Labra et al., 2002; Labra, Beltrán, et al., 2001; Labra, Escobar, et al., 2001; Labra & Niemeyer, 1999). Lipids in precloacal secretions of 20 species of *Liolaemus* include mainly alkanes, long-chain fatty acids, and steroids, with cholesterol and five fatty acids appearing in all species, but with clear interspecific differences in chemical profiles (Escobar et al., 2001; Labra, Escobar, et al., 2001). Small differences in secretions between two populations of *L. fabiani* might be explained by different environmental conditions (Escobar et al., 2003). In *L. wiegmanni*, one of the few lizards where precloacal gland secretions of females have been examined, most compounds (steroids and waxy esters, followed by squalene, alcohols, aldehydes, and carboxylic acids) are shared by both sexes, but there are clear intersexual differences in proportions of many compounds (García-Roa et al., 2016).

In 12 species of spiny lizards of the genus *Sceloporus* (Fam. Phrynosomatidae), femoral secretions are mainly composed of fatty acids, followed by salicylates, aldehydes, alcohols, alkanes, ketones, steroids, etc. (Campos et al., 2020). Species living in colder habitats have greater proportions of unsaturated fatty acids and lower proportions of aldehydes (Campos et al., 2020). Moreover, in secretion of

S. virgatus and related species, two cyclic dipeptides, cyclo(L-Leu-L-Pro) and cyclo(L-Pro-L-Pro), have also been found. These are highly odorous pyrazine derivatives that are discriminated by males and might act as pheromones (Pruett et al., 2016; Romero-Díaz et al., 2020).

The preanal gland secretion of male Hardwick's spiny-tailed lizards (*Uromastix hardwickii*) (Fam. Agamidae) contain fatty acids, triacylglycerols, waxy esters, steroids, and phospholipids, while females have only steroids, and phospholipids (Chauhan, 1986). In the related Egyptian spiny-tailed lizard (*U. aegyptia microlepis*), preanal gland secretions contain mainly steroids and fatty acids, but also terpenoids, ketones, α-tocopherol, aldehydes, and alcohols. Males have higher proportions of fatty acids and α-tocopherol, but lower proportions of ketones than females (Martín et al., 2016). The most abundant compounds would not be stable as substrate scent-marks in the desert hot-arid climate where these lizards inhabit, but secretions could rather be adapted to persist under the more favorable micro-climatic conditions inside burrows where these lizards spend time (Martín et al., 2016).

Lipids in femoral gland secretions have also been examined in Iguanian lizards of other families such as in the Great Basin collared lizard (*Crotaphytus bicinctores*) (Fam. Crotaphytidae), where the main compounds in secretions of males are steroids (mainly two triunsaturated steroids and cholesterol), fatty acids, waxy esters, alcohols, and aldehydes (Martín, Ortega, & López, 2013).

In the yellow-belly gecko (*Hemidactylus flaviviridis*) (Fam. Gekkonidae) the preanal gland secretions of males are composed of fatty acids, triacylglycerols, waxy esters, steroids, and phospholipids (Chauhan, 1986). In the Australian Bynoe's gecko species complex (*Heteronotia binoei*) (Fam. Gekkonidae), precloacal gland secretions, which include steroids and fatty acids, are lineage specific and have diverged relatively more than morphology, suggesting that pheromones may influence behavioral isolation (Zozaya et al., 2019). Among-lineage chemical variation is associated with geographic variation in precipitation but not temperature (Zozaya et al., 2022).

The femoral secretions of males and females of the South African giant girdled lizard or sungazer (*Cordylus giganteus*) (Fam. Cordylidae) contain only semi-volatile chemicals, including fatty acids, alcohols, ketones, esters, and steroids (Louw et al., 2007). Femoral secretions could be important in the social biology of this lizard as it occurs in the related Cape girdled lizards (*C. cordylus*), which detect and respond differentially to male and female pheromones (Cooper et al., 1996).

Many studies have examined the lipophilic fraction of femoral secretions of males of several, mainly European, species of the Fam. Lacertidae (Baeckens, García-Roa, et al., 2017;

Baeckens, Martín, García-Roa, Pafilis, et al., 2018). Lipids in secretions are mainly steroids and fatty acids with, usually, minor quantities of alcohols, squalene, ketones, waxy esters, aldehydes, tocopherol, etc. Phylogenetic macro-evolutionary modeling suggests that lipids in femoral secretions have proliferated across species following highly different compound-specific evolutionary speeds and trajectories (García-Roa, Jara, López, et al., 2017), leading to patterns of presence and proportion of lipids characteristics of each species. This interspecific variation has little evidence of phylogenetic relatedness but is rather explained by the thermal and hydric environment where each species inhabits (Baeckens, Martín, García-Roa, Pafilis, et al., 2018). Variation in pheromone design may result from differential natural selection, tending to optimize signal efficacy in contrasting environments. This is because unfavorable conditions may alter the scent-marks (Martín et al., 2015; Martín & López, 2013). However, there is no evidence that species with intensified sexual selection have more elaborate signals (Baeckens, Martín, García-Roa, & Van Damme, 2018), and overall diet seems a relatively poor predictor of interspecific differences (Baeckens, García-Roa, et al., 2017). Nevertheless, within a given species, experimental changes in diet can modify proportions of compounds in secretions (García-Roa, Saiz, Gómara, et al., 2017; Kopena et al., 2011, 2014).

Cholesterol is the main lipid in femoral secretions of many lacertid species, found, for example, in high proportions in rock lizards (*Iberolacerta* spp.) (López & Martín, 2005c; Martín, Moreira, & López, 2007), wall lizards (*Podarcis* spp.) (Martín & López, 2006e), and common lizards (*Lacerta vivipara*) (Gabirov et al., 2008). However, the great abundance and ubiquity of this compound in secretions are thought to be mainly useful to constitute an unreactive apolar matrix that delivers the compounds that would be the true semiochemicals but that are found in relatively lower concentrations (Escobar et al., 2003; Weldon et al., 2008). In other species, other compounds are the main ones, such as campesterol in *Psammodromus algirus* (Martín & López, 2006c). In spiny-footed lizards (*Acanthodactylus erythrurus* and *A. boskianus*), long-chain alcohols (e.g., hexacosanol and tetracosanol) are the most abundant compounds, followed by steroids (cholesterol and dehydrocholesterol), fatty acids, glycerol monoethers, and other lipids (Khannoob et al., 2011; López & Martín, 2005d). In secretions of “green lizards” (*Lacerta schreiberi*, *L. viridis*, *L. agilis* or *Timon lepidus*), α -tocopherol is the main compound (Baeckens, Martín, García-Roa, Pafilis, et al., 2018; Kopena et al., 2009; López & Martín, 2006; Martín & López, 2010a), which may protect other compounds from oxidation in wet environments. For example, in Tenerife lizards (*Gallotia galloti*), where α -tocopherol is also an important compound in femoral secretions, the two subspecies from more humid areas have secretions with more α -tocopherol during the breeding season than the subspecies from xeric areas (García-Roa, Megía-Palma, Ortega, et al., 2017).

Similarly, other interpopulational differences in secreted lipophilic chemicals may be related to different environments. In Guadarrama wall lizards (*Podarcis guadarramae*), differences in secretions between two nearby and genetically similar populations inhabiting different altitudes and microclimates may reflect selection for the persistency and efficiency of pheromones in each population (Martín et al., 2015; Martín & López, 2006e). Also, relative proportions of some classes of compounds (i.e., ethyl esters of fatty acids, waxy esters, and aldehydes) in secretions of *Ps. algirus* lizards varied along a 2200 m mountain elevational gradient, likely as an adaptation to different local conditions (Martín et al., 2017). In other species, interpopulational differences in lipids might be mainly explained by distinct genetic differences between diverging populations (Gabirov et al., 2008; Khannoob et al., 2013; Martín, López, et al., 2013)

Within each species, and the same population, there is high interindividual variability in relative proportions of compounds. Age class differences are found in *Ps. algirus* (Martín & López, 2006c), where older males have secretions with lower proportions of fatty acids and higher proportions of steroids than younger adult males. This might allow conspecific to assess male age, which is important in social behavior because older males are territorial while younger males adopt a satellite-sneaker strategy (López, Martín, & Cuadrado, 2003; Nisa-Ramiro et al., 2019). Lacertid lizard species with different color morphs within the same population may also have a chemical polymorphism in lipids and proteins in male femoral secretions and be capable of chemosensory recognition between morphs (Brock et al., 2020; López, Moreira, & Martín, 2009; Mangiacotti, Fumagalli, et al., 2019; Pellitteri-Rosa et al., 2014). Proteins in femoral gland secretions of lacertids also show seasonal, inter- and intra-population variability (Ibáñez et al., 2022; Mangiacotti et al., 2017, 2023; Mangiacotti, Pezzi, et al., 2019).

In Carpetan rock lizards (*Iberolacerta cyreni*) (formerly *Lacerta monticola cyreni*), morphological traits and health states of different males are related to the variability in relative proportions of some lipids in their secretions. These relationships suggest that conspecifics could get reliable information on the producer of a scent mark based on chemicals alone, something supported by experimental manipulations of secretions and scent marks (López et al., 2006; Martín & López, 2006a, 2006b, 2007).

Interestingly, an island introduction experiment of Aegean wall lizards (*Podarcis erhardii*) from a predator-rich island to five predator-free islets showed that, after 4 years, femoral secretions of males from the islets had a richer chemical composition and higher proportions of three lipids likely associated with territoriality and mate choice (Donihue et al., 2020). These results suggest that the chemical signal design can shift rapidly and predictably in novel ecological contexts

(i.e., lower predation risk and higher intrasexual competition due to high population densities in the islets).

Femoral gland secretions of wild male tegu lizards (*Salvator merianae*) (Fam. Teiidae) contain cholesterol and high amounts of saturated fatty acids (mainly hexadecanoic and octadecanoic). There are contrasting differences between wild and captive-bred males, which lack cholesterol but present a high amount of linoleic acid, indicating the important influence of different diets on chemical composition of gland secretions (Martín et al., 2011; Nisa-Ramiro et al., 2020).

In whiptail lizards (*Aspidoscelis lineattissimus*) (Fam. Teiidae), femoral secretions are mainly composed of fatty acids and their esters, squalene, and steroids, etc., but the composition varies between populations. Females and males discriminate and are more attracted to lizard scents from their own than from different populations, suggesting that there may be partial pheromone-mediated premating isolation (Raya-García et al., 2020).

6.1.3 Cloacal glands and feces

Chemicals with functions of pheromones are secreted onto the surface of the feces, probably from internal cloacal glands, as feces are deposited by the lizard. Chemicals in feces can be discriminated and may serve for territorial scent marking in some lizards (Aragón et al., 2000; Bull et al., 2001; Bull, Griffin, & Johnston, 1999; Bull, Griffin, & Perkins, 1999; Carpenter & Duvall, 1995; Duvall et al., 1987; López et al., 1998; Wilgers & Horne, 2009). Chemical signals from scat of Australian tree skinks (*Egernia striolata*) and Stoke's skinks (*E. stokesii*) (Fam. Scincidae) have properties of pheromones (Bull, Griffin, & Johnston, 1999; Bull, Griffin, & Perkins, 1999). Feces of each individual seem to have a unique signature, because lizards respond more strongly to scat of unfamiliar conspecifics than to their own scat, independently of their diets (Bull, Griffin, & Johnston, 1999; Bull, Griffin, & Perkins, 1999). Tokay geckos (*Gekko gecko*) can discriminate their own skin and fecal chemicals from those of same-sex conspecifics (Szabo & Ringler, 2023). Similarly, in lacertid lizards, fecal chemicals may provide similar or complementary information to femoral gland secretions. In male rock lizards (*I. cyreni*), both femoral secretions and fecal chemicals allow for self-recognition, familiar discrimination, and signaling body size (Aragón et al., 2000, 2001b; López et al., 1998). Females of *Ps. algirus* lizards can discriminate between young (sneakers) and old (territorial) males based on chemical cues from either femoral secretions or from feces, and in both cases, females show more interest in scents of old males (Nisa-Ramiro et al., 2019).

Specific chemicals in feces with properties of pheromones have not been identified, but they are probably a combination

of several lipids as they are contained in scat extracts made with organic solvents (dichloromethane), and fractionation of the scat with different solvents (pentane and methanol) led to loss of the unique signals needed for individual or self-recognition (Bull, Griffin, & Perkins, 1999; Labra et al., 2002). Male and female *Liolaemus chilensis* are more active when exposed to lipids extracted from male feces than from female feces or control, but show low responsiveness, suggesting that fecal lipids may convey some information for conspecific recognition, but probably other stimuli (e.g., proteins in feces or visual cues) are also needed for a complete behavioral response (Valdecantos et al., 2020). In some lizards and geckos, feces are deposited on visually conspicuous sites (e.g., on higher rocks) or have an aggregated spatial distribution (Baeckens et al., 2019; Carpenter & Duvall, 1995; Duvall et al., 1987; López et al., 1998), such that the visual location of the pellet at long distance might further elicit the search for chemical signals from the scat or nearby scent marks from other glandular sources. This suggests that feces may act as a composite signal (visual and chemical) in territorial marking.

6.1.4 Urodeal glands

Urodeal glands are found in the cloaca of female lizards mainly in the Fam. Cordylidae and Scincidae. These are tubular organs that empty into folds of the cloacal ducts via small orifices (Gabe & Saint-Girons, 1965; Trauth et al., 1987). Glands are active and have more secretions during the breeding season. In broad-headed skinks (*Eumeces laticeps*) the neutral lipid fraction of urodeal glands, consisting of steryl and waxy esters and acylglycerols, has properties of a sex pheromone, eliciting courtship by males (Cooper et al., 1986; Cooper & Garstka, 1987; Trauth et al., 1987). A similar pheromone from the urodeal gland is thought to occur in females of the cordylid black-lined plated lizard (*Gerrhosaurus nigrolineatus*) (Cooper & Trauth, 1992).

6.2 Chemosensory recognition

A plethora of studies measuring TF rates and other behavioral responses to different scent stimuli have found chemosensory discrimination of conspecific chemicals in many lizard species from most taxonomic groups. Pheromones allow lizards to recognize conspecifics from closely related heterospecifics (e.g., Barbosa et al., 2006; Cooper & Pérez-Mellado, 2002; Cooper & Vitt, 1987; Zozaya et al., 2019), males from females (Cooper et al., 1996; Cooper & Trauth, 1992; Cooper & Vitt, 1984), self from other individuals and familiar from unfamiliar individuals (Alberts, 1992a; Alberts & Werner, 1993; Aragón et al., 2001a, 2001b; Bull et al., 2000; Bull, Griffin, & Johnston, 1999;

Graves & Halpern, 1991), and kin from nonkin (Bull et al., 1994, 2001; Lena & de Fraipont, 1998; Main & Bull, 1996; Werner et al., 1987). Even species considered exclusively visually oriented, with predominant visual displays, very low TF rates, and absence of secretory external glands, such as *Anolis* lizards, can detect conspecific chemical cues (Baeckens et al., 2016).

However, few studies have yet documented the chemical basis of pheromonal recognition and whether lizards can discriminate between different compounds in gland secretions (Cooper et al., 2002a, 2002b; Cooper & Pérez-Mellado, 2001; Martín & López, 2006b, 2006d, 2008b). The lacertid Lilford's wall lizard (*Podarcis lilfordi*) discriminates between lipids, proteins, and carbohydrates, and also among different lipids such as glycerol, cholesterol, and oleic, and hexadecanoic acids (Cooper et al., 2002a, 2002b). Female wall lizards (*P. guadarramae*) and rock lizards (*I. cyreni*) discriminate and assess changes in concentrations of several steroids found in femoral secretions of males (Martín & López, 2006b, 2006d). Intersexual differences in responses to different lipids suggest that secretions contain multiple messages (multiple pheromones) with different information and intended receivers (López & Martín, 2012; Martín & López, 2008b). Male Yarrow's spiny lizards (*Sceloporus jarrovii*) can discriminate different concentrations of single compounds (two cyclic dipeptides) from the multiple chemicals found in femoral secretions, and single from combined mixtures of compounds, suggesting that minor changes in presence/absence or abundance of single key compounds in a multi-component signal may affect chemosensory responses (Romero-Díaz et al., 2020, 2021).

6.2.1 Sex and individual recognition

Most lizards from different taxonomic groups can discriminate between scent of male and female conspecifics (e.g., Liolaemidae: Labra & Niemeyer, 1999; Scincidae: Cooper & Vitt, 1984; Gekkonidae: Cooper & Steele, 1997; Mason & Gutzke, 1990; Lacertidae: Cooper & Pérez-Mellado, 2002; López & Martín, 2001a; Cordylidae: Cooper et al., 1996). Very often male and female pheromones elicit different TF rates and reproductive behaviors. For example, male leopard geckos (*E. macularius*) perform aggressive behaviors toward male, but not female, scent and tail vibrations (a courtship behavior) toward female, but not male, scents (Cooper & Steele, 1997). Pheromonal sex discrimination may be season-dependent suggesting their active role in reproduction. For example, during the post-reproductive season, tree-dwelling lizards (*Liolaemus tenuis*) have similar TF rates in conspecific and control enclosures, while during the mating season TF rates are higher and enclosures of females elicit more TF by both sexes (Labra & Niemeyer, 1999). Pheromones also allow lizards to obtain

additional information. For example, male wall lizards (*P. guadarramae*) show sex discrimination and differential responses to scent from gravid and nongravid females (Cooper & Pérez-Mellado, 2002).

The relative importance of visual and chemical cues to identify sex may vary between species, with visual identification being more important in species with sexual dichromatism (see Cooper & Greenberg, 1992). However, chemical stimuli are also clearly involved, and many species may rely more on chemoreception. Experimental manipulation of coloration and scent of male and female wall lizards (*P. guadarramae*) showed that males react more aggressively to intruders impregnated with male scent, independently of their actual sex and coloration (López & Martín, 2001a; López, Martín, & Cuadrado, 2002). Both color and scent are important in eliciting courtship. However, females painted as females and impregnated with female scent are preferentially courted. Males impregnated with female scent do not elicit aggressive responses but are often courted despite their actual sex and coloration. Pheromones seem to be more important than color patterns in sex recognition at close range (López & Martín, 2001a).

True individual recognition in lizards has received little attention, being demonstrated, at least, in wall lizards (Carazo et al., 2008), although several studies suggest discrimination between their own scent and those of other conspecific individuals (Fig. 4) in several skinks (Cooper, 1996; Graves & Halpern, 1991), iguanids (Alberts, 1992a), cordylids (Cooper et al., 1999), lacertids (Aragón et al., 2001b; Mangiacotti et al., 2020; Mangiacotti, Gaggiani, et al., 2019), liolaemids (Labra et al., 2003; Labra, Beltrán, et al., 2001; Labra & Niemeyer, 1999), and anguids (Gonzalo et al., 2004). Furthermore, class-level discrimination between scent of familiar and unfamiliar individual males (Fig. 4) has been found in males of several iguanid (Alberts & Werner, 1993; Glinski & O'Neil Krekorian, 1985) and lacertid lizards (Aragón et al., 2001a, 2001b, 2003; Carazo et al., 2008; López & Martín, 2002). Discrimination between scents of familiar and unfamiliar individuals of the opposite sex occurs in skinks (Cooper, 1996) and geckos (Steele & Cooper, 1997). However, most studies of familiar chemical discrimination were accomplished by housing animals together to create familiar individuals, while there is little direct empirical support for pheromonal discrimination among individuals whose actual social relationships in their natural environment are known. A study that combined field observations and laboratory experiments found that male rock lizards (*I. cyreni*) discriminate between scent of familiar males (those whose home ranges actually overlap in the field) and unfamiliar males (those whose home ranges are far apart) (Aragón et al., 2001a). Behavioral responses to scents of unfamiliar males depend on relative body size differences between males. In contrast, scents of

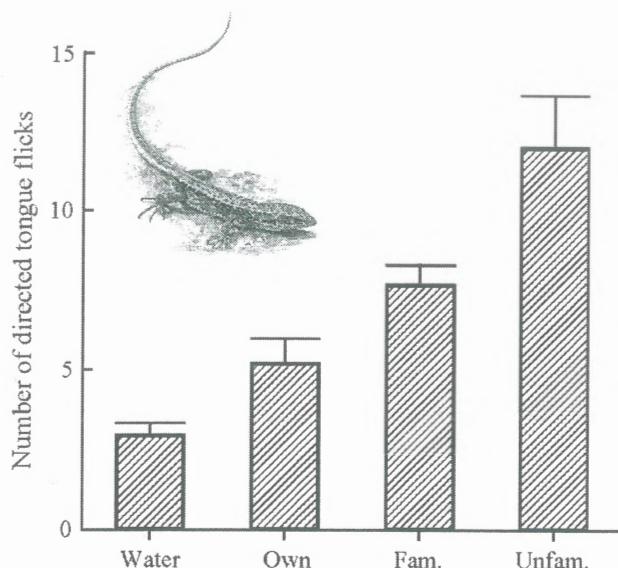


FIG. 4 Individual chemosensory recognition in lizards. Number (mean \pm SE) of directed tongue-flicks emitted in 1 min. by male rock lizards (*Iberolacerta cyreni*) in response to the presentation of cotton swabs bearing deionized water (odorless control), or scent from their own femoral gland secretions, or femoral gland secretions of familiar (Fam.) or unfamiliar males (Unfam.) (J. Martín and P. López, unpublished data).

different individual familiar males elicit different responses, but responses are unrelated to body size, suggesting individual discrimination of familiar neighbors (Aragón et al., 2001a). The ability of territorial lizards to discriminate between neighbors (familiar) and non-neighbors (unfamiliar) may help to stabilize social systems by reducing the frequency and intensity of aggressive encounters between neighbor males (Glinski & O’Neil Krekorian, 1985) or by favoring mate location (Cooper, 1996). Proteins in lizards’ gland secretions, because of their permanent direct relationship with genes, seem the best candidate to convey individual stable information that may allow self and individual recognition (Alberts & Werner, 1993; Mangiacotti et al., 2020; Mangiacotti, Gaggiani, et al., 2019).

6.2.2 Trailing

Pheromone-mediated location of conspecifics has been documented extensively in snakes (see below), but is poorly documented in other reptiles. In lizards, a few studies showed the use of scent trails to locate mating partners and to facilitate mate guarding in male broad-headed skinks (*E. laticeps*) (Cooper & Vitt, 1986), male snow skinks (*Niveoscincus microlepidus*) (Olsson & Shine, 1998), female sleepy lizards (*Tiliqua rugosa*) (Bull & Lindle, 2002), male slow worms (*A. fragilis*) (Gonzalo et al., 2004), and female tegus (*Salvator merianae*) (Richard et al., 2020). However, pheromone trailing might be widespread, because many lizards can detect substrate scent

marks of conspecifics (e.g., Aragón et al., 2001a; Cooper et al., 1996).

Chemosensory recognition of scent trails by males may facilitate location of new females, but may also allow prolonged mate guarding of the female partner (Olsson & Shine, 1998). In snow skinks (*N. microlepidus*), males and females form “pairs” for long periods, and males preferentially follow the scent trail of their vitellogenic female “partner” rather than that of another vitellogenic female, thus maintaining prolonged partnership (Olsson & Shine, 1998). Conversely, during the mating season, female sleepy lizards (*Tiliqua rugosa*) that are more frequently found with their male partner are more likely to follow the scent path of their male partner than less strongly bonded females (Bull & Lindle, 2002). However, after mating has finished, females no longer scent trail. Males do not follow their female partner in any season. These results suggest that female sleepy lizards play an active role in maintaining the partnership and monogamy.

6.2.3 Kin recognition

Kin recognition may allow lizards to recognize offspring and tolerate relatives, maintain stable family associations of mother and offspring or sibling juveniles, reduce kin competition, or avoid inbreeding. Mother-offspring recognition has been found in common lizards (*L. vivipara*) (Lena & de Fraipont, 1998), and the Australian skinks *E. stokesii* and *T. rugosa* (Bull et al., 1994; Main & Bull, 1996). Sibling recognition has been described in hatchling green iguanas (Werner et al., 1987) and juvenile skinks *E. striolata* (Bull et al., 2001). Kin recognition is presumably based on pheromonal cues, but in these studies, the use of additional cues was not discounted.

Kin discrimination suggests that individuals must have unique signals (e.g., Alberts et al., 1993) and that they can discriminate between different signals. The mechanisms that allow kin recognition might be either familiarity with signals of individuals associated with kin in the early part of the life or more interestingly phenotype matching, in which close relatives have genetically similar signals and are recognized just because of that similarity (Waldman, 1987). The highly variable major histocompatibility complex (MHC) may lead to different pheromones (likely proteins) in each individual, but more similar between related individuals, which may be involved in kin and individual recognition and in mate choice (Penn & Potts, 1999).

6.3 Pheromones in mate choice

Female mate choice was thought to be rare in lizards (Olsson & Madsen, 1995, 1998; Tokarz, 1995). Most studies only examined mate choice based on visual traits without

finding indications of mate choice, perhaps because in many cases visual traits do not provide reliable information on the male characteristics useful to females (Olsson & Madsen, 1995, 1998). In contrast, as it occurs in many other animals (Johansson & Jones, 2007), pheromones might provide more reliable and detailed information about males than might be obtained from visual traits alone. However, this possibility has been largely ignored in most studies of mate choice (Tokarz, 1995), despite pheromonal recognition being widespread among lizards.

Some studies have found evidence of female preferences for substrates scent marked by particular individual males, which may suggest active mate choice based on pheromones (e.g., López, Aragón, & Martín, 2003; López & Martín, 2005a; López, Muñoz, & Martín, 2002; Martín & López, 2000, 2006b; Olsson et al., 2003). Femoral secretions seem affected by the “quality” and health state of the individual male that produces them and may transmit chemical information about the characteristics of that male (Martín & López, 2015; Penn & Potts, 1998). By deciding where to establish their home ranges based on chemical characteristics of scent marks of males, females may increase the probability of mating with certain individual males (Fig. 5). The quality of male pheromones could communicate the heritable genetic quality of a male to a female, which in many cases may be the only benefit of mate choice to female lizards, and thereby serve as the basis for adaptive female choice.

In rock lizards (*I. cyreni*), when scent from two males of similar body size is offered, females preferentially associate with the scents of healthy and relatively heavier males (López, Muñoz, & Martín, 2002; Martín & López, 2000, 2006a, 2006b). This suggests that females prefer to stay in areas of males that secrete certain pheromones because those males are of higher genetic “quality” (Martín & López, 2006b).

