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What makes a lizard invasive? Behavioral and neural correlates of invasion success

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WHAT MAKES A LIZARD INVASIVE? BEHAVIORAL AND NEURAL CORRELATES OF
INVASION SUCCESS
LAUREN M. DAVIS

A DEPARTMENT HONORS THESIS SUBMITTED TO THE
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Abstract

To understand what makes an invasive species successful, we must understand the behavioral mechanisms these invaders employ. In this study, I examined traits associated with the “boldness” behavioral syndrome (i.e., aggression, general activity levels, and behavioral flexibility), and the morphology of brain regions associated with those traits. I assessed boldness by conducting a series of four behavioral tests designed to measure aggression towards prey, aggression towards a conspecific, overall activity in an open field test, and flexibility in completing a novel task. I compared these measures in two species pairs: the native green anole (*Anolis carolinensis*; $n = 12$) and the invasive Cuban brown anole (*Anolis sagrei*; $n = 15$), and the native Texas banded gecko (*Coleonyx brevis*; $n = 4$) and the invasive Mediterranean house gecko (*Hemidactylus turcicus*; $n = 8$). I found that the brown anole was “bolder” than the green anole in two of the four behavioral tests conducted, but there was no difference between the two gecko species for any of the behavioral tests conducted. In contrast to my predictions, the native green anole had a larger relative brain mass (a general indicator of behavioral flexibility) and a relative total brain volume than the invasive brown anole. Green anoles also had larger neuron somas in the ventromedial nucleus of the amygdala and the medial cortex (regions associated with the boldness behaviors), contradicting my predictions. However, the Mediterranean house gecko had a larger relative brain mass than the native Texas banded gecko, consistent with the predicted pattern. I also found several within species relationships between behaviors expressed during the trials and the brain regions with which I predicted they would be associated. Together, these results provide one of the first studies of the relationships between brain and behavior in invasion biology.

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Introduction

Invasive species colonize habitats outside of their native range as a result of introduction by human activity or natural range extension. Invasive species may pose threats to ecological processes, reduce biodiversity, damage local economies, and negatively affect human health (Vitousek, D'Antonio, Loope, & Westbrooks, 1996). For example, the presence and behaviors of nonnative organisms can indirectly interfere with the courtship and mating behaviors of native organisms, which can result in the decline of native species populations (e.g., Gamradt, Kats, & Anzalone, 1997). Or, the introduction of novel disease vectors, like invasive mosquitos, can increase disease transmission rates and place strains on public health efforts to eradicate diseases (e.g., Juliano & Lounibos, 2005). Pimentel, Zuniga and Morrison (2005) estimated that economic damages and control efforts associated with the diverse non-native invasive species found in the U.S., including plants, animals, and microbes, cost approximately \$120 billion/year. As a result, humans can undoubtedly benefit from understanding these invasive organisms to prevent their spread and to mitigate their negative impacts.

It is important, then, to identify potential invaders and manage invasive populations. In the past, these efforts have focused largely on understanding the ecological, genetic, and life history characteristics of invasive organisms. Many predictors of invasive ability in plants have been well documented in the literature (see Rejmánek, 2000); however there has been a relatively recent emphasis in research efforts towards understanding consistent predictors of invasive ability in animals. In a meta-analysis designed to identify consistent, independently verified predictors of invasion success across seven different plant and animal taxa, Hayes & Barry (2008) found that climate or habitat match, history of invasion success, and propagule pressure are each consistently correlated with a successful transition from introduction to

establishment within and across taxa. A subsequent study, focusing exclusively on invasion success in reptiles and amphibians, revealed that in addition to climate match and history of invasion success, both genus and family also served as significant predictors of invasion success (Bomford, Kraus, Barry, & Lawrence, 2009). Risk managers and invasion biologists can thus reliably use climate/habitat match, history of invasion success, propagule pressure, and taxonomy to assess the risk of potential invasion events and to develop management plans. However, risk management plans could be improved by focusing on the direct interactions between an invader and its environment during the process of dispersal and spread that can lead to establishment success.

To fully understand the mechanisms underlying invasion success, we need to turn our attention to the behavioral mechanisms employed by invasive species. Understanding the behavioral mechanisms of invasion success enables us to determine how variation in certain behavioral traits influences patterns of dispersal and spread (Holway & Suarez, 1999). For instance, invasive cane toads (*Bufo marinus*) in tropical Australia have accelerated the invasion process by travelling overnight for long distances along roads, allowing them to advance the invasion front much more rapidly than their ancestors that typically travel less frequently with shorter movements (Alford, Brown, Schwarzkopf, Phillips, & Shine, 2009). Moreover, we can use our knowledge of the behavioral mechanisms associated with invasive organisms to create efficient management plans to reduce their spread. Such approaches have been used in the past on the island of Guam to trap the highly invasive brown tree snake (*Boiga irregularis*) by providing artificial visual and olfactory cues mimicking a mouse (Shivik, 1998). However, the application of behavioral studies to conservation biology constitutes a relatively young field of

research, in which we are just beginning to fully understand the behavioral mechanisms underlying invasion success.

Furthermore, to my knowledge no previous studies have examined the relationship between behaviors associated with invasion success and the corresponding brain regions that function in the expression of those behaviors. Thus, the present study is one of the first to directly examine the relationship between behaviors involved in invasion success and brain morphology in a comparative context. In this study, I examined the boldness syndrome in four lizard species (two species pairs, each of which included two related species that vary in invasive ability) to determine which behavioral mechanisms enable invasion success. I also examined the morphology of brain regions associated with those behaviors to determine if correlations between behaviors conferring invasive ability and consistent differences in brain morphology exist.

Behavioral syndromes

One approach to understanding the behavioral mechanisms employed by successful invaders is to examine whether invasive species exhibit consistent behavioral syndromes, or “personalities.” A behavioral syndrome is a suite of correlated behaviors expressed across different contexts (Sih, Bell, Johnson, & Ziemba, 2004a). For instance, the “aggression” syndrome predicts that some individuals consistently display more aggressive behaviors than their conspecifics across different situations. A highly aggressive individual would do well in competitive or antipredator situations, but would perform poorly in a situation such as parental care (Sih, Bell, & Johnson, 2004b). The contexts across which behavioral syndromes occur are broad and variable. An individual can express a behavioral syndrome in different contexts

occurring at the same point in time, in the same context but at different points in time, or in different contexts at different points in time (Sih & Bell, 2008). In other words, behavioral syndromes include the expression of consistent behaviors over time in either similar or different situations.

Behavioral syndromes can provide a useful context for studying invasion biology, especially in a future of increasing global climate and environmental change. The way that individuals of a species interact with their environments through reactions to predators, food resources, habitat, and social or sexual interactions with conspecifics is at least partially controlled by the animals' behavioral syndromes (Real, Reader, Sol, McDougall, & Dingemans, 2007). Consequently, population dynamics and patterns of dispersal are also influenced by behavioral syndromes. Population dynamics and species' patterns of dispersal will undoubtedly change in the future as a result of human-induced environmental changes, and understanding behavioral syndromes in light of invasion ecology can help us determine which species might become successful invaders. These environmental changes include habitat loss and fragmentation, increased human harvesting, and exposure to novel abiotic factors such as chemicals, artificial lights or climate change. Potential behavioral responses to these human-induced changes include responding to novel 'enemies' such as predators, pathogens, competitors, and abiotic stressors; novel resources like food and habitat; and timing of biological events such as migration or reproduction (Sih, Cote, Evans, Fogarty, & Pruitt, 2012). As populations of a species are forced to cope with these human-induced changes, we can expect to see the emergence of new invasive species that are better suited to cope with these unfamiliar, or novel, environments. Thus, an understanding of the behavioral mechanisms employed by species to cope with novel environments produced by human actions, and the ensuing changes in

population dynamics, will enable us to better design and execute management plans for populations of both invasive and threatened species.

Boldness

The boldness syndrome may provide a particularly useful context in which to consider invasion success. I define boldness as the propensity of an individual to explore novel contexts (Rehage & Sih, 2004; Wilson & Godin, 2009). The boldness syndrome correlates aggressiveness, exploratory behavior, and high levels of activity (Sih *et al.*, 2004b), and also entails behavioral flexibility. These behaviors all seem to play an important role in the growth and dispersal of animal populations, which are key events in the establishment of an invasive population. The boldness syndrome has been well documented in numerous diverse taxa, including the fishing spider (*Dolomedes triton*; Johnson & Sih, 2007); the European house cricket (*Acheta domesticus*; Wilson *et al.*, 2010); birds such as the dark eyed junco (*Junco hyemalis thurben*; Atwell *et al.*, 2012) and the greater rhea (*Rhea americana*; de Azevedo & Young, 2006); lizards such as the Namibian rock agama (*Agama planiceps*; Carter, Heinsohn, Goldizen, & Biro, 2012) and the Iberian wall lizard (*Podarcis hispanica*; Rodríguez-Prieto, Martín & Fernández-Juricic, 2010); and several species of fish including the three-spined stickleback (*Gasterosteus aculeatus*; Huntingford, 1976), the small South American cichlid fish (*Nannacara anomala*; Brick & Jakobsson, 2002), and the bluegill sunfish (*Lepomis macrochirus*; Wilson & Godin, 2009).

