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Biological Conservation 121 (2005) 91–98

BIOLOGICAL CONSERVATION

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Physiological evidence for reproductive suppression in the introduced population of brown tree snakes (*Boiga irregularis*) on Guam

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Received 10 February 2004; received in revised form 14 April 2004; accepted 15 April 2004

Abstract

Introduced species are an increasingly pervasive problem. While studies on the ecology and behavior of these pests are numerous, there is relatively little known of their physiology, specifically their reproductive and stress physiology. One of the best documented introduced pest species is the brown tree snake, *Boiga irregularis*, which was introduced onto the Pacific island of Guam sometime around World War II. The snake is responsible for severely reducing Guam's native vertebrates. We captured free-living individuals throughout the year and measured plasma levels of stress and sex hormones in an effort to determine when they were breeding. These data were compared to reproductive cycles from a captive population originally collected from Guam. Free-living individuals had chronically elevated plasma levels of the stress hormone corticosterone and basal levels of sex steroids and a remarkably low proportion were reproductively active. These data coincide with evidence that the wild population may be in decline. Captive snakes, had low plasma levels of corticosterone with males displaying a peak in plasma testosterone levels during breeding. Furthermore, we compared body condition between the free-living and captive snakes from Guam and free-living individuals captured from their native range in Australia. Male and female free-living snakes from Guam exhibited significantly reduced body condition compared to free-living individuals from Australia. We suggest that during the study period, free-living brown tree snakes on Guam were living under stressful conditions, possibly due to overcrowding and overexploitation of food resources, resulting in decreased body condition and suppressed reproduction.

Keywords: Brown tree snake; *Boiga irregularis*; Introduced pest; Guam; Stress

1. Introduction

Invasive pest species are one of the most pervasive problems being addressed in conservation efforts today (Mack et al., 2000; Vitousek et al., 1996). As such many studies have investigated the impacts that invasive species have had on the ecology, behavior, and physiology of native species (D'Antonio and Dudley, 1995). While there have been a number of studies investigating the

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0006-3207/$ - see front matter
doi:10.1016/j.biocon.2004.04.012

ecology of the invader, little is known of the physiology of the invader. We are beginning to gain a better understanding of what makes a good invader, but for conservation efforts to be successful we need to know as much as possible about the physiology of the invader. Gaining a general understanding of the breeding biology and the stresses the invader is exposed to could assist focusing management efforts (Wingfield et al., 1997). For example, efforts to interrupt breeding would be most successful during the animal's breeding season.

We know essentially nothing about the stressors faced by invasive species when they invade a new environment. Potential initial stressors could include a lack of shelter, adverse climate, and a lack of food availability. After an invader is established, potential stressors could include overcrowding and the loss of prey as native species are extirpated. When levels of stress are high, the standard vertebrate response is to activate the hypothalamic–pituitary–adrenal axis and release adrenocorticosteroids into the bloodstream (Wingfield and Romero, 2001). As stress and reproduction are generally thought to oppose one another, periods of high stress could disrupt reproduction (Greenberg and Wingfield, 1987; Moore and Jessop, 2003). This may include a decrease in plasma sex steroid levels when stress and plasma andrenocorticosteroid levels increase (Moore and Jessop, 2003; Moore et al., 2000a). The chronic or long-term effects of stress can include complete inhibition of the reproductive system (Wingfield and Ramenofsky, 1999). Plasma levels of corticosterone, the primary adrenocorticosteroid in reptiles, have been shown to be a good estimate of the amount of physiological stress to which the animal is exposed (e.g. Romero and Wikelski, 2001). This study attempts to address if an invader is stressed in a new environment and what effect this is having on reproduction. For this we chose to investigate the brown tree snake, *Boiga irregularis*, on Guam.