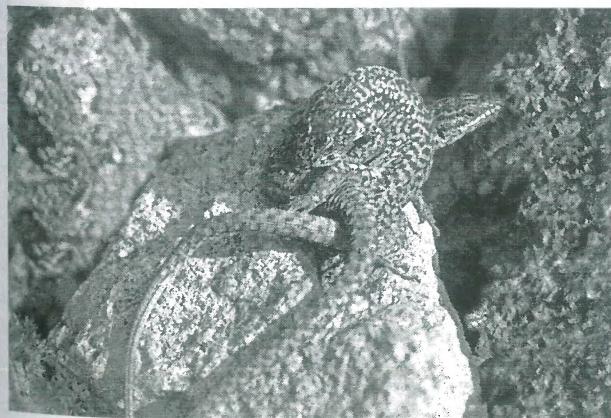


FIG. 5 Mating of male and female rock lizards (*Iberolacerta cyreni*). The male is biting the ventrolateral side of the female. The female is accepting the mating of this particular individual male, while advances by other individual males may be actively rejected. (Photograph by J. Martin.)

Female lizards may obtain other information from male pheromones. Female sand lizards (*Lacerta agilis*) prefer to associate with scent of males with an MHC genotype more different than their own (Olsson et al., 2003). A similar way to avoid inbreeding might function in other lizard species (Bull & Cooper, 1999). Selective mating with non-kin or unrelated pairs may confer genetic benefits because the new combinations of immunocompetence in offspring will defend them more effectively against evolving parasites (Penn & Potts, 1999).

When scents of two male rock lizards (*I. cyreni*) of different ages are offered, females associate preferentially with scents of older males (López, Aragón, & Martín, 2003). This suggests that females can assess the males’ age by their pheromones, and that females prefer using areas scent-marked by older territorial males (i.e., that have “proved their ability to survive”). Thus, females may increase their opportunities to mate with old males or may avoid harassment by sneaking young males. This agrees with field observations on females mating with old males, and rejection of advances by young males (López, Aragón, & Martín, 2003).

However, there is little knowledge of the role of specific chemicals (pheromones) as sexual signals in mate choice. Female rock lizards (*I. cyreni*) show stronger chemosensory responses and prefer the scent of individual males that allocate more cholesta-5,7-dien-3-ol (provitamin D₃), ergosterol (provitamin D₂) and oleic acid to femoral secretions, which suggests that females use these chemicals to choose between males’ scents (Martín & López, 2006b, 2010b). Females can discriminate these compounds, and changes in their concentration, from similar chemicals (i.e., cholesterol) also found in males’ secretions. Moreover, females, but not males, are more attracted to areas experimentally manipulated to increase the proportion of ergosterol in natural scent marks of males (Martín & López, 2006b, 2012). These results suggest that femoral secretions with higher proportions of these compounds might be reliable advertisements of the quality of a male that females could use to select mates. For example, cholesta-5,7-dien-3-ol is often found in the skin, where it will transform into vitamin D₃ after exposure to sun UV-B irradiation (Carman et al., 2000). Vitamin D₃ is essential in calcium metabolism and immune system regulation (Fraser, 1995; Hayes et al., 2003). Therefore, allocating provitamin D to femoral secretions might require diverting vitamin D from metabolism. Moreover, very often, the synthesis of vitamin D₃ in the skin is not sufficient to meet physiological requirements, and lizards require dietary intake of vitamin D as an essential nutrient (Ferguson et al., 2005). Thus, diet quality may affect quality of pheromones. After experimental supplementation of dietary vitamin D, male rock lizards (*I. cyreni*) increase the proportion of provitamin D₃ in

femoral secretions, which renders their scent marks more attractive to females (Martín & López, 2006a). This suggests that allocation of this provitamin to secretions is costly and dependent on the ability of a male to obtain enough vitamin D in the diet, which may explain why females select these males. Similar results were found concerning α -tocopherol (=vitamin E), a vitamin with important antioxidant and immunoregulatory metabolic functions, in femoral secretions of male green lizards (*L. viridis*) (Brigelius-Flohe & Traber, 1999). Areas scent marked by males with experimentally increased vitamin E (after a dietary supplementation) were preferred by females (Kopena et al., 2011).

6.3.1 Evolutionary mechanisms

Empirical research on the mechanisms that confer reliability to sexual signals has focused almost exclusively on visual signals, but little is known about the mechanisms that may maintain pheromones as reliable sexual signals. Some theoretical models of mate choice without direct benefits predict that signals used in sexual selection can only be evolutionarily stable if they are honest and condition-dependent or costly to the signaler and if the cost is correlated with the signaler's quality (Grafen, 1990; Pomiankowski, 1988; Zahavi, 1975). One possible cost is the energetic expenditure of producing and maintaining the immune system to fight against parasites, which may have a major effect on condition, thus creating a trade-off because resources

are used by both the condition-dependent sexual advertisement and the immune system (Sheldon & Verhulst, 1996; Wedekind & Folstad, 1994). Therefore, only individuals in good condition could mount a strong immune defense and produce an extravagant sexual ornament (Westneat & Birkhead, 1998).

Evidence for parasite-mediated sexual selection has been found in many animals that use visual ornaments to attract females. Studies with rodents and insects suggest that parasitic infections and immunocompetence may also affect the information content of pheromones (Penn & Potts, 1998; Rantala et al., 2002, 2003). Parasites and health state may affect pheromones of lizards too. For example, male common wall lizards (*Podarcis muralis*) with fewer parasites and greater immune responses have femoral secretions with higher proportions of two esters of oleic acid (Martín et al., 2008). Thus, pheromones might function in parasite-mediated sexual selection and females might use these relationships to select males. For example, female wall lizards (*P. guadarramae*) select scents of males with higher proportions of cholesta-5,7-dien-3-ol in femoral secretions (López & Martín, 2005a) (Fig. 6). This could be explained because proportions of cholesta-5,7-dien-3-ol in males are related to their immune response, such that only males with a good immune system might allocate higher amounts of this chemical to signaling. Similarly, scents of healthier *Ps. algirus* males are more attractive to females and chemical analyses showed that parasite load and the immune response

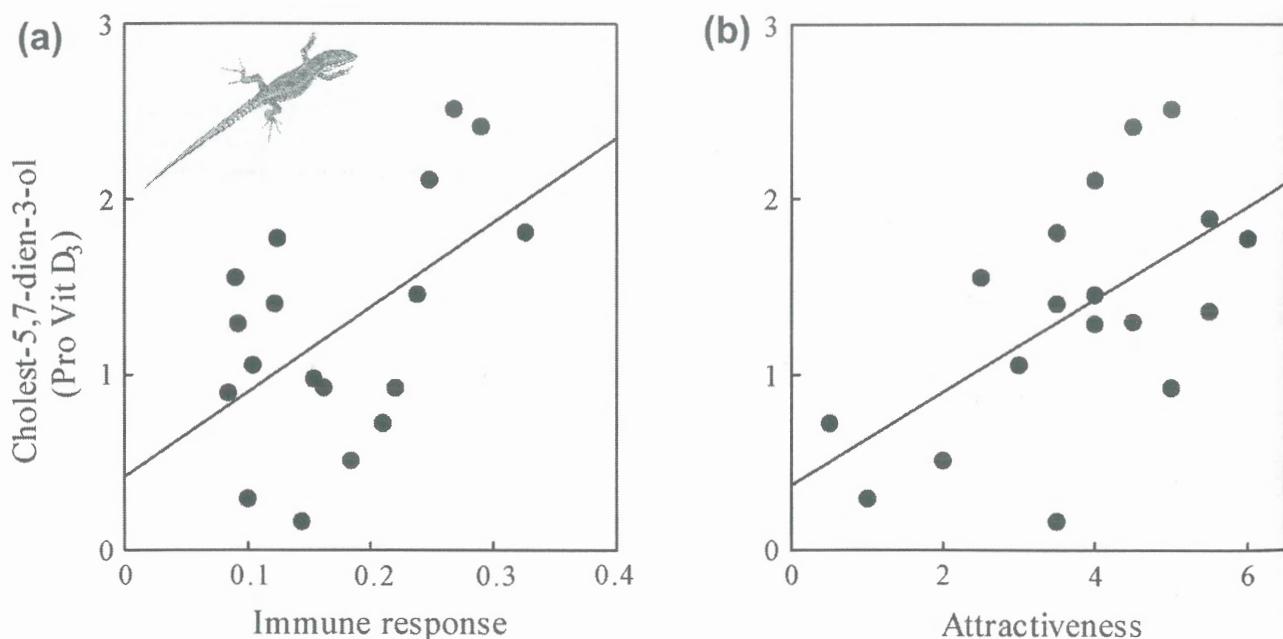


FIG. 6 Female mate choice in lizards. Relationships between the relative proportions of cholesta-5,7-dien-3-ol (pro vitamin D₃) in femoral gland secretions of male Iberian wall lizards (*Podarcis guadarramae*) and (A) the magnitude of their T-cell immune response (estimated with the phytohaemagglutinin, PHA, delayed-type hypersensitivity test), or (B) the attractiveness of their pheromones to females (estimated from the number of females that preferred substrates scented by a particular male) (J. Martín and P. López, unpublished data).

are related to the proportions in male secretions of some lipids (alcohols and fatty acids), which females can discriminate (Martín, Civantos, et al., 2007).

Pheromones might be honest signals if there were a tradeoff between sexual advertisement and the immune system, independent of the negative effects of parasites. In an experiment, the immune system of male wall lizards (*P. guadarramae*) was challenged with a bacterial antigen (lipopolysaccharides, LPS, from the cell wall of the bacterium, *E. coli*), without pathogenic effects, to explore whether immune activation per se affected chemical ornaments. The immune activation results in decreased proportions of provitamin D₃ in femoral secretions (López, Gabirot, & Martín, 2009), which renders scent of males less attractive to females (López & Martín, 2005a). This suggests the existence of a trade-off between physiological regulation of the immune system and the allocation of essential nutrients with important metabolic functions (e.g., vitamins or other “costly” lipids) to sexual chemical ornaments.

Another possible cost of pheromone production may be related to the immunosuppressive effects of T (Folstad & Karter, 1992; Wedekind & Folstad, 1994). The experimental elevation of T levels may result in decreased immune responses (e.g., Belliure et al., 2004). Given that gland secretions are under direct androgenic control (Alberts, Pratt, & Phillips, 1992; Alberts, Sharp, et al., 1992), producing pheromones may require increasing circulating T levels with the subsequent immunosuppressive effects. Thus, only high-quality males could increase T to produce pheromones without detrimental effects on their immune system. This was tested in an experiment where T levels of male wall lizards (*P. guadarramae*) were increased (Martín, López, et al., 2007). Females discriminate and show greater responses to femoral secretions of T-implanted males than to control males, likely because T increases the amount or concentration of chemical signals. However, T supplementation also induces qualitative changes in lipid composition of secretions (i.e., proportions of cholesta-5,7-dien-3-ol decrease) (Martín, López, et al., 2007), which might be related to the effect of T in modulating immunity and lipid biochemistry (e.g., Lacy et al., 2002; Sheridan, 1994). Further, females prefer any male able to maintain higher proportions of cholesta-5,7-dien-3-ol in secretions, regardless of the T-manipulation (Martín, López, et al., 2007). Thus, because this steroid is negatively affected by T, there might be trade-offs between increasing T levels to increase the production of chemical secretions to attract females and maintaining metabolism.

Other theories of mate choice propose that males may evolve non-honest signals that exploit the sensory system of females (e.g., Fuller et al., 2005). A preexisting sensory bias for food chemicals might explain the chemosensory preferences of females for male scents. When the hunger

levels of female rock lizards (*I. cyreni*) are manipulated, food-deprived females exhibit increased chemosensory responses to chemical stimuli from both invertebrate prey and femoral secretions of males, which may depend on provitamin D₃, a lipid found in both prey and males' scent, but not to control water (Martín & López, 2008a). Moreover, hungry females spend more time on male scent marks with experimentally increased provitamin D₃, whereas for females with satiated hunger, this effect is not so clear. These results suggest that preexisting sensory bias for essential nutrients (e.g., provitamin D₃) may be the origin of female responses to similar male pheromones. However, sensory traps might later evolve into honest signals if they are differentially costly for males and only high-quality males can afford them (Macías-García & Ramirez, 2005). Therefore, a preexisting sensory bias for essential nutrients may further allow the evolution and maintenance of similar honest pheromones used in mate choice.

6.4 Pheromones in agonistic interactions and dominance between males

Male competition over females often favors the evolution of male attributes that confer fighting ability and correlated conspicuous status-signaling traits (Andersson, 1994). When males of different dominance statuses exhibit different badges, individuals may use these badges to judge relative fighting ability and to modulate their own behavior accordingly, avoiding costs of escalated aggressive interactions (Enquist & Leimar, 1983). In some lizards, pheromones may signal a male's dominance status, or related characteristics such as body size, through productivity rates and/or the quality of the secretions (Alberts, Pratt, & Phillips, 1992; López, Martín, & Cuadrado, 2003; Moreira et al., 2006). Substrate scent marks can provide information on the individuality of familiar males, but also on body size and associated fighting ability of unfamiliar males (Aragón et al., 2000, 2001a, 2001c, 2003; Carazo et al., 2007; Labra, 2006). Pheromones from dominant green iguana males (*I. iguana*) can elevate corticoid levels in juveniles, which indicates increased stress that may suppress growth and assertion displays in juveniles (Alberts, Jackintell, & Phillips, 1994). Male wall lizards (*P. liolepis*) are attracted to areas scent-marked by males of higher competitive ability (i.e., larger size), but not to areas of smaller males (Carazo et al., 2007). This shows that males can assess rival competitive ability (i.e., rival size) from scent marks, and suggests that scent marks do not function as chemical barriers to deter intruders, but that they may rather inform on the quality of a male and his territory.

Before and during agonistic encounters between males, species, and individuals recognition and assessment of the relative fighting abilities of potential opponents based on

pheromones may be useful to make fighting decisions (Cooper & Vitt, 1987). The assessment of the probable outcome of future encounters with the same familiar individual may be the best way of decreasing costs of fighting (López & Martín, 2001b). Experimental manipulations of the scent of male wall lizards (*P. guadarramae*) suggest that pheromones are involved in rival recognition during agonistic interactions, reducing fighting with males bearing manipulated familiar scents (López & Martín, 2002). Moreover, male rock lizards (*I. cyreni*) use scent matching, comparing the scent of substrate marks with the scent of any male they encounter nearby, to assess the ownership status of these males and to decide the fighting strategy (López & Martín, 2011). Intruder males decrease fighting intensity with presumably territory owners because the probability of winning a fight to these males is low. Therefore, pheromonal recognition mechanisms may reduce the intensity and costs of fighting, playing an important role in the organization of social systems.

Signaling social dominance may also be useful in other contexts. Male rock lizards (*I. monticola*) (formerly *Lacerta monticola monticola*) may use scent from femoral secretions and copulatory plugs for self-discrimination and for estimating the dominance status of other males (Moreira et al., 2006). Substrate scent marks may function to establish dominance hierarchies, while copulatory plugs and femoral secretions might allow males to scent-mark the female body during mating, which might influence reproductive decisions under selective pressures of sperm competition.

However, the chemical basis of rival assessment is little known, but this may be related to changes in concentrations of some chemicals in scents related to fighting ability. Dominant male green iguanas (*I. iguana*) produce larger amounts of femoral gland secretions than subordinates (Alberts, Pratt, & Phillips, 1992). Proportions of cholesterol in femoral secretions of male rock lizards (*I. cyreni*) change with body size (López et al., 2006). Males discriminate and respond aggressively to cholesterol stimuli depending on their own body size (Martín & López, 2007, 2008b). Moreover, in staged encounters in neutral cages between two unfamiliar and size-matched males, focal males lose more agonistic interactions against males manipulated to increase cholesterol in their scent than in control tests (Martín & López, 2007). Dominant male rock lizards (*I. monticola*) produce femoral secretions with higher proportions of two alcohols (hexadecanol and octadecanol) than subordinates (Martín, Moreira, & López, 2007). Males discriminate and respond aggressively toward hexadecanol according to their own dominance status, suggesting that hexadecanol may be a reliable status badge.

Nevertheless, there is little knowledge regarding the mechanisms and evolutionary relationships between dominance

status and pheromones. In lizards, the production of gland secretions (Alberts, Pratt, & Phillips, 1992), lipid metabolism (Sheridan, 1994) and male aggressiveness and dominance (Moore & Lindzey, 1992) are all dependent on androgen levels. The immunosuppressive effect of T (Belluire et al., 2004; Folstad & Karter, 1992; Wedekind & Folstad, 1994) may provide a link between dominance status and pheromones, such that only genuinely dominant males could maintain a good immune response and produce pheromones of dominance. For example, male rock lizards (*I. monticola*) with greater immune responses have higher dominance status and higher proportions of hexadecanol in secretions (Martín, Moreira, & López, 2007). Social costs may also be imposed by the targeted receivers. Thus, if the status signal is incongruent with behavior, deception may be detected and punished by genuinely dominant individuals (Martín & Forsman, 1999; Olsson, 1994). Therefore, subordinates might be better off avoiding signaling their dominance status (i.e., not allocating certain lipids to secretions).

7 SQUAMATA: SNAKES

7.1 Secretory glands and potential pheromones

7.1.1 Skin

Chemical analyses of the skin or shed skins of many snake species have found a diversity of compounds such as fatty acids, steroids, alcohols, aldehydes, waxy esters, hydrocarbons, and methyl ketones (e.g., Jacob et al., 1993; Mason et al., 1987, 1989; Roberts & Lillywhite, 1980; Schell & Weldon, 1985; reviewed in Mason, 1992; Weldon et al., 2008). Lipids in the skin are mainly considered useful to avoid water loss (e.g., Burken et al., 1985), but some lipids may play an important role as pheromones as suggested by observations of trailing, aggregation and courtship (e.g., Mason, 1993). In amphibious sea snake species (*Laticauda* spp.) skin lipid profiles differ between species, and this allows interspecific chemosensory recognition and species isolation within sympatric congeners (Shine et al., 2002).

The homologous series of relatively nonvolatile long-chain saturated and ω -9-cis-unsaturated methyl ketones from the skin lipids of female garter snakes (*Thamnophis sirtalis parietalis*) have been characterized chemically and synthesized in the laboratory, and their role in attracting males and eliciting courtship is well studied (Garstka & Crews, 1981; Mason, 1993; Mason et al., 1987, 1989, 1990; see below). The expression of these pheromones in females results from the activation by the feminizing estradiol, while it is inhibited by T in males (Parker & Mason, 2012, 2014). Similar series of ketones have been described in the skin of brown tree snakes (*Boiga irregularis*) (Murata et al., 1991). If male snakes do not detect this sexual attractiveness

of female pheromones, then courtship will not be initiated and subsequent mating will not occur (Greene & Mason, 1998; Mason, 1993). Also, there is interpopulational variation in female garter snake pheromones (i.e., unsaturated methyl ketones), associated with preferences of males for following scent trails and courting females of their own population, suggesting that some pheromone-mediated sexual isolation exists among populations (LeMaster & Mason, 2003).

7.1.2 Cloacal scent glands

Almost all snake species have a pair of elongated glands, located in the base of the tail, which secrete mainly lipids through two ducts located along the cloacal orifice. Secretions are mainly considered as defensive malodorous chemicals discharged to deter predators or even alarm pheromones for conspecifics (Graves & Duval, 1988; Weldon et al., 1991, 2008). However, cloacal secretions may also have other functions. Scents from the cloacal area and the skin may allow species recognition in several Neotropical snakes (Gabirot et al., 2012). Also, to reject unpreferred mates, female brown tree snakes (*B. irregularis*) can release cloacal secretions that inhibit male courtship (Greene & Mason, 2000, 2003). When cloacal secretion of females is experimentally added to a substrate, males decrease time and intensity of courtship to females, while cloacal secretion of males has no effect (Greene & Mason, 2003). These data suggest that volatile pheromones in cloacal secretions of females may regulate the events leading to copulation.

7.1.3 Nasal glands

These are a pair of glands, found only in some colubrid snakes of the Subfam. Psammophiinae, which are located lateral to the nasal cavity and that secrete close to the external nares (de Haan, 2003; Dunson et al., 1978). Secretions, consisting of lipids and proteins, are extended over the skin and serve to protect against water evaporation, but also it has been suggested that these snakes might perform self-rubbing to enable subsequent scent marking of substrate and conspecific individuals (de Haan, 2003).

7.2 Chemosensory recognition

7.2.1 Sex discrimination and trailing

Most snakes have the chemosensory ability to follow substrate-deposited scent trails for many functions, such as to locate sexual partners, overwintering hibernacula or hidden prey, or avoiding conspecifics or predators (e.g., Constanzo, 1989; Ford, 1986; Ford & Low, 1984; Greene et al., 2001; LeMaster & Mason, 2001; reviewed in Mason, 1992; Parker & Mason, 2011). The ability to orient and locate mates based on pheromonal cues can have significant

consequences to reproductive success (Shine, O'Donnell, et al., 2005). Females of many snake species, within at least five families, produce pheromone trails that males follow to locate females during the mating season (e.g., Ford & Low, 1984; LeMaster et al., 2001; LeMaster & Mason, 2001). Pheromone trails inform on the species identity, sex, sexual attractiveness, mating status, and travel direction of the individual signaler (Ford, 1982; Ford & Schofield, 1984; O'Donnell et al., 2004). For example, male red-sided garter snakes (*T. s. parietalis*) can detect and follow skin lipid trails of females but do not follow male trails. This suggests that the female pheromone responsible for trailing is the same sexual attractiveness skin pheromone that releases male courtship (LeMaster & Mason, 2001).

However, most research on this topic was based on artificial situations, testing the ability of captive snakes to follow continuous trails over structurally simple homogeneous substrates. A field study tested the response of adult garter snakes (*T. s. parietalis*) to pheromone trails in nature showed that during the spring breeding season, males detect and follow female trails, whereas neither sex displays trailing behavior during the autumn migration to the hibernacula (LeMaster et al., 2001). Another field experiment showed that male garter snakes follow a female's trail less successfully if she has recently been followed by rival males, suggesting that trailing males obscured the cues left by the female (Shine, Webb, et al., 2005). Also, trail-following males switch from chemical to visual cues as soon as they are close to a female, enhancing speed but decreasing the accuracy of mate location (Shine, Webb, et al., 2005). Because garter snakes mate in large aggregations, pheromones may be so widely distributed throughout the den area that they may be of little use in locating females. Males also use other visual (body size, muddiness) and thermal cues (very cold snakes are often females) to discriminate between males and females; but after a mating ball forms around a female, pheromones may be the most important cue (Shine & Mason, 2001).

The importance of pheromones for mate location and identification may also depend on the habitat of each species. In arboreal and semi-aquatic habitats, the possibility of depositing a continuous terrestrial pheromone trail is difficult. For example, males of the tree-snake (*B. irregularis*) and the semi-aquatic Burmese Python (*Python bivittatus*) follow single female trails but do not discriminate between male and female trails (Greene et al., 2001; Richard et al., 2019). Although female *B. irregularis* have methyl ketones in the skin, these are insufficient to elicit maximum male reproductive behaviors (Parker et al., 2018). This suggests that nonterrestrial taxa may not emit substrate sex-attractant pheromonal signals and that volatile pheromones may be more important. For example, male northern watersnakes (*Nerodia sipedon*) successfully follow both the terrestrial

trails and volatile signals from estrous females (Jellen et al., 2022). However, male turtle-headed sea snakes (*Emydocephalus annulatus*) use visual cues (size, movement, and coloration) to locate and assess whether any snake-shaped objects are potential sexual partners, while pheromonal skin lipids are important only after the male comes into physical contact with the stimulus (Shine, 2005).

7.2.2 Mate assessment

Pheromones may have a major role in reproductive behavior of many snake species (Andren, 1982; Greene & Mason, 1998; Parker & Mason, 2011). Red-sided garter snakes (*T. s. parietalis*) in Manitoba (Canada) court and mate in large aggregations after emergence from overwintering dens and before dispersing to their summer ranges (Mason, 1993; Shine, Elphick, et al., 2001). Chemoreception is the primary means by which male garter snakes assess another snake's species, population, sex, body size, condition, and mated status (Devine, 1977a; LeMaster & Mason, 2003; Mason, 1993; O'Donnell et al., 2004; Shine, O'Connor, & Mason, 2000a; Shine, Olsson, & Mason, 2000; Shine, Phillips, et al., 2003a).

Pheromonal cues not only enable males to discriminate between suitable and unsuitable mates, but pheromones may also provide many other types of information. For example, male garter snakes prefer to court larger females because they produce more or larger offspring. In laboratory tests, males prefer to display courtship behavior to skin lipid extracts from large females, showing that pheromones allow males to determine female size (LeMaster & Mason, 2002). In the field, males not only direct more courtship to longer and heavier-bodied females but also court most vigorously in response to lipids extracted from the skins of such females (Shine, Phillips, et al., 2003b). Chemical analyses showed that smaller females have skin profiles composed predominantly of saturated methyl ketones, while larger females have more unsaturated methyl ketones (LeMaster & Mason, 2002).

However, courtship and mating are size-assortative: larger male snakes court only large females, whereas small males court small and large females. Experimental manipulations of body sizes and scents showed that large male's direct intense courtship only when the stimuli provide both visual and pheromonal evidence of large body size, whereas small males are much less discriminating in both respects (Shine, Phillips, et al., 2003a). Thus, size-assortative mating is generated not by larger males excluding their smaller rivals from the largest females (as occurs in other reptiles), but by a size-related shift in the visual and pheromonal cues that elicit courtship.

Experimental trials showed that papers impregnated with lipids from newly emerged female garter snakes, which

are weak, attract more intense male courtship than the scent of postrecovery dispersing females (Shine, Wall, et al., 2005). Changes in female pheromones are responsible for this declining postemergence female attractivity (Uhrig et al., 2012). Chemoreception may also be used by male garter snakes to assess whether or not a female has recently mated (Devine, 1977b; O'Donnell et al., 2004; Ross & Crews, 1977; Shine, Olsson, & Mason, 2000). Males deposit a thick gelatinous plug that occludes the female cloaca after copulation and physically prevents remating by the female (Shine, Olsson, & Mason, 2000). The plug may also function as a spermatophore, gradually liberating sperm contained in the protein matrix as the plug dissolves (Friesen et al., 2013). Pheromonal cues from copulatory plugs were thought to discourage additional matings from rival males (Ross & Crews, 1977), but experimental work supports that, rather than from the plug, pheromonal information arises from airborne scent from copulatory fluids (Shine & Mason, 2012; Shine, Olsson, & Mason, 2000).

