



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

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Received: 7 February 2018 / Revised: 29 April 2018 / Accepted: 3 May 2018 / Published online: 28 May 2018
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Abstract

Innate antipredator responses are integral for survival in many species, particularly those which lack parental care. Antipredator responses include both active (fight or flight) and passive behaviours (immobility). As the success of antipredator responses directly relates to survival and fitness, investigating the drivers that explain variance in these traits is key to understanding how predation shapes the instinctive behaviour of animals. We quantified innate antipredator behaviour of hatchling Australian water dragons (*Intellagama lesueurii*) immediately after hatching using a model snake to simulate a series of attacks, and scored their behaviour using a fight or flight index. Then we explored which factors were related to dragon antipredator behaviour, such as habitat disturbance, origin population, morphology, and parental genetic effects and phenotype (clutch effects). We developed multiple hypotheses and used model selection to determine which factors drive variation in hatchling antipredator behaviour. Clutch effects explained a significant proportion of variation in innate antipredator responses, suggesting a heritable component. We also found an effect of body size on innate antipredator behaviour: larger hatchlings were more prone to flight behaviour (e.g. short-distance runs and long-distance sprinting), while smaller individuals were more prone to standing their ground and being aggressive (e.g. throat puffing, mouth gaping, biting). Clutch effects also explained a significant proportion of the variance in dragon body size. Our study provides evidence that the innate antipredator responses of water dragons are heritable in origin (directly through clutch effects, and indirectly through body size) and not associated with particular populations or habitat types. We suggest future research examine the survival implications of these responses.

Significance statement

The action an animal takes in response to a predator is a life or death decision, and can be required immediately after birth. These innate antipredator behaviours may be genetically linked, and enable individuals to emerge into their environment with the necessary behaviour to promote survival. We examined what factors drive hatchling lizards to exhibit different innate antipredator behaviour. Our study found that body size affected their innate behaviour: larger hatchlings were more prone to flee and smaller hatchlings were more likely to fight. Interestingly, parental genetics and phenotype (clutch effects) also significantly explained the variation in innate antipredator behaviour, which supports the hypothesis that these behaviours are heritable. Understanding what drives variation is a cornerstone of evolutionary biology, and our findings raise questions about how selection acts on antipredator behaviour and the degree to which they are plastic.

Keywords Behavioural ecology · Clutch effects · Fight or flight · *Intellagama lesueurii* · Water dragon

Communicated by T. Madsen

Electronic supplementary material The online version of this article (<https://doi.org/10.1007/s00265-018-2505-7>) contains supplementary material, which is available to authorized users.

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Introduction

Predation is one of the strongest selective forces shaping a species' evolutionary trajectory (Lima and Dill 1990; Lind and Cresswell 2005; Ferrari et al. 2009). Thus, prey species have evolved a host of behavioural, morphological, and physiological tactics (antipredator responses) to facilitate survival (Greene 1988; Caro 2005, 2014). Specific antipredator behaviours are wide-ranging, and include active (e.g. fight or flight; Eilam 2005; Foster et al. 2015) and passive behaviour (e.g. death feigning or immobility; Eilam 2005; Santos et al. 2010). The use of a specific antipredator response can be context-dependent (Vanhooydonck and Van Damme 2003; Ferrari et al. 2009). For example, antipredator behaviour varies between habitat types in 11 species of lacertid lizards: open or vertical microhabitats are associated with fleeing while vegetated habitats promote immobility (Vanhooydonck and Van Damme 2003). Antipredator behaviour can incur biologically relevant costs like increased energy expenditure, decreased foraging and basking opportunities, injury, or tail autotomy (Ydenberg and Dill 1986; Lima and Dill 1990; Cooper and Wilson 2008; Bateman et al. 2014; Kuo and Irschick 2016). Overreactions can lead to squandered resources and opportunities, while responses that underreact to a threat can result in mortality. As such, an individual's ability to correctly employ an effective antipredator behaviour, within a particular situation, is key for survival (Bateman et al. 2014) and can directly relate to overall fitness (but see review by Lind and Cresswell 2005).

