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Adaptive ecology of the King's skink, *Egernia kingii*, in response to varying levels of predation risk, with a focus on caudal autotomy

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Declaration

To the best of my knowledge and belief this thesis contains no material previously published by any other person except where due acknowledgment has been made. This thesis contains no material which has been accepted for the award of any other degree or diploma in any university.

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The research presented and reported in this thesis was conducted in compliance with the National Health and Medical Research Council Australian code for the care and use of animals for scientific purposes 8th edition (2013). The proposed research study received animal ethics approval from the Curtin University Animal Ethics Committee, Approval Number **ARE2017-12.** Additional approvals were provided by the Western Australian Department of Biodiversity, Conservation and Attractions, Approval Numbers 08-001238 and FO25000158, Rottnest Island Authority (Chapters 3, 6 & 7), Department of Defence (Chapter 3) City of Joondalup (Chapter 3), City of Stirling (Chapter 6) and the Western Australia Museum (Chapters 4 & 5).

Statement of Contribution by Others

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The study presented in Chapter 2 was accepted in the peer-reviewed journal '*Biological Reviews*' on the 25th May 2020.

BARR, J. I., SOMAWEERA, R., GODFREY, S. S., GARDNER, M. G. & BATEMAN, P. W. (2020). When one tail isn't enough: abnormal caudal regeneration in lepidosaurs and its potential ecological impacts *Biological Reviews. Online early.* https://doi.org/10.1111/brv.12625

JIB, RS and PWB conceived the idea and designed the methodology. I collected the data; I analysed the data with guidance from all authors; I wrote the manuscript and all authors contributed to the revisions of the manuscript.

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JIB and PWB designed the experiment. JIB and CAB collected the data. All Authors assisted with the interpretation of the data. I wrote the manuscript, all authors contributed to revisions of the manuscript.

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Thanks for everything Ma. This is for you.

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General Abstract

Predation pressures shape a species' morphology, ecology and behaviour both ontogenetically and over evolutionary time. Anti-predation strategies allow the individual to avoid predation but can have high associated future costs. Caudal autotomy, the ability to drop a portion of the tail, is used by many species of lizards as an effective anti-predation strategy. Species with intra-vertebral autotomy, the more common and ancestral type within lizards, possess pre-formed planes of weakness within a series of their caudal vertebrae. Once autotomised, the tail is then regenerated over time with a cartilage rod lacking autotomy planes replacing the original vertebrae. Caudal autotomy, although an effective strategy in avoiding predation, incur high future costs to the individual, from both the physical loss of the tail until it is regenerated, as well as the energy directed to regeneration. As such, the use of caudal autotomy is known to change ontogenetically in species, as well as predation pressure, with juveniles often relying more on caudal autotomy than do adults, who may have other anti-predation tactics. In this thesis I investigated how caudal autotomy changes in the King's skink *(Egernia kingii*), a large scincid: 1) ontogenetically – as the individual matures, and 2) evolutionarily – examining isolated populations under different predator pressures, both morphologically and behaviourally.

Predation pressure were assessed using representative soft clay models across several sites varying in predator diversity, with higher attack rates correlated to avian, but not overall, predator diversity. However, this was likely influenced by nesting avian species at one of the sites. No difference in attack rates were observed ontogenetically, with adult and juvenile representative clay models being attacked at similar rates.

Presence of intra-vertebral fracture planes, and the ability to autotomise, were confirmed for both juvenile and adult individuals by micro-CT analysis, and were not restricted ontogenetically. Juveniles, however, invested more into longer relative tail length compared to adults, suggesting a potential ontogenetic reliance on autotomy through presenting a larger target to redirect attacks away from the body. Likelihood of autotomy was not influenced by ontogeny or by predation pressure, with individual perception of threat being the main factor influencing the likelihood of autotomy.

The phenomenon of re-regeneration was also identified as a potential alternative strategy to minimise future costs of autotomy and regeneration, with higher incidences and proportions of tail as re-regenerated tissue correlated to increased predator diversity, particularly mammals.

Caudal autotomy and regeneration do not always go according to plan, and I provide the most comprehensive review to date on abnormal regeneration for lepidosaurs, discussing the potential ecological effects of abnormal caudal regeneration, and potential future research in this area.

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List of abbreviations

3D: Three dimensional

Al: Aluminium

AIC: Akaike information criterion

CFL: *Caudifemoralis longus* muscle

CI: Confidence interval

CMCA: Centre for Microscopy, Characterisation and Analysis

CP: Coastal Perth

C.S: Cross section

CT: Computerised tomography

DBCA: Department of Biodiversity, Conservation and Attractions

DOD: Department of Defence

E. kingii*:* Egernia kingii

GI: Garden Island

GLM: General linear model

GLMM: General linear mixed model

ha: Hectares

HW: Head width

LM: Linear model

No.: Number of

Pers. obs: Personal observation

PI: Penguin Island

QQ: Quantile-quantile

RI: Rottnest Island

RIA: Rottnest Island Authority

RL: Regeneration length

SD: Standard deviation

SVL: Snout to vent length

TL: Tail length

Unpub. Data: Unpublished data

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Chapter 1. General introduction

Figure 1.1: King's skink (*Egernia kingii)*

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1.1 Introduction

Predator-prey interactions are amongst the strongest selective pressures that can shape a species' evolution. Anti-predation strategies, be they morphological, behavioural or physiological, can increase the immediate survival of the individual, allowing them to escape from a predation event, or avoid being detected altogether (Arnold, 1984; Stevens, Stubbins & Hardman, 2008; Ruxton, 2009). For example; morphological traits such as armour to protect against predators is found across all taxa: arthropods [e.g. Orthoptera, Bateman & Fleming (2013); Isopoda, Schmalfuss (1984)], mammals [e.g. pangolins, (Yang *et al.*, 2012; Wang *et al.*, 2016)] and reptiles [e.g. lizards, (Broeckhoven *et al.*, 2018)]. Similarly, there are a range of behavioural traits such as deliberate camouflage e.g. spider crabs of the family Majidae decorating their bodies with vegetation (Wicksten, 1993; Thanh *et al.*, 2003), and physiological traits such as the Bombardier beetle (*Pheropsophus jessoensis*) using explosive chemical irritants to deter predation (Dean *et al.*, 1990; Sugiura & Sato, 2018) or autohaemorrhaging (blood-squirting) behaviour in arthropods (e.g. Orthoptera, Bateman & Fleming (2009a); Plecoptera, Benfield (1974) and reptiles (e.g. snakes, (Hoefer, Mills & Robinson, 2019); lizards, Sherbrooke & Middendorf III (2001)).

Anti-predation strategies allow potential prey to avoid being eaten but can have short- or long-term fitness costs for individuals (Arnold, 1988; Cooper & Blumstein, 2015; Bels & Russell, 2019). Selection for highly resolved abilities to determine the degree of threat can thus influence how anti-predator strategies are used, with the aim of reducing potential costs incurred (Lima & Dill, 1990; Stankowich & Blumstein, 2006; Rosier & Langkilde, 2012; Cooper & Blumstein, 2015). For example, vigilance behaviour such as head up and scanning is a common method to detect potential predators by many taxa while foraging (Unck *et al.*, 2009; Cooper & Blumstein, 2015), especially in higher risk habitats (Underwood, 1982; Altendorf *et al.*, 2001; Wolff & Horn, 2003), but is associated with decreased food and energy intake (Sinclair, Arcese & Arcese, 1995; Fortin *et al.*, 2004).

As predator risk for an organism can vary spatially, temporally and ontogenetically (Arnold, 1984; Cooper, Pérez-Mellado & Vitt, 2004; Brock *et al.*, 2015), and as maintenance of both behavioural and physiological anti-predatory tactics can be potentially costly to the individual (Downes, 2001; Naya *et al.*, 2007), tactics can be predicted to vary and change between populations and individuals based on age, sex and degree of predator naivety (Arnold, 1984; Abrams & Matsuda, 1993; Head, Keogh & Doughty, 2002; Cooper *et al.*, 2004; Barbosa & Castellanos, 2005; Abegg *et al.*, 2014). However, how quickly and to what extent anti-predation strategies change can be quite complex and variable. A study by Blumstein, Daniel & Springett (2004) investigating changes in predator recognition in tammar wallabies (*Macropus eugenii*) indicated that in populations with complete predator absence, predator recognition can be lost within as little as 130 years. Pafilis *et al.* (2009), studying tail autotomy as a defence in 15 species of Mediterranean lizard, indicated that this anti-predation strategy is not maintained by overall predation risk, but is influenced primarily by the presence of vipers alone. Additionally, strategies to deal with predators, morphological, behavioural and physiological, can change rapidly (Langkilde, 2009), such as during maturation of the individual (Hawlena *et al.*, 2006; Pafilis & Valakos, 2008). For example, certain species have brightly coloured tails as juveniles which help re-direct attacks away from the body (Cooper & Vitt, 1985; Castilla *et al.*, 1999; Bateman, Fleming & Rolek, 2014), but are lost as they mature (Arnold, 1984; Hawlena *et al.*, 2006).

Caudal autotomy, the ability to willingly shed a portion of the tail, is an effective anti-predation strategy that is used by many lizard species (Arnold, 1984; Bellairs & Bryant, 1985; Pafilis & Valakos, 2008; Bateman & Fleming, 2009b; Emberts, Escalante & Bateman, 2019). Caudal autotomy is classified as either intra-vertebral autotomy or inter-vertebral autotomy (Arnold, 1984). Intra-vertebral autotomy is the more common and ancestral form, where fracture planes are present *within* a series of caudal vertebrae, whereas inter-vertebral autotomy has planes of weakness that occur *between* the vertebrae, in the inter-vertebral space (Woodland, 1920; Bellairs & Bryant, 1985; Arnold, 1988). When grasped by a predator, a portion of the tail can be shed at an autotomy plane, allowing the lizard to break free from the predator’s grasp and escape (Etheridge, 1967; Arnold, 1988). As intra-vertebral autotomy is under a higher degree of neurological control compared to inter-vertebral autotomy, intra-vertebral autotomy has a degree of neural regulation deriving from a decision-making process by the animal, and not just as a result from physical stimulus alone; however, this may vary between species (Bellairs & Bryant, 1985; Cooper *et al.*, 2004; Clause & Capaldi, 2006). Once autotomised, the tail thrashes for a period of time to assist in distracting the predator and aid the escape of the lizard (Dial & Fitzpatrick, 1983; Arnold, 1988). The tail then regenerates over time, with the original vertebrae being replaced with a semi-rigid cartilage rod that lacks autotomy planes (Woodland, 1920; Bellairs & Bryant, 1985; Alibardi, 2010). With the regenerated cartilage rod lacking autotomy planes, subsequent caudal autotomy events have to occur at a more proximal position on the tail, requiring a larger portion of tail to be lost (Bellairs & Bryant, 1985; Arnold, 1988; Bateman & Fleming, 2009b).

Caudal autotomy is, then, an extreme anti-predator tactic involving the voluntary sacrifice of a large part of an individual’s body, and has presumably been under significant evolutionary selection from multiple predators. Although autotomy allows the immediate survival of the individual, there are both short and long-term costs associated with this strategy such as: reduced likelihood of escape from future predation events, either from missing a part of their tail or with a regenerated tail lacking autotomy planes (Arnold, 1984; Downes & Shine, 2001); reduced growth or reproductive output from re-directed energy into regeneration (Vitt, Congdon & Dickson, 1977; Chapple & Swain, 2004), and other varying effects on their ecology (Clause & Capaldi, 2006; Naya *et al.*, 2007; Bateman & Fleming, 2009b; McElroy & Bergmann, 2013). As such, if the selective pressures maintaining caudal autotomy as a defence are removed or reduced, it would be predicted that autotomy itself would soon also be reduced in significance as an anti-predatory tactic. This does indeed appear to occur; for example, populations of the lacertid lizards *Podarcis lilfordi* and *P. hispanica* show an decrease in reliance on a suite of traits associated with caudal autotomy (frequency of voluntary autotomy, latency to autotomise, pressure on the tail needed to induce autotomy, vigour of post-autotomy tail movements, and distance moved by the shed tail post-autotomy) as predation risk decreases across island and mainland sites in Spain (Cooper *et al.*, 2004). For lizard taxa freed from reliance on autotomy, fracture planes appear to be rapidly lost through ossification, generally starting from the most distal position of the tail moving proximally, occurring dorsal to ventral for the vertebra with the fracture plane in the neural arch ossifying first, followed by the centrum (Etheridge, 1967; Bellairs & Bryant, 1985). Loss or reduction of caudal autotomy planes can, to some extent, be associated with tail specialisation, but not always (Fleming, Valentine & Bateman, 2013) and reduced predation risk, where the costs of caudal autotomy outweigh the benefits appears to be the main driver of reduction in autotomic ability (Arnold, 1988; Bateman & Fleming, 2009b; Fleming *et al.*, 2013).

Caudal autotomy research has received much attention with majority of research focussing on either ecological impacts of autotomy, the regeneration process itself, and how ecological factors like predation risk influence caudal autotomy behaviour (see Bateman & Fleming, 2009; Gilbert, Payne & Vickaryous, 2013; Higham *et al.*, 2013; McElroy & Bergmann, 2013; Lozito & Tuan, 2017; Alibardi, 2019; Emberts *et al.*, 2019). Ontogenetic changes in caudal autotomy, at least morphologically regarding loss or reduction of fracture planes, has received some attention (Etheridge, 1967; Arnold, 1984; Arnold, 1988; Russell & Bauer, 1992; Arnold, 1994). However, behavioural studies specifically investigating ontogenetic changes seem to be lacking (Pafilis & Valakos, 2008), and would benefit from investigation (Bateman & Fleming, 2009; Fleming *et al.*, 2013).

The King's skink (*Egernia kingii*) is a large (over 50 cm) skink endemic to the south west of Western Australia, including coastal islands (Cogger, 2014). King's skinks undergo a considerable ontogenetic shift during development, growing from 60-80mm SVL with a mass of 7g as juveniles, to up to 244mm SVL with a mass of 220-360g as adults (Dilly, 2000; Arena & Wooller, 2003).

Juveniles experience higher predation risk than do adults (Bonnet *et al.*, 1999; Aubret *et al.*, 2004), although this can vary with different types of predator species (Pearson, Shine & How, 2002). Adult skinks are large enough to actively defend themselves against certain snake species (Masters & Shine, 2003). This change in potential defence capabilities as King's skinks grow, together with isolated natural populations that vary in exposure to predator species, presents an ideal natural laboratory to test hypotheses investigating how use of caudal autotomy, an effective yet costly anti-predation strategy, changes ontogenetically across a predation-risk gradient.

1.2 Thesis overview

1.2.1 Aims and objectives

The primary objectives of the research in this thesis (Figure 1.2) were to determine how the use of caudal autotomy changes both ontogenetically and across a predation risk gradient, using the King's skink (*Egernia kingii*), a large endemic species to Western Australia, as a model species.

This thesis explores six primary research aims:

1) Quantify the occurrence of, and potential ecological costs of, abnormal regeneration in lepidosaurs (lizards and Tuatara);
2) Investigate if predation risk changes ontogenically using representative soft clay models across a predator risk gradient;
3) Investigate if and how simple caudal morphology shifts ontogenetically exploiting caudal autotomy as an anti-predation strategy
4) Identify if caudal autotomy is morphologically restricted during ontogenetic development
5) Identify how use of caudal autotomy changes behaviourally in natural habitats across an ontogenetic and predation risk gradient
6) Investigate the potential for alternative anti-predation strategies which will minimise long-term costs associated with caudal autotomy

1.2.2 Chapter overview

Caudal autotomy and subsequent regeneration, as well as their associated ecological affects have been the subject of multiple publications and reviews (Clause & Capaldi, 2006; Maginnis, 2006; Bateman & Fleming, 2009b; Alibardi, 2010; Higham, Russell & Zani, 2013; Alibardi, 2017; Lozito & Tuan, 2017; Alibardi, 2019; Emberts *et al.*, 2019); therefore, in Chapter 2, I present an in-depth literature review focused on quantifying the recognised, but previously under-investigated, topic of *abnormal* caudal regeneration, or regenerative multi-furcation (production of multiple tails) for all lepidosaurs (lizards and tuatara) on a global scale and discuss potential benefits and detriments from when regeneration associated with autotomy goes awry. This review was conducted for lepidosaurs making use of both peer-reviewed and non-peer-reviewed literature including social media to identify the taxonomic and population level distribution and frequency of this phenomenon, extracting morphological information on number, size and caudal location of additional tail material and providing a robust discussion about the potential ecological and behavioural effects on individuals' life histories, informed by previous studies on costs and benefits of caudal autotomy and regeneration.

In Chapter 3, I investigated predation risk of King's skinks across different sites with different predators using representative soft clay models, a method for assessing predation risk (see Bateman, Fleming & Wolfe, 2017 for a review). This study highlights the importance for controls in clay model studies, something that many studies before did not include.

In Chapter 4, I investigated how investment into relative tail length changes ontogenetically in the King's skink and propose that this strategy reduces predation risk for juveniles.

In Chapter 5, I investigated if and how caudal autotomy is restricted ontogenetically in the King's skink using CT scanning and identifying presence, absence or ossification of their intra-vertebral autotomy planes.

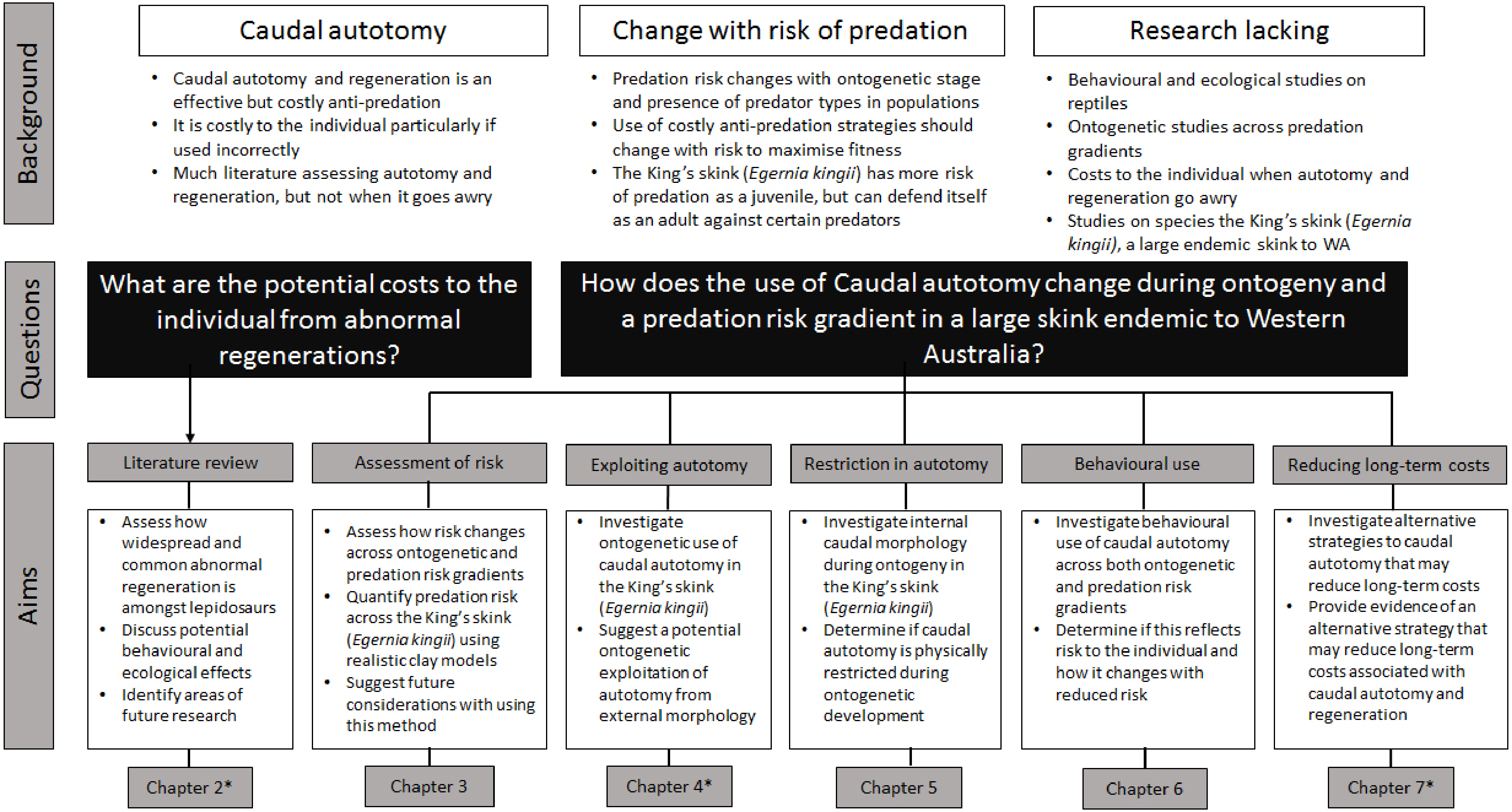
In Chapter 6, I investigated how the decision by skinks to autotomise the tail during a simulated predation event and how long the tail thrashed post-autotomy, both regarded as measures of response to predation risk, changed in isolated natural populations over an ontogenetic and predation risk gradient.

In Chapter 7, I investigated the phenomenon of re-regeneration; the ability to regenerate an already regenerated tail as a potential alternative anti-predation defence strategy to minimise long term costs to the individual associated with caudal autotomy.

Finally, Chapter 8 presents a general discussion of the chapters synthesising the overall finding of my thesis, and potential future directions from this research.

The research from this thesis provides deeper insight into anti-predation strategies, specifically caudal autotomy showing: 1) that anti-predation strategies are complex, 2) that they can be ontogenetically exploited to increase their potential effectiveness, 3) they can be manipulated to reduce their potential future costs, 4) vary based on the individual and not population, and 5) can go awry and potentially have drastic ecological effects to the individual.

Chapters 2, 4 and 7 have been published in peer-reviewed journals, Chapters 3, 5 and 6 are in preparation for submission to peer reviewed journals.



Caudal autotomy

- Caudal autotomy and regeneration is an effective but costly anti-predation
- It is costly to the individual particularly if used incorrectly
- Much literature assessing autotomy and regeneration, but not when it goes awry

Change with risk of predation

- Predation risk changes with ontogenetic stage and presence of predator types in populations
- Use of costly anti-predation strategies should change with risk to maximise fitness
- The King's skink (*Egernia kingii*) has more risk of predation as a juvenile, but can defend itself as an adult against certain predators

Research lacking

- Behavioural and ecological studies on reptiles
- Ontogenetic studies across predation gradients
- Costs to the individual when autotomy and regeneration go awry
- Studies on species the King's skink (*Egernia kingii*), a large endemic skink to WA

What are the potential costs to the individual from abnormal regenerations?

How does the use of Caudal autotomy change during ontogeny and a predation risk gradient in a large skink endemic to Western Australia?

Literature review

- Assess how widespread and common abnormal regeneration is amongst lepidosaurs
- Discuss potential behavioural and ecological effects
- Identify areas of future research

Chapter 2*

Assessment of risk

- Assess how risk changes across ontogenetic and predation risk gradients
- Quantify predation risk across the King's skink (*Egernia kingii*) using realistic clay models
- Suggest future considerations with using this method

Chapter 3

Exploiting autotomy

- Investigate ontogenetic use of caudal autotomy in the King's skink (*Egernia kingii*)
- Suggest a potential ontogenetic exploitation of autotomy from external morphology

Chapter 4*

Restriction in autotomy

- Investigate internal caudal morphology during ontogeny in the King's skink (*Egernia kingii*)
- Determine if caudal autotomy is physically restricted during ontogenetic development

Chapter 5

Behavioural use

- Investigate behavioural use of caudal autotomy across both ontogenetic and predation risk gradients
- Determine if this reflects risk to the individual and how it changes with reduced risk

Chapter 6

Reducing long-term costs

- Investigate alternative strategies to caudal autotomy that may reduce long-term costs
- Provide evidence of an alternative strategy that may reduce long-term costs associated with caudal autotomy and regeneration

Chapter 7*

Figure 1.2: Conceptual flow diagram of research aims and output of the thesis. Asterix indicates published papers.

]

1.3 References

"Every reasonable effort has been made to acknowledge the owners of copyright material. I would be pleased to hear from any copyright owner who has been omitted or incorrectly acknowledged."

ABEGG, A. D., MARIO DA ROSA, C., BORGES, L. M. & ORTIZ, F. R. (2014). Re-encounter of an *Homonata uruguayensis* specimen (Vaz-Ferreira & Sierra De Soriano, 1961) (Squamata: Phyllodactylidae) with tail bifurcation and first record in the state of Rio Grande do Sul, Brazil. *Boletín de la Sociedad zoológica del Uruguay* **23**(2), 74-78.

ABRAMS, P. & MATSUDA, H. (1993). Effects of adaptive predatory and anti-predator behaviour in a two-prey—one-predator system. *Evolutionary Ecology* **7**(3), 312-326.

ALIBARDI, L. (2010). *Morphological and Cellular Aspects of Tail and Limb Regeneration in Lizards : a Model System with Implications for Tissue Regeneration in Mammals*. Springer-Verlag. Heidelberg, Berlin

ALIBARDI, L. (2017). Review: Biological and Molecular Differences between Tail Regeneration and Limb Scarring in Lizard: An Inspiring Model Addressing Limb Regeneration in Amniotes. *Journal of experimental zoology. Part B, Molecular and developmental evolution* **328**(6), 493-514.

ALIBARDI, L. (2019). Tail regeneration in Lepidosauria as an exception to the generalized lack of organ regeneration in amniotes. *Journal of Experimental Zoology Part B: Molecular and Developmental Evolution*, 1-20.

ALTENDORF, K. B., LAUNDRÉ, J. W., LÓPEZ GONZÁLEZ, C. A. & BROWN, J. S. (2001). Assessing Effects of Predation Risk on Foraging Behavior of Mule Deer. *Journal of Mammalogy* **82**(2), 430-439.

ARENA, P. C. & WOOLLER, R. D. (2003). The reproduction and diet of *Egernia kingii* (Reptilia : Scincidae) on Penguin Island, Western Australia. *Australian Journal of Zoology* **51**(5), 495-504.

ARNOLD, E. (1984). Evolutionary aspects of tail shedding in lizards and their relatives. *Journal of Natural History* **18**(1), 127-169.

ARNOLD, E. (1988). Caudal autotomy as a defense. *Biology of the Reptilia* **16**, 235-273.

ARNOLD, E. N. (1994). Investigating the evolutionary effects of one feature on another: does muscle spread suppress caudal autotomy in lizards? *Journal of Zoology* **232**(3), 505-523.

AUBRET, F., BONNET, X., MAUMELAT, S., BRADSHAW, D. & SCHWANER, T. (2004). Diet divergence, jaw size and scale counts in two neighbouring populations of tiger snakes (*Notechis scutatus*). *Amphibia-Reptilia* **25**(1), 9-17.

BARBOSA, P. & CASTELLANOS, I. (2005). *Ecology of predator-prey interactions*. Oxford University Press.

BATEMAN, P., FLEMING, P. & ROLEK, B. (2014). Bite me: blue tails as a 'risky-decoy'defense tactic for lizards. *Current Zoology* **60**(3), 333-337.

BATEMAN, P., FLEMING, P. & WOLFE, A. (2017). A different kind of ecological modelling: the use of clay model organisms to explore predator–prey interactions in vertebrates. *Journal of Zoology* **301**(4), 251-262.

BATEMAN, P. W. & FLEMING, P. A. (2009). There will be blood: autohaemorrhage behaviour as part of the defence repertoire of an insect. *Journal of Zoology* **278**(4), 342-348.

BATEMAN, P. W. & FLEMING, P. A. (2013). Signaling or not-signaling: variation in vulnerability and defense tactics of armored ground crickets (*Acanthoplus Speiseri*: Orthoptera, Tettigoniidae, Hetrodinae). *Journal of Insect Behavior* **26**(1), 14-22.

BELLAIRS, A. & BRYANT, S. (1985). Autotomy and regeneration in reptiles. *Biology of the Reptilia* **15**(5), 301-410.

BELS, V. & RUSSELL, A. (2019). *Behavior of Lizards: Evolutionary and Mechanistic Perspectives*. CRC Press.

BENFIELD, E. F. (1974). Autohemorrhage in Two Stoneflies (Plecoptera) and its Effectiveness as a Defense Mechanism. *Annals of the Entomological Society of America* **67**(5), 739-742.

BLUMSTEIN, D. T., DANIEL, J. C. & SPRINGETT, B. P. (2004). A Test of the Multi-Predator Hypothesis: Rapid Loss of Antipredator Behavior after 130 years of Isolation. *Ethology* **110**(11), 919-934.

BONNET, X., BRADSHAW, D., SHINE, R. & PEARSON, D. (1999). Why do snakes have eyes? The (non-) effect of blindness in island tiger snakes (*Notechis scutatus*). *Behavioral Ecology and Sociobiology* **46**(4), 267-272.

BROCK, K. M., BEDNEKOFF, P. A., PAFILIS, P. & FOUFOPOULOS, J. (2015). Evolution of antipredator behavior in an island lizard species, Podarcis erhardii (Reptilia: Lacertidae): The sum of all fears? *Evolution* **69**(1), 216-231.

BROECKHOVEN, C., EL ADAK, Y., HUI, C., VAN DAMME, R. & STANKOWICH, T. (2018). On dangerous ground: the evolution of body armour in cordyline lizards. *Proceedings of the Royal Society B: Biological Sciences* **285**(1880), 20180513.

CASTILLA, A. M., GOSA, A., GALAN, P. & PEREZ-MELLADO, V. (1999). Green tails in lizards of the Genus *Podarcis*: Do they influence the intensity of predation? *Herpetologica* **55**(4), 530-537.

CHAPPLE, D. G. & SWAIN, R. (2004). Inter-populational variation in the cost of autotomy in the metallic skink (Niveoscincus metallicus). *Journal of Zoology* **264**(4), 411-418.

CLAUSE, A. R. & CAPALDI, E. A. (2006). Caudal autotomy and regeneration in lizards. *Journal of Experimental Zoology* **305**(12), 965-973.

COGGER, H. G. (2014). *Reptiles and Amphibians of Australia*, 7th edition. CSIRO Publishing, Victoria, Australia.

COOPER, J. W. E. & BLUMSTEIN, D. T. (2015). *Escaping From Predators: An Integrative View of Escape Decisions*. Cambridge University Press, Cambridge.

COOPER, W. E., PÉREZ-MELLADO, V. & VITT, L. J. (2004). Ease and effectiveness of costly autotomy vary with predation intensity among lizard populations. *Journal of Zoology* **262**(3), 243-255.

COOPER, W. E. & VITT, L. J. (1985). Blue tails and autotomy: enhancement of predation avoidance in juvenile skinks. *Zeitschrift fuer Tierpsychologie* **70**(4), 265-276.

DEAN, J., ANESHANSLEY, D., EDGERTON, H. & EISNER, T. (1990). Defensive spray of the bombardier beetle: a biological pulse jet. *Science* **248**(4960), 1219-1221.

DIAL, B. E. & FITZPATRICK, L. C. (1983). Lizard tail autotomy: function and energetics of postautotomy tail movement in *Scincella lateralis*. *Science* **219** (4583), 391-393.

DILLY, M. L. (2000). *Factors affecting the distribution and variation in abundance of the King's skink (Egernia kingii) (Gray) in Western Australia*. Honours thesis thesis, Murdoch University.

DOWNES, S. (2001). Trading heat and food for safety: costs of predator avoidance in a lizard. *Ecology* **82**(10), 2870-2881.

DOWNES, S. & SHINE, R. (2001). Why does tail loss increase a lizard's later vulnerability to snake predators? *Ecology* **82**(5), 1293-1303.

EMBERTS, Z., ESCALANTE, I. & BATEMAN, P. W. (2019). The ecology and evolution of autotomy. *Biological Reviews* **94**, 1881-1896.

ETHERIDGE, R. (1967). Lizard caudal vertebrae. *Copeia* **1967**(4), 699-721.

FLEMING, P. A., VALENTINE, L. E. & BATEMAN, P. W. (2013). Telling tails: selective pressures acting on investment in lizard tails. *Physiological and Biochemical Zoology* **86**(6), 645-658.

FORTIN, D., BOYCE, M. S., MERRILL, E. H. & FRYXELL, J. M. (2004). Foraging costs of vigilance in large mammalian herbivores. *Oikos* **107**(1), 172-180.

GILBERT, E. A., PAYNE, S. L. & VICKARYOUS, M. K. (2013). The anatomy and histology of caudal autotomy and regeneration in lizards. *Physiological and Biochemical Zoology* **86**(6), 631-644.

HAWLENA, D., BOOCHNIK, R., ABRAMSKY, Z. & BOUSKILA, A. (2006). Blue tail and striped body: why do lizards change their infant costume when growing up? *Behavioral Ecology* **17**(6), 889-896.

HEAD, M. L., KEOGH, J. S. & DOUGHTY, P. (2002). Experimental Evidence of an Age-Specific Shift in Chemical Detection of Predators in a Lizard. *Journal of Chemical Ecology* **28**(3), 541-554.

HIGHAM, T. E., RUSSELL, A. P. & ZANI, P. A. (2013). Integrative biology of tail autotomy in lizards. *Physiological and Biochemical Zoology* **86**(6), 603-610.

HOEFER, S., MILLS, S. & ROBINSON, N. (2019). Autohaemorrhaging in a Bahamian pygmy boa, *Tropidophis curtus barbouri*. *Herpetological Bulletin* **150**, 39-40.

LANGKILDE, T. (2009). Invasive fire ants alter behavior and morphology of native lizards. *Ecology* **90**(1), 208-217.

LIMA, S. L. & DILL, L. M. (1990). Behavioral decisions made under the risk of predation: a review and prospectus. *Canadian Journal of Zoology* **68**(4), 619-640.

LOZITO, T. P. & TUAN, R. S. (2017). Lizard tail regeneration as an instructive model of enhanced healing capabilities in an adult amniote. *Connective Tissue Research* **58**(2), 145-154.

MAGINNIS, T. L. (2006). The costs of autotomy and regeneration in animals: a review and framework for future research. *Behavioral Ecology* **17**(5), 857-872.

MASTERS, C. & SHINE, R. (2003). Sociality in lizards: family structure in free-living King's Skinks *Egernia kingii* from southwestern Australia. *Australian Zoologist* **32**(3), 377-380.

MCELROY, E. J. & BERGMANN, P. J. (2013). Tail autotomy, tail size, and locomotor performance in lizards. *Physiological and Biochemical Zoology* **86**(6), 669-679.

NAYA, D. E., VELOSO, C., MUÑOZ, J. L. & BOZINOVIC, F. (2007). Some vaguely explored (but not trivial) costs of tail autotomy in lizards. *Comparative Biochemistry and Physiology Part A Molecular & Integrative Physiology* **146**(2), 189-193.

PAFILIS, P., FOUFOPOULOS, J., POULAKAKIS, N., LYMBERAKIS, P. & VALAKOS, E. D. (2009). Tail shedding in Island lizard [LACERTIDAE, REPTILIA]: Decline of antipredator defenses in relaxed predation environments. *Evolution* **63**(5), 1262-1278.

PAFILIS, P. & VALAKOS, E. D. (2008). Loss of caudal autotomy during ontogeny of Balkan Green Lizard, *Lacerta trilineata*. *Journal of Natural History* **42**(5-8), 409-419.

PEARSON, D., SHINE, R. & HOW, R. (2002). Sex-specific niche partitioning and sexual size dimorphism in Australian pythons (*Morelia spilota imbricata*). *Biological Journal of the Linnean Society* **77**(1), 113-125.

ROSIER, R. L. & LANGKILDE, T. (2012). Behavior under risk: how animals avoid becoming dinner. *Nature Education Knowledge* **2**, 8.

RUSSELL, A. & BAUER, A. (1992). The m. caudifemoralis longus and its relationship to caudal autotomy and locomotion in lizards (Reptilia: Sauna). *Journal of Zoology (London)* **227**, 127-143.

RUXTON, G. D. (2009). Non-visual crypsis: a review of the empirical evidence for camouflage to senses other than vision. *Philosophical Transactions of the Royal Society of London B: Biological Sciences* **364**(1516), 549-557.

SCHMALFUSS, H. (1984). Eco-morphological strategies in terrestrial isopods. *Symposium of the Zoological Society of London* **53**, 49- 63.

SHERBROOKE, W. C. & MIDDENDORF III, G. A. (2001). Blood-Squirting Variability in Horned Lizards (*Phrynosoma*). *Copeia* **2001**(4), 1114-1122.

SINCLAIR, A. R. E & ARCESE, P. (1995). *Serengeti II: Dynamics, Management, and Conservation of an Ecosystem*. University of Chicago Press.

STANKOWICH, T. & BLUMSTEIN, D. (2006). Fear in animals: A meta-analysis and review of risk assessment. *Proceedings of the Royal Society B: Biological Sciences* **272**(1581), 2627-34.

STEVENS, M., STUBBINS, C. L. & HARDMAN, C. J. (2008). The anti-predator function of ‘eyespots’ on camouflaged and conspicuous prey. *Behavioral Ecology and Sociobiology* **62**(11), 1787.

SUGIURA, S. & SATO, T. (2018). Successful escape of bombardier beetles from predator digestive systems. *Biology Letters* **14**(2).

THANH, P. D., WADA, K., SATO, M. & SHIRAYAMA, Y. (2003). Decorating behaviour by the majid crab *Tiarinia cornigera* as protection against predators. *Journal of the Marine Biological Association of the United Kingdom* **83**(6), 1235-1237.

UNCK, C. E., WATERMAN, J. M., VERBURGT, L. & BATEMAN, P. W. (2009). Quantity versus quality: how does level of predation threat affect Cape ground squirrel vigilance? *Animal Behaviour* **78**(3), 625-632.

UNDERWOOD, R. (1982). Vigilance Behaviour in Grazing African Antelopes. *Behaviour* **79**(2), 81-107.

VITT, L. J., CONGDON, J. D. & DICKSON, N. A. (1977). Adaptive strategies and energetics of tail autonomy in Lizards. *Ecology* **58**(2), 326-337.

WANG, B., YANG, W., SHERMAN, V. R. & MEYERS, M. A. (2016). Pangolin armor: Overlapping, structure, and mechanical properties of the keratinous scales. *Acta Biomaterialia* **41**, 60-74.

WICKSTEN, M. K. (1993). A Review and a Model of Decorating Behavior in Spider Crabs (Decapoda, Brachyura, Majidae). *Crustaceana* **64**(3), 314-325.

WOLFF, J. O. & HORN, T. V. (2003). Vigilance and foraging patterns of American elk during the rut in habitats with and without predators. *Canadian Journal of Zoology* **81**(2), 266-271.

WOODLAND, W. (1920). Memoirs: some observations on caudal autotomy and regeneration in the gecko (*Hemidactylus flaviviridis*, Rppel), with notes on the tails of *Sphenodon* and *Pygopus*. *Journal of Cell Science* **2**(257), 63-100.

YANG, W., CHEN, I. H., MCKITTRICK, J. & MEYERS, M. A. (2012). Flexible Dermal Armor in Nature. *JOM* **64**(4), 475-485.

Chapter 2. When one tail isn't enough: abnormal caudal regeneration in lepidosaurs and its potential ecological impacts

The study presented in Chapter 2 was accepted in the peer-reviewed journal '*Biological Reviews*' on the 25th May 2020.

Barr, J. I., Somaweera, R., Godfrey, S. S., Gardner, M. G. & Bateman, P. W. (2020). When one tail isn't enough: abnormal caudal regeneration in lepidosaurs and its potential ecological impacts *Biological Reviews. Online early.* https://doi.org/10.1111/brv.12625

Figure 2.1: King's skinks (*Egernia kingii)* with regenerative caudal bifurcations

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Barr, J. I. & Bateman, P. W. (2020). *Egernia kingii* (King's skink): Caudal bifurcation. *Herpetological Review* **51**(2), 331.

2.1 Abstract

Abnormal caudal regeneration, the production of additional tails through regeneration events, occurs in lepidosaurs as a result of incomplete autotomy or a sufficient caudal wound. Despite being widely known to occur, documented events generally are limited to opportunistic single observations — hindering the understanding of the ecological importance of caudal regeneration. Here we compiled and reviewed a robust global database of both peer-reviewed and non-peer reviewed records of abnormal regeneration events in lepidosaurs published over the last 400 years. Using this database, we qualitatively and quantitatively assessed the occurrence and characteristics of abnormal tail regeneration among individuals, among species, and among populations. We identified 425 observations from 366 records pertaining to 175 species of lepidosaurs across 22 families from 63 different countries. At an individual level, regenerations ranged from bifurcations to hexafurcations; from normal regeneration from the original tail to multiple regenerations arising from a single point; and from growth from the distal third to the proximal third of the tail. Species showing abnormal regenerations included those with intra-vertebral, inter-vertebral or no autotomy planes, indicating that abnormal regenerations evidently occur across lepidosaurs regardless of whether the species demonstrates caudal autotomy or not. Within populations, abnormal regenerations were estimated at a mean ± SD of 2.75 ± 3.41% (range 0.1–16.7%). There is a significant lack of experimental studies to understand the potential ecological impacts of regeneration on the fitness and life history of individuals and populations. We hypothesised that abnormal regeneration may affect lepidosaurs *via* influencing kinematics of locomotion, restrictions in escape mechanisms, anti-predation tactics, and intra- and inter-specific signalling. Behaviourally testing these hypotheses would be an important future research direction.

2.2 Introduction

Autotomy, the self-induced shedding of part of the body, occurs in a wide range of taxa (Fleming, Muller & Bateman, 2007; Emberts, Escalante & Bateman, 2019). Perhaps the most familiar example is caudal autotomy – the ability to shed all or a part of the tail – employed by many species of lizards, some snakes (Cooper & Alfieri, 1993; Ananjeva & Orlov, 1994), and even a few other reptiles such as the tuatara (*Sphenodon punctatus*) (Seligmann, Moravec & Werner, 2008), usually as a decoy to avoid a predation event (Arnold, 1988; Bateman & Fleming, 2009). Most reptiles that use caudal autotomy have a series of post-pygal caudal vertebrae with pre-formed intra-vertebral breakage or autotomy planes (Etheridge, 1967; Arnold, 1984). These breakage planes, together with the organisation of associated tissues, result in sections of the tail that can be consciously autotomised allowing escape from threats (Etheridge, 1967; Arnold, 1984). Some lizard taxa, such as agamids, lack these intra-vertebral breakage planes, but still possess the ability to autotomise by having inter-vertebral (between the caudal vertebrae) breakage planes (Arnold, 1984; Bellairs & Bryant, 1985). Other lizard taxa – the varanids, chameleons and isolated members of some other clades – lack either type of fracture plane, and cannot autotomise their tails at all (Etheridge, 1967; Arnold, 1984; Bellairs & Bryant, 1985; Herrel *et al.*, 2012).

2.2.1 Caudal regeneration in lepidosaurs

The ability to regenerate tissue is often, but not exclusively, coupled with autotomy (Lozito & Tuan, 2017; Alibardi, 2019; Barr *et al.*, 2019*a*; Emberts *et al.*, 2019). Evolving and being lost multiple times across taxonomic levels, autotomy and regeneration capacity is significantly influenced by both abiotic and biotic factors (Alibardi, 2017, 2019; Emberts *et al.*, 2019). Lepidosaurs represent an evolutionary mid-point, a terrestrial clade that lack a moulting or metamorphosis stage, often a point of regeneration in other taxa, but have not yet developed an immune and healing system as efficient as that of higher amniotes such as mammals where fast healing through the formation of scar tissue is prioritised over regeneration (Alibardi, 2017, 2019). In lizard taxa with intra-vertebral breakage planes – the more ancestral condition – a broken tail regenerates over time with the bony vertebrae being replaced by a rigid cartilage rod (Woodland, 1920; Bellairs & Bryant, 1985). Inter-vertebral breaks do not possess the same regenerative ability as intra-vertebral breaks and regeneration is often limited, appearing to be more like wound healing with associated scar tissue rather than regeneration (Etheridge, 1967; Arnold, 1984; Schall *et al.*, 1989). The regenerative capacity in species that autotomise inter-vertebrally can still vary considerably, depending on where the injury, autotomy or amputation occurs within the tail (Jamison, 1964; Bellairs & Bryant, 1985; Maginnis, 2006; Alibardi, 2019); additionally, re-regeneration can also subsequently occur from further breaks of the regenerated cartilage rod (Barr *et al.*, 2019*a*).

2.2.3 Cost of tail loss

Tail loss, whilst allowing a lizard to escape from a predatory encounter, also comes with costs to the individual. Costs of caudal autotomy in lizards have been studied extensively over the last century (Woodland, 1920; Arnold, 1984, 1988; Bellairs & Bryant, 1985; Clause & Capaldi, 2006; Bateman & Fleming, 2009; Alibardi, 2010, 2019; Higham, Russell & Zani, 2013). Tails can affect success in mate signalling and courtship (Hamilton & Sullivan, 2005), mate access and mating success (Salvador, Martin & López, 1995), social status (Fox, Heger & Delay, 1990), conspecific aggression and dominance (Schall *et al.*, 1989), protection from predators (Bateman, Fleming & Rolek, 2014) and locomotion (McElroy & Bergmann, 2013), as well as being used to store fat and nutrients for energy (Dial & Fitzpatrick, 1981). Tail loss can, therefore, be costly, and energy investment into regeneration and speed of regeneration can affect growth and reproductive output of individuals (Congdon, Vitt & King, 1974; Dial & Fitzpatrick, 1981; Bellairs & Bryant, 1985) and vary dramatically among species, sex and season, and with importance of the tail to the individual’s ecology (Congdon

et al., 1974; Vitt, Congdon & Dickson, 1977; Alibardi & Meyer-Rochow, 1989; Fleming, Valentine & Bateman, 2013; Alibardi & Meyer-Rochow, 2019).
The majority of research on autotomy in lepidosaurs has focused on the loss of a portion of the tail and regeneration of that tail, both in terms of ecological impacts (Bateman & Fleming, 2009; Higham *et al.*, 2013; McElroy & Bergmann, 2013), and of the process itself (Alibardi, 2010; Sanggaard *et al.*, 2012; Gilbert, Payne & Vickaryous, 2013; Jacyniak, McDonald & Vickaryous, 2017; Lozito & Tuan, 2017; Alibardi, 2019). .

2.2.4 Abnormal caudal regeneration

In both intra- and inter-vertebral autotomising taxa, caudal regeneration can go awry, such that more than one tail grows post autotomy; for example, bifurcation of the tail of the agamid *Paralaudakia (Stellio) caucasius* (Ananjeva & Danov, 1991), and trifurcation of the tail in the lacertid *Algyroides nigropunctatus* (Koleska & Jablonski, 2015). The process of 'abnormal' caudal regeneration, hereafter referring to regeneration deviating from a single tail to produce a furcation event, has received some attention (see Przibram, 1909; Volante, 1923; Bellairs & Bryant, 1985); however, much of this is from older research experimentally investigating the regeneration process and the importance of the ependyma – the glial membrane that lines the spinal cord (Woodland, 1920; Das, 1932; Simpson, 1964; Alibardi, Sala & Miolo, 1988). When this lining is disrupted, either from an incomplete autotomy event or sufficient caudal wound, the regeneration process commences — and if the original tail remains partially attached, the generation of a secondary tail can occur (Simpson, 1964; Lozito & Tuan, 2017). Additionally, this process can be exacerbated following multiple partial wounds producing individuals like the *Salvator merianae* reported by Pelegrin & Leão (2016) with a caudal hexa-furcation (six tails).

2.2.5 Tail loss and regeneration in other reptilian clades

Within other non-lepidosaur reptilian clades, tail loss and regenerative capacity vary (Alibardi, 2019; Emberts *et al.*, 2019). Tail damage and regeneration of damaged tails, including abnormally regenerated tails, has been recorded in testudines (Kuchling, 2005; Rahman, 2011), but it is not known if any testudines have true caudal breakage planes. Tail loss has been recorded in several species of snakes (e.g. Ananjeva & Orlov, 1994; Fitch, 2003) and in at least some is referred to as autotomy (e.g Akani *et al.*, 2002) or as inter-vertebral non-specialised pseudoautotomy (i.e. occurs without regeneration) (Todd & Wassersug, 2010). Although tail loss comes with a cost to mating ability for male garter snakes (*Thamnophis sirtalis parietalis*) (Shine *et al.*, 1999), there is no evidence of regeneration of any sort in snake tails (Arnold, 1984). Within Crocodilia, survival of severe wounds is common, and a few incidences of regeneration have been recorded; one of caudal regeneration in *Melanosuchus niger*, with the individual having a 21.5 cm ‘elongated rod of calcified cartilage’ (Bellairs & Bryant, 1985, p.392), as well as a single observation of caudal bifurcation reported by Ramírez-Bravo, Solis & Stephanie (2020) in *Caiman crocodylus*. This review will, however, focus on abnormal caudal regenerations of lepidosaurs, i.e. lizards and tuatara.

2.2.6 Aims of the review

As our understanding of the costs and benefits of caudal autotomy increases, we are better able to consider the impacts, if any, of abnormal caudal regeneration. However, detailed investigations are lacking on abnormal caudal regeneration in lepidosaurs, its frequency and distribution through the taxon, and potential ecological impacts to individuals or even populations (Passos *et al.*, 2016). Herein, we review available information on regenerative furcation and multi-furcation regeneration events, and any abnormal caudal regeneration producing multiple tails in lepidosaurs. Specifically, we (1) identify and examine records of abnormal caudal regeneration within families and species, (2) assess the occurrence of abnormal regenerations within populations, (3) quantify the number of additional tails and the percentage of tail that abnormal regenerations represent, and (4) using this information, discuss the potential ecological impacts that such regeneration events may have on the fitness and life history of individuals and populations, providing suggestions for future research on this phenomenon.

2.3 Methods

We compiled published records of abnormal caudal regenerations in lepidosaur taxa from peer-reviewed literature and from non-peer-reviewed 'grey' literature, as well as online records on social and popular media, from the earliest available published material (dated 1586) up to July 2019. It has been shown that the inclusion of grey literature in reviews is important, both preventing biases and including relevant, albeit not peer-reviewed data, providing a more robust and well-rounded database (Conn *et al.*, 2003; Bellefontaine & Lee, 2013; Auliya *et al.*, 2016; Cross *et al.*, 2019).

2.3.1 Search parameters

Google Scholar, *Scopus*, *Web of Science* and *BioRxiv* were used to search for published, peer-reviewed literature incorporating the search phrases listed in Table 2.1. The first 15 pages (150 records) of articles (sorted by relevance) were reviewed, or all returns in the case of less than 15 pages. Beyond 150 records, the articles returned were found to be outside the scope of the literature search. Additionally, as most of the multiple regeneration events appear to be single-observation reports, *Herpetological Review* short notes from 2000 to June 2019 were searched for additional records using the find function for 'ifid', relating to bifid and trifid tails and 'furcat' for multi-furcation events. Internal references for articles were also investigated. The complete endnote library of *Herpetological Review* was also searched for the terminology 'ifid', 'furcat' 'fork' and 'split' to maximise location of any records relating to lizard caudal furcation events. For online social and popular media; *Flickr*, *Instagram* and *Google Image*, as well as requests to several online herpetological groups on *Facebook* were used. Search terminology are outlined in Table 2.1.

