

# Behavioural and Biological Responses of Australian Water Dragons (*Intellagama lesueurii*) to Urbanisation

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Presented for the degree of  
Doctor of Philosophy



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Submitted for examination: 13 April 2018  
Final submission: 7 August 2018



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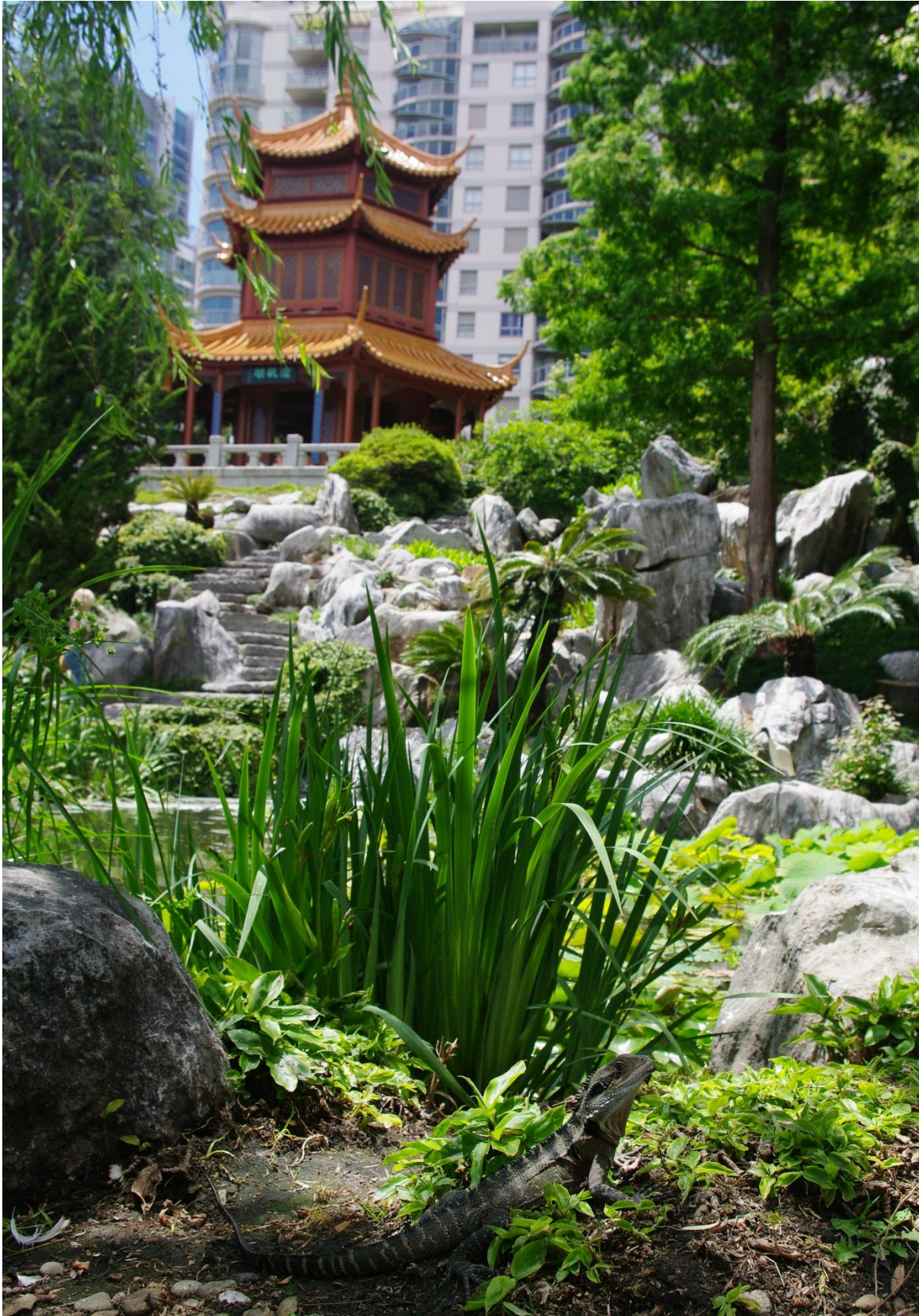
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An Australian water dragon (*Intellagama lesueurii*) living within the Chinese Garden of Friendship at Darling Harbour; an isolated greenspace in the middle of Sydney's central business district.

## Declaration

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I certify that the work contain within this thesis, entitled “Behavioural and Biological Responses of Australian Water Dragons (*Intellagama lesueurii*) to Urbanisation”, has not been submitted for a degree nor has it been submitted as part of the requirements for a degree to any other university or institution other than Macquarie University.

I also certify that this thesis is an original piece of research, and has been written by myself. All assistance provided in conducting and reporting this research has been appropriately acknowledged, and all information sources and literature cited has been properly referenced.

The research presented within this thesis was conducted in accordance with the Macquarie University Animal Ethics Committee (ARA protocol # 2015/023) and Taronga Zoo Animal Ethics Committee (ARA protocol # 3b/08/15). Also, our research was licensed by the New South Wales National Parks and Wildlife Service, Office of Environment and Heritage (scientific license # SL100570).

James Baxter-Gilbert  
Macquarie Student ID: 43811892  
August 2018

## List of Original Publications

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This thesis is based on the following original publications, which are referred to in the text by Roman numerals (I, II, III, IV, V, and VI). Original publications are reported with the permission from their copyright holders where applicable.

- I      Baxter-Gilbert, J., and Whiting, M. J. (2018). Street fighters: Bite force, injury rates, and population density of urban Australian water dragons (*Intellagama lesueurii*). *Submitted*.
  
- II     Baxter-Gilbert, J., Riley, J. L., and Whiting, M. J. (2018). Bold new world: Urbanisation selects for boldness in a lizard. *Submitted*
  
- III    Baxter-Gilbert, J., Riley, J. L., and Whiting, M. J. (2018). Runners and fighters: Clutch effects and body size drive innate antipredator behaviour in hatchling lizards. *Behavioral Ecology and Sociobiology* 72: 97, doi: 10.1007/s00265-018-2505-7
  
- IV    Baxter-Gilbert, J., Riley, J. L., Frère, C. H., and Whiting, M. J. (2018). Anthropogenic selection: Human-altered landscapes drive the development of divergent morphology in an urbanised lizard. *Submitted*.
  
- V      Baxter-Gilbert, J., Mühlenhaupt, M., and Whiting, M. J. (2018). Comparability and repeatability of three commonly used methods for measuring endurance capacity. *Journal of Experimental Zoology Part A: Ecological and Integrative Physiology*, doi: 10.1002/jez.2145
  
- VI    Baxter-Gilbert, J., Riley, J. L., and Whiting, M. J. (2018). Running through the concrete jungle: Performance capacity and trade-offs in an urban-living lizard. *Submitted*.



## Contributors and Division of Labour

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All contributors are ordered alphabetically by last name, following my name. Contributors' initials are as follows: JBG – James Baxter-Gilbert, CF – Celine Frère, MM – Max Mühlenhaupt, JR – Julia Riley, and MW – Martin Whiting.

	<i>Chapter I</i>	<i>II</i>	<i>III</i>	<i>IV</i>	<i>V</i>	<i>VI</i>
Conception and Design	JBG, MW	JBG, MW	JBG, MW	JBG, MW, CF	JBG, MW	JBG, MW
Logistics and Data Collection	JBG	JBG	JBG	JBG	JBG, MM	JBG
Statistical Analysis	JBG	JBG, JR	JBG, JR	JBG, JR	JBG	JBG, JR
Writing and Reporting	JBG, MW	JBG, JR, MW	JBG, JR, MW	JBG, JR, MW, CF	JBG, MM, MW	JBG, JR, MW
Overall Responsibility	JBG	JBG	JBG	JBG	JBG	JBG



A male Australian water dragon (*Intellagama lesueurii*) within the B2B kangaroo yard at Taronga Zoo, Mosman, NSW.

## Acknowledgements

*“Always remember, it’s simply not an adventure worth telling if there aren’t any dragons”*  
– Sarah Ban Breathnach (2009)

The adventure that has culminated in writing this thesis, and which has been fraught with dragons, has been made possible by the most wonderful collection of people. For some of them, it was their direct involvement in the science; slugging it out in waist-deep water in the middle of the night, lunging for lizards falling from trees, and simultaneously being eaten alive by mosquitoes. For others, it was all the things that happen outside of research, the friends, the laughter, and all that comes with trying to explore a new continent in your seemingly minuscule ‘spare time’. It is the people we share our adventures with that really makes them memorable. And, of course, a healthy dose of dragons.

First and foremost, I must express my deepest gratitude to my supervisor Dr Martin Whiting who made this all possible. Martin, thank you for your support, insights, and expertise in all aspects of this project. You welcomed me into the lab long before the start of this research, and your kindness, generosity, and supportiveness has meant so much to Julia and I. I have learnt a lot from you, whether it be how to navigate admin encounters, design assays, develop a question worth answering, or rejig a manuscript to reach more people. I cannot thank you enough for my experience in your wonderful lab, and your mentorship.

I also need to thank all of the members of the Lizard Lab. Théo Damasio and Max Mühlenhaupt, both of you worked so hard on this project. I hope your experience with us at the Lizard Lab provided you with what you were looking for in your internships. This thesis would not be the same without either of you and look forward to reading your theses in the future. Similarly, a big thank you is required for the army of volunteers that worked hard to feed, water, and clean all of the captive dragons over the last three years. Furthermore, to my comrades in science: Julia Riley, Fonti Kar, Dan Noble, Christian Perez, Isabel Damas, Birgit Szabo, Arnaud Badiane, Iván Beltrán, Sergio Narretto, Jodie Gruber, and Kate Umbers - thank you! All of you made this happen. Whether it was helping to figure out a challenge, pondering an idea, battle R code, or even just talking about the latest nerdy thing you just saw online. You guys made the lab “The Lab”. I feel very fortunate to have shared the grad student life with you.

I would also like to thank Dr Culum Brown for all of his logistical help, particularly for the canoe and providing all of the space need for me to build a dragon maternity ward. Similarly, thank you to Dr Celine Frère for all of her insights into urban dragons and their evolutionary ecology. Our conversations, and the wonderful work your lab conducts, contributed greatly to my understanding of these lizards. I also must thank Drs Mariella Herberstein and Andrew Barron for providing their support and advice during my candidature at Macquarie University. Of course, I would not have been able to even begin this PhD project without the support of the Macquarie University International Graduate Student Scholarship and the Natural Sciences and Engineering Research Council of Canada. I also have to thank the National Park Association NSW; the Dragons of Sydney project team (Geetha Ortac, Fran van den Berg, Chantel Benbow, and Steph Clark) were fantastic collaborators. I hope we helped some people gain a deeper appreciation for these amazing lizards. You guys rock!

Beyond directly influencing the thesis itself, many people have contributed my PhD adventure. I have always been a staunch supporter in the idea that good science requires



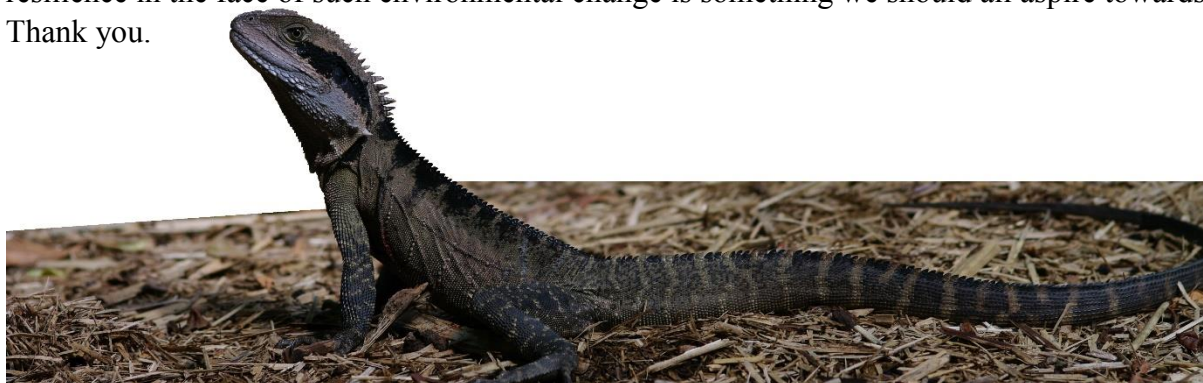
frequent visits to the pub. I must thank Drs Peter Harlow, Glenn Shea, and David Kirshner for all of their bountiful herpetological knowledge shared at our regular Beer Bingers meetings. Our frequent success at trivia nights cemented the idea that a sound understanding of science and reptiles, and a thirst for free beer, is more important than any knowledge of sports, history, or politics. Cheers! Similarly, a big thank you to Stephan Klopper, Jo Ocock, Liv Monck-Whipp, Geoff Hughes, Sean Espinola, and Steve Marks for nights spent relaxing with a nice cocktail or chasing herps at home and abroad. Also I need to thank the wider group of amazing friends, family, and colleagues, both within Australia and around the world. There is far too many of you guys to list by name. Moving around the planet comes with obvious logistical challenges for keeping in touch, but I have definitely found that no matter where I am none of you are no more than a text, call, email, or GIF away. Thank you for keeping in touch from afar or sharing in this adventure when we were able to get together.

Much of my time spent out of academics was with the wonderful people at Taronga Zoo, particularly at Backyard to Bush (B2B). To Simon Brown, thank you. I still must apologise for leaving B2B to start this PhD, but I am so grateful you let me stay on in the capacity I did. To the rest of the B2B team (alphabetised): Holly Caputo, Steve Dalywater, Sam Elton, Brett Finlayson, Paul Hare, Suzie Lemon, Farley Macdonald, Lachlan Manning, Imogen Palmer, Bec Russell-Cook, and everyone else - thanks! I've learned a lot from you guys, some of which was animal related, most of which I cannot mention in polite company, and all of which was an absolute blast!

I particularly want to thank my family- my Mom, my Dad, my brothers, Chris and Matt, and sisters, Kate and Jenn, and all of their growing families. I know that the career path I have chosen generally means I miss regular family functions, like holidays, birthdays, actual births, dinners, camping trips, hockey games, and so on, but I have always felt your love and support. It means a lot. Thanks!

I must of course give a tremendous thank you to, my amazing partner, in life, love, and science, Julia Riley. You have always been a tremendous source of inspiration, love, and knowledge. I am so lucky to be on this adventure with you. You have been involved in every aspect of this project, with advice, collaboration, and endless support. I could not have done this without you. Thank you for everything you do that allows us to keep making our dreams come true. I think we may be the only two people whose honeymoon lasted 4 years and earned them two doctorates. #TeamAwesome #QualantityTime

Lastly, to the scaly beasts of this adventure, the dragons - sorry. I know you hate me. I'll forgive all the biting, tail-whipping, and scratching, if you forgive my intrusion. Your resilience in the face of such environmental change is something we should all aspire towards. Thank you.



## Thesis Summary

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One of the strongest driving forces of evolution is environmental change. Within the modern era, some of the most rapid environmental change has occurred as a result of urbanisation. As such, recent research aims to understand how species are adapting to urban landscapes (urban evolution) and how natural selection is operating in unnatural environments (anthropic selection). This goal was the cornerstone of my thesis research, using Australian water dragons (*Intellagama lesueurii*) to examine how the selective forces within urban environments may shape their natural histories and alter their evolutionary trajectory. I took a multidisciplinary approach to explore water dragon urban evolution, by examining traits related to their behavioural ecology (social interactions and spatial organisation, behavioural traits, and innate antipredator responses; Chapters I, II, & III respectively), morphology (body length and size; Chapter IV), and physiology (performance capacity; Chapters V & VI), using both field observations and laboratory experiments. The basis of most of this thesis' research involved contrasting traits of lizards living in, or originating from, populations varying in their levels of urbanisation (urban, semi-natural, and natural). I also examined the mechanisms that may have caused urban-derived divergence of morphological and behavioural traits (i.e., heritable traits vs phenotypic plasticity). My research identified several urban-derived divergent phenotypes, including increased rates of aggressive encounters, increased boldness, decreased body size paired with increased limb and head size, and decreased endurance capacity. My research also identified that both divergent behavioural (boldness) and morphological (body length and size) traits have a genetic origin, suggesting they are heritable and may be adaptive. Overall, this thesis provides insight into the urban evolution of water dragons in the Sydney region, and provides a framework for using a multidisciplinary approach to rigorously examine mechanistic urban ecology and evolution.

## **General Introduction**

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It has been said that “good fences make good neighbours”, unfortunately humans do not often respect natural boundaries. In fact, we can be terrible neighbours. Even in humanity’s early beginnings, our presence within an ecosystem was often associated with substantial decreases in biodiversity, and this is a trend that continues today (Burney and Flannery 2005; Sandom et al. 2014; Faurby and Svenning 2015). A prominent driver of global biodiversity loss is habitat degradation and fragmentation resulting from human development and urbanisation (Vitousek et al. 1997; McKinney 2002). Furthermore, the global footprint of urban areas is increasing each year and is expected to continue to increase extensively as the human population reaches 9 billion by mid-century (United Nations 2014). Put frankly, the march of human progress has come at the cost of numerous species and ecosystems (Vitousek et al. 1997; McKinney 2002, 2008; Burney and Flannery 2005). This is only one side of the proverbial coin however, and there is hubris in assuming that life on Earth will not respond to the challenges humanity presents. Nature has always found a way, despite catastrophes and massive extinction events (Schulte et al. 2010). This is not to detract from the wholly important task of mitigating the mounting threats that humans pose to the planet. Yet, by studying how natural selection and other evolutionary processes are shaping species in an ever-increasingly anthropogenic world, we are able to examine evolution in real-time and in a globally occurring natural experiment.

### **Urban evolution**

The biodiversity of the Galápagos archipelago aided Charles Darwin’s understanding of how different habitats can drive divergent traits, and subsequently helped form his understanding of natural selection and descent with modification (Sulloway 1984; Hoernle et al. 2002). Similar to the geologic processes that can lead to the formation of new landscapes (e.g., the volcanic and tectonic formation of islands and land bridges in Central and South America; Hoernle et al. 2002) urbanisation gives rise to new environments (Ellis & Ramankutty 2008) and in doing so promotes the diversification of traits and even species (Johnson & Munshi-

South 2017). Urban habitats are often isolated and limited in size, functioning much like islands (Davis and Glick 1978; Soulé et al. 1988; Littleford-Colquhoun et al. 2017), which provides an excellent environment for rapid evolution (Thompson 1998; Millien 2006, 2011). Ecologically, urban landscapes contain novel anthropogenic threats and mortality sources (e.g., traffic, pollutants, fragmentation, and introduced predators; Shochat et al. 2006; McKinney 2008), but can also provide exploitable opportunities and resources (e.g., decreased natural predators, thermal buffers, anthropogenically-subsidised food sources; Ditchkoff et al. 2006; Kowarik 2011; Winchell et al. 2016). Therefore, as wildlife encounters urbanisation, they will also face considerable selective pressure that will either force them to adapt or perish. Thus, urbanisation also is an excellent crucible by which to study evolution in real time (Winchell et al. 2016; Diamond et al. 2017; Johnson & Munshi-South 2017; Sprau & Dingemanse 2017).

Recent research has observed that many species exhibit urban-derived divergent phenotypes (see meta-analysis by Alberti et al. 2017 and review by Shochat et al. 2006; Sih et al. 2011), as well as documented evidence of urban evolution itself (see review by Johnson & Munshi-South 2017). For example, numerous songbird and frog species call more loudly in urban landscapes (theorised to be a response to compensate for anthropogenic noise; Parris et al. 2009; Barnett 2015). Pipistrelle bats (*Pipistrellus pipistrellus*) alter their flight patterns and movement in response to urban light (Hale et al. 2015). Physiologically, tree lizards (*Urosaurus ornatus*) have decreased their stress response and increased their innate immunity to handle the challenges of urban-living (French et al. 2008), while side-blotched lizards (*Uta stansburiana*) increase their reproductive output (clutch and egg size) in urban areas to offset increased adult mortality rates and accelerate their pace of life (Lucas and French 2012). Furthermore, morphological differences have been observed between urban and non-urban songbirds (Evans et al. 2009; Brown and Bomberger Brown 2013) and lizards (Marnocha et al. 2011; Iglesias et al. 2012; Littleford-Colquhoun et al. 2017). For example, populations of cliff swallows (*Petrochelidon pyrrhonota*) living around highway infrastructure exhibit



increased wing length, which is hypothesised to enhance vertical manoeuvring during flight to avoid traffic (Brown and Bomberger Brown 2013). Similarly, urban anoles (*Anolis sagrei*, Marnocha et al. 2011; *Anolis cristatellus*, Winchell et al. 2016) and swampland lashtail lizards (*Gowidon temporalis*; Iglesias et al. 2012) exhibit increased limb length, which could increase their ability to exploit human-altered habitats (e.g., artificial basking, perching, and refuge sites).

Over the last few decades the field of mechanistic urban ecology has grown substantially (Sukopp 1998, Shochat et al. 2006; Forman 2016; Pickett and Cadenasso 2017), yet it has been only recently that framing urban ecology within an evolutionary context has gained traction within the literature (Atwell et al., 2012; Winchell et al. 2016; Alberti et al. 2017; Diamond et al. 2017; Johnson & Munshi-South 2017; Littleford-Colquhoun et al. 2017). Notably, there is a gap in our knowledge as to the mechanisms that are giving rise to urban-derived divergent phenotypes. For example, although several studies have observed morphological differences that are presumed to be adaptive in urbanised bird and lizards (e.g., skull shape and limb length; Irschick et al. 2005; Marnocha et al. 2011; Iglesias et al. 2012; Hutton & McGraw 2016), whether or not these differences are a result of multigenerational selection (heritable adaptation) or within-generation plasticity (phenotypic plasticity) remains unknown. This leaves researchers questioning the drivers behind their results. It has been suggested that within-generation plastic responses may provide a stopgap allowing populations to temporarily persist under urban pressures, creating enough time for natural selection and adaptive heritable traits to evolve (Price et al. 2003; Winchell et al. 2016), however much more investigation is required. As such, the nascent field of urban evolution (Johnson & Munshi-South 2017), although being grounded in long-established biological fields (e.g., evolutionary biology, population ecology, and urban ecology), stands to provide valuable insights into our understanding of evolution both in general and within a changing world.

## Urbanisation and reptiles

For most people the term ‘urban wildlife’ is typically associated with commensal species, like cockroaches (*Blattella germanica*; Owens and Bennett 1982), feral pigeons (*Columba livia*; Sacchi et al. 2002), and rats (*Rattus* sp.; Feng and Himsworth 2014). These are the iconic “urban species”, despite also being invasive pests in most parts of the world (Owens and Bennett 1982; Sacchi et al. 2002; Feng and Himsworth 2014). Yet, a diversity of reptiles call many major Australian cities their home; with some persisting and even thriving, while others are less successful (How and Dell 1994; Tait et al. 2005; Garden et al. 2006; Shea 2010; Wilson and Swan 2017). As such, there is tremendous research potential for urban reptiles to provide valuable insights into the processes of urban evolution.

Unfortunately, reptiles have suffered the largest declines of any vertebrate taxa, with urbanisation having been listed as a major threat to 34% of all imperilled reptile species (Gibbons et al. 2000; Böhm et al. 2013). For this reason the majority of research into the effects of urbanisation on reptile populations has primarily focused on identifying the multitude of threats urban landscapes pose, including: decreased abundance and diversity (Minton 1968; Germaine and Wakeling 2001; Barrett and Guyer 2008; Hamer and McDonnell 2010), decreased genetic diversity and gene flow (Delaney et al. 2010; Cureton et al. 2014), decreased population size (Wolf et al. 2013), decreased body condition (Lomas et al. 2015), increased parasite load (Davis et al. 2012), increased mortality rates (Budischak et al. 2006; Jones and Sievert 2012), and disrupted population demographics (Marchand and Litvaitis 2004). Although increasing levels of urbanisation and human disturbance negatively affect most reptiles, this is not the case for all species and there are reptiles that are quite tolerant to urbanisation. For example, the garden skink (*Lampropholis guichenoti*), delicate skink (*Lampropholis delicata*), and blue-tongue skink (*Tiliqua scincoides*) occur across the greater Sydney region in New South Wales, Australia, regardless of the last 200 years of urbanisation (Shea 2010). Yet, despite our knowledge of the threats urban landscapes pose to reptiles, there is a dearth of information regarding the specific traits, mechanisms, and

adaptations that have allowed urban reptiles to persist. It is this knowledge gap into urban-derived divergent phenotypes and potential adaptations of urban living in reptiles that is the focus of my thesis.

**My study species: the Australian water dragon, *Intellagama lesueurii***

Australian water dragons (*Intellagama lesueurii*) are a large agamid species (maximum snout-vent length of 304 mm; Thompson 1993) with a prominent spinose nuchal and vertebral crest, and a body colour ranging from light olive to brown with dark bands (Fig 1; Wilson and Swan 2017). Free-living individuals reach sexual maturity around 4-5 years of age (Thompson 1993; Harlow and Harlow 1997; Hoskin 2010), and adults exhibit male-biased sexual dimorphism in head and body size (larger in males) and ventral coloration (red in males and brown/tan in females; Cuervo and Shine 2007; Baird et al. 2012). First described in 1831 by J.E. Gray, based on a specimen collected by C.A. Lesueur during the Baudin Expedition of 1800, they are the sole species of their genus; diverging from other Australian agamids approximately 20-23 million year ago (Covacevich et al. 1990; Hugall et al. 2008; Hosking 2010; Amey et al. 2012). The species ranges along Australia's east coast from Cooktown, Queensland, in the north, to Gippsland, Victoria, in the south (Fig. 2; Wilson and Swan 2017). There are also populations near Adelaide, South Australia and Charleville, Queensland that likely result from human intervention (Fig. 2; Tait et al. 2005; Wilson and Swan 2017). Naturally, populations are associated with forested areas and freshwater bodies, with individuals using treed and rocky habitats for foraging and shelter while exploiting waterways for thermoregulation and evasion of predators (Fig 2; Doody et al. 2012; Cogger 2014; Wilson and Swan 2017). These lizards also thrive in major Australia cities, such as Brisbane, Canberra, and Sydney (Littleford-Colquhoun et al. 2017; Wilson and Swan 2017). Previous urban research has demonstrated rapid genetic and morphological diversification within urban-living water dragon populations (Littleford-Colquhoun et al. 2017), suggesting that they are undergoing urban evolution.