Although antipredator behaviour shifts over time due to experience and learning (Lima and Dill 1990; Brown and Laland 2003), the presence of these behaviours at the time of birth or hatching suggests that many species enter the world with a predetermined suite of innate antipredator responses (Herzog and Burghardt 1986; Dalesman et al. 2006). Newborn gartersnakes (*Thamnophis melanogaster*, *T. sirtalis*, *T. butleri*) employ active antipredator behaviour, with differences in the intensity and direction (fighting or fleeing) differing between species, and may be associated with species-specific ecological differences (Herzog and Burghardt 1986). Within a given species, variation in fight or flight response is often tightly linked with morphology. For example, body size and age class in Iberian rock lizards (*Lacerta monticola*) is related to how close a predator can approach an individual; smaller lizards hold their ground longer, whereas larger conspecifics are more prone to fleeing (Martín and López 2003). Also, there can be inter-population differences in innate antipredator responses. Moor frog tadpoles (*Rana arvalis*) exhibit geographically distinct antipredator morphology (tail shape) and activity, which suggests localised population-level differences in tadpole physical and behavioural antipredator responses (Laurila et al. 2006). Overall, innate antipredator responses appear to vary depending on a multitude of factors,

which results in inter species, intra species, and intra-individual variation in this behaviour. This variation demonstrates how predation can result in complex selection on prey (Webb et al. 2001; Martín and López 2003; Eilam 2005).

Our aim was to identify the factors influencing innate antipredator behaviour using Australian water dragons (*Intellagama lesueurii*) as a model species. We collected eggs from gravid female water dragons across 12 sites with varying levels of habitat disturbance from urbanisation, and then incubated these eggs until hatchling emergence under constant laboratory conditions. Immediately after emergence, we exposed hatchlings to a standardised antipredator behavioural assay, which quantified the dragon's fight or flight response. We then asked whether habitat disturbance, morphology, origin population, and maternal/paternal genetic effects and phenotype (clutch effects; Webb et al. 2001), explained the observed variation in innate antipredator response. We developed multiple hypotheses, including both a null and full model for comparison, and conducted model selection to test which factors were driving variation in innate antipredator response. We tested the following hypotheses:

- (1) If innate antipredator response is heritable, then we would expect a significant proportion of the variation in fight or flight score to be explained by an individual's parental genetic effects and phenotype (clutch effects), as well as origin population (population effects);
- (2) If habitat disturbance (urbanisation) has selected for differences in innate antipredator response, then the degree of urbanisation at each hatchling origin population (urban, semi-natural, or natural) will be significantly related to their fight or flight score; and
- (3) If body size influences their innate antipredator response, then an individual's snout-vent length (SVL) will be negatively related to their fight or flight score (e.g. larger lizards should be more prone to fleeing rather than remaining immobile or fighting; Martín and López 2003).

Methods

Study species

Australian water dragons are a large lizard species native to eastern Australia (maximum snout-vent length 304 mm; Thompson 1993). They have a wide-ranging incubation duration (68–120 days; Harlow and Harlow 1997), and sex is temperature-dependent (equal sex ratio produced at 26.5 °C; Harlow 2001; Doody et al. 2006). Water dragons are also relatively long-lived (28–40 years; Harlow and Harlow 1997) and have a generation time of 5 years (Littleford-

Colquhoun et al. 2017). Populations are often associated with forested areas and freshwater bodies in natural areas (Cogger 2014); however, they can also be found in anthropogenic landscapes, such as backyards and urban greenspaces (Littleford-Colquhoun et al. 2017). Recent research has demonstrated rapid genetic and morphological diversification within urban-living populations, suggesting that water dragons are undergoing urban evolution (Johnson and Munshi-South 2017; Littleford-Colquhoun et al. 2017). Also, water dragons exhibit altered behavioural traits in response to human-modified habitats, such as increased boldness in semi-natural (moderate modification) and urban landscapes compared to populations from natural areas (JB-G et al. unpublished data.). Across habitat types, the known predators of water dragons are diverse, including birds (corvids, owls, and raptors), domestic mammals (cats and dogs), fish (eels and trout) and reptiles (lizards and snakes; Doody et al. 2014). Typically, their antipredator behaviour involves bipedal and quadrupedal running, as well as diving and swimming (Bellairs 1970; Doody et al. 2014). However, they will deploy more aggressive strategies, such as puffing up their throat, gaping their mouth, and biting, when cornered or captured (JB-G pers. obs.). Furthermore, water dragons receive no post-emergence parental care which suggests that they are a model species for examining ecologically-and genetically-related drivers for innate antipredator behaviour.

