Does egg incubation temperature impact the long-term behaviour and cognition of bearded dragons (*Pogona vitticeps*)?

UNIVERSITY OF
LINCOLN

Harry Siviter

A thesis submitted in partial fulfilment of the requirements of the University of Lincoln for the MSc by Research

November 2015

Contents

List of figures

Chapter One

Chapter Two

Chapter Three

Chapter Four

General abstract

Egg incubation temperature impacts a range of phenotypes in oviparous reptiles including sex, growth, physiology, behaviour and cognition. However, the long-term behavioural consequences of egg incubation temperature have seldom been studied. This project investigated the long-term effects of egg incubation temperature on the behaviour and cognition of bearded dragons. The first experiment tested the impact of incubation temperature on the growth and foraging behaviour of bearded dragons. The second experiment tested the personality of lizards and the third the gaze following ability of lizards. The results showed that egg incubation temperature influenced the growth and foraging behaviour of bearded dragons. While the personality of the lizards was unaffected, incubation temperature did influence the development of different behavioural traits during ontogeny. The gaze following ability of bearded dragons was unaffected by incubation temperature however the results did show for the first time that a lizard species is capable of gaze following into the distance. The overall results show that incubation temperature impacted upon some of the long-term behaviour of bearded dragons and suggests that egg incubation temperature will influence the lifetime history trajectory of bearded dragons.

Chapter One: 1.1 General Introduction

Behavioural plasticity is defined as 'the capacity of the same genotype to produce a different phenotypic outcome depending upon inputs during development' (Nettle & Bateson, 2015).There are two different types of adaptive developmental plasticity. The first assumes that the information obtained in early environmental circumstances allows animals to adapt their phenotype to the surrounding environment. For example, when gravid female field fall crickets *(Gryllus pennsylvanicus)* are exposed to a wolf spider (*Hogna helluo*) the offspring of the cricket show a heightened immobility response to spiders cues (Storm & Lima, 2010). The second implies that early environmental conditions affect the somatic state of animals and is often referred to as making the 'best of a bad situation' (Nettle & Bateson, 2015). For example, bulb mites (*Rhizoglyphus robini*) that develop on a rich diet display an aggressive phenotype when older, whereas mites raised on poorer diets develop a non-aggressive phenotype (Smallegange, 2011). The interaction between early environmental factors such as temperature and humidity that occur during egg incubation can dramatically affect the phenotypes of oviparous reptiles (Deeming, 2004) and in some cases these factors can affect the long-term phenotypes of reptiles (Burger, 1998; Elphick and Shine, 1998). The characteristics of oviparous reptiles have been shown to be influenced by the smallest of details including (Burger, 1998; Elphick and Shine, 1998), humidity, moisture levels and substrate type (Booth, 2004).

The interaction between incubation temperatures and sex is well studied; temperature has been shown to influence the sex of at least 13 out of 25 crocodilian species (although not all species have been tested), both species of tuataras, approximately 67-85 turtle species out of 245 species analysed, and approximately 37-161 species of lizard species from approximately 3,750 species (Valenzuela, 2004). This phenomenon is called temperature dependent sex determination (TSD) and it has been suggested that it is adaptive as it allows flexibility to environmental change (Deeming and Ferguson, 1989). However, research has shown that the temperatures that select for males or females are species-dependent and no trend has been seen (Deeming, 2004). For example, Deeming and Ferguson (1989), showed in alligators (*Alligator mississippiensis*) that hotter incubation temperatures (33°C) produced males while in various species of chelonians and squamates, higher temperatures produce females (Bull, 1980; Gutzke, 1987). Not all oviparous reptiles have their sex determined by temperature; others are determined by genes, also known as genotypic sex determination (GSD; Valenzuela, 2004). Much previous work has focussed on this aspect of egg incubation, therefore there are large gaps in our knowledge about the impact of egg incubation temperature on other traits.

Egg incubation temperature has been shown to influence the morphology and physiology of oviparous reptiles (Alberts *et al.*, 1997; Elphick and Shine, 1998). For example pine snakes (*Pituophis melanoleucus*) incubated at hotter temperatures took significantly longer to shed their first skin than snakes incubated at colder temperatures (Burger, 1989). The growth of oviparous reptiles is also influenced by incubation temperature. For example, geckos (*Eublepharis macularius*) incubated at hotter temperatures (32.5°C) grow faster than those incubated at colder temperatures (30°C) (Tousignat and Crews, 1995) although the reverse effect is seen in the (*Podarcis muralis*) lizard as lizards incubated at colder temperatures grew faster (Van Damme *et al.*, 1992). Egg incubation temperature has also been shown to affect the body plans of lizards (*Takydromus wolteri*) as lizards incubated at hotter temperatures had significantly shorter and narrower heads than lizards incubated at lower temperatures (Pan and Ji, 2001).

Egg incubation temperature has also been shown to influence the behaviour of oviparous reptiles (Van Damm *et al.*, 1997, Burgess *et al.*, 2006) and particular attention has been given to the anti-predatory behaviours of hatchlings. For example, running speed (see Table 1) appears to differ depending on incubation temperature, but again the differences appear to be species specific; elf skinks (*Nannoscincus maccoyi*) incubated at cooler conditions (20 ± 4°C) run faster than those incubated at warmer temperatures (Downes and Shine, 1999) whereas (*Bassiana duperreyi*) lizards showed the opposite effect with those incubated at hotter temperatures (27 ± 4° C) running faster than those incubated at colder temperatures (20 ± 4° C). These differences persisted for up to 20 weeks (Elphick and Shine 1998). Furthermore, egg incubation temperature can influence predator detection. Downes and Shine (1999) showed that elf skink hatchlings incubated under cooler conditions would respond more strongly to snake scent than lizards incubated at warmer incubation temperatures. This suggests that elf skinks are better at detecting potential predators and quicker to avoid them which in turn could increase their fitness. Egg incubation temperature has also been shown to affect the maintenance behaviours of oviparous reptiles such as righting time in turtles which will decrease the likelihood of predation as the animals will be vulnerable for less time (Ashmore and Janzen, 2003).

Whilst development under optimal conditions should encourage the development of normal cognitive functions, research has shown that when incubated at the limits of incubation temperature, differences in incubation history will influence the cognition of oviparous lizards. Amiel and Shine (2012) suggested that individuals of *Bassiana duperreyi* hatchlings incubated at hotter temperatures (22 ± 7°C) learnt a simple learning discrimination, the location of a safe shelter, more quickly than compared to those incubated at colder temperatures (16 ± 7°C). This effect was replicated in young lizards (10-25 days old) of the same species (Amiel *et al.,* 2014) suggesting that the influence of egg incubation on learning may continue into adulthood. Clark *et al.* (2014) took this research one step further by showing that lizards incubated at hotter temperatures were successful at reversal learning while lizards incubated at colder temperatures could not. This implies that the lizards incubated at hotter temperatures had flexible learning while the cold group did not. For this experiment however, lizards incubated at colder temperatures failed to learn the association while the lizards incubated at hotter temperatures only performed one reversal implying that this may not have been true flexible learning. Taken together, these results suggest that in addition to physiological and behavioral traits, learning ability may be impacted by incubation temperature. However, our understanding of the relationship between incubation temperature and cognitive ability is still in its infancy and needs further investigation.

1.2 Key questions

The majority of work examining the effect of egg incubation temperature on the phenotypes of reptiles has tended to use hatchlings and the long-term effects of egg incubation temperature are rarely considered outside of TSD (Burger, 1992; Van Damme *et* al., 1992; Deeming, 2004). Hatchlings are more likely to be vulnerable to predators so looking at the effect of egg incubation temperature on anti-predator behaviour, such as running speed, is important. However, other types of behaviours will be important for an animal if it is to reproduce thus, the long-term influences of egg incubation temperature on the physiological, behavioural and cognitive phenotypes of oviparous reptiles is something that needs to be investigated further.

Bearded dragons (*Pogona vitticeps*) are a social oviparous species of reptile that are common throughout central Australia. They have a wide range of different behavioural repertoires (Brattstrom, 1971) and have recently been shown to be capable of sophisticated social learning in the form of imitation (Kis *et al.*, 2014). When incubated under recommended conditions (27-30^{o}C), bearded dragons have GSD, however evidence shows that when incubated at extremely hot temperatures bearded dragons have TSD (+33^{o}C) (Holleley *et al* 2015). Therefore, bearded dragons are an ideal species to test for the effect of egg incubation temperature on phenotypic differences due to incubation temperature as long as the lizards are incubated across their normal range (27^{o} C-30^{o} C) as there will not be sex based differences due to incubation temperature.

To examine the impact of egg incubation on behavioural and cognitive phenotypes of bearded dragons, we investigated three key areas. The first investigated the effect of egg incubation temperature on foraging behaviour and growth. Egg incubation temperature has been shown to effect the growth of various different lizards, although no pattern between the relationship between incubation temperature and growth has been found (Deeming, 2004). Given that both the growth and size of reptiles has been shown to be important for fitness it was essential to investigate this. Further, we wanted to examine how this interacted with foraging success. No study has previously looked at the relationship between egg incubation temperature and the foraging behaviour of lizards and there is only one paper which has examined this in snakes. Burger (1991b), showed that pine snakes (*Pituophis melanoleucus)* incubated at cooler incubation temperatures were less able to successfully catch and eat mice when presented with them. If an animal is unable to forage both efficiently and successfully then it is less likely to survive or to pass on its genes (Dunham, 1978). With this in mind Chapter Two investigates the effect of egg incubation temperature on foraging behaviour by providing juvenile bearded dragons with prey items (crickets) and recording the lizards' success in approaching them. The relationship between foraging ability and growth and is then explored.

The second area of interest is the impact of incubation temperature on personality. Personality traits are behavioural traits that are consistent over time and context; they have been shown to affect the fitness and lifetime history trajectories of various different species of animals (Biro and Stamps, 2008). The results of chapter two suggests that egg incubation temperature effect the foraging behaviour of bearded dragons and studies have shown that 'boldness' and increased foraging rate are positively correlated (Ward *et* al., 2004; Kruvers *et al.,* 2009). Thus it is likely that personality may be affected by incubation temperature. To our knowledge, no study has previously investigated this. The findings of this are presented in Chapter Three.

The final area of interested is the impact of incubation temperature on social cognition. Up until recently reptiles were perceived as "stupid and inert" (Wilkinson and Huber, 2012). However, recent research has revealed that reptiles have more complex cognition than previously thought (reviewed by Wilkinson and Huber, 2012). For example red-footed tortoises (*Chelonoidis carbonaria*) have been shown to be capable of spatial cognition that is similar to that of mammals and birds (Wilkinson *et al.,* 2007; Wilkinson *et al 2009;* Mueller-Paul *et al.,* 2012). Further, they are capable of social learning and can learn to solve a detour task by observing the behaviour of a conspecific (Wilkinson *et al.,* 2010). Crucially for this work, bearded dragons have been shown to be capable of sophisticated social learning in the form of imitation (Kis *et al*., 2014) Chapter Four therefore investigates the effects of egg incubation temperature on the social cognition of bearded dragons. Specifically, we investigated gaze following into the distance and geometric gaze following. Being able to follow the gaze of a conspecific offers many potential advantages to animals. It is thought that animals might follow gaze to find potential food sources or to alert to potential predators (Zuberbühler, 2008). Wilkinson *et al.* (2010) showed that the red footed tortoise is capable of following the gaze of a conspecific into the distance suggesting that at least one species of reptile has the cognitive capacity to gaze follow. Egg incubation temperature had been shown to influence the learning ability of bearded dragons (Junttilia *et al.,* in preparation) which could in turn influence their ability to gaze follow and the trajectory at which animals learn to gaze follow throughout ontogeny.

Each chapter will be presented as a stand-alone paper. The findings will be integrated and summed up in a general discussion at the end of the thesis.

Table 1. A review of studies that have tested the effect of egg incubation temperature on the growth and running speed of oviparous reptiles. The arrows in the temperature section show if an increase (arrow pointing up) or a decrease (arrow pointing down) in temperature increases or decreases the growth and speed of oviparous reptiles. If no change occurs than a + is used.

Order	Species	Temperature	Growth	Speed	Reference
Chelonian	*Apalone mutica*	↑		↑	Janzen, 1993
	Chelonia mydas.	↑		↑ (swimming speed)	Burgess, Booth & Lanyon, 2006
	Chelonia mydas (again)	↓		↑	Ischer, Ireland & Booth, 2009
	Dermochelys coriacea	↓		↑	Mickelson & Downie, 2010
	Chrysemys picta bellii	↑		↑	Elnitsky & Claussen, 2006
Crocodilian	*Alligator mississippiensis*	= [1]	↑		Joanen *et al*., 1987
	Crocodylus porosus	↑	↑		Webb & Cooper-Preston, 1989
	Crocodylus niloticus	↑	↑		Hutton, 1987
Lizards	*Eublepharis macularius*	↑	↑		Tousignat & Crews., 1995
	Sceloporus virgaatus	↓		↑	Qualls & Andrews, 1999
	Podarcis muralis	↓	↑		Van Damme *et al*., 1992
	Cyclura nubile	↑	↑		Alberts *et al*., 1997
	Bassiana duperreyi	↑		↑	Elphick and Shine, 1998; Amiel et al., 2013
	Nannoscincus maccoyi	↓		↑	Downes and Shine, 1999
	Lmpropholis delicata	↑		↑	Downes & Shine, 1999

	Saproscincus mustelina	↑		↑	Downes & Shine, 1999
	Amphibolurus muricatus	↓	↑	↓	Esquerre, Keogh & Schwanz, 2014
	Chamaeleo calyptratus	↓	↑	+	Andrews, 2008
	Oligosoma Suteri	↑		↑	Hare, Pledger, Daugherty, 2008
	Anolis carolinensis	↓	↑		Goodman, 2008
Snakes	*Pituophis melanoleucus*	↓		↑	Burger, 1991
	Coluber constrictor	↑		↑	Burger, 1990

[1] Intermediate temperatures had highest growth rates

Chapter Two – Foraging behaviour of juvenile lizards correlates with egg incubation temperature

Abstract

Egg incubation temperature impacts the behaviour of various species of reptiles. Previous experiments have tended to focus on differences in the anti-predator responses of hatchlings incubated at different temperatures. However, survival requires more than evading predators. To date, no study has looked at the long-term impact of egg incubation temperature on the foraging behaviour of lizards. This study investigated this using a clutch of bearded dragon (*Pogona vitticeps*) eggs that were incubated at different temperatures within the natural range; half of them were incubated at a 'hot' temperature (30 ± 3 ^{o}C) and half at a 'cold' temperature (27 ± 3 ^{o}C). The growth and foraging behaviour of the lizards was then compared. The lizards incubated at colder temperatures grew more quickly; however, those incubated at hotter temperatures were significantly better at foraging. These results are the first to show that egg incubation temperature impacts the foraging behaviour of lizards and suggests a trade-off between growth and foraging speed, which could influence a reptile's life history trajectory.

2.1. Introduction

Sensitivity to environmental factors such as egg incubation temperatures allows for the development of a variety of different phenotypes in oviparous reptiles (Deeming 2004). Temperature-dependent sex determination is a well-documented example of this in reptiles (Valenzuela 2004) but, temperature can also impact on hatching success, hatchling growth, physiology, morphology, and locomotion (Elphick and Shine, 1998; Flatt *et al*., 2001; Deeming 2004; Booth 2004; Burgess *et al*., 2006).

Generally, the effects of incubation temperature on behavioural traits have been tested in neonatal animals. For instance, cold-incubated hatchlings of *Bassiana duperreyi* (Scincidae) exhibited more anti-predator behaviours than hot-incubated hatchlings despite comparable running speeds (Flatt *et al*., 2001). Hatchling pine snakes (*Pituophis melanoleucus*) incubated at cool temperatures (22-23°C) took longer to emerge from the egg at hatching, exhibited less anti-predator responses, were less effective at foraging, and were less likely to find shade during a thermoregulation test compared with snakes incubated at hotter temperatures (27-28°C; Burger, 1998). Burger (1991) tested the ability of pine snake hatchlings from two incubation temperatures to trace a scent in a Y-shaped maze where one branch of the maze was baited with a mouse odour while the other was not. Snakes from the hottest incubation temperature (33°C) were attracted more often to the mouse odour than animals incubated at colder temperatures (28°C). By contrast responses to snake scent and the ability to evade snake predation was unaffected by incubation temperature in three lizard species (Downes & Shine 1999). Burger (1991b) also provided live mice to pine snake hatchlings and recorded how quickly the snakes approached and ate the prey species. The snakes incubated at hotter (30-33°C) and medium incubation temperatures (26-28°C) hunted significantly quicker and were more able hunters than those at colder temperatures (21-23°C). In addition, studies on the effect of incubation temperature on learning ability of *B. duperreyi* hatchlings have shown that hot-incubated individuals were significantly better at learning tasks than cold-incubated animals (Amiel & Shine 2012, Amiel *et al*., 2014; Clark *et al*. 2014).

Although some effects of incubation temperature, most obviously sex (Deeming & Ferguson 1991; Valenzuela 2004), have long-lasting (life-time) effects on animals, most studies have only tested the impact on hatchlings (Deeming 2004; Booth 2004). This is despite the potential importance of incubation environment on long-term fitness of individuals. Downes and Shine (1999) looked at the effects of incubation temperature on phenotypic traits such as running speeds and the chemosensory response to a snake scent for up to 42 days post-hatching in three species of Australian lizard, however, it was age rather than incubation environment that had the most impact on behaviour. Warner & Andrews (2002) tested the long-term effects of incubation environment on phenotype of the eastern fence lizard (*Sceloporus undulates)* under field conditions. They found that hydric conditions for eggs during incubation had no effect on survival or phenotypic traits after a year but clutch was a determining factor for these traits. Slow-growing but fast runners had better survival than fast-growing but slower individuals. In contrast Elphick and Shine (1997) showed that juvenile lizards (*Bassiana duperreyi)* incubated at hotter incubation temperature were faster than those incubated at colder temperatures. This shows that only a few studies have examined these key behaviours over long periods of time despite the fact that differences in the phenotypes of reptiles has been shown to affect an individual's fitness and survival rates (Warner & Andrews, 2002).

There are two key areas of these studies that are of concern. Firstly, most studies are conducted on hatchlings so we have large gaps in our understanding of the long-term effects of varying egg incubation temperature on the phenotypic plasticity of oviparous reptiles. Being faster as a hatchling does not necessarily translate to faster speeds as adults (Husak 2006). How long do effects of incubation temperature observed in neonatal individuals persist as they grow, are they ecologically relevant, and will they impact an animal's long-term fitness? Secondly, most tests of locomotor speed are conducted under a degree of duress – hatchling lizards are 'encouraged' to run by people (e.g. Downes & Shine, 1999; Warner & Andrews 2002) rather being allowed to move naturally within the test arena. Hatchlings have even been exposed to direct predation by snakes without any means of escape (Downes & Shine, 1999). If individuals were indirectly encouraged to move would the effect of incubation temperature still remain? Moreover, how long do behavioural effects observed in hatchling snakes (Burger 1991b) persist into juveniles or adults?

In light of these issues we have examined the growth and foraging behaviour of bearded dragons (*Pogona vitticeps*) incubated at two different temperatures. In lizards, there are a variety of advantages to being bigger than a conspecific (Van Damme *et al.,* (1992); Braña, 2000; Ferguson and Fox, 1984). Larger animals are more likely to retain body heat for longer, meaning that they can remain active for longer, (Avery, 1982) and are more likely to survive in times of hardship such as during temperature declines (Ferguson and Fox, 1984). Further, social dominance in lizards is strongly correlated with size in both males and females (Fox, 1983; Tokarz, 1985) allowing dominant lizards to have control over resources, such as basking spots, food, territory and reproductive activity (Stamps, 1977, Stamps, 1984, Trivers, 1976). The ability to forage effectively is also crucial for survival. If an animal cannot forage effectively it is more likely to grow at a slower rate and will take longer to reach sexual maturity, and more likely to die earlier (Dunham, 1978) thus it is less likely that it will pass on its genes. Bearded dragons typically live in deserts and woodland in central Australia where food is often scarce and males will fight for territories and reproductive opportunities (Brattstrom, 1971). Being able to forage effectively will give a lizard a direct advantage over competitors, as their longevity could increase. When bearded dragons are incubated at their optimal range the sex of the lizards is determined by genotypic sex determination (Ezaz *et al.*, 2005; 2011; Holleley *et al.,* 2015) making them an ideal species for this type of experiment.