The brown tree snake is a pest species on the Pacific Island of Guam where it was introduced sometime during or immediately after World War II. After its introduction to Guam, the population erupted, expanding its range across the entire island (Rodda et al., 1992). High densities of this generalist predator have led to the depletion of most of Guam's native vertebrate fauna (Wiles et al., 2003). Fritts and Rodda (1995) reported that all of Guam's surviving endotherm populations consist of less than 1000 individuals and the viability of these populations is questionable. Brown tree snake predation has had an adverse effect on 17 of 18 native species on Guam including various small mammals, lizards, and the extinction or extirpation of 12 bird species (Savidge, 1987; Rodda et al., 1997; Wiles et al., 2003). These negative effects of the brown tree snake could be repeated if the snake gets established on other islands (it has been already seen on Hawaii).

Peak densities of over 100 brown tree snakes per hectare on Guam were measured in the mid-1980s, preceded by apparently continuous population growth from the time of introduction (Rodda et al., 1992, 1999b). Subsequent surveys in the early 1990s found lower peak snake densities (20–50 snakes per hectare) along with more variable levels depending on location on the island (Rodda et al., 1992). It is thought that depleted food resources have led directly to declines in brown tree snake densities on Guam (Fritts and Rodda, 1995). Such declines may be due to adult mortality and/or suppressed reproduction in the population. Wiles et al. (2003) suggest, without presenting data, that a dramatic decline in body condition since 1985 supports the premise that the population is in decline.

In its native habitat of Australia, the brown tree snake displays a cyclic reproductive cycle in both female gamete production and male testicular activity (Bull and Whittier, 1996, 1997; Shine, 1991; Whittier and Limpus, 1996). On Guam, we know little about the seasonal reproductive cycle of the snakes as few mature, reproductively active snakes have been found during sampling (Mathies et al., 2001; Rodda et al., 1999a). Rodda et al. (1999a) estimated that only 0.5% of total captures on Guam were reproductively active females. In histological and morphological samples collected on Guam over a two-year period as part of this study, Mason et al. (unpublished) found that only six of 128 females had vitellogenic follicles. Population data indicate that there is a smaller fraction of mature adults in the Guam population compared to either the native, Australian population or to data for other snake species (Rodda et al., 1999b).

The aim of this study was to examine physiological mechanisms that may explain the low proportion of reproductively active adults on Guam by examining seasonal cycles of reproduction, stress, and body condition of the brown tree snake. We predicted that in the Guam brown tree snakes, low body condition would be accompanied by high levels of stress hormones and low levels of sex steroids in comparison with the native populations from Australia and captive populations. More specifically, we compared plasma levels of sex (testosterone in males, 17β-estradiol and progesterone in females) and stress (corticosterone) steroid hormones between free-living snakes on Guam and breeding captive snakes originally collected on Guam. We also compared body condition between these two groups and free-living individuals from a native population from Australia. The data from this study will complement current management efforts designed to control introduced brown tree snake populations; for example, applied research methods to manage brown tree snake populations include inhibiting reproduction using chemical and immunological fertility control (Brown Tree Snake Control Committee, 1996).

2. Materials and methods

Free-living brown tree snakes on Guam were captured during every month of the year over a two-year period (1991: October–December; 1992: January–March; 1993: April–September). Snakes were caught by hand at night at various sites across the island. For analyses, samples were grouped by month across years. Only reproductively mature adult animals were included in the analyses (51 males and 117 females). These included individuals that exceeded 100 cm in snout-vent length as these individuals are considered adults and reproductively capable (Rodda et al., 1999b). Animals were sacrificed by decapitation rapidly after capture at which point trunk blood samples were collected. The blood samples were collected in less than 5 min from capture and the bodies preserved in 10% buffered formalin. Plasma for the measurement of endogenous steroids was separated by centrifugation and stored at −80 °C until analysis. Body measurements of snout-vent length (SVL) and mass were collected.

We also determined the annual cycle of sex steroids and corticosterone in a captive population of brown tree snakes at Oregon State University. All snakes in the captive colony were mature adults originally collected on Guam in 1993. During sample collection, all individuals went through a breeding season as determined by observations of courtship and ritualized male combat behavior (Greene and Mason, 2000). The snakes were brought into breeding condition starting in mid-January after a five-week period of cooling (Greene and Mason, 1998). Between January and December 1996, we collected monthly blood samples from the seven female and 10 males of the colony. All snakes were bled within 5 min of being disturbed from the caudal vein. As not to confound other behavioral studies being conducted with the same snakes (Greene and Mason, 1998, 2000, 2003; Greene et al., 2001), snakes were not permitted to copulate during the period of study, although courtship behavior was observed throughout the course of the study.

