

AN ABSTRACT OF THE DISSERTATION OF

Heather L. Waye for the degree of Doctor of Philosophy in Zoology presented on April 19, 2007.

Title: Reproductive Biology and Behavior of the Brown Tree Snake (*Boiga irregularis*) on Guam.

Abstract approved: \_\_\_\_\_

Robert T. Mason

While there are many more species of reptiles in the tropics than in temperate latitudes, relatively little is known about the natural history of tropical species of snakes. Even basic information, such as reproductive ecology and behavior, is lacking. Patterns of reproduction in tropical species differ from patterns in temperate species in important ways, such as the duration of gonadal activity and environmental factors that influence the frequency and timing of reproductive bouts. One tropical species, the brown tree snake (*Boiga irregularis*), was accidentally brought to the island of Guam and quickly became established throughout the island. Although this population has been monitored for over twenty years, many aspects of its basic biology, including its reproductive cycle, have yet to be described. The purpose of this dissertation research was to describe the reproductive biology and behavior of brown tree snakes on Guam. I used aggregation and shelter choice trials to determine whether females show aggregation behavior and to identify the cues that elicit aggregation. Reproductive state of the test snakes did not affect their response to the

scent of a single male or female, but did change their response to multiple female scents. Measurements of gonad development and steroid hormones over a four-month period from captive snakes on Guam were compared to those obtained over the same time period from free-living snakes. Reproduction on Guam was found to be extended but seasonal, with females becoming vitellogenic in the latter part of the dry season and into the wet season. I also found that the corticosterone stress response did not vary with sex, size, or body condition, but the response of gonad sex hormones to acute stress was greater in larger snakes. I measured the body condition index and corticosterone levels of brown tree snakes on Guam to determine whether that population still showed the chronic stress and poor condition apparent in an earlier study. Significantly lower levels of corticosterone in all snakes in 2003 suggests that although juveniles did not have significantly improved energy stores they, along with mature males and females, were no longer under chronic levels of stress.

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Reproductive Biology and Behavior of the Brown Tree Snake  
(*Boiga irregularis*) on Guam.

by

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APPROVED:

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Major Professor, representing Zoology

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Chair of the Department of Zoology

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Dean of the Graduate School

I understand that my dissertation will become part of the permanent collection of Oregon State University libraries. My signature below authorizes release of my dissertation to any reader upon request.

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Heather L. Waye, Author

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## DEDICATION

This thesis is dedicated to my parents, Les and Jean Waye, who have supported me wholeheartedly in everything, even when they weren't sure about where I was going with this snake thing.

And to my husband, Peter – if we can write our theses and finish our degrees at the same time without trying to throttle each other, I think we'll be fine.

REPRODUCTIVE BIOLOGY AND BEHAVIOR OF THE BROWN TREE SNAKE (*BOIGA*  
*IRREGULARIS*) ON GUAM.

CHAPTER 1 – INTRODUCTION

Environmental damage caused by humans generally falls into one of three categories: 1) pollution, 2) resource misuse, and 3) introduction of exotic organisms (Coblentz, 1990). “Exotic organisms”, or nonindigenous species, are those that have been brought by humans to areas beyond their native ranges (Kolar & Lodge, 2001). Introductions can be deliberate, because of human immigrants nostalgic for the flora and fauna of home, for example, or in an attempt to control local pests. Other species are brought to new areas for agricultural reasons and later become feral, while still others are accidental hitchhikers in cargo or on ships. Invasive species are those nonindigenous species that have become established and spread from the point of introduction (Kolar & Lodge, 2001). Once exotics are established they often become a permanent component of their new environment (Coblentz, 1990). Invasive species have had a significant impact on ecosystems worldwide, especially island ecosystems. The loss of biodiversity through the introduction of non-native species is second only to the loss due to habitat destruction (Wilcove et al., 1998).

Some introduced species, such as corn, wheat, poultry, and cattle, are beneficial in that they provide a significant portion of the world’s food supply

(Pimentel et al., 2001). However, nonindigenous plants, animals, and microbes are estimated to cost \$137 billion annually in the USA alone, in environmental damage and economic loss (Pimentel et al., 2000). Much of this money is spent on attempts to control insects, feral pigs, exotic plants, and plant pathogens, while insect and pathogen damage to crops and timber trees can cause economic losses. Monetary loss does not include ecological impacts from predation or herbivory on native species, habitat modification or destruction, the introduction or spread of disease, competition with native species, and genetic modification of native species (Simberloff, 2000).

Introduced vertebrates are often the most damaging to island ecosystems; for example, European rats (*Rattus* spp.) have become established on many islands worldwide with disastrous results, and predators such as cats, dogs, and mongooses have been devastating to endemic birds, mammals, and reptiles (Dobson, 1988). For example, New Zealand has lost approximately 40% of its native terrestrial bird species, and many species of reptiles, invertebrates, and plants are threatened, primarily due to invasive species (Clout, 2001). Most of these highly disruptive vertebrates have been mammals, but there are reptile and amphibian species that have had major impacts on the ecosystems to which they were introduced (e.g., Hayes & Jennings, 1986; Burnett, 1997; Kiesecker et al., 2001). One of the best examples of a non-mammalian invasive species is the brown tree snake, *Boiga irregularis*.

### **The Brown Tree Snake as an Invasive Species**

The brown tree snake was accidentally brought to the island of Guam (Fig. 1.1) soon after World War II; the earliest record of *B. irregularis* on Guam is from the

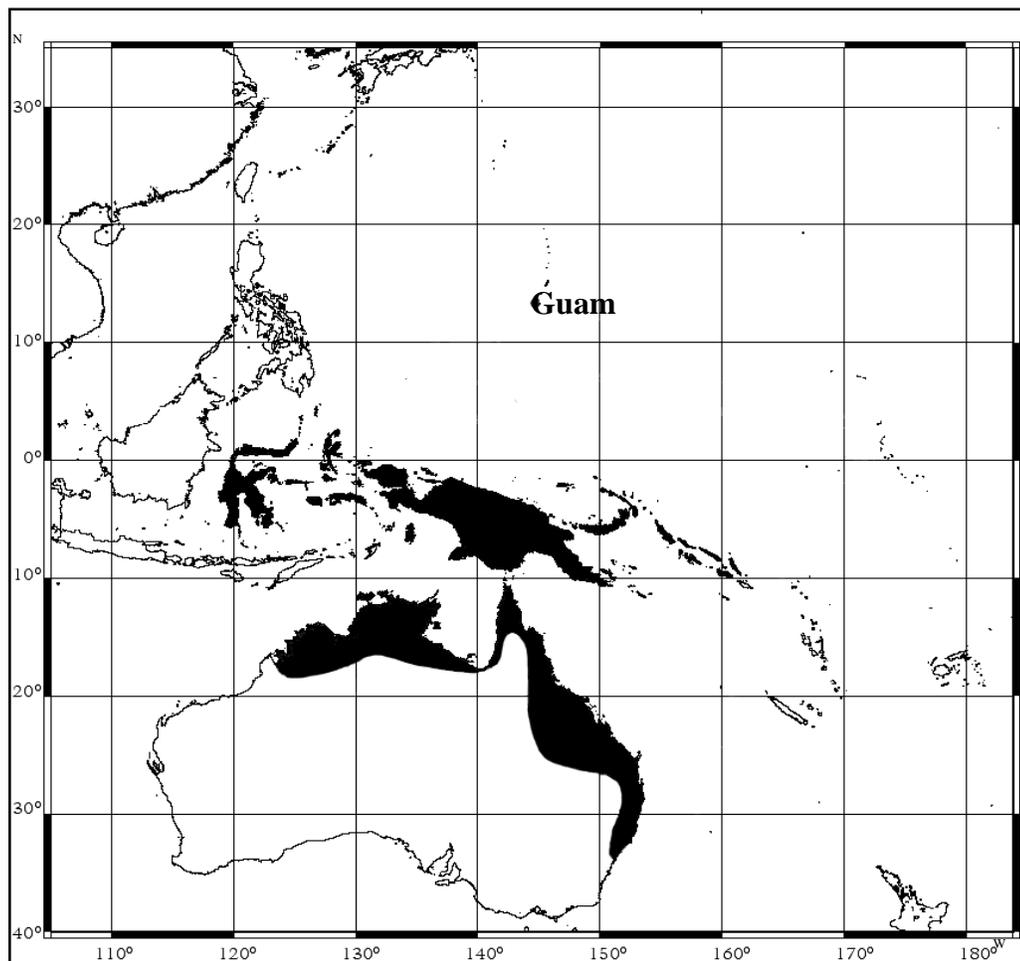


Figure 1.1. Native range and location of the introduced population of *Boiga irregularis* on Guam (based on Cogger, 1994 and Ehmann, 1992).

1950's (Savidge, 1987). The original snakes are thought to have been stowaways in military materiel salvaged from the Admiralty Islands north of New Guinea (Rodda et al., 1992); however, phylogenetic analysis shows that the Guam snakes most likely originated from nearby West Sepik province, Papua New Guinea (Rawlings et al., 1998). The first museum specimen was collected in 1960, and by 1970 the species had spread to the northern end of Guam (Rodda et al., 1992). Brown tree snakes spread

across the island at a rate of approximately 1.6 km/year, and are now found throughout Guam (Savidge, 1987).

Attempts to measure the size of the population of brown tree snakes on Guam were not made before 1985, but there appears to have been a decline in numbers in northern Guam between 1985 and 1989 (Rodda et al., 1992). Rodda et al. (1992) reported peak densities in northern Guam of around 100 snakes per hectare in 1985, while later estimates ranged from 24/ha in 1992 at a site in northern Guam to 49/ha at the putative colonization site in 1990 (Rodda et al., 1999b). No population estimates have been published for the years after 1992. The figure most often quoted is of 13,000 snakes per square mile (Fritts & Rodda, 1988), or 50/ha, which is high for a large-bodied species of snake, although perhaps not for an island population (Rodda & Dean-Bradley, 2002). Small, secretive species, such as *Diadophis punctatus* or *Carphophis vermis*, can reach many hundreds of snakes per hectare, while larger colubrids usually occur at one to 20/ha (Fitch & Echelle, 2006). Wiles et al. (2003) state that current densities of snakes are less than those found several decades ago, although there are no published data to support this statement. There are no reliable records of population trends in central and southern Guam (Fritts, 2002).

### ***Biology of Boiga irregularis***

The genus *Boiga* includes approximately 33 species (Vogel, 2004) of long, thin-bodied snakes with large heads and large eyes. They are nocturnal arboreal snakes, and typically demonstrate a shift in prey from ectotherms to endotherms as they increase in size (Greene, 1989). Species of *Boiga* can be found in Asia, India, sub-

Saharan Africa, northern Australia, New Guinea, Indonesia, and the Philippines (Greene, 1989; Luiselli et al., 1998).

The native range of *Boiga irregularis* covers the eastern and northern coastal areas of Australia and the islands of Papua New Guinea and eastern Indonesia (Fig 1.1) (Cogger, 1994), so this species occurs naturally in temperate, subtropical, and tropical habitats (Bull et al., 1997). Based on mid-dorsal, ventral, and subcaudal scale counts, it appears that brown tree snakes in Australia, Papua New Guinea, and the Solomon Islands belong to a single species with several subspecies (Whittier et al., 2000).

The brown tree snake is long and slender and specialized for arboreal habitat, which in Australia is generally unoccupied by the more prevalent elapid snake species (Shine, 1991). In Australia, brown tree snakes are found in a wide range of habitats, including rainforests and mangroves, wet and dry sclerophyll forests, paperbark swamps, and coastal heaths (Cogger, 1994). Brown tree snakes are found in all habitats on Guam, but seem to prefer the forests (Campbell III, 1999).

In southeast Queensland, Australia, brown tree snakes are encountered more often in late spring and summer, the warmest and wettest months. Snakes are caught least often in the coolest and driest months, especially when temperatures drop below 15°C (Bull & Whittier, 1996). Brown tree snakes are active all year on Guam, but peak activity occurs May through July, when rainfall generally starts to increase (Fritts et al., 1987b). Brown tree snakes are nocturnal, and seek daytime refugia in dark, small spaces (Tobin et al., 1999), which is the reason they often end up in cargo.

The distribution of brown tree snakes in Australia appears to be limited at least in part by temperature (Cogger & Heatwole, 1981), so the constant warm temperature on Guam may alleviate at least one factor that controls the distribution and abundance of this species in other parts of its range (Rodda et al., 1999a). Another limitation to the population size of brown tree snakes in Australia could be the lack of inexperienced prey species. Australian bird and lizard species evolved with brown tree snakes so they may have accumulated defenses against this form of predation. In contrast, prey species on Guam are particularly vulnerable to arboreal generalist predators, as they, like many island species, evolved without this particular threat (Rodda et al., 1997). Competition with other snake species is another possible limitation for brown tree snakes in Australia, although the more recently-arrived colubrids tend to occupy niches that were unexploited by Australian elapids (Shine, 1991).

The brown tree snake is a generalist, both in terms of food and method of foraging. Brown tree snakes use both active searching and ambush foraging modes (Rodda, 1992), and although they are usually arboreal, they also forage on the ground (Cogger, 1994). They use a combination of constriction and venom to subdue prey, although they can easily overpower prey with constriction alone (Rochelle & Kardong, 1993). Brown tree snakes in Australia eat a range of vertebrate species, including lizards, small mammals, birds and eggs, but in New Guinea this species rarely eats birds (Beehler et al., 1986). Males and females seem to have similar diets (Savidge, 1988; Shivik & Clark, 1999b), while smaller individuals tend to eat frogs and lizards, likely due to the smaller size of the prey items (Shine, 1991). On Guam,

juvenile brown tree snakes eat lizards and adults eat mammals and birds (Savidge, 1988). Captive hatchlings on Guam have also consumed grasshoppers (Linnell et al., 1997). Snakes on Guam have been observed eating a wide array of items, including dog food, paper towels, chicken bones and spareribs (Rodda et al., 1999a).

Food supply is thought to be the main limiting factor for brown tree snakes on Guam (Rodda et al., 1999b; Wiles et al., 2003). For example, the body condition of females declined between 1985 and 1990 (Jordan & Rodda, 1994), and since 1989 small lizards have made up the majority of the snakes' diet (Rodda et al., 1999a). Low body condition usually results from a reduction in food (e.g., Romero & Wikelski, 2001) that changes the size of the fat stores in an individual (Bonnet & Naulleau, 1994). Brown tree snakes sampled on Guam in the early 1990's had a significantly lower body composition index (a comparison of the length of the individual to its weight) and higher baseline plasma levels of the stress hormone corticosterone than brown tree snakes from Australia or in captivity (Moore et al., 2005), suggesting that adult snakes are unable to find enough food to maintain body condition.

The reproductive cycle of brown tree snakes on Guam has yet to be described, and is not well understood even in its native range (Shine, 1991; Whittier & Limpus, 1996). Preliminary data suggest that males with mature sperm can be found all year on Guam, but the proportion of males that are undergoing active spermiation varies seasonally (Mason et al. unpub. data). It appears that few snakes of reproductive size (reproductively competent) are actually reproductively active (as determined by analysis of circulating gonadal steroids) (Mathies et al., 2001; Moore et al., 2005), possibly due to their poor body condition. Only a few clutches of eggs on Guam have

been reported; these were laid by captive females between mid-May and mid-July (McCoid, 1994; Linnell, 1997; Waye & Mason, in prep). Timing of mating is not well known anywhere that this species occurs (Whittier & Limpus, 1996). Although it has been suggested that brown tree snakes on Guam can produce more than one clutch in a year (Rodda et al., 1992), and that brown tree snakes on Guam reproduce throughout the year (McCoid, 1994), there is no published evidence to support either of these claims and little precedent for either of these modes in any snake species (Seigel & Ford, 1987). While males with sperm can be found throughout the year in Australia (Bull et al., 1997) and Guam (Mason et al. unpub. data), gonad development in Australian males and females is highly seasonal (Shine, 1991; Whittier & Limpus, 1996; Bull et al., 1997) and there are no published data to suggest that they do otherwise on Guam.

Mating behavior in this species is similar to that of other colubrid snakes (Greene & Mason, 2000). However, males also perform ritualized combat behavior, in which they perform head-jerking and body alignment behaviors, then try to pin each other's head to the ground; generally the larger male wins (Greene & Mason, 2000). Courtship and combat behaviors start only after the snakes tongue-flick each other, indicating that pheromones are important mediators of these behaviors. There is evidence for a female sex attractant pheromone, a male pheromone that stimulates female courtship behavior, a male pheromone that stimulates combat behavior, and, in female cloacal secretions, a courtship-inhibition pheromone (Greene & Mason, 2000; Greene & Mason, 2003). The female sex pheromone is made up of a combination of nonvolatile nonpolar lipids located in the skin of the snake (Greene & Mason, 1998).

Although the skin of female brown tree snakes contains the same long chain saturated and monounsaturated methyl ketones that comprise the sexual attractiveness pheromone of the female red-sided garter snake (*Thamnophis sirtalis parietalis*) (Murata et al., 1991), methyl ketones do not appear to play a role in eliciting male courtship behavior in this species (Greene & Mason, 1998).

Attempts have been made to compile a list of characteristics common to introduced species that became successful invaders, to predict which species are likely to become invaders in the future (Kolar & Lodge, 2001). In general, some of the characteristics that invasive vertebrate species share are: a broad environmental tolerance and wide habitat distribution in its native range; relatively large body size and short generation time; females that are able to colonize alone; high reproductive capability; broad diet; gregariousness, or at least a lack of territoriality, and ability to coexist with human activity and use human activity as a dispersal mechanism (Ehrlich, 1989; Ricciardi & Rasmussen, 1998). *Boiga irregularis* displays many of these characteristics (wide distribution in native range, broad diet, lack of territoriality, human coexistence, and ease of dispersal). Very little is known about the generation time or reproductive capability of this species.

### ***Impact of Boiga irregularis on Guam***

Savidge (1987) examined various hypotheses put forward to explain the disappearance of bird species on Guam, which included impacts from pesticides, natural disturbance, and disease. The correlation between the spread of brown tree snakes on Guam and the disappearance of bird populations and reduction of alternative prey (i.e., introduced mammals) strongly suggests that the brown tree snake

was responsible (Savidge, 1987). The resident avifauna of Guam formerly consisted of 28 species, including four seabirds and seven introduced species (Duckworth et al., 1997). Of the native bird species, 17 have drastically reduced populations and 11 have been extirpated from Guam (Wiles et al., 2003). Breeding populations of seabirds are gone, the remaining species of forest birds are listed as endangered (Rodda et al., 1998), and several of the introduced species also suffered declines, all apparently due to the brown tree snake (Savidge, 1987). The loss of Guam's avifauna has resulted in the removal of pollinators and seed dispersers from the ecosystem. This loss likely has had a great impact on forest growth and regeneration (e.g., Ritter & Naugle, 1999), although the ecology of native plants on Guam before the arrival of brown tree snakes is largely unknown (Perry & Morton, 1999).

The continuing presence of the brown tree snake on Guam complicates efforts to manage endangered bird species. Mariana crows (*Corvus kubaryi*) on Guam successfully reproduce only where nests are protected by barriers (Tarr & Fleischer, 1999). These birds suffer predation from monitor lizards and rats as well as snakes (Wiles et al., 1995). The population of Guam rails (*Rallus owstoni*) was about 80,000 individuals in the 1960s. By 1983 they had mostly disappeared from Guam (Engbring & Pratt, 1985) and the last wild individual was seen in 1986. Reintroduction of this species to Guam will not be possible without the ability to control the effects of brown tree snakes (Savidge, 1987; Haig et al., 1990), although other introduced predators, such as feral cats, pose a significant threat as well (Fritts & Leasman-Tanner, 2001).

Bats are also important pollinators and seed dispersers on islands (Craig, 1993). Two of the three species of bats (the only native mammals on Guam) have

been extirpated, and the third is endangered (Wiles, 1987). However, it is not likely that this decline is due to the brown tree snake. *Pteropus tokudae* was long considered to be rare on Guam (Wiles, 1987), and *Emballonura semicaudata* was likely extirpated due to damage to roosting caves during World War II. The role of the brown tree snake in the disappearance of these bats from Guam is unknown (Fritts & Leasman-Tanner, 2001) and probably minor. The third species, the Marianas fruit bat (*Pteropus mariannus*), was first depleted by overhunting, and then possibly by snake predation (Wiles, 1987; Wiles et al., 1995). Brown tree snakes are thought to be responsible for the very low survival rates of juvenile bats, although the only published evidence to support this claim is the discovery of a single brown tree snake with three small fruit bats in its stomach (Wiles, 1987). The Chamorro people of the Mariana Islands consider fruit bats a delicacy, and many bats are imported to Guam from other islands to meet this demand (Wiles & Payne, 1986). Local and federal laws protect the bats, but poaching is still common and has kept populations from recovering (Wiles, 1987). This species of fruit bat has also steadily declined on Tinian, Rota, and Saipan (Krueger & O'Daniel, 1999), where brown tree snakes have not become established, strongly suggesting that pressure from poaching, and not predation by brown tree snakes, is responsible.

Populations of small mammals (including *Mus musculus*, *Rattus rattus*, and *Suncus murinus*, all introduced species) have declined in certain habitats following the introduction of the brown tree snake (Savidge, 1987). It has been suggested that the snakes spread so rapidly throughout the island because people moved them around to reduce local populations of rats (Rodda et al., 1992), although given the reluctance of

most people to handle large, aggressive snakes, this scenario seems unlikely. The introduced shrew, *Suncus murinus*, was introduced around the same time as the brown tree snake and could be found all over Guam by 1958. Shrew populations have since declined (Fritts & Rodda, 1988). The presence of exotic small mammals probably enabled the brown tree snake to exist on Guam in high numbers after native species disappeared (Savidge, 1987). This hypothesis should be investigated more fully, as it could influence strategies to manage the snakes.

Of 10 species of native lizards, four have been extirpated and five are rare (Fritts & Leasman-Tanner, 2001). Although the snake-eyed skink, *Cryptoblepharus poecilopleurus*, was never common on Guam and the rock gecko (*Nactus pelagicus*) declined due to predation by the introduced shrew, other disappearances are thought to have been caused by the brown tree snake (Rodda & Fritts, 1992). Three skink species and three gecko species that were historically found on Guam, but have not been seen there in recent decades, can still be found on surrounding islets (Perry et al., 1998b). Native geckos have all decreased in abundance, while the introduced common house gecko (*Hemidactylus frenatus*) has become relatively more abundant, probably due to its ability to coexist closely with humans. Two other introduced species, the green anole (*Anolis carolinensis*) and the curious brown skink (*Carlia fusca*), occur most frequently in the diet of brown tree snakes. While *A. carolinensis* has declined on Guam, possibly because they sleep in exposed sites at night, *C. fusca* are still common (Rodda et al., 1999a). The mangrove monitor, *Varanus indicus*, seems to have also declined, but due to its large size it is doubtful that this reduction is due to the brown tree snake (Rodda & Fritts, 1992). The introduction of the poisonous

marine toad (*Bufo marinus*) and feral dogs are more likely to have been the cause (McCoid et al., 1994).

People have lost domestic animals and pets to the snakes (Fritts & McCoid, 1991), and human babies have been bitten and envenomated (Fritts & McCoid, 1999). Between 1986 and 1991, 147 people visiting the Guam Memorial Hospital emergency room were there for snakebite treatment. Roughly 40% of these were under four years of age (Fritts et al., 1990, 1994). While a brown tree snake could not possibly ingest a human, human odor does induce a predatory response in this species, possibly due to human skin components that are similar to those in other mammals (Greene et al., 2002). The venom itself has low proteolytic activity on mammalian tissue (Weinstein et al., 1991) but potent neurotoxic activity on avian tissue (Lumsden et al., 2004), reflecting the evolutionary history of brown tree snakes as primarily bird specialists. Although the bite of this species is considered to be relatively harmless, small children and infants can react to the venom, as they are most likely to be bitten during feeding attempts when the snake is actively trying to introduce venom into its prey (Fritts et al., 1990). Reactions have included localized discoloration and swelling, as well as increased pulse rate, respiratory distress, and lethargy (Fritts et al., 1990, 1994) although the latter group of symptoms could also be explained as the result of hyperventilation due to vigorous and extended crying after being bitten by a snake.

