

Urbanisation and Shifting Phenotypes
Behavioural, Physiological and Cognitive Strategies
of the Indian Rock Agama *Psammophilus dorsalis*

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To

Ma and Baba

DECLARATION

I hereby declare that I am the sole author of this thesis. I authorize Indian Institute of Science to lend this thesis to other institutions or individuals for the purpose of scholarly research.

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Synopsis

Humans directly or indirectly cause changes in the environment, and urbanisation is currently one of the most important threats to biodiversity. Urbanisation exposes organisms to novel pressures that are drastically different from those in their native habitats, as human modification rapidly and dramatically changes natural environments, alters habitats as well as shifts resources and predator communities. Organisms can cope with the novel challenges by modifying their behaviour, physiology, morphology and cognition. To understand the impacts of urbanisation on phenotypic traits, the goal of my research was to study the social and survival strategies of the Indian rock agama, *Psammophilus dorsalis*. Using a combination of field and laboratory-based experiments, I examined differences in signal-receiver dynamics in communication, anti-predator strategies, stress physiology, and spatial learning. Social interactions in this species involve rapid physiological colour changes and behavioural displays. My work shows that colour patterns are diametrically different between courtship and aggressive interactions. Males change their dorsal body region to red, and their lateral body region to black when courting females, whereas these regions turn yellow and orange respectively when fighting with competitive males. Regardless of social context, suburban males express lower colour contrast and are also slower to change colours than rural males. Using robotic lizard stimuli, I found that receiver responses match the population-specific intensity of male signals. For the first time in any lizard species, I find that perception and responsiveness to motion and colour are lateralized in different ways. *Psammophilus dorsalis* is left visual field dominant when responding to social

display colours, but motion stimuli elicit similar responses from both visual field. Along with shifts in colour signalling strategies, stress physiology and social behavioural display was also affected by urbanisation. Suburban males had significantly higher circulating corticosterone levels during both control conditions and immediately following social interactions compared to rural males. Proportion and rate of courtship displays was also significantly lower in suburban males compared to rural males. In the field, escape strategies of males, but not females differed between suburban and rural populations, such that suburban males were more tolerant of simulated predator attacks than rural males. As expected from their cryptic body patterns, females, regardless of habitat, relied more heavily on crypticity rather than flight to minimize predation risk. Suburban males also had stronger cognitive skills, as spatial learning and reversal learning in suburban males was faster than in rural males. In sum, differences in these behavioural, physiological, and cognitive responses of suburban and rural populations of lizards demonstrated in my thesis, indicate human-induced changes in selective pressures that support shifted survival and reproductive strategies. *Psammophilus dorsalis* promises to be an excellent system to further examine the specific selective pressures that shift in urban landscapes. The study of multiple integrated phenotypic traits in response to urbanisation gives a broader perspective as to how a species can flexibly adapt to rapid environmental disturbances, which is currently one of the greatest threats to biodiversity worldwide.

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Introduction

The environment is neither entirely constant nor completely chaotic, but fluctuates and varies in predictable and unpredictable ways over geological, evolutionary and ecological time scales, and this variation in the environment is a strong selective pressure for all organisms. Environmental disturbances can be predictable, like the changing seasons with shifts in temperature, salinity, and humidity. Environments can also be unpredictable, and include variable shifts in predation pressure, resource availability or sudden climatic changes like storms or drought events (Jessop et al., 2013). But apart from more natural environmental change, the rate and extent at which environments are shifting globally is now largely driven by anthropogenic events, such as deforestation, habitat alteration for agricultural use and greenhouse gas induced warming (Vitousek et al., 1997; Grimmond, 2007). Among the various anthropogenic disturbances, urbanisation is one of the most important threats to biodiversity worldwide as it is rapidly expanding and introduces novel stressors to which organisms have not been exposed during their evolutionary history (Macnair, 1991; Shochat et al., 2006; McKinney, 2008). Urbanisation leads to a number of important environmental alterations, including the replacement of natural vegetation and soil with built up artificial structures which are fragmented by roads, shifts in the type and abundance of food resources, increased disturbance due to human activity, changes in predator community and increase in the level of pollution such as noise and chemicals (Sih et al., 2011; Sol et al., 2013). Therefore, urbanisation simultaneously alters habitat structure and increases fragmentation, and changes community dynamics, including predator and resource

diversity and availability, as well as overall disturbance conditions in the environment. Because these environmental alterations are drastic and rapid, they are likely to exceed the tolerance limit of most species (Sih et al., 2011). In fact, one of the first consequences of urbanisation is the reduction in population size of native species and the subsequent demographic changes that increase the probability of local diversity loss (McKinney, 2008; Soule et al., 1986). The effects of urbanisation on wildlife, however has recently gained importance, but there is still much to be understood about the survival strategies and adaptive capacity for species that seem tolerant to this form of human induced modification in their habitat (but see see Luniak, 2004 for mammals; Møller, 2009 for birds).

Organisms have to respond flexibly to changing environments to increase their probability of survival. Traits that can be modified easily, such as morphology (eg. mass), physiology (eg. modulation of hypothalamo-pituitary-adrenal axis), behaviour (eg. anti-predatory and foraging behaviour), and cognitive abilities (eg. learning and neophilia) of animals respond fastest to immediate challenges (Sol et al., 2013; Crispo et al., 2010; Overli et al., 2007). There is now a growing body of scientific evidence describing behavioural modifications that differ between individuals faced with different stressors in their environment, including in the context of urbanisation (see reviews by Sol et al., 2013; Lowry and Wong, 2012; Tuomainen and Candolin, 2011). Changes in biotic and abiotic factors due to human activity can cause modifications to common behaviours such as changing the timing and duration of breeding and altering the diet and foraging pattern depending on the type and abundance of resources (Lowry and Wong, 2012). There can also be modifications in vigilance behaviour and communication in noisy environments

that directly affect fitness related traits, such as survival and reproduction (see Sol et al., 2013 for birds and mammals). Most studies on birds, mammals and reptiles have shown that habituation to humans leads to urban individuals being less risk averse, allowing closer approaches before escaping compared to their rural conspecifics (for mammal Engelhardt and Weladji, 2011; for birds Clucas and Marzluff, 2012; for reptiles Samia et al., 2016). Rapid and unanticipated environmental shifts not only cause animals to respond with behavioural modifications but also with physiological responses. These unpredictable events exert their effects through the response of the hypothalamo-pituitary-adrenal (HPA) axis, commonly also known as the “stress” axis (Wingfield and Kityaski, 2002; Sapolsky et al., 2000). An acute elevation in glucocorticoids by activation of the HPA axis enables vertebrates to cope with immediate environmental challenges (Sapolsky et al., 2000). But prolonged or repeated exposure to stressors leading to a chronic elevation in baseline glucocorticoid levels can have deleterious effects on growth (Hull et al., 2007), immune functions (French et al. 2006, 2007) and reproduction (Moore and Jessop, 2003; Wingfield and Sapolsky, 2003). Changes on glucocorticoids levels in response to urbanisation and anthropogenic disturbance such as ecotourism have been mostly studied in birds with few examples from reptiles (for birds, see Fokidis et al., 2009; Partecke et al., 2005; Mullner et al., 2004; Schoech et al., 2007; Marzluff, 2001; for reptiles, see French et al., 2008; Romero and Wikelski, 2002). However, results from these studies indicate that it is difficult to predict the direction of response as it is species specific and might depend on the duration of exposure to urban environments. Learning abilities and tolerance of novel objects are also likely to be important characteristics in animals residing in urban environments, especially since individuals with better problem

solving abilities and learning skills might perform better in challenging situations. As expected, several successful urban adapted species of birds have been found to have reduced neophobia and increased cognitive skills (Tryjanowski et al., 2016; Lowry and Wong, 2012). Thus, effects of urbanisation on animals have been studied in several different ways, with typical focus on anti-predatory and foraging behaviour, stress physiology and learning about novel food sources. But urbanisation affects the habitat and the social environment as well and therefore should affect social strategies and cognition along with other physiological and behavioural responses. Finally, most of the evidence for flexible and adaptive strategies of urban animals comes from birds and mammals, and reptiles have been largely ignored. Thus, my thesis examined multivariate phenotypic traits of urban and rural populations of an agamid lizard, *Psammophilus dorsalis*, to determine how survival and social strategies change with urbanisation.

1.1 The study system: Indian rock agama, *Psammophilus dorsalis*

Psammophilus dorsalis (Class: Reptilia; Order: Squamata; Suborder: Iguania; Family: Agamidae) is endemic to India and is widely distributed in semi-arid regions of southern peninsular India, where they are found on rocky boulders and sheet rocks interspersed with scrub vegetation (Radder and Saidapur, 2005), as well as in suburban habitats (Balakrishna et al., 2016). In the suburban areas, *P. dorsalis* are generally found in localized clusters around residential construction sites, unbuilt plots, and urban gardens. *Psammophilus dorsalis* is sexually dimorphic with males being larger than females and also generally occupying higher perch sites compared to females (Radder et al., 2005). Males also develop conspicuous physiological colours during the breeding

season, typically from April to August (Radder and Saidapur, 2005), which become dull at the end of the breeding season. Previous studies on diet and foraging strategies of *P. dorsalis* find high overlap of diet (80.3%) between urban and rural populations of lizards, but foraging strategies differed between males wherein rural males had higher rate of movement compared to urban males (Balakrishna et al.,2016).

Study sites for the research reported in this thesis were located in and around the city of Bangalore, India. Suburban study sites ($N = 3$ sites, centroid 13.0422° N and 77.5940° E) were located within city limits and rural study sites were located near Antharagange forest range in Kolar district ($N = 2$ sites, centroid 13.1243° N and 78.0346° E), and each replicate site were approximately 1 km^2 . Replicate sites within each habitat were 5-10 km apart, and the suburban and rural habitats were at least 60 km apart (Fig.1.1). Rural study sites were highly contrasting in habitat characteristics compared to suburban study sites and had no human disturbance in terms of anthropogenic structures. Rural sites were comprised of rocky hills and boulders with interspersed scrub vegetation and were stable in terms of habitat characteristics such as perches or refuges used by lizards. In contrast, suburban sites were mostly empty development plots surrounded by small houses or were in and around construction areas with scrub vegetation. Lizards were only found in suburban sites with partial vegetation cover and low to intermediate anthropogenic built-up structures (Amdekar et al. *in preparation*). The suburban sites selected for the study were also dynamic in habitat characteristics as they kept changing in terms of availability of suitable perches or refuges used by lizards due to anthropogenic activities. The rate of changes in habitat characteristics in suburban areas can be classified as both short-term (example: during

monsoon, the dense vegetation cover in small plots alters perch and refuge sites) and long-term (example: empty plots change to built-up houses in a few months to a year leading to habitat loss for resident lizards in those plots). In rural sites, the boulder field is interspersed with low shrub cover that changes seasonally (short-term), but not long-term for lack of anthropogenic activity and development. Apart from physical characteristics of habitat difference I also predict that suburban and rural sites differ in terms of predation pressure, local lizard density and type of resources (Balakrishna et al. 2016; Amdekar et al. *in preparation*). Both field and laboratory-based experiments were conducted with lizards from rural and suburban sites for this work.

1.2 Broad objectives

The broad objectives of this thesis included comparing social strategies, such as colour communication and hormonal and behavioural responses, of suburban and rural *P. dorsalis* during courtship and competitive encounters. Animals use dynamic physiological colours to change body colour and pattern for communication, thermoregulation or camouflage (Stuart Fox and Mousalli 2009). In Chapter 1, I report the use of physiological colours by *P. dorsalis* during social communication and the ways in which they differ depending on habitat (suburban and rural) and social context (courtship and competition). Efficient signalling requires a signaller who generates, encodes and transmits a message and a receiver who receives and decodes it (Endler, 1993). Decoding requires processing in the brain, and visual information is processed in multiple brain areas. In reptiles and other groups with monocular vision, visual information from each eye is processed independently in the two brain hemispheres (Greenberg, 1982; Bisazza et al., 1998). Thus, in chapter 2, I examined the effects of

brain laterality in detecting complex social signals, which comprise colour and movement components. Social signalling is conducted in variable outdoor environments, and thus habitat conditions and disturbance can affect the different components in complex signals (Endler, 1992). Under such circumstances, effective communication will persist if receivers also match and tune their responsiveness to the intensity of signaller displays. Thus, in chapter 2, I also compared receiver responses to colour signals across suburban and rural populations. Social signalling not only involves colour but also behavioural and physiological responses. The social environment of an animal is one of the primary sources of external stimuli that can induce a physiological stress response. Species physiologically respond to social interactions with the activation of both the HPA and HP-Gonadal axes. Besides the typical social stressors experienced by all lizards, *P. dorsalis* experiences novel disturbances in urban environments. Thus, in chapter 3, I examined behavioural and steroid hormone (corticosterone and testosterone) responses of *P. dorsalis* as an effect of social interaction and habitat disturbance.

Apart from social strategies, I also compared survival strategies of *P. dorsalis*, which include escape responses and learning abilities. Escape strategies of animals are economic decisions, expected to vary as a function of both intrinsic (e.g., performance ability) and extrinsic factors (e.g., level of threat and microhabitat). Anthropogenic disturbance, especially urbanisation, changes a range of environmental factors including habitat characteristics and predation risk. As a consequence of differences in microhabitat structure and repeated exposure to anthropogenic disturbance, I hypothesized that animals in urban environments will be less risk averse than those in rural environments. Thus, in chapter 4, I examined the importance of extrinsic and intrinsic factors to

understand the escape strategies of *P. dorsalis* across an urban-rural gradient. Locating important resources in the environment, such as food sources and safe refuges, are also critical for the survival of animals. One important behavioural tool that animals use to accomplish this task is to learn about the location of these important resources to return later when required. The unpredictability and complexity in structural features of a habitat can influence learning abilities in a species. In the final chapter, I examined differences in learning and reversal learning between suburban and rural individuals of *P. dorsalis* to test my hypothesis that increased cognitive skills are found in animals residing in complex habitats.

In sum, the individual chapters in this thesis are as follows:

Chapter 1: Signalling with physiological colours: effects of social context and urbanisation on colour signalling strategies

Chapter 2: Receiver responses to social display colours: effects of brain laterality and population difference in detecting signal components

Chapter 3: Physiological stress and social behaviour: do urban and rural males differ in their courtship and aggressive interactions?

Chapter 4: A multivariate approach to understanding shifts in escape strategies

Chapter 5: Learning to be safe: effects of urbanisation on learning and reversal learning



Figure 1.1. Suburban (U) and rural (R) study sites and male and female of *Psammophilus dorsalis* (also published in Batabyal, A., Balakrishna, S., & Thaker, M. 2017. A multivariate approach to understanding shifts in escape strategies of urban lizards. Behavioral Ecology and Sociobiology, 71(5), 83.)

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Chapter 1:
**Signalling with physiological colours: effects of social context and urbanisation on
colour signalling strategies**

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2.1 Introduction

Dynamic physiological colours, which are actively modulated by signallers within seconds to minutes, have been documented in a wide range of taxa from crustaceans and cephalopods, to fishes, amphibians and reptiles (Camargo et al., 1999; Hemmi et al., 2006; Hanlon, 2007; Stuart Fox and Mousalli, 2008; Zylinski et al., 2011). Capacity of physiological colour change, however, varies dramatically between species. Aquatic taxa, like cephalopods and fishes, show remarkable abilities to change body colour and pattern within seconds to minutes (Morris et al., 1995; Messenger, 2001). For most terrestrial species, these physiological colour changes are limited to achromatic changes in contrast with lightening or darkening of the skin (e.g. Cooper and Greenberg, 1992; Camargo et al., 1999; Kindermann et al., 2013). For a few well characterised terrestrial vertebrates, physiological colours can shift hues across a wider range of visible wavelengths. In the Chamaeleonidae family, many species are capable of large changes in both achromatic contrast and chromatic contrasts in the red, brown, yellow and green spectral wavelengths (Stuart Fox and Mousalli, 2008; Ligon and McGraw, 2013; Teyssier et al., 2015). Regardless of the specific colour patterns, physiological colour changes for both aquatic and terrestrial taxa depend on context, shifting for camouflage, thermoregulation, and social communication (Messenger, 2001; Stuart Fox and Mousalli, 2009; Zylinski et al., 2011).

Understanding variation in colour signals has been central to the study of animal communication for decades (Hamilton and Zuk, 1982; Andersson, S. and Andersson, M., 1998; Dale, 2006). Given that signals have evolved to attract attention and convey information (Hebets and Papaj, 2005; Partan and Marler, 2005), species that use

physiological colours for communication are able to use particular colour patterns to convey specific information in different social contexts. For example, in three-spined sticklebacks (*Gasterosteus aculeatus*), males shift the intensity of their red nuptial colouration during both male-male and male-female interactions to convey dominance status and male parental ability (Candolin, 2000). Dwarf chameleons (*Bradypodion* sp.) are observed to alter their chromatic contrast to signal dominance or submissiveness during social interactions, regardless of their conspicuousness against the background (Stuart Fox and Mousalli, 2008; Keren-Rotem et al., 2016). Dynamic signals can also be used to convey different information to different receivers. Male mourning cuttlefish (*Sepia plangon*) can lateralize physiological colour changes, such that one side expresses male courtship patterns, while the other expresses female-mimicked patterns. This dual signalling state allows males to court females, while simultaneously distracting a rival male (Brown et al., 2012). Given that a single body region can express more than one chromatic and achromatic state at any given point in time, signallers have the potential to convey multiple messages rapidly and dynamically during courtship and aggressive encounters.

Most studies on physiological colours have illustrated the importance of environmental conditions, focussing on induced patterns to enhance crypticity or conspicuousness against different substrates or backgrounds (Endler, 1992; Stuart Fox and Mousalli, 2009; Clarke and Schluter, 2011; Cole and Endler, 2015, Wong and Candolin, 2015). Individuals should also vary the intensity and brightness of colour patterns for social communication depending on local environmental conditions (e.g. Leal and Fleishmann, 2004; Kelly et al., 2012). For example, in three-spined sticklebacks,

algal turbidity influences the expression of the red nuptial coloration (Wong et al., 2007). Similarly, differences in the intensity of light in xeric and mesic habitats seem to influence the brightness of the statically-coloured dewlaps on *Anolis cristatellus* (Leal and Fleishmann, 2004). Surprisingly very few terrestrial vertebrates have been studied to determine intraspecific population variation in physiological colours across different environmental conditions (but see Boback and Siefferman, 2010). Because they are dynamic, and thus a form of phenotypic plasticity, use of physiological colours can respond to external drivers over very short time scales, and provide a good opportunity to examine how changes in both the physical and social environment influences intraspecific variation in magnitude and range of colour patterns.

Here, I examine the pattern, range and speed of physiological colour change in the Indian rock agama, *Psammophilus dorsalis*, to determine how visual signalling differs between social context and between populations. I explicitly quantified colour changes during social interactions with females as well as males to determine whether this species uses different visual signals for courtship and aggression. *Psammophilus dorsalis* is widely distributed in semi-arid open habitats of southern peninsular India, which include human modified environments. In order to quantify the range (i.e. capacity) of physiological colour changes during social interactions for this species, I included males from both suburban and rural areas, representing two contrasting environmental conditions. Urbanisation alters a large suite of biotic and abiotic factors, from lighting conditions and habitat composition, to levels of social competition and predation risk (Longkore and Rich, 2004; Kempenaers et al., 2010). Mounting evidence suggests that these changes can affect animal communication by affecting the production of signals,

transmission efficiency, as well as receiver reception (Rosenthal and Stuart-Fox, 2012). In this paper, I focus on the physiological colour signals themselves, to determine strategies and variation in their use. In doing so, I examine the relative importance of social context and environment in the expression of physiological colour patterns.

2.2 Methods

Adult lizards were collected from study sites of approximately 1 km² each, located in and around the city of Bangalore, India. Suburban study sites ($N = 3$ sites) were located within city limits and rural study sites were located near Antharagange forest range in Kolar district ($N = 2$ sites). Replicate sites within each habitat were 5-10 km apart, and the suburban and rural habitats were at least 60 km apart. I performed the following experiments during the peak breeding season (April-August) over two consecutive years (2013, 2014). This species is known to court and fight throughout the breeding season (Radder et al., 2006) and has 1-2 clutches a year (Srinivas et al., 1995), and thus were expected to be socially responsive during the experimental trials. Adult male lizards [$N = 25$ total from suburban (7-10 per site); and 25 total from rural (10-15 per site)] were captured by noosing and brought into the laboratory. All males and females used in the study were sexually mature adults. I ensured that all females were non- gravid to minimize variation in receptivity, which was evident by the observation that all females were behaviourally reactive during social trials (e.g., responded to males with head bobs and tail raise).

Wild-caught lizards were housed individually in glass terraria in a dedicated animal housing room that permitted natural temperature and light conditions. Terraria

(60x30x25cm) were lined with disposable paper towels as the substratum, provided with rocks for refuge, and were covered on all sides to minimize disturbance. Individual incandescent basking lights (60 W) above each terraria were turned on from 0800 - 1200 hours, and from 1500 - 1600 hours. Lizards were provided with live ants and field crickets daily for food and water *ad libitum* and were maintained in the laboratory for 7-9 days. Before the start of the experimental trials, mass (g) and snout-to-vent length (SVL in mm) were measured for all individuals using weighing balance and digital callipers respectively. All lizards were returned to the site of capture after the following experimental trials.

To quantify the effect of social stimulus on colour patterns, I exposed each focal male ($N=20$ total suburban and 20 rural, excluding control animals) to a stimulus male and female separately. Stimulus individuals were caught from rural and suburban sites that were different from where focal animals were caught, and therefore were unfamiliar to focal males. Each stimulus individual was randomly paired with 2-3 focal individuals from the same population. Social interactions were staged under full-spectrum lights (Viva-Lite:B22) in testing tanks (95x45x30cm), and began with a 30 min acclimatization period, followed by a 30 min interaction period with one of the stimuli. Males in the control treatment ($N=5$ suburban and 5 rural) were also kept in the same testing tank for 30 min but were not exposed to any social stimuli. Lizards were randomly assigned to control or social test groups, where the order of stimulus (exposure to female or male) was also randomized. All trials were conducted from 08:00 – 12:00 or from 15:00 – 17:00, which are the peak activity periods for this species. Testing tanks were kept behind a blind which enabled us to digitally record the social interactions with minimal

disturbance (using Cannon EOS550D with 18-55mm lens) for subsequent quantification of the speed of colour changes (see details below).