Capture sites, egg collection, and incubation

We sampled lizards from 12 study sites within the greater Sydney area in New South Wales, Australia (see [Supplementary materials](#) for exact location details) that ranged in their level of anthropogenic habitat disturbance (4 urban, 4 semi-natural, and 4 natural). Urban sites had a relatively dense human population, and a landscape that was widely human-modified (e.g. concrete, buildings, gardens, roads). Semi-natural sites were located in urban parkland that contained waterways adjacent to urban areas, and they had a moderate level of human visitation. Natural sites were generally associated with native bushland, waterways with forested riparian zones, and a relatively low human presence.

During October and November of 2015, gravid female dragons engaging in nest searching behaviour (e.g. test digging at nest sites) were captured by hand or noose-pole, palpated for the presence of shelled eggs, and transported to Macquarie University (Sydney, NSW) or retained at Taronga Zoo (Sydney, NSW). Gravid females were placed in 100 L plastic tubs with moist substrate, water, and maintained at an average room temperature of 30 °C (Harlow 2001). Oviposition was induced by administering a dose of 100 mg/kg calcium gluconate (Phebra, Lane Cove, NSW, AU) into the left forearm, whereupon

the lizard was rested for 60 min. The lizards was then administered a shallow intraperitoneal injection of synthetic oxytocin (Syntoncin, Ilium, Glendenning, NSW, AU) at a dose 60 IU/kg (Mader 1996; Harlow and Taylor 2000). Gravid females were held for no more than 72 h, whereupon they were released at their capture site regardless to if they had oviposited or not. This short-term holding allowed us to minimise the potential effect handling and captivity may have on mother stress and, in turn, egg development (e.g. long-term stress during development may result in altered antipredator behaviour in lizards; Uller and Olsson 2006).

Once oviposition was complete, the clutch was placed in a 500 ml plastic container with 115 g of moistened vermiculite (− 150 kPa; 130% water by dry mass of vermiculite; Harlow 2001). The containers were fitted with an oxygen permeable polyethylene membrane (Glad Wrap, Glad Products of Australia, Rhodes, NSW, AU) and placed in incubators set at a constant temperature of 26.5 °C (Harlow 2001). Incubation of the eggs took an average of 68.3 days (± 0.3 SE). Incubators were checked twice daily (approx. 08:00 and 17:00 h), and once a hatchling fully emerged from its egg it was immediately assayed.

Antipredator assay

We quantified the antipredator response of hatchling water dragons ($N=370$; see [Supplementary materials](#) for site-specific sample sizes) by simulating a predatory attack within a large rectangular testing arena (600 W \times 1500 L \times 400 H mm). The base of the arena was lined with a single sheet of blank newsprint (providing traction), while the walls of the arena were a matte black plastic (preventing climbing). We used a 1 m long rubber model of a red-bellied blacksnake (*Pseudechis porphyriacus*), a known predator of water dragons (Doody et al. 2014), mounted to a 2 m long piece of wooden dowel. This setup allowed for the model snake to enter the arena, while the researcher could remain obscured by the arena walls. All assays were conducted in a temperature-controlled room (24 °C).

Hatchlings were removed from their incubation container, and individually placed in the arena for their behavioural assay. The assay involved the model snake being thrust towards the dragon once every 30 s for a total of 10 simulated attacks. The dragon's responses were recorded and categorised as: (1) escape run (fast pace, long distance); (2) evade run (slow pace, short distance); (3) still (freeze/immobile); (4) tail swish (undulation of the tail); (5) throat puff (inflation of the throat and chest-raised stance); (6) gape (open mouth); and (7) bite (dragon's mouth makes contact with the snake). After the assay we measured hatchlings (mass and snout-vent length), microchipped them for identification (using a passive integrated transponder tag), and either released them at the mother's site of capture or retained them

as part of a captive colony at Macquarie University. It was not possible to record data blind, as clutches were incubated with siblings (simulating natural conditions and hatching stimuli) and origin locations of the clutches were required to both release individuals back into their origin populations and to organise the captive colony for future experiments.