Table 2.1: Search terminology and search engines used for generating the database

Search area		**Search terms**
Published literature	*Web of Science*	Lizard bifurcation tail
	Google Scholar	Lizard bifurcated tail
	Scopus	Lizard trifurcation tail
	BioRxiv	Lizard trifurcated tail
		Lizard furcation
		Lizard furcate
		Lizard caudal bifurcation
		Lizard bifurcation tail
		Lizard caudal trifurcation
		Lizard caudal trifurcation
		Lizard bifid tail
		Lizard trifid tail
		Forked tail lizard
		Split tail lizard
		Caudal regeneration anomalies lizard
		Caudal regeneration abnormal lizard
	Herpetological Review (PDF search) (January 2000–June 2019)	'ifid', 'furcat'
	Herpetological Review (endnote library)	'ifid', 'furcat', fork, split

Online social and popular media	*Google Image* search *Flickr* search	Lizard bifurcation tail Lizard fork tail Lizard split tail Lizard trifurcation tail
	Instagram	#forktail #Splittail #bifurcation #trifurcation #twotaillizard #mutantlizard
	Facebook social media groups Herpetological Photography Herpetological Collections and Collections Management Australian Herpetological Photography Australian Society of Herpetologists Inc.	A request was posted on their social media feeds asking for any reports of abnormal regeneration, bifurcation or multifurcation

2.3.2 Database construction

We constructed a database extracting information on: date of publication, family, genus and species, location of observations (or where the specimens were acquired in the case of museum or captive specimens), type of furcation event that occurred (bi-, tri- quadri-, penta-, hexa-), investigation method [observation, X-ray, computerised tomography (CT) etc.] and type of observation (natural, museum, captive and experimental). We also recorded the type of abnormal caudal regeneration that occurred, classifying them into three categories, normal regeneration from the original tail, multiple regenerations from a single point, and regeneration from an already regenerated tail (see Figure 2.2) when stated, described in detail, or could be accurately identified from scientific drawings or photographs (from general difference in regenerated tails such as scale pattern and/or colour/size difference) as well as from X-ray or CT figures. Species identification for online social and popular media photographs were checked with herpetologists familiar with the fauna of the area to ensure appropriate classification.

Where possible, morphometric data for tail length (mm), regeneration length (or lengths, mm) and point of furcation on tail were extracted. Point of furcation was categorised into which third (proximal, middle or distal) of the tail was the furcation event found. Studies that reported on different species within the same record or multiple observations within the same record were treated as individual observations for analysis. Relative furcation percentage, the length of furcation as a percentage of the original tail or primary tail (if a normal regeneration event was present on the original tail), was established from either text descriptions (when measurements were reported) and/or calculated from visual sources (photographs or scientific drawings) as a fraction of the original tail. For multi-furcation events (tri- to hexa-), cumulative percentage of furcation was calculated. Where multiple regenerations occurred at a singular point, the longest length of tail was used as the primary tail in establishing the relative furcation length percentage. Relative furcations that were visually measured as less than 10% of the tail, were recorded as 10% for analysis.

Frequency of furcation was established for records that reported the total numbers of individuals examined in either natural populations or museum specimen collections. For these records we also calculated the percentage of furcation from those individuals exhibiting regeneration. If records reported more than a certain number of individuals examined (e.g. > 350 individuals) that number reported (e.g. 350) was used as the total number of individuals examined to establish frequency of furcation occurrence. As species names can change, the names of all reported species were checked against the reptile database (Uetz, Freed & Hošek, 2019) for the most recent classification of the record's species, genus and family, as well as the taxonomy and number of species for each family. All analysis was performed to species level where possible.

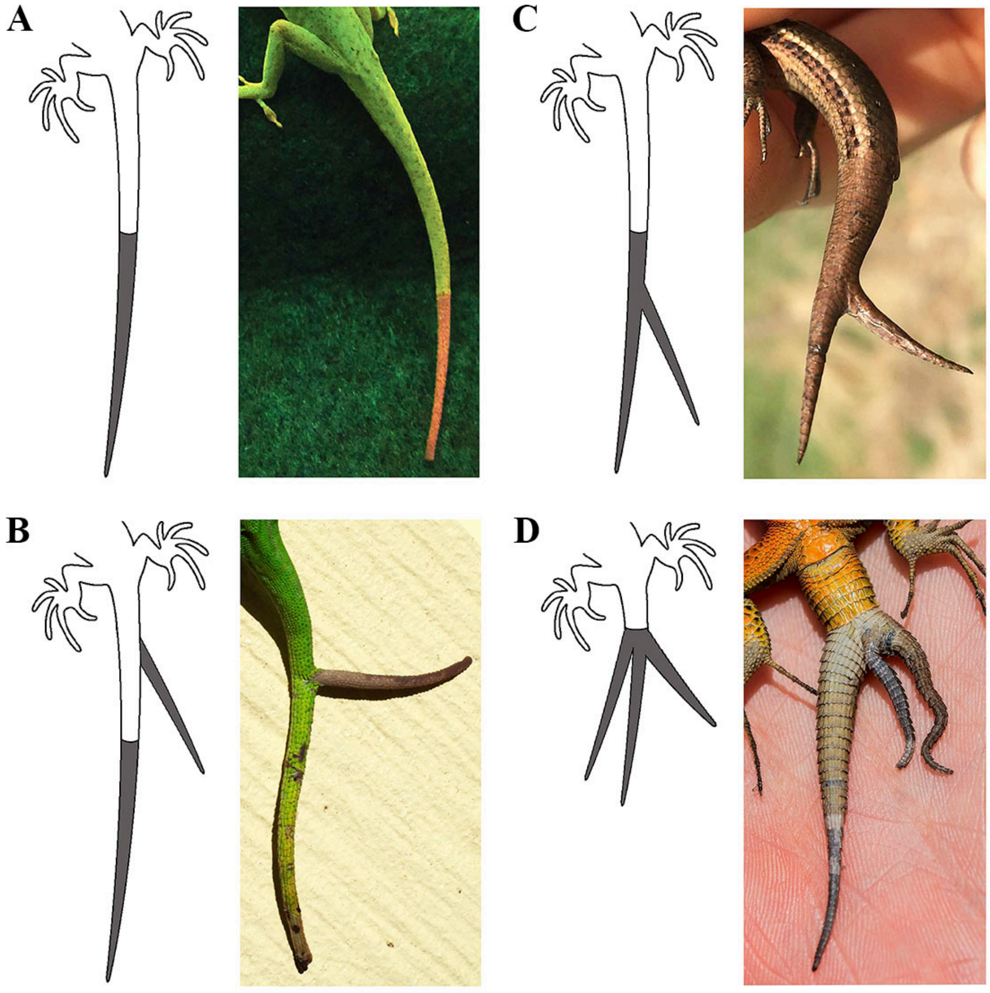

Figure 2.2: Visual representation of abnormal regeneration types and standard regeneration for comparison. (A) Standard regeneration (Ritzman *et al.*, 2012); (B) regeneration from the original tail (photograph: Chris Thawley); (C) regeneration from an already regenerating tail forming multiple tails (photograph: Jules Farquhar); and (D) multiple regenerations from a single point (Koleska & Jablonski, 2015).

2.4 Review of studies reporting on abnormal regeneration

2.4.1 Database search

Our search results from all sources (see Appendix 2) returned 366 records of abnormal regeneration events from over 400 years (1586–2019), with a total of 425 observations across 22 families, 97 genera and 175 species (Figure 2.3). Of the 366 records, 50% were from peer-reviewed works (42.1% journal articles, 7.4% books, 0.5% conference papers), 0.6% from ‘grey’ literature (0.3% unpublished data and 0.3% technical reports), with the majority of records (49.4%) coming from online social and popular media photos. The majority of reports were from species from the USA (90), Australia (60), India (16) and Brazil (12), with the entire data set spanning 63 countries and with 294 of the 366 records able to be linked to the location of the observation (Figure 2.4).

2.4.2 Furcation and regeneration types

The majority of the 425 abnormal regenerations were of bifurcations (366), with 49 occurrences of trifurcation, eight of quadrifurcation and a single observation each for pentafurcation (Chan *et al.*, 1984) and hexafurcation (Pelegrin & Leão, 2016). We were able to calculate, or accurately estimate, the relative (for bifurcation) or cumulative (for multifurcation) percentage of furcation for 306 of the 425 observations (Table 2.2). Individuals exhibiting a single furcation event (bifurcation) had a mean (± SD) additional 35.5 ± 21.6% of tail material in addition to the original or primary tail, with the mean relative furcation length percentage increasing with the number of furcation events (Table 2.2). Of the total 425 observations, we were able accurately to describe the type of regenerative furcation of 303 observations (see Figure 2.2 for categories). Of those 303 observations, 45.9% were described as normal regeneration from the original tail, 28.7% as abnormal caudal

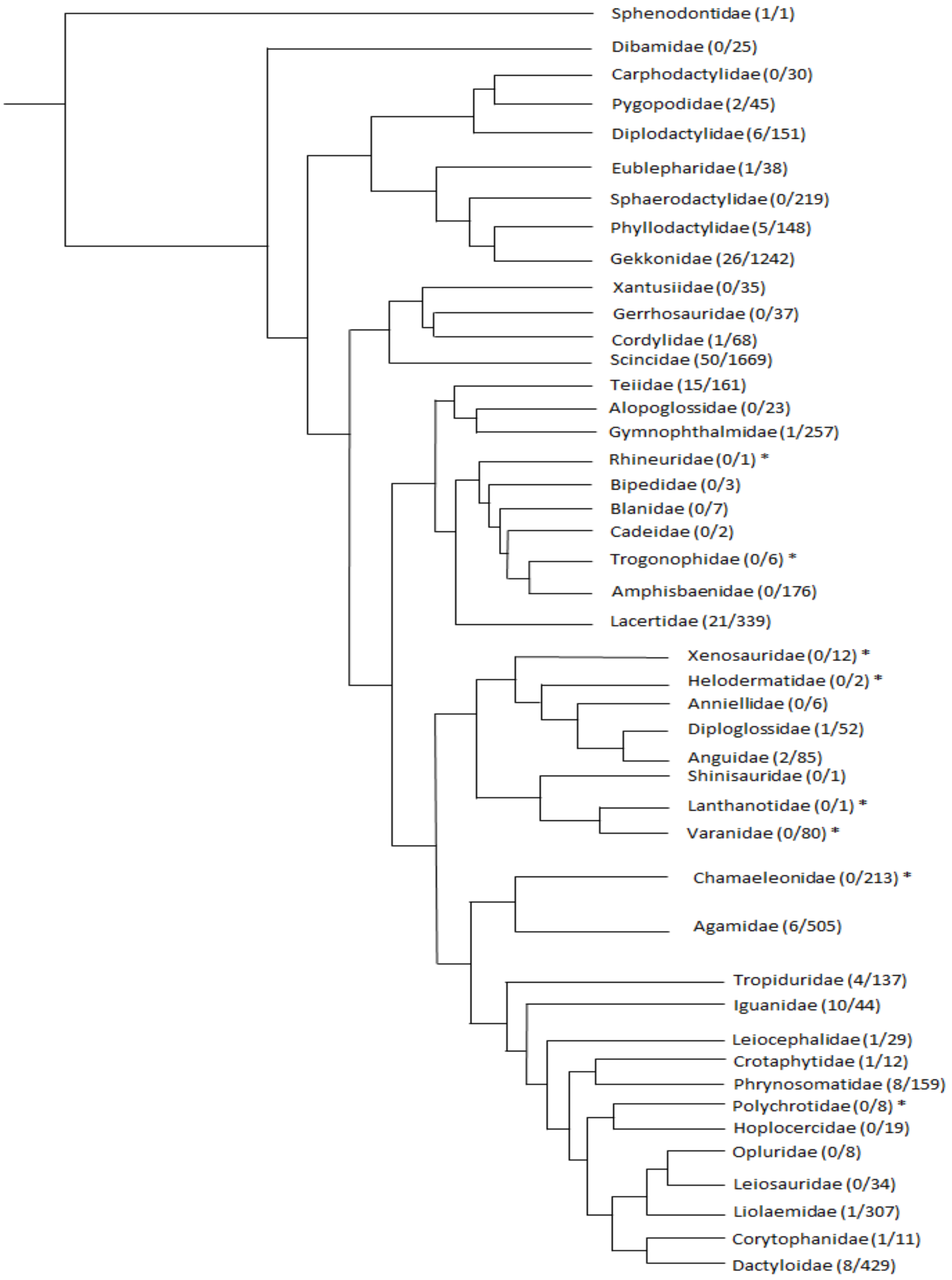

Figure 2.3: Phylogenetic tree adapted from Pyron *et al*. (2013) for families consistent with the most recent classifications from The Reptile Database (Uetz *et al*., 2019; http://www.reptile-database.org, accessed July 27th 2019). Numbers in parentheses are the number of species observed from the literature search to have abnormal regeneration and the number of known species in the family. Asterisks mark families that do not have intra-vertebral or inter-vertebral autotomy planes according to information extracted from literature assessing caudal vertebrae structure (Etheridge, 1967; Hecht & Costelli, 1971; Bellairs & Bryant, 1985; Arnold, 1988; Funk, 2019).

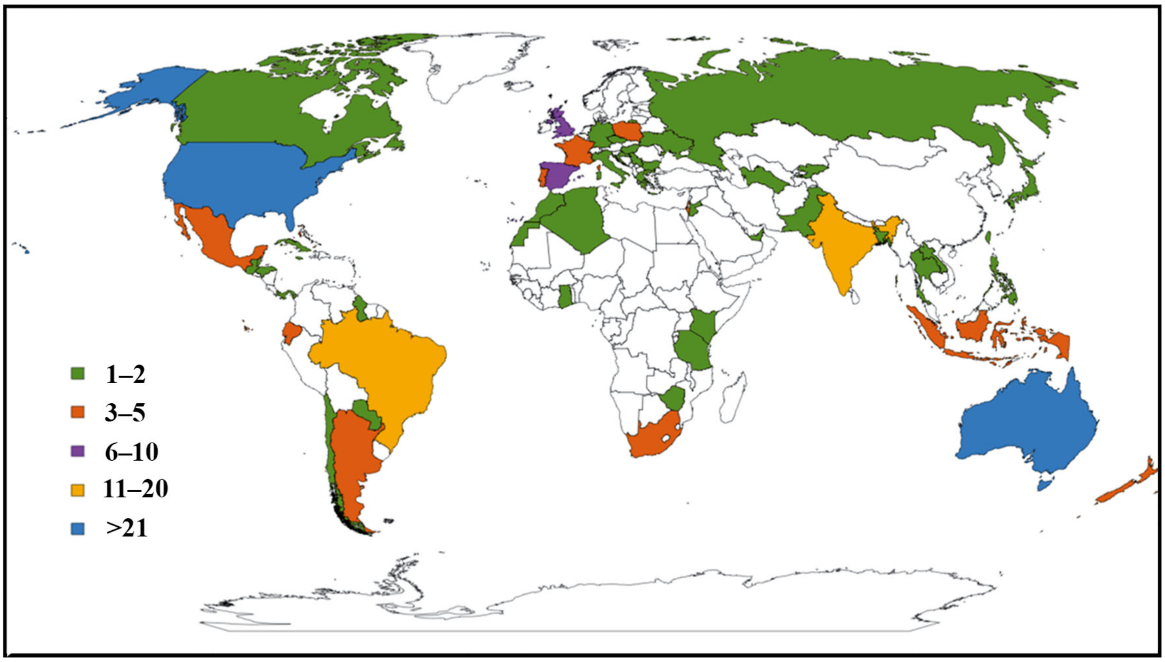

Figure 2.4: Records of abnormal regeneration events reported in lepidosaurs across the world.

regeneration occurring on an already regenerated tail, and 21.8% as having multiple regenerations arising from a single point. Several cases were reported exhibiting a combination of the regeneration types, with two observations (0.7%) having both normal regeneration from the original tail and multiple regenerations from a single point, four (1.3%) having regenerations on already regenerated tails or multiple regenerations from a single point, and five (1.6%) having normal regeneration from the original tail and regeneration on a regenerated tail.

For the abnormal caudal regeneration events where position was identified (*N* = 326), the majority occurred on the distal third of the tail (44.2%) followed by the middle third (32.2%) with the fewest occurring within the proximal third of the tail (22.4%). Three records (0.9%) had abnormal regenerations on both the distal and middle thirds of the tail, with one record (0.3%) occurring on both the proximal and middle sections.

2.4.3 Population frequency

From the 366 records of abnormal caudal regenerations, 47 data sets (34 natural populations, 11 museum specimen collections and two from a combination of both museum and natural observations) across 37 different species were available (Table 2.3). Both studies that combined museum and natural data observed a single furcation in the field and then used a museum collection to assess frequency of occurrence. Mean (± SD) incidence of abnormal regenerations from total population numbers was 2.75 ± 3.41% (range 0.1–16.7%), and 7.01 ± 6.42% (range 0.59–20.0%) for individuals in the population with regenerated tails.

Table 2.2: Relative furcation length (furcation length/tail length) percentages for both bifurcation and multifurcation events. *N* is number of observations where furcation length/s were able to be established, with total number of observations for the furcation categories in parentheses. Mean ± SD and range are reported, with cumulative percentages shown for multifurcation categories.

Furcation	***N***	**Mean ± SD (%)**	**Range (%)**	**Number of families**
Bifurcation	270 (366)	35.2 ± 21.6	4.49–100	20
Trifurcation	29 (49)	47.6 ± 42.8	11.8–160	11
Quadrifurcation	5 (8)	86.8 ± 47.9	30.0 –147	5
Pentafurcation	1 (1)	135 ± NA	–	1
Hexafurcation	1 (1)	322 ± NA	–	1

Table 2.3: Frequency of abnormal regeneration events for populations and museum collections

Source	**Species**	**No. with furcation**	**No. examined**	**No. with regeneration**	**% within population**	**% within population with regeneration**	**Type**
Abegg *et al.* (2014)	*Homonota uruguayensis*	1	1000	–	0.10	NA	Natural
Angeli (2013)	*Pholidoscelis (Ameiva) polops*	5	39	–	12.8	NA	Natural
Baeckens *et al*. (2018)	*Podarcis melisellensis*	1	74	–	1.35	NA	Natural
Barr & Bateman (in press)	*Egernia kingii*	2	254	129	0.79	1.55	Museum
Barr & Bateman (in press)	*Egernia kingii*	1	105	82	0.95	1.22	Natural
Bates (1989)	*Pachydactylus capensis*	1	247	170	0.40	0.59	Museum
Bressi (1999)	*Podarcis siculus*	1	100	–	1.00	NA	Museum

Broadley (1979)	*Ichnotropis capensis*	1	43	–	2.33	NA	Natural
Casas *et al.* (2016)	*Teius teyou*	1	640	108	0.16	0.93	Museum
Cordes & Walker (2013)	*Aspidoscelis velox*	2	>200	–	1.00	NA	Museum
De Andrade *et al.* (2015)	*Hemidactylus agrius*	2	62	–	3.23	NA	Natural
Dudek & Ekner-Grzyb (2014)	*Lacerta agilis*	1	>500	–	0.20	NA	Natural
Dudek & Ekner-Grzyb (2014)	*Zootoca vivipara*	1	>500	–	0.20	NA	Natural
Garcia-Vinalay (2017)	*Hemidactylus frenatus*	4	74	–	5.41	NA	Natural
Gordeev (2017)	*Eremias arguta*	1	12	–	8.33	NA	Natural
Gordeev (2017)	*Lacerta agilis*	1	43	–	2.33	NA	Natural
Hayes *et al.* (2012)	*Cyclura carinata*	4	390	119	1.03	3.36	Natural
Hayes *et al.* (2012)	*Cyclura cychlura*	1	59	6	1.69	16.7	Natural
Hayes *et al.* (2012)	*Cyclura cychlura*	2	310	57	0.65	3.51	Natural
Hayes *et al.* (2012)	*Cyclura cychlura*	1	99	27	1.01	3.70	Natural
Hayes *et al.* (2012)	*Cyclura rileyi*	8	328	198	2.44	4.04	Natural

Hayes *et al.* (2012)	*Cyclura rileyi*	1	57	14	1.75	7.14	Natural
Hayes *et al.* (2012)	*Cyclura rileyi*	1	31	20	3.23	5.00	Natural
Hayes *et al.* (2012)	*Cyclura rileyi*	4	75	32	5.33	12.5	Natural
Hickman (1960)	*Liopholis (Egernia) whitii*	1	350	–	0.29	NA	Natural
Homan (2015)	*Ctenotus robustus*	1	97	–	1.03	NA	Natural
Homan (2015)	*Carinascincus (Niveoscincus) coventryi*	1	124	–	0.81	NA	Natural
Jablonski (2016)	*Ablepharus deserti*	1	30	–	3.33	NA	Natural
Kerr *et al.* (2005)	*Pholidoscelis (Ameiva) erythrocephalus*	1	18	7	5.56	14.3	Natural
Khan (1991)	*Cyrtopodion (Tenuidactylus) kohsulaimanai*	1	23	–	4.35	NA	Museum
Kolenda *et al.* (2017)	*Lacerta agilis*	1	10	–	10.0	NA	Natural
Kolenda *et al.* (2017)	*Lacerta agilis*	1	208	–	0.48	NA	Natural
Kolenda *et al.* (2017)	*Zootoca vivipara*	1	18	–	5.56	NA	Natural

Koleska (2018)	*Teira dugesii*	1	>20	–	5.00	NA	Natural
Magalhães *et al.* (2015)	*Brasiliscincus heathi*	2	12	10	16.7	20.0	Natural
Montanucci (1969)	*Crotaphytus collaris*	1	59	–	1.69	NA	Museum
Najbar & Skawiński (2018)	*Anolis bimaculatus*	1	>100	–	1.00	NA	Natural
Renet (2013)	*Timon lepidus*	2	88	12	2.27	16.7	Natural
Seligmann *et al.* (2008)	*Sphenodon punctatus*	9	172	143	5.23	6.29	Museum
Sorlin *et al.* (2019)	*Podarcis muralis*	2	96	–	2.08	NA	Natural
Tamar *et al.* (2013*a*)	*Acanthodactylus boskianus*	1	391	–	0.26	NA	Natural/Museum
Tamar *et al.* (2013*b*)	*Ophisops elgans*	1	>360	–	0.28	NA	Natural/Museum
Trauth, Walker & Cordes (2014)	*Aspidoscelis sexlineata*	3	201	–	1.49	NA	Museum
Turner *et al.* (2017)	*Plestiodon longirostris*	7	506	–	1.38	NA	Natural

Vergilov & Natchev (2017)	*Ablepharus kitaibelii*	4	415	–	0.96	NA	Natural
Vrcibradic & Niemeyer (2013)	*Notomabuya (Mabuya) frenata*	3	216	178	1.39	1.70	Museum
Walley (1997)	*Plestiodon (Eumeces) anthracinus*	1	350	–	0.29	NA	Museum

2.5 Low occurrence, low reporting or low survivability?

A total of 425 observations of abnormal caudal regeneration across 175 species (Figure 2.3) is almost certainly a significant underestimation of abnormal caudal regeneration occurrences in lepidosaurs. Abnormal caudal regenerations have been observed in lizards for more than 400 years (Gessner, Cambier & Wechel, 1586), with detailed illustrations of lizards bearing bifurcated, trifurcated and even quadrifurcated tails from throughout the 17th and 18th centuries (Figure 2.5). They even receive a special mention in Salvador Dali's autobiography *The secret life of Salvador Dali* where he commented "*The monster of my zoological garden was a lizard with two tails, one very long and normal and the other shorter. This phenomenon was connected in my mind with the myth of bifurcation, which appeared to me even more enigmatic when it manifested itself in a soft and living being*" (Dali, 1942, p. 87).

In the past, abnormal caudal regenerations are likely to have been viewed only as interesting observations and therefore have not commonly appeared in peer-reviewed platforms. For those records that have been published, they are generally published as observational notes in journals such as *Herpetological Review* (32 records for 36 species), with more in-depth ecological studies being limited (Hayes *et al.*, 2012), and potential ecological effects of abnormal regenerations all but absent and only suggested (Wilson, 2012). However, a sharp increase in records of abnormal regeneration in peer-reviewed publications has occurred within the last 10 years (Figure 2.6). Additionally, the increasing use of social media platforms has allowed observations to be recorded and displayed to the online community, with almost half of abnormal caudal regeneration observations (49.4%) for our review coming from online social and popular media photographs.

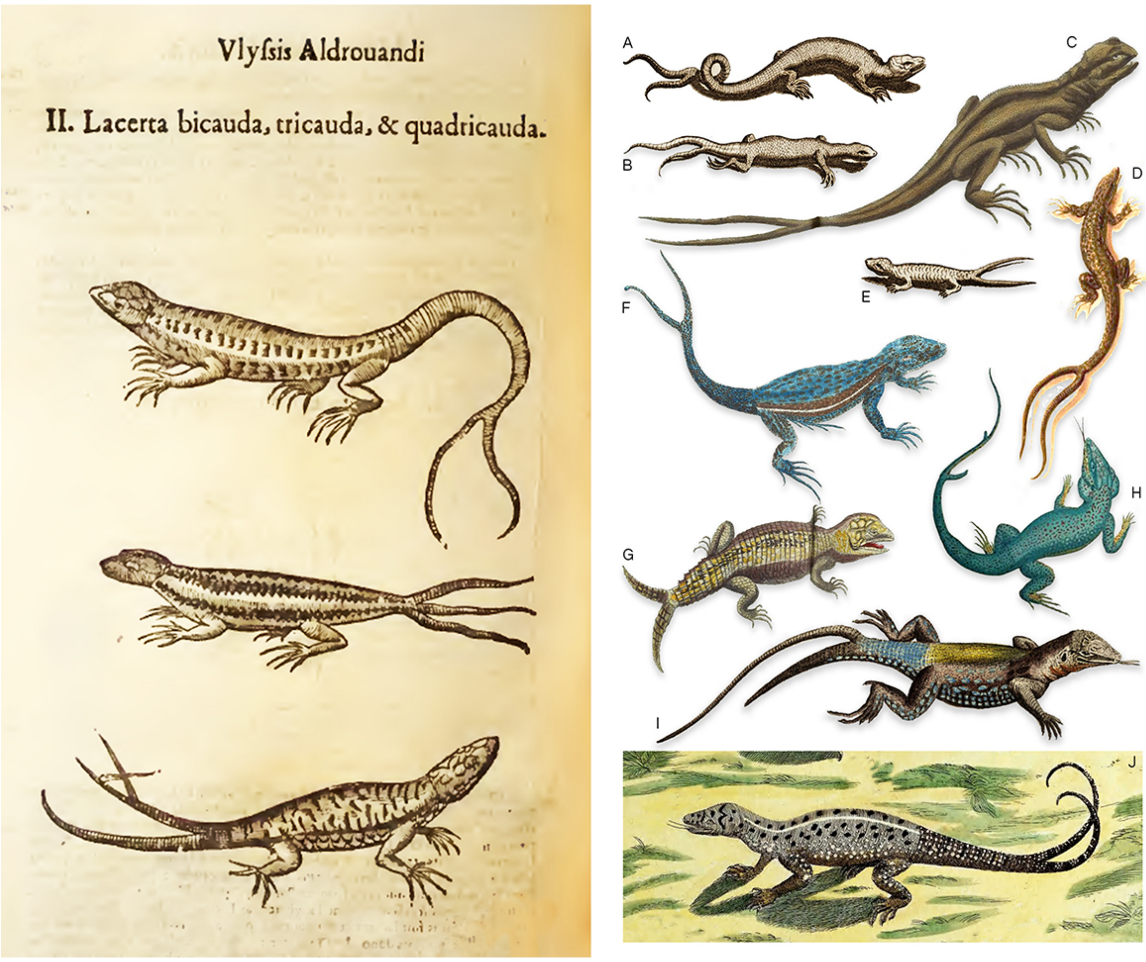

Figure 2.5: Historical illustrations of abnormal regenerations and their nomenclature in lizards. Left: Aldrovandi *et al.* (1642) *Monstrorum historia*. Right: A, B & E, Jonstonus (1678); D, unpublished watercolour by Vlyssis Aldrovandi (*Tavole vol. 004 Unico - Misc. Piante e Animali - Volume miscellaneo contenente 87 tavole di animali, plate 52; available online at* http://aldrovandi.dfc.unibo.it/pinakesweb/main.asp); C, F, G, H & J, Seba *et al.* (1735); I, Edwards (1743)

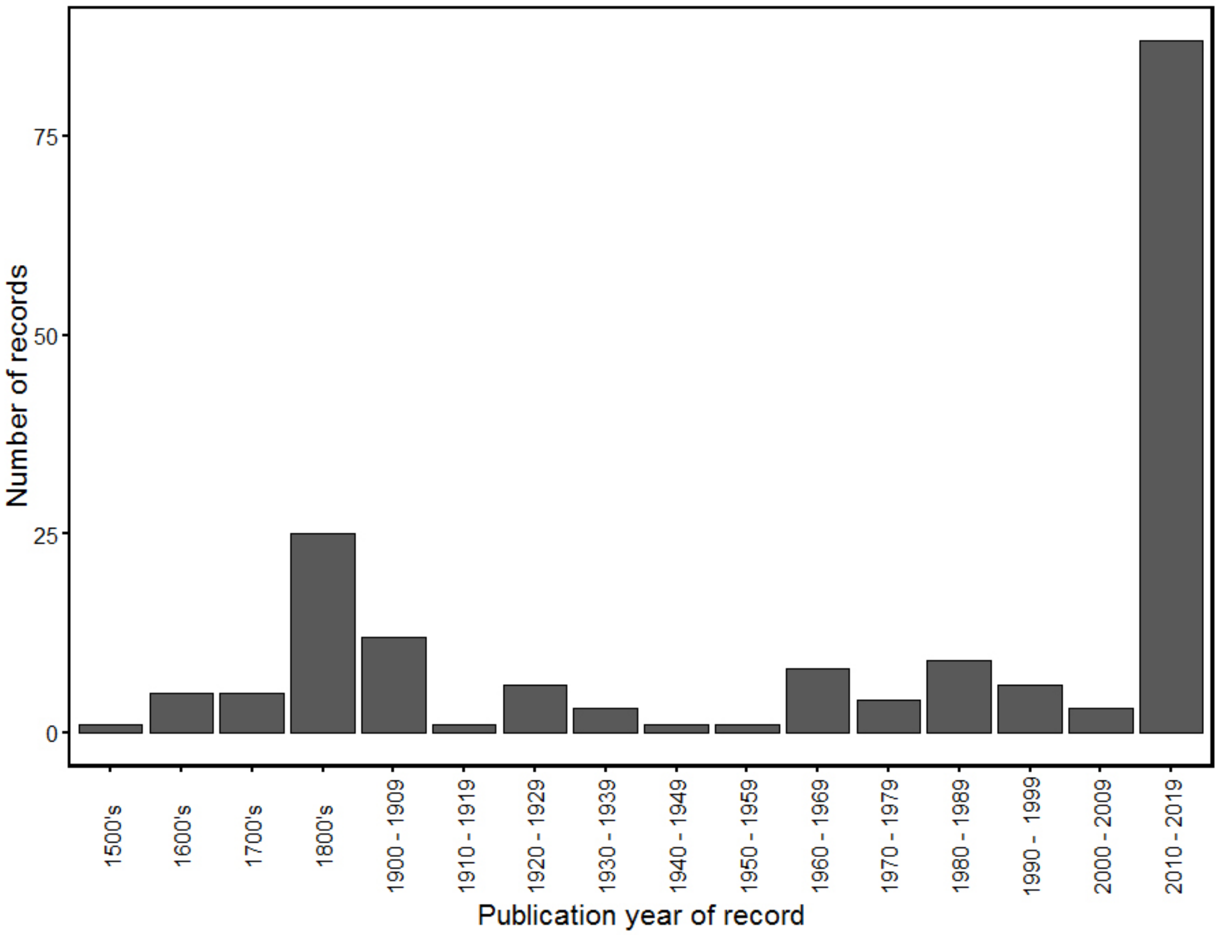

Figure 2.6: Peer-reviewed publication records of abnormal tail regeneration according to year of publication.

Records of abnormal regeneration events came from 63 different countries (Figure 2.4), with the two highest records of occurrence from English-speaking countries: 30.6% from the USA (90 records across 39 species) and 20.4% from Australia (60 records across 41 species), despite other regions such as South America and south-east Asia having higher diversity of lizards (Roll *et al.*, 2017). Language barriers as well as cultural differences in science practice, can hinder records from some parts of the world being published or being available *via* online-platform searches (Møller & Jennions, 2001; Amano, González-Varo & Sutherland, 2016). Our database included multiple records of abnormal regeneration observations from non-English languages including French, German, Italian, Latin and Spanish. However, we are aware, and would like to acknowledge, that some non-English publications may have been missed as a result, although we did make an effort to search for these.

One important aspect that remains uncertain in untangling the frequency of reported abnormal caudal regeneration within populations is whether it is attributed to a low likelihood of a furcation event occurring (see Section 2.6), or to low survivability of individuals with abnormally regenerated tails (see Section 2.7). Below we discuss factors that may contribute to the production of abnormal regeneration, as well as how abnormal regenerations may affect the ecology of the individual, drawing from literature around autotomy and regeneration, and highlight the lack of literature on abnormal regeneration.

2.6 Abnormal regeneration: Occurrences and limitations

2.6.1 Physical limitations

Abnormal regenerations evidently occur across lepidosaurs regardless of whether the species demonstrates intra-vertebral, inter-vertebral or no autotomy planes (Figure 2.3, Table 2.3). There are, however, factors which may influence the likelihood of abnormal regenerations occurring. For example, abnormal regenerations might be predicted to occur primarily from incomplete autotomy, or caudal wounds in species that have reduced or completely lost the ability to autotomise their tails, like that observed in the marine iguana, A*mblyrhynchus cristatus* (Barr *et al*., 2019*b*). Alternatively, species that readily utilise caudal autotomy may be predicted to be less likely to generate abnormal regenerations due to more effective and complete autotomy; however, this requires more data.

Ontogenetic loss of intra-vertebral autotomy planes occurs in numerous species. Planes may be lost entirely or restricted to a certain portion of the vertebrae (Etheridge, 1967; Arnold, 1984, 2009). Muscle attachment at the individual segments may also fortify during ontogeny, further increasing ontogenetic restrictions of autotomy (Arnold, 1984). This loss of autotomy planes, and muscle fortification, may either reduce the likelihood of abnormal regenerations occurring by limiting autotomy, or it may favour the production of abnormal regenerations through incomplete autotomy at a partially ossified or fortified plane, particularly as the ability to generate tail tissue growth is not linked to the ability to autotomise the tail, e.g. marine iguanas (Barr *et al.*, 2019*b*).

Once a lepidosaur autotomises its tail, a continuous cartilage rod is regenerated in place of the bony vertebrae and this lacks autotomy planes (Woodland, 1920; Arnold, 1984; Alibardi, 2010); however, the animal still retains the ability to regenerate from an already regenerated section of the tail following a shearing event, termed re-regeneration (Lozito & Tuan, 2017; Barr *et al.*, 2019*a*). From our review, 28.7% of records included a degree of abnormal re-regeneration. The amount of tail already regenerated, and when the regeneration occurred in the individual's life (juvenile or adult), would influence both where and when during the individual's life new or additional abnormal regenerations could occur. The number and diversity of predators (Barr *et al.*, 2019*a*) and the efficiency of predators (Bateman & Fleming, 2011) are also likely to influence rates of re-regeneration in populations.

The rate that lepidosaurs regenerate their tail, and potentially secondary tails, varies dramatically (Arnold, 1984; Maginnis, 2006). Taxa with intra-vertebral autotomy planes seem to regenerate their tail at a faster rate than those with inter-vertebral autotomy planes (Arnold, 1984) as do taxa that are short-lived and early maturing, and those in which the tail has a higher value (Congdon *et al.*, 1974; Vitt *et al.*, 1977; Alibardi & Meyer-Rochow, 1989, 2019). The degree and depth of the wound or break in the tail can also affect the formation and final size of the abnormal regeneration (Woodland, 1920; Simpson, 1964; Alibardi *et al.*, 1988). Additionally, a certain degree of control over energy prioritisation towards or away from caudal regeneration has been observed: species are able to prioritise energy into caudal regeneration at the expense of growth or reproduction, or *vice versa* forgoing caudal regeneration to prioritise reproduction and growth [see Maginnis (2006) for review]. However, as far as we are aware, neither the mechanism or degree of control over this energy redirection is known, nor if a degree of ‘control’ over regeneration rates of abnormal caudal regenerations is present.

2.6.2 Ease and use of autotomy

The ease of autotomy should be correlated with the costs and benefits to a lepidosaur of autotomising a tail (Arnold, 1988), which would take into account multiple factors contributing to this decision including the animal’s size and its ability to fend off predators. For instance, juveniles tend to rely more heavily on autotomy than do adults for predatory avoidance (Hawlena *et al.*, 2006; Barr *et al.*, 2019*c*), and therefore might be more likely to have higher incidence of complete successful autotomy, or succumb to predation. Adults however, may be more able to fight and defend themselves against certain predators, either due to their increased size, or to ontogenetically developed weaponry (Masters & Shine, 2003; Arbour & Zanno, 2018), and therefore may not be willing to shed their tail as easily (Arnold, 1984; Pafilis & Valakos, 2008). This may lead to an increase in abnormal caudal regeneration from incomplete autotomy in adults, or from acquisition of sufficient wounds from fighting (Vervust *et al.*, 2009). Similarly, more gracile individuals, either within or among species, may rely more heavily on autotomy to avoid predation. However, despite ease of autotomy receiving much attention (see Arnold, 1984, 1988; Bateman & Fleming, 2009), comparable data to assess this are lacking due to: (1) classification methods of robustness (see Bateman & Fleming, 2009); (2) inconsistent or unrealistic stimuli to induce autotomy (Arnold, 1988; Sanggaard *et al.*, 2012); or (3) lack of studies for intra-specific assessment (see Emberts *et al.*, 2019).

2.6.3 Inefficient predation and habitat

Occurrence of autotomy in populations is highly influenced by predator type, predator densities, predation efficiency and habitat (Schoener, 1979; Bateman & Fleming, 2009; Tyler, Winchell & Revell, 2016). Medel *et al.*

(1988) compared efficiency rates of three predators (falcon, snake and teiid lizard) in capturing lizards and found that the falcon was the most efficient, with no lizards escaping or autotomising, whereas the snake and teiid were less efficient, with 10–20% of lizards escaping *via* caudal autotomy. Bateman & Fleming (2011) investigating autotomy in brown anoles (*Anolis sagrei*) found that only 7% of the population exhibited evidence of autotomy in a population exposed to ‘efficient’ feral cat predators, compared to 25% in the population exposed to ‘inefficient’ domestic cats, that were well fed, and more likely to play with the lizards.

As autotomy is influenced by predator type, predation efficiency and habitat, we could expect that abnormal regeneration would also be influenced by these factors. Hayes *et al.* (2012) investigated the effect of invasive rodents (primarily rats) on iguanas *Cyclura* spp., which, as far as we are aware, is the only study providing an ecological context to abnormal regeneration (tail furcation) occurrences. Hayes *et al.* (2012) reported higher frequencies of both tail autotomy (normal autotomy) and tail furcation (abnormal caudal regeneration) in iguana populations exposed to invasive rodents. Predation type, efficiency and density not only influence the frequency of autotomy, but would also likely influence whether abnormal regenerations are likely to occur.

2.7 Abnormal regeneration: Potential behaviour and ecological effects

2.7.1 Locomotion and escape speed

Locomotion and escape speeds are significantly influenced by tail morphology. When lizards lose a portion of their tail to caudal autotomy, the effects may vary but the majority suffer a reduction of speed, particularly for those species with longer tails (McElroy & Bergmann, 2013). In addition, lizards’ locomotory kinematics can change, leading to changes in weight distribution and propulsive forces (Jagnandan, Russell & Higham, 2014), particularly when jumping or on vertical surfaces (Medger, Verburgt & Bateman, 2008; Fleming & Bateman, 2012). Species that inhabit dense vegetation, tight crevices or burrows are likely to suffer restricted freedom of movement, with abnormal regenerations decreasing streamlining and adding physical restriction. For example, the skink *Egernia kingii* inhabits burrows and crevices below roots or rocky substrata (Cogger, 2014), and as illustrated in figure 2.7, the large bifurcation exhibited by an adult *E. kingii* [snout-to-vent length (SVL) 240 mm] representing approximately 54% of the total tail length is a significant addition to the original tail, in both size, mass and width. Wilson (2012) recorded a caudal bifurcation for a *Lerista labialis*, a species known to ‘swim’ through sand. A caudal bifurcation would result in a reduction in streamlining, hindering normal movement through the substrate, preventing both escape from predators, and foraging efficiency.

Abnormal regenerations can also add considerable weight to the tail, and may change the kinematics of an individual’s locomotion, as well as reducing its sprint speed. Although no studies investigating the potential negative effects of abnormal caudal regenerations are known, additional weight is an ethical issue that needs to be addressed with attachment of devices, such as radio transmitters to animals. *A Manual for Wildlife Radio Tracking* by Kenward (2001) reports that adverse effects from tags can occur if they exceed 3–5% of the animal’s body weight, although this may vary for species depending on their locomotion type (arboreal *versus* terrestrial etc.) (Knapp & Abarca, 2009). Abnormal caudal regenerations, the number of furcations, and their position on the tail, are all likely to affect the amount of additional relative weight added to the individual depending on the diameter of the tail tissue, hence altering their locomotory kinetics. For example, an individual with a furcation representing 10% of the original tail length within the proximal third of the tail may have additional weight equivalent to a furcation representing 30% at the distal portion of the tail, depending on the size of the regeneration. Similarly, the same 10% furcation at the proximal third may be equivalent in weight, but not necessarily physical impairment, to several 10% furcation events at the distal end of the tail. Although our review found that majority of furcations did occur within the distal third of the tail (44.2%), high numbers were also found in both the middle (32.2%) and proximal (22.4%) third, with a specimen of the skink *Eutropis indeprensa* (Emerson & Dalabajan, 2018) reported as having an almost entire secondary tail located dorsally to the primary tail. However, size of abnormal regenerations showed considerable variability, presumably as regenerative capacities differ among species and type of autotomy plane and/or wound (Arnold, 1984; Bellairs & Bryant, 1985; Alibardi, 2019).

Figure 2.7: An *Egernia kingii* adult (snout-to-vent length 240 mm) from the Western Australia Museum collection (R36041) exhibiting a large regeneration bifurcation event, confirmed by micro-computerised tomography (insert).

2.7.2 Predation

In many species of lizards, caudal autotomy provides an effective yet costly anti-predation tactic (Arnold, 1988; Bateman & Fleming, 2009) with many species having adaptations to redirect attacks away from the body and towards the tail (Humphreys & Ruxton, 2018) such as brightly coloured tails that contrast with their bodies (Vitt & Cooper, 1986; Hawlena *et al.*, 2006), or ‘motion dazzling’ patterns on their body that affect the predators’ perception

(Murali & Kodandaramaiah, 2016). Other species, often in addition to morphological redirection adaptations, employ behaviour such as tail waving to redirect attacks away from the body (Cooper, 1998; Telemeco, Baird & Shine, 2011). Regardless of the strategy, a multifurcated tail may have benefits, or detriments, regarding predation. Having a multifurcated tail might increase the chances of a predatory attack being deflected towards the tail by providing a larger target (Barr *et al.*, 2019*c*), particularly if the regenerated region has a strong contrasting colour as seen in certain lizards like *Anolis carolinensis* (Ritzman *et al.*, 2012) or *Eutropis indeprensa* (Emerson & Dalabajan, 2018). On the other hand, having a multifurcated tail may also increase the chances of detection, reduce escape ability/speed, and/or increase the chances of the predator capturing/grasping the individual. Increased chances of predation arising from an abnormally regenerated tail may offer an explanation of the low occurrence rates reported in populations (Table 2.3); however, further studies would be required to assess this accurately.

2.7.3 Signalling and courtship behaviour

Tails can play an important role in acquiring potential mates through signalling or assertion of dominance by either visual displays or agonistic interactions. Badges – referring to behaviour or morphological structures that are an indication of size and/or dominance – are often correlated with mate acquisition, territory defence and survival (Qi *et al.*, 2011). In lizards, loss of these badges can negatively affect the individual’s territory acquisition, mating success and social dominance. For example, Fox & Rostker (1982) showed that juvenile *Uta stansburiana* lost social dominance following a loss of two thirds of their tail. A similar loss of social dominance was seen for subadult *U. stansburiana*, but social dominance was restored with artificial restoration of tails for females, but not males (Fox *et al.*, 1990). In addition to signalling badges, tails can be used as weapons in certain reptile species (Arbour & Zanno, 2018). Although best known in reptiles from extinct dinosaurian clades such as stegosaurs and ankylosaurs, in extant species, for example *Agama*, males are known to whip other individuals during agonistic contests (Schall *et al.*, 1989). Furthermore, *A. agama* have the ability to form a variety of clubbed tail structures following an inter-vertebral tail break, which Schall *et al.* (1989) proposed may give those individuals a competitive edge in agonistic interactions. Multifurcation events may affect an individual's ability to signal and/or acquire mates, either from disruption to the standard signalling behaviour or loss of competitive ability in agonistic interactions. However, the opposite may also apply, where a multifurcated tail is more obvious to potential mates, but may also inhibit caudal functionality as a potential weapon in agonistic encounters. Again, our understanding here is hampered by the lack of research into this phenomenon.

2.8 Future directions

Abnormal caudal regeneration has largely been overlooked as an area of scientific study. Generally, studies are limited to single observations (and possibly morphological measurements), the occasional report of occurrence frequency in populations, and almost no ecological studies for either their occurrence or ecological effects (Hayes *et al.*, 2012; Wilson, 2012). In this review, we compiled a large global database of recorded abnormal regeneration occurrences, drawing from the known ecological effects of caudal autotomy and regeneration (as data regarding this for abnormal regenerations is almost non-existent), to provide a discussion on the potential ecological implications of abnormal regenerations. Despite research showing how tail morphology and caudal autotomy affects species ecology (Arnold, 1984; Bateman & Fleming, 2009; Fleming *et al.*, 2013), there are still many significant gaps in our understanding of the ecological and behavioural effects of caudal autotomy and regeneration, let alone abnormal regeneration.

Going forward with research on abnormal regenerations in lepidosaurs there are several simple approaches that would greatly increase our understanding of the ecological effects on the animal: (1) publish records of abnormal regenerations, either in peer-reviewed or non-peer-reviewed platforms. Abnormal caudal regenerations are not just interesting passing observations, they can provide large amounts of scientific information regarding both patterns of occurrence, influence of habitat, and which species, sex or ontogenetic stages might have the higher occurrences of this phenomenon, and face higher consequences from it. (2) Providing morphological measurements and/or a photograph of individuals found during scientific studies/surveys. Inclusion of details such as SVL, tail length, position of furcation, length/s of furcation/s, sex, ontogenetic stage, and mass would be very beneficial. (3) Provide numbers of occurrences for populations or museum collections if appropriate. Both provide valuable information that will help identify how common abnormal regenerations are in populations, and what potential factors may affect the production of abnormal regenerations for species, type or presence of caudal autotomy planes, predation risk of populations, etc. (4) Behaviourally test hypothesised limitations resulting from abnormal caudal regeneration. Experimental manipulation of behaviour such as escape speeds, locomotion, foraging efficiency and habitat navigation to assess potential ecological impacts of abnormal regeneration can be quite easily carried out with addition of artificial silicon-cast or 3D-printed tails. This is paramount in order to assess the degree to which abnormal regeneration is detrimental to the individual, what role it plays in influencing their behavioural ecology, and how multiple tails influence the survival of lepidosaurs.

2.9 Conclusions

(1) This review provides the most comprehensive documentation and discussion of abnormal tail regeneration to date, i.e. regeneration producing a furcation event, in lepidosaurs. Abnormal regenerations have been documented for over 400 years in lepidosaurs, occurring in over 175 different species across 22 of the 45 recognised families, including species that have lost the ability to autotomise their tail. No previous review of autotomy, in any

taxa, has considered the frequency, distribution, or ecological impacts of abnormal regeneration.

(2) Known records of abnormal regenerations are still likely to under-represent its occurrence in lepidosaurs as a result of language barriers, science-practice culture differences, and undervaluing observations for publication, in either peer-reviewed or non-peer-reviewed sources. We strongly encourage the publication of abnormal regeneration observations on either peer-reviewed or non-peer reviewed platforms.

(3) In lepidosaurs, two- to six-tailed individuals have been recorded, with abnormal regenerations able to occur on all regions of the tail (proximal, middle and distal), in both original and previously regenerated regions, and can lead to the addition of 300% cumulative length to the primary tail.

(4) Abnormal regenerations are likely to affect the ecology of individuals severely; however, specific data are absent, and we can only extrapolate conclusions from known effects of autotomy and regeneration.

(5) Future studies regarding abnormal regenerations, and the degree to which they affect an animal's ecology, should focus on detailed reporting of abnormal regenerations, including morphometrics and occurrence in populations, as well as using experimental manipulations to quantify their effects on key fitness behaviours.

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2.11 References

“Every reasonable effort has been made to acknowledge the owners of copyright material. I would be pleased to hear from any copyright owner who has been omitted or incorrectly acknowledged.”

References used in the supporting information (Table A2.1) are identified with asterisks.

*ABEGG, A. D., MARIO DA ROSA, C., BORGES, L. M. & ORTIZ, F. R. (2014). Re-encounter of an *Homonata uruguayensis* specimen (Vaz-Ferreira & Sierra De Soriano, 1961) (Squamata: Phyllodactylidae) with tail bifurcation and first record in the state of Rio Grande do Sul, Brazil. *Boletín de la Sociedad zoológica del Uruguay* **23**(2), 74–78.

AKANI, G. C., LUISELLI, L., M. WARIBOKO, S., UDE, L. & ANGELICI, F. (2002). Frequency of tail autotomy in the African Olive Grass Snake, *Psammophis phillipsii* from three habitats in southern Nigeria. *African Journal of Herpetology* **51**, 143–146.

*ALDROVANDI, U., BERNIA, M. A., TEBALDINI, N., CORIOLANO, G. B. & AMBROSINI, B. (1642). *Vlyssis Aldrouandi patricii Bononiensis Monstrorum historia : cum Paralipomenis historiae omnium animalium*. Typis Nicolai Tebaldini, Bononiae.

*ALIBARDI, L. (2010). *Morphological and Cellular Aspects of Tail and Limb Regeneration in Lizards : a Model System with Implications for Tissue Regeneration in Mammals*. Springer-Verlag Berlin Heidelberg.

ALIBARDI, L. (2017). Review: Biological and Molecular Differences between Tail Regeneration and Limb Scarring in Lizard: An Inspiring Model Addressing Limb Regeneration in Amniotes. *Journal of experimental zoology. Part B, Molecular and developmental evolution* **328**(6), 493–514.

ALIBARDI, L. (2019). Tail regeneration in Lepidosauria as an exception to the generalized lack of organ regeneration in amniotes. *Journal of Experimental Zoology Part B: Molecular and Developmental Evolution*, 1–20.

ALIBARDI, L. & MEYER-ROCHOW, V. (1989). Comparative fine structure of the axial skeleton inside the regenerated tail of some lizard species and the tuatara (*Sphenodon punctatus*). *Gegenbaurs morphologisches Jahrbuch* **135**, 705–716.

ALIBARDI, L. & MEYER-ROCHOW, V. B. (2019). Microscopical observations on the regenerating tail in the tuatara Sphenodon punctatus indicate a tendency to scarring, but also influence from somatic growth. **280**(3), 411–422.

*ALIBARDI, L., SALA, M. & MIOLO, V. (1988). Morphology and electron microscopy of experimentally produced tail regenerates in lizard. *Acta embryologiae et morphologiae experimentalis* **9**(3), 181–194.

AMANO, T., GONZÁLEZ-VARO, J. P. & SUTHERLAND, W. J. (2016). Languages Are Still a Major Barrier to Global Science. *PLoS biology* **14**(12), e2000933–e2000933.

ANANJEVA, N. & ORLOV, N. L. (1994). Caudal autotomy in a Colubrid snake *Xenochrophis piscator* from Vietnam. *Russian Journal of Herpetology* **1**, 169–174.

*ANANJEVA, N. B. & DANOV, R. A. (1991). A rare case of bifurcated caudal regeneration in the Caucasian agama, *Stellio caucasius*. *Amphibia-Reptilia* **12**(3), 343–349.

*ANGELI, N. F. (2013). *Ameiva polops* (Saint Croix Ameiva). Conservation. *Caribbean Herpetology* **45**(1).

*ANGELI, N. F. (2018). On 'lost' indigenous etymological origins with the specific case of the name *Ameiva*. *ZooKeys* **748**, 151–159.

ARBOUR, V. M. & ZANNO, L. E. (2018). The evolution of tail weaponization in amniotes. *Proceedings of the Royal Society B: Biological Sciences* **285**(1871), 20172299.

*ARIANO-SÁNCHEZ, D. & GIL, J. (2016). *Ctenosaura palearis* (Guatemalan Spiny-tailed Iguana)-Tail trifurcation. *Herpetological Review* **47**(3), 463–464.

ARNOLD, E. (1984). Evolutionary aspects of tail shedding in lizards and their relatives. *Journal of Natural History* **18**(1), 127–169.