7.2.3 Pheromonal female mimicry

In red-sided garter snakes (*T. s. parietalis*), there are some males, called "she-males," that mimic females (Mason & Crews, 1985, 1986). Male's direct intense courtship to she-males as well as to females (Shine, Harlow, et al., 2000; Shine, O'Connor, & Mason, 2000b) (Fig. 7). Experiments using hexane extracts showed that this effect is due

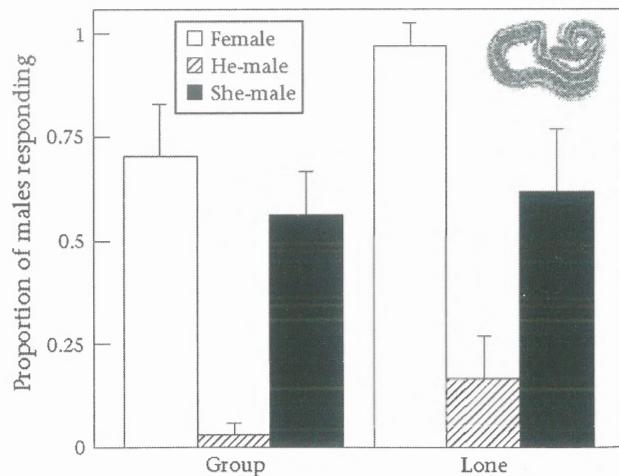


FIG. 7 Pheromonal female mimicry in garter snakes. Relative attractiveness (mean + SE) of females, normal males (he-males), and she-males to free-ranging male garter snakes (*Thamnophis sirtalis parietalis*). Attractiveness was assessed by measuring the proportion of males showing courtship behavior to the "target" snake. Males were tested either when they were part of a "mating ball" (group) or when they were solitary (alone). (Taken from Shine, R., Harlow, P. S., LeMaster, M. P., Moore, I., & Mason R. T. (2000). The transvestite serpent: Why do male garter snakes court (some) other males? *Animal Behaviour*, 59, 349–359.)

to the skin lipids of she-males (Mason et al., 1989). Chemical analyses of female and she-male skin lipid extracts collected during the breeding season reveal a similar pheromone profile, one that is not observed in the skin lipids of males (Mason, 1993). The “she-maleness” is an intrinsic property of a male rather than an artifact of lipid transfer from females (Shine, Harlow, et al., 2000).

However, “she-maleness” is not a permanent characteristic of a particular subset of males, but a transitory phase that most (perhaps all) male snakes pass through after they emerge from the winter den (Shine, Harlow, et al., 2000). Recently emerged males have to restore their physiological performance (e.g., locomotion, fighting, and courtship ability) and spend the first day relatively inactive and without courting females. Interestingly, the experimental application of female skin lipids onto normal males decreases their courtship levels. These results suggest that recently emerged males may benefit from mimicking female skin lipids because it “switches off” the male’s own (energetically expensive) courtship until they restore performance and courtship abilities. Female mimicking during this time might also disadvantage his rivals by distracting them from females and increasing their energy expenditure (Shine, Harlow, et al., 2000).

Nevertheless, she-males provide sufficient attraction and attract vigorous courtship of males only when females are not present, while she-males are abandoned immediately if a real female is found (Shine, Langkilde, & Mason, 2003). This suggests that males search for the most female-like pheromonal stimulus. Costs of misdirected courtship to other males impose selection on the chemosensory abilities of male snakes to discriminate between sexes (Mason et al., 1987, 1989, 1990; Shine, Langkilde, & Mason, 2003). Thus, it was suggested that she-males would attract other males not to obtain mating advantages within mating balls around females, but because while the she-male snakes are cold and slow, courtship warms them and protects them against predatory crows, but when she-males are warm, pheromones volatilize and lost attractiveness (Shine et al., 2012; Shine, Phillips, et al., 2001).

8 SUGGESTIONS FOR FUTURE RESEARCH

The results of experiments that suggest an important role of chemical cues in the reproductive behavior of reptiles prompt a reexamination of how pheromones may affect many social and sexual behaviors, such as mate choice and intrasexual interactions, in other reptilian species. In most previous studies, there was a probable “researcher’s sensory bias” due to the prevalence of visual signals for human observers (even when pheromones might be more important for humans than we admit). However, the importance of the

“hidden” chemical signals should be reconsidered in many cases. The design of new studies and experiments that specifically look for pheromonal effects on reproductive behavior could provide new insights on this subject.

We should increase and use the knowledge of glandular chemicals of reptiles to explore what are the functions of these chemicals and their potential role(s) as pheromones. Therefore, future studies should be multidisciplinary and analyze simultaneously the chemistry of potential pheromones and their effects on behavior of reptiles. This has been traditionally done in studies of chemical ecology of invertebrates, especially those with economic interests such as insect pests, but it is rarely done in studies of vertebrates and particularly reptiles.

Most current studies are based on a few “model” species (e.g., lacertid lizards and garter snakes), but we need to study more species representative of other groups, and in different environmental situations, if we want to know the extent of generalization of the results or whether different alternatives exist. Knowing more about a diversity of species and habitats would allow doing more comparative studies that would lead to a better understanding of the functional and evolutionary role of pheromones in reptiles.

The interaction of chemical signals with other types of signals (visual, acoustic, etc.) should also be considered. Multiple types of signals may be used by the same animal, and we need to understand whether multiple traits signal different characteristics or are redundant. Reptiles are especially good models for this question as many uses both visual and chemical signals, and different types of signals likely deliver similar messages but in different contexts (e.g., long vs short distance or in different microhabitats).

An interesting research field may be to understand the evolution of pheromones as signals. We need to know the evolutionary and physiological mechanisms that allow the receivers to rely on the messages of pheromones (why are pheromones honest signals?) or alternatively, that allow the senders to exploit the sensory physiology of the receivers with the pheromones (sensory bias). This is a current and rapidly growing topic in the study of visual signals based on colorful pigments, especially in birds, but it is little known for chemical signals. However, the mechanisms involved are likely similar and, moreover, that the chemicals used to “construct” visual signals are but a particular subset of chemical signals, and that the only difference is the sensory system (either the eyes or the VNO) used by the receivers to detect the presence and concentration of these chemicals. The studies of reproductive behavior and signaling systems based on pheromones may, thus, be not just a “curiosity” of some “peculiar” animals, but may help to understand how intraspecific communication of most animals has evolved.

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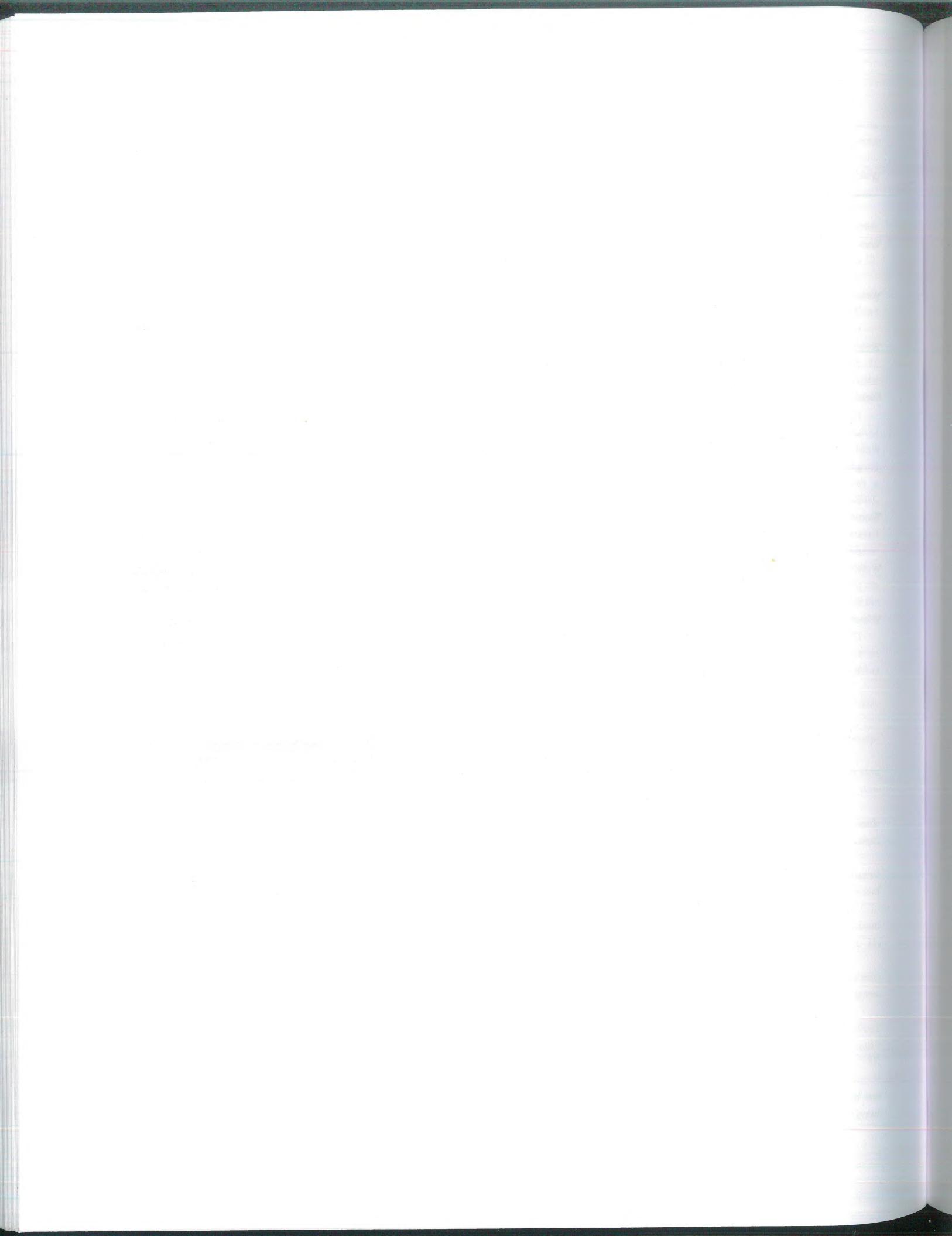
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Chapter 7

Stress and reproduction in reptiles

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ABBREVIATIONS

5-HT	serotonin (5-hydroxytryptamine)
ACTH	corticotropin
AGBG	androgen-glucocorticoid-binding globulin
AVP	arginine vasopressin
AVT	arginine vasotocin
B	corticosterone
CRF	corticotropin-releasing factor (see CRH)
CRH	corticotropin-releasing hormone (same as CRF)
DA	dopamine
DHT	dihydrotestosterone
E ₂	estradiol
Epi	epinephrine
FSH	follicle-stimulating hormone
GABA	γ-amino butyric acid
GluN ₂	glutamatergic NMDA receptor subunit
GnIH	gonadotropin release-inhibiting hormone
GnRH	gonadotropin-releasing hormone
GR	glucocorticoid receptor
GTH	gonadotropin
HPA	hypothalamus-pituitary-adrenal
HPG	hypothalamus-pituitary-gonadal
LH	luteinizing hormone
MR	mineralocorticoid receptor
NE	norepinephrine
NMDA	N-methyl-D-aspartic acid
P ₄	progesterone
PHA	phytohemagglutinin
RU-486	mifepristone
SHBG	sex hormone-binding globulin
SVL	snout-to-vent length

1 INTRODUCTION

1.1 Objectives

This review was written to place the context of integrated stress and reproductive interactions within the framework of reptilian natural life histories and to provide a revised account of what is known about the effects of stress on reproduction in

reptiles, through a survey of recent studies and older literature (Greenberg, 2002; Greenberg & Wingfield, 1987; Guillette et al., 1995; Lance, 1990; Moore & Jessop, 2003; Romero, 2002; Tokarz & Summers, 2011).

1.2 Definitions

Despite the ambiguity of the word “stress” in the vernacular, even in scientific discourse stress may refer to (1) noxious stimuli, (2) physiological and behavioral coping responses to stimuli, and (3) pathological elevation and persistence of physiological and behavioral responses that result in disease (Creel, 2001; Dallman, 2003; McEwen & Wingfield, 2003; Romero, 2004; Wingfield & Ramenofsky, 1999). At present, many stress biologists explicitly refer to stimuli as stressors and adaptive or maladaptive reactions as stress responses (Creel, 2001).

In this review of the effects of stress on reproduction in reptiles, (i) stress is defined as the internal state that occurs when an animal is exposed to a stressor, (ii) stressors as external conditions that are capable of disrupting an animal's homeostasis (maintenance of physiological responses within tolerance limits via feedback mechanisms), and (iii) stress response as the physiological and behavioral mechanisms that act to restore an animal's homeostasis (Pottinger, 1997). Often, the effects of stress on reproduction in reptiles result from exposure to a stressor, followed by activation of neural and physiological stress responses and secretion of glucocorticoids, such as corticosterone, the primary glucocorticoid of reptiles (Hanke & Kloas, 1995). An increase in secretion of corticosterone has been commonly used as an indicator of stress responsiveness (Creel, 2001; Gangloff & Greenberg, 2023; Greenberg & Wingfield, 1987; Moore & Jessop, 2003; Romero, 2004; Wingfield & Ramenofsky, 1999; Warwick et al., 2023), useful because this response has been measured in a broad range of species (both reptilian, and in other classes), and the physiological and behavioral effects are understood.

Stress, or the internal state produced in response to a stressor, engages both stress-related and other nonstress-related neural circuits that integrate specific physiological and behavioral responses (Summers & Winberg, 2006). This integrative response to stressors plays an important role in the concept that the stress response is a strategy by which animals cope with stressful social and physical environments (Koolhaas et al., 1999; McEwen & Wingfield, 2003; described below). The response to stressful environments has an important temporal aspect, making it important to understand the timeline that connects differences between acute and chronic stresses. The definition of acute stress should be determined by the intersection of the timing of neural and physiological stress responses with the behavioral changes that accompany them (Silvestre, 2014). Thus, acute stress is functionally relevant at the specific time point when a neural or physiological component of the stress response has changed from baseline (e.g., increased brain CRF concentrations) and behavioral or motivational change is manifest (e.g., increased sensitivity to aggressive display and/or decline in courtship behavior) after an animal has been exposed to a stressor. This is difficult because in many cases no temporal framework has been established relating neural and physiological responses to stressors with behavioral responses to stressors. However, both social and physical stressors have been linked temporally to neuroendocrine changes in some species of lizards (Korzan & Summers, 2021; Matter et al., 1998; Summers, Forster, et al., 2005; Summers, Korzan, et al., 2005; Summers et al., 1998; Summers, Summers, et al., 2003), and the results of these studies may provide some framework with which to judge the differences between acute versus chronic stress.

The normal temporal pattern of neuroendocrine stress responsiveness in examined lizards appears to be biphasic (see below in Section 2.1 on brain mechanisms mediating stress response for specific details), which may indicate a natural demarcation between acute and chronic stress (Summers, 2002). While the exact timing is likely to be different for each species, and also to be modified by allostatic load (see below), acute stress appears to be evident during motivational and/or behavioral stages that last up to approximately 60 min, whereas chronic stress may functionally be defined by stressors that last from 60 min to many weeks.

Allostasis constitutes a dynamic responsiveness of stress systems resulting in homeostasis through change. Allostatic load, or the cumulative cost of homeostasis through change, connects normal physiology with states in which serious pathophysiology occurs, through “allostatic overload” (McEwen & Wingfield, 2003; Sterling & Eyer, 1988). There are clearly differences between an animal’s responses to predictable and nonlife-threatening stressors and to unpredictable potentially life-threatening stressors. McEwen and Wingfield (2003) used the term “stress” to “describe events

that are threatening to an individual and which elicit physiological and behavioral responses as part of allostasis in addition to that imposed by the normal life cycle.” Wingfield and Ramenofsky (1999) had previously termed these facultative behavioral and physiological responses triggered by unpredictable events in the environment an “emergency life history stage” and noted that these responses appear to be mediated by increases in glucocorticoids.

In general, the concept of allostasis as developed by McEwen and Wingfield (2003) has been favorably received (Dallman, 2003; Romero, 2004; Schulkin, 2003; Walsberg, 2003). Schulkin (2003) noted that the allostatic concept of McEwen and Wingfield brings together both biomedical and ecological approaches to studying stress, and promises to be especially helpful in understanding the biological consequences of stress in an ecological context. Some aspects of the allostatic concept of McEwen and Winfield have received criticism, however. For example, Dallman (2003) has questioned whether the new terms (“allostasis,” “allostatic load,” and “allostatic overload”) are really needed; whether the idea that the allostatic response systems are not essential for maintenance of life is valid; and whether the proposed allostatic response system differs in kind from homeostatic response systems. Dallman (2003) also emphasized the importance of explicitly including central neural mechanisms in any consideration of stressors and the stress response. In the view of Walsberg (2003), the primary weakness of the allostatic concept of McEwen and Wingfield is that it places too much emphasis on the balance between an animal’s energy expenditure and energy intake. Specifically, Walsberg (2003) points out that animals in nature are rarely in energy balance and that an animal’s energy balance can be greatly modified without necessarily inducing stress. Thus, according to Walsberg (2003), energy balance may be of limited utility as an index of physiological stress.

A final matter relating to definitions, concerns the naming of the glands in reptiles that synthesize the stress hormones. In reptiles the steroidogenic tissue that synthesizes the glucocorticoid hormones and the chromaffin tissue that synthesizes the catecholamine hormones are intermingled to various degrees within two paired glands, (Chester Jones & Phillips, 1986; Loftis, 1978; Loftis & Bern, 1972; Norris & Carr, 2020). Because these glands in many reptiles (chelonians, crocodilians, and most snakes) are positioned as in mammals above the kidneys (Norris & Carr, 2020), we will refer to them as adrenal glands.

1.3 Importance of studying reptiles

Extant reptiles consist of tortoises, turtles, and terrapins (order Testudines or Chelonia); the tuatara (order Rhynchocephalia or Sphenodontidae); snakes, lizards, and

amphisbaenids (order Squamata); and the crocodiles consisting of the gavials (or gharials), crocodiles, and alligators (order Crocodylia) (Tudge, 2000; Vitt & Cardwell, 2008). The reptiles are considered to be an important group in vertebrate evolution based on their close relationship to birds and common ancestry with a group that gave rise to mammals (Benton, 2005).

There are several reasons why it is important to study the effects of stress on reproduction in reptiles. The first reason is that such studies are necessary for the conservation of these key vertebrates. Like amphibians, many wild populations of reptiles are declining and in some cases, species are considered to be in danger of extinction (see reviews by Cockrem, 2005; Gibbons et al., 2000; Gregory & Schmid, 2001; Irwin & Irwin, 2006; Jessop, 2001; Jessop et al., 2000, 2002; Tracy et al., 2006; Tyrrell & Cree, 1998; Valverde et al., 1999; Wack et al., 2008; Whittier et al., 1997), and displacement or predation by harmful invasive species such as the brown tree snake, *Boiga irregularis* (Moore et al., 2005). Knowledge obtained from studies of the effects of stress on reproduction in reptiles may also lead to better management of captive reptiles in zoos and more humane treatment of reptiles in laboratory research as well (Langkilde & Shine, 2006).

Squamate reptiles (lizards and snakes) exhibit unique reproductive patterns and have many useful characteristics for studies of the effects of stress on reproduction. For example, males and in some cases, females in some species of lizards and snakes exhibit alternative reproductive tactics (Crews et al., 1983; Knapp, 2004; Moore et al., 1998; Sinervo & Lively, 1996; Summers, 1984b). Species with alternative reproductive tactics may be of special utility in demonstrating how exposure to stressors affects tradeoffs between mating success and individual survival (Knapp & Moore, 1996, 1997a; Lancaster et al., 2008; Miles et al., 2007). Additionally, reproductive physiology of certain reptiles may provide reasonable models for human reproductive events, such as the alternating pattern of ovulation (Geisthövel et al., 1984; Gougeon & Lefevre, 1984; Jones, Guillette Jr., et al., 1983), also found in the green anole, *Anolis carolinensis*, regulated by an alternating pattern of neurotransmitter activity in the hypothalamus (Desan et al., 1992; Jones et al., 1990; Jones et al., 1997).

Finally, it is important to study the effects of stress on reproduction in reptiles because so much remains to be learned. Although it is quite clear that the adrenocortical response to acute stress in reptiles in terms of the release of stress hormones is similar to that observed in other vertebrates (see Moore & Jessop, 2003; Romero, 2002; Wingfield & Sapolsky, 2003), relatively little is known about the mechanisms that mediate the stress response in these vertebrates in terms of cellular receptors, plasma binding proteins, etc. (Moore & Jessop, 2003).

1.4 Relationship of stress to reproduction in reptilian life histories

In considering the effects of stress on reproduction in reptiles, it is important to consider these effects in terms of natural life histories. Although many studies of stress in reptiles and other vertebrates have tended to focus on those instances when animals suddenly experience highly aversive conditions in their environment such as predation attempts or storms, it is necessary to keep in mind that stressful conditions may arise on a day-to-day basis, involve a variety of different stressors, and have an impact on a variety of life history traits. As knowledge of the physiological mechanisms that govern the stress response is still minimal, we believe that studies of the effects of stress on reproduction in reptiles are likely to be of most value in testing hypotheses related to behavior, ecology, and evolution rather than in testing hypotheses related to the proximate mechanisms that govern the stress response. For this reason, we believe that studies that examine the effects of stress on reproduction in reptiles within the context of the life history of these vertebrates are of particular value. Studies of reptiles that have examined, for example, how exposure to stressors affects offspring fitness (Belluire et al., 2004; Preest et al., 2005; Weiss et al., 2007) as well as the possible tradeoff between reproductive success and survival (e.g., French et al., 2007; French & Moore, 2008; Lancaster et al., 2008; Mills et al., 2008) illustrate such a life history approach.

Whereas global climatic changes are likely to influence the reproductive success of whole populations or multiple species, relatively small changes in microhabitat such as the differences in soil moisture or local humidity may adversely affect reproductive function (Jones, Summers, & Lopez, 1983; Summers, 1988; Summers et al., 1985) in a subset of a reptile population. Glucocorticoid stress hormones affect and are in turn affected by an animal's feeding and energetics (Cash & Holberton, 1999; Cote et al., 2006; Woodley et al., 2003). These glucocorticoid-induced changes in feeding and energetics may over time also have an effect on gonadal function (Cote et al., 2006; Moore & Jessop, 2003) and even offspring fitness (Cote et al., 2006; Lancaster et al., 2008; Meylan & Clobert, 2005; Vercken et al., 2007).

Stress responsiveness and coping strategies in reptiles are also affected by predation (Berger et al., 2007; Rödl et al., 2007), by territorial behavior and social rank (Korzan & Summers, 2007, 2021; Korzan et al., 2021), as well as by an individual's sex, because male and female reptiles differ in their natural history (Smith & John-Alder, 1999). For example, whereas both male and female reptiles exhibit aggressive behavior in an attempt to attain territories and elevated social rank, the purpose and advantages of

aggression may be sex-specific (Andrews & Summers, 1996; Korzan, Forster, et al., 2006; Summers et al., 1995). It is important to consider the ecological and social aspects of reptilian life history, because the effect of stress on reproduction will vary based on sex, size, stage of development, social status, opponent recognition, daily and annual cycles, environmental conditions, and food or water availability (Dauphin-Villemant & Xavier, 1987; Forster et al., 2005; Jones, Summers, & Lopez, 1983; Korzan & Summers, 2007, 2021; Licht, 1967; Summers, 1988, 1995; Summers et al., 1997; Summers & Norman, 1988; Summers et al., 1995).

2 MECHANISMS MEDIATING STRESS RESPONSE

2.1 Brain

The release of adrenal glucocorticoid hormones into the plasma is often the primary or only focus of studies on stress and this is also true for studies that examine the effects of stress on reproduction. However, it is critical to keep in mind that the release of glucocorticoid hormones is governed by the actions of the hypothalamic-pituitary-adrenal axis (HPA), that the HPA axis is regulated by both the central and peripheral nervous systems (Capaldo et al., 2003), and that the hormones of the HPA axis in turn may affect the central nervous system (Summers et al., 2000; Summers, Matter, et al., 2003).

No neurotransmitter systems, with the possible exception of those that involve corticotropin-releasing factor (CRF), the pro-opiomelanocortin (POMC)-related peptides adrenocorticotrophic hormone (ACTH), β -endorphin, and melanocyte-concentrating hormone (MCH) or the orexins/hypocretins (Orx) are exclusively and specifically associated with stress; stress-related neurocircuitry is present that overlaps and interacts with other behavioral and reproductive neurocircuits (Korzan & Summers, 2021; Summers & Winberg, 2006). These neurocircuits in mammals are primarily glutamatergic, GABAergic, and CRFergic, and involve limbic brain regions such as the hippocampus, amygdala, bed nucleus of the stria terminalis, hypothalamus, raphé, and locus ceruleus (Curtis et al., 1999; Forster et al., 2008; Herman & Cullinan, 1997; Mo et al., 2008). Similar neurotransmitter systems and brain regions are involved in reptilian stress responses (Bugnon et al., 1984; Øverli et al., 2007; Summers, Forster, et al., 2005; Summers & Winberg, 2006). For example, in the green anole lizard, *Anolis carolinensis*, stress resulting from social interactions in males elevates expression of glutamatergic NMDA receptor subunit (GluN₂) in lizard medial cortex (Meyer et al., 2004) and also influences the relative ratio of GluN_{2A} to GluN_{2B} subunit concentration in this hippocampal region

(Summers, Forster, et al., 2005), thereby modifying receptor function and presumably postsynaptic current. The stress resulting from anoxia modifies cortical cell NMDA current in turtles (*Trachemys picta*: Shin & Buck, 2003) and increases GABA concentrations (*Trachemys scripta elegans*: Hitzig et al., 1985). These glutamate and GABA circuits influence and are also influenced by other transmitter systems, such as serotonin (5-HT), dopamine (DA), and norepinephrine (NE). For example, GABA inhibits 5-HT release in the hippocampus of *Anolis carolinensis* (Summers, 2001; Summers, Matter, et al., 2003). Stress in response to heat exposure stimulates increased brain 5-HT in the lizard, *Agama stellio* (Mohamed & Rahman, 1982). Stress resulting from restraint also stimulates serotonergic activity in numerous brain regions, including nucleus accumbens, hippocampus, amygdala, and locus ceruleus, but surprising not in the raphé, where 5-HT perikarya are located (Emerson et al., 2000; Ling et al., 2009). The stress resulting from social interactions in male lizards rapidly increases serotonergic activity in brain regions associated with stress neurocircuitry (e.g., hippocampus, amygdala, hypothalamus, raphé, and locus ceruleus), in a pattern differentiated by social rank (Korzan & Summers, 2004; Korzan et al., 2001, 2000; Matter et al., 1998; Summers, Forster, et al., 2005; Summers & Greenberg, 1995; Summers, Korzan, et al., 2005; Summers et al., 1998; Summers, Summers, et al., 2003; Summers & Winberg, 2006; Watt et al., 2007). Social interactions and stress hormones follow a similar temporal schedule as rank-defined serotonergic activity (Summers, 2001, 2002; Summers, Watt, et al., 2005), and glucocorticoids both systemically and locally modify 5-HT release and activity in the hippocampus of *Anolis carolinensis* (Summers, 2001; Summers et al., 2000; Summers, Matter, et al., 2003).