Fight or flight index scoring

From the behaviours observed in the antipredator assay we calculated a metric to summarise the dragon's overall antipredator responses, by weighting antithetical responses with antithetical values. Flight responses were given negative values with escape runs (-2) being twice the score of evade runs (-1). The fight responses were assigned positive values increasing in weight with increasing intensity, ranging from tail swish ($+1$), throat puff ($+2$), gape ($+3$), and bite ($+4$). When individuals engaged in a passive antipredator response (immobile), their response was assigned a value of 0. Then, to calculate an overall fight or flight score (FoF) for each individual, the ten response values were averaged (FoF = $[\sum \text{response values}] / n_{\text{response values}}$).

Statistical analyses

All statistical analyses were performed with R version 3.4.2 (R Core Team 2017). Fight or flight behaviour was collected from 370 hatchling water dragons, across 74 clutches and 12 sites within the Sydney region. We used linear mixed models (LMMs) to examine what factors affect antipredator fight or flight responses in hatchling water dragons. LMMs were fitted using the R package 'lme4' (Bates et al. 2015). Prior to analyses, we explored our data to ensure there was no collinearity between predictor variables, missing values, or unexplained outliers following the protocol in Zuur et al. (2010). We used a rank transformation to normalise the fight or flight score (Kar et al. 2016). A set of five candidate LMMs were constructed to compare hypotheses regarding which factors best served to explain innate antipredator response in this species. The assumptions of normality of residuals and heterogeneity of variance were verified for all LMMs (Zuur et al. 2010).

Our first model (1) represented the null hypothesis, and was an intercept-only model with no random effects (fitted with the function 'lm' from the 'stats' R package). Our second model (2) was an intercept-only LMM, which also included the random effects of clutch identity and site. The inclusion of random effects accounts for dependency among measurements of individuals from the same clutch, as well as individuals from the same population (Zuur et al. 2009). Inclusion of random effects also allowed us to estimate the influence clutch and site has on innate antipredator behaviour; all LMMs that included clutch and site identity as a random effect estimated σ^2_{clutch} , the variation among individuals due to maternal or

paternal genetic and non-genetic effects, and σ^2_{site} , the variation among individuals due to localised population effects (Noble et al. 2014). These LMMs also estimated the residual variance (σ^2_r). For models that were best supported, we calculated the proportion of variance explained by clutch and site effects. To accomplish this we first calculated phenotypic variance (σ^2_p), which is the sum of all variance components including σ^2_r (Noble et al. 2014). Then, we estimated clutch effects as $\sigma^2_{\text{clutch}}/\sigma^2_p$ and site effects as $\sigma^2_{\text{site}}/\sigma^2_p$ (Lynch and Walsh 1998; Noble et al. 2014). We calculated 95% confidence intervals for clutch and site effects by bootstrapping the data 1000 times with the 'boot' function from the R package 'boot' (Davison and Hinkley 1997; Cauty and Ripley 2017). Clutch and site effects were considered significant when the 95% confidence intervals did not include 0.

The subsequent candidate models all included the random effects of clutch identity and site. Our third LMM (3) included the categorical predictor variable of urban category. The fourth LMM (4) included the continuous predictor variable of snout-vent length (mm). The fifth (5) LMM was the full model including all fixed and random effects included in the previous models. Initially, in the full LMM, the interaction between urban category and sex was examined, but it was found to be non-significant so the model was re-fitted without the interaction and re-run.

To compare the level of support among our five candidate models, Akaike's Information Criterion (AIC) was used where models with the lowest AIC values have the most support (Burnham and Anderson 2003). Akaike weights (w_i) were also calculated to provide an estimate of the probability that any given model was the best within the candidate set. AIC differences (Δ_i), the difference between the AIC with the lowest value and all other AIC models, were also calculated to quantify the level of support for models (Burnham and Anderson 2003). Models with Δ_i values < 2 are considered to have substantial support (Burnham and Anderson 2003), and thus parameter and variance estimates were inspected and reported for all models with $\Delta_i < 2$. The R function 'confint' from the R package 'Mass' was used to bootstrap 95% confidence intervals for parameter and variance estimates (Ripley 2018). Additionally, the R package 'MuMIn' (Barton 2009) was used to calculate the conditional R^2 value for each model, which we used to assess model fit (Nakagawa and Schielzeth 2013). Conditional R^2 is not sensitive to the number of variables in a model. So, as we state above, AIC was the main variable used for model selection.