The impact of the brown tree snake is not limited to the fauna of Guam; the introduction of this species has had a large economic impact as well. The brown tree snake was the cause of more than 1600 power outages on Guam between 1978 and 1997, and the frequency of outages due to snakes appears to be increasing (Fritts,

2002). Approximately 30% of the power outages on Guam are caused by brown tree snakes on power lines or in substations or generation facilities (Fritts, 2002) with associated losses and costs estimated to be in the millions of dollars (Fritts et al., 1987a; Fritts, 2002). The Animal and Plant Health Inspection Service's Wildlife Service program has estimated the damages to resources caused by brown tree snakes between 1994 and 1997 to total US \$1,225,812 (Bergman et al., 2002).

Although the brown tree snake is claimed to have been the cause of many of the species declines and power disruptions on Guam, there is a tendency to ascribe a wide variety of problems to the snake without evidence to support the charge. In particular, the reasons behind the decline of bat and lizard species are largely unverified; the brown tree snake was likely involved in the disappearance of some of the lizard species, but introduced lizards and small mammals might have played a major role. This has led to a "demonizing" of the snake, encouraging the public to view it as a "super invader", possibly to the detriment of organized efforts to study and understand the species in its niche on Guam. Likewise, complete removal of the brown tree snake from Guam would certainly aid in the management of endangered wildlife and power disruptions, but will probably not completely alleviate these problems. Other introduced species (e.g., rats, cats, monitor lizards) prey on native birds and lizards, and the many tropical storms and typhoons will always challenge the electrical system on Guam.

## **Control Techniques**

The full scope of the impact of brown tree snakes on Guam was not realized until the mid-1980s, so by the time an organized response was launched the snakes were already well established. Many government agencies have been involved in various aspects of the control program on Guam, including the Guam Division of Aquatic and Wildlife Resources, the U.S. Geological Survey, U.S. Fish and Wildlife Service, U.S. Department of Defense, U.S. Department of the Interior, and U.S. Department of Agriculture. Millions of dollars of federal funds have been earmarked for brown tree snake control. For example, in fiscal year 2005 approximately \$5.5 million was allocated to brown tree snake research and interdiction, mostly from the Office of Insular Affairs and the Department of Defense (Colvin et al., 2005). US Code Title 7 (Agriculture) Chapter 111 (Brown Tree Snake Control and Eradication) authorizes appropriations of up to \$10.6 million for activities conducted through various federal agencies for each of the fiscal years 2006 through 2010.

A variety of approaches to brown tree snake management are being developed, including physical control of the snakes, ongoing public education programs and technical meetings and workshops, and a website of information on current efforts and results (Fritts & Leasman-Tanner, 2001). Public education programs alert people to the need to report sightings of snakes on other islands and to the likely consequences of translocating brown tree snakes, and help them to snake-proof their homes (Campbell III et al., 1999). Many of the following techniques for physically managing brown tree snakes on Guam are still under development; exclusion and capture techniques are the only ones currently in use (Campbell III et al., 1999).

One of these capture techniques is to capture snakes by hand (Campbell III et al., 1999). Trained searchers hunt for snakes using spotlights along fence lines in high-risk areas, such as cargo holding areas, the airport, and military properties (Rodda et al., 1998; Vice & Pitzler, 2002). Roughly 3500 to 5000 snakes are caught and destroyed each year on Guam, one-third of these by hand (Vice & Pitzler, 2002). Hand capture is only effective and practical in areas that are accessible and surrounded by chain-link fencing, so its usefulness is limited to specific areas on Guam. However, this technique can be used to partially compensate for the biases in size and sex of snakes produced by trapping methods (Vice & Pitzler, 2002).

Trained dogs and handlers are used on Guam to search for brown tree snakes in cargo intended for destinations thought to be susceptible to brown tree snake introduction, and in associated cargo holding facilities and transport vessels (Engeman et al., 1998b). Dog teams are also in use in Oahu, Hawaii and Saipan, to check cargo arriving from Guam (Engeman et al., 2002), although currently only 60% of military aircraft arriving from Guam are inspected (Fritts & Leasman-Tanner, 2001). The detector-dog program found 34 brown tree snakes during inspections on Guam between 1993 and August 1996 (Engeman et al., 1998a); four more snakes were found over a 10 week period in early 1998 after Supertyphoon Paka (Vice & Engeman, 2000). Capture rates after 1998 have not been reported. Dog teams found between 38% and 80% of the snakes planted in cargo during a series of efficiency tests (Engeman et al., 1998b; Engeman et al., 2002), indicating that this form of screening for stowaways is not effective enough to stand alone as the only method for intercepting snakes. The “sniffer dog” program is popular and highly visible to the

press and public, although in relation to its cost it has intercepted relatively few snakes.

Barriers to brown tree snake movements have been difficult to develop due to the exceptional climbing ability of this species. Snakes can be excluded from power lines using conical guards, guy wires, and smooth poles (Fritts & Chiszar, 1999) and from nesting trees using electrical and mechanical barriers (Aguon et al., 1999). Snake-proof fencing has been used to exclude snakes from areas of one hectare (Campbell III, 1999), although this technique appears effective for very small areas only. After four years of trapping, snakes were still captured within a 23 ha barrier enclosure (Rodda et al., 1992). Several barrier designs have been tested, including vinyl, metal mesh, and masonry with an electrified metal strip (Perry et al., 1998a; Vice & Pitzler, 2002). Of these, pre-stressed concrete barriers were the most effective at excluding brown tree snakes (Rodda et al., 1992). However, a barrier that successfully impedes the movements of snakes must be able to withstand typhoons and chewing by rats, and also be regularly maintained for full effectiveness (Rodda et al., 2002). In addition, most fencing materials do not exclude either small or very large snakes (Campbell III, 1999). Snake barriers can also have unexpected side effects; for example, feral cats were able to use snake barriers to corner and more easily capture reintroduced Guam rails (Rodda et al., 2002).

Trapping programs have been used to reduce the number of snakes around airports and cargo areas since 1993 (Engeman & Vice, 2000). Approximately 2000-3000 traps are in regular use on Guam (Rodda et al., 2002). Much effort has been put into the development and testing of various trap designs, many involving the use of a

live prey item (i.e., mice) inside the trap (described in Engeman & Vice, 2000; Vice & Pitzler, 2002). This trap technology also has potential uses outside of Guam; traps developed for brown tree snake capture on Guam have been used successfully (without bait mice) to trap specific “nuisance” snakes in the suburbs of Melbourne, Australia (Temby & Engeman, 2006).

There are several problems with these traps, however. First, it is expensive and time-consuming to build, set up, and maintain the arrays of fences and traps, and traps and fences must be replaced after each typhoon. Reports of brown tree snakes on Hawaii tend to increase after a typhoon has destroyed the fences around cargo areas in Guam, as seen after Supertyphoon Pongsona, which flattened fences around airports and cargo areas on December 8, 2002 (e.g., “Storm widens door for tree snakes”, Honolulu Star-Bulletin, December 11, 2002). Secondly, the traps use live mice as bait. This raises concerns about animal care and ethics and the possibility of an exotic species of mouse escaping into the environment. In addition, a regular supply of live mice is difficult to obtain on Guam, so at any given time a significant percentage of traps are left unbaited (HLW pers obs. 2003). One option is the use of carrion (Shivik, 1998) or even mechanized mouse lures (Lindberg et al., 2000) as bait, but even traps with live bait are not fully effective at catching snakes (Engeman & Vice, 2000). Very small (less than 600 mm SVL), very large, and gravid snakes are much less likely to be caught by traps (Rodda et al., 2002; Vice & Pitzler, 2002), so a large proportion of the snake population is untrappable, including the actively reproducing component. Finally, food-baited traps may be less successful when the surrounding prey base is plentiful (Rodda et al., 2002). This situation is of great concern in areas such as

enclosures that have been nearly, but not completely, cleared of snakes, and on other islands where traps are placed to monitor for recently-arrived snakes.

Commercially available attractants and repellents have been tested, but Chiszar et al. (1997) found that while some substances were effective in the laboratory, they were much less effective when tested in the field. Products containing capsaicin have no effect on brown tree snakes (Chiszar, 2001), and McCoid et al. (1993) found that Dr. T's Snake-A-Way (7% naphthalene and 28% sulfur) did not repel brown tree snakes. Methyl bromide applied as a fumigant in cargo areas will kill snakes several days after application (Savarie et al., 2005), but this chemical was targeted for complete removal from use in developed countries by 2005 due to its impact on the ozone layer (EPA, 2005). Delivery of acetaminophen in dead mice reduced the number of snakes in a test plot on Guam by up to 50% (Campbell III & Sugihara, 2001; Savarie et al., 2001). Attempts have been made to combine this toxic bait with broadcast techniques to reach inaccessible areas for widespread snake control. For example, dead mice implanted with radiotransmitters and secured to plastic flagging or small plastic parachutes to catch in the tree canopy were dropped from helicopters in test areas. No more than 63% of these mice were eaten by snakes (Shivik et al., 2002). The concern that the endangered Marianas crow will also eat the toxic baits (Shivik et al., 2002) was addressed by Avery et al. (2004) using a different species of crow. They concluded that the impact on the crows could be minimized by changing the bait station design so that crows are prevented from retrieving the bait. The impact of the acetaminophen bait on other native species, or on cats and dogs, has not been determined. As one of the long-term goals of brown tree snake control is the

reintroduction of native birds, chemicals used to kill or repel snakes must also be examined for potential adverse effects on all native and non-target species.

Parasites (Telford, 1999) and diseases (Nichols & Lamirande, 2001) have been examined as means of controlling brown tree snakes on Guam, although the safety and efficacy of these approaches have yet to be determined (Rodda et al., 1998). Brown tree snakes are susceptible to some ophidian paramyxoviruses and to a reovirus, with a mortality rate of 37% for the reovirus and up to 100% mortality with the paramyxoviruses (Nichols & Lamirande, 2001). In its native range, *Boiga irregularis* carry haemogregarine (blood) parasites (Mackerras, 1961; Ewers, 1968), a reptilian hookworm (Schad, 1962), and several different intestinal parasites (Caudell et al., 2002b). Brown tree snakes from Guam do not appear to have any blood parasites (Lamirande et al., 1999), which is consistent with the observation that introduced populations may lose many or most of their parasite species as a result of the translocation (Torchin et al., 2003). Telford (1999) suggests the use of haemogregarines in biological control of snakes on Guam, if they are specific to brown tree snakes and can develop in potential mosquito or mite vectors on Guam. It may take a very high parasite load to produce direct physiological effects on snakes (Brown et al., 2006), but it is possible that lower levels of infection could have a detrimental effect on individuals exposed to other sources of stress (Caudell et al., 2002b). Even if the parasites themselves do not weaken the Guam population, the parasites could be used as a way to introduce other lethal factors into the snakes (Telford, 1999). However, until the accidental transfer of brown tree snakes to other islands can be limited, there is a risk that pathogens or parasites carried by the snakes

could become introduced elsewhere and infect indigenous populations of reptiles.

Therefore, potential pathogens must be screened for possible host range and effects on nontarget species before they are used on brown tree snakes (Howarth, 1999; Caudell et al., 2002b).

Introduction of a snake predator onto Guam is another form of biological control that has been suggested. Predators of brown tree snakes in their native range are not known, although Caudell et al. (2000) found a brown tree snake in the stomach of a red-bellied black snake (*Pseudechis porphyriacus*) and observed a large marine toad (*Bufo marinus*) catch and eat a neonate brown tree snake. It appears that none of the potential predators of brown tree snakes in Australia feed exclusively on this species, or even exclusively on reptiles (Caudell et al., 2002a). On Guam, snakes are killed by monitor lizards, feral pigs, and cats and dogs as well as by humans, but not in large enough numbers to control the snake population (Savidge, 1991). A great deal of controversy surrounds the release of non-indigenous species for biological control (Simberloff & Stiling, 1996; Howarth, 1999), even if this was a viable option on Guam.

Brown tree snakes in the laboratory will follow pheromone trails left by conspecifics. Males likely follow female trails to obtain mating opportunities and follow male trails to engage in combat behavior (Greene et al., 2001). Females will aggregate with other females in hide boxes in the laboratory (Waye and Mason in prep), and probably will follow female trails to find suitable aggregation or egg-laying sites (Greene et al., 2001). Cloacal secretions from a female brown tree snake inhibit courtship behavior of males (Greene & Mason, 2000) and so could be used to repel

them from areas of interest or to disrupt mating. Pheromones may be especially effective as trap enhancers, to lure snakes into traps instead of using a live prey item to bait the trap (Mason, 1999), and may be more likely to catch the very large males or gravid females that traps currently miss. Likewise, synthetic sex pheromone trails leading up to traps could greatly increase their efficacy (Greene & Mason, 1998). Odor-only lures that mimic prey items are less successful than those that also incorporate visual stimuli (Shivik & Clark, 1999a); snakes respond most strongly to a combination of cues when presented with prey (Shivik, 1998) but do not require a visual stimulus to follow the scent of another snake. Also, pheromone-baited traps would attract mature snakes regardless of the availability of the surrounding prey base and thus would be more effective than food-baited traps for monitoring and controlling newly-established populations of snakes. The use of pheromones in insect control has been shown to be ecologically friendly and cost-effective (Shani, 1991, 2000). It would be relatively easy and inexpensive to re-apply snake pheromones after a storm. The application of pheromones in the control of introduced vertebrate species has recently become a reality. Recent advances in the characterization of sea lamprey (*Petromyzon marinus*) pheromones will make possible the control of this introduced species using natural products instead of relying on a toxicant-based control program (Sorensen et al., 2005). Similarly, the recent discovery of a male sex pheromone in round gobies (*Neogobius melanostomus*), another invasive species in the Great Lakes, could lead to more effective control techniques (Corkum et al., 2006). This promising technology should be explored in brown tree snakes as well.

### Are Brown Tree Snakes Likely to Become Established Elsewhere?

Brown tree snakes have appeared on Saipan, Hawaii, Okinawa, Rota, Diego Garcia, Kwajalein, Pohnpei, Tinian (Fig. 1.2), and even in Texas, apparently as accidental hitchhikers from Guam (Fritts et al., 1999). These snakes will readily hide in cargo, cargo containers, and wheel wells of aircraft (Tobin et al., 1999). One snake that arrived on Pohnpei was found dead in a cargo container on a ship from Guam (Buden et al., 2001), and the snakes that made it to Hawaii appeared to have arrived by aircraft from Guam (Rodda et al., 1997). Translocation of brown tree snakes is of especially great concern to the people of Hawaii due to the threat they pose to the remaining native bird species and the tourism and electrical industries (Kraus & Cravalho, 2001). The federal program to prevent translocation of brown tree snakes from Guam to Hawaii was established in January 1995. Seven brown tree snakes were found in Hawaii between 1981 and 1994 (Fritts et al., 1999), while only one was

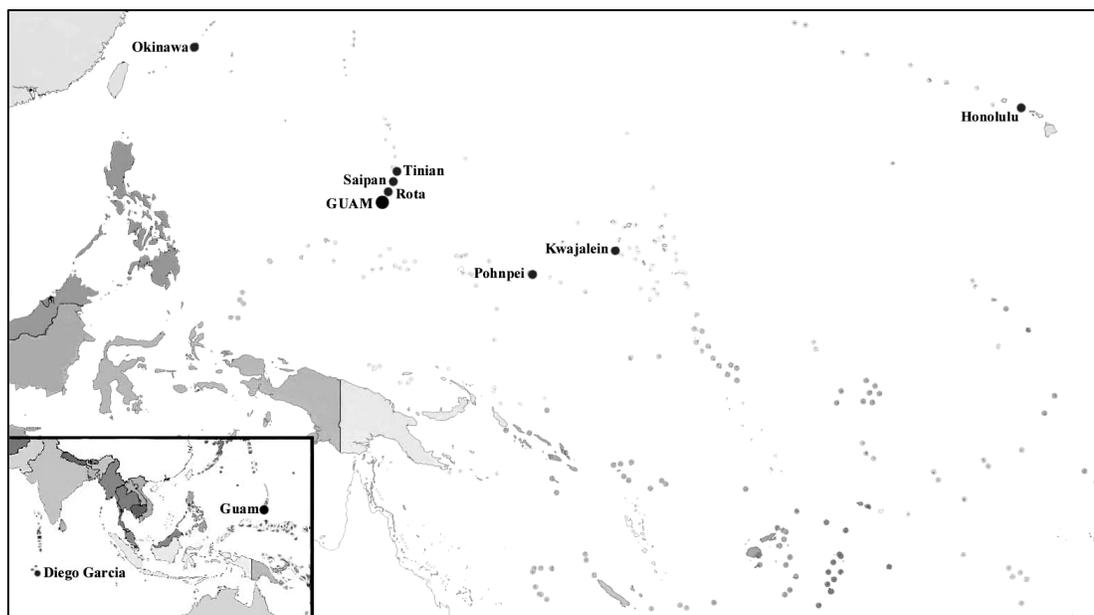


Figure 1.2. Locations where translocated *Boiga irregularis* have been found. The inset shows the location of Diego Garcia in relation to Guam.

discovered between 1995 and 2000 (Kraus & Cravalho, 2001).

However, the brown tree snake is not the only exotic snake that has been found on Hawaii, nor is it the only species with the potential to have such a disastrous effect on native fauna (Kraus & Cravalho, 2001). Greene (1989) points out that the attributes of *Boiga irregularis* that have allowed it to become such a successful invader of Guam (e.g., large gape, ability to climb, nocturnal foraging behavior, known predation on birds and bats) are common to other members of the genus *Boiga*. Snakes belonging to the genera *Thamnophis*, *Boa*, *Python*, *Elaphe*, *Pituophis*, *Lampropeltis*, and *Coluber* have been found on Hawaii. Most were probably smuggled in as part of the pet trade. Many of these species share at least a few of the characteristics that have allowed *B. irregularis* to thrive on Guam (for example, a generalist nocturnal predator with arboreal tendencies) (Kraus & Cravalho, 2001).

The current range of the brown tree snake includes tropical and temperate habitats, which correspond to Class A and Class C on Koeppen's climate map (Fig. 1.3) (FAO-SDRN, 1997). Class A, the tropical zone, is defined by temperatures greater than 18°C in the coldest month. Class C, the temperate zone, is defined as those areas where the average temperature of the coldest month is between -3 and 18°C, and the average temperature of the warmest month is greater than 10°C. Figure 1.3 shows the global distribution of those subzones in which *B. irregularis* is already established.

Although the brown tree snake can be found in the temperate zone (Cf and Cw) in Australia, it is restricted to the coastal areas near the Tropic of Capricorn, and so is unlikely to survive the winters of the Cf subzone in Europe, China, and

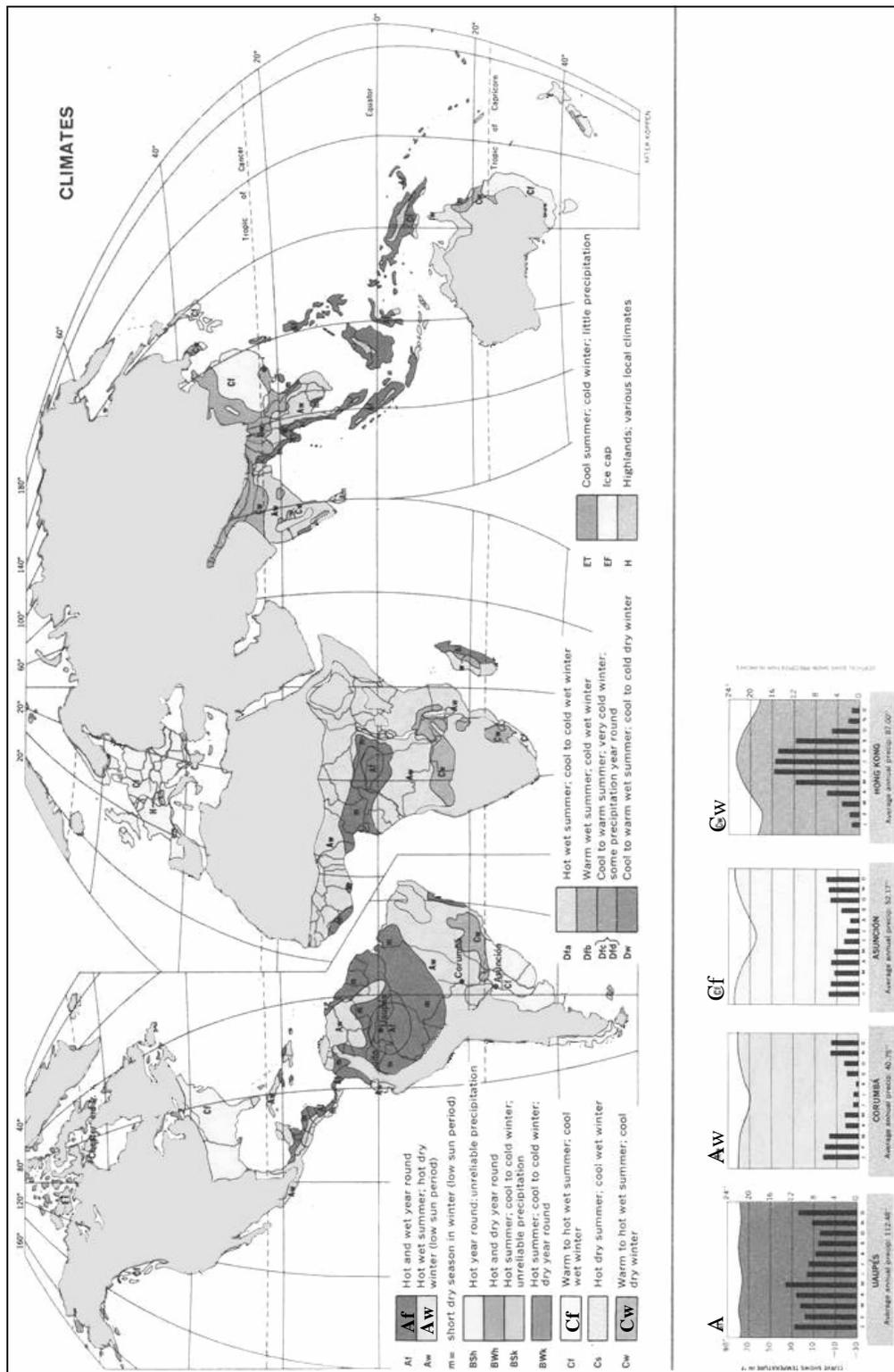


Figure 1.3. Global distribution of Koeppen climatic subzones that encompass the native and introduced range of *Boiga irregularis* (modified from FAO-SDRN 1997).

southeastern USA (except possibly the extreme southern coast). Anderson and others

(2005) examined the thermal preferences of brown tree snakes, but the thermal limits of this species are unknown. Bull and Whittier (1996) found that brown tree snakes near the southern edge of their range in Australia are active when the minimum temperature is above 15°C. In Brisbane, Australia, the minimum temperature is below 15°C for 5 months of the year, but does not drop below 10°C (Bull & Whittier, 1996), so presumably brown tree snakes could survive elsewhere in climates with similar minima. However, snakes from Guam under a cooling regime in the laboratory appeared to be under stress below 18°C (HLW pers obs 2004), so the Guam population may not be able to survive as well as Australian brown tree snakes at lower temperatures.

Many of the larger islands and the continental areas already have robust snake guilds, which means that any brown tree snake that finds its way there will have to compete for a wary prey base and could even become prey itself. For example, while much of central Africa falls within the tropical Aw and Af subzones, native snakes such as the boomslang (*Dispholidus typus*), green mamba (*Dendroaspis angusticeps*) (Brazaitis & Watanabe, 1992), and *Boiga blandingi* (Greene, 1989) are arboreal predators on lizards and birds and would compete with *Boiga irregularis* for food. Others, such as the Cape file snake (*Mehelya capensis*) or the Cape cobra (*Naja nivea*), feed on other snakes (Brazaitis & Watanabe, 1992) and could be potential predators of introduced brown tree snakes.

Therefore, other tropical islands (or island-like habitats on the mainland) are most likely to be the only other locations where introduced brown tree snakes could become established. However, this is not insignificant. These tropical islands, with

their insolated naïve fauna, are also where an introduced predator would have the most devastating effect. There are many islands in the South Pacific with suitable climate and habitat for brown tree snakes (Figs 1.2 and 1.3), with Hawaii as the prime example. This potential threat is tempered by the lack of regular traffic between islands; most of these islands (outside of Micronesia and Hawaii) are without direct flights or cargo ships from Guam or from anywhere within the native range of *Boiga irregularis*. The current lack of any other established populations of introduced brown tree snakes outside of Guam, not even on nearby Rota, Saipan, or Tinian, underscores this point.