For social interactions, 5-HT appears to be an important stress-related neurotransmitter because the inhibition of the reuptake of 5-HT reversed social status in male *Anolis carolinensis* (Larson & Summers, 2001). Stress resulting from hyperosmotic conditions and dehydration influences 5-HT, but also NE, epinephrine (Epi) and corticosterone in the soft-shelled turtle, *Lissemys punctata punctata*, (Mahapatra et al., 1991). Similarly stress resulting from physical restraint elevates central catecholamines in the stress circuitry of the lizard, *Anolis carolinensis* (Ling et al., 2009; Waters et al., 2005). While DA and other central catecholamines are stress-responsive (Korzan, Øverli, & Summers, 2006; Korzan et al., 2001, 2000; Ling et al., 2009), the role for DA appears to be more closely related to memory and motivation (Korzan et al., 2007; Korzan & Summers, 2007). Motivation and memory are important during stress; and brain regions like the nucleus accumbens associated with motivation are modified in reptiles (Summers, Summers, et al., 2003; Summers, Watt,

et al., 2005) and mammals (Lukkes et al., 2008) by activation of stress circuitry and neurotransmitters.

As might be presumed, neural stress responses can be very rapid, and neural effects of stress have been demonstrated as fast as 0.5 min for stress due to social interactions in the mountain spiny lizard, *Sceloporus jarrovi* (Matter et al., 1998), and as rapidly as 0.42–1.50 min for physical or social stressors in the green anole, *Anolis carolinensis* (Emerson et al., 2000; Ling et al., 2009; Waters et al., 2005; Watt et al., 2007). Following a very rapid neural response in *Anolis carolinensis*, a discernable physiological response to stressors is evident within 1.5 min (Ling et al., 2009; Summers, Watt, et al., 2005; Watt et al., 2005). The results from both neural and endocrine measurements of male *Anolis carolinensis* that were exposed to social stressors for an extended period suggest a biphasic stress response with the initial phase ending somewhere between 20 and 60 min (Summers, Summers, et al., 2003; Summers, Watt, et al., 2005). A second neural and endocrine stress response phase begins between 40 and 60 min, depending on social status, brain region examined, and neural or endocrine stress parameter measured (Summers, 2002; Summers, Summers, et al., 2003). The temporal range of the two putative stress phases suggested by these results have a similar time frame to that of the pulsatile secretion of the HPA axis hormones CRF, ACTH, and corticosterone in mammals (Lightman et al., 2002). For example, in rats half of one full pulse phase from nadir to peak of one corticosterone pulse in plasma or brain lasts on the order of 30 min (Droste et al., 2008; Lightman et al., 2002). This pulsatile ultradian pattern defines the normal circadian rhythm, and the frequency of glucocorticoid pulses is increased by chronic stress (Lightman, 2008). In lizards, chronic stress also alters the circadian phase relationship of corticosterone by increasing its frequency (Summers & Norman, 1988).

The normal phase relationships and biphasic pattern of neuroendocrine stress responsiveness observed in many vertebrates including reptiles suggests a natural demarcation between acute and chronic stress (Summers, 2002). While the exact timing is likely to be different for each species, and also to be modified by allostatic load (see below), acute stress appears to begin approximately 0.4 min after exposure of an animal to a stressor and to last up to 60 min, whereas chronic stress may last from 60 min to many weeks.

2.2 Hypothalamic-pituitary-adrenal (HPA) axis

The reptilian endocrine stress response appears to include similar elements to the hypothalamic-pituitary-adrenal (HPA) cascade in other vertebrates, including mammals. One of the key initial elements of the HPA axis and an important central neurotransmitter, CRF has been demonstrated to be present in the brain of a small number of

reptiles including the lizard *Anolis carolinensis* (Ten Eyck et al., 1996), the water snake *Natrix maura maura* (Mancera et al., 1991), and the red eared turtle, *Pseudemys scripta elegans* (Bugnon et al., 1984; Fellmann et al., 1984). The distribution of CRF in the reptilian brain appears to be similar to that of mammals, and specifically includes paraventricular neurons with terminals in the median eminence, suggesting delivery of CRF to the adenohypophysis for the HPA cascade. What is more, in the water snake, *N. m. maura*, CRF is colocalized with arginine vasotocin (AVT) (Mancera et al., 1991), much as it is with the potent synergistic ACTH secretagogue arginine vasopressin (AVP) in mammals (Mouri et al., 1993; Plotsky & Sawchenko, 1987). Additional evidence of reptilian CRF in the HPA cascade comes from the Indian keeled lizard, *Mabuya carinata*, in which a general CRF receptor antagonist (α -helical CRF) blocks stress-related effects on ovarian recrudescence (Ganesh & Yajurvedi, 2002b). In the turtle *Pseudemys scripta elegans*, adrenalectomy was used to demonstrate normal HPA negative feedback on CRF production in the region of the median eminence (Fellmann et al., 1984).

It is likely the reptilian CRF stimulates the cells of the adenohypophysis that produce ACTH (i.e., corticotropes), because such cells have been described in the adenohypophysis of the teiid lizard, *Cnemidophorus lemniscatus* (Del Conte, 1980). Pituitary corticotropes synthesize and release ACTH, which has been demonstrated to stimulate adrenal corticosteroid synthesis and thus release in several lizards (*Sceloporus undulatus*: Carsia & John-Alder, 2003; *Amblyrhynchus cristatus*: Romero & Wikelski, 2006; *Hoplodactylus maculatus*: Preest et al., 2005; *Egernia whitii*: Cartledge & Jones, 2007), the caiman, *Caiman crocodilus* (Gist & Kaplan, 1976), but not in the turtle, *Pseudemys scripta* (Sanford & Stephens, 1988). Unlike ACTH, which is a potent stimulator of glucocorticoid synthesis in reptiles, the natriuretic peptides appear to have an inhibitory effect on glucocorticoid synthesis in reptiles because these peptides inhibited in vitro steroidogenesis in dispersed adrenocortical cells that were obtained from the Eastern fence lizard, *Sceloporus undulatus* (Carsia & John-Alder, 2006).

The primary adrenal glucocorticoid in reptiles is corticosterone (Hanke & Kloas, 1995). Additionally, reptiles appear to possess a glucocorticoid plasma-binding protein (Jennings et al., 2000). Two plasma steroid-binding globulins have been identified in male tree lizards, *Urosaurus ornatus*, and one of these binds androgens and C-21 steroids such as corticosterone and has been termed androgen-glucocorticoid-binding globulin (AGBG); the other is a sex hormone-binding globulin (SHBG) that binds androgens and E₂ with high affinity (Jennings et al., 2000). Moreover, the binding capacity of AGBG (but not of SHBG) has been shown to be significantly greater in

territorial males than in nonterritorial males in *Urosaurus ornatus* (Jennings et al., 2000). The response to corticosterone appears to show sex-related differences in the skink, *Egernia whitii* (Cartledge & Jones, 2007). In addition, the marine iguana, *Amblyrhynchus cristatus*, the European common lizard, *Lacerta vivipara*, and the green anole, *Anolis carolinensis*, show diurnal variation in corticosteroid secretion, which can be modified by stress (Dauphin-Villemant & Xavier, 1987; Romero & Wikelski, 2006; Summers & Norman, 1988).

2.3 Immune system

The hormonal interface between genome and environment necessarily involves immune function, with exogenously elevated corticosterone plus immune challenge (sheep red blood cells) in wild *Lacerta vivipara* affecting fitness and body condition (Meylan et al., 2010). Corticosterone decreased clutch success, juvenile size, and body condition, but enhanced metabolism and catalase activity. However, the immune challenge ameliorated corticosterone effects relative to stamina and hatching date. In wild garter snakes, the heterophil:lymphocyte (H:L) ratio varied by geographic region, but was inversely compared to corticosterone and glucose in response to stress (Gangloff et al., 2017). A positive correlation exists between H:L ratio and corticosterone concentrations in initial blood samples, which is lost over time. The data, therefore, suggest a negative relationship between corticosterone and lymphocyte levels. The relationship between immune function and stress reactivity is well established in mammals, and the sparse data on reptiles suggest that more studies on the influence of cytokines on the HPA axis hormones, as well as the reverse relationships, are warranted.

2.4 Hypothalamic-pituitary-gonadal (HPG) axis

The hypothalamic-pituitary-gonadal (HPG) cascade in reptiles is similar to that described in other vertebrates (Licht & Porter, 1987). As in mammals, norepinephrine (NE) appears to play an important role in ovarian function in female lizards (Jones et al., 1990). In the green anole lizard *Anolis carolinensis*, noradrenergic activity in the contralateral hypothalamus predicts the pattern of ovarian growth and ovulation (Desan et al., 1992), which in female anoles entails the ovulation of a single egg at a time and an alternating pattern in egg production between the left and right ovaries in successive ovulations (Smith et al., 1972). This hypothalamic regulatory control of ovarian function in *Anolis carolinensis* appears to be informed by afferent input from ovaries to brain (Jones et al., 1997). Ovariectomy influences not only noradrenergic activity but also hemisphere specific serotonergic and dopaminergic activity as well.

Work in the leopard gecko, *Eublepharis macularius*, suggests that the most ubiquitous form of gonadotropin-releasing hormone (GnRH) in vertebrates, type II or chicken II (GnRH II or cGnRH₂) shows gene, peptide, and receptor expression in reptiles (Ikemoto et al., 2004; Ikemoto & Park, 2003). However, as in other vertebrate classes there are multiple GnRH isoforms in reptiles. For example, Ikemoto and Park (2007) have identified two distinct forms of GnRH and three forms of GnRH receptor in the leopard gecko, *Eublepharis macularius*. However, the green anole lizard, *Anolis carolinensis*, may be limited to just one form, cGnRH₂ (Lescheid et al., 1997). According to Tsai and Licht (1993a) turtles such as *Trachemys scripta* have at least 2 GnRH types (cGnRH_{1&2}), with cGnRH₁ being the most abundant type in the median eminence. In addition to GnRH, a second neurohormone, which is part of the RFamide peptide family, gonadotropin-release inhibiting hormone (GnIH), acts to inhibit gonadotropin release and appears to be present in reptiles as well as other vertebrates (Tsutsui & Osugi, 2009; Tsutsui et al., 2018).

A variety of GnRH subtypes are capable of stimulating LH release from pituitary gonadotropes in the turtle, *Trachemys scripta* (Tsai & Licht, 1993b). Surprisingly, subcutaneous implants of the GnRH agonist deslorelin acetate in female veiled chameleons (*Chamaeleo calyptratus*) did not effectively limit reproductive function, an effect GnRH negative-feedback mechanisms by high-dose agonists would typically produce (Cermakova et al., 2023). The pituitary gonadotropes of other reptiles, such as in the North African spiny-tailed lizard, *Uromastyx acanthinura*, have been characterized as having only FSH-like secretory capacity, at least as far as immunoreactivity to human LH and FSH antibodies may demonstrate (Hammouche et al., 2007). In the Indian keeled lizard, *M. carinata*, the physiological effects of FSH in males including the full complement of gonadotropic and steroidogenic effects are subject to inhibition by stress and glucocorticoids (Yajurvedi & Menon, 2005; Yajurvedi & Nijagal, 2000). In female *M. carinata*, FSH also stimulates ovarian function (recrudescence) but is inhibited (as in mammals) by β-endorphin (Ganesh & Yajurvedi, 2003).

Although two gonadotropins, homologous to FSH and LH, are part of the reptilian HPG axis, early studies had difficulty demonstrating their separate reproductive functions (Licht et al., 1976; Licht et al., 1979). In the green sea turtle, *Chelonia mydas*, FSH and LH in females were demonstrated to be distinctly regulated, and have different functions in mating and oviposition, suggesting distinctive roles in oviposition (Licht et al., 1979). Similarly, in a more recent study in the lizard *Calotes versicolor*, a combined treatment of LH and FSH was found to be more effective in stimulating testicular recrudescence than FSH alone, suggesting distinct functions in males (Vijaykumar et al., 2002).

In addition to the role of gonadotropins in stimulating gametogenesis and gonadal steroid hormone synthesis, there is some exciting evidence from a study of the lizard *Uta stansburiana* that these pituitary hormones may also act directly to modulate a variety of physiological, morphological, and behavioral traits (Mills et al., 2008).

There is some evidence that the seasonality of reproductive function in reptiles is mediated by changes at the level of the hypothalamus (in terms of the areas that regulate GnRH release and thus pituitary gonadotropin release) rather than at the level of the gonads (in terms of gonadal sensitivity to the actions of gonadotropin). For example, FSH treatment in the green anole, *Anolis carolinensis*, was effective in stimulating testicular growth in males during the so-called refractory period, which occurs immediately after testicular regression in the late summer and fall (Summers, 1984a).

3 EFFECTS OF STRESS ON REPRODUCTIVE FUNCTION

3.1 Neurotransmitters

As brain NE, DA, and 5-HT systems have been demonstrated to influence gonadal function in reptiles (Desan et al., 1992; Jones et al., 1990, 1997), it is likely that stressor-induced changes in these neurotransmitter systems also affect reproductive processes. In the green anole, *Anolis carolinensis*, the stress associated with social interactions has been found to diminish telencephalic serotonergic and dopaminergic activity, but elevated brainstem serotonergic activity suggests negative feedback in the 5-HT system, which probably occurs at the level of the raphe (Summers et al., 1997). The courtship behavior and ovarian cycles of these socially stressed subordinate females were also reduced relative to controls (Summers et al., 1995). Serotonergic activity also affects male courtship behavior in the red-sided garter snake, *Thamnophis sirtalis parietalis*, as indicated by the finding that a combined treatment with corticosterone and with a 5-HT_{2A}-receptor antagonist reduced male courtship behavior (Lutterschmidt et al., 2004).

3.2 Reproductive hormones

One of the more common adverse effects of a prolonged stress response is the disruption of reproductive physiology and behavior (Greenberg & Wingfield, 1987; Wingfield & Sapolsky, 2003; Warwick et al., 2023). Among other effects, stress hormones released from the hypothalamic-pituitary-adrenal axis can act at multiple levels to inhibit the secretion and/or action of hormones necessary for gonadal function and reproductive behavior (Lance et al., 2004).

There is evidence in many species of reptiles that plasma androgen levels in males decline after exposure to stressors (see Fig. 1 and Table 1). The effects of exogenous corticosterone treatment on testosterone levels in males have also been examined in the tree lizard, *Urosaurus ornatus* (Knapp & Moore, 1997a). Tree lizards are of special interest because they have two alternative male reproductive morphs: nonterritorial males with orange dewlaps ("orange males") and territorial males with orange dewlaps containing a central bluish-green spot ("orange-blue males") (Moore & Thompson, 1990). Knapp and Moore (1997a) treated males of both morph types with exogenous

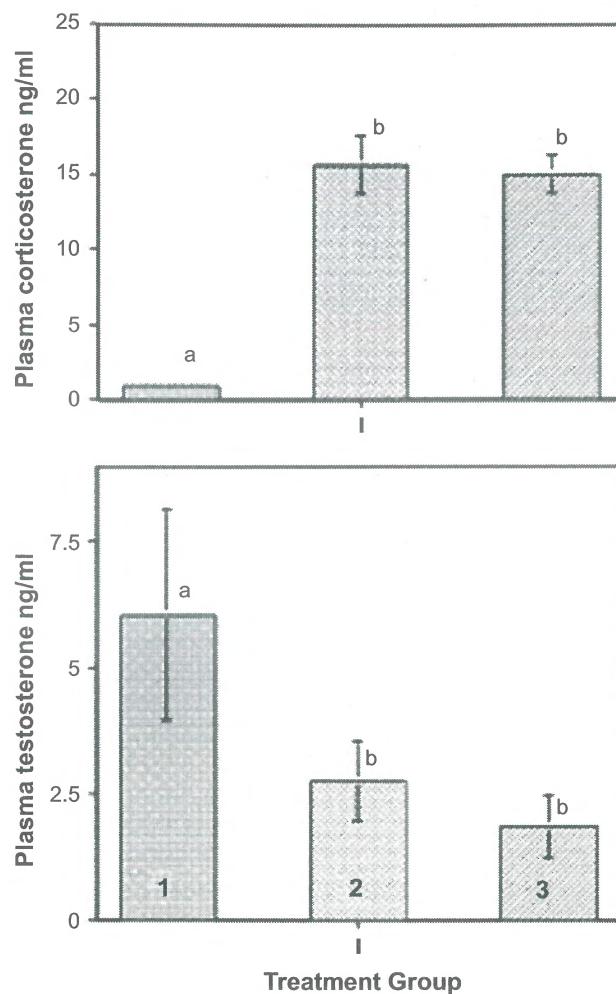


FIG. 1 Mean plasma corticosterone (\pm SEM) in ng/mL (upper panel) and mean plasma T (\pm SEM) in ng/mL (lower panel) in male alligators immediately after capture and at 2 h postcapture. Group 1 ($n = 18$; left bar) represents samples immediately at capture, group 2 ($n = 18$; middle bar) represents samples collected at 2 h following the immediate sample, and group 3 ($n = 18$; right bar) represents samples collected at 2 h only. Lower case letters when different indicate differences are highly significant ($P < .001$). (Reproduced from Lance, V. A., Elsey, R. M., Butterstein, G., & Trosclair, P. L. I. (2004). Rapid suppression of testosterone secretion after capture in male American alligators (*Alligator mississippiensis*). General and Comparative Endocrinology, 135, 217–222.)

TABLE 1 Relative changes in plasma testosterone levels in males following exposure to stressors or stress hormones in different groups of reptile

Species	Relative changes in plasma testosterone (T) levels	Reference
Painted turtle (<i>Chrysemys picta</i>)	T levels declined from initial values within 24 h of capture	Licht et al. (1985)
Musk turtle (<i>Sternotherus odoratus</i>)	T levels declined 35%–60% from initial values within 24 h of capture, depending on season	Mendonça and Licht (1986)
Snapping turtle (<i>Chelydra serpentina</i>)	T levels increased above initial values within 24 h of capture but declined below initial values by 168 h postcapture	Mahmoud et al. (1989)
Snapping turtle (<i>Chelydra serpentina</i>)	T levels gradually declined from initial values within 24 h of capture in males obtained in June and July, regardless of temperature or phase of spermatogenic cycle	Mahmoud and Licht (1997)
Hawksbill turtle (<i>Eretmochelys imbricata</i>)	T levels did not decline significantly from initial values in immature males at 1 and 5 h postcapture	Jessop et al. (2004)
American alligator (<i>Alligator mississippiensis</i>)	T levels were lower than initial values at all sample time periods after capture, with T levels being 50% of initial values by 4 h and less than 10% by 24 h postcapture	Lance and Elsey (1986)
American alligator (<i>Alligator mississippiensis</i>)	T levels declined in subadult males treated with ACTH or with saline within 6 h of treatment	Mahmoud et al. (1996)
American alligator (<i>Alligator mississippiensis</i>)	T levels in juvenile males from a contaminated lake were lower than from a control lake but were no different than initial values after 2 h of restraint following capture	Guillette, Crain, et al. (1997)
American alligator (<i>Alligator mississippiensis</i>)	T levels in subadult and adult males decreased from initial values by 2 h postcapture	Lance et al. (2004)
Tuatara (<i>Sphenodon punctatus</i>)	T levels did not differ significantly from controls within 3 h of capture but were lower in males exposed to repeated blood sampling for 24 h	Cree, Guillette, Cockrem, and Joss (1990)
Shingleback skink (<i>Tiliqua [Trachydosaurus] rugosa</i>)	T levels did not differ significantly from controls within 2–3 min or 24 h of capture, although androgens were abnormally low in males held in captivity for several months	Bourne et al. (1986)
Brown anole (<i>Anolis sagrei</i>)	T levels were lower after 7 days of exposure to CORT delivered from subcutaneous pellets than in placebo-treated males	Tokarz (1987)
Tree lizard (<i>Urosaurus ornatus</i>)	T levels in males (orange-blue morph) that were held in collecting bags were not significantly different from initial values after 10 min but were lower by 240 min, and T levels in males exposed to captivity in cages for days declined slightly but nonsignificantly	Moore et al. (1991)
Side-blotched lizard (<i>Uta stansburiana</i>)	T levels in males that were exposed to CORT delivered from Silastic implants for at least 7 days were lower than in sham-treated males	DeNardo and Licht (1993)
Italian wall lizard (<i>Podarcis sicula sicula</i>)	T levels declined from initial values within 48 min of capture in males obtained in April, May, and June	Manzo et al. (1994)
Tree lizard (<i>Urosaurus ornatus</i>)	T levels in males that were captured and physically restrained for 10 or 30 min were negatively correlated with CORT levels in males of the orange morph, and T levels in males of both the orange and orange-blue morphs that had received exogenous CORT from dermal patches for a day before capture were lower than in controls with orange males having lower T levels than orange-blue males	Knapp and Moore (1997a)
Keeled Indian Mabuya (<i>Mabuya carinata</i>)	T levels were lower in males who received FSH and CORT injections over 30 days than in males who received only FSH injections	Yajurvedi and Nijagal (2000)
Common gecko (<i>Hoplodactylus maculatus</i>)	T levels in males did not differ significantly from initial values at 4 and 24 h postcapture	Cree et al. (2003)
White's skink (<i>Egernia whitii</i>)	T levels were lower than initial values at 60 and 240 min postcapture in males held in cloth bags following capture but T levels did not differ significantly from initial values in males held in captivity from 1 to 28 days	Jones and Bell (2004)

TABLE 1 Relative changes in plasma testosterone levels in males following exposure to stressors or stress hormones in different groups of reptile—cont'd

Species	Relative changes in plasma testosterone (T) levels	Reference
Keeded Indian Mabuya (<i>Mabuya carinata</i>)	T levels in males exposed for 30 days to a variety of stressors or to a variety of stressors and FSH injections were lower than in males that received only FSH injections	Yajurvedi and Menon (2005)
Red-sided garter snake (<i>Thamnophis sirtalis parietalis</i>)	T levels in males that were captured in the spring and held in cloth bags for up to 4 h were lower at 2 h postcapture than in control males that were captured and bled within 90 s of capture	Moore et al. (2001)
Red-sided garter snake (<i>Thamnophis sirtalis parietalis</i>)	T levels in males that were captured and subjected to serial blood sampling were lower than initial values at 4 h postcapture in males captured in the summer, and at 4 h in males captured in the fall, but were not significantly different from initial values at any sample time in males that were captured in the early or late spring	Moore et al. (2001)
Red-spotted garter snake (<i>Thamnophis sirtalis concinnus</i>)	T levels in males that were captured and subjected to serial blood sampling were lower than initial values at 1 h postcapture in males captured in the spring of 1995 and of 1999 and at 1 h postcapture in males captured in the fall of 1999, but were not significantly different from initial values in males captured in the summer of 1999	Moore et al. (2001)
Brown tree snake (<i>Boiga irregularis</i>)	T levels in males captured in the field from a wild population were lower in monthly samples than in males in a captive population	Moore et al. (2005)

ACTH, corticotropin; CORT, corticosterone; FSH, follicle-stimulating hormone; T, testosterone.

corticosterone or sesame oil (placebo) delivered via dermal patches (see Knapp & Moore, 1997b). The plasma levels of corticosterone in males of both morphs that received corticosterone patches did not differ significantly at the end of the experiment, and importantly the corticosterone levels were similar to those observed in male *Urosaurus ornatus* exposed to stressors (Knapp & Moore, 1997a; Moore et al., 1991). The testosterone levels in males of both morph types that received corticosterone were significantly lower than in controls; however, testosterone levels in males of the nonterritorial orange morph were significantly lower than in males of the territorial orange-blue morph. Similar to tree lizards, the relationship between plasma corticosterone levels and plasma androgen levels in males differs with male mating tactics in the collared lizard *Crotaphytus collaris* (Baird & Hews, 2007), although it is unclear to what extent this difference is mediated by stress. According to Baird and Hews (2007), plasma corticosterone levels in male *Crotaphytus collaris* were negatively correlated with both testosterone and dihydrotestosterone levels in territorial males, but did not covary significantly with these androgens in nonterritorial males.

The finding in most studies that exposure of male reptiles to stressors increases circulating corticosterone levels and decreases circulating androgen levels is important because it suggests a means by which stress can inhibit male reproductive function. It also suggests that the energetics-hormone vocalization model proposed by Emerson (2001)

for amphibians might be useful in understanding the effects of stress in male reptiles because many display behaviors in male reptiles are androgen-dependent.

Very little information is available on what effect exposure to stressors has on other reproductive hormones in male reptiles. Licht et al. (1985) reported that in the painted turtle, *Chrysemys picta*, plasma FSH levels declined significantly in males within a day of capture. And Cree, Guillette, Cockrem, Brown, et al. (1990) in a study of the tuatara, *Sphenodon punctatus*, found that plasma progesterone levels in males were significantly elevated at 3, 12, and 24 h post capture and concluded that the increase in plasma progesterone following exposure of males to capture was most likely due to a stress-induced increase in adrenal synthesis of progesterone.

Surprisingly, relatively few studies have investigated the effects of stress on the reproductive hormones of female reptiles. The results from available studies suggest that the effects of stress on plasma levels of estradiol and progesterone vary by species and by female reproductive condition.

In the snapping turtle, *Chelydra serpentina*, females that were captured in June had significantly elevated plasma progesterone levels 6 h after capture and significantly elevated estradiol levels 24 h after capture (Mahmoud et al., 1989). The levels of these two steroid hormones returned to baseline within 7 days of capture. When analyzed by female

reproductive condition, gravid females had a significant increase in estradiol whereas nongravid females did not (Mahmoud et al., 1989). Although progesterone levels increased significantly in both gravid and nongravid female *Chelydra serpentina*, the increase in progesterone was unexpectedly greater in nongravid females than in gravid females (Mahmoud et al., 1989). It was suggested that the adrenal gland as well as ovarian follicles were the sources of the measured progesterone (Mahmoud et al., 1989).