Further, instead of studying neonates we examined the foraging activity in animals at 18 weeks of age. Differences in growth were observed and as a result the 'cold group' was tested at the same age and the same mass as the animals from the hotter temperature ('hot group'). This allowed us to investigate the effects of egg incubation temperature on behavior when groups were matched for both age and size. The growth of the lizards were also recorded and compared between lizards from different incubation temperatures. Live house crickets (*Acheta domesticus*) were used as prey to test for differences in the foraging ability of the juvenile bearded dragons. Given that pine snakes incubated at hotter temperatures were better at foraging than those from colder incubation temperatures (Burger, 1991b), we hypothesized that the 'hot group' of lizards would be more successful than the 'cold group' at foraging and that this would be unaffected by the mass of the individuals.

2.2. Methodology

(a) Animals

A clutch of 14 eggs were laid by a captive female bearded dragon sired by only one male. The clutch was then split into two groups of seven eggs at random. They were subsequently incubated in plastic boxes with vermiculite (Booth, 2004). The 'hot group', was incubated in a heat-controlled room maintained at an average temperature of 30°C (± 3°C) whilst the 'cold group' of eggs were incubated in a second heat-controlled room at an average temperature of 27°C (± 3°C). Water was added to the substrate surrounding the eggs as necessary. Animals in the 'hot group' hatched between 58 and 60 days (mean ± SD = 58.6 ± 0.3 days) after being laid, while the 'cold group' hatched between 83 and 91 (mean ± SD = 88.9 ± 1.0) days after being laid (t = -33.947, P = 0.001).

After hatching, the lizards were all housed in the same room and maintained at a temperature of 29°C (± 3°C). Initially the lizards were kept in groups in small glass vivariums (30 x 20 x 20 cm) and were later moved into bigger ones (145 x 48 x 60 cm). Hatchlings all received food *ad lib*, however, as they got older all food was weighed and balanced meaning that the 'hot' and 'cold' group received the same amount of food. Lizards incubated at different temperatures were kept separately. The lizards were sexed by vent inspection at 15 weeks of age and both groups contained 4 males and 3 females. The lizards were weighed (in grams) using an electronic scale (Ohaus precision plus TP2000) every week from two weeks after hatching and throughout the testing period. The mass of the animals was recorded for up to 52 weeks after hatching however this data (18 weeks

+) was not included as egg laying makes it less reliable has the mass of bearded dragons drop significantly once an animal lays eggs .

(b) Experimental set-up and apparatus

An arena measuring 128 x 32.5 cm with 19 cm opaque high walls, was used for the foraging experiment. The floor (wooden) of the arena was marked into 25cm segments up to the test distance of 1.0m; these distances were used to calculate the speed of the lizards. A digital video camera (Sony HDR-CX22OE) was placed above the arena on a tripod and all trials were recorded. The testing room was maintained at 28.5^{o}C (± 1^{o}C).

(c) Procedure

The 'hot group' were tested 18 weeks after hatching. The 'cold group' were tested at 15 weeks, when they attained the same mass as the 'hot group,' and again at 18 weeks when they were the same age as the hot group.

All animals were habituated to the arena. During habituation trials, the lizards were individually placed at one end of the testing arena and a cricket (a favoured food item) was put at the opposite end. If the subject moved towards, caught and ate the cricket in three consecutive trials, each of which was a maximum of 3 minutes long, then it was considered habituated (Kis *et al*, 2014). This continued until all animals were habituated, which took 2 days.

At the start of each experimental trial the lizard was placed in the starting position, which was behind the zero line for speed calculation (see Figure 1). A transparent plastic container containing a cricket was then placed in the arena 1.0 m from the lizard. The lizard had 3 minutes to approach the cricket. Once the lizard was within 5 cm of the box, a second cricket was presented to the lizard as a reward and the stimulus cricket was removed. The size of the cricket (1.4 cm in length) was kept constant for all trials. If the lizard did not approach the cricket within the 3-minute trial time then the stimulus cricket was removed and the lizard was deemed to have been unsuccessful in that trial. To ensure that the lack of approach was not due to issues with motivation a second cricket was still presented. If the lizard did not eat the cricket then the trial was excluded from the analysis but the trial was not repeated. Each lizard received 4 trials, irrelevant of success. This resulted in the 'hot group' having 27 usable trials the 'cold group' – same mass had 24 trials and the 'cold group' – same age had 28 trials. The order in which the lizards were tested was randomly selected daily using a random number generator. Lizards from all treatments were tested between the hours of 9.00-16.00; the hot group were tested over three days while the cold groups over two days.

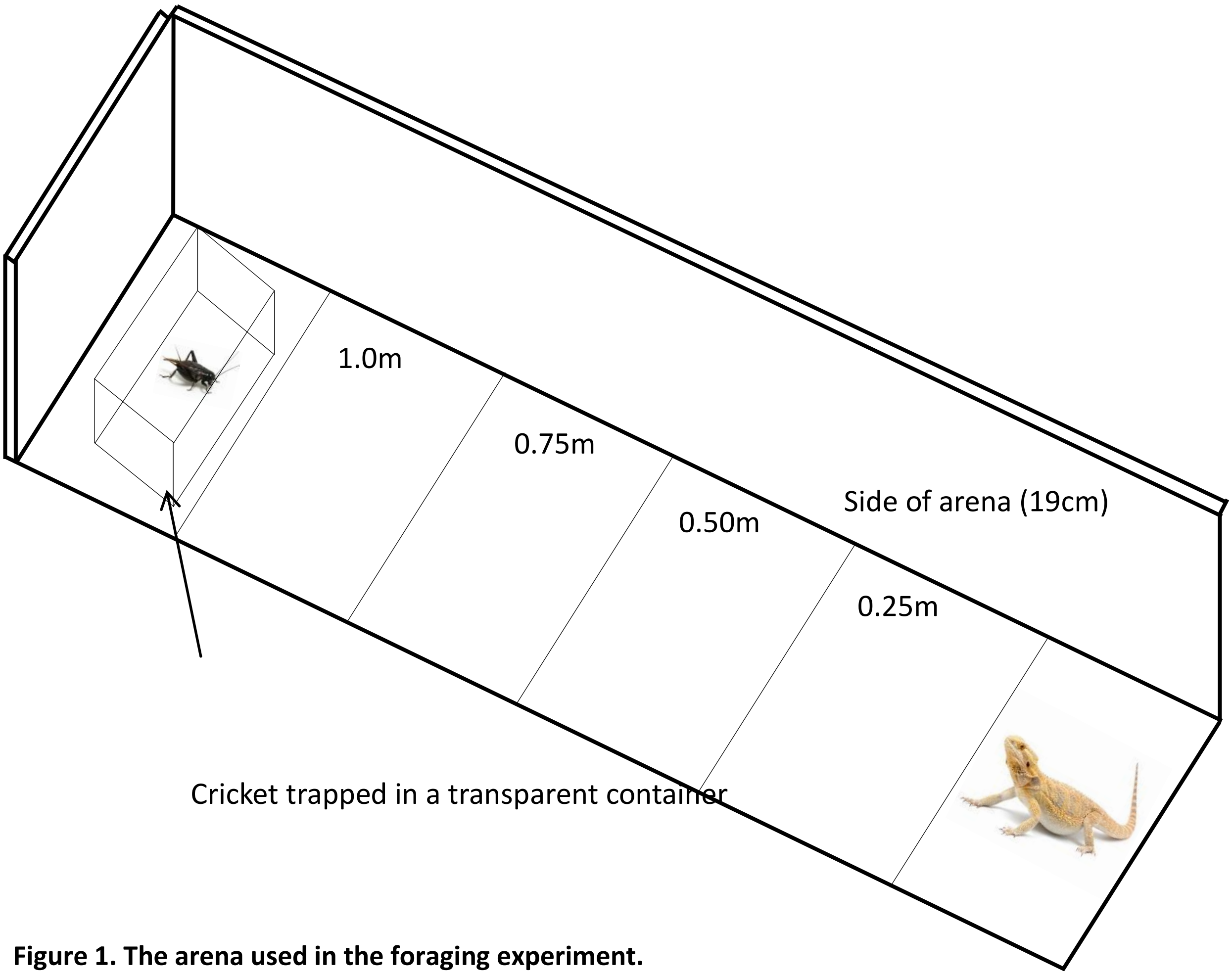

Figure 1. The arena used in the foraging experiment.

(d) Data analysis

The video for each trial was analysed using Avidemux 2.6 software. The time taken for each lizard to move across each 25cm segment of the arena was recorded and converted to speeds (m/s). A general linear model was used to analyse the effects of incubation treatment on mass. The mass of the lizards was the dependant variable while the age, temperature and the interaction between temperature and age were included as fixed factors. The individual lizard was included as a random factor in the model. Average foraging success (successful or unsuccessful) was calculated for each animal by assigning each trial a pass of fail mark (1 or 2) and the effect of incubation treatment was tested with a Kruskal Wallis test. Mann-Whitney U tests were used to test pairwise differences in the successful trial completion data from the treatment groups. A general linear model, with post-hoc Tukey tests, was used to test for differences in the speed of the treatment groups. The running speed (metres per second) was used as the dependent variable while the treatment, distance and the interaction between the two were used as fixed factors. The individual lizard was used as a random variable. Lizards tested at different times were considered as being in different 'treatment' groups.

All data was analysed using SPSS (version 23) with the exception n of the general linear modelling whereby Minitab (version 16.2.4) was used.

2.3. Results

(a) Impact of temperature on mass.

All animals were a similar mass at hatching, but by 10 weeks-of-age the lizards in the 'cold group,' were heavier, a trend that continued as both groups got older (Figure 2). General linear modelling indicated that mass was significantly affected by the age of the animal ($F_{1,187}$ = 726.76, $p < 0.001$). More interestingly, the temperature at which the eggs were incubated also had a significant effect on mass ($F_{1,187}$ = 15.68, $p < 0.001$) . There was also a significant effect of the individual within the model ($F_{12,187}$ = 6.21, $p < 0.001$) and a significant interaction between temperature and age ($F_{1,187}$ = 122.24, $p < 0.001$). This means that the 'cold group' had significantly quicker growth than the 'hot group.'

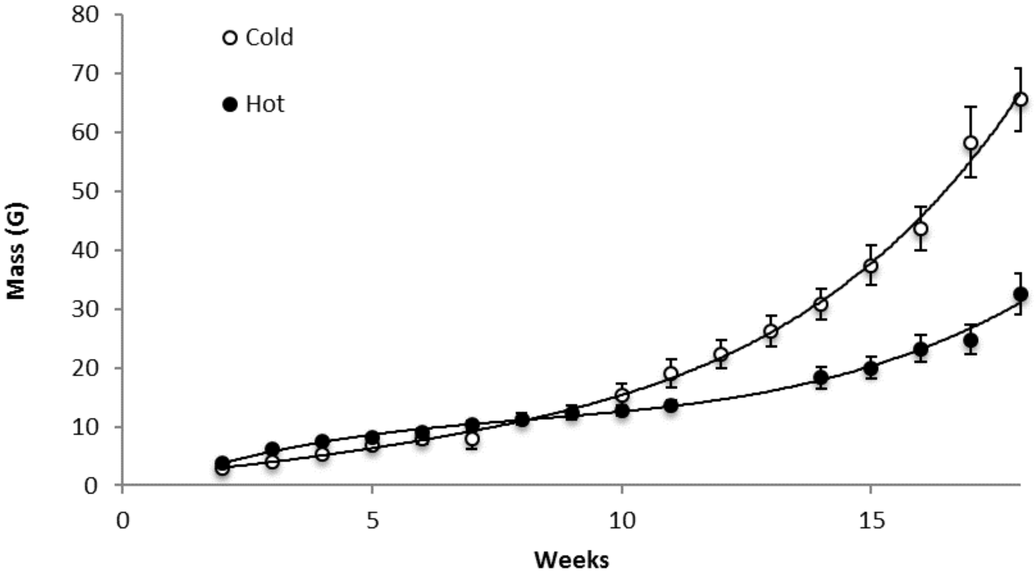

Figure 2. Average mass (± SE) of bearded dragons incubated at 27°C and 30°C from 2 to 18 weeks from hatching (with least square regression lines calculated by excel).

(b) Test completion

The lizards in the 'hot-group' were 100% successful at this task (27/27 usable trials). By comparison, the 'cold-group' of bearded dragons failed to complete 9 trials out of 24 usable trials (38%) when they were matched for mass and failed to complete 11 trials out of 28 usable trials (39%) when they were matched for age. An analysis of the mean foraging success showed a significant difference between the lizards from different treatment groups (Kruskal-Wallis test: $\chi^2 = 6.484$, $df = 2$, $p =$

0.039). Mann-Whitney tests revealed that the hot-incubated group was significantly more successful than the cold-incubated animals at both the same mass ($U = 7.0$, $n_1 = 7$, $n_2 = 6$, $p = 0.014$) and age ($U = 10.5$, $n_1 = 7$, $n_2 = 7$, $p = 0.025$). The cold-incubated lizards did not alter significantly at different ages indicating that their behaviour was consistent across time ($U = 18.5$, $n_1 = 6$, $n_2 = 7$, $p = 0.709$.)

(c) Speed to finish trial

Generally the speed of the lizards decreased as they approached the cricket (Figure 3). Measures of speed over the four different segments showed that the 'hot-group' was quicker than the 'cold-group' at both the same age and same mass over the first 3 intervals (Figure 3). General linear modelling revealed that treatment and distance both significantly affected running speed of the subjects (treatment: $F_{2,45} = 5.82$, $p = 0.013$; distance: $F_{3,45} = 20.69$, $p < 0.001$). However, as the speed of the bearded dragons progressively decreased as they neared the cricket and in the last 25cm the lizards moved comparatively slowly in all three conditions. Interestingly, as the 'cold-group' got older they ran at slower speeds (Figure 3). In addition, the interaction between distance and treatment was also significant ($F_{6,45} = 3.23$, $p = 0.016$).

ANOVA tests revealed significant differences in speeds between the treatment groups recorded at 0.25 m ($F_{2,15} = 7.134$, $p = 0.007$), 0.50 m ($F_{2,15} = 4.320$, $p = 0.033$) but neither at 0.75 m ($F_{2,15} = 3.596$, $p = 0.053$) nor at 1.0 m ($F_{2,15} = 1.607$, $p = 0.233$). Tukey tests showed but the 'hot group' was not significantly different with the 'cold group' at the same mass but they were significantly faster than the 'cold group' when they were the same age at 0.25 m (p = 0.006), 0.50 m (p = 0.026) , 0.75 m (p = 0.045) but not at 1.0 m (0.211).

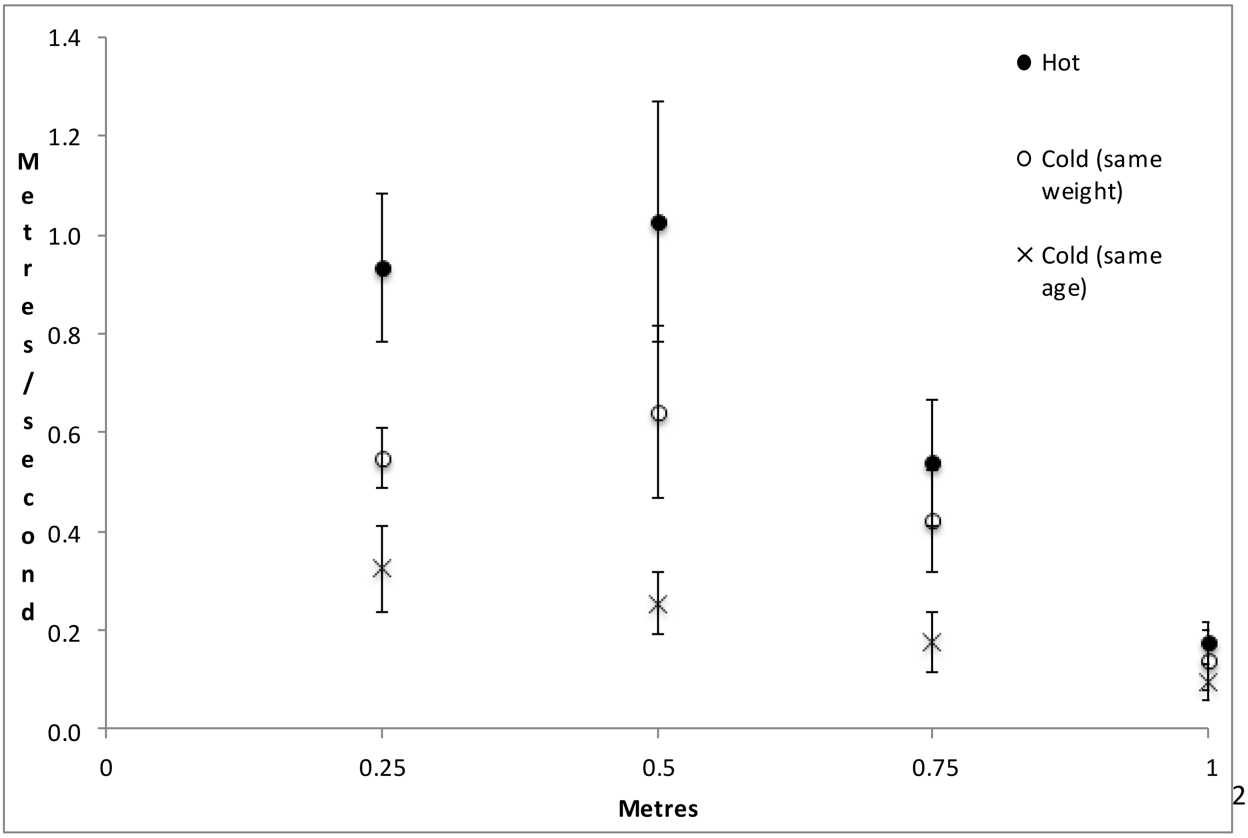

Figure 3. Average (± SE) of running speeds (m·s^{-1}) for bearded dragons in the different treatments measured at each 0.25 m intervals over the course of a 1 m track (with least square regression lines calculated by excel)

2.4. Discussion

This experiment shows that egg incubation temperature impacts upon the post-hatching growth of bearded dragons. Those incubated at a cooler temperature were significantly heavier than those incubated at a hotter temperature from 10 weeks of age showing for the first time that egg incubation temperature influenced the long-term growth of bearded dragons. This trend continued until at least 18 weeks. Further, the foraging success of juvenile bearded dragons was significantly affected by egg incubation temperature. The 'hot group' completed more trials than the 'cold group' when the animals were at both the same age and the same mass but were only significantly quicker than the 'cold group' when tested at the same age. This implies that the 'hot group' were better at foraging than the 'cold group'. Despite this, the 'cold group' were heavier, suggesting that there may be a potential trade-off between a lizard's size and its foraging ability. These results however support work by Burger (1991b) on snakes and suggests that incubation temperatures could have a long-term influence the foraging ability of a wide variety of oviparous reptiles although it is unknown if the same potential trade-off is seen in pine snakes.

Egg incubation temperature has been shown to impact the growth of various different species of reptiles (Deeming, 2004). Previous studies have revealed that egg incubation temperature impacts on the mass and size of lizard's hatchlings in various ways, such as affecting body plan, head size, tail length and other traits (Alberts *et al.*, 1997; Deeming, 2004). It would be expected that animals at colder temperatures would perhaps be bigger as hatchlings take longer to hatch and have more time to absorb nutrients from the yolk sac (Andrews, 2004). Alberts *et al.*, (1997) showed that there were no differences in Cuban rock iguanas hatchlings (*Cyclura nubila*) incubated at different temperatures but lizards incubated at hotter temperatures (31^{o}C) had quicker post hatching growth speeds in their first year from hatching with the differences persisting for up to 14 months. Van Damme *et al.,* (1992) showed the opposite effect in the wall lizard hatchlings (*Podarcis muralis*) with lizards incubated at colder temperatures (24^{o}C) having quicker growth post hatching compared with lizards incubated at hotter temperatures (28^{o}C). In lizards there are numerous advantages to being larger than a conspecific such as dominance over resources and better survival rates over winter (Fox, 1983; Ferguson and Fox, 1984; Tokarz, 1985). Our results showed that bearded dragons incubated at colder incubation temperatures grew more quickly than lizards incubated at hotter incubation temperatures. Smaller animals are likely to be more vulnerable to potential predators meaning that quicker growth rates, as were seen in the lizards incubated at colder temperatures could be advantageous (Deeming, 2004). However, despite this bearded dragons incubated at hotter incubation temperatures were quicker at foraging than lizards incubated at colder temperatures. Egg incubation temperature has also been shown to impact the running speed of various different oviparous reptiles (Downes & Shine, 1999; Warner & Andrews 2002) however these experiments encouraged animals to run under a degree of duress rather than testing appetitive behaviour (Deeming, 2004). The results of the present study show that egg incubation temperature has long-term effects on the running speeds of bearded dragons even when animals were not 'encouraged to move' by a human experimenter. This is likely to be important for individual fitness suggesting that when food resources are scarce lizards incubated at hotter temperatures will outperform lizards incubated at colder temperatures in regards to foraging success. The results of the present experiment suggest that there is a potential trade-off between the growth and foraging speed in relation to egg incubation temperature. This could imply that egg incubation temperature affect the life-history trajectory of bearded dragons.