Body condition of each individual was defined as the residual from the linear regression of mass on snout-vent length. For the regression analysis, we included all captive and free-living individuals from Guam as well as free-living individuals (25 males and 22 females) captured from a variety of sites in the snakes, native range of Australia. Body condition was then compared by ANOVA and between groups, within sex differences were determined using Tukey–Kramer HSD post-hoc analysis.

Plasma levels of testosterone, progesterone, estradiol, and corticosterone were determined by radioimmunoassay. Field collected samples were analyzed according to Grassman and Hess (1992). Briefly, sex steroids were measured following extraction with diethyl ether and chromatographic separation on Sephadex LH-20 microcolumns while corticosterone was assayed directly after extraction. All samples were corrected for individual extraction efficiency. Plasma volumes from 10 to 90 μl were used in all assays. All samples were analyzed in single assays with the following intra-assay variation: testosterone 1.5%, estradiol 5%, progesterone 5.5%, corticosterone 9.5%. The average water blanks were all less than 10 pg/tube for all assays. Captive samples were analyzed according to Moore et al. (2000b). These samples were all extracted in diethyl ether and chromatographed on celite microcolumns. Plasma volumes of 5–10 μl were used. Limits of detection were ~1.0 ng/ml for corticosterone and ~0.3 ng/ml for testosterone, estradiol and progesterone. The intra- and interassay variations for these assays were all less than 15% (Lerner and Mason, 2001). Differences in plasma hormone levels between months were determined by ANOVA in the free-living population and by repeated measures ANOVA in the captive population. Direct statistical comparisons of hormone levels between the free-living and captive snakes were not performed due to the differences in assay protocols and single versus repeated sampling protocols.

3. Results

Within each sex there was a significant positive relationship between snout-vent length and mass in a combined analysis for snakes from Guam (free-living and captive) and Australia (males: linear regression, $r^2 = 0.89$, $p < 0.0001$; females: linear regression, $r^2 = 0.74$, $p < 0.0001$). For males, body condition (defined as each individual's residual from this regression) was significantly different between these three groups of snakes (Fig. 1; ANOVA, $F_{2,82} = 7.58$, $p < 0.001$) with the Australian snakes being significantly greater than the free-living snakes from Guam (Tukey–Kramer HSD, $p < 0.05$). For females, body condition was significantly different between the three groups of snakes (Fig. 1; ANOVA, $F_{2,142} = 16.39$, $p < 0.0001$) with the Australian and captive snakes from Guam being significantly greater than the free-living snakes from Guam (Tukey–Kramer HSD, $p < 0.05$).

Male free-living snakes from Guam showed significant seasonal differences in plasma concentrations of testosterone (Fig. 2(a); ANOVA: $F_{10,40} = 2.34$, $p = 0.028$) with June being significantly different from March and August (Tukey–Kramer HSD, $p < 0.05$). In free-living male snakes there were no significant differences between the months in plasma concentrations of corticosterone (Fig. 2(b); $F_{10,36} = 1.80$, $p = 0.095$).

Female free-living snakes from Guam did not show seasonal differences in estradiol (Fig. 3(a); ANOVA: $F_{11,102} = 0.73$, $p = 0.71$) or progesterone (Fig. 3(b);