In the tuatara, *Sphenodon punctatus*, progesterone levels were significantly higher in vitellogenic females that were captured in January and subjected to acute stress (i.e., capture and 3 h of confinement) than in free-roaming females (Cree, Guillette, Cockrem, Brown, et al., 1990). However, the plasma levels of estradiol and testosterone in these captive females did not differ significantly from those found in freely roaming females (Cree, Guillette, Cockrem, Brown, et al., 1990).

In adult female alligators (*Alligator mississippiensis*) that were captured during the breeding season and subjected to restraint and blood sampling for 48 h, plasma estradiol levels decreased significantly below initial values by 22 h after capture and then remained unchanged (Elsey et al., 1991). Corticosterone secretion had a biphasic pattern in that plasma corticosterone continued to rise for up to 16 h, then declined and then rose again significantly from 24 to 48 h (Elsey et al., 1991).

Stress may inhibit ovarian estradiol synthesis in the Indian keeled lizard, *M. carinata* (Ganesh & Yajurvedi, 2002a). Female *M. carinata* treated for 30 days with FSH (to induce ovarian recrudescence) and exposed daily to stressors (handling, chasing, and noise) had significantly lower serum estradiol levels than did females that were treated with FSH alone (Ganesh & Yajurvedi, 2002a).

In contrast to studies that have reported an effect of short-term stress on female reproductive hormones, no similar effect has been observed in female red-sided garter snakes, *Thamnophis sirtalis parietalis* (Whittier et al., 1987). Whittier et al. (1987) reported that the plasma levels of estradiol, progesterone, testosterone, and corticosterone in female *Thamnophis sirtalis parietalis* did not differ significantly in females that were captured in the fall, allowed to hibernate in the laboratory, and placed under summer-like condition in the laboratory than in females that were captured in the field after emergence from hibernation, maintained under fluctuating conditions, and then transferred to the laboratory during the first 5 weeks after emergence. Because the hormone cycles observed in these two groups of females were so similar, it was suggested that female garter snakes may be relatively insensitive to the disruption of neuroendocrine events that are induced by handling and captivity over time periods of hours and days (Whittier et al., 1987).

Moore et al. (2005) have suggested that an introduced population of brown tree snakes, *Boiga irregularis*, on Guam

may be experiencing stress-induced reproductive suppression because of possible overcrowding and overexploitation of food resources (Moore et al., 2005). Three lines of evidence have been advanced to support this claim (Moore et al., 2005). First, male and female *B. irregularis* captured on Guam have been found to have a significantly lower body condition relative to freely living snakes captured from the native range in Australia, and females (but not males) also have been found to have a significantly lower body condition relative to a captive population of snakes maintained at Oregon State University. Second, previous studies of a wild population of *B. irregularis* on Guam found relatively few breeding individuals (Mathies et al., 2001; Rodda et al., 1999). The third line of evidence for a stress-induced inhibition of reproduction in the Guam population of *B. irregularis* is based on the monthly baseline levels of plasma corticosterone and sex steroids in free-living and captive males and females during the annual cycle. Moore et al. (2005) found that plasma corticosterone levels were higher and plasma testosterone levels were lower in field-captured males than in captive males in most months. Similarly, plasma corticosterone levels were higher and plasma estradiol and progesterone levels lower in field-captured females than in captive females (Moore et al., 2005, Fig. 2). Moore et al. (2005) suggested that these differences in baseline hormone levels are consistent with the hypothesis that the Guam populations of *B. irregularis* are living under stressful conditions and are thus reproductively suppressed. If true, this would be an example of stress directly inhibiting reproductive activity in a wild reptile, something that Guillette et al. (1995) stated had not been documented in a reptile at the time of their review. However, although the observed baseline levels of corticosterone and sex steroids are indeed compatible with the hypothesis that *B. irregularis* in Guam are being reproductively suppressed, it must be kept in mind that it is extremely difficult to draw strong inferences when comparing the plasma levels of hormones from free-living animals to those from captive animals. Thus, additional studies are needed before the hypothesis that *B. irregularis* in Guam are being reproductively suppressed because of stress can be accepted.

3.3 Gonads

The effects of stress on gonadal function in reptiles have been considered in several excellent reviews (Greenberg & Wingfield, 1987; Guillette et al., 1995; Pottinger, 1997). Since the publication of these reviews, there is additional information on how stress affects gametogenesis in the Indian keeled lizard, *M. carinata* (Ganesh & Yajurvedi, 2002a, 2002b; Ganesh & Yajurvedi, 2003; Yajurvedi & Menon, 2005; Yajurvedi & Nijagal, 2000). Similar studies of the effects of stress on gonadal function in other lizards as well as crocodilians, snakes, turtles, and the tuatara are needed.

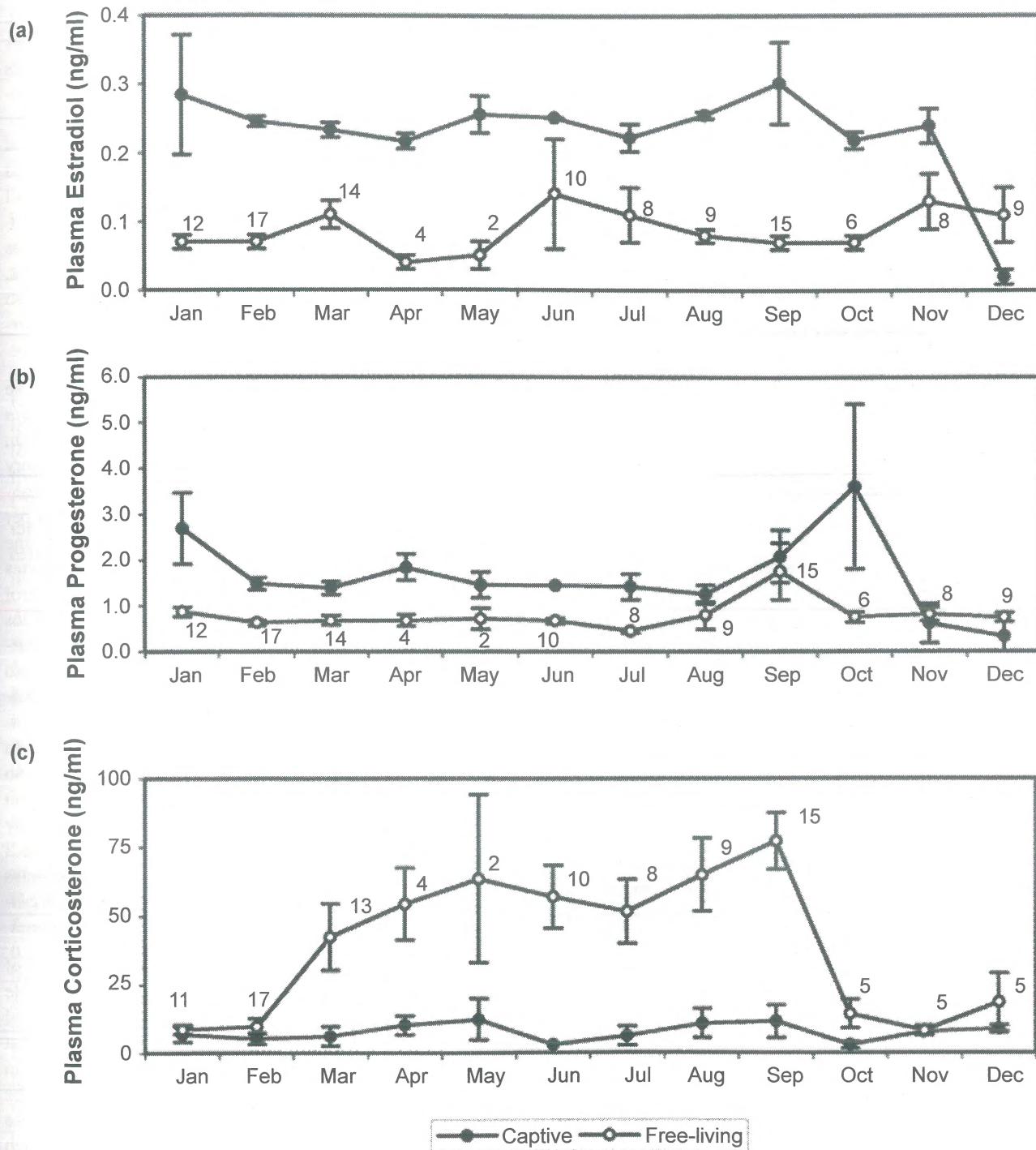


FIG. 2 Annual cycles of (A) estradiol (E_2), (B) progesterone (P_4), and (C) corticosterone (CORT) for female captive and freely living populations of brown tree snakes (*Boiga irregularis*) from Guam. Numbers next to the points are the sample sizes for the free-living group. Sample size for the captive group was seven for all months. (Reproduced from Moore, I. T., Greene, M. J., Lerner, D. T., Asher, C. E., Krohmer, R. W., Hess, D. L., Whittier, J., & Mason, R. T. (2005). Physiological evidence for reproductive suppression in the introduced population of brown tree snakes (*Boiga irregularis*) on Guam. Biological Conservation, 121, 91–98.)

3.3.1 Ovarian function

There is convincing evidence that stress affects ovarian function in lizards (Crews, 1974; Ganesh & Yajurvedi, 2002a, 2002b, 2003; Morales & Sánchez, 1996; Summers, 1995; Summers & Norman, 1988; Summers et al., 1995). In

the green anole, *Anolis carolinensis*, social factors affect the rate of environmentally induced ovarian recrudescence in females (Crews, 1974). Crews (1974) found that reproductively inactive female *Anolis carolinensis* that were exposed to stimulatory environmental conditions and dominant males exhibiting more intermale aggressive behavior

than courtship behavior had a reduced rate of ovarian recrudescence (percentage of females with yolk follicles and median ovarian condition) in comparison to control females. The inhibition of ovarian recrudescence in female *Anolis carolinensis* that were exposed to intermale aggressive behavior was not due to the absence of male courtship behavior because females that were exposed to castrated males, which did not court females, exhibited normal ovarian recrudescence (Crews, 1974). Thus, exposure of females to male aggressive behavior during the period of ovarian recrudescence appears to be a social stressor in *Anolis carolinensis*.

Female density, another social stressor, has also been shown to affect the rate of ovarian recrudescence in female *Anolis carolinensis* (Summers et al., 1995). Seasonally nonreproductive female *Anolis carolinensis* that were housed in a group of five females and one male as well as exposed to stimulatory environmental conditions for one month had smaller ovaries and less oviductal mass than did females that had been housed singly with a male (Summers et al., 1995). Interestingly, this inhibitory effect of female density was found to be size-dependent as females weighing more than 2.7 g or more exhibited normal ovarian recrudescence independent of whether they were housed with females (Summers et al., 1995). Additional evidence indicates that female body size is an important factor governing the effects of social stressors during ovarian recrudescence in female *Anolis carolinensis* (Summers, 1995). In a study in which female *Anolis carolinensis* were treated with corticotropin (ACTH) once a day for 3 weeks, ovarian and oviductal growth was inhibited in small females (<2.6 g) but enhanced in large females (>2.6 g; Summers, 1995).

Environmental stressors have also been shown to influence the rate of environmentally induced ovarian recrudescence in female *Anolis carolinensis*. Seasonally reproductively inactive female *Anolis carolinensis* that were housed under relatively low humidity conditions (<35%) as well as a stimulatory temperature and photoperiod regime had reduced ovarian growth and oviductal growth in comparison to females that were housed under relatively high humidity conditions (>80%) and stimulatory environmental conditions (Summers, 1988; Summers & Norman, 1988). It was suggested that dehydration of females in the relatively low humidity treatment group might have acted directly to inhibit reproduction or indirectly via the release of stress hormones (Summers & Norman, 1988).

Captivity appears to be a potent stressor in many reptiles (reviewed in Gangloff & Greenberg, 2023; Romero, 2002). Morales and Sánchez (1996) found that captivity-induced stress disrupted female follicular growth and vitellogenesis in the tropical sharp-mouthed lizard, *Anolis pulchellus*. Vitellogenesis, the synthesis of yolk protein precursors such as vitellogenin and their processing and deposition by growing oocytes, is a fundamental process in the development of

ovarian follicles in reptiles (reviewed in Guraya, 1989). Female *Anolis pulchellus* that had been exposed to 4 days of captivity had significantly less vitellogenin synthesis and mRNA in the liver and lower plasma levels of vitellogenin than control females (Morales & Sánchez, 1996). These negative effects of captivity on vitellogenesis and ovarian development in female *A. pulchellus* were reduced or prevented in females that were treated with estradiol during captivity, a finding that suggests that the stress associated with captivity suppressed estradiol synthesis, which is required to stimulate vitellogenesis (Morales & Sánchez, 1996). Exposing females to the stress elicited by capture also affects ovarian function in the snapping turtle, *Chelydra serpentina* (Mahmoud & Licht, 1997).

The effects of stress on seasonal ovarian development have also been examined in the Indian keeled lizard, *M. carinata* (Ganesh & Yajurvedi, 2002a, 2002b; Ganesh & Yajurvedi, 2003). Female *M. carinata* that were exposed to stimulatory environmental conditions and to physical stressors (handling, chasing, and noise applied randomly five times per day for 1 month) during the recrudescence phase of the ovarian cycle had significantly reduced numbers of oocytes and primordial follicles compared to untreated control females and completely lacked vitellogenic follicles (Ganesh & Yajurvedi, 2002a). Moreover, female *M. carinata* that were exposed to the same stressors but were also treated with follicle-stimulating hormone (FSH) had significantly fewer oogonia, primary oocytes, and primordial follicles than did females that were treated only with FSH. In addition, females exposed to stressors as well as FSH had no vitellogenic follicles and had significantly lower serum estradiol levels and liver weights than females treated only with FSH. Based on these findings Ganesh and Yajurvedi (2002a) suggested that the growth of vitellogenic follicles was inhibited by a stress-induced decrease in estradiol synthesis that was not prevented by FSH treatment.

Two lines of evidence suggest that the inhibitory effects of stress on ovarian development in FSH-treated female *M. carinata* are mediated by the activation of the adrenal gland (Ganesh & Yajurvedi, 2002a). First, females that were exposed to stressors had a significant increase in the nuclear diameter of the steroidogenic cells of the adrenal cortex; and second there was a significant decrease in the number of islands of white pulp in the spleen, which is the main site of lymphocyte production. Ganesh and Yajurvedi (2002a) suggested that the stress-induced activation of the adrenal gland in female *M. carinata* stimulated corticosterone synthesis, and the resulting increase in corticosterone may have inhibited ovarian development and suppressed the immune system by decreasing lymphocyte production.

Stress-induced inhibition of ovarian recrudescence in female *M. carinata* requires the activation of the hypothalamic-

pituitary-adrenal axis (Ganesh & Yajurvedi, 2002b). Treatment of female *M. carinata* with an antagonist (α -helical CRF₉₋₄₁) to CRF largely prevented the inhibition of ovarian and oviductal development in females that were exposed to stressors during the recrudescence phase of the ovarian cycle (Ganesh & Yajurvedi, 2002b). Specifically, females exposed to both stressors and the CRF antagonist had significantly heavier ovaries and oviducts (relative to body size), significantly more oocytes, primordial follicles, and stage II follicles (previtellogenic follicles) than did females exposed only to stressors. Moreover, unlike the females who were exposed only to stressors and lacked vitellogenic follicles (stage IV and stage V), females who were exposed to stressors but who also received the CRF antagonist had vitellogenic follicles. The exposure of female *M. carinata* to stressors also adversely affected ovarian germinal bed activity because females exposed only to stressors had significantly fewer oocytes and primordial follicles (Ganesh & Yajurvedi, 2002b). Treatment with the CRF antagonist also inhibited the stress-induced increase in the nuclear diameter of the steroidogenic adrenal cortical cells and the decrease in number of islands of white pulp in the spleen (Ganesh & Yajurvedi, 2002b).

A further indication of the involvement of the HPA in the stress-induced inhibition of seasonal recrudescence in *M. carinata* is the finding that treatment of females with β -endorphin inhibits both seasonal and FSH-induced ovarian recrudescence (Ganesh & Yajurvedi, 2003). Endogenous opioids including β -endorphin are released as part of the stress response (reviewed in Drolet et al., 2001). Treatment of female *M. carinata* with beta-endorphin daily for 30 days during the period of seasonal ovarian recrudescence inhibited vitellogenesis compared to control females treated with distilled water (Ganesh & Yajurvedi, 2003). Germinal bed activity was also inhibited in the ovaries of females that received either the 0.5 or 1.0 mg (but not 0.1 mg) dose of β -endorphin in that they had significantly fewer primordial follicles than controls. When administered concurrently with FSH, β -endorphin also inhibited FSH-induced ovarian recrudescence and vitellogenesis. This finding led Ganesh and Yajurvedi (2003) to suggest that β -endorphin in female *M. carinata* may be having an inhibitory effect at the level of the ovary independent of an effect on the release of pituitary FSH. These investigators speculated that β -endorphin treatment might have prevented vitellogenesis by inhibiting estradiol synthesis by ovarian follicles (Ganesh & Yajurvedi, 2003). Interestingly, β -endorphin treatment stimulated rather than inhibited in vitro estradiol synthesis by ovaries obtained from the Italian wall lizard, *Podarcis sicula sicula* (Polzonetti-Magni et al., 1994). This difference in the effects of β -endorphin treatment may be due to the fact that the Ganesh and Yajurvedi (2003) study was an in vivo study that used relatively high doses (i.e., 0.1, 0.5, or 1.0 mg) of

β -endorphin, whereas the Polzonetti-Magni et al. (1994) study was an in vitro study that used a relatively low dose (4 pmol) of β -EP.

3.3.2 Testicular function

Exposure to stressors or the stress hormone corticosterone has been shown to affect testicular function in several species of lizards (Dunlap & Schall, 1995; Manzo et al., 1994; Tokarz, 1987; Yajurvedi & Menon, 2005; Yajurvedi & Nijagal, 2000) and in a turtle (Mahmoud & Licht, 1997). However, no significant relationship was found to exist between testicular function and either social status (dominant versus subordinate males) or relative corticosterone levels (high versus low) in the green anole, *Anolis carolinensis* (Greenberg et al., 1984). Although male *Anolis carolinensis* that were judged in male-male pairings to be subordinate individuals had significantly lower corticosterone levels than did males that were judged to be dominant they did not differ from dominant males in spermatogenic stage as assessed by the relative proportions of spermatogonia, primary spermatocytes, spermatids, and spermatozoa in the seminiferous tubules (Greenberg et al., 1984). It is unclear why there was no significant difference in spermatogenic stage between subordinate and dominant males given that they differed significantly in corticosterone levels. One possible explanation is that corticosterone levels in subordinate males may not have been elevated for a sufficient period of time to affect testicular function. Another possibility put forth by Greenberg et al. (1984) is that it might be highly adaptive for subordinate males not to differ from dominant males in spermatogenic stage because it would permit them to breed relatively quickly either because they successfully supplanted other dominant males after aggressive interactions or because the former dominant males were removed by predators. A similar phenomenon has been noted in birds in which the gonads and accessory organs of animals that are exposed to short-term stressors due to storms are maintained in a near functional state so that birds are able to renest quickly after the storm is over (Wingfield & Romero, 2001).

Although the study by Greenberg et al. (1984) did not find an effect of stress due to social interactions on testicular function in *Anolis carolinensis*, studies of other lizards have demonstrated that exposure to stressors or to corticosterone adversely affects testicular function. For example, in the brown anole, *Anolis sagrei*, reproductively active males that were implanted for 3 weeks with corticosterone pellets had significantly reduced testis weight, less spermatogenic activity, and significantly lower plasma testosterone levels than did placebo-treated controls (Tokarz, 1987). Similarly, in the fence lizard, *Sceloporus occidentalis*, males who were implanted with corticosterone had a significant reduction in testosterone levels and testis mass compared

to males who received empty implants (Dunlap & Schall, 1995). This is a reasonable result given that the transition of spermatocytes into spermatozoa is known to be highly androgen-dependent in reptiles (Lance, 1984).

Environmental stressors have also been demonstrated to inhibit testicular activity in male *M. carinata* (Yajurvedi & Menon, 2005). Yajurvedi and Menon (2005) exposed male *M. carinata*, which were at the beginning of the recrudescence phase of the annual testicular cycle, to either mild acute stressors (handling, chasing, noise applied randomly for 5 min each, five times a day), to FSH (on alternative days), to stressors plus FSH, or to saline (treatment control) for 30 days. Males that were exposed to the stressors had significantly fewer secondary spermatocytes and comparatively fewer spermatozoa in the seminiferous tubules than did treatment-control males (Yajurvedi & Menon, 2005). The effects of exposure to stressors were much more evident in males who had also been treated with FSH in that they had significantly fewer primary and secondary spermatocytes as well as spermatids than did males who were treated with just FSH. However, they did not differ significantly in number of spermatogonia. Males that were exposed to both stressors and FSH also had significantly lower serum testosterone levels than in FSH-treated males, a finding that may indicate that stress in male *M. carinata* inhibits the later stages of spermatogenesis (i.e., formation of secondary spermatocytes) and spermiogenesis by decreasing testosterone secretion. Based on these findings, Yajurvedi and Menon (2005) argued that stress in male *M. carinata* has less of an effect on spermatogonial proliferation and entrance into meiosis (forming primary spermatocytes) than it does on the later stages of spermatogenesis (formation of secondary spermatocytes) and spermiogenesis. This is a reasonable argument in light of the evidence that spermatogonial proliferation and early stages of meiosis in reptiles are FSH-dependent processes, whereas the later stages of spermatogenesis and spermiogenesis are largely androgen-sensitive processes (Lance, 1984). Thus, there is evidence for a stage-dependent response of spermatogenic cells to stress in male *M. carinata* (Yajurvedi & Menon, 2005). It should also be noted that normal adrenal activity is apparently necessary for the recrudescence and maintenance of testicular activity in male *M. carinata* because spermatogenic activity and testicular steroidogenesis are depressed in adrenalectomized males (Yajurvedi & Chandramohan, 1993). It has been hypothesized that the decline in testicular function following adrenalectomy in male *M. carinata* is due to a decrease in pituitary gonadotropin release and subsequent decline in testosterone secretion (Yajurvedi & Chandramohan, 1993). The finding that treatment of adrenalectomized male *M. carinata* with LH and FSH normalized spermatogenic and steroidogenic activity supports this hypothesis (Yajurvedi & Chandramohan, 1994).

3.4 Behavior

Exposure of animals to unpredictable environmental stressors elicits what has been traditionally termed an acute stress response but more recently has been termed an “emergency life history stage” (Wingfield et al., 1998; Wingfield & Ramenofsky, 1999; Wingfield & Romero, 2001). An emergency life history stage consists of a suite of facultative behavioral and physiological responses that are largely mediated by the activation of the hypothalamic-pituitary-adrenal axis and release of glucocorticoids (Landys et al., 2006; Wingfield et al., 1997; Wingfield et al., 1998; Wingfield & Ramenofsky, 1999). During an emergency life history stage (=physiological state C, Wingfield et al., 1998) an animal might abandon its current life history state, especially if it is a relatively energetically expensive state such as reproduction, and adopt an alternative strategy such as moving away, seeking refuge, or seeking refuge and then moving away if conditions do not improve (Wingfield & Ramenofsky, 1999). This concept that animals in an emergency life history stage change their behavior in response to the short-term effects of glucocorticoids (i.e., over minutes to hours) has been demonstrated especially well in field studies of birds in which individuals that were exhibiting reproductive behavior changed their behavior in response to rapid and unexpected environmental perturbations such as severe storms (e.g., see Wingfield, 1988; Wingfield & Romero, 2001).

To our knowledge, there are no studies of reptiles comparable to those of birds that have documented a change in reproductive behavior in free-living animals that is due to an emergency life history stage caused by unpredictable, short-term environmental stressors. There are, however, studies of reptiles that have sought to ascertain whether male or female reptiles alter their reproductive behavior when treated experimentally with corticosterone to elevate the levels of this stress hormone to the levels that would likely occur in an emergency life history stage. Using this experimental approach, corticosterone treatment has been shown to inhibit male aggressive and territorial behavior in lizards (DeNardo & Licht, 1993; DeNardo & Sinervo, 1994a, 1994b; Tokarz, 1987) and male mating behavior in snakes (Lutterschmidt et al., 2004; Moore & Mason, 2001).

In many species of lizards, male territorial behavior is an important factor affecting male mating success (reviewed in Olsson & Madsen, 1998; Stamps, 1983), and several studies have shown that corticosterone treatment inhibits aspects of male territorial behavior (DeNardo & Licht, 1993; DeNardo & Sinervo, 1994a, 1994b; Tokarz, 1987). In the brown anole, *Anolis sagrei*, males that were implanted with corticosterone pellets exhibited significantly fewer displays, approaches, and bites in response to

stimulus males than did placebo-treated controls; they also erected a dorsal crest less frequently and had significantly lower plasma testosterone levels than did placebo-treated males (Tokarz, 1987). Similarly, DeNardo and Licht (1993) in a laboratory study of the side-blotched lizard, *Uta stansburiana*, found that corticosterone-implanted males in comparison to saline-implanted males had significantly reduced display and attack behavior when challenged by an intruder male as well as significantly lower circulating testosterone levels. The inhibitory effects of corticosterone on aggressive behavior in male *U. stansburiana* appear to be mediated directly by corticosterone rather than by a corticosterone-induced lowering of testosterone levels because males treated with both corticosterone and testosterone implants still showed reduced male aggressive behavior (DeNardo & Licht, 1993). Although corticosterone treatment inhibited male aggressive behavior, it did not inhibit male courtship or copulatory behavior when corticosterone-treated males were tested with estrogen-treated and thus presumably sexually receptive females (DeNardo & Licht, 1993). Corticosterone treatment has also been found to inhibit territorial behavior in free-living male *U. stansburiana* (DeNardo & Sinervo, 1994a). Corticosterone-implanted male *U. stansburiana* were found to be significantly less active and had significantly smaller home ranges than saline-implanted males, but only when their neighbors were saline-treated and thus normally aggressive males (DeNardo & Sinervo, 1994a). Because corticosterone-implanted males lost space to saline-implanted males, DeNardo and Sinervo (1994a) suggested that elevated corticosterone levels put males at a competitive disadvantage. Corticosterone treatment has also been found to inhibit territorial behavior in male *U. stansburiana* even when males are treated with testosterone (DeNardo & Sinervo, 1994b). Male *U. stansburiana* that were implanted with testosterone had a significant increase in activity and home range size compared to saline-implanted males, whereas males that received both testosterone and corticosterone implants had a reduction in activity and in home-range size similar to that previously reported for males that were implanted only with corticosterone (DeNardo & Sinervo, 1994b).