The mechanisms that control differences in individual phenotypes are not that well understood. It has been suggested that embryonic sensitivity to environmental conditions, such as temperature, is adaptive as each individual egg in a clutch will have individual phenotypic differences as a consequence of temperature variance which will alter the somatic state of each animal (Deeming and Ferguson, 1989, Deeming and Ferguson, 1991). Deeming and Ferguson (1989) suggested that crocodilian embryos that develop under different incubation temperatures produce hatchlings that are pre-adapted to a variety of different environments, which is likely to increase the likelihood of survival and future reproduction (Deeming & Ferguson 1989). A recent study that examined the stomach contents of wild bearded dragons found that termites (*Isoptera*) accounted for 94.6% their diet (Oonincx *et al.*, 2015). Termite biomass and terminate foraging activity increases significantly with an increase in soil moisture levels (Abensperg-Traun and de Boer, 1990) while hotter temperatures will result in a reduction of soil moisture and a reduction in potential prey for bearded dragons. This suggests that in hotter environments food resources will be scarce implying that foraging ability will be crucial for survival meaning that bearded dragons incubated at hotter incubation temperatures could have a direct advantage. In contrast to this, when food abundance is high bearded dragons incubated at colder incubation temperature could have the advantage as they have quicker growth rates. Social dominance and size are positively correlated (Fox, 1983; Tokarz, 1985) meaning that lizards incubated at colder incubation temperatures to dominate resources such as food and reproductive activity (Stamps, 1977, Stamps, 1984, Trivers, 1976). Reptilian egg incubation temperature is directly related to the temperature of the external environment and the results of the present experiment suggest that egg incubation temperature will produce animals that are more suited to the environment which they are emerging into.

Our study showed that there is a potential trade-off between growth and foraging speed 18 weeks after hatching despite the lizards been tested at temperature that was intermediate to incubation temperature. Thus lizards incubated at a certain temperature may be more suited to emerge into an environment with a similar temperature compared with lizards incubated at other temperatures. Evidence to support this idea can be found in the literature as sun skink lizards (*Lampropholis delicata)* and weasel skinks (*Saproscincus mustelina)* incubated at hotter temperatures ran faster than lizards incubated at colder temperatures at warm temperatures but stopped more when tested at colder temperature compared to lizards incubated at colder incubation temperatures (Downes and Shine, 1998). Elphick and Shine (1999) also showed that lizards (*Bassiana duperreyi)* incubated at hotter incubation temperatures ran faster at warmer body temperatures than lizards incubated at colder temperatures. Evidence is also provided by the present study as lizards incubated at colder temperatures grew bigger more quickly and large reptiles are better at retaining heat and more suited to cold environments (Avery, 1982). Environmental conditions will of course change seasonally however this might give oviparous reptiles an early advantage (Adolph, 1993). Undoubtedly, due to slight differences in the environmental factors for each individual egg within a clutch there will be individual differences in the phenotypes of reptiles from the same clutch with different animals being more suited to different environments. The results of the current study are the first to suggest that egg incubation temperature impacts upon the behaviour and growth of bearded dragons which in turn suggests that egg incubation temperature is likely to affect the life history trajectories of the animals.

In sum, our results suggest that there were no differences between the mass of the lizards in early development, however as the lizards developed differences in growth rates did occur. The results also showed that the running speed of lizards can be tested in other ecologically relevant experiments such as foraging ability and suggest a new methodology for testing running speed. This is the first experiment to demonstrate that the foraging ability of a lizard species is impacted by egg incubation temperature.

Chapter Three: Early environmental factors that occur during embryonic development influence the behavioural traits of oviparous reptiles.

Abstract

Long-term personality traits, i.e. differences in the behaviour of individual animals of the same species that are consistent over time and context, such as ‘boldness’ have been shown to be heritable and experimentally repeatable in various species. Interestingly, research is showing that early environmental factors that occur during ontogeny can also impact the ‘personality’ of animals. Manipulating reptilian egg incubation temperature is an ideal way to investigate this further, as it has been shown to impact the physiology, morphology, behaviour, and even the cognition of certain oviparous reptiles. Bearded dragon (*Pogona vitticeps*) eggs were incubated at two different average temperatures and the hatchlings were raised under the same environmental conditions. Individual lizards were tested with a novel object and a novel environment test to see whether egg incubation temperature has a long-term impact on the ‘boldness’ of juvenile lizards. The results showed that in the novel object experiment, egg incubation temperature did affect the time spent in close proximity to the novel object as well as activity levels however, in the novel environment locomotion was not influenced by incubation temperature. The results were not repeatable suggesting that egg incubation temperature did not influence the personality of bearded dragons. This suggests that egg incubation temperature impacts the behavioural traits of lizards at different stages in ontogeny.

3.1 Introduction

The term 'personality' describes differences in behavioural traits between different individual animals from the same species that are consistent over time and context (Carere and Eens, 2005). Animal personality has been shown to be heritable (Brown, 2007) meaning that these behaviours are subject to natural and sexual selection (Smith and Blumstein, 2008; van Oers *et al.,* 2008). Although animal personality has an underlying genetic component (Van Oers *et al*, 2005), it can also be heavily influenced by environmental factors. Boldness, the willingness of an animal to take risks in a novel situation, is the most common personality trait studied. For instance, rainbow trout (*Onchorhyncus mykiss*) adjust their 'boldness' levels based on observing shy or bold conspecifics (Frost *et al.*, 2007) and tropical poeciliid fish (*Brachyraphis episcopi)* have different levels of 'boldness' based on environmental factors, such as localised predation pressure (Brown, Jones and Braithwaite, 2005).

Egg incubation temperature affects sex determination, growth, behaviour and pigmentation in many reptiles (Deeming, 2004) and so incubation conditions could impact on boldness. 'Boldness' has been shown to have implications for an animal's life history trajectory across a range of species (Biro and Stamps, 2008) and can affect current and future reproductive success (Wolf *et al.*, 2007). Various personality traits have been shown in different species of reptiles. For example, darker melanin-based colouration in eastern Hermann's tortoises (*Eurotestudo boettgeri*) correlates with aggressive behaviour and boldness (Mafli, 2009) which could in turn be important for food acquisition. Male Eastern water skinks (*Eulamprus quoyii*) are bolder than female skinks, which improved their performance in a spatial cognition task (Carazo, 2014). If egg incubation temperature influences the boldness of reptiles this will impact the fitness and life history trajectory of oviparous reptiles affecting survival and future reproduction.

Despite the relative abundance of research into animal personality traits, the relationship between early life experiences that occur during embryonic development and the resulting impact on animal personality post-hatching has received little attention. The work that is available suggests that this is a promising avenue for research. Artificial manipulation to increase androgen levels in yolks of black-headed gull (*Larus ridibundus*) eggs resulted in a positive correlation between personality traits such as 'aggression' (Müller *et al.*, 2009). Prenatal, maternal effects, such as stress levels, have been shown to elevate glucocorticoids levels in fish and bird eggs (damselfish fish , *Pomacentrus amboinensis*: McCormick, 2006; Japanese quall, *Coturnix japonica:* Bertin *et al.*, 2009) showing that egg laying females can change the hormone composition of their eggs which can in turn effect an animal's personality (Bertin *et al.*, 2009).

The most obvious example of early environmental factors influencing the phenotype of oviparous species is in species with temperature-dependent sex determination (Janzen *et al.,* 1998;

Valenzuela, 2004). We know that sex can impact upon the personality of an individual (Carazo, 2014). Further, egg incubation temperature affects the behaviour (Burger, 1989; Burgess, 2006) and the cognition (Amiel and Shine, 2012; Amiel, Lindström and Shine, 2013; Clark *et al.,* 2014) of various reptile species. In this experiment we investigate the impact of egg incubation temperature on personality in lizards. This study looked at the effect of egg incubation temperature on the boldness of bearded dragons (*Pogona vitticeps*) an oviparous lizard found in central Australia. We used a novel object test and a novel environment test to investigate personality differences between animals incubated at different temperatures. In contrast to some other studies investigating animal personality, this experiment tested both the short-term repeatability of the behavioural traits of the animals, over the course of 5-6 days and the long-term, over the course of five months. Our previous work suggests that bearded dragons incubated at hotter temperatures are quicker at approaching prey than lizards at colder temperatures (Siviter *et al.*, in preparation a; chapter 2). Given the correlation between foraging and boldness seen in other species (Ward *et* al., 2004; Kruvers *et al.,* 2009) we hypothesised that lizards incubated at hotter egg temperatures would be bolder than lizards incubated at colder egg incubation temperatures.

3.2 Methodology and design

(a) Animals

This study was conducted using 13 bearded dragons hatched from the same clutch where the laying female only had access to one male (Siviter *et al*., in preparation a). Half of the eggs were randomly chosen to be incubated at 30 ± 3^{o}C, the 'hot group,' and the other half were incubated at 27 ± 3^{o}C, the 'cold group.' Once the lizards had hatched the environmental conditions, such as provision of food, housing and room temperature, were the same for all lizards. Over the course of testing all lizards were between the ages of 10-15 months, animals were sexually mature and had previous experimental experience (see chapter two). The 'hot group' contained 7 lizards with 4 males and 3 females while the 'cold group' contained 6 lizards, 3 male and 3 female. If a female was gravid she was temporarily removed from the experiment and tested again after she had laid her eggs. The average mass and body size of the 'hot' and the 'cold groups' were similar during testing (10 months: $t = -0.76$, $p = 0.324$; 12 months: $t = 0.96$, $p = 0.359$: 15 months: $t = 1.03$, $p = 0.324$)

Novel object experiment

(b) Experimental design

The experimental arena was a wooden box measuring 76.5 x 73cm and 19cm high with black meshing placed over the top. The box was visually split into four quadrants (38.25 x 36.5cm) one of which contained the novel item. Four objects, all initially novel to the lizards and all of which were a similar size (mean ±SD; 13.62 ± 2.43), were used in this experiment. Two objects were used for test one and two for test two. The objects were always placed in the same quadrant in the arena, 54cm from the starting position of the lizards. The room was maintained at 28.5 ± 1^{o}C by portable electric heaters. A digital camera (Sony HDR-CX22OE) attached to a tripod was placed above the arena so that the whole arena area could be recorded during the experiment.

(c) Procedure

All animals were habituated to the environment prior to testing. During habituation a single lizard was placed into the arena and left for 15 minutes for 3 successive days. After this a mealworm was placed on the opposite side of the enclosure. The lizard had 5 minutes to reach the mealworm. If the lizard ate the mealworm within 5 minutes of it being placed on 3 consecutive days then it was considered habituated. If the lizard failed to eat the mealworm within 5 minutes the habituation procedure was repeated until the lizard ate on three consecutive days. This continued until all the animals were habituated.

To ensure that the behavioural traits being measured were consistent across time the lizards received four different test trials with four different novel objects. The lizards were first tested 10 months after hatching with either one of two novel objects (counterbalanced across animals; test 1). At the start of each trial a lizard was placed on a marker on the opposite side of the arena to the object, facing towards it. Each trial lasted 20 minutes and each lizard was exposed to the first novel object for four consecutive days. This allowed us to measure their responses to the repeated exposure to an originally novel object. After being repeatedly exposed to the original novel object lizards would receive two more trials of 5 minutes, one with a new novel object and one with the now ‘familiar’ object. 5 months later the experiment was repeated with two different novel objects; this will be referred to as test 2.

(d) Data collection and statistical analysis

Solomon coder was used to analyse the behaviour of the lizards. Only the first 5 minutes of each video was analysed to allow direct comparison with all trials. The amount of time that each lizard spent in the area surrounding the novel object was recorded. This was considered as the animal being in close proximity to the object. When any part of a lizard, was on the line surrounding the object it was considered to be inside the quadrant with the exception of the tail of the lizard. The time the lizard spent moving (locomotion) was also recorded for each animal. The lizard was considered to be moving if it lifted a leg off the ground and the body position moved. A lizard was not considered to be moving if only the head of the animal moved. All videos were coded by a coder who was blind to the experimental and incubation conditions

The data from the novel object experiment was not normally distributed and therefore non-parametric tests were used. A linear mixed effects model was used to see if there was an effect of incubation temperature on proximity to the ‘familiar’ object. The dependant variable was time spent in close proximity to the object and the fixed factors were locomotion, temperature and the interaction between the two. The random factors were month and animal. The same formula was used to determine if temperature influenced the time spent in close proximity to the novel object. Wilcoxon tests were used to test whether the behaviours were consistent and repeatable over time when comparing data sets (in the short term and long term) with average values being used from both novel objects and compared over time. All p-values are given as exact values to control for the small sample size.

Novel environment experiment

(e) Experimental design

The experiment was identical to the previous experiment with some small exceptions. Instead of four novel objects, four different novel environments were used. Arena A measured 76.5 x 73 cm with pink patterned wrapping paper along the walls and sawdust on the floor, by contrast, arena B was rectangular (105 x 38 cm) and contained leopard print wall paper and a plastic bubble-wrap floor. In the later experiments two different arenas were used. Arena C measured 77 x 38 cm with a laminated, patterned floor with a comic book wallpaper design. Arena D was 63 x 40 cm and contained a randomly patterned wallpaper containing and the floor was covered with paper cuttings. The lizards were first tested 12 months (test 3) after hatching; this was repeated again 3 months later, when the lizards were 15 months old (test 4).

(f) Procedure

The procedure for the novel environment experiment was identical to that of the novel object experiment with the exception that the lizards were not habituated to the environment prior to starting.

Data collection and data analysis.

A linear mixed effects model was also used to see if there were differences between groups in the responses of the repeated exposure to the originally novel but now ‘familiar environment’. The dependant variable was the time spent in locomotion and the fixed factors were trial and temperature while the random factors were animal and month. The same formula was used to determine if temperature influenced time spent in locomotion in a novel environment. Wilcoxon tests were used to test whether the behaviours were consistent and repeatable over time when comparing data sets (in the short term and long term) with average values being used from the novel environment and familiar environment compared over time

3.3 Results

Repeated exposure to an originally novel object

Figure 1 shows that the 'hot group' spent more time in close proximity to the 'familiar' object in all of the repeated exposure trials when the lizards were 10 months old. When the lizards were tested 5 months later it appeared that both groups of lizards spent similar amounts of time in close proximity to the novel object (Figure 2). The results did show that over both time periods egg incubation temperature did have an effect on the time spent in close proximity to the novel object (t = 2.46, df = 122.41, p = 0.015). The results also showed that there was a significant effect of incubation temperature on the locomotion of the lizards (t = 4.66, df = 122.05, p < 0.001) and there was also a significant quadratic relationship of locomotion and time spent in close proximity to the object (t = -3.94, df = 122.59, p = 0.001). This suggests that incubation temperature affected locomotion and lizards that moved around an intermediate about of time spent more time in close proximity to the object. There was no effect of trial on the results suggesting that the animals did not habituate to the novel object (p > 0.05).

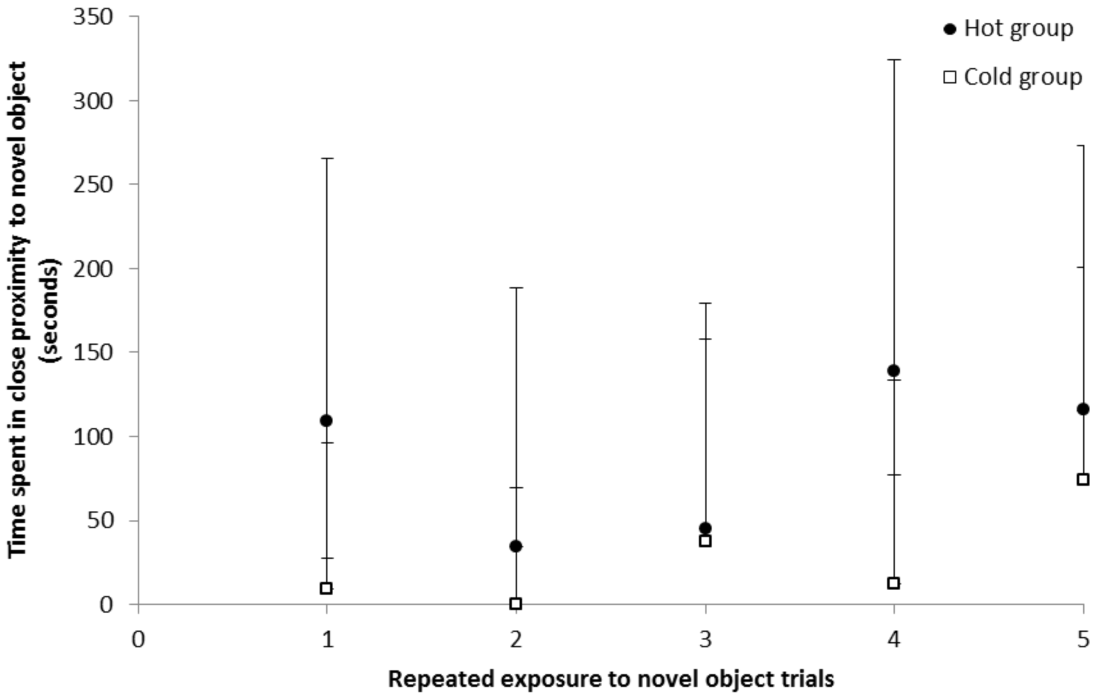

Figure 1. Trial 1 shows the median time spent in close proximity to an originally novel object for both the 'hot' and the 'cold group' (± the interquartile range) when tested at 10 months of age.

Trials 2-5 shows the median time spent (± the interquartile range) in close proximity to the object during e repeated exposure over the trials. .

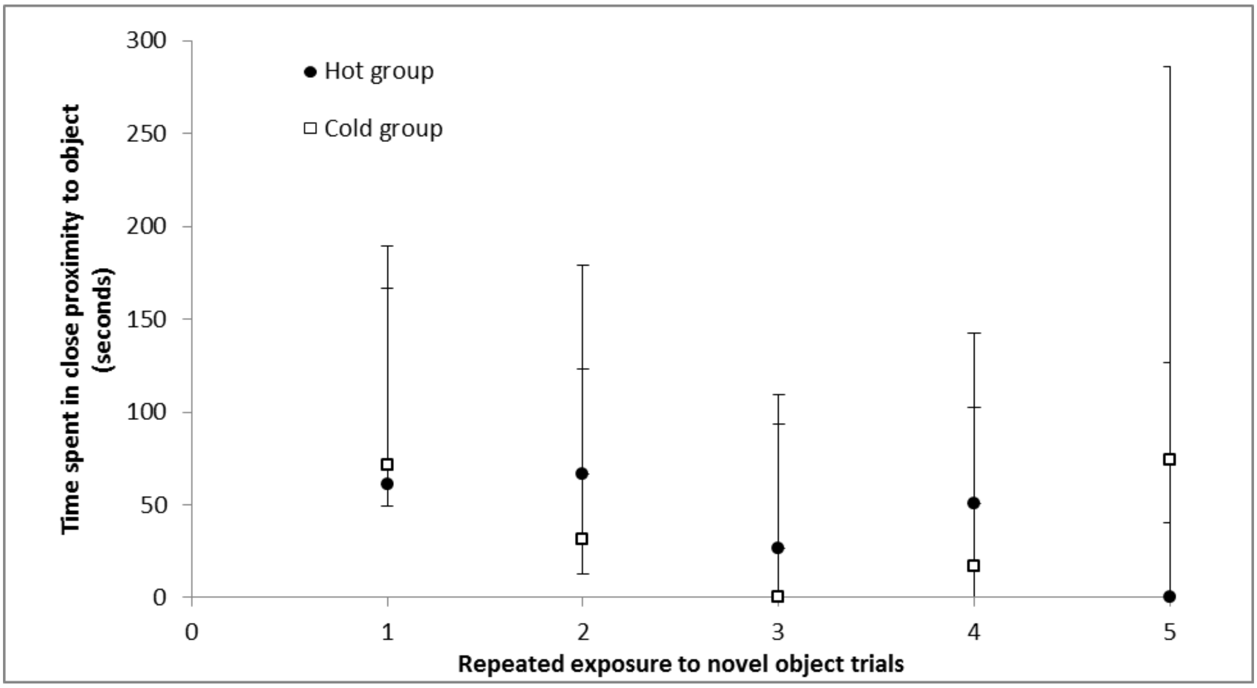

Figure 2. Trial 1 shows median time spent in close proximity to an originally novel object for both the 'hot' and the 'cold group (± the interquartile range). Trials 2-5 show median time spent in close proximity to the object during repeat exposure trials when the lizards were tested at 15 months of age.