Data availability The datasets generated during and/or analysed during the current study are not publicly available due to logistical constraints, but are available from the corresponding author on reasonable request.

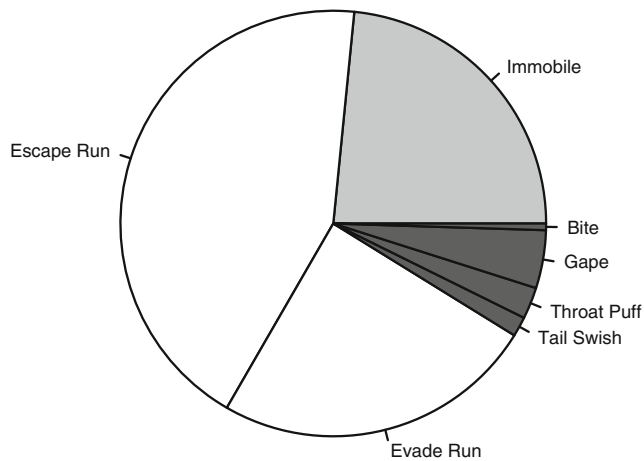


Fig. 1 The cumulative proportion of innate antipredator behaviours elicited by hatchling water dragons when presented with a simulated snake attack. Passive antipredator behaviour, immobility, is shaded light grey (23.4% of responses). Flight responses include escape runs (43.3%) and evade runs (24.5%), which are filled with white. Fight responses (shaded in dark grey) included tail swish (1.5%), throat puff (2.4%), gape (4.4%), and bite (0.5%)

Results

Overall, water dragon hatchlings exhibited passive antipredator behaviour (immobility) during 23.4% of encounters with the model snake. Active antipredator responses, that were divided into flight (both escape and evade runs) and fight behaviour (tail swish, throat puff, gape, and bite), occurred during 67.6 and 8.8% of encounters, respectively. Specifically, of the flight responses, escape runs were observed the most often (43.2%) and, of the fight responses, gape was the most common (4.4%; see Fig. 1 for a full breakdown on the proportions of antipredator responses).

Models 2 and 4 (Table 1) were best supported during model selection. The $\Delta_{M4-M2} = 0.357$, which is < 2 , suggesting they both suitably explain dragon antipredator responses. The models explained 41% (model 2) and 49% (model 4) of the data (Table 1). Model 4 had the greatest support because of its smaller AIC value, higher w_i , and higher conditional R^2 value (Table 1).

Table 1 Model selection of linear mixed models (LMMs) representing alternative hypotheses explaining variance in water dragon antipredator behaviour. Included are the number of parameters (k), log-likelihood of

Model no.	Model R code*	k	LL	Conditional R^2	AIC	Δ_i	w_i
1 (null)	~ 1	0	-517.916	0	1039.833	0	0.003
3	~ urban.cat+(1 clutch_identity) + (1 site)	1	-511.426	0.152	1034.853	4.980	0.036
5 (full)	~ urban.cat+svl + (1 clutch_identity) + (1 site)	2	-509.788	0.190	1033.576	6.257	0.068
2	~ 1 + (1 clutch_identity) + (1 site)	0	-511.006	0.149	1030.012	9.821	0.407
4	~ svl + (1 clutch_identity) + (1 site)	1	-509.828	0.189	1029.655	10.178	0.486

*Model 1 was fitted using the function 'lm' using the 'stats' R package, and models 2–4 were fitted using the function 'lmer' using the R package 'lme4'

Coefficient estimates from model 4 indicate that water dragon snout-vent length was negatively related to their fight or flight score (Table 2); smaller dragons were more prone to a more aggressive response (fight), while larger dragons had a higher escape response (flight; Fig. 2). We also saw significant clutch effects (Table 2), which suggests that parental genetic and non-genetic effects explained a moderate level of variation in dragon antipredator response (Table 2). The variance estimates from model 2 also support this latter finding (Table 2; Fig. 3).