Body colour quantification

I quantified physiological body colour changes in two ways, using reflectance spectrometry and standardized photography. During the 30 min social interactions and for control males (no social interactions), I took repeated measurements of percent reflectance of the dorsal and lateral sides of the body. Reflectance measurements of a 2mm² area of skin surface was taken through a custom-made flexible fibre-optic probe, angled at 45° relative to the body, that was connected to a spectrometer (JAZA2474, Ocean Optics) and light source (PX lamp, Ocean Optics). Spectral measurements were corrected against white and black standards (as per White et al., 2015). Because colour changes occur rapidly in this species and is affected by stress and handling, I mounted the probe against the body of the lizard with Velcro tape before the start of the acclimation period (see Fig 2.1). This enabled us to take repeated measurements of colour without handling the lizard and disturbing the social interactions.

Thus, for each focal male, I recorded spectral reflectance of the dorsal and lateral body regions at least six times at 5 min intervals during the 30 min social interaction period. Reflectance spectra from each time point were averaged across every 5nm wavelength range and I selected the maximum colour spectra from each individual for subsequent visual modelling. I chose the maximum spectra because I wanted the highest magnitude of colour change expressed. Chromatic (dS) and achromatic (dL) contrasts from these spectra were calculated to determine whether the maximum spectra could be

discriminated in the perceptual colour space of an agamid lizard, as defined by the quantum catches of receptors in cone cells, taking into account spectral sensitivity of each cone receptor type and receptor noise of a typical agamid eye (as per Barbour et al., 2002; Teasdale et al., 2013; Rankin and Stuart-Fox, 2015 and Yewers et al., 2015). Model calculations are detailed in Stuart-Fox and Mousalli (2003) and Teasdale, Stevens and Stuart-Fox (2013). Background reflectance for all data was standardized against a brown background and irradiance conditions were set as standard sunlight without shade. Thus, for each individual, I calculated the maximum change in chromatic and achromatic contrast values for each body part (dorsal and lateral) during male-male interactions, male-female interactions and control conditions.

To quantify the speed maximum physiological colour change during social interactions, I used the digital videography data and extracted images of the focal male at 10sec intervals. For each image, I calculated hue and chroma values (see details below) and used those colour parameters to calculate the speed of the maximal shifts in colour for dorsal and lateral bands in both social contexts for suburban and rural males. Methods for hue and chroma calculations from photographs were as per Stevens et al., 2007 (see also Smith et al., 2016). In brief, a photograph of a colour checker (X-rite model: MSCCPP), with grey/white/black card with 20% grey reflectance was taken with the same camera under the same lighting conditions to linearize and equalize photographs before analysis (Stevens et al., 2007). From all digital images, I extracted the red (R), green (G), and blue (B) values of a standard-sized square patch (0.5 cm) of the dorsal and lateral body regions using Matlab script written by J. Endler (Mathworks Inc.). The R, G, and B values were used to calculate hue and chroma (as per Stevens et al., 2007; Smith et

al., 2016). The time interval of the maximum change in hue (shift between yellow and orange-red on dorsal band) or chroma (shift between orange and black on lateral band) was calculated as the speed of physiological colour change.

This species is not covered under the Schedules of the Indian Wildlife (Protection) Act, therefore collection permits are not required. All capture, handling, and experiment protocols were approved by the Institutional Animal Ethics Committee at the Indian Institute of Science (CAF/Ethics/394/2014).

Statistical analyses

I compared the chromatic and achromatic contrasts of dorsal and lateral bands separately, to determine if social context (male-male, male-female, control), population (suburban and rural), and body condition (scaled mass index) affect the magnitude of colour expression, using a linear mixed-effects model, with replicate sites and individual focal male ID as random effects (R package: lme4 and lmerTest: Bates, Maechler, Bolker and Walker, 2014; Kuznetsova, Brockhoff and Christensen, 2015). Body condition was calculated from mass and SVL as the scaled mass index (SMI) based on the central principle of scaling (see Peig and Green, 2009). I also compared the rate of maximal colour change on the dorsal and lateral bands to determine differences across social context (male-male, male-female), population (suburban and rural), and body condition (SMI) using a linear mixed-effects model, with replicate sites and individual focal male ID as random effects. For all these analyses, I included an interaction term between social context and population, and present the F test for lmer results (type III with Satterthwaite approximation for degrees of freedom). Where relevant, I performed post hoc

comparisons for all interaction effect terms using Tukey contrasts (R package multcomp: Hothorn et al., 2013). Variation in colour responses between replicate sites within populations (SD was < 0.5 for all tests) and between individual focal males (SD was < 2 for all tests) were negligible, and thus I pooled population data for all figures. All data were analysed in R version 3.0.3 (R core team 2014).

2.3 Results

Spectral data show no UV reflectance on any body region for this species, and thus physiological colour changes in *P. dorsalis* during social interactions occur within the visible range of the spectrum (400-700nm; Figure 2.1). During staged interactions with females, 19 rural males and 15 suburban males (of 20 each) showed colour changes on their dorsal and lateral bands that were at least 4 times greater than that seen during control conditions (chromatic contrast range for controls = 0.10-0.70 dorsal; 0.42-0.60 lateral). During staged interactions with males, 14 rural males and 11 suburban males (of 20 each) showed colour changes that were also at least 4 times greater than during control conditions. Subsequent analyses of dorsal and lateral colour changes were restricted to only those lizards described above, as they responded to social interactions with detectable colour changes.

I find no significant effect of body condition (SMI) on the chromatic and achromatic contrast of either the dorsal (chromatic: $F_{1,7}=0.002$, $P=0.961$; achromatic: $F_{1,26}=0.317$, $P=0.577$) or lateral (chromatic: $F_{1,41}=0.004$, $P=0.948$; achromatic: $F_{1,41}=2.255$, $P=0.065$) body regions for both populations in all social contexts. Body condition also did not affect the rate of either dorsal or lateral colour change (Dorsal:

$F_{1,32}=0.331$, $P=0.568$; Lateral: $F_{1,27}=0.078$, $P=0.786$). Instead, I find that the physiological colours on both the dorsal and lateral bands of males changed independently depending on social context, with some variation between populations (detailed results below).

Magnitude of physiological colour change

In general, when males of *P. dorsalis* interact with females, the dorsal band changes from patchy yellow (typical neutral colour) to orange or red, while the lateral band changes from patchy orange (typical neutral colour) to black (Fig. 2.1a). During interactions with other males, the dorsal band changes to yellow, while the lateral band changes to an even bright orange (Fig. 2.1b). Under controlled conditions with no social encounters, the dorsal band remains a patchy or dull yellow-brown while the lateral band remains dull orange-brown, with little changes during the 30 min observation period (Fig. 2.1c).

Regardless of the specific hues that are expressed, chromatic contrasts of the colours on the dorsal band and the lateral band were affected by an interaction between social context and population (Fig. 2.2; Dorsal: $F_{2,23}=3.18$, $P=0.060$; Lateral: $F_{2,41}=4.87$, $P=0.012$). Chromatic contrast of the dorsal band in rural males was highest during male-female interactions compared to male-male interactions (post hoc $z=-3.58$, $p=0.004$) or control conditions (post-hoc $z=6.79$, $p<0.001$). Chromatic contrast of rural males during male-male interaction was also significantly higher than during control conditions (post hoc $z=2.91$, $p=0.040$). Among suburban males, chromatic contrast of the dorsal band was not significantly different between social contexts (post-hoc $z=-0.62$, $p=0.988$), but were

higher than that seen during control conditions [post-hoc $z=2.81$, $p=0.054$ (male-male); $z=3.76$, $p=0.002$ (male-female)]. Rural and suburban males differed significantly in the chromatic contrast of their dorsal band during male-female interactions only ($z=-3.42$, $p=0.007$). Thus, during interactions with females, rural males turned a richer shade of red (hue: $675\pm 4.82\text{SE}$; chroma: $2.43\pm 0.16\text{SE}$) compared to suburban males (hue: $667\pm 2.77\text{SE}$; chroma: $2.00\pm 0.05\text{SE}$). For the lateral band, rural males increased their chromatic contrast significantly during male-female interactions compared to during male-male interactions ($z=-5.71$, $p<0.001$) and control conditions ($z=6.59$, $p<0.001$). Urban males increased the chromatic contrast of their lateral band similarly during both social interactions compared to control conditions [$z=4.69$, $p<0.001$ (male-female); $z=3.79$, $p=0.002$ (male-male)]. No significant difference was observed between populations in the chromatic contrast of the lateral band.

Achromatic contrast (brightness) of dorsal band was significantly affected by social context ($F_{2,33}=9.84$, $P<0.001$), such that achromatic contrast during male-female interaction was significantly higher compared to control conditions ($z=3.46$, $p=0.001$). No significant differences in achromatic contrast of lateral band was seen across social contexts or between populations.

Speed of physiological colour change

Speed of the maximal colour change on the dorsal band was affected by an interaction between social context and population ($F_{1,3}=26.137$, $P=0.011$). Both rural and suburban males changed the colour of their dorsal band significantly faster during male-male interactions compared to male-female interactions ($z=-2.90$, $p=0.017$ (rural); $z=-$

13.25, $p < 0.001$ (suburban); Fig. 2.3a). But population differences in the speed of colour change was only apparent during male-female interactions wherein rural males were significantly quicker to change their dorsal band than suburban males (dorsal: $z = 5.07$, $p < 0.001$). Although all males initiated colour change within 10 sec during any social interaction, the time taken by suburban males to maximally change their dorsal colour during interactions with females ranged from 260 to 1200 sec whereas the longest time taken by a rural male to maximally shift its dorsal colour was 400 sec (Fig. 2.3a).

Speed of the maximal colour change on the lateral band was affected independently by population and social context (population: $F_{1,18} = 7.287$, $P = 0.014$; social context: $F_{1,9} = 23.628$, $P < 0.001$). Similar to dorsal colour change, speed of the colour change on the lateral band was also significantly faster during male-male compared to male-female interactions and rural males changed colour faster than suburban males (Fig. 2.3b).

2.4 Discussion

Our study reports conspicuous and rapid colour change in an agamid lizard species, which involves independent shifts in colour on two body regions during social interactions. Not only do I add a new genus to the list of animals that utilize physiological colours, I show that colour change is dramatic, among the most rapid, and specific to the social context. During encounters with females, the dorsal band on males of *P. dorsalis* physiologically changes from dull yellow to bright orange/red and the lateral band shifts from patchy brown to black. During encounters with other males, the dorsal band becomes bright yellow and the lateral band shifts to orange. These colour patterns result

in diametrically different levels of chromatic contrast, such that males show higher chromatic contrast overall when courting females than when interacting with other males. Speed of the colour changes, however, is faster during male-male interactions than during male-female interactions. Although it is common to find population differences in static colours (Endler 1991; Marshall et al., 2015), I also find that the nature of this social colour communication differs across populations in anthropogenically-disturbed landscapes, suggesting that variation in physiological colours is influenced by environmental conditions as well as social context.

Many animals use visual signals during social communication, typically showing context-specific displays for courtship and competition. In many agamid lizards, static or developmentally fixed colours affect the probability of winning intraspecific competitive encounters (Stuart Fox and Johnston, 2005; Healey et al., 2007; Hamilton et al., 2013) or attracting mates (e.g. LeBas and Marshall, 2000; Hamilton and Sullivan, 2005). But unlike static colour signals, which cannot change during social interactions, physiological colours provide a wider communication range for species. During social interactions, males of the panther chameleon, *Furcifer pardalis*, shift their skin colour patches to an excited state during both courtship and competition contexts (Teyssier et al., 2015). I find that males of *P. dorsalis* show rapid colour changes similar to chameleons, but have two distinct bands on their body, as opposed to irregular patches, that change during social interactions. Despite the numerous descriptions of colour changes in agamas (e.g., Inoue and Inoue, 1977; Anibaldi et al., 1998; Norfolk et al., 2010; Langkilde and Boronow, 2012; Smith et al., 2016), no study to date has found different colour patterns during courtship and aggressive interactions. Males of *P. dorsalis* use the same body regions to

signal differently to males and females, shifting within seconds to minutes across large values of percent reflectance (from 20 – 80%) along a wide visible spectral range (hue). Thus, physiological colour change is a major signalling modality in this species. The strikingly different colour patterns expressed suggest that males may be conveying different information to conspecific males and females.

Visual signalling strategies of *P. dorsalis* involve both contrast and speed. Colour changes on males of *P. dorsalis* showed highest chromatic contrast during courtship interactions, but were faster (with lower chromatic contrast) during aggressive interactions. Bright and intense colours with high contrast are considered preferred qualities of potential mates for many vertebrates (e.g. Hill, 2006; Dubuc et al., 2014; Fukuda and Karino, 2014), but the extensive evidence comes from studies of species with static colour patches. Our results suggest that high chromatic contrast in colours during courtship displays (red dorsal + black lateral) may also be a signal of male quality for females. Conversely, the chromatic contrast of colour patterns during aggressive encounters (yellow dorsal + orange lateral) and during neutral non-social contexts (patchy yellow dorsal + patchy orange-brown lateral) are both low, which may enable males to more rapidly shift from neutral to aggressive displays. Rate of colour change may be a signal of male dominance in *P. dorsalis*, similar to *Chameleo calyptratus*, where the rate of colour change is correlated to winning fights (Ligon and McGraw, 2013). Determining the specific information conveyed with the speed and the colour patterns used during social interactions needs further investigation.

Psammophilus dorsalis not only expresses rapid colour change that is specific to the social context, but I find some population variation in the timing and magnitude of

colour change that may be related to urbanisation. Phenotypes of species that live in urban environments show major shifts in a range of ways, including changes in physiological, behavioural and morphological traits (French et al., 2008; Lazic' et al., 2013; Sol, Lapiedra and González-Lagos, 2013; Hutton and McGraw, 2016). Effects of urbanisation on animal communication has also been documented, especially in song structure and plumage colour of avian species and in social signalling in fish species (Wood and Yezerinac, 2006; Jones et al., 2010; Nemeth et al. 2013; Tringali and Bowman, 2015; Wong and Candolin, 2015). To date, the effect of urbanisation on visual signaling in lizards is unknown (but see Tuomainen and Candolin, 2011; Sol et al. 2013; Bateman and Fleming, 2014; Hutton and McGraw, 2016 for urbanisation effects on other behaviours). I find that during courtship encounters, male lizards in suburban habitats showed duller colours and were generally slower to attain maximal colour shifts than rural males. If courtship colour changes are honest indicators of male quality, then the duller suburban males may be of lower quality than rural males, beyond the expected differences due to body condition. Furthermore, population differences were not similar for all colour bands on males, suggesting that some signals (black lateral band) are more robust to environmental variation than others.

The intensity of visual colour signalling can be influenced by mechanistic, as well as ecological and social factors. Yellow-red pigment colouration has been documented in many animals and is attributed to the synthesis of pteridines and acquisition of carotenoids from the diet (Grether et al., 2001; Hill et al., 2002; Sefc et al., 2014). But for *P.dorsalis*, dietary overlap between urban and rural populations is high (80%) and the only notable difference is in the number of ants consumed (Balakrishna et al., 2016),

which are not likely to contribute to yellow-red pigmentation. Thus, differences in the dorsal colour pattern between suburban and rural males of *P. dorsalis* is unlikely to be due to dietary differences. Ecological and social factors in the anthropogenically-disturbed and natural habitats, however, can affect colouration in animals. Unlike rural habitats, urban areas are more heterogeneous in a range of ways, from more varied substrate types and different microhabitat conditions (Gomez and Thery, 2004; Leal and Fleishmann, 2004), to higher unpredictability and greater changes in habitat structure (Gilbert, 1991). In our study area, habitat fragmentation has resulted in higher local densities in the suburban population (Amdekar and Thaker, 2016), and potentially habituating males to repeated close-range social interactions. Thus, low colour contrasts in suburban males might reflect a reduction in the need to intensively signal in high density social conditions. Composition and abundance of predator communities might also be different in suburban habitats compared to rural areas (e.g. Thorington and Bowmann, 2003; Amdekar and Thaker, unpublished for *P. dorsalis*). A combination of variable microhabitat conditions, changes in local densities, and differences in predation pressure may interact to influence the intensity of visual signals, potentially shifting communication strategies of *P. dorsalis* in these remnant urbanised habitats.

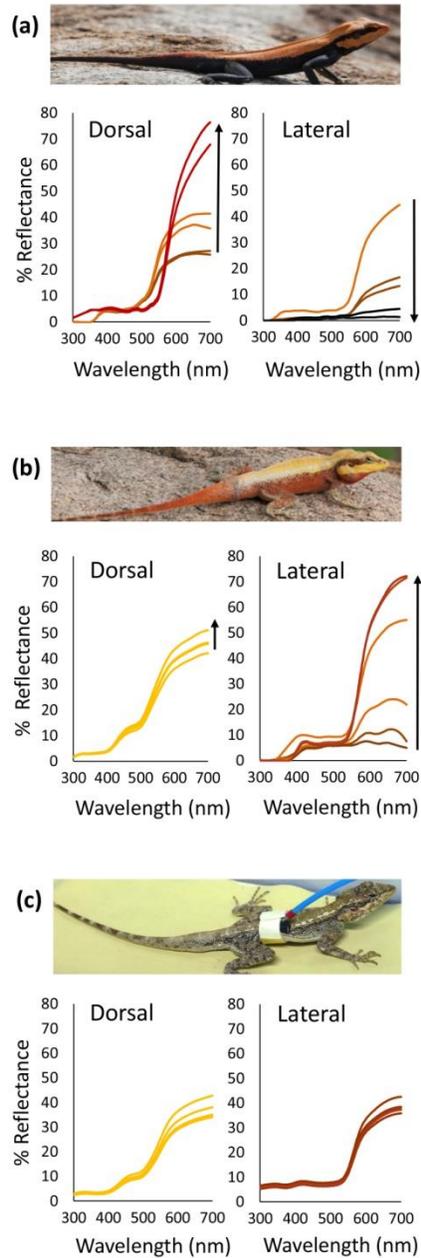


Figure 2.1. Rapid physiological colour change in a typical male of *P. dorsalis* during social interactions. (a) During male-female interactions, the dorsal band shifts from patchy yellow to red and the lateral band shifts from patchy brown to black. (b) During male-male interactions, the dorsal band shifts to yellow, and the lateral band shifts from patchy brown to orange. (c) During control non-social conditions, the dorsal and lateral bands show little change from patchy brown. The arrows indicate direction of colour change over a 30-min interval.

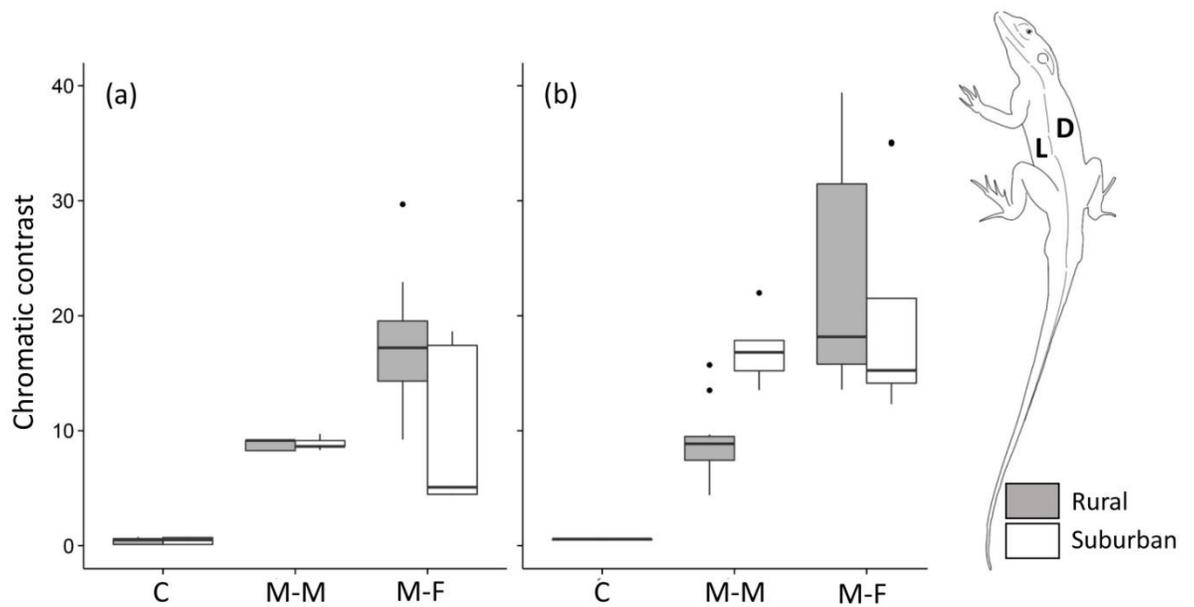


Figure 2.2. Chromatic contrast of the maximal physiological colour on males of *P. dorsalis* during male-female interactions (M-F), male-male interactions (M-M) and control conditions (C). Chromatic contrast of the (a) dorsal band differed significantly between social contexts and between urban and suburban populations, while that of the (b) lateral band differed significantly between social contexts only. Shown are box-plots, with medians, quartiles, 5th and 95th percentiles and extreme values.

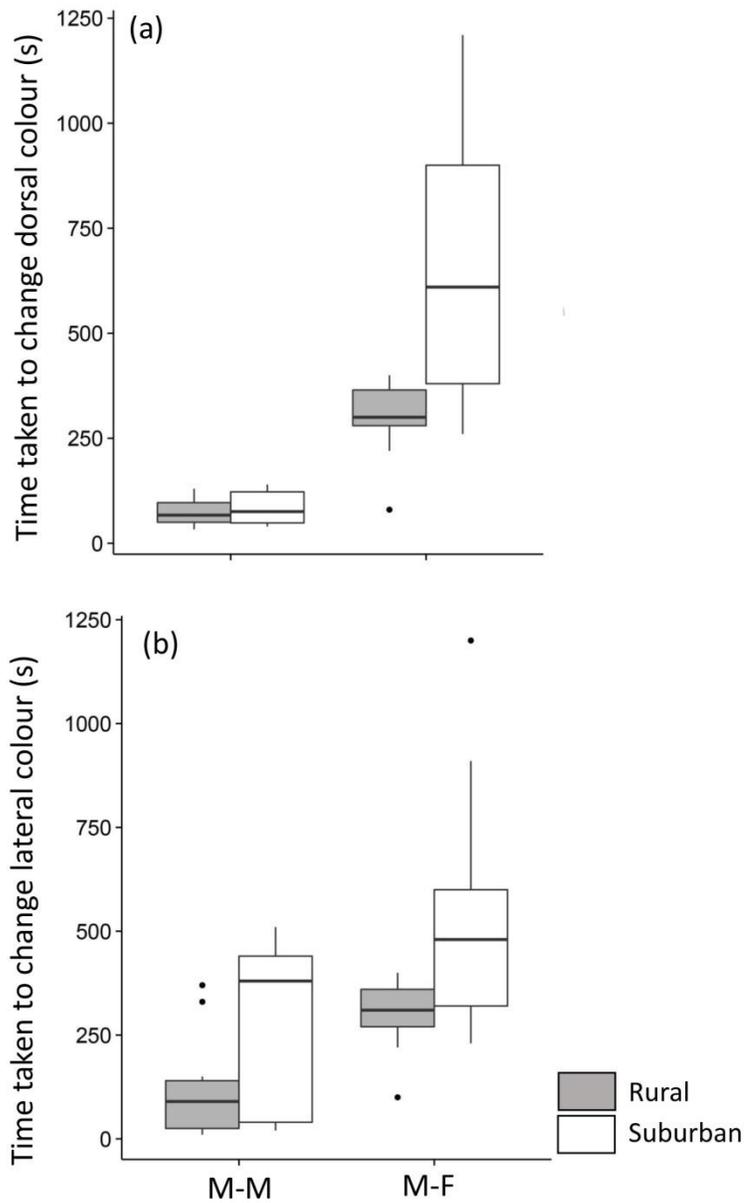


Figure 2.3. Time taken (s) to attain a maximal colour shift during male-female interactions was significantly faster for rural males of *P. dorsalis* compared to suburban males for the (a) dorsal band and (b) lateral band. Shown are box-plots, with medians, quartiles, 5th and 95th percentiles and extreme values.

