2017

Using model systems to investigate the effects of captivity on phenotypic variation: implications for captive breeding programmes

Stephanie Kirsten Courtney Jones
University of Wollongong

UNIVERSITY
OF WOLLONGONG
AUSTRALIA

School of Biological Sciences

Using model systems to investigate the effects of captivity on phenotypic variation: implications for captive breeding programmes.

A thesis submitted in fulfilment of the requirements for the award of the Degree

Doctor of Philosophy

From

University of Wollongong

Stephanie Kirsten Courtney Jones, BSc, MSc

March, 2017

ABSTRACT

Captive breeding programmes (CBPs) offer a method for preventing the extinction of threatened species by assisting with species recovery, primarily by generating animals for reintroduction and supplementing wild populations. However, CBPs often have difficulty establishing self-sustaining populations, unable to maintain consistent reproduction and survivorship in captivity for reintroducing animals back into the wild. A contributing factor leading to this issue may be captive conditions producing phenotypes that differ from wild phenotypes. These phenotypic changes may lead to captive individuals having reduced survivorship, as well as reduced reproductive success, both in captivity and following reintroduction. Ultimately, a range of factors will determine the success of reintroductions; however, the phenotypic changes occurring in captivity, and how this may impact reintroduction success remains largely unknown. In this thesis, I outline how an animal's phenotype may contribute to the success or failure of CBPs, and in turn, reintroduction success. I used a mammalian and an amphibian species as models to examine phenotypic changes in captivity and specifically looked at developmental, morphological and behavioural phenotypes.

While the effects of captivity on behavioural and morphological phenotypes have been widely reported, few studies have compared differences between captive-reared and wild animals, the transgenerational effects on behavioural and morphological phenotypes, and potential differences between sexes in response to captivity, which are particularly relevant for determining reintroduction success. Using house mouse (*Mus musculus*) as a model species, I determined whether behavioural and morphological phenotypes in captive-reared and wild-caught animals differed. Specifically, for behavioural phenotypes, I sought to determine whether the boldness and activity behavioural type of captive-reared and wild-caught animals differed, whether these behavioural types were subject to transgenerational effects in captivity, and whether there were sex-specific differences in behavioural types. To do this, I examined the boldness and activity behavioural types displayed in a novel environment. I used an open field test (OFT) to simulate a novel environment. Mice reared in a captive environment were found to differ in their boldness and activity behavioural type compared with their wild-caught conspecifics. After one generation, there was evidence

for transgenerational effects in captivity on some behavioural traits but not behavioural type. Four behavioural traits (Perimeter: max speed, Perimeter: average speed, Mean speed, Distance) were driving the compositional differences in behavioural type between captive-reared F_4 and captive-reared F_5 females, and there was no evidence that changes in behavioural type were dependent on sex. Importantly however, behavioural type did differ between wild-caught females and males, suggesting that captivity resulted in the loss of sex specific behaviours. To determine whether the morphology of captive-reared and wild-caught animals differed, I compared the external and internal morphology of captive-reared and wild-caught animals, tested whether morphology was subject to transgenerational effects in captivity, and compared morphology between sexes in animals from both captive and wild environments. To do this, external body morphological trait measurements were made, and macroscopic dissection of organs conducted, to quantify morphological differences between wild-caught and captive-reared mice. External traits included body mass, skull length, snout to vent length, tail length and foot length (right hind leg). Internal traits included weights of brain, liver, kidney, heart, lungs, testes/ovaries, spleen, stomach, caecum, small- and large-intestine and the lengths of the small- and large-intestine. I found an absence of changes in external morphology masked internal morphological changes; there was a significant effect of rearing environment on kidney, spleen and caecum mass and small intestine length. There was also evidence for transgenerational effects in morphology between captive generations, however, only in internal morphology, and only in females; five morphological traits (brain, kidneys, stomach, caecum and ovaries) were driving compositional differences in internal morphology between captive-reared F_4 and captive-reared F_5 females. Morphological changes were also evident within the acclimation period, suggesting that phenotypic plasticity contributed to the rapid changes in morphology. Further, morphology significantly differed depending on sex, indicating that sexual dimorphism was maintained in captivity.

I then examined the genetic mechanisms underpinning the observed transgenerational effects in the captive-reared house mouse (*Mus musculus*) population using broad sense heritability analyses including mid parent- and single parent-offspring regressions. Specifically, I measured the heritability of boldness and activity behavioural types as well as internal morphology. Slopes for boldness and activity were all positive,

indicating a low to moderate degree of heritability. The slopes for internal morphology were undetectable. Although none of the heritability estimates were statistically significant, likely due to small sample sizes, my findings suggest that the potential for genetic change in captivity might vary considerably between traits, with some but not all phenotypic traits displaying some degree of heritability, which may allow for rapid adaptation to captive conditions. Traits that were not highly heritable may be strongly influenced by environmental conditions and are likely to display a high degree of plasticity. Continuing to explore the potential for traits to evolve in captivity may help inform captive breeding and reintroduction programmes.

Using the striped marsh frog (*Limnodynastes peronii*) as a model species, I examined how environmental conditions experienced in captivity influenced phenotypic traits. Food availability and temperature are known to trigger phenotypic change, however, the interactive effects between these factors are only beginning to be considered. The aim of this study was to examine the independent and interactive effects of long-term stochastic food availability and water temperature on larval survivorship, growth and development of the striped marsh frog, *Limnodynastes peronii*. To evaluate the effects of food availability and temperature, I exposed tadpoles to one of six experimental treatments (referred to as 1. Constant 18°C, 2. Constant 22°C, 3. Constant 26°C, 4. Stochastic 18°C, 5. Stochastic 22°C and 6. Stochastic 26°C) across a 14-week experimental period. Throughout the 14-week experimental period, I monitored the survivorship, development and growth of individual tadpoles in each experimental treatment on a weekly basis. This included recording the number of tadpoles surviving, the number of tadpoles reaching metamorphosis, the time taken to reach metamorphosis and tadpole growth. Changes in food availability mediated the effects of temperature, with slower larval growth and higher survivorship in stochastic food availability treatments. These findings suggest that interactions between environmental factors can influence anuran growth, development and survivorship. Furthermore, identifying the phenotypic traits that change and the specific mechanisms (i.e. the abiotic and biotic factors) associated with phenotypic change in captivity, can help managers develop and refine approaches used in captive-breeding and reintroduction programmes.

Overall, my results have shown that captivity can result in changes to phenotypic traits. In addition, some but not all phenotypic traits may be heritable, allowing for rapid

adaptation to captive conditions. For other traits that did not display a shift in response to captive conditions, this may indicate such traits being strongly influenced by environmental conditions and displaying a high degree of plasticity. Further, the environmental conditions in captivity can alter developmental trajectories and survivorship. From an applied perspective, understanding how environmental factors interact to cause phenotypic change may assist with conservation by improving the number of individuals generated in captive breeding programmes. These results contribute to our understanding of the role of phenotypic variation in captive breeding programmes.

THESIS CERTIFICATION

Certification

I, Stephanie K. Courtney Jones, declare that this thesis, submitted in fulfilment of the requirements for the award Doctor of Philosophy, in the School of Biological Sciences, University of Wollongong, is wholly my own work unless otherwise referenced or acknowledged. This document has not been submitted for qualifications at any other academic institution.

Signed

Dated: 2 March 2017

DECLARATION

This work contains no material which has been accepted for the award of any other degree or diploma in any university or other tertiary institution to Stephanie K. Courtney Jones and, to the best of my knowledge and belief, contains no material previously published or written by another person, except where due reference has been made in the text. I give consent to this copy of my thesis when deposited in the University Library, being made available for loan and photocopying, subject to the provisions of the Copyright Act 1968.

The author acknowledges that copyright of published works contained within this thesis (as listed below) resides with the copyright holder (s) of those works. I also give permission for the digital version of my thesis to be made available via the University's digital research repository, the Library catalogue, the Australasian Digital Theses Program (ADTP) and also through web search engines, unless permission has been granted by the University to restrict access for a period of time.

This thesis contains four data chapters, each of which is written as a manuscript for publication including referencing style, and are therefore intended to stand-alone. With this, some information may be redundant or repeated. While the candidate made substantial contributions to the manuscripts and is fully responsible for the work presented in this thesis, where the first person is used in the manuscripts it is used in the plural ('we') to reflect contributions from co-authors.

Dr Phillip Byrne was involved in designing the project, guided data collection and provided comments on drafts of all chapters. All data used in this thesis were collected during the period of the PhD. Contributions by co-authors are detailed for each manuscript below.

Chapter 2: Effect of captivity on house mouse behaviour in a novel environment: implications for conservation practices.
Courtney Jones, S.K. Munn, A.J. and Byrne, P.G.

SKCJ, AJM and PGB developed the experimental approach; SKCJ performed the experiment; SKCJ performed the data analysis; SKCJ wrote the manuscript; SKCJ and PGB finalised the manuscript for submission. The paper was submitted to *Applied Animal Behaviour Science* in **June 2016**, and resubmitted following revision in **October 2016**, accepted **January 2017**.

Chapter 3: Effect of captivity on morphology in mice: negligible changes in external morphology mask significant changes in internal morphology.
Courtney Jones, S.K. Munn, A.J. and Byrne, P.G.

SKCJ, AJM and PGB developed the experimental approach; SKCJ performed the experiment; SKCJ performed the data analysis; SKCJ wrote the manuscript; SKCJ and PGB finalised the manuscript for submission. The paper was prepared for submission to *Journal of Morphology*.

Chapter 4: What role does heritability play in transgenerational phenotypic responses to captivity? Implications for managing captive populations.
Courtney Jones, S.K. and Byrne, P.G.

SKCJ and PGB developed the experimental approach; SKCJ performed the experiment; SKCJ performed the data analysis; SKCJ wrote the manuscript; SKCJ, and PGB finalised the manuscript for submission. The paper was submitted to *Journal of Animal Breeding and Genetics* in **February 2017** and is currently in review.

Chapter 5: Long-term changes in food availability mediate the effects of temperature on growth, development and survival in striped marsh frog larvae: implications for captive breeding programmes.
Courtney Jones, S.K. Munn, A.J., Penman, T.D. and Byrne, P.G.

SKCJ, AJM and PGB developed the experimental approach; SKCJ performed the experiment; SKCJ, PGB and TDP performed the data analysis; SKCJ wrote the manuscript; SKCJ, AJM, TDP and PGB finalised the manuscript for submission. The

paper was submitted to *Conservation Physiology* in **February 2015**, and resubmitted following revision in **May 2015**, accepted **May 2015**.

CONFERENCES AND OTHER PUBLICATIONS

In addition to the submitted manuscripts listed above, during the course of my PhD I have published three journal articles external to my thesis and presented data from this thesis at national and international conferences. I also organised and chaired a symposium on Conservation Behaviour. A summary of this activity is provided below.

Publications

Williamson SA, Courtney Jones SK, and Munn AJ (2014) Is gastrointestinal plasticity in king quail (*Coturnix chinensis*) elicited by diet-fibre or diet-energy dilution? Journal of Experimental Biology **217:** 1839 – 1842

Courtney Jones SK, and Munn AJ (2013) Caecal abnormality in a layer hen (*Gallus gallus forma domestica*) not accompanied by deficits in digestive performance or egg productivity. Animal Science Journal **84:** 97 – 100

Courtney Jones SK, Cowieson A, Williamson SA and Munn AJ (2013) No effect of short-term exposure to high-fibre diets on the gastrointestinal morphology of layer hens (*Gallus gallus domesticus*): body reserves are used to manage energy deficits in favour of phenotypic plasticity. Journal of Animal Physiology and Animal Nutrition **97:** 868 – 877

Conference presentations

S Courtney Jones and P Byrne (2016) Applications of conservation behaviour: the effects of captivity. Ecological Society of Australia Conference. Fremantle, Australia.
Awarded the Society for Conservation Biology prize for best spoken paper on conservation.

S Courtney Jones, A Munn and P Byrne (2016) Applications of conservation behaviour: the effects of captivity on behaviour. North American Congress for Conservation Biology. Madison, WI, USA.

S Courtney Jones, A Munn and P Byrne (2016) The application of conservation behaviour to conservation practices: A case study on the effects of captivity on behaviour in a model species. Oceania Congress for Conservation Biology Conference. Brisbane, Australia.

S Courtney Jones, T Penman, A Munn and P Byrne (2015) Long-term changes in food availability mediate the effects of temperature on growth, development and survival in Anuran larvae: implications for captive breeding programmes. Australian Society of Herpetology. Lake Eildon, Australia

Symposia

S Courtney Jones (2016) Conservation behaviour: Putting behavioural ecology theory in conservation practice. Ecological Society of Australia. Fremantle, Australia. Symposia organiser and chair.

ACKNOWLEDGEMENTS

A PhD is no solitary endeavour and would not be possible without the support, advice and assistance in the scientific process; these are some of the people I thank.

Firstly, to my supervisor, although somewhat unknowingly in my undergraduate studies, I began pestering Dr. Phillip Byrne about phenotypic plasticity in lectures. There are audio recordings to prove it. Unsatisfied to leave those questions alone, I wandered through Africa, zoos, volunteering and it wasn't until a chance hallway encounter many years later that my ideas could be developed into a tangible project that you and I finally began to answer those questions. Thank you for supporting me along my journey; it certainly has been a challenge, but through your wisdom, guidance, patience, enthusiasm and attention to detail, you have taught me to be a scientist. Your passion for conservation is unrivalled and knowledge is exceptional.

To my committee – many thanks for the time you have invested in this project and for your comments on the many previous drafts of this thesis. To Associate Prof. James Wallman, many thanks for coming on board as a co-supervisor and providing invaluable advice and guidance on the write-up of this thesis. Professor Kristine French, thank you for supporting me throughout my candidature, for your insights, sage words of advice and offering endless pearls of wisdom on seemingly any topic. To Dr. Katarina Mikac and Prof. Sharon Robinson, thank you for your ongoing support throughout the years. It has been empowering to see strong intelligent women passionate about science and education. To Dr. Trent Penman, for providing valuable statistical advice and analyses. To Dr. Adam Munn, for providing access to equipment and comments on draft manuscripts. To Dr. Tracy Maddocks, thank you for all your outstanding advice, technical assistance with experimental set up and animal husbandry throughout the experiments and our chats not only helped shape the experimental approach, but more importantly kept me sane.