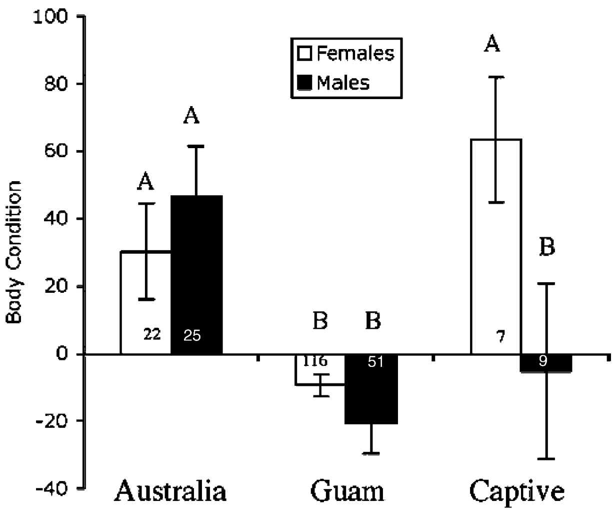

Fig. 1. Body condition (residuals from the linear regression of mass on snout-vent length) of free-living male and female brown tree snakes from the native range in Australia, the introduced population on Guam, and the captive population at Oregon State University. Bars represent means and standard errors. Statistical analyses were limited to within each sex and different letters above bars denote significant differences between groups.

ANOVA: $F_{11,102} = 1.49$, $p = 0.15$). Free-living females did show significant monthly differences in plasma corticosterone concentration (Fig. 3(c); ANOVA: $F_{11,92} = 6.64$, $p < 0.0001$) with the following significant differences between months (Tukey–Kramer HSD, $p < 0.05$; January differs from June, August, September; February differs from June, August, September; September differs from October, November, December).

Captive males had a peak in testosterone during March and April (repeated measures ANOVA: $F_{11,76} = 2.055$, $p = 0.040$), when male courtship activity was at its peak (Greene and Mason, 1998, 2000). Captive female snakes displayed no monthly progesterone (repeated measures ANOVA: $F_{11,40} = 1.070$, $p = 0.426$) or estradiol (repeated measures ANOVA: $F_{11,40} = 0.701$, $p = 0.725$) cycle. Neither males (repeated measures ANOVA: $F_{11,70} = 1.278$, $p = 0.261$) nor females (repeated measures ANOVA: $F_{11,78} = 0.877$, $p = 0.567$) had annual cycles of corticosterone.

4. Discussion

In this study, we have documented chronically elevated levels of corticosterone and basal levels of sex steroids in an introduced population of brown tree snakes on Guam. This supports previous studies that have failed to find significant numbers of breeding individuals in the wild population (Mathies et al., 2001; Rodda et al., 1999a) and suggested that individuals in