The boldness syndrome may arise from differences in fitness strategies (i.e., individuals emphasizing future reproductive success vs. current reproductive success), which should result in the evolution of systematic differences in risk-taking behavior. Individuals that prioritize current

reproductive success (i.e., r-selected organisms) should display riskier behaviors across all contexts than individuals with high expectations for future reproductive success (i.e., K-selected organisms) that have to survive in relatively good condition until the time of reproduction. Consequently, these differences in life-history strategy may have, in part, resulted in the persistence and evolution of the boldness syndrome (Wolf, Van Doorn, Leimar, & Weissing, 2007). We might infer that species attempting to maximize current reproductive success have been selected to display riskier/bolder behaviors, which I suggest might confer invasive ability. In conjunction with this idea, Sol *et al.* (2012) demonstrated that high population growth rates improve the likelihood of establishment success by invaders when propagule size is small and when the invaded and native ranges match one another in climate and habitat. These results further support the notion that life-history strategy, in general, may confer invasive ability.

A review by Biro & Stamps (2008) indicated that boldness, activity rates, and aggressiveness are all positively related to food intake rates, productivity (i.e., the generation of new biomass via growth or reproduction), and other life-history traits in a wide range of taxa, suggesting that boldness functions as an adaptation to improve an individual's fitness. For example, a study examining the invasive crayfish *Pacifastacus leniusculus* found a positive correlation between aggressiveness and foraging activity that allows *P. leniusculus* to achieve and maintain high densities, which increases prey consumption and invader dispersal as a result (Pintor, Sih, & Kerbey, 2009). These results suggest that behaviors associated with the boldness syndrome (aggression and activity) likely influence population dynamics and contribute to invasive ability. Understanding if and how boldness influences invasive ability would enable us to make connections between an organism's behavior, fitness, and life history strategy to provide a more holistic view on the characteristics of an invasive organism.

Boldness behaviors and invasive ability

We can use the boldness syndrome as a context for understanding animal personality traits and behavioral mechanisms that likely influence an organism's success during various stages of the invasion process. The process of dispersal throughout a novel environment imposes high risks and costs to the disperser. Accordingly, animals that are bolder, more aggressive, and exploratory often take greater risks to gain greater rewards, in which they prioritize speed over accuracy in decision making while exploring novel environments (Sih & Del Giudice, 2012). Furthermore, success during different phases of the invasion process (i.e., departure, dispersal, and establishment) may depend on different behaviors within the boldness syndrome. For instance, locomotion and spatial orientation abilities likely play a larger role during the dispersal phase of the invasion process, while aggression and social behavior probably have a greater effect during establishment (Cote, Clobert, Brodin, Fogarty, & Sih, 2010).

Within the boldness syndrome, aggression, overall activity levels, and exploratory behavior are all positively correlated with one another, and bold individuals consistently display these behaviors across different contexts over time. I expect that these "boldness" behaviors consistently influence an individual's propensity to explore novel environments, and consequently influence dispersal behavior and invasion success. Below, I focus on these three components of boldness (aggression, overall activity levels, and behavioral flexibility) in relation to invasion success across a variety of taxa to illustrate the connection between the boldness syndrome and invasive ability.

The success of an invasive species in establishing a new population depends on the organisms' interactions with native species through competition and predation, in which highly

aggressive species are often successful invaders. For instance, the invasive freshwater amphipod *Gammarus pulex* actively replaces populations of the native *G. duebeni celticus* through relatively high levels of aggression towards its congener (Dick, Elwood, & Montgomery, 1995). Differences in levels of aggression displayed between *G. pulex* and *G. duebeni celticus* result in differential rates of predation, by which *G. pulex* can replace predated *G. duebeni celticus*. Additionally, a study by Duckworth & Badyaev (2007) showed that the coupling of dispersal and aggression enabled western bluebirds (*Sialia mexicana*) to displace less aggressive mountain bluebirds (*Sialia currucoides*) by natural range extension over the last 30 years. Interestingly, they also observed that aggression rapidly decreased within a few generations of western bluebirds once species replacement was complete. These findings suggest that highly aggressive western bluebirds are selected to disperse to new breeding grounds along the invasion front (while less aggressive individuals remain in the old territory), in which they are successful in displacing native mountain bluebirds. Overall, aggressive behaviors seem to provide a mechanism by which invaders can outcompete native individuals for valuable resources and territories, in addition to causing differential predation on native species. As a result, aggressive behaviors likely confer an advantage to invaders during dispersal and the establishment of new populations.

Multiple studies have indicated that exploratory behavior and high activity rates contribute significantly to the boldness syndrome and may contribute to invasive ability. A study examining the grouping tendencies and exploratory behavior of the invasive delicate skink (*Lampropholis delicata*) and the non-invasive garden skink (*L. guichenoti*) found that the invasive delicate skink was significantly more exploratory and more likely to find pathways through a novel environment to resources than the non-invasive garden skink (Chapple,

Simmonds, & Wong, 2011). Another study conducted by Rehage & Sih (2004) examined the link between dispersal, boldness, and invasiveness in four *Gambusia* (mosquitofish) species that vary in invasive ability and found that the highly invasive *G. affinis* and *G. holbrooki* dispersed more quickly and traveled a greater distance from an experimental stream than their congeners, *G. hispaniolae* and *G. geiseri*. Furthermore, they found that *G. affinis* and *G. holbrooki* were significantly bolder (measured as the proportion of fish out of refuge) than the non-invasive *G. hispaniolae* but not *G. geiseri*, providing partial support for their prediction that invasive *Gambusia* would be bolder than their non-invasive congeners. These results generally suggest that successful invaders are more exploratory and more active in novel environments. Moreover, activity levels and exploratory behavior serve as components of a broader life-history strategy that links these behaviors to feeding rate, metabolic expenditures, and predation risk, which have consequences for individual fitness and for population dynamics (Sih *et al.*, 2004a). As such, the examination of exploratory behavior and activity levels in invasive organisms could reveal valuable information about behavioral mechanisms that contribute to invasion success.

In novel environments, invaders are presented with new challenges of resource acquisition, predator defense, and reproduction that likely differ from their native habitat. Individuals that can readily learn to cope with these new challenges (i.e., behaviorally flexible individuals) can modify their behavior to accommodate a new environment, giving them an advantage in establishing new populations. Many studies exploring the relationship between behavioral flexibility and survival/population establishment in novel environments focus on innovation frequency in behavior and cognitive ability (Sol, Duncan, Blackburn, Cassey, & Lefebvre, 2005; Sol & Lefebvre, 2000; Lefebvre, Reader, & Sol, 2004). Accordingly, relative forebrain size is frequently considered as a metric of behavioral flexibility, operating under the

assumption that a larger neural substrate provides a greater information-processing capacity (Sol, Timmermans & Lefebvre, 2002; Sol *et al.*, 2012).

In an examination of 69 introduced bird species, Sol *et al.* (2002) found that species with relatively larger brains (measured as total brain mass) and a higher frequency of foraging innovations were more likely to succeed in the introduction phase of the invasion process than species with smaller brains and a lower frequency of foraging innovations. Furthermore, a later study conducted by Sol *et al.* (2005) found that innovation frequency was positively associated with brain size among avian families, and that innovation frequency was also positively associated with invasion potential. They ultimately concluded that large brains appear primarily to help birds respond to novel conditions by enhancing their cognitive skills, and that large/elaborated brains function to deal with changes in the environment. Likewise, Amiel, Tingley & Shine (2011) have documented the relationship between larger relative brain size and the establishment success of human-introduced amphibians and reptiles at the family level: invasion potential increased with increasing average residual brain mass per family. These studies provide strong evidence that increased behavioral flexibility conferred by larger brains and improved cognitive ability help invaders establish new populations in novel environments.

Brain morphology of bold behaviors

To my knowledge, this is the first study to examine relationships between brain morphology and invasive ability. Consequently, there is relatively little information in the literature regarding which brain regions might play an important role during the invasion process. I propose that regions functioning in invasive ability will be those that regulate and function in the expression of behaviors correlated by the boldness syndrome.

Specifically, brain regions involved in fear and aggression, behavioral flexibility and exploratory behavior, and social display behaviors all likely contribute to the expression of the boldness syndrome and possibly underlie invasion success. In the process of exploring a novel environment, spatial learning undoubtedly plays a large role in an organism's ability to forage and learn territorial boundaries. Similar to birds and mammals, the hippocampal formation of the reptilian brain, the medial cortex (CxM), is critical to spatial cognition and building relational memory representations of allocentric space that enable the reptile to learn their surrounding environment (Rodríguez *et al.*, 2002). A greater capacity for spatial memory would enable invaders to readily establish new populations in novel environments.

As discussed above, elevated levels of aggression can also confer greater invasive ability by enabling invaders to outcompete and displace native species. Across several vertebrate taxa, the Social Behavior Network (medial amygdala, lateral septum, preoptic area, anterior hypothalamus, ventromedial hypothalamus, and the periaqueductal gray of the midbrain) activates and regulates various social behaviors (Newman, 1999) that may play an important role during the invasion process. Specifically, the amygdala and the preoptic area (POA) both regulate aggressive behaviors that are critical to the invasion process. The amygdala plays an important role in territorial aggression and defensive or flight behaviors (Newmann, 1999). In many reptiles, the amygdala is homologous to posterior dorsal ventricular ridge (PDVR), in which the ventromedial nucleus (VMN) is most prominent in green anole lizards (Greenberg, Scott & Crews, 1984). The preoptic area (POA) regulates antagonistic display behaviors (Wheeler & Crews, 1978), which likely plays an essential role during the social integration of an invader during the establishment phase of the invasion process.