Discussion

Our research exposed hatchlings to a simulated predatory attack immediately after emergence from their eggs, allowing us to quantify behaviours that were not confounded by prior experience with predators, social interactions, or the world outside of an individual's egg. The antipredator behaviour we observed (fleeing, fighting, or remaining immobile) were innate—the outcome of genetic effects. We determined that the variation in these innate antipredator responses was related to clutch effects, as well as individual body size (SVL). Interestingly, dragon origin population (hypothesis 2) did not explain significant variance in innate antipredator response, nor was the level of habitat disturbance related to an individual's fight or flight response. Thus, innate antipredator behaviour of water dragons does not differ between populations in the Sydney region or habitat-linked environmental factors, including urbanisation. In fact, our findings suggest that water dragon's innate antipredator responses depend on the individual—related to both an individual's body size (hypothesis 3) and clutch effects (hypothesis 1; parental genetic effects and phenotype; Webb et al. 2001).

The variation we observed in hatchling water dragon antipredator responses was significantly explained by clutch effects, which provides insight into the mechanism by which these innate behaviours are conferred. Not only are these innate antipredator behaviours heritable (Brodie 1989; Åbjörnsson et al. 2004; Sih et al. 2004), but siblings also may have a higher

the model (LL), AIC values, AIC differences (Δ_i), Akaike weights (w_i), and model fit (conditional R^2). LMMs for which $\Delta_i < 2$ are bold, and model outputs from these models are reported in Table 2

Table 2 Coefficient and variance estimates from linear mixed effect models with support (see Table 1). Included are associated 95% confidence intervals (CIs) for parameter and variance estimates, t values, and p values. Bold estimates reflect significant effects, which were determined using p values for coefficient estimates and if 95% CIs do not include 0 for variance estimates

Model 4 \sim svl + (1 clutch_identity) + (1 site)					
Fixed effects	β	Lower 95% CI	Upper 95% CI	t value	p
Intercept	3.575	0.933	5.974	2.846	0.004
Snout-vent length	-0.074	-0.124	-0.019	-2.825	0.005
Random effects	σ^2	Lower 95% CI	Upper 95% CI		
Clutch identity	0.355	0.171	0.488		
Site	0.186	0.000	0.361		
Residuals	0.894	0.820	0.968		
Model 2 \sim 1 + (1 clutch_identity) + (1 site)					
Fixed effects	β	Lower 95% CI	Upper 95% CI	t value	p
Intercept	0.032	-0.132	0.202	0.379	0.705
Random effects	σ^2	Lower 95% CI	Upper 95% CI		
Clutch identity	0.339	0.156	0.484		
Site	0.171	0.000	0.332		
Residuals	0.907	0.834	0.980		

likelihood of expressing comparable behaviours (Arnold and Bennett 1984; Sih et al. 2004). Similar findings have been reported in garter snakes (*Tradix*), with high variability in antipredator responses at the population-level but significant litter effects (between siblings), suggesting heritability (Arnold and Bennett 1984). Behavioural traits associated with antipredator responses, such as aggression, boldness, and exploration, can also be heritable (Ariyomo et al. 2013; Petelle et al. 2015). Additionally, non-genetic maternal experience, predatory exposure, and/or stress hormones affect antipredator behaviours of offspring in a host of taxa, such as fish (Giesing et al. 2010), insects (Storm and Lima 2010), and snakes (Robert et al. 2009). Our findings support the growing body of research providing evidence for the impact of parental

genetic and non-genetic effects on the antipredator behaviour of their offspring. After interpreting this result, we wanted to further investigate (post hoc) the influence of clutch effects on body size—the other factor that was significantly related to innate antipredator response. Clutch effects also explained a significant proportion of variance in hatchling dragon body size (42% of the variation in snout-vent length; Fig. 3). This suggest there are two avenues by which parental genetic and non-genetic effects are driving antipredator responses: (1) directly, through behaviour and (2) indirectly, through body size.