This research has been conducted with the support of the Australian Postgraduate Award and was made possible by the generous support of the University of Wollongong and the Centre for Sustainable Ecosystem Solutions. Thank you to the Australian Society of Herpetology, Society of Conservation Biology, Ecological Society of Australia and University of Wollongong for access to travel grants to attend scientific

conferences within Australia and overseas. Special thanks to the Sydney Chapter of the Society of Conservation Biology and the ongoing support from the inspired, strong, intelligent women behind the chapter.

This thesis was composed of two major experiments, both of which involved daily husbandry, behavioural assays, dissections, data collection and entry. Thanks to all the volunteers involved with the tadpole project assisting with daily husbandry and counting each individual on an almost daily basis, in no particular order Elizabeth Price, Sean Williamson, Alana Johnson, Aidan Johnson, Aimee Bullock, Adam Skidmore. Special thanks to Alexandra Leslie, James Lidsey, Emma McInerney and Alexandra May.

Thanks to all volunteers in the mouse project, for helping out with the daily husbandry and in general enthusiasm for conservation based work, Allison Binley, Michael Davies, Suzanne Eacott, Aidan Johnson, James Lidsey, Gary Maidman, Alexandra May, Caitlin Nicholls, Mitchell Stares and Mathew Stewart. To Prof. Rob Brooks and Alyssa Gibson at University of New South Wales for providing valuable advice and the established captive House mouse population. Special thanks to Peter Oaks at Oaks Organics for allowing me onto his beautiful property to trap mice.

I also need to thank those who helped with ideas and commented on draft manuscripts including Jess Bramley-Alves, Chris Caldow, Luke Collins, Faye Courtney, Urtzi Enriquez-Urzelai, Amy-Marie Gilpin, Martin Hing, Laura Lopez, Aidan Johnson, Emma McInerney, Pierre-Olivier Montiglio, Owen Price, Sharon Robinson, Sarah Simmonds, Melinda Waterman and the anonymous reviewers. Thanks to the technical officers in the School of Biological Sciences for laboratory and field support. Thank you to Prof. Phillip Seddon and Dr. Tim Jessop for examining my thesis and all of their valuable insights.

Thank you to the postgraduate cohort at University of Wollongong, it’s been a rewarding and enjoyable couple of years and your support and advice is valued without measure, in particular Ben Gooden, Luke Collins, Matt Rees and Mathew Stewart. On a more personal note, thanks to Biz and my family and friends who’ve championed me from start to finish. Thank you for all the cups of tea, popcorn and yarns.

"In every remote corner of the world there are people… who have devoted their lives to saving threatened species. Very often, their determination is all that stands between an endangered species and extinction.

But why do they bother? Does it really matter if the Yangtze river dolphin, or the kakapo, or the northern white rhino, or any other species live on only in scientists' notebooks?

Well, yes, it does. Every animal and plant is an integral part of its environment: even Komodo dragons have a major role to play in maintaining the ecological stability of their delicate island homes. If they disappear, so could many other species. And conservation is very much in tune with our survival. Animals and plants provide us with life-saving drugs and food, they pollinate crops and provide important ingredients or many industrial processes. Ironically, it is often not the big and beautiful creatures, but the ugly and less dramatic ones, that we need most.

Even so, the loss of a few species may seem irrelevant compared to major environmental problems such as global warming or the destruction of the ozone layer. But while nature has considerable resilience, there is a limit to how far that resilience can be stretched. No one knows how close to the limit we are getting. The darker it gets, the faster we're driving.

There is one last reason for caring, and I believe that no other is necessary. It is certainly the reason why so many people have devoted their lives to protecting the likes of rhinos, parakeets, kakapos, and dolphins. And it is simply this: the world would be a poorer, darker, lonelier place without them."

– Mark Carwardine

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

1 INTRODUCTION: THE EFFECTS OF CAPTIVITY ON PHENOTYPIC VARIATION

1.1 Captive breeding programmes

Captive breeding programmes (hereafter CBPs) are increasingly relied upon as an important conservation tool for threatened species management (Conde *et al.*, 2011). Captive breeding programmes provide a controlled environment for the rearing, maintenance and preservation of many species challenged by key threatening processes in the wild (Bryant *et al.*, 1999; Thomas *et al.*, 2004). Fischer and Lindenmayer (2000) reported that only 13% of all reintroductions with a captive source population were ultimately considered successful, with success determined as self-sustaining populations following reintroduction. For example, captive-born carnivores have significantly lower survivorship (32% survival rate) compared to wild-born carnivores (53% survival rate) following reintroduction (Jule *et al.*, 2008). These are alarming statistics considering that captive breeding is the primary recovery action for many threatened species.

Currently, the central focus of many captive breeding programmes is identifying and countering adverse genetic changes that occur in captivity. These typically include factors such as loss of genetic diversity, inbreeding depression and genetic adaptations to captivity, all of which can compromise individual viability and the success of reintroduction programmes (Frankham 2008; Williams and Hoffman 2009). However, captive conditions often represent an environment vastly removed from wild conditions, and as such, differing selection pressures arise, often resulting in reduced individual fitness upon reintroduction (Mathews *et al.*, 2005).

Reasons for failure vary greatly and are typically considered on a case-by-case basis. However, failures have been attributed to the excision of natural behavioural repertoires and/or changes in the animals' physiology or morphology (Snyder *et al.*, 1996; Birkhead *et al.*, 2006; Teixeira *et al.*, 2007). Tarszisz *et al.* (2014) conducted a detailed review of reintroduction failures and reported that while 78% of studies described behavioural phenotypes, only 9% of studies described physiological phenotypes in their reintroduction attempts. The success of reintroductions with captive sourced populations may be improved through pre-release screening for suitable traits (e.g. Mathews *et al.*, 2005), or pre-release training to reinforce appropriate behaviours (e.g.

Shier and Owings, 2006). To date, however, the assessment of phenotypic traits in CBPs has been limited and may be a key factor in the poor success of reintroductions due to the reduced fitness of individuals in captivity. In this thesis, I suggest that reintroduction success might be substantially improved by incorporating an understanding of phenotypic traits into management programmes, and by starting to make holistic assessments of trait change in captivity (Tarszisz *et al.*, 2014).

1.2 The cause and effect of phenotypic variation

Changes in the natural, sexual and artificial selective pressures that increase fitness in captivity can lead to a directional shift in phenotypic traits away from the wild phenotype towards an optimal mean trait value for captive conditions (McDougall et al., 2006; McPhee and McPhee 2012). In concert, the uniform and unchallenging environments in captivity may cause rapid losses in genetic and phenotypic variation (Mathews et al., 2005; e.g. Briscoe *et al.*, 1992). Phenotypic variation is widely recognised as a contributing factor to population persistence; multiple phenotypes (polyphenism) expressed within a population allow adaptation to environmental fluctuations via sub populations (Kussell and Leibler 2005). A theoretical framework study, which modelled the means and variances of phenotypes in response to environmental changes, determined that long term productivity of a functional group with similar resource requirements and predators was higher, with high phenotypic variation (Norberg *et al.*, 2001). While short-term productivity was lower with high phenotypic variation, this was due to the presence of sub-optimal individuals, with phenotypic variance linearly associated with the ability to respond to environmental change (Norberg *et al.*, 2001). Relating this theoretical knowledge to CBPs, we can infer that by maximising phenotypic variation we could improve the overall long-term productivity of the captive population and maximise its ability to respond to environmental change upon release.

A growing number of studies have demonstrated that changes in selective pressure, and loss of phenotypic variation (leading to phenotypic homogeneity) in captive populations, has been attributed to poor reintroduction success (Snyder *et al.*, 1996; Fischer and Lindenmayer 2000; O'Regan and Kitchener 2005). Moreover, the degree of phenotypic homogeneity may increase with each captive generation, leading to phenotypes vastly removed from the wild phenotype (Wisely *et al.*, 2002; McPhee

2004a, b). These phenotypic changes in response to captivity may lead to captive individuals having reduced survivorship compared with their wild conspecifics, as well as reduced reproductive success following reintroduction (Philippart 1995; Anthony and Blumstein 2000; Johnson *et al.*, 2014). Furthermore, habituation to captive conditions and insufficient challenges during the rearing process may not adequately prepare captive bred individuals to challenges encountered in novel environments. For example, Christie *et al.* (2012) compared wild born and first-generation hatchery wild steelhead (*Oncorhynchus mykiss*) in captive and wild conditions and reported that first-generation hatchery fish exhibited increased reproductive success in captive conditions. However, the offspring of hatchery fish had reduced fitness in wild conditions, suggesting that an adaptation to captivity occurred within one generation, and that there may have been selection for traits maladaptive for wild conditions. These case studies, along with a multitude of others, exemplify how phenotypic changes can occur as a result of differences between captive and wild environmental conditions, and draw attention to the fact that these changes are likely to reflect differences in evolutionary processes (Kohane and Parsons 1988; Snyder *et al.*, 1996).

It is apparent that loss of phenotypic variation may have profound consequences, but to date only a few studies have attempted to investigate why loss of phenotypic variation occurs in captivity. Phenotypic homogeneity in captivity may occur as a result of uniform and unchallenging environments (Mathews *et al.*, 2005). Furthermore, the accompanying changes in evolutionary processes occurring within captivity, such as relaxed selective pressures or directional selection for a suite of traits favoured in captivity (McDougall *et al.*, 2006; McPhee and McPhee 2012), can potentially contribute to release failure (Kohane and Parsons 1988; Snyder *et al.*, 1996). While there is a general acknowledgment of the potential "domestication" of animals in captivity (O'Regan and Kitchener 2005), to my knowledge limited research has been conducted to identify and potentially reduce adaptations to captivity, and most research has been conducted in birds (e.g. Munkwitz *et al.*, 2005; Maxwell and Jamieson, 1997). Moreover, studies attempting to investigate morphological and physiological adaptations to captivity are notably lacking (O'Regan and Kitchener 2005; Tarszisz *et al.*, 2014). Given the potential for phenotypic traits to change in response to selection pressures and environmental conditions that occur in captivity, it is imperative to gain

an understanding of how phenotypic change occurs in CBPs, as this knowledge may substantially improve reintroduction success.

1.3 The effects of captivity on phenotypic traits

Provided a captive source population has high phenotypic variation (i.e. is phenotypically diverse), it might not matter if a proportion of the captive population is unsuitable for release, as long as there has been pre-release screening for suitable traits (e.g. Mathews *et al.*, 2005), or pre-release training to reinforce appropriate behaviours (e.g. Shier and Owings, 2006) and identify suitable founders for reintroductions. Numerous studies have investigated the behavioural, physiological and morphological adaptations of animals to captivity (Table 1.1; Carducci and Jakob 2000; Geiser and Ferguson 2001; Slade *et al.*, 2014). However, few studies have explicitly compared phenotypic differences between captive-reared and wild-caught animals. Using an 'adaptive baseline' provides the ability to demonstrate and track the effects of captivity. That is, the scale of phenotypic plasticity, the direction of change, and the specific phenotypic traits that change (Mathews *et al.*, 2005; DeGregorio *et al.*, 2013; Jarvie *et al.*, 2015). For example, a comparison between captive-bred and wild caught feathertail gliders (*Acrobatus pygmaeus*) found captive-bred individuals had longer activity periods and less frequent torpor bouts (Geiser and Ferguson 2001). Indeed, characterisation of phenotypes such as behaviour is now being used as criterion for selecting animals suitable for reintroduction (Bremner-Harrison *et al.*, 2004). Given the potential range of phenotypic traits that could change in captivity, studies attempting to investigate the influence of captivity should aim to compare a variety of phenotypic traits, including behavioural and morphological traits with wild-caught animals. Such research will provide important insights into the types of traits most susceptible to change, if the direction and magnitude of changes differ between phenotypic traits, and, ultimately, whether these trait changes have implications for post-release fitness (McPhee 2004a, b; McDougall *et al.*, 2006; McPhee and Carlstead 2010).

When considering how phenotypic traits change in captivity, it is also important to consider the effect of sex. It is well established that phenotypic traits can differ between the sexes due to sexual selection favouring different trait values in each sex (Schuett *et al.*, 2010; Fresneau *et al.*, 2014). In general, it is expected that intra- and inter-sexual selection (i.e. male-male competition and female mate choice) will favour sex-specific

behaviours and sexual dimorphism (Hedrick and Temeles 1989; Kokko 2005; McPherson and Chenoweth 2012). Differences between captive and natural environments, such as reduced competition for resources and artificial selection for animals suited to captivity, inadvertently lead to phenotypic change; in turn this may lead to changes to, or a reduction of, sexual dimorphism and behavioural differences (Table 1.1; O'Regan and Kitchener 2005; McPherson and Chenoweth 2012). For example, a study investigating the effects of captivity on morphology in American mink (*Mustela vison*) found a reduction of sexual dimorphism in body size and craniometric variation (Lynch and Hayden 1995). Given that sexual selection in phenotypic traits is evident across various taxa, captive-based research would benefit from determining the effects of captivity on the strengths and targets of sexual selection, and resultant phenotypic differences between the sexes.

Table 1.1 A detailed breakdown of the phenotypic traits and experimental factors considered in fifty one case studies investigating the effects of captivity on phenotypic traits. Phenotypic traits are separated into four distinct categories: Behavioural (B), morphological (M), physiological (P) or life-history traits (L). Experimental factors included whether multiple generations (Multi. Gen.), sex (Sex), wild comparisons (Wild comp.), were considered. Further, if animals were reintroduced (Reintro.), whether a pre-release assessment (Pre-release) was conducted.