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Chapter 2

Receiver responses to social display colours: effects of brain laterality and population difference in detecting signal components

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3.1 Introduction

“indeed a conscious system in its own right, perceiving, thinking, remembering, reasoning, willing, and emoting. . . both the left and the right hemisphere may be conscious simultaneously in different, even in mutually conflicting, mental experiences that run along in parallel”

— Roger Wolcott Sperry, 1974

Lateralization of the brain, wherein the left and right hemispheres show independent functions, has been well documented in a range of taxa (Bisazza et al., 1998; Byrne et al., 2004; Rogers, 2002). Early studies with humans found dominant activity for speech or language in the left hemisphere, while the right hemisphere dominates during spatial and emotional tasks (Galaburda et al., 1978). In many invertebrates and vertebrates, performance tasks including social behaviours, foraging and anti-predatory behaviour, vocalisation, response to novel stimuli, and handedness are lateralized (Bisazza et al., 1998; Rogers, 2002; Vallortigara and Rogers, 2005). Although hemispheric brain areas control the opposite sides of the body, functional overlap of information between the two hemispheres does occur via commissural connections (Bisazza et al., 1998). High levels of information transfer between the brain hemispheres are found in primates and other mammals (Parsons and Rogers, 1993), but other vertebrates such as birds have fewer commissural connections between brain hemispheres (Bisazza et al., 1998; Lustig, 2012). Inter-hemispheric commissural connections are weakest in fish, reptiles, and amphibians, resulting in high functional laterality and hemispheric dominance for a range of tasks (Bisazza et al., 1998; Lustig, 2012).

Like most of sensory information, visual information is processed in multiple regions of the brain. In vertebrates, full or partial decussation of the optic nerve occurs at the optic chiasm and thus projections are either fully contralateral, or in some cases both contralateral and ipsilateral in each hemisphere. In mammals, partial decussation of the optic nerve and high level of hemispheric connectivity exists, and thus visual information from both eyes are processed simultaneously in the cerebral cortex of both hemispheres with functional overlap of information (Denenberg, 1981). In birds, fish, reptiles, and amphibians, full decussation of the optic nerve occurs, resulting in contralateral projections from each eye (Greenberg, 1982; Bisazza et al., 1998; Denenberg, 1981). Since these groups of animals mostly have independent monocular vision from each eye, visual information from each eye is processed independently in the two brain hemispheres. Study of the brain and visual system in *Anolis* lizards indicate that a vast majority of the retinal fibres project contralaterally to the opticus tementii with little ipsilateral connections to the thalamus (Deckel, 1995). The small inter hemispheric projections that are present are unlikely to allow high level functional integration of information across the two hemispheres (Greenberg, 1982). Thus, to control for conflicting responses elicited by two stimuli, hemispheric dominance for different tasks is important (Vallortigara and Rogers, 2005). Hemispheric biases during social interactions in particular has been reported in olive fruit flies (Benelli et al., 2015), fish (Cantalupo et al., 1996; Sovrano et al., 2001), lizards (Hews and Worthington, 2001; Hews et al., 2004) and mammals (Austin and Rogers, 2012). A consistent left eye bias during aggressive and courtship interactions for tetrapod vertebrates has emerged from several studies (Andrew and Rogers, 2002; Bisazza et al., 1998; Hews and Worthington, 2001; Hews et

al., 2004; Deckel, 1995). Left visual field dominance during aggressive interactions suggests that the right hemisphere responds actively during competitive interactions (Greenberg, 1982; Deckel, 1995).

Many animals especially lizards use postural and colour displays during social interactions with conspecifics (Sullivan et al., 2007). The posteriolateral placement of the eye prevents binocular vision in lizards and thus provides an advantage for testing lateralized responses during social displays. Specific behavioural and colour displays are used by several lizard species groups, such as chameleons (StuartFox and Mousalli, 2008; Ligon and McGraw, 2013; Teyssier et al., 2015), anoles (Deckel, 1995) and agamids (Zambre and Thaker, 2017; Chapter 1). For example, lateralization in social behaviour has been reported from male *Anolis sp.*, male *Urosaurus ornatus* and female *Sceloporus virgatus*, all of which show left eye bias (or right hemispheric dominance) during aggressive interactions (Deckel, 1995; Hews and Worthington, 2001; Hews et al., 2004). Social displays in many vertebrates, including lizards, however are multi component, comprising of colour and behavioural displays (Lebas and Marshall, 2000; Arbutnott and Crespi, 2009). Complexity of signalling can thus encompass either multiple components or even variation in signal components across different habitats. Signalling in a disturbed habitat might cause alterations in the original signal by affecting some components more than others, such as colours used for visual signalling (Wong et al., 2007; Chapter 1). Under such circumstances, effective communication may require receivers to also match the signaller displays for communication to persist effectively.

I examined laterality in the Indian rock agamid *Psammophilus dorsalis* to determine whether motion and colour elicit lateralized responses in receivers. I also

examined if the responses of male and female receivers, independent of laterality, match the known variation in signaller displays across populations. Males of *P. dorsalis* have complex social signalling that involves physiological colours (Chapter 1) and conspicuous behavioural displays (see Chapter 3 and Deodhar, 2017). During the breeding season, typically from April to August, males develop conspicuous physiological colours on the dorsal and lateral regions of their body which can shift dynamically within minutes depending on the type of social interaction (Chapter 1). In general, when males of *P. dorsalis* interact with females, the dorsal band changes from patchy yellow (typical neutral colour) to orange or red, while the lateral band changes from patchy orange (typical neutral colour) to black (see Chapter 1). During interactions with other males, the dorsal band changes to yellow, while the lateral band changes to an even bright orange (see Chapter 1). As is typical for most agamid lizards, *P. dorsalis* also shows head bob and push up displays during inter- and intrasexual social interactions (Pandav et al., 2007; Deodhar, 2017). *Psammophilus dorsalis* are also found across urban and rural habitats and males of these populations show marked differences in social signals in terms of colour displays. Urban males are duller and paler in their dorsal colours during courtship displays and are slower to change colour (see Chapter 1). In this study, I examined laterality in receiver responses to social signals with the use of robotic stimuli that mimicked two key components: (1) multiple speeds of head bobbing behaviour (slow, medium, fast), and (2) multiple colours (red, yellow, black and grey). The visual grasp reflex (Nava et al., 2012; Zambre and Thaker, 2017) of receivers in response to controlled robotic stimuli exposed simultaneously in both visual fields allow us to determine visual field bias for movement and colour stimuli. The method of using

robotic stimulus to elicit responses has been previously shown in many lizards and thus is a robust method to examine receiver responses (Nava et al 2009; Nava et al., 2012; Clark et al., 2015; Zambre and Thaker, 2017). By recording receiver responses to paired stimuli, I can determine whether and to what degree complex social signal processing are lateralized, as well as whether receivers from different populations differ in their responsiveness to social signals. Under natural conditions, behavioural displays by males are directed at both male and female receivers, and thus I expect both sexes to respond similarly to robotic movement. Given the context specific display of colours, I expect responses to robotic colour stimuli to be lateralized and specific to the sex of the receiver, such that females respond more strongly to red and black whereas males respond to yellow and red. Independent of laterality, I also expect receiver responses to match the intensity of signals between populations.

3.2 Methods

Designing of robotic stimuli

I designed robotic stimuli to closely mimic two key aspects of the social display of *P. dorsalis* males: motion and colour. To minimize the complexity of the stimuli, robotic stimuli were not meant to mimic the entire lizard body, but was a cylinder (3.5 cm diameter x 4 cm length), approximately the size of a *P. dorsalis* male's head that varied in colour and moved in a manner that mimicked a typical head bob display (Figure.3.1). To correctly match colour and motion to the natural responses of males, I first caught wild sexually mature males and females during the peak breeding season and brought them to the laboratory, where they were housed individually in a dedicated lizard housing

facility. After a 24-hour habituation period, males were allowed to interact undisturbed for 30 min with a conspecific male or female ($N=20$ for each type of social interaction, Batabyal and Thaker unpublished). These social encounters were staged in large glass tanks (95x45x30cm) under full spectrum lighting behind a blind, and were digitally recorded from above. From these videos, I extracted all occurrences of headbob displays and quantified the rate of headbob, measured as the time it took for a male to lift and return his head to the original position (i.e. perform a single head bob). Speed of headbob displays by males ranged from 1.25 – 2 Hz during interactions with females and from 0.75 – 1.5Hz during interactions with males with an amplitude ranging between 20-30 degrees from the viewing conspecific. Thus, I designed the robotic stimuli to move at one of three rates: slow (0.5Hz), medium (1Hz) and fast (2Hz) with an amplitude of 30 degrees centered at the test lizard's nearest eye, which reflected the range of head bob speeds showed by males during male-male (medium) and male-female (fast) interactions.

During social interactions, males of *P. dorsalis* express red (dorsal and lateral regions), yellow (dorsal region only), and black (lateral region only) physiological colours (Chapter 1). To ensure that the colours on the robotic model were within the natural range and perceptually similar to the spectral reflectance of males, I used visual modelling to compare the red, yellow and black paints on the robots to the reflectance displayed by *P. dorsalis* during social interactions. Natural spectral reflectance for social colours were obtained from Chapter 1 data, and spectral reflectance for the robotic colours were taken using a spectrophotometer (Ocean Optics Jaz), following methods detailed in Zambre and Thaker, 2017 and Chapter 1. Visual modelling was done using the PAVO package in R statistical software (Maia et al., 2013) using spectral sensitivity

states of the agamids *Ctenophorus ornatus* and *C. decresii* species for the analysis (from Barbour et al., 2002 and Yewers et al., 2015 respectively). Visual systems in diurnal lizards are conserved and therefore the spectral sensitivities of *Ctenophorus* species serve as a suitable alternative, since those of *P. dorsalis* are unknown (Olsson et al., 2013). Irradiance values for visual modelling was fixed as standard daylight irradiance conditions ('D65') provided in the PAVO package. I also applied the von Kries transformation as described in Endler and Mielke (2005) to account for light adaptation. Euclidean distances between red, yellow, and black obtained from averaged natural spectra and from the robotic stimuli indicated that stimulus colours were similar to natural spectra of *P. dorsalis* (Euclidean distances between colour pairs: average natural black and model black=0.024; average natural orange-red and model red=0.362; average natural yellow and model yellow=0.164, also see Figure.3.2).

Experimental design of receiver responses

Receiver responses for motion and colour were measured during the breeding season from April to August 2015 using wild-caught adult males and females of *P. dorsalis*. All animals were housed individually in glass terraria (60x30x25cm) in a dedicated animal housing room that permitted natural temperature and light conditions. Terraria were lined with disposable paper towels as the substratum, provided with rocks for refuge, and were covered on all sides to minimize disturbance. Lizards were acclimatized for 2 days before the start of trials. Animals were provided with live ants and field crickets daily for food and water *ad libitum* and were maintained in the laboratory for 7-9 days.

Receiver responses were measured using a paired choice trial. I designed narrow glass testing tanks (35x11x35 cm) that restricted the movement of lizards such that they were facing the front throughout the trial. The front and back of the tank were opaque to minimize disturbance and the long lateral sides were transparent with brown removable sliders. One robotic stimulus was placed on each of the two lateral sides of the tank with standard brown cardboard as the background to mimic the natural signalling conditions. This entire setup was kept under full spectrum lighting conditions in the laboratory, and was behind a blind to minimize disturbance. Two behavioural responses were recorded during each trial: (1) the choice made by each focal animal, measured as the movement of the head in the direction of the preferred stimulus, and (2) the latency to make that choice (Nava et al., 2009; Nava et al., 2012).

To determine responses to movement rates, males ($N=42$) and females ($N=35$), were given a choice between the following robotic pairs: slow(0.5Hz), medium (1Hz), or fast (2Hz) moving neutral grey stimulus paired with a stationary neutral grey stimulus. To quantify laterality in receiver responses to moving stimuli, I exposed all animals to the robotic stimuli in both left and right visual fields, in random order with a 24hr time gap between trials. Thus, the same pairing was repeated for all lizards with the stimuli positions switched. Results from the motion trials (see Results below) were used to determine the speed with the lowest variation in response times for both males and females. This speed of motion (1 Hz) was then set for the colour trials as the standard speed for all pairings. To determine responses to stimulus colour, males ($N=40$, for 6 colour trials) and females ($N=32$, for 6 colour trials) were given a choice between the following robotic pairs: a red, black, or yellow moving stimulus paired against a moving

grey stimulus. Similar to the motion trials, the positions of the coloured and grey stimuli was initially randomized and then switched.

All behavioural trials were digitally recorded with an overhead camera and the preferred choice was classified when the lizard completely turned its head towards one stimulus. Digital recordings were used to determine the latency of each choice. Before the start of each choice trial, lizards were allowed to bask individually under a 60 W incandescent light bulb for at least 30 min to reach preferred body temperatures before being transferred to the testing tank. Lizards were allowed 10 mins to acclimate in the testing tank before the beginning of each trial, during which the lateral sides were blocked with brown cardboard. When the boards were removed, each lizard was exposed to two stimuli simultaneously on either side. Lizards were allowed 4 hours in their home tanks between successive trials and same individual was tested for maximum of 2 trials in one day. One set of lizards (Males: $N=42$, Females: $N=35$) were exposed to all motion trials and a different set of lizards were exposed to all colour stimuli (Males: $N=40$, Females: $N=32$). Responses of lizards were separated according to habitat (Suburban and Rural) for further analyses (see Statistical analyses and Results).

Statistical analyses

I first scored responses of individuals as 0 (no response) and 1 (response) based on whether they turned towards a stimulus, for all types of stimuli (3 speeds or 3 colours) and for all visual fields of exposure (left or right). To determine if the stimulus elicited lateralised responses (turn to one side over another), I used generalised linear mixed effects models (R package: `glmmADMB`, Bolker et al. 2012) with a binomial

distribution, wherein the response variable was scored as 0 or 1, and stimulus type (slow, medium, fast), sex (males and females), and visual field (left or right) were fixed factors with lizard ID as a random effect. I first constructed a global model with sex, stimulus type, and visual field of exposure as three-way interaction factor (model1: Response ~ Sex * Stimulus * Visual_field, random=1|ID). I then contrasted simplified models with two-way interactions of the fixed factors against this global model to determine the most parsimonious model (Anova). An additive model of sex, stimulus type, and visual field of exposure, with an additional interaction term of sex and stimulus (model2: Response ~ Sex + Stimulus + Visual_field + Sex:Stimulus, random=1|ID), sufficiently explained the response of lizards (Anova comparison of model1 and model2: $P=0.761$).

I ran a similar glmm (glmm ADMB) for colour stimuli (black, red, yellow vs grey). Responsiveness to colour was best predicted by a global model with sex, type of colour, and visual field as three-way interaction (model1: Response ~ Sex * Colour * Visual_field, random=1|ID). Removal of the three way interaction term resulted in a significant difference between the models (model2: Response ~ Sex + Colour + Visual_field + Sex:Colour + Colour:Visual_field+ Sex:Visual_field, random=1|ID; Anova comparison between model1 and model2: $P<0.001$). Thus, the three-way factor of sex*colour*visual_field was an important predictor of whether lizards turned towards a coloured stimulus. To better understand these interactions, I separated the data into males and females and analysed sex-specific responses to colour and visual field as a two-way interaction model (Response~Colour*Visual_field, random=1|ID). For both colour and motion stimuli I performed contrasts between interacting factors in the glmm models using lsmeans (Lenth, 2016).

For those individuals that responded, I then compared the latency to respond to a moving stimulus to determine whether sex (male, female), speed of stimulus (slow, medium, fast), and visual field (left, right) affected how quickly an animal responds by using a linear mixed effects model with individual ID as a random effect. Similarly, I compared the latency to respond to a colour stimulus using a linear mixed effects model with sex, type of colour (black, red, yellow) and visual field as fixed factors and individual ID as random effect (R package: lme4 and lmerTest (Bates et al., 2014; Kuznetsova, A., Brockhoff, P. B., & Christensen, 2016)). As I found left visual field dominance for colour (see Results) I subdivided response of left visual field of males and females according to habitat (urban and rural) and compared the latency to respond to colour stimulus using a linear mixed effects model with type of colour and habitat as fixed factors and individual ID as random effect. For these analyses, I included an interaction term between the three fixed factors and presented the F test for lmer results that predicted the latency to respond (type III with Satterthwaite approximation for degrees of freedom). Where relevant, I performed post hoc comparisons for all interactions using lsmeans (R package lsmeans: (Lenth, 2016)). Variation due to individual ID was low for all tests ($SD < 1.00$). All data analyses were performed using R studio version 3.4.2 (R core team 2016).

3.3 Results

Response to a moving stimulus

When given a choice between a moving stimulus and a stationary one, all lizards that responded chose the moving stimulus, regardless of motion speed. The response of

lizards, measured as whether or not they turned to the stimulus (0 or 1), was best explained by an interaction of sex and stimulus speed, and not visual field (z ratio=1.19, $p=0.071$, Table 3.1). When given a choice between a stationary stimulus and a slow moving stimulus, more males turned towards the moving stimulus than females (z ratio=-2.92, $p=0.040$, Figure 3.3, Table 3.1). More females turned towards the moving stimulus when it was of medium speed compared to slow speed (z ratio=2.79, $p=0.054$, Figure 3.3, Table 3.1).

Latency to respond to a moving stimulus was significantly affected by an interaction between sex and speed of the stimulus (interaction $F_{2,334}=3.20$, $P=0.041$), but not the visual field ($F_{1,334}=0.13$, $P=0.717$). Further post hoc comparisons showed that males responded fastest to the medium speed compared to the fast speed (post hoc $p=0.007$, Figure 3.3). Females responded similarly to all speeds (post hoc $p>0.07$ for all pairwise comparisons, Figure 3.3).

Response to a coloured stimulus

During the colour trials, all lizards that responded turned towards the coloured stimulus over the grey one, indicating that they could detect differences between these stimuli. The proportion that responded, however, depended on the type and position of the colour stimulus, with more males and females being responsive when the coloured stimulus was in their left visual field compared to right (Table 1). When comparing sex-specific responses across colour stimuli, a greater number of males responded to red and yellow colours when they were seen from their left visual field compared to right (Red: z ratio= 3.09, $p=0.001$; Yellow: z ratio=2.23, $p=0.025$, Figure 3.4, Table 3.1). Females showed similar responses to red and black colours, wherein a greater number of females

responded to these colours when they were seen from their left visual field compared to right (Red: z ratio= 1.97, $p=0.047$; Black: z ratio=3.43, $p< 0.001$, Figure 3.4, Table 3.1).

Latency to respond to a colour stimulus was affected by an interaction between sex and the type of colour (interaction $F_{2,288}=4.48$, $P=0.012$), as well as an interaction between type of colour and visual field (interaction $F_{2,288}=4.73$, $P=0.009$). Post hoc comparisons showed that males responded fastest to red compared to yellow (post hoc $p=0.028$, Figure 3.4). Latency for females to respond were similar across coloured stimuli (post hoc $p > 0.20$ for all pairwise comparisons, Figure 3.4). Laterality, or left visual field bias, in the latency to respond was observed only for yellow stimuli for both males (mean \pm s.e.m: 31.54 \pm 1.84 sec for left vs 44.50 \pm 1.97 sec for right; post hoc $p<0.001$) and females (23.40 \pm 1.79 sec for left vs 37.42 \pm 2.20 sec for right; post hoc $p<0.001$).

Population differences in receiver responses

Based on the responses above, populations comparisons were done with only left visual field responses (Suburban: Male=20, Female=15; Rural: Male=20, Female=17). Suburban and rural population differences in response to colour stimulus was found for females only and not males (Figure 3.5). Latency to respond to a moving colour stimulus by females was affected by an interaction between habitat and colour ($F_{2,55}=5.02$, $P=0.009$). Post hoc comparisons showed that suburban females were slower to respond to red colour compared to rural females (post hoc $p=0.009$, Figure 3.5b).

3.4 Discussion

Social displays in *P. dorsalis* are complex, involving physiological colours and behaviour displays. When stimulus components are separated, I found that males and

females show lateralized responses for colours and not for moving stimuli. Regardless of the speed of the stimulus, all lizards chose the moving stimulus over the stationary one in both visual fields. In response to colour, more males and females responded when the colour stimulus was on left visual field than on right. The results are in general congruence with previous evidence across taxa for right hemispheric preference in social aggression, although I show for the first time in lizards that all social display colours, including courtship displays, have a left visual field bias. I also find some population differences in how quickly receivers respond to some colour stimuli, which correspond to inter-population variation in the signals themselves.