## **Conclusion**

The brown tree snake has been firmly established on Guam for at least three decades, and is still a major ecological and economic problem. A plan to increase development of Anderson Air Force base on Guam (e.g., “US military plans pose threat to Guam's endangered birds”, Los Angeles Times, December 25, 2004) will enhance its importance as a center of operations in the South Pacific. This expansion will lead to increased traffic between Guam and other locations, and a greater possibility of translocating brown tree snakes. It may be possible to trap most of the snakes out of cargo areas and keep them at low densities within these well-defined areas (Engeman & Linnell, 1998), but control on a larger scale may not be feasible (Perry et al., 1998a). Any one management technique would likely be effective on only part of the snake population (Campbell III et al., 1999). Effective control of brown tree snakes on Guam requires a multi-faceted approach that involves techniques

to exclude snakes from important areas and prevent their spread, trapping and population control throughout the island, control of other exotic species that could be additional prey items, and arguably most important, research to describe the basic ecology and reproductive biology of this species on Guam. The successful use of pheromones in sea lamprey control underscores the usefulness of research in this area.

The establishment of brown tree snakes on Guam, while disruptive and destructive, offers a unique opportunity for research on invasive species and island colonizations. The genetics of this population has still to be described, as well as related issues such as founder effects, genetic drift, and adaptation to new environments. Virtually no investigations have been made as to the effects of the loss of avian and lizard species on the insects or plants of Guam.

As argued by Greene (1989), *Boiga irregularis* is not the only species of *Boiga* that could have caused such havoc on Guam, although it was able to exploit the situation more fully than might have been possible for many other species. It can be considered a model invasive species; it is a generalist (in terms of food and habitat preferences), it is apparently able to survive in relatively high densities, it is hardy and can survive long periods without eating, and last (but certainly not least), it is able to take advantage of humans moving around the planet to spread to new habitat. Whether *B. irregularis* is able to become established outside of its native range and Guam remains to be seen, but it is a strong reminder that global transportation of organisms, whether accidental or deliberate, means that any species has the potential to become an invader.

## **Summary of Research and Experimental Goals**

The overlying goal of the following studies was to describe the reproductive behavior and reproductive biology of a tropical snake, the introduced population of brown tree snakes on Guam. There are many more species of tropical snakes than temperate ones, but the tropical species have not been as well studied. Even basic information, such as reproductive biology and behavior, is sparse and unknown for the majority of species. The brown tree snake is a good model for the study of tropical snakes, as it is native to a range of habitats from temperate Australia to tropical Papua New Guinea and Guam. This distribution presents the opportunity to make comparisons within the species between temperate and tropical populations and native and introduced populations.

I started by examining behaviors that are mediated by reproductive pheromones. The reaction of both females and males to the skin lipids of others is important if pheromones are to be used in the management of these snakes, and in understanding the behavior of this species. Previous research (Greene et al., 2001) showed that males will follow pheromone trails left by other males and by females, apparently with the intention of combat with the males and mating the females. However, the response of female brown tree snakes to the skin lipids of other females and males was unknown. Chapter Two describes the response of female brown tree snakes to the presence or the scent of other brown tree snakes, using aggregation behavior as the bioassay.

The results of the aggregation and shelter choice experiments showed the importance of the reproductive state of the females. Chapter Three describes the

reproductive cycle of brown tree snakes on Guam, using hormones and histological examination of gonads. A previous study of this population (Moore et al., 2005) found that a small proportion of adults of reproductive size were reproductively active, and all snakes had very high levels of corticosterone. Therefore, I established a supplemental feeding experiment to determine whether the lack of reproduction was due to insufficient food. If food was limiting, these experimental snakes would then add individuals that were reproductively active to the sample used to determine reproductive cycles.

The elevated concentrations of corticosterone found by Moore et al. (2005) prompted the exploration of the acute stress response, to determine what levels of corticosterone would indicate chronic stress, and how stress affects gonadal sex steroid hormones. In Chapter Four, I subjected free-living snakes to capture stress for different lengths of time, and compared the results between the sexes, over the five months of the sampling period, and for different body conditions to determine variables that influence the acute stress response in this species.

The size-weight data collected in 1992 and 1993 and my data collected in 2003 presented a good opportunity to test the conclusion put forward in Weatherhead and Brown (1996). They stated that the direct measurement of body condition is more accurate than estimation using a body condition index, and advocated the use of more than one measure of condition. In Chapter Five I compared body condition and plasma levels of corticosterone between the two sampling periods, and examined annual variation in precipitation on Guam to determine whether environmental conditions could have contributed to the apparent change in physical condition.

Successful management of an invasive species depends on having some basic knowledge of its natural history, especially its reproductive behaviors and biology.

The introduced population of brown tree snakes on Guam provides a unique opportunity to study tropical snakes and contribute to that growing body of research, while exploring the ways in which basic biological investigations can provide information that will hopefully assist in the management of an invasive vertebrate.

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CHAPTER 2

THE ROLE OF VISUAL AND CHEMOSENSORY CUES IN FEMALE BROWN TREE SNAKE  
AGGREGATION BEHAVIOR.

Heather L. Waye and Robert T. Mason

## **Abstract**

Snakes are thought to lack social behaviors, but many snakes do form aggregations. Some aggregations occur because of common interest in a location, and others for some benefit from grouping with other individuals. However, it is not clear whether snakes aggregate based on chemosensory cues or due to the presence of other individuals. We examined aggregation in female brown tree snakes (*Boiga irregularis*) to determine whether this species shows aggregation behavior and which cues elicit aggregation. We used shelter-choice trials with pheromone-scented boxes and aggregation trials to observe the responses of the snakes to other individuals and to scent cues alone. Females aggregated when tested with other females.

Reproductive state of the test snakes did not affect their response to the scent of a single male or female, but did change their response to multiple female scents. This change in response to the scent of other females, but not to the presence of other females, suggests that some female social behaviors differ depending on whether only chemical cues are present.

## **Introduction**

Although snakes are generally thought of as lacking social behaviors (Brattstrom, 1974), the tendency for individual snakes to come together and form aggregations has been well documented (reviewed by Gregory et al., 1987). The proximate cause of an aggregation can be placed into one of two categories: individual snakes may be simultaneously attracted to the features of a particular site, or they may be attracted to the presence of another snake. Examples of habitat features that could

draw snakes together include suitable hibernation (Gregory, 1984) or oviposition sites (Brown & Shine, 2005), thermoregulation, or high densities of prey (Arnold & Wassersug, 1978). In these examples, an important environmental factor is spatially restricted in occurrence, so snakes occur at these specific locations in greater numbers than in the surrounding habitat. Therefore, an individual would be at that location regardless of the presence of other snakes. On the other hand, there are many potential benefits that could cause a snake to seek out and be in close proximity to other snakes. Snakes in aggregations potentially benefit from physical contact with each other. For example, grouped snakes have been shown to minimize water loss (Graves et al., 1986) and temperature loss (Aleksiuk, 1977) compared to single snakes. In addition, these two categories are not always mutually exclusive, and a particular aggregation may be the result of a combination of factors (Gregory et al., 1987).

The formation of social aggregations is mediated mainly through chemosensory cues, with the vomeronasal organ processing chemical stimuli (Heller & Halpern, 1982b). These cues are pheromones, chemical signals emitted by one individual that affect the behavior or physiology of others of the same species (Karlson & Luscher, 1959). The response to these cues can vary between species. Water snakes (*Nerodia r. rhombifera*) avoid scent cues left by conspecifics (Porter & Czaplicki, 1974), while garter snakes (*Thamnophis* spp.) can be found in multi-species aggregations (Gregory, 1975) and usually prefer scent cues left by conspecifics (e.g., Porter & Czaplicki, 1974; but not Wayne & Gregory, 1993). Snakes will prefer sites previously occupied by other snakes ("habitat conditioning" Burghardt, 1983), and the cues they use to determine previous occupancy appear to be components of skin lipids

that are left on the substrate (e.g., Graves et al., 1991). Even when another snake is not currently at that site, these signs of occupancy could indicate that the site is suitable for use by another snake. It appears that the vomeronasal system may play a primary role in aggregation behavior (Burghardt, 1980; Heller & Halpern, 1982b), but the relative contributions of visual and chemosensory cues have yet to be examined (Mason, 1992).

While aggregation and trailing of conspecific cues in snakes has been well established, virtually all research has involved temperate species. Environmental factors such as temperature or water loss may not be limiting to species in tropical climates, and thus other factors (social or environmental) may be more important in controlling aggregative behaviors. Studies of home range sizes and movements in tropical snakes have shown that some species may avoid conspecifics (Webb & Shine, 1997; Whitaker & Shine, 2001; Fitzgerald et al., 2002), while others show communal nesting and overwintering behaviors (Whitaker & Shine, 2001).

The brown tree snake (*Boiga irregularis*), is a nocturnal, arboreal colubrid from northern and eastern Australia and Papua New Guinea (Cogger, 1994). This species was accidentally introduced to the island of Guam around the end of World War II, with devastating effects on the ecology and economy of the island (Rodda et al., 1992). There are a few published reports of aggregations of brown tree snakes of unknown sex in Australia (Covacevich & Limpus, 1973; Hoser, 1980) and on Guadalcanal Island (Pendleton, 1947). Greater understanding of the social behavior of brown tree snakes would allow better targeting of management efforts and expand our knowledge of tropical snake social behaviors. Male brown tree snakes will follow

skin lipid trails of conspecific males and females (Greene et al., 2001), but the social preferences of the females of this species have not been addressed. We conducted a series of aggregation trials to determine whether females would prefer to occupy shelters in groups or would avoid contact with other females. Although this experiment would tell us whether or not the snakes would aggregate, it would not determine whether aggregating snakes simply preferred the same shelter (site-specific factors) or if they really do prefer to cluster with other snakes (social factors). A series of two-shelter choice trials were performed to determine whether these snakes aggregate based on chemical cues left by other snakes, or if the physical presence of another snake in the shelter is required.

## **Methods**

Snakes used in this study were collected on Guam in 2000 and housed under laboratory conditions (Greene et al., 1997) for at least two years prior to the start of the behavior trials. The colony originally consisted of five males and 18 females. Females ranged in size from 119 to 160 cm SVL (180 to 670 g), and males from 166 to 185 cm SVL (530 to 1000 g). Snakes were individually housed in Plexiglas cages (Mason et al., 1991) with branches for climbing and hide boxes suspended from the roof of the cages. Cages were kept in a microprocessor-controlled environmental chamber with photoperiod 12L:12D, photophase temperatures of 26.7°C, scotophase temperatures of 25.0°C and 80% relative humidity. Trials were conducted between June 2002 and February 2006, and we increased the humidity in the environmental chamber in October 2005 to initiate folliculogenesis.

For all of the following behavior trials, snakes were placed in an enclosed 1.95 m<sup>3</sup> testing arena constructed of clear Plexiglas. Snakes were introduced into the arena at 15:00 and left until 10:00 the following morning to allow time for the snakes to explore their surroundings before the lights came back on and they chose a refuge for the lighted hours (Tobin et al., 1999). The arena was washed with soap and hot water, rinsed, and allowed to dry between trials. Hide boxes in the arena were clear plastic boxes (40.5 x 28 x 15 cm) with a 6 cm x 8 cm hole in one end of the opaque lid to provide an entrance on top of the box. After each trial, the boxes and lids were washed with soap and hot water, rinsed, and allowed to dry before the next trial.

### ***Aggregation Trials***

For each aggregation trial, six hide boxes were placed in the arena as shown in Fig. 2.1., with the openings on top facing the outside walls of the arena, and the boxes flush against the walls. Each box was clean and devoid of any bedding. Female snakes were placed in the center of the arena sequentially, in no particular order, through a window in the front of the arena. At the end of each trial the number of snakes and the identity of each snake in each hide box was recorded, and the snakes returned to their respective cages. Sixteen females were used in each of ten trials between June and September 2002. To increase the sample size, six additional trials using 13 of the same females were completed between June and August 2004, to match the seasonality of the previous trials.

### ***Shelter Choice Trials***

Shelter-choice trials were conducted using two of the hide boxes, one placed in each of the two back corners of the test arena (Fig. 2.1b). Each box was lined on the

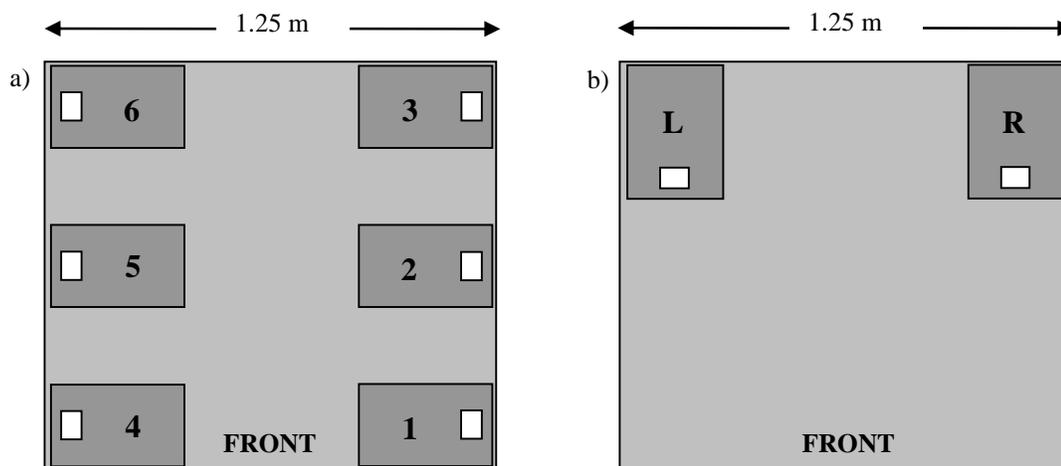


Figure 2.1. Top view, showing location and numbering of hide boxes in the test arena for a) aggregation and b) shelter choice trials.

bottom with two new paper towels, either clean or scented as described below.

*Three scented females* – These trials were conducted between October 2004 and February 2005 (nonreproductive), and again in December 2005 (reproductive) after the increase in humidity and change in the reproductive state of the female snakes. One of the hide boxes (unscented) was lined with clean paper towels and the other was occupied by three females (selected at random without replacement, but not including the individual tested). Each scent-donating snake was introduced into the box through the opening in the lid and allowed to move around in the box and on the paper towel substrate for two minutes. The snake was held by its tail to avoid allowing its cloaca to enter the box, thus restricting the scent in the box to skin lipids only. The three snakes were put in the box one at a time, and the box then placed in one of the randomly chosen back corners of the test arena. The unscented box was placed in the other back corner and the test snake placed at the center front of the arena. Each

female was also tested with two unscented boxes to test for bias in the experimental design.

*One scent snake (male or female)* – The design of these trails was the same as above, except the scented box was temporarily occupied by one female or one male. These trials were conducted between March and May 2005 (nonreproductive), and again between January and May 2006 (reproductive). Each female was tested once with an unscented hide box and a box scented by another female, and was also tested once with an unscented box and a box scented by a male. The scent-donating snakes were chosen in no particular order, as were the test females.

### ***Statistics***

*Aggregation trials* – Five trials in which 25% or more of the snakes were not in any hide box at the end of the trial were removed from analysis. The distribution of the number of times each number of snakes was recorded was compared for the earlier series of trials (in 2002) and the later trials (2004). There was no significant difference (Chi-square contingency table,  $p > 0.25$ ) so the 11 trials were pooled for subsequent analysis. Chi-square goodness of fit was used to compare the observed distribution to a binomial distribution (uniform), a Poisson distribution (random), and a negative binomial distribution (clustered or aggregated). Classes with small expected frequencies were pooled to make the expected frequencies at least 3 (Krebs, 1989). The standardized Morisita index ( $I_p$ ) (Smith-Gill, 1975) is considered one of the best and most widely used measures of dispersion (Krebs, 1989). It is particularly popular because it is independent of abundance and mean density (Elliott, 1977). The index varies from -1.0 to +1.0 with 0 signifying a random distribution, negative values

indicating uniformity, and positive values indicating aggregation. The 95% confidence limits are at -0.5 and +0.5 (Krebs, 1989; Hurlbert, 1990). The goodness of fit calculations and standardized Morisita index were calculated using program NEGBINOM (Krebs, 1989).

*Shelter choice trials* – The Chi-square goodness of fit (using the Yates correction for continuity) was used to compare the observed results to the values expected if the hide boxes were selected at random (Zar, 1984).

## **Results**

### ***Aggregation Trials***

The standardized Morisita index indicated a clumped or aggregated distribution ( $I_p = 0.51$ ,  $p < 0.05$ ). The distribution of the observed frequencies was significantly different from the binomial distribution ( $X^2 = 172.43$ ,  $p < 0.0001$ ) and the Poisson distribution ( $X^2 = 71.26$ ,  $p < 0.0001$ ), therefore the observed frequencies do not have either a uniform or random distribution. The observed distribution is also significantly different from a negative binomial distribution with the same mean ( $\mu = 2.18$ ), variance ( $s^2 = 6.27$ ), and estimated  $k = 0.81$  ( $X^2 = 17.10$ ,  $p = 0.009$ ) (Fig. 2.2). The best fit is the negative binomial distribution. Although the negative binomial distribution is frequently used to describe aggregation, many patterns of aggregation do differ significantly from this distribution (Krebs, 1989; Hurlbert, 1990).

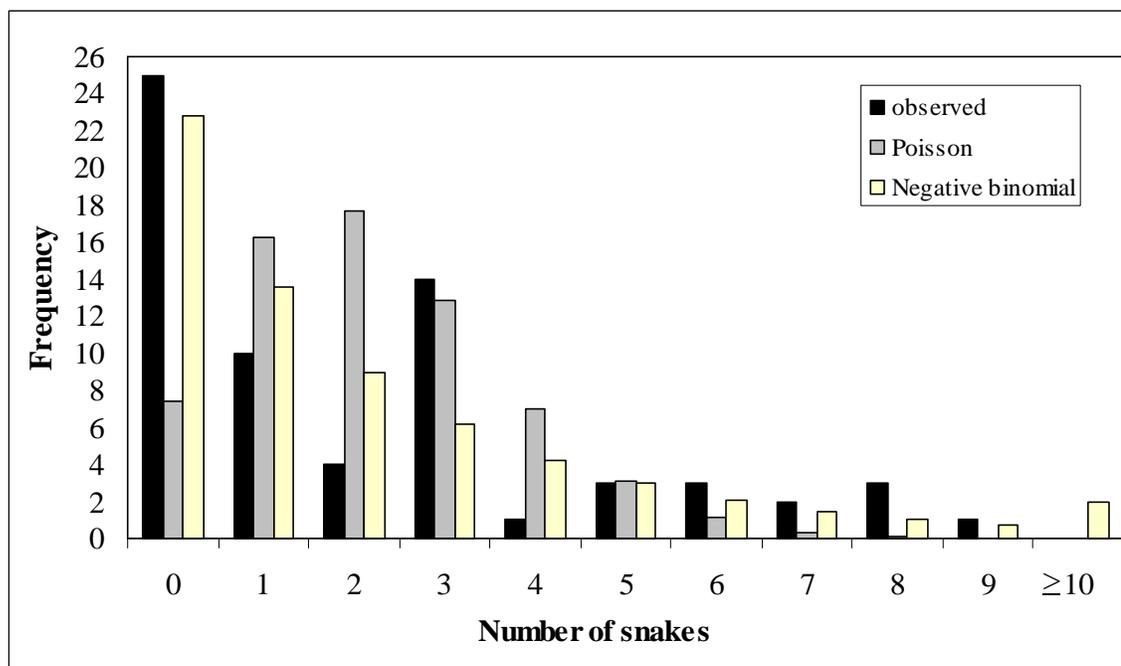


Figure 2.2. Observed distribution of the frequency of each grouping size of snakes, and the corresponding Poisson and negative binomial distributions calculated using the mean and variance of the observed distribution.

### *Shelter Choice Trials*

*Three scented females* - The trials with two empty boxes showed no significant bias in occupancy ( $X^2 = 2.0$ ,  $df = 1$ ,  $p = 0.157$ ) (Table 2.1). Nonreproductive females chose the unscented box over the scented box ( $X^2 = 6.31$ ,  $df = 1$ ,  $p = 0.012$ ), while reproductive females were generally found in the scented box, although this was not statistically significantly different from no preference ( $X^2 = 0.77$ ,  $df = 1$ ,  $p = 0.381$ ). However, the results for reproductive snakes was significantly different from the results for nonreproductive snakes ( $X^2 = 18.97$ ,  $df = 1$ ,  $p = 0.0001$ ).

*One scented female* - Female snakes chose the scented hide box over the unscented box both when they were nonreproductive ( $X^2 = 9.38$ ,  $df = 1$ ,  $p = 0.002$ ) and when they were reproductively active ( $X^2 = 6.31$ ,  $df = 1$ ,  $p = 0.012$ ) (Table 2.1).

Table 2.1. Results of shelter choice trials using female brown tree snakes, with one hide box scented and the other one left clean. The number of successes is defined as the number of times a snake chose the scented box (except in the bias trial, where it is the number of times a snakes chose the box on the left side of the arena).

<b>Trial</b>	<b>No. of snakes</b>	<b>No. successes</b>	<b>p-value</b>
<b>One scent female</b>			
Nonreproductive	13	12	0.002
Reproductive	13	11	0.012
<b>One scent male</b>			
Nonreproductive	12	4	0.234
Reproductive	12	4	0.234
<b>Three scent females</b>			
<i>Bias trial</i>	13	9	0.157
Nonreproductive	13	2	0.012
Reproductive	13	8	0.381

*One scent male* – Nonreproductive female snakes were found in the unscented box more often than in the one scented by a male, but this was not significant ( $X^2 = 1.42$ ,  $df = 1$ ,  $p = 0.234$ ). Reproductive females also did not show a significant preference ( $X^2 = 1.42$ ,  $df = 1$ ,  $p = 0.234$ ).

## Discussion

Communal nesting is widespread throughout the squamates, and is demonstrated by a range of species in several snake families including the Colubridae (reviewed by Graves & Duvall, 1995). For example, rough green snakes (*Ophiodrys*

*aestivus*) nested communally even when presented with abundant homogeneous nest sites (Plummer, 1981), while keelback snakes (*Tropidonophis mairii*) preferred nest boxes containing old conspecific eggshells (Brown & Shine, 2005). Snakes benefit from sharing oviposition sites mostly through the quality of the nesting site, which may have better thermal or hydric characteristics than other potential sites (Brown & Shine, 2004).

Little is known about the reproductive biology of brown tree snakes, but it appears that the increase in humidity and rainfall at the start of the wet season is the cue to initiate ovarian recrudescence (Shine, 1991; Whittier & Limpus, 1996). In this study, the increase in irrigation and humidity in the environmental chamber triggered follicular development in captives. This change in reproductive condition, as determined by the presence of enlarged follicles, did not affect their preference for the box scented by one other female, nor did it change their reaction to the male-scented box. However, it did change the response to the multiple-scent box, which suggests that this response is at least partially dependent on reproductive state, unlike the response to the scent of a single female or male.

The overall preference for the multiple-scented box after the onset of vitellogenesis could reflect communal nesting behavior, although the majority of the females were not actually gravid and did not go on to deposit eggs. Each female was housed with a male overnight to give them the opportunity to mate, but it appears that at least some of the females did not accept the courtship overtures of that male. Therefore, it seems more likely that reproductive females chose the box with multiple female scents because they were looking for another mating opportunity. Male brown

tree snakes follow the scent trails of females (Greene et al., 2001) so females willing to mate might seek out groupings of other females as a way to increase encounters with suitable males.

Likewise, the avoidance of multiple female scents while the snakes were nonreproductive could be an attempt to avoid male harassment by avoiding groupings of females that might attract courting males. Both reproductive and nonreproductive females also showed a tendency to avoid hide boxes that had been occupied by males. Females of other snake species have been shown to avoid males outside of the mating season (LeMaster et al., 2001; Whitaker & Shine, 2001), and during the mating season (Shine et al., 2004). Male brown tree snakes perform combative wrestling behaviors with each other, and courtship behavior with a female is similar, including head-lifting displays and the male pinning the female's head (Greene & Mason, 2000). Females can discourage a courting male by releasing a courtship inhibition pheromone with their cloacal secretions (Greene & Mason, 2000; Greene & Mason, 2003), although the male will continue to follow and weakly court the female (Greene & Mason, 2000). Adult males of this species grow to be significantly longer and heavier than females (Cogger, 1994), so a female might find it difficult to reject a suitor without some effort and risk to herself. A female that is willing to mate can reject an unsuitable male, but nonreproductive females might attempt to avoid male attention altogether and therefore avoid the scents of males and groups of other females.