If we expect native and invasive individuals to differ in their expression of boldness behaviors, then we might also expect them to differ in the morphology of brain regions associated with those behaviors (e.g., the CxM, VMN, or POA). Consequently, individuals that display more bold behaviors would have larger brain regions that control those behaviors than individuals who display fewer or less bold behaviors. As a parallel example, adult male zebra finches (*Poephila guttata*) show a marked increase in the volumes of telencephalic nuclei that are involved in song learning behaviors (HVC, RA, and area X) in comparison to age-matched females who do not sing (Bottjer, Glaessner, & Arnold, 1985), indicating that frequently used brain regions tend to be larger in volume. Furthermore, frequently used brain regions typically possess larger, denser neurons that reflect a greater number of afferent neural projects to that region, enabling the region to process information more efficiently (Wade, 2011). As such, we might expect invasive individuals to have larger, denser neurons in brain regions associated with the expression of boldness behaviors.

Study system

This study aimed to determine the behavioral mechanisms associated with invasive ability across two pairs of species: the invasive Cuban brown anole (*Anolis sagrei*) and the native green anole (*Anolis carolinensis*), and the invasive Mediterranean house gecko (*Hemidactylus turcicus*) and the native Texas banded gecko (*Coleonyx brevis*).

Lizards are appropriate study organisms for this study because they are abundant in the field and are relatively easy to catch and handle; they can also be housed in an animal care facility with relative ease and minimal cost. Furthermore, lizards display a broad array of behaviors that enable them to adapt to various environments. The green anole (Family:

Dactyloidae) is a trunk-crown anole (inhabiting the canopies and upper portions of tree trunks) and is endemic to the southeastern United States, ranging from Florida to Texas with introduced populations occurring in the Hawaiian Islands (Lovern, Holmes & Wade, 2004). The brown anole (Family: *Dactyloidae*) is a trunk-ground anole (perching on lower portions of tree trunks and the ground surrounding trees) and is endemic to Cuba and the Bahamas. However, the brown anole was introduced to the Florida Keys in the late 1800s, accelerating its range extension northward throughout Florida in the 1970s. Today, the brown anole has successfully established numerous populations in Florida, Georgia, Texas, southern California, Hawaii, Grand Cayman, Taiwan, and Grenada (Kolbe *et al.*, 2004). Recent studies have indicated that the co-occurrence of native green anoles and invasive brown anoles in natural urban environments causes green anoles to perch higher in trees and decreases the population density of green anoles (Edwards & Lailvaux, 2012), indicating that the presence and behaviors of invasive brown anoles can directly impact native green anole populations.

The Texas banded gecko (Family: *Gekkonidae*) inhabits semi-arid, rocky terrains and is endemic to southwest Texas and the Chihuahuan desert of northern Mexico (Dixon, 2009). The Mediterranean house gecko (Family: *Gekkonidae*) is endemic to the Mediterranean basin (southern Europe and northern Africa), but records indicate that it first appeared in Key West, Florida in 1910. This species is highly invasive and has established populations all across the southern U.S. (Alabama, Arizona, Arkansas, southern California, Florida, Georgia, Louisiana, Maryland, Mississippi, Missouri, New Mexico, Oklahoma, South Carolina, Texas, and Virginia), as well as parts of Panama, Mexico, and Cuba (Rödder & Lötters, 2009). The Mediterranean house gecko's invasion success in Texas may be attributed to low predation pressures, minimal interspecific competition, and its life history which maximizes survival at all ages (Selcer, 1986).

Using a set of four behavioral tests to measure aggression, activity, and behavioral flexibility, I tested for differences between the behaviors of invasive and native individuals and for correlations between behavior and brain morphology. I measured behaviors specifically associated with the boldness syndrome (i.e., aggression, activity rates, and exploratory behavior). Then I measured brain mass and examined the morphology of brain regions associated with behaviors comprising the boldness syndrome (amygdala, medial cortex, and preoptic area) to determine if species that vary in invasive ability also differ in behavioral displays and brain morphology. I hypothesized that highly invasive species display more bold behaviors and have larger, denser neurons in the associated brain regions than less invasive species. Specifically, I predicted that the invasive lizards would display (i) higher levels of overall activity in an open-field test, (ii) greater behavioral flexibility and exploratory behavior in a divider challenge, (iii) more aggression towards prey, and (iv) higher levels of aggression towards conspecifics. I also predicted that invasive lizards would have a larger brain-to-body mass ratio, and would have a larger VMN, CxM, and POA containing larger, denser neurons than the native lizards. In frequently used brain regions, larger, denser neurons indicate that the region efficiently processes more information as the result of a greater number of neural afferent projections to the region (Wade, 2011). Furthermore, I predicted that correlations exist between the display of bold behaviors and the morphology of the corresponding brain regions within each species.

Materials and Methods

Study Organisms

To assess relative differences among native and non-indigenous invasive lizards in “bold” behaviors and brain morphology, I compared the invasive Cuban brown anole to the native green anole and the invasive Mediterranean house gecko to the native Texas banded gecko (Figure 1). Lizards were caught either by hand or with a noose, and only adult male lizards were included in the study. I focused on males in this study because male lizards generally display higher levels of activity and aggression. Twelve green anoles were collected in June 2013 from their native range in Bexar County, Texas (Snout Vent Length (SVL) = 55-68 mm), and 15 brown anoles were caught in May 2013 from an invaded range in Volusia County, Florida (SVL = 51-63 mm). Four Texas banded geckos were caught in June 2014 from their native range in Brewster County, Texas (SVL = 46-49 mm), and eight Mediterranean house geckos were caught in June 2014 from an invaded range in Bexar County (SVL = 47-55 mm). All lizards were acclimated for at least 10 days and no more than 16 days in Trinity University’s Animal Care Facility before beginning behavioral testing. All procedures used in this study were approved by Trinity University’s Animal Research Committee.

Animal Care

Lizards were housed according to the Herpetological Animal Care and Use Committee (HACC) guidelines and a protocol for care and housing of *Anolis* lizards (Sanger, Hime, Johnson, Diani & Losos, 2008). Lizards were housed individually in clear, plastic cages (43 x 22 x 30 cm³) containing R’zilla terrarium liner (Zilla, Franklin WI 53132) and either a wooden perch for anoles (to provide a vertical surface on which to perch because anoles are arboreal) or a

piece of crumpled packing paper for geckos (to provide refuge during the day because geckos are nocturnal).

Anole lizard cages were partially exposed to 15-20W full spectrum UV heat lamps (Fluker's Sunspot) on a 13:11 L:D cycle, and partially covered using a thin piece of Styrofoam to provide shade. Following natural weather patterns in San Antonio, temperatures in the animal care facility where the anoles were housed ranged from 20.6 – 40.4°C, and humidity ranged from 16 – 87%. The geckos were housed without a UV heat lamp, but the facility lights were set on a 13:11 L:D cycle. Temperatures in the (climate controlled) animal care facility ranged 26.0 – 28.5°C and humidity ranged 34 – 69%. All lizards were watered daily and fed a diet of two crickets dusted in calcium powder every other day (with exception for specific days of behavioral testing, described below).

Behavioral Tests

I conducted a series of four behavioral tests with each lizard: a test measuring aggression towards prey, a test measuring aggression towards a conspecific (two replicates per individual), an open-field test measuring overall activity, and a divider challenge measuring behavioral flexibility. With the exception of the aggression-towards-conspecifics test (described below), each behavioral test was conducted once per individual. Behavioral tests were conducted on anoles between the hours of 0900 and 1700 in an outdoor grassy area near the animal care facility. Observations for each test were made from behind a blind at a distance of approximately 5 m from the testing arena to prevent the observer's presence from affecting the behavior of the lizard. Behavioral tests were conducted on geckos between the hours of 2100 and 0100 inside the animal care facility using Maxxima LED red-light flashlights (*MF-37*, Hauppauge NY 11788)

for observation in the dark. Individual lizards were never tested more than twice in one 24 h period, in order to minimize the effects of human handling on the behaviors displayed by lizards during testing, and to reduce the effects of one test on another. In total, these tests were conducted within a one-week period.

The order of trials differed arbitrarily between the anole and gecko groups, but the order of trials was consistent within each group. For anoles, I conducted the aggression-towards-prey tests first, followed by an open-field test. I then conducted a divider challenge and the first replicate of the aggression-towards-conspecifics test on the second day, and the second replicate of the aggression-towards-conspecifics test was conducted on the third day. The aggression-towards-conspecific test was likely the most stressful test because of the forced encounter between males, so replicates of the test were conducted on separate days to reduce the effects of stress on the lizard's behavior and to minimize the effects of one aggression trial on the other. For geckos, the open field test was conducted on the first night. The first replicates of the aggression-towards-conspecifics trial were conducted on the second night, and half of the geckos were tested in the divider challenge. The second replicates of the aggression-towards-conspecifics trial were conducted on the third night, and the other half of the geckos were tested in the divider challenge. The aggression-towards-prey trial was conducted last, concluding behavioral testing on the geckos.

I measured aggression towards prey by timing the latency for a lizard to catch a cricket (Wilson & Godin, 2009). Prior to testing, all lizards were maintained on two-cricket/day diet (greater than the standard diet for lizards in captivity; Sanger *et al.*, 2008) for the four days preceding the trial to ensure that the lizards were not hungry at the time of testing. This allowed me to measure aggression without hunger serving as a confounding variable. For this test, lizards

were acclimated for 10 min under an opaque shelter (24 oz. Ziplock container covered by duct tape) on one side of the lizard's home cage (43 x 22 x 30 cm³), and a cricket was hidden under a small paper cup on the opposite side of the cage. After the acclimation period, both containers were removed and the latency period for the lizard to catch the cricket was recorded. The trial ended when the lizard caught the cricket, or after 30 min (whichever occurred sooner). In trials that reached 30 min, I assigned that individual a maximum latency score of 1800 sec.