Lateralization was not observed for general moving stimuli for both males and females. This suggests that motion alone might not be a sufficient component for recognition of social displays, especially since motion is also associated with many other stimuli such as with predators or prey. A variety of species across taxa are found to be more reactive to predators seen in their left visual field than right (Vallortigara et al., 1999). In contrast to leftward responses for predators, toads and a variety of bird species are more likely to attack prey viewed from the right hemifield (Vallortigara et al., 1998; Clayton and Krebs, 1994; Ventolini et al., 2005). These rightward biases for foraging response is only apparent when the food needs to be discriminated from similar targets and handled with precision, and not for simplified prey objects (Robins and Rogers, 2004). Though the movement pattern for conspecific displays and movement of predators or prey might be different, a general motion stimulus in the environment might be sufficient to attract attention across both visual fields. Further processing of motion stimuli for precise responses might invoke laterality when the organism needs to respond

with elaborate behavioural reactions. In my study, I found that motion that mimics general lizard head bob behaviour without any association with display colours, elicits similar responses from both visual hemifields, thus strongly supporting the hypothesis that *P. dorsalis* might use motion to attract attention of receivers. Laterality in signal processing is only seen when motion is associated with specific social display colours.

My results show that proportion of male responses to red and yellow colours and female responses to red and black are lateralized. There are two key conclusions that can be drawn from these results. First, receiver responses match the physiological display colours of male *P. dorsalis* during competition and courtship interactions. Males of *P. dorsalis* display red dorsal and black lateral body colours during courtship and yellow dorsal and red lateral body colours during competitive interactions. Thus, males are considerably less responsive to black and females are less responsive to yellow, which are the colours that are not typically displayed to them during social interactions. These sex differences suggest that responses were not generalised for all colours but right brain hemisphere dominance is seen for relevant social display colours only. Second, my study shows right hemispheric dominance for all social colours. The phenomenon of right hemispheric dominance during aggressive displays is commonly found in other taxa such as fish, birds, and lizards (Bissaza et al., 1998; Worthington and Hews, 2001; Hews et al., 2004). But very few studies have examined lateralization in both courtship and aggressive contexts for a single species. In a now classic experiment, hemispheric dominance in chicks was detected by injecting cyclohexamide or glutamate in left and right brain hemispheres which blocked proper development. Elevated levels of attack and copulation were observed following injection in the left and not the right brain

hemisphere, suggesting that the right hemisphere activated attack and copulation while left hemisphere suppresses it (Howard et al., 1980). In my study, this might be the reason why visual grasp reflex was found to be stronger when such social display colours, which generally elicit an aggressive or courtship response from receivers, was presented in the left visual field compared to right.

Along with laterality in receiver responses for social display colours, *P. dorsalis* females across suburban and rural habitats differ in their latency to respond to specific colours. During courtship interactions under natural conditions, the dorsal red colour on males differ between suburban and rural populations, such that suburban males have lower colour contrast and a higher variation in their dorsal red (Chapter 1). Variation in red colour quality in males may explain why female receivers from rural populations were faster to respond to red compared to suburban females. Signals, receiver responses, and the signalling environment are highly coupled and thus a shift in one of the factors can causes the others to alter as well (Endler, 1992). When signals are composed of multiple components and the fluctuating and changing signalling environment affects one component more than the other, receivers can shift their reliability from a signal component that is malleable for a signal component that is more robust against environmental fluctuations. In case of *P. dorsalis*, signaller males from disturbed urban habitats, seem to have one colour component (dorsal red) that is more affected and variable than the other (lateral black) (results from Chapter 1 showing dorsal red in males from suburban sites have lower contrast and greater variation in colour compared to males from rural sites, Figure 2.2a). Receiver responses, wherein female receivers respond faster to black followed by red in suburban habitats, suggest that black is a more

robust signal in that habitat (Figure 3.5b). Further studies are needed to understand what each colour component of the signal conveys and how environmental fluctuations that leads to signal variation might affect mating opportunities and ultimately fitness.

Table 3.1. Proportion of individuals that responded to different motion and colour stimuli when viewed from the left vs right visual field.

Stimulus	Type	Sex	Visual field	
			Left	Right
Motion	Slow	Female	0.57	0.51
		Male	0.95	0.83
	Medium	Female	0.85	0.77
		Male	0.95	0.83
	Fast	Female	0.77	0.60
		Male	0.73	0.59
Colour	Black	Female	1	0.40
		Male	0.77	0.72
	Red	Female	1	0.45
		Male	0.92	0.29
	Yellow	Female	0.66	0.57
		Male	0.84	0.41

Proportion was calculated from 35 females and 42 males tested in the motion trials and 32 females and 40 males tested in the colour trials. Significant laterality (bias towards left visual field) by males and females was seen for specific colours, as indicated in **bold**.



Figure 3.1. Experimental setup in which two stimuli were simultaneously placed on either side of the testing tank, where the test lizard was introduced. The lateral view shows the stimulus from the perspective of the lizard.

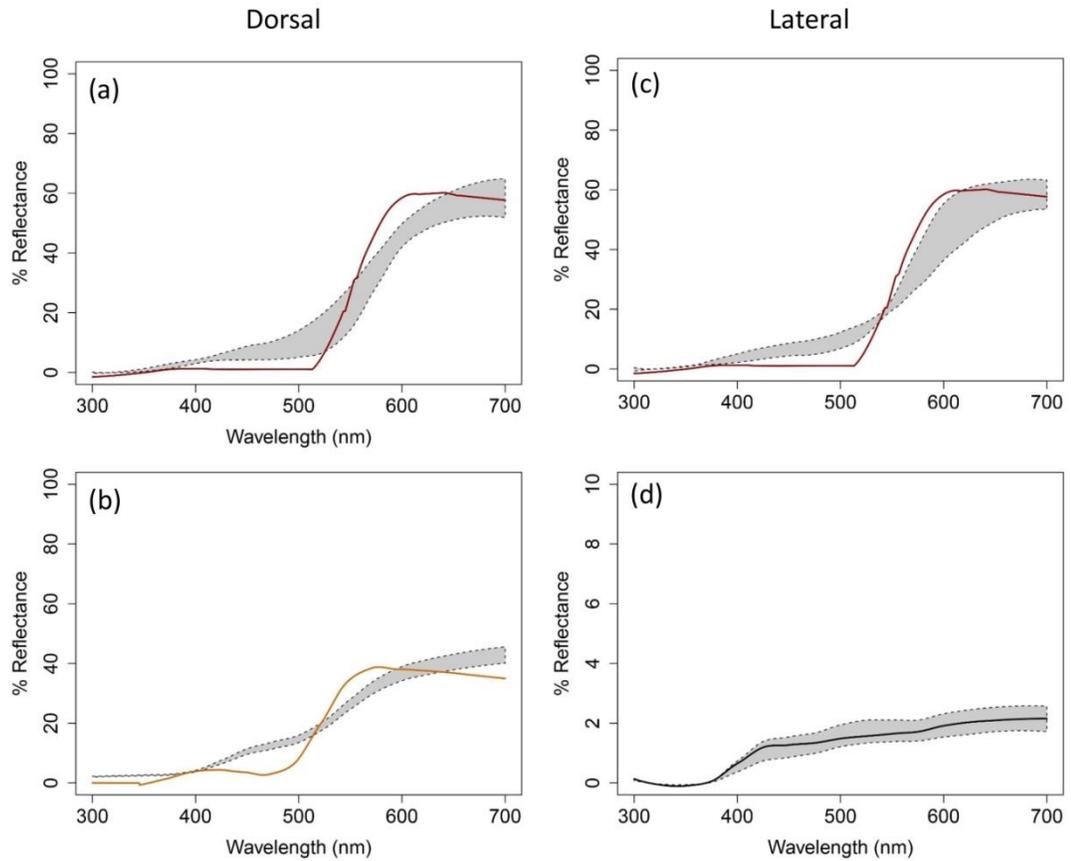


Figure 3.2. Reflectance spectra of typical natural variation in dorsal (a) red and (b) yellow, and lateral (c) red, and (d) black colours in *P. dorsalis* (grey) and the corresponding model colours (solid lines) used on the robotic stimuli.

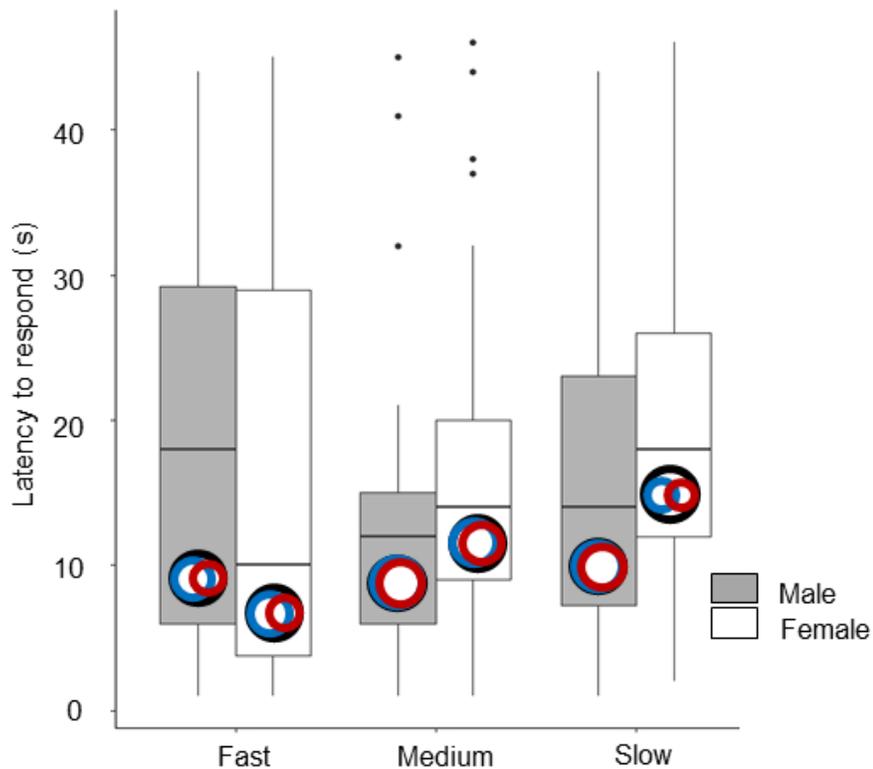


Figure 3.3. Latency to respond to a moving stimulus (Fast, Medium, Slow) from both visual fields combined for males (N= 42, grey boxes) and females (N= 35, white boxes). Boxplots show medians, quartiles, 5th and 95th percentiles and extreme values. There was no significant laterality in response to different motion stimuli, as illustrated by the inner circles within boxplots which represent the proportion of individuals that turned to the moving stimulus when it was on left visual field (blue circle) compared to when it was on right visual field (red circle). Diameters of the circles equal the proportion of individuals.

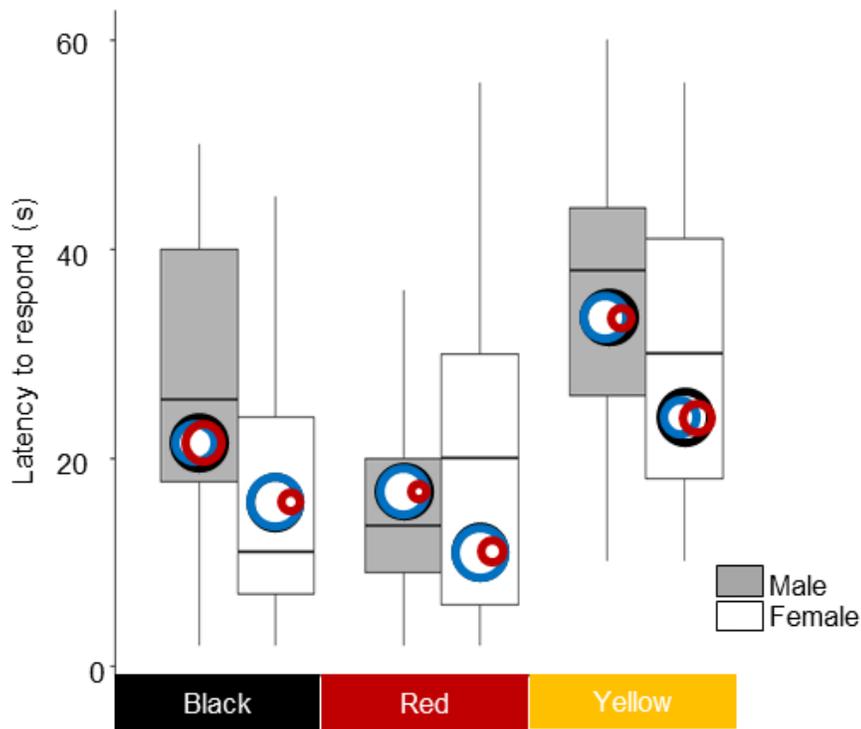


Figure 3.4. Latency to respond to a colour stimulus (Black, Red, Yellow) from both visual fields combined for males (N= 40, grey boxes) and females (N= 32, white boxes). Boxplots show medians, quartiles, 5th and 95th percentiles and extreme values. The inner circles within boxplots represent the proportion of individuals that turned to the colour stimulus when it was on the left visual field (blue circle) compared to when it was on the right visual field (red circle). Diameters of the circles equal the proportion of individuals. Note that significant laterality (bias towards left visual field) was observed for males in response to Red and Yellow colours and for females in response to Black and Red colours.

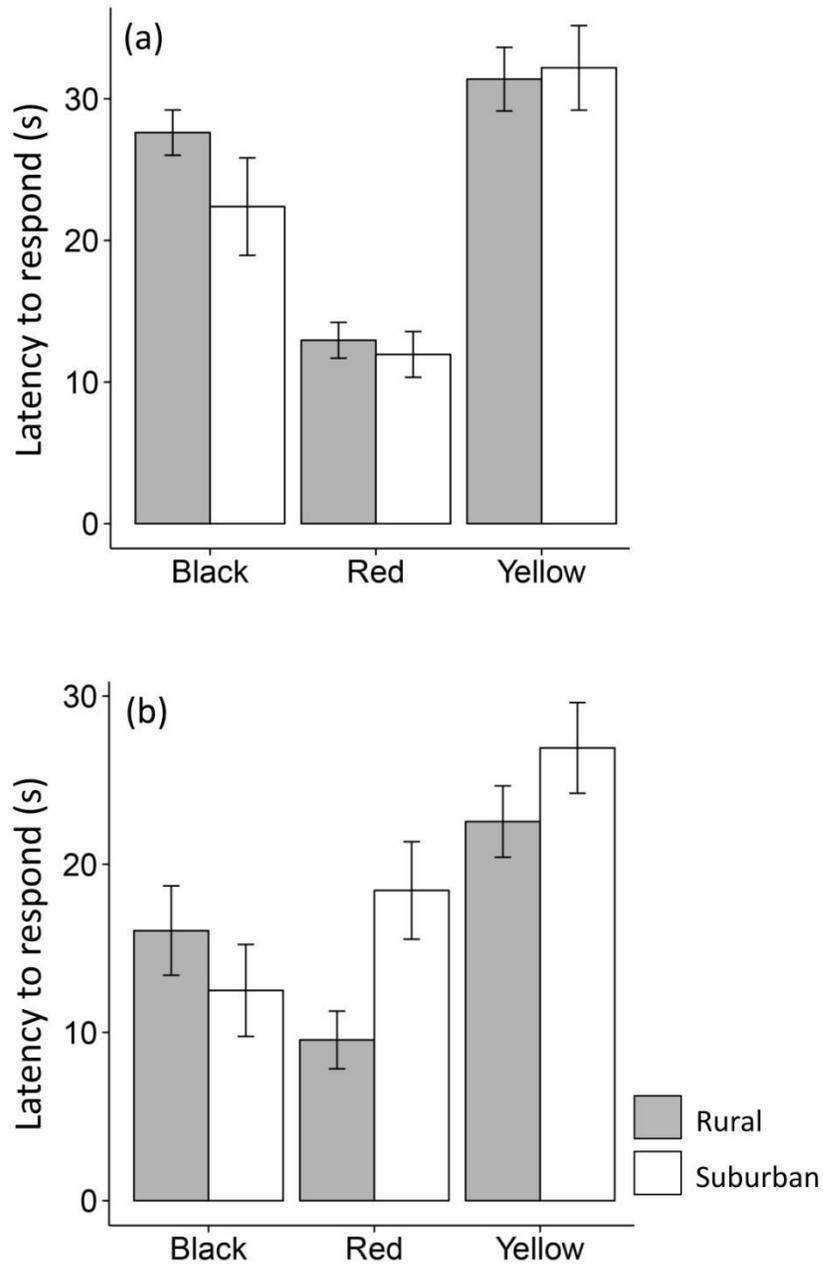


Figure 3.5. Latency to respond (mean \pm se) to a colour stimulus (black, red, yellow) for (a) males and (b) females from suburban and rural populations. Responses are only for left visual field where grey bars represent rural and white bars represent suburban populations.

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Chapter 3

Physiological stress and social behaviour: do urban and rural males differ in their courtship and aggressive interactions?

4.1 Introduction

Most animals respond to predictable and unpredictable changes in their environment by flexibly adjusting their physiology and behaviour. The activation of the hypothalamo-pituitary-adrenal (HPA) axis is one of the most robust, generalized, and consistent mechanisms by which animals respond to various environmental and ecological stressors such as shifts in temperature, humidity, predation pressure, conspecific density and resource availability (Sapolsky et al., 2000). Secretion of glucocorticoid hormones from an activated HPA axis mediates a suite of behavioural and physiological responses that are expected to have immediate adaptive functions that reduce the impact of potential stressors and increase the chance of survival (Moore and Jessop, 2003; Breuner and Hahn, 2003; Kitaysky et al., 1999). Glucocorticoids and other secreted hormones of the HPA axis (e.g. Oxytocin, ACTH, CRF) directly affect the central nervous system, cardiovascular system, immune system, and also reproductive system to stimulate a range of effects including enhancing memory consolidation, cardiovascular tone, immune activation, and altering reproductive behaviour (reviewed in Sapolsky et al., 2000; Wingfield and Sapolsky, 2003). The mechanistic interactions between the HPA axis and multiple other physiological pathways enable animals to respond rapidly to various challenges (Sapolsky et al., 2000). For example, in males of many squamates reptiles, the close link between HPA and hypothalamo-pituitary-gonadal (HPG) axes often leads to correlated changes in circulating androgen hormone levels along with up or down regulation of corticosterone in response to environmental stressors (Moore et al., 1991; Knapp and Moore, 1997).

The social environment of an animal is one of the primary sources of external stimuli that can induce a physiological stress response. The frequency, type of interaction, and social or dominance status of the individual can affect the HPA axis activity. Many species physiologically respond to social interactions with the activation of both the HPA and HPG axes (DeNardo and Licht, 1993; Knapp and Moore, 1997; Creel et al., 2013). The magnitude of change in circulating hormone levels and the timing of those changes, however, varies between individuals, morphs, and between species (Knapp and Moore, 1995; Knapp and Moore, 1996; Creel et al., 2013). For example, males of the less aggressive morph in the tree lizard, *Urosaurus ornatus*, show elevated plasma corticosterone levels and depressed testosterone levels after winning a staged male-male encounter. In contrast, the more aggressive territorial morphs of *U. ornatus* show no difference in plasma corticosterone or testosterone levels compared to control males who did not engage in social interactions (Knapp and Moore, 1996). In *Anolis carolinensis*, winners of a fight have elevated and sustained high circulating androgens within 1hr of a male-male interaction, whereas losers had depressed androgen levels even after 1 week (Greenberg and Crews, 1990). Thus, social encounters not only elicit changes in circulating glucocorticoid and androgenic steroid hormone levels, these hormones can further mediate behaviour during and after the social interaction.

Apart from natural environmental and social stressors, many species are now experiencing novel disturbances due to anthropogenic pressures. One common anthropogenic pressure is urbanisation, wherein animals are not only exposed to high human activity, but experience changes in habitats and microclimate, as well as altered food and predator communities (Tuomainen and Candolin, 2011). Species that persist in

urban habitats are forced to cope with these unpredictable and often sudden changes in the environment, which requires flexible behavioural and physiological responses (Tuomainen and Candolin, 2012; Sol et al., 2013), typically mediated by the activation of the HPA axis. Individuals may thus react by either heightening responsiveness and performance or by constraining expression of certain traits depending on the challenges encountered (Killen et al., 2013). By fragmenting natural habitats, urbanisation increases the frequency, distribution, and intensity of multiple perturbation factors that all interact to influence free living animals. Not surprisingly, many studies have found differences in circulating glucocorticoid levels and behaviour in animals as a consequence of living in urban environments, but the intensity and direction of responses vary between species (Partecke et al., 2006; Fokidis et al., 2009; French et al., 2010; French et al., 2008). Lack of a consistent directional response (i.e. elevated glucocorticoid levels in urban areas) strongly indicates the importance of understanding the specific ecological context as well as inter-individual differences in risk perception and responsiveness.

I conducted a fully factorial study to determine the effects of both social interactions and urbanisation on the physiology and social behaviour of the Indian rock agama *Psammophilus dorsalis*. I specifically quantified behaviour and steroid hormone levels (corticosterone and testosterone) of males during intersexual and intrasexual interactions to determine whether courtship and aggressive contexts elicit different responses. Behavioural responses were measured during the staged social encounters, and the magnitude and time-course of hormonal responses were quantified repeatedly for up to 30 min after the social interactions. As the first study to test the prediction that

urbanisation affects social interactions in a reptile species, I explicitly compared the physiological and behavioural responses of males from suburban and rural populations.

4.2 Methods

Psammophilus dorsalis is sexually dimorphic (SVL range: males = 95 – 140 mm and females = 75 – 96 mm) and males also develop conspicuous colouration during the breeding season, typically from April to August, which become dull at the end of the breeding season (Radder et al., 2005). During social interactions, males of *P. dorsalis* express dynamic physiological colour changes on their dorsal and lateral body parts that shift from yellow to red or from orange to black respectively depending on the social context (Chapter 1).

Experimental design

I performed the following experiments during the breeding season (April-August) over two consecutive years (2013, 2014). Free ranging male lizards ($N=32$ from suburban, 32 from rural) were captured by noosing and brought into the laboratory, where they were housed individually in glass terraria (60x30x25cm). All terraria had disposable absorbent paper as the substratum and rocks for refuge, and were covered on all sides to minimize disturbance. Terraria were maintained in a room that permitted natural temperature and lighting conditions. Individual 60 W incandescent basking lights were turned on from 0800 - 1200 hours, and from 1500 - 1600 hours, and lizards were provided with live ants, field crickets, and fresh water daily. All lizards were maintained

in the laboratory for 7-9 days and were returned to the site of capture after experimental trials were complete.