However, females do prefer to occupy shelters that are currently occupied by other females. The immediate benefits of physical contact with other snakes could outweigh the risk of encountering a male when not reproductively active. Brown tree

snakes show increased activity in the wet season throughout their range (Bull & Whittier, 1996; Rodda et al., 1999) and appear to be limited in distribution by moisture and temperature (Rodda et al., 1999). Although captive snakes were housed under conditions of high humidity, they often have trouble shedding and could potentially reduce water loss through physical contact with other snakes (Graves et al., 1986). The actual presence of another snake in a shelter provides a set of cues (visual, tactile, chemosensory, and olfactory) that elicit different behaviors than the scent alone. At least some component of social grouping behavior is based on chemosensory cues; female brown tree snakes selected refuges previously occupied by one, but not three, other females, and had different responses to the male and female scent cues.

Female brown tree snakes demonstrated a tendency to group together during the aggregation trials, but the relatively large number of groups of three snakes was unexpected. Studies of aggregation in the laboratory typically offer a relatively small number of shelters, which increases the likelihood of observing aggregation (Gregory, 2004), but presumably would also result in groupings that are artificially large. Snakes are generally found in groups of two to four in a natural environment, and only rarely did they occur in larger aggregations (Gregory, 2004). A large aggregation may be more likely to attract unwanted attention from predators or to deplete local resources, while smaller groups of snakes might balance out the benefits of physical contact with any associated costs. Therefore, the predominance of small groupings could be the result of an attempt to optimize the benefits and drawbacks of aggregation.

On the other hand, the high incidence of groups of three could indicate that

there was more than one statistical population in the group of snakes; that is, individuals with different responses to a particular scent at the same time. This possibility has not been explored in most snake aggregation papers, but it is certainly implied in their data. For example, Heller and Halpern (1982a) reported that most, but not all, test snakes returned to a shelter after disturbance, but did not discuss why this might be. In our study, differing responses may be related to reproductive state; it appears that few brown tree snakes of reproductive size on Guam are actually reproductively active (Mathies et al., 2001; Moore et al., 2005) and so every snake may not respond to a particular scent cue with the same behavior. The presence of more than one statistical population is supported by the lack of fit of the aggregation data to a negative binomial distribution, which is often used as a model of a typical clustered distribution. Many aggregated distributions observed in nature fail to fit the negative binomial distribution, so the standardized Morisita index is often used to test for aggregated dispersion (e.g., Malhado & Petrere Jr., 2004; Pooler & Smith, 2005; Otero et al., 2007).

Rodda et al. (1999) stated that, based on combinations of brown tree snakes in traps, there was no apparent social structure to aggregations on Guam. However, once a snake had entered a trap (presumably while pursuing the food bait) it could not leave and therefore had no control over which other snakes shared occupation of the trap. Rodda et al. (1999) did find fewer heterosexual and more homosexual combinations than expected, which is supported by the results of our study. We found that females actively preferred to aggregate with other females, but were not attracted to the scent of males, while it has been shown that males follow the scent of males and females

(Greene et al., 2001). Overall, these preferences should result in more female-female groups and male-male groups than would be expected, as observed by Rodda et al. (1999).

Female brown tree snakes appear to have complex social behaviors, which are dictated at least in part by reproductive state. Some grouping behavior appears to be unrelated to reproduction, and instead may be a function of hydric conditions or other microhabitat features. The particular behavior pattern depends on the cues present: the physical presence of other individuals provides a very different stimulus than pheromonal cues alone.

The use of pheromones to control a vertebrate pest species has already proven effective (Li et al., 2002; Sorensen et al., 2005) and could certainly be attempted in the case of brown tree snakes on Guam. A better understanding of social behaviors (and reproductive biology in general) is necessary before better techniques can be developed for the management of endangered or pest species; for example, this information could be used to refine the timing of sampling efforts and enhance the usefulness of pheromones in management. This study has shown that females would likely be found in small, short-term aggregations on Guam, and the use of pheromones to attract snakes to traps may work well at all times for males, but would perhaps be only seasonally effective for females.

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CHAPTER 3

A COMPARISON OF THE REPRODUCTIVE BIOLOGY OF CAPTIVE AND FREE-LIVING  
BROWN TREE SNAKES (*BOIGA IRREGULARIS*) ON GUAM.

Heather L. Waye and Robert T. Mason

## **Abstract**

Very little is known about reproduction in tropical snakes, even though many more species of snakes occur in the tropics than are found in temperate habitats. We endeavored to describe the reproductive cycle of the brown tree snake (*Boiga irregularis*), a colubrid species native to Australia and New Guinea and with an introduced population on Guam. Previous studies found that few reproductively competent snakes were reproductively active, and this lack of reproduction appeared to be due to near-starvation conditions on Guam. We housed male and female brown tree snakes in semi-natural enclosures on a schedule of supplemental feeding to determine whether improved body condition would allow the snakes to become reproductively active. Measurements of gonadal development and concentrations of plasma sex steroid hormones and corticosterone were collected over a four-month period and compared to those obtained over the same time period from free-living snakes. Reproduction on Guam is extended but seasonal, with females becoming vitellogenic in the latter part of the dry season and into the wet season. The timing of reproduction of brown tree snakes on Guam appears to depend on the female cycle, while males are capable of mating at any time of year. Eggs appear to be oviposited to maximize optimal hydric conditions.

## **Introduction**

While there are many more species of reptiles in the tropics than in temperate latitudes, relatively little is known about the natural history of tropical species of snakes (Vitt, 1987; Brown & Shine, 2002). Even basic information, such as

reproductive ecology and behavior, is lacking, although recent studies have started to address this deficiency (Aldridge et al., 1995; Shine & Keogh, 1996; Brown et al., 2002; Brown & Shine, 2006). Patterns of reproduction in tropical species differ from patterns in temperate species in important ways, such as the duration of gonadal activity and those environmental factors that influence the frequency and timing of reproductive bouts (Brown & Shine, 2002), and there is likely to be wide variety in the patterns of reproductive cycles in tropical snakes (Fitch, 1982). Species sharing the same environment can differ greatly in aspects of their reproductive biology (Brown & Shine, 2002) and closely related species can have similar reproductive characteristics while in very different habitats (Aldridge et al., 1995). This suggests that reproductive processes, while influenced and entrained by local environmental conditions, are ultimately phylogenetically conserved between populations (Brown et al., 2002).

The brown tree snake (*Boiga irregularis*) is a large arboreal member of the family Colubridae native to northern and eastern Australia and Papua New Guinea (Cogger, 1994). This species was accidentally introduced onto the island of Guam during or after World War II and quickly became established throughout the island (Savidge, 1987). Ten species of native forest birds and six species of native lizards have been extirpated from the island mainly due to predation by this snake (Rodda et al., 1999b). This snake has also had a large economic impact, mainly by causing power outages and eating pets and domesticated animals (Rodda et al., 1997). Of particular concern is that the snakes will also become established in Hawai'i, where there have been at least eight verified sightings of brown tree snakes that were accidentally brought there in cargo from Guam (Kraus & Cravalho, 2001).

The brown tree snake is a good model for the study of tropical colubrids, primarily because it can be found in a range of habitats from temperate Australia to tropical Papua New Guinea and Guam, which allows for comparisons both between subtropical and tropical and between native and introduced populations. However, many brown tree snakes on Guam that are large enough to breed are not reproducing; even though they are apparently reproductively competent, they are not reproductively active (Mathies et al., 2001; Moore et al., 2005). Brown tree snakes on Guam have also been reported to have chronically high levels of the stress hormone corticosterone and significantly lower body condition than brown tree snakes from Australia or those in captivity (Moore et al., 2005). The adaptive stress response in the short term results in an increase in the mobilization of fat stores to provide energy and the suppression of reproductive behavior while increasing foraging and escape behavior (reviewed by Wingfield et al., 1998). However, prolonged stimulation of the stress response can suppress growth and the immune system, and completely inhibit reproduction (Guillette et al., 1995; Wingfield et al., 1998). Many of the larger prey species on Guam are greatly reduced in numbers or completely extirpated, so it is possible that larger brown tree snakes are now unable to find enough food to support reproduction and are even on the verge of starvation (Moore et al., 2005).

An important factor in understanding the geographic spread and establishment of an invasive species is knowledge of its reproductive biology (Whittier & Limpus, 1996). Reproduction in brown tree snakes in northeastern Australia has been described through the examination of museum specimens and the measurement of follicle size and testicular volume (Shine, 1991; Whittier & Limpus, 1996; Bull et al.,

1997), but the seasonality of the reproductive cycle of this species on Guam is still unknown. Sex and stress steroid hormones and gonads of captive snakes with an augmented diet were compared to those of wild-caught snakes to test the hypothesis that an inadequate prey base is limiting reproductive activities on Guam, and to increase the number of samples of reproductively active adults. A better understanding of the reproductive biology of this population is greatly needed (Rodda et al., 1999a), for management purposes and to expand our insight into reproduction in tropical reptiles in general.

## **Methods**

In 2003, 80 snakes (40 male, 40 female) were housed in semi-natural enclosures at the University of Guam from May through September. Each snake was at least 100 cm snout-vent length (SVL), as snakes this size are usually reproductively mature (Rodda et al., 1999a). Snakes were housed individually in wood-frame cages (45 cm H x 30 cm W x 60 cm D) with screen on front and back to allow free air flow and solid sides and top and bottom to minimize cues from nearby snakes. The snakes were exposed to ambient temperature and humidity (verified with an indoor/outdoor thermometer) and natural light cycle. Each cage contained a plastic container with a hole cut in the side for a hide box and a bowl of water for drinking and soaking *ad libitum*.

Males and females were assigned at random to one of two feeding groups, High and Low. Snakes in the High group were initially fed 10% of their body weight at capture in mice every 4 weeks, then in mid-July this was increased to 10% of their

capture weight every 2 weeks. The Low group was initially fed 5% of capture body weight every 4 weeks, and then 10% every 4 weeks starting in mid-July. Every month each snake was weighed and a blood sample (300  $\mu$ l) drawn within 3 minutes of disturbance from the caudal vein using a heparinized 1-cm<sup>3</sup> syringe and 25-g needle.

Free-living snakes on Guam were caught by hand at night from April through September 2003, and blood was drawn from the caudal vein within 3 minutes of capture. All blood samples were placed in a cooler with an ice pack until return to the field laboratory, after which time they were centrifuged and the plasma removed. Plasma was stored at -20°C until the samples could be shipped to Oregon State University where they were stored at -70°C until assayed. Mass (g) and snout-vent length (SVL) (cm) were recorded, and after the snakes were sacrificed, the fat bodies were weighed and testes and ovaries collected and preserved in 10% buffered formalin.

### ***Histology***

The number and length of ovarian follicles and the length and diameter of the testes were measured to the nearest 0.1mm. Testes were examined following the techniques of Clesson et al. (2002). In short, testes were dehydrated in progressive alcohols, cleared in xylene, embedded in paraffin, and cut into 10  $\mu$ m sections using a rotary microtome. Sections were affixed to slides with a gelatin mixture and stained with Harris hematoxylin, Biebrich scarlet/orange II, and fast green. The tubule diameter and epithelial height of 10 seminiferous tubules in each testes were measured to the nearest 0.1  $\mu$ m using an ocular micrometer. Only tubules that appeared circular in cross-section were measured. The presence of sperm in the lumen of the

seminiferous tubules and in the epididymis was noted.

### ***Radioimmunoassay***

Plasma levels of testosterone and corticosterone were determined for male snakes and 17  $\beta$  -estradiol and corticosterone for females by radioimmunoassay following the procedure described by Moore et al. (2000). Plasma volumes of 4 to 200  $\mu$ l were used, and samples were incubated with testosterone antibody T-3003 or 17 $\beta$ -estradiol antibody E-6006 from Wein, or corticosterone antibody B3-163 from Esoterix Endocrinology. Intraassay variation was 14% for 17  $\beta$  -estradiol, 15% for testosterone, and 15% for corticosterone. Interassay variation was 16% for 17  $\beta$  -estradiol, 17% for testosterone, and 18% for corticosterone. Limits of detection were ~ 0.01 ng/ml for testosterone, ~ 0.04 ng/ml for corticosterone, and ~ 0.003 ng/ml for 17  $\beta$  -estradiol.

### ***Statistics***

To determine whether the two feeding regimes resulted in differences between the two groups of snakes, we used two-way repeated measures ANOVAs (with high and low food groups and months as variables) to determine the source of variation in mass, corticosterone, and estradiol or testosterone. We used adjusted mass, calculated as the mass of each snake at time of capture scaled to 100, and subsequent measurements scaled relative to the initial mass. A two-way ANOVA without interactions (with the three groups and months as variables) was used to determine sources of variation in corticosterone, estradiol, and testosterone. Natural log-transformed testosterone, estradiol, corticosterone, and adjusted mass were used in the two-way ANOVAs and two-way repeated measures ANOVAs. The Kruskal-Wallis

one-way ANOVA on ranks was used to compare testosterone and estradiol concentrations, average tubule diameter, epithelial height, and follicle size between months. The Mann-Whitney rank sum test was used to compare testosterone concentrations, sampling dates, tubule diameter, and epithelial height between males with sperm and without sperm. Pairwise comparisons were made using the Tukey test. All statistical analyses were performed using Jandel SigmaStat version 3.1 software package (Jandel Corporation). Analyses were considered statistically significant when  $p < 0.05$ .

## **Results**

### ***Food groups***

*Females* – There was a significant difference in mass due to group ( $F = 36.301$ ,  $p < 0.001$ ) and month ( $F = 12.422$ ,  $p < 0.001$ ), and a significant interaction between group and month ( $F = 23.627$ ,  $p < 0.001$ ). For the High food group, mass in September was significantly higher than other months ( $p < 0.001$ ), and for the Low food group, mass in June was significantly higher than the other months ( $p < 0.001$ ). There was no significant difference in levels of estradiol between the Low and High food groups ( $F = 0.002$ ,  $p = 0.965$ ) or between months ( $F = 1.428$ ,  $p = 0.241$ ). Corticosterone did not differ significantly between the Low and High groups ( $F = 0.964$ ,  $p = 0.336$ ), but did differ between months ( $F = 7.238$ ,  $p < 0.001$ ) (Fig. 3.1). This difference in corticosterone levels was between June and September ( $p < 0.001$ ) and June and July ( $p = 0.003$ ).

*Males* - There was a significant difference in mass due to group ( $F = 20.321$ ,  $p < 0.001$ ) and month ( $F = 12.565$ ,  $p < 0.001$ ), and a significant interaction between group and month ( $F = 25.384$ ,  $p < 0.001$ ). For the High food group, mass in September was significantly higher than the other months ( $p < 0.001$ ) and August was significantly higher than June ( $p < 0.001$ ) and July ( $p = 0.023$ ), but for the Low food group there were no significant differences between months. There was no significant

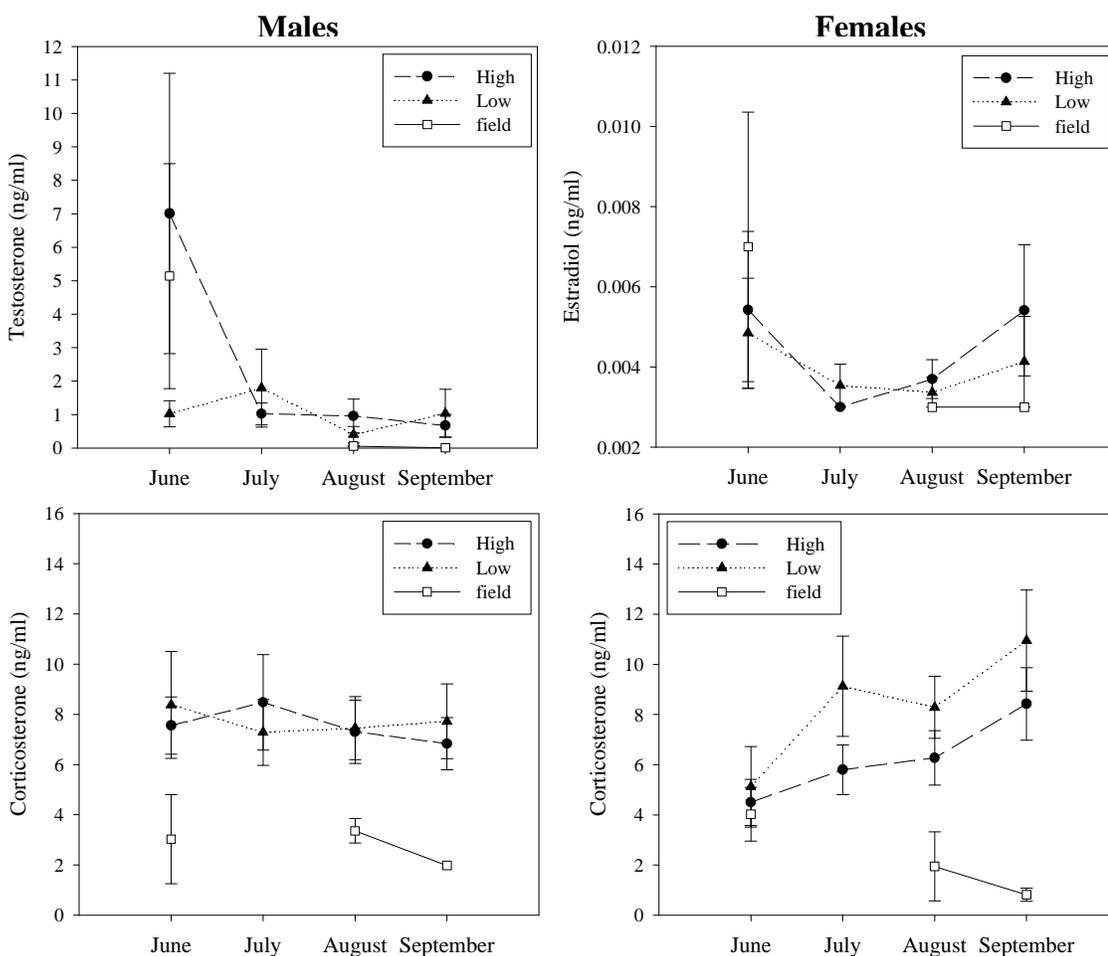


Figure 3.1. Mean plasma concentrations (and standard error) of gonadal sex steroid hormones and corticosterone in free-living male and female brown tree snakes and snakes in two food groups over a four-month period. One data point in the female estradiol August sample (0.56 ng/ml) was removed for the construction of this graph.

difference in levels of testosterone between the Low and High food groups ( $F = 0.677$ ,  $p = 0.418$ ) but there was between months ( $F = 11.962$ ,  $p < 0.001$ ). Testosterone levels were significantly different between June and the other months (all  $p < 0.001$ ). Corticosterone did not differ significantly between food groups ( $F = 0.231$ ,  $p = 0.635$ ) or months ( $F = 0.139$ ,  $p = 0.936$ ) (Fig. 3.1).

### ***Food groups and free-living snakes***

The data for the two food groups was compared to the field data (free-living snakes 90 cm SVL and longer) to determine whether they could be pooled for further analysis.

*Females* – There was no significant difference in corticosterone between months ( $F = 1.131$ ,  $p = 0.339$ ) but there was a significant difference between groups ( $F = 5.544$ ,  $p = 0.005$ ). Estradiol concentrations did not differ between groups ( $F = 0.0958$ ,  $p = 0.908$ ), or months ( $F = 1.493$ ,  $p = 0.220$ ). Although there was a peak in August, it was mainly due to one snake with a very high concentration of estradiol (0.56 ng/ml); most of the rest of the samples were below the limit of detection.

*Males* – There was no significant difference in corticosterone between months ( $F = 0.144$ ,  $p = 0.933$ ) but there was a significant difference between groups ( $F = 6.956$ ,  $p = 0.001$ ). Testosterone concentrations did not differ between groups ( $F = 2.325$ ,  $p = 0.102$ ) but did differ between months ( $F = 4.325$ ,  $p = 0.006$ ).

Testosterone and estradiol for the three groups were pooled for further analyses. The difference in corticosterone due to groups, for both males and females, precluded the addition of food group data to field data.

Testosterone concentrations varied significantly between months ( $p = 0.034$ ).

Levels in June were much higher than other months, July and September were also high, while April, May and August were low (Fig. 3.2). Few males had concentrations of testosterone greater than 10 ng/ml, and most of these were in June (few in July and Sept). Levels of testosterone were significantly higher for males with sperm in the lumen of the seminiferous tubules than males without sperm ( $p < 0.001$ ), and for males with sperm in the epididymis than males without ( $p < 0.001$ ). There was no significant difference in sampling date between those with sperm in the seminiferous tubules and those without ( $p = 0.746$ ), but there was for sperm in the epididymis ( $p =$

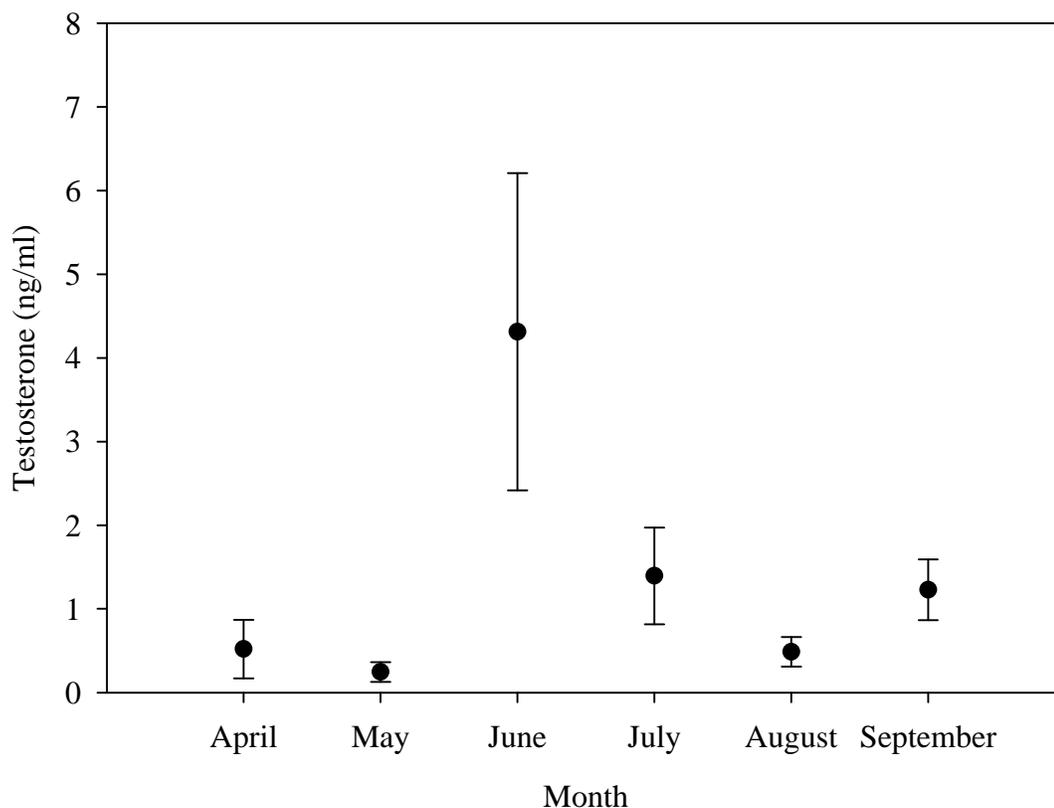


Figure 3.2. Mean plasma concentration of testosterone (and standard error) for male brown tree snakes at least 90 cm SVL sampled over a six-month period on Guam.

0.033). Testosterone was significantly correlated with SVL ( $r = 0.537$ ,  $p = 0.0004$ ), average diameter of the seminiferous tubules ( $r = 0.586$ ,  $p < 0.00001$ ), and average height of the tubule epithelium ( $r = 0.534$ ,  $p = 0.0005$ ). The average tubule diameter did not vary by month ( $p = 0.126$ ), nor did the average epithelial height ( $p = 0.082$ ). The average epithelial height and tubule diameter were significantly higher for males with sperm in the epididymis or in the seminiferous tubules ( $p < 0.001$  for both). The smallest male with sperm present in either the seminiferous tubules or the epididymis was 103 cm SVL.

Estradiol concentrations did not vary significantly between months ( $p = 0.680$ ), and many of the samples were below the limit of detection. The largest follicle measured for each female was significantly correlated to SVL ( $r = 0.395$ ,  $p = 0.003$ ), and to the concentration of estradiol ( $r = 0.743$ ,  $p < 0.0001$ ). The average size of the largest follicle did not vary with month ( $p = 0.259$ ). Follicles were categorized based on size (Bull et al., 1997), with follicles less than 3 mm indicating a nonreproductive snake, follicles 3 to 10 mm indicating early vitellogenesis, and those larger than 10 mm indicating late vitellogenesis. Follicles larger than 10 mm were found in May through September, but those larger than 20 mm were only found in late June through September (Fig. 3.3). The smallest snake with follicles greater than 3 mm was 89.5 cm SVL.