Taxa	Species	B	M	P	L	Multi. Gen.	Sex	Wild comp.	Reintro.	Pre-release	Ref
Mammals											
	Bank vole *Clethrionomys glareolus*	✓						✓			[1]
	Meadow vole *Microtus pennsylvanicus*	✓					✓	✓			[2]
	Southern brown bandicoot *Isoodon obesulus fusciventer*			✓				✓			[3]
	Oldfield mouse *Peromyscus polionotus subgriseus*	✓	✓			✓		✓			[4, 5]
	European otter *Lutra lutra*				✓	✓	✓	✓	✓		[6]
	Feathertail glider *Acrobates pygmaeus*	✓	✓	✓			✓	✓			[7]
	Numbat *Myrmecobius fasciatus*		✓	✓				✓			[8]
	House mouse *Mus musculus*		✓		✓		✓	✓	✓	✓	[9]
	Swift fox *Vulpes velox*	✓			✓	✓	✓		✓	✓	[10]
	Black footed ferret *Mustela nigripes*	✓	✓			✓	✓	✓			[11-13]

Taxa	Species	B	M	P	L	Multi. Gen.	Sex	Wild comp.	Reintro.	Pre-release	Ref
Mammals											
	European mink *Mustela lutreola*				✓		✓		✓		[14]
	Golden lion tamarin *Leontopithecus rosalia*	✓				✓		✓			[15]
	Lion *Panthera leo*		✓				✓	✓			[16, 17]
	Tiger *Panthera tigris*		✓				✓	✓			[17]
	Cavy *Cavia aperea*	✓		✓			✓	✓			[18]
	Meerkat *Suricata suricatta*	✓						✓			[19]
	Spotted hyaena *Crocuta*	✓					✓	✓			[20]
	Coyote *Canis latrans*	✓						✓			[21]
	Bighorn sheep *Ovis canadensis*				✓			✓	✓		[22]
	Tasmanian devil *Sarcophilus harrisii*		✓		✓				✓		[23]
Birds											
	Loggerhead shrike *Lanius ludovicianus*	✓							✓	✓	[24]
	Brown teal *Anas chlorotis*		✓				✓	✓			[25]
	Dark-eyed junco *Junco hyemalis*		✓	✓				✓			[26]

Taxa	Species	B	M	P	L	Multi. Gen.	Sex	Wild comp.	Reintro.	Pre-release	Ref
Birds											
	Mallard *Anas platyrhynchos*	✓	✓		✓		✓	✓	✓		[27]
	Red junglefowl *Gallus gallus*	✓	✓				✓				[28, 29]
	Houbara bustard *Chlamydotis macqueenii*	✓	✓					✓	✓		[30, 31]
	Rufous-crested bustard *Eupodotis ruficrista*		✓								[31]
	White-bellied bustard *Eupodotis senegalensis*		✓								[31]
	Blue tit *Cyanistes caeruleus*	✓					✓	✓	✓	✓	[32]
	Attwater's Prairie chicken *Tympanuchus cupido attwateri*	✓			✓		✓		✓	✓	[33]
	Mountain chickadee *Poecile atricapillus*	✓	✓				✓	✓	✓		[34]
Fish											
	Guppy *Poecilia reticulata*		✓	✓		✓	✓	✓	✓		[35-37]
	Steelhead *Oncorhynchus mykiss*	✓	✓		✓	✓		✓	✓		[38, 39]
	Atlantic salmon *Salmo salar*				✓	✓		✓	✓		[40]
	Amargosa river pupfish *Cyprinodon diabolis*		✓	✓	✓						[41]
	Electric fish *Gnathonemus petersii*			✓			✓				[42]

Taxa	Species	B	M	P	L	Multi. Gen.	Sex	Wild comp.	Reintro.	Pre-release	Ref
Reptiles											
	Ratsnake *Elaphe obsoleta*	✓						✓		✓	[43]
	Tuatara *Sphenodon punctatus*	✓	✓	✓	✓		✓	✓	✓	✓	[44]
	Lacertid lizard *Psammodromus algirus*	✓			✓			✓	✓		[45]
	Otago skink *Oligosoma otagense*	✓	✓	✓				✓			[46]
Amphibians											
	Mallorcan midwife toad *Alytes muletensis*		✓		✓	✓					[47]
Invertebrates											
	Field cricket *Gryllus campestris*	✓					✓	✓			[48]
	Jumping spider *Phidippus audux*	✓						✓			[49]
	Milkweed bug *Oncopeltus fasciatus*		✓		✓	✓					[50]
	Puget blue butterfly *Icaricia icarioides blackmorei*		✓		✓	✓	✓				[51]

1.4 Multiple generations in captivity: effect on phenotypic traits

Phenotypic traits that are subject to fitness costs in captivity are predicted to shift away from the wild phenotype with each subsequent generation in captivity, leading to changes in life history traits including reproductive success and survivorship (Connolly and Cree 2008). The transgenerational shift in traits that increases fitness in captivity can be expected with change in the strength and targets of selection in captivity (McPhee 2004a, b; McPhee and McPhee 2012). These transgenerational effects on phenotypes in captivity may result from transgenerational plasticity or genetic changes, such as heritable genetic mutations (Chakravarti *et al.* 2016; Evans *et al.* 2014; Richards *et al.* 2010; Martos *et al.* 2015). If transgenerational effects result from transgenerational plasticity, environmental factors that the parental generation experiences will trigger particular trait expressions in offspring (e.g. maternal effects or epigenetic variation; Keller *et al.*, 2001; Dor and Lotem 2009). Further, there is emerging evidence that transgenerational shift in traits can occur quickly. McPhee (2004b) tested for directional and relaxed selection in populations of oldfield mice (*Peromyscus polionotus subgriseus*) maintained in captivity for differing periods (2, 14 and 35 generations) and found an increased magnitude of change in cranial and mandibular size and shape with each subsequent generation maintained in captivity. These findings have important implications as they suggest that captivity can impose changes in selective pressures, and that over multiple generations, these shifts can lead to the captive phenotype differing from the wild phenotype (O'Regan and Kitchener 2005; McDougall *et al.*, 2006; McPhee and Carlstead 2010). However, despite potential for trait change in the captive environment, few studies have examined the effects of captivity on phenotypic traits across multiple generations (Table 1.1; McPhee 2004b). Understanding and controlling transgenerational effects may be able to mitigate the effects of captivity, influencing the success of offspring in the wild. However, this requires a better understanding of transgenerational effects (Richards *et al.*, 2010; Evans *et al.*, 2014; Chakravarti *et al.*, 2016).

1.5 Stimulating phenotypic variation in captivity: Approaches and implications

Selection in captivity should favour phenotypic traits that promote reproductive success of individuals (Smith 1978; Bull *et al.*, 2004). This may also capitulate itself by selecting for easier to handle animals that increase breeding (Mason *et al.*, 2013).

However, if CBPs aim to release the captive animals into a novel environment, the initial captive phenotype may not be the optimal phenotype for release situations (Fischer and Lindenmayer 2000; Ford 2002). For example, populations of released captive-bred mallards (*Anas platyrhynchos*) showed preference to anthropogenic food sources and, despite similar time budgets, never achieved an equivalent body condition of wild birds (Champagnon *et al.*, 2012). Consequently, there was lower survival probability in captive-released mallards. Such examples demonstrate that a single phenotype displayed across an entire captive-bred population may not be optimal in both captive and in natural environments following reintroduction (Shoval *et al.*, 2012). Consequently, it may be critical to identify methodologies to reduce the phenotypic changes occurring in captivity and maximise the potential for reintroduction success. One approach to improve the likelihood for success upon release may be to increase phenotypic variation within a population. This may be possible by increasing the expression of multiple phenotypes (polyphenism) or phenotypic plasticity, with the outcome of expressing phenotypes more suitable for the release environment.

Phenotypic plasticity is the ability of an organism to change its phenotype in response to varying abiotic and biotic environmental factors (Miner *et al.*, 2005). When faced with dynamic environmental conditions, some organisms can readily respond by changing phenotypes, allowing for a range of optimal phenotypes to be produced in response to multiple environments (DeWitt *et al.*, 1998). If the optimal phenotype can change with environmental condition, this presents an adaptive advantage that can improve organismal fitness (De Jong 2005; Reed *et al.*, 2010). Phenotypic variation is likely to be reinforced by the species' level of phenotypic plasticity. That is, upon release into a novel environment, the individual has the ability to rapidly change their phenotype (Zalewski and Bartoszewicz 2012).

Currently, captivity provides an environment resembling a static 'ideal' environment and does not necessarily provide the required environmental fluctuations or challenges that encourage the expression of a diverse range of phenotypes, or the generation of stochastic phenotype switching (Kussell and Leibler 2005; Mathews *et al.*, 2005). If the CBP has the aim to provide animals for reintroduction, I suggest that the CBP rearing methodologies should increase the feature of the environmental characteristics of the reintroduction or translocation environments within captive conditions (Thomas, 2011;

Tarszisz et al., 2014). These challenges, dependent on the recommended CBP approach, should be either provided continuously or stochastically. Challenges may include exposure to the original cause of decline (Fischer and Lindenmayer 2000), environmental heterogeneity (West-Eberhard 1989), parasitism (Summers *et al.*, 2003) or seasonal changes such as food availability. For example, exposure to parasites has the ability to generate polyphenism to promote variation in reproductive traits, such as courtship displays and genital morphology (Summers *et al.*, 2003). The ability to rapidly produce multiple phenotypes via induced plastic changes in morphology, behaviour and physiology in response to challenges and novel environments is likely to increase the likelihood for survival (Price *et al.*, 2003; Pfennig *et al.*, 2010). As such, phenotypic plasticity has been identified as a key driver for the origin of novel phenotypes, divergence amongst populations and influencing the patterns of emerging diversity (Pfennig *et al.*, 2010). Given the static 'ideal' captive environments, CBPs may not provide adequate conditions to promote such phenotypic plasticity or rather marginalise phenotypes, and as a result, released individuals may have a decreased likelihood of survival.

1.6 Model species

Model species provide a suitable alternative to examining the phenotypic changes in captivity and provide valuable information for applying to endangered species' captive breeding (Table 1.1; Fischer and Lindenmayer 2000). This may include invasive procedures to determine the effects of captivity on phenotypic traits, such as internal morphology. For example, to understand the proximate mechanisms of phenotypic change, such as rapid alterations in morphology and behaviour in the endangered Devil's Hole pupfish (*Cyprinodon diabolis*), a surrogate species Amargosa River pupfish (*Cyprinodon nevadensis amargosae*) was used to examine how environmental conditions influence morphological development (Lema and Nevitt 2006). Furthermore, captive populations may not yield suitable sample sizes (Réale *et al.*, 1999; van Oers *et al.*, 2004; van Oers *et al.*, 2005). In light of these limitations, the use of model species can be a suitable alternative and provide valuable information for endangered species (Mathews *et al.*, 2005). For this thesis, I used a mammalian and an amphibian species as models to investigate phenotypic responses to captivity, specifically looking at developmental, morphological and behavioural phenotypes.

*1.6.1 Mammalian model species: house mouse (*Mus musculus*)*

Approximately twenty five percent of all mammals are at risk of extinction (Di Marco *et al.*, 2012). Many mammalian species require captive breeding due to habitat loss or degradation, introduced predators, competitors and exposure to disease (Frankham 2008). Small mammals, such as rodents, are an ideal model group to understand the phenotypic responses of terrestrial mammals to captivity (Dew-Budd *et al.*, 2016). Rodents are easily maintained in captivity, and, due to short generation times, transgenerational studies can occur over short periods (O'Regan and Kitchener 2005). For these reasons, rodents such as house mouse are being increasingly used as a model to address questions related to small mammal captive breeding and reintroduction (O'Regan and Kitchener 2005; Paproth 2011; Slade *et al.*, 2014). In my thesis, I used the house mouse (*Mus musculus*) as a model species for small mammals. *Mus musculus* is small rodent species that shares several life-history traits in common with other small mammals, including short generation time, large litter sizes, iteroparity, polygamous mating strategies, sexual dimorphism and early age at maturity (Glucksmann 1974; Millar and Zammuto 1983; Stearns 1983; Promislow and Harvey 1990; Austad 1997; Latham and Mason 2004).

*1.6.2 Amphibian model species: striped marsh frog (*Limnodynastes peronii*)*

Amphibians are declining faster than any other vertebrate group (Stuart *et al.*, 2004; Gascon *et al.*, 2007) and captive breeding programmes have been established for various amphibian species (Stuart *et al.*, 2004; Gascon *et al.*, 2007; Griffiths and Pavajeau 2008). However, many of these programmes have been unable to consistently generate large populations of healthy individuals. Empirical studies have now begun to address this issue by investigating how phenotypic traits such as growth, development and survivorship are influenced by various factors in the captive environment (Álvarez and Nicieza 2002; Christy and Dickman 2002; Ogilvy *et al.*, 2012; Mantellato *et al.*, 2013). To establish optimal captive rearing environments for threatened amphibian species, exploring factors influencing growth and development in model species with analogous life histories to endangered species may provide a useful first step towards identifying optimal rearing conditions. For example, the establishment of *ex-situ* breeding programmes for threatened amphibian species, *Geocrinia alba* and *Geocrinia vitellina* were expedited by studying the growth and development of the common frog,

G. rosea (Mantellato *et al.*, 2013). In my thesis, I used the striped marsh frog (*Limnodynastes peronii*) as a model species for aquatic frog species in the family Myobatrachidae that are listed as critically endangered under the IUCN Red List in Australia (IUCN, 2016; Hero *et al.*, 2006). *Limnodynastes peronii* is a common Australian frog species with a wide distribution along the east coast, extending from cool temperate regions in Victoria to the tropical regions of northern Queensland (Wilson 2001). Many threatened anurans are temperate-zone pond-breeding species in which larvae experience marked fluctuations in temperature and food availability over extended developmental periods (i.e. >2 months). Larval *L. peronii* are found in various aquatic environments that experience a broad range of nutritional and temperature conditions, making *L. peronii* an ideal model species in which to examine the effects of several different variables (e.g. food availability in combination with temperature variation) on various phenotypic traits, including growth and development and survivorship (Niehaus *et al.*, 2006).