To quantify the effect of social stimulus on behaviour and hormonal responses, I exposed each focal male ($N=20$ from suburban and 20 from rural, excluding control animals) to a stimulus male and a sexually mature female separately. Stimulus males and females were caught from rural and suburban sites that was different from where focal animals were caught. Focal males were exposed to staged social encounters with stimulus animals from the same habitat type. Each behavioural trial began with a 30 min acclimatisation period in a testing tank followed by a 30 min interaction period with one of the stimuli. Social interactions were staged in testing tanks (95x45x30cm) that were lined on the bottom with a rubberized sheet marked with 4x4 cm grids. A different control set of males ($N=12$ from suburban and 12 from rural) were also kept in the same testing tank for 30 min but were not exposed to any social stimuli. Focal male lizards were randomly assigned to social and control groups. All trials were video recorded with Canon 550D for subsequent quantification of behaviour and to minimize disturbance by observers. After social interactions and blood sampling (see below), lizards were allowed 24 hrs to recover in their home tanks before being exposed to the second social stimulus. The order of stimulus (exposure to female or male) was randomized. All experiments were conducted from 08:00 – 12:00 or from 15:00 – 17:00, which are the peak activity periods for this species. Before the start of the experimental trials, mass (g) and snout-to-vent length (mm) were measured for all individuals using a weighing balance and digital callipers respectively. All lizards were returned to site of capture after the experimental trials.

Behavioural responses

From the 30-min video recording of social encounters with males and females, I recorded the following behaviours (similar to Carpenter, 1962; Radder et al., 2006).

Simple push-ups and head bobs: Push-up consists of raising and lowering the head and trunk by the straightening of the forelimb. It is mostly accompanied by head bobs. Head bobs are of two types in this species. A single head bob is performed when the animal raises its head ~1 cm from initial position and lowers it. A double head bob or shudder is performed when the animal raises and lowers its head twice in quick succession.

Crouch walk: This behaviour has not been described previously in published literature. A male performs a low walk by moving forward slowly with a continuous head shudder. This behaviour has been exclusively observed when males encounter females.

Bite: One individual grips any body part of another with its mouth.

Mount: One individual holds down the other either fully or partially by climbing on top of it.

Hormonal responses

After the first behavioural trial, I took a single blood sample from each focal male at one of the following time points: 0min, 10min, 20min or 30min post trial ($N=5$ individuals /time point, see Appendix Fig. A.4.1). Blood samples were obtained from the

retro-orbital sinus using heparinized microcapillary tube (following Thaker et al., 2009). A second blood sample was taken at the same time point after the second behavioural trial 24 hours later, resulting in two blood samples per individual. As a comparison, one blood sample was also obtained from the control animals (no social stimulus) at the same time points: 0min, 10min, 20min or 30min post acclimation ($N=3$ individuals /time point). Once collected, blood samples were centrifuged and the plasma was stored at -20°C until analysis. Enzyme-Immuno Assay kits (Arbor Assay Corticosterone kit no. 13A052 and 14A011; Testosterone kit no. 13A029 and 14A091) were used to measure the circulating corticosterone and testosterone levels in the plasma. EIA kits were first optimized as per Wada et al., 2007, and I subsequently analysed at a dilution ratio of 1:100 for corticosterone and 1:140 for testosterone. For both hormones, samples were run in duplicate and a total of 12 assays were run along with a duplicate lab standard in each assay. For corticosterone, the intra-plate coefficient of variation was 0.12-8.72 and the inter-plate coefficient of variation was 9.51. For testosterone, the intra-plate coefficient of variation was 0.04-9.81 and inter-plate coefficient of variation was 8.47.

Statistical analyses

I first compared the proportion of individuals that showed at least one overt behavioural response during each social interaction separately using a two-sample test for equality of proportion (Chi squared test) with continuity correction across the two habitats. For those individuals that showed at least one behavioural response (Suburban: $N=12$; Rural: $N=16$), I ran a generalised linear model (negative binomial distribution for

count data) with the most common behavioural response (head bob) with habitat and body condition (see below) of interacting individuals (males or females) as fixed factors. Body condition was calculated from mass and snout-vent length as the scaled mass index based on the central principle of scaling (see Peig & Green, 2009). I compared the change in circulating corticosterone levels as a function of social context (male-male, male-female, control), time (0, 10, 20, 30 min post-interaction period) and habitat (suburban and rural) using a three way ANOVA with Tukey's post-hoc comparison wherever relevant. I compared circulating testosterone levels in the same way using three-way ANOVA but used the ARTool (Assigned rank transformation) package in R (2016) as testosterone data was non-normal. Outliers of excessively low corticosterone and high testosterone levels (beyond 4 standard deviations from the mean) were excluded before analysis (Corticosterone: Rural: $N=2$; Testosterone: Rural: $N=1$, Suburban: $N=4$). Tukey's post-hoc comparisons were done using the lsmeans package (Lenth, 2016) in R. To quantify the effect of body condition on circulating hormone concentration I first ran a linear regression (for corticosterone) and a generalised linear model (gamma distribution) for testosterone as testosterone had a non-normal distribution. As no effect of body condition was found on hormone levels, all further analysis was done without body condition as a factor.

All statistical analyses were conducted using R Studio (3.0.1) statistical software. This study was approved by the Institutional Animal Ethics Committee of Indian Institute of Science (CAF/Ethics/394/2014).

4.3 Results

Behavioural responses

In staged encounters with both males and females, a higher proportion of rural males were behaviourally reactive compared to suburban males. During encounters with females, 65% of rural males showed behavioural displays, whereas only 20% of suburban individuals did ($\chi^2 = 6.54$, $P = 0.010$). During encounters with males, 55% of rural males showed some social displays, while only 25% of suburban males did ($\chi^2 = 2.60$, $P = 0.1$).

There was no effect of habitat, body condition of focal male, or body condition of intruder male on head bob behavioural display during male-male social interaction (glm: $P > 0.05$, Fig.4.1). Biting behaviour was shown by more suburban males (4/9 males) compared to rural males (2/13); but on the contrary, mounting was displayed by more rural males (6/13) compared to suburban males (2/9).

During male-female encounters, a significant effect of habitat was found for head bob display (glm: $z = -3.18$, $P = 0.001$, Fig. 4.1) such that rural males performed more head bob displays compared to suburban males. But no effect of body condition of the focal male ($z = 1.35$, $P = 0.175$) or the interacting female ($z = 1.01$, $P = 0.311$) was observed. Around 50% of the individuals from both the populations (Rural: 9/16 and Suburban: 5/12) performed crouch walk behaviour but the frequency of the behavioural display per trial per individual was low compared to head bob displays.

Hormonal responses

Corticosterone was found to be significantly affected by the interaction between habitat and time ($F_{3,76} = 4.79$, $P = 0.004$). The data was subset according to treatments

(Control, Male-male and Male-female) for further two-way ANOVAs with post hoc interactions.

Under control conditions (no social encounter), corticosterone was significantly higher in suburban males compared to rural males ($F_{1,16}= 5.96$, $P=0.026$, Fig. 4.2). But no effect of time or any interaction between habitat and time was found. During male-male encounters, a significant effect of habitat was observed ($F_{1,29}= 22.81$, $P<0.001$) wherein suburban males had higher circulating levels of corticosterone compared to rural males (post hoc $p<0.001$, Fig. 4.2a).

During male-female encounters, both time ($F_{3,31}= 3.72$, $P=0.021$) and habitat ($F_{1,31}= 22.48$, $P<0.001$) significantly influenced circulating corticosterone levels. Suburban males had significantly higher circulating levels of corticosterone compared to rural males (post hoc $p<0.001$, Fig. 4.2b) and the difference in hormone level was greatest between 0 and 20 mins post interaction (post hoc $p=0.01$, Fig. 4.2b).

Similar to corticosterone, testosterone was also affected by an interaction of habitat and time ($F_{3,68}= 10.80$, $P<0.001$). Further subdividing the data according to interactions showed that under male-male encounters, testosterone was affected by time ($F_{3,27}= 3.52$, $P=0.028$, Fig. 4.3a) but during male-female encounters, there was an interaction effect between time and habitat ($F_{3,26}= 5.70$, $P=0.003$, Fig. 4.3b). After an interaction with a female, suburban males had significantly high levels of testosterone (compared to control conditions) which declined significantly by 20 min post interaction (20min: post hoc $p=0.007$, 30min: post hoc $p=0.032$, Fig. 4.3b). Rural males maintained testosterone at an elevated level till 30 min post interaction.

4.4 Discussion

I found that social encounters elicit a behavioural response and induce changes in steroid hormone levels in *P. dorsalis* as expected. Differences in corticosterone and testosterone levels, however, were largely driven by population differences in the males rather than the type of social interaction. Suburban males had significantly elevated levels of corticosterone both under control conditions and following intra- and intersexual interactions compared to rural males. Testosterone levels were elevated in both suburban and rural males following social interactions. After courtship interactions with females, suburban males had higher testosterone levels, which rapidly declined to control levels within 20 min post interaction, whereas in rural males no decline in elevated testosterone level was observed till 30 min post interaction. Along with differences in hormonal responses between populations, I also found that the proportion and intensity of courtship behaviour was affected by urbanisation. A significantly lower proportion of suburban males displayed courtship behaviour and the intensity of display was also lower compared to rural males. This study provides the first evidence that urbanisation alters the social responses of a reptile species.

The role of corticosterone and testosterone in responding to the demands of social interactions has been investigated in a variety of reptiles. Results from these investigations suggest that the behavioural, physiological, and environmental context in which a social encounter occurs is key to understanding the role of steroid hormones in social interactions. For example, male Eastern fence lizards, *Sceloporus undulatus*, increase circulating corticosterone levels in response to male or female encounters during the breeding season but not during the non-breeding season, and only encounters with

males result in an increase in plasma testosterone levels (Smith and John-Alder, 1999). In my study, changes in both corticosterone and testosterone levels was found to be similar during courtship and competitive contexts within populations. Rural males maintained low corticosterone levels across control conditions and even during courtship and competitive encounters. By contrast, suburban males had higher corticosterone levels during control conditions compared to rural males, and elevated their corticosterone levels after both courtship and competition with a peak at 20 min post interaction. Testosterone levels were elevated by both rural and suburban males following social interactions. While suburban males rapidly lowered their testosterone levels to control levels within 20 min, rural males maintained elevated testosterone levels up till 30 min after any social interaction. These patterns of hormonal responses within populations seems to suggest that the energetic demands during both inter- and intrasexual interactions may be similar. I speculate that the elevation of corticosterone and decline of testosterone following a social encounter in suburban males can be mechanistically explained by the different need for energy mobilisation. After an energetically demanding behavioural encounter, corticosterone is elevated to mobilise energy to restore homeostasis (Moore and Jessop, 2003; Wingfield et al., 2001). Correspondingly, testosterone level declines to minimized the re-occurrence of immediate and energetically demanding social behaviour (Moore and Jessop, 2003; Wingfield et al., 2001). My data with *P. dorsalis* supports this negative correlation between these steroid hormones especially at 20 min post interaction when corticosterone is highest and testosterone is lowest (Appendix A.4.2).

Variation in the duration and magnitude of the adrenocortical or corticosterone response to the same type of stressor may occur and these differences in responsiveness reflect a change in the sensitivity of the HPA axis to stressors which is termed as adrenocortical modulation (Wingfield and Romero, 2001). A number of studies have demonstrated that the physiological state of individuals, such as differences in body condition, reproductive state, age, sex, social status and even the type and number of steroid hormone receptors can modulate the adrenocortical response (e.g., Dunlap and Schall, 1995; Grassman and Hess, 1992; Knapp and Moore, 1996; Jennings et al., 2000). In addition, differences in the external environment such as variation in rainfall, temperature, food availability, humidity, or general habitat alterations by anthropogenic disturbances can affect individual adrenocortical modulation (e.g., French et al., 2010; Dunlap and Wingfield, 1995; Moore et al., 2001). In my study, the difference in hormonal responses and social behaviour between populations is not due to difference in body condition or age or reproductive status. These differences can be due to long-term (life-time) physiological changes arising from habitat disturbance causing shifts in predation pressure, conspecific density or resource availability. Behavioural differences or coping styles to social stressors might be different between suburban and rural individuals due to shifts in conspecific density and territory overlap in suburban areas, making suburban lizards more tolerant towards other males (personal observation and Amdekar and Thaker unpublished). High local density of conspecifics in suburban areas might also lead to lower motivation during staged courtship encounters as availability of females is generally higher. I observe that along with low responsiveness during courtship encounters, *P. dorsalis* from suburban habitats also have duller low contrast

colours during courtship displays (Chapter 1). Suburban individuals of *P. dorsalis* show similar physiological responses to that seen by non-aggressive, non-territorial individuals of other species that typically show “reactive coping styles” with low aggression and high stress hormone levels (DeNardo and Licht, 1993; Koolhaas et al., 1999). On the other hand, rural individuals of *P. dorsalis* seem to show a proactive coping style, with lower corticosterone levels and elevated testosterone levels (Koolhaas et al., 1999), likely because competitive encounters might require greater responsiveness and aggression since territories are larger and have less overlap (Deodhar, 2017). Such frequent male-male encounters during the breeding season is known to inhibit corticosterone elevation in other lizard species (Knapp and Moore, 1995), which enables testosterone release to be more responsive to immediate social requirements. Thus, when high demand for frequent aggressive encounters exist, it can be energetically efficient and necessary to down-regulate adrenocortical responses (Moore and Jessop, 2003; Wingfield and Sapolsky, 2003). Here I show that urbanisation alters social interactions, such that behavioural and physiological responses are altered to reflect an alternative reactive coping style, fundamentally different from the proactive style of the territorial and aggressive *P. dorsalis* male in his natural habitat.

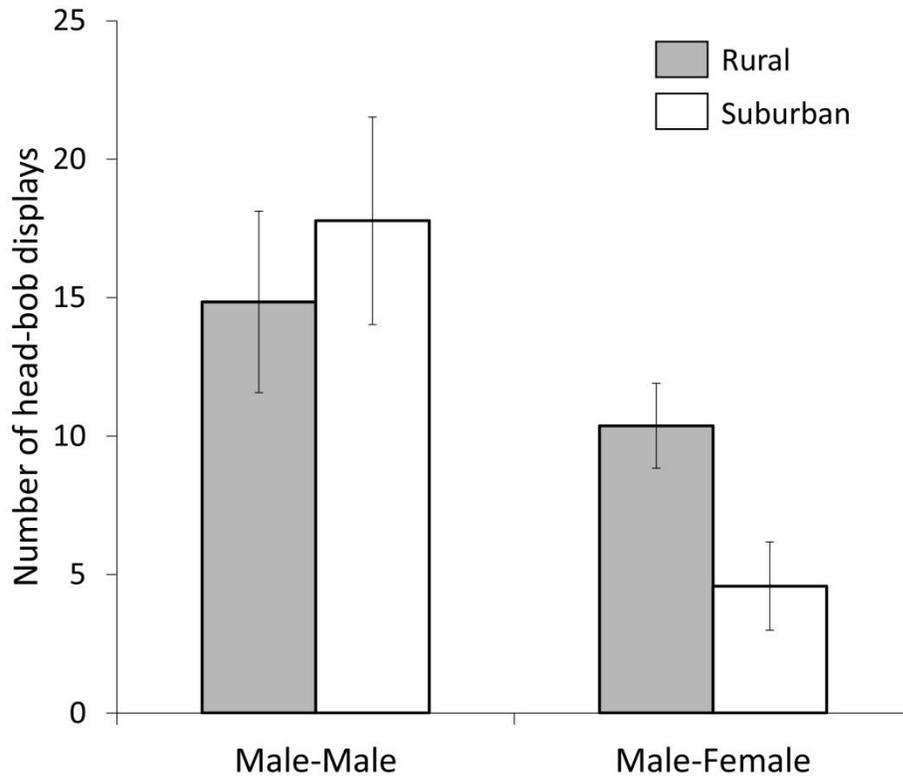


Figure. 4.1. Number of times head bob displays were shown by suburban and rural males during each male-male and male-female staged encounter. Rural males displayed significantly greater number of head bobs compared to suburban males during male-female interaction. Shown are bar plots with mean and ± 1 standard error.

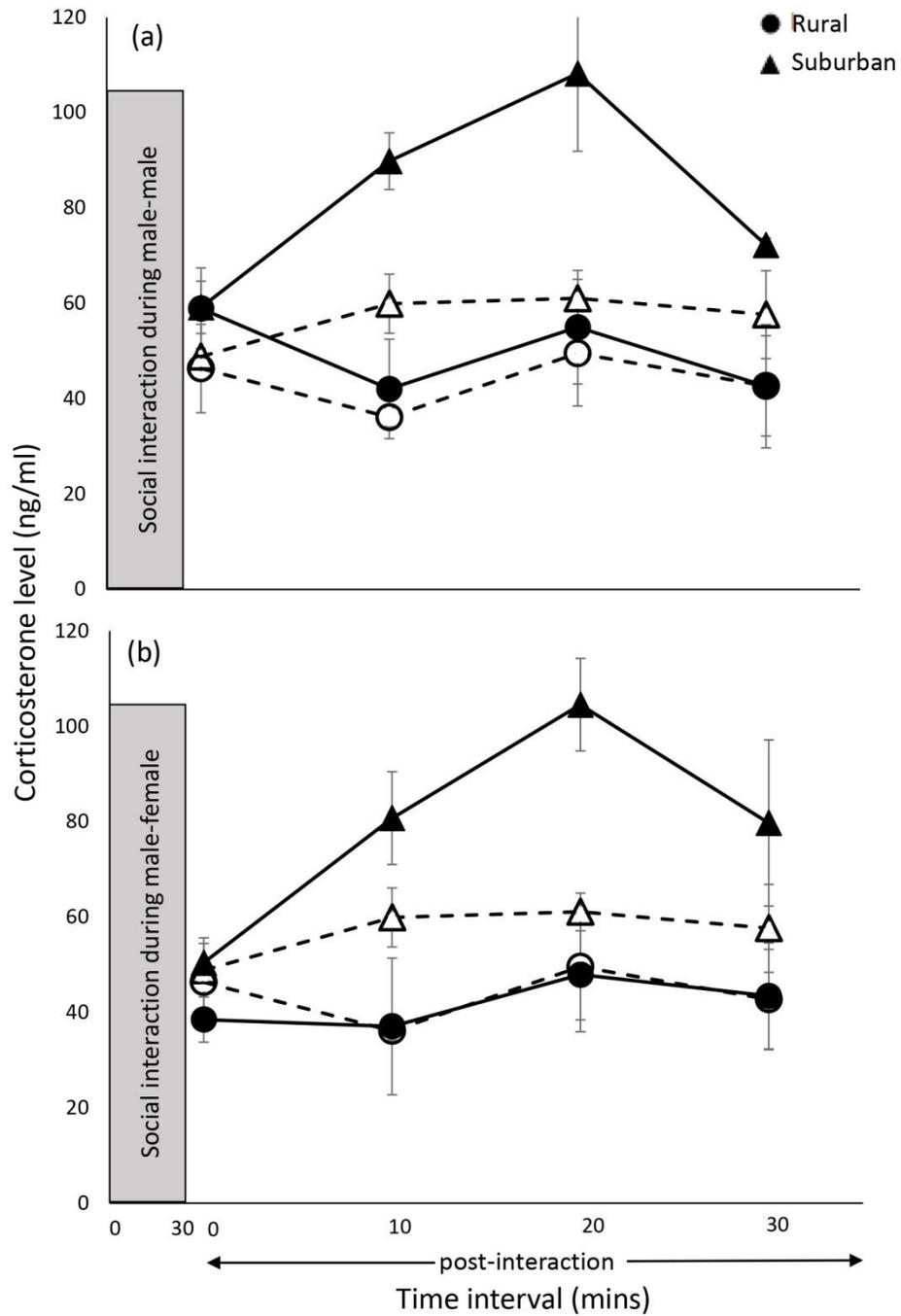


Figure 4.2. Change in corticosterone levels across time after staged (a) Male-male and (b) Male-female interactions. Open symbols denote hormone levels during control conditions for both populations across time. Error bars are ± 1 SE.