## **Discussion**

Tropical habitats are often regarded as aseasonal because there is little annual variation in temperature and photoperiod; however, strong changes in precipitation

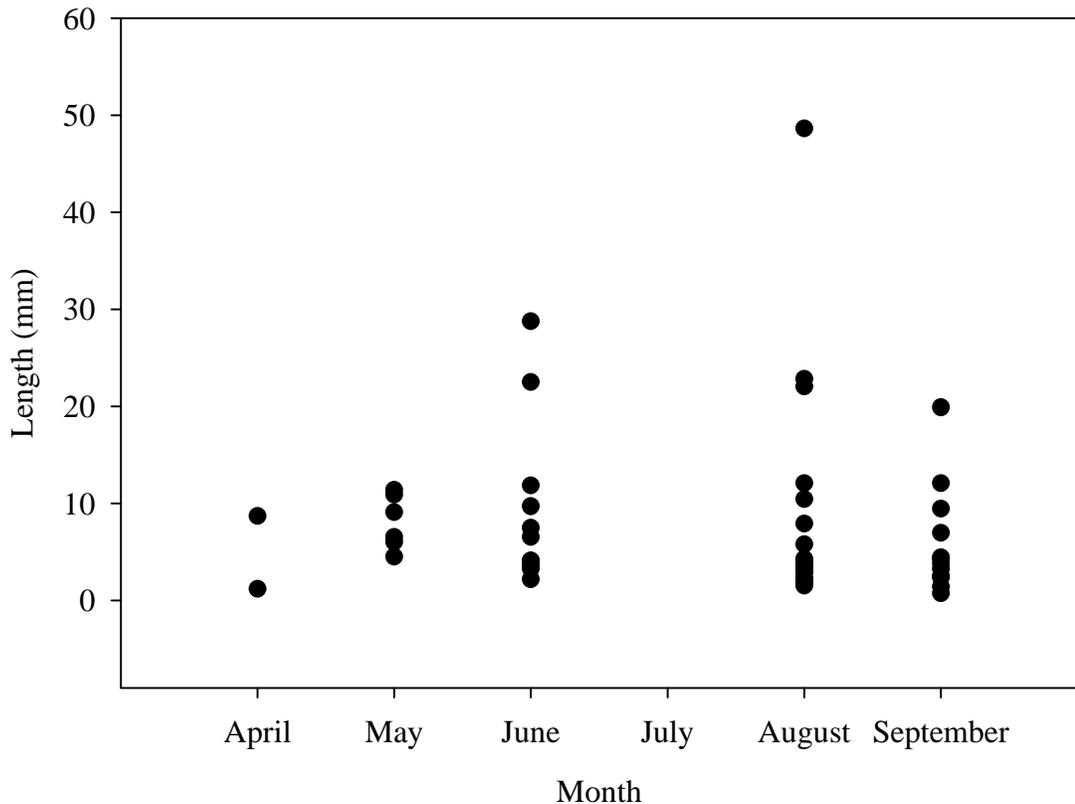


Figure 3.3. Length of largest follicle for female brown tree snakes at least 90 cm SVL sampled over a six-month period on Guam.

over the year can create different environmental conditions throughout the year. This variation in rainfall and resultant fluctuations in prey availability and hydric conditions can lead to seasonal changes in local abundance and behavior in many tropical species, including snakes (Brown et al., 2002; Brown & Shine, 2006). For example, brown tree snakes in southeast Queensland, Australia, are encountered more often in the warmest and wettest months and are caught least often in the coolest and driest months (Bull & Whittier, 1996) (Fig. 3.4). Brown tree snakes on Guam are active year-round, but the months of peak activity appear to be May, June and July, the months leading into the wet season (Fritts et al., 1987; Rodda et al., 1999a) (Fig. 3.5).

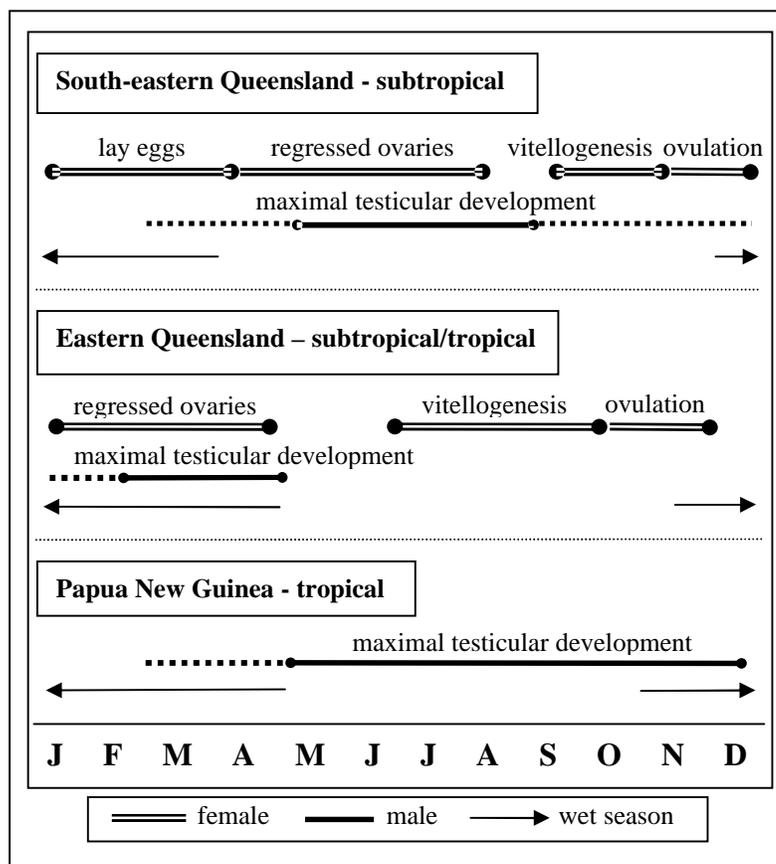


Figure 3.4. Seasonality of brown tree snake reproductive activities in subtropical and tropical areas (from Shine 1991, Whittier and Limpus 1996, and Bull *et al.* 1997).

*Boiga irregularis* in Australia have a dissociated pattern of reproduction; males have regressed testes when female ovarian development is at its peak, suggesting sperm storage in the male (Whittier & Limpus, 1996), as females do not have sperm storage structures in the oviduct (Bull *et al.*, 1997) (Fig. 3.4). Males in Queensland have mature sperm in the testes between May and August, when the snakes are inactive. Most males are in late recrudescence stage in December to April (the wet season), but males with sperm in the *ductus deferens* (and thus capable of inseminating females) are found throughout the year (Bull *et al.*, 1997), which is

consistent with most tropical snakes (Seigel & Ford, 1987). Males from tropical Papua New Guinea have maximal testicular development throughout the dry season and into the wet season and testicular regression between January and March. Sperm is also present in the *ductus deferens* throughout the year in this population (Bull et al., 1997).

Therefore, the production of sperm by brown tree snakes in subtropical climates has a strong seasonality; even in tropical Papua New Guinea, few males undergo spermiation in the final months of the wet season (Bull et al., 1997). On Guam, males undergoing spermiation were found in June through September, and given the high proportion of males with active testes in September (58% of the males examined in that month) it seems likely that spermatogenesis continued through at least October (Fig. 3.6) and possibly through December (Aldridge & Arackal, 2005).

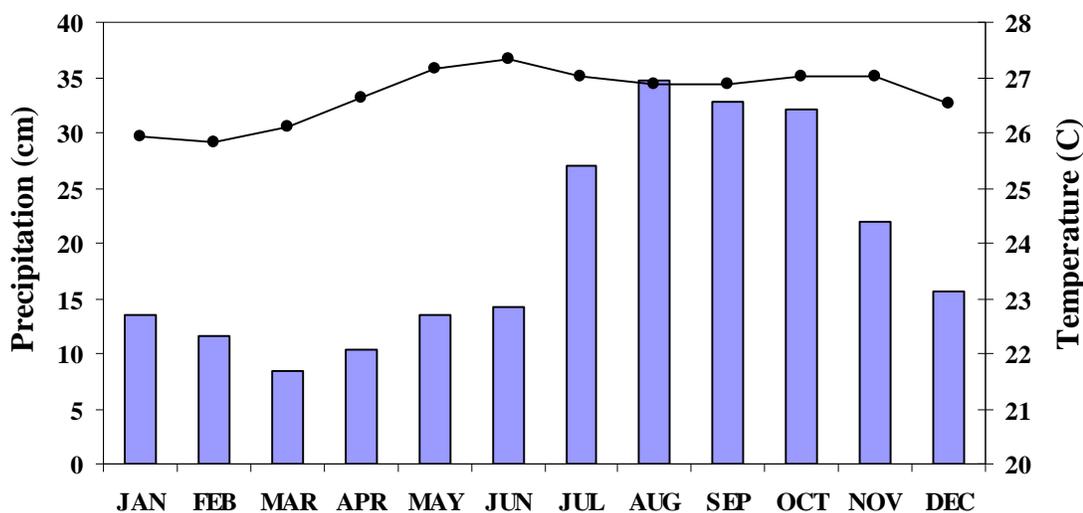


Figure 3.5. Average rainfall (bars) and temperature (line) recorded at Andersen AFB, Guam, for the years 1953 to 2002 (from the Western Regional Climate Center). The wet season, characterized by an increase in rain, generally runs from July through November.

Only two males had sperm in the epididymis in late June; most with stored sperm were collected in August and September. Previous studies (Moore et al., 2005; Savidge et al., 2007) found that males with mature sperm can be found all year on Guam, with the proportion of males that are undergoing active spermiogenesis varying seasonally.

Spermatogenesis seems to be similar to the pattern seen in Papua New Guinea, with peak testicular development falling between June and December, but with some males producing sperm in the other months as well.

Testosterone levels are expected to be highest when spermiogenesis occurs (Lance, 1984), but while testosterone was significantly elevated in males undergoing spermiogenesis, the peak in testosterone was in June and July. This was also the case for males sampled in 1991-1993 (Moore et al., 2005), and could indicate a period of courtship, mating and combat behavior. Male snakes in captivity at Oregon State University had peak levels of testosterone when courtship activity was greatest (Moore

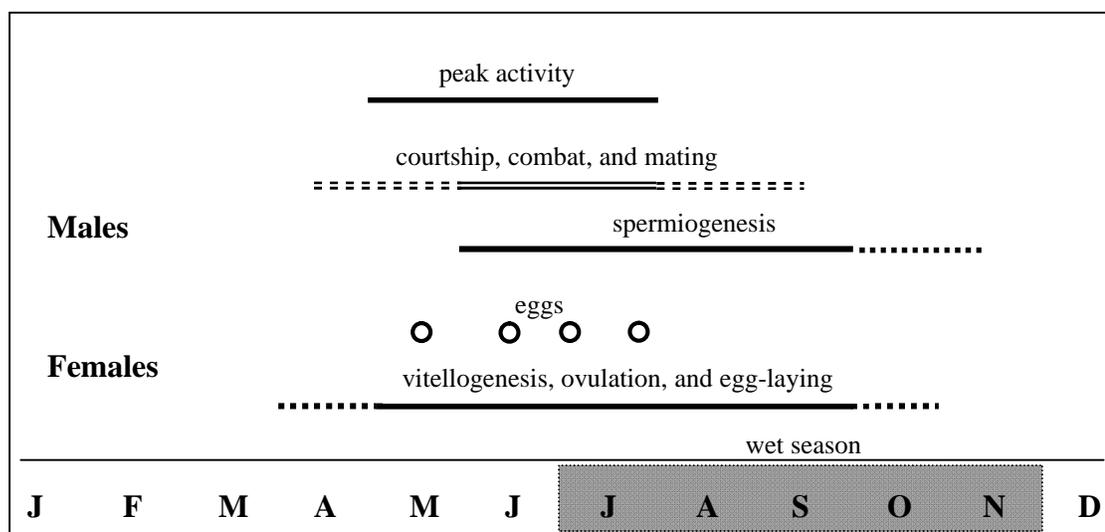


Figure 3.6. Seasonality of male and female brown tree snake reproductive activities on Guam. Dotted lines indicate hypothetical activity.

et al., 2005). The average testosterone levels in August and September in this study might have been as great as those in June if more free-living snakes had been sampled; the captive snakes did not have the opportunity to perform behaviors associated with reproduction and thus their testosterone levels were not maximally elevated (Yang & Wilczynski, 2002).

Female brown tree snakes in Queensland undergo vitellogenesis in the dry season and start to ovulate by the start of the wet season. They lay eggs throughout the wet season (Shine, 1991; Whittier & Limpus, 1996; Bull et al., 1997) although there are reports of females ovipositing before the wet season is fully underway (Brian James pers. comm). The seasonality of female reproduction in Papua New Guinea is unknown.

Females with ovarian follicles in the late vitellogenic stage (greater than 10 mm) were found on Guam in May through September. Follicles were largest in August, although two females laid eggs in captivity in mid-June and late July. There was an increase in corticosterone in captive females between June and September, corresponding with a slight increase in estradiol (Fig. 3.1). Estradiol levels were positively correlated to follicle size, as would be expected (estradiol increases with follicle size, and drops after ovulation)(Licht, 1984), and the increase in corticosterone also indicates that vitellogenesis was underway, as higher levels of plasma corticosterone may have been related to energy mobilization required for vitellogenesis (Grassman & Crews, 1990; Wilson & Wingfield, 1992).

Only two other clutches of eggs on Guam have been reported; these were laid by captive females in mid-May (Linnell et al., 1997) and early July (McCoid, 1994).

Although all of these records fall within a two month period, the enlarged follicles observed in August and September indicate that eggs are laid over a roughly six-month period (Fig. 3.6). Females with enlarged follicles or oviductal eggs have been reported to be found in almost every month, and it has been suggested that this is evidence that reproduction is aseasonal on Guam (McCoid, 1994; Rodda et al., 1999a; Savidge et al., 2007). Seigel and Ford (1987) and Zug et al. (1979) state that although aseasonal reproduction in tropical snakes is possible, a conservative approach to the existing data is recommended. While it remains speculative whether or not females can reproduce throughout the year, the proportion of females that are reproductive is highly likely to vary seasonally, as seen in another species of *Boiga* (Luiselli et al., 1998) and in other tropical snakes (Shine, 1986; Seigel & Ford, 1987; Solorzano & Cerdas, 1989; Janeiro-Cinquini et al., 1993; Aldridge et al., 1995; Madsen & Shine, 1996; Shine & Keogh, 1996; Luiselli et al., 2002; Brown & Shine, 2006). Thus, this population appears to have an attenuated reproductive season with the majority of the clutches oviposited during the wet season.

Female brown tree snakes have an associated pattern of reproduction, in that mating occurs when their gonadal steroids are at their peak (Whittier & Tokarz, 1992). Based on observations of captive snakes, it appears that brown tree snakes mate approximately 30 to 40 days before eggs are deposited (pers. obs., Barnett, 1993; Mathies et al., 2004), and that females ovulate approximately 30 days before egg laying (Mathies et al., 2004). We observed mating in captivity; females oviposited only after mating and late vitellogenic females that did not mate later resorbed their follicles (pers. obs.). In Queensland, a large increase in male activity about a month

before the start of the wet season (Bull & Whittier, 1996) could be a sign of mating activity (Whittier & Limpus, 1996), although mating has not been directly observed in the wild for this species (Bull et al., 1997). If mating on Guam follows the same seasonal cues as in Queensland, it should occur just before the start of the rainy season, or in May and June. This coincides with the period of peak snake activity (Fritts et al., 1987) and peak testosterone production (Fig. 3.6). However, the eggs laid in May and June indicate that some mating activity could occur as early as April, and enlarged follicles in August and September mean that mating could occur as late as September, so mating may happen over a six month period with a peak in activity early in the wet season.

The timing of reproductive activities of oviparous reptiles in tropical environments is thought to depend either on biotic factors (e.g., avoidance of predation or maximal prey availability) or abiotic factors (e.g., optimal conditions for egg development) (Brown & Shine, 2006). For at least some tropical snake species, seasonal timing of female reproductive activities is influenced by environmental conditions that provide the best conditions for incubation. Males may have a flexible and almost aseasonal cycle, in which they store sperm when not actually mating, to adapt to the timing of the female cycle (Aldridge & Duvall, 2002). Species that lay their eggs underground have peak egg-laying at the end of the wet season, when the soil is moist enough to provide optimal hydric conditions for developing embryos without danger of flooding the nest (Brown & Shine, 2006). Others nest in tree hollows during the wet season (Shine, 1991; Brown et al., 2005) where flooding is less likely but high humidity is necessary to provide moisture for the eggs. Brown tree

snakes appear to lay their eggs in holes in trees or cliffs (Rodda et al., 1999a), and therefore would require the high levels of humidity characteristic of the wet season to provide sufficient hydric conditions for their eggs.

The main obstacle to describing the reproductive ecology of brown tree snakes has been the difficulty in finding reproductive females. They are likely to be inactive when gravid (Rodda et al., 1999a), but females with yolking follicles should still be active and relatively easy to sample. Very few snakes of reproductive size are reproductively active on Guam. For example, Mathies et al. (2001) found only 2 out of 33 females were reproductively active, and another survey conducted between 1991 and 1993 found only 10 out of 220 female brown tree snakes of adult size contained vitellogenic follicles (unpub. data). We found in this study that the percentage of adult females that were reproductively active was similar to that reported for the native range; 22% of the females of adult size sampled on Guam during this study had follicles over 10mm, while 20% of the females of mature body size in the Queensland study had enlarged follicles or oviductal eggs (Whittier & Limpus, 1996). Some species of temperate snakes reproduce biennially instead of every year (Seigel & Ford, 1987), and the frequency of reproduction is probably dependent on resource availability (Seigel & Fitch, 1985). Brown tree snakes could have similar constraints on feeding and foraging, which would limit the number of females reproducing in a given year (Whittier & Limpus, 1996). Populations of brown tree snakes in Australia are most likely limited by prey availability (Whittier & Limpus, 1996; Rodda et al., 1999b) and it appears that while brown tree snakes on Guam are also limited by food, it could be to no greater degree than seen in their native range.

Supplemental feeding did not make as much of a difference in the reproductive physiology of the experimental snakes as we expected. Free-living snakes on Guam in 2003 did not appear to be as food-stressed as those sampled in 1991-1993, although regular feeding seemed to enhance the reproductive state of some of the individuals involved. Testosterone and estradiol did tend to be higher in captive snakes than free-living snakes in August and September, but this difference was not statistically significant. A longer period of supplemental feeding might have provided significant results, and attempts to thoroughly describe brown tree snake reproduction on Guam will be more likely to succeed if a supplemental feeding component is included.

In conclusion, although this study cannot conclusively rule out mating and egg-laying throughout the entire year, it is highly likely that a large component of reproduction is strongly seasonal and influenced by the wet season. Male brown tree snakes on Guam differ from those in Queensland in the seasonality of testicular activity, and appear to be more comparable to males in Papua New Guinea in that spermiogenesis is not limited to the dry season. However, the female cycle is more informative in terms of describing the reproductive biology of this species on Guam, as the male cycle is probably flexible and determined by the reproductive state of females. Future studies should focus sampling efforts on female snakes to determine the seasonality of reproduction on Guam and the environmental cues that control it.

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CHAPTER 4

MAGNITUDE AND VARIABILITY OF THE HORMONAL RESPONSE TO CAPTURE STRESS IN  
BROWN TREE SNAKES (*BOIGA IRREGULARIS*) ON GUAM.

Heather L. Waye and Robert T. Mason

**Abstract**

Plasma concentrations of glucocorticoids are often used as an indicator of stress and physiological condition of individuals in a population. A previous study showed high baseline levels of corticosterone and low concentrations of sex steroid hormones in brown tree snakes on Guam. With the aim of determining baseline levels and the magnitude of the acute stress response we recorded plasma concentrations of corticosterone and sex steroid hormones of brown tree snakes on Guam in 2003. We found seasonality in the corticosterone stress response in males but not in females. The corticosterone stress response did not vary with sex, size, or body condition of the snakes, but the response of gonadal sex hormones to acute stress was greater in larger snakes. Testosterone and estradiol concentrations changed significantly in response to acute stress. Therefore, only free-living snakes should be used for examination of sex steroid concentrations. Baseline corticosterone levels recorded by the earlier study were much higher than the acute stress levels in this study. It appears that conditions on Guam have changed dramatically over time and measurements of corticosterone are one useful tool for tracking these changes.

**Introduction**

The vertebrate stress response, characterized by the release of glucocorticoids from the adrenal cortex, produces physiological and behavioral changes that shift resources to those processes necessary for survival of the current challenge (reviews by Greenberg & Wingfield, 1987; Guillette et al., 1995; Buchanan, 2000; Sapolsky et al., 2000). The acute stress response, produced by short-term stress, shuts down

digestion and behaviors related to feeding and mating, while increasing mobilization of energy stores through gluconeogenesis. However, chronic, long-term stress can lead to cessation of reproduction, suppression of the immune system, muscle protein loss, suppression of growth, and neural damage.

Measurements of plasma concentrations of glucocorticoids are often used as an indicator of stress and condition of individuals in a population, as they can be correlated with body condition (Romero & Wikelski, 2001), and are used in wildlife management as a physiological index to measure response to environmental extremes and human disturbance (Millsbaugh & Washburn, 2004; Walker et al., 2005). Such measurement could also be used to monitor the resilience of an introduced species. This data could be used to determine the species' suitability to, and potential impact on, a new environment and possibly identify stressors that could be exploited towards its management. For example, increased stress is known to suppress the production of sex steroid hormones and thus reproduction (Moore & Jessop, 2003b), so high levels of glucocorticoids could indicate that individuals that would otherwise be reproductively competent are not reproductively active.

*Boiga irregularis*, the brown tree snake, was accidentally introduced to the island of Guam near the end of World War II (Savidge, 1987; Rodda et al., 1992). It quickly spread throughout the island, eradicating most of the native bird and lizard species (Rodda et al., 1992; Wiles et al., 2003). Plasma concentrations of sex steroid hormones and the glucocorticoid corticosterone were measured for snakes in this population in 1992 and 1993 (Moore et al., 2005) as part of a study investigating the previously reported low proportion of reproductively active snakes (Rodda et al.,

1999; Mathies et al., 2001; Moore et al., 2005). Snakes in that study had routinely high baseline levels of corticosterone, indicating chronic stress, and very few had concentrations of gonadal sex steroids above a basal level (Moore et al., 2005). This lack of reproductively active adults precluded description of the reproductive cycle of the population, which would have contributed significantly to management efforts that aim to control this species on Guam.

Baseline levels of corticosterone in brown tree snakes on Guam appeared to be very high in 1992 and 1993, up to 110 ng/ml (Moore et al., 2005), but were they higher than might be expected in a free-living population? Lizards such as *Podarcis sicula* (Manzo et al., 1994) and *Amphibolurus ornatus* (Baverstock & Bradshaw, 1975) had up to 150 ng/ml corticosterone upon capture, depending on the season (reviewed by Tyrrell & Cree, 1998). We measured the plasma concentrations of corticosterone and sex steroid hormones of brown tree snakes on Guam in 2003 in order to determine baseline levels and the magnitude of the acute stress response. Before we can describe the reproductive cycle and use corticosterone as a measure of the physiological condition of this population, we must establish what level of corticosterone would indicate that this population is under stress and how increased concentrations of corticosterone affect levels of sex hormones in this species.

## **Methods**

Ninety-five free-living snakes on Guam were caught by hand at night from May through September 2003. Blood samples (300  $\mu$ l) were drawn within 3 minutes of capture from the caudal vein using heparinized 1-cm<sup>3</sup> syringes and 25-g needles.

The snakes were then placed singly into heavy canvas bags (31 x 67 cm) until the second sample. Male and female snakes were assigned to one of four groups, with the intention of obtaining equal numbers of males and females in each group over the sampling period. The bags were folded to restrict movement of the snakes inside, and were kept at ambient temperature until the second sample. The second blood sample was taken either 1, 4, 8, or 24 h after initial capture. Blood samples were placed in a cooler with an ice pack until return to the field laboratory, then centrifuged and the plasma removed. Plasma was stored at -20°C until the samples could be shipped to Oregon State University and once there, stored at -70°C until assayed. Mass (g) and snout-vent length (SVL) (cm) of each snake were recorded.