Novel object experiment

The results revealed that egg incubation temperature significantly affected the amount of time the lizards spent in close proximity to the novel object (Temperature: t = -2.75, df = 48.76, p = 0.016) with the 'hot group' spending more time in close proximity to the novel object in test 1 but not test 2 (Figure 3 and Figure 4). The results also showed that with the first 3 novel objects the 'hot group' spent more time in locomotion when compared to the 'cold group' (Figure 5). The time that the lizards spent in locomotion was also significantly different for lizards incubated at different incubation temperatures (Locomotion: t = 4.69, df = 2.95, p < 0.001) and there was a significant interaction between locomotion and temperature (t = -2.75, df = -2.08, p = 0.008). The effects of month and individual lizard were used as a random factor in model and were not significant (p > 0.05). This shows that egg incubation temperature influenced both the time that the lizards spent in close proximity to the novel object and the locomotion of the lizards.

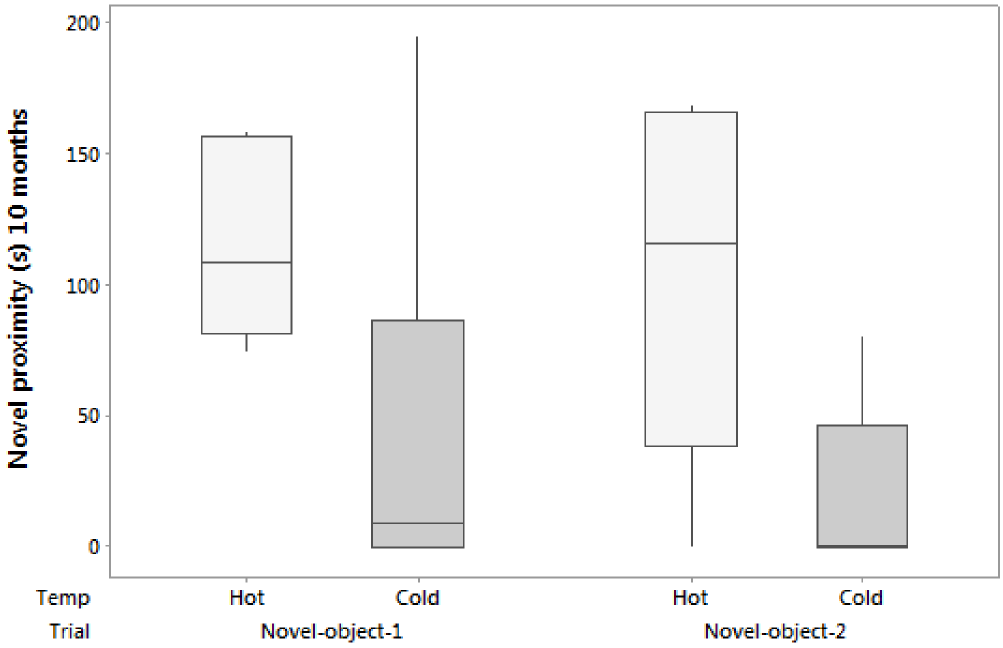

Figure 3. Median time spent (± interquartile range) in the quadrant containing the novel object over the course of 5 minutes for the 'hot' and 'cold' group when the animals were first tested with two novel objects at 10 months of age (test 1).

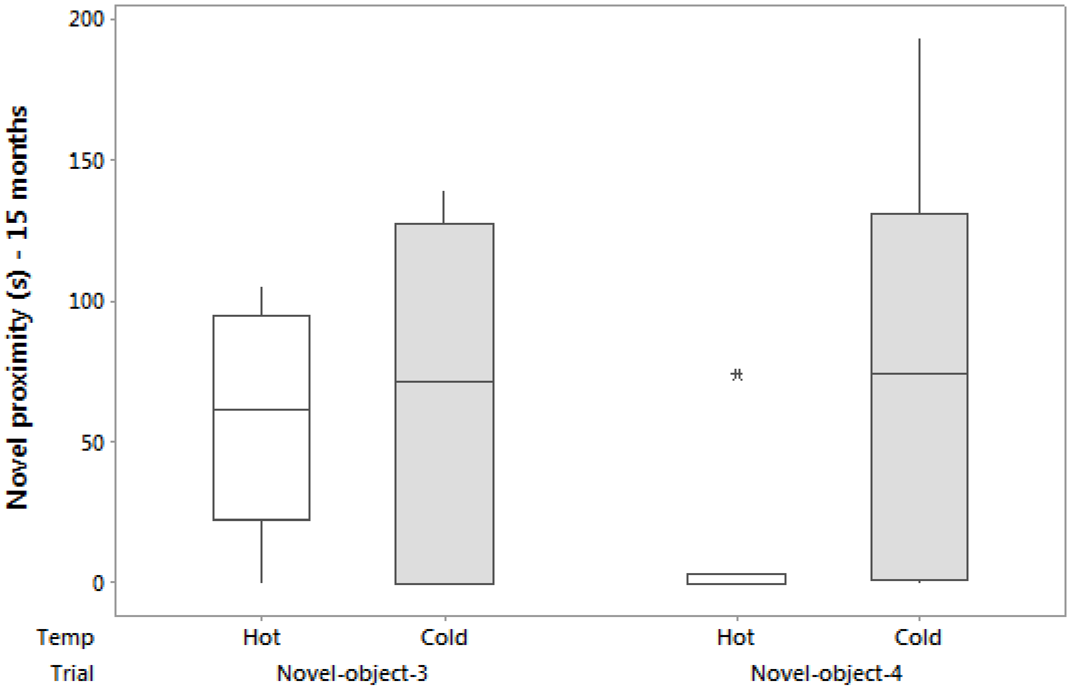

Figure 4. Median time spent in seconds (± interquartile range) in the quadrant containing the novel object over the course of 5 minutes for the 'hot' and 'cold' group when the animals were tested with two novel objects for a second time, 5 months after previously being tested (test 2).

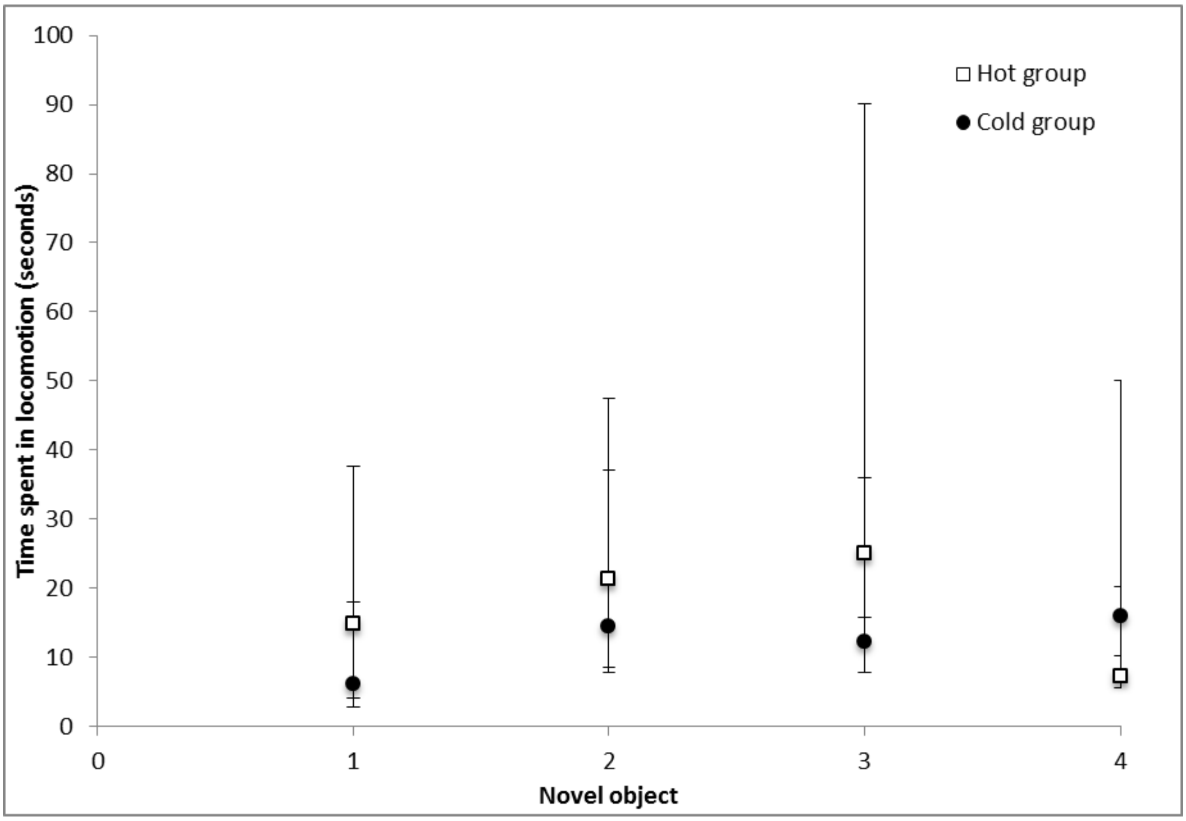

Figure 5. Median time spent in locomotion in the 4 novel object experiments (± the inter quartile range). Novel object 1 and 2 were tested at 10 months after hatching and novel objects 3 and 4 were tested at 15 months after hatching.

Time differences in the novel object experiment

When the lizards were tested originally in test 1, there were no significant differences in the trial 1 proximity to novel objects for the 'hot group' (Wilcoxon x^2 = -0.34, df = 7, p = 0.813) or the 'cold group' (Wilcoxon x^2 = -0.73, df = 6, p = 0.625) over the course of 4-5 days suggesting that the behaviour was repeatable (Figure 3). The lizards proximity to the novel object was also repeatable over the course of 4-5 days for both the 'hot group' (x^2 = -1.75, df = 6, p = 0.125) and the 'cold group' (x^2 = -1.21, df = 6, p = 0.313) in test 2 when the lizards were tested at 15 months after hatching.

To see if the lizard's willingness to be in close proximity to a novel object was consistent over time (5 months) the average response to the two novel objects was taken from each test and compared using a Wilcoxon. The 'behaviour of the 'hot group' was not repeatable (x^2 = -2.37, df = 7 p = 0.016) and neither was the 'cold group' (x^2 = -2.20, df = 6, p = 0.031).

Repeated exposure to an originally novel environment

Figure 6 shows that during the repeated exposure trials, when the lizards were tested originally at 12 months the 'hot group' spent more time in locomotion when in the familiar environments but 3 months later there were no differences between the lizard incubated at different temperatures

(Figure 7). The results confirmed this and showed that there was no significant effect of incubation temperature on the locomotion in now ‘familiar’ environment (Temperature: t = -1.10, df = -8.05, p = 0.292) and there was no significant effect of trial (Trial: t = 2.00, df = 3.74, p = 0.053).

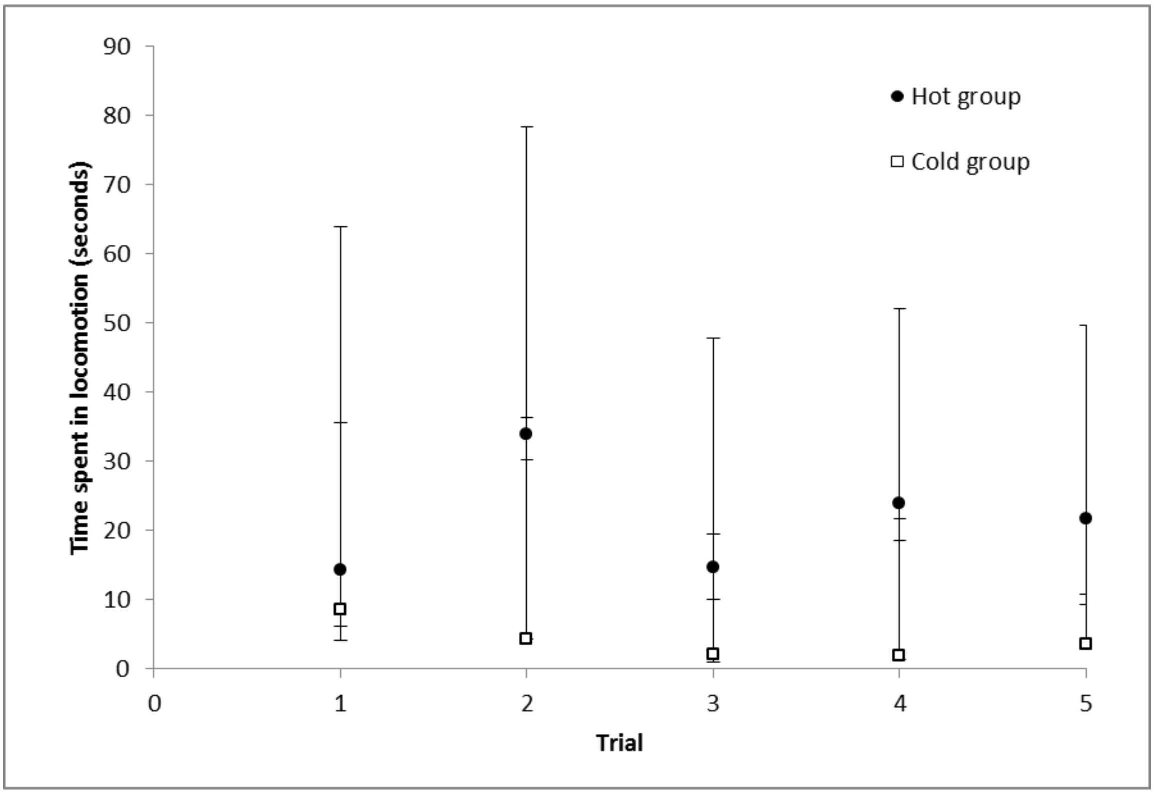

Figure 6. Trial 1 shows the median time spent in locomotion for both the ‘hot’ and the ‘cold group (± the interquartile range) when the lizards were tested at 12 months after hatching Trials 2-5 show the time spent in locomotion when repeatedly exposed to the originally novel environment.

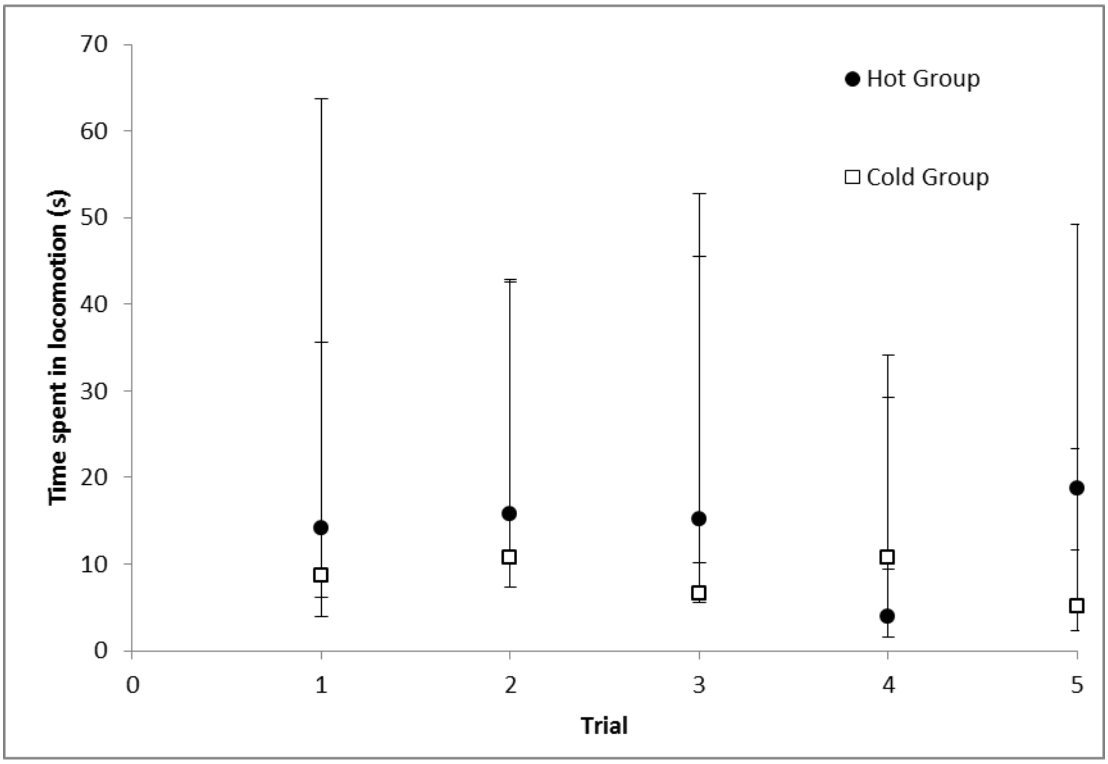

Figure 7. Trail 1 shows the median time spent in locomotion (± interquartile range) for both the 'hot' and the 'cold group' in an originally novel environment when the lizards were tested at 15 months after hatching). Trials 2-5 show time the medium time spent moving in repeated exposure trials to the environment.

Novel environment experiment

Lizards incubated at hotter incubation temperatures moved around more in a novel environment when tested at 12 months of age (test 3; Figure 8. When the lizards were tested again in two different novel environment 3 months later, the lizards from each group moved around the novel environment a similar amount (Figure 9, test 4) and the results showed that overall there was no significant effect on incubation temperature on the locomotion of bearded dragons in a novel environment (Temperature: $t = 0.28$, $df = 8.25$, $p = 0.279$).

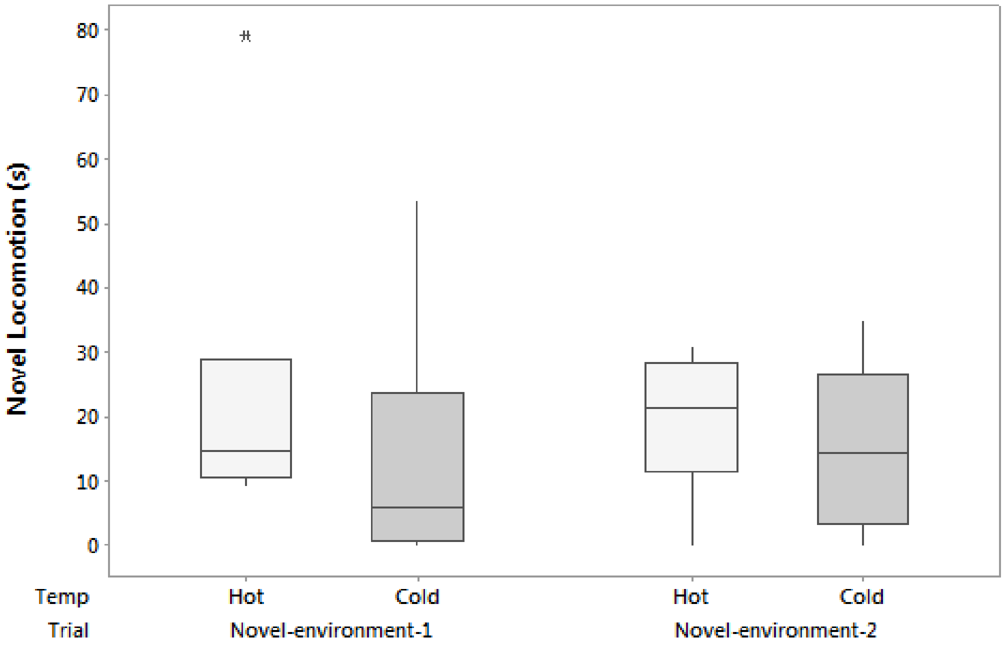

Figure 8. Median (± inter quartile range) time spent in locomotion for the ‘hot’ and ‘cold’ group in a novel environment when the lizards were tested for the first time in two novel environments at 12 months after hatching (test 3).