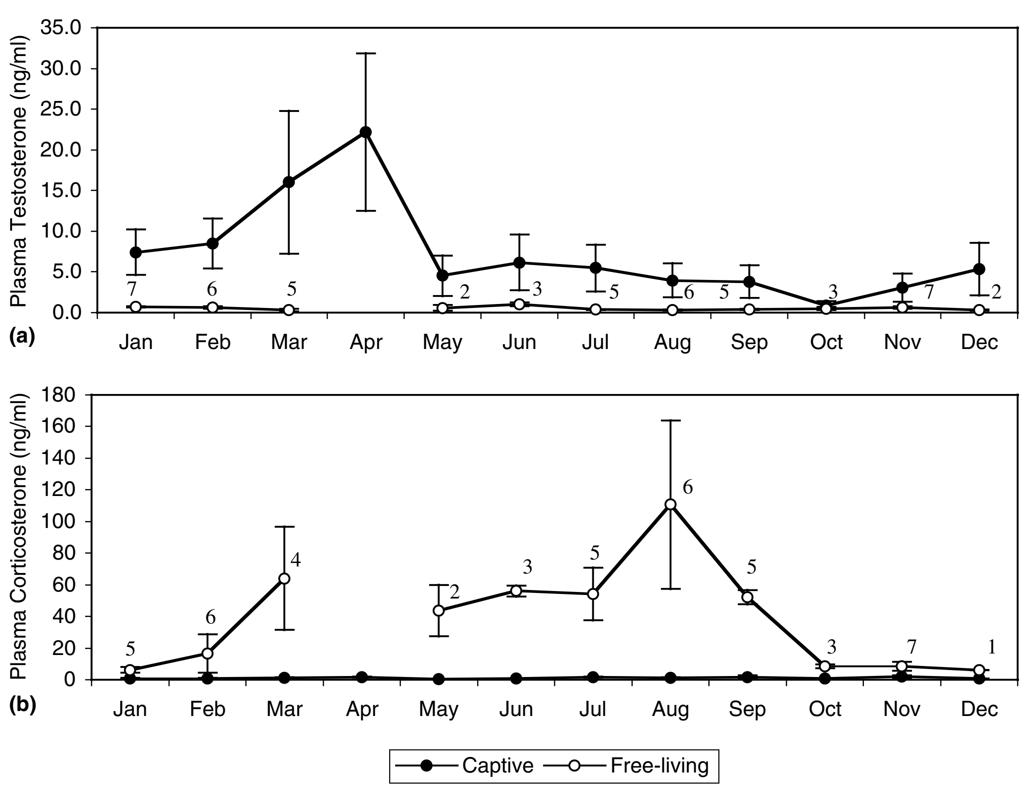

Fig. 2. Annual cycles of (a) testosterone and (b) corticosterone for male captive and free-living populations of brown tree snakes from Guam. Numbers next to the points are the sample sizes for the free-living group. Sample size for the captive group was nine for all months.