ARNOLD, E. (1988). Caudal autotomy as a defense. *Biology of the Reptilia* **16**, 235–273.

ARNOLD, E. (2009). Investigating the evolutionary effects of one feature on another: Does muscle spread suppress caudal autotomy in lizards? *Journal of Zoology* **232**, 505–523.

AULIYA, M., ALTHERR, S., ARIANO-SANCHEZ, D., BAARD, E. H., BROWN, C., BROWN, R. M., CANTU, J.-C., GENTILE, G., GILDENHUYS, P., HENNINGHEIM, E., HINTZMANN, J., KANARI, K., KRVAVAC, M., LETTINK, M., LIPPERT, J., *ET AL*. (2016). Trade in live reptiles, its impact on wild populations, and the role of the European market. *Biological Conservation* **204**, 103–119.

*BADIANE, A. (2017). Trifurcation caudale chez le lézard des murailles *Podarcis muralis* (Laurenti, 1768) (Squamata : Lacertidae). *Bulletin de la Société Herpétologique de France* **163**, 106–107.

*BAECKENS, S., LEIRS, W. & SCHOLLIERS, J. (2018). *Podarcis melisellensis* (Dalmatian Wall Lizard). Tail bifurcation. *Herpetological Review* **94**, 746.

*BANTA, B. H. (1963). An anomalous California Red-backed Alligator lizard. *Turtox News* **41**, 207.

*BARR, J. I. & BATEMAN, P. W. (in press). *Egernia kingii* (King's skink): caudal bifurcation. *Herpetological Review*.

BARR, J. I., BOISVERT, C. A., SOMAWEERA, R., TRINAJSTIC, K. & BATEMAN, P. W. (2019*a*). Re-regeneration to reduce negative effects associated with tail loss in lizards. *Scientific Reports* **9**(1), 18717.

*BARR, J., GILSON, L. N., SANCHEZ GARZON, D. F. & BATEMAN, P. W. (2019*b*). *Amblyrhynchus cristatus* (Marine Iguana). Tail regeneration and bifurcation. *Herpetological Review* **50**(3), 567.

BARR, J. I., SOMAWEERA, R., GODFREY, S. S. & BATEMAN, P. W. (2019*c*). Increased tail length in the King's skink, *Egernia kingii* (Reptilia: Scincidae): an anti-predation tactic for juveniles? *Biological Journal of the Linnean Society* **126**(2), 268–275.

*BATEMAN, H. & CHUNG-MACCOUBREY, A. (2013). *Aspidoscelis exsanguis* (Chihuahuan Spotted Whiptail), *Aspidoscelis neomexicana* (New Mexico Whiptail), *Aspidoscelis uniparens* (Desert Grassland Whiptail) Bifurcated tails. *Herpetological Review* **44**(4), 663.

BATEMAN, P. & FLEMING, P. (2009). To cut a long tail short: a review of lizard caudal autotomy studies carried out over the last 20 years. *Journal of Zoology (London)* **277**(1), 1–14.

BATEMAN, P. W. & FLEMING, P. A. (2011). Frequency of tail loss reflects variation in predation levels, predator efficiency, and the behaviour of three populations of brown anoles. *Biological Journal of the Linnean Society* **103**(3), 648–656.

BATEMAN, P., FLEMING, P. & ROLEK, B. (2014). Bite me: blue tails as a 'risky-decoy'defense tactic for lizards. *Current Zoology* **60**(3), 333–337.

*BATES, M. F. (1989). Tail-break frequency, tail size and the extent of caudal autotomy in the Cape thick-toed gecko, *Pachydactylus capensis capensis* (Sauria: Gekkonidae). *NAVORSINGE VAN DIE NASIONALE MUSEUM BLOEMFONTEIN* **6**(7), 225–239.

*BAXTER-GILBERT, J. & RILEY, J. (2015). *Intellagama lesuerii lesuerii* (Eastern Water Dragon). Trifid Tail. *Herpetological Review* **46**, 433–434.

*BELLAIRS, A. & BRYANT, S. (1985). Autotomy and regeneration in reptiles. *Biology of the Reptilia* **15**(5), 301–410.

BELLEFONTAINE, S. & LEE, C. (2013). Between Black and White: Examining Grey Literature in Meta-analyses of Psychological Research. *Journal of Child and Family Studies* **23**, 1378–1388.

*BRESSI, N. (1999). European Sauria in the Herpetological Collection of the Trieste Natural History Museum *Natura Croatica* **8**(3), 345–366.

*BRINDLEY, H. (1894). On a specimen of *Hemidacylus gledovii*, Murray, with bifid renewed tail. *Journal of the Bombay Natural History Society* **9**, 30–33.

*BRINDLEY, H. (1898). Some cases of caudal abnormality in *Mabuya carinata* and other lizards. *Journal of the Bombay Natural History Society* **11**, 680–689.

*BROADLEY, D. G. (1978). African *Trachylepis punctatissima (=Mabuya striata punctatissima). Occasional papers of the National Museums and Monuments of Rhodesia. Series B, Natural sciences* **6**, 45–79.

*BROADLEY, D. G. (1979). A field Study of Two Sympatric 'Annual' Lizards (*Genus Ichnotropis*) in Rhodesia. *South African Journal of Zoology* **14**(3), 133–138.

*BROCK, K. & BELASEN, A. (2014). *Podarcis erhardii* (Erhard's Wall Lizard). Bifurcated tail, post-autotomy. *Herpetological Review* **45**, 332.

*CABANAS, R. (1946). Nota sobre un caso de hiperregeneracion de la cola de (*Lacerta ocellata*). *Boletin de la Sociedad Espanola de Historia Natural* **489-10**, 525–526.

*CALORI, L. (1858). Sullo scheletro della Lacerta viridis, sulla riproduzione della coda nelle lucertole, ecc. *Memorie della Reale Accademia delle Scienze dell'Istituto di Bologna* **9**, 345–382.

*CAMPER, B. T. & CAMPER, J. D. (2017). *Anolis equestris* (Knight Anole). Tail trifurcation. *Herpetological Review* **48**(3), 634.

*CASAS, M. H., ARÁOZ, E. & MONTERO, R. (2016). Prevalence of autotomy and tail regeneration in *Teius teyou* (Reptilia, Teiidae). *Journal of Herpetology* **50**(3), 449–456.

*CHAN, J. G., YOUNG, L. L., CHANG, P., SHERO, C. M. & WATTS, C. (1984). Morphological anomalies of two geckos, *Hemidactylus frenatus* and *Lepidodactylus lugubri*s, and the toad, *Bufo marinus,* on the island of Hawaii. In *Proceedings of the Fifth Conference in Natural Sciences Hawaii Volcanoes National Park*, Honolulu. Honolulu (HI).

*CHANDRA, M. & MUKHERJEE, R. (1980). The occurrence of bifurcated tail in agama lizard *agama tuberculata* from simla hills himachal pradesh india. *The journal of the Bombay Natural History Society* **77**, 343.

*CHÁVEZ-VILLAVICENCIO, C. & TABILO-VALDIVIESO, E. (2017). Anomalía en el crecimiento de la cola de *Liolaemus tenuis* (Duméril & Bibron 1837) (Reptilia, Squamata, Liolaemidae). *Boletín Chileno de Herpetología* **4**, 10–11.

*CLARK, W. H. (1973). A San Joaquin fence lizard with a bifid tail. *HISS News-Journal* **1**(5), 158.

CLAUSE, A. R. & CAPALDI, E. A. (2006). Caudal autotomy and regeneration in lizards. *Journal of Experimental Zoology* **305**(12), 965–973.

COGGER, H. G. (2014). *Reptiles and Amphibians of Australia*, 7th edition. CSIRO Publishing, Victoria, Australia.

*COLWELL, G. J. (1992). *Microlophus bilineatus* (Galapagos lava lizard). Morphology. *Herpetological Review* **23**(4), 118.

CONGDON, J. D., VITT, L. J. & KING, W. W. (1974). Geckos: adaptive significance and energetics of tail autotomy. *Science* **184**(4144), 1379–1380.

CONN, V. S., VALENTINE, J. C., COOPER, H. M. & RANTZ, M. J. (2003). Grey literature in meta-analyses. *Nursing research* **52**(4), 256–261.

COOPER, W . E. (1998). Reactive and anticipatory display to deflect predatory attack to an autotomous lizard tail. *Canadian Journal of Zoology* **76**(8), 1507–1510.

COOPER, W. E. & ALFIERI, K. J. (1993). Caudal autotomy in the eastern garter snake, *Thamnophis s. sirtalis*. *Amphibia-Reptilia* **14**(1), 86–89.

*CORDES, J. E. & WALKER, J. M. (2013). *Aspidoscelis velox* (plateau striped whiptail). Bifurcation. *Herpetological Review* **44**(2), 319.

*COZENDEY, P., CAMPOS, M. P. S., LANNA, F. M., DE AMORIM, J. & DE SOUSA, B. (2013). *Ophiodes striatus* (Striped Worm Lizard). Bifurcated tail. *Herpetological Review* **44**(1), 145–146.

CROSS, S. L., TOMLINSON, S., CRAIG, M. D., DIXON, K. W. & BATEMAN, P. W. (2019). Overlooked and undervalued: the neglected role of fauna and a global bias in ecological restoration assessments *Pacific Conservation Biology* **25**(4), 331–341.

*CROUCH, C. R. (1969). Odd Fellow. *Wildlife in Australia* **6**, 101.

DALI, S. (1942). *The Secret Life of Salvador Dalí*. Dover Publications.

*DAS, G. M. (1932). Observations on the trifid tails in two specimens of *Hemidactylus flaviviridis* with a note on the artificial regeneration of double and triple tails of the Tokhak Lizard *Gecko verticillatus*. *Journal of the Bombay Natural History Society* **35**, 657–662.

*DAWBIN, W. H. (1962). The Tuatara in its natural habitat. *Endeavour* **11**(81), 16–24.

*DE ANDRADE, M. J. M., LOPES, J. R. I., DE SALES, R. F. D. & FREIRE, E. M. X. (2015). *Hemidactylus agrius* (Country leaf–toed gecko): Polydactyly and tail bifurcation. *The Herpetological Bulletin* **131**, 28–29.

*DE NOBLEVILLE, L. D. A. (1756). *Histoire naturelle des animaux: Suite de la matiere medicale*. Desaint & Saillant.

DIAL, B. E. & FITZPATRICK, L. C. (1981). The energetic costs of tail autotomy to reproduction in the lizard *Coleonyx brevis* (Sauria: Gekkonidae). *Oecologia* **51**(3), 310–317.

*DUDEK, K. & EKNER-GRZYB, A. (2014). Field observation of two-tailed sand lizard *Lacerta agilis* Linnaeus, 1758 and a common lizard *Zootoca vivipara* Jacquin, 1787 in Poland. *Slovenia Naturae* **16**(1), 65–66.

*DUGÈS, A. (1829). *Mémoire sur les espèces indigènes du genre Lacerta*. Annales Sciences Naturelles, Paris.

*EDWARDS, G. (1743). *A natural history of uncommon birds : and of some other rare and undescribed animals, quadrupedes, fishes, reptiles, insects, etc*. Printed for the author, at the College of Physicians in Warwick-Lane, London.

*ELLIS, R. J. (2015). *Ctenotus grandis grandis* (Grand Ctenotus). Bifurcation. *Herpetological Review* **46**(256).

EMBERTS, Z., ESCALANTE, I. & BATEMAN, P. W. (2019). The ecology and evolution of autotomy. *Biological Reviews* **94**, 1881–1896.

*EMERSON, Y. & DALABAJAN, A. C. (2018). Tail bifurcation in *Eutropis indeprensa* on Palawan Island, Philippines. *South East Asia Vertebrae Records* **1**, 6–7.

*ESPASANDÍN, I. (2017). Cola bífida en *Anguis fragilis* (Linnaeus, 1758). *Boletín de la Asociación Herpetológica Española* **28**(2), 11–12.

ETHERIDGE, R. (1967). Lizard caudal vertebrae. *Copeia*, 699–721.

*EVANS, S. & BELLAIRS, A. (1983). Histology of a triple tail regenerate in a gecko, *Hemidactylus persicus*. *British journal of herpetology* **6**(9), 319–322.

*EVERSMANN, E. (1858). Erinnerungen aus einer Reise in's Ausland 1857 – 1858. *Bulletin de la Société impériale des naturalistes de Moscou* **3**, 265–304.

*FILADELFO, T., SOEIRO, M., COELHO, D. & HAMDAN, B. (2017). *Phyllopezus pollicaris* (Brazilian Gecko): Tail bifurcation. *Herpetological Review* **48**, 656.

FITCH, H. S. (2003). A comparative study of loss and regeneration of lizard tails. *Journal of Herpetology* **37**(2), 395–399.

FLEMING, P. A. & BATEMAN, P. W. (2012). Autotomy, tail regeneration and jumping ability in Cape dwarf geckos (*Lygodactylus capensis*) (Gekkonidae). *African Zoology* **47**(1), 55–59.

FLEMING, P. A., MULLER, D. & BATEMAN, P. W. (2007). Leave it all behind: a taxonomic perspective of autotomy in invertebrates. *Biological Reviews* **82**(3), 481–510.

FLEMING, P. A., VALENTINE, L. E. & BATEMAN, P. W. (2013). Telling tails: selective pressures acting on investment in lizard tails. *Physiological and Biochemical Zoology* **86**(6), 645–658.

*FORBES, R. B. (1961). An unusual specimen of the lizard *Cnemidophorus perplexus*. *Turtox News* **39**(8), 221.

FOX, S. & ROSTKER, M. A. (1982). Social Cost of Tail Loss in *Uta stansburiana*. *Science* **218**(4573), 692–693.

FOX, S. F., HEGER, N. A. & DELAY, L. S. (1990). Social cost of tail loss in *Uta stansburiana*: lizard tails as status-signalling badges. *Animal Behaviour* **39**(3), 549–554.

*FRAISSE, P. (1885). *Die Regeneration von Geweben und Organen bei den Wirbelthieren, besonders Amphibien und Reptilien*. T. Fischer, Cassel.

FUNK, R. S. (2019). 112 - Tail Amputation. In *Mader's Reptile and Amphibian Medicine and Surgery (Third Edition)*. (eds S. J. Divers and S. J. Stahl), pp. 1113–1115.e1. W.B. Saunders, St. Louis (MO).

*GACHET, H. (1834). MÉMOIRE sur la reproduction de la queue des reptiles *sauriens*. *Actes de la Société Linnéenne de Bordeaux Zoologie* **6**(36), 213–259.

*GANDLA, C. & SRINIVASULU, C. (2015). A two-tailed Indian gaint leaf-toed gecko (*Hemidactylus giganteus*). *Taprobanica* **7**, 263–265.

*GARCIA-VINALAY, A. (2017). *Hemidactylus frenatus* tail bifurcation. *Mesoamerican Herpetology* **4**, 635.

*GESSNER, C., CAMBIER, R. & WECHEL, J. (1586). *Conr. Gesneri Tigurini medicinæ et philosophiae professoris in Schola Tigurina, Historiæ animalium liber II : qui est de quadrupedibus ouiparis*. Ex officina typographica Ioannis Wecheli. Impensis Roberti Cambieri, Francofurdi.

*GHOSH, A. & BANERJEE, K. (2019). *Eutropis bibronii* (Seashore skink). Tail bifurcation. *Herpetological Review* **50**(3), 573–574.

*GIEBEL, C. G. (1864). Eine doppelschwänzige Eidehse. *Zeitschrift für die gesammten Naturwissenschaft* **24**, 48–49.

GILBERT, E. A., PAYNE, S. L. & VICKARYOUS, M. K. (2013). The anatomy and histology of caudal autotomy and regeneration in lizards. *Physiological and Biochemical Zoology* **86**(6), 631–644.

*GOGLIATH, M., PEREIRA, L., NICOLA, P. & RIBEIRO, L. (2012). *Ameiva ameiva* (Giant Ameiva). Bifurcation. *Herpetological Review* **43**(1), 129.

*GOGOI, M., KUNDU, S., GOSWAMI, J., SAIKIA, D. & PANDEY, N. (2018). First record of tail bifurcation in Tokey Gecko (*Gekko gecko*) from the Kaziranga, Assam, India: a field observation. *International Journal of Experimental Research and Review* **15**, 5–8.

*GOIN, C. J. & GOIN, O. B. (1971). *Introduction to Herpetology*, Second edition. W. H. Freeman and Company, San Francisco, USA.

*GORDEEV, D. (2017). Cases of incomplete autotomy and tail regeneration abnormality of the steppe-runner (*Eremias arguta* (PALLAS, 1773) and sand lizard (*Lacerta agilis* (LINNEAEUS, 1758) in the Volgograd region. *Current Studies in Herpetology* **17**(1), 3–9.

*GRÄPER, L. (1909). Über eine dreischwänzige Eidechse mit sieben Schwanzskeleten. *Archiv für Entwicklungsmechanik der Organismen* **27**(4), 640–652.

HAMILTON, P. S. & SULLIVAN, B. K. (2005). Female mate attraction in ornate tree lizards, *Urosaurus ornatus*: a multivariate analysis. *Animal Behaviour* **69**(1), 219–224.

HAWLENA, D., BOOCHNIK, R., ABRAMSKY, Z. & BOUSKILA, A. (2006). Blue tail and striped body: why do lizards change their infant costume when growing up? *Behavioral Ecology* **17**(6), 889–896.

*HAYES, W. K., IVERSON, J. B., KNAPP, C. R. & CARTER, R. L. (2012). Do invasive rodents impact endangered insular iguana populations? *Biodiversity and conservation* **21**(7), 1893–1899.

HECHT, M. K. & COSTELLI JR, J. (1971). The postcranial osteology of the lizard Shinisaurus: the appendicular skeleton. *Herpetologica* **27**(1), 87–98.

HERREL, A., MEASEY, G., VANHOOYDONCK, B. & TOLLEY, K. (2012). Got it clipped? The effect of tail clipping on tail gripping performance in Chameleons. *Journal of Herpetology* **46**, 91–93.

*HEYBORNE, W. & MAHAN, A. (2017). *Hemidactylus frenatus* (Common House Gecko). Tail bifurcation. *Herpetological Review* **48**, 437–438.

*HEYBORNE, W. & MCMULLIN, K. (2016). *Sceloporus uniformis* (Yellow-backed Spiny Lizard). Bifurcated tail. *Herpetological Review* **47**, 674.

*HICKMAN, J. L. (1960). Observations on the skink lizard *Egernia whitii* (Lacepede). *Papers and Proceedings of the Royal Society of Tasmania* **94**, 111.

HIGHAM, T. E., RUSSELL, A. P. & ZANI, P. A. (2013). Integrative biology of tail autotomy in lizards. *Physiological and Biochemical Zoology* **86**(6), 603–610.

*HIROTA, S. (1895). Notes on the scink with an accessory tail. *Dobutsugaku zasshi* **7**, 107–115.

*HOMAN, P. (2015). A record of four regrowing tails in a large striped skink C*tenotus robustus* from yan yean, Victoria. *Victorian Naturalist* **132**(1), 12–15.

*HORA, S. L. (1926). Notes on lizards in the Indian Museum. I. On the unnamed collection of lizards of the family Geckonidae. *Rocords of the Indian Museum* **28**, 128–193.

HUMPHREYS, R. K. & RUXTON, G. D. (2018). What is known and what is not yet known about deflection of the point of a predator's attack. *Biological Journal of the Linnean Society* **123**(3), 483–495.

*JABLONSKI, D. (2016). Tail bifurcation in a Desert Lidless Skink (*Ablepharus deserti*) from Kyrgyzstan. *IRCF Reptiles & Amphibians* **23**(3), 171–172.

*JABLONSKI, D. & REICHSTEIN, L. (2019). *Cryptoblepharus pannosus* (Ragged Snake-eyed Skink). Tail trifurcation. *Herpetological Review* **50**, 783–784.

JACYNIAK, K., MCDONALD, R. P. & VICKARYOUS, M. K. (2017). Tail regeneration and other phenomena of wound healing and tissue restoration in lizards. *The Journal of Experimental Biology* **220**(16), 2858–2869.

JAGNANDAN, K., RUSSELL, A. P. & HIGHAM, T. E. (2014). Tail autotomy and subsequent regeneration alter the mechanics of locomotion in lizards. *The Journal of Experimental Biology* **217**(21), 3891–3897.

JAMISON, J. P. (1964). Regeneration subsequent to intervertebral amputation in lizards. *Herpetologica* **20**(3), 145–149.

*JONSTONUS, J. (1678). *A description of the nature of four-footed beasts : with their figures engraven in brass*. Printed for the widow of John Jacobsen Schipper, and Stephen Swart, Amsterdam.

KENWARD, R. (2001). *A Manual for Wildlife Radio Tagging*. Academic Press.

*KERR, A. M., POWELL, R. & PARMERLEE, J. S. (2005). *Ameiva erythrocephala* (Teiidae) on Sint Eustatius, Netherlands Antilles: Baseline data on a small population in a severely altered habitat. *Caribbean Journal of Science* **41**(1), 162–169.

*KHAN, M. (1991). A new *Tenuidactylus* gecko from the Sulaiman Range, Punjab, Pakistan. *Journal of Herpetology* **25**(2), 199.

KNAPP, C. & ABARCA, J. (2009). Effects of radio transmitter burdening on locomotor ability and survival of Iguana hatchlings. *Herpetologica* **65**, 363–372.

*KNIGHT, M. (1965). *Reptiles in Britain*. Brockhampton Press Ltd, Leicester.

*KOLENDA, K., WIECZOREK, M., NAJBAR, A., NAJBAR, B. & SKAWIŃSKI, T. (2017). Limb malformation and tail bifurcation in sand lizards (*Lacerta agilis*) and common lizards (*Zootoca vivipara*) from Poland. *Herpetology Notes* **10**, 713–716.

*KOLESKA, D. (2018). First record of tail bifurcation in *Asaccus gallagheri* from the United Arabian Emirates. *Herpetology Notes* **11**, 115–116.

*KOLESKA, D. & JABLONSKI, D. (2015). Tail trifurcation recorded in *Algyroides nigropunctatus* (Duméril & Bibron, 1839). *Ecologica Montenegrina* **3**, 26–28.

*KOLESKA, D. & JABLONSKI, D. (2018). A four-tailed *Iguana delicatissima* (Squamata: Iguanidae) on Petite Terre, Guadeloupe (Lesser Antilles, Caribbean Region). *Phyllomedusa* **17**, 157–159.

*KOLESKA, D., KULMA, M. & VRABEC, V. (2017*a*). *Teira dugesii* (Madeiran Wall Lizard). Tail bifurcation. *Herpetological Review* **48**(2), 440–441.

*KOLESKA, D., SVOBODOVÁ, V., HUSÁK, T., KULMA, M. & JABLONSKI, D. (2017*b*). Tail bifurcation recorded in *Sauromalus ater*. *Herpetology Notes* **10**, 363–364.

KUCHLING, G. (2005). Bifid tail regeneration in a turtle, *Emydura sp* (Testudines : Chelidae). *Chelonian Conservation and Biology* **4**, 935–937.

* KUKUSHKIN, O. (2018). On anomalies of the caudal regeneration and autotomy in *Mediodactylus danilewskii* (Reptilia: Sauria: Gekkonidae). *Current Studies in Herpetology* **18**(3), 180-187.

*KUMBAR, S. (2011). *Hemidactylus Flaviviridis* (House Gecko): Bifurcation. *Herpetological Review* **42**(1), 94.

*LA CÉPÈDE, C. (1799). *Histoire naturelle des quadrupèdes ovipares et des serpens. Tome II.* Saugrain, Paris.

*LAVER, H. (1879). A two tailed lizard. *Science gossip* **15**, 232.

*LOVERIDGE, A. (1923). Notes on East African Lizards collected 1920–1923, with the description of two new species of *Agama lionotus*. *Proceedings of the Zoological Society of London* **93**(4), 935–969.

LOZITO, T. P. & TUAN, R. S. (2017). Lizard tail regeneration as an instructive model of enhanced healing capabilities in an adult amniote. *Connective Tissue Research* **58**(2), 145–154.

*MAGALHÃES, F., CAMURUGI, F., SILVEIRA-FILHO, R., MÂNGIA, S. & MOURA DA CONCEIÇÃO, B. (2015). *Brasiliscincus heathi* (Brazilian Mabuya). Tail bifurcation. *Herpetological Review* **46**(4), 624–625.

MAGINNIS, T. L. (2006). The costs of autotomy and regeneration in animals: a review and framework for future research. *Behavioral Ecology* **17**(5), 857–872.

* MARCGRAVE, G. (1648). *Historiae Naturalis Brasiliae Lugdun*. Batavorum, apud Franciscum Hackium et Amstelodami apud Lud. Johannes de Laet, Elzevierium, Lugdun. Batavorum.

*MARCHANT, M. (1718). *Histoire de l'Académie Royale des Sciences*. Imprimerie Royale, Paris.

*MARIA, M. & AL-RAZI, H. (2018). Observation of tail bifurcation in *Hemidactylus frenatus* (Schlegel, 1836). *Herpetology Notes* **11**, 953–954.

*MARTINS, R. L., PEIXOTO, P., FONSECA, P. H., MARTINELLI, A., SILVA, W. & PELLI, A. (2013). Abnormality in the tail of the collated lizard *Tropidurus* gr. *torquatus* (Iguania, Tropiduridae) from Uberaba city, Minas Gerais State, Brazil. *Herpetology Notes* **6**, 369–371.

MASTERS, C. & SHINE, R. (2003). Sociality in lizards: family structure in free-living King's Skinks *Egernia kingii* from southwestern Australia. *Australian Zoologist* **32**(3), 377–380.

*MATA-SILVA, V., ROCHA, A., GANDARA, A. & JOHNSON, J. (2010). *Cophosaurus texanus* (Greater Earless Lizard). Multiple tails. *Herpetological Review* **41**(3), 352–353.

*MATA-SILVA, V., ROCHA, A., JOHNSON, J. & WILSON, L. (2013). *Urosaurus bicarinatus* (Tropical Tree Lizard). Bifurcation. *Herpetological Review* **44**(4), 686–687.

MCELROY, E. J. & BERGMANN, P. J. (2013). Tail autotomy, tail size, and locomotor performance in lizards. *Physiological and Biochemical Zoology* **86**(6), 669–679.

*MCKELVY, A. & OZELSKI-MCKELVY, A. (2012). *Gekko monarchus* (Warty House Gecko) Bifurcation. *Herpetological Review* **43**(1), 132.

*MCKELVY, A. & STARK, C. (2012). *Plestiodon fasciatus* (Common Five Lined Skink) Bifurcation. *Herpetological Review* **43**(1), 138.

MEDEL, R., JIMÉNEZ, J. E., FOX, S. & JAKSIC, F. (1988). Experimental evidence that high population frequencies of lizard tail autotomy indicate inefficient predation. *Oikos* **53**(3), 321–324.

MEDGER, K., VERBURGT, L. & BATEMAN, P. W. (2008). The influence of tail autotomy on the escape response of the Cape dwarf gecko, *Lygodactylus capensis*. *Ethology* **114**(1), 42–52.

*MENDES, D., SILVA-NETO, A., SOBRAL, R. & RAFAEL, J. A. (2020). *Trachylepis atlantica* (Noronha Skink). Tail bifurcation. *Herpetological Review* **50**(4), 793.

*MITCHELL, J., MCDANIEL, W. & MCDANIEL, J. (2012). *Plestiodon inexpectatus* (Southeastern Five-lined Skink). Bifurcation. *Herpetological Review* **43**(4), 650.

*MODRÝ, D., NECAS, P., RIFAI, L., BISCHOFF, W., HAMIDAN, N. & AMR, Z. (2013). Revision of the Levantine "*Lacerta*" laevis/kulzeri-Complex: 3. The

Rock Lizard of Wadi Ramm, *Phoenicolacerta kulzeri khazaliensis* ssp. n. *Vertebrate Zoology* **63**, 307–312.

MØLLER, A. P. & JENNIONS, M. D. (2001). Testing and adjusting for publication bias. *Trends in Ecology & Evolution* **16**(10), 580–586.

*MONSISBAY, M. I. & OLCHA, M. A. (2016). Reporte de bifurcación de la cola en *Anolis porcatus* (Squamata: Dactyloide): Report of tail bifurcation in *Anolis porcatus* (Squamata: Dactyloidae). *Revista Cubana de Ciencias Biológicas* **5**(2), 1–4.

*MONTANUCCI, R. (1969). Remarks upon the Crotaphytus-Gambelia controversy (Sauria: Iguanidae). *Herpetologica* **25**(4), 308–314.

*MONTEIL, M. (1880). *Présentation d'un lézard bifid*. Association française pour l'avancement des sciences, Paris.

*MÜLLER, H. (1852). Eine Eldechse, Lacerta viridis, mit zwi über einander gelagerten Shwänzen, wlche belde als das Prodict einer überrelchen und durch den felnern Bau des Wiedererzeugten bemerkenswerthen Reproductionskraft erchelnen. *Verhandlungen Physikal Medicin Gesellschaft in Würzburg* **2**, 66–70.

MURALI, G. & KODANDARAMAIAH, U. (2016). Deceived by stripes: Conspicuous patterning on vital anterior body parts can redirect predatory strikes to expendable posterior organs. *Royal Society Open Science* **3**(6), 160057.

*NAJBAR, A. & SKAWIŃSKI, T. (2018). *Anolis bimaculatus* (panther anole). Tail bifurcation. *Herpetological Review* **49**(2), 325.

*NEEDHAM, J. T. & LAVIROTTE, L. A. (1750). *Nouvelles observations microscopiques: aves des découvertes intéressantes sur la composition et la décomposition des corps organisés*. Chez L.-É. Ganeau, Paris.

*NEWMAN, D. (1987). *Tuatara*. John McIndoe & Department of Conservation, New Zealand.

*OFORI, B. Y., MARTEY, P., MUSAH, Y. & ATTUQUAYEFIO, D. (2018). Tail bifurcation in the African rainbow lizard (*Agama agama* Linnaeus, 1758) from Ghana, West Africa. *Herpetology Notes* **11**, 843–845.

PAFILIS, P. & VALAKOS, E. D. (2008). Loss of caudal autotomy during ontogeny of Balkan Green Lizard, *Lacerta trilineata*. *Journal of Natural History* **42**(5–8), 409–419.

*PASSOS, D., PINHEIRO, L., GALDINO, C. & ROCHA, C. (2014). *Tropidurus semitaeniatus* (Calango de Lagedo). Tail bifurcation. *Herpetological Review* **45**(1), 138.

*PASSOS, D. C., FONSECA, P. H. M., DE VIVAR, P. R. R., KANAYAMA, C. Y., TEIXEIRA, V. P. & MARTINELLI, A. G. (2016). Tail trifurcation in the lizard *Salvator merianae* (Squamata: Teiidae) investigated by computer tomography. *Phyllomedusa: Journal of Herpetology* **15**(1), 79–83.

*PELEGRIN, N. & LEÃO, S. (2016). Injured *Salvator merianae* (Teiidae) regenerates six tails in central Argentina. *Cuadernos de Herpetología* **30**(1), 21–23.

*PERRAULT, M. (1688). *Essais de physique, ou Recueil de plusieurs traitez touchant les choses naturelles. Tome IV.* De l'Academie Royale des Sciences, docteur en medecine de la faculté de Paris, Paris.

*PHEASEY, H., SMITH, P., BROUARD, J. & ATKINSON, K. (2014). *Vanzosaura rubricauda* (Red-tailed Vanzosaur). Bifurcation and trifurcation. *Herpetological Review* **45**(1), 138–139.

*PIANA, G. D. (1894). Ricerche sulla poidattilia aquisita determinata sperimentalmente neir Tritoni e sulle code soprannumerarie nella lucertola. *Ricerche fatte nel Laboratorio di Anatomia Normale Della R. Universita di Roma* **4**, 65–71.

*POLA, L. & KOLESKA, D. (2017). Tail bifurcation in Common wall lizard (Podarcis muralis Laurenti, 1768) from Liguria, Italy. In *9th Workshop on Biodiversity (Jevany, Czech Republic 8–9 July 2017). Proceedings of the 9th Workshop on biodiversity*, pp. 93–97.

*PORTA, G. D. (1619). *Jo Baptistae Portae Neapolitani, Magiae Naturalis Libri Viginti: In Quibus Scientiarum Naturalium divitiae, & deliciae demonstrantur*. Aubrii & Schleichius.

*PRZIBRAM, H. (1909). *Experimental-Zoologie: eine Zusammenfassung der durch Versuche ermittelten Gesetzmässigkeiten tierischer Wieder-Erzeugung (Nachwachsen, Umformung, Missbildung). Regeneration*. F. Deuticke, Leipzig, Wien.

PYRON, R. A., BURBRINK, F. T. & WIENS, J. J. (2013). A phylogeny and revised classification of Squamata, including 4161 species of lizards and snakes. *BMC Evolutionary Biology* **13**(1), 93.

QI, Y., WAN, H., GU, H. & WANG, Y. (2011). Do displays and badges function in establishing the social structure of male toad-headed lizards, *Phrynocephalus vlangalii*? *Journal of Ethology* **29**(2), 381–387.

*QUATTRINI, D. (1953). Superrigenerazione della coda nei rettili. *Monitore zoologico italiano* **62**, 466–470.

*QUELCH, J. J. (1890). Four tailed lizard. *The Journal of the Royal Agricultural & Commercial Society of British Guiana* **4**, 148–149.

RAHMAN, S. C. (2011). *Chelydra serpentina* (Common Snapping Turtle). Bifid tail. *Herpetological Review* **42**(2), 265.

RAMÍREZ-BRAVO, O. E., SOLIS, J. M. & STEPHANIE, T. (2020). *Caiman crocodylus* (Spectacled caiman). A rare case of caudal secondary regeneration. *Herpetological Review* **51**(1), 117–118.

*REDI, F. (1684). *Osservazioni di Francesco Redi accademico della Crusca intorno agli animali viventi che si trovano negli animali viventi*. per Piero Matini, all'insegna del Lion d'oro.

*RENET, J. (2013). Two cases of tail abnormality in the Ocellated Lizard *Timon lepidus lepidus* (Daudin, 1802) in south-eastern France (Bouches-du-Rhône). *Nature de Provence* **2**, 99–101.

RITZMAN, T. B., STROIK, L. K., JULIK, E., HUTCHINS, E. D., LASKU, E., DENARDO, D. F., WILSON-RAWLS, J., RAWLS, J. A., KUSUMI, K. & FISHER, R. E. (2012). The gross anatomy of the original and regenerated tail in the Green Anole (*Anolis carolinensis*). **295**(10), 1596–1608.

ROLL, U., FELDMAN, A., NOVOSOLOV, M., ALLISON, A., BAUER, A. M., BERNARD, R., BÖHM, M., CASTRO-HERRERA, F., CHIRIO, L., COLLEN, B., COLLI, G. R., DABOOL, L., DAS, I., DOAN, T. M., GRISMER, L. L., *ET AL*. (2017). The global distribution of tetrapods reveals a need for targeted reptile conservation. *Nature Ecology & Evolution* **1**(11), 1677–1682.

*RYDER, J. A. (1893). The inheritance of modifications due to disturbances of the early stages of development, epecially in the Japanese dmesticated rces of Gold-Carp. *Proceedings of the Academy of Natural Sciences of Philadelphia* **45**(1), 75–94.

SALVADOR, A., MARTIN, J. & LÓPEZ, P. (1995). Tail loss reduces home range size and access to females in male lizards, *Psammodromus algirus*. *Behavioral Ecology* **6**(4), 382–387.

SANGGAARD, K. W., DANIELSEN, C. C., WOGENSEN, L., VINDING, M. S., RYDTOFT, L. M., MORTENSEN, M. B., KARRING, H., NIELSEN, N. C., WANG, T., THØGERSEN, I. B. & ENGHILD, J. J. (2012). Unique structural features facilitate lizard tail autotomy. *PloS one* **7**(12), e51803–e51803.

SCHALL, J. J., BROMWICH, C. R., WERNER, Y. L. & MIDLEGE, J. (1989). Clubbed regenerated tails in *Agama agama* and their possible use in social interactions. *Journal of Herpetology* **23**(3), 303–305.

SCHOENER, T. W. (1979). Inferring the properties of predation and other injury-producing agents from injury frequencies. *Ecology* **60**(6), 1110–1115.

*SCOTT, A. F. (1982). *Eumeces fasciatus* (Five-lined Skink). Morphology. *Herpetological Review* **13**(2), 46.

*SEBA, A., BOERHAAVE, H., GAUBIUS, H. D., MASSUET, P., JANCOURT, L., MUSSCHENBROEK, P. V., ARTEDI, P. & TANJÉ, P. (1735). *Locupletissimi rerum naturalium thesauri accurata descriptio, et iconibus artificiosissimis expressio, per universam physices historiam : opus, cui, in hoc rerum genere, nullum par exstitit*. Apud J. Wetstenium, & Gul. Smith, & Janssonio-Waesbergios, Amstelaedami.

*SELIGMANN, H., MORAVEC, J. & WERNER, Y. L. (2008). Morphological, functional and evolutionary aspects of tail autotomy and regeneration in the 'living fossil' *Sphenodon* (Reptilia: Rhynchocephalia). *Biological Journal of the Linnean Society* **93**(4), 721–743.

SHINE, R., OLSSON, M. M., MOORE, I. T., LEMASTER, M. P. & MASON, R. T. (1999). Why do male snakes have longer tails than females? *Proceedings of the Royal Society B: Biological Sciences* **266**(1434), 2147–2147.

*SIMPSON., S. B. (1964). Analysis of tail regeneration in the lizard *Lygosoma laterale*. I. Initiation of regeneration and cartilage differentiation: The role of ependyma. *Journal of Morphology* **114**(3), 425–435.

*SINGH SOOD, M. (1939). A peculiar case of caudal abnormality in *Hemidactylus flaviviridis* rüppel. *Proceedings of the Indian Academy of Sciences - Section B* **9**(5), 316–322.

* SLOTOPOLSKY, B. (1921). *Beiträge zur Kenntnis der Verstümmelungs-und Regenerationsvorgänge am Lacertilierschwanze*, Zurich.

*SORLIN, M., GANGLOFF, E., KOUYOUMDJIAN, L., CORDERO, G. A., DARNET, E. & AUBRET, F. (2019). *Podarcis muralis* (Common Wall Lizard). Tail bifurcation. *Herpetological Review* **50**(2), 377–378.

*STARK, G., DAVID, D. & LEWIN, A. (2018). *Acanthodactylus aegyptius* (Egyptian Fringed-fingered Lizard). Tail bifurcation. *Herpetological Review* **49**(2), 324–325.

*TAMAR, K., MAZA, E. & MEIRI, S. (2013*a*). *Acanthodactylus boskianus* (Bosk's Fringe Fingered Lizard). Bifurcation. *Herptelogical Review* **44**(1), 135–136.

*TAMAR, K., MAZA, E. & MEIRI, S. (2013*b*). *Ophisops elegans* (Snake-Eyed Lizard). Bifurcation. *Herptelogical Review* **44**(1), 146.

TELEMECO, R., BAIRD, T. & SHINE, R. (2011). Tail waving in an Australian scincid lizard (*Bassiana duperreyi*) functions to deflect attacks rather than as a pursuit-deterrent signal. *Animal Behaviour* **82**, 369–375.

*TERNI, T. (1915). Atudio anatomico di una coda doppia *Gongylus ocellatus*, Wagl. *Archivo Italianto di Anatomia e di Embriologica* **14**(2), 290–314.

*TEYNIÉ, A. (2004). Notes on reptiles of Nam Lan conservation area in Phongsaly Province of Lao PDR. *Societé d'Histoire Naturelle*.

TODD, J. & WASSERSUG, R. (2010). Caudal pseudoautotomy in the Eastern Ribbon Snake Thamnophis sauritus. *Amphibia-Reptilia* **31**, 213–215.

*TOFOHR, O. (1903). Gabelschwänzige Eidechsen. *Blätter für Aquarien- und Terrarienkunde* **14**, 319–320.

*TOFOHR, O. (1905). Gabelschwänzige Eidechsen. *Blätter für Aquarien- und Terrarienkunde* **16**, 233–235.

*TORNIER, G. (1897). Über experimentell erzeugte dreischwänzige Eidechsen und Doppelgliedmassen von Molchen. *Zoologischer Anzeiger* **20**, 356–362.

* TORNIER, G. (1901). Neues über das natürliche Entstehen und experimentelle Erzeugen überzähliger und Zwillingsbildungen. *Zoologischer Anzeiger* **24**, 633–658.

*TRAUTH, S. E., WALKER, J. M. & CORDES, J. E. (2014). *Aspidoscelis sexlineata sexlineata* (Six-lined Racerunner). Supernumerary caudal anomalies and a bifid tail. *Herpetological Review* **45**(3), 492–493.

*TUMLISON, R., CONNIOR, M. B., ROBISON, H., MCALLISTER, C., DURDEN, L. A., SAUGEY, D. A. & SASSE, B. (2015). Vertebrate natural history notes from Arkansas. *Journal of the Arkansas Academy of Science* **60**, 106–115.

*TURNER, H., GRIFFITHS, R. A., GARCIA, G. & OUTERBRIDGE, M. (2017). *Plestiodon longirostris* (Bermuda Skink). Tail bifurcation. *Herpetological Review* **48**(1), 198–199.

TYLER, R. K., WINCHELL, K. M. & REVELL, L. J. (2016). Tails of the city: caudal autotomy in the Tropical lizard, *Anolis cristatellus*, in urban and natural areas of Puerto Rico. *Journal of Herpetology* **50**(7), 435–441.

UETZ, P., FREED, P. & HOŠEK, J. (eds.) (2019). The Reptile Database, http://www.reptile-database.org, accessed July 27th 2019

*VALMONT DER BOMARE, M. (1775). *Dictionnaire raisonné universel d'histoire naturelle Tome V*. Chez Brunet, Paris.

*VERGILOV, V. & NATCHEV, N. (2017). First record of tail bifurcations in the snake-eyed skink (*Ablepharus kitaibelii* Bibron & Bory de Saint-Vincent, 1833) from Pastrina hill (northwestern Bulgaria). *Arxius de Miscel·lània Zoològica* **15**, 224–228.

VERVUST, B., VAN DONGEN, S., GRBAC, I. & VAN DAMME, R. (2009). The mystery of the missing toes: extreme levels of natural mutilation in island lizard populations. *Functional Ecology* **23**(5), 996–1003.

*VINCENT, H. (1877). Note sur les Lézards à plusieurs queues. *Bulletin Société d'étude des sciences naturelles de Nîmes* **5**, 123–127.

VITT, L. J., CONGDON, J. D. & DICKSON, N. A. (1977). Adaptive strategies and energetics of tail autonomy in Lizards. *Ecology* **58**(2), 326–337.

VITT, L. J. & COOPER JR, W. E. (1986). Tail loss, tail color, and predator escape in Eumeces (Lacertilia: Scincidae): age-specific differences in costs and benefits. *Canadian Journal of Zoology* **64**(3), 583–592.

*VOLANTE, F. (1923). Contributo alla conoscenza della superrigenerazione nei rettili. *Monitore zoologico italiano* **34**, 185–201.

*VRCIBRADIC, D. & NIEMEYER, J. (2013). *Mabuya frenata*, *M. macrorhyncha*. Tail bifurcation. *Herpetological Review* **44**(3), 510–511.

*VYAS, R. (2016). Bifid tails in two Indian lizards. *IRCF Reptiles & Amphibians* **23**(2), 14–26.

*WAGNER, P., INEICH, I., LEACHÉ, A. D., WILMS, T. M., TRAPE, S., BÖHME, W. & SCHMITZ, A. (2009). Studies on African Agama VI. Taxonomic status of the West African Agama (Sauria: Agamidae) with prominent tail crests: *Agama boulengeri* Lataste 1886, *Agama insularis* Chabanaud, 1918 and *Agama cristata* Mocquard, 1905. *Bonner zoologische Beiträge* **56**(4), 239–253.

*WALKER, J. M. & FLANAGAN, A. (2019). *Cnemidophorus lemniscatus* (Rainbow Whiptail). Caudal wound and bifurcation. *Herpetological Review* **50**(2), 370–371.

*WALLEY, H. D. (1997). On the occurrence of bifurcation in the scincid lizard *Eumeces anthracinus*. *Bulletin of the Maryland Herpetological Society* **33**(4), 178–180.

*WILSON, S. K. (2012). *Australian Lizards: A Natural History*. CSIRO Publishing, Victoria, Australia.

*WOODLAND, W. (1920). Memoirs: some observations on caudal autotomy and regeneration in the gecko (*Hemidactylus flaviviridis*, Rppel), with notes on the tails of *Sphenodon* and *Pygopus*. *Journal of Cell Science* **2**(257), 63–100.

*YANKANCHI, S. & KUMBAR, S. (2016). Bifid tail in *Hemidactylus prashadi* (Smith, 1935). *Reptile Rap* **18**, 34–35.

Chapter 3. Assessing ontogenetic change in predation risk for a lizard with clay models: confounding effects of site and predators, and limitations of the method

The study presented in this chapter is in preparation for submission to review within the peer-reviewed literature.

Barr, J.I., Somaweera, R., Godfrey, S.S., Bateman, P.W. Assessing ontogenetic change in predation risk for a lizard with clay models: confounding effects of site and predators, and limitations of the method. *In preparation*.

Figure 3.1: Black-shouldered kite *(Elanus axillaris)*

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3.1 Abstract

Predator-prey interactions are often complex and can be difficult to identify, particularly in small or cryptic species. Representative soft clay models have become a common tool in assessing risk of predation, particularly in reptiles. The King's skink (*Egernia kingii)* is a large (up to 244mm SVL) lizard that undergoes a significant ontogenetic shift in size, from a small gracile juvenile, to a robust adult capable of defending itself against some comparatively large predators. We hypothesised that predators would preferentially target smaller individuals, as safer prey, with higher attack rates observed with increasing predator densities. Soft clay models of juvenile and adult skinks, and control clay balls were deployed across four sites varying in diversity of predators for five days and checked twice daily for signs of attacks by predators. A total of 157 attack events were recorded from 2678 model check observations (5.86%) with the majority of the 540 attack marks being caused by birds. Contrary to our hypotheses, no significant differences in attack events or intensity was observed between the three model types; however, there were significant differences in attack rates between sites, with Penguin Island having the most attacks indicating a large aggressive response by nesting avian species. This study highlights the need for controls in clay model studies, which have largely been absent from past studies, as well as advising caution in data interpretation when only considering single sites.

3.2 Introduction

Predation risk for a species can vary dramatically between populations based on the abundance and diversity of predator's present (Abrams & Matsuda, 1993; Barbosa & Castellanos, 2005). Additionally, as animals grow and other features change ontogenetically, their predation risk can also change (Lima & Dill, 1990; Hawlena *et al.*, 2006). In many species, including lizards, juveniles frequently face higher predation pressure compared to adults due to juveniles' smaller body size and mass (Glaudas, Winne &

Fedewa, 2006; Bateman & Fleming, 2009; Fresnillo, Belliure & Cuervo, 2016). Juveniles often have a limited ability to defend themselves against predators compared to adults, which may have more effective defensive/offensive morphological structures, such as horns or body armour (Ferguson & Fox, 1984; Stankowich, 2012; Somaweera, Brien & Shine, 2013; Arbour & Zanno, 2018). As such, selective pressure on juveniles generally favour crypsis, or, if foraging requirements force them into the open, to rely more on diversionary tactics such as dazzle-striping (Hawlena *et al.*, 2006; Kuriyama & Hasegawa, 2017), blue tails (Cooper & Vitt, 1985; Bateman, Fleming & Rolek, 2014) or imitation of noxious beetles (Huey & Pianka, 1977).

Dietary studies and field observations have limitations in assessing the predation risk for small, difficult to observe species (Thaker *et al.*, 2011; Kutt, 2012; Cross *et al.*, 2020); however, visually representative soft clay models of prey organisms offer an alternative method to assess predation risk (Bateman, Fleming & Wolfe, 2017; Nordberg & Schwarzkopf, 2019). Marks and indentations from claws or teeth left in clay models by predators, often aided by camera traps and fauna survey data, provide information about the predation risk of a species (Webb & Whiting, 2005; Fresnillo, Belliure & Cuervo, 2015; Bateman *et al.*, 2017). The majority of studies using clay models have focussed on the effects of conspicuous colouring (Castilla *et al.*, 1999; Bateman *et al.*, 2014; Fresnillo *et al.*, 2015) or the effects of shelter and/or habitat type (Webb & Whiting, 2005; Oversby *et al.*, 2018; Hansen *et al.*, 2019; Nordberg & Schwarzkopf, 2019; Costa, Coroller & Salvidio, 2020), and not directly on body size reflecting ontogenetic change. Among very limited studies that consider size of clay models, Bittner (2003) found that models representing juvenile garter snakes (*Thamnophis sirtalis*) were attacked more often than were larger, adult-size models.

The King's skink, *Egernia kingii,* is a large-bodied skink distributed along the south west coast of Western Australia including many of the offshore islands

(Cogger, 2014). Juvenile King's skinks appear to be more susceptible to predation than adults (Bonnet *et al.*, 1999; Aubret *et al.*, 2004), presumably due to substantial ontogenetic change in their size and weight (Arena & Wooller, 2003). When born, King's skinks measure approximately 60-80 mm SVL, with a mass of 7 g (Arena & Wooller, 2003), but upon maturity, they have an SVL of up to 244 mm (Storr, 1978) and a mass of more than 360 g (Dilly, 2000). Adult King's skinks are able to actively defend themselves and fight or chase away some potential predators (Masters & Shine, 2003). Furthermore, younger King's skinks have proportionally longer tails than do adults, suggesting a greater reliance on autotomy as means of predator escape (Barr *et al.*, 2019). As predation in reptiles (and other taxa) has been shown to be size dependent (e.g. Ferguson & Fox, 1984; Forsman, 1993) we therefore hypothesised that predators of King's skinks will prefer to predate smaller, juvenile skinks as they are safer to attack.

Here we assess ontogenetic changes in predation risk in the King's skink using representative soft clay models across several island and mainland sites varying in perceived predation risk. We predicted that: 1) sites with a higher diversity of predators will have higher attack rates; that 2) models of juveniles would have a higher attack rate compared to models of adults, being attacked *sooner* and *more often* than would models of adults; and 3) that predators relying on visual cues, such as birds, will be responsible for the majority of attacks.

3.3 Methods

Representative soft clay models (Figure 3.2) for adult King's skinks (SVL 200 mm, tail length 200 mm), juvenile King's skinks (SVL 100 mm, tail length 100 mm) and a control ball of clay (diameter 100 mm) were hand rolled from 500 g pre-packaged Plastiplay^{TM} brown modelling clay. Four sites were chosen for this study; Rottnest Island (-32.006214°, 115.514878°), Garden Island (-32.218796°, 115.679058°), Penguin Island (-32.305646°, 115.691014°) and

Perth coast (-31.815103°, 115.734666°), all located within 50km of Perth, Western Australia. The three islands, all originally part of the same limestone shelf, became isolated from both one another and the mainland approximately 5000-8000 years ago (Playford, 1988; Hughes, 2012) and all vary in both terrestrial and avian potential predators (Table 3.1).

Predation risk was established based on the diversity of potential predators at the different sites (Cooper, Pérez-Mellado & Vitt, 2004; Pafilis *et al.*, 2009; Itescu *et al.*, 2017) taken from literature, online sources and direct observations (Table 3.1). Online fauna databases NatureMap (DBCA, 2007) and Atlas of Living Australia (ALA, 2019) were searched for observations, with species noted as 'present' in the area if there had been five or more observations between 2014 and 2020. A search radius of 5km was used for identifying potential avian predators, with potential terrestrial predators as only those occurring within sites. Additionally, species were classified as 'present' from the authors' personal observation while in the field, as well as from unpublished fauna survey data (City of Joondalup, *unpub data*). Species identified as potential predators were then checked against literature for records of predating lizards; for snakes (Bonnet *et al.*, 1999; Pearson, Shine & How, 2002; Aubret *et al.*, 2004), birds (Marchant & Higgins, 1990) and mammals (Coman, 1973; Crawford, 2010; Bamford, 2012).