1.7 Thesis aims

Ultimately, a range of factors will determine the success of reintroductions; however, incorporating an understanding of phenotypic traits and assessment of trait change in captivity may be a key factor in reintroduction success. In this thesis, I outlined how an animal's phenotype may contribute to the success or failure of CBPs and, in turn, reintroduction success, with a specific focus on the changes to behaviour, morphology, and growth and development that occur in captivity. Further, I explored how manipulating environmental conditions in captivity can be used to promote phenotypic plasticity and the potential for inducing the expression of favourable phenotypic traits in populations of captive-bred species.

My thesis had three main aims: 1) to determine the effect of captivity on phenotypic traits, including growth, developmental, morphological and behavioural phenotypes; 2) to measure the heritability of phenotypic traits to illuminate the potential for rapid adaptation to captivity; and 3) to better understand how environmental conditions in captivity interact to change phenotypic traits and how these phenotypic changes may improve the number and viability of individuals generated in captive breeding programmes.

1.8 Thesis outline

Chapters in this thesis follow a journal article structure. As a result, methods are described sequentially in each chapter, with reference made to previous chapters where necessary. This structure has resulted in some degree of overlap, particularly in the methods sections for Chapter 2, 3 and 4.

In **Chapter 2,** I have used house mouse (*Mus musculus*) as a model species to determine whether behaviour in captive-reared and wild-caught animals differs. While the effects of captivity on behaviour have been widely reported, few studies have compared differences between captive-reared and wild animals, the transgenerational effects on behaviour, and potential differences between sexes in response to captivity. Even fewer studies have examined behavioural types (a composition of behavioural traits) displayed in novel environments, which are particularly relevant for determining reintroduction success. Mice reared in a captive environment were found to differ in their boldness and activity behavioural type compared with their wild-caught conspecifics. After one generation, there was evidence of transgenerational effects in captivity on some behavioural traits but not the behavioural type, and there was no evidence that changes in behavioural type were dependent on sex. Importantly, however behavioural type did differ between wild-caught females and males, suggesting that captivity resulted in the loss of sex specific behaviours. These findings contribute to a small but growing body of evidence that captivity can result in a change of behavioural type and the loss of sex-specific behaviours, and phenotypic plasticity might have a significant influence on behavioural types across captive generations.

In **Chapter 3,** I have used the house mouse (*Mus musculus*) as a model species to test whether i) external and internal morphology differ between captive and wild animals; ii) morphology was subject to transgenerational effects in captivity; and iii) morphology differed between the sexes in animals from captive versus wild environments. While captivity is known to cause changes in external morphological traits, captivity can also drive changes in internal morphology. However, few studies have explicitly compared morphological differences between captive and wild animals, and even fewer have examined internal morphology. In this chapter I provide evidence to suggest that subtle external changes can mask more pronounced internal changes, and that phenotypic plasticity may have a significant influence on morphology across captive generations, as

well as between sexes. A key discussion point is that changes in internal morphology could have severe and unforeseen effects on the viability of captive animals following release.

In **Chapter 4,** I have examined how the captive phenotype can shift away from the wild phenotype with each subsequent generation in captivity, via transgenerational effects. There is emerging evidence that controlling transgenerational effects may be able to mitigate the potentially detrimental effects of captivity, influencing the success of offspring in the wild. However, it remains largely unknown whether transgenerational changes occur via genetic mechanisms of inheritance (i.e. heritability). The overall aim of this study was to investigate the heritability of phenotypic traits using house mouse (*Mus musculus*) known to display transgenerational effects. Chapter 4 investigates what factors may be driving transgenerational effects in captivity. The findings presented in this chapter suggest that some, but not all, phenotypic traits may display some degree of heritability, and demonstrate an evolutionary potential for the rapid adaptation to captive conditions. For other phenotypic traits, heritability, was very low, or even undetectable, which suggests that some phenotypic traits are strongly influenced by environmental conditions, and are likely to display a high degree of plasticity. The main conclusion of this chapter is that identifying mechanisms that drive transgenerational effects, such as heritability occurring in captivity, may be important for the development of control measures to regulate adaptations to captivity.

In **Chapter 5**, I have examined the independent and interactive effects of long-term stochastic environmental conditions in captivity, specifically food availability and water temperature, on larval, growth, development and survivorship of the striped marsh frog (*Limnodynastes peronii*). While the independent effects of food availability and temperature on growth and development in larval species are well established, the interactive effect of these factors on growth, development and survival to maturity is only just beginning to be considered, with evidence emerging to suggest that such interactions can alter developmental trajectories. Changes in food availability mediated the effects of temperature, with slower larval growth and higher survivorship in stochastic food availability treatments. These findings suggest that interactions between environmental factors can influence anuran growth, development and survivorship.

Such advances have the potential to improve the output of amphibian captive breeding programmes and assist with amphibian conservation.

The **General Discussion (Chapter 6)** section synthesises all chapters and makes management recommendations based on my findings. I discuss the findings and how they contribute to the current knowledge of captive breeding programmes and reintroductions, and consider the wider implications and future directions of my findings for the role of phenotypic variation in captive breeding programmes. This chapter is intended primarily for captive breeding specialists, and is intended to aid in the development of strategies for managing phenotypic change and maintenance in captive breeding programmes.

1.9 References

Álvarez D and Nicieza AG (2002) Effects of temperature and food quality on anuran larval growth and metamorphosis. Functional Ecology **16:** 640 – 648

Anthony LL and Blumstein DT (2000) Integrating behaviour into wildlife conservation: the multiple ways that behaviour can reduce Ne. Biological Conservation **95:** 303 – 315

Austad SN (1997) Comparative aging and life histories in mammals. Experimental Gerontology **32:** 23 – 38

Birkhead TR, Pellatt EJ, Matthews IM, Roddis NJ, Hunter FM, McPhie F and Castillo-Juarez H (2006) Genic capture and the genetic basis of sexually selected traits in the zebra finch. Evolution **60:** 2389 – 2398

Bremner-Harrison S, Prodohl P and Elwood RW (2004) Behavioural trait assessment as a release criterion: boldness predicts early death in a reintroduction programme of captive-bred swift fox (*Vulpes velox*). Animal Conservation **7:** 313 – 320.

Briscoe DA, Malpica JM, Robertson A, Smith GJ, Frankham R, Banks RG and Barker JSF (1992) Rapid loss of genetic variation in large captive populations of *Drosophila* flies: implications for the genetic management of captive populations.. Conservation Biology **6:** 416 – 425

Bryant EH, Backus VL, Clark ME and Reed DH (1999) Experimental tests of captive breeding for endangered species. Conservation Biology **13:**1487 – 1496

Bull JJ, Pfennig DW and Wang I-N (2004) Genetic details, optimization and phage life histories. Trends in Ecology and Evolution **19:** 76 – 82

Carducci JP and Jakob EM (2000) Rearing environment affects behaviour of jumping spiders. Animal Behaviour **59:** 39 – 46

Chakravarti LJ, Jarrold MD, Gibbin EM, Christen F, Massamba-N'Siala G, Blier PU and Calosi P (2016) Can trans-generational experiments be used to enhance species resilience to ocean warming and acidification? Evolutionary Applications **9:** 1133 – 1146

Champagnon J, Guillemain M, Elmberg J, Massez G, Cavallo F and Gauthier-Clerc M. (2012) Low survival after release into the wild: assessing "the burden of captivity" on mallard physiology and behaviour. European Journal of Wildlife Research **58:** 255 – 267

Christie MR, Marine ML, French RA and Blouin MS (2012) Genetic adaptation to captivity can occur in a single generation. Proceedings of the National Academy of Sciences **109:** 238 – 242

Christy MT and Dickman CR (2002) Effects of salinity on tadpoles of the green and golden bell frog (*Litoria aurea*). Amphibia-Reptilia **23:** 1 – 11

Conde DA, Flesness N, Colchero F, Jones OR and Scheuerlein A (2011) An emerging role of zoos to conserve biodiversity. Science **331:** 1390-1391

Connolly JD and Cree A (2008) Risks of a late start to captive management for conservation: phenotypic differences between wild and captive individuals of a viviparous endangered skink (*Oligosoma otagense*). Biological Conservation **141:** 1283 – 1292

De Jong G (2005) Evolution of phenotypic plasticity: patterns of plasticity and the emergence of ecotypes. New Phytologist **166:** 101 – 118

DeGregorio BA, Weatherhead PJ, Tuberville TD and Sperry JH (2013) Time in captivity affects foraging behavior of ratsnakes: implications for translocation. Herpetological Conservation and Biology **8:** 581 – 590

Dew-Budd K, Jarnigan J and Reed LK (2016) Genetic and sex-specific transgenerational effects of a high fat diet in *Drosophila melanogaster*. PLoS ONE **11:** e0160857

DeWitt TJ, Sih A and Wilson DS (1998) Costs and limits of phenotypic plasticity. Trends in Ecology and Evolution **13:** 77 – 81

Di Marco M, Cardillo M, Possingham HP, Wilson KA, Blomberg SP, Boitani L and Rondinini C (2012) A novel approach for global mammal extinction risk reduction. Conservation Letters **5:** 134 – 141

Dor R and Lotem A (2009) Heritability of nestling begging intensity in the house sparrow (*Passer domesticus*). Evolution **63:** 738 – 748

Evans ML, Wilke NF, O'Reilly PT and Fleming IA (2014) Transgenerational effects of parental rearing environment influence the survivorship of captive-born offspring in the wild. Conservation Letters **7:** 371 – 379

Fischer J and Lindenmayer DB (2000) An assessment of the published results of animal relocations. Biological Conservation **96:** 1 – 11

Ford MJ (2002) Selection in captivity during supportive breeding may reduce fitness in the wild. Conservation Biology **16:** 815 – 825

Frankham R (2008) Genetic adaptation to captivity in species conservation programs. Molecular Ecology **17:** 325 – 333

Fresneau N, Kluen E and Brommer JE (2014) A sex-specific behavioral syndrome in a wild passerine. Behavioral Ecology **25:** 359 – 367

Gascon C, Collins JP, Moore RD, Church DR, McKay JE and Mendelson JR III (eds) (2007) Amphibian conservation action plan. IUCN/SSC Amphibian Specialist Group. Gland, Switzerland and Cambridge, UK.

Geiser F and Ferguson C (2001) Intraspecific differences in behaviour and physiology: effects of captive breeding on patterns of torpor in feathertail gliders. Journal of Comparative Physiology B **171:** 569 – 576

Glucksmann A (1974) Sexual dimorphism in mammals. Biological Reviews **49:** 423 – 475

Griffiths RA and Pavajeau L (2008) Captive breeding, reintroduction, and the conservation of amphibians. Conservation Biology **22:** 852 – 861

Hedrick AV and Temeles EJ (1989) The evolution of sexual dimorphism in animals: hypotheses and tests. Trends in Ecology and Evolution **4:** 136 – 138

Hero J-M, Morrison C, Gillespie G, Roberts JD, Newell D, Meyer E, McDonald K, Lemckert F, Mahony M, Osborne W and Hines H (2006) Overview of the conservation status of Australian frogs. Pacific Conservation Biology **12:** 313-320

IUCN 2016. The IUCN Red List of Threatened Species. Version 2016-2. <http://www.iucnredlist.org>. Downloaded on 31 October 2016

Jarvie S, Senior AM, Adolph SC, Seddon PJ and Cree A (2015) Captive rearing affects growth but not survival in translocated juvenile tuatara. Journal of Zoology **297:** 184 – 193

Johnson DW, Grorud-Colvert K, Sponaugle S and Semmens BX (2014) Phenotypic variation and selective mortality as major drivers of recruitment variability in fishes. Ecology Letters **17:** 743 – 755

Jule KR, Leaver LA and Lea SE (2008) The effects of captive experience on reintroduction survival in carnivores: a review and analysis. Biological Conservation **141:** 355 – 363

Keller LF, Grant PR, Grant BR and Petren K (2001) Heritability of morphological traits in Darwin's finches: misidentified paternity and maternal effects. Heredity **87:** 325 – 336

Kohane MJ and Parsons PA (1988) Domestication: evolutionary change under stress. In Hecht MK, and Wallace B, editors. Evolutionary Biology (23: 31-48). Springer US, Boston, MA.