Body size has an effect on lizard antipredator responses; however, the direction of the effect appears to be species-dependent, and influenced by ecological and thermal factors, as well as age (Martín and López 2003; de Barros et al. 2010). Our findings, that smaller dragons were more prone to an aggressive response (fight), while larger dragons had a higher escape response (flight), supported our prediction (hypothesis 3). Our finding was similar to that seen in Iberian rock lizards, with larger individuals, related to an older age-class, fleeing predators sooner than smaller conspecifics (Martín and López 2003). This was attributed to smaller lizards being less conspicuous to predators and the thermal cost of fleeing to a refuge being higher for smaller individuals that cool more quickly (Martín and López 2003). However, the trend of larger lizards fleeing while smaller conspecifics hold their ground or become aggressive is reversed in other species. For example, larger tegu lizards (*Tupinambis merianae*), also related to an older age-class, are more likely to be aggressive compared to smaller conspecifics (de Barros et al. 2010). Yet, these results are more pronounced at lower body temperatures when smaller lizards are more likely to flee to compensate for reduced thermal-dependent physiological performance (de Barros et al. 2010). In our study, we were able to remove the confounding effects of thermal influence age, and prior

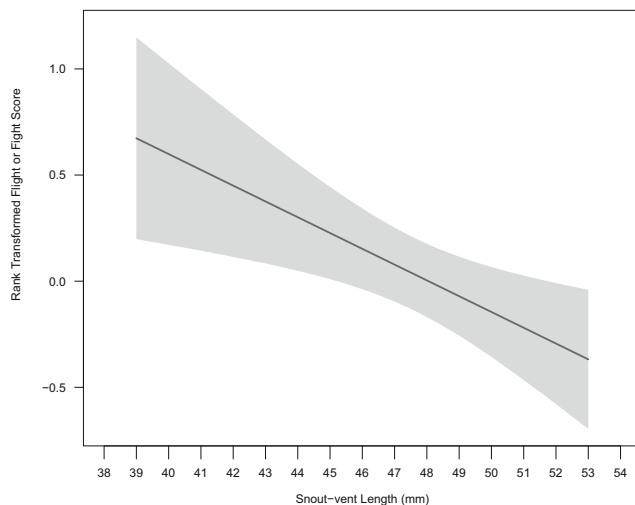


Fig. 2 The relationship between flight and fight score (rank transformed) and snout-vent length of water dragons. The larger the fight or flight value the more aggressive the dragon's response was, and, conversely, the smaller the value the greater the flight response of the dragon. The shaded polygon represents predicted 95% confidence intervals around the predicted fitted line from supported model 4 (see Table 1)

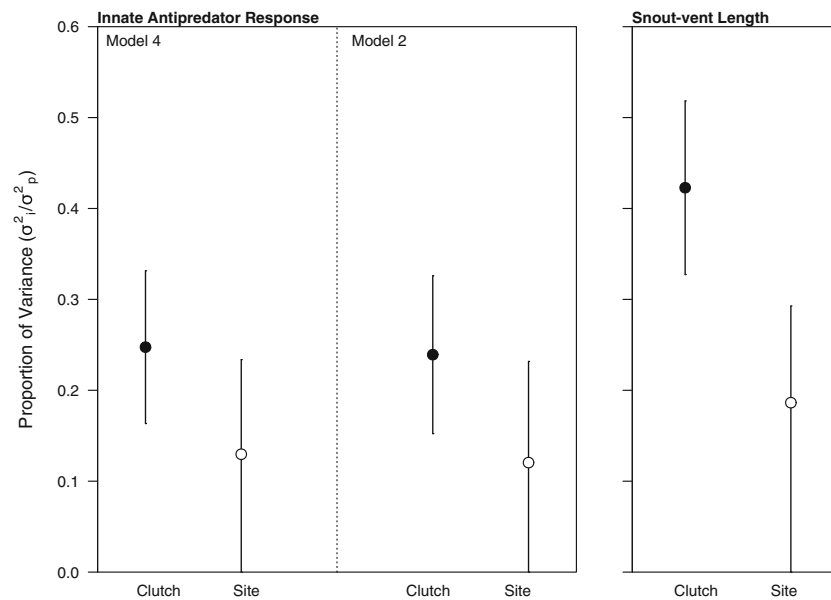


Fig. 3 Estimates of the proportion of variance in innate antipredator response that is explained by clutch ($\sigma^2_{\text{clutch}}/\sigma^2_{\text{p}}$) and site ($\sigma^2_{\text{site}}/\sigma^2_{\text{p}}$) effects within supported (a) model 4 and (b) model 2 (see Table 1). Post hoc we also estimated the proportion of variance in snout-vent length that is explain by clutch and site effects using an intercept-only linear