The natural history of water dragons may allow them to have resisted the negative effects of urbanisation, and in doing so provide them the opportunity to adapt to the novel environment. For example, they are a dietary generalist and are able to exploit a diverse array of food items, including algae, bird faeces, crustaceans, lizards (including juvenile conspecifics), fish, scorpions, seaweed, small mammals, as well as native and introduced insects and plants (Mackay, 1959; Anonymous, 1976; Clifford & Hamley, 1982; Greer, 1990; Wilson & Knowles, 1992; Meek et al., 2001; Baxter-Gilbert 2014; Frère et al. 2015). I have also observed them consuming a variety of cooked human foods (e.g., hamburger, hot chips, and popcorn), pet foods, and pest species (e.g., locust, *Locusta migratoria*; pers. obs.). In public areas such as picnic sites, they are known to actively approach humans and scavenge food dropped or thrown to the ground (pers. obs.). Beyond dietary habits, other aspects of their biology allow water dragons to persist in altered habitats. For example, during development they have a highly variable incubation duration (68-120 days; Harlow and Harlow 1997), which can facilitate successful incubation to occur over a range of thermal and environmental conditions. That being said, sex is temperature-dependent (equal sex ratio produced at 26.5 °C; Harlow 2001) and a stable population structure may still require specific nest site selection. They are also habitat generalists, although typically being found in treed riparian habitats in natural landscapes (Cogger 2014; Wilson and Swan 2017), they are able to persist in agricultural areas, backyards, coastal cliffs, degraded forests, and urban greenspaces, which may or may not have open freshwater bodies (pers. obs.). This suggests that although waterways are common in natural habitats, it is not necessarily a limiting factor for the species. Finally, anecdotal evidence suggests that these lizards exhibit a relatively high degree of cognitive ability, which they may use to exploit human-modified landscapes. Over the course of my research I have observed a free-living adult female water dragon who was able to identify the time of day that insects escape an exhibit at Taronga Zoo. This individual appeared to form a daily routine around utilising this subsidised food source, by entering a building at approximately 09:00 h and waiting for a zookeeper to open the insect exhibit. She



then would forage on any insects that escaped during cleaning, and once the exhibit was closed the lizard would exit the building until the next day (Baxter-Gilbert pers. obs.). The lizard was not deliberately rewarded for this behaviour, in fact she would regularly be ushered out of the building. Similarly, urban wildlife like raccoons (*Procyon lotor*) use their problem solving skills to exploit anthropogenic resources, and have higher cognitive abilities than their rural counterparts (MacDonald and Ritvo 2016). Urban wildlife can learn how to navigate human transportation infrastructure to maximise foraging opportunities (e.g., feral dog, *Canis familiaris*, learning to navigate the Moscow subway network to ride between denning grounds and foraging sites; Lemon 2015). Overall, this species' generalist predisposition may have facilitated their perseverance in urban areas, both behaviourally and biologically, and, in turn, may have allowed evolutionary processes to select for adaptive urban-divergent traits that enable their success in urban areas.

My research addresses several aspects of water dragon behaviour and biology, and compares individuals from populations living in natural habitats to those from moderately human-modified (semi-natural) and heavily human-modified (urban) habitats to explore the presence of urban-derived divergent phenotypes. Natural sites were associated with large protected green spaces and national parks, often with waterways and treed shorelines, and a relatively low direct human presence or footprint. Semi-natural sites had natural features, such as waterways with treed shorelines, but were close to, or surrounded by, urban/suburban areas, and had a moderate daily human presence. Urban sites had a landscape that was predominantly human-modified (e.g., buildings, concrete, gardens, roads, etc.), and a dense resident local human population. The purpose of this thesis is to contrast the water dragon populations living within these distinct site categories to address gaps in our knowledge as to how urban landscapes are changing the wildlife that reside within them. My research included both laboratory and field-based studies, and examined the mechanisms that may be driving the formation of urban-derived divergent traits. My thesis is structured into six separate empirical chapters, and I provide a brief description of each study's aim below.

## Thesis Aims

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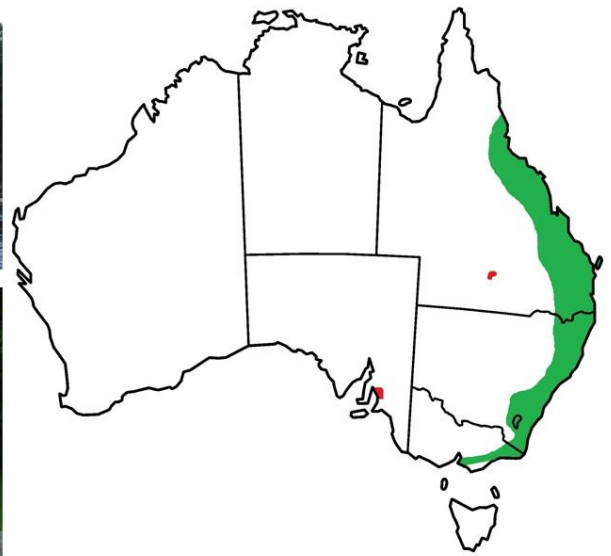
The main objective of my thesis is to quantify the biological traits of water dragons, which have been altered in response to urbanisation. My aim was to determine if these urban-derived divergent traits are a result of within-generation experience (e.g., learning or phenotypic plasticity) or urban evolution (e.g., genetic/heritable traits selected over time). These goals will be accomplished by addressing the following six research foci:

- I) Assess the effect of urbanisation on dragon population density and social interactions;
- II) Examine the effects of urbanisation on heritable behavioural traits;
- III) Determine if urbanisation has altered innate anti-predator responses;
- IV) Test the drivers of morphological variation between urban and natural populations, and determine if variation is a result of phenotypic plasticity or heritable origin;
- V) Assess the repeatability and comparability of different endurance metrics for water dragons, and related this to their use in performance capacity research;
- VI) Examine if urbanisation has resulted in altered performance capacities, which may provide an advantage for surviving in urban habitats.

Five of the six chapters (I, II, III, IV, and VI) deal directly with urban-derived divergent phenotypes and how these traits may provide urbanised populations of dragons an advantage. The remaining chapter (V) was used to inform my methodology for Chapter VI, as well as research in this field. All but two chapters are in preparation for publication, and have been formatted for potentially appropriate journals. Chapter III has been published in *Behavioral Ecology and Sociobiology* and Chapter V has been published in the *Journal of Experimental Zoology*. Each empirical chapter is written and formatted as a standalone piece for publication, and as such there is some repetition.



**Figure 1.** Two free-living adult Australian water dragons residing within the Taronga Zoo grounds; one of which is a female (lower left) and the other a male (upper right).



**Figure 2.** Australian water dragons (*Inellagama lesueurii*) are found in a diverse range of habitats, including natural areas (upper left) and urban landscapes (lower left). Its natural range extends along Australia's east coast (range map on right adapted from Wilson and Swan 2017; green-shading denotes native range and red-shading denotes introduced populations).

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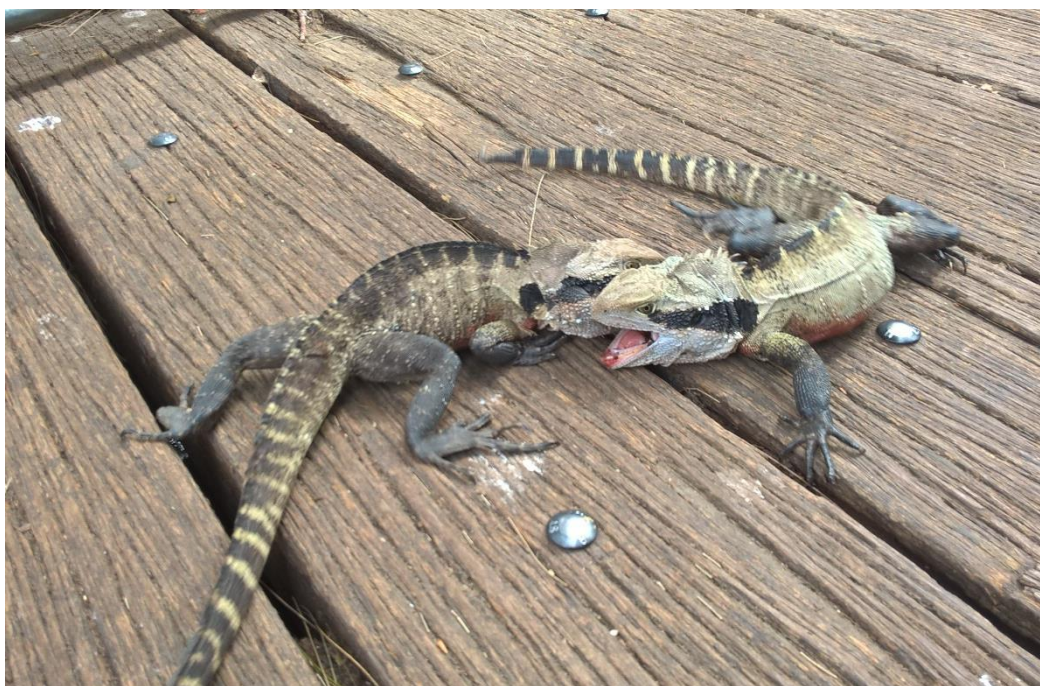
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## Chapter I

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Street fighters: Bite force, injury rates, and population density of urban

Australian water dragons (*Intellagama lesueurii*)



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The following manuscript is being prepared for publication. It follows the format of the journal *Austral Ecology*. Tables, figures, and supplementary material follow this manuscript's references.

## Abstract

In an increasingly urbanised world, it is imperative that we understand how wildlife responds to this novel anthropogenic landscape, both at the individual- and population-level.

Urbanisation generally reduces biodiversity, but can also favour particular species and increase their abundance relative to wild populations. When population density increases, so too does the frequency and cost of social interactions. We studied Australian water dragons (*Intellagama lesueurii*), a species common in urban, semi-natural and natural areas, to firstly test the prediction that urban populations occur at higher densities, and then determine the consequences of urbanisation for combat rates (quantified using wounding) and bite force. We established that urban populations are denser than ones from semi-natural and natural habitats. We also recorded significantly more wounds in females from urban populations than females from both natural and semi-natural populations. Urban males also had significantly higher incidence of wounding than males from natural populations. We did not find a difference in male or female bite force among any populations across the urban-natural gradient. Overall, we found evidence that urbanisation results in a higher population density of water dragons and more frequent conspecific combat, but this was not associated with an increase in bite force. These findings suggest that: 1) there may be a physiological cost to living in urban habitats related to increased contest rates and wounding, 2) altered social interactions may have implications on fitness, and 3) urban-derived selective forces have not increased water dragon combat performance capacity.

## Introduction

Competition for mates and limited resources has a profound impact on the behavioural and social interactions of many vertebrates and invertebrates (Greenberg & Crews 1983; Ostfeld 1990; Maher & Lott 2000; Losin *et al.* 2016). These fundamental processes can be exacerbated when habitat is disturbed or fragmented, like during urbanisation (see review by Banks *et al.* 2007). Compared to undisturbed habitats, urban landscapes frequently contain a host of novel ecological challenges, including altered food webs, altered thermal regimes, anthropogenic mortality sources, increased vectors for disease and parasites, limited suitable habitat, and novel predators (McIntyre 2000; Shochat *et al.* 2006; Hamer & MacDonnell 2008). All these factors can influence social interactions and spatial organisation in wildlife (see review by Maher & Lott 2000). Furthermore, urban habitats are also often isolated or limited in size, functioning much like islands (Davis & Glick 1978; Soulé *et al.* 1988; Littleford-Colquhoun *et al.* 2017), and provide an excellent opportunity to examine the behavioural and spatial ramifications of urbanisation on wildlife within a natural experiment (Banks *et al.* 2007).

Some species are able to thrive in urban areas because they utilise human resources, and this often results in higher population densities compared to conspecific populations in natural habitats (termed ‘urban exploiters’; Blair 1996; Francis & Chadwick 2012; Teixeira *et al.* 2016). A higher population density, however, can present a unique set of challenges, such as increased social interactions, which may require adaptation if urban populations are to persist (Shochat *et al.* 2006; Alberti *et al.* 2017). Many urbanised species have responded to the challenges of finite suitable habitat behaviourally, with populations becoming more crowd-tolerant (i.e., increased home-range overlap and decreased conspecific aggression). This phenomenon has been reported in some mammals (Davison *et al.* 2009), lizards (Johnston & Bouskila 2007), and snakes (Mitrovich *et al.* 2009; Corey & Doody 2010). For example, urban coyotes (*Canis latrans*) increase home range overlap and group size as an adaptive response to clumped urban resources (Atwood & Weeks 2003), as do coachwhip

snakes (*Masticophis flagellum*) in human-disturbed areas (decreased home range size and distance to their nearest neighbour; Mitrovich *et al.* 2009). Alternatively, animals in denser urban environments may become more aggressive towards conspecifics, as reported in some birds (Abert's towhees, *Melospiza aberti*, and curve-billed thrashers, *Toxostoma curvirostre*; Fokidis *et al.* 2011) and lizards (Cuban rock iguana, *Cyclura nubile*; Lacy & Martins 2003). Although effective in the short-term, these altered behavioural responses may be a stop-gap that allows species to persist in urban areas until natural and sexual selection can drive mechanistic urban adaptations, like morphological and physiological responses, that confer fitness benefits (Shochat *et al.* 2006). Adaptive traits related to conspecific contest competition, such as enhanced bite force and other animal weaponry (structures specifically used in contests; Emlen 2008), could be used to respond to altered population densities and social interactions (Kneill 2009). For example, increased population density of wall lizards (*Podarcis sicula*) in an island habitat resulted in a higher frequency of wounding and also, a higher bite force (Vervust *et al.* 2009). Bite force has been suggested as a key determinant of contest outcome in lizards (Herrel *et al.* 2001; Lailvaux *et al.* 2004; Vanhooydonck *et al.* 2005; Husak *et al.* 2006), and is therefore predicted to be the target of selection when contest competition drives access to territories and mates. For example, higher bite force in male collared lizards (*Crotaphytus collaris*) correlates with an individual's ability to win contests against rivals and increased inferred fitness (Husak *et al.* 2009); however alternative mating tactics that avoid costly contests also have fitness benefits (see Baird *et al.* 2007; Braun 2016). Therefore, if urban populations are adapting to the disrupted social interactions and spatial organisation caused by urban habitats, then we expect both behavioural adaptations (crowd-tolerance or aggressive encounters) and physiological responses, to occur.

We examined the effect of urbanisation on Australian water dragons (*Intellagama lesueurii*), a lizard species thriving across a variety of human-modified habitats, ranging from natural bushland to urban cores. We examined nine water dragon populations varying in their level of urbanisation (urban, semi-natural, and natural), to determine if urbanisation has



altered this species' social interactions and population density. Adult water dragons are sexually dimorphic, with adult males demonstrating a larger body length, mass, and a proportionally larger head (Thompson 1993; Harlow 2001; Gardiner *et al.* 2014). Male water dragons are also combative, with some contests lasting upwards of two hours (Baird *et al.* 2012; Baird 2013). They engage in contests to establish and defend territories or adopt a satellite non-territorial reproductive tactic until an opportunity arises to challenge a territorial male or a territory becomes available (Baird *et al.* 2012, 2014). Conversely, female water dragons do not defend territories and tend to be more aggregative; an individual's home range overlaps with multiple males and females, and females tend to form strong social associations with one another unrelated to kinship (Strickland *et al.* 2014). As such, we expected urbanisation to affect the rate of social interactions of sexes differently. We predict that population density will increase with urbanisation. If this is the case, then we expect that male urban water dragons will not become crowd-tolerant, and instead, engage in higher rates of combat, which will result in more wounds in urban habitats than semi-natural and natural habitats. In contrast, we expect that the wounding rate in females will be similar among habitat types. In addition, if urban males do exhibit more wounds, due to more frequent combat, then we predict higher bite force for males within urban habitats compared to semi-natural and natural habitats. In contrast, females are likely not under the same selective pressures, due to different social and spatial organisation (Strickland *et al.* 2014), and so we expect that female bite force will be similar among habitat types.

## **Methods**

### ***Study Species and Sites***

Australian water dragons are large agamid lizards (maximum snout-vent length [SVL]: 304 mm; Thompson 1993) distributed along Australia's east coast, and are common around bodies of freshwater within forested areas (Cogger 2014). However, they are also found in urban areas (Littleford-Colquhoun *et al.* 2017) and their distribution overlaps a majority of the

Australian human population (approx. 80%; Australian Bureau of Statistics 2011). Previous research has documented distinct genetic and morphological difference between urban water dragon populations (Littleford-Colquhoun *et al.* 2017), suggesting that urban areas may be altering phenotypic traits, such as behaviour and biology.

We collected dragons at nine sites (three urban, four semi-natural, two natural) in a 50 km radius within the greater Sydney area in New South Wales, Australia, from October 2015 to March 2017 (see Supplementary Material for detailed locations). Urban sites had a landscape that was widely human-modified (e.g., buildings, concrete, gardens, roads, etc.), and a dense local human population. Semi-natural sites had natural features, such as waterways with treed shorelines, but were close to urban/suburban areas and had a moderate daily human presence (e.g., park visitors). Natural sites, although not completely free from human disturbance, were associated with large green spaces, waterways with treed shorelines, and a relatively low direct human presence or footprint.

### ***Population Density Index***

We created a population density index using line transect counts (Overton 1971; Vervust *et al.* 2009). Survey dates ranged across the dragon's post-nesting active season (December to March) during the summer months of 2016-17 and 2017-18. Surveys were conducted mid-morning on days when the weather was optimal for lizard basking (clear or partially clear skies with an air temperature between 25 - 35 °C). We performed five surveys along a fixed transect at each of the nine sites, and recorded the number of dragons observed within 10 m of the primary surveyor; which remained the same during each survey for consistency. Transects ranged from 0.30–3.00 km in length (mean 1.08 km  $\pm$  0.13 SE), and followed a linear pathway (either walking trail or waterway) depending on the site and terrain. Typically, surveys took between 1-2 hours, depending on terrain and number of dragons sighted.

### ***Handling, Morphology, Wounding, and Bite Force***

All dragons used in the wounding and bite force analysis ( $n = 190$ ) were captured either by hand or using a noose-pole, and sampling was conducted both during the day and night (depending on terrain and location). Once captured, adult dragons were sexed using secondary sexual characteristics (e.g., relative head size and chest colouration). We measured individual snout-vent-length (SVL) with a clear plastic ruler ( $\pm 1$  mm), and jaw length and width was measured using a set of digital Vernier callipers ( $\pm 0.01$  mm; Whitworth, Brisbane, Australia). We recorded the dragon's surface body temperature with a handheld laser thermometer ( $\pm 0.1^\circ\text{C}$ ; RIT310, Ryobi, Doncaster, Australia) held to the dragon's abdomen prior to measuring bite force (Berg *et al.* 2015). Bite force was measured using an isometric Kistler force transducer (type 9203; Kistler Inc. Wintherthur, Switzerland), mounted on a retort stand, and connected to a Kistler charge amplifier (type 5995). All animals were coerced to bite on two parallel plates (fixed at a distance of 3 mm), by stroking both sides of the dragon jaw simultaneously with the researcher's index finger and thumb. Each individual dragon was tested three consecutive times, and the maximum bite force recorded and used in analyses (Anderson *et al.* 2008).

Fresh wounds, scars, obvious broken bones, and missing digits were counted and recorded as a measure of wounding rate (tally of the total number of injuries per individual). The purpose of collecting this information was to glean which individuals were being injured more often, as a proxy for the rate of conspecific combat. As such, and to maintain statistical power, we pooled injuries of different types into one metric 'wounding'. Of the injuries, the most commonly recorded wounds were V-shaped scars over the lower back, hips, and base of the tail, and scarring around the head and jowls, which are indicative of combat (Fig. 1). We also commonly recorded missing digits. We did not include broken and regenerating tails or tail tips, as injuries such as these have a higher likelihood of being related to predation attempts (Hayes *et al.* 2012). Once measured, the lizards were immediately released back at their site of capture.

## *Analyses*

Data are presented as means  $\pm$  standard error (SE). All summary statistics and data visualizations were calculated from raw data, and unless otherwise stated they were predicted from the models. All statistical tests were conducted in R version 3.2.3 (R Core Team 2016). Before beginning our analyses, we explored each dataset following the protocol outlined in Zuur *et al.* (2010). During our data exploration, we did not find any outliers or strong collinearity between our predictor variables within the same model.

### *Population Density Index*

We compared population densities between habitat types using a generalised linear mixed effects model with a Poisson distribution using the function *glmer* within the R package *lme4* (Bates *et al.* 2015). The total number of dragons observed per survey was the response variable which we offset by the transect distance (km). We included the fixed effect of habitat type (categorical with three levels: urban, semi-natural, and natural), and the random intercept of site to control for dependencies within our data relating to population-specific differences. For this and all models below, we ensured that model assumptions were met and that Poisson models were not overdispersed (as per Zuur *et al.* 2010). To generate contrasts among habitat types we used the function *lsmeans* from the *lsmeans* R package (Lenth 2016). *P* values generated for comparisons among habitat type were corrected using Tukey's HSD using a multiplicity adjustment (Lenth 2016).

### *Wounding*

We examined difference in wounding separately for each sex (female and male), because of the known sexual differences in both ecology, morphology, and social behaviour (Thompson 1993; Baird *et al.* 2012, 2014; Strickland *et al.* 2014). The data was zero-inflated with 44% of females and 16% males not having wounds. Therefore, we used a zero-inflated mixed-effect

Poisson regression, with the function *glmmadmb* in the R package *glmmADMB* (Skaug et al. 2014), to test for differences in wounding rates among habitat types (categorical with three levels: urban, semi-natural, and natural). We controlled for differences in body size by SVL (mm) as a fixed effect, and, as above, we accounted for dependencies in our data related to population-specific effects by including the random intercept of site. We conducted pairwise comparisons between habitat types using the same protocol as above (*lsmeans* from the *lsmeans* R package; Lenth 2016).

### *Bite Force*

We used linear mixed effect models (with a Gaussian distribution using the function *lmer* from the R package *lme4*; Bates *et al.* 2015) to examine differences in the maximum bite force (N) of water dragons among habitat types. Again, we examined each sex separately. We controlled for differences in body size (SVL; mm) and body temperature (°C) by including these variable as fixed effects. Both maximum bite force and SVL were log-transformed in the analysis, due to a non-scalar linear relationship between these two variables (Lailvaux et al. 2004). We also included a random intercept for site, and tested contrasts between all habitat types using the same method as above (Lenth 2016).

## **Results**

### *Population Density Index*

Urban areas had significantly higher population densities than both semi-natural ( $\beta = -0.868 \pm 0.263$ ,  $z = -3.302$ ,  $P = 0.003$ ) and natural areas ( $\beta = -1.094 \pm 0.290$ ,  $z = -3.780$ ,  $P = 0.001$ ; Fig. 2; see supplementary material for specific densities). Population densities did not differ significantly between semi-natural and natural areas ( $\beta = -0.226 \pm 0.309$ ,  $z = -0.731$ ,  $P = 0.745$ ; Fig. 2).

### *Wounding Rate*

In both male and female water dragons, the wounding rates were significantly, positively related to SVL (females:  $\beta = 0.025 \pm 0.009$ ,  $z = 2.93$   $P = 0.003$ ; males:  $\beta = 0.020 \pm 0.003$ ,  $z = 6.52$ ,  $P < 0.001$ ). This relationship was controlled for in our examination of wounding rate (results below).

On average, female water dragons were observed with  $1.2 \pm 0.5$  wounds (median = 1, range = 0 to 6). The wounding rate for female water dragons was significantly higher in semi-natural than natural areas (Table 1), and significantly higher in urban than natural areas (Table 1). The wounding rate for female dragons was not significantly different between urban and semi-natural areas (Table 1).

On average, male water dragons were observed with  $4.3 \pm 0.4$  (median = 3, range = 0 to 18) wounds. The wounding rate for male dragons was significantly higher in urban compared to natural areas (Table 1). The wounding rate for male dragons between natural and semi-natural areas, and semi-natural and urban areas was not significantly different (Table 1).

### *Bite Force*

Female water dragons had an average maximum bite force of  $47.9 \pm 2.9$  N (ranging from 4.9 to 176.5 N), and males had an average maximum bite force of  $317.9 \pm 18.0$  N (ranging from 30 to 642 N). Maximum bite force did not differ significantly among habitat types in either female or male water dragons (Table 2; Fig 3b). Body size (log-transformed SVL) was significantly related to female ( $\beta = 4.414 \pm 0.696$ ,  $t = 6.643$ ,  $P < 0.001$ ) and male maximum bite force ( $\beta = 7.033 \pm 0.544$ ,  $t = 12.936$ ,  $P < 0.001$ ), while body temperature was not (female:  $\beta = 0.000006 \pm 0.009$ ,  $t = 0.0006$ ,  $P = 0.100$ ; male:  $\beta = -0.002 \pm 0.007$ ,  $t = -0.257$ ,  $P = 0.798$ ).

## Discussion

Urban water dragons occurred in higher densities than conspecifics living in semi-natural and natural habitats. Furthermore, both male and female water dragons from urban populations have significantly higher wounding rates than natural populations. Females from urban population also bore significantly more wounds than semi-natural populations. These findings support our predictions that urban water dragons live in higher population densities than their counterparts in wild populations and male water dragons within these urban areas are engaging in higher rates of male-male combat, while our prediction regarding female wounding rate (i.e., no difference) was not supported. As expected, we did not observe an increase in female bite force related to urbanisation; however, contrary to our expectations, we also did not find evidence for increased bite force in urban males. This suggests that although water dragons are living in higher density, and engaging in conspecific fights more often, this has not resulted in stronger selection on bite force in urban individuals.

Human-subsidised resources and altered predator-prey relationship are factors that cause the population density of particular species, often termed ‘urban-exploiters’, to increase in urbanised habitats (Blair 1996; Francis & Chadwick 2012; Teixeira et al. 2016). Urban water dragon population densities were significantly higher than densities in natural habitats, which suggests water dragons may fit the classification of an urban exploiter. Traditionally, much of the research into urban exploiting species has focused on birds (Gering & Blair 1999; Shochats 2004; Kark *et al.* 2007; Evans *et al.* 2011; Meillère *et al.* 2015); however, arthropods (McIntyre 2000) and mammals (Prange *et al.* 2004; Teixeira et al. 2016) have garnered some attention. Generally, native reptile species are underrepresented in studies examining urban exploiters; yet there are observations of higher-than-natural population densities of urbanised lizards and turtles (Henle 1990; Souza & Abe 2000; Germaine & Wakeling 2001; Johnston & Bouskila 2007). For example, tree dtella geckos (*Gehyra variegata*) occur in a density an order of magnitude higher on buildings compared to their



natural habitat (trees) due to a higher prey abundance (Henle 1990), which follows assertions that increased resources can result in increased population density in urban areas (Blair 1996; Francis & Chadwick 2012). Similarly, water dragons may be able to exploit the vast array of potential food sources in urban areas, as they are generalist omnivores (Baxter-Gilbert 2014). However, although an increase in population density in urban areas may appear to be beneficial for a species' persistence, living in higher densities may come with substantial costs (e.g., body condition, clutch size, and lifespan; Shochat 2004) or require divergent behavioural or morphological phenotypes to promote urban survival (Tomkins & Brown 2004; Banks *et al.* 2007; Knell 2009).