Kokko H (2005) Treat'em mean, keep'em (sometimes) keen: evolution of female preferences for dominant and coercive males. Evolutionary Ecology **19:** 123 – 135

Kussell E and Leibler S (2005) Phenotypic diversity, population growth, and information in fluctuating environments. Science **309:** 2075 – 2078

Latham N and Mason G (2004) From house mouse to mouse house: the behavioural biology of free-living *Mus musculus* and its implications in the laboratory. Applied Animal Behaviour Science **86:** 261 – 289

Lema SC and Nevitt GA (2006) Testing an ecophysiological mechanism of morphological plasticity in pupfish and its relevance to conservation efforts for endangered Devils Hole pupfish. Journal of Experimental Biology **209:** 3499 – 3509

Lynch JM and Hayden TJ (1995) Genetic influences on cranial form: variation among ranch and feral American mink *Mustela vison* (Mammalia: Mustelidae). Biological Journal of the Linnean Society **55:** 293 - 307

Mantellato L, Gaikhorst G, Kruger R, Vitali S and Robertson H (2013) Growth and development of captive *Geocrinia rosea* (Myobatrachidae): a rare species analogue. Zoo Biology **32:** 374 – 380

Martos SN, Tang WY and Wang Z (2015) Elusive inheritance: transgenerational effects and epigenetic inheritance in human environmental disease. Progress in Biophysics and Molecular Biology, **118:** 44 – 54

Mason G, Burn CC, Dallaire JA, Kroshko J, McDonald Kinkaid H and Jeschke JM (2013) Plastic animals in cages: behavioural flexibility and responses to captivity. Animal Behaviour **85:** 1113 – 1126

Mathews F, Orros M, McLaren G, Gelling M and Foster R (2005) Keeping fit on the ark: assessing the suitability of captive-bred animals for release. Biological Conservation **121:** 569 – 577

Maxwell JM and Jamieson IG (1997) Survival and recruitment of captive-reared and wild-reared takahe in Fiordland, New Zealand. Conservation Biology **11:** 683 – 691

McDougall P, Réale D, Sol D and Reader S (2006) Wildlife conservation and animal temperament: causes and consequences of evolutionary change for captive, reintroduced, and wild populations. Animal Conservation **9:** 39 – 48

McPhee ME (2004a) Generations in captivity increases behavioral variance: considerations for captive breeding and reintroduction programs. Biological Conservation **115:** 71 – 77

McPhee ME (2004b) Morphological change in wild and captive oldfield mice *Peromyscus polionotus subgriseus*. Journal of Mammalogy **85:**1130 – 1137

McPhee ME and Carlstead K (2010) The importance of maintaining natural behaviors in captive mammals. In: Kleiman DG, Thompson KV, and Baer CK, editors. Wild mammals in captivity: principles and techniques for zoo management. (pp. 303-312) University of Chicago Press, Chicago

McPhee ME and McPhee NF (2012) Relaxed selection and environmental change decrease reintroduction success in simulated populations. Animal Conservation **15:** 274 – 282

McPherson FJ and Chenoweth PJ (2012) Mammalian sexual dimorphism. Animal Reproduction Science **131:** 109 – 122

Millar JS and Zammuto RM (1983) Life histories of mammals: an analysis of life tables. Ecology **64:** 631 – 635

Miner BG, Sultan SE, Morgan SG, Padilla DK and Relyea RA (2005) Ecological consequences of phenotypic plasticity. Trends in Ecology and Evolution **20:** 685 – 692

Munkwitz NM, Turner JM, Kershner EL, Farabaugh SM and Heath SR (2005) Predicting release success of captive-reared loggerhead shrikes (*Lanius ludovicianus*) using pre-release behavior. Zoo Biology **24:** 447 – 458

Niehaus AC, Wilson RS and Franklin CE (2006) Short- and long-term consequences of thermal variation in the larval environment of anurans. Journal of Animal Ecology **75:** 686 – 692

Norberg J, Swaney DP, Dushoff J, Lin J, Casagrandi R and Levin SA (2001) Phenotypic diversity and ecosystem functioning in changing environments: a theoretical framework. Proceedings of the National Academy of Sciences **98:** 11376 – 11381

O'Regan HJ and Kitchener AC (2005) The effects of captivity on the morphology of captive, domesticated and feral mammals. Mammal Review **35:** 215 – 230

Ogilvy V, Preziosi RF and Fidgett AL (2012) A brighter future for frogs? The influence of carotenoids on the health, development and reproductive success of the red-eye tree frog. Animal Conservation **15:** 480 – 488

Paproth A (2011) Measuring fitness loss in captive breeding programs: implications for threatened species recovery. Masters Thesis. University of Melbourne, Melbourne, Australia

Pfennig DW, Wund MA, Snell-Rood EC, Cruickshank T, Schlichting CD and Moczek AP (2010) Phenotypic plasticity's impacts on diversification and speciation. Trends in Ecology and Evolution **25:** 459 – 467

Philippart JC (1995) Is captive breeding an effective solution for the preservation of endemic species? Biological Conservation **72:** 281 – 295

Price TD, Qvarnström A and Irwin DE (2003) The role of phenotypic plasticity in driving genetic evolution. Proceedings of the Royal Society of London. Series B: Biological Sciences **270:** 1433 – 1440

Promislow DEL and Harvey PH (1990) Living fast and dying young: a comparative analysis of life-history variation among mammals. Journal of Zoology **220:** 417 – 437

Réale D, Festa-Bianchet M and Jorgenson JT (1999) Heritability of body mass varies with age and season in wild bighorn sheep. Heredity **83:** 526 – 532.

Reed TE, Waples RS, Schindler DE, Hard JJ and Kinnison MT (2010) Phenotypic plasticity and population viability: the importance of environmental predictability. Proceedings of the Royal Society B: Biological Sciences **277:** 3391 – 3400

Richards CL, Bossdorf O and Pigliucci M (2010) What role does heritable epigenetic variation play in phenotypic evolution? BioScience **60:** 232 - 237

Schuett W, Tregenza T and Dall SRX (2010) Sexual selection and animal personality. Biological Reviews **85:** 217 – 246

Shoval O, Sheftel H, Shinar G, Hart Y, Ramote O, Mayo A, Dekel E, Kavanagh K and Alon U (2012) Evolutionary trade-offs, pareto optimality, and the geometry of phenotype space. Science **336:** 1157 – 1160

Slade B, Parrott ML, Paproth A, Magrath MJL, Gillespie GR and Jessop TS (2014) Assortative mating among animals of captive and wild origin following experimental conservation releases. Biology Letters **10:** 20140656

Smith JM (1978) Optimization theory in evolution. Annual Review of Ecology and Systematics **9:** 31 – 56

Snyder NFR, Derrickson SR, Beissinger SR, Wiley JW, Smith TB, Toone WD and Miller B (1996) Limitations of captive breeding in endangered species recovery. Conservation Biology **10:** 338 – 348

Stearns SC (1983) The influence of size and phylogeny on patterns of covariation among life-history traits in the mammals. Oikos **41:** 173 - 187

Stuart SN, Chanson JS, Cox NA, Young BE, Rodrigues AS, Fischman DL and Waller RW (2004) Status and trends of amphibian declines and extinctions worldwide. Science **306:** 1783 – 1786

Summers K, McKeon S, Sellars J, Keusenkothen M, Morris J, Gloeckner D, Pressley C, Price B and Snow H (2003) Parasitic exploitation as an engine of diversity. Biological Reviews **78:** 639 – 675

Tarszisz E, Dickman CR and Munn AJ (2014) Physiology in conservation translocations. Conservation Physiology **2:** cou054.

Teixeira CP, de Azevedo CS, Mendl M, Cipreste CF and Young RJ (2007) Revisiting translocation and reintroduction programmes: the importance of considering stress. Animal Behaviour **73:** 1 – 13

Thomas CD (2011) Translocation of species, climate change, and the end of trying to recreate past ecological communities. Trends in Ecology & Evolution **26:** 216 – 221

Thomas CD, Cameron A, Green RE, Bakkenes M, Beaumont LJ, Collingham YC, Erasmus BFN, de Siqueira MF, Grainger A, Hannah L, Hughes L, Huntley B, van Jaarsveld AS, Midgley GF, Miles L, Ortega-Huerta MA, Townsend Peterson A, Phillips OL and Williams SE (2004) Extinction risk from climate change. Nature **427:** 145 – 148

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

van Oers K, Drent PJ, de Goede P and van Noordwijk AJ (2004) Realized heritability and repeatability of risk-taking behaviour in relation to avian personalities. Proceedings of the Royal Society of London B: Biological Sciences **271:** 65 – 73

West-Eberhard MJ (1989) Phenotypic plasticity and the origins of diversity. Annual Review of Ecology and Systematics **20:** 249 – 278

Williams SE and Hoffman EA (2009) Minimizing genetic adaptation in captive breeding programs: a review. Biological Conservation **142:** 2388 – 2400

Wilson RS (2001) Geographic variation in thermal sensitivity of jumping performance in the frog *Limnodynastes peronii*. Journal of Experimental Biology **204:** 4227 – 4236

Wisely SM, Ososky JJ and Buskirk SW (2002) Morphological changes to black-footed ferrets (*Mustela nigripes*) resulting from captivity. Canadian Journal of Zoology **80:** 1562 – 1568

Zalewski A and Bartoszewicz M (2012) Phenotypic variation of an alien species in a new environment: the body size and diet of American mink over time and at local and continental scales. Biological Journal of the Linnean Society **105:** 681 – 693

1.9.1 Table 1.1 References

1. Mathews F, Orros M, McLaren G, Gelling M and Foster R (2005) Keeping fit on the ark: assessing the suitability of captive-bred animals for release. Biological Conservation **121:** 569 – 577

2. Kozuch AE and McPhee ME (2014) Effects of environmental complexity and temporary captivity on foraging behavior of wild-caught meadow voles. Journal of Applied Animal Welfare Science **17:** 157-171

3. Larcombe AN and Withers PC (2007) Effects of long-term captivity on thermoregulation, metabolism and ventilation of the southern brown bandicoot (Marsupialia: Peramelidae). Journal of Comparative Physiology B **177:** 229 – 236

4. McPhee ME (2004) Morphological change in wild and captive oldfield mice *Peromyscus polionotus subgriseus*. Journal of Mammalogy **85:**1130 – 1137

5. McPhee ME (2004) Generations in captivity increases behavioral variance: considerations for captive breeding and reintroduction programs. Biological Conservation **115:** 71 – 77

6. Sjöåsen T (1996) Survivorship of captive-bred and wild-caught reintroduced European otters *Lutra lutra* in Sweden. Biological Conservation **76:** 161 – 165

7. Geiser F and Ferguson C (2001) Intraspecific differences in behaviour and physiology: effects of captive breeding on patterns of torpor in feathertail gliders. Journal of Comparative Physiology B **171:** 569 – 576

8. Cooper CE and Withers PC (2012) Does season or captivity influence the physiology of an endangered marsupial, the numbat *Myrmecobius fasciatus*? Journal of Mammalogy **93:** 771 - 777

9. Slade B, Parrott ML, Paproth A, Magrath MJL, Gillespie GR and Jessop TS (2014) Assortative mating among animals of captive and wild origin following experimental conservation releases. Biology Letters **10:** 20140656

10. Bremner-Harrison S, Prodohl P and Elwood RW (2004) Behavioural trait assessment as a release criterion: boldness predicts early death in a reintroduction programme of captive-bred swift fox (*Vulpes velox*). Animal Conservation **7:** 313 – 320.

11. Wisely SM, Ososky JJ and Buskirk SW (2002) Morphological changes to black-footed ferrets (*Mustela nigripes*) resulting from captivity. Canadian Journal of Zoology **80:** 1562 – 1568

12. Wisely SM, Santymire RM, Livieri TM, Marinari PE, Kreeger JS, Wildt DE and Howard J (2005) Environment influences morphology and development for in situ and ex situ populations of the black-footed ferret (*Mustela nigripes*). Animal Conservation **8:** 321 – 328

13. Biggins DE, Vargas A, Godbey JL and Anderson SH (1999) Influence of prerelease experience on reintroduced black-footed ferrets (*Mustela nigripes*). Biological Conservation **89:** 121 – 129

14. Maran T, Põdra M, Põlma M and Macdonald DW (2009) The survival of captive-born animals in restoration programmes – case study of the endangered European mink *Mustela lutreola*. Biological Conservation **142:** 1685 – 1692

15. Stoinski TS, Beck BB, Bloomsmith MA and Maple TL (2003) A behavioral comparison of captive-born, reintroduced golden lion tamarins and their wild-born offspring. Behaviour **140:** 137 – 160

16. Saragusty J, Shavit-Meyrav A, Yamaguchi N, Nadler R, Bdolah-Abram T, Gibeon L, Hildebrandt TB and Shamir MH (2014) Comparative skull analysis suggests species-specific captivity-related malformation in lions (*Panthera leo*). PLoS ONE **9:** e94527.

17. Hartstone-Rose A, Selvey H, Villari JR, Atwell M and Schmidt T (2014) The three-dimensional morphological effects of captivity. PLoS ONE **9:** e113437

18. Künzl C, Kaiser S, Meier E and Sachser N (2003) Is a wild mammal kept and reared in captivity still a wild animal? Hormones and Behavior **43:** 187 – 196

19. Hollén LI and Manser MB (2007) Persistence of alarm-call behaviour in the absence of predators: a comparison between wild and captive-born meerkats (*Suricata suricatta*). Ethology **113:** 1038 – 1047

20. Benson-Amram S, Weldele ML and Holekamp KE (2013) A comparison of innovative problem-solving abilities between wild and captive spotted hyaenas, *Crocuta crocuta*. Animal Behaviour **85:** 349 – 356

21. Shivik JA, Palmer GL, Gese EM and Osthaus B (2009) Captive coyotes compared to their counterparts in the wild: does environmental enrichment help? Journal of Applied Animal Welfare Science **12:** 223 – 235

22. Ostermann SD, Deforge JR and Edge WD (2001) Captive breeding and reintroduction evaluation criteria: a case study of peninsular bighorn sheep. Conservation Biology **15:** 749 – 760

23. Rogers T, Fox S, Pemberton D and Wise P (2016) Sympathy for the devil: captive-management style did not influence survival, body-mass change or diet of Tasmanian devils 1 year after wild release. Wildlife Research **43:** 544 – 552

24. Munkwitz NM, Turner JM, Kershner EL, Farabaugh SM and Heath SR (2005) Predicting release success of captive-reared loggerhead shrikes (*Lanius ludovicianus*) using pre-release behavior. Zoo Biology **24:** 447 – 458

25. Moore SJ and Battley PF (2006) Differences in the digestive organ morphology of captive and wild brown teal *Anas chlorotis* and implications for releases. Bird Conservation International **16:** 253 – 264

26. Casto JM, Val Nolan J and Ketterson ED (2001) Steroid hormones and immune function: experimental studies in wild and captive dark-eyed juncos (*Junco hyemalis*). The American Naturalist **157:** 408 – 420

27. Champagnon J, Guillemain M, Elmberg J, Massez G, Cavallo F and Gauthier-Clerc M. (2012) Low survival after release into the wild: assessing "the burden of captivity" on mallard physiology and behaviour. European Journal of Wildlife Research **58:** 255 – 267

28. Håkansson J and Jensen P (2005) Behavioural and morphological variation between captive populations of red junglefowl (*Gallus gallus*) – possible implications for conservation. Biological Conservation **122:** 431 - 439