I measured overall activity levels and exploratory behavior among individual lizards by conducting an open-field test (Trník & Albrechtová, 2011), in which a single lizard was placed in a mesh arena (63 x 39 x 37 cm³) with a grid drawn on five of the six walls of the arena. The lines of this grid served as boundaries that allowed us to quantify lizard movement during the trials. The sixth wall of the arena was made of clear plastic and functioned as the roof of the arena in this trial to limit the lizard's movements to the five mesh walls. The floor of the arena (where the lizard began the trial) contained a grid of 20 squares, in which the six squares in the center of the grid were demarcated from the surrounding 14 squares (along the perimeter of the arena) by red lines to create an interior zone and a periphery zone (Figure 2). Before beginning a trial, the lizard was placed under an opaque shelter (as above) in the center of the six interior squares and allowed to acclimate for 10 min. Upon removing the opaque shelter, I observed the lizard for 20 min and recorded the total number of boundaries crossed by the lizard during the trial and the amount of time it spent in the six interior squares, the 14 periphery squares and on the walls of the arena. I only counted a boundary as crossed when the lizard had moved all four limbs across the boundary line. A lizard's total number of boundaries crossed and time spent in the interior squares, as opposed to in the periphery squares or on the walls, were used as metrics of boldness. Lizards that crossed more total boundaries were considered to be more active, and

lizards that spent more time in the six interior squares were considered to be bolder than lizards that spent more time in the periphery squares or on the walls. While open field tests serve as good systems for studying fear and anxiety in animals, we can also measure boldness as a metric of time spent out of refuge (Rehage & Sih, 2004), or as time spent out in the open as opposed to on the walls.

I conducted a divider challenge to measure behavioral flexibility, in which I timed the latency for a lizard to cross an unfamiliar divider to reach the opposite side of the arena, which contained a set of valuable resources (Chappel *et al.*, 2011). For this test, a wire mesh divider was attached to a mesh arena ($63 \times 39 \times 37 \text{ cm}^3$) to divide the arena into two sections, separating one third of the cage from the other two-thirds of the cage. The lizard began the trial on the smaller side of the arena that did not contain any resources, while the larger side of the arena contained a cricket and either a perch (for anoles) or a crumpled piece of paper (for geckos) which was taken directly from the lizard's home cage and served to incentivize the lizard to cross the divider. At the bottom of the divider, a portion of PVC tube (7.62 cm long and 7.0 cm wide) was inserted to create a tunnel connecting the two sides of the arena. Furthermore, a 5 cm space was left between the roof of the cage and the top of the divider so that the lizard could climb over the divider to the other side of the arena (Figure 3). Before beginning the trial, the lizard was placed under an opaque shelter (as above) on the small side of the arena and allowed to acclimate for 10 min. Upon removing the container, the lizard was observed for 20 min, or until it crossed the divider. I recorded the lizard's latency-to-cross and the method for crossing to the other side (crawling through the tube vs. climbing over the divider). If the lizard did not cross the divider, then its latency to cross was recorded as 1200 sec.

Lastly, I measured aggression towards a conspecific by counting aggressive displays in arena trials in which two lizards are given the opportunity to challenge each other for a single perch (for anoles) or a piece of crumpled paper (for geckos; Lailvaux, Herrel, VanHooydonck, Meyers & Irschick 2004). Lizards were randomly paired and were not matched for size prior to testing, so each lizard completed two aggression-towards-conspecific tests to obtain an average measure of aggression for each lizard. For this test, two lizards were acclimated for 10 min under an opaque shelter on opposite sides of a mesh arena ($63 \times 39 \times 37 \text{ cm}^3$) containing a perch in the middle (Figure 4). Following the acclimation period, both shelters were removed and each lizard was observed for 10 min. I recorded the latency to the first display of aggression and all other instances of aggressive displays. The number of pushups and dewlap extensions performed were counted for anoles, and the number of head raises, tail wags, aggressive postures, pushups, head jerks, back arches, and licks were recorded for geckos (Marcellini, 1977). In the event that the two lizards locked jaws, the trial was ended immediately and the two lizards separated from each other.

Brain morphology

To assess relative differences among native and non-native invasive lizards in brain morphology, I examined overall brain size and the morphology of three nuclei associated with the bold behaviors: the ventromedial nucleus (VMN), the pre-optic area (POA), and the small cells of the medial cortex (CxM,sc). Upon completion of all behavioral tests, I measured the snout-vent length (SVL) of each lizard to the nearest 0.5 mm using a ruler and massed every lizard to the nearest 0.1 g using a Pesola scale. Lizards were euthanized via rapid decapitation to collect brain tissues. Immediately after removal from the skull and handling tissues only by the

brainstem, each brain was massed on weigh paper using a scale, flash frozen in cold isopentane, and stored at -80°C until further use. As an indicator of behavioral flexibility, I measured the average brain-to-body mass ratio for each species (Amiel *et al.*, 2011; Sol, Bacher, Reader & Lefebvre, 2008).

Each frozen brain was sectioned coronally into four alternate series at $20\mu\text{m}$ using a Leica cryostat, and mounted onto SuperFrost Plus (Fisher Scientific; Hampton, NH) slides that were stored at -80°C . Two series of the sectioned tissues (i.e., at $40\mu\text{m}$ intervals) were dehydrated, cleared with xylene, and stained using thionin. Thionin stains allow us to visualize only the neuron soma by the presence of a clearly defined nucleolus. Stained slides were coded prior to examination to avoid researcher's bias during data collection. I then examined the cross-sectional area of the stained neuron somas and their density in the amygdala (VMN), small cells of the medial cortex (CxM,sc), and preoptic area (POA) using an Eclipse Ni-U Nikon microscope with an OS-Fi2v color camera at 400X magnification and the NIS Elements software. I determined the average soma area of each individual by measuring 25 arbitrarily chosen neuron somas in the rostrocaudal center of the brain for both the left and right hemisphere of the brain, for a total of 50 neurons per region. These values were averaged for statistical analysis. I calculated the neuron density of the VMN and the POA by counting the number of distinct neurons within an $80 \times 80\mu\text{m}$ area of the same section from which I measured the soma area. I also determined the total volume of the VMN, POA, and CxM in the right hemisphere for each individual by measuring the cross sectional area of the region at $80\mu\text{m}$ intervals and multiplying the volume of each measured section by the intersection distance (Figures 5 & 6).

To standardize brain size as a function of lizard size, I measured brain mass and total brain volume. I used the Cavalieri method to estimate total brain volume from a systematic-

random series of 9-32 thionin stained sections, measuring the area of every eighth 20 μm section per individual (Mouton, 2002). I then multiplied the volume of each brain section measured by the intersection distance to calculate the volume of the whole brain.

Statistical Analyses

A series of Kolgoromov-Smirnov tests indicated that the brain and behavioral data in this study were non-normal. Thus, I conducted species comparisons for each behavioral test and data on metrics of brain morphology using Mann-Whitney U tests. To reduce the number of variables in subsequent analyses, I calculated principal component (PC) scores from a PC analysis on variables measuring aggression towards conspecifics, retaining those axes with an eigenvalue greater than 1 to conduct species comparisons. Correlational analyses were conducted within each species to test for significant relationships between the behavioral measures of boldness and the measures of neuron size and density in the brain regions associated with those specific behaviors. Prior to analysis of brain measures, I conducted correlation analyses within species to determine relationships between total brain volume and region volume, neuron soma size, and neuron density in the VMN, POA, and CxM. Where correlations existed, I standardized measures by total brain volume for further analysis. The alpha level for all tests was set at 0.05, and I considered p -values of less than 0.10 to be marginally significant.

Results

Preliminary statistical analysis

In Mediterranean house geckos, total brain volume was positively correlated with CxM volume ($r(8) = 0.884, p < 0.01$), and total brain volume was positively correlated with VMN density in Texas banded geckos ($r(4) = 0.988, p = 0.012$). No other significant relationships existed between total brain volume and the region volume, neuron size, or neuron density of the VMN, POA, or CxM within species.

I conducted a principal component analysis to reduce the number of variables analyzed in correlational analyses using behaviors displayed during the aggression-towards-conspecifics trials. For anoles, the first two PC axes extracted from display behaviors observed during the aggression-towards-conspecifics test explain 81.1% of the variance in these behavioral data (Table 1). The first axis loads strongly and positively for pushups and dewlaps, and the second axis loads strongly and positively for pushups and the time to first display. For geckos, the first four PC axes extracted from display behaviors observed during the aggression-towards-conspecifics test explain 90.1% of the variance (Table 2). The first axis loads strongly and positively for head jerks and licks, and it loads strongly and negatively for head raises and postures. The second axis loads strongly and positively for head raises, postures, and licks, and it loads strongly and negatively for tailwags. The third axis loads strongly and positively for the time to first display, and it loads strongly and negatively for back arches. Lastly, the fourth axis loads strongly and positively for the time to first display.

Behavior

In the open field test, brown anoles crossed more total boundaries than green anoles ($U = 47, p = 0.037$; Figure 7). However, brown and green anoles did not differ in the amount of time they spent in the interior squares of the open field test arena. Furthermore, I did not find any differences between Mediterranean and Texas banded geckos in the total number of boundaries crossed or the amount of time spent in the interior squares of the open field test arena.