For many species, suitable urban habitats are finite in size (Davis & Glick 1978; Soulé *et al.* 1988) and increased population density within these areas may require individuals to become either more crowd-tolerant (Davison *et al.* 2009; Mitrovich *et al.* 2009; Corey & Doody 2010) or more aggressive towards conspecifics (Lacy & Martins 2003; Fokidis *et al.* 2011) to compensate for increased conspecific interactions. We found no reduction in wounding rate between urban water dragon populations and the other habitat types, so we can infer from our findings they are not becoming more crowd-tolerant. Instead, we found there was a significant increase in wounding of both urban female and male water dragons compared to conspecifics from natural areas, which suggests they are being exposed to increasing rates of aggressive encounters. Our findings reflect what has been found in wall lizards (*Podarcis sicula*; Vervust *et al.* 2009) and Eurasian badgers (*Meles meles*; Macdonald *et al.* 2004), wherein higher population density increased male wounding rates. But, in contrast, fan-fingered geckos (*Ptyodactylus guttatus*) that live on buildings and occur at a density 195 times higher than natural populations living on rock faces and caves, which do not exhibit an increase in aggressive encounters (Johnston & Bouskila 2007). These geckos instead used mate guarding and social-sorting to compensate for the dramatically inflated population density (Johnston & Bouskila 2007). It is likely that the behavioural responses a particular species exhibits due to increased urban population density (e.g., increased crowd-

tolerance or aggression) is related to the degree of sexual selection from contest competition; however, this postulate requires further research. Interestingly, the increased wounding rate in females suggest that in urban areas where population density is higher, and suitable habitat is finite, females may be either altering their normal social behaviour to become more aggressive (choosing to engage in more conspecific combat). Previous research in collar lizards has seen female aggressive encounters increase when key resources are limited (elevated rock perching, Baird and Sloan 2003; Baird 2013). An alternative rationale, however, is that increase female wounding a merely a result of individuals suffering the brunt of male-based aggression or biting through potential coercion from males. Future studies focused on determining the demographics of contest instigators is required to better understand the source of increased wounding rates in urban female water dragons. Overall, our findings suggest that urban living can result in higher rates of wounding of both males and females, reflecting increased conspecific aggression, and suggests that there may be a substantial physiological cost of living in urban areas.

Population density is often a key factor in the evolution of divergent phenotypes, such as sexual size dimorphism (Stamps 1983) and male weaponry (Tomkins & Brown 2004; Knell 2009). Increased population densities of male European earwigs (*Forficula auricularia*) on islands has resulted in higher levels of male competition, which in turn has selected for exaggerated weaponry (pincer size and head shape; Tomkins & Brown 2004). This positive relationship between contest competition and population density has also been observed in insects (Tomkins & Brown 2004; Buzatto *et al.* 2012) and reptiles (Vervust *et al.* 2009); although a negative relationship has been observed in frogs (Buzatto *et al.* 2015; Lüpold *et al.* 2017) and ungulates (Jorgenson *et al.* 1998; Kruuk *et al.* 2002). For example, male quacking frogs (*Crinia georgiana*) use their forearms during pre-copulatory male combat (Buzatto *et al.* 2015). At low density these frogs increase combat success and mating opportunities by enlarging their forearms, however at high density their arms are reduced in size, with energy

being diverted to increased sperm production and quality instead (Buzatto *et al.* 2015). Interestingly, we did not detect any significant relationship, positive or negative, between water dragon bite force and urbanisation, for either females (as predicted) or males (contrary to our predictions). A potential explanation for a lack of positive directional selection on male urban water dragon bite force could be related to morphological and physiological constraints. Male water dragons naturally engage in male combat and territoriality (Baird *et al.* 2012; Baird 2013), thus selection may have already driven this species to its functional maxima, and any exaggerations in weaponry may be detrimental or not physiologically or morphologically possible (Kokko & Brooks 2003). However, further investigation into the kinematics and physiological constraints of water dragon skull morphology, musculature, and bite force are required to test this hypothesis. Anecdotally, we observed two separate instances of fatal male-combat both related to bite wounds. In one case a male suffered widespread deep tissue damage along the mid-body and limbs leading to a fatal infection, while in the other case a male had a large section of his lower jaw removed during combat (Fig 4). This suggests that the bite force in male water dragons is already a significant weapon, and increased bite force may not actually provide any further advantage. Furthermore, investment in animal weaponry is to incur a trade-off and can come at a cost to other morphological structures. For example, in multiple species of *Onthophagus* dung beetles, horn size is traded-off against the size of antennae, eyes, testes, and wings (Emlen 2001; Simmons & Emlen 2006). Alternatively, selection may be operating on other sexual traits, beyond male-combat weaponry, such as sperm competition (Buzatto *et al.* 2015; Lüpold *et al.* 2017) or density-dependant alternative mating tactics (Kokko & Rankin 2006).

Overall, although our study found that urban water dragons are living at higher densities, suggesting they are an urban exploiting species (Blair 1996; Francis & Chadwick 2012), they engage in more conspecific contests. This has not, however, translated into increased bite force, suggesting that the investment of head musculature is likely to be costly and may constrain additional selection on head size and head muscles. An alternative means

through which selection may be driving increased combat potential is through jaw muscle stamina, rather than maximal bite force. We recommend future research examine if increased urban population density and aggressive encounters has driven selection for increased combat endurance. What is clear, is that more research is required to uncover how these urbanised lizards are responding to not only a novel ecosystem, but also a novel social and spatial landscape and how this may effect mating systems and population sex ratios. In general this study contributes to the small, but growing, field of mechanistic urban ecology (Shochat *et al.* 2006), and furthers our understanding of an iconic Australian reptile as it persists in an ever-changing urban world.

### **Acknowledgements**

We would like to thank P. Bolton, T. Damasio, C. Fryns, G. Hughes, F. Kar, S. Klopper, L. Monk-Whipp, M. Mühlenhaupt, and D. Noble for their assistance in the field, as well as all our colleagues at the Lizard Lab at Macquarie University for their support. Thanks to J. Riley for assistance with statistical analysis, and editing previous versions of this manuscript. We would also like to thank Taronga Zoo, notably S. Brown and the team at Backyard to Bush, P. Harlow, and the veterinary staff at the Taronga Wildlife Hospital who performed the post-mortem necropsies. This research was supported by Macquarie University and Natural Sciences and Engineering Research Council of Canada. Experimental protocols were approved by the Macquarie University Animal Ethics Committee (ARA # 2015/023), Taronga Zoo Animal Ethics Committee (ARA # 3b/08/15), and New South Wales National Parks and Wildlife Services (scientific license # SL100570).

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A water dragon basking on a wooden deck outside the B2B office at Taronga Zoo

## Tables

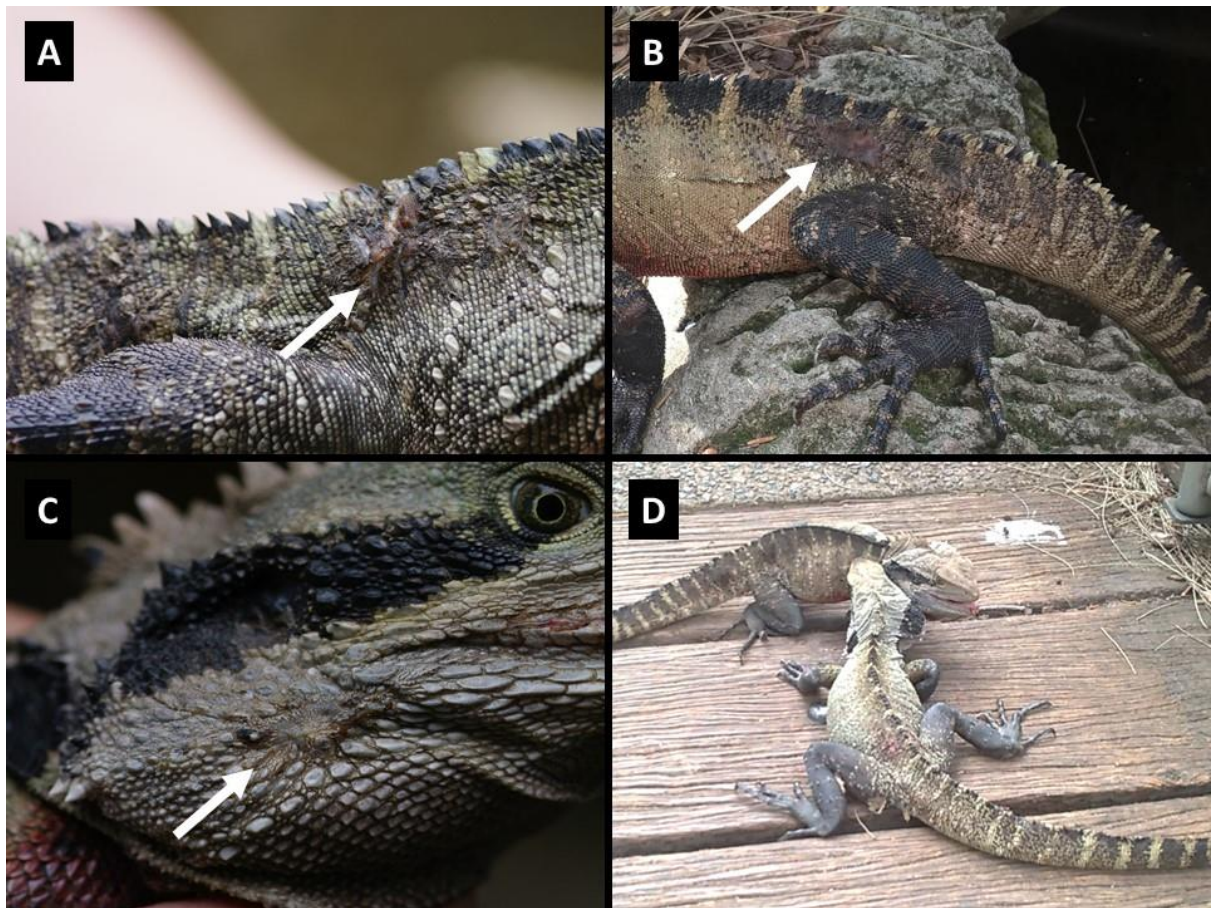
**Table 1.** The wounding rate compared among habitat types in both female and male water dragons. Zero-inflated mixed-effect Poisson regressions were performed separately for each sex. Parameter estimates are on the latent (log) scale, significant pairwise comparisons are bolded, and p-values are corrected for multiple comparisons using a Tukey HSD multiplicity adjustment ( $P_{corr}$ ).

Habitat type	Females				Males			
	$\beta$	$SE$	$z$	$P_{corr}$	$B$	$SE$	$z$	$P_{corr}$
Natural vs. Semi-Natural	<b>-0.996</b>	<b>0.352</b>	<b>-2.830</b>	<b>0.013</b>	-0.395	0.345	-1.146	0.486
Natural vs. Urban	<b>-0.802</b>	<b>0.344</b>	<b>-2.331</b>	<b>0.052</b>	<b>-1.129</b>	<b>0.320</b>	<b>-3.533</b>	<b>0.001</b>
Semi-natural vs. Urban	0.194	0.443	0.438	0.900	-0.734	0.455	-1.611	0.241

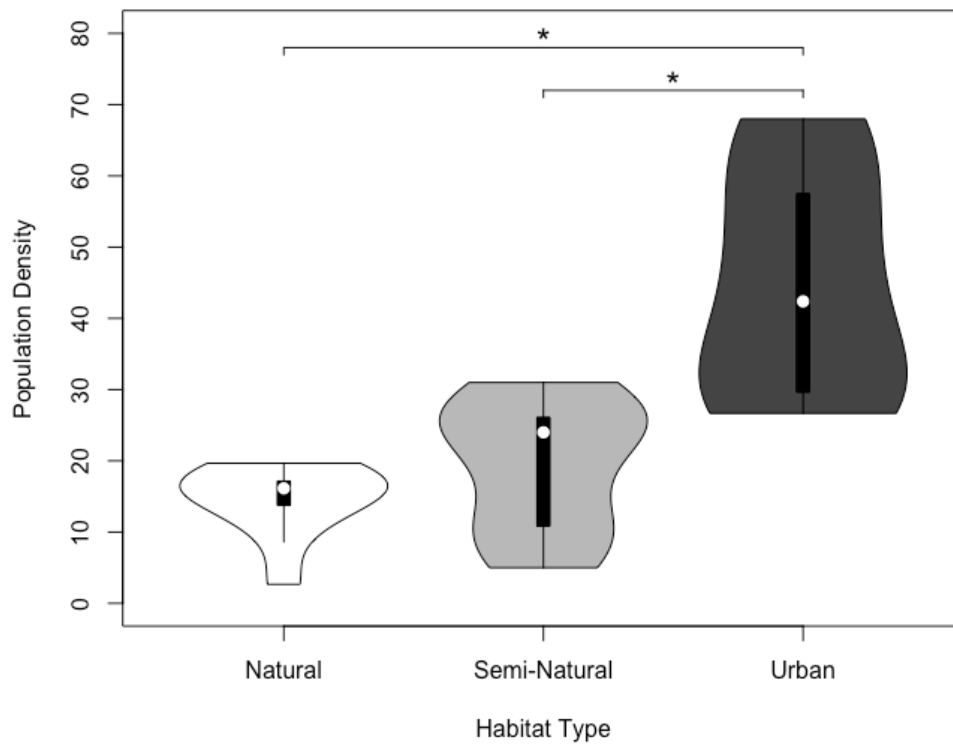
**Table 2.** Pairwise comparisons of the effect of habitat type on maximum bite force (N) measured in female and male water dragons. Linear mixed effect models were performed separately for each sex. Parameter estimates are on the latent (log) scale and p-values are corrected for multiple comparisons using a Tukey HSD multiplicity adjustment ( $P_{corr}$ ).

Habitat type	Females				Males			
	$\beta$	$SE$	$z$	$P_{corr}$	$\beta$	$SE$	$z$	$P_{corr}$
Natural vs. Semi-Natural	0.157	0.364	0.431	0.903	0.097	0.083	1.177	0.467
Natural vs. Urban	-0.372	0.349	-1.068	0.534	0.070	0.080	0.871	0.659
Semi-natural vs. Urban	-0.529	0.310	-1.707	0.202	-0.028	0.077	-0.360	0.931

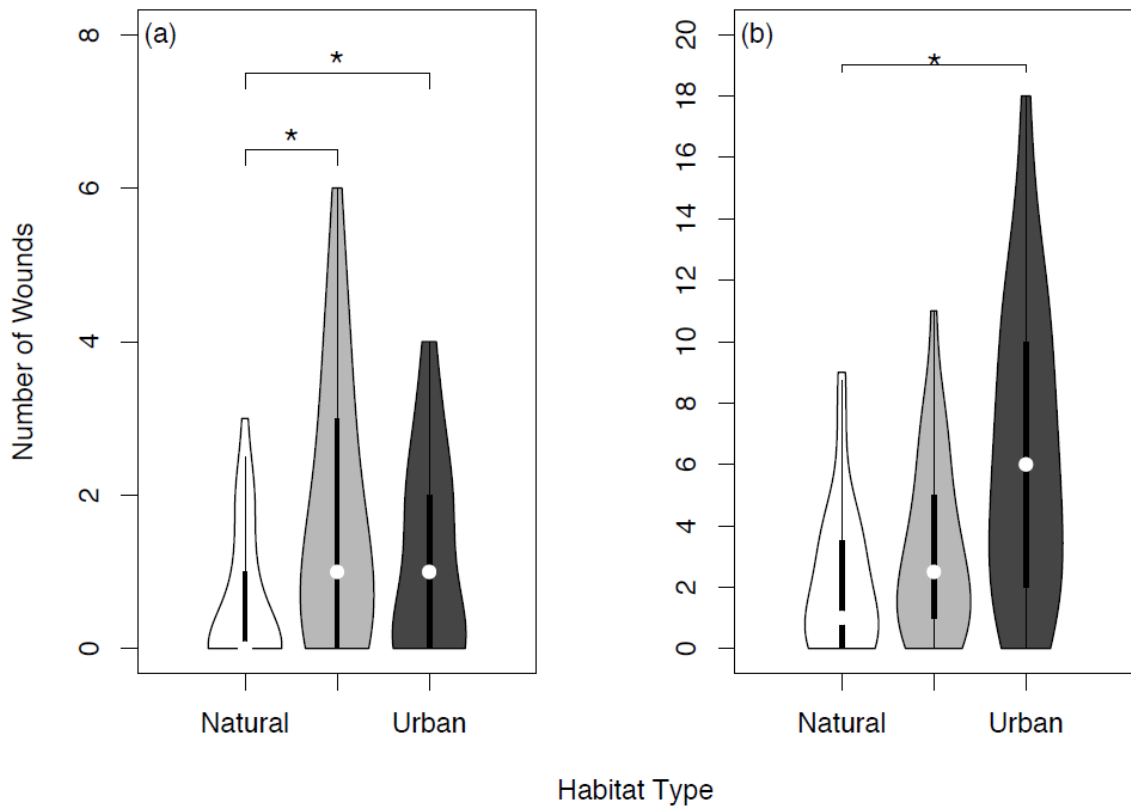
## Figures



**Figure 1.** Wounds observed on Australian water dragons, including bite marks over the hips and tail (A and B), and jowls (C). Many of the scars observed were a direct result on conspecific aggression, like male combat (D).



**Figure 2.** Water dragon population densities (individuals/km) across habitat types. We observed significantly higher densities of dragons in urban than both semi-natural and natural areas. Significant comparisons are shown using bars with an asterisk (\*), and plotted data are raw. Treatment medians are represented with a white dot, the interquartile range is represented with a thick, black rectangle, 95% confidence intervals are represented with a thin, black line. A density plot of each site's population density is perpendicular, on the left and right, to each line diagram.



**Figure 3.** The wounding rate for (a) female and (b) male Australian water dragons across habitat types. Plotted data are raw, and significant comparisons (that were determined using separate zero-inflated mixed-effect Poisson regressions that controlled for SVL differences by including this factor as a covariate) are shown using bars and an asterisk (\*). Treatment medians are represented with a white dot, the interquartile range is represented with a thick, black rectangle, 95% confidence intervals are represented with a thin, black line. A density plot of each individual's wounding rate and bite force, respectively, is perpendicular, on the left and right, to each line diagram.





**Figure 4.** During combat a male water dragon had the front section of his lower jaw removed and a complete separation of the mandibular symphysis. This injury would have likely resulted in mortality, and as such, the animal was euthanized. Photo courtesy of D. Pritchard.

## Supplementary Material

### Methods

#### *Sites*

Australian water dragons were captured and measured at 9 sites in a 50 km radius around the Greater Sydney Area in New South Wales, Australia, from October 2015 to March 2017. The urban sites were: 1) the Manly to Shelly Beach boardwalk near Manly (33.79° S, 151.29° E), 2) the Taronga Zoo grounds near Bradleys Head (33.84° S, 151.24° E), 3) the Chinese Garden of Friendship near Darling Harbour (33.88° S, 151.20° E), and 4) the Macquarie University Campus near Marsfield (33.77° S, 151.11° E). The semi-natural sites were: 1) Lane Cove National Park near Chatswood West (33.79° S, 151.14° E), 2) Brown's Watering Hole near North Epping (33.76° S, 151.11° E), and 3) Lake Parramatta near North Parramatta (33.47° S, 151.00° E). The natural sites were: 1) the Grose River near Yarrimundi (33.62° S, 150.67° E), and 2) the Nepean River near Emu Plains (33.46° S, 150.39° E).

### Table

**SM Table 1:** The estimated density of water dragons in different habitat types, calculated from the number of dragons observed during a survey, divided by the transect length (km).

Habitat Category of Site	N	Mean dragons per linear km	SE
Natural	2	14.61	1.59
Semi-Natural	3	19.30	2.32
Urban	4	44.58	3.51

## Chapter II

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Bold new world: Urbanization selects for boldness in a lizard



James Baxter-Gilbert, Julia L. Riley, and Martin J. Whiting

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The following manuscript is being prepared for publication. It follows the format of the journal *Behavioural Ecology*. Tables, figures, and supplementary material follow this manuscript's references.

## Abstract

Urban environments are novel landscapes that markedly alter animal behavior. Divergence in behavior in response to urbanization may provide advantages in navigation, exploiting resources, and surviving under urban selective forces. Relatively few studies however, have identified population-level behavioral changes in response to urbanization that are not confounded by rearing environment and prior experience (e.g., an urban upbringing). We used the Australian water dragon (*Intellagama lesueurii*) to test whether populations under varying levels of urbanization have heritable behavioral types or whether behavior is best explained by plasticity. Eggs from urban, semi-natural, and natural populations were hatched in the lab and the hatchlings were reared in the lab under standardized conditions (a common-garden experiment) to remove the potentially confounding effects of rearing environment and prior experience on behavior. We then assayed individual behavioral traits (boldness, exploration, and neophilia) five times across their first year of development. We compared behavioral traits, as well as consistency in behavior, between urban, semi-natural, and natural populations. Levels of neophilia or exploratory behavior were similar among all populations. Dragons from semi-natural populations however, were significantly bolder than those from natural populations, and urban dragons were bolder than dragons from natural populations, although this trend was not significant. Dragons from semi-natural and urban populations had similar boldness scores, suggesting a biologically relevant difference in boldness between them and natural populations. To the best of our knowledge, this is the first demonstration of an urban-derived divergent behavioral trait (boldness) in a vertebrate, which is disentangled from the confounding effects of rearing environment and prior experience.

## Introduction

Novel landscapes can expose individuals to challenges that may substantially alter their behavior (Sol et al. 2013; Alberti et al. 2017; Lapiedra et al. 2017). This action is typified by the behavioral shifts of animals living within urban environments (Shochat et al 2006; Garroway and Sheldon 2013; Lowry et al. 2013; Sol et al. 2013), and has been documented in birds (Atwell et al. 2012), mammals (Lyons et al. 2017), reptiles (Peterman and Ryan 2009), and spiders (Kralj-Fišer et al. 2017). These urban-derived divergent behaviors can include altered anti-predator responses (McCleery 2009; Blumstein 2014), foraging behavior (Geggie and Fenton 1985; Shochat et al. 2004; Short and Petren 2008), behavioral thermoregulation (Peterman and Ryan 2009), and mate calling/songs (Parris et al. 2009; Barnett 2015). Furthermore, changes in behavioral traits (e.g., boldness, neophilia, and exploration behavior) may also provide advantages to navigating and exploiting urban environments (Kralj-Fišer et al. 2017). Boldness reflects an individual's propensity to take risks; bolder individuals may be more active in novel, urban landscapes and situations, which could increase their time spent foraging, mate searching, or defending a territory (Réale et al., 2007; Sol et al. 2013; Sprau and Dingemanse 2017). Similarly, neophilia – an individual's willingness to engage with novel stimuli or objects – could provide substantial advantages within an urban environment by increasing their ability to exploit novel resources (e.g., food sources or shelter; Miranda et al. 2013). Finally, an individual's propensity to explore could influence their success in urban environments because it provides individuals with important information about their environment (Dingemanse et al. 2002; Lapiedra et al. 2017).

Although altered behavior and behavioral traits has been seen in numerous urban-living species, the specific mechanism driving the formation of urban-derived divergent behavioral traits remains unclear. Behavioral plasticity has been suggested to aid urban-dwelling individuals increase their exploitation of urban resources and decrease the costs associated with urban habitats (e.g., anthropogenic environmental, ecological, and physiological stress; Ditchkoff et al. 2006; Partecke et al. 2006; French et al. 2008; Atwell et

al. 2012; Lucas and French 2012; Kralj-Fišer et al. 2017). Alternatively, if these behavioral traits are heritable, and provide an advantage in urban environments, then selection may favour them in urban populations. Recent research has suggested that urban evolution is driving the persistence of species in heavily human-modified habitats (Johnson and Munshi-South 2017). Yet, even though divergent behavior in urban-living populations is likely adaptive, previous research has struggled to determine if these behaviors are a result of selection or plasticity (Diamond 1986; Møller 2008; Lowry et al. 2013; Sol et al. 2013; Alberti et al. 2017). Recently, heritability of behavioral traits (e.g., boldness and aggression) has been documented for several urban-dwelling bird species (Evans et al. 2010; Müller et al. 2013; Holtmann et al. 2017; Sprau and Dingemanse 2017). Paradoxically, flight-capable birds should experience weaker selection within urban environments compared to terrestrial species, because they are able to rapidly vacate urban habitats. In contrast, less vagile terrestrial species are physically tied to specific locations within urban environments (Brown 1978; Wiens and Donoghue 2004; Lyons et al. 2017). Research into heritable behavioral traits in terrestrial urban species remains rare (but see Kralj-Fišer and Schneider 2012), but they are a model system that could greatly enhance our understanding of the full extent to which urban environments are shaping animal behavior.

The Australian water dragon (*Intellagama lesueurii*) is a lizard species common throughout the eastern seaboard of Australia (Cogger 2014). Water dragons are common in urban areas and appear to have successfully exploited human-altered landscapes, where some populations have experienced rapid morphological evolution (Littleford-Colquhoun et al. 2017). This species is therefore a good model for testing whether behavior may play a role in their success in urban environments. Specifically, we used a common garden experiment, which removed the confounding effects of rearing environment and prior experience, to test whether urban environments have selected for a particular behavioral traits or whether plasticity best explains variation in behavior in relation to urbanization. We raised hatchling dragons from eggs collected from mothers living in urban, semi-natural, and natural

populations, and repeatedly quantified their behavioral traits (boldness, neophilia, and exploration) over their first year of life. We predicted higher levels of boldness, neophilia, and exploration in individuals from urban and semi-natural origin populations compared to their natural-living counterparts. If these predictions are upheld, this would constitute evidence for heritable behavioral divergence in urbanized populations. We also examined if behavioral traits were consistent throughout development, and compared their consistency among origin population categories (urban, semi-natural, and natural). Consistency in behavioral traits across time suggests strong, constant selection for a particular behavioral type within an environment (Dingemanse and Réale 2005; Bell 2012).

## **Methods**

### ***Study Species***

Water dragons are a large (maximum snout-vent length: 304 mm; Thompson 1993) agamid lizard. They are relatively long-lived species (28-40 years; Harlow and Harlow 1997, Griffiths 2006) with a generation time of 5 years (Littleford-Colquhoun et al. 2017). Water dragons are naturally found in forested areas associated with creeks, rivers, and other freshwater bodies (Cogger 2014); however, they are also common in urban parklands and other green spaces (Littleford-Colquhoun et al. 2017).