Each site had 25 triads of models (adult, juvenile and control ball) deployed within an approximately 1500 m² grid. Each triad was spaced ~10m apart, with individual models within triads set 30-50cm from one another. Models of juveniles were tethered to paper plates using fishing line which were then covered by ground substrate to prevent their removal by predators (Castilla *et al.*, 1999; Bateman *et al.*, 2014). Models were deployed between September and October 2017. All triads of models were deployed in the early afternoon on the first day, checked twice daily (morning ~ 8am and afternoon ~2 pm) for four days, and collected at the morning check on the fifth day. At each check, for each model, we recorded; if it had been attacked or not

(Yes/No) as well as the number, and type of indentations present. All attacked models were photographed. After each check, indentations in the models were smoothed over so that each recording reflected the number and type of fresh attacks. Marks were classified as bird (V/U shape peck or stab marks), rodent (incisor marks), lizard (recessed homodont teeth), tammar wallaby (*Macropus eugenii*) (Garden Island) or quokka (*Setonix brachyurus*) (Rottnest Island), and invertebrate (stippling pattern) (Figure 3.3). Marks not characteristic of attacks (such as footprints from walking over the model, or marks from vegetation) were recorded, but omitted from data analysis. Skink models that had legs removed, were noted as a single attack on the legs as the total number could not be verified. In some instances, models had large numbers of multiple overlapping attacks that were difficult to quantify: these were included for attack event analysis (attacked yes/no) data, but not for the attack count analysis. On Penguin Island, four juvenile models over the five days were attacked severely, being torn in two, with part or all the model missing, despite being tethered to paper plates. These individuals were not replaced and were removed from subsequent observations and analysis (n = 22). Garden Island recorded no attack events, only having five marks from tammar wallabies of 675 observations and therefore were not included in the analysis but are discussed.

3.3.1 Statistical analysis

All analyses were conducted in R studio version 1.1.383 (R Development Core Team, 2013). General linear mixed models (GLMM) were used to assess ontogenetic change in predation risk using the glmer function of the lme4 package (Bates *et al.*, 2014). Two models were developed, with the following response variables; 1) whether the model was attacked or not (binomial, link = "logit") using all observations, 2) number of attacks (Gamma, link = “inverse”) only including attack observations. Soft clay model sets were included as a random factor for all three GLMMs. Fixed effects for the two GLMM’s were set as site, model type, day, time of check (am/pm), and a site: model interaction effect. Models that were unable to have attack numbers counted accurately, due to high number of overlapping attacks, were not included in the attack intensity analysis. The most appropriate model was selected based on the lowest AIC using the drop1 function from the lme4 package (Bates *et al.*, 2014) with the complete model being the most appropriate and used in both cases. Appropriateness of the GLMMs were assessed using a Hosmer and Lemeshow goodness of fit test (hoslem.test function) for the attack event (binomial) GLMM, with residual and QQ plots used for attack intensity and attack position (Gamma) GLMMs. Chi-Square and p-values were obtained using the Anova function from the Car package (Fox *et al.*, 2017). Post hoc analysis were performed using the emmeans function in package emmeans (Lenth, 2019). Analysis of attacker type between sites was assessed using a Fisher test.

Table 3.1: Potential predator species to King's skinks (*Egernia kingii*) across the study sites. Presence records based from ATLAS of Living Australia (ALA, 2019) accessed 4th June 2020, Nature maps (NM) (DBCA, 2007) accessed 4th June 2020, City of Joondalup Whitfords Nodes fauna survey 2016 (City of Joondalup, *unpub. data*) and J. Barr *pers. obs*.

Species	**Penguin Island**	**Rottnest Island**	**Garden Island**	**Coastal Perth**
Mammals				
Cat *(Felis catus)*	-	Historical (2002)	Historical (1998)	X
Fox *(Vulpes vulpes)*	-	-	Historical (1996/7)	X
Reptiles				
South west carpet python *(Morelia spilota imbricata)*	-	-	X	-
Tiger snake *(Notechis scutatus)*	-	-	X	-
Dugite *(Pseudonaja affinis)*	-	X	-	X
Aves				
Collared sparrowhawk	-	X	-	-

(Accipiter cirrocephalus)				
Brown goshawk *(Accipiter fasciatus)*	X	X	-	X
Swamp harrier *(Circus approximans)*	X	-	-	-
Australian raven (*Corvus coronoides)*	X	X	X	X
Kookaburra (*Dacelo novaeguineae*)	X	-	-	X
Black-shouldered kite *(Elanus axillaris)*	X	X	-	X
Nankeen kestre*l* *(Falco cenchroides)*	-	X	-	X
Whistling kite *(Haliastur sphenurus)*	X	-	-	-
Australian little eagle *(Hieraaetus morphnoides)*	X	X	-	-
Osprey/Eastern osprey *(Pandion haliaetus/cristatus)*	X	X	X	X
Total number of predator species	Eight	Eight	Four	Nine
References	J. Barr *pers. obs*; DBCA, 2007; ALA, 2019	J. Barr *pers. obs*; DBCA, 2007; ALA, 2019	J. Barr *pers. obs*; DBCA, 2007; ALA, 2019	J. Barr *pers. obs*; DBCA, 2007; City of Joondalup, *unpub. data*; ALA, 2019

Figure 3.2: Example of representative clay models. Adult (top), juvenile tethered to plate (right) and control ball (left).

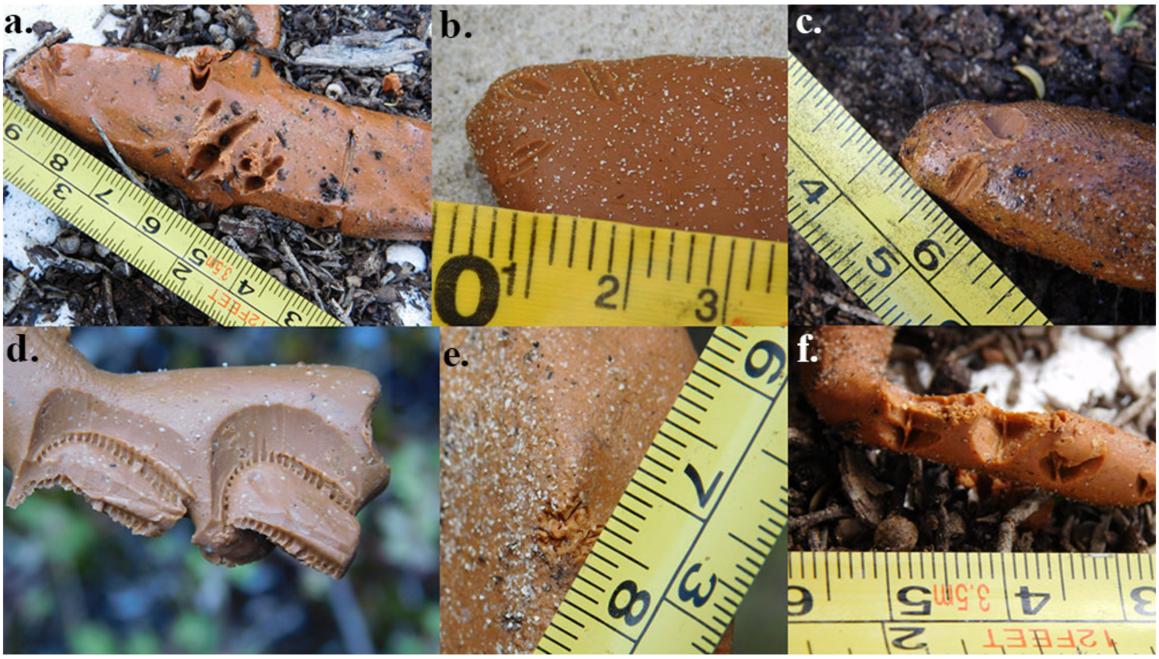

Figure 3.3: Predator attack indentations on representative soft clay models soft clay models. Bird peck (a.), rodent incisor (b.), tammar wallaby (c.), lizard (d.), invertebrate (e.), quokka (f.).

3.4 Results

A total of 157 attack events were recorded from 2678 model check observations (5.86%) across the four sites. Sites significantly differed in both the probability of attack events and number of attacks (Table 3.2) with Penguin Island having both significantly higher probability of attack events (Figure 3.4a) and average number of attacks (Figure 3.4b) compared to Rottnest Island and Coastal Perth.

3.4.1 Clay models within sites

A significant difference between the clay models within sites was detected from the Site:Model interaction effect for the probability of attack events, but not for the average attack intensity (Table 3.2). Adult models had a higher probability of being attacked on Rottnest Island compared to control balls, and at Coastal Perth compared to juveniles, although probability of attack events between adult model and juveniles compared to the control balls at Coastal Perth, did not differ (Table 3.3, Figure 3.5). No difference in attack event probability was detected between model types on Penguin Island (Table 3.3, Figure 3.5).

3.4.2 Clay models between sites

All three model types had higher attack probabilities on Penguin Island compared to Rottnest Island, with juvenile and control balls being attacked more compared to Coastal Perth site (Table 3.4, Figure 3.5). No difference in proportion of attack events for the three model types was observed between Rottnest and Coastal Perth sites.

3.4.3 Probability of attack events over time

Attack event probability generally decreased with each day for all three sites that recorded attack events (Figure 3.6a), with majority of attack events recorded at the morning check for Penguin Island and Coastal Perth, but Rottnest having similar attack events at both checks (Figure 3.6b). Juvenile models had slightly more attack events recorded on the first morning check for both Penguin Island and Rottnest Island, with adult models recording more attacks for the Coastal Perth site (Figure 3.7).

3.4.4 Attacker type

The type of attackers significantly differed between sites (P < 0.001, Figure 3.8), with majority of the 157 attack events from birds (86.62%), followed by rodents (10.83%), invertebrates (1.91%) and lizards (0.64%). Of the 157 attack events, 134 were able to have the number of attacks adequately quantified, with a total of 540 attacks across the four sites. Penguin Island had the most attacks per model (average ± SD = 5.33 ± 4.58) followed by Rottnest Island (2.88 ± 3.34) and Coastal Perth (1.97 ± 1.16), with no attacks on Garden Island.

Table 3.2: GLMMs investigating fixed effects on proportion of attack events and number of attacks on models.

Measure	Fixed effect	df	ChiSq	P-value
Probability of attack events	Site	2	23.1	**< 0.001***
	Model	2	4.86	0.088
	Site: Model	4	11.4	**0.022**
	Day	4	37.4	**< 0.001***
	Time	1	33.8	**< 0.001***
Number of attacks	Site	2	24. 4	**< 0.001***
	Model	2	3.88	0.143
	Site: Model	4	1.73	0.785
	Day	4	3.21	0.523
	Time	4	0.424	0.515

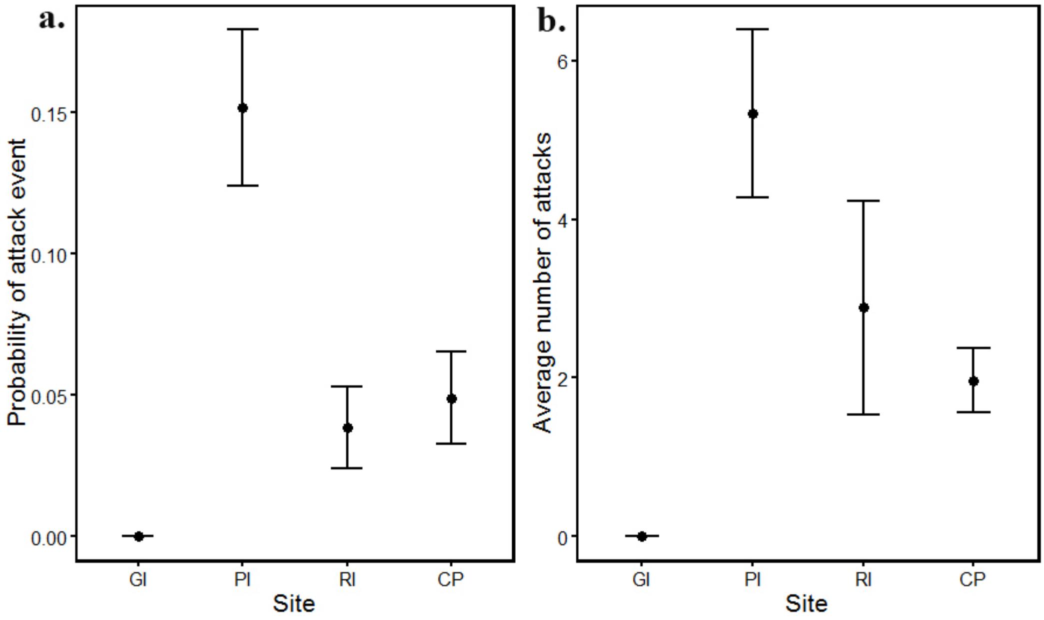

Figure 3.4: Probability of an attack event (a.) and average number of attacks per model (b.) on soft clay models for research sites Garden Island (GI), Penguin Island (PI), Rottnest Island (RI) and Coastal Perth (CP). Average ± 95% CI error bars shown. No attacks were observed on Garden Island.

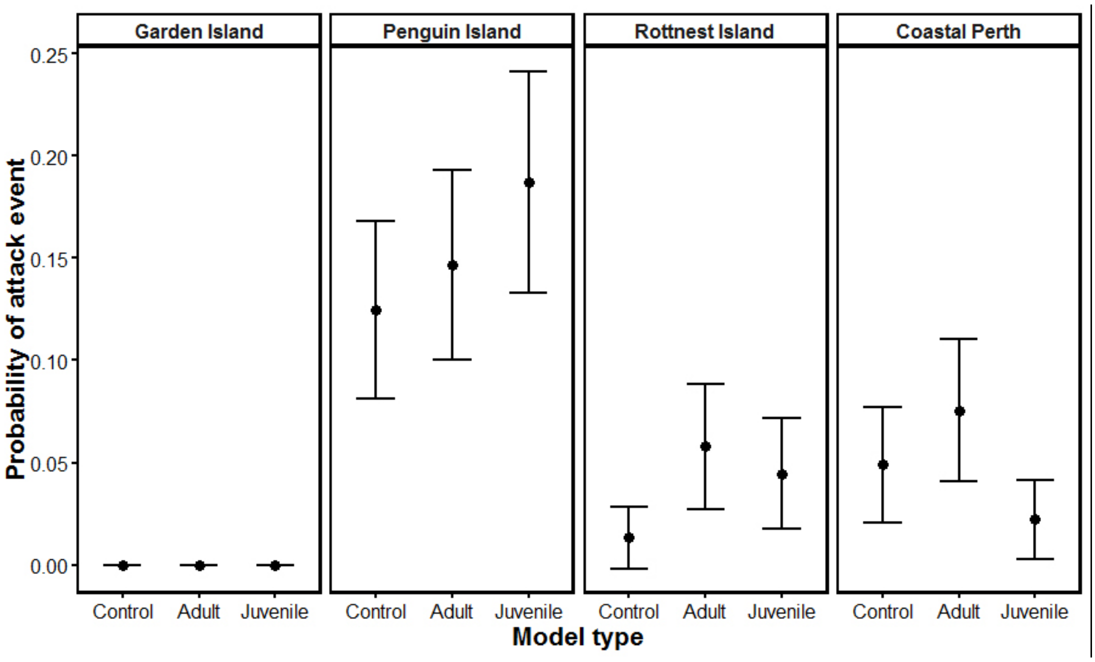

Figure 3.5: Probability of attack events between soft clay models (adult, juvenile and control balls) within research sites Garden Island, Penguin Island, Rottnest Island and Coastal Perth. Average ± 95% CI error bars shown. No attacks were observed on Garden Island.

Table 3.3: Post hoc testing for the proportion of attack events GLMM investigating differences in attack events between soft clay models (adult, juvenile and control), within sites (Penguin Island, Rottnest Island and Coastal Perth). No attacks were observed on Garden Island.

Site	Comparison	Z-stat	P-value
Penguin Island	Adult - Control	0.746	0.736
	Adult - Juvenile	- 1.18	0.465
	Control - Juvenile	- 1.89	0.141
Rottnest Island	Adult - Control	2.42	**0.048**
	Adult - Juvenile	0.67	0.781
	Control - Juvenile	- 1.92	0.134
Coastal Perth	Adult - Control	1.21	0.443
	Adult - Juvenile	2.57	**0.027**
	Control - Juvenile	1.53	0.274

Table 3.4: Post hoc testing for the proportion of attack events GLMM investigating difference in attack rate between sites (Penguin Island (PI), Rottnest Island (RI) and Coastal Perth (CP)) for soft clay models (adult, juvenile and control). No attacks were observed on Garden Island.

Model	Comparison	Z-stat	P-value
Control	PI - RI	3.71	**< 0.001**
	PI - CP	2.36	**0.048**
	RI -CP	- 1.97	0.119
Adult	PI - RI	2.59	**0.026**
	PI - CP	1.94	0.126
	RI - CP	- 0.71	0.758
Juvenile	PI - RI	3.78	**< 0.001**
	PI - CP	4.42	**< 0.001**
	RI -CP	1.12	0.501

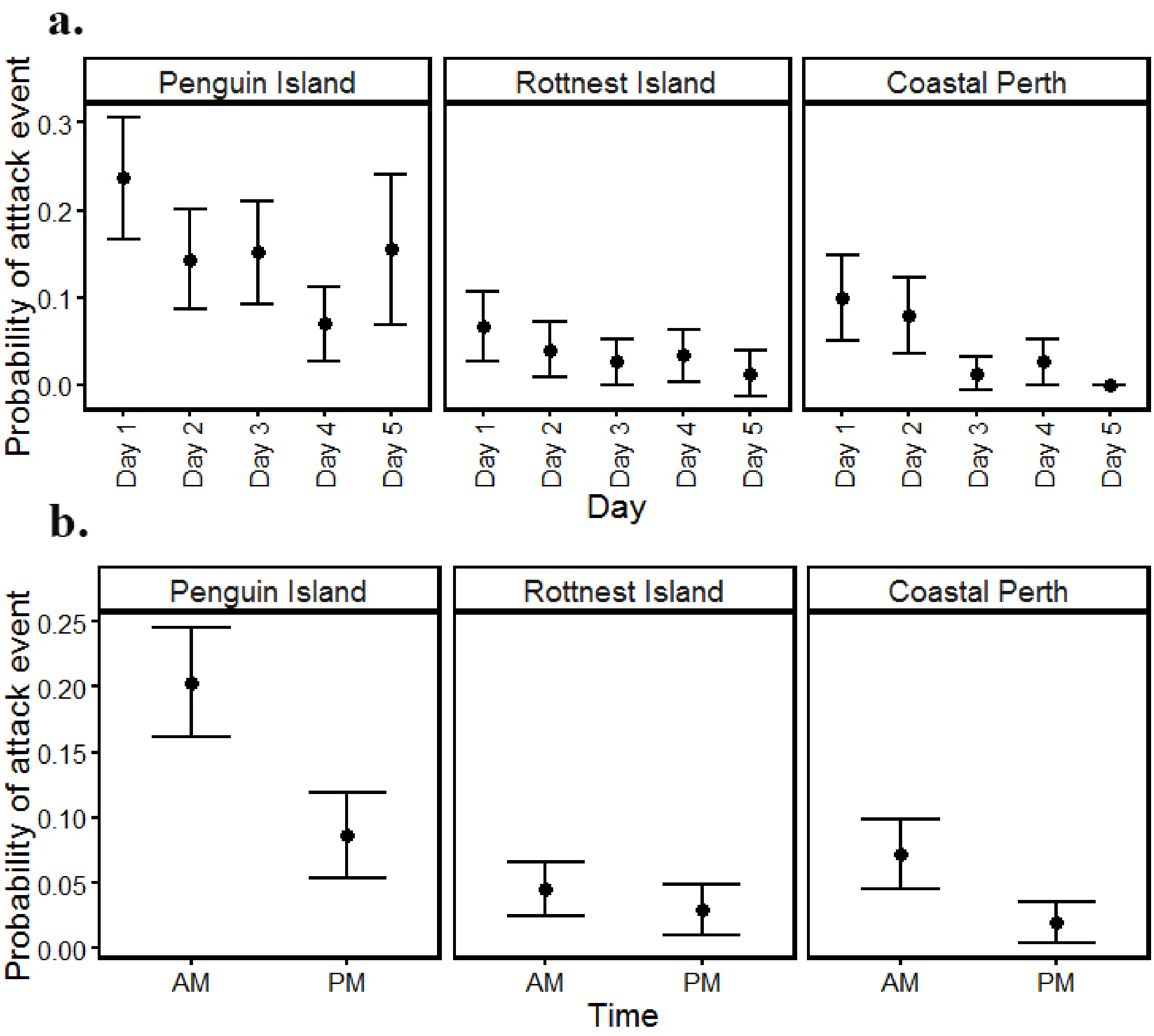

Figure 3.6: Probability of attack events occurring over the duration of the study (a.) and morning or afternoon checks (b.). No attacks were observed on Garden Island.

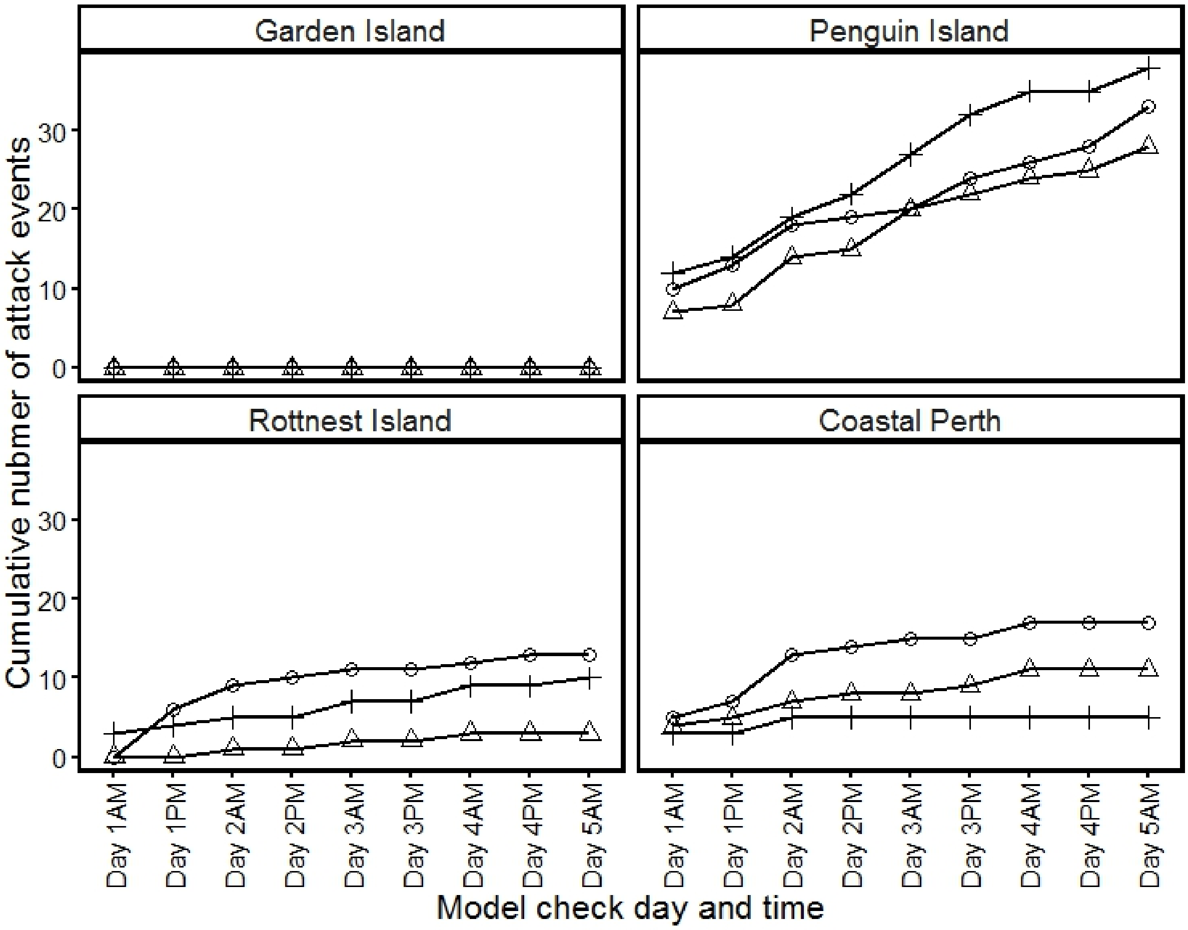

Figure 3.7: Cumulative number of attack events on adult-sized clay models (O), juvenile-sized clay models (+) and control clay balls (Δ) scored twice a day (AM and PM) over five consecutive days. No attacks were observed on the models deployed at Garden Island.

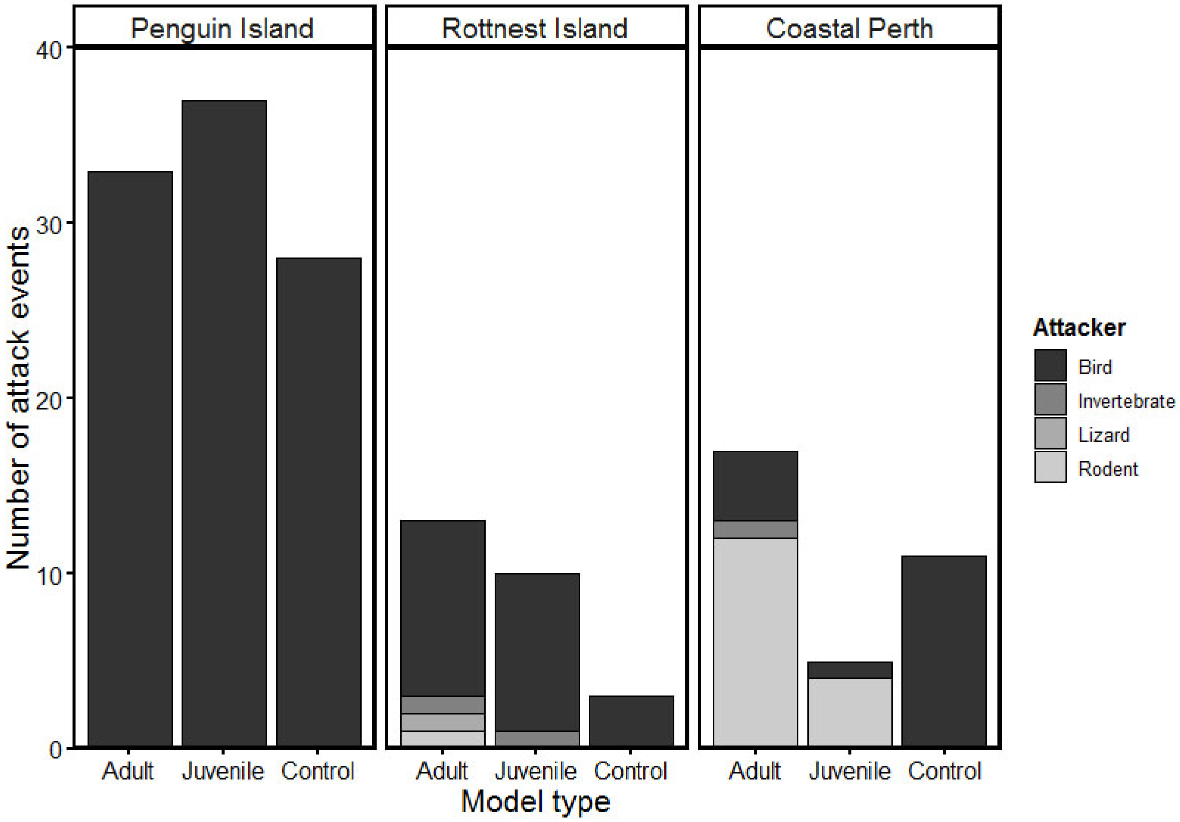

Figure 3.8: Classification of attack events by attacker type across the three research sites for the three model types. No attacks were observed on Garden Island.

3.5 Discussion

We predicted that: 1) sites with a higher diversity of predators will have higher attack rates, with 2) models of juveniles having a higher attack rate compared to models of adults, being attacked *sooner* and *more often* than models of adults and 3) that predators relying on visual cues, such as birds, will be responsible for the majority of attacks. Only the third prediction was unequivocally supported –the majority of the attack indentations at all sites were made by birds, with models on Penguin Island being attacked the most.

Significant differences in rates of attack between sites were recorded; however, higher attack rates were not observed at the sites with overall presumed higher predation risk, but were correlated with the number of avian species occurring at the sites. Juvenile models received slightly more attack events than did the other model types at the initial check for Penguin Island and Rottnest Island, but overall, they were not attacked significantly more or sooner than were adult models. Apart from Rottnest Island, control balls showed similar attack rates to both adult and juvenile models, suggesting that 'predatory' responses at the other sites were partly initiated by curiosity.

3.5.1 Difference in attack rates

Overall, attack rates did not correlate to increased species diversity, but did follow a pattern consistent with number of avian predatory species present at the sites. Penguin Island, with the highest number of potential avian predatory species, recorded the highest probability and number of attacks compared to the other three sites, followed by Rottnest Island and Coastal Perth, then Garden Island with the lowest number of avian predator species. However, overall predation risk can be affected by a multitude of factors (Barbosa & Castellanos, 2005; Andersson, Wallander & Isaksson, 2009; Bateman & Fleming, 2011; Frey *et al.*, 2018). We therefore advise caution with interpreting results, particularly with representative clay models, and discuss potential influencing factors and limitations below.

Aggressive interactions between heterospecifics can increase risk of sustaining damage to individuals, but are not an active attempt to predate the animal (Ducey, Schramm & Cambry, 1994). Although Penguin Island had the highest avian predation diversity, only two marks were recorded as claw marks, with a large majority of marks more representative of narrow beaks. Several species of bird, including Bridled Terns (*Onychoprion anaethetus*), Silver Gulls (*Chroicocephalus novaehollandiae*) and Little Penguins (*Eudyptula minor*) nest on Penguin Island. Adult King's skinks are known to predate on the eggs of these birds (Nicholls, 1974; Meathrel & Klomp, 1990), and in turn the birds are known to be aggressive toward King's skinks (J. Barr *pers. obs*). Although adult skinks are responsible for predating on eggs, bird aggression is likely directed to skinks in general, as opposed to a particular ontogenetic stage. However, there was a non-significant higher attack rate aimed at lizard models, with attacks also aimed at the control balls, suggesting that the birds were aggressive to all unknown objects at the site (King, Hemsworth & Coleman, 2003), exacerbated by the nesting season (Rößler, Pröhl & Lötters, 2018).

Rottnest Island and Coastal Perth sites recorded similar numbers of attacks (Figure 3.4) although the type of marks differed, with Rottnest Island having mainly avian attacks and Coastal Perth having rodent attacks, with both sites recording markings from invertebrates (Figure 3.8). Rodents are noted to predate on certain species of lizard, including skinks. For example, a study by Thibault *et al.* (2017) in New Caledonia found that 19.1% of gut contents (n = 283) from two *Rattus* species contained remnants of at least 12 skink species. Rodents are also known to be aggressors to larger lizards and their presence has been correlated with damaging attacks in natural populations. For example, a study by Hayes *et al.* (2012) investigating the impact of invasive mammals on isolated populations of several *Cyclura* iguanids found that tail damage, both resulting in autotomy events of regenerative furcations was highly correlated to the presence of rodents. Although invertebrates are recorded to predate on lizard species (Keehn & Feldman, 2018; Nordberg & Schwarzkopf, 2019) these observations were only minor (Figure 3.8).

By contrast, no attacks at all were recorded on Garden Island. Although having the lowest number of potential avian predator species (Table 3.1), the lack of avian predation marks may have been the result of the location of the representative clay model deployment. Many southern areas of Garden Island where the models were deployed are quite densely populated by the invasive Arum Lily (*Zantedeschia aethiopica*). This plant is known to be toxic to native herbivores, not being grazed when established (Keighery, 1997), and although evidence of *E. kingii* was seen in the area, both *E. kingii* and potential predators may be less likely to inhabit these areas.

Rottnest Island was the only site to show a significant difference between one of the model types and the control, with lizard models having significantly higher attack rate than did the control (Figure 3.5, Table 3.3). Although several species of avian predators are present on Rottnest Island, the Australian Raven (*Corvus coronoides*) has established a large population across the island (Rottnest Island Authority, 2015; Oversby *et al.*, 2018). Ravens are known for their intelligence (Emery & Clayton, 2004), and are likely to have better cognitive ability for shape recognition. A recent study by Oversby *et al.* (2018) investigating predation on Rottnest bobtail skinks (*Tiliqua rugosa konowi*) recorded that soft clay models of this species were attacked primarily by Ravens with soft clay lizard models being attacked significantly more than were control balls. Although Ravens are known as being primarily scavengers, they still predate other vertebrates, including lizards, and can have a large effect on native species (Rowley & Vestjens, 1973; Dorfman & Read, 1996; Stevenson, 2011; Barr, 2019).

3.5.2 Limitations of clay models

A review by Bateman *et al.* (2017) indicated that the use of clay models to provide an insight into assessing predation risk for small vertebrates from visually orientated predators is a valuable technique. However, it also highlighted limitations, and suggested caution with data interpretation. One issue that is highlighted by Bateman *et al.* (2017), and often overlooked by clay model studies, is the use of clay controls. Our study also emphasises the need for controls. Without the use of controls, our results would have been interpreted differently, suggesting that both adult and juvenile models had high risk of predation (although overall higher attack rates on Penguin Island), and that adult models experienced higher risk than did juvenile models at the Coastal Perth site. On Penguin Island, all model types, including the control ball, were attacked significantly more than at the other two sites that recorded attacks (Table 3.4, Figure 3.5). Four of the 25 juvenile models were torn apart, with both bodies and limbs being peppered with beak marks, and several more models having limbs torn from the models. This type of attack was not observed at any other of the other three sites. Finally, while some differences in attack rate between the sites are easy to interpret – the nesting birds at Penguin Island for example – if one carried out this experiment only at the Rottnest site, or the Coastal Perth site, or the Garden Island site, one might come to very different conclusions to predatory behaviour and the validity of the use of clay models, despite much of the predator diversity apparently being shared between the sites. Pairing of clay models with camera traps would also be suggested in future studies in order to quantify aggressive versus predation interactions and increase accuracy of data interpretation.

Another issue is that the models do not 'behave' as live lizards would, that might allow avoidance or deflection of predatory attacks. For example, juvenile *Acanthodactylus beershebensis* with bright blue tails foraged more actively than those with faded colouration, but also employed deflective tail displays whilst doing so (Hawlena *et al.,* 2006). Additionally, perception of realism from visual or olfactory cures differs for predators, and how they view potential prey. Representative clay models for example, may not be an effective means of assessing predation risk in snakes that may rely more on olfactory cues (Bateman *et al.*, 2017). Avian predators see in a different light spectrum to humans (Hart, 2001), with many predatory birds use ultraviolet (UV) reflections to identify prey markers (Lind *et al.*, 2013; Mitkus *et al.*, 2018). Despite this, UV reflectance in assessing predation with clay models is not often performed, and may vary based on the prey item being represented (Bateman *et al.*, 2017). Additionally, many raptors are known to rely on their keen vision to identify potential prey whilst scanning (Jones, Pierce Jr & Ward, 2007; Land & Nilsson, 2012; Mitkus *et al.*, 2018). This may affect their ability to distinguish stationary clay models as a prey item (Kane & Zamani, 2014), although foraging methods and visual abilities do vary between species (Berger-Tal & Saltz, 2016; Potier *et al.*, 2016).

3.6 Conclusion

Representative soft clay models are a useful tool in assessing predation risk and predatory-prey dynamics; however, they are not without their limitations. Therefore, caution is advised with data interpretation. In this study a significant difference in number of attacks were observed between sites, with the highest number of attacks observed at the site with the highest number of avian predator species. However, this difference may have been compounded by aggressive responses from nesting seabirds. Differences in attack rate towards models and controls, and in total number of attacks, between sites suggests that future studies need to be cautious in the interpretation of their results if only one site is used. However, accuracy of data interpretation will be strengthened by the use of controls, camera traps and making the models as 'realistic' visually and olfactory as possible.

3.7 Acknowledgements

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3.8 References

“Every reasonable effort has been made to acknowledge the owners of copyright material. I would be pleased to hear from any copyright owner who has been omitted or incorrectly acknowledged.”

Abrams, P. & Matsuda, H. (1993). Effects of adaptive predatory and anti-predator behaviour in a two-prey—one-predator system. *Evolutionary Ecology* **7**(3), 312-326.

ALA. (2019). Atlas of Living Australia http://www.ala.org.au, Accessed 4 June 2020.

Andersson, M., Wallander, J. & Isaksson, D. (2009). Predator perches: a visual search perspective. *Functional Ecology* **23**(2), 373-379.

Arbour, V. M. & Zanno, L. E. (2018). The evolution of tail weaponization in amniotes. *Proceedings of the Royal Society B: Biological Sciences* **285**(1871), 20172299.

Arena, P. C. & Wooller, R. D. (2003). The reproduction and diet of *Egernia kingii* (Reptilia : Scincidae) on Penguin Island, Western Australia. *Australian Journal of Zoology* **51**(5), 495-504.

Aubret, F., Bonnet, X., Maumelat, S., Bradshaw, D. & Schwaner, T. (2004). Diet divergence, jaw size and scale counts in two neighbouring populations of tiger snakes (*Notechis scutatus*). *Amphibia-Reptilia* **25**(1), 9-17.

Bamford, M. (2012). Cat Predation and Suburban Lizards: A 22 Year Study at a Suburban Australian Property. *The Open Conservation Biology Journal* **6**, 25-29.

Barbosa, P. & Castellanos, I. (2005). *Ecology of predator-prey interactions*. Oxford University Press.

Barr, J. I. (2019). Predation of a Buff-banded Rail *Hypotaenidia philippensis* by Australian Ravens *Corvus coronoides* on Penguin Island, Western Australia. *Australian Field Ornithology* **36**, 93-94.

Barr, J. I., Somaweera, R., Godfrey, S. S. & Bateman, P. W. (2019). Increased tail length in the King’s skink, *Egernia kingii* (Reptilia:

Scincidae): an anti-predation tactic for juveniles? *Biological Journal of the Linnean Society* **126**(2), 268–275.

BATEMAN, P. & FLEMING, P. (2009). To cut a long tail short: a review of lizard caudal autotomy studies carried out over the last 20 years. *Journal of Zoology (London)* **277**(1), 1-14.

BATEMAN, P., FLEMING, P. & ROLEK, B. (2014). Bite me: Blue tails as a 'risky-decoy'defense tactic for lizards. *Current Zoology* **60**(3), 333-337.

BATEMAN, P., FLEMING, P. & WOLFE, A. (2017). A different kind of ecological modelling: the use of clay model organisms to explore predator–prey interactions in vertebrates. *Journal of Zoology* **301**(4), 251-262.

BATEMAN, P. W. & FLEMING, P. A. (2011). Frequency of tail loss reflects variation in predation levels, predator efficiency, and the behaviour of three populations of brown anoles. *Biological Journal of the Linnean Society* **103**(3), 648-656.

BATES, D., MAECHLER, M., BOLKER, B. & WALKER, S. (2014). lme4: Linear mixed-effects models using Eigen and S4. *R package version* **1**(7).

BERGER-TAL, O. & SALTZ, D. (2016). *Conservation Behavior Applying Behavioral Ecology to Wildlife Conservation and Management*.

BITTNER, T. (2003). Polymorphic Clay Models of Thamnophis Sirtalis Suggest Patterns of Avian Predation. *OHIO J SCI* **103**, 62-66.

BONNET, X., BRADSHAW, D., SHINE, R. & PEARSON, D. (1999). Why do snakes have eyes? The (non-) effect of blindness in island tiger snakes (*Notechis scutatus*). *Behavioral Ecology and Sociobiology* **46**(4), 267-272.

CASTILLA, A. M., GOSA, A., GALAN, P. & PEREZ-MELLADO, V. (1999). Green tails in lizards of the Genus *Podarcis*: Do they influence the intensity of predation? *Herpetologica* **55**(4), 530-537.

CITY OF JOONDALUP. *Unpub data*. Whitfords Nodes fauna survey 2016.

COGGER, H. G. (2014). *Reptiles and Amphibians of Australia*, 7th edition. CSIRO Publishing, Victoria, Australia.

COMAN, B. (1973). The diet of red foxes, Vulpes vulpes L., in Victoria. *Australian Journal of Zoology* **21**(3), 391-401.

COOPER, W. E., PÉREZ-MELLADO, V. & VITT, L. J. (2004). Ease and effectiveness of costly autotomy vary with predation intensity among lizard populations. *Journal of Zoology* **262**(3), 243-255.

COOPER, W. E. & VITT, L. J. (1985). Blue tails and autotomy: enhancement of predation avoidance in juvenile skinks. *Zeitschrift fuer Tierpsychologie* **70**(4), 265-276.

COSTA, A., COROLLER, S. & SALVIDIO, S. (2020). Comparing day and night predation rates on Lizard-like clay models. *Herpetological Conservation and Biology* **15**, 198-203.

CRAWFORD, H. (2010). *A comparison of the red fox (Vulpes vulpes) and feral cat (Felis catus) diets in the south west region of Western Australia*. Honours thesis, Murdoch University.

CROSS, S., CRAIG, M., TOMLINSON, S. & BATEMAN, P. (2020). I don't like crickets, I love them: invertebrates are an important prey source for varanid lizards. *Journal of Zoology* **310**(4), 323-333.

DBCA. (2007). NatureMap: Mapping Western Australia's Biodiversity (https://naturemap.dbca.wa.gov.au/). Department of Biodiversity, Conservation and Attractions, Accessed on 4 June 2020.

DILLY, M. L. (2000). *Factors affecting the distribution and variation in abundance of the King's skink (Egernia kingii) (Gray) in Western Australia*. Honours thesis thesis, Murdoch University.

DORFMAN, E. J. & READ, J. (1996). Nest predation by corvids on cormorants in Australia. *Emu-Austral Ornithology* **96**(2), 132-135.

DUCEY, P. K., SCHRAMM, K. & CAMBRY, N. (1994). Interspecific Aggression Between the Sympatric Salamanders, Ambystoma maculatum and Plethodon cinereus. *The American Midland Naturalist* **131**(2), 320-329.

EMERY, N. J. & CLAYTON, N. S. (2004). The mentality of crows: convergent evolution of intelligence in corvids and apes. *science* **306** (5703), 1903-1907.

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**(2), 342-349.

FORSMAN, A. (1993). Survival in Relation to Body Size and Growth Rate in the Adder, Vipera berus. *Journal of Animal Ecology* **62**(4), 647-655.

FOX, J., WEISBERG, S., ADLER, D., BATES, D., BAUD-BOVY, G., ELLISON, S., FIRTH, D., FRIENDLY, M., GORJANC, G. & GRAVES, S. (2017). Package 'car'.

FRESNILLO, B., BELLIURE, J. & CUERVO, J. J. (2015). Red tails are effective decoys for avian predators. *Evolutionary Ecology* **29**(1), 123-135.

FRESNILLO, B., BELLIURE, J. & CUERVO, J. J. (2016). Ontogenetic shifts in risk behaviours are related to body size and coloration in spiny-footed lizards. *Animal Behaviour* **119**, 165-172.

FREY, D., VEGA, K., ZELLWEGER, F., GHAZOUL, J., HANSEN, D. & MORETTI, M. (2018). Predation risk shaped by habitat and landscape complexity in urban environments. *Journal of Applied Ecology* **55**(5), 2343-2353.

GLAUDAS, X., WINNE, C. T. & FEDEWA, L. A. (2006). Ontogeny of Anti-Predator Behavioral Habituation in Cottonmouths (Agkistrodon piscivorus). *Ethology* **112**(6), 608-615.

HANSEN, N. A., SATO, C. F., MICHAEL, D. R., LINDENMAYER, D. B. & DRISCOLL, D. A. (2019). Predation risk for reptiles is highest at remnant edges in agricultural landscapes. *Journal of Applied Ecology* **56**(1), 31-43.

HART, N. S. (2001). Variations in cone photoreceptor abundance and the visual ecology of birds. *J Comp Physiol A* **187**(9), 685-97.

HAWLENA, D., BOOCHNIK, R., ABRAMSKY, Z. & BOUSKILA, A. (2006). Blue tail and striped body: why do lizards change their infant costume when growing up? *Behavioral Ecology* **17**(6), 889-896.

HAYES, W. K., IVERSON, J. B., KNAPP, C. R. & CARTER, R. L. (2012). Do invasive rodents impact endangered insular iguana populations? *Biodiversity and conservation* **21**(7), 1893-1899.

HUEY, R. B. & PIANKA, E. R. (1977). Natural selection for juvenile lizards mimicking noxious beetles. *Science* **195**(4274), 201-3.

HUGHES, M. (2012). Blending a heritage of recreation and tourism with conservation of natural heritage: an example from Penguin Island, Western Australia. *Journal of Heritage Tourism* **7** (1), 1-11.

ITESCU, Y., SCHWARZ, R., MEIRI, S., PAFILIS, P. & CLEGG, S. (2017). Intraspecific competition, not predation, drives lizard tail loss on islands. *Journal of Animal Ecology* **86**(1), 66-74.

JONES, M. P., PIERCE JR, K. E. & WARD, D. (2007). Avian vision: a review of form and function with special consideration to birds of prey. *Journal of Exotic Pet Medicine* **16**(2), 69-87.

KANE, S. A. & ZAMANI, M. (2014). Falcons pursue prey using visual motion cues: new perspectives from animal-borne cameras. *The Journal of Experimental Biology* **217**(2), 225-234.

KEEHN, J. E. & FELDMAN, C. R. (2018). Predator attack rates and anti-predator behavior of side-blotched lizards (Uta stansburiana) at Southern California wind farms, USA. *Herpetological Conservation and Biology* **13**(1), 194-204.

KEIGHERY, G. (1997). Arum Lily as an environmental weed in Western Australia-overview of distribution and threat to natural systems. In *Proceedings of a workshop held at HMAS Stirling, Garden Island, Western Australia: Arum Lily (Zantedeschia aethiopica)*. CRC for Weed management Systems, Adelaide, Australia.

KING, T., HEMSWORTH, P. H. & COLEMAN, G. J. (2003). Fear of novel and startling stimuli in domestic dogs. *Applied Animal Behaviour Science* **82**(1), 45-64.

KURIYAMA, T. & HASEGAWA, M. (2017). Embryonic developmental process governing the conspicuousness of body stripes and blue tail coloration in the lizard Plestiodon latiscutatus: Developmental color pattern formation in lizard. *Evolution & Development* **19**, 29-39.

KUTT, A. (2012). Feral cat (Felis catus) prey size and selectivity in north-eastern Australia: Implications for mammal conservation. *Journal of Zoology* **287**(4), 292-300.

LAND, M. F. & NILSSON, D. E. (2012). *Animal Eyes*. OUP Oxford.

LENTH, R. (2019). emmeans: Estimated Marginal Means, aka Least-Squares Means.

LIMA, S. L. & DILL, L. M. (1990). Behavioral decisions made under the risk of predation: a review and prospectus. *Canadian Journal of Zoology* **68**(4), 619-640.

LIND, O., MITKUS, M., OLSSON, P. & KELBER, A. (2013). Ultraviolet sensitivity and colour vision in raptor foraging. *The Journal of Experimental Biology* **216**, 1819-26.

MARCHANT, S. & HIGGINS, P. J. (1990). *Handbook of Australian, New Zealand & Antarctic birds / S. Marchant and P.J. Higgins (co-ordinators)*. Melbourne : Oxford University Press, Melbourne.

MASTERS, C. & SHINE, R. (2003). Sociality in lizards: family structure in free-living King's Skinks *Egernia kingii* from southwestern Australia. *Australian Zoologist* **32**(3), 377-380.

MEATHREL, C. E. & KLOMP, N. I. (1990). Predation of little penguin eggs by King's skinks on Penguin Island, Western Australia. *Corella* **14**(4), 129-130.

MITKUS, M., POTIER, S., MARTIN, G. R., DURIEZ, O. & KELBER, A. (2018). Raptor Vision. Oxford University Press.

NICHOLLS, C. A. (1974). Double-brooding in a Western Australian Population of the Silver Gull, Larus novaehollandiae Stephens. *Australian Journal of Zoology* **22**(1), 63-70.

NORDBERG, E. J. & SCHWARZKOPF, L. (2019). Predation risk is a function of alternative prey availability rather than predator abundance in a tropical savanna woodland ecosystem. *Scientific Reports* **9**(1), 7718.

OVERSBY, W., FERGUSON, S. M., DAVIS, R. A. & BATEMAN, P. W. (2018). Bad news for bobtails: understanding predatory behaviour of a resource-subsidised corvid towards an island endemic reptile *Wildlife Research* **45**(7), 595-601.

PAFILIS, P., FOUFOPOULOS, J., POULAKAKIS, N., LYMBERAKIS, P. & VALAKOS, E. D. (2009). Tail shedding in Island lizard [LACERTIDAE, REPTILIA]: Decline of antipredator defenses in relaxed predation environments. *Evolution* **63**(5), 1262-1278.

PEARSON, D., SHINE, R. & HOW, R. (2002). Sex-specific niche partitioning and sexual size dimorphism in Australian pythons (*Morelia spilota imbricata*). *Biological Journal of the Linnean Society* **77**(1), 113-125.

PLAYFORD, P. E. (1988). *Guidebook to the geology of Rottnest Island*. Geological Society of Australia, WA Division and the Geological Survey of Western Australia.

POTIER, S., BONADONNA, F., KELBER, A., MARTIN, G. R., ISARD, P.-F., DULAURENT, T. & DURIEZ, O. (2016). Visual abilities in two raptors with different ecology. *The Journal of Experimental Biology* **219**(17), 2639-2649.

R DEVELOPMENT CORE TEAM. (2013). R: A language and environment for statistical computing In R Foundation for Statistical Computing, Vienna, Austria.

RÖßLER, D. C., PRÖHL, H. & LÖTTERS, S. (2018). The future of clay model studies. *BMC Zoology* **3**(1), 6.

ROTTNEST ISLAND AUTHORITY. (2015). Terrestrial management Strategy (ed. R. I. Authority).

ROWLEY, I. & VESTJENS, W. J. (1973). The comparative ecology of Australian corvids. V. Food. *CSIRO Wildlife Research* **18** (1), 131-155.

SOMAWEERA, R., BRIEN, M. & SHINE, R. (2013). The Role of Predation in Shaping Crocodilian Natural History. *Herpetological Monographs* **27**(1), 23-51.

STANKOWICH, T. (2012). Armed and dangerous: Predicting the presence and function of defensive weaponry in mammals. *Adaptive Behavior* **20**, 32-43.

STEVENSON, C. (2011). *Ecological impacts of Australian Ravens on bush bird communities on Rottnest Island*. Honours thesis, Murdoch University.

STORR, G. (1978). The genus *Egernia* (Lacertilia, Scincidae) in Western Australia. *Records of the Western Australian Museum* **6**(2), 147-187.

THAKER, M., VANAK, A. T., OWEN, C. R., OGDEN, M. B., NIEMANN, S. M. & SLOTOW, R. (2011). Minimizing predation risk in a landscape of multiple predators: effects on the spatial distribution of African ungulates. **92**(2), 398-407.

THIBAULT, M., BRESCIA, F., JOURDAN, H. & VIDAL, E. (2017). Invasive rodents, an overlooked threat for skinks in a tropical island hotspot of biodiversity. *New Zealand Journal of Ecology* **41**, 74-83.

WEBB, J. K. & WHITING, M. J. (2005). Why don't small snakes bask? Juvenile broad-headed snakes trade thermal benefits for safety. *Oikos* **110**(3), 515-522.

Chapter 4. Increased tail length in the King's skink, *Egernia kingii* (Reptilia: Scincidae): an anti-predation tactic for juveniles?

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Figure 4.1: An autotomised King's skink (*Egernia kingii*) tail found in the field on Garden Island

4.1 Abstract

Caudal autotomy is an adaptive, but costly, anti-predation strategy used by many lizard species. As predation risk varies with ontogenetic life stage, it can be predicted that the use of costly anti-predation mechanisms would also change if they are no longer required. Here, we assess ontogenetic change in relative tail length and degree of caudal autotomy in the King's skink (*Egernia kingii*), a large skink endemic to Western Australia. We found that younger individuals invested more in relative tail length than did older ones, with younger individuals also having a higher degree of their tail comprised of regenerated tissue. This appears to reflect an ontogenetic shift in the risk of predation for this species, with larger, more mature individuals capable of actively defending themselves against certain predator types and therefore decreasing their reliance on a costly anti-predation strategy compared to juveniles.