### ***Radioimmunoassay***

Plasma levels of testosterone (T) and corticosterone (B) were determined for male snakes and 17 $\beta$ -estradiol (E<sub>2</sub>) and B for females by radioimmunoassay following the procedure described by Moore et al. (2000). Briefly, plasma volumes of 4 to 200  $\mu$ l were equilibrated overnight with the tritiated hormones of interest (Amersham). Each sample was extracted twice in 2 ml diethyl ether, and dried in a warm water bath under a stream of nitrogen gas. The extracts were resuspended in 10% ethyl acetate in isooctane, and were chromatographed through individual celite columns to separate the steroid fractions and neutral lipids. The purified eluates were dried and resuspended in buffer. For the assay, all samples, including serial dilutions and 100% bounds, were incubated overnight with 100  $\mu$ l of antibody (testosterone antibody T-3003 or estradiol antibody E-6006 from Wein, or corticosterone antibody B3-163 from Esoterix Endocrinology) and 100  $\mu$ l of tritiated steroid. Unbound steroid was

separated using dextran-coated charcoal and the bound steroid decanted into scintillation vials, resuspended in 3.5 ml scintillation fluid, incubated for 12 h, and counted on a Beckman LS1800 scintillation counter. Intraassay variation was 14% for estradiol, 15% for testosterone, and 15% for corticosterone. Interassay variation was 16% for estradiol, 17% for testosterone, and 18% for corticosterone. Limits of detection were ~ 0.01 ng/ml for T, ~ 0.04 ng/ml for B, and ~ 0.003 ng/ml for E<sub>2</sub>.

### *Statistical Analyses*

Two-way analysis of variance (ANOVA) was used to examine the effect of treatment group and sex on difference in log-transformed pre-stress B or square root-transformed B difference (B concentrations were transformed to normalize data). One-way ANOVA (F statistic) or the nonparametric Kruskal-Wallis ANOVA on ranks (H statistic) were used to compare pre- and post-treatment hormone levels between treatment groups. A body condition index (BCI) was calculated as the residual of each individual on the linear regression of log SVL on log mass. The Mann-Whitney rank sum test was used to compare concentrations of B between positive BCI and negative BCI groups. Paired t-tests or the nonparametric Wilcoxon Signed Rank tests were used to compare pooled samples of hormones before and after capture stress. Spearman Rank Order correlations were used to examine relationships between hormone levels, snake SVL, and body condition. Average hormone levels in each month were compared using the Kruskal-Wallis one-way ANOVA on ranks. All statistical analyses were performed using Jandel SigmaStat version 3.1 software package (Jandel Corporation). Analyses were considered statistically significant when  $p < 0.05$ .

## Results

A total of 47 males (61.5 – 142 cm SVL) and 48 females (58.0 – 119 cm SVL) were sampled immediately after capture and again after either 1, 4, 8, or 24 h.

Fourteen males and 11 females were in the 1 h group, 13 males and 14 females in the 4 h group, 13 males and 9 females in the 8 h group, and 7 males and 14 females in the 24 h group.

Pre-stress concentrations of B did not differ significantly between males and females ( $F = 1.380$ ,  $p = 0.243$ ) or between treatment groups ( $F = 1.587$ ,  $p = 0.198$ ).

The change in B after stress also did not differ significantly between males and females ( $F = 2.320$ ,  $p = 0.132$ ) or between treatment groups ( $F = 0.952$ ,  $p = 0.419$ ).

Concentrations of B increased significantly by 1 hr after the snakes were placed in bags, and remained high through 24 hr of bagging (Fig. 4.1).

Body condition residuals (BCI) for males and females were each divided into two groups, negative BCI and positive BCI. There was a significant difference in B pre-stress between negative and positive BCI ( $p = 0.002$ ), but not post-stress ( $p = 0.944$ ). The change in B was also not significantly different between the two groups ( $p = 0.621$ ) (Fig. 4.2).

### *Males*

Initial levels of B were not significantly different between the four sampling groups ( $F = 0.717$ ,  $p = 0.548$ ) so they were combined (pre-stress samples). The levels of B at 1, 4, 8, and 24 hours after the initial sample were also not significantly different from each other ( $F = 1.035$ ,  $p = 0.387$ ), and were also pooled (post-stress samples). The concentration of B in the pre-stress samples was significantly lower

than that in the post-stress samples ( $p < 0.001$ ) (Fig. 4.1). Corticosterone increased approximately 10-fold in the first hour of confinement stress, then declined after 4 hours of stress.

Pre-stress concentrations of T were not significantly different between groups ( $H = 4.729$ ,  $p = 0.193$ ) and neither were the post-stress samples ( $H = 3.246$ ,  $p = 0.335$ ). There was no significant difference between the pre-stress and post-stress concentrations of T ( $p = 0.264$ ) (Fig. 4.1). Many of the samples, both pre-stress and post-stress, were at the limit of detection of the assay (0.01 ng/ml).

*Size* - Corticosterone concentrations were not significantly correlated with

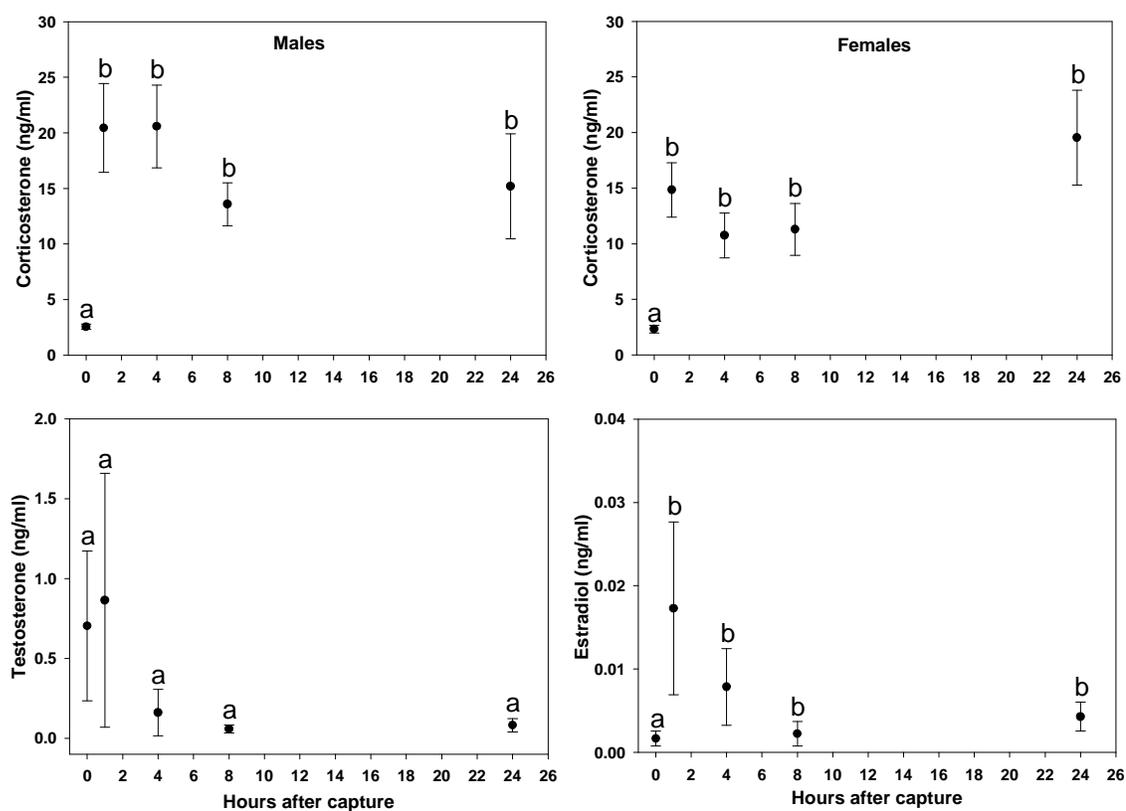


Figure 4.1. Mean plasma concentrations (and standard errors) of corticosterone, testosterone, and  $17\beta$ -estradiol in male and female brown tree snakes on Guam before and after acute confinement stress.

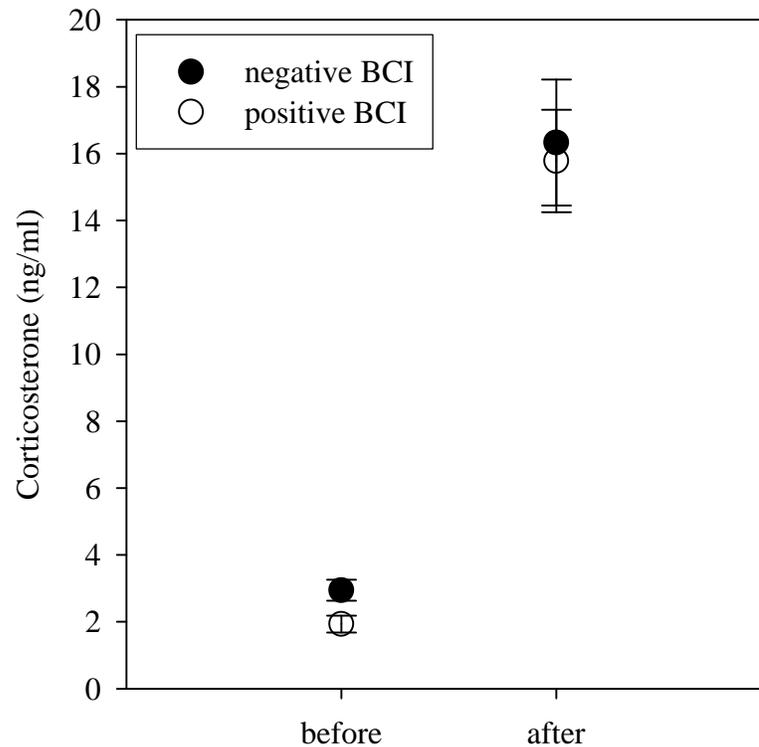


Figure 4.2. Mean plasma corticosterone (and standard errors) for male and female brown tree snakes grouped by negative body condition and positive body condition, before and after acute stress.

body length (pre-stress B,  $p = 0.205$ ; post-stress B,  $p = 0.272$ ; B difference,  $p = 0.266$ ).

Testosterone concentrations were higher in longer snakes both before ( $r = 0.496$ ,  $p < 0.001$ ) and after ( $r = 0.595$ ,  $p < 0.001$ ) stress, and the decrease in T was greater in longer snakes ( $r = -0.597$ ,  $p < 0.01$ ).

*Date* – There was no significant difference in T difference between months ( $p = 0.208$ ) (Fig. 4.3), and although B difference did vary between months, these differences were not significant ( $p = 0.070$ ) (Fig. 4.4).

*Condition* – Body condition declined throughout the sampling period ( $r = -0.513$ ,  $p < 0.001$ ), but there was no significant correlation between the length of captured males and body condition ( $p = 0.361$ ). Body condition was also positively

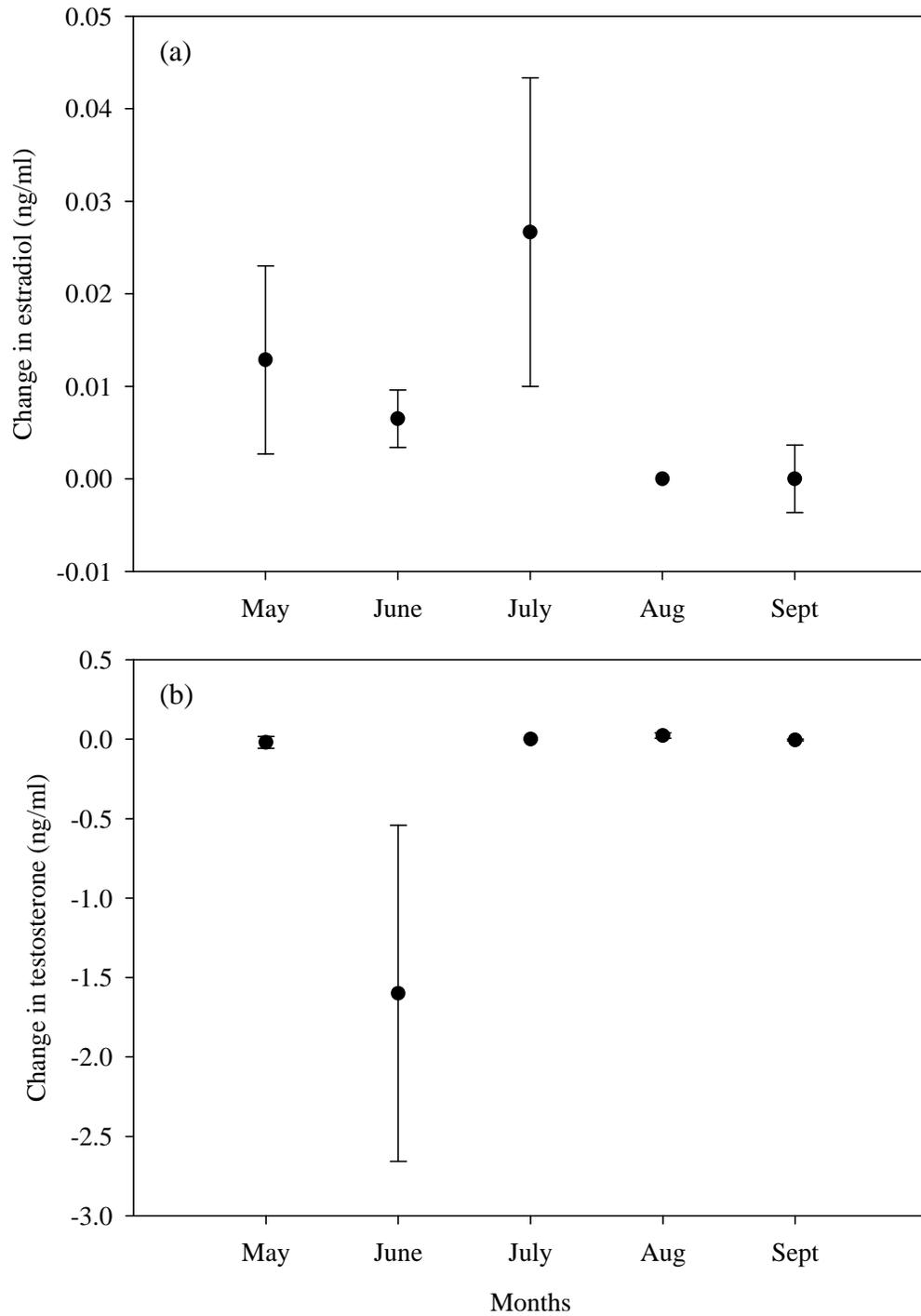


Figure 4.3. Mean change and standard error in (a)  $17\beta$ -estradiol in females and (b) testosterone in males, following acute stress, for five months of sampling in 2003.

correlated with initial concentrations of T ( $r = 0.372$ ,  $p = 0.01$ ) but not with concentrations of T post-stress ( $p = 0.361$ ). Snakes with better body condition also

had a larger decrease in T post-stress ( $r = -0.488$ ,  $p < 0.001$ ). There was no significant correlation between body condition and concentrations of B (pre-stress B,  $p = 0.555$ ; post-stress B,  $p = 0.894$ ; B difference,  $p = 0.928$ ).

### *Females*

Pre-stress concentrations of B were not significantly different between the four groups ( $H = 3.292$ ,  $p = 0.349$ ) and were pooled, as were the four post-stress samples ( $F = 1.866$ ,  $p = 0.149$ ). The concentration of B in the pre-stress sample was significantly lower than in the post-stress sample ( $t = -7.419$ ,  $p < 0.001$ ) (Fig. 4.2). There was a seven-fold increase in mean B in the first hour of confinement stress, a

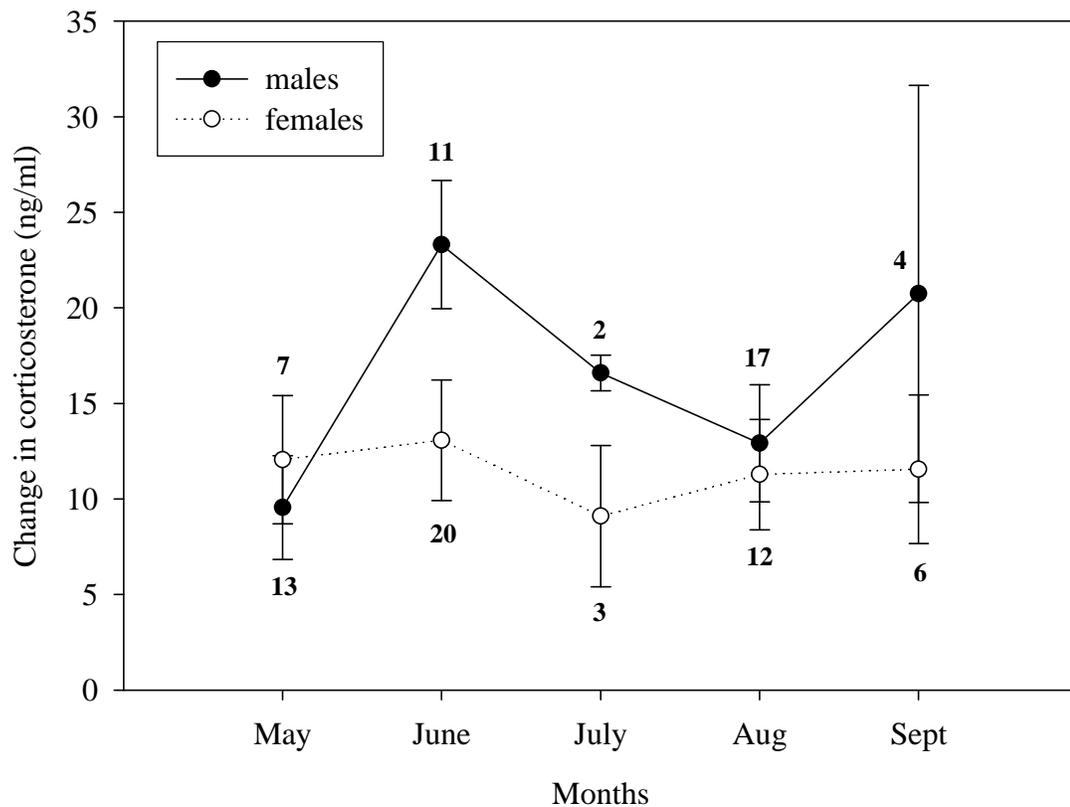


Figure 4.4. Mean change (and standard error) in plasma corticosterone for male and female brown tree snakes following acute stress. The sample size for each month is shown above and below the data points.

slight drop by 4 hours, and a 10-fold increase over the initial B concentration by 24 hours of confinement.

Pre-stress concentrations of E<sub>2</sub> were not significantly different between groups (H = 1.146, p = 0.766) and were pooled. The four post-stress samples were also pooled (H = 0.881, p = 0.830). There was a significant post-stress increase in E<sub>2</sub> (p = 0.003) (Fig. 4.2). Many of the samples were at the limit of detection of the assay (0.003 ng/ml).

*Size* – Concentrations of B were not significantly correlated with length (pre-stress B, p = 0.653; post-stress B, p = 0.510; B difference, p = 0.718). Although pre-stress concentrations of E<sub>2</sub> were not correlated with length (p = 0.592), E<sub>2</sub> increased with length post-stress (r = 0.339, p = 0.019), and the change in E<sub>2</sub> also significantly increased with length (r = 0.355, p = 0.013).

*Date* – There was a significant difference in the change in levels of E<sub>2</sub> between months (p = 0.036) (Fig. 4.3), but not in B difference (p = 0.992) (Fig. 4.4).

*Condition* - Body condition declined over the sampling period (r = -0.360, p = 0.012), and showed a positive correlation with pre-stress E<sub>2</sub> (r = 0.329, p = 0.022) and a negative correlation with pre-stress B (r = -0.439, p = 0.002). There was no correlation between body condition and change in E<sub>2</sub> (p = 0.999) or in B (p = 0.305), or between body condition and post-stress levels of E<sub>2</sub> (p = 0.711) or B (p = 0.682).

## **Discussion**

A review of seasonal changes in glucocorticoids in reptiles found that 70% of the species surveyed showed seasonal changes in baseline levels of corticosterone

(Romero, 2002). Those species that did show seasonality usually had peak levels of corticosterone during the breeding period. Most tropical reptiles have seasonal reproduction, but the actual timing of the breeding period can vary considerably between species even in the same location (Brown & Shine, 2006). The results presented here showed no seasonality in baseline levels of corticosterone in male and female brown tree snakes on Guam. This lack of seasonality differs from a previous study on Guam (Moore et al., 2005), which found a dramatic decrease in corticosterone between October and February, from the end of the wet season and through the dry season. The difference in seasonality between this study and the previous one is likely due to differences in body condition. Snakes in the earlier study had poor body condition compared to those in captivity and in the native range in Australia (Moore et al., 2005), and compared to snakes sampled in 2003 (Waye and Mason in prep). Seasonal changes in food availability or humidity may have presented a more stressful challenge in that year than in 2003.

Baseline levels of corticosterone were negatively correlated with body condition in females, but not in males. Corticosterone levels were not significantly different between the sexes, so females did not show a greater stress response than males, but perhaps female plasma levels of corticosterone are more dependent on physiological condition. For example, good body condition is necessary for reproduction in female asp vipers (*Vipera aspis*), as activities associated with female reproduction are energetically expensive. Condition is less important for males, and males in poor condition may nevertheless show active courtship (Aubret et al., 2002). Similarly, a positive correlation between corticosterone and hematocrit (as an indicator

of dehydration) was found in female bearded dragons (*Pogona barbata*) but not in males (Amey & Whittier, 2000). Body condition of males and females declined from May to September, but there was no corresponding increase in baseline corticosterone, indicating that the snakes were in good enough physical condition that this decline in condition was not stressful.

The acute stress response in reptiles can vary depending on the season, or the size, age, condition, or sex of an individual (reviewed by Moore & Jessop, 2003b). Sex-based changes in glucocorticoids differ dramatically between species. In some species females show a greater response to stress (Zerani et al., 1991), in other species males have a greater response (Jessop, 2001), but in other species there is no difference between the sexes (Lance & Elsey, 1986; Tyrrell & Cree, 1998; Romero & Wikelski, 2001). Individuals with poor body condition tend to have a larger increase in glucocorticoids in response to stress, but this is not the case for all reptile species (Moore & Jessop, 2003b).

In this study, plasma concentrations of corticosterone did increase significantly with capture stress in both male and female brown tree snakes. Males and females started at a baseline average around 2 ng/ml and increased to approximately 11 to 20 ng/ml after 4 hours of captivity, which is within the range reported for other reptiles (reviewed by Tyrrell & Cree, 1998). This increase did not vary significantly with length of snake, sex, or body condition, and for females there was no difference between months. Males did show a greater increase in corticosterone in June and September than in the rest of the sampling period. The greater increase in corticosterone in June, when taken with the higher levels of testosterone at that time,

suggests a breeding season near the start of Guam's wet season (which is July through December). The second peak in stress corticosterone in September is more likely a product of the great variability in the stress response shown by these snakes. The change in corticosterone over all of the snakes sampled ranged from a decrease of 7 ng/ml to an increase of 62 ng/ml, with no correlation to body condition, sex, duration of stress, or size of snake. The four males sampled in September varied widely in their response to acute stress and so the seemingly large corticosterone peak is likely no more than a sampling artifact.

While we found that free-ranging snakes had a baseline average of 2.5 ng/ml corticosterone and an average of 20 ng/ml corticosterone after acute stress, a study in July 2000 (Mathies et al., 2001) recorded concentrations of 5 to 10 ng/ml corticosterone in free-ranging snakes and up to an average of 60 ng/ml after acute stress. Although there were differences in sampling techniques and radioimmunoassay procedures between these two studies, it appears that background levels of corticosterone and the magnitude of the stress response were similar. Baseline corticosterone levels in 1992 and 1993 were highest in August, at an average of 110 ng/ml (Moore et al., 2005), at least twice as high as the acute stress levels in the later studies. It appears that brown tree snakes are capable of plasma concentrations of corticosterone much higher than those produced by the acute stress protocols used by us and by Mathies et al (2001). The extremely high levels in the 92/93 study are probably related to poor body condition (Moore et al., 2005). The snakes sampled in 2003 were in relatively good condition which may have dampened their stress response (Moore & Jessop, 2003b).