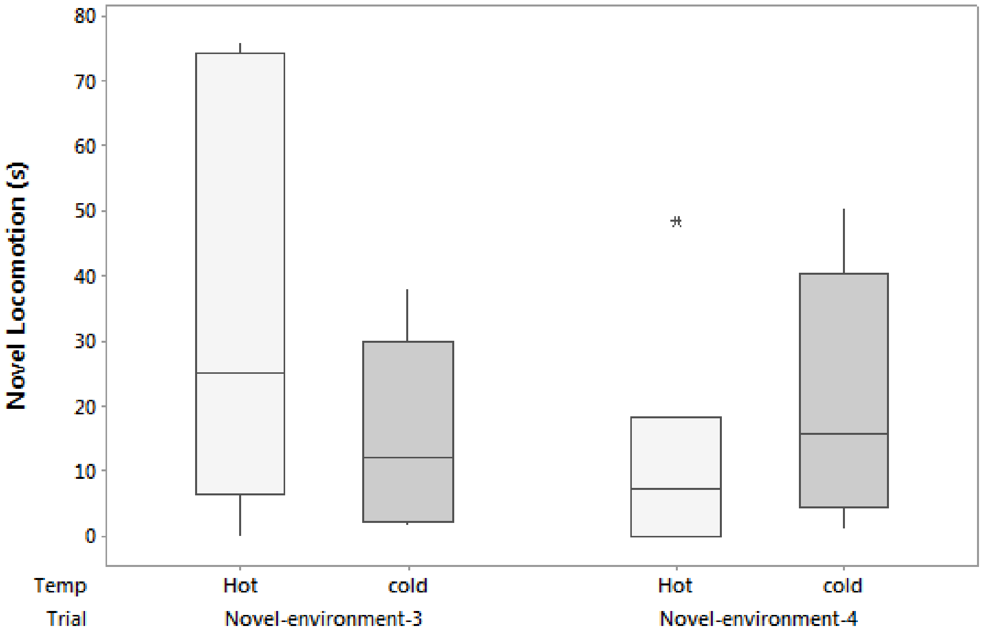

Figure 9. Median(±inter quartile range) time spent in locomotion for the ‘hot’ and ‘cold’ group in a novel environment when the lizards were tested in two different novel environments 3 months after originally being tested (test 4).

Time differences in the novel environment experiment

The results showed that in test 1 the behaviour of the lizards was repeatable in the short term across 4-5 days for the ‘hot group’ (x^2 = -1.36, df = 6, p = 0.219) and the ‘cold group’ (x^2 = -0.41, df = 6, p = 0.813). For test 2 ‘hot group’ (x^2 = -0.73, df = 6, p = 0.563) and the ‘cold group’ (x^2 = -2.02, df = 5, p = 0.063).

The results of a Wilcoxon test showed that the ‘behaviour of the ‘hot group’ was not significantly different over the course of 3 months (x^2 = -1.01, df = 7 p = 0.375) as was the behaviour of the ‘cold group’ (x^2 = -9.43, df = 6, p = 0.438).

3.4 Discussion

The results of the novel object experiment showed that the ‘hot group’ spent more time in close proximity to the novel object compared to the ‘cold group’ in test 1 however, this was not repeatable five months later in test 2. As well as this, in the presence of the first three novel objects the ‘hot group’ appeared to spend more time in locomotion than the ‘cold group’. The novel environment experiment showed that there was no difference between the locomotion of lizards incubated at different temperatures either. It is surprising that the locomotion of the lizards was significantly affected by incubation temperature in the novel object experiment but not the novel environment experiment. One possible explanation for this is that egg incubation temperature influences the development of certain behavioural traits at different stages in ontogeny. The results from test 1 suggest that egg incubation temperature influenced both the proximity to the novel object and the activity of the lizards, with lizards incubated at hotter temperatures being more active and ‘bolder’. Boldness and activity levels are often positively correlated (Van Oers, 2005). The novel environment experiment was conducted two months later meaning that it is possible that the behavioural traits had changed over time. However, for behavioural traits such as boldness to be defined as personality they need to be repeatable across time and context. Our results show that in the novel object experiment the time spent in close proximity to the novel object lizards was repeatable in the short-term but not the long-term. This would suggest that egg incubation temperature does not influence the long-term personality of lizards but instead influences the development in behavioural traits at different stages in ontogeny. This therefore implies that egg incubation temperature influenced the behavioural traits of bearded dragons at different stages throughout ontogeny.

Egg incubation temperature has been shown to affect the development of oviparous reptiles with the growth, physiology and behaviour all being influenced by incubation temperature (Deeming, 2004). For example, egg incubation temperature impacts upon the growth rates of oviparous reptiles at different stages in ontogeny. Alberts *et al.,* (1997) showed that in Cuban rock iguana’s (*Cyclura* *nubile*) growth rates were effected by incubation temperature with lizards incubated at hotter temperatures (31^{o}C) having quicker growth rates for up to 14 months. In contrast to this Siviter *et al.,* (in preparation a, chapter two) found that the growth rates of bearded dragons incubated at different temperatures were different at different stages in their development. The results showed that lizards incubated at colder temperature grew heavier faster than those incubated at a hotter temperature, suggesting differences in the ontogeny of the lizards. Developmental changes in the behaviour of oviparous reptiles as a result of incubation history have rarely been examined, with most previous studies only testing the behaviour of hatchlings meaning the long-term effects of incubation temperature on behaviour are largely unknown, with some exceptions (Elphick and Shine, 1998). The results of our experiment show that egg incubation temperature influences the behavioural traits of bearded dragons for up to at least 10 months from hatching and suggest that developmental differences in behavioural traits are likely to be due to differences in incubation history. The fact that the influence of incubation temperature were stronger while the animals were younger could suggest that incubation temperature is 'prepares' oviparous reptiles for the environment which they will emerge into. However, the results from test 2 show a large variance in the results between novel object 3 and novel object 4 suggesting that to investigate this further another repetition on the novel object experiment would be advisable. Previous experiments testing the personality of animals generally only test the repeatability of behavioural traits over short periods of time (e.g. Frost *et al.*, 2007; Short and Petren, 2008). Interestingly, the results of the present experiment showed that certain behavioural traits were repeatable in the short-term but not in the long-term. Had this experiment failed to test the long-term repeatability then a different interpretation would have been placed on the results.

The mechanisms that cause temperature-dependent differences in the phenotypes of oviparous reptiles are still not fully understood (Valenzue, 2004; Deeming, 2004). One possibility is that the relationship between egg incubation temperature and the phenotype of oviparous species of reptiles is adaptive as it allows a clutch of eggs to be suited to a wide variety of different environments (Deeming and Ferguson, 1991). The environment that the lizards are incubated in will reflect the environment that they are emerging into and it has been suggested that this is adaptive, as animals will emerge into an environment that they are better adapted to (Siviter *et al.,* in preparation a; chapter 2). It has been suggested that the sensitivity of oviparous reptiles to environmental factors may make animals more flexible in dealing with changing environments and could be a valuable defence mechanism for tackling human induced climate change.

Personality traits such as boldness and activity have been shown to be linked with food acquisition in a range of different species (Biro and Stamps, 2008). The results of the present experiment suggest that lizards incubated at different temperatures will behave differently at different stages of their development. Interestingly, Siviter *et al.,* (in preparation a; chapter 2) showed that lizards incubated at hotter temperatures were better at foraging than lizards incubated at colder temperatures while lizards incubated at colder temperatures had higher growth rates. The results from the present experiment suggest that the 'hot group' were 'bolder' and more active when tested at 10 months from hatching compared with the 'cold group'. A positive correlation between foraging intake and activity/boldness has been seen in a range of species, for example, active *Solea solea* fish were shown to have a higher feed intake when compared to less active fish (Mas-Muñoz *et al.,* 2011) and great tits (*Parus major*) that sang more actively were more explorative and suggested to be better at foraging (Naguib *et al.,* 2010). The results from the present experiment could suggest that there could be a positive correlation between higher incubation temperatures, foraging ability and certain behavioural traits, although this would require further research. Keogh *et al.,* (2012) showed that the activity rates of a polygynous lizard (*Eulamprus heatwolei*) and reproductive rates were positively correlated with males that are active in mate searching increasing individual fitness. However, when resources are plentiful lizards that are less bold may have an advantage over active lizards as they may have a higher likelihood of predation (Carter *et al.,* 2010) and will potentially use more energy. Cooler egg incubation temperatures can produce heavier and larger lizards (Deeming, 2004) suggesting that lizards incubated at these temperatures will be able to dominate resources (Tokarz, 1985). The 'cold group' were less active than the 'hot group' suggesting that bearded dragons incubated at lower temperatures may have quicker growth rates as they use less energy. The present study shows that throughout development egg incubation temperature influences the developmental differences in the behaviour traits of oviparous reptiles; this suggests that egg incubation temperature influences the life history and fitness of individual oviparous reptiles and produces reptiles that are able to adjust to a flexible environment.

In conclusion, the results showed that egg incubation temperature did not impact the long-term 'boldness' of bearded dragons but rather egg incubation temperature influenced the development of the behavioural traits of lizards at early stages in ontogeny.

Chapter Four- Bearded dragons are capable of following the gaze of conspecifics into the distance but not geometrically

Abstract

Gaze following is the ability to co-orient with a conspecific and is thought to be highly adaptive. There are two types of gaze following that are often referred to, gaze following into the distance and gaze following around a barrier also known as geometric gaze following. Research suggests gaze following into the distance does not involve complex cognition while geometric gaze following does. Egg incubation temperature has been shown to influence the learning ability of oviparous reptiles which could potentially influence gaze following ability. This experiment investigated three questions. 1) Can bearded dragons follow the gaze of a conspecific into the distance; 2) can bearded dragons geometrically gaze follow and 3) does egg incubation temperature influence bearded dragons' ability to gaze follow. The results showed that bearded dragons are capable of following the gaze of a conspecific into the distance but not geometrically. There was also no difference in the gaze following ability of lizards incubated at different egg incubation temperatures. The fact that the lizards were not capable of geometrically gaze following provides more evidence that these two different types of gaze following involve different levels of cognitive abilities with geometric gaze following involving more complex cognition. The results are the first to show that a lizard species is capable of gaze following into the distance, suggesting that gaze following into the distance is more common than previously thought.

4.1 General Introduction

Egg incubation temperature has been shown to influence the phenotypes of various different species of oviparous reptiles (Deeming, 2004) and can even affect the behaviour and cognition of reptiles (Amiel and Shine, 2012). For example, lizards (*Bassiana duperreyi)* incubated at hotter temperatures (22 ±7.5^{o}C) were better at learning a simple learning discrimination than lizards incubated at colder temperatures (16 ±7.5^{o}C) (Amiel and Shine, 2012). It has also been suggested lizards (*Bassiana duperreyi)* incubated at hotter temperatures were capable of greater cognitive flexibly than lizards incubated at colder temperatures (Clark *et al.,* 2014). The ability to gaze follow has many potential advantages; co-orienting with conspecifics may aid learning about potential food sources, in predator detection and increase the observations of important social interactions (Emery, 2000; Zuberbühler, 2008). Gaze following is thought to contain both innate and learnt elements, thus if egg incubation temperature influences the learning ability of oviparous reptiles then it could impact on the ability to gaze follow. Two different modes of gaze following are typically observed, gaze following into the distance and geometric gaze following. Gaze following into the distance refers to an animal's ability to follow the gaze of a conspecific or a hetero-specific to a location in space (Emery *et al*., 2000). Geometric gaze following, whereby an animal has to change its own line of sight in order to follow the gaze of another (e.g. around a barrier) is thought to be more complex (Loretto *et al.*, 2010) as the animal either has to learn that barriers will impair sight (Tomasello *et al.,* 1999) or understand the visual representation of the animal gaze (Povinelli and Eddy, 1996). Given that gaze following into the distance is not thought to require complex cognition it has been suggested that this trait should be relatively common throughout the animal kingdom while geometric gaze following will be less frequently observed. For example Loretto *et al.,* (2010) showed that the northern bald ibis (*Geronticus eremita*) were able to follow the gaze of a conspecific into the distance but not around a barrier and Burkart & Heschl (2006) showed that common marmosets (*Callithrix jacchus*) could follow the gaze of a human experimenter into the distance but not around a barrier. In addition, Schoegl *et al.,* (2007) also showed that common ravens (*Corvus corax*) (that can geometrically gaze follow) would rapidly habituate to the gaze of a human experimenter when following the gaze of a conspecific into the distance however they did not habituate to the gaze of a conspecific looking around a barrier, suggesting two different modes for gaze following into the distance and geometric gaze following.

The development of gaze following into the distance and gaze following around a barrier appear to develop at different stages in ontogeny. The ability to gaze follow around a barrier will undoubtedly have important learning elements to it and the development of gaze following throughout ontogeny is well studied in animals including humans (Brooks and Meltzoff, 2005).

Ravens, for example, could gaze follow into the distance at around 8 weeks of age however they could not follow the gaze of a conspecific around a barrier until they were approximately 8 months old which is around the time they become independent from their parents (Schloegl *et al*., 2007). This same trend was seen throughout the ontogeny of wolves (*Canis lupus*) (Range & Virányi, 2011) and evidence has shown the human infants can acquire the skill to gaze follow into the distance at as soon as two months of age (Scaife and Bruner, 1975) and only become capable of geometric gaze in-between 12-18 months of age (Moll and Tomasello, 2004). These results suggest that if an animal is socially housed and repeatedly exposed to the gaze of a conspecific that an animal can learn to follow the gaze of a conspecific.

The central bearded dragon (*Pogona vitticeps*) can be found throughout central Australia and is considered a social species of reptiles with several social interactions being recorded (Carpenter, 1970; Brattstrom, 1971). Males and females will have overlapping territories and will often share basking spots while males will defend territories from other males. Previous studies (Siviter *et al.,* in preparation a; Siviter *et al.,* in preparation b) have shown that egg incubation temperature can affect the long-term behaviour of bearded dragons. This experiment looked at the differences in the gaze following ability of bearded dragons with different incubation histories. We ran two separate experiments. Experiment one tested whether bearded dragons are capable of following the gaze of a conspecific into the distance while experiment two tested whether bearded dragons can follow the gaze of a conspecific around a barrier. Recent research has shown that egg incubation temperature influenced the learning ability of bearded dragons, with lizards incubated at colder temperatures (27^{o}C ± 3) performing better than lizards incubated at a hotter temperatures (30^{o}C ± 3) (Junttilia *et al.,* in preparation). It was hypothesized that lizards incubated at colder incubation temperatures will perform better in both experiment one and experiment two compared with lizards incubated at hotter incubation temperatures.

Experiment 1: Gaze following into the distance

4.2 Introduction

The orienting response model refers to animals following the gaze of a conspecific into the distance and contains both learnt and innate elements and one hypothesis suggests that some animals, including humans are 'hard-wired' to gaze follow (Emery, 2000). However, it is widely accepted that this type of gaze following does not require high levels of cognitive ability as it is common throughout the animal kingdom (Povinelli and Eddy, 1996; Tomasello, 1999; Loretto *et al.,* 2010). For example numerous primate species (apes *Pan troglodytes, Pongo pygmaeus* : Tomasello *et al.,* 1998; Itakura, 1996), Old world monkeys (*Cercocebus atys torquatus*, *Macaca mulatta*, *M. arctoides*, *M. nemestrina*: Tomasello *et al.,* 2001; *Macaca arctoides* Anderson and Mitchell, 1999), birds (Northern bald ibises *Geronticus eremita*: Loretto, *et al.,* 2010; common raven: *Corvus corax* : Schloegl *et al.,* 2007) and one species of reptile (red footed tortoises (*Geochelone carbonaria*) : Wilkinson *et al.,* 2010) have all been shown to be capable of gaze following into the distance. It still remains unclear if the ability to follow gaze into the distance is unique to tortoises or if it is more general across reptiles.

This experiment tested to see whether bearded dragons could follow the gaze of a conspecific into the distance. We tested whether they could follow the gaze of a conspecific to the side and upwards and whether egg incubation temperature influenced this ability. It has been suggested that looking to the side and looking upwards have different functions. For example looking up has been described as an anti-predator response for spotting aerial predators (Bugnyar *et al*., 2004) while following the gaze of a conspecific to the side might be more relevant in social or hunting situations (Range and Viranyi, 2011). Bearded dragons are a social species of reptiles and have recently being shown to be capable of sophisticated social learning, namely imitation, (Kis *et al*, 2014) which is often thought to be the pinnacle of social learning. Given that bearded dragons incubated at colder incubation temperatures were better at learning a simple learning discrimination (Junttilia *et al.,* in preparation) than lizards incubated at hotter incubation temperatures it was predicted that bearded dragons would have different levels of gaze following ability at different stages in ontology.

4.3 Methodology

Egg incubation temperature

A clutch of eggs, sired by one male, was randomly split into half and assigned to the 'hot group' and the 'cold group'. The 'hot group' were incubated at an average temperature of 30 ± 3°C and the 'cold group' were incubated at an average temperature of 27 ± 3°C. The eggs were held in plastic boxes with a vermiculate substrate and kept moist (Booth, 2004).

Animals

Once hatched the animals were housed in as similar environments as possible and maintained at an average temperature of 29 ± 3° C. The animals were socially housed, in vivariums that were 150 x 60 x 60cm, with each vivarium containing a UV light, heat lamp and shelters. There were a total of 13 animals used in this experiment with the 'hot group' containing 7 lizards, 4 males and 3 females, while the 'cold group' contained 6 lizards, 3 males and 3 females. All animals had previous experimental experience (e.g. Siviter *et al.,* in preparation a, Siviter *et al.,* in preparation b) and were subadults when tested (46-51 weeks old). There were no differences in mass of the lizards at the time of this experiment and all animals were sexually mature.

Experimental set up

The experimental setup consisted of a rectangular arena measuring 118cm x 37cm x 19cm. A computer monitor was positioned in one end of the arena, which was used to present video stimuli to the observer animal (Figure 1). The video footage showed a bearded dragon doing one of three things; looking straight (the control), looking to the side and looking upwards (see descriptions of videos below).

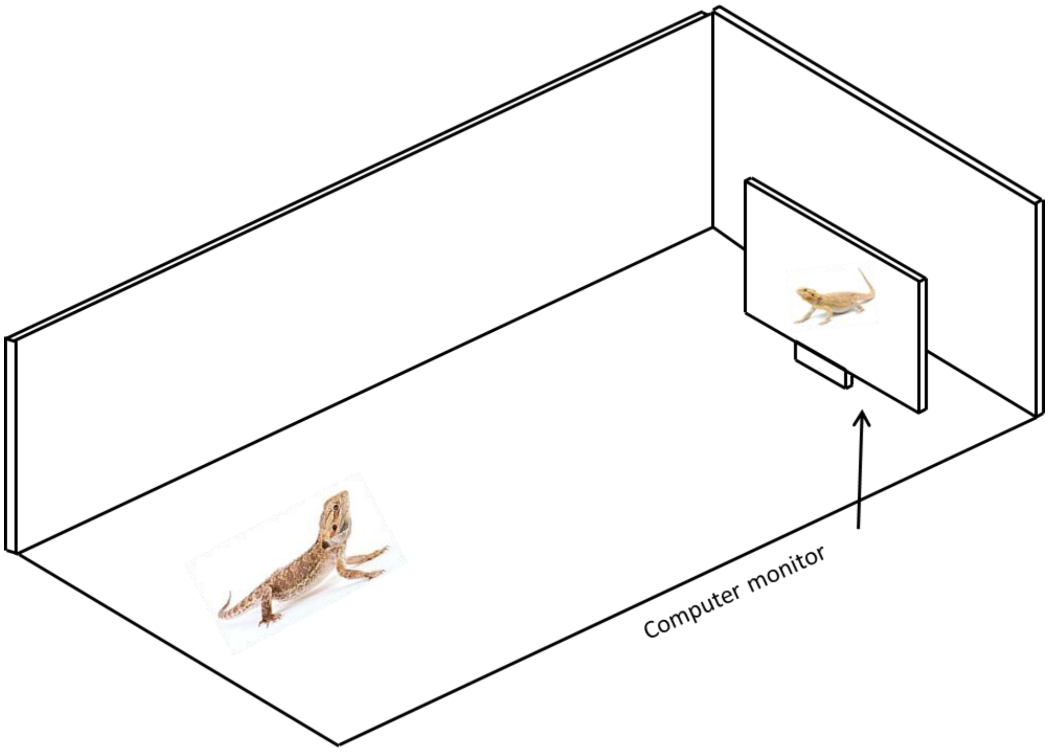

Figure 1. Experimental apparatus for experiment 1.