Corticosterone treatment also affects male locomotor performance and endurance in *Uta stansburiana* (Miles et al., 2007). Male *U. stansburiana* (blue morph) that were given long-term implants of corticosterone had increased locomotor performance (stamina) and a lower resting metabolic rate in comparison to sham-treated control males (Miles et al., 2007). It was suggested that this observed corticosterone-induced increase in locomotor performance and decrease in resting metabolic might be adaptive by increasing the ability of subordinate males to move to new areas and to conserve energy necessary for survival (Miles et al., 2007).

Stress-induced increases in plasma corticosterone levels may affect how a reptile responds in future social interactions. The blocking of corticosterone synthesis with injections of the drug metyrapone has been shown to inhibit male habituation to repeated aggressive stimuli in the green anole, *Anolis carolinensis* (Yang & Wilczynski, 2003). This finding, if confirmed, would fit with the idea that elevated levels of corticosterone play an important role in modifying an animal's subsequent social behavior.

In the copperhead, *Agristodon contortrix*, a nonterritorial species of snake, males compete with other males for priority access to females (Schuett & Grober, 2000; Schuett et al., 1996). Several studies of male *A. contortrix* have examined whether the losers and winners of stage fights differ in hormone profiles (Schuett & Grober, 2000; Schuett et al., 1996). One hour after staged male-male fights, losers of fights were found to have significantly higher plasma levels of corticosterone than did winners of fights, or in males of a control group in which males were paired with a female, although losers and winners as well as control males did not differ significantly in plasma testosterone levels (Schuett et al., 1996). Schuett and Grober (2000) have also shown in a subsequent study of male *A. contortrix* that the plasma levels of corticosterone and lactate 60 min after fights were significantly higher in losers than in winners of fights or in control males that were not used in fights (Fig. 3). Schuett and Grober (2000) suggested that losers of fights had higher levels of lactate and corticosterone than winners because of differences in psychoneuroendocrine factors rather than because of differences in exercise during fights. These investigators also proposed that the elevated levels of corticosterone in losers might retard metabolic recovery and perhaps inhibit subsequent aggressive behavior (Schuett & Grober, 2000).

Corticosterone treatment or a stress-induced increase in corticosterone levels have also been found to inhibit mating behavior in several species of snakes (Lutterschmidt et al., 2004; Moore & Mason, 2001; Schuett, 1996). Corticosterone injections, for example, have been found to inhibit male courtship behavior in the red-sided garter snake, *Thamnophis sirtalis parietalis* (Lutterschmidt et al., 2004; Moore & Mason, 2001). Red-sided garter snakes are unusual because male courtship behavior is not dependent upon the activational effects of sex-steroid hormones (Crews, 1984, 1991; Crews et al., 1984). Moore and Mason (2001) in a study of *Thamnophis sirtalis parietalis* found that corticosterone treatment suppressed male mating behavior in a threshold manner. These investigators reported that the proportion of males that courted unmated, sexually attractive females in an arena was significantly lower in males that had been captured one month after spring emergence and treated with intraperitoneal injections of either 25, 50, or 100 mg (but not 10 mg) of corticosterone

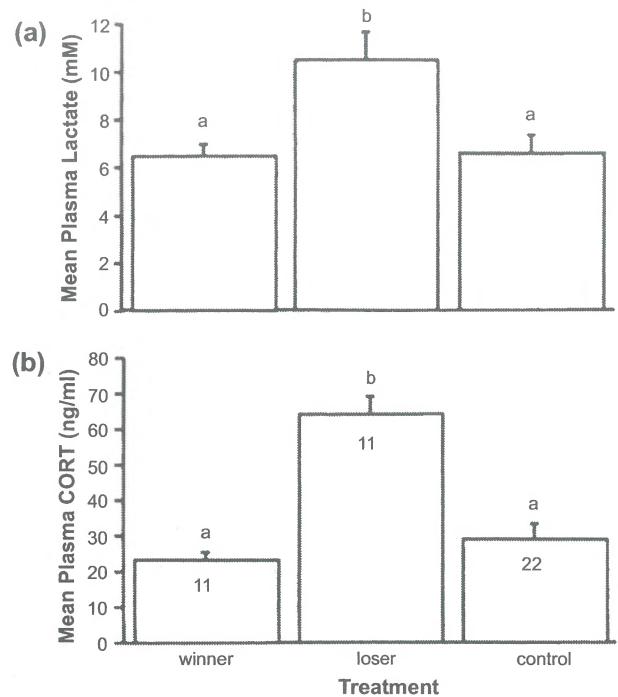


FIG. 3 Effects of fight outcome on (A) mean (\pm SEM) plasma lactate and (B) mean plasma corticosterone (abbreviated B or CORT) in male copperhead snakes (*Akistrodon contortrix*). Sample sizes for both histograms are provided in (B). Letters above error bars represent significant differences, $P < .05$. (Reproduced from Schuett, G. W., & Grober, M. S. (2000). Post-fight levels of plasma lactate and corticosterone in male copperheads, *Akistrodon contortrix* (Serpentes, Viperidae): Differences between winners and losers. *Physiology and Behavior*, 71, 335–341.)

than in vehicle-treated or untreated males (Fig. 4). The inhibitory effects of corticosterone on male sexual behavior in *Thamnophis sirtalis parietalis* noted in the Moore and Mason (2001) study do not appear to be mediated via a corticosterone-induced lowering of testosterone levels because corticosterone treatment had no effect on plasma testosterone levels.

Corticosterone injections significantly reduced courtship behavior in a dose-dependent manner in free-living male *Thamnophis sirtalis parietalis* that were captured during the month following their emergence from hibernacula (Lutterschmidt et al., 2004), a time when mating and plasma testosterone levels are declining (Krohmer et al., 1987). In addition to examining the effects of corticosterone treatment, Lutterschmidt et al. (2004) also investigated what effects the hormone melatonin and the serotonergic type 2A (5-HT_{2A}) receptor antagonist, ketanserin, have on male courtship behavior in *Thamnophis sirtalis parietalis*. The experiments were designed to determine whether melatonin modulates the behavioral and hormonal responses of males to exogenous corticosterone. Males in four pretreatment groups received a single intraperitoneal injection of vehicle, 0.03 mg melatonin (low dose), 0.3 mg melatonin (high

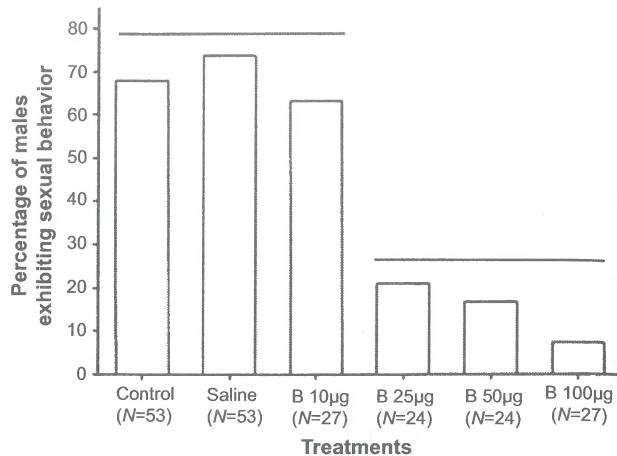


FIG. 4 Percentage of male red-sided garter snakes, *Thamnophis sirtalis parietalis*, exhibiting sexual behavior in groups of males that were treated with different doses of corticosterone (B), saline, or were untreated. Groups underneath the solid lines are not significantly different from one another. (Reproduced from Moore, I. T., & Mason, R. T. (2001). Behavioral and hormonal responses to corticosterone in the male red-sided garter snake, *Thamnophis sirtalis parietalis*. *Physiology & Behavior*, 72, 669–674.)

dose), or ketanserin (0.045 mg). Thirty minutes after pretreatment, males within each group received either no corticosterone (vehicle), 15 mg of corticosterone, or 60 mg of corticosterone and then were exposed in an arena to unmated, sexually attractive females. Within each pre-treatment group corticosterone treatment significantly decreased average courtship scores (Lutterschmidt et al., 2004). Moreover, pretreatment with melatonin before corticosterone treatment further suppressed male courtship behavior. Melatonin pretreatment when followed by vehicle also significantly inhibited male courtship behavior. These findings led Lutterschmidt et al. (2004) to suggest that melatonin and corticosterone have an additive inhibitory effect on male courtship behavior in *Thamnophis sirtalis parietalis*. Pretreatment of male *Thamnophis sirtalis parietalis* with the 5-HT_{2A} receptor antagonist ketanserin followed by corticosterone treatment reduced male courtship behavior. However, pretreatment with ketanserin when followed by vehicle treatment did not reduce male courtship behavior. The inhibitory effects of melatonin and corticosterone on male courtship behavior do not appear to be mediated by androgens because treatment with either melatonin or corticosterone did not significantly affect the circulating concentrations of testosterone or of 5 α -dihydrotestosterone. Based on the observed effects of melatonin, corticosterone, and ketanserin, Lutterschmidt et al. (2004) argued that corticosterone and melatonin modulate male courtship behavior in *Thamnophis sirtalis parietalis* via their actions on a serotonergic neural system. However, it should be noted that Lutterschmidt and Mason (2005) in a subsequent study of male *Thamnophis sirtalis parietalis*

found no evidence that melatonin plays a role in the modulation of the stress response.

A stress-induced increase in plasma corticosterone also appears to inhibit male courtship behavior in the copperhead, *A. contortrix* (Schuett, 1996). In this species of snake, males fight for priority access to females during the two mating periods in the spring and late fall (Schuett, 1996; Schuett et al., 1996). In staged fights between male *A. contortrix*, males that lost fights in comparison to males that won fights exhibited significantly less courtship behavior in trials with females, which occurred 30 min after fights (Schuett, 1996). This inhibition of male courtship behavior in losers was relatively long-lasting because it was still present when losers were retested at 24 h postfights, although courtship scores did recover by 7 days postfights (Schuett, 1996).

To our knowledge, there are no examples in reptiles of females terminating or modifying their reproductive behavior in response to short-term stressors. However, there are examples of females continuing to maintain reproductive behaviors even when exposed to extreme stressors (Moore & Jessop, 2003). In the green turtle, *Chelonia mydas*, for example, females that had been recently seriously injured by sharks have been found to maintain nesting behaviors (Jessop, 2000). Similarly, in the red-sided garter snake, *Thamnophis sirtalis parietalis*, females have been observed to mate despite sustaining injuries or being exposed to adverse environmental conditions (Moore, 1999; Whittier et al., 1987). Elsey et al. (1990) also noted that in the American alligator, *Alligator mississippiensis*, plasma corticosterone levels in females that were actively defending their nests (in breeding pens) did not differ significantly from females that were not attending their nests. There are several possible reasons for why females might be less likely than males to modify their reproductive behavior in response to short-term stress. First, female reptiles have a greater energy investment in their eggs than do males. Thus, it may benefit females more than males to maximize their current reproductive effort, even though under certain situations it could entail costs to female survival (Moore & Jessop, 2003). Second, females in comparison to males may require much higher levels of plasma corticosterone before there is a change in female behavior in response to a stressor (Moore & Jessop, 2003).

4 SEASONAL CHANGES IN CIRCULATING CORTICOSTERONE LEVELS

4.1 Baseline and stress levels

Baseline and stress-induced glucocorticoid levels vary seasonally in some species of reptiles (reviewed in Romero,

2002; Romero & Reed, 2005). These authors suggest that corticosterone "samples collected in less than 2 min can be assumed to reflect unstressed concentrations with a high degree of confidence, and samples collected within 3 min most likely reflect unstressed concentrations." It is important to note, however, that upstream secretagogues are likely to be released much faster, and that measuring wild population corticosterone levels is difficult. Populations exposed to chronically stressful stimuli make it difficult to know if capture and sampling are altering the levels.

A novel approach to circumvent this issue was utilized by Baxter-Gilbert et al., 2014 measuring turtle corticosterone levels from the claws of freshly killed specimens on a major roadway. Although males had higher corticosterone levels on average compared to females, comparing claw trimmings of painted turtles (*Chrysemys picta*) from control sites against specimens impacted on roadways yielded no difference in corticosterone levels (Baxter-Gilbert et al., 2014).

Temperature is a critical environmental factor influencing reproduction and hormonal responses for all species, however, specifically for poikilotherms temperature dictates activity levels, especially metabolism (Newell, 1966), and neuronal function (Kerkut & Taylor, 1958; Prosser & Nelson, 1981). This is also true for baseline glucocorticoid concentrations in lizards (Racic et al., 2020). Eastern fence lizards (*Sceloporus undulatus*), acclimated to four different temperatures 22°C, 29°C, 33°C, and 36°C had baseline and stress-induced corticosterone concentrations which increased as temperature increased (Fig. 5), suggesting that metabolic rate was a critical factor. Moreover, a threshold may exist for significant stress-induced corticosterone secretions to occur. At 29°C, lizards produced a robust increase in corticosterone in response to a restraint stressor, an effect that was not evident in *Sceloporus undulatus* held at 22°C (Fig. 5). Interestingly, corticosterone concentrations measured from wild animals caught at various ambient temperatures were not correlated with body temperature (Racic et al., 2020; Fig. 5). The data suggest that field capture methods are stressful enough to occlude the metabolic effect clearly evident in the laboratory. Additionally, field conditions may contribute to significant other factors, including those modifying metabolism (eating, reproductive behavior, being chased by a predator, or fighting with a conspecific), that may have muted the effect of ambient/body temperature influence on hormonal secretion.

It was also noted by Romero (2002), however, that in some species of reptiles, a stress-induced increase in corticosterone might not occur until after 10 min of exposure to a stressor. However, a number of studies in the lizard *Anolis carolinensis* have demonstrated that a stressor may induce a

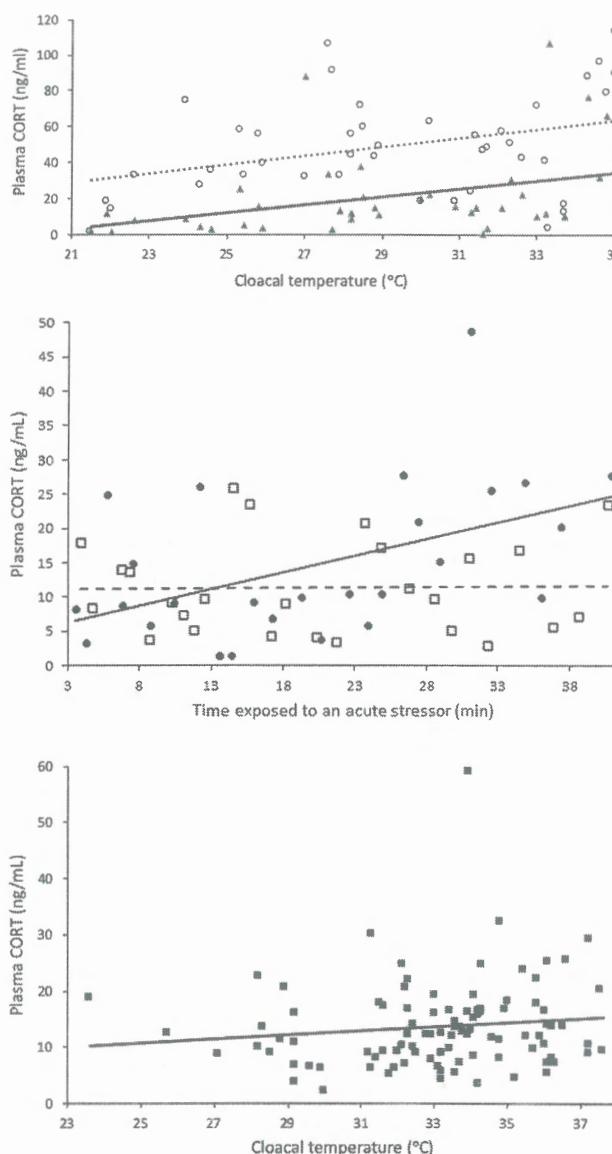


FIG. 5 (Top) Baseline (solid line and triangles) and stress-induced (dotted line and open circles) plasma corticosterone concentrations of *Sceloporus undulatus* lizards ($n=41$) at different body temperatures in the laboratory. (Middle) Plasma corticosterone concentrations of lizards following exposure to a stressor for different lengths of time (encompassing both time in a cloth bag and time required to obtain blood) in a warm (solid line and circles) or a cool room (dashed line and open squares). (Bottom) Baseline plasma corticosterone concentrations versus body temperature of lizards measured at the time of capture from the field. (Reproduced from Racic, A., Tylan, C., & Langkilde, T. (2020). Effects of temperature on plasma corticosterone in a native lizard. *Scientific Reports*, 10, 16315.)

significant elevation in plasma corticosterone levels within 1.5 min and that the speed of this response may be dependent on territorial or social status (Ling et al., 2009; Summers, Watt, et al., 2005; Watt et al., 2005). Therefore, Romero (2002) has suggested that “baseline” is a more appropriate term than “basal” for describing glucocorticoid levels in

animals that were sampled within several minutes of capture because basal levels would be those in unstressed animals before capture.

It has been suggested that seasonal baseline glucocorticoid levels meet the demands normally associated with a predictable life-history stage (physiological state B), whereas stress-related levels of glucocorticoids respond to life-threatening and unpredictable events associated with an emergency life-history stage (physiological state C) (Landys et al., 2006; Wingfield et al., 1997, 1998; Wingfield & Ramenofsky, 1999; Wingfield & Romero, 2001). Most studies have found that diel and seasonal elevations in baseline glucocorticoid levels are much lower than stress-related levels, and it is thought that these baseline levels of glucocorticoids play a key role in the regulation of feeding, locomotor activity, and energy metabolism associated with predictable life-history cycles (Landys et al., 2006). Baseline glucocorticoid levels also mediate important ontogenetic transitions (Wada, 2008).

4.2 Prebreeding, breeding, and postbreeding periods

To make it easier to discern possible patterns in a data set with high variability in assay methods, sampling times after animal capture, and type of glucocorticoid measured, Romero (2002) ranked glucocorticoid levels, measured within a season, as being either high, medium, or low relative to the levels at other seasons for the same sex and species (Table 2). The seasons were prebreeding, breeding, postbreeding, and other. Seasons were assigned different relative ranks for glucocorticoid levels only when a significant difference was present between seasons in measured glucocorticoid levels. On the other hand, if glucocorticoid concentrations did not vary significantly with season, a ranking of medium was assigned to each season measured for glucocorticoid levels.

Baseline corticosterone levels in reptiles varied seasonally for the majority of examined species with seasonal reproductive cycles and plasma corticosterone levels were most often highest during the breeding season relative to other seasons (Romero, 2002). The assertion that baseline corticosterone levels in many reptiles tend to be highest during the breeding season is strengthened by the results of earlier studies that showed that the size (mass and/or volume) of the adrenal glands was greatest in animals sacrificed during the breeding season (Romero, 2002; see references in Guillette et al., 1995). Manzo et al. (1994) found that in male *Podarcis sicula sicula* the in vitro pattern of adrenal gland corticosterone secretion paralleled the in vivo pattern of corticosterone secretion in that the highest secretion rates occurred in May during the mating phase.

TABLE 2 Relative baseline and stressor-induced corticosterone levels during the breeding and nonbreeding seasons in different groups of reptile

Species	Sex	Relative plasma corticosterone (CORT) levels	Reference
Loggerhead sea turtle (<i>Caretta caretta</i>)	Male and female	Baseline and stressor-induced CORT in small turtle caught by trawl were higher in the summer breeding season than in the winter nonbreeding season, and in small turtles baseline CORT was not significantly different between season but stressor-induced CORT was higher in the breeding season	Gregory et al. (1996)
Galápagos tortoise (<i>Geochelone nigra</i>)	Male	Baseline CORT was higher in the breeding season than in the nonbreeding season in animals living under seminatural conditions	Schramm et al. (1999)
Gopher tortoise (<i>Gopherus polyphemus</i>)	Male and female	Baseline CORT did not differ significantly during the active period from May through October, a period that include most of the mating season	Ott et al. (2000)
Desert tortoise (<i>Gopherus agassizii</i>)	Male and female	Baseline CORT was higher in the breeding season than in the nonbreeding season in captive animals	Lance et al. (2001)
Green sea turtle (<i>Chelonia mydas</i>)	Male	Stressor-induced CORT was lower in migrating breeding males than in nonbreeding males	Jessop et al. (2002)
American alligator (<i>Alligator mississippiensis</i>)	Female	Baseline CORT did not vary significantly among sampled months or with reproductive stage	Guillette, Woodward, et al. (1997)
Tuatara (<i>Sphenodon punctatus</i>)	Male and female	Baseline CORT varied among the four season of the year and in the females varied between reproductive states	Tyrrell and Cree (1998)
European common lizard (<i>Lacerta vivipara</i>)	Male and female	Baseline CORT in males did not vary significantly during the active period and baseline CORT in females was higher in the breeding season and was influenced by female reproductive stage	Dauphin-Villemant et al. (1990)
Whiptail lizard (<i>Cnemidophorus uniparens</i>)	Female	Baseline CORT was lower in the breeding season in the nonbreeding season	Grassman and Crews (1990)
Six-lined race runner (<i>Cnemidophorus sexlineatus</i>)	Male and female	Baseline CORT in males was higher in the breeding season than in the nonbreeding season and baseline CORT in females did not differ significantly between seasons	Grassman and Hess (1992a)
Side-blotched lizard (<i>Uta stansburiana</i>)	Female	Baseline CORT was higher in the breeding season than in the nonbreeding season and female reproductive mass was positively correlated with CORT levels	Wilson and Wingfield (1992)
Italian wall lizard (<i>Podarcis sicula sicula</i>)	Male	Baseline and stressor-induced CORT were higher in the breeding season than in the nonbreeding season	Manzo et al. (1994)
Side-blotched lizard (<i>Uta stansburiana</i>)	Male and female	Baseline CORT was higher in the breeding season than in the nonbreeding season	Wilson and Wingfield (1994)
Western fence lizard (<i>Sceloporus occidentalis</i>)	Male and female	Baseline CORT did not differ significantly between seasons and stressor-induced CORT was higher during the breeding season in April, with greater responses in peripheral than central populations of lizards	Dunlap and Wingfield (1995)
Common gecko (<i>Hoplodactylus maculatus</i>)	Female	Baseline CORT varied significantly with season but was independent of reproductive state	Girling and Cree (1995)
Brown anole (<i>Anolis sagrei</i>)	Male	Baseline CORT was lower in the breeding season than in the nonbreeding season	Tokarz et al. (1998)
Eastern fence lizard (<i>Sceloporus undulatus</i>)	Male	Stressor-induced CORT was higher in the breeding season than in the nonbreeding season in males exposed to male and female conspecifics	Smith and John-Alder (1999)
Bearded dragon lizard (<i>Pogona barbata</i>)	Male and female	Baseline CORT did not vary significantly with time of year or reproductive condition	Amey and Whittier (2000)

Continued

TABLE 2 Relative baseline and stressor-induced corticosterone levels during the breeding and nonbreeding seasons in different groups of reptile—cont'd

Species	Sex	Relative plasma corticosterone (CORT) levels	Reference
Galapagos marine iguana (<i>Amblyrhynchus cristatus</i>)	Female	Baseline CORT was higher in the breeding season than in the nonbreeding season	Rubenstein and Wikelski (2005)
Green anole lizard (<i>Anolis carolinensis</i>)	Male	Baseline CORT was higher in the postbreeding season than in the breeding season in both lightweight and heavy-weight males	Husack et al. (2007)
Texas horned lizard (<i>Phrynosoma cornutum</i>)	Male and female	Baseline CORT was higher in both sexes in the breeding season than in the nonbreeding season, and stressor-induced CORT in females was higher in the breeding season in the nonbreeding season	Wack et al. (2008)
Eastern fence lizard (<i>Sceloporus undulatus</i>)	Female	Baseline CORT was higher in the breeding season than in the nonbreeding season, whereas stressor-induced CORT did not differ significantly between the seasons	Phillips and Klukowski (2008)
Red-sided garter snake (<i>Thamnophis sirtalis parietalis</i>)	Male	Baseline CORT was higher in the breeding season upon emergence from the den than in the nonbreeding season	Krohmer et al. (1987)
Red-sided garter snake (<i>Thamnophis sirtalis parietalis</i>)	Male	Baseline CORT was higher in the spring breeding season than in the nonbreeding season, and stressor-induced CORT was higher in the summer at 1 h postcapture	Moore et al. (2001)
Red-spotted garter snake (<i>Thamnophis sirtalis concinnus</i>)	Male	Baseline CORT was lower in the breeding season than in the nonbreeding season	Moore, Lerner, et al. (2000)
Red-spotted garter snake (<i>Thamnophis sirtalis concinnus</i>)	Male	Baseline CORT did not differ significantly between the spring breeding season and the nonbreeding season in 1999 and stressor-induced CORT was higher in the spring of 1993 and 1999 at 4 h postcapture and in the summer and fall of 1999 at 1 h postcapture	Moore and Mason (2001)
Western diamondback rattlesnake (<i>Crotalus atrox</i>)	Female	Baseline CORT was higher in the breeding season than in the nonbreeding season and varied with stage of the reproductive cycle	Taylor et al. (2004)

Stress-induced levels of corticosterone in reptiles also tend to vary seasonally and to be elevated during the breeding season. Thus, glucocorticoid levels may be relatively high during reproduction to meet the increased energetic demands associated with this life history stage (reviewed in Harshman & Zera, 2007). During the breeding season a short-term increase in glucocorticoid levels due to adverse weather conditions might inhibit an animal's reproductive behavior and induce dispersal behavior (Wingfield et al., 1998; Wingfield & Ramenofsky, 1999; Wingfield & Romero, 2001). Similar permissive effects of glucocorticoids have been demonstrated for aggressive territorial behavior in *Anolis carolinensis* (Summers, Watt, et al., 2005), which may suggest a specific purpose for elevated plasma corticosteroid concentrations during the breeding season, at least in males. The permissive effects of glucocorticoids are believed to be mediated by baseline glucocorticoid levels acting via the low-affinity mineralocorticoid receptor (Type I, MR), whereas the stimulatory, suppressive, and preparatory effects of glucocorticoids are for the most part thought to be mediated by stress-levels of glucocorticoids acting via the high-affinity glucocorticoid receptor (Type II, GR) (Romero, 2004; Sapolsky et al., 2000). However, the

necessary corticosteroid contribution to territorial aggressive behavior in *Anolis carolinensis* appears to be mediated by GR, since the GR antagonist mifepristone inhibits early stages of aggression (Summers, Watt, et al., 2005).