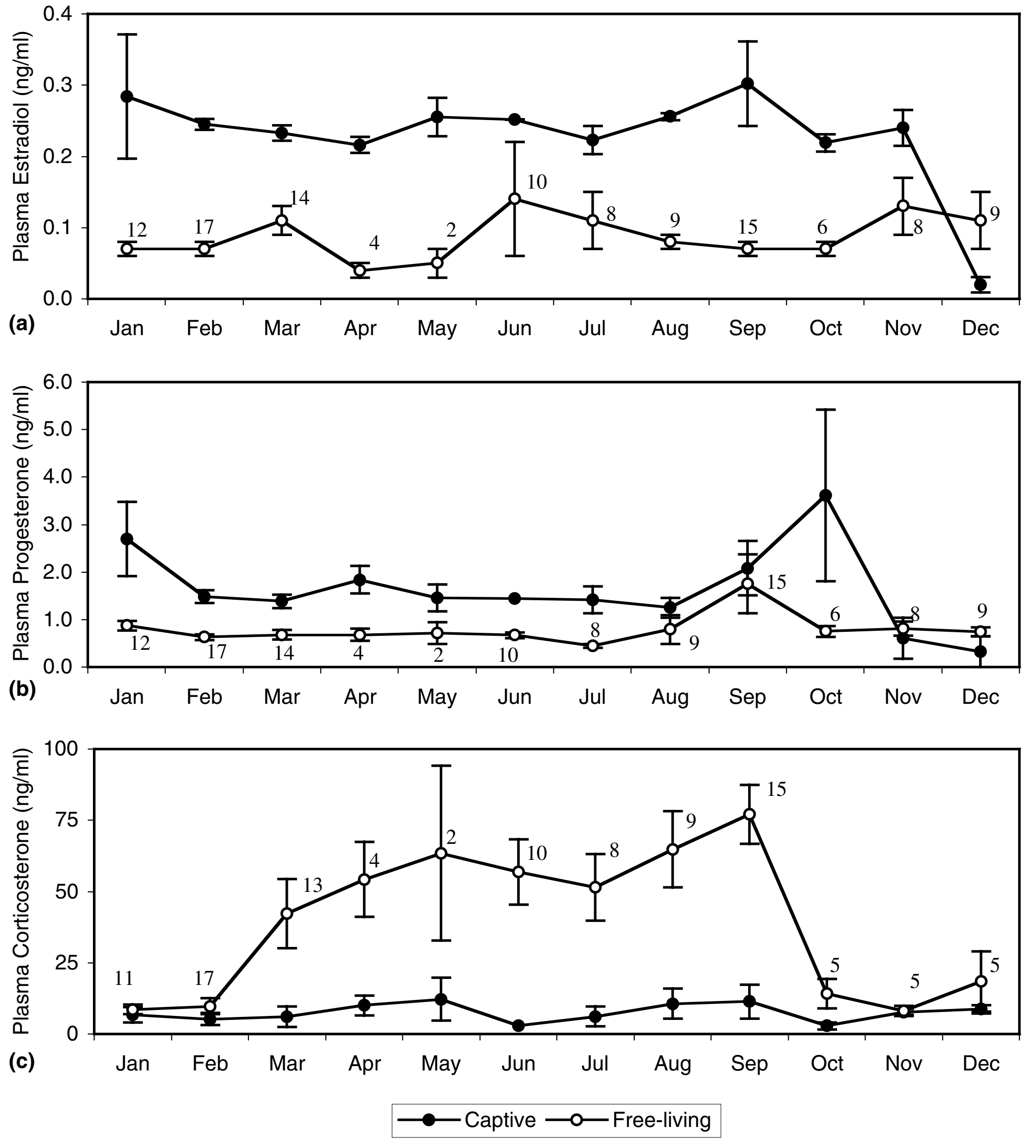

Fig. 3. Annual cycles of: (a) estradiol, (b) progesterone, and (c) corticosterone for female captive and free-living populations of brown tree snakes from Guam. Numbers next to the points are the sample sizes for the free-living group. Sample size for the captive group was seven for all months.

the population are in poor body condition (Wiles et al., 2003). The captive snakes exhibited a sex steroid cycle similar to those previously reported in other snakes (Moore et al., 2000b; Taylor et al., 2004). The lack of a sex steroid cycle in the free-living population, combined with the lack of gametogenesis (Mason et al., unpublished), suggests that most individuals within the population on Guam were not reproductively competent during the period this study was conducted. Depressed body condition and elevated plasma corticosterone levels in the free-living animals suggest that a lack of food resources was placing individuals under chronic stress resulting in suppression of the reproductive system. These results suggest a mechanism, and could possibly serve as a prediction, of a population crash.

Stress and reproduction have classically been thought to be in a reciprocal relationship (Greenberg and Wingfield, 1987). This is especially true when animals are experiencing high levels of stress which can directly suppress reproduction at multiple levels. Studies have looked at the levels of physiological stress, and its results, faced by native species in response to a variety of factors (e.g. Romero and Wikelski, 2001). However, to our knowledge no studies have addressed the level of stress faced by an introduced species despite the fact that, by definition, the pest species is invading a novel area with a host of new challenges.

Multiple lines of evidence suggest that the baseline levels of corticosterone in free-living individuals were above normal seasonal variation and are high enough to elicit an "emergency life history stage" and suppression of reproduction (Wingfield et al., 1998). Corticosterone levels in the range of normal seasonal variation are often positively associated with plasma sex steroid levels in reptiles (Moore and Jessop, 2003; Moore et al., 2000b). In contrast, extremely elevated levels of corticosterone are usually associated with suppressed sex steroid levels in snakes and other reptiles (Moore et al., 2000a, 2001; Guilette et al., 1995). The elevated baseline corticosterone levels we report for the free-living population are similar to the highest levels reported for free-living brown tree snakes when captured and exposed to a variety of stressful stimuli (Mathies et al., 2001). Corticosterone has also been shown to directly suppress courtship behavior in snakes (Moore and Mason, 2001). These lines of evidence suggest that free-living brown tree snakes on Guam were chronically and pathologically stressed during the study period.

In this study, we used two different techniques to obtain and measure plasma steroid levels from snakes: single point samples following decapitation of free-living individuals versus repeated samples from caudal vein of captive individuals. Previous research indicates that rapidly obtaining blood samples does not affect plasma steroid levels (Moore et al., 2001) and different handling techniques have been shown to have little effect on measurements of plasma steroid levels (Taylor and Schuett, 2004). Since all samples in this study were collected within 5 min of capture, the differences in sampling protocols would not have differential effects on the values obtained. Because of these different sampling protocols (single versus repeated samples), we remain conservative and have refrained from direct statistical comparisons of the plasma hormone levels between these populations and in our interpretation of these data. In addition to the handling differences, the plasma samples were assayed for steroids using slightly different protocols. For both protocols the steroids were extracted from the plasma using organic solvents and the extracts were then purified by chromatography. While analyzing the same samples using different techniques can give different results and therefore warrants caution, experimental evidence confirms that purification of the sample using organic extraction and chromatography, as was conducted in both our protocols, removes differences otherwise associated with protocols much more different than the ones we used (Stanczyk et al., 2003).