Procedure

All lizards were habituated to their environment. Each lizard received two ten-minute habituation trials. Mealworms were placed inside the arena and the animals were considered habituated if they ate all the food on two consecutive trials. All the lizards were habituated within two trials. Animals were tested over the course of five days with each animal receiving one session a day containing 3 trials. Each trial was separated by an inter-trial interval of at least 10 minutes during which the lizard was returned to its enclosure. Each animal received 9 trials in total, 3 trials per condition. The order of the trials was counterbalanced between animals and across sessions.

The observer animal was placed 60cm from the monitor facing towards the screen. If the observer was not looking at the screen (classed as at least one eye facing towards the screen) or the lizard moved off before the video was played then the trial was terminated and repeated on another day, although the other trials from the sessions could still be used. Exactly 5 seconds after the bearded dragon had been placed in the arena a video from one of the three conditions was presented to the observer lizard (see below). For this experiment three different stimuli were presented to the subjects. The videos presented a female bearded dragon that was unfamiliar to the observer lizard and showed her 1) moving her head up 2) moving her head sideways and 3) stationary and facing forward which was used as a control (see below for details). Once the videos had played the video stopped on the last frame. The average length of the video clips was 3.8 seconds and a different video was used in each trial meaning that each animal only saw each video once. The same female bearded dragon was used as the demonstrator animal in each trial. The response from the lizard was recorded for 5 seconds after the video had started after which the trial was terminated. All trials were recorded on a video camera (Sony HDR-CX22OE) and were analysed using VLC media player. As bearded dragons do not have visible sclera, head orientation was used to assess the direction in which the dragon looked and is commonly used for assessing gaze direction (e.g. Loretto *et al*, 2010).

Look up condition: The video showed the demonstrator lizard looking up either by moving its head upward or by turning its head and positioning one eye upwards. An observer lookup was counted if the observer dragon looked up by either extending its head and neck upwards or by turning its head so one eye was directed upwards in the 5s following the stimulus presentation.

Look sideways condition: The stimuli were the same as those in the look up condition with the exception that the observer animals observed footage of the demonstrator looking into the distance to the side. The observer lizard was considered to be looking sideways if its head moved sideways in either direction. Bearded dragons have binocular vision meaning that in order to follow the gaze of a conspecific the lizard could move its head to the side in either direction.

Control Stationary demonstrator: To control for the influence of the presence of a conspecific we included a condition in which the demonstrator faced towards to observer and did not show a shift in gaze direction. When presented with the control condition, observers were expected to show fewer shifts in their gaze.

Data

All trials were coded from video recordings and a response was only included if it occurred within 5 seconds of the stimulus being presented. The data was analysed using a general linear mode that tested for the effect of temperature and test condition on gaze. For the looking up condition if a lizard looked up in a trial it was coded as 1 and if it did not look up it was coded as 2. In the looking to the side condition if a lizard looked to the side it was coded as a 1 if it did not it was coded as a 2. These numbers were used as the dependant variable in a general linear model while incubation temperature and test condition were used as fixed factors as was the interaction between them. The individual was used as a random factor. Ten percent of the data was coded by a second individual who was naïve to the conditions of the experiment and inter observer reliability was good (Cohens k = 0.892, p = 0.001)

4.4 Results

Looking up into the distance condition:

The lizards looked up on average 30.76% of time in looking up trials but only 12.82% of time in control trials (Figure 2). Despite this there was no significant difference between the looking up condition and the control when all the animals were examined. ($F_{1,23}$ = 3.56, p = 0.072). The 'hot group' spent on average 28.57% of trials looking up when the demonstrator lizard did compared to the 'cold group' that spent 33.33% (Figure 2). There was no effect of incubation temperature on average number of trials that the lizards looked up ($F_{1,23}$ = 0.52, p = 0.490) and there was no interaction between incubation temperature and test condition ($F_{1,22}$ = 0.47, p = 0.503).

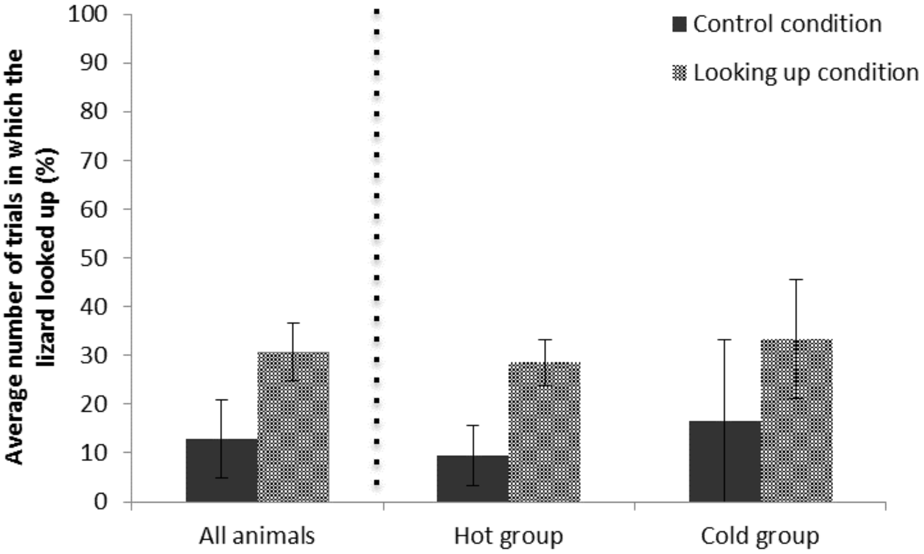

Figure 2. Mean % of trials that the lizards looked up in (±SE) for the control condition and the looking up condition.

Looking sideways into the distance condition:

The lizards looked to the side in 87.17% of the look sideways trials compared to the control trials where they spent 53.84% looking to the side and this difference was significant overall ($F_{1,23} = 8.37$, p = 0.008). The 'hot group' spent on average 90.47% of trials looking sideways when the demonstrator lizard looked to the side while the 'cold group' spent on average 85.71% of time trials looking to the side, (Figure 3) but there was no statistical difference between the groups ($F_{1, 23} = 0.38$, p = 0.543). There was no interaction between incubation temperature and test condition ($F_{1,22} = 0.17$, p = 0.686).

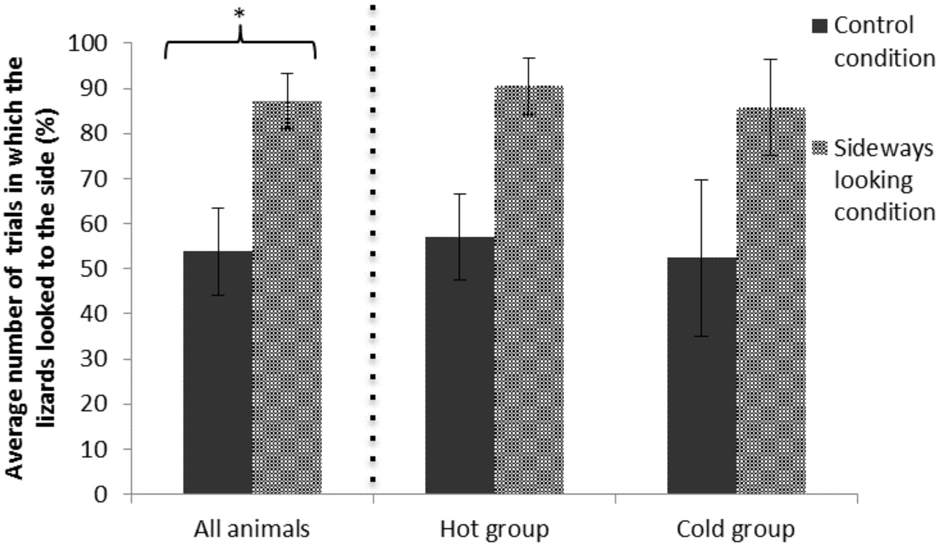

Figure 3. Mean % of trials in which the lizards looked to the side (± SE) for the control condition and the sideways looking condition.

4.5 Discussion

The results did show for the first time that a lizard species is capable of following the gaze of a conspecific into the distance, meaning that two different species of reptiles have now been shown to be capable of following the gaze of conspecifics (Wilkinson *et al.,* 2010). This suggests that the ability to gaze follow into the distance is possibly more common in reptiles than previously thought. The results of this experiment are also the first to show that a reptile species is capable of gaze following to the side. The results showed no difference between lizards incubated at different temperatures in their ability to follow the gaze of a conspecific. Interestingly the lizards readily followed the gaze of a conspecific to the side but not upwards. It is possible that the two types of gaze serve different functions. Bearded dragons are a social species that are vulnerable to aerial predators (Marchant & Higgins 1993). Following the gaze of a conspecific upwards has been determined as a cue for spotting potential aerial predators meaning that it is surprising that lizards do not readily follow the gaze of a conspecific upwards (Bugynar *et al*., 2004). However, bearded dragons, like many other lizards (Greer 1989), freeze when a predator is observed. It is possible that if the lizard was to look up it might attract the attention of aerial predators.

The results showed no effect of egg incubation temperature on gaze following into the distance, although lizards incubated at colder temperatures did appear to have greater variation in looking than lizards incubated at hotter temperatures. Although egg incubation temperature did not influence the ability of lizards to gaze follow into the distance it is possible that incubation temperature would perhaps influence other types of gaze following therefore in experiment two we tested the same lizards in a geometric gaze following experiment.

Experiment 2 – Geometric gaze following

4.6 Introduction

Gaze following into the distance, is thought to reflect a social facilitated response which does not require complex cognition (Povinelli and Eddy, 1996). Geometric gaze following, in which an animal is able to co-orient with a conspecific around a barrier is thought to be more cognitively demanding and implies that the animal has either learnt that barriers impair sight or and that the animal understands the visual perception of another animal (Tomasello *et al.,* 1999; Bräuer *et al.,* 2005, Loretto *et al.,* 2010). Gaze following into the distance is relatively common throughout the animal kingdom, however, geometric gaze following is not and only a few species have been shown to be capable of geometric gaze following (chimpanzees *Pan troglodytes*: Bräuer *et al.,* 2005: ravens Bugnyar *et al.*, 2004; wolves, Range and Viranyi, 2011).

The bearded dragons used in this experiment were socially housed in vivarium's containing multiple barriers in the form of shelters meaning that the animals had ample opportunity to learn the role of a barrier. Bearded dragons have also been shown to use shelters to hide after confrontation (Carpenter *et al.*, 1970). To our knowledge, no species of reptile has been shown to follow the gaze of a conspecific around a barrier therefore experiment two aimed to answer two questions: 1) Can bearded dragons follow the gaze of a conspecific around a barrier and 2) does egg incubation temperature affect bearded dragons' ability to geometrical gaze follow. Based on the same rationale as experiment one it was hypothesized that the lizards incubated at colder incubation temperatures will move around the barrier more when the demonstrator lizard looked behind the barrier.

4.7 Methodology

Experimental set up

The same animals that were used in experiment one were used in this experiment, they were 15 weeks older (61-66 weeks) at the time of testing. The arena used for this measured 73x73cm and a computer monitor upon which the stimuli were presented was placed at one end of the arena. In addition to this a visual barrier (41 x 16cm) was placed so that the lizard could see the screen but the barrier obstructed the view of the lizard to one side of the arena (Figure 4).

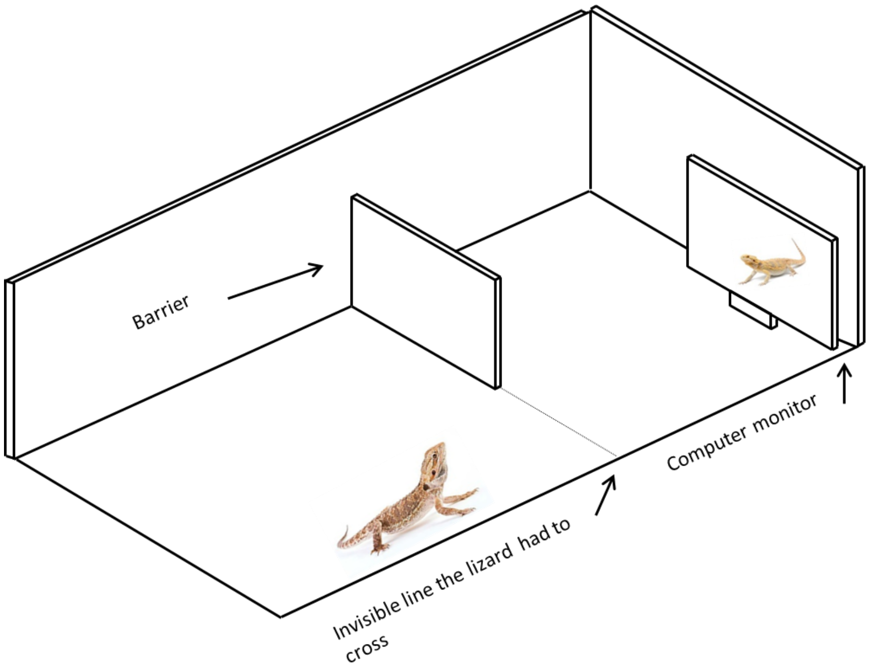

Figure 4. Experimental apparatus used in experiment 2.

Procedure

The procedure was the same as used in experiment one with the addition of the barrier, which was used in all conditions. The stimuli were similar to those used in the previous experiment. After all the videos had been played the lizards had 55 seconds in the arena until the experimenter terminated the trial. With the added exception of the increase in trial time the procedure was the same as used in experiment one. There was also an addition of an extra condition;

Looking sideways behind the barrier: The demonstrator lizard looked sideways behind the barrier. This meant that the observer lizard had to reposition itself in order to follow the gaze of the demonstrator lizard.

Statistical analysis.

The direction of gaze was taken from the first 5 seconds after the video had been played. This was analysed for all conditions. For the gaze following into the distance part of this experiment the same statistics were used as in experiment one. The videos were analysed blind to the condition. . Ten percent of the data was analysed by a second individual who was blind to condition and the Inter observer reliability was good (Cohens k = 0.913, p = 0.001). When coding the videos, the lizard was considered to have moved past the barrier if its head moved past the barrier so it could clearly follow the gaze of the demonstrator lizard or if the observer animal climbed over the barrier in the 60 seconds after the video had been played (Figure 4). If the lizard was considered to have passed the barrier it was coded as 1 and if it did not pass the barrier it was coded as 2 and the coding was same for all conditions. The data was analysed using a general linear model and the movement around the barrier was used as the dependant variable while incubation temperature and trial as well as the interaction between them were used as fixed factors. The individual was used as a random factor.

4.8 Results

Looking upwards into the distance:

The lizards looked up in 66.66% of the trials when the demonstrator did compared to 30.76% of trials in the control condition which was significantly different ($F_{1,23}$ = 10.89, p = 0.003). The 'hot group' looked up on average in 76.19% of trials in the compared to the 'cold group' that spent on average 55.55% of trials looking up. However there was no significant effect of incubation temperature ($F_{1,23}$ = 1.44, p = 0.242; Figure 5) and no significant interaction between incubation temperature and test condition ($F_{1,22}$ = 0.466, p = 0.502).

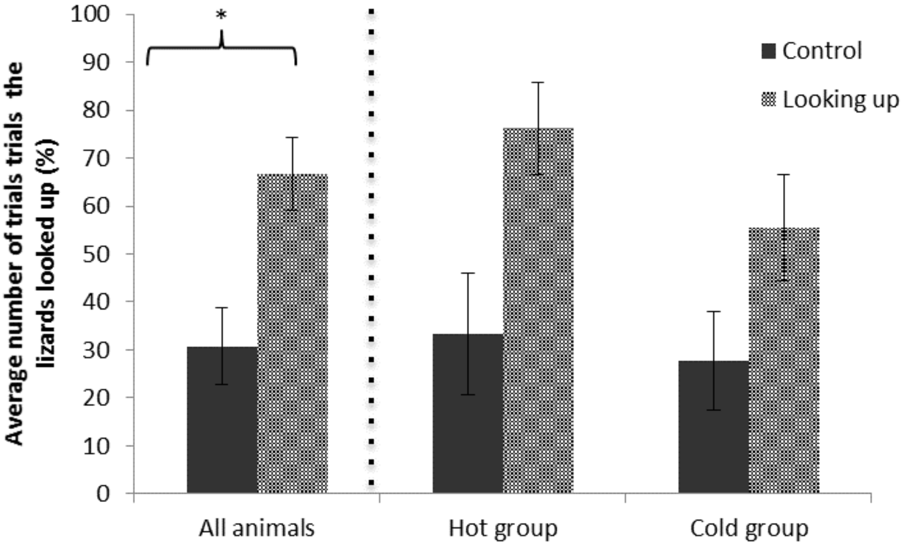

Figure 5. Mean % of trials that the lizards look upwards (± SE) for the control condition and looking up condition for experiment 2.

Sideways looking into the distance:

The lizards looked to the side in 92.30% of trials compared to 61.53% in the control and this difference was significant ($F_{1,23}$ = 21.49, p = 0.001). The 'hot group' spent on average 95.30% of the trials looking to the side when the demonstrator lizard did compared to the 'cold group' that spent 88.88% of trials and there was no significant effect of incubation temperature ($F_{1,23}$ = 0.29, p = 0.597; Figure 6). There was also no significant interaction between incubation temperature and test condition ($F_{1,22}$ = 0.002, p = 0.965).

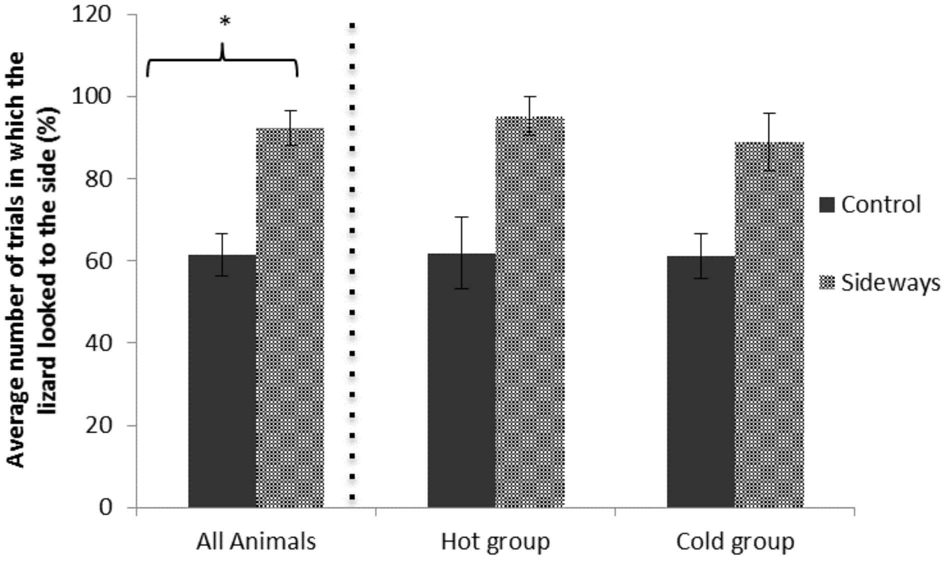

Figure 6. Mean % of looks trials in which the lizard looked to the side (± SE) for the control group and the looking sideways condition for experiment 2.