29. Håkansson J and Jensen P (2008) A longitudinal study of antipredator behaviour in four successive generations of two populations of captive red junglefowl. Applied Animal Behaviour Science **114:** 409 – 418

30. Islam MZ, Singh A, Basheer MP, Judas J and Boug A (2013) Differences in space use and habitat selection between captive-bred and wild-born houbara bustards in Saudi Arabia: results from a long-term reintroduction program. Journal of Zoology **289:** 251 – 261

31. Bailey TA, Mensah-Brown EP, Samour JH, Naldo J, Lawrence P and Garner A. (1997) Comparative morphology of the alimentary tract and its glandular derivatives of captive bustards. Journal of Anatomy **191:** 387 – 398

32. Herborn KA, Macleod R, Miles WTS, Schofield ANB, Alexander L and Arnold KE (2010) Personality in captivity reflects personality in the wild. Animal Behaviour **79:** 835 – 843

33. Burns-Cusato M and Morrow ME (2003) Fear in the captive-bred ttwater's prairie chicken as an indicator of postrelease survival. International Journal of Comparative Psychology **16:** 95 – 110

34. Freas CA, Bingman K, Ladage LD and Pravosudov VV (2013) Untangling elevation-related differences in the hippocampus in food-caching mountain chickadees: the effect of a uniform captive environment. Brain, Behavior and Evolution **82:** 199 – 209

35. van Oosterhout C, Smith AM, Hänfling B, Ramnarine IW, Mohammed RS and Cable J (2007) The guppy as a conservation model: implications of parasitism and inbreeding for reintroduction success. Conservation Biology **21:** 1573 – 1583

36. Faria PJ, van Oosterhout C and Cable J (2010) Optimal release strategies for captive-bred animals in reintroduction programs: experimental infections using the guppy as a model organism. Biological Conservation **143:** 35 – 41

37. Burns JG, Saravanan A and Helen Rodd F (2009) Rearing environment affects the brain size of guppies: lab-reared guppies have smaller brains than wild-caught guppies. Ethology **115:** 122 – 133

38. Kostow KE (2004) Differences in juvenile phenotypes and survival between hatchery stocks and a natural population provide evidence for modified selection due to captive breeding. Canadian Journal of Fisheries and Aquatic Sciences **61:** 577 – 589

39. Lee JSF and Berejikian BA (2008) Effects of the rearing environment on average behaviour and behavioural variation in steelhead. Journal of Fish Biology **72:** 1736 – 1749

40. Evans ML, Wilke NF, O'Reilly PT and Fleming IA (2014) Transgenerational effects of parental rearing environment influence the survivorship of captive-born offspring in the wild. Conservation Letters **7:** 371 – 379

41. Lema SC and Nevitt GA (2006) Testing an ecophysiological mechanism of morphological plasticity in pupfish and its relevance to conservation efforts for endangered Devils Hole pupfish. Journal of Experimental Biology **209:** 3499 – 3509

42. Landsman RE (1991) Captivity affects behavioral physiology: plasticity in signaling sexual identity. Experientia **47:** 31 – 38

43. DeGregorio BA, Weatherhead PJ, Tuberville TD and Sperry JH (2013) Time in captivity affects foraging behavior of ratsnakes: implications for translocation. Herpetological Conservation and Biology **8:** 581 – 590

44. Jarvie S, Senior AM, Adolph SC, Seddon PJ and Cree A (2015) Captive rearing affects growth but not survival in translocated juvenile tuatara. Journal of Zoology **297:** 184 – 193

45. Santos T, Pérez-Tris J, Carbonell R, Tellería JL and Díaz JA (2009) Monitoring the performance of wild-born and introduced lizards in a fragmented landscape: implications for *ex situ* conservation programmes. Biological Conservation **142:** 2923 – 2930

46. Connolly JD and Cree A (2008) Risks of a late start to captive management for conservation: phenotypic differences between wild and captive individuals of a viviparous endangered skink (*Oligosoma otagense*). Biological Conservation **141:** 1283 – 1292

47. Kraaijeveld-Smit FJL, Griffiths RA, Moore RD and Beebee TJC (2006) Captive breeding and the fitness of reintroduced species: a test of the responses to predators in a threatened amphibian. Journal of Applied Ecology **43:** 360 – 365

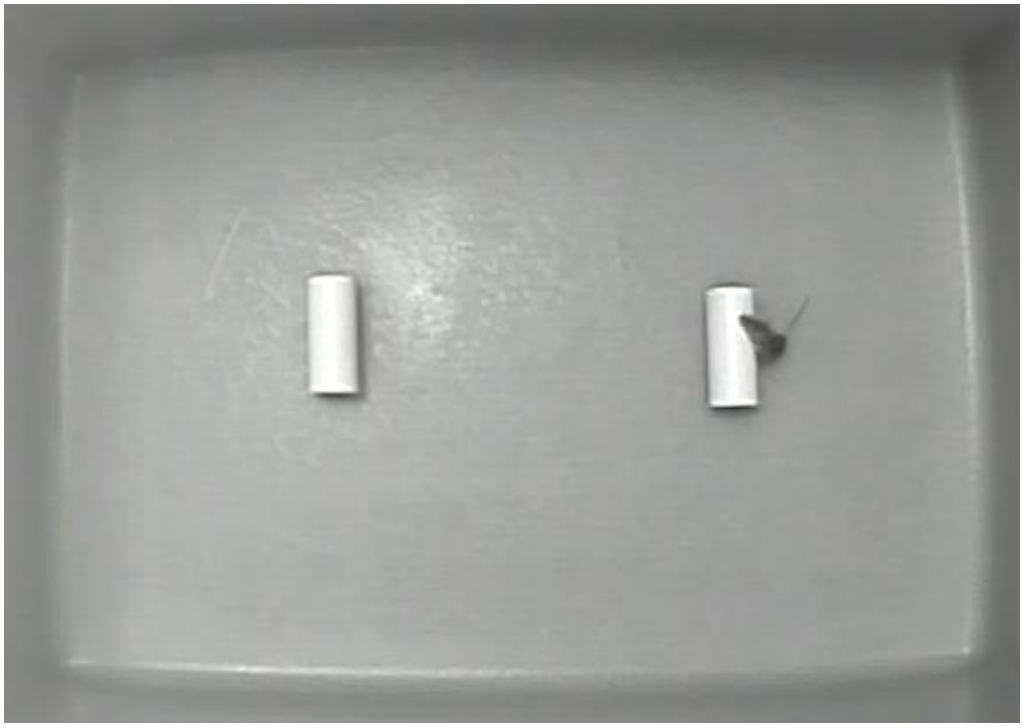
48. Fisher DN, James A, Rodríguez-Muñoz R and Tregenza T (2015) Behaviour in captivity predicts some aspects of natural behaviour, but not others, in a wild cricket population. Proceedings of the Royal Society B: Biological Sciences **282:** 20150708

49. Carducci JP and Jakob EM (2000) Rearing environment affects behaviour of jumping spiders. Animal Behaviour **59:** 39 – 46

50. Rodriguez-Clark KM (2004) Effect of captivity on genetic variance for five traits in the large milkweed bug (*Oncopeltus fasciatus*). Heredity **93:** 51 – 61

51. Schultz CB, Dzurisin JD and Russell C (2009) Captive rearing of puget blue butterflies (*Icaricia icarioides blackmorei*) and implications for conservation. Journal of Insect Conservation **13:** 309 – 315

2 EFFECT OF CAPTIVITY ON HOUSE MOUSE BEHAVIOUR IN A NOVEL ENVIRONMENT: IMPLICATIONS FOR CONSERVATION PRACTICES



2.1 Abstract

Captive breeding programmes offer a method for preventing the extinction of threatened species but often have difficulty establishing self-sustaining populations, with CBPs unable to maintain consistent reproduction and survivorship in captivity to allow for the reintroduction of animals. This difficulty can arise because the behaviour of captive-reared animals differs from wild animals. While the effect of captivity on animal behaviour has been widely reported, few studies have explicitly compared differences between captive-reared and wild-caught animals. Even fewer have examined behavioural types (a composition of behavioural traits) displayed in novel environments, which is particularly relevant for determining reintroduction success. Further, the transgenerational effects on behavioural type, and potential differences between sexes in response to captivity, remain almost completely unknown. Using house mouse (*Mus musculus*) as a model for small mammals, I tested whether boldness and activity behavioural types (boldness: an individual's reaction to risky situations including novel environments; activity: general activity level of an individual) displayed in a novel environment differed between captive-reared and wild-caught animals. In addition, it was tested whether behavioural types were subject to transgenerational effects in captivity, and whether there were sex-specific differences in behavioural types. I used an open field test to simulate a novel environment. Captive-reared mice were found to have differing boldness and activity behavioural types compared to wild-caught mice. There was marginal evidence for transgenerational effects on behavioural type in captivity, but three behavioural traits displayed a shift away from wild behaviours. Furthermore, behavioural types of individuals in captivity did not differ depending on sex, however behavioural type did differ between wild-caught females and males. These findings suggest that captivity can result in behavioural changes and loss of sex-specific behaviours. In addition, phenotypic plasticity may have a significant influence on behavioural type. This knowledge may be critical for developing methods to improve small mammal reintroduction programmes.

Key words: Captive breeding, behaviour, reintroduction, phenotypic plasticity, captivity, transgenerational effects

2.2 Introduction

Captive breeding programmes (hereafter CBPs) are increasingly relied upon as an important conservation tool for threatened species management (Conde *et al.*, 2011). Captive breeding programmes provide a controlled environment for the rearing, maintenance and preservation of many species challenged by key threatening processes in the wild (Thomas *et al.*, 2004; Bryant *et al.*, 1999). However, captive populations often produce behavioural phenotypes that differ from wild populations (Snyder *et al.*, 1996). Behaviour has been shown to be highly heritable in captivity, leading to a shift away from the wild behavioural phenotype with each subsequent generation maintained in captivity (Araki et al. 2009; McPhee 2004; McPhee and McPhee 2012; Ariyomo et al. 2013; Dingemanse et al. 2002; Drent et al. 2003). Furthermore, these behavioural changes may lead to captive individuals having reduced survivorship compared with their wild conspecifics, as well as reduced reproductive success following reintroduction (Johnson *et al.*, 2014; Anthony and Blumstein, 2000; Philippart 1995). It is understood that the captive environment induces changes to the behavioural phenotype, but identifying specific mechanisms that cause such changes can be challenging, largely due to a multitude of abiotic and biotic differences between captive and natural environments. For instance, differences in behavioural phenotypes between captive-reared and wild individuals have been associated with environmental enrichment, habitat complexity and social learning environment (*see* Shier and Owings 2006; Bremner-Harrison *et al.*, 2004; Geiser and Ferguson 2001; Carducci and Jakob 2000).

While the effects of the captive environment on behaviour have been widely reported (Snyder *et al.*, 1996), few studies have quantified the particular composition of behavioural traits that an individual expresses (hereafter referred to as behavioural type; Bell 2007) in comparison to a control group of wild animals. Using an ‘adaptive baseline’ provides the ability to demonstrate and track the effects of captivity. That is, the scale of behavioural plasticity, the direction of change, and the specific behavioural traits that change (Jarvie *et al.*, 2015; Mathews *et al.*, 2005). For example, in a study comparing the behaviour of captive-bred versus wild-caught bank voles (*Clethrionomys glareolus*) it was found that captive-bred individuals displayed some wild-caught nest building and burrowing behaviours. However, captive-bred individuals were unable to

utilise key food resources, and were less dominant in their interactions with conspecifics than wild-caught individuals. As a consequence, the captive-bred individuals were determined unsuitable for release (Mathews *et al.*, 2005).

Of note, few studies have attempted to investigate behavioural types that may impact the fitness of individuals following reintroduction (Moseby *et al.*, 2014; Smith and Blumstein 2008; McDougall *et al.*, 2006).

Testing behaviour in a novel environment (e.g. open field test) is a commonly used tool for determining behavioural types, such as activity or boldness (Réale *et al.*, 2007; Yuen *et al.*, 2015; Rosemberg *et al.*, 2011). Consequently, measuring behavioural types in a novel environment, and quantifying any changes resulting from maintenance in captivity may provide a valuable approach for increasing the success of captive-breeding and reintroduction programmes. Indeed, behavioural characterisation has been used as a criterion for selecting animals for reintroduction (Bremner-Harrison *et al.*, 2004; Mathews *et al.*, 2005). Specifically, boldness and activity relate to the tendency of an individual to take risks and explore novel environments (Réale *et al.*, 2007; Coleman and Wilson 1998). In addition, boldness has been used to predict the probability that individuals survive and reproduce following reintroduction (Herborn *et al.*, 2010; Wilson and Godin 2009). If changes in these behavioural types occur in captivity, the probability of an individual's survival and reproductive success might decline, and in turn, impact the likelihood that the reintroduction programme is successful. Based on optimality theory, an optimal level of boldness and activity would be expected for any given species in any given environment, with extremes on the axes of variation (shy-bold; inactive-active) being costly and selected against (Herborn *et al.*, 2010). Boldness and activity can affect performance and fitness, and by determining these behavioural types, this information may be used to determine an individual's suitability for release (Mathews *et al.*, 2005). Further, knowledge of behavioural changes occurring in captivity may be used to develop strategies to alleviate problems associated with domestication (Mason *et al.*, 2013), or the effect of captivity on behaviours considered important for reintroduction success (McDougall *et al.*, 2006).