mixed-effect model (LMM) that included the random effects of clutch identity and site. Proportion of variance ($\sigma^2_i/\sigma^2_{\text{p}}$) was estimated using the protocol described in the main text. Error bars around estimates are 95% confidence intervals (CIs) and estimates were considered significant if their 95% CIs not include 0

experience, to experimentally examine innate antipredator responses within a laboratory setting. A potential rationale for our findings are that larger hatchling dragons may be more conspicuous to predators (Martín and López 2003), however they may also have a performance advantage for fleeing, with respect to running and jumping ability (as seen in *Anolis* sp.; Losos 1990). Thus, it may benefit larger individuals to flee rather than fight or remain immobile. Conversely, smaller hatchling dragons would be less visible to predators and may rely on immobility to avoid detection, as seen in other lizards (Martín and López 2003), snakes (Kissner et al. 1997), and frogs (Martín et al. 2005; Bateman and Fleming 2014). However, immobility becomes a riskier strategy as predators get closer (Martín et al. 2005). If immobility fails to conceal a hatchling, there is a point where escape by flight may no longer be a viable option. As such, an individual may be forced to resort to a more aggressive tactic and engage in a fight response. Furthermore, smaller lizards (e.g. *Anolis carolinensis*) have been seen to employ a ‘hawk strategy’ and increase aggression and fight instigation against larger conspecifics in size-asymmetric contests, despite their low contest success rates (10%; Jenssen et al. 2005); a trend seen across numerous taxa (see reviews by Just and Morris 2003; Morrell et al. 2005).

Overall, we were able to determine several key drivers explaining water dragon innate antipredator behaviour. We determined that parental genetic effects and phenotype (clutch effects) explained a significant amount of variation in both body size and innate antipredator response, which supports the

hypothesis that innate antipredator behaviours are linked to heritable behavioural, morphological, and physiological traits (Arnold and Bennett 1984; Brodie 1989; Webb et al. 2001; Thaker et al. 2009). Conversely, we did not find that habitat disturbance or origin population were factors related to dragon innate antipredator response. This suggests that within a population, although antipredator response has a heritable component (Arnold and Bennett 1984; Brodie 1989; Sih et al. 2004), variability in water dragon antipredator behaviour remains high. Understanding what drives variance in a trait, particularly one that is so intimately linked to fitness, is a fundamental challenge in evolutionary biology. The fact that hatchling water dragons exhibit a range of innate antipredator responses raises interesting questions about how selection acts on antipredator behaviour and the degree to which it is plastic. Future studies that test for repeatability of antipredator behaviour over the course of development will be highly beneficial, as would an examination of incubation effects (e.g. mean temperature, fluctuating temperature, humidity, and nest mate proximity).

Acknowledgements We would like to thank the many individuals who assisted in the fieldwork: P. Bolton, T. Damasio, C. Fryns, G. Hughes, F. Kar, S. Klopper, L. Monk-Whipp, M. Mühlhaupt, and D. Noble, as well as Taronga Zoo and the rest of the members of Macquarie University Lizard Lab. We would also like to thank two anonymous reviewers whose contributions improved this article.

Funding information This research was supported by Macquarie University (scholarship awarded to JB-G) and Natural Sciences and Engineering Research Council of Canada (scholarship awarded to JB-G).

Compliance with ethical standards

Ethical approval Lizard capture and our experimental protocols followed animal ethics guidelines that were approved by both the Macquarie University Animal Ethics Committee (ARA no. 2015/023) and Taronga Zoo Animal Ethics Committee (ARA no. 3b/08/15). Our research was approved by the New South Wales National Parks and Wildlife Service, Office of Environment and Heritage (Licence no. SL100570).

Conflict of interest The authors declare that they have no conflict of interest.

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