Green anoles caught crickets in the aggression-towards-prey test marginally faster than invasive brown anoles ($U = 49.5, p = 0.076$), however there were not any differences between native and invasive geckos, nor were there any differences between native and invasive species in the latency period to cross a novel divider in the divider challenge. However, marginally more brown anoles (71.43%) actually crossed the divider than green anoles (33.33%; $\chi^2 = 3.77, p = 0.052$). Mediterranean house geckos and Texas banded geckos did not differ in the proportion of lizards that actually crossed the divider. Lastly, I did not find any differences between native and invasive anoles in the time to first display or the number of aggressive displays performed during the aggression-towards-conspecifics test. However, Texas banded geckos performed marginally more tail wags during the aggression-towards-conspecifics test than Mediterranean house geckos ($U = 5, p = 0.073$). There were no other differences in the number of aggressive displays performed or the time to first display between Texas banded and Mediterranean house geckos.

Brain

Green anoles had a larger relative brain mass than brown anoles ($U = 19, p < 0.01$; Figure 8), as well as a larger relative total brain volume ($U = 11, p < 0.01$; Figure 9). Green anoles also had larger neuron somas in the POA ($U = 20, p = 0.043$; Figure 10) and the CxM ($U = 21, p = 0.029$; Figure 11) than brown anoles. Green anoles also had marginally larger neuron somas in

the VMN ($U = 15, p = 0.091$) than brown anoles, and marginally larger volume of the CxM ($U = 22, p = 0.065$). However, green and brown anoles did not differ in the length of the third ventricle, the volume of the VMN or POA, nor the neuron density of the three regions examined.

Mediterranean house geckos had a larger relative brain mass than Texas banded geckos ($U = 2, p = 0.016$; Figure 8), as well as a larger relative total brain volume ($U = 0, p < 0.01$; Figure 9). Texas banded geckos also had marginally denser neurons in the POA than Mediterranean house geckos ($U = 0, p = 0.095$). Mediterranean house geckos and Texas banded geckos did not differ in the neuron soma density of the VMN or CxM, nor did they differ in the neuron soma size or volume of the three examined regions.

Brain and behavior

To test whether the morphology of brain regions associated with particular bold behaviors varied in association with those behaviors within each species, I ran a series of correlation analyses between measures of brain morphology and behavior. In the open field test, I predicted that measures of the brain regions associated with spatial orientation (CxM,sc) would be positively associated with the total number of boundaries crossed, and that lack of fear (VMN) would be positively associated with the amount of time spent out in the open. In green anoles, the VMN volume was positively related to the amount of time spent in the interior squares of the open field test arena ($r = .711, p = 0.048$; Table 3), and VMN soma size was marginally, negatively related to the total number of boundaries crossed. In brown anoles, there was a marginally negative relationship between the VMN soma density and the amount of time spent in the interior squares of the open field test arena ($r = -0.54, p = 0.089$), and there was a negative relationship between the CxM,sc soma size and the amount of time spent in the interior

squares of the open field test arena ($r = -0.643$, $p = 0.045$; Table 3). In Texas banded geckos, there was a negative relationship between the VMN soma size and the total number of boundaries crossed during the open field test ($r = -0.991$, $p < 0.01$; Table 4). There were no significant relationships between open field test behaviors and VMN or CxM morphology within Mediterranean house geckos.

In the divider challenge, I predicted that there would be a positive association between the latency to cross the divider and behavioral flexibility (relative brain mass), as well as with spatial orientation (CxM). There was a positive relationship between CxM volume and the latency period to cross the divider for Mediterranean house geckos ($r = 0.871$, $p < 0.01$; Table 5). However, I found a positive correlation between CxM volume and total brain volume in Mediterranean house geckos, in which CxM volume and that latency period were no longer significantly correlated when controlling for total brain volume. There were no other significant relationships between the latency to cross the divider and relative brain mass or the morphology of the CxM within green anoles, brown anoles, or Texas banded geckos.

In the aggression-towards-conspecifics test, I predicted that there would be a positive association between the time to first display and aggression (VMN), as well as between the number of aggressive displays performed and antagonistic display behavior (POA). There was a marginally negative relationship between VMN soma size and PC2 within green anoles. There was a negative relationship between PC1 and POA neuron density for brown anoles ($r = -0.802$, $p = 0.030$; Table 6). In the aggression-towards-conspecifics test for Mediterranean house geckos, there was a positive relationship between PC2 and the POA soma size ($r = 0.822$, $p = 0.023$) and a positive relationship between PC1 and POA neuron density ($r = 0.942$, $p = 0.016$). There was also a negative relationship between PC4 and the VMN soma size ($r = -0.789$, $p = 0.020$), and

there was a negative relationship between PC3 and POA volume ($r = -0.890$, $p = 0.043$). Lastly, there was a marginally positive relationship between VMN soma density and PC4 within Mediterranean house geckos ($r = 0.687$, $p = 0.060$; Table 7). There were no significant associations between behaviors measured during the aggression-towards-conspecifics challenge and the morphology of the VMN or POA within Texas banded geckos.

Lastly, I predicted that there would be a positive association between the latency to catch a cricket in the aggression-towards-prey challenge and aggression (VMN) within species. However, I did not find any significant associations between these variable in any of the four species.

Discussion

This study is one of the first of its kind to explore the behavioral and neural mechanisms associated with invasive ability. I predicted that invasive lizard species would display bolder behaviors than their native counterparts, and that they would also have a larger VMN, POA, and CxM, each containing larger, denser neurons. I found that invasive brown anoles were more active in the open field test than native green anoles and that more brown anoles completed the divider challenge than green anoles, providing partial support for my predictions. However, I did not find any other differences in behavior between anoles or between geckos. Thus, high overall activity levels and exploratory behavior may be important for anoles in the invasion process (especially in the process of dispersal; Cote *et al.*, 2010), but aggression may have less of an effect.

I also found that native green anoles had a larger relative brain mass and relative total brain volume than the invasive brown anoles, contradicting my predictions. Furthermore, native green anoles had larger neuron somas in the POA and CxM_{sc} than the invasive brown anoles, also contradicting my predictions. The green anole has successfully established populations on the Hawaiian (Lovern *et al.*, 2004) and Ogasawara Islands (Toda, Takahashi, Nakagawa, & Sukigara, 2010), but it has yet to expand its range outside of these regions. This semi-invasiveness could possibly explain the lack of differences observed in the aggression behavioral trials and the trends observed in brain morphology. Yet, in agreement with my predictions, the invasive Mediterranean house geckos had a larger relative brain mass and relative total brain volume than the native Texas banded geckos.

Thus, I found some general support for my hypothesis that invasive lizards would have different brain morphologies in comparison to their native counterparts in the direction that I

predicted for geckos but in the opposite direction than I predicted for anoles. It is likely worthwhile to consider whether the brain regions in question regulate behavior through excitatory or inhibitory signaling. For instance, in the mammalian brain, local inhibitory circuits regulate fear memory acquisition in the amygdala (Ehrlich *et al.*, 2009). Consequently, larger, denser neurons in a certain brain region do not necessarily translate to an up regulation of behaviors associated with that region, which could potentially explain some of the opposite trends observed between brain morphology and behavior within lizard species in this study.

I found several significant relationships between behavior and the brain regions I predicted would be associated with these behaviors within species. In the open field test, I found relationships between brain morphology and behaviors expressed during the test (amount of time in interior squares and total boundaries crossed) for both native green and invasive brown anoles. Native green anoles possessing a larger VMN spent more time in the interior squares of the open field test arena (an indicator of boldness). Additionally, green anoles with large neuron somas in the VMN crossed fewer total boundaries during the open field test than individuals with smaller VMN somas. Invasive brown anoles with larger CxM,sc somas spent less time in the interior squares of the open field test arena. While brown anoles were more active than green anoles overall during the open field test, I did not find any correlations between overall activity levels, neuron soma size or density, or volume of the VMN or CxM within brown anoles. It is possible that another brain region not included in this study, such as the nucleus accumbens, plays a greater role in regulating overall activity levels, which likely plays an important role in the invasion process. Past studies have revealed that an injection of dopamine to the mouse nucleus accumbens initiates locomotor responses in an open field test (Mogenson, Jones, & Yim, 1980).

As such, the nucleus accumbens could play an important role in regulating overall activity levels and initiating dispersal responses.

Furthermore, I found that native Texas banded geckos with larger VMN somas were less active overall during the open field test than conspecifics with smaller VMN somas, indicating that the VMN plays a role in the overall activity levels or exploration speed in banded geckos. I did not find any significant relationships between the morphology of the VMN or CxM and behaviors expressed during the open field test in Mediterranean house geckos.

There was also no correlation between the amount of time lizards spent in the interior squares of the open field test arena and the total number of boundaries crossed during the trial, indicating that an individual's boldness response to the novel environment did not significantly affect its overall level of activity throughout the trial. Presumably, individuals who spent more time in the interior squares of the open field test arena at the beginning of the trial were responding boldly, as opposed to remaining motionless out of fear. Furthermore, from my own observations, lizards that spent time in the interior squares of the open field test arena spent time scanning the environment and were not completely frozen.

In the divider challenge, I found that Mediterranean house geckos with a larger CxM took longer to complete the divider challenge than individuals with a smaller CxM, contradicting my prediction that spatial orientation mediated by the CxM would be positively associated with exploratory behavior.