4.2 Introduction

Many animals employ specialised tactics to escape predators. Autotomy, the ability to shed part of the body to escape a predator, is an extreme example. This anti-predatory trait is found across a multitude of taxa (Maginnis, 2006; Fleming, Muller & Bateman, 2007). In particular, caudal autotomy – the shedding of part of the tail - is found in many squamates, including lizards, tuatara and some snakes (Arnold, 1988; Cooper & Alfieri, 1993; Clause & Capaldi, 2006; Seligmann, Moravec & Werner, 2008). Lizards with advanced caudal autotomy ability possess breakage planes within a series of their caudal vertebrae (Etheridge, 1967; Bellairs & Bryant, 1985). Once autotomised, the tail regenerates with a rigid cartilage rod lacking autotomy planes in place of the vertebrae (Woodland, 1920; Alibardi, 2010). For the tail to be voluntarily shed again (e.g. following another predation attempt), it would need to autotomise at a plane more proximal to the body (Arnold, 1984; Bateman & Fleming, 2009). Thus, it has been suggested that a longer tail could allow for more opportunities of autotomy and escape from predators than would be possible for individuals with shorter tails (Bateman & Fleming, 2009; Fleming, Valentine & Bateman, 2013).

The adaptive value of caudal autotomy is likely to be context dependent as it can be energetically costly to regrow the tail (Vitt, Congdon & Dickson, 1977). The concept that larger species of lizards are more capable of defending themselves than smaller ones, and as a result may not need to rely on such extreme tactics as autotomy is outlined in Arnold (1984). However, this is highly likely to depend on the individual's size in relation to their average predator, as well as their risk of predation, as indicated by Fleming *et al.* (2013). In their review, Fleming *et al.* (2013) reported that more gracile lizard species tend to have longer tails than robust individuals, reflecting the prediction that investment in tails is influenced by potential predation pressure.

Additionally, anti-predation tactics, including caudal autotomy, can change ontogenetically (Barbosa & Castellanos, 2005; Creer, 2005). Adults often have fewer predators than juveniles due to an increase in body size, and/or the development of defensive weaponry, reducing the number of predatory taxa able to subdue them (Saporito *et al.*, 2010; Stankowich, 2012). Juvenile Balkan green lizards, *Lacerta trilineata*, are willing to rapidly drop their tails, but adults lose the ability (or at least their willingness) to autotomise their tail (Pafilis & Valakos, 2008). The use of autotomy as an anti-predation tactic as juveniles can be enhanced through redirection of predation attempts (Humphreys & Ruxton, 2018). Several species of lizards possess brightly coloured tails, or 'motion dazzling' patterns that help redirect predator attacks away from their bodies as juveniles, but as they mature, the colour fades to become uniform with the rest of the animal (Vitt & Cooper Jr, 1986; Castilla *et al.*, 1999; Fitch, 2003; Hawlena *et al.*, 2006; Bateman, Fleming & Rolek, 2014; Murali & Kodandaramaiah, 2018). These colour changes may also be associated with behavioural changes. Juvenile lizards that forage in the open more than adults are more exposed to visual predators, and thus having a tail that is more brightly coloured than the body would be adaptive as part of the ‘risky decoy’ hypothesis (Bateman *et al.*, 2014). Similarly, lizard species that actively forage also tend to have longer tails (Fleming *et al.*, 2013). Therefore, just as investment in tail length as a defence mechanism may alter between species, we hypothesise that this should also be true for life stages: if it were adaptive to invest in a longer tail during the ontogenetic stages most vulnerable to predation, then tail length should be proportionally longer in juveniles than adults in lizard species that rely on other defence tactics when they mature. This is particularly likely if both resource and energy allocation to different body parts for both growth and regeneration are costly (Barbosa & Castellanos, 2005). Here, we assess ontogenetic shifts in tail length and prevalence of caudal regeneration in the King’s skink, *Egernia kingii*, to investigate potential shifts in anti-predation tactics between life stages.

The King’s skink is a large (up to 550 mm total length), uniformly coloured skink endemic to Western Australia (Storr, 1978; Cogger, 2014). At birth, individuals measure 60 – 80 mm with a mean mass of 7 g. Individuals take approximately five years to mature, reaching a snout to vent (SVL) up to 244 mm (Storr, 1978) with an average weight of between 220 - 360 g, resulting in a 30-50 fold increase in weight (Dilly, 2000; Arena & Wooller, 2003). Juveniles have been found to experience higher predation risk than adults (Aubret *et al.*, 2004), and adults have been reported to actively chase and defend themselves against certain predators (Masters & Shine, 2003). For long-lived species, like *E. kingii*, where there is a change from relatively small, gracile juveniles to larger, robust adults, we hypothesise that there will be an ontogenetic change in investment in tail length that reflects predictions based on potential predation pressure and concomitant reliance on autotomy (Fleming *et al.*, 2013). Specifically, we predict that:

1) As *E. kingii* individuals grow, they will reduce their investment in their tail, such that adults will have proportionally shorter tails, reflecting reduced reliance on the tail as an anti-predator device through autotomy.

2) Furthermore, if the adaptiveness of autotomy varies with life stage, we predict that this will be reflected in patterns of tail regeneration. That is, more

adults will show evidence of regeneration than juveniles, due to a longer period of time available to them in which to lose tails, but will also have lower relative proportion of their tail consisting of regenerated tissue, indicating that autotomy occurred when they were younger.

4.3 Methods

We studied 300 preserved specimens from the Western Australia Museum. For each specimen, we recorded the snout-vent length (SVL), tail length (TL) and any regeneration length (RL) to the nearest millimetre using a flexible material tape measure. Incidence of regeneration (the proportion of samples with regeneration) was calculated for each age class, as was the proportion of regeneration (RL/TL). Mass was not recorded due to potential inaccurate measurements from preservation methods. Tail regeneration was identified from multiple morphological indications including evident colour change in a region of the tail, change in scale pattern of a caudal region or narrowing of a tail segment. Specimens that had their tail tip or part of the tail missing were excluded from analysis as this might reflect post-mortem tail loss. Individuals that had their complete (intact), original (without regeneration) tail were used to assess SVL and TL correlation, as well as having their relative tail lengths (TL/SVL) calculated for comparison among the age classes.

As *E. kingii* are long-lived species, individuals were classified into four age categories to comprehensively investigate ontogenetic changes: neonates (SVL 60 - 80 mm), less than two years old (80 mm < SVL < 150 mm), two - four years old (150 mm < SVL < 180 mm) and four plus year old (SVL > 180 mm), as indicated by Arena (1986). *E. kingii* lack evident secondary sexual characteristics; therefore, sex was determined from head width (HW) to SVL ratios for adults only (SVL ≥ 185 mm) as indicated by Arena & Wooller (2003) for males (≥ 0.163) and females (≤ 0.154). A total of 24 individuals fell between these ratios and as a result had their sex classified as unknown and were not included in analyses when comparing the sexes.

All analyses were conducted in R studio version 1.1.383 (R Development Core Team, 2013). Linear models (LM's) were used to assess the effect of age class on skinks' relative tail length (TL/SVL) and correlation between SVL and TL for the age classes. Regression statistics for the SVL and TL model were acquired via the summary function, and residuals extracted via the residual function. To understand if age classes had similar variation within their age groups, a homogeneity of variance were assessed using a Levene test. General linear models (GLM's) were used to assess the influence of age class on the incidence of regeneration (binomial family) and the proportion of regeneration (Gamma family) and Wilcoxon tests were used to compare these measures between sexes.

P-values were derived using either F-tests (for linear models) or Wald chi-square tests (for generalised linear models) calculated using the function 'Anova' in the package 'car' (Fox *et al.*, 2017). Differences among age groups were analysed using post hoc Tukey tests via the glht function in the package multcomp (Hothorn *et al.*, 2017). Data distributions were examined using frequency histograms, with residual and QQ plots for appropriateness of LM's and GLM's.

4.4 Results

4.4.1 Body size and Tail length correlations

Of the 300 *E. kingii* specimens assessed, 110 (36.7%) had their complete original tail, 130 (43.3%) showed evidence of regeneration, and 60 specimens (20.0%) were missing varying degrees of their tail (excluded form analysis). Of the 110 individuals with their intact, original tail a strong significant correlation between SVL and TL was observed, but varied for the individual age classes (Table 4.1). Correlation strength increased from neonates to less than two year old's, followed by a gradual decrease thereafter as the animals matured (Figure 4.2). Residuals extracted from the linear model showed a significant difference in variation among the age groups (F= 25.6, P < 0.001) (Figure 4.4), with the two - four year old and four years plus age groups having much larger variation than did the neonates and the less than two year age categories. Skink age classes differed significantly in their average relative tail lengths (TL/SVL ratios) ($F = 15.1$, df $= 3$, $P < 0.001$). Less than two year old's ($t = 6.291$, $P < 0.001$) and two-four year old's ($t = 2.96$, $P < 0.05$) had significantly longer relative tail lengths compared to the four plus age category, with relative tail length clearly decreasing as the animal matures (Figure 4.3). There was no significant difference in relative tail length between adult males and females ($t = -1.18$, df $= 21.7$, $P = 0.251$).

Table 4.1: TL and SVL relationship equation, correlation and significance for *Egernia kingii* and their specific age categories as displayed in Figure 4.2

Age category	Sample size	Linear relationship	Correlation (R^2 value)	R^2 significance
All age classes	110	$Y = 1.06x + 27.2$	0.838	$P < 0.001$
Newborn	23	$Y = 0.934x + 27.8$	0.277	$P < 0.001$
Less than two years	34	$Y = 1.31x - 4.32$	0.816	$P < 0.001$
Two – four years	20	$Y = 2.77x - 246$	0.474	$P = 0.001$
Four plus years	33	$Y = 1.23x - 14.6$	0.206	$P < 0.01$

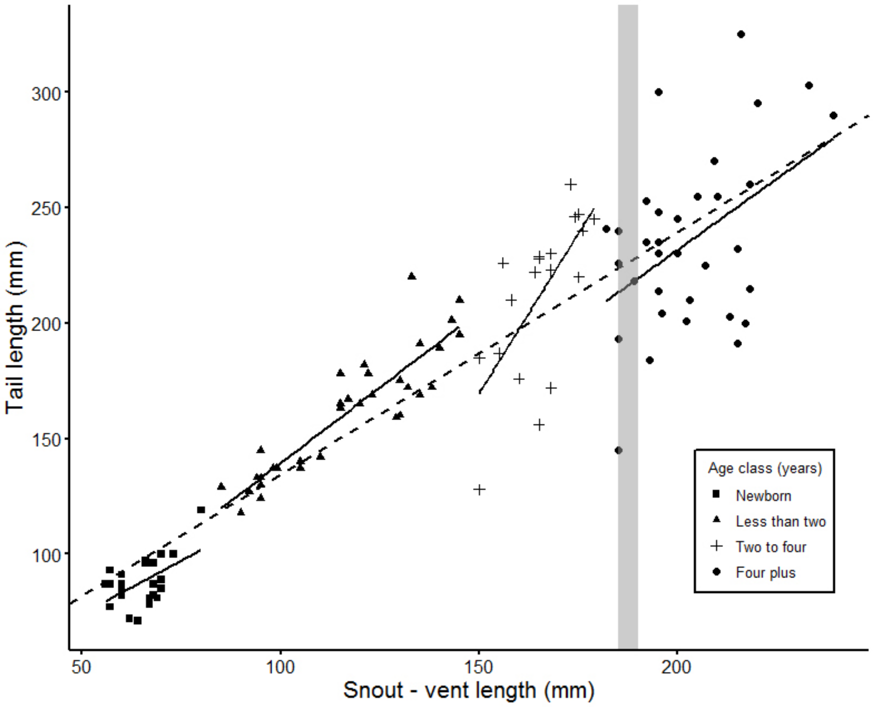

Figure 4.2: Correlation between snout to vent Length (SVL) and tail length (TL) for all samples (dashed line) and different *Egernia kingii* age classes (solid lines) with intact tails (n = 110). Shaded section represents the SVL range of skinks when they reach maturity (Arena & Wooller, 2003).

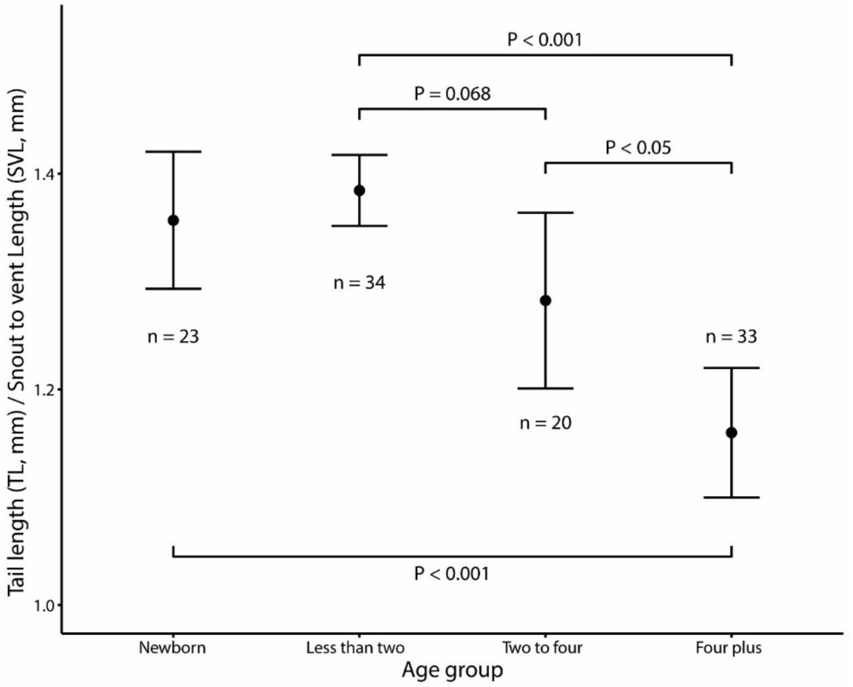

Figure 4.3: Comparison of relative tail length (tail length (TL) / snout to vent Length (SVL)) for different *Egernia kingii* age classes with intact tails (n = 110). Average ± 95% CI shown with number of specimens in each age class (n). P - values as indicated from post hoc Tukey test of the model.

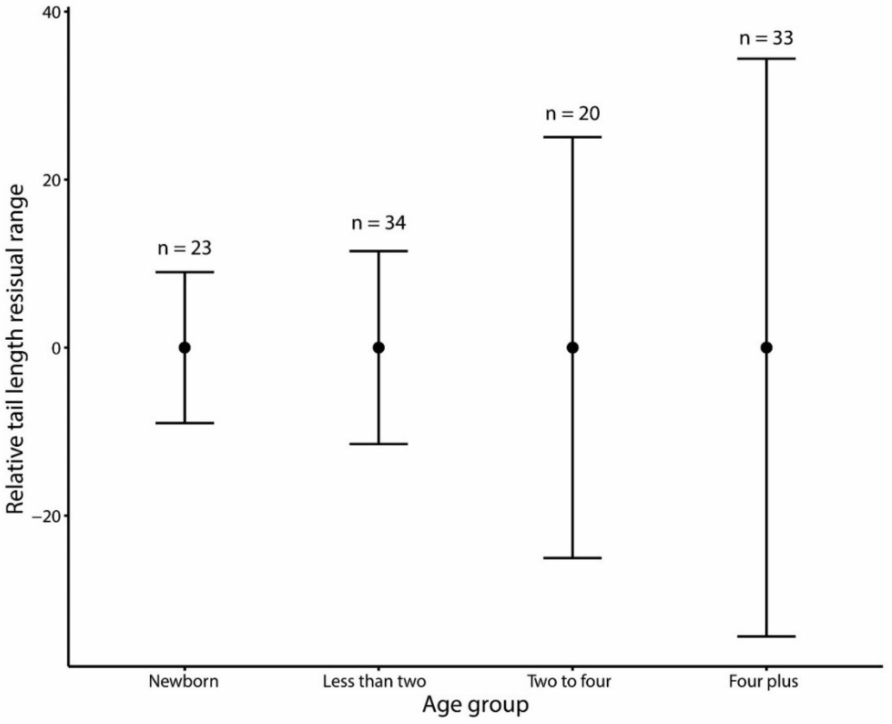

Figure 4.4: Variation in the residuals of the relative tail length of *Egernia kingii* specimen age groups with intact tails (n = 110) extracted from the linear model. Average ± SD shown with number of specimens in each age class (n).

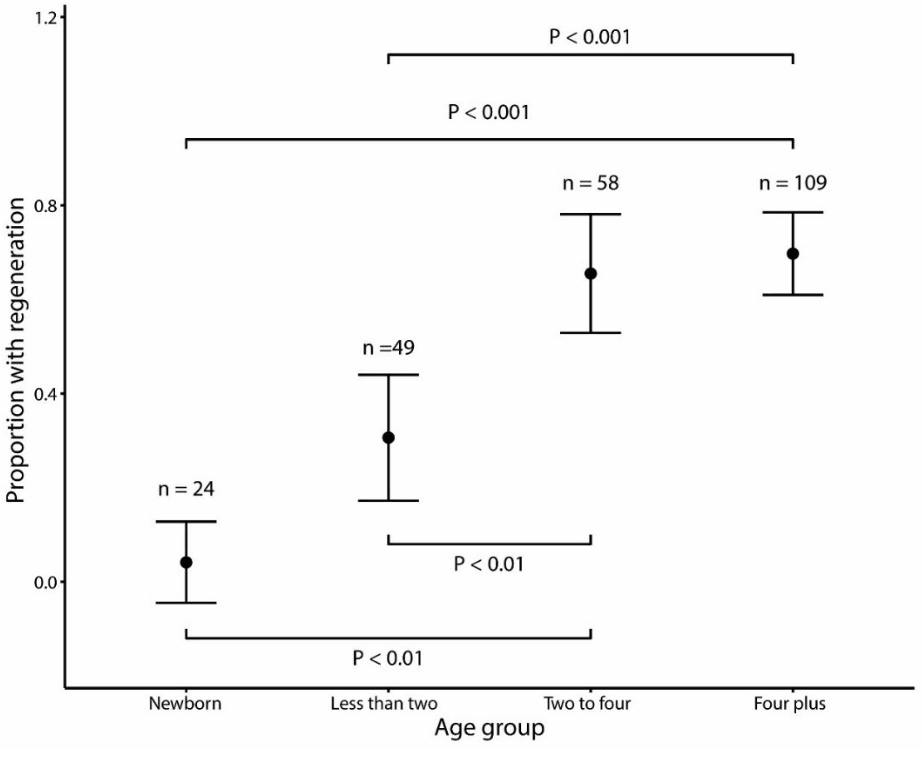

Figure 4.5: Proportion of *Egernia kingii* specimens exhibiting regeneration for each age class. Average ± 95% CI shown with number of specimens in each age class (n). P - values as indicated from post hoc Tukey test.

4.4.2 Incidence and degree of regeneration

Incidence of regeneration significantly different among age classes ($\chi^2 = 54.0$, df = 3, $P < 0.001$) for the 130 specimens that had regenerated tails. The incidence of regeneration increased with age, with a smaller incidence of regeneration in the neonate and less than two year old categories than the two - four year old and four years plus age categories (Figure 4.5). No significant difference in the proportion of tail comprised of regenerated tissue was detected among age categories ($\chi^2 = 5.43$, df = 2, $P = 0.143$). Younger *E. kingii* had a larger portion of their total tail length that comprised still regenerating tissue compared to those of the more mature skinks (Figure 4.6); however, the difference among age classes was not significant. There was no significant difference in the incidence of regeneration (W = 936, P-value = 0.124, n = 81), or the percentage of tail regenerated (W = 438, p-value = 0.319, n= 55) between the males and females.

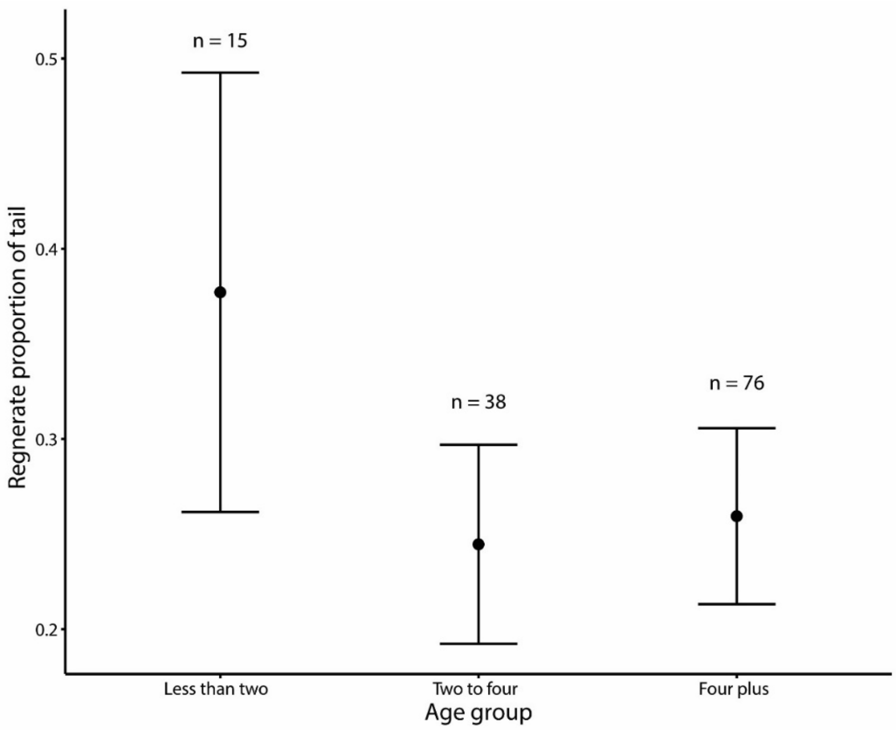

Figure 4.6: Comparison of the proportion of regeneration to the total tail length of the age classes for *Egernia kingii* that exhibited caudal regeneration. Average ± 95% CI shown with number of specimens in each age class (n). Neonates are not included; only one skink in this category showed evidence of tail regeneration.

4.5 Discussion

Our results suggest that there is an ontogenetic shift in relative tail length as *E. kingii* age, with younger individuals investing more in tail length than do adults, but that there is no difference between adult males and females. Our predictions regarding patterns of tail regeneration reflecting ontogenetic variation in reliance on autotomy in *Egernia kingii* were also supported: younger individuals (not including neonates) had a larger relative proportion of tail comprised of regenerating tissue than did older individuals, with the overall incidence of regeneration increasing as the animals aged. Regenerated tissue mainly accumulated within the first two years of life (only the one neonate showed evidence of regeneration) and eventually plateaued. Although multiple factors can influence tail length and regeneration patterns (Vitt *et al.*, 1977; Bateman & Fleming, 2009; Fleming *et al.*, 2013), we suggest that this change in relative tail length and regeneration is most likely to be influenced by a change in predation risk during ontogeny, which we discuss below.

Egernia kingii ontogenetically change from small, gracile lizards to large robust ones (Arena & Wooller, 2003). Arena (1986) found that after *E. kingii* reach maturity they go through a period of reduced SVL growth, but a marked rate of evenly distributed growth in body mass, resulting in a larger and more robust animal. As a result, adults seem to be large enough to actively defend themselves against some predators. Adult *E. kingii* have been recorded fighting with and chasing away both tiger snakes, *Notechis scutatus* (Masters & Shine, 2003) and Rottnest Island dugites, *Pseudonaja affinis exilis* (J. Barr, *pers. obs.*). Juvenile *E. kingii* are reported to experience higher predation risk than adults (Arena, 1986; Aubret *et al.*, 2004), and are more likely to flee quicker from threats than are adults (Masters & Shine, 2003; J. Barr, *pers. obs.*). However, as caudal autotomy is used as an anti-predation tactic in life, it serves no function until the individual is born (Moffat & Bellairs, 1964).Our data suggest that investment in tail length is most important within the first two years of their life for *E. kingii,* perhaps when they are most vulnerable, where utilisation of autotomy would be most valuable. As they mature, ontogenetic increases in size and robustness may allow adult *E. kingii* to adopt a more active anti-predation behavioural defence strategies, and as a result they may not need to resort to the costlier process of caudal autotomy.

However, once mature, *E. kingii* are still susceptible to some predators, being recorded in the stomachs of South-west carpet python, *Morelia spilota imbricata* (Pearson, Shine & How, 2002). Although younger individuals had a stronger investment in their relative tail length than older skinks (Figure 4.2), the residual data (Figure 4.5) indicates that there is substantial variation in relative tail length among older individuals (Four plus age class), with some individuals having longer tails than others. This variation in relative tail length among older individuals might reflect differences in predation risk between different environments; if some populations are exposed to high predation pressure from different types of predators once mature, caudal autotomy may still be utilised an effective anti-predation mechanism.

Predators of *E. kingii* include highly venomous elapids (Aubret *et al.*, 2004), which use a combination of their highly potent venom and constriction to subdue their prey (Shine & Schwaner, 1985). In addition to active defence against such predators, *E. kingii* may also possess immunity to venomous predators. Three relatives of *E. kingii (E. striolata*, *E. cunninghami* and *E. whitii*) all display high resistances to large doses of sympatric snake venoms, including the tiger snake, and the eastern brown snake *Pseudonaja textilis* (Minton & Minton, 1981). Lizards that escape venomous snakes may still receive a considerable dose of venom if bitten, but may have sufficient immunity to survive the encounter (Minton & Minton, 1981). The ontogenetic stage or mass of the test animals were not recorded in Minton & Minton (1981), and, therefore, inference of ontogenetic resistance in lizard species could not be assessed. However, many elapids undergo ontogenetic shifts in their venom composition and potency, correlated to dietary shifts, with saurophagous juvenile snakes having a more potent venom against lizards compared to adults that are more diet generalists (Cipriani *et al.*, 2017; Wolfe, Bateman & Fleming, 2018). If younger *E. kingii* are more susceptible to sympatric elapid venoms, and have a reduced ability to struggle free, it would be adaptive to provide a larger relative target in the tail to directing the attack away from the body and allow the use of caudal autotomy to stop venom entering the blood stream, and to escape from the predator.

Anti-predation mechanisms such as tail autotomy may also be employed to escape conspecific aggression (Pafilis *et al.*, 2009). For example, autotomy rates in two gecko species, *Hemidactylus turcicus* and *Mediodactylus kotschyi*, were positively correlated with gecko density, suggesting that increased intraspecific aggression was the main driver (Itescu *et al.*, 2017). Reliance on tail autotomy and, therefore, investment in relative tail length, may aid younger lizards of species that are subjected to conspecific aggression. Skinks in the *Egernia* radiation (Gardner *et al.*, 2008) range from solitary to highly stable family groups (Chapple, 2003). Although not studied in *E. kingii*, infanticide of young is known to occur in related group-living species such as *E. stokesii* (Lanham & Bull, 2000; O'Connor & Shine, 2004). *Egernia kingii* is thought to be group-living, with up to four adults sharing a common core home range, in addition to multiple sub adults and juveniles (Chapple, 2003; Masters & Shine, 2003; Barr, 2016). Increased investment in relative tail length may aid younger skinks to both escape from predators and from aggressive mature conspecifics.

Lizard tails can have a wide range of specialised functions including intraspecific competition (Murphy & Mitchell, 1974; Schall *et al.*, 1989; Tsellarius & Tsellarius, 1997), signalling (Johnson & Brodie Jr, 1974; Vitt & Cooper Jr, 1986; Cooper, 2001), and as prehensile tails for gripping (Losos, Walton & Bennett, 1993). Tail specialisations can also undergo ontogenetic changes; for example, several skink species have bright conspicuously-coloured tails as juveniles that appear to have a role in directing attacks toward the tail and away from the body (Cooper & Vitt, 1985; Hawlena *et al.*, 2006; Pafilis & Valakos, 2008; Bateman *et al.*, 2014; Fresnillo, Belliure & Cuervo, 2016). As the skinks mature, this deflecting colouration is lost. Although the original tails of *E. kingii* do not show a distinct contrast from their bodies, regenerated tails in juvenile *E. kingii* are much darker in colour than the intact parts (J. Barr, pers. obs.). As the individual and the regenerated portion of the tail matures, the distinct colour contrast appears to fade, resembling the colour of the original tail (J. Barr, pers. obs.). This distinct contrast, in both *E. kingii* and other species that exhibit similar contrasting regeneration, may act to redirect subsequent predator attacks towards the regenerating distal tail as juveniles. This in turn would minimise the amount of remaining original tail that would be lost in subsequent attacks and contributing towards an adaptive economy of autotomy (Cromie & Chapple, 2013). However, further investigation would need to be conducted in order to identify if the contrasting colour of the regenerate, as observed in other lizard species, aids in redirecting attacks away from their original tail.

In conclusion, our study of *E. kingii* indicates that they undergo an adaptive ontogenetic shift in tail investment, with higher utilisation of caudal autotomy within the first two years of life. They favour a longer relative tail length when younger, which provides a larger target for predators to attack, and rely on caudal autotomy to allow escape a predator. As they mature, they are more capable of active defence, having relatively shorter tails and are more able to escape predation events without relying on autotomy. This however, is likely to have a degree of plasticity, and be influenced by type or diversity of predator/s present in their environment (Pearson *et al.*, 2002).

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4.7 References

“Every reasonable effort has been made to acknowledge the owners of copyright material. I would be pleased to hear from any copyright owner who has been omitted or incorrectly acknowledged.”

LIBARDI, L. (2010). *Morphological and Cellular Aspects of Tail and Limb Regeneration in Lizards : a Model System with Implications for Tissue Regeneration in Mammals*. Springer-Verlag, Heidelberg, Berlin

ARENA, P. (1986). *Aspects of the biology of the King's skink Egernia kingii (Gray)*. Honours thesis thesis, Murdoch University.

ARENA, P. C. & WOOLLER, R. D. (2003). The reproduction and diet of *Egernia kingii* (Reptilia : Scincidae) on Penguin Island, Western Australia. *Australian Journal of Zoology* **51**(5), 495-504.

ARNOLD, E. (1984). Evolutionary aspects of tail shedding in lizards and their relatives. *Journal of Natural History* **18**(1), 127-169.

ARNOLD, E. (1988). Caudal autotomy as a defense. *Biology of the Reptilia* **16**, 235-273.

AUBRET, F., BONNET, X., MAUMELAT, S., BRADSHAW, D. & SCHWANER, T. (2004). Diet divergence, jaw size and scale counts in two neighbouring populations of tiger snakes (*Notechis scutatus*). *Amphibia-Reptilia* **25**(1), 9-17.

BARBOSA, P. & CASTELLANOS, I. (2005). *Ecology of predator-prey interactions*. Oxford University Press.

BARR, J. (2016). *Social structure and the influence of personality traits in the King’s skink (Egernia kingii) on Penguin Island, Western Australia*. Honours thesis thesis, Murdoch University.

BATEMAN, P. & FLEMING, P. (2009). To cut a long tail short: a review of lizard caudal autotomy studies carried out over the last 20 years. *Journal of Zoology (London)* **277**(1), 1-14.

BATEMAN, P., FLEMING, P. & ROLEK, B. (2014). Bite me: Blue tails as a ‘risky-decoy’defense tactic for lizards. *Current Zoology* **60**(3), 333-337.

BELLAIRS, A. & BRYANT, S. (1985). Autotomy and regeneration in reptiles. *Biology of the Reptilia* **15**(5), 301-410.

CASTILLA, A. M., GOSA, A., GALAN, P. & PEREZ-MELLADO, V. (1999). Green tails in lizards of the Genus *Podarcis*: Do they influence the intensity of predation? *Herpetologica* **55**(4), 530-537.

CHAPPLE, D. G. (2003). Ecology, life-history, and behaviour in the Australian scincid genus *Egernia*, with comments on th evolution of complex sociality in lizards. *Herpetological Monographs* **17**(1), 145-180.

CIPRIANI, V., DEBONO, J., GOLDENBERG, J., JACKSON, T. N. W., ARBUCKLE, K., DOBSON, J., KOLUDAROV, I., LI, B., HAY, C., DUNSTAN, N., ALLEN, L., HENDRIKX, I., KWOK, H. F. & FRY, B. G. (2017). Correlation between ontogenetic dietary shifts and venom variation in Australian brown snakes (*Pseudonaja*). *Comparative Biochemistry and Physiology Part C Toxicology & Pharmacology* **197**, 53-60.

CLAUSE, A. R. & CAPALDI, E. A. (2006). Caudal autotomy and regeneration in lizards. *Journal of Experimental Zoology* **305**(12), 965-973.

COGGER, H. G. (2014). *Reptiles and Amphibians of Australia*, 7th edition. CSIRO Publishing, Victoria, Australia.

COOPER, W. E. (2001). Multiple roles of tail display by the Curly-Tailed Lizard *Leiocephalus carinatus*: pursuit deterrent and deflective roles of a social signal. *Ethology* **107**(12), 1137-1149.

COOPER, W. E. & ALFIERI, K. J. (1993). Caudal autotomy in the eastern garter snake, *Thamnophis s. sirtalis*. *Amphibia-Reptilia* **14**(1), 86-89.

COOPER, W. E. & VITT, L. J. (1985). Blue tails and autotomy: enhancement of predation avoidance in juvenile skinks. *Zeitschrift fuer Tierpsychologie* **70**(4), 265-276.

CREER, D. A. (2005). Correlations between ontogenetic change in color pattern and antipredator behavior in the racer, *Coluber constrictor*. *Ethology* **111**(3), 287-300.

CROMIE, G. L. & CHAPPLE, D. G. (2013). Is partial tail loss the key to a complete understanding of caudal autotomy? *Austral Ecology* **38**(4), 452-455.

DILLY, M. L. (2000). *Factors affecting the distribution and variation in abundance of the King's skink (Egernia kingii) (Gray) in Western Australia*. Honours thesis thesis, Murdoch University.

ETHERIDGE, R. (1967). Lizard caudal vertebrae. *Copeia* **1967**(4), 699-721.

FITCH, H. S. (2003). A comparative study of loss and regeneration of lizard tails. *Journal of Herpetology* **37**(2), 395-399.

FLEMING, P. A., MULLER, D. & BATEMAN, P. W. (2007). Leave it all behind: a taxonomic perspective of autotomy in invertebrates. *Biological Reviews* **82**(3), 481-510.

FLEMING, P. A., VALENTINE, L. E. & BATEMAN, P. W. (2013). Telling tails: selective pressures acting on investment in lizard tails. *Physiological and Biochemical Zoology* **86**(6), 645-658.

FOX, J., WEISBERG, S., ADLER, D., BATES, D., BAUD-BOVY, G., ELLISON, S., FIRTH, D., FRIENDLY, M., GORJANC, G. & GRAVES, S. (2017). Package 'car'.

FRESNILLO, B., BELLIURE, J. & CUERVO, J. J. (2016). Ontogenetic shifts in risk behaviours are related to body size and coloration in spiny-footed lizards. *Animal Behaviour* **119**, 165-172.

GARDNER, M. G., HUGALL, A. F., DONNELLAN, S. C., HUTCHINSON, M. N. & FOSTER, R. (2008). Molecular systematics of social skinks: phylogeny and taxonomy of the *Egernia* group (Reptilia: Scincidae). *Zoological Journal of the Linnean Society* **154**(4), 781-794.

HAWLENA, D., BOOCHNIK, R., ABRAMSKY, Z. & BOUSKILA, A. (2006). Blue tail and striped body: why do lizards change their infant costume when growing up? *Behavioral Ecology* **17**(6), 889-896.

HOTHORN, T., BRETZ, F., WESTFALL, P., HEIBERGER, R. M., SCHUETZENMEISTER, A., SCHEIBE, S. & HOTHORN, M. T. (2017). Package 'multcomp'. Obtenido de http://cran. stat. sfu. ca/web/packages/multcomp/multcomp. pdf.

HUMPHREYS, R. K. & RUXTON, G. D. (2018). What is known and what is not yet known about deflection of the point of a predator's attack. *Biological Journal of the Linnean Society* **123**(3), 483-495.

ITESCU, Y., SCHWARZ, R., MEIRI, S., PAFILIS, P. & CLEGG, S. (2017). Intraspecific competition, not predation, drives lizard tail loss on islands. *Journal of Animal Ecology* **86**(1), 66-74.

JOHNSON, J. A. & BRODIE JR, E. D. (1974). Defensive behaviour of the western banded gecko, *Coleonyx variegatus*. *Animal Behaviour* **22**, 684-687.

LANHAM, E. J. & BULL, C. (2000). Maternal care and infanticide in the Australian skink, *Egernia stokesii*. *Herpetological Review* **31**(3), 151-152.

LOSOS, J. B., WALTON, B. M. & BENNETT, A. F. (1993). Trade-offs between sprinting and clinging ability in Kenyan Chameleons. *Functional Ecology* **7**(3), 281-286.

MAGINNIS, T. L. (2006). The costs of autotomy and regeneration in animals: a review and framework for future research. *Behavioral Ecology* **17**(5), 857-872.

MASTERS, C. & SHINE, R. (2003). Sociality in lizards: family structure in free-living King's Skinks *Egernia kingii* from southwestern Australia. *Australian Zoologist* **32**(3), 377-380.

MINTON, S. A. & MINTON, M. R. (1981). Toxicity of some Australian snake venoms for potential prey species of reptiles and amphibians. *Toxicon* **19**(6), 749-755.

MOFFAT, L. A. & BELLAIRS, A. D. A. (1964). The regenerative capacity of the tail in embryonic and post-natal lizards (*Lacerta vivipara*). *Journal of Embryology and Experimental Morphology* **12**(4), 769-786.

MURALI, G. & KODANDARAMAIAH, U. (2018). Body size and evolution of motion dazzle coloration in lizards. *Behavioral Ecology* **29**(1), 79-86.

MURPHY, J. B. & MITCHELL, L. A. (1974). Ritualized combat Behavior of the Pygmy Mulga Monitor lizard, *Varanus gilleni* (Sauria: Varanidae). *Herpetologica* **30**(1), 90-97.

O'CONNOR, D. E. & SHINE, R. (2004). Parental care protects against infanticide in the lizard *Egernia saxatilis* (Scincidae). *Animal Behaviour* **68**(6), 1361-1369.

PAFILIS, P., MEIRI, S., FOUFOPOULOS, J. & VALAKOS, E. (2009). Intraspecific competition and high food availability are associated with insular gigantism in a lizard. *Naturwissenschaften* **96**(9), 1107-1113.

PAFILIS, P. & VALAKOS, E. D. (2008). Loss of caudal autotomy during ontogeny of Balkan Green Lizard, *Lacerta trilineata*. *Journal of Natural History* **42**(5-8), 409-419.

PEARSON, D., SHINE, R. & HOW, R. (2002). Sex-specific niche partitioning and sexual size dimorphism in Australian pythons (*Morelia spilota imbricata*). *Biological Journal of the Linnean Society* **77**(1), 113-125.

R DEVELOPMENT CORE TEAM. (2013). R: A language and environment for statistical computing in R Foundation for Statistical Computing, Vienna, Austria.

SAPORITO, R. A., ISOL, A. M., MACCACHERO, V. C., CONDON, K. & DONNELLY, M. A. (2010). Ontogenetic scaling of poison glands in a dendrobatid poison frog. *Journal of Zoology (London)* **282**(4), 238-245.

SCHALL, J. J., BROMWICH, C. R., WERNER, Y. L. & MIDLEGE, J. (1989). Clubbed regenerated tails in *Agama agama* and their possible use in social interactions. *Journal of Herpetology* **23**(3), 303-305.

SELIGMANN, H., MORAVEC, J. & WERNER, Y. L. (2008). Morphological, functional and evolutionary aspects of tail autotomy and regeneration in the 'living fossil' *Sphenodon* (Reptilia: Rhynchocephalia). *Biological Journal of the Linnean Society* **93**(4), 721-743.

SHINE, R. & SCHWANER, T. (1985). Prey constriction by venomous snakes: A review, and new data on Australian species. *Copeia* **1985**(4), 1067-1071.

STANKOWICH, T. (2012). Armed and dangerous: Predicting the presence and function of defensive weaponry in mammals. *Adaptive Behavior* **20**, 32-43.

STORR, G. (1978). The genus *Egernia* (Lacertilia, Scincidae) in Western Australia. *Records of the Western Australian Museum* **6**(2), 147-187.

TSELLARIUS, A. & TSELLARIUS, E. (1997). Behavior of *Varanus griseus* during encounters with conspecifics. *Asiatic Herpetological Research* **7**, 108-130.

VITT, L. J., CONGDON, J. D. & DICKSON, N. A. (1977). Adaptive strategies and energetics of tail autonomy in Lizards. *Ecology* **58**(2), 326-337.

VITT, L. J. & COOPER JR, W. E. (1986). Tail loss, tail color, and predator escape in Eumeces (Lacertilia: Scincidae): age-specific differences in costs and benefits. *Canadian Journal of Zoology* **64**(3), 583-592.

WOLFE, A. K., BATEMAN, P. W. & FLEMING, P. A. (2018). Does urbanization influence the diet of a large snake? *Current Zoology* **64**(3), 311-318.

WOODLAND, W. (1920). Memoirs: Some Observations on Caudal Autotomy and Regeneration in the Gecko (*Hemidactylus flaviviridis*, Rppel), with Notes on the Tails of *Sphenodon* and *Pygopus*. *Journal of Cell Science* **2**(257), 63-100.

Chapter 5. Split ends: Caudal autotomy fractures planes in a large scincid lizard, *Egernia kingii* during ontogenetic development

The study presented in this chapter is in preparation for submission to review within the peer-reviewed literature.

Barr, J.I., Boisvert, C. A., Trinajstic, K.& Bateman, P.W. Caudal autotomy fractures planes in a large scincid lizard, *Egernia kingii* during ontogenetic development. *In preparation.*

Figure 5.1: South west carpet python *(Morelia spilota imbricata*)

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5.1 Abstract

Caudal autotomy, the ability to drop a portion of the tail, is widely used in many species of lizards as an effective anti-predation strategy. In some, generally larger species, intra-vertebral fracture planes within the caudal vertebrae can ossify during ontogenetic growth, resulting in the loss of ability to autotomise the tail. As an individual grows their risk of predation can decrease (e.g. from having a larger body size), allowing them to use less costly strategies for defence, such as clawing or biting, as opposed to costly strategies like caudal autotomy. The King's skink, *Egernia kingii*, is a large lizard growing up to over half a meter and is endemic to the south west of Western Australia and surrounding islands. It undergoes a significant morphological change through ontogeny, changing from a gracile juvenile to a large robust adult capable of fighting and chasing away certain predators. Previous research has indicated that juvenile *E. kingii* rely more on autotomy that do adults. In this study, we use micro-CT to investigate the caudal morphology of *E. kingii* to determine if caudal fracture planes are lost or restricted ontogenetically. The total number of caudal vertebrae between individuals vary from 44 to 50, with pygal vertebrae numbering 5-6, and post pygal 39 – 44. There was no evidence that fracture planes were lost ontogenetically. The retention of caudal autotomy planes in adults indicates the ability to utilise caudal autotomy as a last option to avoid predation or high intra- or inter-specific aggression. However, other factors such as strengthening of caudal muscle attachments between autotomisable sections of the tail, or regulating the conscious decision by the individual to autotomize, may be in place to mitigate the unnecessary costs associated with premature caudal autotomy, particularly as an adult.

5.2 Introduction

Caudal autotomy, the ability to voluntarily shed a portion of a tail, is an effective but costly anti-predation strategy present in many species of lizards (White, 1925; Arnold, 1984; Bateman & Fleming, 2009; Emberts, Escalante

& Bateman, 2019). Species with intra-vertebral caudal autotomy, the more common and ancestral type, have a series of caudal vertebrae – the post-pygal vertebrae – containing pre-formed fracture planes within the vertebrae (Etheridge, 1967; Arnold, 1984). These vertebrae are preceded by a series of non-autotomisable vertebrae – the pygal vertebrae – usually 4-19 depending on the species, which are associated with the attachment of the *Caudifemoralis longus* muscle (CFL), and, in males, the muscles of the reproductive organs, the hemipenes (Hoffstetter & Gasc, 1969; Bellairs & Bryant, 1985; Russell & Bauer, 1992; Arnold, 1994; Zani, 1996). Once autotomised, the tail is regenerated over time with the original bony vertebrae replaced by a rigid cartilage rod lacking autotomy planes (Woodland, 1920; Alibardi, 2010). The number of autotomisable post-pygal vertebrae and their distribution along the tail vary among species (Holder, 1960; Etheridge, 1967; Hoffstetter & Gasc, 1969; Bellairs & Bryant, 1985; Arnold, 1994). This variation, in both number and position along the tail, influences the use of caudal autotomy as an anti-predation strategy by restricting the amount of tail that can be autotomised, as well as the number of successive times caudal autotomy events can occur (Arnold, 1988; Bateman & Fleming, 2009).

As both tail loss and regeneration are costly to the individual (Vitt, Congdon & Dickson, 1977; Dial & Fitzpatrick, 1981; Dial & Fitzpatrick, 1984; McElroy & Bergmann, 2013), ability or willingness to use caudal autotomy can change over time, both ontogenetically, and on an evolutionary scale for species or populations in relation to predation risk (Etheridge, 1967; Arnold, 1984; Pafilis & Valakos, 2008). Juvenile lizards generally experience higher predation risk compared to adults, relying more on caudal autotomy (Hawlena *et al.*, 2006; Bateman, Fleming & Rolek, 2014; Barr *et al.*, 2019b), with adults able to defend themselves against certain predators either due to their larger size or more effective armouring (Arnold, 1984; Masters & Shine, 2003). Juveniles can also have characteristics to amplify the effectiveness of caudal autotomy such as brightly coloured tails (Cooper & Vitt, 1985; Hawlena *et al.*, 2006) or a relatively larger tail (Barr *et al.*, 2019b) re-directing attacks away from the body. The ontogenetic loss of fracture planes through fusion of the caudal vertebrae has been described as occurring dorsal to ventral, from the neural arch extending toward the centrum, beginning at the most distal vertebrae in the tail and proceeding proximally (Etheridge, 1967). Etheridge (1967) described vertebrae as no longer being autotomous when the fusion point reaches the transverse process.

Autotomy planes are present in most species of the family Scincidae, with individuals relying heavily on caudal autotomy as an anti-predation mechanism, with the exception of the ‘Egernia group’ (Arnold, 1984). The ‘Egernia group’, comprised of the genera *Tiliqua, Cyclodomorphus, Corucia, Egernia, Bellatorias, Liopholis* and *Lissolepis* (Gardner *et al.*, 2008), include species that have lost their autotomy planes evolutionarily (*E. depressa and E. stokesii*), lose their planes ontogenetically (*Corucia zebrata*, *T. rugosa and T. scincoides*), and those who retain autotomy planes as adults (*E. cunninghami, L. inornata*, *T. gigas* and *T. nigrolutea*) (Etheridge, 1967a; Arnold, 1984; Russell & Bauer, 1992; Arnold, 1994). The loss of autotomy planes, either evolutionary or ontogenetically, is associated with species that develop tail specialisation, either as alternative anti-predation strategies such as in *E. depressa and E. stokesii*, armouring and fat storage in *T. rugosa*, or prehensility in *C. zebrata* (Etheridge, 1967; Arnold, 1984; Zani, 1996; Chapple, 2003).

The King’s skink (*Egernia kingii)* is a large skink (up to 550 mm total length) endemic to the south west of Western Australia and surrounding islands. As it grows *E. kingii* changes from a small gracile juvenile (7g, SVL 60-80 mm), to a large robust adult (between 220 – 360g, up to 244mm SVL) (Storr, 1978; Dilly, 2000; Arena & Wooller, 2003). Individuals reach sexual maturity between 185 and 190mm SVL at about 5 years of age (Arena & Wooller, 2003). Juveniles have higher predation risk than adults (Aubret *et al.*, 2004), although this varies depending on the predator type (Pearson, Shine & How, 2002), with adults known to actively defend themselves against certain types of predators (J. Barr *pers. obs*; Masters & Shine, 2003). Juveniles also show high reliance on caudal autotomy compared to adults, and may exploit this anti-predation mechanism by investing more in relative tail length than do adults to redirect attacks away from the body (Barr *et al.*, 2019b). As there seems to be a behavioural shift away from reliance on autotomy as *E. kingii* ages, it might be predicted that they also lose the physical ability to shed their tails. *Egernia kingii* therefore provides a valuable model to investigate ontogenetic changes in caudal morphology, and to explore if fracture planes are lost or restricted as adults as reflection of their anti-predation tactics.

Although radiographs have been successfully used in identifying caudal fracture planes, there are limitations, particularly in specimens with thicker skin or dermal armouring (Etheridge, 1967). Radiography was not sufficient to investigate fracture planes for all caudal vertebrae in *E. kingii* (J. Barr, *unpub. data.)*, however micro-computed tomography (micro-CT) has been shown to be a valuable tool in assessing caudal fracture planes in lizards (Kuhn *et al.*, 2008; Barr *et al.*, 2019a). In this study we aim to 1) investigate the caudal morphology of *E. kingii* for both the pygal and post-pygal vertebrae, 2) identify the pattern and degree of fracture planes present, and 3) assess if the fracture planes are lost or restricted ontogenetically to certain regions of the tail using micro-computer tomography.

5.3 Methods

5.3.1 Specimens

Six preserved *E. kingii* specimens from the Western Australia Museum (WAM) (R61436, R62232, R78064, R84662, R132059 & R151388) and one deceased specimen donated by the Rottnest Island Authority (RIA) were selected to investigate their caudal osteology and presence of fracture planes. The specimen obtained from RIA was frozen at (-20 °C) and preserved in 100% ethanol. Museum specimens were formalin-fixed and stored in 100% ethanol. Specimens were selected during measurement of

WAM's preserved *E. kingii* collection for another study (Barr *et al.*, 2019b) with selected specimens matching criteria of those which were 1) in good condition, 2) with their original tail intact (no regeneration) and 3) that were not rigidly preserved to enable easy manipulation during micro CT scanning. Snout to vent length (SVL) and tail length (TL) of specimens were measured using a flexible fabric measuring tape to the nearest mm. Relative tail length was calculated as TL/SVL from the measurements, with relative number of autotomisable vertebrae per tail length (number of vertebrae with fracture planes/TL) established from confirmation of fracture planes presence from micro-CT.

5.3.2 CT scanning and analysis

Samples were scanned individually using a SkyScan 1176 scanner; Bruker micro-CT, Kontich, Belgium) at the Centre for Microscopy, Characterisation and Analysis (CMCA), University of Western Australia, Western Australia. The CT scans were performed at 18 µm resolution (65 kV, 385µA, 300ms, 1 mm Al filter, 0.5° rotation step, no frame averaging, 360˚ scan) producing 2000 * 1336-pixel images. CT images were reconstructed in NRecon v1.7.1.0 (Bruker micro-CT) using the modified Feldkamp cone- beam algorithm (Gaussian smoothing kernel (2), ring artefact correction (20), beam hardening correction (30%) and threshold for defect pixel masking (0-5%)). 3D models were constructed and manipulated in Avizo 2019.4 (Thermo Fisher Scientific). The caudal vertebral column of all specimens was examined using the Ortho Slice function for both coronal and sagittal cross sections, with presence or absence of an autotomy plane in the centrum and neural arch marked as present or absent (see Figure 5.2).

5.4 Results

The micro-CT scans of the seven *E. kingii* specimens representing juveniles and adults (SVL 132mm – 204mm) reported in Table 5.1 show the average (± SD) number of caudal vertebrae in the *E. kingii* specimens was 46 ± 2 and ranged from 44 to 50. The first 5-6 caudal vertebrae lacked fracture planes, with the start of the post-pygal series of vertebrae (i.e. those with autotomy planes) at either the 6th or 7th caudal vertebrae. The total number of post-pygal vertebrae varied between 39-44 (average ± SD = 41± 2). Autotomy planes were not lost or restricted to certain regions of the tail during ontogeny for the specimens examined, with fracture planes being evident in both the centrum and neural arches of the post-pygal vertebrae for both juveniles and adults (Figure 5.2). Mean relative tail length (mm: TL/SVL) was slightly higher in juveniles (1.31 ± 0.06) compared to adults (1.27 ± 0.04) as was the number of post-pygal vertebrae for the tail length (0.21 ± 0.06, 0.16 ± 0.01).