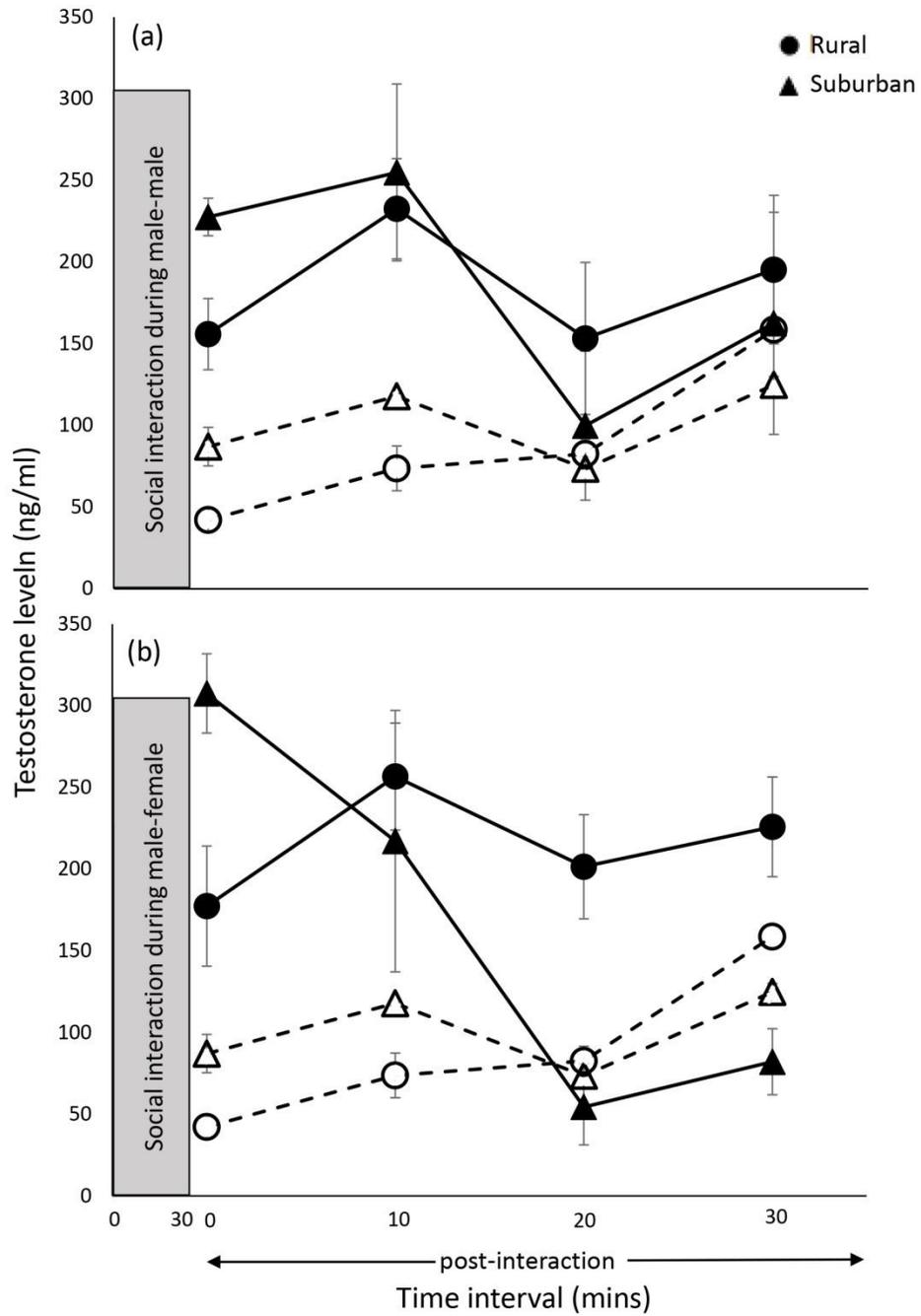


Figure 4.3. Change in testosterone levels across time after staged (a) Male-male and (b) Male-female interactions. Open symbols denote hormone levels during control conditions for both populations across time. Error bars are ± 1 SE

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Appendix

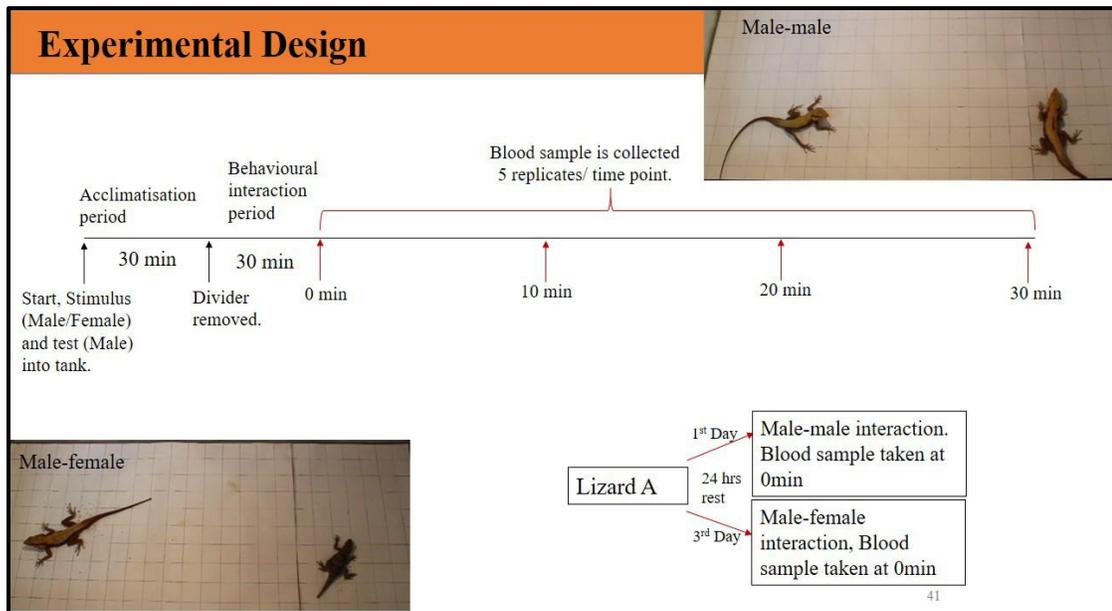


Figure A.4.1. Template of experimental design

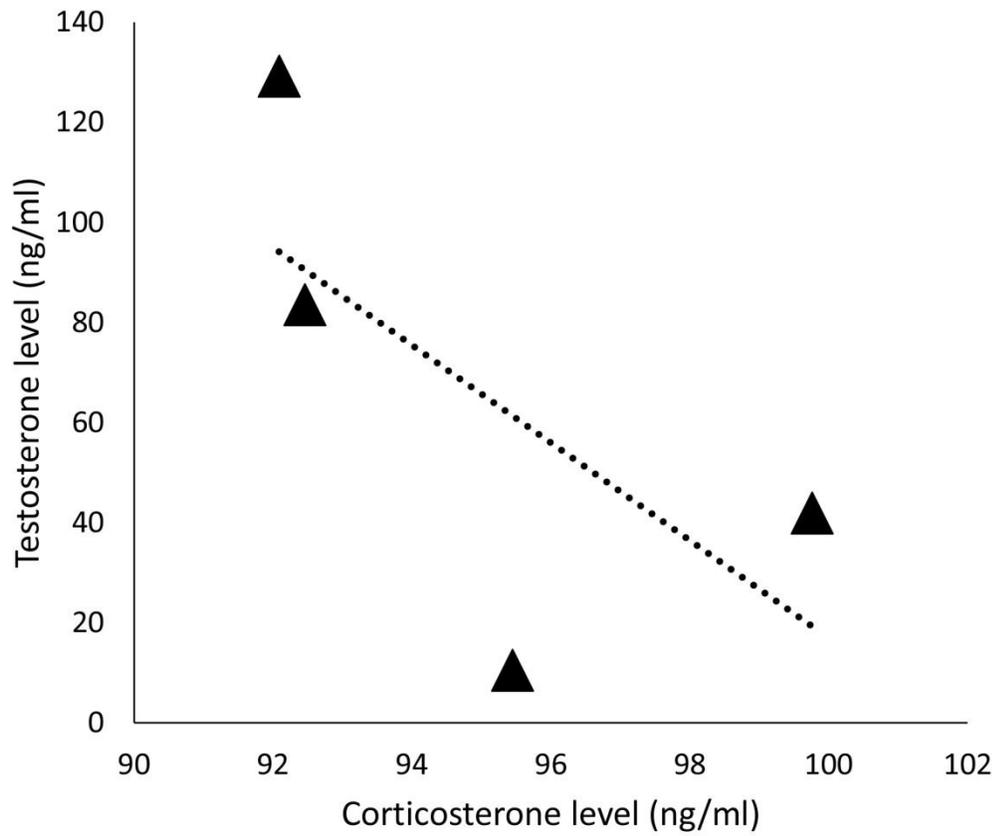


Figure. A.4.2. Corticosterone and testosterone levels (ng/ml) of suburban males 20minutes post social interaction with females

Chapter 4

A multivariate approach to understanding shifts in escape strategies

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5.1 Introduction

All organisms experience environmental fluctuations and shifts over ecological and evolutionary time scales. The speed and unpredictability of environmental changes caused by urbanisation, however, is unprecedented (McKinney, 2002; Schlaepfer et al., 2002). Urbanisation rapidly alters natural habitats of animals, from reducing and fragmenting the landscape with anthropogenic structures, to altering the availability and type of food sources as well as the predator community (McKinney, 2002; DeStefano and DeGraaf, 2003; Ditchkoff et al., 2006). Due to the rapid and dramatic change in these environmental factors, many species are not able to tolerate urbanisation, which often leads to a loss of biodiversity (Marzluff et al., 2008). Some species, however, have greater plasticity and can tolerate such environmental changes by altering their behaviours. As a first-response mechanism, behavioural modifications to novel human induced challenges can prevent individuals from suffering high fitness losses and aid in population survival.

Predation is one of the most ubiquitous threats to the survival of organisms and thus a diverse range of anti-predatory strategies have evolved to minimize the risk of mortality (Lima and Dill, 1990; Cooper and Blumstein, 2015). In response to an approaching predator, the most typical response in animals is to flee. Flight initiation distance (FID), defined as the distance between a potential threat and the prey at the onset of escape, has therefore been a widely used measure of anti-predator responsiveness (Ydenberg and Dill, 1986; Cooper and Blumstein, 2015). Since FID is an economic decision, FID will typically increase as predation risk (cost of remaining) increases, and will decrease as the cost of escape increases (Ydenberg and Dill, 1986). The decision of

when to escape an approaching predator is influenced by numerous intrinsic and extrinsic factors. Extrinsic factors such as the level of threat associated with the predator, ease of escape, and distance from refuge (Engelhardt and Weladji, 2011; Fleming and Bateman, 2014), along with intrinsic factors such as physiological or performance ability (Herrel et al., 2011; Qi et al., 2014) and level of habituation to risk (Brown and Chivers, 2005; Engelhardt and Weladji, 2011) can all affect escape strategies of animals.

Escape decisions are crucial to an individual's fitness (Cooper and Frederick, 2007) and these decisions may depend upon the intrinsic capacity to escape predation, such as the degree of crypticity, the propensity and rate of learning and performance capabilities. Performance abilities such as sprint speed can aid in escape responses and this often differs depending on the sex, morph, and habitat of animals (Qi et al., 2014). In *Anolis sagrei*, longer limbed individuals sprint faster on broader surfaces and thus preferentially use broader surfaces as perches (Calsbeek and Irschick, 2007). Thus, habitat structure can be tightly associated with the escape capabilities in animals. Ability and rate of learning is also expected to differ between species and even between individuals of a species because environmental factors such as predation pressure, microhabitat characteristics, and exposure to anthropogenic disturbances interact to influence habituation or learning (Runyan and Blumstein, 2004; Ellenberg et al., 2009). Several studies have found that frequent exposure to low-risk novel threats, such as human activity, results in habituation of escape responses through learning (Frid and Dill, 2002; Engelhardt and Weladji, 2011).

Among the many extrinsic conditions that influence escape responses, anthropogenic environmental disturbances should strongly affect risk perception and

escape strategies of animals (Shochat et al., 2006). Animals living in urban habitats experience novel physical and microhabitat structures that are generally more varied in type, such as cement walls, metal sheets, and ornamental plants that are not found in natural undisturbed habitats. Natural habitats are often more homogeneous, with less variation in the type of physical structures (typically rocks and boulders for perch and refuge) and a higher proportion of native vegetation (Young and Jarvis, 2001; Balakrishna et al., 2016). For many terrestrial animals, the availability and type of refuge is a strong extrinsic factor that affects escape strategies (e.g. Martin & Lopez, 1995; Schooley et al., 1996; Cooper and Whiting, 2007). Escape theory predicts that FID will increase with distance to refuge (Ydenberg and Dill, 1986), which has been supported by empirical data on many terrestrial vertebrates, including lizards (Samia et al., 2015), birds (Guay et al., 2013) and mammals (Stankowich and Blumstein, 2005; Engelhardt and Weladji, 2011).

Many studies so far, especially in lizards, have examined the importance of either extrinsic factors or intrinsic factors on escape responses. Using the Indian rock agama *Psammodromus dorsalis*, I investigated the effect of urbanisation on escape strategies taking into account both intrinsic and extrinsic factors that might affect escape decisions. As a consequence of differences in habitat structure and repeated exposure to human activity (resulting in habituation), I hypothesize that lizards in urban environments will be less risk averse than lizards in rural environments. Specifically, I expect suburban lizards to perch at lower heights, and when approached, have shorter flight initiation distances and choose closer refuges than rural lizards. For a subset of individuals from both rural and suburban areas, I also simulated repeated predator attacks to test the rate of

habituation, with the prediction that suburban lizards will more rapidly habituate to a low risk human threat. Since intrinsic performance ability can directly influence escape decisions, I measured the sprint speed of wild-caught lizards from both suburban and rural environments to determine if running speed was a factor in the escape strategies of suburban lizards. Finally, I expected sex differences in anti-predator responses and sprint speed, regardless of habitat, as sexes are dimorphic and dichromatic, with starkly different levels of crypticity.

5.2 Methods

Study species and sites

Psammophilus dorsalis males develop conspicuous dynamic colouration which becomes dull at the end of the breeding season, while females maintain cryptic colouration year-round (AB and MT unpublished data). I conducted this study across three different suburban areas located in Bangalore city and two rural areas located near Antharagange forest range of Kolar district approximately 60km from the suburban study sites. Replicate sites within suburban and rural areas were at least 5 km apart. The suburban area comprised of human settlements interspersed with little scrub vegetation, whereas the rural habitat comprised of rocky boulders and scrub vegetation (Balakrishna et al., 2016).

Escape behaviour

I measured the escape behaviour of adult males ($N = 20$ per area, i.e. $N = 40$ total) and females ($N = 20$ per area, i.e. $N = 40$ total) during peak activity periods from 0900-

1100 and 1500-1700 hours in the post breeding season from August to October 2014. By August most females had laid their last clutch of the year. To avoid the potential effects of gravidity on anti-predatory behaviour and sprint speed, I verified the non-gravid status of females by careful observation based on abdomen shape. Before the start of each field trial, the observers (AB and SB) scanned the study areas and located lizards that were sitting on a perch and not actively engaged in foraging or social activities. Only lizards that were perched at approximately 1 - 1.5 m above ground-level and were initially 10-12 m away from the observer were selected for simulated predator attacks. All “predator attacks” were simulated by the same observer (AB) wearing the same coloured clothes (dull olive green) on sunny days only, and involved approaching each lizard at an approximate speed of 1.5m/s directly in a straight line where there was no obstruction of visual field between the lizard and the attacker. Using binoculars from at least 20m from the lizard, the second observer (SB) recorded the location of the initial perch and the path taken to the refuge. After the attack, the following parameters were measured: (1) flight initiation distance (FID) as the distance between the attacker and the initial perch when the lizard started escape, (2) hiding duration, (3) perch height and type, (4) refuge site, and (5) perch-to-refuge distance. If the focal lizard returned to the same perch or to a perch within a 2m radius of the first attack by 10 min, a second and third predator attack was simulated in the same way. I restricted my statistical analysis of repeated attacks to only lizards that were attacked twice, because only 50% of rural lizards (of 40) and 20% of suburban lizards (of 40) returned to their perch in time for three attacks. Repeated attacks of focal lizards were completed within 30 min and all focal lizards attacked

during the same day were at least 200m apart to prevent disturbance and repeated sampling of individuals.

Sprint speed

To measure sprint speed, a different set of lizards from the same suburban and rural habitat were captured by noosing and transported to the laboratory in cloth bags ($N = 26$ males and 25 females from suburban; 26 males and 24 females from rural). I first measured mass and SVL of all individuals and then housed them in glass tanks (60 x 30 x 25 cm) lined with a sandy substratum, in a room with natural lighting and ambient temperature (28 - 30°C). Additional heat for basking was provided using 60W incandescent bulbs that were suspended above each tank and turned on for 2- 3 hours a day. Water was provided *ad libitum* and crickets and grasshoppers were provided daily. Lizards were habituated to laboratory conditions for 2 days before sprint speed was measured. Before the trials, all lizards were first allowed to attain their preferred body temperatures (cloacal temperature was 30 - 34°C measured using thermometer) by basking in a thermal gradient for 30 min. Each lizard was then placed individually on a race track (4.2 m length x 0.1m width x 0.4m height) that was fitted with an overhead camera and lined with fine-grained sandpaper. Lizards were stimulated to run at their maximal speed by gently tapping the base of the tail with a soft brush. All lizards were induced to run three times with a 15 min recovery period between each run. To obtain a measure of burst sprints similar to antipredator escape responses, without subsequent exhaustion, I used a short race track (~ 60 SVL of an average female; 30 SVL of an average male). I also determined that fifteen minutes was a sufficient recovery period

between successive runs, as over 65% of lizards performed their fastest sprints during the second or third run. Any trial in which the lizard did not run beyond 1 m, after 10 tail taps were excluded. Video recordings of all runs from each lizard were used to calculate sprint speed; measured as the time taken to travel across a 0.75m length along the middle of the track. I used the single fastest sprint speed from all three runs by each individual in subsequent statistical analyses. To minimize observer bias, blinded methods were used for measurements of sprint speed, but it was not possible to record data blind for escape responses and habitat characteristics as these involved measurements in the field.

Statistical analyses

I performed repeated measures ANOVAs to determine the effect of attack number (1, 2), habitat (suburban, rural) and sex (male, female) on FID and hiding duration separately. For hiding duration data, I performed an aligned rank transformation before analysis, as the data was non-normal (using ARTool package 2016). For both hiding duration and FID, I included a 2-way interaction term between habitat and sex. I used data from the first attack only to determine the effects of habitat and sex on FID, hiding duration, perch-to-refuge distance and perch height (separate two-way ANOVAs with an interaction term). Hiding duration and perch-to-refuge distance from the first attack were transformed using aligned rank based transformation before analysis as data was non-normal (ARTool package 2016). I also compared body size of lizards as a function of habitat and sex using two-way ANOVA with an interaction term. I first examined the relationship between SVL and sprint speed by computing an overall Pearson's correlation analysis. I then controlled for body size using an Analysis of Covariance (ANCOVA) to

determine the effect of habitat and sex on sprint speed (with interaction). Tukey's post hoc analyses were performed wherever relevant. All data analyses were performed in R studio version 3.2.1 (R core team 2015).

5.3 Results

Escape responses

In response to the first attack, FID was significantly affected by an interaction between habitat and sex (ANOVA: $F_{1,76}=14.22$, $P<0.001$). Rural males had significantly longer FIDs than suburban males (Mean \pm 1 SE: Rural: 3.86 ± 0.22 m; Suburban: 2.42 ± 0.16 m; post hoc $p< 0.001$, Fig. 5.1) and all females (Mean \pm 1 SE: Rural: 2.11 ± 0.22 m; Suburban: 2.13 ± 0.14 m; post hoc $p< 0.001$, Fig. 5.1). Thus, males from suburban areas initiated escape later than rural males, allowing closer approaches of the predator before running. Hiding duration after the first attack for those lizards that were located again was similar across individuals (Mean \pm 1 SE: Rural male: 165.55 ± 26.52 sec; Rural female: 123.8 ± 28.38 sec; Suburban male: 236.4 ± 20.82 sec; Suburban female: 166.92 ± 30.74 sec), with no significant difference between habitats (ANOVA: $F_{1,47}=2.56$, $P=0.115$) or sexes (ANOVA: $F_{1,47}=2.54$, $P=0.117$).

For those lizards that were attacked twice ($N = 48$ total), FID was influenced by an interaction between habitat, sex and attack number (ANOVA: 3 way interaction: $F_{1,43}=7.47$, $P= 0.009$, Fig. 5.2). To further examine the individual effects of each factor, I classified the responses separately for males and females, and found that FID was influenced by an interaction between habitat and attack number for males (ANOVA: $F_{1,19} = 11.39$, $P= 0.003$, Fig. 5.2) but not females (ANOVA: $F_{1,24} = 0.26$, $P= 0.615$, Fig. 5.2).

With the second attack, FID significantly decreased in rural males (ANOVA: $F_{1,12}=41.78$, $P < 0.001$, Fig. 5.2) but not suburban males (ANOVA: $F_{1,7}=5.39$, $P= 0.053$, Fig. 5.2). The FID of females was not significantly affected by habitat or attack number (ANOVA: Habitat: $F_{1,24}=0.87$, $P= 0.360$; Attack number: $F_{1,24}=0.36$, $P= 0.552$, Fig. 5.2).

Similarly, hiding durations across multiple attacks for those lizards that were located again was not significantly affected by either sex (ANOVA: $F_{1,32}=0.01$, $P= 0.970$), habitat (ANOVA: $F_{1,32}=1.45$, $P= 0.232$) or attack number (ANOVA: $F_{1,32}=0.32$, $P= 0.569$). Thus all lizards maintained similar hiding durations, regardless of habitat, sex or repeated attacks.

Habitat characteristics

Given that escape behaviours can be influenced by key local habitat characteristics, I compared perch characteristics (type and height) and refuge characteristics (type and perch-to-refuge distances) of lizards in both habitats. Suburban areas have higher variation in the type of perches and refuges compared to rural areas. Lizards in rural areas ($N = 40$) perched on rocks and small boulders (100%), and took refuge in rock crevices (75%) or scrub vegetation (25%) when attacked. By contrast, lizards in suburban areas ($N = 40$) were found perched on wall ledges (75%), rocks (10%), brick piles (7.5%), drain trenches (2.5%), and house roofs (5%). Refuges for lizards in suburban habitats included all the various structures listed above (87.5%) as well as scrub and ornamental vegetation (12.5%).

Regardless of perch type, perch height of lizards was influenced by an interaction between habitat and sex (ANOVA: $F_{1,76} = 4.69$, $P = 0.033$, Fig. 5.3a). In rural areas, males were found on significantly higher perches compared to females (Mean \pm 1 SE: Rural male: 7.62 ± 0.79 m; Rural female: 4.2 ± 0.64 m; post hoc $p < 0.001$, Fig. 5.3a). Perch heights were also higher for rural males compared to suburban males (post hoc $p = 0.004$, Fig. 5.3a), but not for females (Mean \pm 1 SE: Suburban male: 4.90 ± 0.21 m; Suburban female: 3.85 ± 0.32 m). As expected, the relative location of refuges from perches influenced escape decisions. In response to the first attack, perch-to-refuge distance of a fleeing lizard was significantly shorter in suburban habitats compared to rural habitat (ANOVA: $F_{1,76} = 45.41$, $P < 0.001$, Fig. 5.3b), with no significant differences between the sexes (ANOVA: $F_{1,76} = 0.36$, $P = 0.550$, Mean \pm 1 SE: Rural male: 0.86 ± 0.06 m; Rural female: 0.92 ± 0.10 m; Suburban male: 0.37 ± 0.10 m; Suburban female: 0.53 ± 0.13 m).

Body size and sprint speed

I found a significant interaction between habitat and sex for SVL ($F_{1,97} = 17.72$, $P < 0.001$). As expected, males were significantly larger than females regardless of habitat (both post hoc $p < 0.001$). Between habitats, suburban males were significantly larger than rural males (post hoc $p < 0.001$; Mean \pm 1 SE: Rural males: 104.46 ± 2.25 mm; Suburban males: 125.15 ± 1.79 mm) but there was no significant difference in SVL between females across populations (post hoc $p = 0.202$; Mean \pm 1 SE: Rural females: 85.16 ± 1.43 mm; Suburban females: 90.36 ± 1.13 mm).

In general, lizards with larger SVL had faster sprint speed (Correlation coefficient = 0.34, $p < 0.001$). After controlling for this body size effect (covariate term;

$F_{1,93}=0.26$, $P=0.608$), I find that sprint speed of lizards was only affected by sex ($F_{1,93}=21.17$, $P=0.001$). Males ran faster than females (Mean \pm 1SE: Rural male: 2.85 \pm 0.16 m/s; Rural female: 2.14 \pm 0.10 m/s; Suburban male: 2.94 \pm 0.11 m/s; Suburban female: 2.45 \pm 0.11 m/s; Fig. 5.4). There was no significant effect of habitat (ANCOVA: $F_{1,93}=2.31$, $P=0.131$) or the interaction between habitat and sex in predicting sprint speed ($F_{1,93}=0.49$, $P=0.484$).