Lastly, I also found several within species relationships between the morphology of the VMN or the POA and the principal components of the aggression-towards-conspecifics challenge. I found that brown anoles with denser neurons in the POA scored lower on PC1 than brown anoles with less dense neurons in the POA, and that green anoles with larger neuron

somas in the VMN scored lower on PC2. Additionally, I found several relationships between aggressive behaviors displayed by Mediterranean house geckos towards conspecifics and the morphologies of the VMN and POA. I found a negative relationship between VMN soma size and PC4 of the principle component analysis on the aggressive behaviors displayed by geckos, and a negative relationship between POA volume and PC3. I also found positive relationships between house gecko POA soma size and PC2, as well as between POA soma density and PC1. Perhaps, larger denser neurons in the house gecko POA are sufficient to regulate behaviors independently of POA volume, explaining the opposite trends between neuron size, density, and region volume observed here. Clearly, the relationships between aggression, display behavior, and brain morphology in Mediterranean house geckos are complex.

It is likely that the boldness behaviors are governed by shared neural mechanisms in different regions of the brain, making it difficult to parse out the specific relationships between behavior and brain morphology. Specifically, structures of the Social Behavior Network share cognitive resources that result in an overlap of the circuitry responsible for certain behaviors (Newman, 1999). Thus, it is possible that individual differences in correlated behaviors exist because of individual differences in the neural mechanisms governing those behaviors (Sih & Del Giudice, 2012). I suggest that variation in the expression of aggressive behaviors of the boldness syndrome may result from variations in the structure of the VMN and POA due to the overlap of their circuitry in the Social Behavior Network.

Conclusions

Overall, I found several mixed results that both support and contradict my predictions. For anoles, overall activity levels may serve as a reliable indicator of invasive ability, whereas

total brain size may serve as a reliable indicator of invasive ability for geckos. Initially, I aimed to find generalizable differences in behavior and brain morphology that may be associated with invasion success in reptiles (Van Kleunen, Dawson, Schlaepfer, Jeschke, & Fischer, 2010). However, my results indicate that the differences in brain morphology both between and within species may not be generalizable across taxa. In reality, anoles and geckos differ substantially in their taxonomy and ecology, which likely poses different challenges and selection pressures on individuals of different species during the invasion process. With regards to taxonomy, Bomford *et al.* (2009) found that both family and genus significantly predict invasion success in reptiles, in which *Hemidactylus* geckos qualify as some of the world's most successful invasive reptiles. Furthermore, anoles are diurnal and geckos are nocturnal, which likely results in different foraging strategies and behaviors. Also, anoles are highly territorial (Stamps & Krishnan, 1998) whereas some geckos tend to form clusters around clumped resources (e.g., insects surrounding artificial lights), or at retreat sites during daylight hours (Kearny, Shine, Comber, & Pearson, 2001). Consequently, we might expect the evolution of different behavioral syndromes or corresponding brain regions in response to these differing ecological parameters. As a result, relationships between behavior and brain morphology in anoles may not apply to geckos, and vice versa.

However, I did find a number of significant relationships between boldness behaviors and the brain regions with which I predicted they would be associated. For instance, there were several significant correlations between the morphological measures of the POA and principal components representing antagonistic display behaviors in the aggression-towards-conspecifics challenge for both invasive brown anoles and invasive Mediterranean house geckos. These

results suggest that the preoptic area plays some role during the invasion process. However, further studies are needed to elucidate these relationships.

By understanding the behavioral and neural differences between invasive and non-invasive lizards, we may better prevent and predict future vertebrate invasions. We may also be able to better predict and identify which organisms will be viable in the face of global environmental and climate change. The ability of species to reach new climatically suitable areas will largely be influenced by both habitat loss and fragmentation and the arrival of new invasive species (Thomas *et al.*, 2004). An understanding of the behavioral mechanisms and neural correlates of invasion success will enable us to design appropriate management plans that conserve and promote ecological health.

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Table 1. Principle component analysis on display behaviors observed during the anole aggression-towards-conspecifics test.

Variable	PC Axis	
	1	2
Time to first display	-0.270	0.884
Pushup	0.695	0.567
Dewlap	0.860	-0.180
% variance explained	43.2	37.9
Eigenvalue	1.30	1.14

Table 2. Principle component analysis on display behaviors observed during the gecko aggression-towards-conspecifics test.

	PC Axis			
	1	2	3	4
Time to first display	0.282	-0.195	0.631	0.674
Head raise	-0.563	0.761	-0.031	-0.106
Tail wag	-0.403	-0.704	0.105	-0.400
Posture	-0.549	0.605	0.376	0.140
Head jerk	0.883	0.131	0.248	-0.253
Back arch	0.186	0.034	-0.794	0.481
Lick	0.775	0.530	-0.023	-0.258
% variance explained	32.5	25.4	17.8	14.4
Eigenvalue	2.28	1.78	1.24	1.01

Table 3. Pearson correlation values for relationships between open field test behaviors, ventromedial nucleus morphology, and medial cortex morphology in anoles.

	Species	VMN soma size	VMN soma density	VMN volume	CxM soma size	CxM volume
Time in interior squares (sec)	Brown	0.845	-0.540*	0.061	-0.643**	-0.332
	Green	0.034	0.093	0.711**	-0.043	0.112
Total boundaries crossed	Brown	0.244	-0.706	-0.238	-0.272	0.025
	Green	-0.627*	0.210	-0.125	-0.306	-0.341

Note. $p < 0.1$ *, $p < 0.05$ **, $p < 0.01$ ***

Table 4. Pearson correlation values for relationships between open field test behaviors, ventromedial nucleus morphology, and medial cortex morphology in geckos.

	Species	VMN soma size	VMN soma density	VMN volume	CxM soma size	CxM volume
Time in interior squares (sec)	House	-0.266	0.183	0.074	0.128	0.070
	Banded	0.328	0.264	-0.755	-0.316	-0.670
Total boundaries crossed	House	0.505	-0.358	-0.282	0.334	-0.129
	Banded	-0.991***	0.853	-0.243	-0.805	-0.054

Note. $p < 0.1$ *, $p < 0.05$ **, $p < 0.01$ ***

Table 5. Pearson correlation values for relationships between the divider challenge latency to cross time, relative brain size, and medial cortex morphology in geckos.

	Species	Brain-to-body mass ratio	CxM soma size	CxM volume	CxM volume/tot brain vol
Latency to cross divider (sec)	House	-0.336	-0.261	0.871***	0.560
	Banded	0.268	0.004	-0.488	-0.197

Note. $p < 0.1$ *, $p < 0.05$ **, $p < 0.01$ ***

Table 6. Pearson correlation values for relationships between the principle component axes for display behaviors during the aggression-towards-conspecifics test, ventromedial nucleus morphology, and preoptic area morphology in anoles.

	Species	VMN soma size	VMN soma density	VMN volume	POA soma size	POA soma density	POA volume
PC1	Brown	0.694	0.435	-0.011	-0.105	-0.802**	0.069
	Green	-0.456	-0.281	-0.375	0.217	-0.467	0.313
PC2	Brown	-0.707	-0.508	0.551	-0.287	0.467	0.406
	Green	-0.610*	-0.551	-0.118	0.330	0.149	-0.179

Note. $p < 0.1$ *, $p < 0.05$ **, $p < 0.01$ ***

Table 7. Pearson correlation values for the relationships between the principle component axes for display behaviors during the aggression-towards-conspecifics test, ventromedial nucleus morphology, and preoptic area morphology in geckos.

	Species	VMN soma size	VMN soma density	VMN volume	POA soma size	POA soma density	POA volume
PC1	House	--	-0.584	0.135	-0.274	0.942**	0.164
	Banded	0.649	-0.794	0.248	-0.597	--	-0.720
PC2	House	0.405	-0.223	0.062	0.822**	0.065	-0.448
	Banded	-0.494	0.254	-0.170	-0.819	--	-0.833
PC3	House	-0.338	0.380	-0.595	0.244	0.392	-0.890**
	Banded	0.798	-0.429	-0.063	0.743	--	0.955
PC4	House	-0.789**	0.687	-0.201	-0.354	-0.656	0.419
	Banded	0.531	-0.571	-0.053	-0.583	--	-0.540

Note. $p < 0.1$ *, $p < 0.05$ **, $p < 0.01$ ***



Figure 1. The four lizard species used in this study; (a) the native green anole (*Anolis carolinensis*; n = 12), (b) the invasive Cuban brown anole (*Anolis sagrei*; n = 15), (c) the native Texas banded gecko (*Coleonyx brevis*; n = 4), and (d) the invasive Mediterranean house gecko (*Hemidactylus turcicus*; n = 8). Comparisons were made between the anoles and between the geckos.

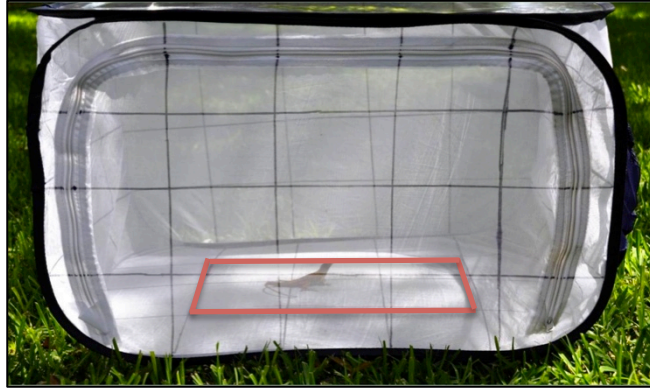


Figure 2. The testing arena used in the open-field test to measure levels of overall activity during a 20 min observation period. Lizards were acclimated for 10 min underneath an opaque shelter in the center of the six interior squares that are demarcated (exaggerated by the red line here) from the 14 periphery squares on the floor of the arena. The total number of boundaries crossed, the amount of time spent in the 6 interior squares, and the amount of time spent on the walls were recorded.