The increase in plasma glucocorticoid hormones in response to stress is typically associated with a decrease in gonadal sex steroid hormones (Greenberg & Wingfield, 1987; Guillette et al., 1995). Testosterone in reptiles usually drops in response to acute stress (e.g., Moore et al., 2000; Moore et al., 2001; Jones & Bell, 2004; Lance et al., 2004), but not in every species (e.g., Knapp & Moore, 1997; Cree et al., 2000). Male brown tree snakes followed the general pattern in that testosterone decreased with acute stress when baseline testosterone concentrations were above basal levels. There was a greater decline in testosterone after acute stress in large males that had high body condition residuals, but these were the individuals that had high baseline levels of testosterone.

The effect of acute stress on estradiol has not been as well studied as the effects on testosterone, and the few results that have been observed vary considerably. Female reptiles and birds generally show a decrease in estrogen after acute stress, but the acute stress response is often suppressed during the breeding season (Wingfield et al., 1998; Moore & Jessop, 2003a). Female alligators (*Alligator mississippiensis*) showed a decrease in estradiol with capture and confinement during the breeding season (Elsey et al., 1991), while vitellogenic or gravid tree lizards (Woodley & Moore, 2002) and vitellogenic tuatara (Cree et al., 1990) showed no change in estradiol after handling stress. We found that plasma estradiol levels increased significantly in female brown tree snakes after acute stress. Baseline concentrations of estradiol were slightly higher in May through July, and the increase in estradiol after stress was significantly higher during this time period. High levels of estradiol in the months leading into the wet season could indicate that the females are undergoing

vitellogenesis (Bona-Gallo et al., 1980; Bonnet et al., 1994), but even the highest concentration of estradiol measured in this study was no greater than the average basal levels of estradiol found in brown tree snake by Moore et al. (2005). Therefore, it seems likely that female brown tree snakes had baseline levels of estradiol that were only coincidentally higher after acute stress due to very low concentrations of estradiol compounded with measurement error. Further acute stress experiments with vitellogenic females could clarify the relationship between stress and estradiol in this species.

Mathies et al. (2001) concluded that blood samples taken from brown tree snakes collected from traps could not be used to examine seasonal hormonal cycles, as corticosterone levels were elevated and it was unclear how this would affect sex steroid levels. Few adults in their study had elevated testosterone or progesterone concentrations, but those samples were taken after a period of confinement in a trap. It appears likely that individuals with high levels of sex hormones before entering the traps would have much lower levels by the time they were sampled after at least one night of confinement, and Mathies et al. (2001) did not collect any corresponding “before stress” blood samples. Although using trapped snakes for studies on reproduction would certainly increase the ease of collection and sampling, and greatly enhance the number of snakes that could be examined, their hormone concentrations would not be representative of the free-ranging population and not very informative.

Low baseline levels of corticosterone, especially compared to an earlier study, and the lack of correlation between the stress response and body condition, indicates that brown tree snakes on Guam are not under chronic stress (at least not in 2003 when

the samples were taken). As this is an introduced species, it is important to monitor the physiological condition of this population to determine whether it is thriving or declining. It appears that samples from either male or female free-living snakes of any size could be used to monitor baseline levels of stress in this population. Body condition of the snakes will likely affect baseline corticosterone levels and the acute stress response, and therefore should be measured in conjunction with hormone sampling. Seasonality of sampling could have an effect on corticosterone measurements, but this could be addressed by sampling throughout the year or by comparing samples from the same time of year. Ongoing sampling of baseline and acute levels of corticosterone and sex hormones would provide information necessary for more accurate monitoring of this population and would offer clues to reproductive seasonality that will assist future research on reproductive cycles on brown tree snakes and on tropical snakes in general.

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## CHAPTER 5

COMBINATIONS OF BODY CONDITION MEASUREMENTS ARE MORE INFORMATIVE THAN  
CONVENTIONAL CONDITION INDICES: TEMPORAL VARIATION IN BODY CONDITION AND  
CORTICOSTERONE IN BROWN TREE SNAKES (*BOIGA IRREGULARIS*).

Heather L. Wayne and Robert T. Mason

**Abstract**

The body condition index is a common method for quantifying the energy reserves of individual animals. Because good body condition is necessary for reproduction in many species, body condition indices can indicate the potential reproductive output of a population. Body condition is related to glucocorticoid production, in that low body condition is correlated with high concentrations of corticosterone in reptiles. We compared the body condition index and plasma corticosterone levels of brown tree snakes on Guam in 2003 to those collected in 1992/93 to determine whether that population still showed the chronic stress and poor condition apparent in the earlier study. We also examined the relationship between fat mass, body condition, and plasma corticosterone concentrations as indicators of physiological condition of individuals in the population. Body condition was significantly higher in 2003 than in the earlier sample for mature male and female snakes, but not for juveniles. The significantly lower levels of corticosterone in all three groups in 2003 suggests that although juveniles did not have significantly improved energy stores they, along with the mature males and females, were no longer under chronic levels of stress. Although the wet season of 2002 was unusually rainy, low baseline levels of corticosterone measured in 2000 indicate that the improved body condition of snakes in 2003 is likely the result of long-term changes in prey populations rather than annual variation in response to environmental conditions.

**Introduction**

The study and management of animal populations requires information about

the factors affecting population dynamics. Information on the relative condition of individuals in a population over time is necessary to predict potential reproductive output or an impending increase or decline in numbers. Body condition, an important measure of the fitness of an animal, usually refers to the relative amount of energy stores compared to some measure of body size (Green, 2001). Energy stores can be allocated to maintenance, growth or to reproduction (Perrin & Sibly, 1993; Heino & Kaitala, 1999; Madsen & Shine, 2002), so a population with many animals in poor condition could indicate that individuals of reproductive size are not reproductively active and the population might be declining.

Male snakes generally invest little energy in their offspring, but the activities necessary to obtain matings (e.g., searching for mates, male-male combat) can be energetically expensive (e.g., Devine, 1984; Duvall et al., 1993; Bonnet & Naulleau, 1996; Shine & Mason, 2005). Although males in poor condition may be able to mate, they may be less able to search or compete for females (Aubret et al., 2002). Females of some species need an initial investment of energy for reproduction and may require several years to accumulate the necessary stores before they can reproduce (Aubret et al., 2002) while other species forage while they are reproducing (Naulleau & Bonnet, 1995). In all species, an adequate supply of food is crucial to provide the energy needed for reproduction.

The fat reserves, or condition, of an individual snake is usually estimated using the residuals from a regression of body mass on body length (e.g., Bonnet & Naulleau, 1996; Shine & Madsen, 1997; Moore et al., 2001; Aubret et al., 2002; Brown et al., 2002). However, because condition may vary heteroskedastically with body size,

direct measurements of body fat are considered to be a more accurate measure of condition (Weatherhead & Brown, 1996). Even so, several studies have shown that only half of the variation in condition scores is due to variation in fat mass (Weatherhead & Brown, 1996; Madsen & Shine, 2002); much of the remaining variance may be due to energy storage in liver or muscle (Madsen & Shine, 2002).

Plasma concentrations of the steroid hormone corticosterone, the primary glucocorticoid in snakes, are also related to body condition. The elevated levels of corticosterone that are observed during times of stress produce physiological and behavioral changes, such as suppression of digestion and reproduction, and mobilization of fat stores for energy (reviews by Greenberg & Wingfield, 1987; Guillette et al., 1995; Buchanan, 2000). High baseline levels of plasma glucocorticoids are often used as an indicator of chronic stress in a population and have been correlated with body condition (Romero & Wikelski, 2001); individuals with low body condition tend to have high levels of plasma corticosterone (Moore et al., 2000).

The brown tree snake (*Boiga irregularis*), a large arboreal member of the family Colubridae, is native to northern Australia and Papua New Guinea (Cogger, 1994). This species was accidentally brought to the island of Guam near the end of World War II and quickly spread throughout the island (Savidge, 1987). Ten species of forest birds and six species of lizards have been extirpated from the island mainly due to predation by this invasive snake (Rodda et al., 1999b). A survey of this population in 1992 and 1993 found that these snakes had highly elevated baseline concentrations of corticosterone and low body condition compared to captive snakes

and brown tree snakes from the native range in eastern Australia (Moore et al., 2005). The conclusion of that study was that the snakes, having drastically reduced the larger-bodied bird and mammal prey on Guam, were food-stressed and possibly near starvation. In addition, very few snakes of reproductive size were reproductively active, another indication that this population was under chronic stress. We compared the body condition index and plasma corticosterone levels of brown tree snakes on Guam in 2003 to those collected in 1992/93 to determine the relationship between fat mass, body condition, and plasma corticosterone concentrations as indicators of physiological condition of individuals in the population, and to determine whether the poor body condition and chronic stress in 1992/93 were still present in 2003.

## **Methods**

Free-living snakes on Guam were caught by hand at night from April through September 2003, and blood samples (300  $\mu$ l) were collected within 3 minutes of capture from the caudal vein using a heparinized 1-cm<sup>3</sup> syringe and 25-g needle. All blood samples were placed in a cooler with an ice pack until return to the field laboratory, centrifuged and the plasma removed. Plasma was stored at -20°C until the samples could be shipped to Oregon State University and once there, stored at -70°C until assayed. Mass (g) and snout-vent length (SVL) (cm) were recorded, and after the snakes were sacrificed the fat bodies were weighed to the nearest 0.01g. The amount of fat in each snake was converted to a percentage of the total body weight.

Plasma corticosterone levels for brown tree snakes collected between 1991 and 1993 were collected using similar methods (Moore et al., 2005). The SVL and mass

for snakes measured from April to September 1992 and 1993 were added to the 2003 data to generate the body condition index (BCI) of each individual as an estimate of the energy stores or fat reserves.

The BCI of each individual was calculated as the residual score from the general linear regression of ln-transformed mass against ln-transformed SVL (Fig. 5.1). The data set was then divided into three groups; snakes 90 cm SVL and greater (males and females), and those less than 90 cm SVL (juveniles) as the approximate size where brown tree snakes undergo an ontogenetic shift in diet from small ectothermic to larger endothermic prey (Greene, 1989). The combined data set consisted of a total of 128 juveniles, 156 females, and 106 males.

### ***Radioimmunoassay***

Plasma levels of corticosterone were determined using radioimmunoassay following the procedure described by Moore et al. (2005). Briefly, plasma volumes of 4 to 200  $\mu$ l were equilibrated overnight with tritiated corticosterone (Amersham). Each sample was extracted twice in 2 ml diethyl ether, and dried in a warm water bath under a stream of nitrogen gas. The extracts were resuspended in 10% ethyl acetate in isooctane, and were chromatographed through individual celite columns to separate the steroid fractions and neutral lipids. The purified elutes were dried and resuspended in buffer. For the assay, all samples, including serial dilutions and 100% bounds, were incubated overnight with 100  $\mu$ l of antibody (corticosterone antibody B3-163 from Esoterix Endocrinology) and 100  $\mu$ l of tritiated steroid. Unbound steroid was separated using dextran-coated charcoal and the bound steroid decanted into scintillation vials, resuspended in 3.5 ml scintillation fluid, incubated for 12 h, and

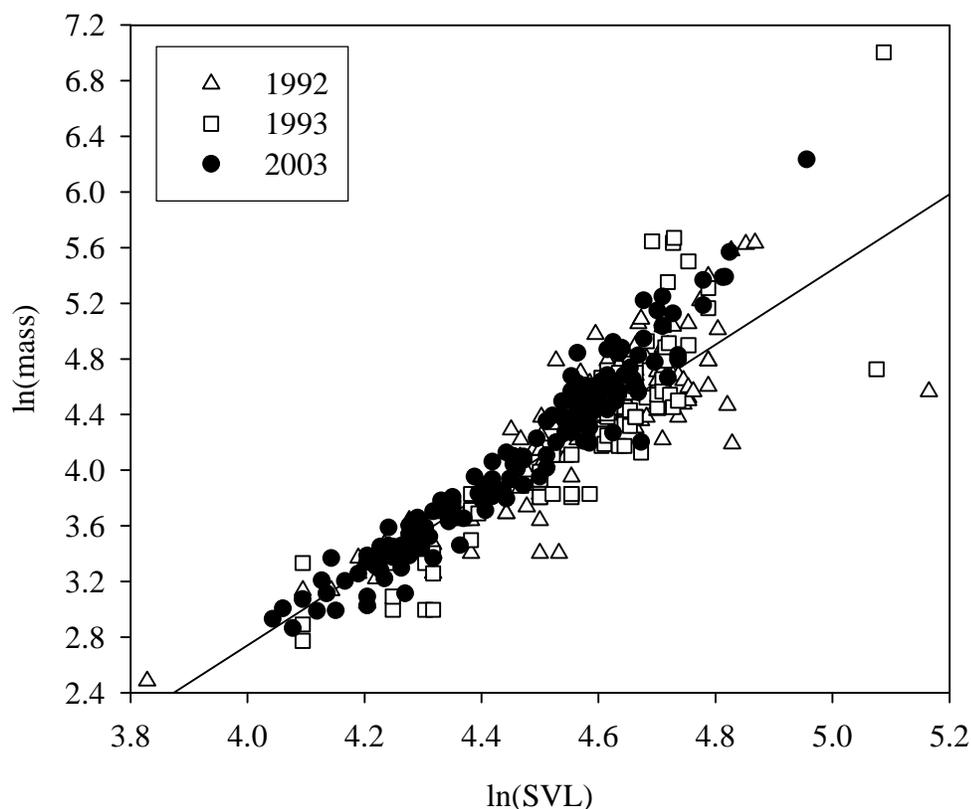


Figure 5.1. Linear regression of ln-transformed mass on ln-transformed SVL for brown tree snakes on Guam in 1992, 1993, and 2003.

counted on a Beckman LS1800 scintillation counter. Intraassay variation was 15% and interassay variation was 18%. The limit of detection for corticosterone was  $\sim 0.04$  ng/ml. Although the 1992/93 samples and the 2003 samples were not assayed together, they were assayed in the same laboratory using similar materials and protocols.

### ***Statistics***

A two-way ANOVA, with group and year as the factors and body condition as the dependent variable, was used to examine BCI between years and groups, and pairwise multiple comparisons using the Tukey Test were used to find the sources of variation. A two-way ANOVA, with year and group as the factors, was used to

compare corticosterone levels. Spearman Rank Order correlation was used to test for correlations between BCI, corticosterone levels, percent fat, and date of sample. All statistical analyses were performed using Jandel SigmaStat version 3.1 software package (Jandel Corporation). Analyses were considered statistically significant when  $p < 0.05$ .

## **Results**

Snakes in the 2003 sample tended to have greater mass for their length than snakes in the 1992 and 1993 samples (Fig. 5.1). Body condition varied significantly among years ( $F = 18.346$ ,  $p < 0.001$ ) and among groups ( $F = 3.168$ ,  $p = 0.043$ ). There was no statistically significant interaction between years and groups ( $F = 2.092$ ,  $p = 0.081$ ).

Adult female BCI differed from all juveniles in 1992 ( $p = 0.003$ ), 1993 ( $p < 0.001$ ), and 2003 ( $p < 0.001$ ), while adult male BCI differed from all juveniles in 1993 ( $p = 0.002$ ) and 2003 ( $p < 0.001$ ), but not in 1992 ( $p = 0.178$ ). Male and female BCI did not differ ( $p = 0.223$ ). Males had significantly different BCI between 2003 and 1992 ( $p < 0.001$ ) and 2003 and 1993 ( $p = 0.006$ ), but not between 1993 and 1992 ( $p = 0.405$ ). Female BCI was significantly different between 2003 and 1992 ( $p = 0.016$ ), but not between 2003 and 1993 ( $p = 0.188$ ) or 1992 and 1993 ( $p = 0.3$ ). Juvenile BCI was not significantly different between any of the three years (Fig. 5.2).

Corticosterone concentrations were significantly different among years ( $F = 73.319$ ,  $p < 0.001$ ) but not between groups ( $F = 2.412$ ,  $p = 0.092$ ). Corticosterone levels were significantly different between 2003 and 1993 ( $p < 0.001$ ) and between

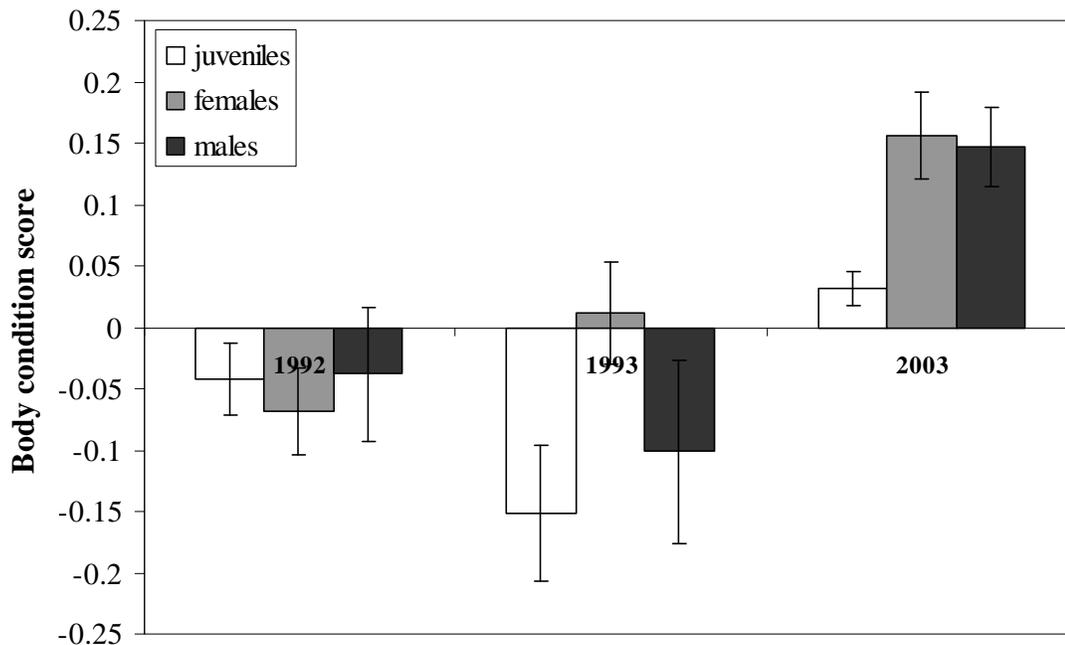


Figure 5.2. Body condition of free-living male, female, and juvenile brown tree snakes on Guam in 1992, 1993, and 2003. Bars represent means and standard errors.

2003 and 1992 ( $p < 0.001$ ), but not between 1992 and 1993 ( $p = 0.999$ ) (Fig. 5.3).

Body condition was significantly correlated to corticosterone ( $r = -0.447$ ,  $p < 0.0001$ ). Body condition in 2003 was also significantly correlated to percent fat in females ( $r = 0.481$ ,  $p < 0.0001$ ) and males ( $r = 0.419$ ,  $p < 0.0001$ ), and had a significant negative correlation with date for both females ( $r = -0.342$ ,  $p = 0.0045$ ) and males ( $r = -0.450$ ,  $p < 0.0001$ ).

## Discussion

Body condition of mature brown tree snakes on Guam was significantly greater in 2003 than in 1992 and 1993; juvenile body condition also improved, but not significantly. Brown tree snakes in Australia show a shift in diet at around 80 to 90

cm SVL, with larger snakes eating more endothermic prey (birds and mammals) and fewer ectothermic prey (lizards and frogs) than smaller snakes (Greene, 1989). On Guam, medium to large snakes originally preyed on birds and small mammals, while small to medium snakes ate small lizards (Savidge, 1988). Much of the larger, endothermic prey species became scarce or have completely disappeared, so adult snakes now subsist on small lizards (Rodda et al., 1999a) and some of the introduced species of birds and small mammals (Rodda et al., 1999b). The change in BCI between years suggests a change in prey availability, although it is uncertain whether the increase was in endothermic prey species or lizards. The slight increase in juvenile BCI could indicate that their access to food species did not improve as greatly as it did for adults. Adult snakes with their larger gape would be able to manage a wider range of prey types than small snakes and would be better able to take advantage of an

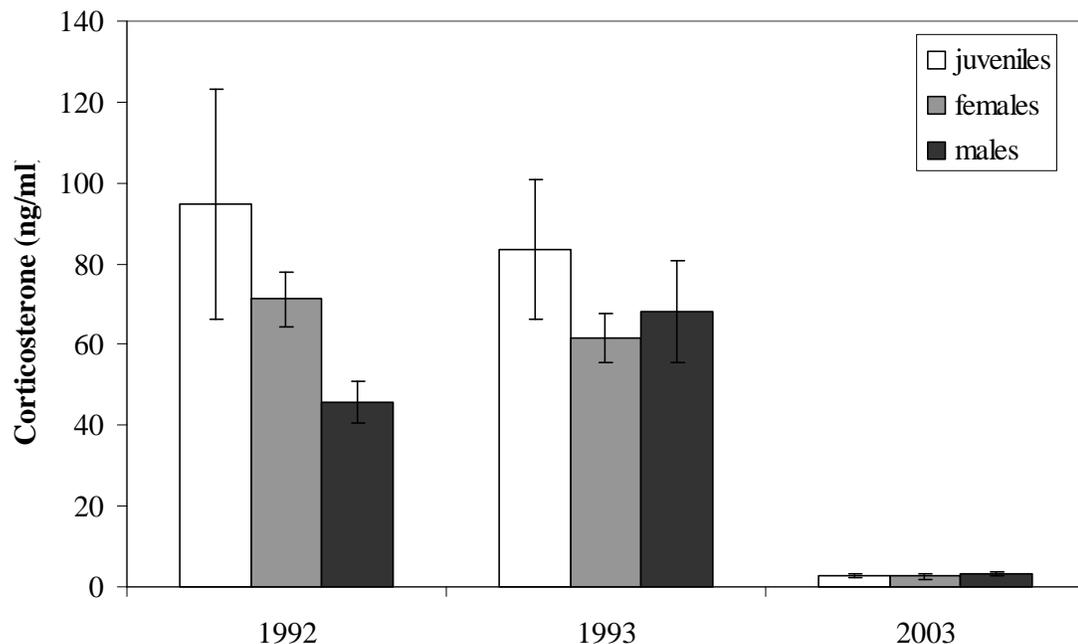


Figure 5.3. Plasma levels of corticosterone for juvenile, female, and male brown tree snakes sampled in different years on Guam. Bars represent means and standard errors.

increase or change in prey type. Alternatively, juveniles could have benefited from the increase in prey as much as the adults, but allocated much of that extra energy to growth (i.e. length) instead of to accumulating fat stores or for reproductive effort (Madsen & Shine, 2002).

The body condition index, while a good estimator of relative condition, is more informative when paired with additive indicators (Weatherhead & Brown, 1996), such as corticosterone concentrations or fat body measurements. Baseline levels of plasma corticosterone were significantly lower in 2003 than in 1992/93 for all three groups of snakes, and there was a significant negative correlation between body condition and corticosterone. This decrease in corticosterone and increase in body condition indicates that food was more available in 2003 and that the snakes were not food-stressed. Although the increase in juvenile body condition from the earlier sample was not significant, corticosterone levels of juveniles were very low in 2003. This observation lends credence to the idea that the slight increase in juvenile body condition, while mature snakes showed a significant improvement, was due to differences in energy allocation between juveniles and adults rather than a disparity in prey availability.

Brown tree snakes might not achieve high body condition scores relative to other species, as they are arboreal snakes that benefit from having long, slender bodies and probably do not ever accumulate large reserves of fat (Lillywhite & Henderson, 1993; Naulleau & Bonnet, 1995). Females of another species of semi-arboreal snake regularly initiated vitellogenesis with few body reserves, and instead relied on active foraging to provide energy for reproduction (Naulleau & Bonnet, 1995). The

maximum amount of fat found in those snakes was no more than 4% of the total body mass (Naulleau & Bonnet, 1995); brown tree snakes in 2003 had fat bodies weighing up to 10% of the total body mass, although most were in the 4 to 6% range. It might not take much extra food to increase the body condition of a brown tree snake from the near-starvation conditions in 1993 to the more robust snakes in 2003.