### ***Field Collection, and Husbandry***

In the spring (October/November) of 2015 we collected gravid, female water dragons from 12 sites (four urban, four semi-natural, and four natural) within a 50 km radius within the greater Sydney area in New South Wales, Australia (see Supplementary Materials for exact location details). Urban sites had a dense local human population, and a landscape that was widely human-modified (e.g., concrete, buildings, gardens, roads, etc.). Semi-natural sites were protected green spaces (national and regional parkland) that contained waterways adjacent to urban areas, and they had a moderate human presence (park visitors). Natural sites, although

not completely free from human disturbance, were generally associated with native bushland, waterways with treed shorelines, and a relatively low human presence.

Upon capture, we transported females to Macquarie University (Sydney, NSW), or if captured at Taronga Zoo (Sydney, NSW) they were held there, and then oviposition was induced (see Supplementary Materials for details). Clutches of eggs were identically incubated throughout development (at a constant temperature of 26.5°C allowing for an equal sex-ratio; Harlow 2001). Upon emerging, hatchlings were randomly allocated to one of 15 common garden enclosures (initial experimental group: N = 96 but group size decreased because of mortality or escape; see below). The enclosures (6.2 m<sup>2</sup> plastic tubs lined with sand, and containing tile refuges, hardwood dowel perches, and a small pool) were outdoors within a predator-exclusion net, which allowed for natural weather and photoperiods common to the Sydney region (see SM Fig. 1). Throughout the experiment all dragons experienced identical housing, husbandry conditions (fed crickets 3 times weekly), thermal conditions, and water *ad libitum*.

### ***Behavioral Assays***

We assayed three behavioral traits (exploration, boldness, and neophilia) five times over the dragons' first year of life (once every 2 months, excluding the winter brumation period of July and August). Behavioral trait assays were conducted indoors over 3 days, and consisted of 1 assay per day. During each of the five rounds of assays, we were not always able to re-capture all lizards, resulting in some variation in sample sizes (Table 1). Our experimental room was not large enough to house all dragons at once, so we conducted assays in four batches (maximum of 32 individuals per batch, two batches per day, and 6 days total). Assays took place in a temperature controlled room, set to the dragon's preferred body temperature of 30°C (Hosking 2010), unless otherwise stated. During assays dragons were individually housed and behaviors were remotely video recorded using a security camera system (CCTV security systems, Melbourne, Victoria). Each behavioral assay (exploration, boldness,



neophilia) was scored from the videos by a single researcher to ensure consistency, with the video scorer being blind to the lizard's origin population (see below for scoring criteria).

### *Day 1: Exploration Behavior*

Our measure of exploration behavior quantified the amount of time (s) a dragon spent moving in a novel arena, and how much time they spent stationary or hiding within a refuge. To assay these behaviors we introduced dragons into a novel environment, similar to an open-field test (Archer 1973; Perals et al. 2017; Riley et al. 2017). The testing arenas were always the same size (rectangular arenas; 690 W × 470 L × 455 H mm) and had two black refuge boxes (120 W × 175 L × 38 H mm) at opposite ends. We varied the substrate between each of the five repeated measures (plain paper, eucalyptus mulch, sugar cane mulch, topsoil, and pine-bark mulch; see SM Fig. 2A) to ensure the arena was novel each time. At the beginning of each trial, we introduced the dragon into the arena within a central, containment refuge. The dragon was allowed to acclimate within this refuge for 5 min, whereupon the refuge was lifted, and the assay began (Riley et al. 2017). Each exploration assay ran for 30 min. The dragons then remained in these enclosures for the duration of the assay period (3 days).

From video recordings, we scored: (i) time spent moving (sec) and (ii) time spent hiding in a refuge (s). These two measures were combined using a principal component analysis (PCA) to create a score that reflected dragon exploration behavior (Joliffe 2002). The PCA was performed using the princomp function in R v 3.0.3 (R Core Team 2016). These two behaviors were highly correlated and loaded opposite to each other on a single component (Table 2) that explained 59% of the variation in the data. We used this first principal component (PC1) in further analyses as our 'exploration score'; as the value decreases it reflects more explorative behavior (i.e., an individual moves more and spends less time hiding).

### *Day 2: Boldness*

Our measure of boldness was the amount of time (sec) it took a dragon to leave an unfavourable refuge after a simulated predatory attack. We created a thermal difference within the testing arena by lowering the temperature in the experimental room to 22°C, and positioning a heat lamp directly over one of the refuge boxes, creating a ‘hot’ refuge (Carazo et al. 2014; Riley et al. 2017). We also positioned an ice pack beneath the enclosure, directly under the other refuge, creating a ‘cold’ refuge (Carazo et al. 2014; Riley et al. 2017; see SM Fig. 2B). At the beginning of each trial, we introduced the dragon into the arena within a central, containment refuge and left it to acclimate for 10 minutes. We then simulated a predatory attack by removing the containment refuge and ‘chasing’ the dragon with a blue, gloved hand until it entered the ‘cold’ refuge (Riley et al. 2017). Then we remotely video recorded the dragon’s behavior for 1 hour. We measured a lizard’s boldness as the amount of time (sec) it took the dragon to leave the ‘cold’ refuge. This value was our ‘boldness score’; with lower times indicating higher boldness. If the dragons did not exit the refuge within the duration of the trial, we assigned it a value of 3600 s.

### *Day 3: Neophilia*

Our neophilia assay quantified how close (cm) a dragon would approach a novel object. Within each enclosure a bullseye (10, 20, 30, and 40 cm diameter rings surrounding a central 5 cm diameter circle) was printed on paper and taped to the base of the arena (prior to all assays beginning; see SM Fig. 2). The two refuge boxes from the previous assay were removed, and a novel object was placed at the centre of the bullseye (see SM Fig. 2C). The objects chosen are common refuse items found in urban areas, and was different across each assay period. The novel object were: 1) unused 350 ml paper coffee cups, 2) unused aluminium 160 ml pie tins, 3) empty 600 ml water bottle, 4) unopened bag of 19 g potato chips, and 5) unopened 330 ml soft drink can. Similar to the previous two assays, each dragon was placed within a central containment refuge at the start of an assay, and left for 10 min to

acclimate. To begin the assay the central containment refuge was removed, and individuals were left for 30 min to interact with the novel object.

From the videos of the neophilia assay we noted the proximity of the individual to the novel object using the rings of the bullseye to indicate distance to the object (e.g., outer-most ring = 20 cm and inner-most ring = 5 cm). Dragons that climbed the novel object were given a score of 0 cm, and individuals beyond the outermost ring were assigned a score of 25 cm. The closest distance a dragon approached the novel object over the 30 minute period represented its ‘neophilia score’ and was the value used in our analysis; lower scores indicate a higher level of neophilia.

## ***Statistical Analyses***

### *Behavioral Traits*

Before analysis we explored our data following the protocol detailed in Zuur et al. (2010). We found that two of our three behavioral traits, boldness and neophilia, followed a normal distribution and had no outliers. We used a rank transformation to normalize our exploration score (Kar et al. 2016). We also determined there was no strong collinearity between model predictor variables.

We examined differences in dragon behavioral traits using linear mixed effect models (LMM, using the function *lmer* in the *lme4* R package; Bates et al. 2015; R Core Team 2016). We ran separate LMMs for each of the three behavioral traits. The LMMs with exploration and neophilia as the response variable included the fixed effects of dragon age (continuous), origin population type (categorical: natural, semi-natural, or urban), and batch (categorical: 1, 2, 3 or 4). The LMM with boldness as the response variable had the additional continuous fixed factor of time spent scaring the lizard (sec). Continuous fixed factors were mean-centred using a z-transformation before analysis, which standardizes the variables and facilitates interpretation of main effects in the presence of interactions (Schielzeth 2010). In all LMMs, we accounted for dependencies within our data from sampling each lizard repeatedly (random

intercept and slope for lizard identity across age), sampling individuals from the same litter, (random intercept for lizard clutch), the same captive enclosures (random intercept for tub identity), and the same study population (random intercept for study site). To allow comparisons among all origin site types, we re-levelled the reference for origin population category and re-ran the model (Nakagawa 2004). The assumptions of normality of residuals, for both fixed and random effects, and heterogeneity of variance were verified for all LMMs (Zuur et al. 2009),  $\alpha$  was set at 0.05, and the R function *confint* was used to bootstrap 95% confidence intervals for parameter estimates. We also calculated unconditional means and 95% CIs (corrected for non-independence) for each origin population type using the function *Effect* in the R package *effects* (Fox 2003; Fox and Hong 2009). Assessment of unconditional means and the magnitude of their differences (i.e., effect size) can reflect biological significance (Nakagawa and Cuthill 2007; Gerstner et al. 2017).

### *Consistency in Behavior*

We examined the consistency of an individual's behavioral traits to investigate if repeatability was affected by origin population type. To accomplish this, we first subsetted the data by origin population category, resulting in three separate datasets. For urban-origin dragons, we had 126 observations from 28 individuals across 17 clutches, 14 enclosures, and 4 populations. For semi-natural dragons, we had 225 observations of exploration, 228 observations of boldness and neophilia, respectively, from 52 individuals across 32 clutches, 15 enclosures, and 4 populations. For natural-origin dragons, we had 82 observations of exploration and 83 of boldness and neophilia, respectively, from 23 individuals across 14 clutches, 13 enclosures, and 4 populations.

We calculated adjusted repeatability ( $R_{adj|age}$ ; Biro and Stamps 2015) for each origin population type while controlling for the same covariates that were within their respective LMMs (Nakagawa and Schielzeth 2010; Biro and Stamps 2015). We calculated 95% confidence intervals by bootstrapping the data 1000 times with the *boot* function from the R

package *boot* (Davison and Hinkley 1997; Canty and Ripley 2017).  $R_{adj|age}$  was considered significantly more than what would occur by chance alone if the 95% confidence intervals did not overlap 0. We compared  $R_{adj|age}$  between treatments by examining overlap between natural, semi-natural, and urban site 95% CIs. Theoretically,  $R_{adj|age}$  ranges between 0 (individuals never expressing the same trait value over repeated measures) and 1 (individuals always expressing the same trait value over repeated measures; Nakagawa and Schielzeth 2010), although the average repeatability observed in the field of animal behavior is 0.37 (Bell et al. 2009).

## Results

### *Behavioral Traits*

Exploration did not significantly differ among origin population category (Table 3; comparison between semi-natural and urban:  $\beta = 0.231$ , 95%  $CI = -0.066$  0.581,  $t\text{-value} = 1.429$ ,  $P = 0.153$ ), and neither did neophilia (Table 3; comparison between semi-natural and urban:  $\beta = -0.628$ , 95%  $CI = -3.173$ , 2.130,  $t\text{-value} = -0.445$ ,  $P = 0.656$ ; Fig. 1). Dragons were significantly bolder if they originated from semi-natural sites than natural sites (Table 3). Boldness did not significantly differ between urban and natural sites (Table 3), nor semi-natural and urban sites ( $\beta = 10.810$ , 95%  $CI = -408.829$ , 448.212,  $t\text{-value} = 0.048$ ,  $P = 0.962$ ). However, both the semi-natural and urban dragons exited the hide (the metric for boldness) at approximately 8.3 min (492 s and 502 s, respectively) sooner than individuals in the natural origin population category (Fig. 1); thus there may be ecological relevance to this finding.

### *Consistency in Behavior*

Dragons from natural populations had high repeatability in exploration behavior ( $R_{adj|age} = 0.50$ , 95%  $CI = 0.13$ , 0.86), while exploration was not significantly repeatable in dragons from urban ( $R_{adj|age} = 0.25$ , 95%  $CI = 0$ , 0.51) and semi-natural ( $R_{adj|age} = 0.03$ , 95%  $CI = 0$ ,

0.29) populations. Repeatability was not significantly different among origin population types.

Dragons originating from urban populations had moderate levels of repeatability in boldness ( $R_{adj|age} = 0.32$ , 95%  $CI = 0.02, 0.63$ ), while boldness was not significantly repeatable in dragons from semi-natural ( $R_{adj|age} = 0.18$ , 95%  $CI = 0, 0.41$ ) and natural ( $R_{adj|age} = 0.25$ , 95%  $CI = 0, 0.61$ ) populations. Repeatability of boldness was also not different among origin population types.

Neophilia of dragons was not significantly repeatable in any origin population (urban:  $R_{adj|age} = 0.05$ , 95%  $CI = 0, 0.34$ ; semi-natural:  $R_{adj|age} = 0.06$ , 95%  $CI = 0, 0.30$ ; natural:  $R_{adj|age} = 0$ , 95%  $CI = 0, 0.45$ ). We observed no difference in repeatability among origin population types.

## Discussion

Dragons from semi-natural populations were significantly bolder than conspecifics from natural populations, and dragons from urban populations mirrored this trend. We did not detect differences in dragon neophilia or exploration among origin population types (urban, semi-urban, and natural). Exploration behavior in individuals from natural populations was highly repeatable ( $R_{adj|age}$ , within-individual repeatability over time; Biro and Stamps 2015), and boldness in individuals from urban populations was moderately repeatable. All other behavioral traits were not repeatable, and dragons did not differ in their behavioral consistency across origin population types.

We found that water dragons from semi-natural populations were significantly bolder than those from natural origin populations. Also, a difference between urban and natural origin populations, although non-significant, trended in the same direction. Logically, we would have expected these results to occur in reverse order, such that lizards were bolder in relation to the extent of urbanization experienced by their origin population. Interestingly, the parameter estimates reflect this logic. The difference in boldness between urban and natural

populations (parameter estimates and effect sizes) was actually slightly greater (by 10 sec) than the difference in boldness between semi-natural and natural environments (Table 3; Fig. 1). The lack of significance is likely a consequence of greater variance in boldness among individuals within the urban environment (Table 3). We suggest that the difference in boldness between dragons from urban and natural origin population categories is likely still biologically relevant, because both the semi-natural and urban dragons exited the hide at approximately 8.3 min (492 sec, 502 sec respectively), sooner than individuals in the natural origin population type. Our findings support the previously established concept that wildlife persisting in, or colonising, urban environments, have a tendency to be bolder (as reviewed in Lowry et al., 2013; Miranda et al., 2013; Sol et al., 2013). However, whether increasing levels of boldness associated with urban areas is a heritable trait or plastic response is difficult to disentangle in previous studies.

This study represents, to the best of our knowledge, the first demonstration of an urban-derived behavioral trait in a vertebrate that is solely associated with an individual's inherent traits, without being confounded by an urban developmental environment or prior experience. Our findings align with recent studies that demonstrate urbanization may be selecting for heritable behavioral traits (e.g., increased aggression and boldness, Evans et al. 2010; Müller et al. 2013; Holtmann et al. 2017; Sprau and Dingemanse 2017). Increased boldness in urban areas may promote fitness, through facilitating increased foraging and mating opportunities. This fitness benefit may drive selection for enhanced boldness within novel environments (Dingemanse and Réale 2005; Réale et al. 2007). For example, increased boldness can provide invasive lizard species such as mourning geckos (*Lepidodactylus lugubris*), which are colonising novel urban environments, a substantial advantage through increased foraging ability and competitive superiority (Short and Petrens 2008).

We did not find differences in neophilia and exploration among origin population categories. These behavioral traits may not be strongly selected for within Sydney urban environments. Alternatively, there may have been a flaw in the trial design (e.g., testing arena

size or means of measuring) or that there are ontogenetic changes in the timing of expression of these behaviors (e.g., dragons do not express variation in neophilia or exploration behavior until they are older). Urban, wild-caught brown anoles (*Anolis sagrei*) are bolder, less aggressive, and more exploratory compared to natural origin populations (Lapiedra et al. 2017); however, in this study they could not rule out the effects of rearing environment and prior experience on the behaviors they were observing. There is the potential that certain behavioral traits, such as boldness, are genetically-linked and may play a larger role for urban success, compared to other traits such as exploration and neophilia. Or, perhaps that behavioral traits such as exploration and neophilia are more plastic in their expression and may react differently in urban habitat. For example, there is a positive correlation between boldness and aggression in song sparrows (*Melospiza melodia*), but this relationship breakdowns in urban areas (Scales et al. 2011). Overall, more research is necessary to understand the selective forces that are shaping the behavior of urban wildlife (Lowry et al. 2013).

The low within-individual repeatability of the behavioral traits we measured, suggests that temporal consistency of exploration, boldness, and neophillia is not favoured within the environments we sampled. Yet, there were two exceptions to this general finding: exploration in individuals from natural populations was significantly and highly repeatable, while boldness in individuals from urban environments was significantly, but moderately, repeatable. This finding suggests that within these environments there is consistent selection on these traits, which results in within-individual consistency in exploration and boldness in natural and urban populations, respectively. Interestingly, boldness is repeatable in urban populations, which further supports our assertion urban selection favours bolder individuals. For the other origin population categories and behavioral traits, where repeatability was not significantly different from zero – one rationale may be that juvenile behavioral traits may be quite plastic in these habitats and that young lizards alter their behavioral traits over time throughout development (Favati et al. 2016; Riley et al. 2017). A lack of consistency in



behavioral traits across development has previously been found in birds (*Gallus gallus*; Favati et al. 2016) and lizards (*Egernia striolata*; Riley et al. 2017), which suggest if a similar study to ours was to be conducted on adult dragons, more consistent behavioral traits may be evident. Currently, the lack of repeatability in our behavioral traits suggests that there is no fixed personality (consistent individual variation in behavior across time; Sih et al. 2004; Bell 2012), at this life-stage of water dragons across all habitats. Interestingly, formation of fixed personalities, related to urban-derived behavioral syndromes, has been documented in adult brown anoles (Lapiedra et al. 2017), and we suggest that further research on adult water dragon personality across urban populations is required to determine if they would yield comparable results. Future studies should investigate development of personality from juvenile to adult life-stages, a typically under-studied topic (Stamps and Groothuis 2010), to examine which behavioral traits are shaped by experience in urban environments, in contrast to what is determined through inheritance at birth (e.g., boldness in our study).

Urban landscapes are both expanding globally, and are a major contributor to biodiversity loss (McKinney 2002; Seto et al. 2012). In light of the novel landscapes humanity has created (Ellis and Ramankutty 2008), it is imperative we understand the role of urban evolution in allowing wildlife to adapt to an increasingly urban world (Dingemanse and Réale 2005; Lowry et al. 2013; Holtmann et al. 2017; Johnson and Munshi-South 2017). Furthermore, we need to understand how these divergent behavioral traits can be applied to conservation action and wildlife management to enable species to survive urbanization, which is currently a major threat to wildlife worldwide (Greggor et al. 2016). Our study provides the first evidence suggesting a genetically-linked, urban-derived divergent behavioral trait (boldness) in a vertebrate, removed from the confounding effects of developmental environment. This important finding underscores the need for further research in the field of behavioral urban ecology, which would serve to further determine the direct heritability of urban-derived behavioral traits, as well as the adaptive role these traits play in urban survival.

Overall, urban landscapes provide an excellent opportunity to understand the role of behavior in evolution, particularly in novel environments.

### **Acknowledgements**

We would like to thank P. Bolton, C. Fryns, F. Kar, S. Klopper, and D. Noble for their assistance in field, T. Damasio and M. Mühlenhaupt for their assistance in the lab, and P. Harlow for providing his insights into this topic. This research was supported by an International Macquarie University Research Excellence Scholarship and a Natural Sciences and Engineering Research Council of Canada Postgraduate Scholarship (awarded to JBG). Experimental protocols were approved by the Macquarie University Animal Ethics Committee (ARA # 2015/023), Taronga Zoo Animal Ethics Committee (ARA # 3b/08/15) and New South Wales National Parks and Wildlife Services (scientific license # SL100570).

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An adult male water dragon basking on a graffiti-covered rock

## Tables

**Table 1.** Number of dragons sampled for each round of behavioral assays split across each type of origin population (natural, semi-natural, and urban).

Origin Site Type	Round 1	2	3	4	5	All rounds
Natural	20	20	16	15	12	83
Semi-natural	49	51	45	43	40	228
Urban	28	26	25	24	23	126
All dragons	97	97	86	82	75	

**Table 2.** Component loadings from our principal component analysis (PCA) for the behaviors scored from our water dragon (*Intellagama lesueurii*) exploration assays. The PCA for the exploration assay consisted of two behaviors (time spent moving in a novel environment, and the time spent hiding in a refuge the new environment). These traits were measured for 103 dragons five times within each dragon's first year of life ( $N_{\text{obs}} = 433$ ).

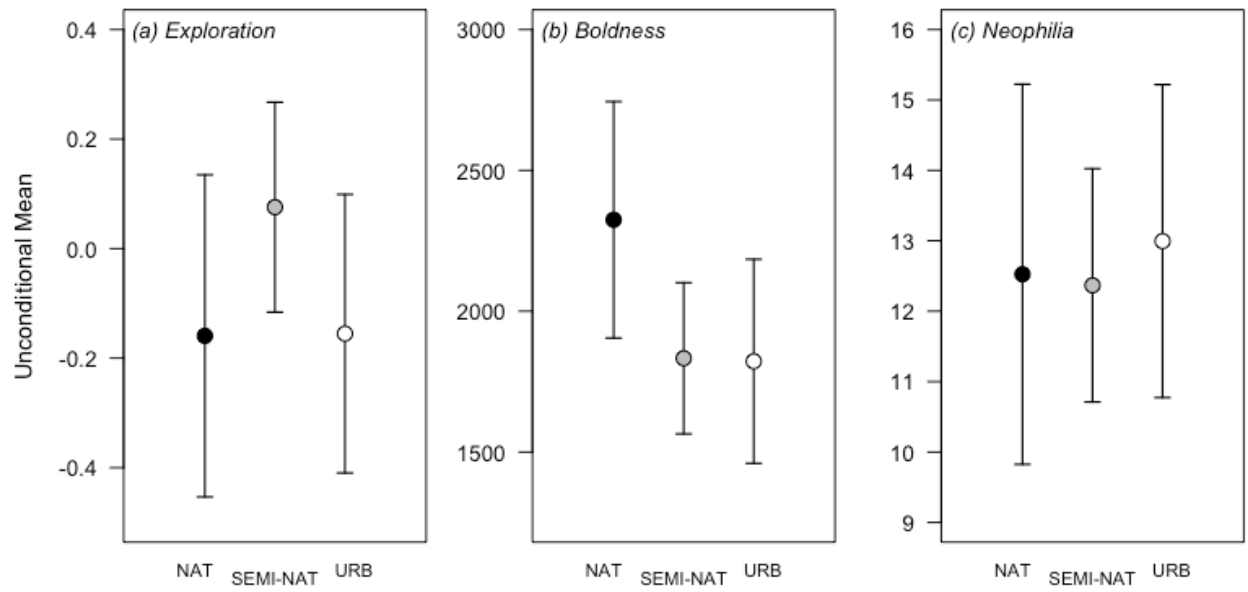
Behaviour	Component one loadings
Exploration	
Time spent moving (s)	-0.707
Time spent hiding in a refuge (s)	0.707

1 **Table 3.** Outcomes of linear mixed effect models testing if behavioral traits (exploration, boldness, and neophilia) were affected by dragon origin  
2 site type (natural: NT, semi-natural: SN, and urban: UB). If fixed factors were not included in the analysis this is represented with three hyphens  
3 (---).

Fixed Effects	Exploration					Boldness					Neophilia				
	<i>N<sub>obs</sub> = 433, N<sub>juv</sub> = 103, N<sub>mom</sub> = 63, N<sub>tub</sub> = 15, N<sub>site</sub> = 12</i>					<i>N<sub>obs</sub> = 437, N<sub>juv</sub> = 103, N<sub>mom</sub> = 63, N<sub>tub</sub> = 15, N<sub>site</sub> = 12</i>					<i>N<sub>obs</sub> = 437, N<sub>juv</sub> = 103, N<sub>mom</sub> = 63, N<sub>tub</sub> = 15, N<sub>site</sub> = 12</i>				
	<i>B</i>	<i>2.5%</i>	<i>97.5%</i>	<i>t-value</i>	<i>P</i>	<i>β</i>	<i>2.5%</i>	<i>97.5%</i>	<i>t-value</i>	<i>P</i>	<i>β</i>	<i>2.5%</i>	<i>97.5%</i>	<i>t-value</i>	<i>P</i>
Intercept	-0.288	-0.629	0.055	-1.656	0.098	<b>2520.372</b>	<b>2067.041</b>	<b>3014.053</b>	<b>10.155</b>	<b>&lt;0.001</b>	<b>10.859</b>	<b>7.100</b>	<b>14.018</b>	<b>6.871</b>	<b>0.000</b>
Age	<b>0.210</b>	<b>0.124</b>	<b>0.287</b>	<b>4.791</b>	<b>&lt;0.001</b>	-75.231	-196.861	34.867	-1.177	0.239	<b>-2.652</b>	<b>-3.753</b>	<b>-1.601</b>	<b>-5.346</b>	<b>0.000</b>
Origin (SN; ref = NT)	0.235	-0.090	0.577	1.320	0.187	<b>-491.589</b>	<b>-982.453</b>	<b>-43.196</b>	<b>-1.959</b>	<b>0.050</b>	-0.157	-3.390	3.198	-0.097	0.923
Origin (UB; ref = NT)	0.004	-0.416	0.402	0.021	0.984	-502.400	-1085.466	41.771	-1.797	0.072	0.471	-3.260	4.518	0.265	0.791
Batch (2; ref = 1)	0.240	-0.101	0.581	1.447	0.148	-337.156	-852.754	107.125	-1.431	0.152	<b>3.747</b>	<b>0.565</b>	<b>6.641</b>	<b>2.470</b>	<b>0.014</b>
Batch (3; ref = 1)	0.183	-0.120	0.475	1.295	0.195	-194.761	-608.259	222.427	-0.977	0.329	<b>2.615</b>	<b>-0.089</b>	<b>5.249</b>	<b>2.016</b>	<b>0.044</b>
Batch (4; ref = 1)	0.128	-0.327	0.526	0.612	0.540	<b>-613.325</b>	<b>-1203.419</b>	<b>11.032</b>	<b>-2.106</b>	<b>0.035</b>	-1.112	-4.929	2.348	-0.559	0.576
Time spent scaring	---	---	---	---	---	<b>277.775</b>	<b>153.078</b>	<b>392.119</b>	<b>4.413</b>	<b>&lt;0.001</b>	---	---	---	---	---
Random Effects	$\sigma^2$	<i>2.5%</i>	<i>97.5%</i>			$\sigma^2$	<i>2.5%</i>	<i>97.5%</i>			$\sigma^2$	<i>2.5%</i>	<i>97.5%</i>		
Identity (Intercept)	0.139	0.041	0.291			208640.400	31915.713	512528.290			0.269	0.001	9.197		
Age (Slope)	0.038	0.000	0.099			65675.700	60.913	248259.968			5.541	0.193	14.590		
Clutch (Intercept)	0.077	0.000	0.182			269941.700	0.000	508816.130			9.788	0.006	17.687		
Tub (Intercept)	0.000	0.000	0.029			6674.700	0.000	88672.273			0.000	0.000	4.203		
Site (Intercept)	0.005	0.000	0.057			0.000	0.000	102263.299			0.013	0.000	5.552		
Residual	0.617	0.519	0.739			1060541.800	886950.857	1263617.613			79.914	64.496	90.495		

4  
5

**Figure**



**Figure 1.** Unconditional means and 95% CIs of each behavioral trait, (a) exploration, (b) boldness, and (c) neophilia, for each origin population category (black, grey, and white points represent natural (NAT), semi-natural (SEMI-NAT), and urban (URB) populations, respectively) of water dragons.