Seasonal changes in baseline levels of corticosterone occur in relation to reproduction (Taylor et al., 2004; Wack et al., 2008). The results of these studies are consistent with the idea that baseline corticosterone levels at least in females are elevated during the breeding season in comparison to the nonbreeding season (Fig. 6).

5 MODULATION OF STRESS RESPONSE DURING REPRODUCTION

5.1 Evidence

One of the more important advances in our understanding of how animals respond to stressors was the finding that the stress response in some situations can be modulated (Wingfield et al., 1998; Wingfield & Ramenofsky, 1999; Wingfield & Romero, 2001). The process by which animals modulate changes in the rate, duration, and magnitude of

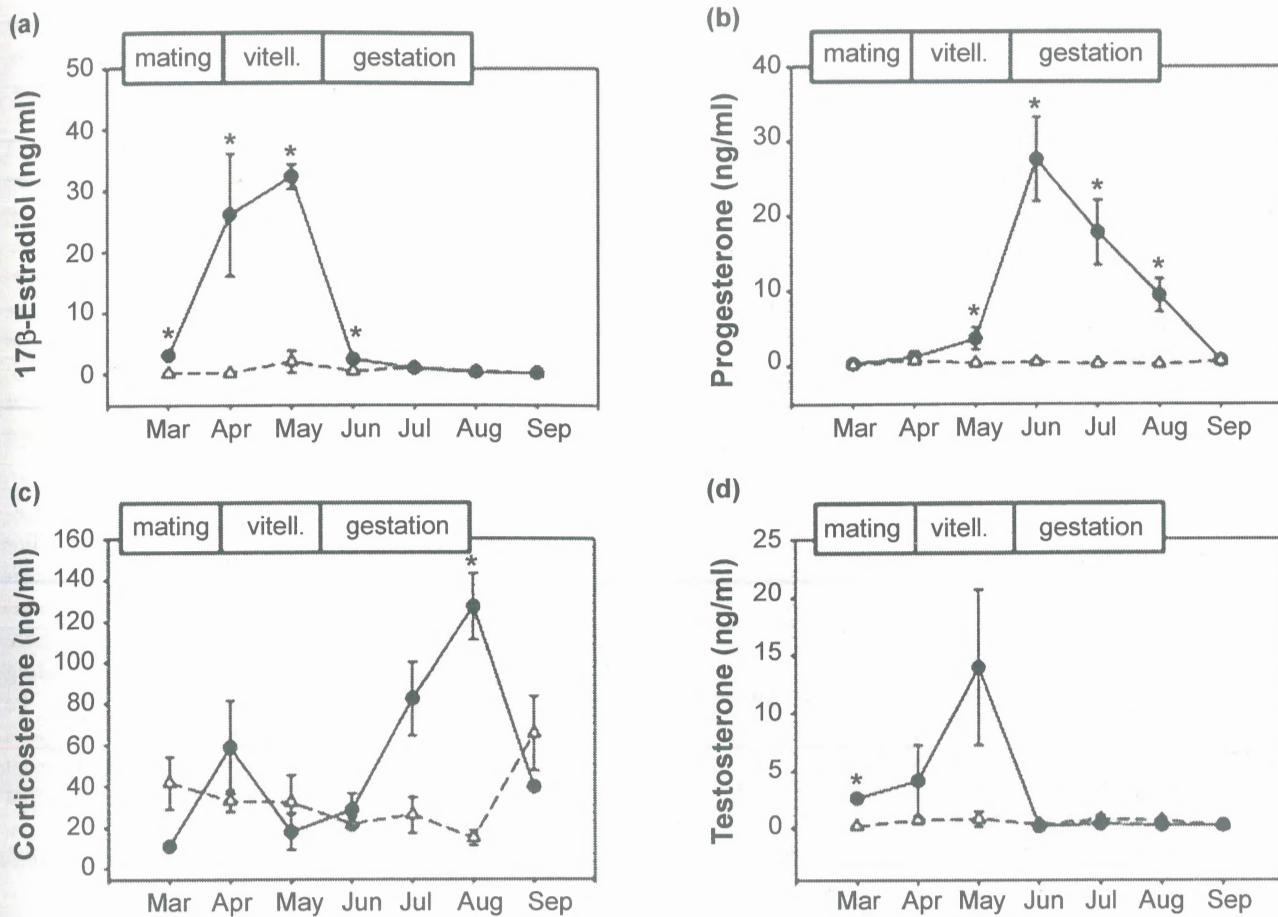


FIG. 6 Monthly plasma levels (\pm SEM) of (A) 17β -estradiol (E_2), (B) progesterone (P_4), (C) corticosterone (CORT), and (D) testosterone (T) in reproductive (black circles, solid lines) and nonreproductive (white triangles, dashed lines) female western diamondback rattlesnakes (*Crotalus atrox*). Hormone levels in nonreproductive snakes were relatively low throughout the year but were high in reproductive snakes at certain times in the reproductive cycle (significant differences are marked with *). Approximate timing of spring mating, vitellogenesis (vitell.), and gestation are indicated in the bars above the graphs. (Reproduced from Taylor, E. N., DeNardo, D. F., & Jennings, D. H. (2004). Seasonal steroid hormone levels and their relation to reproduction in the western diamond-backed rattlesnake, *Crotalus atrox* (serpentes: viperidae). General and Comparative Endocrinology, 136, 238–337.)

glucocorticoid release has been termed adrenocortical modulation (Wingfield & Ramenofsky, 1999; Wingfield & Romero, 2001). The ability of animals to modulate the activity of their hypothalamic-pituitary-adrenal axis in response to environmental and social stressors may be particularly important during energetically demanding activities such as reproduction (Wingfield et al., 1998).

In reptiles, as in other vertebrates, a number of factors such as sex, age, body condition, health, season, population, and reproductive state have been found to affect the likelihood that an animal will modulate its stress response (reviewed in Moore & Jessop, 2003). Here, we will consider the evidence for adrenocortical modulation in reptiles in terms of its impact on reproduction.

A difference in the stress response of breeding versus nonbreeding animals has been documented in turtles (Jessop, 2001; Jessop et al., 2000) and snakes (Cease et al., 2007; Moore et al., 2001; Moore, Lerner, et al., 2000). The

response of females to acute stress as measured by circulating corticosterone levels has been found to be significantly suppressed during the breeding season in the green turtle, *Chelonia mydas* (Jessop, 2001; Jessop et al., 2000) and in the hawksbill turtle, *Eretmochelys imbricata* (Jessop, 2001). Males of these species show a similar reduced stress response during the breeding season, but to a much lesser extent than in females (Jessop, 2001). The adrenocortical response of males to capture also appears to be modulated in the red-sided garter snake, *Thamnophis sirtalis parietalis* (Cease et al., 2007; Lutterschmidt & Mason, 2005; Moore et al., 2001). Moore, LeMaster, and Mason (2000) reported that male *Thamnophis sirtalis parietalis* that were captured during the spring mating season had an increase in plasma corticosterone levels and a decrease in plasma testosterone levels after 4 h of capture relative to baseline levels of these hormones. However, in a subsequent study, Moore et al. (2001) found that male *Thamnophis sirtalis parietalis* that were also captured

during the spring did not have a significant increase in corticosterone levels or a significant decrease in testosterone relative to baseline levels after 1 or 4 h of capture stress (Moore et al., 2001). Moore et al. (2001) suggested that these apparently contradictory results might be explained by differences in when animals were captured, as the initial baseline corticosterone levels in the earlier study were significantly lower than in their 2001 study, although initial testosterone levels were similar. This illustrates the difficulty discussed in Romero (2004) in trying to compare stress responses in a situation in which the compared populations differ in baseline levels of glucocorticoids. It also bears on the question of whether it is more meaningful to compare the absolute stress-induced glucocorticoid levels or the percent change from baseline levels (see Romero, 2004). In another study of *Thamnophis sirtalis parietalis*, corticosterone and testosterone levels in males did not significantly change relative to baseline levels after 4 h of capture in males that were captured during the spring or during the fall when males have a brief period of mating before entering their dens (Lutterschmidt & Mason, 2005).

Actively courting male *Thamnophis sirtalis parietalis* that had emerged from the winter den in the spring have a significantly reduced stress response compared to males that have ceased courting and are dispersing from the den to feeding grounds (Cease et al., 2007). Specifically, courting males captured at the den or within the den perimeter in Manitoba, Canada did not exhibit an increase in plasma corticosterone levels when measured at either 1 or 4 h after capture, whereas dispersing males captured approximately 0.6 m from the den did exhibit a significant increase in corticosterone.

Several studies have examined the adrenocortical stress response of the red-spotted garter snake, *Thamnophis sirtalis concinnus* (Lutterschmidt & Mason, 2005; Moore et al., 2001). The red-spotted garter snake, *Thamnophis sirtalis concinnus*, lives at mid-latitudes and has a longer breeding season than does the red-sided garter snake, *Thamnophis sirtalis parietalis*, that lives in more northern latitudes (Lutterschmidt & Mason, 2005; Moore et al., 2001). Male *Thamnophis sirtalis concinnus* that were captured in western Oregon during the spring, summer, and fall had a similar elevation in plasma corticosterone levels after 1 or 4 h of capture as compared to baseline levels, whereas plasma testosterone levels only declined in males captured during the spring (Moore et al., 2001). Thus, the stress-induced decline in testosterone levels in animals that were captured in the spring appears to be suppressed during the summer and fall when males are undergoing spermatogenesis (Moore et al., 2001).

In a study of the Texas horned lizard, *Phrynosoma cornutum*, Wack et al. (2008) examined the relationship between plasma corticosterone concentrations and sampling time (time from capture) in adult females captured in the breeding season (April–May), egg-laying season (June),

and nonbreeding season (July–September); and in adult males captured in the breeding season and nonbreeding season (June–September). No overall significant relationship was found between sampling times and corticosterone levels across season and sex. Although a significant positive correlation between corticosterone levels and sampling times was detected within nonbreeding males, this relationship became nonsignificant with the removal of one outlier value from the data set. Corticosterone levels and sampling times were, however, significantly and positively related within females during the egg-laying season. Because basal corticosterone levels were already elevated in egg-laying females, it was suggested that the HPA axis during the egg-laying period might be more sensitive to an acute stressor (Wack et al., 2008).

The adrenocortical response to stressors can vary with specific reproductive events in some reptiles (see Table 3). For example, in the olive ridley sea turtle, *Lepidochelys olivacea*, breeding females undergoing mass nesting (arribada) as well as females that were solitary nesters responded more slowly to turning stress than did basking, nonnesting females (Valverde et al., 1999). On the other hand, in the green turtle, *Chelonia mydas*, the stress response of females that abandoned their nests because of competition for nest sites did not differ from that observed in females that persisted in their nesting activities (Jessop & Harmann, 2004). In the tree lizard, *Urosaurus ornatus*, stressed vitellogenic females had significantly higher levels of corticosterone than did gravid females (Woodley & Moore, 2002). And Cartledge and Jones (2007) observed that in the viviparous lizard, *Egernia whitii*, postovulatory females had the most conservative response to stress, and although gestating females showed a large initial response it was not prolonged. In marked contrast, in the viviparous gecko, *Hoplodactylus maculatus*, the corticosterone response of pregnant females to capture and confinement stressors was not suppressed in comparison to that observed in non-pregnant, vitellogenic females (Cree et al., 2003).

Sex differences in the response of breeding individuals to stress resulting from capture has been documented (Table 4) in the green turtle, *Chelonia mydas* (Jessop, 2001); hawksbill turtle, *Eretmochelys imbricata* (Jessop, 2001); olive ridley sea turtle, *Lepidochelys olivacea* (Valverde et al., 1999); tree lizard, *Urosaurus ornatus* (Woodley & Moore, 2002); gecko, *Hoplodactylus maculatus* (Cree et al., 2003); skink, *Egernia whitii* (Cartledge & Jones, 2007); and red-sided garter snake, *Thamnophis sirtalis parietalis* (Moore, Lerner, et al., 2000; Whittier et al., 1987). In all of these species, females had lower circulating corticosterone levels than males in response to the stress induced by capture (Table 4). In contrast, in the lizard, *Cnemidophorus sexlineatus*, plasma corticosterone levels were significantly higher following acute stress (handling) in intact females than in intact males (Grassman & Hess, 1992b).

TABLE 3 Adrenocortical modulation of the stress response in relation to reproductive condition in different groups of reptiles.

Species	Modulation	Corticosterone (CORT) levels and reproductive condition	Reference
Loggerhead sea turtles (<i>Caretta caretta</i>)	Possible	CORT levels did not increase in several large turtle captured during the summer breeding season	Gregory et al. (1996)
Olive ridley sea turtle (<i>Lepidochelys olivacea</i>)	Yes	In nesting female turtle (both mass "arribada" and solitary nesters), CORT levels increased more slowly than in nonbreeding basking turtles within 2 h of the turtles being turned onto their backs	Valverde et al. (1999)
Green turtle (<i>Chelonia mydas</i>)	Yes	In breeding females either to capture or to capture and an ecological heat stressor, CORT levels were lower than in nonbreeding females exposed only to capture	Jessop et al. (2000)
Green turtle (<i>Chelonia mydas</i>)	Yes	CORT levels were lower in breeding females than in nonbreeding females after capture	Jessop (2001)
Hawksbill turtle (<i>Eretmochelys imbricata</i>)	Yes	CORT levels were lower in breeding females than in nonbreeding females after capture	Jessop (2001)
Bearded dragon lizard (<i>Pogona barbata</i>)	No	CORT levels were relatively low after capture and were unrelated to female reproductive stage	Cree et al. (2000)
Tree lizard (<i>Urosaurus ornatus</i>)	Yes	After capture and 10 min of handling, CORT levels in vitellogenic but not gravid females were higher than in control females bled immediately after capture	Woodley and Moore (2002)
Common gecko (<i>Hoplodactylus maculatus</i>)	No	CORT levels in response to capture and confinement did not differ significantly between vitellogenic females and pregnant females	Cree et al. (2003)
White's skink (<i>Egernia whitii</i>)	Yes	CORT levels varied with female reproductive stage with postovulatory females showing the smallest increase in CORT within 4 h of capture	Cartledge and Jones (2007)
Texas horned lizard (<i>Phrynosoma cornutum</i>)	Yes	CORT levels in breeding males, unlike nonbreeding males, did not increase in response to capture and handling	Wack et al. (2008)
Red-sided garter snake (<i>Thamnophis sirtalis parietalis</i>)	Yes	Pre- and poststress CORT levels did not differ significantly in reproductively active males captured at or near the den but did differ in dispersing males captured approximately 0.6 km from the den	Cease et al. (2007)

TABLE 4 Sex differences in adrenocortical stress response following exposure to stressors in different groups of reptiles

Species	Relative plasma corticosterone (CORT) levels	Reference
Red-eared slider turtle (<i>Trachemys scripta elegans</i>)	Males and females did not differ significantly in CORT levels at any sampling time after capture and handling	Cash et al. (1997)
Olive ridley sea turtle (<i>Lepidochelys olivacea</i>)	Breeding females had lower CORT levels than mating males 20 min after turtle had been turned onto their backs	Valverde et al. (1999)
Green sea turtle (<i>Chelonia mydas</i>)	Females had lower CORT levels than males in breeding turtle after capture, restraint, and blood sampling	Jessop (2001)
Hawksbill turtle (<i>Eretmochelys imbricata</i>)	Females had lower CORT levels than males in breeding turtle after capture, restraint, and blood sampling	Jessop (2001)
Hawksbill turtle (<i>Eretmochelys imbricata</i>)	In immature turtles, males and females did not differ significantly in CORT levels after exposure to a capture protocol causing stress	Jessop et al. (2004)
American alligator (<i>Alligator mississippiensis</i>)	Females had lower CORT levels than males in adult alligators from both wild and captive population with 15 min of capture, restraint, and blood sampling	Elsey et al. (1990)

Continued

TABLE 4 Sex differences in adrenocortical stress response following exposure to stressors in different groups of reptiles—cont'd

Species	Relative plasma corticosterone (CORT) levels	Reference
Australian freshwater crocodile (<i>Crocodylus johnstoni</i>)	Males and females of several age classes did not differ significantly in CORT levels after capture, restraint and blood sampling	Jessop et al. (2003)
Tuatara (<i>Sphenodon punctatus</i>)	Males and vitellogenic females did not differ significantly in CORT levels after capture and confinement for 3 h	Tyrrell and Cree (1998)
Six-lined racerunner (<i>Cnemidophorus sexlineatus</i>)	Females had higher CORT levels than males in intact, reproductively mature lizards after being handled for 30 min	Grassman and Hess (1992b)
Marine iguana (<i>Amblyrhynchus cristatus</i>)	Males and females did not differ significantly in CORT levels after capture and restraint for 15 of 30 min	Romero and Wikelski (2001)
Tree lizard (<i>Urosaurus ornatus</i>)	Vitellogenic females had higher CORT levels than males after capture and handling for 10 min	Woodley and Moore (2002)
Common gecko (<i>Hoplodactylus maculatus</i>)	Vitellogenic females had higher CORT levels than males following capture and confinement for 24 h	Cree et al. (2003)
Yellow-bellied water-skink (<i>Eulamprus heatwolei</i>)	Females consistently had at least threefold higher CORT levels than males in reproductively active lizards 1 h following exposure to a variety of potential stressors	Langkilde and Shine (2006)
White's skink (<i>Egernia whitii</i>)	Gestating and postovulatory females had lower CORT levels than reproductively quiescent males after capture and being held in cloth bags for up to 240 min	Cartledge and Jones (2007)

In addition to body condition, an animal's health can affect its response to acute stress. For example, in the western fence lizard, *Sceloporus occidentalis*, corticosterone levels in reproductively active males 1 h after capture and confinement in a cloth bag were higher in males that were infected with a malarial parasite (*Plasmodium mexicanum*) than in uninfected males (Dunlap & Schall, 1995).

Moore and Jessop (2003) posed the question of why it might be advantageous for amphibians and reptiles to suppress their adrenocortical response to stressors during reproduction. They suggested that like in some species of desert and arctic birds (see Wingfield & Romero, 2001), species of reptiles with relatively short breeding seasons may maximize their reproductive success by suppressing their adrenocortical response to stress to continue to breed even in the face of severe stressors. There is some support in reptiles for this suggestion. Male garter snakes captured from a northern population with a relatively short breeding season have been found to exhibit a less intense adrenocortical response to capture than males captured from a southern population with a longer breeding season (Moore et al., 2001). However, as pointed out in Moore and Jessop (2003) the duration of the reproductive season is unlikely to explain why breeding females in at least three species of sea turtles have a reduced or slower adrenocortical response to capture than do nonbreeding female because these reptiles typically have extended breeding seasons and, in some cases, breed throughout the year.

Thus, adrenocortical modulation in sea turtles might be an example of a phylogenetic constraint (Moore & Jessop, 2003). On the other hand, the failure of sea turtles to reduce the magnitude of their stress response might be adaptive if elevated levels of stress hormones are beneficial to reproduction.

5.2 Mechanisms

One important but largely unanswered question is how animals including reptiles modulate their stress responses (Moore & Jessop, 2003; Romero, 2004). In other words, what are the physiological mechanisms that cause a stress response to be suppressed under some conditions but not others? It has been suggested that the type and numbers of glucocorticoid receptors, glucocorticoid plasma binding proteins, and/or activity of enzymes such as 11 β -hydroxysteroid dehydrogenase that convert glucocorticoids to inactive metabolites might play a key role in the modulation of the adrenocortical stress response (Breuner & Orchinik, 2002; Landys et al., 2006; Moore & Jessop, 2003; Romero, 2004; Wingfield & Romero, 2001). This is because they affect the plasma levels of glucocorticoids, their action on target tissues, as well as the physiology of the glucocorticoid negative feedback systems in the brain and pituitary (Fietta & Fietta, 2007; Moore & Jessop, 2003; Romero, 2004).

Glucocorticoid receptors do appear to have similar brain distribution as in mammals and other vertebrates based on

immunochemical staining using antibodies to mammalian receptors (Summers et al., 1994). Thus, we can assume that reptiles are similar to other vertebrates in having a high affinity mineralocorticoid (Type I) receptor and a low affinity glucocorticoid (Type II) receptor that bind glucocorticoids (Landys et al., 2006; Romero, 2004), and perhaps a nongenomic membrane glucocorticoid receptor as well (Borski, 2000; Moore & Evans, 1999).

Reptiles do appear, however, to possess a glucocorticoid plasma binding protein (Jennings et al., 2000). Two plasma steroid-binding globulins have been identified in male tree lizards, *Urosaurus ornatus*, and one of these binds androgens and C-21 steroids such as corticosterone and has been termed androgen-glucocorticoid binding globulin (AGBG); the other is a sex hormone-binding globulin (SHBG) that binds androgens and estradiol with high affinity (Jennings et al., 2000). Moreover, the binding capacity of AGBG (but not of SHBG) is significantly greater in territorial males than in nonterritorial males in *Urosaurus ornatus* (Jennings et al., 2000). This difference in binding capacity of AGBG may explain why free corticosterone levels in nonterritorial males are higher than in territorial males during a stress response and why testosterone levels are depressed to a greater extent in nonterritorial males than in territorial males (Jennings et al., 2000).

Lutterschmidt and Mason (2005) examined the effects of ketanserin (a serotonergic type 2A receptor antagonist), 5-hydroxytryptophan (a precursor in 5-HT and melatonin synthesis), and melatonin (either a low or high dose) on the hormonal responses (changes in corticosterone and androgen levels) of males to capture in two populations of garter snakes (*Thamnophis sirtalis parietalis* and *Thamnophis sirtalis concinnus*). These agents had no significant effect on the plasma levels of corticosterone or androgens in male *Thamnophis sirtalis parietalis* that were captured during the spring and subjected to the stress associated with capture for 4 h. However, treatment of male *Thamnophis sirtalis concinnus* with ketanserin prior to 4 h of capture prevented the significant decline in androgen levels that normally occur in males of this subspecies that are captured during the spring. Based on these results, Lutterschmidt and Mason (2005) suggested that a serotonin-regulated system (but not melatonin) might modulate the activity of the hypothalamic-pituitary-gonadal axis during the physiological stress response of the garter snake.

6 FITNESS EFFECTS OF STRESS DURING REPRODUCTION

6.1 Adults

As pointed out by Greenberg and Wingfield (1987), the physiological stress response cannot be assumed to be

invariably harmful, and may effectively be beneficial, and thus stress depending on a variety of factors may have negative or positive fitness consequences. Congruent with this idea is the finding that stress in reptiles can have either negative or positive effects on survival and other components of fitness depending on species (Cote et al., 2006; Romero & Wikelski, 2001), sex (Cote et al., 2006), and even alternative mating strategies (Comendant et al., 2003; Lancaster et al., 2008).

The effects of stress, either positive or negative, are likely to be especially important during periods of reproduction because of the high energetic costs associated with successful reproduction (see review by Harshman & Zera, 2007). These energetic costs in female reptiles include the energetic costs of gamete production as well as the indirect costs of reproduction including the increased metabolic costs of maintenance or activity (Angilletta & Sears, 2000; French et al., 2007; French & Moore, 2008; Olsson et al., 2000; Shine, 2003; Zani et al., 2008). Although gamete production in male reptiles is less costly than in females (French & Moore, 2008), there are also relatively high costs associated with male reproductive behavior, which depending on species may include the costs of producing displays, acquiring and guarding territories, and aggressive interactions with other males (French & Moore, 2008; Marler & Moore, 1988, 1989; Sinervo et al., 2000). Because of the relatively high costs of reproduction, trade-offs may occur between reproductive activities and activities such as somatic growth, self-maintenance, and immune function (Berger et al., 2005; French et al., 2007; French & Moore, 2008; Lancaster et al., 2008; Mills et al., 2008). Although the production of offspring during a given reproductive event is obviously a key component to an individual's fitness, it is also true that an individual's long-term survival will affect their fitness (French & Moore, 2008).

Trade-offs have been shown to exist between reproductive activity and the functioning of the immune system in a number of species of lizards. In a study of the highly polygynous Galápagos marine iguana, *Amblyrhynchus cristatus*, Berger et al. (2005) measured the immune response of males of three distinct behavioral and morphological types (territorial, satellite, and bachelor) during the peak of the breeding season. Berger et al. (2005) captured adult male *Amblyrhynchus cristatus* and injected them in the toe-web of the back foot with either phytohemagglutinin (PHA) or with saline (control). After initial treatment and measurements, males were released and recaptured just before the next measurement period (at 6, 12, 18, 24, and 48 h after injection) so that the responses to PHA could be ascertained in free-living animals under natural conditions. The large, highly ornamented territorial males that secure the majority of matings (95%) were found to have a lower immune

response than the smaller satellite males that attempt to force copulations with females or the nonreproductive bachelor males (Berger et al., 2005). Territorial males also had significantly lower body condition and higher plasma corticosterone levels than did satellites or bachelors (Berger et al., 2005). Moreover, males of all three types that had their corticosterone levels elevated experimentally by being exposed to restraint or corticosterone injections had reduced immune responses as compared to free-living animals (Berger et al., 2005). These findings are consistent with the idea that elevated corticosterone levels can suppress immune function in a reptile during reproduction.