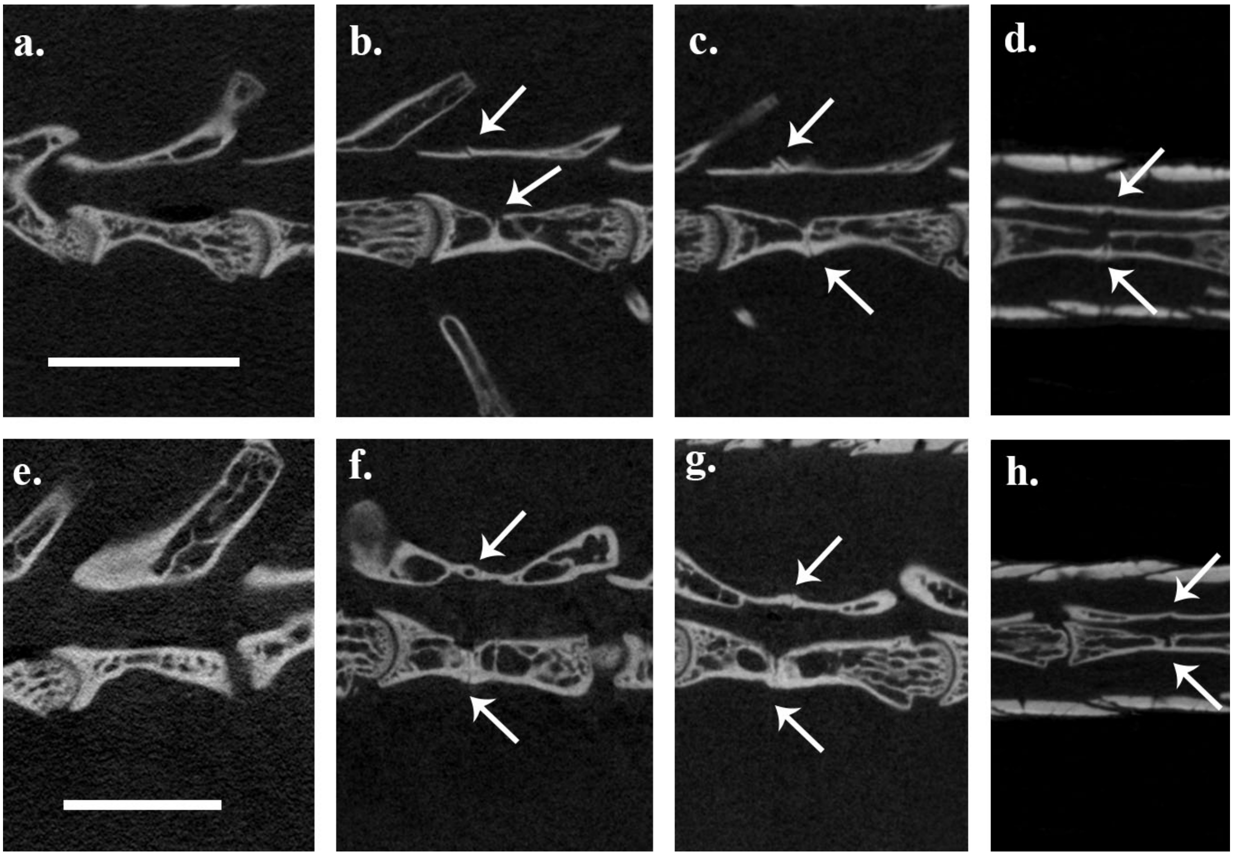

Figure 5.2: Sagittal C.S of *Egernia kingii* caudal vertebrae from reconstructed micro-CT images showing fracture planes (arrow) in the neural arch (top arrows) and centrum (bottom arrows). Images for a juvenile (top row) specimen (R62232) and an adult (bottom row) specimen (RI adult). Pygal vertebra without fracture planes and post-pygal vertebrae with fracture planes at proximal, middle, and distal positions of the tail (a., b., c., d. for the

juvenile and e., f., g., h for the adult). White bar represents 5 mm for the individual specimens.

5.6 Discussion

Our results show that autotomy planes are not lost or restricted ontogenetically in *E. kingii*, with fracture planes present throughout the post-pygal caudal vertebrae of all specimens examined. The number of pygal vertebrae without fracture planes was relatively uniform at 5 or 6 vertebrae, with the presence of the first fracture plane observed on either the 6th or 7th caudal vertebrae. The total number of caudal vertebrae varied slightly between specimens and ontogenetic stage, as did overall tail length with juveniles having slightly longer relative tail length than adults (Barr *et al.*, 2019b), as well as a higher number of pygal vertebrae for tail length. The location of first fracture plane, number of vertebrae with, and number of vertebrae without fracture planes, as well as the total number of vertebrae fall within the previous reported values for other Scincidae species (Etheridge, 1967).

Costly anti-predation strategies are often subject to ontogenetic and evolutionary change due to altered predation risk (Cooper, Pérez-Mellado & Vitt, 2004; Blumstein & Daniel, 2005; Pafilis & Valakos, 2008; Cooper & Blumstein, 2015). Juvenile lizards generally experience higher predation risk than adults, but this may vary based on population, and type and number of predator species present (Pearson *et al.*, 2002; Aubret *et al.*, 2004; Pafilis *et al.*, 2009). Caudal fracture planes were retained ontogenetically in *E. kingii*; however, the ability and ease of dropping a portion of the tail can be influenced by other factors (Arnold, 1988). Fracture planes within the vertebrae, along with caudal muscle and other tissues, are arranged within the tail as autotomisable units (Woodland, 1920; Bellairs & Bryant, 1985; Gilbert, Payne & Vickaryous, 2013; Lozito & Tuan, 2017). Ossification of fracture planes is the most common change in ontogenetic loss of autotomy,

Table 5.1: Caudal vertebrae data for *Egernia kingii* specimens scanned by micro-CT.

Specimen #	Ontogenetic stage	SVL (mm)	TL (mm)	No. caudal vertebrae	No. pygal vertebrae	Caudal vertebrae # with first fracture plane	No. post-pygal vertebrae
R132059	Juvenile	132	172	47	6	7	41
R78064	Juvenile	145	195	47	6	7	41
R62232	Juvenile	150	185	44	5	6	39
R151388	Juvenile	165	229	50	6	7	44
R61436	Adult	185	226	46	5	6	41
R84662*	Adult	192	253	46	5	6	41
RI adult*	Adult	205	258	44	5	6	39

*R84662 tail was broken posthumously at caudal vertebrae vertebra #25 and RI adult tail was broken posthumously at caudal vertebra #9 however, other changes, both morphological and neurological, occur ontogenetically, influencing the ease and likelihood of autotomy. Increased muscle attachment, strength to the myosepta and reduction in planes of weakness in the skin all affect the ease of autotomy and, although generally observed with ossification of autotomy planes ontogenetically, can occur in instances where intra-vertebral autotomy planes are still present (Arnold, 1984; Fox, Perea-Fox & Castro-Franco, 1994). Intra-vertebral autotomy is also a process that is under conscious neurological control (Slotopolsky, 1921; Bellairs & Bryant, 1985; Elwood, Pelsinski & Bateman, 2012). Although generally a level of physical stimulus is required to initiate autotomy (Arnold, 1984; Bellairs & Bryant, 1985) perception of threat can influence the degree of ease and latency that lizards drop their tail (Cooper *et al.*, 2004; Pafilis *et al.*, 2009) and may change ontogenetically with perceived predation risk. In these instances, modulating the conscious decision of whether to drop a portion of the tail, but still retaining the morphological structures for caudal autotomy such as fracture planes, may maximise the benefits but minimise the costs associated with caudal autotomy, depending on the threat faced.

In the absence of predators, strategies primarily used to avoid them, such as caudal autotomy, may be maintained due to intra- or inter-specific aggression (Pafilis, Pérez-Mellado & Valakos, 2008; Bateman & Fleming, 2009; MacDougall *et al.*, 2020), often exacerbated in denser populations such as on islands (Siliceo-Cantero *et al.*, 2017). For example, tail break frequencies in *Mediodactylus kotschyi*, increased with higher gecko abundance, but not predator richness (Itescu *et al.*, 2017). *Egernia kingii* occur on many offshore islands, and on some, such as Penguin Island, which is free of terrestrial predators, can occur in large numbers – up to 667-950 ha^{-1} (Arena, 1986; Langton, 2000; Browne, 2014). However, aggressive interactions, both among adult lizards, particularly during the mating period (J. Barr *pers. obs.*; Arena, 1986), and from nesting shore birds, is evident (J. Barr *pers. obs*). Intra- and inter-specific aggressive interactions may influence the retention of facture planes during ontogeny in *E. kingii*, even with a relaxation in predation risk.

In summary, there is evidence from many lizard species that ontogenetic restriction or loss of caudal autotomy planes occur with decreasing predation risk, either at the population level or because of large shift in size and mass as they mature, allowing reliance on alternative, less costly means compared to caudal autotomy, such as biting, clawing and fighting. However, we found no evidence that caudal fracture planes were lost ontogenetically in the large scincid, *E. kingii* during ontogeny, despite undergoing a large (30- 50-fold) increase in weight as individuals mature. Retention of caudal fracture planes is, we contend, likely to be a result of:1) intra- and inter-specific aggression, and 2) fitness costs associated with caudal autotomy regulated at the neurological level (e.g. decision to autotomise or not), not from loss of fracture planes. Further investigation into the neurological control of caudal autotomy and how changes in strength of muscle attachments between fracture planes occur during ontogeny would allow for more definitive conclusions regarding the ontogenetic retainment of caudal fracture planes.

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5.8 References

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ALIBARDI, L. (2010). *Morphological and Cellular Aspects of Tail and Limb Regeneration in Lizards : a Model System with Implications for Tissue Regeneration in Mammals*. Springer-Verlag, Heidelberg, Berlin

ARENA, P. (1986). *Aspects of the biology of the King's skink Egernia kingii (Gray)*. Honours thesis thesis, Murdoch University.

ARENA, P. C. & WOOLLER, R. D. (2003). The reproduction and diet of *Egernia kingii* (Reptilia : Scincidae) on Penguin Island, Western Australia. *Australian Journal of Zoology* **51**(5), 495-504.

ARNOLD, E. (1984). Evolutionary aspects of tail shedding in lizards and their relatives. *Journal of Natural History* **18**(1), 127-169.

ARNOLD, E. (1988). Caudal autotomy as a defense. *Biology of the Reptilia* **16**, 235-273.

ARNOLD, E. N. (1994). Investigating the evolutionary effects of one feature on another: does muscle spread suppress caudal autotomy in lizards? *Journal of Zoology* **232**(3), 505-523.

AUBRET, F., BONNET, X., MAUMELAT, S., BRADSHAW, D. & SCHWANER, T. (2004). Diet divergence, jaw size and scale counts in two neighbouring populations of tiger snakes (*Notechis scutatus*). *Amphibia-Reptilia* **25**(1), 9-17.

BARR, J. I., BOISVERT, C. A., SOMAWEERA, R., TRINAJSTIC, K. & BATEMAN, P. W. (2019a). Re-regeneration to reduce negative effects associated with tail loss in lizards. *Scientific Reports* **9**(1), 18717.

BARR, J. I., SOMAWEERA, R., GODFREY, S. S. & BATEMAN, P. W. (2019b). Increased tail length in the King’s skink, *Egernia kingii* (Reptilia: Scincidae): an anti-predation tactic for juveniles? *Biological Journal of the Linnean Society* **126**(2), 268–275.

BATEMAN, P. & FLEMING, P. (2009). To cut a long tail short: a review of lizard caudal autotomy studies carried out over the last 20 years. *Journal of Zoology (London)* **277**(1), 1-14.

BATEMAN, P., FLEMING, P. & ROLEK, B. (2014). Bite me: blue tails as a ‘risky-decoy’defense tactic for lizards. *Current Zoology* **60**(3), 333-337.

BELLAIRS, A. & BRYANT, S. (1985). Autotomy and regeneration in reptiles. *Biology of the Reptilia* **15**(5), 301-410.

BLUMSTEIN, D. T. & DANIEL, J. C. (2005). The loss of anti-predator behaviour following isolation on islands. *Proceedings of the Royal Society of London B: Biological Sciences* **272**(1573), 1663-1668.

BROWNE, C. (2014). *King's skinks (Egernia kingii) abundance and juvenile survival unaffected by temporal change or presence of invasive black rats (Rattus rattus) on Penguin Island, Western Australia*. Honours thesis thesis, The University of Western Australia

CHAPPLE, D. G. (2003). Ecology, life-history, and behaviour in the Australian scincid genus *Egernia*, with comments on th evolution of complex sociality in lizards. *Herpetological Monographs* **17**(1), 145-180.

COOPER, J. W. E. & BLUMSTEIN, D. T. (2015). *Escaping From Predators: An Integrative View of Escape Decisions*. Cambridge University Press, Cambridge.

COOPER, W. E., PÉREZ-MELLADO, V. & VITT, L. J. (2004). Ease and effectiveness of costly autotomy vary with predation intensity among lizard populations. *Journal of Zoology* **262**(3), 243-255.

COOPER, W. E. & VITT, L. J. (1985). Blue tails and autotomy: enhancement of predation avoidance in juvenile skinks. *Zeitschrift fuer Tierpsychologie* **70**(4), 265-276.

DIAL, B. E. & FITZPATRICK, L. C. (1981). The energetic costs of tail autotomy to reproduction in the lizard *Coleonyx brevis* (Sauria: Gekkonidae). *Oecologia* **51**(3), 310-317.

DIAL, B. E. & FITZPATRICK, L. C. (1984). Predator escape success in tailed versus tailless *Scinella lateralis* (Sauria: Scincidae). *Animal Behaviour* **32**(1), 301-302.

DILLY, M. L. (2000). *Factors affecting the distribution and variation in abundance of the King's skink (Egernia kingii) (Gray) in Western Australia*. Honours thesis thesis, Murdoch University.

ELWOOD, C., PELSINSKI, J. & BATEMAN, B. (2012). *Anolis sagrei* (Brown Anole). Voluntary autotomy. *Herpetological Review* **43**(4), 642-642.

EMBERTS, Z., ESCALANTE, I. & BATEMAN, P. W. (2019). The ecology and evolution of autotomy. *Biological Reviews* **94**, 1881-1896.

ETHERIDGE, R. (1967). Lizard caudal vertebrae. *Copeia* **1967**(4), 699-721.

FOX, S., PEREA-FOX, S. & CASTRO-FRANCO, R. (1994). Development of the tail autotomy adaptation in lizards under disparate levels of predation at high and low elevations in Mexico. *The Southwestern Naturalist* **39**, 311-322.

GARDNER, M. G., HUGALL, A. F., DONNELLAN, S. C., HUTCHINSON, M. N. & FOSTER, R. (2008). Molecular systematics of social skinks: phylogeny and taxonomy of the *Egernia* group (Reptilia: Scincidae). *Zoological Journal of the Linnean Society* **154**(4), 781-794.

GILBERT, E. A., PAYNE, S. L. & VICKARYOUS, M. K. (2013). The anatomy and histology of caudal autotomy and regeneration in lizards. *Physiological and Biochemical Zoology* **86**(6), 631-644.

HAWLENA, D., BOOCHNIK, R., ABRAMSKY, Z. & BOUSKILA, A. (2006). Blue tail and striped body: why do lizards change their infant costume when growing up? *Behavioral Ecology* **17**(6), 889-896.

HOFFSTETTER, R. & GASC, J.-P. (1969). Vertebrae and ribs of modern reptiles. *Biology of the Reptilia* **1**(5), 201-310.

HOLDER, L. A. (1960). The comparative morphology of the axial skeleton in the Australian Gekkonidae. *Journal of the Linnean Society of London, Zoology* **44**(297), 300-335.

ITESCU, Y., SCHWARZ, R., MEIRI, S., PAFILIS, P. & CLEGG, S. (2017). Intraspecific competition, not predation, drives lizard tail loss on islands. *Journal of Animal Ecology* **86**(1), 66-74.

KUHN, G., GRUBER, P., MÜLLER, R. & RUHLI, F. (2008). Three-dimensional evaluation of structures in small bones by Micro-CT: Tail fracture planes of autotomizing lizards (Scincidae and Gecconidae families). *Internet Journal of Biological Anthropology* **1**(2).

LANGTON, J. (2000). *Population biology of the King's skink (Egernia kingii) (Gray) on Penguin Island, Western Australia*. Honours thesis thesis, Murdoch University.

LOZITO, T. P. & TUAN, R. S. (2017). Lizard tail regeneration as an instructive model of enhanced healing capabilities in an adult amniote. *Connective Tissue Research* **58**(2), 145-154.

MACDOUGALL, M. J., VERRIÈRE, A., WINTRICH, T., LEBLANC, A. R. H., FERNANDEZ, V. & FRÖBISCH, J. (2020). Conflicting evidence for the use of caudal autotomy in mesosaurs. *Scientific Reports* **10**(1), 7184.

MASTERS, C. & SHINE, R. (2003). Sociality in lizards: family structure in free-living King's Skinks *Egernia kingii* from southwestern Australia. *Australian Zoologist* **32**(3), 377-380.

MCELROY, E. J. & BERGMANN, P. J. (2013). Tail autotomy, tail size, and locomotor performance in lizards. *Physiological and Biochemical Zoology* **86**(6), 669-679.

PAFILIS, P., FOUFOPOULOS, J., POULAKAKIS, N., LYMBERAKIS, P. & VALAKOS, E. D. (2009). Tail shedding in Island lizard [LACERTIDAE, REPTILIA]: Decline of antipredator defenses in relaxed predation environments. *Evolution* **63**(5), 1262-1278.

PAFILIS, P., PÉREZ-MELLADO, V. & VALAKOS, E. (2008). Postautotomy tail activity in the Balearic lizard, Podarcis lilfordi. *Die Naturwissenschaften* **95**, 217-21.

PAFILIS, P. & VALAKOS, E. D. (2008). Loss of caudal autotomy during ontogeny of Balkan Green Lizard, *Lacerta trilineata*. *Journal of Natural History* **42**(5-8), 409-419.

PEARSON, D., SHINE, R. & HOW, R. (2002). Sex-specific niche partitioning and sexual size dimorphism in Australian pythons (*Morelia spilota imbricata*). *Biological Journal of the Linnean Society* **77**(1), 113-125.

RUSSELL, A. & BAUER, A. (1992). The m. caudifemoralis longus and its relationship to caudal autotomy and locomotion in lizards (Reptilia: Sauna). *Journal of Zoology (London)* **227**, 127-143.

SILICEO-CANTERO, H., ZÚÑIGA-VEGA, J. J., RENTON, K. & GARCIA, A. (2017). Assessing the relative importance of intraspecific and interspecific interactions on the ecology of anolis nebulosus lizards from an island vs. A mainland population. *Herpetological Conservation and Biology* **12**, 673-682.

SLOTOPOLSKY, B. (1921). *Beiträge zur Kenntnis der Verstümmelungs-und Regenerationsvorgänge am Lacertilierschwanze*, Zurich.

STORR, G. (1978). The genus *Egernia* (Lacertilia, Scincidae) in Western Australia. *Records of the Western Australian Museum* **6**(2), 147-187.

VITT, L. J., CONGDON, J. D. & DICKSON, N. A. (1977). Adaptive strategies and energetics of tail autonomy in Lizards. *Ecology* **58**(2), 326-337.

WHITE, C. P. (1925). Regeneration of the lizard's tail. *The Journal of Pathology* **28**(1), 63-68.

WOODLAND, W. (1920). Memoirs: some observations on caudal autotomy and regeneration in the gecko (*Hemidactylus flaviviridis*, Rppel), with notes on the tails of *Sphenodon* and *Pygopus*. *Journal of Cell Science* **2**(257), 63-100.

ZANI, P. A. (1996). Patterns of caudal-autotomy evolution in lizards. *Journal of Zoology* **240**(2), 201-220.

Chapter 6. Drop it or not: individual perception of threat, not predation risk drives autotomy in King's skinks (*Egernia kingii*)

The study presented in this chapter is in preparation for submission to review within the peer-reviewed literature.

Barr, J.I., Somaweera, R., Godfrey, S.S., Bateman, P.W. Drop it or not: individual perception of threat, not predation risk drives autotomy in King's skinks (*Egernia kingii*). *In preparation*

Figure 6.1: Two juvenile King's skinks (*Egernia kingii*) with regenerated tails © James Barr

6.1 Abstract

Anti-predation strategies, morphological and behavioural, change both ontogenetically and over evolutionary time. Caudal autotomy, the ability to drop a portion of the tail, is an effective, but costly anti-predation strategy used by many species of lizards. Several aspects of caudal autotomy including latency to autotomise and tail thrashing post autotomy, vary between species, ontogenetic stage of individuals, and predation risk as perceived by the individual lizard. We assessed willingness to autotomise a tail, and how it changes ontogenetically in three populations of King's skinks (*Egernia kingii*) at sites that varied in the diversity of potential predators in Perth, Western Australia. We attempted to induce caudal autotomy in 56 wild-caught individuals, ranging from juveniles to mature adults. We predicted that latency of autotomy, and post autotomy thrash time would change with diversity of potential predators (a proxy for predation risk) and with age/size of skink. However, there was neither a significant influence of site or ontogenetic stage on the likelihood of autotomy, nor did post-autotomy thrash time differ among sites. Tails with a larger portion of regeneration thrashed for longer, likely as a result from a difference in proportional mass composition of original and regenerated tissues. Tails from skinks that recorded a higher surface temperature thrashed to exhaustion sooner, while individuals that struggled had a higher likelihood of autotomising their tails. We suggest that individual threat perception, in this case how much the individual struggled, not general population level predation risk may be a more important factor in caudal autotomy behaviour in this large scincid lizard.

6.2 Introduction

Anti-predation strategies are paramount to survival (Cooper & Blumstein, 2015). These strategies change through both ontogenetic and evolutionary time, as well as being influenced by the level of perceived predation risk (Arnold, 1984; Blumstein, Daniel & Springett, 2004; Barbosa & Castellanos, 2005). Caudal autotomy, the ability to shed a portion of a tail, is an effective but costly anti-predation strategy used by many species of lizards (Bellairs & Bryant, 1985; Arnold, 1988; Bateman & Fleming, 2009). The tail regenerates over time, with a rigid cartilage rod replacing the original bony vertebrae (Bellairs & Bryant, 1985; Alibardi, 2010; Lozito & Tuan, 2017; Barr *et al.*, 2019a). When autotomised, the tail thrashes for a period of time to further distract the predator away from the lizard (Bellairs & Bryant, 1985; Arnold, 1988), and although the thrash time can be negatively affected by portion of regeneration (Naya *et al.*, 2007), this varies (Simou *et al.*, 2008). Intra-vertebral autotomy, the most common form of caudal autotomy, involves shedding the tail at pre-formed breakage planes within a series of caudal vertebrae, and is under conscious neurological control of the individual (Slotopolsky, 1921; Bellairs & Bryant, 1985). More so, certain species are able to autotomise their tail to a perceived threat before physical contact is made (Elwood, Pelsinski & Bateman, 2012).

Although better than death from predation, caudal autotomy and subsequent regeneration can have considerable negative effects on the individual (Maginnis, 2006), such as growth (Congdon, Vitt & King, 1974; Vitt, Congdon & Dickson, 1977; Lynn, Borkovic & Russell, 2013), reproductive output (Dial & Fitzpatrick, 1981), ability to avoid further predation events (Downes & Shine, 2001) and escape speed (McElroy & Bergmann, 2013). Perhaps due to the costly nature of caudal autotomy, its use is highly variable between species, populations, and life stages. For example, the ability (or willingness) to drop a tail can vary between sexes (Fox, Conder & Smith, 1998), be lost by lizards as they mature (Etheridge, 1967; Arnold, 1984; Fox, Conder & Smith, 1998; Pafilis & Valakos, 2008), or decrease with reduced predation risk (Fox, Perea-Fox & Castro-Franco, 1994; Cooper, Pérez-Mellado & Vitt, 2004; Pafilis *et al.*, 2009), but can also be maintained in high density populations with high conspecific aggression (Itescu *et al.*, 2017). Alternatively, autotomy frequency in multiple Mediterranean lizard species has been shown to correlate with the presence of a single predator species (vipers) rather than overall predation risk from multiple predator types (Pafilis *et al.* (2009).

Ontogenetic changes in caudal autotomy, at least at the morphological level investigating reduction or loss of fracture planes is well documented (Etheridge, 1967; Arnold, 1988; Russell & Bauer, 1992), however, studies investigating ontogenetic changes in autotomy behaviour are few (Fox, Perea-Fox & Castro-Franco, 1994; Pafilis & Valakos, 2008). The King's skink (*Egernia kingii*; Scincidae) is a large lizard growing up to 244 mm snout to vent length (SVL) endemic to mainland South West Western Australia and surrounding Islands (Storr, 1978; Cogger, 2014). Juveniles face higher predation risk than adults (Bonnet *et al.*, 1999; Aubret *et al.*, 2004). Adults attempt to actively defend themselves against certain predators (Masters & Shine, 2003), although, this is likely to be influenced by the diversity of potential predators at a site (Pearson, Shine & How, 2002). Juvenile King's skinks have longer tails than do adults, with a larger proportion of their tail regenerated, suggesting that they rely more heavily on caudal autotomy for protection than do adults (Barr *et al.*, 2019b). However, adults still retain the ability to autotomise their tails (Barr *et al.*, 2019b).

In this study we assess how the use of caudal autotomy changes ontogenetically in *E. kingii* across three populations that vary in predation risk indicated by diversity of predator types. We predicted that: 1) individuals at sites with a higher predation risk will autotomise their tails more readily than will individuals at sites with lower predation risk; 2) that smaller (juvenile) individuals will autotomise their tails more readily than will adults, and 3); that autotomised tails with higher degree of regenerated tissue will have a shorter thrash time.

6.3 Methods

6.3.1 Study sites

Three sites varying in predator diversity were chosen for this study (Table 6.1). Penguin Island (-32.305658 S, 115.691089 E), Rottnest Island (-31.999479° S, 115.527712° E) and a coastal mainland site in Perth (-31.868450° S, 115.752560° E: 'Coastal Perth' hereafter) are all located within c. 50 km of Perth city in Western Australia. Penguin and Rottnest Islands were originally part of a coastal limestone shelf and became isolated from one another and the mainland c. 5000-8000 years ago (Playford, 1988; Hughes, 2012).

6.3.2 Predation risk

Predation risk was established based on the diversity of potential predators at the different sites (Cooper *et al.*, 2004; Pafilis *et al.*, 2009; Itescu *et al.*, 2017) taken from literature, online sources and direct observations (Table 6.1). Online fauna databases NatureMap (DBCA, 2007) and Atlas of Living Australia (ALA, 2019) were searched for observations, with species noted as 'present' in the area if there had been five or more observations between 2014 and 2020. A search radius of 5km was used for identifying potential avian predators, with potential terrestrial predators as only those occurring within sites. Additionally, species were classified as 'present' from the authors' personal observation while in the field, as well as from personal communications from local rangers. Species identified as potential predators were then checked against literature for records of predating lizards; for snakes (Bonnet *et al.*, 1999; Pearson *et al.*, 2002; Aubret *et al.*, 2004), birds (Marchant & Higgins, 1990) and mammals (Coman, 1973; Crawford, 2010; Bamford, 2012).

6.3.3 Trapping and measurements

Skinks were trapped between January and March 2018 and 2019 using medium Elliot traps ™ baited with universal bait (sardines, peanut butter and oats). Traps were set between the hours of 0900-1400, covered in hessian to prevent overheating and placed under vegetation. Individual skinks at

Penguin Island were identified from PIT tags implanted previously (Browne, 2014; Barr, 2016). All ‘new’ individuals from all three sites were implanted with ISO FDX-B Mini Microchips (1.4mm x 8mm) in the inguinal region (DBCA, 2017) after autotomy testing was completed.

Table 6.1: Potential predator species of King’s skinks (*Egernia kingii*) across the study sites. Presence records based from ATLAS of Living Australia (ALA, 2019) accessed 4th June 2020, Nature maps (NM) (DBCA, 2007) accessed 4th June 2020, J. Taylor personal communication (City of Stirling) and J. Barr *pers. obs*.

Species	**Penguin Island**	**Rottnest Island**	**Coastal Perth**
Mammals			
Cat *(Felis catus)*	-	Historical (2002)	X
Fox *(Vulpes vulpes)*	-	-	X
Reptiles			
South west carpet python *(Morelia spilota imbricata)*	-	-	-
Tiger snake *(Notechis scutatus)*	-	-	-
Dugite *(Pseudonaja affinis)*	-	X	X
Aves			
Collared sparrowhawk *(Accipiter cirrocephalus)*	-	X	-
Brown goshawk *(Accipiter fasciatus)*	X	X	X
Swamp harrier *(Circus approximans)*	X	-	X
Australian raven (*Corvus coronoides)*	X	X	X
Kookaburra	X	-	X

(*Dacelo novaeguineae*)			
Black shouldered kite *(Elanus axillaris)*	X	X	-
Nankeen kestre*l* *(Falco cenchroides)*	-	X	-
Whistling kite *(Haliastur sphenurus)*	X	-	X
Australian little eagle *(Hieraaetus morphnoides)*	X	X	X
Osprey/Eastern osprey *(Pandion haliaetus/cristatus)*	X	X	-
Total number of predator species	Eight	Eight	Seven
References	J. Barr Pers. Obs; DBCA, 2007; ALA, 2019	J. Barr Pers. Obs; DBCA, 2007; ALA, 2019	J. Barr Pers. Obs; DBCA, 2007; ALA, 2019, J. Taylor, *pers. comm.*

Skinks were transported from the field in individual calico bags within an insulated transport container back to the research quarters on both island sites, with individuals from the Coastal Perth site being transported back to Curtin University for measuring and autotomy testing. Skinks were weighed (± 5 g) using a Pesola 500g spring balance (± 0.3%). Snout to vent length (SVL), tail length (TL) and tail regeneration length, the length tail that was regenerated tissue, (RL) was measured using a plastic ruler to the nearest mm. Both relative tail length (TL/SVL) and relative regeneration length (RL/TL) were calculated. Sex was determined for individuals via hemipene probing (Brown, 2012) as King's skinks lack clearly visible secondary sexual characteristics and hemipene extrusion proves difficult with the larger individuals of this species (Arena, 1986). King's skinks, being quite a large and strong skink, can thrash and wriggle (J. Barr, *pers. obs.).* As such, all morphological measures were repeated three times and the average was used in analysis.

6.3.4 Autotomy assays

All morphological measures were taken prior to autotomy testing, with only individuals that had less than half their tail comprised of regenerated tissue selected for autotomy assays. Tails of individuals were measured to half their length using a plastic ruler to the nearest mm and marked with a non-toxic paint pen (Sharpie™). The width at this mid-tail point was measured to the nearest half mm using General ® 150mm metric dial callipers. At this mid-tail point, individuals were squeezed using a pair of General ® 150mm metric dial callipers to two thirds the original width to simulate an attempted predation event for 60 seconds, or until autotomy occurred. To standardise the perception of predator identity to the skinks and have objective measurements, all autotomy assays were conducted by JIB wearing similar field clothing and leather safety gloves (IRONCLAD RANCHWORKS ®), with all autotomy assays being conducted in opaque plastic tubs (630 mm *430 mm*370 mm). For each skink it was recorded if they bit at the callipers (no: 0; yes:1) during the simulated predation event, and allocated a struggle score for the 60 seconds or until autotomy occurred (0 = no movement during squeeze, 1 = normal movement, 2= above normal movement/some vigorous struggling for part of the 60s and 3 = vigorous movement /thrashing for majority of the 60s). For those individuals that autotomised their tail, latency to autotomise in seconds was recorded. Thrash time of autotomised tails were recorded from the time of autotomy, until the tail had undergone a 10 second period with no movement. Surface temperature of the skinks just prior to autotomy testing was measured using a Gastools Infrared non-contact thermometer (focal spot ratio 10:1) at an approximate distance of 20 cm on their upper dorsal surface. All assays were conducted between 1200 and 1700 and were video-taped on a Sony Handy cam XR 150 at 25fps.

6.3.5 Statistical analysis

All analyses were conducted in R studio version 1.1.383 (R Development Core Team, 2013). A general linear model (GLM) was used to analyse (1) the likelihood of autotomizing their tail (0 = did not autotomize, 1 = autotomized) in the 60 second trial using the glm function (binomial, link = "logit") for all individuals subjected to autotomy testing. Linear models (LM's) were used to analyse (2) latency to autotomise in seconds and (3) thrash time in minutes for individuals that autotomised their tails using the lm function. Fixed effects for the first and second models were Site, SVL, regeneration present (Y/N), skink surface temperature (°C), struggle score, if they bit at the threat (Y/N). Fixed effects for third model looking at latency to autotomise were site, SVL, relative regeneration, and skink surface temperature (°C).

The most appropriate model was selected based on the lowest AIC using the drop1 function from the lme4 package (Bates *et al.*, 2014), with the complete models being used for all three models. Collinearity of fixed effects were checked using the vif function from the lsr package (Navarro, 2015). Distributions of the data were assessed both visually using histograms and assisted by the fitdist function from fitdistrplus package (Delignette-Muller & Dutang, 2015). Appropriateness of the GLM model was assessed using a Hosmer and Lemeshow goodness of fit test (hoslem.test function), with residual and QQ plots used to assess both LM's. The Anova function from the Car package (Fox *et al.*, 2017) was used to obtain Chi-Square and p-values for the GLM, as well as F statistics and p-values for the LM's.

6.3.6 Ethical statement

All research was carried out in accordance with the Animal Ethics Office of Curtin University (ARE2017-12) and DBCA regulation 17 licence (08-001238-1).

6.4 Results

A total of 56 skinks across three sites (Penguin Island = 21, Rottnest Island = 18, Coastal Perth = 17) ranging from 90 mm to 217 mm SVL (mean ± SD = 176 ± 35.3) underwent autotomy testing. Of the total 56 skinks, 17 (Penguin Island = six, Rottnest Island = eight, Coastal Perth = three) autotomised their tails. Of the 17 skinks that autotomised their tail, mean latency to autotomy was 19.2 ± 12.3 seconds (± SD; range = 2 – 40), and mean tail thrash time of autotomised sections was 6.68 ± 2.31 minutes (± SD; range = 3.1 – 12.5).

Overall, SVL and site did not influence whether King's skinks autotomised their tails (Table 6. 2). Individuals that struggled more had a higher likelihood of autotomising their tail ($\chi^2 = 25.4$, df = 3, $P < 0.001$, Figure 6.2). No effect on likelihood to autotomise was observed for presence of regeneration, skink temperature or if they bit at the callipers (Table 6.2). Latency to autotomise was not influenced by site, SVL, presence of regeneration, skink temperature, struggle score or if they bit at the callipers (Table 6.2). Degree of regeneration had a significant effect on thrash time (Table 6.2), with tails that had larger amounts of relative regeneration thrashing longer (Figure 6.3). Autotomised tails from skinks with higher initial body surface temperatures thrashed for shorter periods than did those from skinks of a lower temperature (Table 6.2, Figure 6.4), but site and SVL did not significantly influence thrash time of the autotomised tail.

Table 6.2: Summary of GLM and LM results and fixed effects assessing Autotomy (binomial), latency to autotomise (seconds) and tail thrash duration (min).

GLM model	Fixed effect	χ^2 value	F - Value	Df	P - value
Autotomy (binomial)	Site	1.74	-	2	0.419
	SVL (mm)	0.001	-	1	0.982
	Regeneration (yes/no)	0.040	-	1	0.840
	Skink Temp (°C)	0.632	-	1	0.427
	Struggle score	27.7	-	3	**< 0.001**
	Bit (yes/no)	2.04	-	1	0.153
Latency to autotomise (sec)	Site	-	0.471	2	0.650
	SVL (mm)	-	1.35	1	0.912
	Regeneration (yes/no)	-	0.017	1	0.465
	Skink Temp (°C)	-	0.138	1	0.720
	Struggle score	-	0.278	1	0.610
	Bit (yes/no)	-	3.30	1	0.103
Thrash time (min)	Site	-	2.54	2	0.124
	SVL	-	2.92	1	0.116
	Relative regeneration	-	9.56	1	**0.010**
	Skink Temp (°C)	-	5.98	1	**0.032**

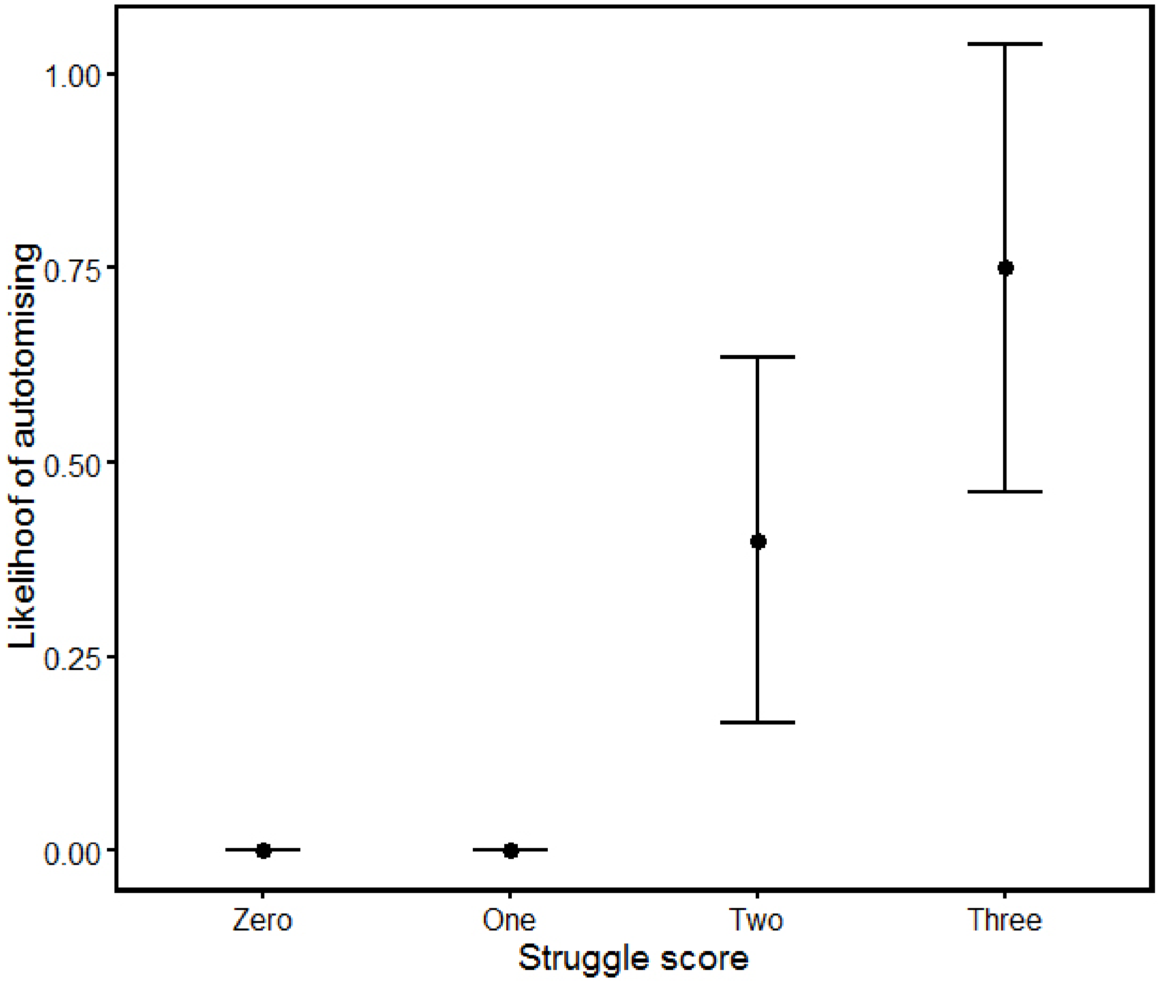

Figure 6.2: Effect of struggling on the likelihood of autotomy. (Zero = no movement during simulated predation event, One = normal movement, Two= above normal movement and/or some vigorous struggling for part of the 60s and Three = vigorous movement and/or thrashing for majority of the 60s). Average ± 95% CI error bars shown.

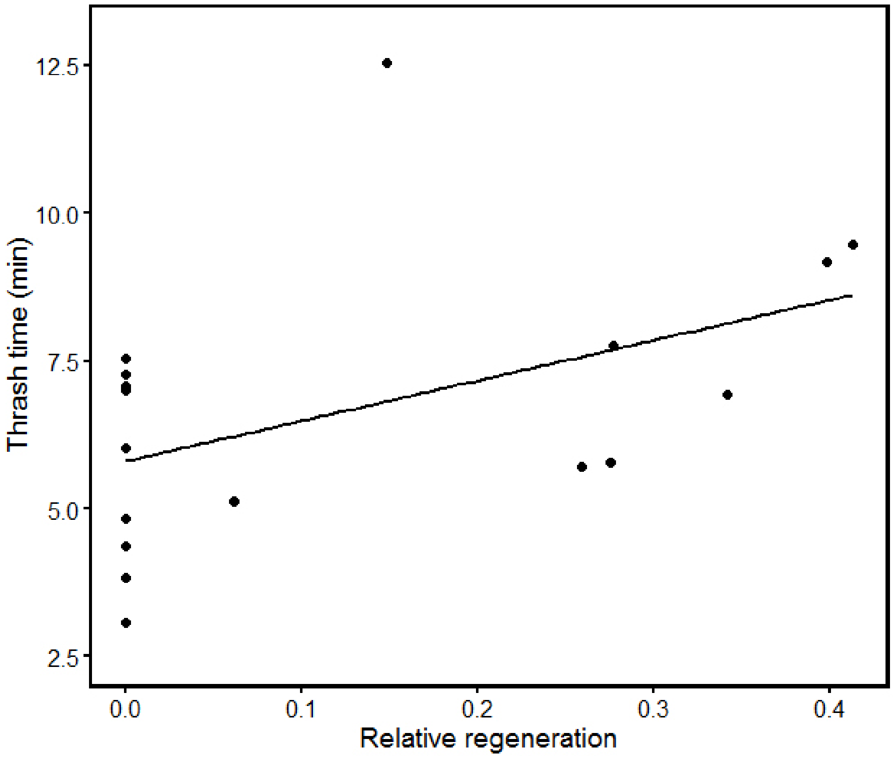

Figure 6.3: Effect of relative regeneration (Regeneration length (mm) / Tail length (mm)) on thrash time of the autotomised tail.

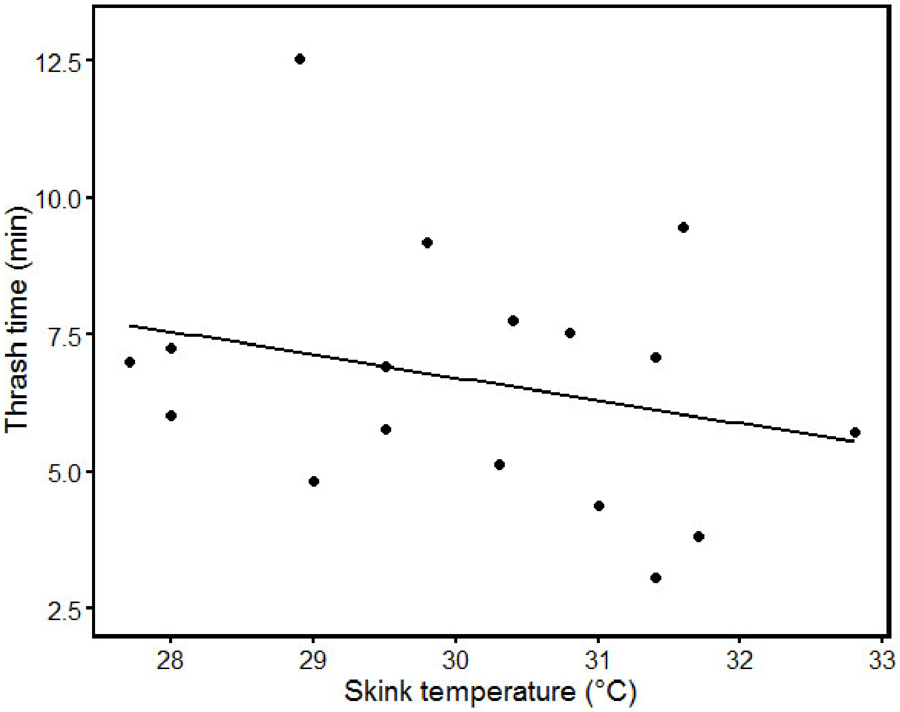

Figure 6.4: Effect of skink temperature (°C) on thrash time (min) for the autotomised tail.

6.5 Discussion

Contrary to our predictions, individual skinks from higher predation risk environments were not more likely to autotomise their tails than were individuals from lower predation risk environments. The most influential factor on whether an individual chose to shed a tail was how much that individual struggled during the behavioural assay. Post-autotomy tail thrash time was affected by degree of regeneration; however, longer not shorter thrash times were observed with increasing proportion of regeneration. Skink surface temperature also affected tail thrash time, with tails thrashing to exhaustion occurring sooner for skinks who had recorded a higher surface body temperature prior to autotomising.

Overall, we found no difference in the autotomy behaviour of *E. kingii* either with varying predation risk or as they grew ontogenetically. These findings contrast with Cooper *et al.* (2004) where *Podarcis* lacertids showed greater ease of autotomy, greater likelihood to autotomise and longer post-autotomy tail thrash as predation risk increased across islands and a mainland Mediterranean site. However, ecological factors, other than predation risk can influence caudal autotomy behaviour within species and populations (Bateman & Fleming, 2009). For example, a study of autotomy rates of two gecko species, *Mediodactylus kotschyi* and *Hemidactylus turcicus,* across multiple locations with varying predation risk found that autotomy rates were more influenced by the density of the study species, and not by predation risk or predator abundance (Itescu *et al.* (2017). High densities of lizards, particularly in insular environments, can evidently result in high levels of intra-specific aggression (Vervust *et al.*, 2009; Itescu *et al.*, 2017), where strategies usually employed to avoid predation may be used to avoid conspecific aggression, as well as potential predators. On Penguin Island, *E. kingii* occur in high densities: ~650-950 ha^{-1} (Arena, 1986; Langton, 2000; Browne, 2014), and aggressive intra-specific interactions are often observed (J. Barr, *pers. obs.*). Additionally, adults of several other *Egernia* species have been documented exhibiting high levels of intra-specific aggression, particularly to unrelated juveniles (Chapple, 2003) and adults of the Otago skink (*Oligosoma otagense*) have been recorded cannibalising juveniles in high density populations (Elangovan *et al.*, 2020). This intra-specific aggression is likely to be, in addition to predator diversity, a significant influence on caudal autotomy behaviour, particularly in highly dense insular environments, and may explain why variation in autotomy behaviour between sites was not observed in this study.

Perception of threat, as well as previous exposure to threat, can significantly influence anti-predation decisions (Downes, 2002; Cooper & Blumstein, 2015; Ortega, Mencía & Pérez-Mellado, 2018; Pears, Emberts & Bateman, 2018). Caudal autotomy, at least for intra-vertebral autotomy, is a conscious decision and neurologically regulated (Slotopolsky, 1921; Bellairs & Bryant, 1985; Clause & Capaldi, 2006), with threat perception influencing the behaviour and decisions to autotomise their tail. In this study willingness to autotomise was strongly influenced by a lizard’s struggle score, with the more the individual struggled and wanted to escape from the perceived threat, the more quickly they dropped their tail (Figure 6.3). Cooper *et al.* (2004) recorded rolling behaviour while investigating latency to autotomy in *Podarcis lilfordi* and *Podarcis hispanica,* assessing it as an alternative escape tactic, not as an influencer of autotomy. Struggling as a measure of perceived threat and influencer of autotomy appears to be under investigated for lizards (Bateman & Fleming, 2009), and should be included in future studies. Presence of tail regeneration, which would be indicative of a previous autotomy event from a potential threat, did not, however, influence caudal autotomy behaviour in this study. Although presence of regeneration would indicate previous exposure to a threat resulting in caudal autotomy, the frequency of exposure cannot be established based on presence of regeneration, or amount of tail regenerated as each previous autotomy event occurs at a more proximal position on the tail, although see Barr *et al.* (2019a). A single threat encounter may result in the whole tail or only a small portion being autotomised and regenerated depending where on the tail the predator grabbed. Additionally, the autotomy event may have resulted from an intra-specific interaction and not an attempted predation event.

Following autotomy, the thrashing and movement of the dropped tail distracts predators, hence assisting in the lizard's escape (Congdon *et al.*, 1974; Dial & Fitzpatrick, 1984; Arnold, 1988). The morphology and physiology of the original tail can differ substantially from those of a regenerated tail (Bellairs & Bryant, 1985; Alibardi, 2010; Lozito & Tuan, 2017). Most obviously, the highly articulated vertebral column in the original tail is replaced by a ridged cartilage rod during regeneration, which can ossify over time (Woodland, 1920; Bellairs & Bryant, 1985). Additionally, the caudal muscles of the original and regenerated tail differ in type, attachment to the skeleton and electrical activity, with muscles of the original tails designed for resistance to fatigue, and those of the regenerated tails for distraction with higher contractile kinetics and power output (Meyer, Preest & Lochetto, 2002; Higham *et al.*, 2013; Alibardi, 2015). In this study *E. kingii* tails with a higher degree of regeneration thrashed for longer (Figure 6.3). This is most likely explained by 1) a difference in relative mass of original tail tissue and regenerating tissue in the autotomised tail affecting the efficiency of the energetics and thrash time until exhaustion (Naya *et al.*, 2007; Cooper & Smith, 2009), or 2) either age and/or site specific adaptions to tail thrash duration from energy stores and/or lactate dehydrogenase (LDH) activity (Meyer *et al.*, 2002) compounded by lower samples sizes within sites of individuals that autotomised their tails. Furthermore, post-autotomy tail thrash times were lower for individuals that recorded higher surface temperatures (Figure 6.4). Higher temperatures would likely increase the metabolic rate for muscle movement in the autotomised tail, perhaps allowing it to thrash more vigorously, but to exhaustion earlier. Further quantification between trade-off with movement duration and ferocity would be fruitful.

Anti-predation strategies, particularly costly ones, can change with risk from predation and non-predatory aggressive threats across evolutionary and ontogenetic time. In this study we found that individual perception and choice, not overall predation risk, influenced the likelihood of caudal autotomy in *E. kingii*, indicated by how much the individuals struggled during the simulated predation event. We found that tails with a higher relative length of regeneration thrashed for longer than did intact tails, but was most likely affected by different mass of regenerated and original tissue of the autotomised tail, compounded with potential local adaptations, and tails from skinks that recorded higher surface temperature thrashed to exhaustion earlier. Future research into the effects of relative regeneration for both length and mass of tissue would allow more definitive conclusions to be drawn.

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6.7 References

"Every reasonable effort has been made to acknowledge the owners of copyright material. I would be pleased to hear from any copyright owner who has been omitted or incorrectly acknowledged."

ALA. (2019). Atlas of Living Australia http://www.ala.org.au, Accessed 4th June 2020.

ALIBARDI, L. (2010). *Morphological and Cellular Aspects of Tail and Limb Regeneration in Lizards : a Model System with Implications for Tissue Regeneration in Mammals*. Springer-Verlag, Heidelberg, Berlin.

ALIBARDI, L. (2015). Regenerating tail muscles in lizard contain Fast but not Slow Myosin indicating that most myofibers belong to the fast twitch type for rapid contraction. *Tissue and Cell* **47**(5), 533-40.

ARENA, P. (1986). *Aspects of the biology of the King's skink Egernia kingii (Gray)*. Honours thesis thesis, Murdoch University.

ARNOLD, E. (1984). Evolutionary aspects of tail shedding in lizards and their relatives. *Journal of Natural History* **18**(1), 127-169.

ARNOLD, E. (1988). Caudal autotomy as a defense. *Biology of the Reptilia* **16**, 235-273.

AUBRET, F., BONNET, X., MAUMELAT, S., BRADSHAW, D. & SCHWANER, T. (2004). Diet divergence, jaw size and scale counts in two neighbouring populations of tiger snakes (*Notechis scutatus*). *Amphibia-Reptilia* **25**(1), 9-17.