5.4 Discussion

I examined the escape strategies of the agamid *P. dorsalis* as a function of multiple extrinsic and intrinsic factors, with the main aim designed to understand the effect of urbanisation on antipredator responses. First, suburban and rural habitats differed in multiple ways that directly influenced escape decisions. Compared to lizards in undisturbed rural areas, lizards in suburban areas used lower perches that varied greatly in type, and chose refuges that were more varied and closer to their perches. As expected, the FID of suburban lizards was lower than rural lizards, but only for males. Apart from habitat characteristics, intrinsic factors like the propensity to habituate to humans (but not sprint speed) also differed between habitats. When attacked multiple times in the field, rural but not suburban males decreased their FIDs significantly across subsequent attacks. For some escape responses, sex differences were more apparent than population differences, such that urbanisation affected the escape strategies of males more than females.

Animals living in urban habitats face multiple challenges including a greatly altered abiotic environment with different types of perch and refuge sites. According to

optimal escape theory (Ydenberg and Dill, 1986), refuge distances greatly influence escape responses. I find that lizards use refuge sites that are significantly closer to their perch in suburban areas than in rural areas. Apart from refuge distances, perch and refuge types were also highly variable in suburban areas, as lizards utilized artificial anthropogenic structures in the environment. In fact, the higher perch heights of lizards in rural areas compared to suburban areas is unlikely to be a function of perch availability, as building walls and ledges are taller than the boulders in the natural rural habitat (AB and MT personal observation). Instead, higher perches in open rocky natural habitats allow for better vigilance, which would offset the costs of being more conspicuous and having fewer refuge options (Krams, 2001). Access to a complex structural landscape, with more perch and refuge options (Prosser et al., 2006) supports the seemingly riskier strategies of suburban lizards, which allow the closer approach of attackers before flight. Although suburban lizards had larger body sizes than rural lizards, and sprint speed was generally correlated with body size (here, see also Losos, 1990; Garland and Losos, 1994), I find no significant differences in intrinsic performance ability (sprint speed) between suburban and rural lizards.

According to the threat sensitivity hypothesis (Helfman, 1989; Chivers et al., 2001) prey animals should balance the costs of escape from the risk of attack, and adjust their anti-predatory response based on the magnitude of predatory threat. Studies have found lower abundance of native predators in urban areas (McKinney, 2008; Shochat et al., 2006), which should reduce overall perceived risk. However, the increase in human disturbance, presence of domesticated animals, and changes in predator species composition can potentially replace the threat from natural predators. Only when human

activity is perceived as a low-risk novel threat, frequent exposure should result in habituation through learning (Labra and Leonard, 1999; Frid and Dill, 2002; Webb and Blumstein, 2005; Engelhardt and Weladji, 2011; Bateman and Fleming 2014). Given this, the reduction in FID found in several studies for animals living in human disturbed habitats, including *P. dorsalis* (this study), is not surprising. All these studies however, compare the escape strategies of suburban and rural populations after a single attack, and is therefore a measure of habituation to lifetime exposure to the low-risk urban threats. Such habituation is necessary for survival in novel urban environments, as it allows individuals to decrease the cost of fleeing and increase time for other activities, such as foraging and social behaviour (Rodriguez et al., 2011). Unlike most other studies of escape responses based on lifetime experience, I also measured the ability to learn from repeated attacks over a single day. Male lizards from the rural habitat were the only ones that decreased their FID over subsequent attacks, clearly indicating that *P. dorsalis* is capable of learning about threats in a very short timescale (similar to Marcellini and Jenssen, 1991; Thaker et al., 2010; Batabyal et al., 2014). The lack of short-term habituation (i.e. reduction in FID) in suburban males further supports the prediction that lifetime exposure to human disturbance in urbanised areas can result in consistently high threat tolerance, with little required change in response to repeated directed exposures.

Like most sexually dimorphic species, males and females use different antipredator strategies that correspond to their morphology. In *P. dorsalis*, males are larger and conspicuously coloured, whereas females are smaller and cryptically patterned. Performance traits have been found to be sex dependent in dimorphic species, with females typically having slower sprint speed than males (Garland and Losos, 1994;

Irschick et al., 2008; Van Damme et al., 2008). The cryptic female colouration and smaller body size of *P. dorsalis* supports an antipredator strategy that allows for closer approach from predators (i.e. shorter FID), with little change over repeated attacks. Thus, *P. dorsalis* females prefer crypsis over early fleeing (i.e. long FID) to avoid detection from predators (Schwarzkopf and Shine, 1992; Stiller and McBrayer, 2013). Females also use lower perches than males in general, which not only influences their foraging and diet choices (Balakrishna et al., 2016), but also supports their escape strategies.

In this study I show that suburban habitats support a shift in antipredator strategies, that is mostly driven by habituation to human exposure and modifications in habitat structure. I suggest that urbanisation, with the associated suite of ecological changes, may be a strong selective force in shaping the responses of species, as changes in escape strategies seem to enable urban lizards to adjust to the novel environment. What remains to be understood is how urban environments are perceived by animals, and the specific ecological factors that drive altered survival strategies.

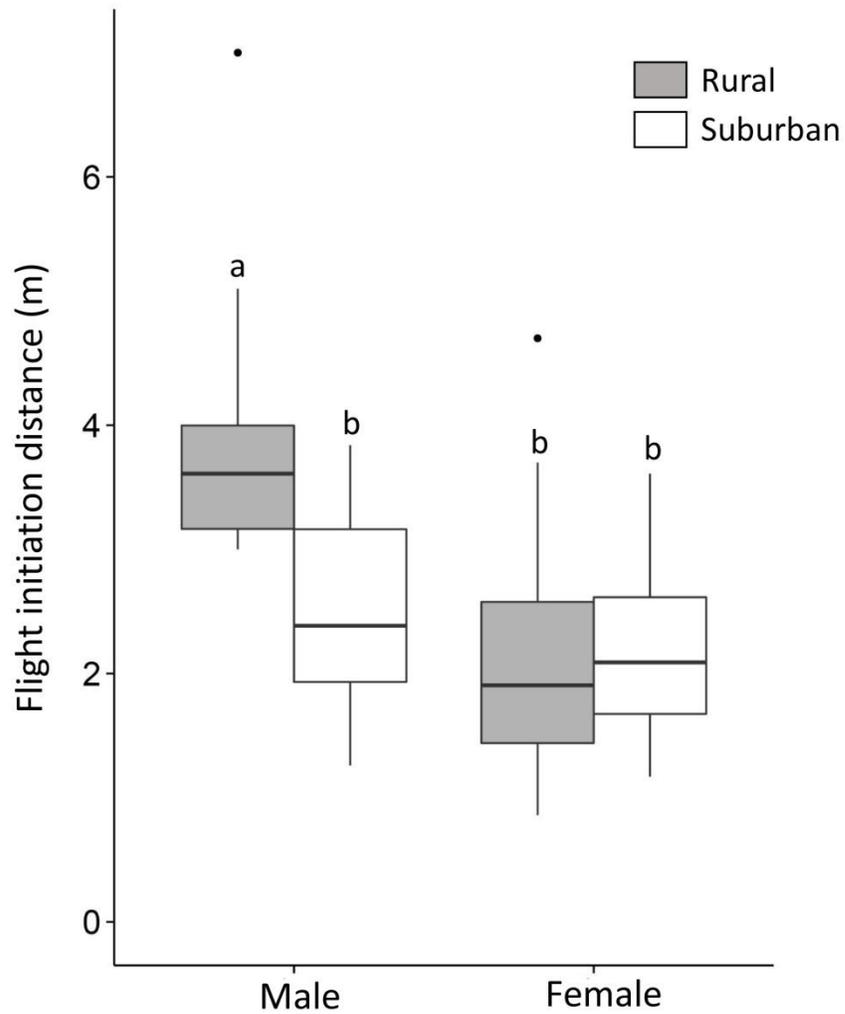


Figure. 5.1. Flight initiation distance (m) of *Psammophilus dorsalis* after one attack was highest in males from rural areas, compared to males from suburban areas and all females. Shown are box-plots, with medians, quartiles, 5th and 95th percentiles, and extreme values. Different letters indicate significant post-hoc differences at $P < 0.01$

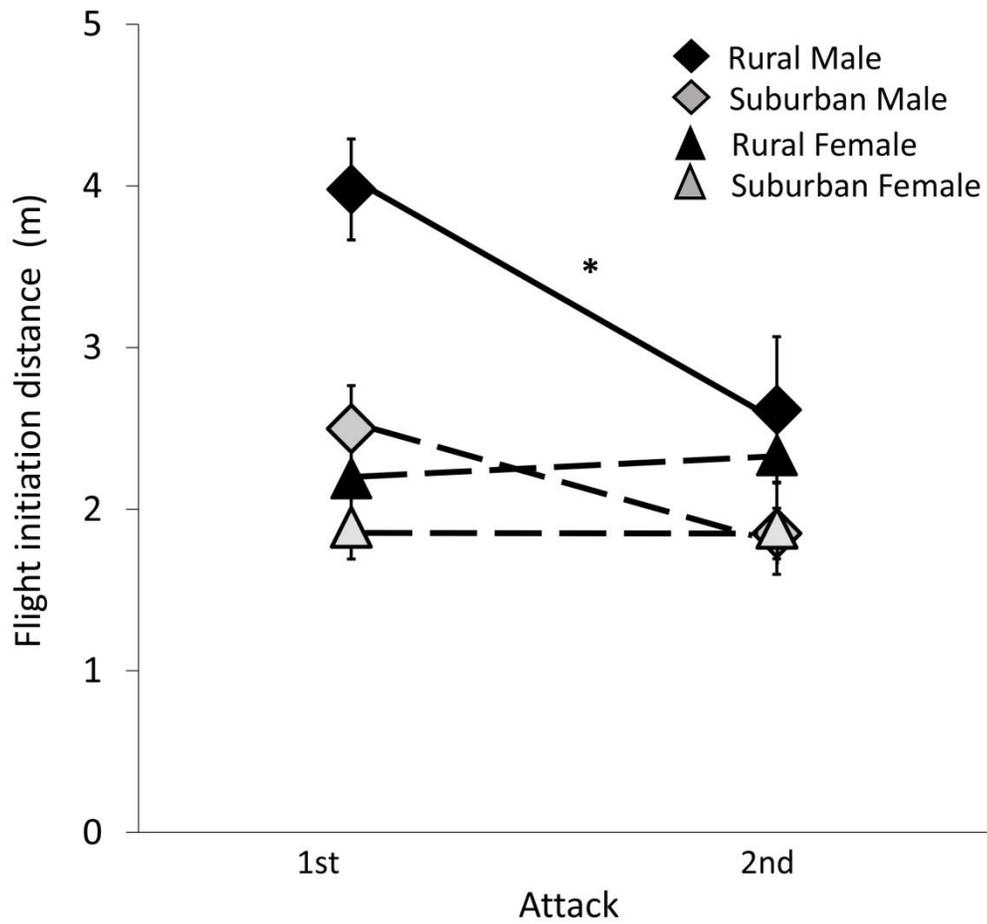


Figure 5.2. In response to a repeated attack, only rural males of *Psammophilus dorsalis* decreased their flight initiation distance (FID; mean \pm 1SE). * denotes post-hoc significant difference at $P < 0.001$

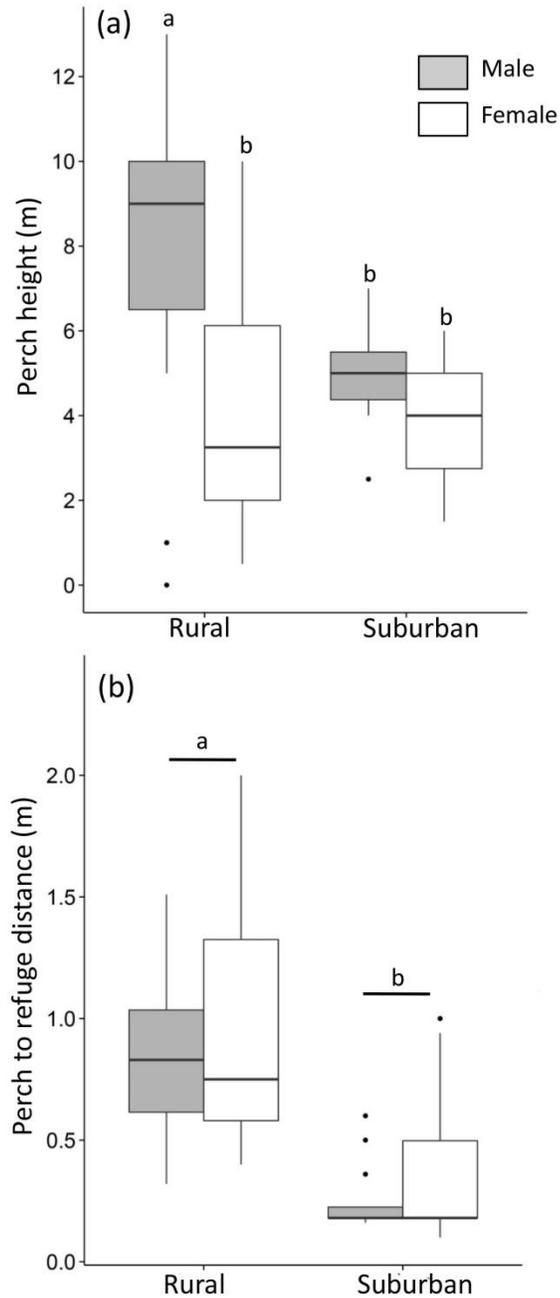


Figure 5.3. (a) Perch height and (b) Perch to refuge distances of *Psammophilus dorsalis* as a function of habitat (suburban, rural) and sex (male, female). Shown are box-plots, with medians, quartiles, 5th and 95th percentiles, and extreme values. Different letters indicate significant post-hoc differences at $P < 0.005$

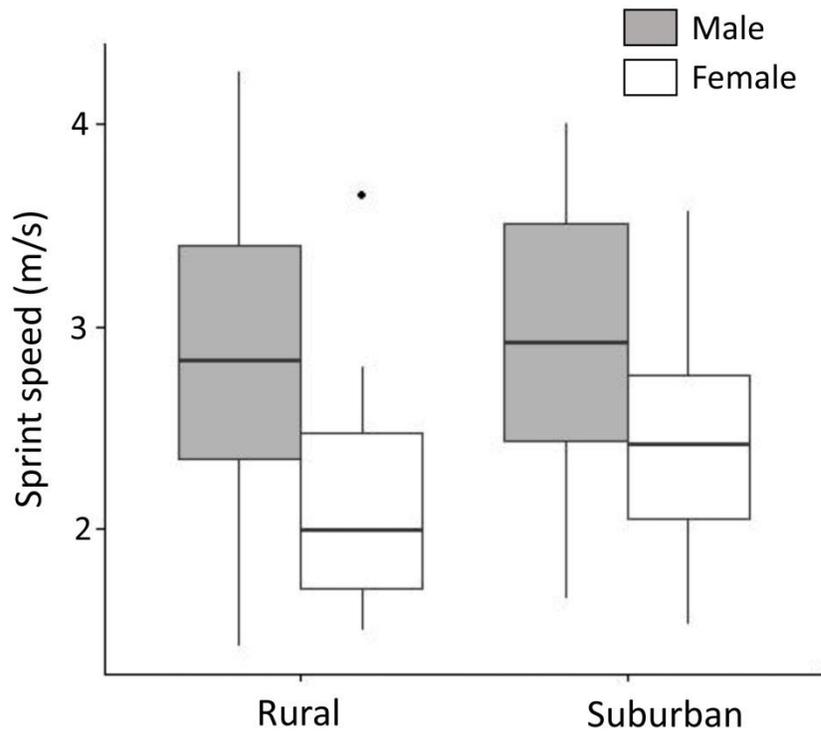


Figure 5.4. Sprint speed of *Psammophilus dorsalis* differed only between males and females, and not between habitats. Shown are box-plots, with medians, quartiles, 5th and 95th percentiles, and extreme values.

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Chapter 5

Learning to be safe: effects of urbanisation on learning and reversal learning

6.1 Introduction

Learning, in response to new stimuli or experience in new environments (Brown and Chivers, 2005), can be measured as a change in behaviour over time that enhances the ability to respond to altered conditions (Dukas, 1998). Locating important resources in the environment, such as food sources and safe refuges are critical for the survival of animals. One important behavioural tool that animals use to accomplish this task is to learn about the location of these important resources to return later when need arises (Paulissen, 2008; LaDage et al., 2012). Studies of navigation on birds and mammals have shown that they are capable of using a number of strategies to locate their specific goal. To identify rewarding or non-rewarding resources, such as food location, shelter and risky areas animals can either use a single cue, path integration or learning a series of environmental cues to form a spatial navigation map (Wilkinson and Huber, 2012). Reptiles can either use the direct properties of the resource such as texture, colour, or odour, or it can utilize external cues that help in guiding an animal towards or away from the target resource that are not necessarily a property of the target resource itself (Day et al., 2003; Noble et al., 2012).

Learning about food, refuge, and threat aversion by reptiles involves use of both associative or non-associative learning skills like spatial or social learning (Shanbhag et al. 2010; Stone et al., 2000; Davis and Burghart, 2011; Lopez et al., 2001; Noble et al., 2012). Aversive learning of predators or predation threat is a highly relevant ecological scenario for an animal and animals are expected to learn faster about a predation threat than other ecological pressures, because slow learning might prove to be costly and lethal (Thaker et al., 2010; Marcellini and Jenssen, 1991). In response to predation risk, many

squamate reptiles use shelters or refuges and thus knowledge about the location of an escape retreat is critical for effective antipredator responses. Thus, spatial learning about location of refuges in their environment is necessary and preferable in order to rapidly learn how to evade threat, and can be used as an effective motivator to study learning capabilities of species (Day et al., 2003). To recognize and remember safe refuges, knowledge about the local habitat is necessary (Paulissen, 2008; Noble et al., 2012). For example, Noble et al. (2012) showed that *Eulamprus quoyii* is capable of exhibiting flexible spatial learning about safe refuges in response to simulated predatory attacks in outdoor enclosures wherein their housing and learning trials were conducted in the same arena. To navigate to safe refuges, squamate reptiles use various types of cues like position or feature cues and distal or local cues in their environment, which differ across species (Day et al., 2003; Paulissen, 2008; LaDage et al., 2012).

The unpredictability and complexity of the environment has been hypothesised to increase cognitive skills in fish (Mackney and Hughes, 1995; Brydges et al., 2008), birds (Roth and Pravosudov, 2009; Pravosudov and Smulders, 2010; Freas et al., 2012) and mammals (Barton and Harvey, 2000), but there exists no literature on the effects of habitat complexity or environmental disturbance on learning abilities in lizards. Very few studies have compared learning abilities across lizard populations, but evidence supports that differences in developmental conditions, especially incubation temperature, affects learning capabilities (Amiel and Shine, 2012; Clark et al., 2013 for lizard). Habitat stability across different populations of a species can also influence learning as has been observed in individuals that have developed under complex and variable environmental conditions to have quick and flexible learning skills (Brydges et al., 2008; Roth et al.,

2010). Behavioural flexibility or the ability to develop new responses to novel stimuli or modify existing responses have been observed in mostly mammals and birds which exploit unpredictable resources and thus alter motor skills, spatial learning, associative learning or reversal learning (Sol et al., 2002; Dunbar et al., 2007). Recently such behavioural flexibility has also been reported from *Anolis evermanni* which shows flexibility across multiple cognitive tasks including solving novel motor task and reversal learning (Leal and Powell, 2012). Individuals developing in a complex dynamic environment might exhibit such behavioural flexibility that facilitate their survival. One such environmental complexity arises due to urbanisation, where typical spatial features or landmarks which might serve as cues for navigation are highly altered by novel man-made structures. The Indian rock agama *Psammophilus dorsalis*, which is commonly found in both suburban and rural habitats, faces such challenges. Here, I provide the first empirical test of the prediction that urbanisation selects for better learning abilities in lizards. The two extreme habitat conditions that this species lives in differ in some key ways. The natural habitat of this species in rural areas of India are composed of rocky hills interspersed with dry scrub vegetation, with no anthropogenic structures and minimal human activity. By contrast, the suburban habitat is mostly composed of unbuilt plots, construction areas surrounded by man-made structures, and houses with some scrub vegetation and trees. Thus, lizards residing in the suburban habitat have to frequently encounter the risk of selecting safe from unsafe refuges as new structures arise which coincide with high level of human activity. Rapid alteration of spatial features also leads to changes in resource availability and distribution, as well as suitable basking sites. By contrast, the rural habitat is stable in terms of spatial features and does not show rapid

shifts during the lifetime of a lizard. Thus, I predict that these key differences in stability of the local environment would affect the learning and reversal learning rates of *P. dorsalis* for a skill that is key to its survival: recognition and utilization of a safe refuge in response to simulated predator attacks.

6.2 Methods

I wild-caught males of *P. dorsalis* from rural ($N= 14$) and suburban areas ($N= 16$) during April to July, which is the peak activity season for this species and housed them in the laboratory for up to 16-18 days for the learning trials. All males were sexually mature adults (SVL = 121.11 ± 2.05 mm) and were of similar age (in their first breeding season). Each lizard was housed individually in a large rectangular enclosure (80x45x30cm) that also served as the testing enclosure. Enclosures were maintained indoors under ambient and visible spectrum white lamps for lighting. Each enclosure was lined with paper towel as substratum and petri dishes were placed to provide water. Lizards were fed with crickets before the start of trials each day.

The associative learning trials were set up such that each enclosure had two identical refuges constructed from PVC pipes that were cut longitudinally (15 cm long x 5 cm radius) and placed at the two ends of the enclosure. A red square (7x7cm) was placed on the wall behind one refuge to serve as spatial cue for the safe refuge. Each individual lizard was subjected to two learning tasks. In the first task (learning trials), lizards had to learn the location of the safe refuge. I simulated predatory attacks by gently tapping the tail of the lizard with a brush which made the animal seek refuge. Lizards were continually “attacked” until it chose the safe refuge. If the lizard entered the unsafe

(incorrect) refuge, I would lift that refuge and simulate attack. Once the lizard entered the safe refuge, it was undisturbed until the next trial. Each animal was attacked three times in a day with a gap of 3-4 hrs in between. Learning task continued for 7 days (20 trials), and a lizard was classified as having learned the safe refuge if it chose the safe refuge as a first choice (after the first attack of the trial) five times in a row or across five consecutive trials.