## Supplementary Materials

### Methods

#### *Sites*

Gravid water dragons were collected at 12 sites in a 50 km radius around the greater Sydney area in New South Wales. The urban sites were: the Manly to Shelly Beach boardwalk in Manly (33.79° S, 151.29° E;  $N_{\text{hatchling}} = 5$ , from 3 clutches), the Taronga Zoo grounds in Bradleys Head (33.84° S, 151.24° E;  $N_{\text{hatchling}} = 13$ , from 8 clutches), Chinese Garden of Friendship in Darling Harbour (33.88° S, 151.20° E;  $N_{\text{hatchling}} = 7$ , from 4 clutches), and Macquarie University Campus in Marsfield (33.77° S, 151.11° E;  $N_{\text{hatchling}} = 3$ , from 2 clutches). The semi-natural sites were: Lane Cove National Park near Chatswood West (33.79° S, 151.14° E;  $N_{\text{hatchling}} = 13$ , from 8 clutches), Brown's Watering Hole near North Epping (33.76° S, 151.11° E;  $N_{\text{hatchling}} = 10$ , from 6 clutches), Lake Parramatta near North Parramatta (33.47° S, 151.00° E;  $N_{\text{hatchling}} = 11$ , from 7 clutches), and Manly Reservoir near Manly Vale (33.46° S, 151.15° E;  $N_{\text{hatchling}} = 13$ , from 9 clutches). The natural sites were: the Grose River near Yarrimundi (33.62° S, 150.67° E;  $N_{\text{hatchling}} = 6$ , from 5 clutches), Lion Island near Pearl Beach (33.27° S, 151.19° E;  $N_{\text{hatchling}} = 7$ , from 4 clutches), Nepean River (33.78° S, 150.64° E;  $N_{\text{hatchling}} = 8$ , from 5 clutches), and Rickaby's Creek near Bligh Park (33.37° S, 150.47° E;  $N_{\text{hatchling}} = 1$ , from one clutch).

#### *Handling, Husbandry, and Egg Collection*

Dragons were captured by hand or noose-pole, palpated for the presence of eggs, and then transported to Macquarie University (Sydney, NSW) or retained at Taronga Zoo (Sydney, NSW; if captured on their grounds). The females were placed in 100 L plastic tubs with moist substrate, drinking water, and kept at an average room temperature of 30 °C, and held for no more than 3 days. Oviposition was induced by administering a dose of 100 mg/kg calcium gluconate (Phebra, Lane Cove, NSW, AU) into the left forearm, whereupon the lizards was rested for 60 minutes. The lizard was then administered a shallow intraperitoneal injection of

synthetic oxytocin (Syntocin, Ilium, Glendenning, NSW, AU) at a dose 60 IU/kg (Mader 1996; Harlow and Taylor 2000). Once oviposition was complete, entire clutches were placed in 500 ml plastic containers with 115 g of moistened vermiculite (-150 kPa; 130% water by dry mass of vermiculite; Harlow 2001). The containers were fitted with an oxygen permeable polyethylene membrane (Glad Wrap, Glad Products of Australia, Rhodes, NSW, AU) and placed in incubators set at 26.5 °C, allowing for equal sex ratios during development (Harlow 2001). The adult female dragons were returned to their site of capture and released after oviposition. Incubation of the eggs took an average of 68.3 days (+/- 0.3 SE), where upon hatchling occurred, and hatchlings were measured and either released at their mother's capture site or allocated into our experimental colony on Macquarie University campus.

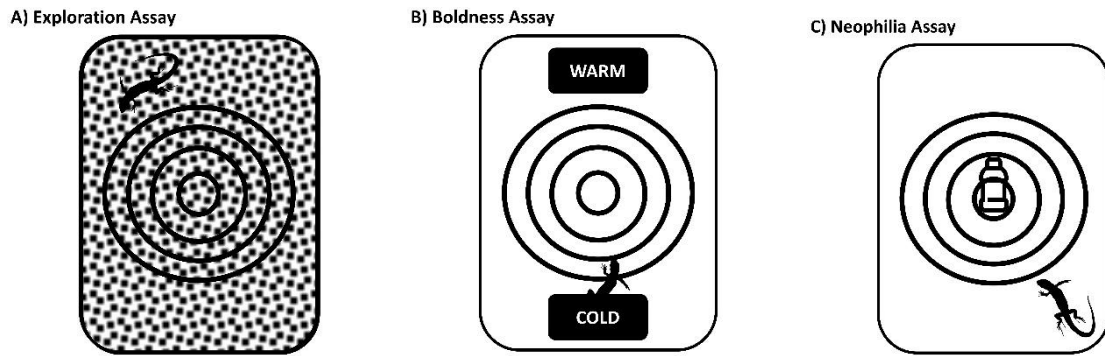
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## SM Figures



**SM Figure 1:** Common garden rearing setup, specifically within-enclosure design (A) and overall outdoor housing setup (B) located on the Macquarie University campus, Sydney, New South Wales, Australia.



**SM Figure 2:** The experimental setup for the three behavioral trait assays: A) exploration assay (mottled background represents a novel substrate added to the enclosure every time a lizard was tested), B) boldness assay (the lizard was chased into the cold hide and time to see how long it would wait before exiting), and C) neophilia assay (the water bottle in the centre of the diagram represent a novel object added to the lizards enclosure, with the individual's proximity to the object determined using concentric rings separated 5 cm apart). See Methods for more details regarding assay scoring.



### Chapter III

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Runners and fighters: Clutch effects and body size drive  
innate antipredator behaviour in hatchling lizards



James Baxter-Gilbert, Julia L. Riley, and Martin J. Whiting

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The following manuscript has been published in *Behavioural Ecology and Sociobiology*, and is presented in its published form.

Pages 80-88 of this thesis have been removed as they contain published material. Please refer to the following citation for details of the article contained in these pages.

Baxter-Gilbert, J., Riley, J. L., and Whiting, M. J. (2018). Runners and fighters: clutch effects and body size drive innate antipredator behaviour in hatchling lizards. *Behavioral Ecology and Sociobiology*, 72, Article 97.

DOI: 10.1007/s00265-018-2505-7

## Supplementary Material

### Methods

#### *Sites*

Gravid Australian water dragons were collected at 12 sites in a 50 km radius around the Sydney, New South Wales. The urban sites were: 1) the Manly to Shelly Beach boardwalk in Manly (33.79° S, 151.29° E;  $N_{\text{hatchling}} = 26$ ), 2) the Taronga Zoo grounds in Bradleys Head (33.84° S, 151.24° E;  $N_{\text{hatchling}} = 38$ ; SM Fig. 1), 3) the Chinese Garden of Friendship in Darling Harbour (33.88° S, 151.20° E;  $N_{\text{hatchling}} = 28$ ), and 4) the Macquarie University Campus in Marsfield (33.77° S, 151.11° E;  $N_{\text{hatchling}} = 9$ ). The semi-natural sites were: 1) Lane Cove National Park near Chatswood West (33.79° S, 151.14° E;  $N_{\text{hatchling}} = 59$ ), 2) Brown's Watering Hole near North Epping (33.76° S, 151.11° E;  $N_{\text{hatchling}} = 37$ ), 3) Lake Parramatta near North Parramatta (33.47° S, 151.00° E;  $N_{\text{hatchling}} = 28$ ; SM Fig. 2), and 4) Manly Reservoir near Manly Vale (33.46° S, 151.15° E;  $N_{\text{hatchling}} = 54$ ). The natural sites were: 1) the Grose River near Yarrimundi (33.62° S, 150.67° E;  $N_{\text{hatchling}} = 45$ ; SM Fig. 3), 2) Lion Island near Pearl Beach (33.27° S, 151.19° E;  $N_{\text{hatchling}} = 16$ ), 3) Nepean River (33.78° S, 150.64° E;  $N_{\text{hatchling}} = 23$ ), and 4) Rickaby's Creek near Bligh Park (33.37° S, 150.47° E;  $N_{\text{hatchling}} = 7$ ).

## SM Figures



**SM Figure 1:** An adult male (foreground) and female (background) water dragon in the garden beds at the Taronga Zoo urban study site. Urban sites had a relatively dense human population, and a landscape that was widely human-modified (e.g., concrete, buildings, gardens, roads, etc.).





**SM Figure 2:** The semi-natural study site at Lake Parramatta, a greenspace completely surrounded by urban sprawl. Semi-natural sites were protected green spaces (parkland) that contained waterways adjacent to urban areas, and they had a moderate amount of human visitors.



**SM Figure 3:** The natural study site on the Grose River. Natural sites, were generally associated with native bushland, waterways with forested riparian zones, and a relatively low human presence.

## Chapter IV

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Anthropic selection: Human-altered landscapes drive the development of divergent morphology in an urbanised lizard



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The following manuscript is being prepared for publication. It follows the format of the *Journal of Evolutionary Biology*. Tables, figures, and supplemental material follow this manuscript's references.

## Abstract

Wildlife living within urban areas are faced with a novel set of challenges resulting in strong selection, which can lead to population-level changes as species strive to adapt. We examined morphological variation of Australian water dragons (*Intellagama lesueurii*) from urban and natural populations, both in the field and under experimental conditions. If an urban-derived divergent morphology has arisen through selection, we predict distinct morphological differences between dragons from urban and natural areas in both adult and hatchling life-stages. We found that urban hatchlings were morphologically distinct from natural populations (shorter body length and longer limb size), as were urban adult males (shorter body length). We then experimentally reared hatchlings from urban and natural origin populations in urban- and natural-style enclosures (2 x 2 factorial design) to determine if morphological differences were related to either the individual's origin population (urban vs. natural; heritable traits) or the habitat experienced through development (urban vs. natural; phenotypic plasticity). Hatchlings from urban populations raised in urban habitats had significantly slower growth rates compared to individuals from natural populations raised in natural habitats. In contrast, individuals from natural populations raised in urban habitats and urban populations raised in natural habitats were no different to the individuals from natural populations raised in natural habitats. Our findings suggests urban-derived divergent morphological phenotypes are heritable but their expression is only maintained within the urban environment, with individuals returning to the species' default morphology if this genotype-environment interaction does not occur.

## Introduction

Habitat destruction and urbanisation have introduced a host of novel ecosystems to the world (Ellis & Ramankutty, 2008). These transformed landscapes, like urban areas, can be fraught with threats and sources of mortality (e.g., novel predators, traffic, pollutants, fragmentation), but can also provide exploitable opportunities and resources for certain species (e.g., decreased natural predators, thermal buffers, novel food sources; Ditchkoff et al., 2006; Kowarik, 2011; Winchell et al., 2016). As wildlife perseveres within, or recolonises, urban areas, there should be considerable novel selective forces within these substantially altered ecosystems (Atwell et al., 2012; Winchell et al., 2016; Diamond et al., 2017; Littleford-Colquhoun et al., 2017; Sprau & Dingemanse, 2017). Recent research has observed species exhibiting urban-derived divergent phenotypes (see meta-analysis by Alberti et al., 2017), as well as documented evidence of urban evolution (see review by Johnson & Munshi-South, 2017). Thus, urban areas can be considered a natural experiment, from which we can gain an understanding of how anthropic selection - when natural selection operates in human-altered environments— impacts wildlife.

Populations living in urban and natural areas differ in behaviour, physiology, and morphology (Parris et al., 2009; Bonier, 2012; Littleford-Colquhoun et al., 2017). For example, birds and frogs call more loudly in urban landscapes to compensate for anthropogenic noise (Parris et al., 2009; Barnett, 2015), and some species of bats alter their flight patterns and movement in response to urban light (Hale et al., 2015). Physiologically, some lizard populations have altered their stress response and innate immunity to handle the challenges of urban-living (French et al., 2008). Provided these responses are adaptive (Nemeth & Brumm, 2010), they may provide the basis for species to rapidly adjust to an ever-expanding anthropogenic landscape (McKinney, 2002; Seto et al., 2012). Although our understanding of the impacts urban environments have on wildlife is growing, there is little experimental research examining whether urban-derived divergent phenotypes are a result of selection through heritability, phenotypic plasticity, or an interplay between the two.



Studying the morphology of urban wildlife is a promising avenue for determining the mechanism for urban-derived divergent phenotypes. There is a close association between morphology and environment (Collette, 1961; Arnold, 1983). The relationship between body size and habitat structure directly impacts performance, and expression of morphological traits can arise rapidly through both phenotypic plasticity and natural selection (birds, Milá et al., 2008; fish, Berner et al., 2008; lizards, Losos, 1990; Losos et al., 1997, 2000; Kolbe & Losos, 2005). Dramatic environmental and ecological change has been seen to drive rapid phenotypic and genomic shifts in anoles (*Anolis* spp., Stuart et al., 2014; Winchell et al., 2016; Campbell-Staton et al., 2017). Similarly, species within urban landscapes exhibit divergent morphological phenotypes (Diamond et al., 2017; Sprau & Dingemanse, 2017). Body and limb length increases in lizard populations living in urban areas (brown anole, *Anolis sagrei*, Marnocha et al., 2011; northern water dragon, *Lophognathus temporalis*, Iglesias et al., 2012). Urban-derived changes in morphology are typically assumed to be an adaptive response to differences in habitat structure, resource availability, and microclimate between urban and natural environments (Marnocha et al., 2011; Iglesias et al., 2012; Lowe et al., 2014). However, much less is known about the mechanism that drives the differences between natural and urban populations (e.g., heritable adaptation or plastic responses), which has left researchers questioning the drivers behind their results (Irschick et al., 2005; Marnocha et al., 2011; Iglesias et al., 2012; Hutton & McGraw, 2016).

The Australian water dragon (*Intellagama lesueurii*) thrives in both urban and natural landscapes along Australia's eastern coastline (Cogger, 2014), and populations from different habitats are likely exposed to different selective forces. In fact, a recent study demonstrated that city dragon populations in the centre of Brisbane CBD (Queensland, Australia) are undergoing both rapid genetic and morphological diversification (Littleford-Colquhoun et al., 2017). Thus, water dragons provide a unique opportunity to examine the mechanisms that drive morphological differences between urban- and natural-living populations. Here, we first used field observations to examine if there were differences in adult dragon morphology

between urban and natural populations. If a difference in morphology is detectable at hatching this suggests a heritable/genetic mechanism. We then raised hatchling water dragons in a 2 x 2 factorial experiment within simulated ‘urban-style’ and ‘natural-style’ environments to test if morphological differences between urban and natural dragons are the product of phenotypic plasticity and/or selection. We predicted that if urban-derived divergent morphology was a result of anthropic selection driving urban evolution (Johnson & Munshi-South, 2017), then the habitat treatments would not impact body length and size. Instead, morphological differences would relate to the population of origin (urban or natural), which would demonstrate a heritable driver rather than a plastic response.

## **Methods**

### ***Study Species and Field Collection***

The Australian water dragon is a large agamid lizard (maximum snout-vent length of 304 mm; Thompson, 1993) with a lifespan of (28-40 years; Harlow & Harlow, 1997; Griffiths, 2006) and generation time of 5 years (Littleford-Colquhoun et al., 2017). They are adept at both climbing and swimming, water dragons are common around bodies of freshwater and are typically associated with forested areas (Cogger, 2014). Interestingly, this species’ distribution overlaps with the majority of the Australian human population (approx. 80%; Australian Bureau of Statistics, 2011), and populations of water dragons can be found in several major cities and around numerous human settlements (e.g., urban greenspaces, botanical gardens, zoos, and backyards).

We collected lizards from seven sites (four urban, three natural) within a 50 km radius within the greater Sydney area in New South Wales, Australia, from October 2015 to March 2017 (see Supplementary Material for detailed locations, handling, and husbandry methods). Urban sites had a dense local human population and a landscape that was widely human-modified (e.g., concrete, gardens, roads, buildings, etc.). Natural sites, although not free from human disturbance, were generally riparian greenspaces consisting of waterways with

wooded shorelines, native vegetation, and a comparatively low human presence (see Supplementary Material for site-specific details).

For the field component of our study, adult males ( $n = 74$ ) and females ( $n = 123$ ) were weighed, measured (see below), and then released at their site of capture. During the nesting season, gravid females were collected, brought to our lab, induced to oviposit (see Supplementary Material for details), and then post-oviposition released at their site of capture. Clutches of eggs were identically incubated throughout development (see Supplementary Material for details), and, upon emerging, most hatchlings (excluding habitat treatment individuals; see below) were immediately measured and released back at the mother's site of capture (hatchling group,  $n = 200$ ). The experimental component of our study saw a subset of the hatchlings ( $n = 97$ ) allocated to habitat treatments where they were housed on-site at Macquarie University and regularly measured over their first year of life (see below).

### ***Morphological Measurements***

Our morphological measurements followed Littleford-Colquhoun et al. (2017). These included snout-vent length (SVL), jaw width (JW), jaw length (JL), upper forearm length (UFL), lower forearm length (LFL), upper hind limb length (UHL), lower hind limb length (LHL), and total hind foot length (HFL; see Supplementary Material Fig. S1). All measurements (except SVL) were made with digital Vernier callipers ( $\pm 0.01$  mm), and SVL was measured with a clear plastic ruler ( $\pm 1$  mm). For adults and released hatchlings, these morphometric measurements were taken either post-capture or post-hatching (before release), respectively. Hatchlings in the habitat treatment experiment were measured six times over their first year of life (beginning after the hatching season, and every 60 days thereafter). Before statistical analysis we log-transformed all of these variables (Winchell et al. 2016); this ensured allometric relationships were linear.

### ***Habitat Treatment Experiment***

We raised hatchlings that were born in 2016 in outdoor enclosures to replicate the weather and climate they would naturally experience around Sydney, New South Wales. Enclosures were 5 x 2.5 m, and constructed within a netted predator exclusion area. Natural-style enclosures were outfitted with natural vegetation (e.g., grasses, weeds, and shrubs), wooden perches of varying diameter (5 - 50 mm), a shaded area, and a 150 L plastic pool (Fig. 1.A). Urban-style enclosures were outfitted with hard, flat surfaces (paving stones, concrete blocks, steel sheets, and roofing tiles), eucalypt mulch, a shaded area, and the same type of plastic pool as the natural treatment (Fig. 1.B). Both the natural- and urban-style enclosures were replicated three times, for a total of six enclosures. Each enclosure held individuals from natural origin populations ( $n = 8$ ) and urban origin populations ( $n = 8$ ), for a total of 16 dragons per enclosure. Siblings were split equally into natural- and urban-style enclosures, thereby controlling for maternal and clutch effects. Each enclosure experienced the same husbandry procedures (e.g., fed three crickets per dragon three times a week). Dragons were housed in these enclosures for their first year of life.

### ***Statistical Analysis***

All statistical tests were conducted in R version 3.2.3 (R Core Team, 2016). Before starting analyses, we explored each dataset following the protocol outlined in Zuur et al. (2010). We did not find any unexplainable outliers or strong collinearity between our predictor variables. To summarise all dragon morphological measurements, excluding SVL, we performed a PCA analysis separately for each dataset (i.e., hatchlings, adult males, adult females, and habitat-treatment hatchlings datasets) with the R package 'princomp'. All measurements were highly correlated, and negatively loaded on the first component (explaining 52-96% of the variation in measures; see Table 1). We used this first principal component (PC1) in further analyses as our 'body size' metric: as the value of PC1 increases, the size of limbs and the head increases.

In all cases, model assumptions of normality of residuals and homogeneity of variance were verified.

### *Hatchlings*

We sampled 198 hatchlings from 39 unique clutches across four populations (two urban [n = 85] and two natural [n = 117]). We used linear mixed effects models to examine differences in hatchling SVL and body size between urban and natural populations with the R package ‘lme4’ (Bates, 2015). Models examining differences in SVL included the fixed factor of urban category (categorical with two levels: urban or natural), and the random intercepts of origin population and mother identity to incorporate dependency among observations of lizards from the same population or the same clutch, respectively. Models examining differences in body size contained the same fixed and random factors, but also included the additional fixed factor of SVL to standardise this variable with respect to body length.

### *Adults*

We measured 197 adult water dragons (123 females, and 74 males) from seven populations in the greater Sydney area (four urban and three natural). Identical linear mixed effects models were performed separately for both sexes, because this species is sexually dimorphic (Baird et al., 2012). Furthermore, in preliminary analyses, we found that males were larger in SVL than females ( $\beta = 0.191$ ,  $t_{2,192} = 17.353$ ,  $P < 0.001$ ), and had larger limbs and heads (PC1; that was relative to SVL) than females ( $\beta = 1.508$ ,  $t_{2,191} = 7.978$ ,  $P < 0.001$ ). For both sexes, linear mixed effects models that examined differences in adult SVL included the fixed factors of urban category (categorical with two levels: urban or natural), and the random intercepts of origin population to incorporate dependency among observations of lizards from the same population. Models examining differences in adult body size contained the same fixed and random factors as above, but also included the additional fixed factor of SVL to standardise this variable with respect to body length.

## *2 x 2 Factorial Experiment*

The number of juveniles from each treatment combination that were measured varied across time (Table 2). These juveniles were from 24 unique clutches, across six populations (three urban [ $n = 49$ ] and three natural [ $n = 48$ ]), and were raised in a total of six enclosures (three natural- and three urban-style). Linear mixed effects models that examined differences in juvenile SVL included the fixed factors of age (continuous) and habitat treatment (categorical with four levels: urban origin with urban rearing [uU], natural origin with urban rearing [nU], natural origin with natural rearing [nN], and urban origin with natural rearing [uN]). The model also included random intercepts of juvenile identity, origin population, mother identity, and rearing enclosure to incorporate dependency among observations of the same lizard, the same population, the same clutch, and the same captive rearing environment, respectively. Models examining differences in the habitat treatment juvenile's body size contained the same fixed and random factors, but also included the additional fixed factor of SVL to standardise this variable with respect to body length. To test for differences between all treatment-comparisons, we re-levelled the fixed factor of habitat treatment until all comparisons were examined.

## **Results**

### ***Hatchling Morphology***

Hatchlings from urban sites were smaller in SVL at birth than hatchlings from natural sites ( $\beta = -0.047$ ,  $t_{2,195} = -3.799$ ,  $P < 0.001$ ), and had a significantly different body size than hatchlings from natural sites ( $\beta = 1.431$ ,  $t_{2,194} = 3.561$ ,  $P < 0.001$ ). Specifically, hatchlings from urban areas had longer limbs and larger heads than those from natural areas (Fig. 2). The model for body size controlled for hatchling SVL, which was positively related to body size ( $\beta = 31.709$ ,  $t_{1,194} = 15.895$ ,  $P < 0.001$ ).

## ***Adult Morphology***

Females did not differ in SVL ( $\beta = -0.112$ ,  $t_{2, 120} = -0.987$ ,  $P = 0.324$ ) between urban and natural sites. Female body size, relative to SVL, also was not different between natural and urban sites ( $\beta = 2.480$ ,  $t_{2, 119} = 0.848$ ,  $P = 0.397$ ). The model for body size controlled for SVL, because body size was significantly related to a female's SVL ( $\beta = 0.131$ ,  $t_{1, 119} = 14.367$ ,  $P < 0.001$ ).

Interestingly, male SVL was smaller in urban sites than natural sites ( $\beta = -0.048$ ,  $t_{2, 71} = -2.155$ ,  $P = 0.031$ ). Male body size, relative to SVL, did not differ between urban and natural sites ( $\beta = -0.021$ ,  $t_{2, 70} = -0.072$ ,  $P = 0.943$ ). The model for body size controlled for SVL, and was significantly related to a male's SVL ( $\beta = 24.205$ ,  $t_{1, 70} = 22.758$ ,  $P < 0.001$ ).

## ***2 x 2 Factorial Experiment***

As to be expected, all juveniles increased in SVL as well as limb and head measures (body size) as they aged (SVL:  $\beta = 0.001$ ,  $t_{1, 492} = 16.636$ ,  $P < 0.001$ ; body size:  $\beta = 0.001$ ,  $t_{1, 492} = 4.706$ ,  $P < 0.001$ ). Juvenile initial SVL, and grow rate of SVL, did not significantly differ among habitat treatments ( $P$  for all treatment comparisons  $> 0.05$ ; Table 3).

Juvenile body size was significantly related to their SVL ( $\beta = 0.012$ ,  $t_{1, 492} = 82.533$ ,  $P < 0.001$ ). Initially, juvenile body size (relative to SVL) did not significantly differ among habitat treatments ( $P$  for all treatment comparisons  $> 0.05$ ; Table 4). Yet, the dragons from urban populations that were raised in an urban treatment grew slower than all other treatments with respect to body size (comparisons with nN and nU were significant, while between uN was marginally significant; Table 4). No other comparisons among treatments were significantly different (Table 4).

## **Discussion**

We determined that the urban-derived divergent morphology of water dragons stems from a genetic/heritable origin, providing the foundation for anthropic selection to drive urban

evolution (Johnson & Munshi-South, 2017). However, we also saw an effect of environment on the maintenance of the phenotypic expression of morphology, suggesting a genotype-environment interaction (Via & Lande, 1985). Genotype-environment interactions occur when heritable traits arise within specific populations, through selection, mutation, or other means, but whose expression is mediated environmentally (Via & Lande, 1985; Fernández & López-Fanjul, 1997; Ghalambor et al., 2007; Grishkevich & Yanai, 2013). Our habitat treatment experiment demonstrated that juvenile lizards from urban populations raised in urban-style enclosures (uU) grew significantly slower (body size) than all other treatments (nN, uN, and nU). Interestingly, all individuals from natural populations (nN and nU) and individuals from urban population raised in natural-style habitats (uN), had a similar growth rate, body length (SVL) and body size. These results suggest that although an individual's morphology and growth rate is a consequence of genetic factors, it also has to be reinforced through habitat use or cues. Without both aspects present, an individual's body size remains similar to a natural form (returning to a default setting). This assertion is further supported by our field data, which showed that although hatchlings from urbanised populations were significantly smaller (SVL) with comparably longer limbs and larger heads than individuals from natural populations, this was not always the case for adults. Free-living urban adult males were also significantly smaller (SVL) than males from natural areas, although their body size (PC1) did not differ significantly, and adult females did not differ in body length or size between urban and natural populations. As such, although free-living urban individuals enter the world with an urban-derived divergent morphology, the maintenance of these altered body lengths and sizes relies on environmental feedback, which could also appear to differ demographically due to sex-specific differences in habitat use (Baird et al., 2012, Gardiner et al., 2014).