BAMFORD, M. (2012). Cat Predation and Suburban Lizards: A 22 Year Study at a Suburban Australian Property. *The Open Conservation Biology Journal* **6**, 25-29.

BARBOSA, P. & CASTELLANOS, I. (2005). *Ecology of predator-prey interactions*. Oxford University Press.

BARR, J. (2016). *Social structure and the influence of personality traits in the King's skink (Egernia kingii) on Penguin Island, Western Australia*. Honours thesis thesis, Murdoch University.

BARR, J. I., BOISVERT, C. A., SOMAWEERA, R., TRINAJSTIC, K. & BATEMAN, P. W. (2019a). Re-regeneration to reduce negative effects associated with tail loss in lizards. *Scientific Reports* **9**(1), 18717.

BARR, J. I., SOMAWEERA, R., GODFREY, S. S. & BATEMAN, P. W. (2019b). Increased tail length in the King's skink, *Egernia kingii* (Reptilia: Scincidae): an anti-predation tactic for juveniles? *Biological Journal of the Linnean Society* **126**(2), 268–275.

BATEMAN, P. & FLEMING, P. (2009). To cut a long tail short: a review of lizard caudal autotomy studies carried out over the last 20 years. *Journal of Zoology (London)* **277**(1), 1-14.

BATES, D., MAECHLER, M., BOLKER, B. & WALKER, S. (2014). lme4: Linear mixed-effects models using Eigen and S4. *R package version* **1**(7).

BELLAIRS, A. & BRYANT, S. (1985). Autotomy and regeneration in reptiles. *Biology of the Reptilia* **15**(5), 301-410.

BLUMSTEIN, D. T., DANIEL, J. C. & SPRINGETT, B. P. (2004). A Test of the Multi-Predator Hypothesis: Rapid Loss of Antipredator Behavior after 130 years of Isolation. *Ethology* **110**(11), 919-934.

BONNET, X., BRADSHAW, D., SHINE, R. & PEARSON, D. (1999). Why do snakes have eyes? The (non-) effect of blindness in island tiger snakes (*Notechis scutatus*). *Behavioral Ecology and Sociobiology* **46**(4), 267-272.

BROWN, D. (2012). *A Guide to Australian Skinks in Captivity*. Reptile Pub., Burleigh, QLD.

BROWNE, C. (2014). *King's skinks (Egernia kingii) abundance and juvenile survival unaffected by temporal change or presence of invasive black rats (Rattus rattus) on Penguin Island, Western Australia*. Honours thesis thesis, The University of Western Australia

CHAPPLE, D. G. (2003). Ecology, life-history, and behaviour in the Australian scincid genus *Egernia*, with comments on th evolution of complex sociality in lizards. *Herpetological Monographs* **17**(1), 145-180.

CLAUSE, A. R. & CAPALDI, E. A. (2006). Caudal autotomy and regeneration in lizards. *Journal of Experimental Zoology* **305**(12), 965-973.

COGGER, H. G. (2014). *Reptiles and Amphibians of Australia*, 7th edition. CSIRO Publishing, Victoria, Australia.

COMAN, B. (1973). The diet of red foxes, Vulpes vulpes L., in Victoria. *Australian Journal of Zoology* **21**(3), 391-401.

CONGDON, J. D., VITT, L. J. & KING, W. W. (1974). Geckos: adaptive significance and energetics of tail autotomy. *Science* **184**(4144), 1379-1380.

COOPER, J. W. E. & BLUMSTEIN, D. T. (2015). *Escaping From Predators: An Integrative View of Escape Decisions*. Cambridge University Press, Cambridge.

COOPER, J. W. E. & SMITH, C. S. (2009). Costs and economy of autotomy for tail movement and running speed in the skink *Trachylepis maculilabris*. *Canadian Journal of Zoology* **87**(5), 400-406.

COOPER, W. E., PÉREZ-MELLADO, V. & VITT, L. J. (2004). Ease and effectiveness of costly autotomy vary with predation intensity among lizard populations. *Journal of Zoology* **262**(3), 243-255.

CRAWFORD, H. (2010). *A comparison of the red fox (Vulpes vulpes) and feral cat (Felis catus) diets in the south west region of Western Australia*. Honours thesis, Murdoch University.

DBCA. (2007). NatureMap: Mapping Western Australia's Biodiversity (https://naturemap.dbca.wa.gov.au/). Department of Biodiversity, Conservation and Attractions, Accessed on 4 June 2020.

DBCA. (2017). Permanent marking of vertebrates using microchips (ed. c. a. a. Department of Biodiversity).

DELIGNETTE-MULLER, M. L. & DUTANG, C. (2015). fitdistrplus: An R package for fitting distributions. *Journal of Statistical Software* **64**(4), 1-34.

DIAL, B. E. & FITZPATRICK, L. C. (1981). The energetic costs of tail autotomy to reproduction in the lizard *Coleonyx brevis* (Sauria: Gekkonidae). *Oecologia* **51**(3), 310-317.

DIAL, B. E. & FITZPATRICK, L. C. (1984). Predator escape success in tailed versus tailless *Scinella lateralis* (Sauria: Scincidae). *Animal Behaviour* **32**(1), 301-302.

DOWNES, S. & SHINE, R. (2001). Why does tail loss increase a lizard's later vulnerability to snake predators? *Ecology* **82**(5), 1293-1303.

DOWNES, S. J. (2002). Does responsiveness to predator scents affect lizard survivorship? *Behavioral Ecology and Sociobiology* **52**(1), 38-42.

ELANGOVAN, V., CREE, A., MONKS, J. & GODFREY, S. (2020). *Oligosoma otagense* (Otago Skink). Aggression and cannabalism. *Herpetological Review* **51**(1), 129.

ELWOOD, C., PELSINSKI, J. & BATEMAN, B. (2012). *Anolis sagrei* (Brown Anole). Voluntary autotomy. *Herpetological Review* **43**(4), 642-642.

ETHERIDGE, R. (1967). Lizard caudal vertebrae. *Copeia* **1967**(4), 699-721.

FOX, J., WEISBERG, S., ADLER, D., BATES, D., BAUD-BOVY, G., ELLISON, S., FIRTH, D., FRIENDLY, M., GORJANC, G. & GRAVES, S. (2017). Package 'car'.

FOX, S., CONDER, J. & SMITH, A. (1998). Sexual Dimorphism in the Ease of Tail Autotomy: Uta stansburiana with and without Previous Tail Loss. *Copeia* **1998**, 376.

FOX, S., PEREA-FOX, S. & CASTRO-FRANCO, R. (1994). Development of the tail autotomy adaptation in lizards under disparate levels of predation at high and low elevations in Mexico. *The Southwestern Naturalist* **39**, 311-322.

HIGHAM, T., LIPSETT, K., SYME, D. & RUSSELL, A. (2013). Controlled Chaos: Three-Dimensional Kinematics, Fiber Histochemistry, and Muscle Contractile Dynamics of Autotomized Lizard Tails. *Physiological and biochemical zoology : PBZ* **86**, 611-30.

HUGHES, M. (2012). Blending a heritage of recreation and tourism with conservation of natural heritage: an example from Penguin Island, Western Australia. *Journal of Heritage Tourism* **7** (1), 1-11.

ITESCU, Y., SCHWARZ, R., MEIRI, S., PAFILIS, P. & CLEGG, S. (2017). Intraspecific competition, not predation, drives lizard tail loss on islands. *Journal of Animal Ecology* **86**(1), 66-74.

LANGTON, J. (2000). *Population biology of the King's skink (Egernia kingii) (Gray) on Penguin Island, Western Australia*. Honours thesis, Murdoch University.

LOZITO, T. P. & TUAN, R. S. (2017). Lizard tail regeneration as an instructive model of enhanced healing capabilities in an adult amniote. *Connective Tissue Research* **58**(2), 145-154.

LYNN, S., BORKOVIC, B. P. & RUSSELL, A. (2013). Relative apportioning of resources to the body and regenerating tail in juvenile leopard geckos

(*Eublepharis macularius*) maintained on different dietary rations. *Physiological and Biochemical Zoology* **86**(6), 659-668.

MAGINNIS, T. L. (2006). The costs of autotomy and regeneration in animals: a review and framework for future research. *Behavioral Ecology* **17**(5), 857-872.

MARCHANT, S. & HIGGINS, P. J. (1990). *Handbook of Australian, New Zealand & Antarctic birds / S. Marchant and P.J. Higgins (co-ordinators)*. Melbourne : Oxford University Press, Melbourne.

MASTERS, C. & SHINE, R. (2003). Sociality in lizards: family structure in free-living King's Skinks *Egernia kingii* from southwestern Australia. *Australian Zoologist* **32**(3), 377-380.

MCELROY, E. J. & BERGMANN, P. J. (2013). Tail autotomy, tail size, and locomotor performance in lizards. *Physiological and Biochemical Zoology* **86**(6), 669-679.

MEYER, V., PREEST, M. R. & LOCHETTO, S. M. (2002). Physiology of Original and Regenerated Lizard Tails. *Herpetologica* **58**(1), 75-86.

NAVARRO, D. J. (2015). Learning statistics with R: A tutorial for psychology students and other beginners. Version 0.5. University of Adelaide, Adelaide, Australia

NAYA, D. E., VELOSO, C., MUÑOZ, J. L. & BOZINOVIC, F. (2007). Some vaguely explored (but not trivial) costs of tail autotomy in lizards. *Comparative Biochemistry and Physiology Part A Molecular & Integrative Physiology* **146**(2), 189-193.

ORTEGA, Z., MENCÍA, A. & PÉREZ-MELLADO, V. (2018). Antipredatory behaviour of a mountain lizard towards the chemical cues of its predatory snakes. *Behaviour* **155** (10-12), 817–840.

PAFILIS, P., FOUFOPOULOS, J., POULAKAKIS, N., LYMBERAKIS, P. & VALAKOS, E. D. (2009). Tail shedding in Island lizard [LACERTIDAE, REPTILIA]: Decline of antipredator defenses in relaxed predation environments. *Evolution* **63**(5), 1262-1278.

PAFILIS, P. & VALAKOS, E. D. (2008). Loss of caudal autotomy during ontogeny of Balkan Green Lizard, *Lacerta trilineata*. *Journal of Natural History* **42**(5-8), 409-419.

PEARS, J. B., EMBERTS, Z. & BATEMAN, P. W. (2018). The Scent of Danger: the Impact of Predator Chemical Cues on Emergence from Refuge and Willingness to Autotomize Limbs in the House Cricket (Acheta domesticus). *Journal of Insect Behavior* **31**(4), 416-426.

PEARSON, D., SHINE, R. & HOW, R. (2002). Sex-specific niche partitioning and sexual size dimorphism in Australian pythons (*Morelia spilota imbricata*). *Biological Journal of the Linnean Society* **77**(1), 113-125.

PLAYFORD, P. E. (1988). *Guidebook to the geology of Rottnest Island*. Geological Society of Australia, WA Division and the Geological Survey of Western Australia.

R DEVELOPMENT CORE TEAM. (2013). R: a language and environment for statistical computing In R Foundation for Statistical Computing, Vienna, Austria.

RUSSELL, A. & BAUER, A. (1992). The m. caudifemoralis longus and its relationship to caudal autotomy and locomotion in lizards (Reptilia: Sauna). *Journal of Zoology (London)* **227**, 127-143.

SIMOU, C., PAFILIS, P., SKELLA, A., KOURKOULI, A. & VALAKOS, E. D. (2008). Physiology of Original and Regenerated Tails in Aegean Wall Lizard (Podarcis erhardii). *Copeia* **2008**(3), 504-509.

SLOTOPOLSKY, B. (1921). *Beiträge zur Kenntnis der Verstümmelungs-und Regenerationsvorgänge am Lacertilierschwanze*, Zurich.

STORR, G. (1978). The genus *Egernia* (Lacertilia, Scincidae) in Western Australia. *Records of the Western Australian Museum* **6**(2), 147-187.

VERVUST, B., VAN DONGEN, S., GRBAC, I. & VAN DAMME, R. (2009). The mystery of the missing toes: extreme levels of natural mutilation in island lizard populations. *Functional Ecology* **23**(5), 996-1003.

VITT, L. J., CONGDON, J. D. & DICKSON, N. A. (1977). Adaptive strategies and energetics of tail autonomy in Lizards. *Ecology* **58**(2), 326-337.

WOODLAND, W. (1920). Memoirs: some observations on caudal autotomy and regeneration in the gecko (*Hemidactylus flaviviridis*, Rppel), with notes on the tails of *Sphenodon* and *Pygopus*. *Journal of Cell Science* **2**(257), 63-100.

Chapter 7. Re-regeneration to reduce negative effects associated with tail loss in lizards

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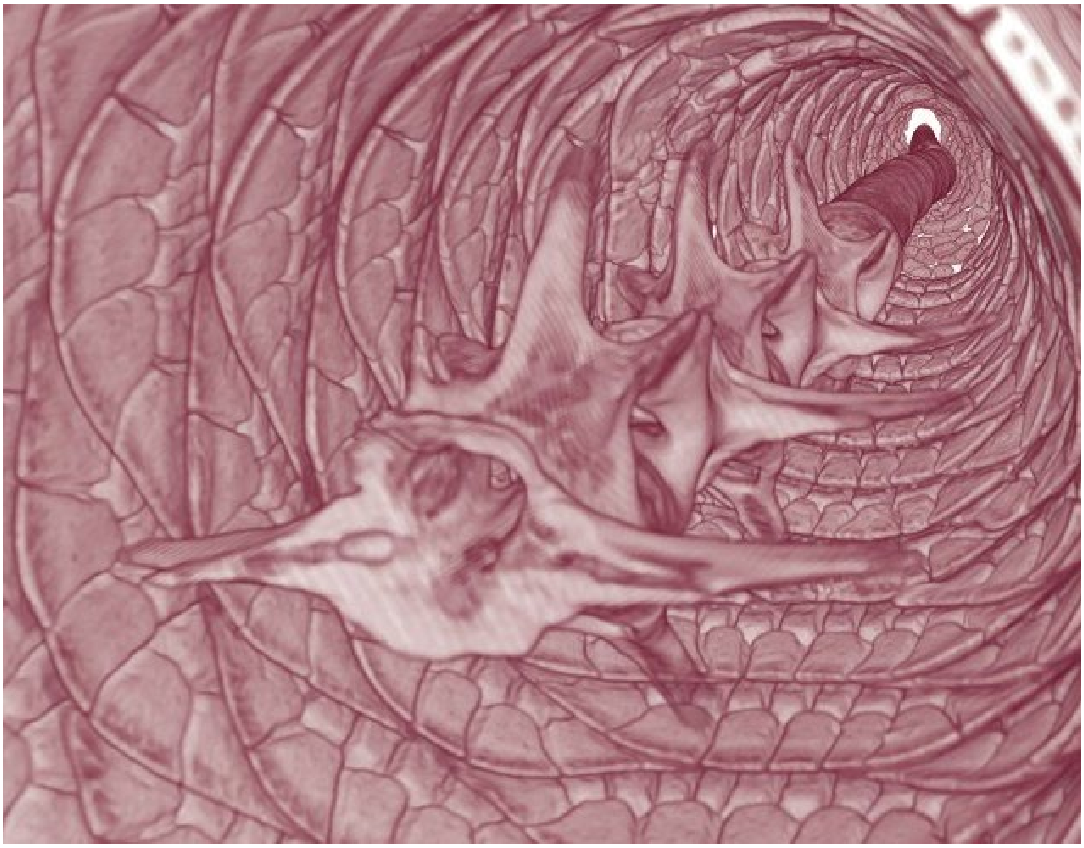

Figure 7.1: Looking inside an autotomised tail – micro-CT scan

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7.1 Abstract

Many species of lizards use caudal autotomy, the ability to self-amputate a portion of their tail, regenerated over time, as an effective anti-predation mechanism. The importance of this tactic for survival depends on the degree of predation risk. There are however, negative trade-offs to losing a tail, such as loss of further autotomy opportunities with the regenerated tail vertebrae being replaced by a continuous cartilaginous rod. The common consensus has been that once a tail has been autotomised and regenerated it can only be autotomised proximal to the last vertebral autotomy point, as the cartilage rod lacks autotomy planes. However, anecdotal evidence suggests that although the regenerated portion of the tail is unable to autotomise, it can re-regenerate following a physical shearing event. We assessed re-regeneration in three populations of the King's skink (*Egernia kingii*), a large lizard endemic to south-west Western Australia and surrounding islands. We show that re-regeneration is present at an average of 17.2% in all three populations and re-regenerated tissue can comprise up to 23.3% of an individual's total tail length. The ability to re-regenerate may minimise the costs to an individual's fitness associated with tail loss, efficiently restoring ecological functions of the tail.

7.2 Introduction

Caudal autotomy is a highly effective anti-predation strategy for squamates, ancestral for all modern taxa and for which we have fossil evidence from Early Permian captorhinids (Price, 1940; LeBlanc *et al.*, 2018). Caudal autotomy, and associated mechanisms, appear to have been lost and re-gained in multiple lizard taxa, depending on the ecological importance of their tail (Arnold, 1984; Dial & Fitzpatrick, 1984; Bellairs & Bryant, 1985). In some species, caudal autotomy is selected against ontogenetically, with fracture planes ossifying as the individuals mature (Etheridge, 1967; Arnold, 1984). Post-autotomy, an individual's tail regenerates, with the original bony vertebrae replaced by a rigid cartilage rod that partially ossifies over time

(Woodland, 1920; Alibardi, 2010; Lozito & Tuan, 2017). Although losing a portion of a tail can have a range of immediate and long term consequences (see Arnold, 1984; Bateman & Fleming, 2009; McElroy & Bergmann, 2013) for reviews), the regenerated tail can restore certain ecological functions associated with the original tail (Vitt, Congdon & Dickson, 1977; Martín & Salvador, 1993; Zamora-Camacho *et al.*, 2016).

7.2.1 Anatomy and morphology of caudal autotomy

There are two ways of shedding a tail: inter-vertebral autotomy, occurring when the tail breaks across inter-vertebral spaces at a point of weakness (Arnold, 1984; Babcock & Blais, 2001), and intra-vertebral autotomy – the ancestral and more frequent form – occurring at pre-formed breakage planes within a series of caudal vertebrae, termed post-pygal vertebrae (Etheridge, 1967; Bellairs & Bryant, 1985). Intra-vertebral autotomy is under more complex neurological control of the individual compared to inter-vertebral autotomy, with some species able to autotomise their tail without a physical stimulus (Bellairs & Bryant, 1985; Elwood, Pelsinski & Bateman, 2012). The tails of species with intra-vertebral autotomy are constructed as autotomisable segments; however, the cartilage rod that regenerates after autotomy lacks breakage planes and therefore cannot be autotomised, with future autotomy events having to occur at the next most proximal vertebrae of the original tail (Etheridge, 1967; Bellairs & Bryant, 1985; Chapple & Swain, 2004; Bateman & Fleming, 2009; Losos, 2009; Lozito & Tuan, 2017). In addition to the regenerated tail differing from the original in terms of internal morphology, the external tail in many species shows a narrowing at the point of autotomy, as well as changes in scale pattern and colour from the original tail (Seligmann, Moravec & Werner, 2008).

7.2.2 Regeneration after autotomy events

It has been assumed that, as the cartilage tube has no breakage planes, lizards cannot autotomise and regenerate sections of already regenerated tails but must instead autotomise the tail closer to the base each time; e.g. “the regenerated tail … lack[s] intravertebral autotomy fracture planes … and, therefore, subsequent autotomies must take place more proximally” (Bateman & Fleming, 2009); “[L]izards that experience repeated tail autotomy must lose their tails progressively closer to the tail base …” (Chapple & Swain, 2004); “When a tail regenerates, the new portion is made of a rod of cartilage and thus lacks the intravertebral breakage planes that enable an unregenerated tail to autotomize” (Losos, 2009, p154). However, it may not be as simple as this. Although autotomy and regeneration are primarily, and efficiently, used together, autotomy is not required for caudal regeneration to occur (Simpson Jr, 1964; Gilbert, Payne & Vickaryous, 2013; Lozito & Tuan, 2017; Barr *et al.*, 2019). Lizards possess the ability to regenerate a cartilage rod and associated tail from an already regenerated portion of their tail, after a shearing event through the cartilage rod, such as a bite from a predator. This regrowth phenomenon, termed re-regeneration, has, as far as we are aware, only been recorded anecdotally (Brindley, 1894; Cooper & Smith, 2009; Lozito & Tuan, 2017) and may further enhance the capacity of regeneration to reduce negative effects associated with caudal autotomy such as time and energy trade-offs to growth and reproduction (Congdon, Vitt & King, 1974; Vitt *et al.*, 1977; Dial & Fitzpatrick, 1981; Lynn, Borkovic & Russell, 2013).

Here we present evidence of re-regeneration in King’s skinks (*Egernia kingii*), a large (up to 244 mm SVL, 550 mm total length) scincid lizard endemic to the south west of Western Australia and its surrounding Islands (Storr, 1978; Cogger, 2014). Although juveniles appear to rely more on caudal autotomy than do adults, adults still possess the ability to autotomise their tails (Barr *et al.*, 2018). In this study we investigate 1) the occurrence and use of re-regeneration across three isolated populations of *E. kingii* that vary in predation risk, 2) assess the internal morphology of re-regeneration using micro CT technology, and 3) discuss the potential mitigating effects of re-regeneration as well as its use in restoring tail function for lizard ecology.

7.2 Methods

7.2.1 Field data

Morphological data for *E. kingii* were collected from three locations along the coast of Western Australia, Rottnest Island (-31.999421°, 115.527540°), Penguin Island (-32.305839°, 115.691340° and Coastal Mainland (-31.868445°, 115.752549°) between 2017 and 2019. General morphological measurements including snout to vent length (SVL), tail length (TL) and regeneration lengths (RL) were measured to the nearest mm using a plastic ruler. Total regeneration length (length of the whole regenerate) as well as length of individual regeneration segments (primary, secondary or tertiary) were recorded. For analysis, three cases with a tertiary regeneration were included as part of the secondary regeneration length. Percentage of re-regeneration occurrence in populations was established, as well as the percentage of the total tail length comprised of re-regeneration and percentage of total regeneration length comprised of re-regeneration for each individual. All statistics were performed in RStudio Version 1.1.383 (R Development Core Team, 2013).

7.2.3 Re-regeneration specimen and CT analysis

For micro CT a single autotomised tail was collected from an adult (SVL 198 mm) in February 2018 on Rottnest Island, Western Australia. The sample was frozen and then preserved in 100 % ethanol after taking a 1 cm tail tip for genetics sampling. The sample was scanned using a micro-CT (SkyScan 1176 scanner; Bruker micro-CT, Kontich, Belgium) at the Centre for Microscopy, Characterisation and Analysis (CMCA), University of Western Australia, Western Australia. The CT scan was performed at 18 µm resolution (50 kV, 500µA, 390ms, 0.5 mm Al filter, 0.5° rotation step, 360° scan and two frame averaging) producing 2000 * 1336-pixel images. CT images were reconstructed in NRecon v1.7.1.0 (Bruker micro-CT) using the modified Feldkamp cone- beam algorithm (Gaussian smoothing kernel (2), ring artefact correction (8), beam hardening correction (30%) and threshold for defect pixel masking (3%)). The spinal column was manually selected as a volume of interest (VOI) within CTAnalyser software v1.17.7.2 (Bruker micro-CT). 3D model was recreated in CTvox v3.3.0 r1403 (Bruker micro-CT) and coronal C.S of the model acquired from digital manipulation of the 3D model.

7.2.4 Ethical statement

All research was carried out in accordance with the Animal Ethics Office of Curtin University (ARE2017-12) and Department of Biodiversity, Conservations and Attractions (DBCA) regulation 17 licence (08-001238-4) for capture and handling of animals.

7.3 Results

The changes in external morphology associated with regeneration, tail width and scale colour, are evident for both the primary regeneration (Figure 7.2, section 3) and the re-regeneration event (Figure 7.2, section 5) of an autotomised tail. The CT scan 3D reconstruction of the vertebral column (Figure 7.2) shows, from left to right, the distal fractured (half) vertebra from the recent autotomy event (1.), two original vertebrae with fracture planes present (2.), the partial vertebra from the previous, older autotomy event where the cartilage regeneration has been anchored to the vertebra post-fracture (3.), followed by the older more mature (primary) regenerated tissue (4.), point of secondary regeneration to the primary (5.), and newest (secondary) regenerated tissue (6.), both of which are externally ossified and lack autotomy planes. Coronal and transverse C.S taken from the CT scan highlight the difference in the internal structure of the regenerated tissues, specifically the degree of ossification of the primary regenerated tissue (4.) and secondary regenerated tissue (6.), with the primary regenerated tissue being more ossified than is the secondary regenerated tissue. This is further highlighted by the angled sagittal C.S of the primary and secondary regeneration, with the primary regenerated tissue showing a solid outer sheath, and the secondary regenerated tissue having a distinct outer and inner sheath, with both exhibiting a hollow inner core for the spinal cord tissue (5.).

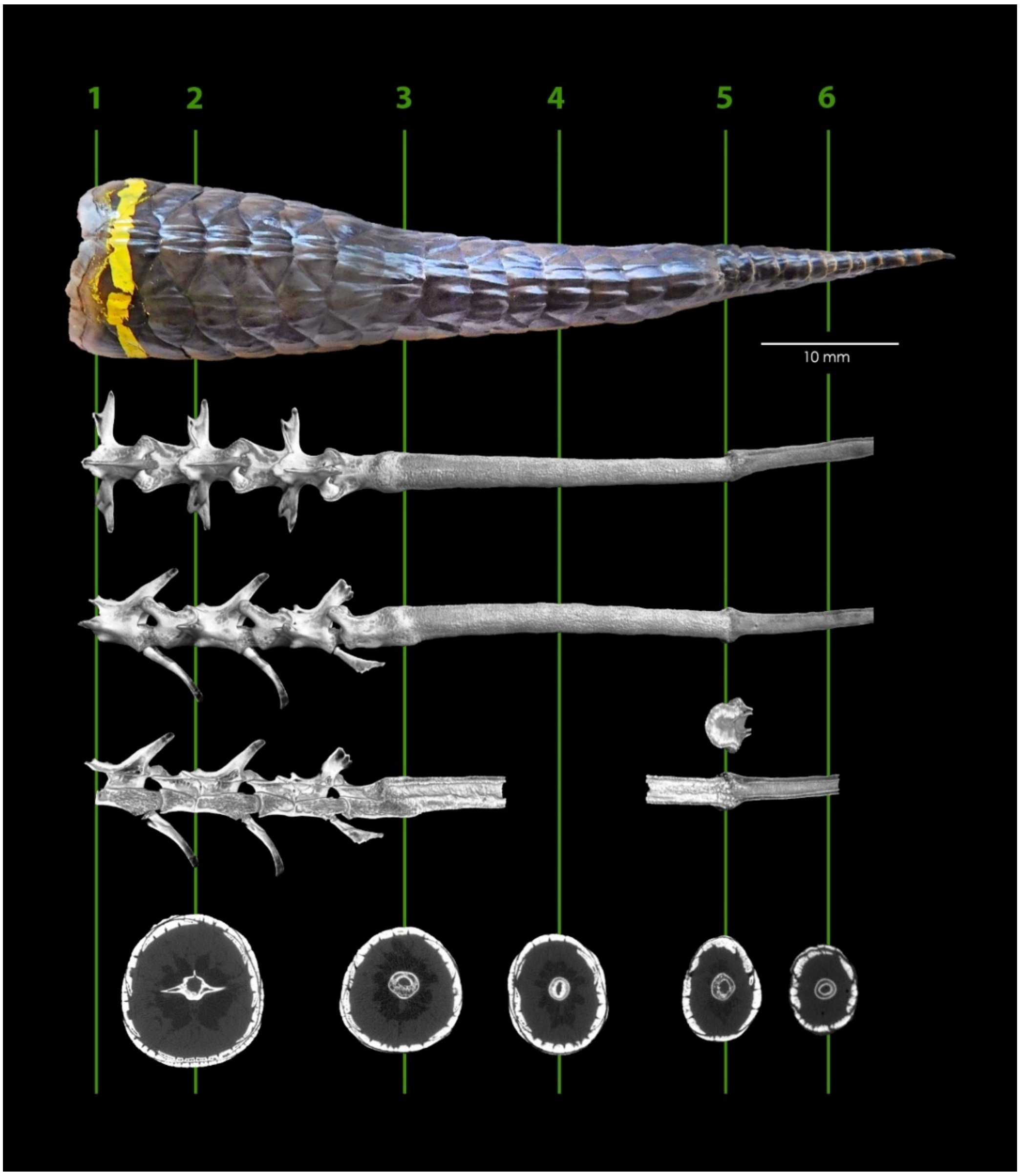

Figure 7.2: Autotomised tail and 3D model reconstruction from micro CT of *Egernia kingii* showing the fractured vertebra (1.), two intact vertebrae (2.), vertebra and primary regeneration fusion point (3.), primary cartilage regeneration (4.), fusion point of primary and secondary cartilage regenerations (5.), and secondary cartilage regeneration (6.) Transverse C.S below correspond to lines on diagram. 1 cm tail tip taken for genetics is missing from the 3D model.

Field data gathered across the three sites indicated that re-regeneration events were not isolated (Table 7.1). Higher proportions of re-regeneration were observed in sites with terrestrial predators (Coastal Mainland and Rottnest Island), compared to the site with no terrestrial predators (Penguin Island), with higher levels of overall regeneration observed in the Penguin Island and Coastal Mainland sites (Figure 7.3). Re-regeneration events occurred at an average of 17.2% for all individuals captured across the three sites (range 13.3% - 25.0%), and in 23.5% (range 17.1 - 46.2%) of individuals that had undergone a regeneration event. Percentage of re-regeneration represented on average (± SD) 18.0 ± 14.8 % of the total tail length and 38.5 ± 20.6 % of the total regenerated length.

Table 7.1: Summary statistics of *Egernia kingii* populations for the number of individuals caught at each site: those that had regenerated tails and those that had re-regenerated tails; the percentage that the re-regeneration contributed to the total tail (original and regenerated tissue), and the regenerated tissue only

Metric	**All sites**	**Rottnest Island**	**Penguin Island**	**Coastal Mainland**
Number caught/ with regeneration/ with re-regeneration	157/115/27	24/13/6	105/82/14	28/21/7
Percentage of total tail length (mean ± SD) that the re-regeneration comprised	18 ± 14.8%	21.2 ± 16.2%	14 ± 11.1%	23.3 ± 19.3%
Percentage of regeneration length (mean ± SD) that the re-regeneration comprised	38.5 ± 20.6%	42.8 ± 18.2%	29.9 ± 18.4%	51.9 ± 20.7 %

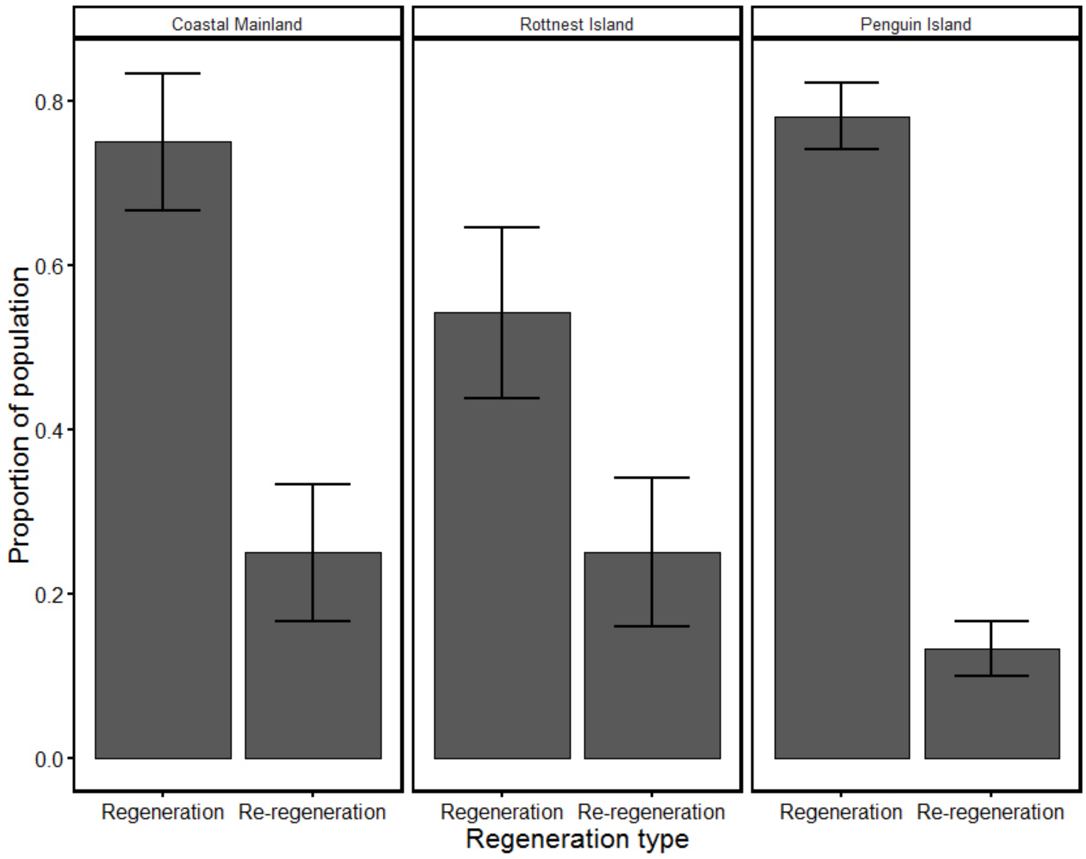

Figure 7.3: Comparison of proportion of regeneration and re-regeneration of tail tissue for the three study sites from highest predation risk (Coastal Mainland) to lowest predation risk (Penguin Island). Mean ± se are reported.

7.5 Discussion

Losing a tail comes with costs, although these are less severe than being killed by a predator. These costs, whether they be to locomotion (Martin & Avery, 1998; Fleming & Bateman, 2012; Zamora-Camacho *et al.*, 2016), anti-predation behaviour (Cooper & Vitt, 1985; Bateman, Fleming & Rolek, 2014) or even to social status (Fox, Heger & Delay, 1990), can be minimised through regeneration of the tail. Here, we have presented unequivocal evidence, through micro CT, that further regeneration of tail tissue is possible if a lizard loses part of the regenerated tail, something that has only been anecdotally recorded before (Vitt *et al.*, 1977; Cooper & Smith, 2009; Lozito & Tuan, 2017). From field data we show that re-regeneration occurs in, and is not an isolated occurrence, in *E. kingii*. Additionally, re-regeneration is known to occur in other species, as seen in *Bellatorias major* (Scincidae) (Figure 7.4), a species related to *E. kingii.* The ability for re-regeneration, such as we demonstrate here, is also likely to aid in restoration of certain behavioural and ecological functions of the tail, and subsequently increase fitness and survival.

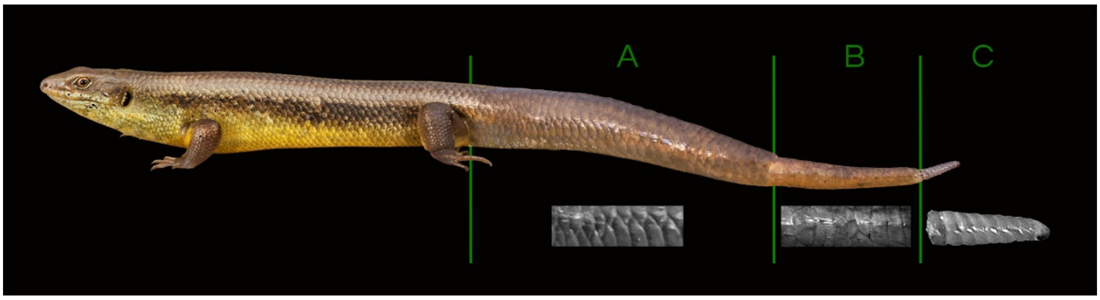

Figure 7.4: Re-regeneration event in a *Bellatorias major* at Cape York, Queensland, showing the external morphology changes associated with the original (A), regenerated (B) and re-regenerated (C) sections of the tail (photo Ryan Francis).

Lizard taxa that rely heavily on their tail, either as an anti-predation tactic or for locomotion, will incur higher costs for not having a tail (Dial & Fitzpatrick, 1984; Downes & Shine, 2001; Medger, Verburgt & Bateman, 2008; McElroy & Bergmann, 2013), and therefore would be predicted to invest more energy both into tail development (Fleming, Valentine & Bateman, 2013; Barr *et al.*, 2018) and into quicker, and more efficient regeneration (Naya *et al.*, 2007; Lynn *et al.*, 2013). Re-regeneration may be more beneficial to populations or species with increased predation risk. Both our sites with terrestrial predators showed higher occurrences of re-regeneration, with the Coastal Mainland site having a higher proportion of re-regenerated tail tissue (Table 7.1). Additionally, three individuals at the Coastal Mainland site had tertiary regenerations on their tails, indicating further re-regeneration events. Invasive mammals, particularly the European red fox (*Vulpes vulpes*) and feral cat (*Felis catus*) on reptile populations in Australia (Woinarski *et al.*, 2018). Our Coastal Mainland site, which is near an urban area, had the highest diversity of predators, including feral cats, dogs, and until recently, red foxes in the area (J. Taylor, pers. comm). As a caveat, intra-specific male-male aggression can also produce high autotomy rates in populations (Pafilis *et al.*, 2009; Itescu *et al.*, 2017). Penguin Island, although lacking terrestrial predators, is known to have high densities of *E. kingii,* and male-male aggression may contribute to the similar regeneration proportion observed in our Coastal Mainland site population (Figure 7.3).

Tail regeneration post-autotomy, as well as re-regeneration of the regenerated tissue post-trauma, requires time and energy, and is dependent on other energetic demands that the lizard faces. Recorded rates of caudal regeneration vary considerably between species, ranging from 0.2 mm per day to 2 mm per day (Hughes & New, 1959; Jamison, 1964; Arnold, 1984), with some species like *Anniella pulchra* (Anniellidae) regenerating much slower (4.1mm in 11 months) (Miller, 1944). As the loss of a tail can have a range of negative effects, it has been proposed that a species will balance the costs of regeneration against requirements for reproductive output (Vitt *et al.*, 1977). Species that are short lived and mature early will prioritise reproductive output over regeneration, while species that are long lived and mature late, with potential future reproductive seasons will do the opposite (Vitt *et al.*, 1977). Older individuals of the gecko *Coleonyx variegatus* (Eublepharidae) prioritised energy investment in tail regeneration and less into growth than did younger individuals, which investing more energy in body growth and less in tail regeneration (Dial & Fitzpatrick, 1981). Furthermore, adult *C. brevis* females prioritise energy into egg production at the expense of tail regeneration (Dial & Fitzpatrick, 1981).

Re-regeneration is likely to benefit the individual and minimise long term ecological costs associated with caudal autotomy. Firstly, having the ability to regenerate from an already regenerated tail will ensure that an individual does not permanently have a severely reduced tail length following a physical shearing event. Secondly, as a smaller portion of tail would be regenerating, as opposed to if the individual was forced to autotomise a larger portion of tail at the next proximal autotomy plane, both time and energy for regeneration would be reduced. Thirdly, the time an individual would be with a shorter tail would also be reduced. Here, we have presented data on additional regenerative ability in lizards, re-regeneration. We have indicated that, at least in *E. kingii*, this is; 1) not an isolated event and 2) can comprise a large portion of the individual's tail. Although the regenerated cartilage rod lacks autotomy planes, and its shedding therefore not likely to be under the same conscious control as intra-vertebral autotomy (Woodland, 1920; Alibardi, 2010; Lozito & Tuan, 2017), we suggest that re-regeneration may provide an additional component in mitigating the negative effects of caudal autotomy on an individual's fitness, particularly in populations with high predation risk. Predator size, type and efficiency, i.e. whether attacks tend to be fatal or directed at the tail, may also influence the likelihood or re-regeneration events occurring (Medel *et al.*, 1988; Bateman & Fleming, 2011). More research on an ecological comparison of the effects of regeneration and re-regeneration is likely to be fruitful.

7.6 Data availability

The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

7.7 Author Contributions Statement

JIB and PWB designed the experiment. JIB carried out the field work. JIB and CAB undertook the CT-Scanning. JIB, CAB, RS, KT and PWB all assisted with the interpretation of the data. JIB wrote the main manuscript text, with JIB, CAB, RS, KT and PWB all contributing to the subsequent drafts. RS prepared Figure 7.2 and 7.4, and JIB prepared Figure 7.3.

7.8 Competing interests

The author(s) declare no competing interests.

7.9 Acknowledgements

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7.10 References:

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ALIBARDI, L. (2010). *Morphological and Cellular Aspects of Tail and Limb Regeneration in Lizards : a Model System with Implications for Tissue Regeneration in Mammals*. Springer-Verlag, Heidelberg, Berlin

ARNOLD, E. (1984). Evolutionary aspects of tail shedding in lizards and their relatives. *Journal of Natural History* **18**(1), 127-169.

BABCOCK, S. & BLAIS, J. (2001). Caudal vertebral development and morphology in three salamanders with complex life cycles (*Ambystoma jeffersonianum*, *Hemidactylium scutatum*, and *Desmognathus ocoee*). *Journal of Morphology* **247**(2), 142-159.

BARR, J., GILSON, L. N., SANCHEZ GARZON, D. F. & BATEMAN, P. W. (2019). Amblyrhynchus cristatus (Marine Iguana). Tail regeneration and bifurcation. *Herpetological Review* **50**(3), 567.

BARR, J. I., SOMAWEERA, R., GODFREY, S. S. & BATEMAN, P. W. (2018). Increased tail length in the King’s skink, *Egernia kingii* (Reptilia: Scincidae): an anti-predation tactic for juveniles? *Biological Journal of the Linnean Society* **126**(2), 268–275.

BATEMAN, P. & FLEMING, P. (2009). To cut a long tail short: a review of lizard caudal autotomy studies carried out over the last 20 years. *Journal of Zoology (London)* **277**(1), 1-14.

BATEMAN, P., FLEMING, P. & ROLEK, B. (2014). Bite me: blue tails as a ‘risky-decoy’defense tactic for lizards. *Current Zoology* **60**(3), 333-337.

BATEMAN, P. W. & FLEMING, P. A. (2011). Frequency of tail loss reflects variation in predation levels, predator efficiency, and the behaviour of three populations of brown anoles. *Biological Journal of the Linnean Society* **103**(3), 648-656.

BELLAIRS, A. & BRYANT, S. (1985). Autotomy and regeneration in reptiles. *Biology of the Reptilia* **15**(5), 301-410.

BRINDLEY, H. H. (1894). On a specimen of *Hemidacylus gledovii*, Murray, with bifid renewed tail. *Journal of the Bombay Natural History Society* **9**, 30 - 33.

CHAPPLE, D. G. & SWAIN, R. (2004). Inter-populational variation in the cost of autotomy in the metallic skink (*Niveoscincus metallicus*). *Journal of Zoology (London)* **264**(4), 411-418.

COGGER, H. G. (2014). *Reptiles and Amphibians of Australia*, 7th edition. CSIRO Publishing, Victoria, Australia.

CONGDON, J. D., VITT, L. J. & KING, W. W. (1974). Geckos: adaptive significance and energetics of tail autotomy. *Science* **184**(4144), 1379-1380.

COOPER, J. W. E. & SMITH, C. S. (2009). Costs and economy of autotomy for tail movement and running speed in the skink *Trachylepis maculilabris*. *Canadian Journal of Zoology* **87**(5), 400-406.

COOPER, W. E. & VITT, L. J. (1985). Blue tails and autotomy: enhancement of predation avoidance in juvenile skinks. *Zeitschrift fuer Tierpsychologie* **70**(4), 265-276.

DIAL, B. E. & FITZPATRICK, L. C. (1981). The energetic costs of tail autotomy to reproduction in the lizard *Coleonyx brevis* (Sauria: Gekkonidae). *Oecologia* **51**(3), 310-317.

DIAL, B. E. & FITZPATRICK, L. C. (1984). Predator escape success in tailed versus tailless *Scinella lateralis* (Sauria: Scincidae). *Animal Behaviour* **32**(1), 301-302.

DOWNES, S. & SHINE, R. (2001). Why does tail loss increase a lizard's later vulnerability to snake predators? *Ecology* **82**(5), 1293-1303.

ELWOOD, C., PELSINSKI, J. & BATEMAN, B. (2012). *Anolis sagrei* (Brown Anole). Voluntary autotomy. *Herpetological Review* **43**(4), 642-642.

ETHERIDGE, R. (1967). Lizard caudal vertebrae. *Copeia* **1967**(4), 699-721.

FLEMING, P. A. & BATEMAN, P. W. (2012). Autotomy, tail regeneration and jumping ability in Cape dwarf geckos (*Lygodactylus capensis*) (Gekkonidae). *African Zoology* **47**(1), 55-59.

FLEMING, P. A., VALENTINE, L. E. & BATEMAN, P. W. (2013). Telling tails: selective pressures acting on investment in lizard tails. *Physiological and Biochemical Zoology* **86**(6), 645-658.

FOX, S. F., HEGER, N. A. & DELAY, L. S. (1990). Social cost of tail loss in *Uta stansburiana*: lizard tails as status-signalling badges. *Animal Behaviour* **39**(3), 549-554.

GILBERT, E. A., PAYNE, S. L. & VICKARYOUS, M. K. (2013). The anatomy and histology of caudal autotomy and regeneration in lizards. *Physiological and Biochemical Zoology* **86**(6), 631-644.

HUGHES, A. & NEW, D. (1959). Tail regeneration in the geckonid lizard, *Sphaerodactylus*. *Development* **7**(3), 281-302.

ITESCU, Y., SCHWARZ, R., MEIRI, S., PAFILIS, P. & CLEGG, S. (2017). Intraspecific competition, not predation, drives lizard tail loss on islands. *Journal of Animal Ecology* **86**(1), 66-74.

JAMISON, J. P. (1964). Regeneration subsequent to intervertebral amputation in lizards. *Herpetologica* **20**(3), 145-149.

LEBLANC, A. R. H., MACDOUGALL, M. J., HARIDY, Y., SCOTT, D. & REISZ, R. R. (2018). Caudal autotomy as anti-predatory behaviour in Palaeozoic reptiles. *Scientific Reports* **8**(3328), 2045-2322.

LOSOS, J. B. (2009). *Lizards in an Evolutionary Tree: Ecology and Adaptive Radiation of Anoles*. University of California Press, Berkeley, California, USA.

LOZITO, T. P. & TUAN, R. S. (2017). Lizard tail regeneration as an instructive model of enhanced healing capabilities in an adult amniote. *Connective Tissue Research* **58**(2), 145-154.

LYNN, S., BORKOVIC, B. P. & RUSSELL, A. (2013). Relative apportioning of resources to the body and regenerating tail in juvenile leopard geckos (*Eublepharis macularius*) maintained on different dietary rations. *Physiological and Biochemical Zoology* **86**(6), 659-668.

MARTIN, J. & AVERY, R. A. (1998). Effects of tail loss on the movement patterns of the lizard, *Psammodromus algirus*. *Functional Ecology* **12**(5), 794-802.

MARTÍN, J. & SALVADOR, A. (1993). Tail loss reduces mating success in the Iberian rock-lizard, *Lacerta monticola*. *Behavioral Ecology and Sociobiology* **32**(3), 185-189.

McElroy, E. J. & Bergmann, P. J. (2013). Tail autotomy, tail size, and locomotor performance in lizards. *Physiological and Biochemical Zoology* **86**(6), 669-679.

Medel, R., Jiménez, J. E., Fox, S. & Jaksic, F. (1988). Experimental Evidence That High Population Frequencies of Lizard Tail Autotomy Indicate Inefficient Predation. *Oikos* **53**(3), 321-324.

Medger, K., Verburgt, L. & Bateman, P. W. (2008). The influence of tail autotomy on the escape response of the Cape dwarf gecko, *Lygodactylus capensis*. *Ethology* **114**(1), 42-52.

Miller, C. M. (1944). Ecologic Relations and Adaptations of the Limbless Lizards of the Genus Anniella. *Ecological Monographs* **14**(3), 271-289.

Naya, D. E., Veloso, C., Muñoz, J. L. & Bozinovic, F. (2007). Some vaguely explored (but not trivial) costs of tail autotomy in lizards. *Comparative Biochemistry and Physiology Part A Molecular & Integrative Physiology* **146**(2), 189-193.

Pafilis, P., Foufopoulos, J., Poulakakis, N., Lymberakis, P. & Valakos, E. D. (2009). Tail shedding in Island lizard [LACERTIDAE, REPTILIA]: Decline of antipredator defenses in relaxed predation environments. *Evolution* **63**(5), 1262-1278.

Price, L. I. (1940). Autotomy of the tail in Permian Reptiles. *Copeia* **1940**(2), 119-120.

R Development Core Team. (2013). R: a language and environment for statistical computing In R Foundation for Statistical Computing, Vienna, Austria.

Seligmann, H., Moravec, J. & Werner, Y. L. (2008). Morphological, functional and evolutionary aspects of tail autotomy and regeneration in the ‘living fossil’ *Sphenodon* (Reptilia: Rhynchocephalia). *Biological Journal of the Linnean Society* **93**(4), 721-743.

Simpson Jr, S. (1964). Analysis of tail regeneration in the lizard *Lygosoma lateral*e. I. Initiation of regeneration and cartilage differentiation: the role of ependyma. *Journal of Morphology* **114**(3), 425-435.

Storr, G. (1978). The genus *Egernia* (Lacertilia, Scincidae) in Western Australia. *Records of the Western Australian Museum* **6**(2), 147-187.

VITT, L. J., CONGDON, J. D. & DICKSON, N. A. (1977). Adaptive strategies and energetics of tail autonomy in Lizards. *Ecology* **58**(2), 326-337.

WOINARSKI, J., MURPHY, B., PALMER, R., LEGGE, S., DICKMAN, C., DOHERTY, T., EDWARDS, G., NANKIVELL, A., READ, J. & STOKELD, D. (2018). How many reptiles are killed by cats in Australia? *Wildlife Research* **45**(3), 247-266

WOODLAND, W. (1920). Memoirs: some observations on caudal autotomy and regeneration in the gecko (*Hemidactylus flaviviridis*, Rppel), with notes on the tails of *Sphenodon* and *Pygopus*. *Journal of Cell Science* **2**(257), 63-100.

ZAMORA-CAMACHO, F. J., RUBIÑO-HISPÁN, M. V., REGUERA, S. & MORENO-RUEDA, G. (2016). Does tail regeneration following autotomy restore lizard sprint speed? Evidence from the lacertid psammodromus algirus. *Herpetological Journal* **26**, 213-218.

Chapter 8. General discussion

Figure 8.1: Bridled Tern (*Onychoprion anaethetus*)

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8.1 Summary of findings

In this thesis, I demonstrate how caudal autotomy is a highly complex anti-predation strategy for a large lizard that undergoes ontogenetic changes in predation risk, as well as how this strategy can be exploited to maximise its effectiveness and minimise future costs. Additionally, I show that caudal autotomy and subsequent regeneration do not always go according to plan, and provide a comprehensive assessment and discussion of regenerative multi-furcation, referred to as ‘abnormal regeneration’ within lepidosaurs. In this discussion, I summarise the main findings of this thesis and discuss future directions of research for caudal autotomy and regeneration. From my research, I perceive areas of further research into caudal autotomy that will help bridge the knowledge gaps in this field, particularly how this costly but effective anti-predation strategy changes ontogenetically, behaviourally, morphologically and phylogenetically; as also the previously understudied area of abnormal regeneration, and how this may affect the behavioural ecology and fitness of individuals.

In Chapter 2, I provided a robust and comprehensive global review on abnormal regeneration in lepidosaurs (lizards and tuatara, where multiple tails are produced from abnormal regeneration events). I identified 366 records from both peer-reviewed and non-peer reviewed sources that spanned 63 different countries and represented 175 different species from 22 of the 45 lepidosaur families. However, this is still likely to be an underestimation. Studies investigating and reporting abnormal regeneration, as shown in Chapter 2 have been identified in published literature since the 16th century (Gessner, Cambier & Wechel, 1586), but have only begun to peak within the last decade (Figure 2.6). The majority of peer-reviewed studies are short observational notes, with occasional reporting of morphological measurements and/or frequency of occurrence in populations or collections (Table 2.3). I also identified that abnormal regeneration can result in the individual having two to six tails, creating up to 300% of additional tail material. This is likely to have considerable implications for the behavioural ecology and fitness of the individual, but few studies have investigated the area of abnormal regeneration in more detail (Przibram, 1909; Volante, 1923; Bellairs & Bryant, 1985), with even fewer studies providing an ecological context (Hayes *et al.*, 2012), and the subsequent potential negative effects to the individual (Wilson, 2012).