Figure 3. The testing arena used in the divider challenge to measure behavioral flexibility and exploratory behavior in (a) anoles and (b) geckos. The mesh screen divided one-third of the arena where the anole was acclimated for 10 min underneath an opaque shelter before beginning the trial from the two-thirds of the arena containing valuable resources – a perch and a cricket for anoles (a) and a piece of crumpled paper and a cricket for geckos (b). Lizards could crawl through a PVC tube (7.62 cm long, 7.0 cm wide) or over the mesh screen to reach the resources. Lizards were given a period of 20 min to complete the challenge. The time at which the lizard crossed to the other side of the arena was recorded.



Figure 4. The testing arena used to measure aggression towards conspecifics in (a) anoles and (b) geckos. Lizards were acclimated for 10 min underneath opaque shelters on opposite sides of the arena. Both individuals were observed for 10 min, during which all aggressive displays (see text for details) and the time of the first aggressive display performed were recorded.

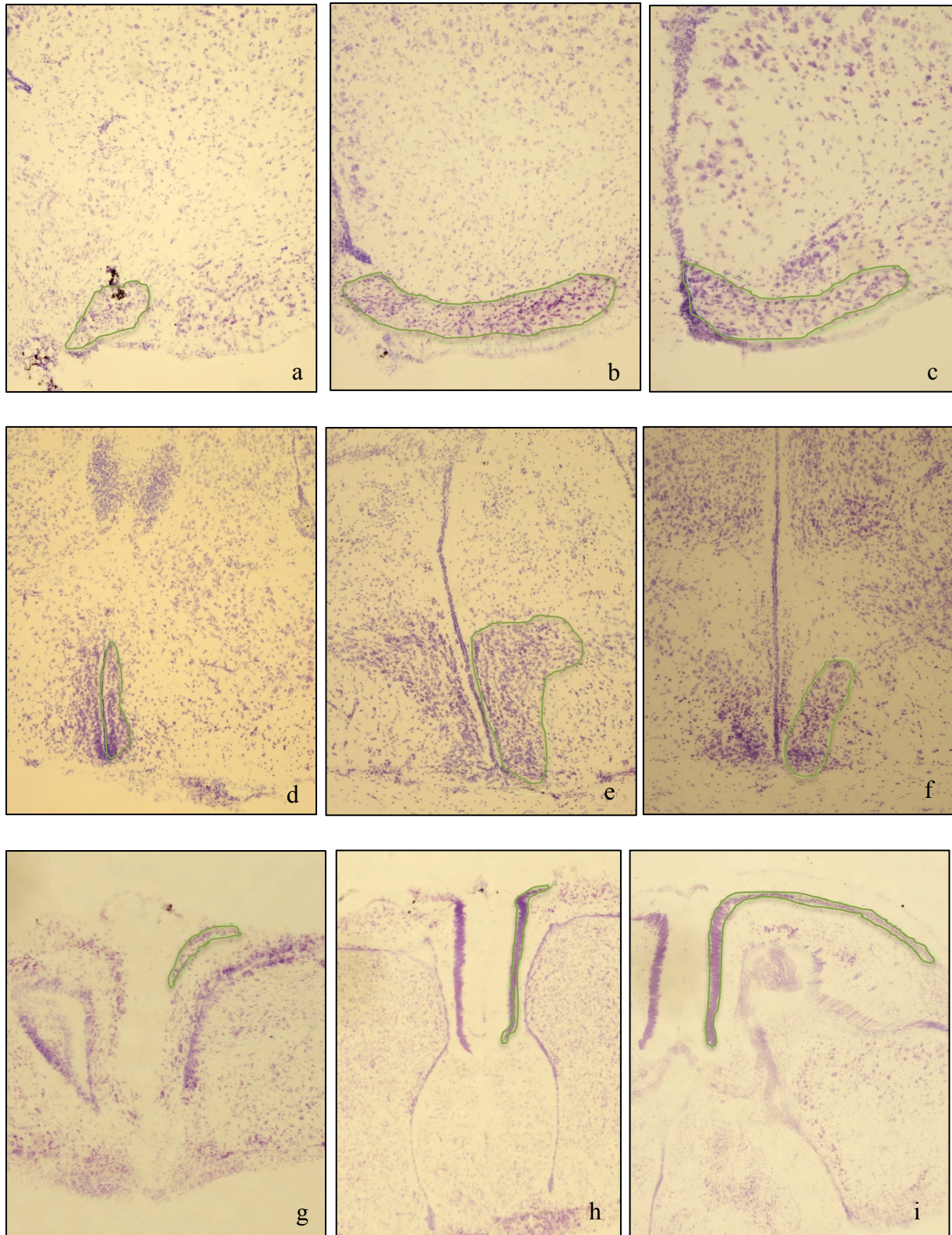


Figure 5. Cross-sections of the anole (a) rostral VMN, (b) medial VMN, (c) caudal VMN, (d) rostral POA, (e) medial POA, (f) caudal POA, (g) rostral CxM, (h) medial CxM, and (i) caudal CxM.

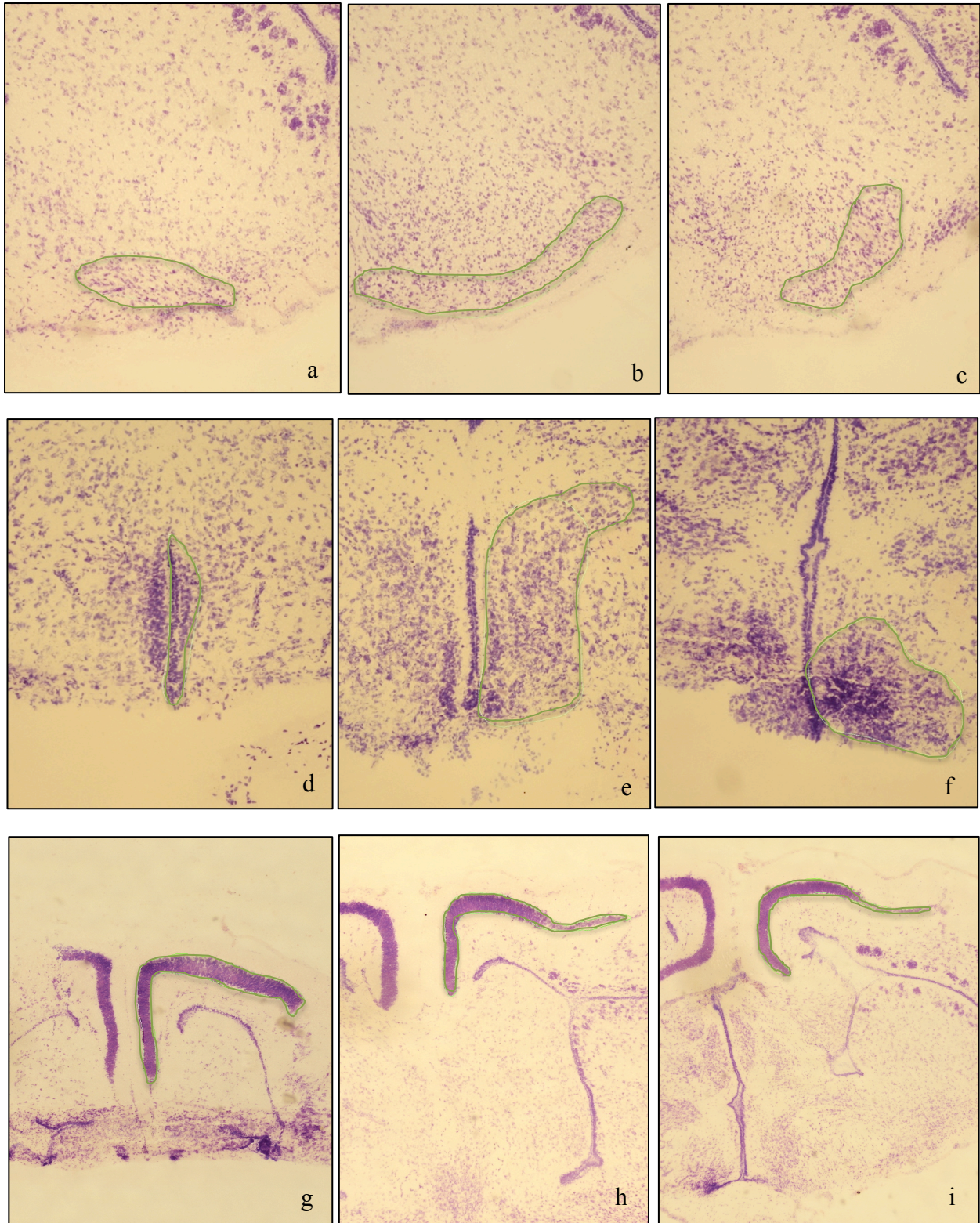


Figure 6. Cross-sections of the gecko (a) rostral VMN, (b) medial VMN, (c) caudal VMN, (d) rostral POA, (e) medial POA, (f) caudal POA, (g) rostral CxM, (h) medial CxM, and (i) caudal CxM.