How directional selection and phenotypic plasticity alter behavioural traits in the captive environment is only beginning to be investigated (Evans *et al.*, 2014; Nelson *et al.*, 2013). Developmental plasticity in behaviour allows individuals to alter their

behavioural traits to suit their captive environment. In contrast, transgenerational effects in the captive environment influence the behavioural traits passed from parents onto offspring (Evans *et al.*, 2014). Due to changes in the strengths and targets of selection in captivity, and the heritable nature of behavioural traits, a shift in behaviour that increases fitness in the captive environment can be expected (McPhee 2004). Therefore, one might expect behaviour to shift away from the wild behavioural phenotype with each subsequent generation in captivity. Indeed, there is a growing body of evidence for transgenerational behavioural changes occurring in captivity. Previous research has shown that animals maintained in captivity for multiple generations usually display a consistent directional shift in behaviour away from the wild phenotype. Furthermore, these transgenerational behavioural changes have been shown to increase fitness within the captive environment (Johnson *et al.*, 2014; Mason *et al.*, 2013; Christie *et al.*, 2012; McPhee 2004). Commonly reported transgenerational behavioural changes include loss of anti-predator responses and reduced exploratory behaviour (Håkansson and Jensen 2008). For example, refuge-seeking behaviour of Oldfield mice (*Peromyscus polionotus subgriseus*) decreased in frequency with an increasing number of generations maintained in captivity (McPhee 2004).

The way behavioural traits change in captivity, and the direction of transgenerational effects, could depend on a multitude of factors, but one of the most important is likely to be sex. It is well established that behavioural types can differ between the sexes due to sexual selection favouring different trait values in each sex (Fresneau *et al.*, 2014; Schuett *et al.*, 2010). In general, it is expected that intra- and inter-sexual selection (male-male competition and female mate choice) will favour bolder and aggressive males and shy and discriminant females (Kokko 2005). However, such effects might be species- or taxon-specific. For example, a study investigating the effect of reproductive tactics on behavioural syndromes (i.e. personality) in African striped mice (*Rhabdomys pumilio*) found consistent sex-based differences in activity, boldness, exploration and aggression (Yuen *et al.*, 2015). Given that sexual selection in behavioural types is evident across various taxa, captive-based research stands to benefit enormously from exploring the effects of captivity on the strengths and targets of sexual selection, and resultant behavioural differences between the sexes. A small number of behavioural studies on captive populations have examined the effects of captivity and sex on

behaviour (*see* Benson-Amram *et al.*, 2013; Herborn *et al.*, 2010; Mathews *et al.*, 2005; Bremner-Harrison *et al.*, 2004). Of these studies, only one examined the interaction between rearing environment and sex on behaviour, therefore more studies are required.

The overall aim of this study was to investigate whether behaviour in captive-reared and wild-caught animals differ using house mouse (*Mus musculus*) as a model species. To address this overall aim, I had three specific aims i) to compare behavioural types displayed by captive-reared and wild-caught individuals in a novel environment; ii) to determine whether behavioural types are subject to transgenerational effects in the captive environment; and iii) to examine the behavioural types displayed by each sex. The respective predictions for these aims were i) the captive-reared animals would display differing trait values for boldness and activity behavioural types compared to wild-caught individuals; ii) the behavioural type would be subject to transgenerational effects in the captive rearing environment, with captive-reared individuals displaying behavioural types that do not significantly differ from their captive-reared parents, but do significantly differ from wild-caught individuals; and iii) the behavioural types would differ depending on sex. Further, the behavioural type displayed by each sex will be consistent across captive-reared and wild-caught individuals, with captive-reared animals displaying differing trait values for boldness and activity behavioural types regardless of sex.

2.3 Methods

2.3.1 Ethical note

This study was conducted under University of Wollongong Animal Ethics Approval AE13/17.

2.3.2 Study species

The house mouse (*M. musculus*) is a small rodent species widespread throughout the world. The species has a short generation time, has an iteroparous reproductive strategy, displays clear sex roles, polygamous mating strategies and can be easily maintained in captivity. For these reasons, it is being increasingly used as a model to address questions related to small mammal captive breeding and reintroduction (Slade *et al.*, 2014; Paproth 2011).

2.3.3 Housing

All individuals (wild-caught and captive-reared) were maintained separately in opaque plastic cages (32 x 18 x 12 cm; MB1 Mouse Box, Wiretainers Pty Ltd., Melbourne, Victoria, Australia) with a metal top. Wood shavings were used as cage substrate and all cages were provided with bedding material (shredded paper) and a 6 x 4 cm cardboard tube (toilet paper roll) for shelter. Water and food (Vella Stock Feeds brand Rat and Mouse Nut; The Vella Group, Glendenning, New South Wales, Australia) were available ad libitum. Ad libitum food quantities were determined as 20 grams of food per 100 g of body mass supplied daily (Hubrecht and Kirkwood 2010). Room temperature was maintained at 22 ± 2°C on a reversed 12: 12 light: dark cycle, with full spectrum UV light provided. Housing conditions were based on conditions supplied to the original wild-caught founder generation and average temperatures in the field during the study period. Humidity was not controlled, but was monitored daily and recorded as 75 ± 10%. Animals were monitored daily, with cages cleaned once a week by removing the occupant and placing them in a round escape-proof container (54 x 52 cm; Spacepac Industries Pty. Ltd., Wollongong, NSW, Australia) then placing them in a new cage.

2.3.4 Captive-reared parent generation (captive-reared F_4*)*

Eleven sexually mature virgin male *M. musculus* and fifteen sexually mature virgin females were sourced from a captive population maintained at University of New South Wales, Sydney under Ethics Permit UNSW Reg. No. 12/88A. All individuals were third or fourth generation captive-maintained mice born between late-2012 and mid-2013. All animals had unrelated parents and grandparents from multiple litters that were descendants of an original wild-caught founder generation consisting of 42 females and 45 males captured between March and May 2011 at an agricultural site in the western Sydney area (34°4′36.48″S, 150°34′15.6″E).

Prior to this study, the captive-reared F_4 mice were housed in a temperature (19 - 25°C) and light controlled room (12: 12 hr reverse light cycle, lights on at 9:00 AM AEST). Humidity was not controlled but was ~30% (A. Gibson, personal communication, 17 January 2014). Males were housed separately at weaning to avoid aggression and physical injury but female siblings were housed together in groups of up to three

individuals. All animals were provided with food and water ad libitum. Mice were checked three times a week for changes in body condition, behaviour and injuries.

For this study, captive-reared F_4 individuals were collected from University of New South Wales on January, 17, 2014 and transported to the Ecological Research Centre at the University of Wollongong, Wollongong (34°24′24″S 150°52′46″E). Mice were weighed (grams) on digital scales (Mettler-Toledo PJ3600, Mettler-Toledo Ltd., Port Melbourne, Australia) and then housed individually (*see* 2.3.3 Housing). Mice were acclimated in the individual housing for a maximum of 21 days (male: average 11 ± 2 days; female: average 16 ± 5 days; due to the restrictions in processing mice through the behavioural characterisation). Once acclimated, the captive-reared F_4 mice were then entered into the behavioural characterisation assay (*see* 2.3.7 Behavioural Characterisation) before breeding the captive-reared F_5 generation.

2.3.5 Captive-reared offspring generation (captive-reared F_5*)*

Pedigree mapping was used to ensure unrelated individuals from the founder generation were paired so that captive-reared F_5 females and males had unrelated parents and grandparents. Each monogamous breeding pair was held together for one week in standard caging (*see* 2.3.3 Housing). Water and food (Vella Stock Feeds brand Rat and Mouse Nut; The Vella Group, Glendenning, New South Wales, Australia) were available ad libitum, and temperature and light: dark cycles were uniform to those provided for the F_4 acclimation period.

Once mated, the captive-reared F_4 dams were monitored to check for young. Mice were checked once a day, commencing ten days following the male being removed, with the monitoring period lasting an average of 10 ± 2 days. Offspring were housed with their mother until they were weaned at 25 days of age; weaning age was kept uniform across all litters to reduce differences in maternal investment post-pregnancy. At 25 days of age, the captive-reared F_4 dam was removed from the breeding cage, and the litter was then housed for two days under ad libitum conditions to reduce stress on the litter following removal of the dam. Offspring were then housed individually in standard caging (*see* 2.3.3 Housing). Upon entry into the individual housing, individuals had their sex confirmed (13 males and 14 females for this study).

2.3.6 Wild-caught population

Eight sexually mature males and fifteen sexually mature females *M. musculus* were captured in October – November 2014, at the same agricultural site in the western Sydney area (34°4′36.48″S, 150°34′15.6″E) as the source founder population for the captive-reared F_4 generation. Elliott traps (30 x 10 x 8 cm; Sherman Traps Inc., Florida, USA) were set inside and outside sheds and surrounding vegetation. These were checked, emptied and reset daily in the early morning approximately 8:00 AM AEST. Elliott traps were baited with honey and peanut butter rolled oat balls. Once captured, animals were transported to the Ecological Research Centre at the University of Wollongong, Wollongong (34°24′24″S 150°52′46″E) and were housed in the same caging as the captive-reared generations (*see* 2.3.3 Housing). Mice were weighed (grams) upon entry into the individual housing. To match the acclimation period of the captive-reared F_4 individuals and account for the possible effects of the stress of captivity, wild-caught mice were acclimated for a maximum of 21 days (male: average 11 ± 2 days; female: average 16 ± 5 days) prior to behavioural characterisation.

2.3.7 Behavioural characterisation

Behavioural characterisation occurred at sexual maturity for all wild-caught, captive-reared F_4 and captive-reared F_5 individuals (Captive-reared F_4 = 26; Captive-reared F_5 = 27; Wild-caught = 23). To ensure no effects of mating on behavioural characterisation, both captive-reared F_4 and captive-reared F_5 behavioural characterisations were conducted when individuals were virgins. As I was unable to determine whether wild-caught mice were virgins, all wild caught mice were acclimated for a maximum period of 21 days to reduce any effects of potential mating.

Behavioural characterisations for captive-reared F_4, captive-reared F_5 individuals and wild-caught individuals were conducted in late Australian Spring/early Summer and in late Autumn/early Winter. As behavioural analyses were unable to be run simultaneously for all populations, I assumed acclimation period would account for any confounding effects associated with season. To determine how individuals displayed behavioural traits along the bold/shy and active/inactive axes of variation of the active and bold behavioural types, 14 behavioural traits were used (Table 2.1). These traits have previously been used to determine boldness or activity in the following empirical

studies: Augustsson *et al.*, (2005); Augustsson and Meyerson (2004); McPhee (2004). For full ethogram see Table 2.1.

Table 2.1 Ethogram of behaviours measured in open field test.

Behavioural trait	Behavioural measure description	Functional category
Distance (m)	Total distance covered in OFT	Activity
Meandering (°/m)	Absolute turn angle/Total distance travelled	Boldness
Mean speed (m/s)	Average speed during OFT	Activity
Maximum speed (m/s)	Maximum speed reached during OFT	Activity/Boldness
% Time mobile	% Total time spent mobile (Animal is in motion)	Activity
% Time active	% Total time spent active (Animal is mobile or performing some other behaviour)	Activity
% Time freezing	% Total time spent freezing (Animal is not moving, may be performing some other behaviour)	Boldness
Jumping: total number	Total count of jumps in OFT	Boldness
In tunnel: total time (s)	Total time spent in the tunnels (May include or exclude tail)	Boldness
% Centre: total time spent	% Time spent in the centre of the arena	Boldness
Centre: mean speed (m/s)	Average speed in centre zone of OFT	Activity
Centre: maximum speed (m/s)	Maximum speed in centre zone of OFT	Activity/Boldness
Perimeter: mean speed (m/s)	Average speed in perimeter zone of OFT	Activity
Perimeter: maximum speed (m/s)	Maximum speed in perimeter zone of OFT	Activity/Boldness

2.3.8 Apparatus

I used an Open Field Test (henceforth OFT) to determine the behavioural types individuals would display in a novel environment. The OFT arena was constructed from an opaque rectangular LDPE plastic tank with an arena size of 90 x 60 cm with 60 cm high walls (Spacepac Industries Pty. Ltd., Wollongong, NSW, Australia). Two PVC tunnels (6 x 4 cm) were placed in the central part of the arena at opposite ends (located 10 cm from the arena walls) to simulate shelter. Above each arena (n= 4), a video camera (PRO-735 Camera, Swann Systems, Melbourne, Australia) was placed to record the entire OFT trial. Recorded videos were stored on a Digital Video Recorder (DVR8-4100, Swann Systems, Melbourne, Australia) and behaviour was analysed using ANY-maze® software (Stoelting Co., U.S.A). This analysis software is routinely used in vertebrate behavioural characterisation (see Rosemberg *et al.*, 2011; Brenes *et al.*, 2009; Walf and Frye 2007). The location and behaviours (duration) of the mice for the entire duration of the OFT were recorded. Trials were conducted at the same time of day and were conducted in the dark half of the light cycle. At the conclusion of the OFT observation period, a test subject was removed from the OFT arena and the OFT arena and shelters were thoroughly cleaned using 70% EtOH to remove any traces of animal scents.

2.3.9 OFT Procedure

Individual mice were transferred to the OFT arena and were placed in the estimated central point of the OFT arena. Following an acclimation period (2 minutes), behaviour was recorded for 20 minutes (1200 seconds). Fourteen behavioural traits were measured (Table 2.1).

2.3.10 Statistical Analysis

2.3.10.1 Multivariate analysis

To examine the effects of rearing environment on the behaviour of mice, I used multivariate analyses with Primer 7 (Clarke and Gorley 2015) and PERMANOVA+ B version (Anderson and Gorley 2007). This non-parametric analysis accounts for any potential issues with small sample sizes. Of note, sample sizes used in this present study were comparable with other studies of this nature (Slade *et al.*, 2014; Paproth 2011;

Bremner-Harrison *et al.*, 2004; McPhee 2004; Geiser and Ferguson, 2001). To remove the effects of body mass on behaviour, I calculated the residuals of a least squares regression of each behavioural trait on body mass. I then normalised the behavioural trait data so that all behavioural traits would take values within the same limits (-2 to +2 to cover all entries). To test whether behavioural type varied between rearing environment and sex, a two factor PERMANOVA was used on the 14 behavioural traits, the factors were rearing environment (3 levels orthogonal and fixed; wild-caught; captive-reared F_4 and captive-reared F_5) and sex (2 levels orthogonal and fixed; female and male) were used with acclimation period (number of days) as covariate. Interaction factors between acclimation period, rearing environment and sex were included to account for any interactive effects. Compositional differences in behavioural types between wild-caught; captive-reared F_4 and captive-reared F_5 were visualised using non-metric multidimensional scaling (nMDS) ordinations. All analyses used Euclidean similarity measures. Similarity percentage (SIMPER) analysis was used to identify the behavioural traits contributing most strongly to the compositional changes in behavioural type detected.