Short- and long-term effects of caudal autotomy and subsequent regeneration are well studied; with losing a portion of a tail affecting locomotion (see McElroy & Bergmann, 2013 for a review), social status (Fox, Heger & Delay, 1990), and future predation events (Downes & Shine, 2001). Additonally, energy diverted to regeneration can affect growth and reproduction (Congdon, Vitt & King, 1974; Vitt *et al.*, 1977; Bellairs & Bryant, 1985). Given the significant body of knowledge regarding negative effects of caudal autotomy and regeneration on the individual, it is reasonable to assume that abnormal regeneration would also significantly affect their ecology. Chapter 2 provides the most comprehensive assessment of abnormal regeneration to date, with an extensive discussion focussed on ecological context, knowledge gaps, and future directions for abnormal regeneration reporting and research.

Predator-prey dynamics are highly complex (Barbosa & Castellanos, 2005). In Chapter 3, I attempted to assess ontogenetic changes in predation risk for lizards using representative clay models across several sites that varied in potential predator diversity. I observed no difference in attack rates between juvenile and adult model types. However, the importance of control clay balls was evident, despite often being overlooked in clay model studies (Bateman, Fleming & Wolfe, 2017). Attack rates as predicted were primarily from avian species, with attack rates on models correlated with number of potential avian predator species, but not overall predator diversity. Therefore, based on my research, I advise caution in interpretation of assessing predation risk from representative clay models. One site that received the most attacks was likely to have been severely biased from several nesting non-predatory bird species, directing aggressive behaviour towards new objects within the area, as attacks did not vary between the three model types. I recommend that when undertaking future studies using representative clay models, additional measures should be implemented to minimise misinterpretation of attack data. These measures include use of control clay balls, pairing of camera traps with representative models, taking measures of predatory species abundance, and making representative clay models as real as possible, including UV reflectance and scent marking (Bateman *et al.*, 2017; Rößler, Pröhl & Lötters, 2018).

Reliance on caudal autotomy has been demonstrated to vary ontogenetically with juveniles of certain species having brightly coloured tails, which are lost as they mature, to re-direct attacks away from their body (Cooper & Vitt, 1985; Castilla *et al.*, 1999; Hawlena *et al.*, 2006; Bateman, Fleming & Rolek, 2014). In Chapter 4, I investigated if *Egernia kingii,* which lack bright coloured tails, undergo an ontogenetic shift in relative tail length, with juveniles having longer tails that potentially provide a larger target for predators, directing attacks away from the body (Barr *et al.*, 2019b). From museum specimens, I found that individuals with intact, original tails had longer relative tails as juveniles than did full grown adults. Younger individuals that had a regeneration event had a larger portion of their tail comprising regenerated tissue, indicating a higher reliance on caudal autotomy as an anti-predation strategy. However, residual variances of relative tail length for individuals with intact, original tails increased with age categories, which most likely indicates that adults in some populations exposed to particular predators still rely on their tail for caudal autotomy.

Intra-vertebral autotomy, characterised by the presence of pre-formed fracture planes within a series of caudal vertebrae can become ossified ontogenetically in certain species resulting in the loss or restriction of caudal autotomy (Etheridge, 1967; Arnold, 1984; Bellairs & Bryant, 1985; Arnold, 1988). In Chapter 5, using micro-CT, I investigated if fracture planes within the caudal vertebrae are lost or restricted ontogenetically in King's skinks as they age. All individuals scanned, both juveniles and adults, had fracture planes present within all post-pygal caudal vertebrae, indicating that they retain the osteological ability to autotomise their tail, and that it was not restricted to a portion of the tail. Although loss of fracture planes is associated with loss of caudal autotomy, non-osteological changes such as strengthening of muscle attachment to the vertebrae can occur ontogenetically and restrict the ease of autotomy (Arnold, 1984; Fox, Perea-Fox & Castro-Franco, 1994). As such, histological assessment of muscle fibres and attachment in combination with osteology analysis should be considered in the future to identify how caudal autotomy can be limited on a finer scale.

Ease and willingness to drop a portion of the tail varies with predation risk (Cooper, Pérez-Mellado & Vitt, 2004; Pafilis *et al.*, 2009; Itescu *et al.*, 2017) and with ontogeny (Pafilis & Valakos, 2008), with post-autotomy tail thrash time being effective in predator distraction (Dial & Fitzpatrick, 1983), and varying based on the composition of regenerated tissue (Meyer, Preest & Lochetto, 2002; Naya *et al.*, 2007; Simou *et al.*, 2008). In Chapter 6, I investigated how caudal autotomy behaviour varied ontogenetically and between populations with different predator types. Contrary to my predictions, I found that individual perception of threat, as measured by struggling intensity of the lizard when handled, was the main influencer of willingness to autotomise, not ontogenetic stage nor predator diversity. Threat perception is a significant influential factor that can affect the appropriate use of anti-predation strategies (Stankowich & Blumstein, 2006; Cooper & Blumstein, 2015), but appears to be lacking from autotomy research beyond predation risk of natural populations (Bateman & Fleming, 2009). No difference in thrash time was observed between site or between ontogenetic stage, but post-autotomy tails with higher relative regeneration thrashed for longer. Regenerated muscle fibres differ from that of the original tail, favouring higher contractile force for distraction, with original tails favouring fatigue resistance (Meyer *et al.*, 2002; Higham *et al.*, 2013a; Alibardi, 2015). Although thrash times comparing between regenerated and original tails are reported to vary (Meyer *et al.*, 2002; Naya *et al.*, 2007; Simou *et al.*, 2008) the pattern observed in Chapter 6 likely reflects a difference in mass, not length, of regenerated and original tissue and should be considered in the future.

Once a tail has been autotomised, a continuous cartilage rod regenerates in place of the bony vertebrae and can ossify over time (Woodland, 1920; Bellairs & Bryant, 1985). As the regenerated rod lacks autotomy planes, subsequent conscious autotomy events, occurring at an intra-vertebral fracture plane, have to occur at a more proximal position (Arnold, 1988), limiting its use and effectiveness. In Chapter 7, I investigate re-regeneration i.e. a regeneration event occurring on a regenerated tail from a shearing event. I found that, on average, higher occurrence of re-regeneration, as well as the percentage of tail it comprised, was found at the sites with terrestrial predators, the highest being at a coastal mainland site that has several species of mammalian predators. By providing ecological context, I suggest that this phenomenon is a potential adaptive anti-predation strategy, just like autotomy of intact tail material, although it is likely to depend on the robustness of the prey and on the type of predator (Barr *et al.*, 2019a). The King’s skink is a robust and large scincid, known to be able to fend off certain snake predators (Masters & Shine, 2003). As intra-vertebral caudal autotomy is a neurologically controlled, conscious process (Bellairs & Bryant, 1985; Clause & Capaldi, 2006), the individual retains control over the decision to autotomise a portion of its tail, as demonstrated in Chapter 6. Individuals, depending on the predator type and perceived threat, may choose to fight and, if grabbed on an already regenerated tail, a shearing event may occur at a lower position of the tail, breaking the tail through the regenerated part, as opposed to a conscious autotomy of the tail at a more proximal position.

This, although potentially increasing the immediate risk of being predated, would have long term energy savings associated with regeneration.

As demonstrated in this thesis, caudal autotomy is a highly complex and variable anti-predation strategy that can be exploited and manipulated to potentially maximise its effectiveness, and minimise associated costs. However, as reported in Chapter 2, it can go astray and have the potential for large consequences to the individual's behavioural ecology. Caudal autotomy has been a well-studied area of research for over 100 years (Przibram, 1909; Woodland, 1920; Vitt *et al.*, 1977; Bateman & Fleming, 2009; Higham, *et al.*, 2013b; Barr *et al.*, 2019a) yet despite this many significant knowledge gaps remain. Below, I discuss several important areas which I believe will add to and greatly improve our understanding of caudal autotomy, and I provided suggestions for future research.

8.2 Future directions

As indicated by Emberts *et al.* (2019) in a recent review, evolutionary changes in fracture planes, how they are lost or evolve within a phylogenetic context, is lacking. Although general observations regarding fracture plane absence, presence and ontogenetic loss has been investigated in representatives of lizard families, this is highly variable (see Etheridge, 1967; Arnold, 1994; Zani, 1996). Loss of fracture planes ontogenetically and over evolutionary time is complex, being associated with tail importance (Arnold, 1984; Bateman & Fleming, 2009; Fleming, Valentine & Bateman, 2013). The sub family Egerniinae represents a small phylogenetic grouping of approximately 60 species from eight genera (*Egernia, Bellatorias, Liopholis, Lissolepis, Tiliqua, Tribolonotus, cyclodomorphus* and *Corucia* (Gardner et al., 2008; Uetz, Freed & Hošek, 2019; While et al., 2015). Within this group, species exhibit a wide range of tail specialisations and loss of autotomy, both for entire species e.g. *Corucia zebrata* and *Egernia depressa* (Etheridge, 1967), ontogenetically reduced e.g. *Tiliqua rugosa* and *Tiliqua scincoides*

(Russell & Bauer, 1992) or retained such as observed from this thesis within *Egernia kingii* (Chapter 5). Additionally, members of the ‘Egernia group’ have species that vary considerably in size, both between species, and ontogenetically within species, as well time until maturity (two- five years) (Chapple, 2003). This small phylogenetic grouping possesses a wide variety of traits that make them an ideal grouping to investigate change in caudal autotomy morphologically, phylogenetically, physiologically and behaviourally, both evolutionary and ontogenetically.

As demonstrated in Chapter 2, ‘abnormal regeneration’, despite being known about for over 400 years, has received little attention, specifically in regard to an ecological context (Gessner *et al.*, 1586; Przibram, 1909; Bellairs & Bryant, 1985; Hayes *et al.*, 2012). As I explain within Chapter 2, the addition of extra mass and structure of tail material to the individual is likely to affect its behavioural ecology, but, as far as I am aware, no study has investigated this. *Egernia kingii*, like many species, resides in rocky outcrops between crevices, or amongst tree roots (Chapple, 2003) and is therefore required to navigate physically restrictive terrain. Species like *E. kingii* that have to navigate physically restrictive terrain provide ideal study species to behaviourally investigate how abnormal regeneration would affect their behavioural ecology, in particular for escape from predators. Behavioural studies could potentially be conducted with the addition of a 3D printed or silicon cast secondary tail attached with strapping tape or super glue, similarly to how radio transmitters are attached to the lateral tail base of lizards (Kenward, 2001; Barr, 2016).

8.3 References

“Every reasonable effort has been made to acknowledge the owners of copyright material. I would be pleased to hear from any copyright owner who has been omitted or incorrectly acknowledged.”

ALIBARDI, L. (2015). Regenerating tail muscles in lizard contain Fast but not Slow Myosin indicating that most myofibers belong to the fast twitch type for rapid contraction. *Tissue and Cell* **47**(5), 533-40.

ALIBARDI, L. (2019). Tail regeneration in Lepidosauria as an exception to the generalized lack of organ regeneration in amniotes. *Journal of Experimental Zoology Part B: Molecular and Developmental Evolution*, 1-20.

ARNOLD, E. (1984). Evolutionary aspects of tail shedding in lizards and their relatives. *Journal of Natural History* **18**(1), 127-169.

ARNOLD, E. (1988). Caudal autotomy as a defense. *Biology of the Reptilia* **16**, 235-273.

ARNOLD, E. N. (1994). Investigating the evolutionary effects of one feature on another: does muscle spread suppress caudal autotomy in lizards? *Journal of Zoology* **232**(3), 505-523.

BARBOSA, P. & CASTELLANOS, I. (2005). *Ecology of predator-prey interactions*. Oxford University Press.

BARR, J. (2016). *Social structure and the influence of personality traits in the King’s skink (Egernia kingii) on Penguin Island, Western Australia*. Honours thesis thesis, Murdoch University.

BARR, J. I., BOISVERT, C. A., SOMAWEERA, R., TRINAJSTIC, K. & BATEMAN, P. W. (2019a). Re-regeneration to reduce negative effects associated with tail loss in lizards. *Scientific Reports* **9**(1), 18717.

BARR, J. I., SOMAWEERA, R., GODFREY, S. S. & BATEMAN, P. W. (2019b). Increased tail length in the King’s skink, *Egernia kingii* (Reptilia: Scincidae): an anti-predation tactic for juveniles? *Biological Journal of the Linnean Society* **126**(2), 268–275.

BATEMAN, P., FLEMING, P. & ROLEK, B. (2014). Bite me: blue tails as a ‘risky-decoy’defense tactic for lizards. *Current Zoology* **60**(3), 333-337.

BATEMAN, P., FLEMING, P. & WOLFE, A. (2017). A different kind of ecological modelling: the use of clay model organisms to explore predator–prey interactions in vertebrates. *Journal of Zoology* **301**(4), 251-262.

BATEMAN, P. W. & FLEMING, P. A. (2009). To cut a long tail short: a review of lizard caudal autotomy studies carried out over the last 20 years. *Journal of Zoology (London)* **277**(1), 1-14.

BELLAIRS, A. & BRYANT, S. (1985). Autotomy and regeneration in reptiles. *Biology of the Reptilia* **15**(5), 301-410.

CASTILLA, A. M., GOSA, A., GALAN, P. & PEREZ-MELLADO, V. (1999). Green tails in lizards of the Genus *Podarcis*: Do they influence the intensity of predation? *Herpetologica* **55**(4), 530-537.

CHAPPLE, D. G. (2003). Ecology, life-history, and behaviour in the Australian scincid genus *Egernia*, with comments on th evolution of complex sociality in lizards. *Herpetological Monographs* **17**(1), 145-180.

CLAUSE, A. R. & CAPALDI, E. A. (2006). Caudal autotomy and regeneration in lizards. *Journal of Experimental Zoology* **305**(12), 965-973.

CONGDON, J. D., VITT, L. J. & KING, W. W. (1974). Geckos: adaptive significance and energetics of tail autotomy. *Science* **184**(4144), 1379-1380.

COOPER, J. W. E. & BLUMSTEIN, D. T. (2015). *Escaping From Predators: An Integrative View of Escape Decisions*. Cambridge University Press, Cambridge.

COOPER, W. E., PÉREZ-MELLADO, V. & VITT, L. J. (2004). Ease and effectiveness of costly autotomy vary with predation intensity among lizard populations. *Journal of Zoology* **262**(3), 243-255.

COOPER, W. E. & VITT, L. J. (1985). Blue tails and autotomy: enhancement of predation avoidance in juvenile skinks. *Zeitschrift fuer Tierpsychologie* **70**(4), 265-276.

DIAL, B. E. & FITZPATRICK, L. C. (1983). Lizard tail autotomy: function and energetics of postautotomy tail movement in *Scincella lateralis*. *Science*, 391-393.

DOWNES, S. & SHINE, R. (2001). Why does tail loss increase a lizard's later vulnerability to snake predators? *Ecology* **82**(5), 1293-1303.

EMBERTS, Z., ESCALANTE, I. & BATEMAN, P. W. (2019). The ecology and evolution of autotomy. *Biological Reviews* **94**, 1881-1896.

ETHERIDGE, R. (1967). Lizard caudal vertebrae. *Copeia* **1967**(4), 699-721.

FLEMING, P. A., VALENTINE, L. E. & BATEMAN, P. W. (2013). Telling tails: selective pressures acting on investment in lizard tails. *Physiological and Biochemical Zoology* **86**(6), 645-658.

FOX, S., PEREA-FOX, S. & CASTRO-FRANCO, R. (1994). Development of the tail autotomy adaptation in lizards under disparate levels of predation at high and low elevations in Mexico. The Southwestern Naturalist **39**, 311-322.

FOX, S. F., HEGER, N. A. & DELAY, L. S. (1990). Social cost of tail loss in *Uta stansburiana*: lizard tails as status-signalling badges. *Animal Behaviour* **39**(3), 549-554.

GARDNER, M. G., HUGALL, A. F., DONNELLAN, S. C., HUTCHINSON, M. N. & FOSTER, R. (2008). Molecular systematics of social skinks: phylogeny and taxonomy of the *Egernia* group (Reptilia: Scincidae). *Zoological Journal of the Linnean Society* **154**(4), 781-794.

GESSNER, C., CAMBIER, R. & WECHEL, J. (1586). *Conr. Gesneri Tigurini medicinæ et philosophiae professoris in Schola Tigurina, Historiæ animalium liber II : qui est de quadrupedibus ouiparis*. Ex officina typographica Ioannis Wecheli. Impensis Roberti Cambieri, Francofurdi.

HAWLENA, D., BOOCHNIK, R., ABRAMSKY, Z. & BOUSKILA, A. (2006). Blue tail and striped body: why do lizards change their infant costume when growing up? *Behavioral Ecology* **17**(6), 889-896.

HAYES, W. K., IVERSON, J. B., KNAPP, C. R. & CARTER, R. L. (2012). Do invasive rodents impact endangered insular iguana populations? *Biodiversity and conservation* **21**(7), 1893-1899.

HIGHAM, T., LIPSETT, K., SYME, D. & RUSSELL, A. (2013a). Controlled Chaos: Three-Dimensional Kinematics, Fiber Histochemistry, and Muscle Contractile Dynamics of Autotomized Lizard Tails. *Physiological and biochemical zoology : PBZ* **86**, 611-30.

HIGHAM, T. E., RUSSELL, A. P. & ZANI, P. A. (2013b). Integrative biology of tail autotomy in lizards. *Physiological and Biochemical Zoology* **86**(6), 603-610.

ITESCU, Y., SCHWARZ, R., MEIRI, S., PAFILIS, P. & CLEGG, S. (2017). Intraspecific competition, not predation, drives lizard tail loss on islands. *Journal of Animal Ecology* **86**(1), 66-74.

KENWARD, R. (2001). *A Manual for Wildlife Radio Tagging*. Academic Press.

MASTERS, C. & SHINE, R. (2003). Sociality in lizards: family structure in free-living King's Skinks *Egernia kingii* from southwestern Australia. *Australian Zoologist* **32**(3), 377-380.

MCELROY, E. J. & BERGMANN, P. J. (2013). Tail autotomy, tail size, and locomotor performance in lizards. *Physiological and Biochemical Zoology* **86**(6), 669-679.

MEYER, V., PREEST, M. R. & LOCHETTO, S. M. (2002). Physiology of Original and Regenerated Lizard Tails. *Herpetologica* **58**(1), 75-86.

NAYA, D. E., VELOSO, C., MUÑOZ, J. L. & BOZINOVIC, F. (2007). Some vaguely explored (but not trivial) costs of tail autotomy in lizards. *Comparative Biochemistry and Physiology Part A Molecular & Integrative Physiology* **146**(2), 189-193.

PAFILIS, P., FOUFOPOULOS, J., POULAKAKIS, N., LYMBERAKIS, P. & VALAKOS, E. D. (2009). Tail shedding in Island lizard [LACERTIDAE, REPTILIA]: Decline of antipredator defenses in relaxed predation environments. *Evolution* **63**(5), 1262-1278.

PAFILIS, P. & VALAKOS, E. D. (2008). Loss of caudal autotomy during ontogeny of Balkan Green Lizard, *Lacerta trilineata*. *Journal of Natural History* **42**(5-8), 409-419.

PRZIBRAM, H. (1909). *Experimental-Zoologie: eine Zusammenfassung der durch Versuche ermittelten Gesetzmässigkeiten tierischer Wieder-Erzeugung (Nachwachsen, Umformung, Missbildung). Regeneration*. F. Deuticke, Leipzig, Wien.

RÖßLER, D. C., PRÖHL, H. & LÖTTERS, S. (2018). The future of clay model studies. *BMC Zoology* **3**(1), 6.

RUSSELL, A. & BAUER, A. (1992). The m. caudifemoralis longus and its relationship to caudal autotomy and locomotion in lizards (Reptilia: Sauna). *Journal of Zoology (London)* **227**, 127-143.

SIMOU, C., PAFILIS, P., SKELLA, A., KOURKOULI, A. & VALAKOS, E. D. (2008). Physiology of Original and Regenerated Tails in Aegean Wall Lizard (Podarcis erhardii). *Copeia* **2008**(3), 504-509.

STANKOWICH, T. & BLUMSTEIN, D. (2006). Fear in animals: A meta-analysis and review of risk assessment. *Proceedings of the Royal Society B: Biological Sciences* **272**(1581), 2627-34.

UETZ, P., FREED, P. & HOŠEK, J. (eds.) (2019). The Reptile Database, http://www.reptile-database.org, accessed June 19th 2020

VITT, L. J., CONGDON, J. D. & DICKSON, N. A. (1977). Adaptive strategies and energetics of tail autonomy in Lizards. *Ecology* **58**(2), 326-337.

VOLANTE, F. (1923). Contributo alla conoscenza della superrigenerazione nei rettili. *Monitore zoologico italiano* **34**, 185-201.

WHILE, G. M., CHAPPLE, D. G., GARDNER, M. G., ULLER, T. & WHITING, M. J. (2015). Egernia lizards. *Current Biology* **25**(14), R593-R595.

WILSON, S. K. (2012). *Australian Lizards: A Natural History*. CSIRO Publishing, Victoria, Australia.

WOODLAND, W. (1920). Memoirs: some observations on caudal autotomy and regeneration in the gecko (*Hemidactylus flaviviridis*, Rppel), with notes on the tails of *Sphenodon* and *Pygopus*. *Journal of Cell Science* **2**(257), 63-100.

ZANI, P. A. (1996). Patterns of caudal-autotomy evolution in lizards. *Journal of Zoology* **240**(2), 201-220.

Appendix 1. Copyright statements

Chapter 2. When one tail isn't enough: abnormal caudal regeneration in lepidosaurs and its potential ecological impacts

To Whom it May Concern, I James Ian Barr collected and analysed the data; I led the writing of the manuscript; all authors contributed to the writing and revisions of the manuscript for the following publication:

BARR, J. I., SOMAWEERA, R., GODFREY, S. S., GARDNER, M. G. & BATEMAN, P. W. (2020). When one tail isn't enough: abnormal caudal regeneration in lepidosaurs and its potential ecological impacts *Biological Reviews. Online early.* https://doi.org/10.1111/brv.12625

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Chapter 3. Assessing ontogenetic change in predation risk for a lizard with clay models: confounding effects of site and predators, and limitations of the method

To Whom it May Concern, I James Ian Barr collected; and analysed the data with the guidance of SSG; I led the writing of the manuscript; all authors contributed to the writing and revisions of the manuscript for the following publication:

Barr, J. I., Somaweera, R., Godfrey, S. S. & Bateman, P. W. Assessing ontogenetic change in predation risk for a lizard with clay models: confounding effects of site and predators, and limitations of the method. *In preparation for submission*.

Chapter 4. Increased tail length in the King’s skink, *Egernia kingii* (Reptilia: Scincidae): an anti-predation tactic for juveniles?

To Whom it May Concern, I James Ian Barr collected; and analysed the data with the guidance of SSG; I led the writing of the manuscript; all authors contributed to the writing and revisions of the manuscript for the following publication:

Barr, J. I., Somaweera, R., Godfrey, S. S. & Bateman, P. W. (2019). Increased tail length in the King’s skink, *Egernia kingii* (Reptilia: Scincidae): an anti-predation tactic for juveniles? *Biological Journal of the Linnean Society* **126**(2), 268–275. https://doi.org/10.1093/biolinnean/bly196

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Chapter 5. Split ends: Caudal autotomy fractures planes in a large scincid lizard, *Egernia kingii* during ontogenetic development

To Whom it May Concern, I James Ian Barr collected and analysed the data; I led the writing of the manuscript; all authors contributed to the writing and revisions of the manuscript for the following publication:

BARR, J. I., BOISVERT, C. A., TRINAJSTIC, K. & BATEMAN, P. W. Caudal autotomy fractures planes in a large scincid lizard, Egernia kingii during ontogenetic development. *In preparation for submission*.

Chapter 6. Chapter 6. Drop it or not: individual perception of threat, not predation risk drives autotomy in King's skinks (*Egernia kingii*)

To Whom it May Concern, I James Ian Barr collected; and analysed the data with the guidance of SSG; I led the writing of the manuscript; all authors contributed to the writing and revisions of the manuscript for the following publication:

BARR, J. I., SOMAWEERA, R., GODFREY, S. S. & BATEMAN, P. W. Drop it or not: individual perception of threat, not predation risk drives autotomy in King's skinks (*Egernia kingii*). *In preparation for submission*.

Chapter 7. Re-regeneration to reduce negative effects associated with tail loss in lizards

To Whom it May Concern, I James Ian Barr collected and analysed the data; I led the writing of the manuscript; all authors contributed to the writing and revisions of the manuscript for the following publication:

Barr, J. I., Boisvert, C. A., Somaweera, R., Trinajstic, K. & Bateman, P. W. (2019). Re-regeneration to reduce negative effects associated with tail loss in lizards. *Scientific Reports* **9**(1), 18717. https://doi.org/10.1038/s41598-019-55231-6

Appendix 2. When one tail isn't enough: abnormal caudal regeneration in lepidosaurs and its potential ecological impacts – Online supplementary table included with Barr *et al.*, *In press*.

Table A2.2: Records of abnormal regeneration from the compiled database. Species, type of abnormal regeneration and record reference are reported. References that discussed the concept of abnormal regeneration but did not provide original records are indicated in the Abnormality section as 'discussed'. Nomenclatures of species names consistent with the most recent classifications from The Reptile Database (Uetz *et al*., 2019; http://www.reptile-database.org, accessed July 27th 2019).

Family	Species	Abnormality	Reference
Agamidae	*Agama lionotus*	Bifurcation	Wagner *et al.* (2009)
	Agama picticauda	Bifurcation	Ofori *et al.* (2018)
	Bronchocela cristatella	Trifurcation	Brindley (1898)
	Intellagama lesueurii	Bifurcation	Tofohr (1903); Online media source
		Trifurcation	Baxter-Gilbert & Riley (2015)
	Laudakia tuberculata	Bifurcation	Chandra & Mukherjee (1980)
	Paralaudakia caucasius	Bifurcation	Ananjeva & Danov (1991)
Alopoglossidae	–	–	–
Amphisbaenidae	–	–	–
Anguidae	*Anguis fragilis*	Bifurcation	Espasandín (2017)
	Elgaria multicarinata	Bifurcation	Banta (1963); Online media source

	Elgaria spp.	Bifurcation	Online media source
Anniellidae	–	–	–
Bipedidae	–	–	–
Blanidae	–	–	–
Cadeidae	–	–	–
Carphodactylidae	–	–	–
Chamaeleonidae	–	–	–
Cordylidae	*Platysaurus broadleyi*	Bifurcation	Online media source
Corytophanidae	*Basiliscus vittatus*	Bifurcation	Online media source
Crotaphytidae	*Crotaphytus collaris*	Bifurcation	Montanucci (1969)
Dactyloidae	*Anolis bimaculatus*	Bifurcation	Najbar & Skawiński (2018)
	Anolis carolinensis	Bifurcation	Tumlison *et al.* (2015); Online media source
	Anolis cristatellus	Bifurcation	Online media source
	Anolis distichus	Trifurcation	Online media source
	Anolis equestris	Trifurcation	Camper & Camper (2017)
	Anolis grahami	Trifurcation	Brindley (1898)
	Anolis porcatus	Bifurcation	Monsisbay & Olcha (2016)
	Anolis sagrei	Bifurcation	Goin & Goin (1971) Online media source
	Anolis sagrei	Quadrifurcation	Online media source
Dibamidae	–	–	–
Diplodactylidae	*Amalosia rhombifer*	Bifurcation	Online media source
	Bavayia spp.	Bifurcation	Online media source

	Hesperoedura reticulata	Bifurcation	Online media source
	Nebulifera robusta	Bifurcation	Online media source
	Rhacodactylus auriculatus	Bifurcation	Online media source
	Strophurus williamsi	Bifurcation	Online media source
Diploglossidae	*Ophiodes striatus*	Bifurcation	Cozendey *et al.* (2013)
Eublepharidae	*Eublepharis macularius*	Bifurcation	Online media source
Gekkonidae	*Afrogecko porphyreus*	Bifurcation	Online media source
	Christinus guentheri	Trifurcation	Online media source
	Christinus marmoratus	Trifurcation	Crouch (1969); Online media source
	Cyrtopodion kohsulaimanai	Bifurcation	Khan (1991)
	Gehyra dubia	Bifurcation	Online media source
	Gehyra mutilata	Bifurcation	Online media source
	Gehyra spp.	Bifurcation	Online media source
	Gekko gecko	Bifurcation	Gogoi *et al.* (2018); Online media source
	Gekko monarchus	Bifurcation	McKelvy & Ozelski-McKelvy (2012)
	Gekko vittatus	Bifurcation	Online media source
	Hemidactylus agrius	Bifurcation	De Andrade *et al.* (2015)
	Hemidactylus bowringii	Bifurcation	Teynié (2004)
	Hemidactylus brookii	Bifurcation	Vyas (2016)
		Trifurcation	Hora (1926)
		Bifurcation	Woodland (1920);

Hemidactylus flaviviridis		Singh Sood (1939); Kumbar (2011); Online media source
	Trifurcation	Das (1932)
Hemidactylus frenatus	Bifurcation	Chan *et al.* (1984); Garcia-Vinalay (2017); Heyborne & Mahan (2017); Maria & Al-Razi (2018); Online media source
Hemidactylus giganteus	Bifurcation	Gandla & Srinivasulu (2015)
Hemidactylus gleadovii	Bifurcation	Brindley (1894)
Hemidactylus mabouia	Bifurcation	Loveridge (1923)
Hemidactylus persicus	Trifurcation	Evans & Bellairs (1983)
Hemidactylus prashadi	Bifurcation	Yankanchi & Kumbar (2016)
Hemidactylus spp.	Bifurcation	Online media source
Hemidactylus turcicus	Bifurcation	Online media source
Heteronotia binoei	Bifurcation	Online media source
Lepidodactylus lugubris	Bifurcation	Online media source
	Trifurcation	Online media source
	Pentafurcation	Chan *et al.* (1984)
Lepidodactylus spp.	Bifurcation	Online media source
Lygodactylus verticillatus	Trifurcation	Das (1932)

	Mediodactylus kotschyi	Bifurcation	Kukushkin (2019)
	Pachydactylus capensis	Bifurcation	Bates (1989)
		Trifurcation	Bates (1989)
	Phelsuma madagascariensis	Bifurcation	Online media source
Gerrhosauridae	–	–	–
Gymnophthalmidae	*Vanzosaura rubricauda*	Bifurcation	Pheasey *et al.* (2014)
		Trifurcation	Pheasey *et al.* (2014)
Helodermatidae	–	–	–
Hoplocercidae	–	–	–
Iguanidae	*Amblyrhynchus cristatus*	Bifurcation	Barr *et al.* (2019*b*)
	Ctenosaura palearis	Trifurcation	Ariano-Sánchez & Gil (2016)
	Ctenosaura similis	Bifurcation	Online media source
	Cyclura carinata	Bifurcation	Hayes *et al.* (2012)
		Trifurcation	Hayes *et al.* (2012)
	Cyclura cychlura	Bifurcation	Hayes *et al.* (2012)
	Cyclura rileyi	Bifurcation	Hayes *et al.* (2012)
		Trifurcation	Hayes *et al.* (2012)
		Quadrifurcation	Hayes *et al.* (2012)
	Dipsosaurus dorsalis	Bifurcation	Online media source
	Iguana delicatissima	Quadrifurcation	Koleska & Jablonski (2018)
	Iguana	Bifurcation	Online media source
	Sauromalus ater	Bifurcation	Koleska *et al.* (2017*b*)
Lacertidae	*Acanthodactylus aegyptius*	Bifurcation	Stark *et al.* (2018)

Acanthodactylus boskianus	Bifurcation	Tamar *et al.* (2013*a*)
Acanthodactylus erythrurus	Trifurcation	Online media source
Algyroides nigropunctatus	Trifurcation	Koleska & Jablonski (2015)
Eremias arguta	Bifurcation	Gordeev, 2017
Gallotia atlantica	Bifurcation	Online media source
Gallotia caesaris	Bifurcation	Online media source
Gallotia galloti	Trifurcation	Online media source
Ichnotropis capensis	Bifurcation	Broadley (1979)
Lacerta agilis	Bifurcation	Giebel, 1864; Tornier, 1897; Brindley, 1898; Dudek & Ekner-Grzyb, 2014; Gordeev, 2017; Kolenda *et al.*, 2017; Online media source
Lacerta agilis	Trifurcation	Gräper (1909)
Lacerta viridis	Bifurcation	Aldrovandi *et al.* (1642); Müller (1852); Tornier (1897); Tofohr (1903); Alibardi, Sala & Miolo (1988); Online media source
	Trifurcation	Volant (1923)
Ophisops elegans	Bifurcation	Tamar *et al.* (2013*b*)

Phoenicolacerta kulzeri	Bifurcation	Modrý *et al.* (2013)
Podarcis erhardii	Bifurcation	Brock & Belasen (2014) Online media source
Podarcis filfolensis	Bifurcation	Online media source
Podarcis melisellensis	Bifurcation	Baeckens *et al.* (2018)
Podarcis muralis	Bifurcation	Gachet (1834); Tofohr (1903, 1905); Slotopolsky (1921); Volante (1923); Quattrini (1953); Alibardi *et al.* (1988); Bressi (1999); Alibardi (2010); Pola & Koleska (2017); Sorlin *et al.* (2019); Online media source
	Trifurcation	Badiane (2017)
Podarcis siculus	Bifurcation	Tofohr (1905); Alibardi *et al*. (1988); Bressi (1999); Alibardi (2010)
Podarcis spp.	Bifurcation	Online media source
Teira dugesii	Bifurcation	Koleska *et al.* (2017*a*)
Timon lepidus	Bifurcation	Vincent (1877); Renet (2013)
Unknown	Bifurcation	Aldrovandi *et al.* (1642); Tofohr (1903);

			Online media source
		Trifurcation	Aldrovandi *et al.* (1642)
		Quadrifurcation	Aldrovandi *et al.* (1642)
	Zootoca vivipara	Bifurcation	Laver (1879); Knight (1965); Dudek & Ekner-Grzyb (2014); Kolenda *et al.* (2017); Online media source
		Trifurcation	Tornier (1897); Knight (1965)
Lanthanotidae	–	–	–
Leiocephalidae	*Leiocephalus carinatus*	Bifurcation	Online media source
Leiosauridae	–	–	–
Liolaemidae	*Liolaemus tenuis*	Bifurcation	Chávez-Villavicencio & Tabilo-Valdivieso (2017)
Opluridae	–	–	–
Phrynosomatidae	*Cophosaurus texanus*	Trifurcation	Mata-Silva *et al.* (2010)
	Holbrookia elegans	Bifurcation	Online media source
	Sceloporus occidentalis	Bifurcation	Clark (1973); Online media source
	Sceloporus spp.	Bifurcation	Online media source
	Sceloporus uniformis	Bifurcation	Heyborne & McMullin (2016)
	Sceloporus variabilis	Bifurcation	Online media source
	Urosaurus bicarinatus	Bifurcation	Mata-Silva *et al.* (2013)
	Urosaurus ornatus	Bifurcation	Online media source
	Uta stansburiana	Bifurcation	Online media source

Phyllodactylidae	*Asaccus gallagheri*	Bifurcation	Koleska (2018)
	Homonota uruguayensis	Bifurcation	Abegg *et al.* (2014)
	Phyllopezus pollicaris	Bifurcation	Filadelfo *et al.* (2017)
	Tarentola mauritanica	Bifurcation	Tofohr (1903); Online media source
	Thecadactylus rapicauda	Bifurcation	Online media source
Polychrotidae	–	–	–
Pygopodidae	*Delma borea*	Trifurcation	Online media source
Pygopodidae	*Lialis burtonis*	Bifurcation	Online media source
Rhineuridae	–	–	–
Scincidae	*Ablepharus deserti*	Bifurcation	Jablonski (2016)
	Ablepharus kitaibelii	Bifurcation	Vergilov & Natchev (2017)
	Brasiliscincus heathi	Bifurcation	Magalhães *et al.* (2015)
	Carinascincus coventryi	Bifurcation	Homan (2015); Online media source
	Chalcides ocellatus	Bifurcation	Tofohr (1903); Terni (1915); Cabanas (1946)
	Chalcides sepsoides	Bifurcation	Tofohr (1905)
	Concinnia queenslandiae	Trifurcation	Online media source
	Cryptoblepharus boutonii	Bifurcation	Hirota (1895)
	Cryptoblepharus buchananii	Bifurcation	Online media source

Cryptoblepharus pannosus	Trifurcation	Jablonski & Reichstein (2019)
Cryptoblepharus vittatus	Quadrifurcation	Online media source
Ctenotus grandis	Bifurcation	Ellis (2015)
Ctenotus labillardieri	Bifurcation	Online media source
Ctenotus leonhardii	Bifurcation	Online media source
Ctenotus robustus	Bifurcation	Wilson (2012); Online media source
	Quadrifurcation	Homan (2015)
Ctenotus schomburgkii	Bifurcation	Online media source
Ctenotus spaldingi	Bifurcation	Online media source
Egernia cunninghami	Bifurcation	Online media source
	Trifurcation	Online media source
Egernia kingii	Bifurcation	Barr & Bateman (2020)
Emoia caeruleocauda	Bifurcation	Online media source
Eulamprus heatwolei	Bifurcation	Online media source
Eulamprus quoyii	Bifurcation	Online media source
Eutropis allapallensis	Bifurcation	Vyas (2016)
Eutropis bibronii	Bifurcation	Ghosh & Banerjee (2019)
Eutropis carinata	Bifurcation	Brindley (1898)
Eutropis indeprensa	Bifurcation	Emerson & Dalabajan (2018)
Lamprolepis smaragdina	Bifurcation	Online media source
Lampropholis delicata	Bifurcation	Online media source

Lampropholis guichenoti	Bifurcation	Online media source
Lampropholis mirabilis	Bifurcation	Online media source
Leiolopisma telfairi	Bifurcation	Brindley (1894)
Lerista bougainvillii	Bifurcation	Homan (2015)
Lerista labialis	Bifurcation	Wilson (2012)
Liopholis modesta	Bifurcation	Online media source
Liopholis whitii	Bifurcation	Hickman (1960)
Notomabuya frenata	Bifurcation	Vrcibradic & Niemeyer (2013)
	Trifurcation	Vrcibradic & Niemeyer (2013)
Oligosoma lichenigerum	Bifurcation	Online media source
Plestiodon anthracinus	Bifurcation	Walley (1997)
Plestiodon fasciatus	Bifurcation	Scott (1982); McKelvy & Stark (2012); Online media source
Plestiodon inexpectatus	Bifurcation	Mitchell *et al.* (2012); Online media source
Plestiodon laticeps	Bifurcation	Online media source
	Trifurcation	Online media source
Plestiodon longirostris	Bifurcation	Turner *et al.* (2017)
Plestiodon spp.	Bifurcation	Online media source
Plestiodon tetragammus	Bifurcation	Online media source
Pseudemoia entrecastauxii	Bifurcation	Online media source

	Pseudemoia pagenstecheri	Bifurcation	Online media source
	Psychosaura macrorhyncha	Bifurcation	Vrcibradic & Niemeyer (2013)
	Saproscincus challengeri	Bifurcation	Online media source
	Saproscincus mustelinus	Bifurcation	Online media source
	Scincella lateralis	Bifurcation	Online media source
		Trifurcation	Simpson (1964)
	Trachylepis atlantica	Bifurcation	Mendes *et al.* (2020)
	Trachylepis striata	Bifurcation	Broadley (1978)
Shinisauridae	–	–	–
Sphaerodactylidae	–	–	–
Sphenodontidae	*Sphenodon punctatus*	Bifurcation	Dawbin (1962); Newman (1987); Seligmann *et al.* (2008); Alibardi (2010)
Sphenodontidae		Trifurcation	Online media source
Teiidae	*Ameiva*	Bifurcation	Gogliath *et al.* (2012); Online media source
	Aspidoscelis exsanguis	Bifurcation	Bateman & Chung-MacCoubrey (2013)
	Aspidoscelis inornatus	Bifurcation	Forbes (1961)
	Aspidoscelis neomexicana	Bifurcation	Bateman & Chung-MacCoubrey (2013)
		Bifurcation	Trauth *et al.* (2014)

	Aspidoscelis sexlineatus	Trifurcation	Trauth *et al.* (2014)
		Quadrifurcation	Trauth *et al.* (2014)
	Aspidoscelis spp.	Bifurcation	Online media source
	Aspidoscelis tigris	Bifurcation	Online media source
	Aspidoscelis uniparens	Bifurcation	Bateman & Chung-MacCoubrey (2013)
	Aspidoscelis velox	Bifurcation	Cordes & Walker (2013)
	Cnemidophorus lemniscatus	Bifurcation	Walker & Flanagan (2019)
	Pholidoscelis erythrocephalus	Bifurcation	Kerr *et al.* (2005)
	Pholidoscelis griswoldi	Bifurcation	Online media source
	Pholidoscelis polops	Bifurcation	Angeli (2013)
	Salvator merianae	Trifurcation	Passos *et al.* (2016)
		Hexafurcation	Pelegrin & Leão (2016)
	Teius teyou	Bifurcation	Tornier (1897); Casas *et al.* (2016)
	Tupinambis teguixin	Trifurcation	Brindley (1898)
		Quadrifurcation	Quelch (1890)
	Unknown	Bifurcation	Marcgrave (1648)
Trogonophidae	–	–	–
Tropiduridae	*Microlophus bivittatus*	Bifurcation	Colwell (1992)
	Microlophus delanonis	Bifurcation	Online media source
	Tropidurus semitaeniatus	Bifurcation	Passos *et al.* (2014)
	Tropidurus torquatus	Bifurcation	Martins *et al.* (2013)
Varanidae	–	–	–

Xantusiidae	–	–	–
Xenosauridae	–	–	–
Unknown	Unknown	Bifurcation	Gessner, Cambier & Wechel, 1586; Porta (1619); Jonstonus (1678); Redi (1684); Perrault (1688); Marchant (1718); Seba *et al.* (1735); Edwards (1743); Needham & Lavirotte (1750); Valmont der Bomare (1775); La Cépède (1799); Dugès (1829); Calori (1858); Eversmann (1858); Monteil (1880); Ryder (1893); Piana (1894); Tornier (1901)
		Trifurcation	Porta (1619); Redi (1684); Seba *et al.* (1735); La Cépède (1799); Dugès (1829); Eversmann (1858); Tornier (1901)

NA	NA	Discussed	de Nobleville (1756); Fraisse (1885); Przibram (1909); Bellairs & Bryant (1985); Angeli (2018)

Appendix 3. Publications arising from this research

Appendix 3.1 Peer reviewed papers

Barr, J. I., Somaweera, R., Godfrey, S. S., Gardner, M. G. & Bateman, P. W. (2020). When one tail isn't enough: abnormal caudal regeneration in lepidosaurs and its potential ecological impacts *Biological Reviews. Online early.* https://doi.org/10.1111/brv.12625

Barr, J. I., Boisvert, C. A., Somaweera, R., Trinajstic, K. & Bateman, P. W. (2019). Re-regeneration to reduce negative effects associated with tail loss in lizards. *Scientific Reports* **9**(1), 18717. https://doi.org/10.1038/s41598-019-55231-6

Barr, J. I., Somaweera, R., Godfrey, S. S. & Bateman, P. W. (2019). Increased tail length in the King's skink, *Egernia kingii* (Reptilia: Scincidae): an anti-predation tactic for juveniles? *Biological Journal of the Linnean Society,* ***126***(2), 268–275. https://doi.org/10.1093/biolinnean/bly196

Appendix 3.2. Peer reviewed papers not included in thesis

Barr, J. I. & Bateman, P. W. (2020). *Egernia kingii* (King’s skink): Caudal bifurcation. *Herpetological Review* **51**(2), 331.

Barr, J. I. (2019). Predation of a Buff-banded Rail *Hypotaenidia philippensis* by Australian Ravens *Corvus coronoides* on Penguin Island, Western Australia. *Australian Field Ornithology* **36**, 93-94.

Barr, J., Gilson, L. N., Sanchez Garzon, D. F. & Bateman, P. W. (2019). *Amblyrhynchus cristatus* (Marine Iguana). Tail regeneration and bifurcation. *Herpetological Review* **50**(3), 567.

Barr, J. I., Somaweera, R. & Bateman, P. W. (2018). Ctenotus fallens (West Coast Ctenotus). Anomolous death through use of modelling clay for research. *Herpetological Review* **49**(2), 327-328.

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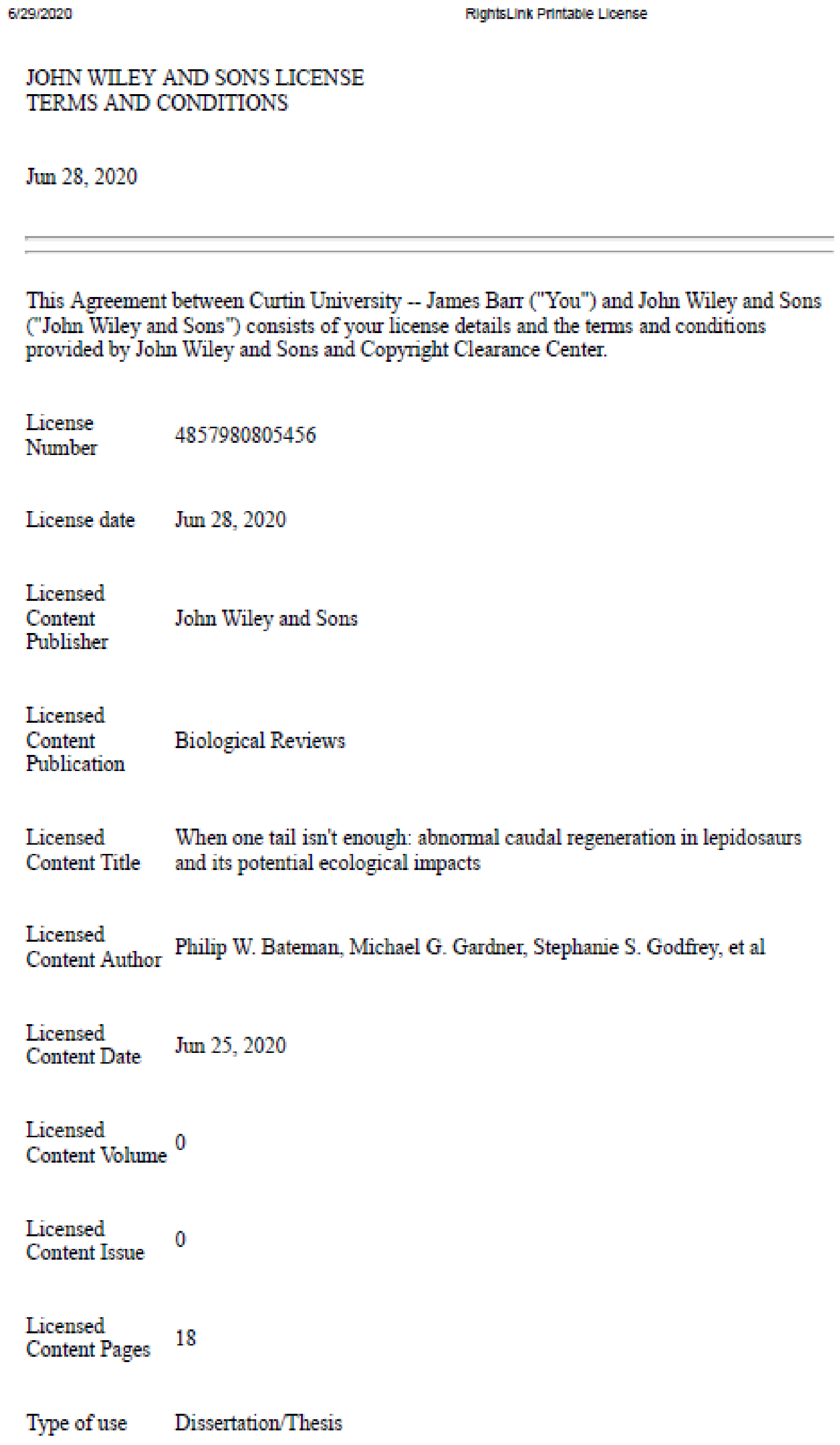

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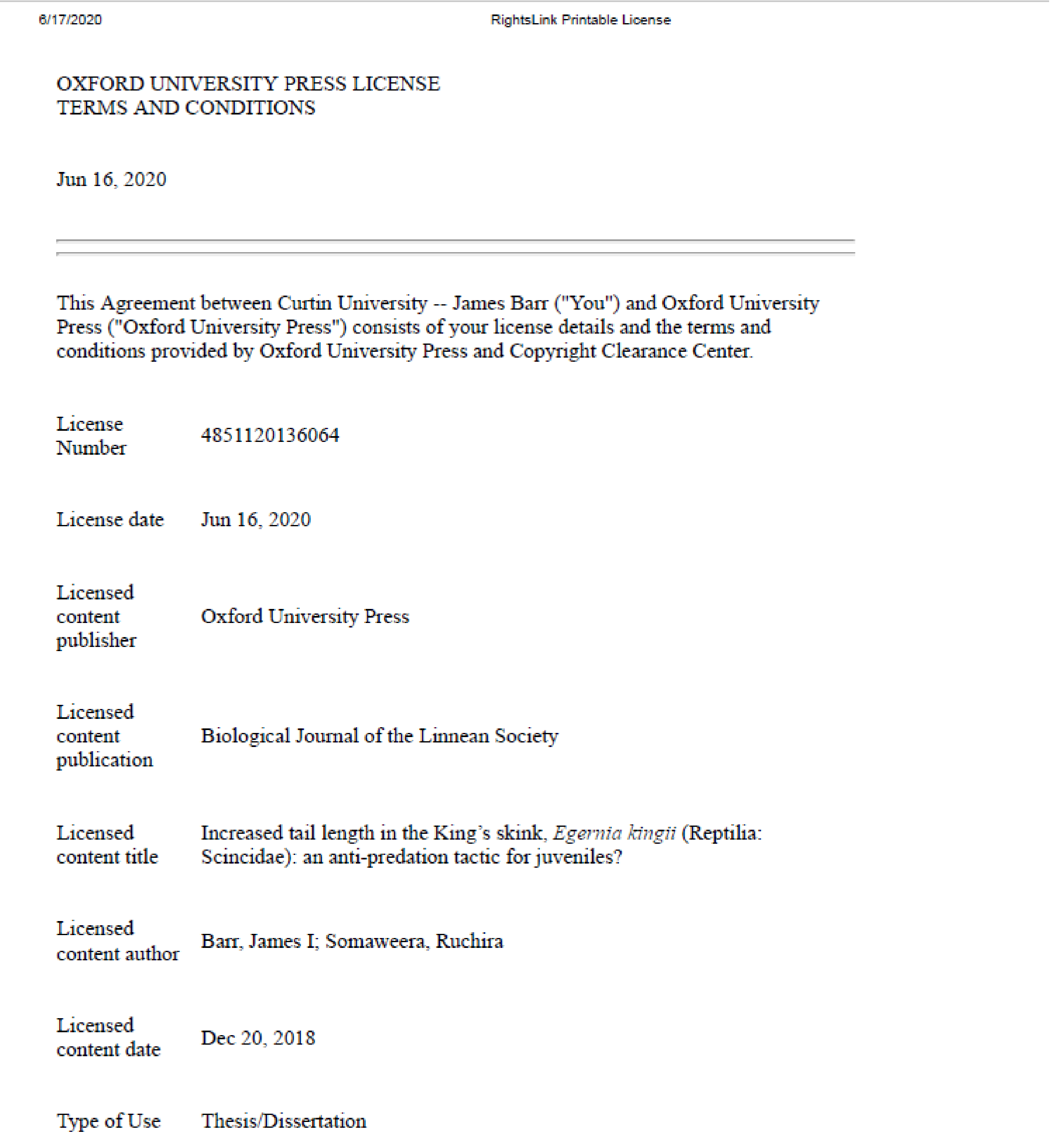

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