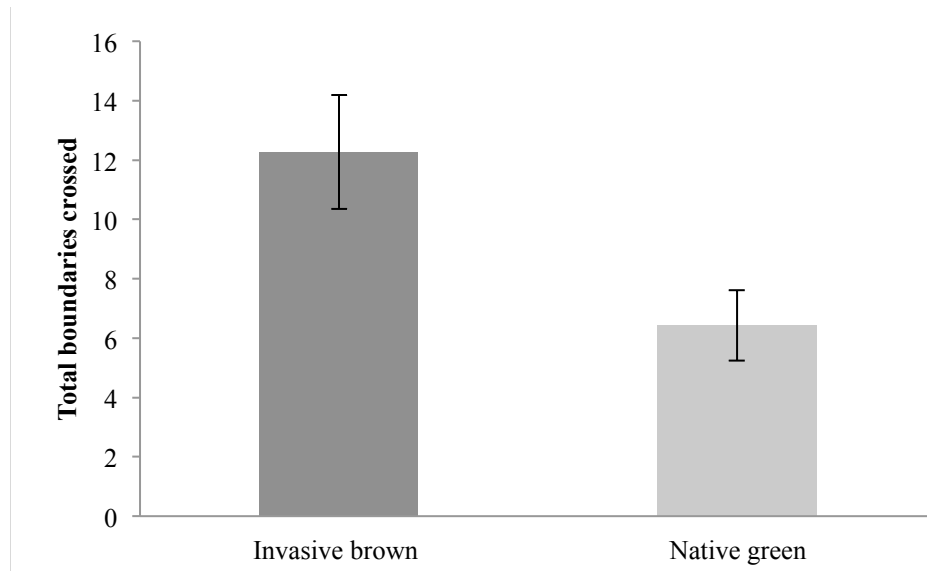


Figure 7. Invasive brown anoles crossed more boundaries during a 20 min open field test than native green anoles. Error bars indicate ± 1 S.E.

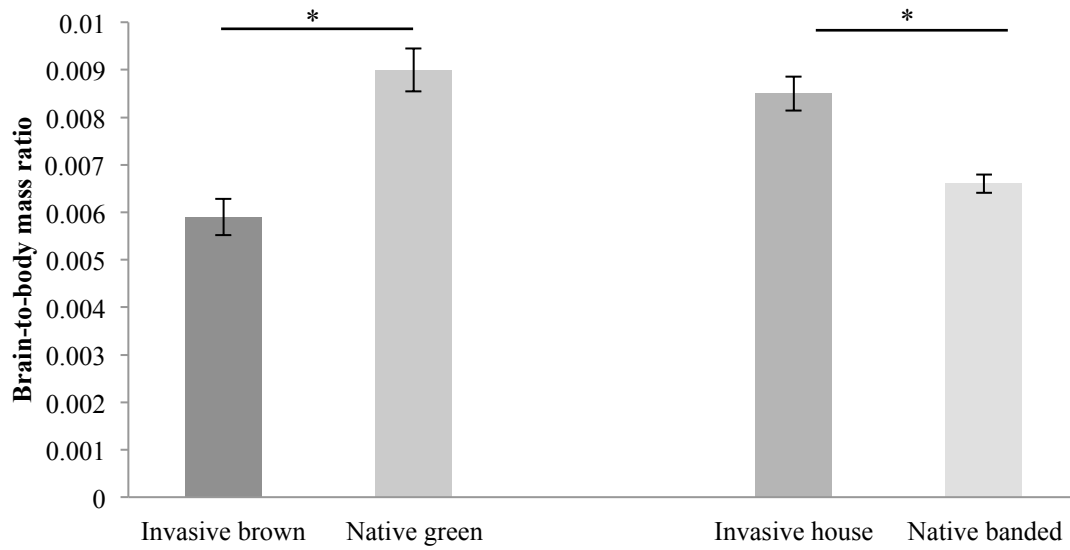


Figure 8. Native green anoles had a larger relative brain mass than invasive brown anoles, and invasive Mediterranean house geckos had a larger relative brain mass than native Texas banded geckos. Asterisks indicate a significant difference with an alpha level of 0.05. Error bars indicate +/- 1 S.E.

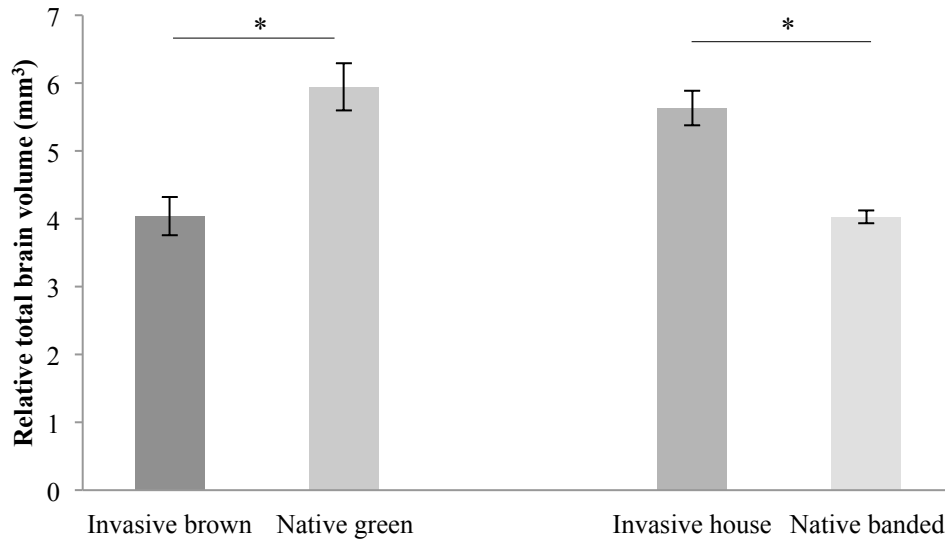


Figure 9. Native green anoles had a larger relative total brain volume than invasive brown anoles, and invasive Mediterranean house geckos had a larger relative total brain volume than native Texas banded geckos. Asterisks indicate a significant difference with an alpha level of 0.05. Error bars indicate +/- 1 S.E.

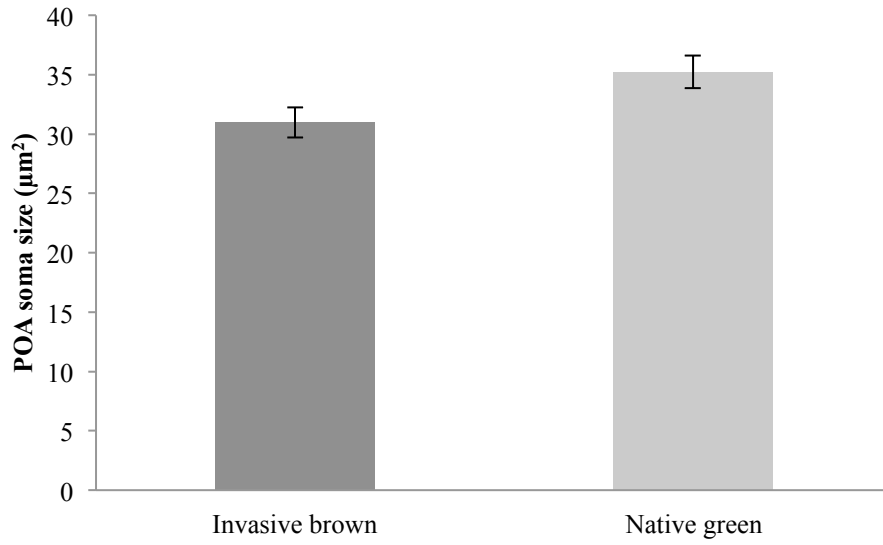


Figure 10. Native green anoles had neurons with a larger cross-sectional area in the preoptic area than invasive brown anoles. Error bars indicate \pm one standard error.

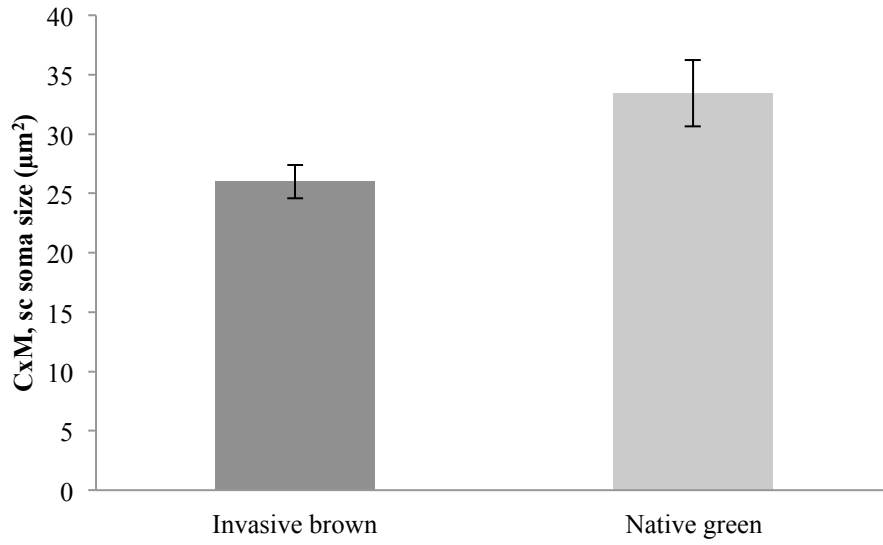


Figure 11. Native green anoles had neurons with a larger cross-sectional area in the small cells of the medial cortex than invasive brown anoles. Error bars indicate +/- one standard error.