Individuals who exhibited learning, which I define as having chosen the safe refuge as first choice for five consecutive trials within 7 days, were subjected to the second task, which was reversal learning. Reversal learning is a task where the positive and negative contingencies are switched with respect to previous learning task so that individuals have to reverse previously created associations. Thus, for the reversal learning task, the refuge without the red local cue served as the new “safe” refuge. The same attack protocol was used to perform these trials. Reversal learning trials were also performed for 7 consecutive days (3 trials per day) and a gap of 24hrs was given between learning and reversal learning tasks. All trials were conducted during the peak activity hours of the lizard (0900-1200 and 1500-1700). *Psammophilus dorsalis* do not have femoral pores so it was assumed that they do not rely on chemical cues for locating refuges, and to ensure that refuge characteristics themselves do not influence shelter choice, the refuges were switched every alternate day.

During each trial I recorded the (i) first refuge chosen by the lizard, (ii) number of incorrect choices made before choosing the safe refuge, and (iii) latency to choose the safe refuge. If a lizard was already found inside the safe refuge at the start of any trial, it was considered to have chosen the safe refuge and the latency to choose that safe refuge

was recorded to be zero seconds. This criterion of learning is biologically relevant as individuals prefer to remain inside refuges during the night, and for general safety. However, to ensure that this criterion of choice did not inflate my results, analysis with and without these trials were performed and no statistical difference in the final conclusion was observed. Thus, the results reported below include all trial data.

Statistical analysis

Latency to select the safe refuge and the number of incorrect choices made were correlated responses (Pearson's correlation coefficient=0.726, $P < 0.001$) so I selected the number of incorrect choices as the response variable for further analysis. I tested whether mean number of incorrect choices made before selecting the safe refuge significantly decreased with time as this pattern would indicate that lizards were learning. I used generalised linear mixed model (GLMM, lme4 package in R) with number of incorrect choices as the dependant variable and trial number and habitat (suburban and rural) as fixed factors with individual ID as a random effect. I also compared the proportion of individuals from suburban and rural habitats that showed learning or reversal learning by using a chi square test for equality of proportion with continuity correction. Finally, I compared the time taken to exhibit learning between the learning trial set (Task 1) and reversal learning trial set (Task 2) for suburban and rural individuals using two-way ANOVA with signed rank transformation (as data was non-normal) to test if learning and reversal learning occur at similar rates. Variation due to individual ID was negligible ($SD < 0.1$) for all analysis.

6.3 Results

Proportion of males that showed learning and reversal learning was similar across habitat (Learning: $\chi^2 = 0.69$, $P = 0.405$, Figure 6.1a; Reversal learning: $\chi^2 = 1.75$, $P = 0.185$, Figure 6.1b). When comparing how quickly lizards learnt the location of the safe refuge in both tasks, I found suburban males to be faster than rural males ($F_{1,19} = 11.17$, $P = 0.003$, Fig.6.2) and all lizards to be faster in learning task than reversal learning task ($F_{1,19} = 10.98$, $P = 0.003$, Fig.6.2). Number of incorrect choices during learning (Task 1) was found to be affected by trial number ($F_{19,539} = 5.01$, $P < 0.001$) and habitat ($F_{1,33} = 8.53$, $P = 0.006$), such that number of incorrect choices decreased with trial numbers and was significantly lower in suburban males compared to rural males (Fig. 6.3). Similarly, during reversal learning (Task 2), number of incorrect choices was found to be significantly affected by trial number ($F_{19,494} = 11.08$, $P < 0.001$) and habitat ($F_{1,26} = 8.67$, $P = 0.006$). Number of incorrect choices made before selecting the new safe refuge in Task 2 decreased with trial number for all lizards, with rural males being slower to select safe refuge compared to suburban males (Fig.6.3).

6.4 Discussion

In this study I provide evidence that *Psammophilus dorsalis* is capable of exhibiting rapid and flexible learning ability when identifying safe from unsafe refuges and also in reversal learning when the contingencies are switched. This study adds support to the growing evidence that squamates are not poor in learning abilities as was thought for many decades (Burghardt, 1977; Wilkinson and Huber, 2012), but are in fact capable of exhibiting behavioural flexibility in terms of rapid learning and unlearning of relevant resources. In my study, 93 percent males of *Psammophilus dorsalis* exhibited

learning about safe refuge within 2-7 days. Recent research on various learning capabilities in lizards show that use of relevant ecological methodology and learning criteria is important in understanding cognitive abilities of a species. Spatial learning about refuges has been shown in turtles, snakes and lizards, with different species employing different strategies for learning (Lopez et al., 2001; Holtzman et al., 1999; Stone et al., 2000; LaDage et al., 2012; Noble et al., 2012). Animals can use intra-maze or local spatial cues and extra-maze or distal spatial cues, as well as natural orientation related to sun compass to locate their refuge (see Lopez et al., 2001 for *Pseudemys scripta* and LaDage et al., 2012 for *Uta stansburiana*; Noble et al., 2012; Beltrami et al., 2010; Foa et al., 2009). Unlike the hippocampus of birds and mammals that helps in formation of spatial representation of the environment, reptiles do not possess a hippocampus and thus learning abilities have been found to be differ between species and orders (Holtzman et al., 1999; Day et al., 1999; Lopez et al., 2000). In my study, the mechanism of spatial learning remains unknown, but lizards had only two options: either they used multiple features of the arena such as position of the local cue (red square) and petri-dish inside the trial arena to orient spatially towards the goal location, or they associated the local cue to the goal location. Interestingly *P. dorsalis* also exhibited rapid reversal learning wherein 70 percent of the males learnt by 7-19 trials which is unlike most studies in which typically less than 50 percent of the trial population shows reversal learning and they require more attempts (trials) to do so (Day et al., 1999; Clark et al., 2013; Noble et al., 2012; Leal and Powell, 2012). The enhanced learning skill exhibited by *P. dorsalis* might be attributed to it being an agamid which may have higher cognitive skills compared to skinks or iguanids, where most of the lizard learning literature has

been generated from (but see Gaalema, 2011 on *Varanus rudicollis* with $N=2$). More likely, however, *P. dorsalis* has a wide geographical distribution that includes a variety of habitats that supports greater flexibility in adjusting to environmental challenges.

Learning and memory allows animals to adjust their behaviour to adapt to changing environments and thus cope with some degree of unpredictability. Thus, in habitats that are predictable and relatively stable with little change in habitat characteristics, individuals show reduced flexibility in learning skills or exhibit stronger long-term memory compared to individuals of the same species that reside in fluctuating environments where they need to form short term memory about relevant resources as it keeps changing (see Mackney and Hughes, 1995 for sticklebacks). Unlike the stable rocky or boulder fields of rural habitats, suburban India comprises habitats that are unstable and dynamically change in structure and composition during the lifetime of a lizard. Lizards in suburban environments in my study area face frequent challenges with respect to finding safe refuges and basking sites, as their local environments within their territories frequently change due to human development (see Fig 1.1). Consequently, in support of my prediction, I find that populations of *P. dorsalis* differ significantly in their learning skills, as suburban lizards were faster in both learning and reversal learning compared to the rural lizards. Though the proportion of individuals exhibiting learning and reversal learning was not different across habitats, the time taken by suburban males to learn about safe refuge during both the tasks was significantly faster than that taken by rural males. Similar to studies in which better learning capabilities were found in individual birds living in harsher environments, I find that lizards residing in unpredictable urban habitats are faster learners (Pravosudov and Clayton, 2002).

Psammophilus dorsalis is a territorial species that most likely uses knowledge of spatial locations for various tasks such as locating food, potential mates, guarding their territory, and hiding from predators. In this study I also show that populations of *P. dorsalis* residing in stable rural and dynamic urban habitats differ in their learning abilities about safe refuges. Not only do urban and rural lizards differ in this one cognitive trait but also in their general escape strategies, as suburban individuals choose closer artificial refuges and are habituated to human exposure thus making them less risk averse than rural lizards (Chapter 4). Though my study was not designed to understand the mechanism involved in learning, higher circulating corticosterone in the suburban individuals that I find (thesis chapter 3) might facilitate faster learning abilities. In the eastern fence lizard (*Sceloporus undulatus*), physiologically higher corticosterone levels enhance aversive learning and memory consolidation (Thaker et al., 2009). Thus, along with shifts in multiple phenotypic strategies (Chapter 1, Chapter 3, and Chapter 4), survival and persistence in urban habitats may require higher and more flexible cognitive skills as well. This increased cognitive ability could be a result of either microevolutionary differences between populations, or individual lifetime experience or a combination of both.

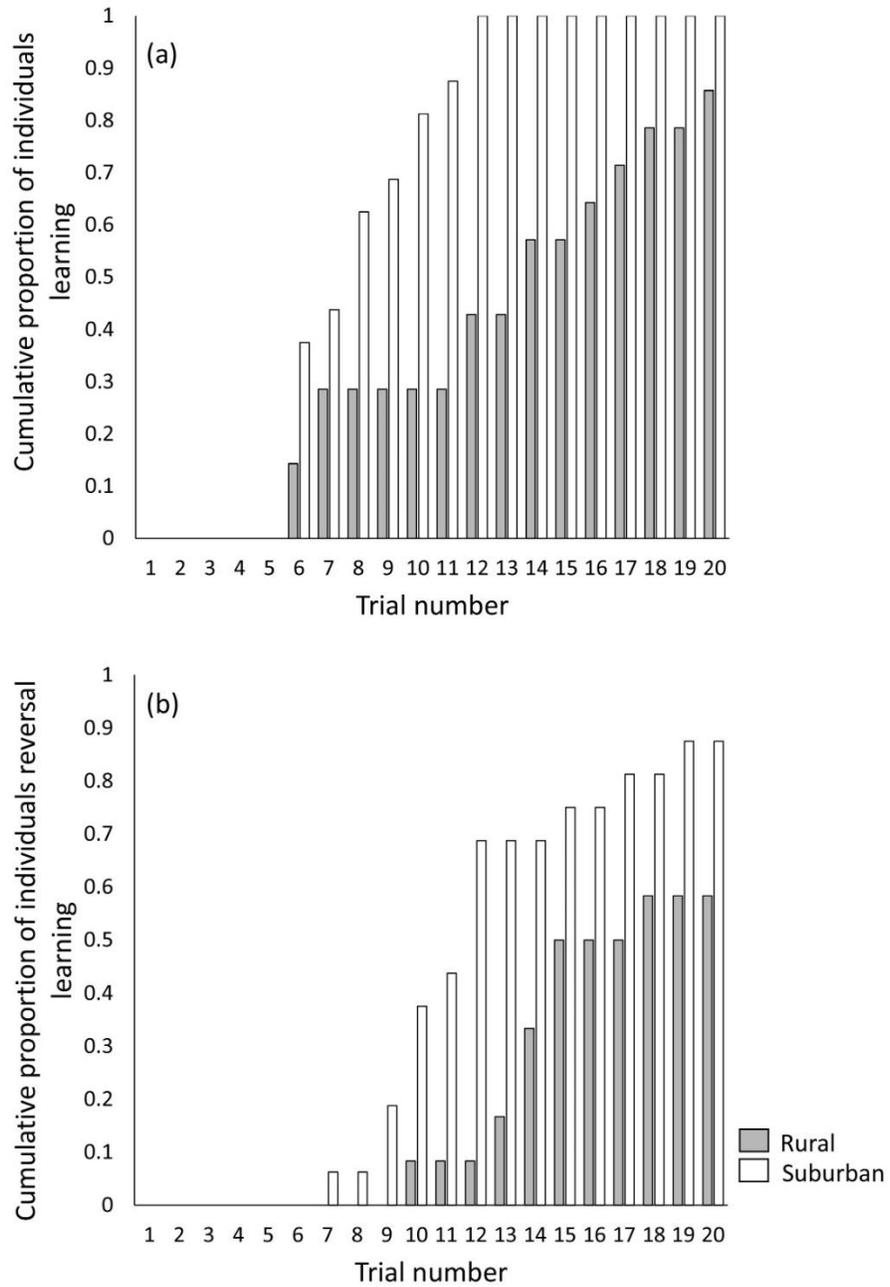


Figure 6.1. Cumulative proportion of individuals from rural and suburban habitats showing a) learning and b) reversal learning across trials

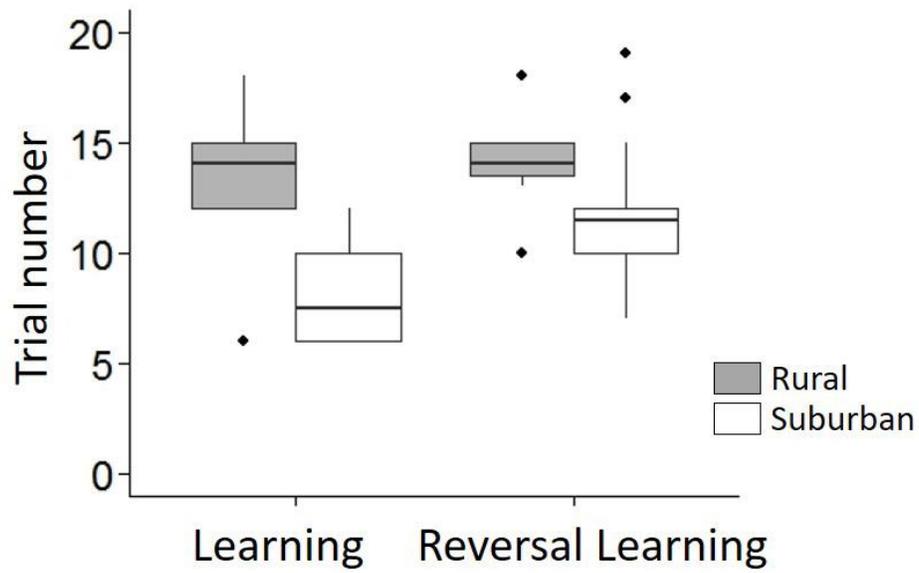


Figure 6.2. Number of trials taken by rural (grey) and suburban (white) lizards to exhibit learning and reversal learning. Shown are boxplots with medians, quartiles and 5th and 95th percentiles and extreme values.

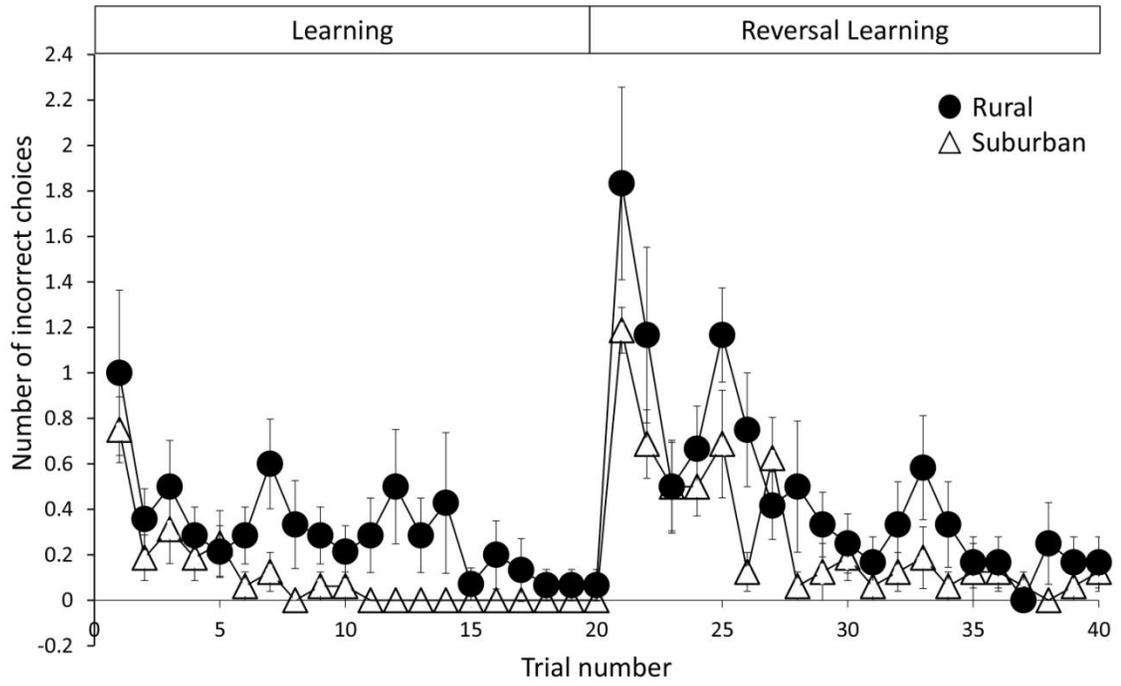


Figure 6.3: Number of incorrect choices made before selecting safe refuge across trials for suburban and rural individuals during both learning and reversal learning task. Shown are means \pm 1se

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Conclusion

Urban environments expose wildlife to new selection pressures to which they have not been exposed in their evolutionary history. But organisms can respond to these new selection pressures by modifying their behaviour, physiology and cognitive abilities to adjust flexibly and survive urbanisation. Urbanisation influences different behaviours in an individual by affecting the factors that determine fitness of the individual such as ability to find food, avoid predation, interact with conspecifics and face novel challenges (Lowry and Wong 2012). Urbanisation is generally thought to have a negative effect on fitness, mainly because habitat destruction and alteration leads to increased mortality and lowered reproductive success (McKinney 2008). But for some species, urbanised landscapes might be tolerable and thus these species are found to coexist with moderate anthropogenic disturbance (Lowry and Wong 2012; Miranda et al. 2013). Understanding the suite of phenotypic traits that enable survival in urbanised environments by a lizard was the focus of this thesis.

Using field based and experimental approach, I found evidence of shifts in multiple phenotypic traits between two populations of the Indian rock agama *Psammophilus dorsalis* that is found across an urbanisation gradient in and around the city of Bangalore, India. This species shows shifts in behaviour, physiology and cognitive abilities in urbanised areas, which enable it to flexibly adjust to novel challenges. My research broadly examined the survival and social strategies of individuals between suburban and rural populations of this species. In the first two chapters of the thesis, I examined colour signalling from the signaller and receiver perspective. Males of *P.*

dorsalis showed rapid physiological colour change during social signalling and it was starkly different between courtship and competitive interactions. For this work, I developed novel tools to measure physiological colour change and receiver responses. I used a customised probe attached to a spectrophotometer that was attached to the animal to measure rapid colour change without disturbance from handling stress. Colour contrast was analysed using visual modelling to integrate the visual system of a typical agamid lizard. From these controlled measures of social colours, I found that suburban males expressed lower colour contrast than rural males and were also slower to change colours during courtship interactions. Thus along with social context affecting physiological colour signal, I provide the first evidence that social signalling through dynamic colour change in terrestrial vertebrates is also affected by urbanisation. In chapter 2, I used robotic models to examine receiver responses to the multicomponent colour signalling display of *P. dorsalis* that has both a movement and colour component to it. Processing of visual information occurs differently in the two halves of the brain and so I also examined the effect of brain laterality in signal response. *Psammophilus dorsalis* showed right hemispheric dominance in responding to colour components of the signal but no laterality was found in response to the movement component. A difference in the latency to respond to red colour was observed in females from different populations, wherein rural females were faster in responding to red compared to suburban females. This difference in the intensity of receiver responses seem to match the intensity of colour between populations of males. In Chapter 1, I found that suburban males have lower dorsal red colour contrast than rural males, and the population of urban males show higher variation in the dorsal colour. Thus, red on males may not be a reliable and

consistent signal for females in suburban environments. I also find shifts in physiology and social behaviour in *P. dorsalis* such that suburban males showed lower proportion of display and lower rates of display during courtship interactions compared to rural males. I also collected blood samples from males from both populations across different time points after staged social interactions to measure corticosterone and testosterone levels. I found that suburban males maintained an elevated level of corticosterone under control conditions and also increased their corticosterone level 20 min after social interactions, whereas rural males maintained low corticosterone levels under all social interactions and control condition. Testosterone levels were elevated by both rural and suburban males immediately following social interactions but suburban males reduced their hormone levels within 20 min post interaction while rural males maintained the elevated level until at least 30 min post interaction. Thus, I found shifts in both physiology and social behavioural displays in populations residing in suburban habitats, which suggests a population shift in coping or reaction styles to various stressors in the disturbed environment.

I also compared survival strategies of *P. dorsalis* across suburban and rural habitats to understand how suburban lizards avoid predators or learn about novel challenges in their disturbed environment. To quantify escape responses of *P. dorsalis*, I conducted a field study in which I compared flight initiation distance, type and distance of refuges used, perch sites and hiding duration of both males and females from suburban and rural habitats in response to a simulated predator attack on field. I also measured sprint speed of lizards from both populations in the laboratory by inducing them to run on a custom-built race track. In the field, flight initiation distance of suburban males was

significantly shorter compared to rural males and these males also perched at lower heights and used closer refuges. Changes in habitat structure and habituation to humans in urban environments might have led to difference in escape responses and microhabitat use in suburban lizards, despite no physiological difference in sprint speed compared to rural lizards. I also found that females of *P. dorsalis*, who are cryptically patterned, might be using camouflage as an antipredator strategy rather than early escape to evade predation, as they show no difference in flight initiation distance across suburban and rural populations. The structural features and disturbance levels in an urban habitat can shift rapidly during the lifespan of a lizard, as human structures are built and human activity increases. It thus becomes very important to learn about safe and unsafe refuges in such a dynamic environment. I tested learning and reversal learning abilities of *P. dorsalis* males from suburban and rural habitats by simulating predator attacks under laboratory conditions, where animals were housed in large enclosures with spatial cues to help them in navigation between artificially safe and unsafe refuges. In congruence with my prediction that habitat instability facilitates faster learning capability, I found that suburban lizards learn and unlearn faster about safe refuges compared to rural lizards.

Thus, my thesis provides a broad perspective on the different phenotypic traits that can shift in individuals facing novel urban challenges. The results from my thesis highlights the importance of having an integrative approach while examining the effects of urbanisation on a species. Most studies to date report effects on either behaviour or physiology or a single phenotypic trait at a time. This creates a problem in assessing the overall effects on a species as predictions based on single phenotypic traits might not be correct. A species might be affected negatively in some aspects of its behaviour, such as

foraging or social communication, but might be better in avoiding predators or learning about novel food in an urban landscape. The sum of these multiple interacting phenotypic effects could result in a welladapted urban organism. What remains a challenge is determining the specific selection pressures that differ in urban environments compared to unmodified natural areas. By expanding research into the phenotypic strategies of urban wildlife and the challenges they face, we will gain a better understanding of which species are likely to successfully inhabit cities and towns and which will ultimately be excluded from them. Thus, similar studies in other species that show flexibility in phenotypic traits and reside in urban habitats, will be an important step to understand how animals cope with our urbanising world.

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