Stress has also been demonstrated to inhibit wound healing in the tree lizard, *Urosaurus ornatus* (French et al., 2006; French & Moore, 2008). French and Moore (2008) argued that wound healing can be used as a measure of innate immune function and that wound healing is a highly biological relevant process in *Urosaurus ornatus* because the majority of individuals in the field have a scar from some type of wounding due to intrasexual combat or predation attempts (unpublished data of Knapp and Moore cited in French & Moore, 2008). Wound healing in male and female *Urosaurus ornatus* has been documented to vary significantly with reproductive stage as well as with context (field or laboratory) (French & Moore, 2008). French and Moore (2008) captured adult *Urosaurus ornatus* of both sexes, assessed their reproductive condition, wounded them by removing a small circle of skin (3.5 mm punch) from the dorsal surface of the pelvis, and either returned them to the field or housed them in the laboratory. In both sexes, the rate of wound healing (as indicated by the percentage of wound that had healed after 10 days) was slowest during the most resource-intense stage of reproduction (French & Moore, 2008). In treated females that had been returned to the field, the rate of wound healing was significantly less in females with vitellogenic ovarian follicles than in prereproductive females, gravid females (females with oviductal eggs), and postreproductive females (French & Moore, 2008). However, in treated females that had been housed in the laboratory, vitellogenic females had a rate of wound healing similar to that of females in other reproductive stages (French & Moore, 2008). In males, the rate of wound healing was significantly less in males that were in the mid-reproduction period than in males that were in either the early- or late-reproduction periods for males that had been housed in the laboratory after treatment, but not in males that had been returned to the field (French & Moore, 2008). To explain why wound healing in males and females differed with context, French and Moore (2008) suggested that females might be food-limited in the field but not in the laboratory. Thus, an abundance of food could explain why females housed in the laboratory could undergo ovarian vitellogenesis and heal wounds at a normal rate. Food

availability had been found in an earlier study to be a key factor affecting the ability of female *Urosaurus ornatus* to sustain reproduction and immune function (French et al., 2007). Female *Urosaurus ornatus* that were housed individually in the laboratory and given unlimited access to food sustained both reproduction and immune system function as measured by ovarian follicle mass and rate of cutaneous wound healing, whereas females that had either limited food or no food did not (French et al., 2007). In addition, French et al. (2007) found no significant effect of wounding on the concentrations of testosterone, estradiol, or corticosterone in their female subjects; nor did they find a significant interaction between wound healing and diet treatment for any of the measured hormones. To explain why reproductively active male *Urosaurus ornatus* in the laboratory but not in the field exhibited depressed wound healing, French and Moore (2008) suggested that some other social factor associated with laboratory housing rather than food availability per se caused reproductively active males to have a slower rate of wound healing. In agreement with previous results (French et al., 2007), French and Moore (2008) detected no significant relationship between plasma levels of estradiol, testosterone, and corticosterone and healing rate for individuals in any reproductive stage or context. Although some studies have reported that testosterone has immunosuppressive actions (reviewed in Roberts et al., 2004), French and Moore (2008) concluded that testosterone is unlikely to be an important factor mediating wound healing in *Urosaurus ornatus*.

In the side-blotched lizard, *Uta stansburiana*, males as well as females exhibit genetically based reproductive strategy polymorphisms (Lancaster et al., 2008; Sinervo & Lively, 1996). Female *Uta stansburiana* within a population exhibit either of two alternative mating strategies. Orange-throated females, (hereafter termed orange females), are "r-strategists" because they produce relatively large clutches of small-sized offspring, whereas yellow-throated females (hereafter termed yellow females) are "k-strategists" because they lay relatively small clutches of large-sized offspring (Lancaster et al., 2008). Lancaster et al. (2008) experimentally manipulated corticosterone levels (high-end basal, stress-related, or unchanged) in adult females as well as the timing of corticosterone exposure to determine the effects of this stress hormone on female survival and reproductive success in relationship to female mating strategy. As predicted the effects of corticosterone treatment differed with female mating strategy. Corticosterone linearly affected survival into the reproductive period but differentially by female mating strategy. The chances of survival decreased in orange females with increasing corticosterone levels, whereas the chances of survival increased in yellow females with increasing corticosterone levels (Lancaster et al., 2008). However, in those females that survived, the

chances of reproducing increased in orange females with increasing corticosterone levels, whereas the chances of reproducing decreased in yellow females with increasing corticosterone levels (Lancaster et al., 2008). Thus, corticosterone appears to mediate a current versus future reproductive trade-off in the two female mating strategies in *Uta stansburiana* (Lancaster et al., 2008).

The differential nature of the tradeoffs observed in female *Uta stansburiana* after manipulation of corticosterone levels may at least in part enhance the competitive ability of females in each mating strategy because corticosterone levels have been shown to increase in both orange and yellow females in nature in response to crowding by conspecifics (Comendant et al., 2003). Thus, according to Lancaster et al. (2008) r-strategist orange females, which face increased survival costs with increasing corticosterone levels, may benefit from laying eggs earlier in the breeding season because they produce relatively large clutches with small hatchlings which may do better by emerging earlier in the breeding season when there is likely to be a lower density of competitors than later in the breeding season. The same is not true for K-strategy yellow females because they do not suffer increased survival costs with increasing corticosterone levels and because they produce relatively small clutches with large hatchlings which may be able to compete successfully for resources under the high-density conditions that are likely to occur later in the breeding season (Lancaster et al., 2008).

The best direct evidence of the negative effects of long-term stress on adult survival in reptiles comes from a study of the Galápagos marine iguana, *Amblyrhynchus cristatus*, by Romero and Wikelski (2001). Although Romero and Wikelski (2001) did not compare animals based on reproductive condition but rather selected animals randomly, they found that during the El Niño of 1998 when *Amblyrhynchus cristatus* on most islands of the Galápagos faced famine because of reduced algae forage, baseline and stress-induced corticosterone levels were significantly higher in iguanas on five of the six examined islands than they were in the year following the El Niño event. Most of the observed differences in basal corticosterone levels between the El Niño and El Niña periods were due to iguanas with body condition indices of 35 or less. Significantly, the corticosterone levels measured after 15 min of handling predicted survival of iguanas on islands that were differentially affected by El Niño conditions (Romero & Wikelski, 2001).

Cote et al. (2006) have assessed the effects of experimentally elevated levels of corticosterone on the survival of adult common lizards, *Lacerta vivipara*, by comparing corticosterone-treated with placebo-treated lizards. These investigators monitored the survival and behavior of corticosterone-treated and of placebo-treated lizards that

were housed in outdoor enclosures from September to April. Corticosterone-treated males had a significantly higher survival rate than placebo-treated males. The survival of corticosterone-treated females did not differ from that of placebo-treated females. In addition, corticosterone treatment tended to increase energy expenditure, food intake, and daily activity (via changes in behavioral time budget). Based on these findings, Cote et al. (2006) suggested that increased corticosterone does not reduce survival but acts either directly or indirectly to promote longer-term survival. Thus, according to Cote et al. (2006) elevated corticosterone levels appear to be an adaptive response to environmental stressors in *Lacerta vivipara*.

Guillette et al. (1995) in an earlier review of the effects of stress in reptiles noted that stress could delay or even prevent oviposition or parturition in reptiles. Difficulty in successfully ovipositing (laying eggs) in oviparous species or parturition (giving birth) in viviparous species can have negative fitness effects especially in chelonians and squamates where it most frequently occurs (Frye, 1991). The effects of stress on the timing of oviposition or parturition in reptiles can be mediated not only by changes in the plasma levels of hormones such as corticosterone but also by the activation of the sympathetic nervous system (see references in Guillette et al., 1995).

6.2 Mothers and offspring

In a study of the viviparous New Zealand common gecko, *Hoplodactylus maculatus*, Cree et al. (2003) exposed pregnant females to corticosterone and found that this treatment harmed fetuses and prevented successful births. Specifically, four out of nine examined embryos from two of five female *Hoplodactylus maculatus* that were treated with corticosterone pellets during pregnancy showed developmental abnormalities at three weeks and none of the corticosterone-treated females successfully gave birth at the beginning of the fourth week (Cree et al., 2003). The significance of this finding, however, is unclear because gravid females were exposed inadvertently to potentially nonphysiological levels of corticosterone (360 ng/mL at 3 weeks). In another study of *Hoplodactylus maculatus* in which corticosterone levels in ACTH-treated pregnant females were within the physiological range seen during acute stress, females had normal pregnancies (Preest et al., 2005). The corticosterone levels in ACTH-treated female *Hoplodactylus maculatus* (mean = 62 ng/mL) were sevenfold greater than basal levels and were similar to levels observed in pregnant females following capture and confinement (Preest et al., 2005). However, these elevated levels of corticosterone did not significantly affect the duration and success of pregnancy (Preest et al., 2005). Meylan and Clobert (2005) also found no effect of

corticosterone treatment of pregnant females on the number of live births in the viviparous common lizard, *Lacerta vivipara*.

In the oviparous eastern three-lined skink, *Bassiana duperreyi*, the transdermal application of corticosterone to gravid females caused females to oviposit their eggs sooner (on average 7.5 days earlier) and at an earlier embryonic stage than did control females that were treated with oil vehicle (Radder et al., 2008). At oviposition, corticosterone-treated females had significantly higher corticosterone levels (mean = 246.2 ng/mL) than did control females (79.0 ng/mL), although corticosterone levels in the egg yolk of offspring were unaffected by maternal treatment. Radder et al. (2008) posited that the observed premature oviposition by corticosterone-treated females might be adaptive in terms of protecting the eggs from the possible harmful effects of corticosterone. On the other hand, in the side-blotched lizard, *Uta stansburiana*, corticosterone treatment of gravid females did not accelerate or delay the timing of when eggs were laid (Lancaster et al., 2008). But there is preliminary evidence that superphysiological levels of corticosterone present in egg yolk at the time of oviposition reduce hatching success in *Uta stansburiana* (Johnston and Moore, unpublished data; Lucas and Weiss, unpublished date; cited in Weiss et al., 2007), a finding that was attributed to the gross detrimental effects of corticosterone on the embryo rather than on the hatching event itself (Weiss et al., 2007).

The effects of treating oviposited eggs with corticosterone rather than treating gravid females have been studied in the tree lizard, *Urosaurus ornatus* (Weiss et al., 2007). The rationale behind this study was the finding that RU-486 (mifepristone), a progesterone and glucocorticoid antagonist, when given to nearly full-term embryos adversely affected hatching success in a dose-dependent manner. This finding led Weiss et al. (2007) to hypothesize that corticosterone plays an important role in the successful hatching of viable *Urosaurus ornatus*. These investigators found that oviposited eggs that were treated with corticosterone on day 30 of incubation hatched significantly sooner than vehicle-treated eggs and untreated eggs. Based on this finding, Weiss et al. (2007) suggested that corticosterone produced in the embryo in response to environmental stressors might mobilize energy stores needed to fuel hatching and/or to stimulate lung maturation in *Urosaurus ornatus*. More broadly, they suggested that embryos within oviposited eggs might be able to detect environmental stressors and accelerate their time of hatching to escape these stressors. The results of Weiss et al. (2007) are noteworthy because they raise the issue of the relative importance of corticosterone derived from the mother versus corticosterone that is synthesized by the developing embryo itself. The extent to which glucocorticoids pass from

mothers to their eggs and the functional significance of such a transfer is an important area of research in oviparous species (Radder, 2007; Romero, 2004).

6.3 Offspring

Maternal stress in a variety of vertebrates has been shown to affect offspring quality (reviewed in Francis et al., 1996; Weinstock, 2001). Several studies have explored the effects of maternal stress on offspring fitness in lizards. Glucocorticoid exposure in reptiles appears to affect both fitness and sex ratios of reptilian offspring (Geffroy & Douhard, 2019), with most undifferentiated gonads sensitive to environmental factors during the middle third of the incubation period. In two studies that report experimental corticosterone induction of primary sex ratio change, treatment led to male sex differentiation (Iungman et al., 2015; Warner et al., 2009).

In the viviparous European common lizard, *Lacerta vivipara*, De Fraipont et al. (2000) found that treating pregnant females with corticosterone had a significant effect on the tendency of their offspring to disperse from their natal area. This is an important finding because dispersal may affect offspring survival (reviewed in Warner & Shine, 2008). In the De Fraipont et al. (2000) study, offspring of corticosterone-treated females were found to move in lower numbers to an enclosure that did not house their mother compared to the offspring of control females. Based largely on this finding De Fraipont et al. (2000) concluded that increased prenatal maternal corticosterone inhibits dispersal (and thus promotes philopatry) and suggested that corticosterone is one of the proximate mechanisms in the prenatal control of juvenile dispersal in *Lacerta vivipara*. According to these investigators if relatively high plasma corticosterone levels decrease the likelihood of maternal survival in *Lacerta vivipara*, then offspring fitness could be increased by not dispersing because of the absence of mother-offspring competition (De Fraipont et al., 2000). Belliure et al., 2004 also reported that the offspring of corticosterone-treated females in *Lacerta vivipara* spent significantly less time moving, a finding consistent with the earlier report of De Fraipont et al. (2000) that the offspring of corticosterone-treated females tended to exhibit philopatry. It is important to note that in the Belliure et al. (2004) study corticosterone was delivered to pregnant females transdermally (see Knapp & Moore, 1997b), whereas in the earlier study of De Fraipont et al. (2000) pregnant females received Silastic implants containing crystalline corticosterone. Meylan et al. (2002) also found evidence in *Lacerta vivipara* that prenatal corticosterone treatment (via transdermal delivery, see Meylan et al., 2003) decreased the tendency of offspring to disperse; however, in this study, there was an interaction

between maternal condition (corpulence) and prenatal corticosterone treatment, with dispersal only decreasing in offspring of corpulent, corticosterone-treated females. Similarly, Meylan et al. (2004) found that the dispersal of offspring from small-sized (young) mothers who had been treated with corticosterone was significantly greater than for the offspring from large-sized (old) mothers who had been treated with corticosterone. Taken together these findings suggest that the tendency of offspring to disperse in *Lacerta vivipara* is mediated by an interaction between corticosterone levels and the prenatal body condition of the mother. Interestingly, the duration of maternal exposure to corticosterone in *Lacerta vivipara* has also been found to affect the dispersal strategy of offspring in a manner that is determined by clutch sex ratio (Vercken et al., 2007). In female-biased clutches, relatively prolonged exposure of mothers to corticosterone during gestation promoted philopatry, whereas shorter exposure to corticosterone enhanced dispersal, a result that was considered to be consistent with kin competition theory (Vercken et al., 2007). Juvenile size, weight, and body condition also varied with the timing and duration of maternal exposure to corticosterone (Vercken et al., 2007).

Corticosterone exposure in pregnant females influences other aspects of offspring fitness, such as decreased sprint speed but normal endurance in *Lacerta vivipara*, in comparison to the offspring of placebo-treated control females (Meylan & Clobert, 2004). These investigators proposed that the lower sprint speed of offspring of corticosterone-treated females could be a behavioral adjustment rather than a shift in physiological performance because offspring required more stimuli to run. Belluire et al. (2004) also noted in *Lacerta vivipara* that prenatal corticosterone increased the time spent basking by juveniles from large mothers but not small mothers, a finding that again illustrates the importance that the body condition of pregnant females plays in modulating the effects of corticosterone. However, offspring quality was not affected by maternal corticosterone in a study of the gecko, *Hoplodactylus maculatus*, in which pregnant females were treated with ACTH to increase their plasma corticosterone levels to those levels observed during acute stress (Preest et al., 2005). The offspring of ACTH-treated female *Hoplodactylus maculatus* did not differ in sprint speed or in body growth from offspring of placebo-treated females (Preest et al., 2005). Although it is difficult to compare the findings of different studies because they vary in so many ways, it seems likely that there are species differences in the effects of maternal corticosterone on offspring fitness in reptiles.

In a study of the European common lizard *Lacerta vivipara*, Meylan and Clobert (2005) not only recorded the morphological characteristics of offspring from corticosterone- and from placebo-treated females at birth but also importantly

followed the growth and survival of offspring in the field until they hibernated. These investigators found that the offspring of corticosterone-treated females compared to the offspring of placebo-treated females had shorter snout-vent lengths (SVL), a leaner body condition (residual from a regression of mass against SVL), and a lower growth rate before hibernation. Significantly, Meylan and Clobert (2005) also found that corticosterone treatment of pregnant female *Lacerta vivipara* affected the probability of offspring survival, and in a sex-specific manner. Juvenile males born from corticosterone-treated females had a higher survival rate than males that were born from placebo-treated females. On the other hand, juvenile females born from corticosterone- or placebo-treated mothers did not differ significantly in survival rate. Meylan and Clobert (2005) concluded that corticosterone treatment of pregnant females does not result in low quality offspring in *Lacerta vivipara*. Moreover, Uller et al. (2005) found that in *Lacerta vivipara* corticosterone treatment of pregnant females did not affect clutch size, offspring sex ratio, or offspring sexual dimorphism (i.e., number of ventral scales). Although sex ratio significantly affected the number of ventral scales in offspring, Uller et al. (2005) found no significant interaction between maternal treatment (either corticosterone or sham treatment) and sex ratio on the number of offspring ventral scales.

7 CONCLUSIONS AND RESEARCH DIRECTIONS

7.1 Conclusions

Exposure of reptiles to a variety of types of stressors causes an elevation in the circulating levels of corticosterone, the most important glucocorticoid in reptiles. This stress-induced increase in corticosterone is often associated with changes in the circulating levels of gonadal steroids. In male reptiles, an increase in plasma corticosterone above baseline levels is usually followed by a decrease in baseline testosterone levels (e.g., Lance et al., 2004). The relationship between plasma corticosterone levels and the levels of estradiol and progesterone following exposure of female reptiles to a stressor varies greatly with species and female reproductive state. However, exposure of females to stressors may lower estradiol levels in some species (e.g., Elsey et al., 1991; Ganesh & Yajurvedi, 2002a). In the Indian keeled lizard corticosterone treatment or exposure to stressors inhibits gonadal recrudescence in females (Ganesh & Yajurvedi, 2002a) as well as males (Yajurvedi & Menon, 2005; Yajurvedi & Nijagal, 2000). Importantly, other hypothalamic-pituitary-adrenal axis factors such as CRF and β -endorphin play a key role in the stress-induced inhibition of gonadal recrudescence (Ganesh & Yajurvedi, 2002b; Ganesh & Yajurvedi, 2003).

Stress in reptiles can inhibit male behaviors that are necessary for successful reproduction. Corticosterone treatment, for example, inhibits male territorial behavior in many species of lizards. Moreover, corticosterone appears to inhibit male behavior by mechanisms that are independent of changes in circulating androgens. There is also evidence from studies of the green anole suggesting that stressor-induced increases in plasma corticosterone might affect how males respond in future social interactions (Korzan, Forster, et al., 2006; Yang & Wilczynski, 2003). Interestingly, in species of reptiles such as the tree lizard with alternative mating strategies stress-induced changes in glucocorticoid and testosterone levels may be a hormonal means of switching male reproductive tactics and thus behaviors (Knapp, 2004; Knapp & Moore, 1996, 1997a; Moore et al., 1998). Stress may also inhibit male courtship behavior in some reptiles. Corticosterone treatment or the stress associated with losing fights has been found to inhibit male courtship behavior in several species of snakes (Lutterschmidt et al., 2004; Moore & Mason, 2001; Schuett, 1996). In these studies, corticosterone apparently inhibited male behavior without affecting plasma androgen levels (Schuett & Grober, 2000; Schuett et al., 1996).

Baseline corticosterone levels in the majority of examined species of reptiles with seasonal reproductive cycles have been found to be highest during the breeding season relative to other seasons. The noted increase in seasonal baseline glucocorticoid levels in most reptiles during the reproductive season suggests that elevated levels of stress hormones are necessary, and perhaps beneficial for reproduction, although exceptionally high glucocorticoid concentrations may inhibit reproductive events. For example, the increase in the baseline levels of corticosterone levels in females at the end of gestation that has been observed in the western-diamondback rattlesnake might serve to mobilize energy for both vitellogenesis and parturition (Taylor et al., 2004). This would be consistent with the energy mobilization hypothesis of Wingfield and Ramenofsky (1999). In addition, a study of the Texas horned lizard found that baseline corticosterone levels in gravid females were highest during the egg-laying period, and this elevation in corticosterone levels during this period might be due to stressors associated with digging multiple nests to find an appropriate place to oviposit eggs (Wack et al., 2008). Stress-induced levels of corticosterone may also be highest during the breeding season, although this is based on the results of relatively few studies (Moore & Jessop, 2003).

The magnitude of the stress response during reproduction may be reduced in some reptiles. In the red-sided garter snake, for example, males suppress their stress response during reproduction (e.g., Moore et al., 2001). In females, the magnitude of the acute stress response also varies with specific stages of female reproduction in some species of lizards

(e.g., Cartledge & Jones, 2007; Dauphin-Villemant & Xavier, 1987; Woodley & Moore, 2002) and turtles (e.g., Valverde et al., 1999). The mechanism for these differences may be encoded in blood telomere length, which in long-lived migrating turtle species (*Dermochelys coriacea*) changes depending on exposure to environmental stress (Plot et al., 2012). Telomere length does not differ initially when individuals are hatched, but shortening of blood telomere length and high telomerase activity is observed during the first few years of this fast-growing, long-lived, species. It is unclear if the high telomerase activity is protecting blood telomere attrition during this period. Environmental stressors accumulated during long sea migrations (2 vs 3 years) in *Dermochelys coriacea* turtles impact reproductive output depending on telomere length (Plot et al., 2012).

Much remains to be learned about the mechanisms that act to modulate the extent of the stress response in reproducing reptiles. The mechanism dampening the stress response of nesting females to capture in olive ridley sea turtles appears to be at the level of the brain rather than at the level of the pituitary or adrenal glands because females that showed little response to the stress associated with capture exhibited a marked increase in corticosterone levels when treated with ACTH (Valverde et al., 1999). A serotonin-regulated system within the brain may also be of importance in modulating the response of the hypothalamic-pituitary-gonadal axis to the stress response because male banded garter snakes treated with a serotonergic type 2A receptor antagonist before capture prevented a stress-induced decline in androgen levels following capture (Lutterschmidt & Mason, 2005). A plasma-binding protein that binds both androgens and C-21 steroids such as corticosterone has been identified in a lizard (Jennings et al., 2000).

The relatively high costs of reproduction may be increased in reptiles that are exposed to stress (e.g., Berger et al., 2005; French et al., 2007; French & Moore, 2008; Lancaster et al., 2008; Mills et al., 2008). In the side-blotched lizard, corticosterone levels may mediate the tradeoff between fitness and reproduction in a mating strategy-dependent manner (Lancaster et al., 2008). Corticosterone has also been shown to affect pregnancy success and/or timing of oviposition in some lizards (e.g., Cree et al., 2003; Radder et al., 2008; Weiss et al., 2007) but not in others (Lancaster et al., 2008; Meylan & Clobert, 2005; Preest et al., 2005). Furthermore, in the tree lizard, an oviparous species, glucocorticoids given to nearly full-term embryos adversely affected hatching success in a dose-dependent manner and stimulated oviposited eggs to hatch significantly sooner (Weiss et al., 2007). Corticosterone treatment of mothers also affects the fitness of offspring in the common European lizard, a viviparous species, by affecting dispersal, reducing sprint speeds, and causing sex-specific changes in the probability of survival (e.g., Belluire et al., 2004; De Fraipont et al., 2000; Meylan & Clobert, 2004, 2005; Meylan

et al., 2004; Uller et al., 2005; Vercken et al., 2007). However, exposing mothers to stress during the prenatal period does not appear to affect clutch size, sex ratio, or sexual dimorphism in these lizards (Uller et al., 2005). The effects that stress during pregnancy may have on offspring fitness are likely to vary with species because treatment with ACTH failed to affect offspring sprint speed or growth rate in a viviparous gecko (Preest et al., 2005).

7.2 Research directions

Although it is generally assumed that exposure to stressors has an inhibitory effect on reproduction, relatively little empirical evidence exists, or has been gained in the last decade, to support this assumption in freely living reptiles (see Guillette et al., 1995). As most work has been based largely on the results of studies of captured animals, additional studies are needed to assess the effects of environmental and social stressors on the reproductive activity of freely living reptiles. An example of this type of approach is seen in a study that examined reproductive activity and corticosterone levels in a population of freely living brown tree snakes that are believed to be living in stressful environmental conditions (Moore et al., 2005). It is also important in future studies of free-living reptiles to investigate whether a tradeoff between reproduction and survival occurs in reptiles that have been exposed to natural stressors rather than to corticosterone treatment (see Lancaster et al., 2008), and the extent to which individuals differ in the modulation of their stress response during reproduction (see Lendvai, Giraudeau, & Chastel, 2007).

A series of studies of a viviparous lizard documented that corticosterone treatment of pregnant females influences offspring behavior, physiological performance, and offspring survival (e.g., Vercken et al., 2007). However, studies are needed to verify that the fitness and survival of offspring are affected when free-living mothers are exposed to naturally occurring stressors. In addition, studies are needed that assess the extent to which glucocorticoids pass from mothers to their eggs and the functional significance of such a transfer (Radder, 2007).

Even though many potential pitfalls exist in using corticosterone levels to evaluate the health of a population (see Romero, 2004), the levels of plasma corticosterone are an important factor in successfully assessing the relative health of populations of wild birds (see Kitaysky et al., 2007) and of wild reptiles (Romero & Wikelski, 2001). Additional studies of this nature are still needed to confirm that circulating corticosterone levels can be used to assess the relative reproductive health of wild populations of reptiles. Moreover, the data from such studies may also serve as useful benchmarks to assess whether captive populations of reptiles are stressed. For those studies that do focus on glucocorticoid stress responses, Breuner et al. (2008) argued that they should

include measures of performance and fitness and incorporate and test “ideas of context-dependency, coping strategies, and possible fluctuating selection pressures when considering the fitness benefits of the acute [glucocorticoid] response.”

There is a clear need for future studies that examine the proximate mechanisms of the stress response, and how they follow the laws of mass action, in reptiles. A plasma protein that binds glucocorticoids has been identified in a lizard (Jennings et al., 2000); however, studies are needed to establish the presence, location, and number of these receptors in other reptiles. Studies of the enzymes that mediate the synthesis and inactivation of corticosterone are also needed. In addition to studies that focus on the actions of corticosterone there is also a need to investigate other components of the stress response.

Even today, the majority of studies that have investigated the effects of stress on reproduction in reptiles have focused on the actions of corticosterone. Moreover, there has been a tendency, without substantiating evidence, to interpret differing sensitivities to corticosterone as indicative of species differences in stress responsiveness. However, upstream stress molecules of either central or endocrine origin (e.g., 5-HT, NE, CRH, ACTH) may have significantly different effects, and the entire endocrine stress axis may behave differently than corticosterone alone. Dallman (2003) and others have argued over two decades ago that central neural mechanisms must be considered in addition to other physiological components of the stress response, but rarely in studies of reptiles have these mechanisms been considered within the framework of stress and reproduction. Neural mechanisms at the level of the brain rather than at the level of the pituitary gland or adrenal gland appear to play a key role in dampening the adrenocortical stress response in nesting female sea turtles (Valverde et al., 1999). While in some squamates 5-HT may modulate HPG activity during the physiological stress response, differential telencephalic and brainstem serotonergic activity induced by social stress predicts gonadal function (Lutterschmidt & Mason, 2005; Summers et al., 1997). We suggest that studies of these central neural mechanisms, as well as the elements of concentration, receptor/binding protein density, affinity, and the laws of mass action, in reptiles, are clearly required if we are to understand better how the stress response can be modulated in relationship to reproductive stage and function.

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