This interaction between heritable traits and urban habitats suggests a potential mechanism that allows water dragons to adapt to urban areas. It has been suggested that when phenotypic plasticity is heritable, a higher degree of plasticity is selected for within



environments that differ the most from where a phenotype originally evolved (Lande, 2009, Chevin & Lande, 2011). As such, populations living within starkly novel environments, such as urban areas, should display more phenotypic plasticity (Chevin & Lande, 2011). Thus, an increased likelihood of heritable phenotypic plasticity provides the framework for urban-adaptive genotype-environment interactions to take place. Previous research has seen urban-derived genotype-environment interactions in the thermal physiology of fungi, insects, and plants (see review by Chown & Duffy, 2015), suggesting that this interaction is particularly important in urban evolution (Johnson & Munshi-South, 2017) and taxonomically wide ranging. Genotype-environment interaction may be particularly important for adaption within urban areas because these landscapes are prone to rapid structural and ecological change, both negatively (further anthropogenic development) and positively (re-greening efforts; Standish et al., 2013). As such, populations experiencing urbanisation may use genotype-environment interactions as a genetically built-in failsafe, allowing individuals to return to their default morphology if the aspects of the urban environment driving phenotypic divergence are not experienced during development or if urbanised individuals are immigrating back into more natural areas. This would explain both our experimental findings and field observations, and suggests that genotype-environment interactions may reflect a bet-hedging strategy provided water dragons an advantage during the transition from their native habitats to urban areas.

Mechanistically, the urban-derived divergent morphology we observed in the free-living hatchling dragons (longer limbs and larger heads) may provide insight as to why these traits arise in urbanised populations. Longer limbs are associated with the use of broader surfaces in the lizard genus *Anolis* (Losos & Sinervo, 1989; Marnocha et al., 2011), *Tropidurus* (Kohlsdorf et al., 2001), and other Australian agamids (e.g., *Lophognathus temporalis*, Iglesias et al., 2012). Urbanisation increases the amount of flat impervious surfaces within habitats (Dale & Frank, 2014; Barnett, 2015), thus it is not surprising that anthropic selection favours this morphology in urban landscapes. Yet, over their first year, the uU juvenile body size was significantly slower compared to the other habitat treatments. This

resulted in comparatively shorter limbs, which is contrary to the original body size of the free-living hatchlings at emergence. This finding complicates our hypothesis that this morphology is adaptive for climbing on particular substrates (see Chapter VI). In regards to urban-derived divergent head size (jaw length and width), either natural selection or sexual selection, or both, can impact these traits because head shape in lizards generally relates to bite force and habitat (Herrel et al., 2001), contest outcome (Husak et al., 2006; Lappin et al., 2006), and diet (Herrel et al., 2006). Urbanisation can increase urban water dragon population density which results in greater crowding and more aggressive interactions (Chapter I), and increased head size may play a role in signalling and communication use during social encounters (e.g., head-bobbing, Baird et al. 2012) or combat endurance (e.g., jaw muscle stamina). However, more investigations are required into the behavioural and ecological factors influencing body size (e.g., performance advantages) across water dragon ontogeny.

Urban environments are globally ubiquitous, continually expanding, and a major threat to biodiversity (McKinney, 2002; Seto et al., 2012). As species grapple with surviving urbanisation, it is quite likely that an array of mechanisms will be employed change with these challenging new landscapes. Adaptation through selection, phenotypic plasticity, and genotype-environmental interactions may work synergistically to facilitate survival (Via & Lande, 1985; Nussey et al., 2005; Alberti et al., 2017; Johnson & Munshi-South, 2017). Plastic responses may provide a stopgap allowing populations to temporarily persist under urban pressures, creating time for natural selection to act upon adaptive heritable traits (Price et al., 2003; Winchell et al., 2016). Certainly, more research is required to validate these assertions (i.e., further genetic analysis, cross-breeding experiments between populations, etc.) and also to delineate other avenues of adaptation such as behavioural, cognitive, ecological, and physiological variation between urbanised and natural-living populations. Furthermore, studies examining the survival and fitness of free-living populations of urbanised individuals in natural and urban settings could provide valuable evolutionary insight, as to the advantages of these urban-derived divergent phenotypes. This research

builds on the idea that although urbanisation is a major threat to biodiversity (Venter et al., 2006), it can also create unique opportunities for natural selection, resulting in adaptive responses that allow certain species to persist and sometimes thrive in an increasingly human world.

## **Acknowledgements**

We would like to thank the many individuals who assisted in fieldwork: P. Bolton, T. Damasio, C. Fryns, G. Hughes, F. Kar, S. Klopper, L. Monk-Whipp, M. Mühlenhaupt, and D. Noble, as well as Taronga Zoo and the Lizard Lab animal care volunteers. This research was supported by Macquarie University and Natural Sciences and Engineering Research Council of Canada. Experimental protocols were approved by the Macquarie University Animal Ethics Committee (ARA # 2015/023), Taronga Zoo Animal Ethics Committee (ARA # 3b/08/15), and New South Wales National Parks and Wildlife Services (scientific license # SL100570).

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## Tables

**Table 1.** Loadings from our principal component analysis (PCA) for the eight log-transformed morphometric measures used to summarise water dragon body size in hatchlings, wild adult males and females, as well as juveniles within the habitat treatment experiment.

	Hatchlings	Females	Males	Cross-Foster
Morphometric measurements	PC1 Loadings			
Jaw Width	0.383	0.340	0.341	0.378
Jaw Length	0.376	0.259	0.377	0.379
Upper Forearm Length	0.329	0.370	0.310	0.368
Lower Forearm Length	0.393	0.476	0.434	0.381
Upper Hindlimb Length	0.404	0.410	0.428	0.379
Lower Hindlimb Length	0.374	0.338	0.369	0.382
Total Hindfoot Length	0.384	0.032	0.371	0.377
Component Variance	6.702	3.556	4.879	6.717
Percent of Variance Explained	64.950	50.802	69.700	95.952

**Table 2.** Samples sizes for our cross-fostered juvenile water dragons (urban origin with urban rearing [uU], natural origin with urban rearing [nU], natural origin with natural rearing [nN], and urban origin with natural rearing [uN]) across six sampling periods, which occurred over their first year of life (bi-monthly).

Habitat treatments	Sample size					
<i>Round</i>	<i>1</i>	<i>2</i>	<i>3</i>	<i>4</i>	<i>5</i>	<i>6</i>
nN	24	22	16	16	16	17
uN	25	25	20	22	20	21
nU	24	22	22	22	21	21
uU	24	22	22	20	20	18

**Table 3.** Comparisons between **(a)** snout-vent length (SVL) of habitat treatments (urban origin with urban rearing [uU], natural origin with urban rearing [nU], natural origin with natural rearing [nN], and urban origin with natural rearing [uN]), and **(b)** how SVL changed as individual's aged. Coefficient estimate differences ( $\beta$ ), t-values ( $t$ ), and p-values ( $P$ ) are presented. Significant differences are represented with an asterisk to the right of the p-value.

(a) Differences in snout-vent length (fixed effect)			
<i>Habitat treatments</i>	$\beta$	$t$	$P$
nN vs. uN	0.016	0.480	0.964
nN vs. nU	0.006	0.352	0.985
nN vs. uU	0.022	0.640	0.919
uN vs. nU	-0.010	-0.302	0.991
uN vs. uU	0.006	0.379	0.982
nU vs. uU	0.016	0.490	0.961
(b) Differences in how snout-vent length changed over time (interaction effect)			
nN vs. uN	< 0.001	0.308	0.999
nN vs. nU	< 0.001	1.317	0.552
nN vs. uU	< 0.001	1.384	0.510
uN vs. nU	< 0.001	1.052	0.719
uN vs. uU	< 0.001	1.131	0.671
nU vs. uU	< 0.001	0.093	0.100

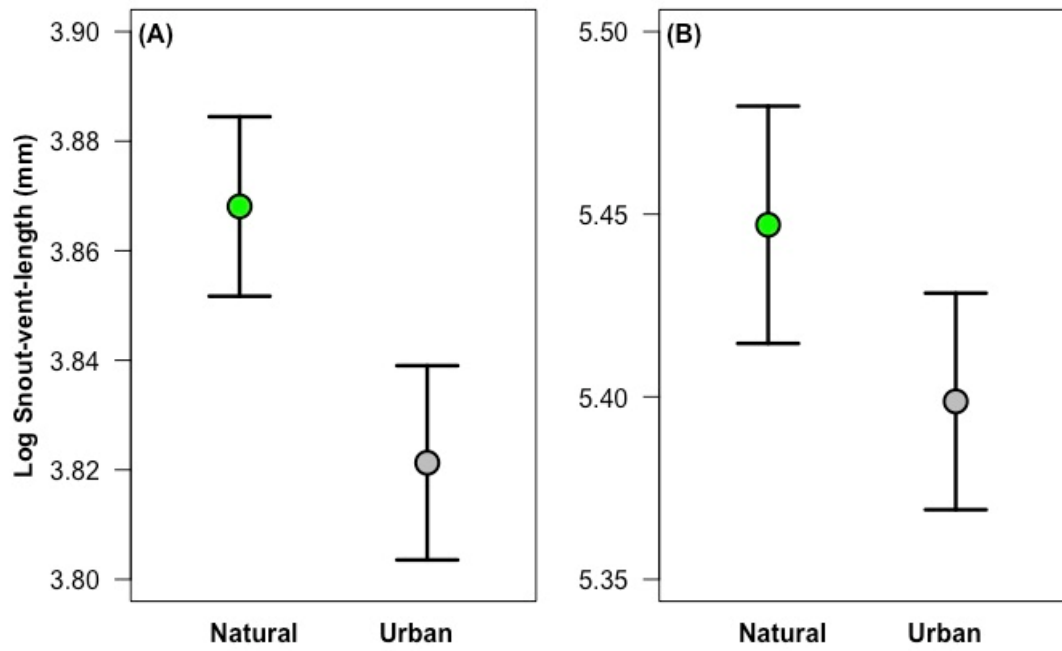
**Table 4.** Comparisons between **(a)** body size (PC1; see Table 1) of habitat treatments (urban origin with urban rearing [uU], natural origin with urban rearing [nU], natural origin with natural rearing [nN], and urban origin with natural rearing [uN]), and **(b)** how body size changed as individual's aged. The model included SVL as a covariate to control for size difference among individuals. Coefficient estimate differences ( $\beta$ ), t-values ( $t$ ), and p-values ( $P$ ) are presented. Significant differences are represented with an asterisk to the right of the p-value.

<b>(a) Differences in body size (fixed effect)</b>			
<i>Habitat treatments</i>	<i>B</i>	<i>t</i>	<i>P</i>
uN vs. nN	0.091	0.776	0.862
uN vs. nU	0.016	0.135	0.999
uN vs. uU	0.100	1.516	0.434
nN vs. nU	-0.075	-1.099	0.692
nN vs. uU	0.010	0.083	0.100
nU vs. uU	0.085	0.727	0.882
<b>(b) Differences in how body size changed over time (interaction effect)</b>			
uN vs. nN	< -0.001	-0.284	0.992
uN vs. nU	< -0.001	-0.297	0.991
uN vs. uU	0.0008	2.515	0.0590
nN vs. nU	< -0.001	0.000	1.000
nN vs. uU	0.0009	2.674	0.039*
nU vs. uU	0.0009	2.801	0.027*

## Figures



**Figure 1.** The setup for the ‘natural’ (A) and ‘urban’ (B) treatments. All replicates were identical in design.



**Figure 2.** Urban populations (grey circle) of (a) hatching, and (b) adult male water dragons have smaller mean snout-vent lengths than natural populations (green circle). Circles are predicted fitted means, and error bars are predicted standard error.

## Supplementary Material

### Methods

#### *Sites*

Water dragons were collected at seven sites in a 50 km radius around Sydney New South Wales, Australia from October 2015 to March 2017. The urban sites were: 1) the Manly to Shelly Beach boardwalk in Manly (33.79° S, 151.29° E;  $n_{\text{MALE}} = 11$ ,  $n_{\text{FEMALE}} = 17$ ,  $n_{\text{HATCHLING}} = 57$ ), 2) the Taronga Zoo grounds in Bradleys Head (33.84° S, 151.24° E,  $n_{\text{MALE}} = 16$ ,  $n_{\text{FEMALE}} = 22$ ,  $n_{\text{HATCHLING}} = 61$ ), 3) the Chinese Garden of Friendship in Darling Harbour (33.88° S, 151.20° E;  $n_{\text{MALE}} = 10$ ,  $n_{\text{FEMALE}} = 13$ ,  $n_{\text{HATCHLING}} = 16$ ), and 4) the Macquarie University Campus in Marsfield (33.77° S, 151.11° E;  $n_{\text{MALE}} = 2$ ,  $n_{\text{FEMALE}} = 1$ ,  $n_{\text{HATCHLING}} = 0$ ). The Manly to Shelly beach boardwalk (established in 1898) is has a narrow section of native, introduced, and non-native vegetation running along a cliff edge, boarded by dense suburban sprawl to one side and the ocean to the other. Taronga Zoo (established in 1916) is a heavily modified landscape comprised of garden beds and animal enclosures, it has a mixture of native, introduced and non-native vegetation. The Chinese Garden of Friendship (established in 1988) is a large garden located in the central business district of Sydney, it is comprised of almost entirely non-native vegetation. Macquarie University Campus (established in 1964) has a number of open green spaces with a mix of native, introduced and non-native vegetation, as well as garden beds.

The natural sites were: 1) the Grose River near Yarrimundi (33.62° S, 150.67° E;  $n_{\text{MALE}} = 11$ ,  $n_{\text{FEMALE}} = 24$ ,  $n_{\text{HATCHLING}} = 53$ ), 2) Lane Cove National Park near Chatswood West (33.79° S, 151.14° E,  $n_{\text{MALE}} = 18$ ,  $n_{\text{FEMALE}} = 34$ ,  $n_{\text{HATCHLING}} = 98$ ), and 3) Brown's Watering Hole near North Epping (33.76° S, 151.11° E;  $n_{\text{MALE}} = 5$ ,  $n_{\text{FEMALE}} = 10$ ,  $n_{\text{HATCHLING}} = 14$ ). The Grose River is a waterway flowing out of the Blue Mountains and connecting to the Hawksbury River (via the Nepean River), it is riparian habitat dominated by native vegetation. Both the Lane Cove National Park and Brown's Watering Hole sites are located in

Lane Cove National Park (approximately 6 km apart), and are riparian habitat dominated with native and introduced vegetation.

### ***Handling, Husbandry, and Egg Collection***

All adult dragons were captured by hand or noose-pole, and gravid females were palpated for the presence of eggs. Once measured, all adult males and non-gravid females were immediately released at their site of capture. During nesting season (October to December each year), gravid females were then transported to Macquarie University (Marsfield, NSW), or retained at Taronga Zoo (Sydney, NSW), and placed in 100 L plastic tubs with moist substrate, water, and an average room temperature of 30 °C. To induce egg laying, gravid females were administered a dose of 100 mg/kg calcium gluconate (Phebra, Lane Cove, NSW, AU) into the left forearm, then given a 60 minute rest followed by a shallow intraperitoneal injection of synthetic oxytocin (Syntocin, Ilium, Glendenning, NSW, AU) at a dose 60 IU/kg (Mader 1996; Harlow and Taylor 2000). Once oviposition was complete, entire clutches were placed in 500 ml plastic containers with 115 g of moisten vermiculite (-150 kPa; 130% water by dry mass of vermiculite; Harlow 2001) and fitted with an oxygen permeable polyethylene membrane (Glad Wrap, Glad Products of Australia, Rhodes, NSW, AU). The containers were then placed in incubators set at 26.5 °C, allowing for equal sex ratios during development (Harlow 2001). The adult female dragons were returned to their site of capture and released after oviposition. Incubation of the eggs took an average of 68.3 days (+/- 0.3 SE), where upon hatchling occurred, and hatchlings were measured and either released at their mother's capture site or allocated to the 2 x 2 factorial experiment.

### **References**

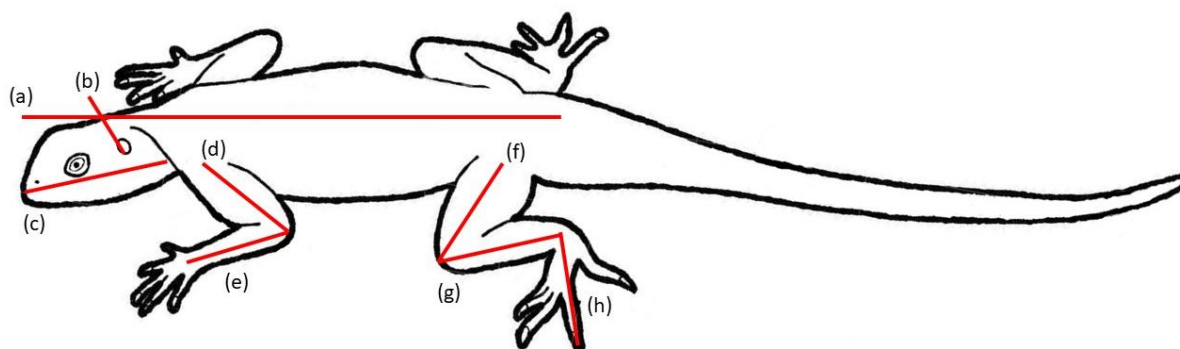
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## SM Figures



**Figure S1.** The locations of the morphological measures taken: a) snout-vent length (SVL), b) jaw width (JW), c) jaw length (JL), d) upper forearm length (UFL), e) lower forearm length (LFL), f) upper hind limb (UFL), g) lower hind limb (LHL), and h) total hind foot length (HFL). SVL was measured with a clear plastic ruler ( $\pm 1$  mm), and all others were measured with a set of digital Vernier callipers ( $\pm 0.01$  mm)

## Chapter V

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### Comparability and repeatability of three commonly-used methods for measuring endurance capacity



James Baxter-Gilbert, Max Mühlenhaupt, and Martin J. Whiting

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The following manuscript has been published in the *Journal of Experimental Zoology Part A: Ecological and Integrative Physiology* and is presented in its published form. It was also featured on the cover of the journal (above). This study, although not directly related to urban ecology, informed the methods used in Chapter VI.

Pages 121-129 of this thesis have been removed as they contain published material.  
Please refer to the following citation for details of the article contained in these pages.

Baxter-Gilbert, J., Mühlhaupt, M., & Whiting, M. J. (2017). Comparability and repeatability of three commonly used methods for measuring endurance capacity. *Journal of Experimental Zoology Part A: Ecological and Integrative Physiology*, 327(10), 583-591.

DOI: 10.1002/jez.2145

## Chapter VI

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Running through the concrete jungle:

Performance capacity and trade-offs in an urban-living lizard



James Baxter-Gilbert, Julia L. Riley, and Martin J. Whiting

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The following manuscript is being prepared for publication. It follows the format of the *Journal of Evolutionary Biology*. Tables, figures, and supplemental materials follow this manuscript's references.

## Abstract

Wildlife living within urban areas provides an excellent opportunity to examine the impacts of novel selective forces within changing ecosystems. If selection is driving divergent phenotypes, this suggests these altered traits may provide urban-living individuals an adaptive advantage. We examined whole-organism performance in Australian water dragons (*Intellagama lesueurii*), a large agamid lizard with divergent morphological traits in urban areas, to determine if urban individuals have performance advantages in endurance or sprint speed, or have a clinging advantage on artificial substrates (concrete) compared to natural material (tree bark). To test if performance advantages were related to an individual's origin population (suggesting a genetic basis) or the habitat it was raised within (suggesting phenotypic plasticity), we experimentally raised lizards from urban and natural populations in either urban- or natural-style environments (2 x 2 factorial design). We also examined if there were trade-offs between performance measures (endurance vs. sprint speed, clinging to concrete vs. clinging to tree bark). We found a trade-off between endurance capacity and sprint speed, but not between the two clinging measures. We did not find any performance advantages for urban lizards; suggesting no urban adaptation for altered performance abilities. In fact, lizards from urban populations reared in an urban-style environment had a significantly lower endurance capacity than lizards from natural populations reared in natural-style environments. This study underscores the importance of considering performance capacity when examining urban evolution, divergent phenotypes of urban living populations, and the need for further research in the field of mechanistic urban ecology.

## Introduction

Urban habitats are exceptional crucibles for studying evolutionary ecology (Shochat et al. 2006; Bonier 2012; Winchell et al. 2016). Urbanisation dramatically transforms landscapes, and can alter the selective forces affecting remnant populations (Alberti et al. 2017), resulting in physiological (French et al. 2008; Atwell et al. 2012; Bonier 2012) and morphological traits (Marnocha et al. 2011; Littleford-Colquhoun et al. 2017) that are divergent from individuals living in natural landscapes (urban-derived divergent phenotypes). For example, both dark-eyed juncos (*Junco hyemalis*) and tree lizards (*Urosaurus ornatus*) decrease their physiological stress response in urban populations (French et al. 2008; Atwell et al. 2012). Urban dwelling leaf-cutter ants (*Atta sexdens*) dramatically increase their heat tolerance compared to wild-living conspecifics (Angilletta Jr. et al 2007). Urbanised landscapes are also associated with altered body size and/or shape in several species of lizard (tree lizards, French et al. 2008; *Anolis sagrei*, Marnocha et al. 2011; *Lophognathus temporalis*, Iglesias et al. 2012; *Anolis cristatellus*, Winchell et al. 2016; *Intellagama lesueurii*, Littleford-Colquhoun et al. 2017) and house mice (*Mus musculus*; Slábová and Frynta 2007). These divergent morphological phenotypes associated with urbanised populations are generally thought to be adaptive. Yet how these traits directly provide a mechanistic advantage in urban landscapes, such as performance capacity, is comparatively understudied.

Measures of whole-organism performance can demonstrate how variation in morphology and physiology interrelate, provide advantages within specific environments, and affect an organism's life history and fitness (Huey et al. 1984; Garland Jr and Losos 1994; Irschick et al. 2008; Lailvaux and Husak 2014). Within *Anolis* spp. variation in body size and limb morphology provides both sprinting and jumping performance advantages on perching substrates varying in size and shape, relating to habitat types (Losos and Sinervo 1989; Losos 1990a). Divergent *Anolis* limb morphology has also been seen between urban and natural-living populations (*Anolis cristatellus*; Winchell et al. 2016). Therefore, urban-derived divergent morphological phenotypes may have arisen to provide performance advantages

(e.g., increased sprint speed or endurance capacity) for individuals living in anthropogenic environments, which relate to the ecological demands of their novel habitat (Winchell et al 2016). Similarly, clinging ability also correlates with forelimb morphology in several lizard species adapted for different habitat types (*Liolaemus* spp., *Phymaturus* spp., *Diplolaemus sexcinctus*; Tulli et al. 2011). Urban environments have increased flat impervious surfaces (Dale and Frank 2014, Barnett 2015), so we would expect that the divergent morphology of urban-living wildlife (e.g., lizards; Marnocha et al. 2011; Iglesias et al. 2012; Winchell et al. 2016; Chapter IV) may increase their ability to climb on concrete and other flat surfaces that are common within urban landscapes.

If urban-derived divergent phenotypes are providing performance advantages, such as sprint speed, endurance and clinging ability, within urban habitats, then these may be adaptive and increase fitness. For example, increased sprint speed is positively correlated with increased territory size and number of offspring in male collared lizards (*Crotaphytus collaris*; Husak et al. 2006). However, selection favouring specific morphological traits or performance capabilities may result in trade-offs (negative correlation between traits; Lailvaux & Husak 2014). Lacertid lizards trade-off sprint speed and endurance due to physiological differences in muscle power output and fatigue resistance (Vanhooydonck et al. 2001, 2014). As such, we would expect that if the urban environment is driving selection for certain performance abilities, then it may come at a cost to another. Urban environments tend to have limited suitable habitat and space, resulting in urban lizards having decreased home range sizes (e.g., wall lizards, *Podarcis muralis*; Brown et al. 1995). If urban populations have smaller home ranges, distances between mates (Chapter I), and territories to patrol, selection for endurance capacity may be relaxed. Previous research experimentally elevating testosterone in side-blotched lizards (*Uta stansburiana*) observed increases in endurance, activity, and home range size (Sinervo et al. 2000); suggesting that there may be a hormonally-mediated link between home range size and performance ability. Furthermore, a reduction in endurance capacity could potentially promote increased sprint speed, which may

enhance escape from novel predators and other anthropogenic mortality sources (e.g., foxes, humans, domestic animals, and traffic; Koenig et al. 2002). With respect to clinging ability in lizards, variance in physiological processes and morphological features, such as toes pads (Irschick et al. 1996) or claws (Zani 2000), facilitates clinging ability within the specific habitat niche of a species (Tulli et al. 2001). However intra-specific differences in clinging ability may not necessarily require a trade-off, because the physiological mechanisms underpinning clinging to one substance or another may be quite similar (e.g., muscle groups). Rather, any difference in clinging ability would likely be related to morphological differences between habitats (e.g., variation in limb length) that could provide a mechanical advantage. As such, if lizard species in urban areas are exhibiting divergent limb morphology (Iglesias et al. 2012; Littleford-Colquhoun et al. 2017; Chapter IV), then this may be providing a clinging advantage for artificial substrates without compromising the individual's clinging ability on natural substrates.

We examined three measures of performance capacity, sprint speed, endurance, and clinging (two substrates for clinging: concrete, tree bark), in Australian water dragons (*Intellagama lesueurii*), a species that exhibits different morphological phenotypes between urban and natural populations (Chapter IV). Previously we examined if urban-derived divergent morphological phenotypes (head shape, body length, and limb length) was driven by heritable traits related to their origin population (suggesting rapid localised adaptation) or related to the habitat a juvenile was reared within (suggesting phenotypic plasticity; Chapter IV). Lizards from urbanised or natural populations were reared in either urban- or natural-style enclosures (2 x 2 factorial experimental design; see Methods), and we repeatedly measured their morphology over their first year (six times; Chapter IV). Dragons from urban origin populations raised in urban-style habitats had a slower limb growth rate, compared to individuals from natural origin populations raised in natural-style enclosures. In this study, we use these same individuals to test if this difference in limb morphology and body size relates to altered performance capacity. We examined if there were increased performance capacity



for urbanised lizards (related to either origin populations or habitat treatment), and if there were trade-offs between specific performance traits (endurance vs. sprint speed; concrete clinging vs. tree bark clinging) allowing for the promotion of one ability with a corresponding reduction in another. If the known divergent morphological phenotypes of urbanised lizards provide a performance advantage, then we would expect to see: 1) an increased sprinting ability in dragons from urban-origin populations, 2) a decreased endurance ability in dragons from urban-origin populations, 3) an increased concrete clinging ability in dragons from urban origin populations. We also examined trade-offs between performance measures, to allow a more thorough interpretation of our data. Based on previous research, we predicted a negative correlation between sprint speed and endurance capacity (suggesting a trade-off), and a positive correlation between concrete and tree bark clinging ability (suggesting no trade-off, as the physiological underpinnings are the same).