Barrier sideways looking into the distance:

The lizards looked sideways in 89.74% of trials when the demonstrator did so compared to 61.53% in the control trials and this difference was significant ($F_{1,23}$ = 10.24, p = 0.004). The 'hot group' looked sideways in 90.47% of the trials compared to the 'cold group' that looked sideways in 88.88% and there were no differences between lizards incubated at different incubation temperatures ($F_{1,23}$ = 0.18, p = 0.894; Figure 7). There was no significant interaction between temperature and test condition ($F_{1,22}$ = 0.09, p = 0.765)

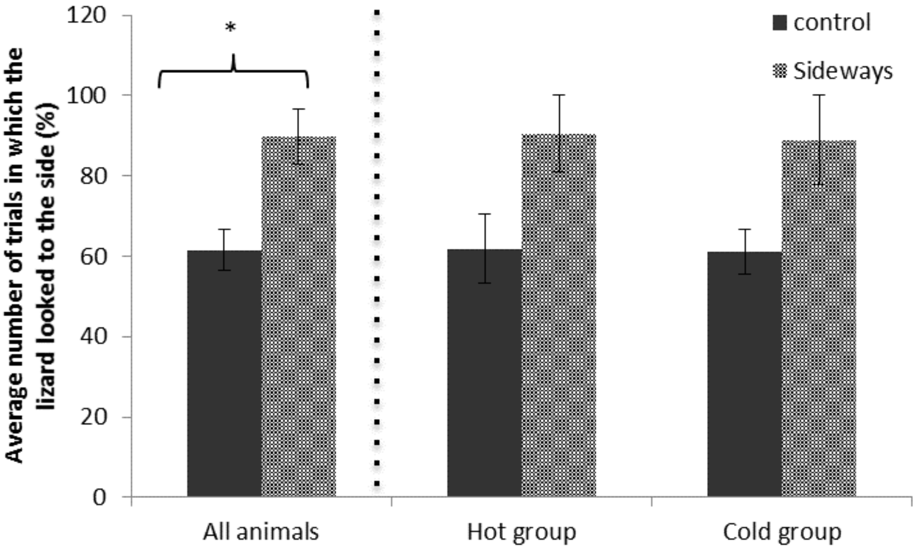

Figure 7. Mean % of trials that the lizard looked to the side (± SE) for the control group and the looking sideways, behind the barrier condition.

Moving around or over the barrier:

The lizards walked around the barrier on average in 48.71% of trials when the demonstrator lizard looked behind the barrier compared to 33.33% in the control trial and this difference was not significant ($F_{1,23}$ = 1.44, p = 0.242; Figure 8). The 'hot group' walked around or over the barrier when the demonstrator animals looked behind the barrier in 57.14% of the trials compared with the 'cold group' that moved around or over the barrier in 38.88% of the trials although this difference was not significant ($F_{1,23}$ = 1.24, p = 0.277; Figure 8). There was also no significant interaction between incubation temperature and test condition ($F_{1,22}$ = 0.09, p = 0.765).

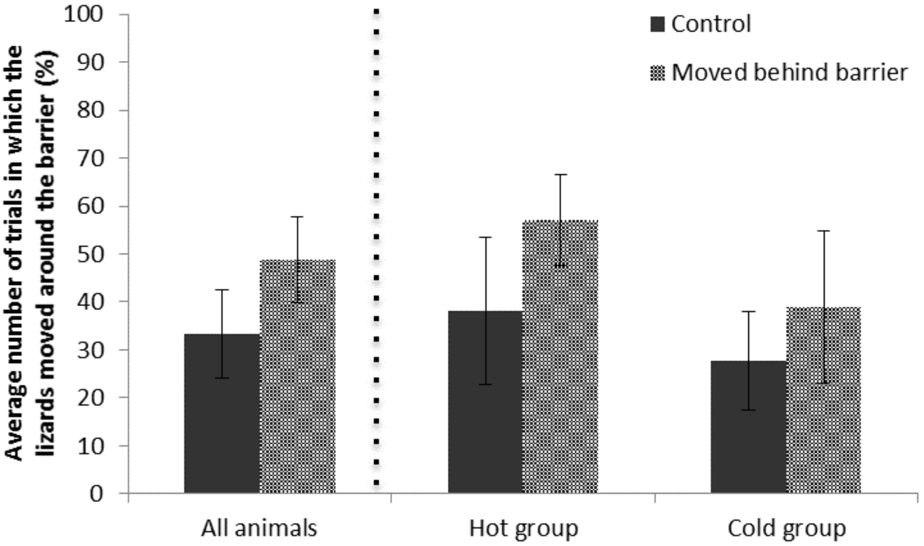

Figure 8. Mean % of trials that the lizards moved around or over the barrier.

4.9 Discussion

The results suggest that bearded dragons are not capable of geometric gaze following despite a slight trend towards bearded dragons moving around the barrier when the demonstrator lizard looked behind it. Interestingly, the 'hot group' did move around the barrier more than the 'cold group' when the demonstrator looked behind the barrier; however this difference was not significant suggesting that the 'hot group' could have just moved more in general. Furthermore, in accordance with the results from experiment 1 there were no differences between the 'hot' and 'cold' groups in their ability to follow the gaze of a conspecific into the distance for any of the looking into the distance conditions. These results suggest that egg incubation temperature does not influence the gaze following ability of adult lizards incubated at different temperatures.

Interestingly and in contrast to the results from experiment one the bearded dragons could follow the gaze of a conspecific look up into the distance. Bearded dragons often freeze when under threat and we previously suggested that the inconsistency of bearded dragons to follow the gaze of a conspecific up was due to this anti-predator response. The results from experiment 1 do show a trend towards the lizards looking up more when a conspecific looked up suggesting that results from experiment 1 could be influenced by the small sample size. The experimental arena was also a slightly different shape meaning that the lizards had a better view of what was above them. It is also possible that something about having the barrier present in the experiment makes the observer look up more. The results could also imply that the trajectory that lizards learn to follow the gaze of a conspecific upwards could be different to that of gaze following to the side. Further experiments would be required to understand why there were differences.

4.10 General discussion

The results of both experiments show that bearded dragons are capable of gaze following into the distance and are the first to show that a lizard species is capable of gaze following suggesting that gaze following maybe more common in reptiles than previously thought. The results also showed that bearded dragons would follow the gaze of a conspecific to the side more readily than upwards, adding evidence to the hypothesis that gaze following to the side and upwards have evolved for different functions. The results also provide evidence that bearded dragons were not capable of geometric gaze following, suggesting that gaze following into the distance and geometric gaze involve different levels of cognitive ability (Loretto *et al.,* 2010).

The results also showed that egg incubation temperature does not influence the gaze following ability of bearded dragons and there are two possible explanations for this. Firstly, as previously mentioned gaze following into the distance requires less complex cognition when compared to geometric gaze following (Povinelli and Eddy, 1996; Tomasello, 1999). Our hypothesis, that bearded dragons incubated at colder incubation temperatures would be better at gaze following, was based on the premise that the lizards incubated at cooler temperatures were better at learning, which in turn could result in an improvement in gaze following ability. Given that gaze following into the distance is relatively simple to learn it is possible that gaze following into the distance is more innate and involves less learning. The results suggest that bearded dragons do not have the capability to gaze follow geometrically, meaning that it is unlikely that geometric gaze following is influenced by egg incubation temperature. Alternatively, egg incubation temperature could affect the gaze following into the distance ability of bearded dragons during early development. Apes, wolves and rooks develop different gaze following abilities at different stages throughout ontogeny (chimpanzees: Bräuer *et al.,* 2005; ravens: Schloegl *et al.*, 2004; wolves: Range and Viranyi, 2011) and research in humans shows that gaze following develops after gaze sensitivity as an infant develops (Brooks and Meltzoff, 2005). The lizards in the present experiment were all around one year old and had ample opportunity to learn to follow the gaze of a conspecific, meaning that the effect of egg incubation temperature on gaze following throughout ontogeny is unknown. In order to determine if egg incubation temperature affects the gaze following ability of bearded dragon during early development it would be necessary to repeat this experiment at different stages of a bearded dragon's life. If bearded dragons were to have different learning abilities then it is possible that the development of bearded dragon gaze following could be influenced.

All species that have been shown to geometrically gaze follow are social species with high levels of maternal care and cooperation, although living in groups is not a guarantee that geometric gaze following will be present (marmosets: Burkart and Heschl, 2004; Northern bald ibises :Loretto *et al.,* 2010). Bearded dragons are a social species (Carpenter, 1970; Brattstrom, 1971) as males and females will have overlapping territories, but they do not cooperate and do not have maternal care. All the lizards in this experiment had extensive experience with barriers and were socially housed and all had been habituated to the environment. They moved past or climbed over the barrier in 33% of control trials suggesting that neophobia was not a factor. These results add to the evidence that gaze following into the distance requires a lower level mechanism compared with geometric gaze following that is more cognitively demanding and supports the hypothesis that there are two modes of gaze following (Schloegl *et al.,* 2004; Lorreto *et al.,* 2010; Range and Virányi, 2011). The results could also suggest that geometric gaze following is possibly only seen in species that live in social groups with higher levels of interactions. In order to test this idea further it would be interesting to compare the gaze following abilities of bearded dragons and *Egernia* lizards that live in family groups (While *et al.,* 2015). It could be hypothesized that *Egernia* lizards, due to their high levels of sociality and extensive experience with using gaze as hatchlings, perform better than bearded dragons in a gaze following experiment.

In conclusion these results show that egg incubation temperature does not influence the gaze following ability of lizards. The results, however, are the first to show that a lizard species is capable of following the gaze of a conspecific into the distance and also provide evidence that bearded dragons cannot geometrically gaze follow. These results provide more evidence that geometric gaze following involves more complex cognition than gaze following into the distance. In order to understand gaze following in bearded dragons better it could be suggested that future studies focus on the development of gaze following throughout ontogeny.

Chapter Five: Summary of findings

The overall results show that egg incubation temperature influenced the growth, foraging ability and the development of behavioural traits of bearded dragons, though their gaze following ability was unaffected. The results reveal, for the first time, that egg incubation temperature influences the long-term growth and behavioural phenotypes of bearded dragons. This could ultimately influence the lizard's life history trajectory.

The results of chapter two showed that incubation temperature influenced the long-term foraging behaviour of bearded dragons with lizards incubated at hotter temperatures foraging more successfully and running faster than lizards incubated at cooler temperatures. There was, however, a potential trade off with growth, as lizards incubated at colder temperatures had quicker growth rates. Previous work with pine snakes hatchlings (*Pituophis melanoleucus*) has shown a similar trend (Burger, 1991 b), suggesting that the influence of incubation temperature on foraging ability could be common to oviparous reptiles. If incubation temperature does impact foraging success then it would also be interesting to see if the same potential trade-off between foraging speed and growth is observed in other oviparous species.

In chapter three we explored the role of incubation temperature on personality. The results showed that at 10 months of age the 'hot group' spent more time in close proximity to the novel object and were more active than the 'cold group.' Although not repeatable, the results suggest that egg incubation temperature could have influenced the development of certain behavioural traits at different stages of ontogeny. The results show that sensitivity to environmental conditions will result in differences in the range of phenotypes of oviparous reptiles which could result in a clutch of eggs being suited to a wide variety of different environments. This implies that bearded dragons incubated at different incubation temperatures will have different life history trajectories. Further, the results show the importance of investigating the relationship between egg incubation temperature and personality in the long-term across ontogeny to ensure the repeatability of the results.

The results from chapter four showed that bearded dragons are capable of gaze following into the distance but no evidence was found suggesting that they were capable of geometric gaze following. This research is the first to show that a lizard species is capable following the gaze of a conspecific and suggests that the two different modes of gaze following require different levels of cognitive ability (Loretto, 2010). Interestingly, egg incubation temperature did not influence gaze following ability this is in stark contrast with the data from the previous chapters. The lizards used in this experiment were sexually mature and it is possible that if they have been tested as hatchlings then the trajectory that develop this ability may differ. Alternatively, it is possible that gaze following is highly adaptive and essential for success and therefore developmental differences are not observed.

What does this mean?

The results clearly show that egg incubation temperature influences the long-term phenotypes of bearded dragons. Chapter two suggests a trade-off between foraging ability and growth while the results of chapter three showed that the behavioural traits of bearded dragons were different at different stages in ontogeny. Growth and foraging ability will be important for the fitness of bearded dragons and behavioural traits such as activity and 'boldness' have been shown to increase food consumption in a range of species (Biro and Stamps, 2008) but have also been linked to increased predation risk (Carter *et al.,* 2010). However, different phenotypes will be suited to different environments. Deeming and Ferguson (1989) suggested that sensitivity to environmental conditions, such as incubation temperature, is adaptive as a clutch of eggs will produce animals that are suited to a wide variety of different environments. The results of this project support this idea and suggest that animals incubated in a certain environment will be more suited to the environment into which they emerge. The results of chapters 2 and 3 suggest that bearded dragons incubated at hotter incubation temperatures will be better at foraging and potentially more active/bolder than lizards incubated at colder incubation temperatures at certain stages in ontogeny. Termites (*Isoptera*) account for (94.6%) of wild bearded dragons diet (Oonincx *et al.,* 2015). Termite abundance and activity decreases in hotter temperatures (Abensperg-Traun & Boer, 1990) and therefore animals hatching into hotter environment will have less prey available. Thus they need to be better hunters. Conversely, at colder incubation temperatures the bearded dragons grew heavier quicker. Being large in cooler environments is thought to be beneficial to lizards as larger lizards can retain heat for longer are more likely to survive winter and can dominate food resources (Avery, 1982; Ferguson and Fox, 1984). However, in order to further test the hypothesis that sensitivity to environmental conditions makes bearded dragons more adapted to their emerging environment, lizards need to be tested in their natural habitat (see Warner and Andrews, 2002). Egg incubation temperature did not influence bearded dragon gaze following ability while research has shown that egg incubation temperature will influence their learning ability (Junttilia *et al*., in preparation). This suggests that egg incubation temperature will affect some cognitive ability of bearded dragons but not gaze following ability. It is possible that gaze following is highly adaptive meaning that differences due to incubation temperature are not observed. The results from this project suggest that egg incubation temperature will affect the life history trajectory of bearded dragons and provide more evidence of the importance of incubation temperature of oviparous reptiles. These results could ultimately be important for *ex-situ* conservation that relies on the artificial incubation of eggs.

Wider Implications

The results show that bearded dragons, incubated within their optimal range, are potentially suited to a wide variety of different environments, which could increase the fitness of the clutch (Deeming and Ferguson, 1989). Further these results suggest that sensitivity to environmental conditions means that individuals emerge into an environment that they are potentially better adapted to. Holleley *et al.,* (2015) showed that extreme incubation temperatures can rapidly change the biology of bearded dragons as bearded dragon incubated at extremely hot temperatures (33^{o}C-36^{o}C) change from having genetic dependant sex determination to temperature dependant sex determination. This suggests that bearded dragons could either be vulnerable to human induced climate change as the biology of the lizards can change rapidly, potentially resulting in the population having a sex bias or the results show that bearded dragons adapt flexibly to environmental change. The results of the current project suggest that bearded dragons alter their phenotypes to a changing environment which could prove as an invaluable defence mechanism in the face of human induced environmental change. Ultimately the impact of human induced climate change on bearded dragons will depend on the speed that the temperature of the environment changes and if the lizards can respond adapt to the new conditions.

Further studies and conclusion

The results of this project show that egg incubation temperature affects a range of different phenotypic traits in bearded dragons. However in order to understand the effects of early environmental factors on the behaviour and cognition of oviparous species further a wide variety of species need to be tested. Currently studies investigating this topic are heavily biased towards a few species. For example the relationship between incubation temperature and the behaviour of pine snakes is extremely well studied (Burger, 1989; Burger, 1991; Burger 1991 b; Burger 1998) while the effects of incubation temperature on the behaviour of crocodilian species is almost unknown (Deeming, 2004). Currently all of the published work investigating the relationship between egg incubation temperature and cognition has been conducted in one species of lizard (*Bassiana duperreyi*) (Amiel and Shine 2012; Amiel *et al.*, 2013; Clark *et al.*, 2014). The effect of incubation temperature on the behaviour of any bird species up until recently was relatively unknown, although now incubation temperature has been shown the influence the growth, body size and locomotor performance of birds (Hepp *et al.,* 2015). Unfortunately due to the relatively few species of oviparous reptiles studied it is also unknown of any possible phylogenetic trends of egg incubation temperature or if the effects of egg incubation temperature are species specific. Some evidence in lizards, however, does suggest that post hatching effects are more likely to be species/ecologically specific rather than having an overall phylogenetic effect. For example, the results of the present experiment suggest that bearded dragons incubated at hotter temperatures ran faster than lizards incubated at colder temperatures. In contrast, lizards (*Nannoscincus maccoyi*) incubated at colder temperatures were faster lizards than lizards incubated at hotter temperatures (Downes and Shine, 1999) while other lizards (*Bassiana duperreyi)* were quicker when incubated at hotter temperatures (Elphick and Shine, 1998). Furthermore, bearded dragons incubated at colder incubation temperatures (27 ± 3) were better at learning a simple learning discrimination than lizards incubated at hotter temperatures (30 ± 3) (Junttilia *et al.,* in preparation); in contrast lizards (*Bassiana duperreyi*) incubated at hotter temperatures (22 ± 7^{o}C) learnt a learning discrimination quicker lizards incubated in cooler conditions (16 ± 7^{o}C) (Amiel and Shine 2012; Clark *et al.,* 2014;). In order to understand the effects of egg incubation temperature on the behaviour and cognition future studies should focus on broadening the species database in order to understand potential phylogenetic trends.

Previous experiments investigating the effects of egg incubation temperature on the phenotypes of oviparous lizards have tended to use hatchlings as a result, very little is known about the long-term effects of incubation temperature (Deeming, 2004). The results of this project show that the impact of egg incubation temperature on the growth and behaviour of bearded dragons were still present in adulthood while the results of chapter 3 showed that the behavioural traits of bearded dragons were different at different stages of ontogeny. Although the behaviour of hatchlings is crucial for survival, in order to reproduce the long term effects of incubation temperature could be essential. This suggests that future studies should focus long-term effects of egg incubation temperature while also testing for the effects of incubation temperature at different stages of ontogeny.

This project suggests that egg incubation temperature will influence the fitness and life history trajectory of bearded dragons. However, the main limitation of this project is that the animals used were captive. Studies have shown that behaviours such as running speed are important for the survival of hatchlings in their natural environment (Warner and Andrews, 2002) while other behavioural traits such as boldness have been shown the influence food intake in the Namibian rock agama lizards (*Agama planiceps*) (Carter, 2010). This suggests that phenotypic traits such as foraging ability and other behavioural traits that are sensitive to incubation period could also influence survival. However in order to understand these further, future experiments should focus on analysing the effects of egg incubation temperature in the wild.

In conclusion, the results showed that egg incubation temperature influenced the long-term phenotypes of bearded dragons effecting growth, behaviour and the behavioural traits throughout ontogeny. The results add to the evidence showing the importance of the environmental conditions that occur during incubation temperature and the resulting impact they have on the fitness of oviparous reptiles.

Acknowledgments

Firstly I would like to thank Anna Wilkinson and Charles Deeming for constant support throughout the project. I would also like to thank Sophie Moszuti, Josh Moss, Benjamin Keep and Stephan Reber for putting up with me in the lab and Anna Frohnwieser for proof reading various parts of this thesis. I would also like to thank Emma Huntbach and Dawn Simpson for helping with animal husbandry. I would also like to thank Adam Welsh, Sarah Placì, and Joanna Rosenberger for analysing and coding numerous videos. Finally I would like to thank my mother and father for encouraging me to follow my ambitions and of course all the bearded dragons for taking part.

References

Abensperg-Traun M A X & Boer E S (1990) Species abundance and habitat differences in biomass of subterranean termites *(Isoptera*) in the wheatbelt of Western Australia. *Australian Journal of Ecology*, 15, 219-226.

Adolph S C & Porter W P (1993) Temperature, activity, and lizard life histories. *American Naturalist*, 142, 273-295

Alberts A C, Perry A M, Lemm J M, Phillips J A (1997) Effects of incubation temperature and water potential on growth and thermoregulatory behaviour of hatchling Cuban rock iguanas (*Cyclura nubi*la), *Copeia*, 1997, 766-776

Amiel J J, Lindström T, Shine R (2014) Egg incubation effects generate positive correlations between size, speed and learning ability in young lizards. *Animal Cognition* 17, 337-347

Amiel J J, Shine R (2012) Hotter nests produce smarter young lizards. *Biology Letters* 8, 372–374

Amiel J J, Tingley R, Shine R (2011) Smart moves: effects of relative brain size on establishment success of invasive amphibians and reptiles. *PLoS One*, *6*, e18277-e18277.

Anderson J R & Mitchell R W (1999) Macaques but not lemurs co-orient visually with humans. *Folia Primatologica*, 70, 17-22.

Anderson J R, Sallaberry P, Barbier H (1995) Use of experimenter-given cues during object-choice tasks by capuchin monkeys. *Animal Behaviour*, 49, 201-208.

Andrews R M (2008) Effects of incubation temperature on growth and performance of the veiled chameleon (*Chamaeleo calyptratus*). *Journal of Experimental Zoology Part A: Ecological Genetics and Physiology*, 309, 435-446.