Differences in body condition and/or population density could explain the differences in plasma hormone levels between the free-living and captive populations of brown tree snakes. Only a few studies in any reptile have investigated the relationships between plasma corticosterone levels and body condition despite their obvious potential interactions (e.g. Romero and Wikelski, 2001; Moore et al., 2000b, 2001; Tokarz et al., 1998). For example, in garter snakes, another colubrid snake, individuals with elevated body condition have significantly lower plasma corticosterone levels than do individuals with depressed body condition (Moore et al., 2000b). High population density has also been associated with elevated plasma levels of corticosterone in reptiles (Elsey et al., 1990). The high population densities of brown tree snakes on Guam have probably resulted in the lack of available food and thus decreased body condition. Studies have documented very low densities of available vertebrate prey species on the island (Fritts and Rodda, 1995).

It is possible that the individuals we caught had abnormally high plasma corticosterone levels and abnormally low plasma sex steroid levels due to a sampling bias. Animals that were in particularly bad condition, low body condition and high plasma corticosterone levels, may have been actively searching for food and thus would have been more visible and caught more readily. However, we feel this is unlikely as we used the same sampling protocol throughout the study and the presence of seasonal variation in corticosterone makes it unlikely that seasonal changes in sex steroids were not noticed because of a sampling bias. It is also worth noting that even during periods of low plasma corticosterone, plasma sex steroid levels were not elevated. With a single exception (estradiol in December), captive snakes had higher monthly means of sex steroids and lower monthly mean levels of corticosterone. Male captive snakes had higher levels of testosterone and lower levels of corticosterone than free-living snakes on Guam (Fig. 2). Female captive snakes had higher plasma levels of progesterone and estradiol as well as lower plasma levels of corticosterone than free-living snakes (Fig. 3). Further, the sex steroid levels we measured in free-living brown tree snakes were similar to those reported by Mathies et al. (2001).

Taken together these studies suggest that the Guam population of brown tree snakes were living under stressful conditions at the time of the study, possibly due to high population densities and reduced food availability, and have suppressed reproduction at multiple levels including steroidogenesis and gametogenesis. The fact that our captive population exhibits very low corticosterone levels, seasonal elevated testosterone levels in males, and courtship behavior in the laboratory supports the idea that what we document in the field is not an artifact of this species but is a biologically relevant phenomena isolated to the Guam population. This introduced population may no longer be growing in size but may have surpassed the carrying capacity of its environment. This knowledge, combined with the applied research methods to manage brown tree snake populations by inhibiting reproduction using chemical and immunological fertility control (Brown Tree Snake Control Committee, 1996) could prove fruitful in ridding the island of the pest and preventing its spread.

Future studies of introduced species should use these techniques, as have been used in conservation efforts on threatened species in the past, as an evaluator and predictor of population status to aid conservation efforts.

Acknowledgements

The collection of field samples was supported by funding from the US Fish and Wildlife Service (USDI 14-16-0009-1577). The laboratory-based research was supported by a National Science Foundation National Young Investigator Award (IBN-9357245), and the Whitehall Foundation (W95-04) to R.T.M. Australian work was supported by an Australian Research Council Grant to J.M.W. and R.T.M. (ARC A0941250) and was conducted under QNPWS Permits T00696; T00713; T00935; T01022, T01248 and 1667; FO/001209/96/SAA; HO/000151/96/SAAF1/000220/98/SAA; Qld DPI Permit 361, 556, 1296 and UQ Animal Ethics Approvals ANAT/338/94/ARC; ANAT/651/94/ARC; ANAT/085/95/ARC; ANAT/645/96/DASH. Radiolabelled steroids were donated by Amersham Pharmacia Biotech, Piscataway, NJ, USA. I.T.M. was supported by a predoctoral Porter Fellowship from the American Physiological Society and a NSF Minority Postdoctoral Fellowship DBI-9904144. M.J.G. was supported by a NIH postdoctoral National Research Service Award (1F32DC00478). Research was conducted under the authority of Oregon State University Institutional Animal Care and Use Committee. All research was conducted in accord with the US Public Health Service 'Policy on Humane Care and Use of Laboratory Animals' and the National Institutes of Health 'Guide to the Care and Use of Laboratory Animals'.

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