## **Methods**

### ***Study Species and Field Sites***

Australian water dragons are large agamid lizards (maximum snout-vent length [SVL] = 304 mm; Thompson 1993) native to eastern Australia (Cogger 2014). Typically water dragons are associated with freshwater bodies (creeks, rivers, dams, and lakes) with forested areas (Cogger 2014), however they are also common in urban greenspaces (Littleford-Colquhoun et al. 2017) and are found a number of Australia's largest cities (Canberra, Brisbane, and Sydney; Wilson and Swan 2017). With the context of locomotion, they are fully adept at climbing, bipedal sprinting, and swimming (Baird et al. 2012; Doody et al. 2014; Cogger 2014).

We collected gravid female water dragons from seven sites (4 urban, 3 natural) within a 50 km radius within the greater Sydney area in New South Wales, Australia (see Supplementary Materials for location details). Natural sites, although not free from human disturbance, were generally associated with treed riparian areas and natural waterways, and a

relatively low human presence. Dragons in natural areas were commonly observed on tree branches and other woody debris (e.g. logs and stumps). Urban sites had a dense local human population, and a landscape that was widely human-modified (e.g., concrete, buildings, gardens, roads). Dragons in urban areas were commonly observed on masonry (e.g., garden walls, walkways, and brickwork) and in planted and managed gardens. We transported females to an indoor captive environment, induced oviposition, and then released them at their site of capture post-oviposition (see Supplementary Materials for details). Clutches of eggs were identically incubated throughout development (26.5 °C; Harlow 2001) and, upon emerging, hatchlings were allocated to a captive habitat treatment (experimental group, N = 59; see below).

### ***Lizard Rearing and Experimental Groups***

All lizards were raised in outdoor enclosures at Macquarie University to replicate comparable weather and climate that origin populations would naturally experience around Sydney. We raised hatchlings in a 2 x 2 factorial experiment with lizards from urban or natural origin populations being reared in natural- and urban-style enclosures (Chapter IV). The purpose of this was to disentangle whether variation in morphology and performance was driven by heritable traits (associated with the individual's origin population and suggesting rapid localised adaptation) or through phenotypic plasticity or prior experience (associated with the habitat treatment). Natural-style enclosures were outfitted with native vegetation, wooden perching of varying diameter (i.e., 5-50 mm), a shaded area, and a water pool. Urban-style enclosures were outfitted with hard, flat surfaces (paving stones, concrete blocks, steel sheets, and roofing tiles), eucalypt mulch, a shaded area, and water pool. Each enclosure (5 x 2.5 m) was constructed within a predator exclusion area. Both the natural- and urban-style enclosures were replicated three times, resulting in a total of six enclosures (Chapter IV). Siblings were split equally across natural- and urban-style enclosures, allowing parental and clutch effects to be controlled for in our experimental design (Chapter IV). The four experimental groups

were: 1) natural origin populations reared in a natural-style enclosure (nN), 2) urban origin populations reared in a natural-style enclosure (uN), 3) natural origin populations in an urban-style enclosure (nU), and 4) urban origin populations in an urban-style enclosure (uU). After 1 year of being reared within these enclosures we measured the performance abilities of the juvenile lizards.

### ***Performance Measures***

Prior to the beginning the performance trials SVL (mm) and mass (g) of each lizard were recorded, and immediately prior to each individual's performance trial their body temperature ( $^{\circ}\text{C}$ ) was also recorded. All performance trials were conducted by the same researcher (JBG) to ensure consistency.

### ***Endurance and Sprint Speed***

Both endurance and sprint speed followed similar methods as described in Noble et al. (2014), with the exception that lizards were given a five day rest period between repeated measures. Each measurement was repeated three times. The sprint speed trial was measured on a 1.5 m long running track lined with a textured rubber mat, marked at 0.25 m intervals (Noble et al. 2014). Lizards were placed at the starting line and stimulated to run by pinching the base of the tail with a blue nitrile gloved hand. We recorded sprint speed using a Panasonic HD video camera (120 fps) and quantified the maximum speed during each trial by determining the shortest time it took the individual to cross between the 0.25 m intervals, which was then transformed into m/s.

The endurance trial immediately followed the sprint speed trial and measured the time (sec) it took a lizard to tire during continuous running (Noble et al. 2014). The endurance arena was constructed from a clear plexiglass box (1.1 x 0.08 m) placed on a human treadmill

set to a fixed running speed of 1.0 km/h (Garland & Else 1987; Noble 2014). During the trial lizards were placed in the first third (0.36 m) of the treadmill, so that as they tired there would be enough time to pinch the base of the tail 10 consecutive times before the lizard was carried along the track and pushed off the end (Garland & Else 1987; Baxter-Gilbert et al. 2018).

### *Clinging Ability*

The two clinging trials occurred two days apart, allowing for one rest day between trials. A trial consisted of three measures of a lizards' ability to grip and hold on to the horizontal surface (i.e., a concrete slab and a section of tree bark) while being pulled backward at a steady pace until it detached. The lizard was attached to a Pesola spring dynamometer (N; medio-line, model #40006) by a 0.5 m length of cotton string tied in a harness anterior to the pelvic girdle. The direction of the pulling was kept level to the substrate (preventing upward and downward pulling forces) and directly to the rear of the lizard (preventing lateral forces). The testing arenas were 0.6 x 0.2 m with a 0.3 m black plastic wall around three of the four sides. The concrete base was an aerated concrete brick (Hebel, PowerBlock). The tree bark base was a 0.6 x 0.2 m section of turpentine (*Syncarpia glomulifera*), a native tree species found throughout the water dragon's distribution in New South Wales. Although both flat surfaces, this measure examined the lizard's ability to adhere to natural and anthropogenic substrates.

### *Statistical Analyses*

Before analysis we explored our data following Zuur et al. (2009), and found that our response variables (endurance, sprint speed, and clinging) adhered to a normal distribution, and had no outliers. There was also no strong collinearity between our model predictor variables.

To fit our mixed effect models (see specific details below) we used the MCMCglmm R package (Hadfield 2010) and performed analyses in R v 3.03 (R Core Team 2016). Mixed effect models were used to incorporate dependency among observations of dragons from the same clutch and enclosures, as well as repeated observations of the same individual in our analyses. This R package uses a Bayesian analysis framework with a Markov chain Monte Carlo (MCMC) sampling approach to fit models. MCMC is used to obtain the distribution of each model parameter, and it requires specification of a probability distribution (prior; Zuur et al. 2013; Gelman et al. 2014). We used inverse Wishart priors for regression and standard deviation parameters ( $V = 1$ ,  $\nu = 0.002$ ; Hadfield 2010). In each model, using a single chain, we generated model parameters 1,500,000 times (iterations), discarded the first 150,000 parameters (burn-in), and sampled every 1,350th parameter (thinning rate). Continuous fixed factors in all models were mean centred prior to analysis. We visually inspected all trace plots from each model to ensure chains were well mixed (i.e., were sampled randomly). We also assessed mixing by ensuring autocorrelation levels were low ( $\text{lag} < 0.1$ ) using the autocorr function, as well as performing Geweke and Heidelberg auto-correlation diagnostics (from the R package coda; Plummer et al. 2015). We also assessed assumptions of normality of residuals and homogeneity of variance for each model (Zuur et al. 2009). In summary, mixing of chains for fixed and random effects was satisfactory for all our models, and visual inspections of plots did not reveal any deviations from homoscedasticity or normality of residuals.

We present pooled posterior modes and 95% credible intervals for each model parameter. Model parameters were considered significant when credible intervals did not include 0, and the pMCMC values were less than 0.05 (Hadfield 2010).

### *Trade-offs in performance metrics*

To assess if there were correlations between performance measures we used two multivariate linear mixed effect models (Dingemanse and Dochtermann 2012; Careau and Wilson 2017). We analysed correlations between endurance (sec) and sprinting speed (body lengths per sec), and clinging to concrete and tree bark (N) in separate models. For the endurance vs. performance model, we included the fixed factors of trial order, experimental group, body temperature, and SVL. For the clinging concrete vs tree bark model, we included the same fixed factors. Both models also included a random intercept and slope for juvenile identity across trial order. We assumed a multivariate normal distribution with a variance-covariance structure specifying the within- and among-individual variance and covariances among performance metrics (Hadfield 2010, Dingemanse and Dochtermann 2012).

#### *Does dragon performance differ across an urban-natural gradient?*

We examined if dragon performance metrics varied across urban-natural experimental groups using four separate identical linear mixed effect models for each response variable. The response variables were endurance (sec), sprint speed (body lengths per sec), clinging to concrete (N), and clinging to tree bark (N). We analysed clinging ability separately for each substrate, because it was significantly lower on concrete than tree bark ( $\beta = -0.523$ , 95% *credible interval* = -0.640, -0.419,  $pMCMC < 0.001$ ).

Fixed factors were trial order (continuous), experimental group (categorical with four levels: nN, nU, uN, and uU), and body temperature (continuous). There were significant trial order effects in our data (Table 2 and 3), potentially due to training effects, and inclusion of them in our models statistically controlled for this potentially confounding variable. We did not include SVL as a fixed effect, although it was significantly correlated with all performance metrics (endurance:  $corr = 0.5$ ,  $\beta = 16.049$ , 95% *credible interval* = 12.312, 21.065,  $pMCMC < 0.001$ ; sprint:  $corr = -0.3$ ,  $\beta = -0.618$ , 95% *credible interval* = -1.083, -0.148,  $pMCMC = 0.012$ ; clinging:  $corr = 0.6$ ,  $\beta = -0.430$ , 95% *credible interval* = 0.318,

0.570,  $pMCMC < 0.001$ ). The reason being that SVL significantly differs among experimental groups (Chapter IV), and this could be the mechanism driving differences in performance. Thus, we did not want to statistically control for SVL in this study.

To incorporate dependency among observations of the same individual we included a random intercept and slope for dragon identity across trial order; and to incorporate the dependency among observations of related dragons and dragons from the same captive rearing environment we included random intercepts for clutch and enclosure. We calculated contrasts between experimental groups that were not tested in the model by default (uN vs. nU, uU vs. nU, and uU vs. uN) by manually comparing posterior distributions from model parameters.

## Results

### *Trade-offs in performance metrics*

At the population level, dragon endurance and sprinting ability was significantly negatively correlated among individuals ( $r_{ind} = -0.979$ , 95% credible interval = -1.341, -0.060), as such as endurance capacity increased, sprint speed decreased and vice versa. In contrast, dragon endurance and sprinting ability was not correlated at the within-individual level (Table 1A).

At the population level, a dragon's ability to grip concrete and tree bark was significantly positively correlated among-individuals ( $r_{ind} = 0.706$ , 95% credible interval = 0.385, 0.878). However, at the within-individual level dragon clinging ability on concrete and tree bark was not correlated (Table 1B).

### *Does dragon performance differ across an urban-natural gradient?*

Dragons from urban origin populations and raised in urban-style enclosures (uU) had a significantly shorter endurance time than dragons from natural populations that were raised in

natural-style enclosures (nN;  $\beta = -16.747$ , 95% *credible interval* = -37.024, 0.485,  $pMCMC = 0.052$ ). The other comparisons of endurance capacity among experimental groups (uN, nU, and nN) were not significantly different (Table 2). Dragons sprint speed (Table 3), clinging ability on concrete (Table 4A) and tree bark (Table 4B) did not differ among experimental groups.

## Discussion

We were unable to detect any performance advantages in urban lizards, related to either urban origin population (uU and uN) or urban habitat treatment (uU and nU), across any of the four metrics we examined (i.e., endurance, sprint speed, and clinging ability on concrete and tree bark). Our predictions were not supported with regard to increased sprint speed (prediction 1) or concrete clinging ability (prediction 3) for urban lizards, but was supported for urban lizards having a decrease in endurance capacity (prediction 2). With respect to performance trade-offs, both our predictions of a trade-off occurring between endurance capacity and sprint speed and no trade-off in clinging ability between concrete and tree bark was supported at the population-level (among individuals); however, it was not evident at the individual-level (within individuals).

The trade-off we observed between endurance capacity and sprint speed at the population-level is similar to findings from lacertid lizards (19 species; Huey et al. 1984; Vanhooydonck et al. 2001, 2014), however the occurrence of this trade-off was contrary to studies from several other species (23 lizard species, Pinch and Claussen 2003; de Albuquerque et al. 2015). Our finding supports theorised trade-offs in performance between endurance and sprint speed based on variation in muscle composition, energetic processes, metabolic pathways, and other physiological differences (Garland Jr. 1984, 2014; Vanhooydonck et al. 2014). It suggests that individual water dragons, regardless of treatment group, consistently differ in morphological and physiological traits that enhance their



sprinting ability while hindering their endurance ability. Interestingly, we did not detect this trade-off when examining correlations between performance scores at the within-individual level. However, we detected an effect of trial order, which may reflect a training effect (increasing performance capacity over time and number of trials; Husak et al. 2016) and this may have influenced correlations between these traits at the within-individual level.

Alternatively, individuals may differ in their motivation between trials, and this could also impact within-individual correlations between traits, although we tried to ameliorate any potential effect by using maximal measures. In regards to clinging ability, we did not see a trade-off between a lizards' ability to hold on to a substrate made of either concrete or tree bark. Instead, they were significantly, positively correlated. This suggests that a lizard's ability to grip and hold on to an artificial substrate (concrete) does not come at a cost to its ability to cling to natural substrates (tree bark). In general, the muscles and physiological mechanisms required for a clawed lizard species to cling to different substrates are essentially the same, and the differences we had predicted between urban and natural lizards was based on the idea that the known morphological differences (Chapter IV) would provide a purely mechanical advantage promoting their ability to cling to concrete. This is different to the physiological and mechanical difference, which is seen between sprint (anaerobic) and endurance (aerobic) locomotion (Huey and Hertz 1982). We did not detect a correlation between clinging to tree bark and concrete at the within-individual level. Our detection of performance trade-offs and correlations at the population-level but not the individual-level underscores the importance of partitioning performance trade-offs at the within- and among-individual levels using multivariate mixed models to fully understand these trade-offs (see Careau and Wilson 2017).

Our findings did not support our hypothesis that urbanisation has selected for any performance advantages (sprint speed, endurance, or clinging ability to concrete or tree bark) related to either the individual's origin population (uU or uN) or rearing habitat (uU or nU). Interestingly, the uU experimental group (urban origin population reared in an urban-style

enclosure) exhibited significantly lower endurance times compared to lizards from the nN experimental group (natural origin population reared in a natural-style enclosure). This demonstrated that urban dwelling lizards from urban origin population had a decreased endurance capacity (supporting our second prediction), however this did not result in a subsequent improvement to this experimental group's (uU) sprinting ability (prediction 1). Previous research on these individuals demonstrates that the uU experimental group had smaller SVL and slower growth rates (Chapter IV), and slower growth rates have been correlated in decreased endurance capacity in common lizard (*Lacerta vivipara*; Clobert et al. 2000). Lacertid lizards have a positive correlation between increased sprint speed and increased limb length (Bauwens et al. 1995), which supports our finding of urban lizards (uU) with shorter limbs not being able to increase their sprint speed performance. Since we did not detect an increase in sprinting speed, the reduction in urban lizard endurance capacity is not likely related to an urban-related performance trade-off, but may merely be a by-product of a decreased growth rate. However, this still does not provide any mechanistic rationale for why uU individuals have are smaller and have a reduced growth rate, while other dragons from urban origin populations (uN) or raised in urban-style enclosures (nU) do not. An alternative explanation is that a reduced endurance ability in urban water dragons is a result of negative directional selection (selection favouring poor performance; Irschick et al. 2008) which may be related to other performance measures we did not examine (e.g., jumping or swimming).

In regards to clinging performance, we did not find any support for our prediction that urban lizards have an increased clinging ability on concrete (prediction 3). Our previous research has demonstrated that the uU experimental group had shorter limbs and SVL compared to nN individuals, and we thought this might provide an increased ability to clinging to anthropogenic substrates. Longer hind limbs and shorter forelimbs have been associated with increased clinging performance in *Anolis* spp. (Losos 1990a,b), while saxicolous scincid lizards, as well as arboreal and saxicolous liolaemid lizards, use longer forelimbs to increase clinging ability (Goodman, 2006; Tulli et al. 2011). This demonstrates

that the limb configuration that promotes benefits in clinging ability is not consistent across species, and likely the limb configuration that promotes clinging ability varies between species in relation to different habitats, body sizes and shapes. However, as no difference in clinging ability was seen between the water dragon experimental groups, it suggests that the divergent morphological phenotype observed in urbanised individuals (uU; Chapter IV) is not related to their clinging ability between natural (native tree bark) and anthropogenic substrates (concrete).

The relationship between morphology, physiology, performance, and environment is well established (Arnold 1983; Losos 1990a,b; Melville and Swain 2000; Calsbeek and Irschick 2007; Goodman et al. 2008; Kaliontzopoulou et al. 2012), as is the presence of divergent morphological phenotypes in urban environments (Slábová & Frynta 2007; French et al. 2008; Marnocha et al. 2011; Iglesias et al. 2012; Hutton and McGraw 2016; Winchell et al. 2016). However, in this study we were unable to detect any performance advantages provided to urban lizard with divergent phenotypes, as a result of either urban origin population or urban-style rearing environment. The presence of divergent phenotype in urbanised populations suggests that either a selective force is driving them, or that the urban environment lacks the selective force to maintain their natural state. Ultimately, we are yet to determine the adaptive reason for the divergent morphometric phenotype seen in urban water dragons (Chapter IV); however it may be related to performance ability we have not measured (climbing, jumping, or swimming), novel mortality sources (Sasaki et al. 2009), or driven by divergent behaviour (Losos et al. 2004) or altered mating systems related to anthropogenic environments (Järvenpää and Lindström 2004; Saaristo et al. 2009). Overall, there is a substantial gap in our knowledge of mechanistic urban ecology (Shochat et al. 2006), and how divergent phenotypes in urban environment affect whole-organism performance, survival, and fitness. Although our study did not detect any performance advantages related to urbanised lizards, we strongly encourage further studies examining divergent phenotypes in urban

environment incorporate measures of performance into their research in an attempt to understand the mechanism and adaptive nature of these morphological changes.

### **Acknowledgements**

We would like to thank the many individuals who assisted in fieldwork (P. Bolton, T. Damasio, C. Fryns, F. Kar, S. Klopper, L. Monk-Whipp, M. Mühlenhaupt, D. Noble, and K. Umbers), as well as C. Wilson and the exceptional husbandry team at Macquarie University's Lizard Lab. This research was supported by an International Macquarie University Research Excellence Scholarships and Natural Sciences and Engineering Research Council of Canada Postgraduate Scholarship (awarded to JBG). Experimental protocols were approved by the Macquarie University Animal Ethics Committee (ARA # 2015/023) and New South Wales National Parks and Wildlife Services (scientific license # SL100570).

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## Tables

**Table 1.** Variance (dark grey shaded), covariance (white shaded) and correlation (light grey shaded) estimates between **(a)** endurance (sec) and sprinting speed (body lengths per sec), and **(b)** clinging (N) to concrete and tree bark for among- and within-individual error with associated 95% credibility intervals (in brackets) testing for correlations between performance metrics in *Intellagama lesueurii*. We bolded variables if the 95% credible intervals did not include 0 to indicate their significance.

### **(a) Endurance versus sprint speed**

*Among individual variances, covariances, and correlations and their associated 95% credibility intervals*

	Endurance	Sprint speed
Endurance	<b>197.207 (0.068, 453.213)</b>	<b>-0.979 (-1.341, -0.060)</b>
Sprint speed	-13.675 (-31.593, 1.310)	<b>2.018 (0.617, 3.720)</b>

*Within individual variances, covariances, and correlations and their associated 95% credibility intervals*

	Endurance	Sprint speed
Endurance	<b>705.530 (548.128, 996.275)</b>	0.007 (-0.233, 0.281)
Sprint speed	0.285 (-11.463, 16.817)	<b>3.440 (2.589, 4.578)</b>

### **(b) Clinging to tree bark versus clinging to concrete**

*Among individual variances, covariances, and correlations and their associated 95% credibility intervals*

	Clinging to tree bark	Clinging to concrete
Clinging to tree bark	<b>0.156 (0.089, 0.250)</b>	<b>0.706 (0.385, 0.878)</b>
Clinging to concrete	<b>0.095 (0.032, 0.148)</b>	<b>0.115 (0.060, 0.195)</b>

*Within individual variances, covariances, and correlations and their associated 95% credibility intervals*

	Clinging to tree bark	Clinging to concrete
Clinging to tree bark	<b>0.164 (0.128, 0.213)</b>	0.056 (-0.118, 0.253)
Clinging to concrete	0.011 (-0.022, 0.040)	<b>0.171 (0.130, 0.211)</b>

**Table 2.** Outcomes of linear mixed-effect models testing if endurance (sec) was affected by experimental group (urban origin and urban environment, uU; natural origin and urban environment, nU; urban origin and natural environment, uN; natural origin and natural environment, nN). Models also include the fixed factors of trial order and body temperature (both mean centred). The models also included a random slope and intercept of dragon identity, and a random intercept of clutch and enclosure. We bold significant variables in each model. For experimental group contrasts we calculated manually by comparing posterior distribution from model estimates, parameter estimates were considered significant when the credible intervals did not include 0 and this is represented in the *pMCMC* column using ‘s’ for significant and ‘ns’ for non-significant. The model included 177 observations of endurance for 59 dragons that came from 22 clutches and 6 enclosures.

<i>Fixed effects</i>	$\beta$	2.5%	97.5%	<i>pMCMC</i>
<b>Intercept</b>	<b>136.834</b>	<b>124.837</b>	<b>150.727</b>	<b>&lt;0.001</b>
<b>Order</b>	<b>14.745</b>	<b>11.623</b>	<b>17.921</b>	<b>&lt;0.001</b>
Treatment (nU; ref = nN)	-10.952	-28.741	6.211	0.220
Treatment (uN; ref = nN)	-13.563	-26.393	8.880	0.288
<b>Treatment (uU; ref = nN)</b>	<b>-16.747</b>	<b>-37.024</b>	<b>0.485</b>	<b>0.052</b>
Treatment (uN; ref = nU)	3.576	-15.074	19.512	ns
Treatment (uU; ref = nU)	-8.979	-24.749	7.481	ns
Treatment (uU; ref = uN)	-15.339	-27.939	7.258	ns
Body temperature	0.717	-3.179	3.974	0.874

<i>Random effects</i>	$\sigma^2$	2.5%	97.5%
Individual	476.876	210.088	771.564
Clutch	0.290	0.000	137.006
Enclosure	-0.262	0.000	75.860
Residual	382.524	296.432	491.032

**Table 3.** Outcomes of linear mixed-effect models testing if sprinting speed (body lengths per s) was affected by experimental group (urban origin and urban environment, uU; natural origin and urban environment, nU; urban origin and natural environment, uN; natural origin and natural environment, nN). Models also include the fixed factors of trial order and body temperature (both mean centred). The models also included a random slope and intercept of dragon identity, and a random intercept of clutch and enclosure. We bold significant variables in each model. For experimental group contrasts we calculated manually by comparing posterior distribution from model estimates, parameter estimates were considered significant when the credible intervals did not include 0 and this is represented in the *pMCMC* column using ‘s’ for significant and ‘ns’ for non-significant. The model included 177 observations of sprinting speed for 59 dragons that came from 22 clutches and 6 enclosures.

<i>Fixed effects</i>	$\beta$	2.5%	97.5%	<i>pMCMC</i>
Intercept	<b>13.028</b>	<b>11.899</b>	<b>14.038</b>	<b>&lt;0.001</b>
Order	<b>0.342</b>	<b>0.092</b>	<b>0.683</b>	<b>0.020</b>
Treatment (nU; ref = nN)	1.182	-0.407	2.326	0.128
Treatment (uN; ref = nN)	-0.248	-1.285	1.390	0.926
Treatment (uU; ref = nN)	1.026	-0.403	2.646	0.196
Treatment (uN; ref = nU)	-0.941	-2.604	0.233	ns
Treatment (uU; ref = nU)	-0.178	-1.383	1.237	ns
Treatment (uU; ref = uN)	1.334	-0.425	2.387	ns
Body temperature	-0.028	-0.230	0.434	0.660

<i>Random effects</i>	$\sigma^2$	2.5%	97.5%
Individual	2.546	1.022	4.922
Clutch	0.005	0.000	0.711
Enclosure	0.009	0.000	0.559
Residual	3.403	2.600	4.346

**Table 4.** Outcomes of linear mixed effect models testing if **(a)** clinging to tree bark (N) and **(b)** clinging to concrete (N) was affected by experimental group (urban origin and urban environment, uU; natural origin and urban environment, nU; urban origin and natural environment, uN; natural origin and natural environment, nN). Models also include the fixed factors of trial order and body temperature (both mean centred). The models also included a random slope and intercept of dragon identity, and a random intercept of clutch and enclosure. We bolded significant variables in each model. We manually calculated experimental group contrasts by comparing posterior distributions from model estimates, parameter estimates were considered significant when the credible intervals did not include 0 and this is represented in the *pMCMC* column using ‘s’ for significant and ‘ns’ for non-significant. Both models included 177 observations of clinging ability for 59 dragons that came from 22 clutches and 6 enclosures.