Andrews R M. (2004) Patterns of embryonic development. In: Deeming, D. C *Reptilian Incubation Environment, Evolution and Behaviour*. Nottingham: Nottingham University Press. 75-100

Ashmore G M & Janzen F J (2003) Phenotypic variation in smooth softshell turtles (*Apalone mutica*) from eggs incubated in constant versus fluctuating temperatures. *Oecologia*, 134, 182-188

Avery R A, Bedford J D, Newcombe C P (1982) The role of thermoregulation in lizard biology: Predatory efficiency in a temperate diurnal basker. *Behavioural Ecology* 1, 261-267

Bertin A, Richard-Yris M A, Houdelier C, Richard S, Lumineau S, Kotrschal K, Möstl E (2009) Divergent selection for inherent fearfulness leads to divergent yolk steroid levels in quail. *Behaviour*, 146, 757-770.

Biro P A & Stamps J A (2008) Are animal personality traits linked to life-history productivity? *Trends in Ecology & Evolution*, 23, 361-368.

Booth D T (2004) Artificial incubation In: Deeming, D. C *Reptilian Incubation Environment, Evolution and Behaviour*. Nottingham: Nottingham University Press. 253-263

Bouwhuis S, Quinn J L, Sheldon B C, Verhulst S (2014) Personality and basal metabolic rate in a wild bird population. *Oikos*, 123, 56-62.

Braña F, Ji X (2000) Influence of incubation temperature on morphology, locomotor performance, and early growth of hatchling wall lizards *(Podarcis murslis). Journal of Experimental Zoology* 286, 422–433.

Brattstrom B H (1971) Social and Thermoregulatory Behavior of the Bearded Dragon, *Amphibolurus barbatu. Copeia,* 3, 484-497

Bräuer J, Call J, Tomasello M (2005) All great ape species follow gaze to distant locations and around barriers. *Journal of Comparative Psychology*, *119*, 145.

Brooks R, Meltzoff A N (2005) The development of gaze following and its relation to language. *Developmental science*, *8*, 535-543.

Brown C, Burgess F, Braithwaite V A (2007) Heritable and experiential effects on boldness in a tropical poeciliid. *Behavioral Ecology and Sociobiology*, 62, 237-243.

Brown, C., Jones, F., & Braithwaite, V. (2005). In situ examination of boldness–shyness traits in the tropical poeciliid, Brachyraphis episcopi. *Animal Behaviour*, 70, 1003-1009.

Bugnyar T, Stöwe M, Heinrich B (2004) Ravens, *Corvus corax*, follow gaze direction of humans around obstacles. *Proceedings of the Royal Society of London B: Biological Sciences*, 271, 1331-1336.

Bull J J (1980) Sex determination in reptiles. *The Quarterly Review of Biology,* 55, 3-21.

Burger J (1989) Incubation temperature has long-term effects on behaviour of young pine snakes (*Pituophis melanoleucus*). *Behavioral Ecology and Sociobiology*, 24, 201-207.

Burger J (1991a) Response to prey chemical cues by hatchling pine snakes (*Pituophis melanoleucus*): effects of incubation temperature and experience. *Journal of chemical ecology*, *17*, 1069-1078.

Burger J (1991b). Effects of incubation temperature on behavior of hatchling pine snakes: implications for reptilian distribution. *Behavioural Ecology and Sociobiology*, 28, 297-303.

Burger J (1998) Effects of incubation temperature on hatchling pine snakes: implications for survival. *Behavioural Ecology*. 43, 11-18.

Burgess E A, Booth D T, Lanyon J M (2006) Swimming performance of hatchling green turtles is affected by incubation temperature. *Coral Reefs*. 25, 341- 349.

Burkart J & Heschl A (2006) Geometrical gaze following in common marmosets (*Callithrix jacchus*). *Journal of Comparative Psychology*, *120*, 120.

Butterworth G & Jarrett N (1991) What minds have in common is space: Spatial mechanisms serving joint visual attention in infancy. *British Journal of Developmental Psychology*, 9, 55-72.

Carazo P, Noble D W, Chandrasoma D, Whiting M J (2014) Sex and boldness explain individual differences in spatial learning in a lizard. *Proceedings of the Royal Society of London B: Biological Sciences*, *281*, doi: 10.1098/rspb.2013.3275

Carere C , Eens M (2005) Unravelling animal personalities: how and why individuals consistently differ. *Behaviour* 142, 1149–1157.

Carpenter C C, Badham J A, Kimble B (1970) Behavior patterns of three species of *Amphibolurus (Agamidae). Copeia, 1970,* 497-505

Carter A J, Goldizen A W, Tromp S A (2010) Agamas exhibit behavioral syndromes: bolder males bask and feed more but may suffer higher predation. *Behavioral Ecology*, 26, doi: 10.1093/beheco/arq036

Clark B F, Amiel J J, Shine R, Noble D W A, Whiting M J. (2014) Colour discrimination and associative learning in hatchling lizards incubated at 'hot' and 'cold' temperatures. *Behavioural. Ecology*. 68, 239-247.

Deeming D C (2004). Post-hatching phenotypic effects of incubation in reptiles. In: Deeming, D. C *Reptilian Incubation Environment, Evolution and Behaviour*. Nottingham: Nottingham University Press. 229-251.

Deeming D C, Ferguson M W. (1989) The mechanism of temperature dependent sex determination in crocodilians. *American Zoologist* 29, 973-985.

Deeming, D. C., & Ferguson, M. W. (1991). *Egg incubation: its effects on embryonic development in birds and reptiles*. Cambridge University Press.

Downes S J, Shine R (1999) Do incubation-induced changes in a lizard's phenotype influence its vulnerability to predators? *Oeclogia* 120, 9-18.

Dunham A E. (1978) Food availability as a proximate factor influencing individual growth rates in the iguanid lizard *Sceloporus merriami*. *Ecology* 59, 770-778

Elnitsky M A & Claussen D L (2006) The effects of temperature and inter-individual variation on the locomotor performance of juvenile turtles. *Journal of Comparative Physiology*, 176, 497-504.

Elphick M J & Shine R (1998) Long-term effects of incubation temperature on the morphology and locomotors performance of hatchling lizards (*Bassiana duperreyi, Scincidae*). *Biological Journal of the Linnean Society*, 63, 429 – 447.

Elphick M J & Shine R (1999) Sex differences in optimal incubation temperatures in a scincid lizard species. *Oecologia*, 118, 431-437.

Emery N J (2000) The eyes have it: the neuroethology, function and evolution of social gaze. *Neuroscience & Biobehavioral Reviews*, 24, 581-604

Esquerré D, Keogh J S, Schwanz E (2014) Direct effects of incubation temperature on morphology, thermoregulatory behaviour and locomotor performance in jacky dragons (Amphibolurus muricatus). *Journal of Thermal Biology*, *43*, 33-39.

Ezaz T, Quinn A E, Miura I, Sarre S D, Georges A, Marshall Graves J A (2005) The dragon lizard *Pogona vitticeps* has ZZ/ZW micro-sex chromosomes. *Chromosome Research* 13, 763-776

Ezaz T, Quinn A E, Sarre S D, O'Meally D, Georges A, Marshall Graves J A (2011) Molecular marker suggests rapid changes of sex-determining mechanisms in Australian dragon lizards. *Chromosome Research* 17, 91-98

Ferguson G W, Fox S F (1984) Annual variation of survival advantage of large juvenile side-blotched lizards*, Uta stansburiana*: its causes and evolutionary significance*. Evolution* 38*,* 342–349

Flatt T, Shine R, Borges-Landeaz P A, Downes S J (2001) Phenotypic variation in an oviparous montane lizard (*Bassiana duperreyi*): the effects of thermal and hydric incubation environments. *Biological Journal of the Linnean Society*, *74*, 339-350.

Fox S F (1983) Fitness, home-range quality, and aggression in *Uta stansburiana*. In Lizard Ecology: Studies of a Model Organism (eds. R.B. Huey, E.R. Pianka & T.W. Schoener), pp. 149-168. Harvard University Press, Cambridge, Massachusetts

Frost A J, Winrow-Giffen A, Ashley P J, Sneddon L U (2007) Plasticity in animal personality traits: does prior experience alter the degree of boldness? *Proceedings of the Royal Society of London B: Biological Sciences*, 274, 333-339

Goodman R M (2008) Latent effects of egg incubation temperature on growth in the lizard *Anolis carolinensis*. *Journal of Experimental Zoology Part A: Ecological Genetics and Physiology*, 309, 525-533

Gutzke W H N (1987) Sex determination and sexual differentiation in reptiles. *Herpetological journal*. 1, 122-125.

Hare K M, Pledger S, Daugherty C H (2008) Low incubation temperatures negatively influence locomotor performance and behavior of the nocturnal lizard *Oligosoma suteri (Lacertidae: Scincidae*). *Copeia*, 2008, 16-22.

Hepp G R, DuRant S E, Hopkins W A (2015) Influence of incubation temperature on offspring phenotype and fitness in birds. *Nests, Eggs, and Incubation: New Ideas about Avian Reproduction*, Oxford University Press 171-178

Holleley C E, O'Meally S D, Sarre J A, Marshall Graves T, Ezaz K ,Matsubara B, Azad X, Zhang A G (2015) Sex reversal triggers the rapid transition from genetic to temperature-dependent sex. *Nature* 523, 79-82.

Husak J F (2006) Does survival depend on how fast you can run or how fast you do run? *Functional Ecology*, 20, 1080-1086

Hutton J M (1987) Incubation temperatures, sex ratios and sex determination in a population of Nile crocodiles (*Crocodylus niloticus*).*Journal of Zoology*, 211, 143-155

Ischer T, Ireland K, Booth D T (2009) Locomotion performance of green turtle hatchlings from the Heron Island Rookery, Great Barrier Reef. *Marine Biology*, 156, 1399-1409

Itakura S (1996) An exploratory study of gaze-monitoring in nonhuman primates. *Japanese Psychological Research*, 38, 174-180.

Janzen F J (1993) The influence of incubation temperature and family on eggs, embryos, and hatchlings of the smooth softshell turtle (*Apalone mutica*). *Physiological Zoology*, 349-373.

Janzen F J, Wilson M E, Tucker J K, Ford S P (1998) Endogenous yolk steroid hormones in turtles with different sex-determining mechanisms. *General and Comparative Endocrinology*, 111, 306-317.

Joanen T, McNeae L, Ferguson M W J (1987) The effects of egg incubation temperature on post-hatching growth of American alligators. In: *Wildlife Management*: *Crocodiles and Alligators.*Webb G J W, Manolis S C & Whitehead P J. (eds), Surry Beatty & Sons Pty LtD, Chipping Norton, NSW, Australia, pp 53-537

Junttilia S, Deeming D C, Wilkinson A (in preparation). The influence of incubation temperature on learning and motivation in an oviparous reptile.

Keogh J S, Noble D W, Wilson E E, Whiting M J (2012) Activity predicts male reproductive success in a polygynous lizard. *PloS* One, doi: 10.1371/journal.pone.0038856

Kis A, Huber L, Wilkinson A (2014) Social learning by imitation in a reptile (*Pogona vitticeps*). *Animal Cognition*, *18*, 325-331.

Kruvers R H, Prins H H T, van Wieren S E, van Oers K, Nolet B A, Ydenberg R C (2009) The effect of personality on social foraging: shy barnacle geese scrounge more. *Proceedings of the Royal Society of London B: Biological Sciences* 277, 601-608

Kurvers R H, Eijkelenkamp B, van Oers K, van Lith B, van Wieren S E, Ydenberg R C, & Prins H H (2009) Personality differences explain leadership in barnacle geese. *Animal Behaviour*, 78, 447-453.

Loretto M C, Schloegl C, Bugnyar T (2010) Northern bald ibises follow others' gaze into distant space but not behind barriers. *Biology letters*, 6, 14-17.

Mafli A, Wakamatsu K, Roulin A (2011) Melanin-based coloration predicts aggressiveness and boldness in captive eastern Hermann's tortoises. *Animal Behaviour*, 81, 859-863.

Marchant S, Higgins PJ (1993) Handbook of Australian, New Zealand and Antarctic Birds. Vol. 2. Raptors to Lapwings. Oxford University Press, Melbourne

Mas-Muñoz, J, Komen H, Schneider O, Visch S W, Schrama J W (2011) Feeding behaviour, swimming activity and boldness explain variation in feed intake and growth of sole (*Solea solea*) reared in captivity. *Plos One,* doi: 10.1371/journal.pone.0021393

McCormick M I (2006) Mothers matter: crowding leads to stressed mothers and smaller offspring in marine fish. *Ecology*, 87, 1104-1109

Mickelson L E.& Downie J R (2010) Influence of incubation temperature on morphology and locomotion performance of Leatherback (*Dermochelys coriacea*) hatchlings. *Canadian Journal of Zoology*, 88, 359-368.

Moll H & Tomasello M (2004) 12-and 18-month-old infants follow gaze to spaces behind barriers. *Developmental Science*, *7*, 1-9.

Mueller-Paul J, Wilkinson A, Hall G, Huber L (2012) Radial-arm-maze behaviour of the red-footed tortoise *(Geochelone carbonaria*). *Journal of Comparative Psychology*, *126*, doi: 10.1037/a0026881

Müller W, Dijkstra C, Groothuis T G (2009) Maternal yolk androgens stimulate territorial behaviour in black-headed gull chicks. *Biology Letters*, 12, doi: 10.1098/rsbl.2009.0283

Naguib M, Kazek A, Schaper S V, Van Oers K, Visser M E (2010) Singing activity reveals personality traits in great tits. *Ethology*, 116, 763-769.

Noble D W, Carazo P, Whiting M J (2012) Learning outdoors: male lizards show flexible spatial learning under semi-natural conditions. *Biology Letters*, 8, 946-948.

Okamoto-Barth S & Tomonaga M (2006) Development of joint attention in infant chimpanzees. *Cognitive development in chimpanzees,* Springer, pp. 155-171

Oonincx D G A B, van Leeuwen J P, Hendriks W H, van der Poel A F B (2015) The diet of free-roaming Australian central bearded dragons *(Pogona vitticeps*). *Zoo biology*. 34, 271-277

O'Steen S & Janzen F J (1999) Embryonic temperature affects metabolic compensation and thyroid hormones in hatchling snapping turtles. *Physiological and Biochemical Zoology*, 72, 520-533.

Pan Z C & Ji X (2001) The influence of incubation temperature on size morphology, and locomotor performance of hatchlings grass lizards (*Takydromus wolteri*). *Acta Ecologica Sinica*, 21, 2031-2038

Pérez-Cembranos A & Pérez-Mellado V (2015) Local enhancement and social foraging in a non-social insular lizard. *Animal Cognition*, 18, 629-637.

Povinelli D J & Eddy T J (1996) Chimpanzees: joint visual attention. *Psychological Science*, 7, 129-135.

Range F & Virányi Z (2011) Development of gaze following abilities in wolves (*Canis lupus*). *PloS One*, DOI: 10.1371/journal.pone.0016888

Ruiz A, Gómez J C, Roeder J J, Byrne,R W (2009) Gaze following and gaze priming in lemurs. *Animal Cognition*, 12, 427-434.

Scaife M & Bruner J S (1975) The capacity for joint visual attention in the infant. *Nature*. 252, 265-266

Schloegl C, Kotrschal K, Bugnyar T (2007) Gaze following in common ravens, *Corvus corax*: ontogeny and habituation. *Animal Behaviour*, 74, 769-778.

Short K H & Petren K (2008) Boldness underlies foraging success of invasive *Lepidodactylus lugubris* geckos in the human landscape. *Animal Behaviour*, 76, 429-437.

Siviter H, Deeming D C, Wilkinson A (a) (in preparation) Foraging behaviour of juvenile lizards correlates with egg incubation temperature.

Siviter H, Deeming D C, Wilkinson A (b) (in preparation) Early environmental factors that occur during embryonic development influence the behavioural traits of oviparous reptiles.

Smallegange I M (2011) Complex environmental effects on the expression of alternative reproductive phenotypes in the bulb mite. *Evolutionary Ecology*, 25, 857–873.

Smith B R, Blumstein D T (2008) Fitness consequences of personality: a meta-analysis. *Behavioral Ecology*. 19, 448

Sol D, Bacher S, Reader S M, Lefebvre L (2008) Brain size predicts the success of mammal species introduced into novel environments. *The American Naturalist*, *172*, S63-S71.

Stamps J A (1977) Social behaviour and spacing patterns in lizards. In Biology of the Reptilia, vol. 7 (eds. C. Gans, & D.W. Tinkle), pp. 265-334. Academic Press, London.

Stamps J A (1984) Rank dependent compromise between growth and predation protection in lizard dominance hierarchies. *Animal Behaviour*, 32, 1101- 1107

Tokarz R R (1985) Body size as a factor determining dominance in staged agonistic encounters between male brown anoles (*Anolis sagrei*). *Animal Behaviour*, 33, 746-753.

Tomasello M, Call J, Hare B (1998) Five primate species follow the visual gaze of conspecifics. *Animal Behaviour*, *55*, 1063-1069.

Tomasello M, Hare B, Agnetta B (1999) Chimpanzees, (*Pan troglodytes*), follow gaze direction geometrically. *Animal Behaviour* 58, 769-777

Tomasello M, Hare B, Fogleman T (2001) The ontogeny of gaze following in chimpanzees, *Pan troglodytes*, and rhesus macaques, *Macaca mulatta*. *Animal Behaviour*, 61, 335-343.

Tousignant A & Crews D (1995) Incubation temperature and gonadal sex affect growth and physiology in the leopard gecko (*Eublepharis macularius*), a lizard with temperature-dependent sex determination. *Journal of Morphology*, 224, 159-170.

Trivers R L (1976) Sexual selection and resource-accruing ability in *Anolis garmani*. *Evolution*, 30, 253-267.

Udell M A, Dorey N R, Wynne C D (2010) The performance of stray dogs (*Canis familiaris*) living in a shelter on human-guided object-choice tasks. *Animal Behaviour*, 79, 717-725.

Valenzuela N & Lance, V. (Eds.). (2004). *Temperature-dependent sex determination in vertebrates* (pp. 131-47). Washington: Smithsonian Books.

Valenzuela N (2004) Temperature-dependant sex determination. In: Deeming, D. C *Reptilian Incubation Environment, Evolution and Behaviour*. Nottingham: Nottingham University Press. 211-227.

Van Damme R, Bauwens D, Braña F, Verheyen R F (1992) Incubation temperature differentially affects hatching time, egg survival, and hatchling performance in the lizard *Podarcis muralis*. *Herpetologica*. 48, 220-228

Van Oers K, De Jong G, Van Noordwijk A J, Kempenaers B, Drent P J (2005) Contribution of genetics to the study of animal personalities: a review of case studies. *Behaviour,* 142, 1185–1206

Ward A J, Thomas P, Hart P J, Krause J (2004) Correlates of boldness in three-spined sticklebacks (*Gasterosteus aculeatus)*. *Behavioral Ecology and Sociobiology*, *55*, 561-568.

Warner D A & Andrews R M (2002) Laboratory and field experiments identify sources of variation in phenotypes and survival of hatchling lizards. *Biological Journal of the Linnean Society*, 76, 105-124

Webb G J W & Cooper P H (1989) Effects of incubation temperature on crocodiles and the evolution of reptilian oviparity. *American Zoologist*, 29, 953- 971

While G M, Chapple D G, Gardner M G, Uller T, Whiting M J (2015) Egernia lizards. *Current Biology*, *25*, 593–595

Wilkinson A & Huber L (2012) Cold-blooded cognition: reptilian cognitive abilities. *The Oxford Handbook of Comparative Evolutionary Psychology. Oxford University Press, New Jersey*, 129-143

Wilkinson A, Chan H M, Hall G (2007). Spatial learning and memory in the tortoise (*Geochelone carbonaria*). *Journal of Comparative Psychology*, 121, 412.

Wilkinson A, Coward S, Hall G (2009) Visual and response-based navigation in the tortoise (*Geochelone carbonaria*). *Animal Cognition*, 12, 779-787.

Wilkinson A, Kuenstner K, Mueller J, Huber L (2010) Social learning in a non-social reptile (*Geochelone carbonaria*). *Biology Letters*, 6, doi: 10.1098/rsbl.2010.0092

Wilkinson A, Mandl I, Bugnyar T, Huber L (2010) Gaze following in the red-footed tortoise (*Geochelone carbonaria*). *Animal Cognition*, 13, 765-769.

Wolf M, Van Doorn G S, Leimar O, Weissing F J (2007) Life-history trade-offs favour the evolution of animal personalities. *Nature*, 447, 581-584.

Zuberbühler K (2008) Gaze following. *Current Biology*, 18. pR453–R455