The condition of snakes captured in 2003 declined from April to September, indicating a relationship between food availability or energy expenditure and seasonally variable environmental conditions. Weather on Guam is affected by the El Niño/Southern Oscillation (ENSO), which causes an exceptionally arid and extended dry season around once every four years on Guam (Lander, 1994). The dry seasons preceding the three sampling years were all moderate El Niño years, but 1992 was the second El Niño year in a row and had lower than average rainfall in the dry season (Fig. 5.4); the following year had the lowest BCI for juveniles and males. Habitats in northern Australia have high temporal variation in prey availability due to the ENSO (Madsen & Shine, 2002), and filesnakes in these habitats show significant annual variation in body condition that is correlated to prey availability and rainfall (Madsen & Shine, 2000). When the amount of precipitation in the wet season prior to each year of sampling is compared to the average monthly rainfall over the last half-century, it is apparent that 2002 had a very rainy, extended wet season (due to Typhoon Chata'an in July and Supertyphoon Pongsona in December) (Fig. 5.4). Environmental conditions can vary dramatically from year to year, and it appears that the condition of the snakes varies widely as well. It could be that the increase in condition of brown tree snakes on Guam in 2003 was a result of the unusually extended wet season the previous year,

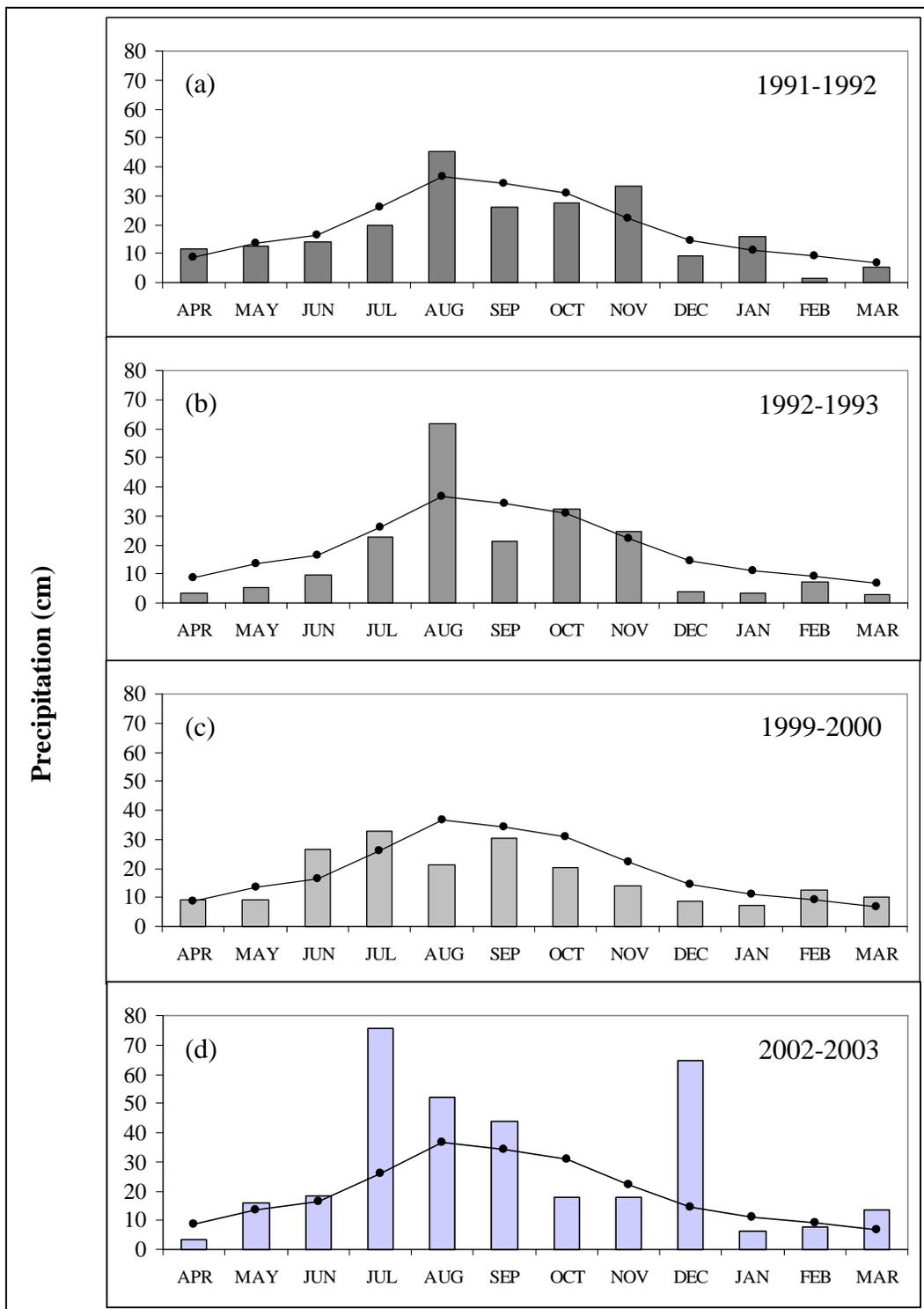


Figure 5.4. Rainfall totals for each month in the years immediately preceding the three sampling years analyzed in this study, (a) 1992, (b) 1993, and (d) 2003, and (c) the sample taken in 2000 (Mathies et al., 2001). The line in each graph is the average total rainfall on Guam for each month between 1948 and 2004. The graph starts with the end of the dry season preceding the sample year (e.g. April 1991 to March 1992).

and not an indication of a long-term shift in prey populations.

On the other hand, it seems likely that the environment on Guam has changed over the last decade. There appears to have been a sharp decline in body condition of brown tree snakes on Guam from 1985 to the late 1990's (Wiles et al., 2003), but free-ranging snakes sampled in July of 2000 had low baseline plasma corticosterone levels (5 to 10 ng/ml) (Mathies et al., 2001) compared to those in 1992/93 (average ~ 60 ng/ml). The wet season prior to the 2000 sample was drier than average, in contrast to the wet season preceding the 2003 sample (Fig. 5.4). It is possible that either the population has now reached a point where it is better able to exploit the resources at hand (fewer large snakes competing for preferred prey), or a new food source is available on Guam and the snakes are taking advantage of an expanded prey base. For example, only two amphibian species were known to be breeding on Guam in the early 1990's, the poisonous Cane Toad (*Bufo marinus*) and the Dwarf Treefrog (*Litoria fallax*). The recent introduction of several additional frog species (including *Eleutherodactylus planirostris* and *Rana nigromaculata*) will likely provide the snakes with a new food source available to both juveniles and adults (Tyrrell et al., 2005).

Weatherhead and Brown (1996) concluded that direct measurement of the condition of an individual is more accurate than an estimation using a body condition index. We found that the combination of corticosterone concentrations and fat mass as measures of condition with residuals from a regression of body mass on body length was much more informative than any one measure alone. The question of whether or not there has been a long-term improvement in body condition of brown tree snakes has important implications for management of this species on Guam. If

the prey population has indeed changed to provide sufficient energy resources for the snakes to reproduce, brown tree snakes will become more numerous. Growth of the brown tree snake population will compromise efforts to reintroduce extirpated species on Guam, and will increase the potential for snake dispersal to other locations through airlines and cargo ships. Snakes that are in better condition may not be as likely to enter food-based traps, so monitoring and eradication programs will be somewhat less effective. The effect of annual variation in rainfall on the population dynamics of prey species should be monitored, as well as the relative condition of brown tree snakes from year to year, to predict changes in the snake population.

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## 6. CONCLUSION

The objective of the research presented in this thesis was to describe the reproductive behavior and reproductive biology of a tropical snake, specifically the introduced population of brown tree snakes on Guam. More specifically, this research had the following goals: 1) to examine the aggregation response of females to visual and chemical cues; 2) to describe the reproductive cycle of brown tree snakes on Guam; 3) to quantify the changes in corticosterone, testosterone, and estradiol in response to acute stress; and 4) to examine changes in physiological condition in this population of snakes between two sampling periods.

This research is significant at a basic research level, because reproductive biology and behavior are largely unknown for most species of tropical snakes. The majority of reptile species live in tropical and subtropical environments, yet much of our understanding of reptile physiology and behavior is based on studies of temperate species. A greater focus on tropical species is needed if we are to fully understand the biology of this group of vertebrates.

These results are also relevant to management efforts. The brown tree snake is an invasive species on the island of Guam, with some potential to invade other tropical islands. The current combination of control techniques is not fully effective for all portions of the population, so the potential use of snake pheromones to bait traps and repel snakes from cargo areas is promising. However, before sex pheromones can be used to control the behaviors of these snakes, we must know more about their reproductive behavior and the timing of reproduction in this population.

Aggregation and shelter choice experiments in Chapter Two demonstrated that female brown tree snakes have differing responses to female and male scents, and that for some cues, this response was dependent on the reproductive condition of the test snakes. Reproductive and nonreproductive females preferred the shelter scented by one other female or by a single male over an unscented shelter. However, while nonreproductive females avoided the shelter scented by three other females, reproductive females did not prefer either shelter. In addition, while nonreproductive females avoided the shelter scented by three other females, they preferred to form aggregations with other females when all of the snakes were placed together. This discrepancy suggests that female brown tree snakes have different responses to the pheromones of conspecifics, depending on the presence of visual or tactile cues and their reproductive state. The use of pheromones as a management tool will more effectively target reproductive females if the timing of application and the composition of individual snakes used as a source for the pheromones are planned to coincide with the reproductive cycle of this population on Guam.

The timing of reproduction of brown tree snakes on Guam was described in Chapter Three, using hormones and gonad development to determine when individuals were reproductively active. It appears that males are capable of dissociated reproduction, in that they produce sperm when females are not receptive and store this sperm until they have an opportunity to mate. Males capable of insemination are present in the population throughout the year. However, there is seasonality to the female cycle, in that females start vitellogenesis near the start of the wet season, and ovulate and oviposit through most of the wet season. Although it is possible that there

are some females that mate and oviposit in every month, the majority of females reproduce seasonally. This seasonality is consistent with what has been seen in the species in its native range in Australia, and with reproductive cycles in tropical snakes in general.

In Chapter Four, I explored the effect of an acute stressor on plasma concentrations of the stress hormone corticosterone and the sex hormones testosterone and estradiol. The stress of confinement produced significant increases in corticosterone after one hour, and longer periods of confinement did not significantly boost this level. The corticosterone response did not differ between males and females, nor did it vary with season or body condition. Testosterone declined with acute stress, especially in large males that had high levels of testosterone to start, but estradiol appeared to increase with stress (although this is likely due to very low concentrations of estradiol and sampling error). Even 24 hours in confinement did not produce the levels of corticosterone measured as background levels in an earlier study, which supports the conclusion that this population showed high levels of chronic stress in the early 1990s.

The body condition index is a common method for quantifying the energy resources of individual animals. Good body condition is necessary for reproduction in many species and can indicate the potential reproductive output of a population. In Chapter Five, I compared the body condition index and plasma corticosterone levels of brown tree snakes on Guam in 2003 to those collected in 1992 and 1993 to determine whether the population still showed the chronic stress and poor condition apparent in the earlier study. This comparison also provided an opportunity to examine the use of

a combination of indicators of condition instead of the body condition index alone.

Body condition was significantly higher in 2003 for mature snakes but not for juveniles, but the significantly lower concentrations of corticosterone in all groups of snakes in 2003 showed that the low body condition in juveniles was not due to stress. Changes in precipitation in sampling years suggest that a long-term change in prey populations is likely the cause of the difference in physiological condition between these two sampling periods.

These results indicate that the timing of reproduction of brown tree snakes on Guam is similar to that seen in other tropical snakes, in that although the breeding season is attenuated, it does show seasonality. Timing and frequency of reproduction appears to be strongly influenced by the environment, both in terms of the time of year that reproduction occurs, and in how the environment affects prey species and energy available for reproduction. In addition, this species shows complex social behaviors that are tied to reproductive state and change depending on the cues received by individual snakes. Collectively, these results provide a foundation for future research on brown tree snake reproductive biology and behavior and suggest intriguing areas of exploration that will contribute to our understanding of reptile physiology and behavior.

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APPENDICES

## APPENDIX 1 – SAMPLE ANOVA TABLES

## Chapter 3

Two Way Repeated Measures ANOVA for female weights, with group and month as factors

Source of Variation	df	SS	MS	F	p
group	1	2010.7	2010.7	36.301	< 0.001
ID	25	1390.8	55.6		
month	3	620.5	206.8	12.422	< 0.001
group x month	3	1180.2	393.4	23.627	< 0.001
<b>residual</b>	73	1215.5	16.7		
<b>total</b>	105	6537.7	62.3		

Two Way Repeated Measures ANOVA for male weights, with group and month as factors

Source of Variation	df	SS	MS	F	p
group	1	2752.9	2752.9	20.321	< 0.001
ID	25	3386.8	135.5		
month	3	819.9	273.3	12.565	< 0.001
group x month	3	1656.4	552.1	25.384	< 0.001
<b>residual</b>	75	1631.4	21.7		
<b>total</b>	107	10330.5	96.5		

## Chapter 4

Two Way ANOVA for corticosterone, with sex and group as factors

Source of Variation	df	SS	MS	F	p
sex	1	0.242	0.242	1.380	0.243
group	3	0.836	0.279	1.587	0.198
group x sex	3	0.037	0.012	0.069	0.976
<b>residual</b>	87	15.270	0.176		
<b>total</b>	94	16.326	0.174		

## Chapter 5

Two Way ANOVA for BCI, with group and year as factors

Source of Variation	df	SS	MS	F	p
group	2	0.432	0.216	3.168	0.043
year	2	2.500	1.250	18.346	< 0.001
group x year	4	0.570	0.143	2.092	0.081
<b>residual</b>	381	25.958	0.0681		
<b>total</b>	389	28.946	0.0744		

Two Way ANOVA for corticosterone, with group and year as factors

<b>Source of Variation</b>	<b>df</b>	<b>SS</b>	<b>MS</b>	<b>F</b>	<b>p</b>
group	2	7055.5	3527.7	2.412	0.092
year	2	214431.1	107215.5	73.319	< 0.001
group x year	4	7658.341	1914.6	1.309	0.268
<b>residual</b>	214	312935.4	1462.3		
<b>total</b>	222	564291.9	2541.9		

Appendix II – BROWN TREE SNAKE SNOUT-VENT LENGTH, MASS, AND FAT MEASUREMENTS

FEMALES

DATE	ID	SEX	SVL(cm)	MASS(g)	FAT(g)	FAT%
4/20/2003	04-20-01	f	101	108	4.14	3.83
4/20/2003	04-20-02	f	90	52	0	0.00
4/24/2003	04-24-01	f	80.5	52	3.47	6.67
5/9/2003	05-09-01	f	69	25	0.64	2.56
5/19/2003	05-19-06	f	91	77.6	6.9	8.89
5/19/2003	05-19-07	f	98	99.8	6.95	6.96
5/19/2003	05-19-09	f	96	99.4	8.8	8.85
5/19/2003	05-19-10	f	103.5	131.5	9.31	7.08
5/21/2003	05-21-01	f	95	90.1	5.79	6.43
5/28/2003	05-28-01	f	95	107.2	10.72	10.00
5/28/2003	05-28-02	f	62.5	22.5	0.75	3.33
6/2/2003	06-02-01	f	58	20.2	0.6	2.97
6/2/2003	06-02-02	f	94.5	75.9	5.27	6.94
6/2/2003	06-02-03	f	68.5	26.7	2.13	7.98
6/2/2003	06-02-04	f	102	137.1	10.21	7.45
6/9/2003	06-09-02	f	62	24.7	1.18	4.78
6/9/2003	06-09-03	f	110	171.7	12.23	7.12
6/9/2003	06-09-04	f	92.5	66.7	3.45	5.17
6/15/2003	06-15-01	f	59	17.5	0.15	0.86
6/16/2003	06-16-01	f	77	37.7	1.61	4.27
6/16/2003	06-16-02	f	71	31.7	1.4	4.42
6/16/2003	06-16-03	f	67	28.2	1.14	4.04
6/16/2003	06-16-04	f	107.5	185		
6/16/2003	06-16-05	f	94.5	78.7	6.55	8.32
6/19/2003	06-19-01	f	97	67.2	1.37	2.04
6/23/2003	06-23-03	f	96	126.8	15.27	12.04
6/23/2003	06-23-04	f	96.5	87.2		
6/23/2003	06-23-08	f	76	43.9	2.44	5.56
6/23/2003	06-23-10	f	93.5	81	6.68	8.25
6/25/2003	06-25-02	f			122.29	
6/30/2003	06-30-02	f	72	29.5	0.51	1.73
6/30/2003	06-30-03	f	87.5	60.2	3.77	6.26
6/30/2003	06-30-05	f	105	113.8	7.24	6.36
6/30/2003	06-30-06	f	111	189.9	16.78	8.84
7/22/2003	07-22-03	f	76	41.4	2.57	6.21
7/22/2003	07-22-04	f	85.5	51.3	1.92	3.74
7/22/2003	07-22-05	f	86	60.4	3.32	5.50
8/6/2003	B4	f	107	66.8	0.47	0.70
8/7/2003	08-07-02	f	96	86.5	4.01	4.64
8/7/2003	08-07-05	f	83	51.2	2.1	4.10
8/7/2003	08-07-06	f	72.5	38	2.45	6.45
8/7/2003	08-07-07	f	72.5	33	1.64	4.97
8/14/2003	08-14-03	f	82.5	49.6	2.18	4.40
8/16/2003	Kf1	f	106	100.6	1.1	1.09
8/16/2003	Kf2	f	98	82	5.08	6.20

## FEMALES con't

DATE	ID	SEX	SVL(cm)	MASS(g)	FAT(g)	FAT%
8/19/2003	08-19-02	f	95	71.6	5.38	7.51
8/19/2003	08-19-03	f	81.5	44	3.12	7.09
8/19/2003	08-19-06	f	73.5	31.3	1.07	3.42
8/19/2003	08-19-07	f	63.5	19.9	0.6	3.02
8/21/2003	08-21-02	f	119	213.9	14.51	6.78
8/21/2003	08-21-03	f	77.5	43.4	1.53	3.53
8/21/2003	08-21-05	f	96	75.5		
8/21/2003	08-21-06	f	72	34.2	1.29	3.77
8/21/2003	08-21-07	f	71.5	22.5	0.56	2.49
8/26/2003	08-26-03	f	82	48.4	2.3	4.75
8/28/2003	08-28-03	f	67	22	0.43	1.95
8/28/2003	08-28-06	f	75	29	1.36	4.69
9/2/2003	09-02-01	f	100	103.4	7.4	7.16
9/2/2003	09-02-02	f	99.5	91.1	6.2	6.81
9/2/2003	09-02-03	f	100.5	100.4	7.7	7.67
9/2/2003	09-02-04	f	101	84.4	4.2	4.98
9/2/2003	09-02-05	f	102	100.9	6.61	6.55
9/2/2003	09-02-06	f	98	74.2	0.52	0.70
9/4/2003	09-04-01	f	67.5	29.1	1.19	4.09
9/4/2003	09-04-02	f	96.5	98.3	8.72	8.87
9/4/2003	09-04-05	f	70	29.2	1.35	4.62
9/11/2003	09-11-01	f	89.5	68.4	4.59	6.71
9/11/2003	09-11-05	f	81	46.1	2.44	5.29
9/11/2003	09-11-06	f	97.5	90.4	7.23	8.00

## MALES

DATE	ID	SEX	SVL(cm)	MASS(g)	FAT(g)	FAT%
4/2/2003	04-02-01	m	96	102		
4/7/2003	04-07-01	m	105.5	105		
4/12/2003	04-12-01	m	101	130		
4/12/2003	04-12-02	m	63	29	1.6	5.52
4/15/2003	04-15-01	m	99	92		
4/22/2003	04-22-01	m	101	104	8.03	7.72
4/22/2003	04-22-02	m	83	58	2.79	4.81
4/22/2003	04-22-03	m	85	62	2.19	3.53
4/24/2003	04-24-02	m	68.5	31.5		
5/9/2003	05-09-02	m	71	27	0.75	2.78
5/14/2003	05-14-01	m	95	95	6.12	6.44
5/19/2003	05-19-01	m	86.5	55	1.7	3.09
5/19/2003	05-19-02	m	61.5	19.8	0.46	2.32
5/19/2003	05-19-03	m	60	21.6	0.4	1.85
5/19/2003	05-19-04	m	69.5	31.7	0.87	2.74
5/19/2003	05-19-05	m	77.5	41.9	2.01	4.80
5/19/2003	05-19-08	m	57	18.7	0.46	2.46
5/27/2003	05-27-01	m	77	38.4	1.44	3.75
5/28/2003	05-28-03	m	73	33.7	1.85	5.49
5/28/2003	05-28-04	m	64.5	24.6	0.87	3.54

## MALES con't

DATE	ID	SEX	SVL(cm)	MASS(g)	FAT(g)	FAT%
5/28/2003	05-28-05	m	77.5	44.9	1.69	3.76
5/28/2003	05-28-06	m	103.5	130.4	11.18	8.57
5/28/2003	05-28-07	m	74	36.2	1.64	4.53
5/28/2003	05-28-08	m	113	168.5	11.54	6.85
5/28/2003	05-28-09	m	93.5	89.6	8.7	9.71
6/5/2003	06-05-01	m	69.5	36.1	1.86	5.15
6/9/2003	06-09-01	m	79	38.6	0.37	0.96
6/9/2003	06-09-05	m	103	126.5		
6/16/2003	06-16-06	m	91	55.4	1.53	2.76
6/18/2003	06-18-01	m	107.5	140.7	6.6	4.69
6/18/2003	06-18-02	m	87.5	58.6	1.1	1.88
6/23/2003	06-23-01	m	142	510		
6/23/2003	06-23-02	m	75	40.6	2.69	6.63
6/23/2003	06-23-05	m	95	96.4	10.37	10.76
6/23/2003	06-23-06	m	73	38.8	2.17	5.59
6/23/2003	06-23-07	m	69	29.5	1.14	3.86
6/23/2003	06-23-09	m	66	25.9	0.77	2.97
6/25/2003	06-25-01	m	103	95.6	3.33	3.48
6/27/2003	06-27-01	m	68	29.4	1.48	5.03
6/30/2003	06-30-01	m	124.5	261.7	23.75	9.08
6/30/2003	06-30-04	m	109.5	118.6	9.92	8.36
7/15/2003	07-08-01	m	104	108.24	7.04	6.50
7/15/2003	07-08-02	m	103	96.37	5.56	5.77
7/15/2003	07-08-03	m	100.5	97.2	6.01	6.18
7/22/2003	07-22-01	m	76.5	41.9	1.99	4.75
7/22/2003	07-22-02	m	68	27.3	0.43	1.58
8/7/2003	08-07-01	m	86	56.9	2.22	3.90
8/7/2003	08-07-03	m	91	60.7	3.52	5.80
8/7/2003	08-07-04	m	74.5	33.8	1.14	3.37
8/14/2003	08-14-01	m	70	31.5	1.17	3.71
8/14/2003	08-14-02	m	68.5	30.6	1.2	3.92
8/14/2003	08-14-04	m	92	80.8	4.45	5.51
8/16/2003	1-07/11	m	123.5	219.4	13	5.93
8/16/2003	3-07/11	m	106.5	124.7	8.39	6.73
8/16/2003	5-07/11	m	114	124.8	3.4	2.72
8/16/2003	19-07/22	m	112	106.2	1.27	1.20
8/19/2003	08-19-01	m	102	71.4	3.17	4.44
8/19/2003	08-19-04	m	72	36.6	1.97	5.38
8/19/2003	08-19-05	m	67	20.6	0.44	2.14
8/21/2003	08-21-01	m	94	71.7	2.73	3.81
8/21/2003	08-21-04	m	114	120.9	3.28	2.71
8/21/2003	08-21-08	m	98	66.3	2.72	4.10
8/21/2003	08-21-09	m	83	45.2	1.83	4.05
8/21/2003	08-21-10	m	82	40.8	1.32	3.24
8/26/2003	08-26-01	m	123	218.7	14.39	6.58
8/26/2003	08-26-02	m	111	153.8	8.1	5.27
8/26/2003	08-26-04	m	67	29.5	1.02	3.46
8/26/2003	08-26-05	m	94	79	3.75	4.75

## MALES con't

DATE	ID	SEX	SVL(cm)	MASS(g)	FAT(g)	FAT%
8/26/2003	08-26-06	m	119	178.8	4.93	2.76
8/28/2003	08-28-01	m	102.5	89.8	7.03	7.83
8/28/2003	08-28-02	m	96	78.3	5.99	7.65
8/28/2003	08-28-04	m	85	44.5	1.63	3.66
8/28/2003	08-28-05	m	78.5	31.8	1.02	3.21
8/28/2003	08-28-07	m	106.5	95.5	3.37	3.53
8/28/2003	08-28-08	m	87.5	48.9	1.75	3.58
9/4/2003	09-04-04	m	98.5	82.9	4.81	5.80
9/11/2003	09-11-02	m	84.5	47.1	1.56	3.31
9/11/2003	09-11-03	m	72.5	36.7	1.65	4.50
9/11/2003	09-11-04	m	70.5	29.1	0.73	2.51