	<i>(a) Clinging to tree bark (N)</i>				<i>(b) Clinging to concrete (N)</i>			
<i>Fixed effects</i>	$\beta$	2.5%	97.5%	<i>pMCMC</i>	$\beta$	2.5%	97.5%	<i>pMCMC</i>
Intercept	<b>3.530</b>	<b>2.868</b>	<b>3.953</b>	<b>&lt;0.001</b>	<b>2.955</b>	<b>2.316</b>	<b>3.586</b>	<b>&lt;0.001</b>
Order	0.009	-0.078	0.070	0.930	0.018	-0.041	0.096	0.552
Treatment (nU; ref = nN)	-0.258	-1.022	0.463	0.432	-0.177	-1.126	0.537	0.516
Treatment (uN; ref = nN)	-0.361	-0.773	0.271	0.308	-0.117	-0.577	0.310	0.538
Treatment (uU; ref = nN)	-0.441	-1.461	0.154	0.116	-0.353	-1.310	0.449	0.274
Treatment (uN; ref = nU)	0.115	-0.716	1.004	ns	0.240	-0.709	0.902	ns
Treatment (uU; ref = nU)	-0.326	-0.757	0.166	ns	-0.232	-0.635	0.255	ns
Treatment (uU; ref = uN)	-0.226	-1.060	0.463	ns	-0.368	-1.110	0.526	ns
Body temperature	-0.043	-0.221	0.128	0.512	-0.038	-0.163	0.072	0.504
<i>Random effects</i>	$\sigma^2$	2.5%	97.5%		$\sigma^2$	2.5%	97.5%	
Individual	0.236	0.098	0.539		0.148	0.050	0.341	
Clutch	0.003	0.000	0.342		0.003	0.000	0.213	
Enclosure	0.020	0.000	0.590		0.056	0.000	0.785	
Residual	0.156	0.118	0.202		0.165	0.119	0.207	



## Supplementary Materials

### Methods

#### *Sites*

Eastern water dragons were collected at 7 sites in a 50 km radius around the greater Sydney area in New South Wales, Australia, from October to December 2015. The urban sites were: 1) the Manly to Shelly Beach boardwalk in Manly (33.79° S, 151.29° E), 2) the Taronga Zoo grounds in Bradley's Head (33.84° S, 151.24° E), 3) the Chinese Garden of Friendship in Darling Harbour (33.88° S, 151.20° E), and 4) the Macquarie University Campus in Marsfield (33.77° S, 151.11° E). The natural sites were: 1) the Grose River near Yarrimundi (33.62° S, 150.67° E), 2) Lane Cove National Park near Chatswood West (33.79° S, 151.14° E), and 3) Brown's Watering Hole near North Epping (33.76° S, 151.11° E).

#### *Handling, Husbandry, and Egg Collection*

All gravid female dragons were captured by hand or noose-pole. Once in hand, gravid females were palpated for the presence of eggs and then transported to Macquarie University (Marsfield, NSW), or retained at Taronga Zoo (Sydney, NSW). Lizards were housed in 100 L plastic tubs with moist substrate and water, for 3 days to allow for oviposition. The room temperature was set to 30 °C (Harlow 2001) to maintain optimum body temperature over the three day oviposition period. To induce egg laying, gravid females were administered a dose of 100 mg/kg calcium gluconate (Phebra, Lane Cove, NSW, AU) into the left forearm, then rested for 60 minute, followed by a shallow intraperitoneal injection of synthetic oxytocin (Syntocin, Ilium, Glendenning, NSW, AU) at a dose 60 IU/kg (Mader 1996; Harlow and Taylor 2000). Once oviposition was complete, entire clutches were placed in 500 ml plastic containers with 115 g of moistened vermiculite (-150 kPa; 130% water by dry mass of vermiculite; Harlow 2001). The containers were fitted with an oxygen permeable

polyethylene membrane (Glad Wrap, Glad Products of Australia, Rhodes, NSW, AU) and placed in incubators set to 26.5 °C, allowing for equal sex ratios during development (Harlow 2001).

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## General Discussion

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The research within this thesis focused on understanding urban-derived divergent behavioural and biological phenotypes of water dragons, and the potential mechanisms causing these traits. To accomplish this, I have examined the differences in population density and social interactions (Chapter I), behavioural traits (Chapter II), innate antipredator responses (Chapter III), morphology (Chapter IV), and performance capacity (Chapter VI) between urban water dragons and their natural-living counterparts. To accomplish the goals of Chapter VI, I also conducted trials to examine the comparability and repeatability of three commonly used methods for measuring endurance capacity (Chapter V), and was able to provide insight into the use of different endurance metrics and study designs in performance research (using water dragons as a model species). Overall, and pertaining more directly to urban evolution, my research has identified several urban-derived divergent phenotypes, including increased rates of male combat (Chapter I), increased boldness (Chapter II), decreased body size paired with increased limb length and head size (Chapter III), and decreased endurance capacity (Chapter VI). Furthermore, in examining the potential mechanisms that caused these traits my research has identified that increased boldness (Chapter II) and divergent urban morphology (Chapter IV) have a genetic origin, suggesting they are heritable and that water dragons are likely undergoing urban evolution.

### Urban-derived divergent phenotypes

Previous research has predicted that abrupt changes in evolutionary trajectory may occur within novel or peripheral environments where there is sufficient gene flow from a native source population (punctuated niche evolution; Holt et al. 2003). This prediction operates on the idea that inflow of genes into sink populations can provide the required variability for these populations to regularly test a gene's adaptive potential within an environment different from the species origin (Holt et al. 2003). This trial and error process of sorting genes within

novel landscapes results in long periods of stable genetic equilibria, until a specific gene demonstrates a strong adaptive advantage and rapidly spreads throughout the sink population (Holt et al. 2003). This prediction is strengthened by recent research that suggests that when phenotypic plasticity is heritable, a higher degree of plasticity is selected for within novel environments (Chevin & Lande, 2011), which creates the potential for increased trait variability in landscapes that are distinctly different from a species' norm. Bolstered trait variability may further increase the likelihood of urbanised populations encountering an adaptive gene/trait that may trigger punctuated niche evolution (Holt et al. 2003) within urban areas.

There are however, several distinct spatial and temporal differences in how gene flow can effect a species adaptive capacity between populations expanding their core range (Kirkpatrick & Barton 1997; Holt et al. 2003) and ones encountering urbanisation. First, the slow growth of urban areas (e.g., Sydney's growth over the last 200 years) would allow for populations experiencing urbanisation to generationally trial traits, as gene flow is slowly reduced over time. This is notably different to species at the periphery of their range abruptly encountering new landscapes (Holt et al. 2003) or exotic species in entirely novel habitats (Kearney et al. 2008). Second, the relative abundance and proximity of natural-living populations interspersed within urban areas, creates more of a mosaic of habitat types (ranging from relatively natural to fully urbanised) and is in contrast to the more directionally linear circumstance of sink populations radiating out of a source population core (Holt et al. 2003). This patchwork of populations within different habitat types would allow for regular, albeit limited, gene flow from multiple potential sources; either through natural process (e.g., immigration through green-space corridors and suburban backyards) or anthropogenic means (e.g., assisted migration, translocation, or escaped/released pets). Overall, the spatial mosaic of populations habitat types surrounding seemingly isolated urban areas may permit limited gene flow, which in turn creates a proverbial 'testing ground' for potential urban-successful

genes. It is likely that this is the foundation from which urban-derived divergent phenotypes are able to give rise within urban populations.

With respect to specific trait types, a recent meta-analysis has determined the presence of global signatures related to increased rates of phenotypic change across a variety of flora and fauna in urban areas (Alberti et al. 2017). This demonstration of expedited phenotypic divergence related to urbanisation further underscores the potential urban landscapes have to dramatically alter species' evolutionary trajectories (Hendry et al. 2008; Partecke et al. 2013; Alberti 2015; Alberti et al. 2017). The driving factors related to an increased rate of urban-derived divergent phenotypes has been collated into five distinct categories: 1) habitat modifications, 2) biotic interactions, 3) heterogeneity, 4) novel disturbances, and 5) social interactions (Alberti et al. 2017). These are all likely major contributors to the urban-derived divergent behavioural and biological traits observed in my thesis.

### ***Behavioural traits***

Urban dragons are bolder, and males engage in higher rates of combat compared to free-living conspecifics from natural habitats (Chapter I and II). These two behavioural responses may produce antithetical results for urban dragon populations, with bolder behaviour likely conferring an advantage to maximise resources and increased combat rates likely incurring a substantial cost. Boldness reflects an individual's propensity to engage in risky behaviour (Réale et al. 2007). Within an urban ecology context, bolder individuals may be more active in urban landscapes allowing them to increase their time spent foraging, mate searching, or defending a territory (Réale et al. 2007; Sol et al. 2013; Sprau and Dingemanse 2017). The increase in boldness is likely a behavioural response to the ecological challenges, such as disrupted biotic interactions (e.g., decreased natural predators and increased novel predators), habitat modifications (e.g., degraded natural resources, fragmentation, novel food sources, pollutants, and thermal buffers), and novel disturbances (humans, traffic, and other infrastructure) observed in urban areas (Ditchkoff et al. 2006; Kowarik 2011; Winchell et al.

2016; Alberti et al. 2017). These factors may exclude or limit shy individuals from performing natural behaviours, such as basking and foraging, and thus provide an advantage for bolder individuals to increase their use of available resources (see meta-analysis by Smith and Blumstein 2008).

The increased population density experienced by water dragon in urban areas has resulted in increase rates of male combat, most likely because encounter rates are higher. This suggests that one of the mechanisms driving urban-derived divergent behavioural traits is social interactions (Alberti et al. 2017). Male water dragons regularly engage in combat to establish and defend territories, but can also adopt a satellite non-territorial tactic if resources are limited (Baird et al. 2012, 2014). Interestingly, the testosterone level of males remains high regardless of territory-holding status in dense populations, suggesting even non-territorial males may be physiologically primed to quickly capitalise on a territory if an opportunity arises to challenge another male (provided conditions are favourable; Baird et al. 2014). However, provided individuals are able maintain territories despite increased contest challenges then increased mating opportunities within urban environment (Baird et al. 2012; Strickland et al. 2014) may offset the energetic costs. More research is required to understand the physiological and reproductive ramifications of increased combat rates and wounding on urban water dragon population stability and structure.

### ***Biological traits***

I also determined that urban water dragons exhibited urban-derived divergent morphology (Chapter IV) and performance capacity (Chapter VI). Urban dragons exhibited smaller body size (snout-vent length; SVL) at hatching and as adult males, and a different body size (limb length and head size) in hatchlings. Furthermore, I observed a decreased growth rate in urban juvenile dragons, but only in individuals from urban origin populations raised in urban habitats. This suggests that a genetic-environmental interaction is required to trigger a differential growth rate, and that without both a genetic precursor (stemming from an urban

adapted origin population) and an environmental cue (urbanised habitat) an urban dragon's body length and size would default to that of individuals from natural populations.

In general, the urban-derived divergent morphology I observed aligns with the expected lizard body size that is selected for in environments with broader surfaces (concrete, brickwork, and other building material). For example, longer limbs have been associated with the use of broader surfaces in multiple lizard clades, like *Anolis* spp. (Losos & Sinervo 1989; Marnocha et al. 2011; Winchell et al. 2016), *Tropidurus* sp. (Kohlsdorf et al. 2001), and *Gowidon* sp. (Iglesias et al. 2012). Changes in morphology related to habitat are typically associated with an increase in performance capacity (Losos & Sinervo 1989), however I was not able to detect such a performance advantage (clinging ability, endurance, or sprint speed) in my research. In fact, the only difference I observed between urban and natural water dragons was that urban water dragons which were smaller (SVL) with a longer limb length and head size, had lower endurance capacity. Thus, the changes in morphology that I observed within urban dragon populations do not overtly appear adaptive (i.e., no performance advantage in the metrics I measured). It is possible, however, that the morphological changes I observed may confer benefits in performance abilities I did not measure (burrowing or jumping), or other factors known to alter body size such as novel mortality sources (Sasaki et al. 2009), divergent behaviours (Losos et al. 2004), or altered mating systems related to anthropogenic environments (Järvenpää and Lindström 2004; Saaristo et al. 2009). My examination into the intricacies of conducting performance measures (Chapter V) demonstrated some of the challenges of conducting performance research (e.g., order effects and under performance), and provided insight into how to overcome them (e.g., mixed modelling and species-appropriate designs). No doubt there are performance ramifications to the altered morphology I documented in water dragons, however, more examination is required.

## **Water dragons, genetically-linked traits, and urban evolution**

In examining the presence of urban-derived divergent phenotypes, I used captive colonies to either manipulate habitat factors, such as in the 2 x 2 factorial experimental rearing environment (Chapters IV and VI), or a standardised habitat, such as in the common garden experiment (Chapter II). In doing so, I removed confounding factors and was able to determine if divergent traits we observed were related to an individual's origin population (i.e., heritable) or habitat-related phenotypic plasticity. As such, I was able to determine that two of the urban-derived divergent traits, increased boldness (Chapter II) and divergent morphologies (Chapter IV), were associated with an individual's origin population, which suggests these traits are heritable. This finding identifies a direct means by which water dragons are undergoing urban evolution through natural selection (Johnson & Munshi-South 2017). This assertion is further strengthened by the fact that my urban study sites (e.g., Taronga Zoo, Manly Beach, and Darling Harbour) are for the most part isolated from one another by dense urban infrastructure and development, which limits or prevents gene flow (Johnson & Munshi-South 2017). The fact that these isolated populations are exhibiting similar trends in urban-derived divergent, heritable traits suggests that they are not merely aberrations of genetic drift operating independently within closed populations (Johnson & Munshi-South 2017), but rather as adaptive traits that selection is acting upon due to similar selective pressures within an urban environment.

Chapter II identified that increased boldness arose from a genetic origin. Boldness is thought to be an adaptive heritable trait for several urban-dwelling bird species (Evans et al. 2010; Atwell et al. 2012; Holtmann et al. 2017; Sprau and Dingemanse 2017). For example, bolder dunnocks (*Prunella modularis*) and great tits (*Parus major*) inhabit urban areas that have a higher rate of human disturbance (e.g., pedestrians, cyclists, vehicles traffic), suggesting a non-random spatial sorting process based on behavioural traits (Holtmann et al. 2017; Sprau and Dingemanse 2017). Such a sorting process could quickly amplify the strength of selection on boldness within urban environments. My findings in Chapter II take



this concept one step further, by eliminating the confounding effects of developmental environment and prior experience (Holtmann et al. 2017) through the use of a common garden experiment. In doing so, I was able to determine that dragons from populations regularly encountering humans (semi-natural and urban) were bolder despite having never experienced an urban environment themselves.

Within Chapter IV, I established that urban-derived divergent morphology was present in adult males, hatchling, and experimentally-raised yearling water dragons. These findings provided insight into the mechanism by which these traits were arising; the experimental-rearing experiment revealed a genetic-environmental interaction (i.e., when heritable traits arise within specific populations, through selection, mutation, or other means, but whose expression is mediated environmentally; Via & Lande, 1985; Ghalambor et al., 2007; Grishkevich & Yanai, 2013). This genetic-environmental interaction resulted in dragons from urban origin populations raised in urban habitats expressing divergent body size and shape through altered growth rates. This finding further supports recent research that found urban populations of water dragons within Brisbane, Australia exhibited distinct genetic and morphological differences (Littleford-Colquhoun et al. (2017)

### **Future research directions**

The findings presented in this thesis have introduced novel avenues of research in the field of urban ecology and evolution. Below I provide a brief list of topics that warrant further investigation.

1. *The cost of living at high population densities* - The level of wounding and combat observed in Chapter I suggests that urbanised water dragons have distinctly different energetic demands compared to conspecifics from natural areas. Previous research found that living in higher densities may come at substantial costs to body condition, clutch size, and lifespan (Shochat 2004), or could alter behavioural or morphological traits (Tomkins and Brown 2004; Banks et al. 2007; Knell 2009). However, research is

needed to address the physiological costs that come with living within an inflated, anthropogenically-subsidised, highly combative population, particularly with respect to fitness (e.g., the life history ramifications of living in a stressful environment) and sexual selection (e.g., increasing density would increase the intensity of sexual selection).

2. *Heritability of urban-derived divergent traits* - A potential novel experimental framework, that could increase our understanding of the heritability of urban-derived divergent traits (i.e., boldness from Chapter II and morphological differences from Chapter IV), is a multigenerational breeding experiment, as well as a use of an animal model once a pedigree is generated (Wilson 2009), to quantify the heritability of these traits in urban and natural environments
3. *How are urban-derived divergent traits adaptive* - Identifying how urban-derived divergent traits are adaptive is the capstone to understanding the role they play in the persistence of urbanised wildlife populations. Optimally, research would first establish that an urban-derived divergent trait exists, then examine if it is heritable, followed by how it is adaptive. This series of research aims would allow for a comprehensive understanding of how selection promotes the trait. Unfortunately, within this thesis I was only able to gain insight into the first two aspects of the process. My research into whether urban-derived divergent morphology resulted in a performance advantage (Chapter VI) did not provide a definitive answer. This may have been because the urban-derived divergent morphology I observed was maladaptive, or I had not examined the correct performance metrics. Either way, much more research into mechanistic urban ecology and evolution is required to gain a fuller picture of the adaptations of wildlife to urban environments (Shochat et al. 2006; Johnson & Munshi-South 2017).

4. *The diversity of innate antipredator responses* - During my examination of the innate antipredator responses of water dragon hatchlings (Chapter III), I was unable to find any differences between urban and natural populations. Instead, a hatchling's innate antipredator response is linked to clutch effects and an individual's body size. This finding raises several research questions. First, although antipredator response has a heritable component (Arnold and Bennett 1984; Brodie 1989; Sih et al. 2004), within population variability in water dragon antipredator behaviour remains high.

Understanding what drives variance in a trait, particularly one that is so intimately linked to survival and fitness, is a fundamental challenge in evolutionary biology. The fact that hatchling water dragons exhibit a large range of innate antipredator responses without selection favouring one or another, raises interesting questions about how selection acts on antipredator behaviour and the degree to which it is plastic. Future studies should also examine the repeatability of antipredator behaviour over the course of development, and the diversity of adult antipredator responses within wild populations.

5. *Cognition and its evolutionary role in urban ecology* - I mentioned in the General Introduction to this thesis, I believe water dragons are intelligent, albeit that this belief is a result of anecdotal evidence and personal observations I have made during my thesis research. Research into animal cognition and urban ecology is gaining traction in the literature, however studies remain rare (see MacDonald and Ritvo 2016). Similarly, the heritable nature of cognition is not well understood (Cole et al. 2012; Smith et al. 2015). As such, studies should examine the role cognition in the success of urban dwelling wildlife, in particular that of water dragons.

## Conclusion

Globally reptiles have, for the most part, suffered greatly due to the effects of urbanisation (Gibbons et al. 2000; Böhm et al. 2013). However, there are reptile species that have taken the challenges humanity presents in their stride, particularly within Australia (How and Dell 1994; Garden et al. 2006; Shea 2010; Wilson and Swan 2017). These urban-dwelling reptiles provide an exceptional opportunity for research to examine the evolutionary ramifications of urbanisation. Although research in urban ecology and evolution has increased recently, there is still a tremendous amount research needed to understand how urbanisation is affecting reptile biodiversity and species richness, as well as how reptiles that remain in urban areas are responding, adapting, and evolving. My research has contributed to the nascent field of urban evolution by examining several forms of urban-derived divergent phenotypes, both behavioural and biological, and examining the means by which they arise. Overall, urbanised water dragons are bolder, engage in combat more often, have lower endurance, and are shorter in body size while having longer limbs and larger heads. My thesis also offers a framework for future studies to build from. Assuming these urban-derived divergent traits are adaptive, it is my hope that long into the future, Sydneysiders will be able to look out and confidently state, “Thar be dragons”.



A water dragon overlooking the Sydney Harbour from Bradley's Head, NSW.

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Both these publications were from data collected during my thesis research. The first natural history note is an account of a male water dragon with a trifold tail that was observed during initial surveys at Taronga Zoo and published in *Herpetological Review* in 2015. The second natural history note is an account of heterospecific coprophagy observed during my second year of fieldwork at the Chinese Garden of Friendship and published in *Herpetofauna* in 2017 (despite that it appeared in the, late, 2014 volume of the journal).

**Baxter-Gilbert, J. H.** and Riley J. L. (2015). *Intellagama lesueurii lesueurii* (Eastern Water Dragon. Trifold tail. *Herpetological Review*, 46, 433-434.

***INTELLAGAMA LESUEURII LESUEURII* (Eastern Water Dragon). TRIFID TAIL.**

On 15 November 2014 a wild adult male Eastern Water Dragon (*Intellagama lesueurii lesueurii*) was captured and removed from a Southern Hairy-nosed Wombat (*Lasiiorhinus latifrons*) enclosure at the Taronga Zoo, Sydney, Australia (33°51'0" S 151°15'0" E). This individual had a trifurcated tail (Fig. 1)

Tail bi-, tri-, and poly-furcation often occurs during the regeneration process that follows autotomy (Sluys et al. 2002. *Stud. Neotrop. Fauna E.* 37:227–231; Galdino et al. 2006. *Phyllomedusa* 5:25–30), and autotomy is typically used as an anti-predator defence during a predation event (Bateman & Fleming 2009 *J. Zool.* 277:1-14). Within the zoo grounds roaming, wild *I. l. lesueurii* regularly enter enclosures and come in contact with a variety of novel domestic and exotic predators, resulting in frequent successful and unsuccessful predation events taking place. For this reason we believe that the trifold tail of this individual may have been caused by regeneration of the tail after a single, or multiple predation attempt(s). However, there is also a high degree of urbanization around the zoo

grounds; as such, pollution may have caused the trifurcation of this tail through developmental deformity, similar to pollution-triggered developmental deformities seen in snakes (Gray et al. 2001. Bull. Chicago Herpetol. Soc. 36:144-148; Gray et al. 2003. Bull. Chicago Herpetol. Soc. 38:4-6; Wallach 2007. Bull. Md. Herpetol. Soc., 43:57-95). Bifurcated tails have been reported for many lizard groups, such as: Agamidae (Brindley 1898. J. Bombay Nat. Hist. Soc. 11:680–689; Ananjeva and Danov 1991. Amphibia-Reptilia. 12:343–356), Scincidae, Lacertidae, Iguanidae (Brindley 1898. *op. cit.*), and Gekkonidae (Woodland 1920. Q. J. Microsc. Sci. 65:63–100). However, to the best of our knowledge, this is the first recorded case of tail trifurcation in *Intellagama lesueurii lesueurii*.



Figure 1 An adult male Eastern Water Dragon (*Intellagama lesueurii lesueurii*) captured at the Taronga Zoo, Sydney, Australia after climbing into a wombat enclosure (A), the individual was found to have grown a trifurcated tail (B) likely due to repeated predation attempts from wildlife living either on zoo grounds or in the surround suburbs.

Pages 178-181 of this thesis have been removed as they contain published material.  
Please refer to the following citation for details of the article contained in these pages.

Baxter-Gilbert, J. (2014). Heterospecific coprophagy in an eastern water dragon,  
*Intellagama lesueurii lesueurii*, (Gray 1831). *Herpetofauna* 44 (1&2), 34-37.

## Appendix II: Presentations given about research from this thesis

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### Conference Presentations

- **Baxter-Gilbert, J. H.** and Whiting, M. J. Shape Up: Variations in Morphology and Performance of Eastern Water Dragons in Response to Urban Landscapes. Australian Society of Herpetologists, Fairbridge, Western Australia, Australia, 20-23 July 2017.
- **Baxter-Gilbert, J. H.** and Whiting, M. J. Surviving the Concrete Jungle: Adaptations by Eastern Water Dragons (*Intellagama lesueurii*) to Urbanisation. Canadian Herpetology Society Annual Conference, Toronto, Canada, 16-19 September 2016
- **Baxter-Gilbert, J. H.** and Whiting, M. J. The Adaptive Responses of Eastern Water Dragons (*Intellagama lesueurii*) to Urbanisation. The 8<sup>th</sup> World Congress of Herpetology, Tonglu, China, 15-21 August 2016.
- **Baxter-Gilbert, J. H.** and Whiting, M. J. Selecting for Godzilla: The Effect of Urban Environments on Dragon Behaviour. The 43<sup>rd</sup> Annual Conference for the Australasian Society for the Study of Animal Behaviour, Katoomba, New South Wales, 5-8 July 2016
- **Baxter-Gilbert, J. H.** and Whiting, M. J. Within the dragons clutches: Examining maternal investment of Eastern Water Dragons across an urban gradient. Australian Society of Herpetologists, Launceston, Tasmania, 15-19 February 2015

### Public Presentations

- **Baxter-Gilbert, J. H.** Eastern Water Dragon Urban Ecology and Adaptive Responses. Guest Lecturer for the Oatley Flora and Fauna Conservation Society, 26 February 2018
- **Baxter-Gilbert, J. H.** The Urban Ecology of Eastern Water Dragons. Invited speaker for the Wild Things speaker series, Bayside Council, Rockdale, New South Wales, 14 June 2017
- **Baxter-Gilbert, J. H.** Dragons of Sydney Harbour. Invited speaker for the National Parks Association. Fortnightly pubic presentations during the summer from January 2016 until April 2018.

### Media Appearances

- Interview on ABC Sydney. Backyards series. 10 February 2018.

### Peer Reviewed Articles

- **Baxter-Gilbert, J. H.**, Riley, J. L., Boyle, S., Lesbarrères, D., and Litzgus, J. D. (2017) Turning the threat into a solution: using roadways to identify mortality risks and species presence in turtles. *Australian Journal of Zoology*, doi: 10.1071/ZO17047
- Riley, J. L., **Baxter-Gilbert, J. H.**, and Litzgus, J. D. (2017) A comparison of three external transmitter attachment methods for snakes. *Wildlife Society Bulletin*, 41: 132-139.
- Davy, C. M., Mastro Monaco, G. F., Riley, J. L., **Baxter-Gilbert, J. H.**, Mayberry, H. W., and Willis, C. K. R. (2016) Conservation implications of physiological carry-over effects in bats recovering from white-nose syndrome. *Conservation Biology*, 31: 615-624.
- Riley, J. L., **Baxter-Gilbert, J. H.**, Guiglielmo, C. G., and Litzgus, J. D. (2016) Scanning snakes to measure condition: a validation of quantitative magnetic resonance. *Journal of Herpetology*, 50: 627-632.
- **Baxter-Gilbert, J. H.**, Riley, J. L., Neufeld, C., Lesbarrères, D., and Litzgus, J. D. (2015) Road mortality responsible for billions for pollinating insect deaths annually. *Journal of Insect Conservation*, 19: 1029-1035.
- **Baxter-Gilbert, J. H.**, Riley, J. L., Lesbarrères, D., and Litzgus, J. D. (2015) Mitigating reptile road mortality: fence failures compromise ecopassage effectiveness. *PLoS ONE*, 10: e0120537.

### Editor Reviewed Natural History Note

- **Baxter-Gilbert, J. H.**, Riley, J. L., Moldowan, P. D., and Litzgus, J. D. (2015) *Sistrurus catenatus catenatus* (Eastern Massasauga Rattlesnake) and *Nerodia sipedon sipedon* (Northern Watersnake) Gut Content and Foreign Object. *Herpetological Review*, 46: 107

Appendix pages 184-188 of this thesis have been removed as they may contain sensitive/confidential content