2.3.10.2 Univariate analyses

Behavioural traits that contributed >10% to compositional changes in behavioural types between wild-caught; captive-reared F_4 and captive-reared F_5 in SIMPER were then analysed using linear mixed effects model (LMMs; Table 2.4) to examine the effects of rearing environment and sex on the behavioural traits in mice. Rearing environment (wild-caught; captive-reared F_4 and captive-reared F_5) and sex (female and male) were the fixed effects, acclimation period (number of days acclimated) was the covariate. An interaction factor between rearing environment and sex was also included. The residuals of a least squares regression of each behavioural trait on body mass were used. For all behavioural data, Tukey's HSD pairwise comparison tests were used for post-hoc comparisons between treatments. All data were analysed in JMP 11.0.0 statistical package.

2.4 Results

2.4.1 Effect of rearing environment and sex on behavioural type

There was a significant interaction between rearing environment and sex (PERMANOVA: Pseudo-F_2 = 3.002, p = 0.008; Table 2.2). Behavioural types significantly differed between individuals from differing rearing environments (PERMANOVA: Pseudo-F_2 = 5.102, p <0.001; Table 2.2) but did not significantly differ between male and female individuals (PERMANOVA: Pseudo-F_2 = 0.415, p = 0.858; Table 2.2). There were no significant interactions between acclimation period, rearing environment and/or sex and there was no significant effect of acclimation period on behavioural type (Table 2.2). SIMPER analysis revealed 8 behavioural traits contributed to the compositional differences in behavioural types between wild-caught; captive-reared F_4 and captive-reared F_5 and sex (only behavioural traits with >10% contribution were considered; *see* 2.6 Supporting Information).

Table 2.2 PERMANOVA analyses comparing effects of rearing environment and sex on behavioural type using multivariate behavioural trait data.

	d.f.	MS	Pseudo-F	P(perm)
Acclimation period x Rearing environment x Sex	2	23.701	1.989	0.058
Acclimation period x Rearing environment	2	11.494	0.964	0.447
Acclimation period x Sex	1	6.920	0.581	0.709
Rearing environment x Sex	2	35.769	3.002	0.008*
Rearing environment	2	60.794	5.102	<0.001*
Sex	1	4.947	0.415	0.858
Acclimation period	1	11.963	1.004	0.375
Residual	64	11.916		

2.4.2 Transgenerational effects in the captive environment on behavioural type

Transgenerational effects in the captive environment were defined as the behavioural type shifting away from the wild phenotype with each subsequent generation in captivity. Behavioural type significantly differed between captive-reared F_5 females and captive-reared F_4 females (PERMANOVA: t_{25} = 1.927, p = 0.013, Table 2.3) and a marginally significant difference occurred between captive-reared F_5 females and wild-caught females (PERMANOVA: t_{25} = 1.542, p = 0.052, Table 2.3). Behavioural type did not significantly differ between captive-reared F_4 females and wild-caught females (Table 2.3). SIMPER analysis revealed that four behavioural traits (Perimeter: max

speed, Perimeter: average speed, Mean speed, Distance) were driving the compositional differences in behavioural type between captive-reared F_5 and captive-reared F_4 females (only behavioural traits with >10% contribution were considered; *see* 2.6 Supporting Information).

Table 2.3 PERMANOVA pairwise tests comparing behavioural type between rearing environments and sex using multivariate behavioural trait data.

Pairwise Tests	t	Den. d.f.	P (perm)
F_5 Female, F_4 Female	1.927	25	0.013*
F_5 Female, Wild Female	1.542	25	0.052
F_4 Female, Wild Female	1.269	26	0.161
F_5 Male, F_4 Male	1.389	20	0.107
F_5 Male, Wild Male	1.429	17	0.096
F_4 Male, Wild Male	2.810	15	<0.001*
F_4 Female, F_4 Male	1.312	22	0.161
F_5 Female, F_5 Male	0.811	23	0.665
Wild Female, Wild Male	1.845	19	0.015*

There were no significant differences between captive-reared F_5 males and wild-caught or captive-reared F_4 males (PERMANOVA: captive-reared F_5 and wild-caught: t_{17} = 1.429, p = 0.096; captive-reared F_5 and captive-reared F_4: t_{20} = 1.389, p = 0.107; Table 2.3). Behavioural type significantly differed between captive-reared F_4 males and wild-caught males (PERMANOVA: t_{15} = 2.810, p <0.001, Table 2.3). SIMPER analysis revealed four behavioural traits were driving the compositional differences in behavioural type between captive-reared F_4 and wild-caught males (% Time active, % Time mobile, Centre: max speed, % Time freezing; *see* 2.6 Supporting Information).

2.4.3 Sex-specific behavioural responses to rearing environment

Pairwise comparisons between males and females in each rearing environment determined only behavioural type significantly differed between wild-caught individuals (PERMANOVA: t_{19} = 1.845, p = 0.015, Table 2.3). Between wild-caught males and females three behavioural traits were driving compositional differences in behavioural type (% Time active; % Time mobile; Centre: max speed; *see* 2.6 Supporting Information).

2.4.4 Effect of rearing environment on behavioural traits

Overall, seven of the eight behavioural traits contributing >10% to compositional differences in behavioural type significantly differed between rearing environments and sex (Table 2.4, *see* 2.6 Supporting Information). There was a significant interaction between rearing environment and sex on % Time spent active and % Time spent mobile (LMM: % Time active: $F_{2, 69}$ = 8.767, p <0.001; % Time mobile: $F_{2, 69}$ = 5.942, p =

0.004; Table 2.4). Compared with wild-caught male mice, captive-reared F_4 male mice spent more time active and mobile (with behavioural traits indicating an increase in activity in captive-reared males). There were no significant differences in time spent active or mobile in captive-reared F_4 and wild-caught female mice. Post-hoc tests demonstrated the transgenerational effects in the captive environment were only evident in males, with % time spent active and mobile significantly differing between captive-reared F_5 and wild-caught mice (Table 2.4, 2.5).

There was a significant difference between individuals from different rearing environments for five behavioural traits: Distance covered, % Time spent freezing, Mean speed, Centre: maximum speed and Perimeter: mean speed (LMMs, Table 2.4). Compared with wild-caught mice, captive-reared F_4 mice covered more distance, spent less time freezing, displayed a faster mean speed and faster mean speed in the perimeter of the OFT arena mobile (with behavioural traits indicating an increase in activity and boldness in captive-reared individuals). In addition, in the centre of the arena, captive-reared F_4 mice displayed a slower maximum speed (Table 2.6). Post-hoc tests demonstrated that transgenerational effects in the captive environment were minimal, with only one behavioural trait (Centre: maximum speed) significantly differing between captive-reared F_5 and wild-caught mice. Conversely, for four behavioural traits (Distance, % Time freezing, Mean speed, Perimeter: mean speed), captive-reared F_5 mice did not significantly differ from wild-caught mice, but did significantly differ from captive-reared F_4 mice (LMMs, Table 2.4, 2.6). There were no significant effects of sex or acclimation period on any behavioural traits (LMMs, Table 2.4).

Table 2.4 Effect of rearing environment and sex on behavioural traits in house mouse. Statistical output from linear mixed effects models (LMMs).

	Rearing Environment x Sex			Rearing environment			Sex			Acclimation period		
Behavioural trait	F	d.f.	p	F	d.f.	p	F	d.f.	p	F	d.f.	p
Distance	0.748	2, 69	0.477	5.409	2, 69	0.006*	0.267	1, 69	0.607	1.048	1, 69	0.309
% Time active	8.767	2, 69	<0.001*	13.009	2, 69	<0.001*	0.967	1, 69	0.328	2.883	1, 69	0.094
% Time mobile	5.942	2, 69	0.004*	11.546	2, 69	<0.001*	1.151	1, 69	0.287	0.758	1, 69	0.387
% Time freezing	2.447	2, 69	0.094	12.947	2, 69	<0.001*	1.586	1, 69	0.212	0.898	1, 69	0.346
Mean speed	0.754	2, 69	0.474	5.411	2, 69	0.006*	0.265	1, 69	0.608	1.044	1, 69	0.310
Centre: maximum speed	0.509	2, 69	0.603	6.031	2, 69	0.004*	1.140	1, 69	0.289	1.352	1, 69	0.249
Perimeter: mean speed	0.264	2, 69	0.768	6.067	2, 69	0.004*	0.145	1, 69	0.704	1.633	1, 69	0.205
Perimeter: maximum speed	1.089	2, 69	0.342	0.365	2, 69	0.695	0.831	1, 69	0.365	0.519	1, 69	0.473

Table 2.5 Interactive effects of rearing environment and sex on behavioural traits in house mouse. Values are raw values mean ± SE.

Behavioural trait	Wild Female (n= 15) Mean ± SE	Wild Male (n= 8) Mean ± SE	Captive F_4 Female (n= 15) Mean ± SE	Captive F_4 Male (n= 11) Mean ± SE	Captive F_5 Female (n= 14) Mean ± SE	Captive F_5 Male (n= 13) Mean ± SE
% Time Active	89.753 ± 1.395^{A}	73.715 ± 4.322^{B}	91.858 ± 2.052^{A}	92.367 ± 1.467^{A}	90.237 ± 1.783^{A}	91.788 ± 2.320^{A}
% Time Mobile	88.046 ± 2.414^{A}	72.289 ± 5.277^{B}	91.727 ± 2.088^{A}	92.367 ± 1.467^{A}	90.120 ± 1.810^{A}	91.238 ± 2.499^{A}

Post-hoc test (Tukey's HSD pairwise comparison tests) for differences among means; means labelled with differing letters are significantly different.

Table 2.6 Effect of rearing environment on behavioural traits in house mouse. Values are raw values mean ± SE.

Behavioural trait	Wild (n= 23) Mean ± SE	Captive F_4 (n= 26) Mean ± SE	Captive F_5 (n= 27) Mean ± SE
Distance	137.106± 15.425^B	189.775± 24.627^A	139.658± 6.372^B
% Time Freezing	49.141± 3.321^B	31.290± 2778^A	41.948± 2.250^B
Mean speed	0.114± 0.013^B	0.158± 0.021^A	0.116± 0.005^B
Centre: maximum speed	1.818± 0.112^B	1.390± 0.058^A	1.619± 0.071^A
Perimeter: mean speed	0.110± 0.012^B	0.166± 0.023^A	0.133± 0.008^B

Post-hoc test (Tukey's HSD pairwise comparison tests) for differences among means; means labelled with differing letters are significantly different.

2.5 Discussion

The aims of this study were threefold. Firstly, to investigate whether behavioural type in a novel environment differed between captive-reared and wild-caught individuals; secondly, to determine whether behavioural changes in captive-reared individuals were subject to transgenerational effects in the captive environment; and thirdly, to determine whether there were differences in behavioural types displayed between the sexes. Mice reared in captivity exhibited a different behavioural type compared with wild-caught conspecifics, providing support for the prediction that captive-reared animals would differ from wild-caught animals. There was evidence for transgenerational effects on behavioural type and as well as on some behavioural traits, providing some support, albeit limited, for the second prediction that the behavioural type would shift away from the wild phenotype with each subsequent generation in captivity. It was found that behavioural type did not significantly differ depending on sex. Furthermore, behavioural type of each sex did not differ in captive environments, but did differ between wild-caught females and males. This finding did not provide any support for the third prediction that each sex would display differing behavioural types.

2.5.1 Effects of captivity on behavioural type displayed in a novel environment

Mice reared in captivity exhibited a different behavioural type compared with wild-caught conspecifics, providing support for the prediction that captive-reared animals would differ from wild-caught animals. My findings provide support for the use of an 'adaptive baseline' by demonstrating the scale of behavioural plasticity occurring; the direction of change; and the behavioural traits that changed (Mathews *et al.*, 2005). In this regard, I suggest that the magnitude and direction of change to behavioural types

(such as boldness and activity used in this study) in an individual may reflect the way the animal behaves in a novel environment following reintroduction (Mason *et al.*, 2013; McDougall *et al.*, 2006).

The effect of captivity on animal behaviour has been reported across a variety of taxa (Wisely *et al.*, 2008; Snyder *et al.*, 1996). Differences in behaviour between captive-reared and wild populations may be expected due to the inherent differences in rearing environments, and associated differences in selection pressures (Mason *et al.*, 2013). However, predicting which behaviours will be affected, and predicting the magnitude and direction of change in a given behaviour can be challenging. Indeed, past studies have shown that the captive behavioural phenotype can remain unchanged, or move toward or away from the wild behavioural phenotype (*see* Champagnon *et al.*, 2012; Augustsson *et al.*, 2005; McPhee 2004; Stoinski and Beck 2004; Geiser and Ferguson 2001; Carducci and Jakob 2000). In this context, changes in behavioural variance in response to captivity may be another useful metric that should be considered when evaluating the behavioural responses to captivity. In general, however, we might expect behavioural type to show adaptations to captivity (Mason *et al.*, 2013). If behaviour in captivity shifts away from the wild behavioural phenotype, it is valuable to determine the ongoing impact of these behavioural changes on individual fitness, particularly if these behavioural changes have consequences for the viability of captive source populations, and/or affect the probability of reintroduction success (Fischer and Lindenmayer, 2000). As such, future research might benefit from investigating whether behavioural changes occurring in captivity are maladaptive under natural conditions.

2.5.2 Transgenerational effects in the captive environment on behavioural type

Between captive generations, there was limited evidence of transgenerational effects on behaviour, with captive-reared female behavioural types showing a marginal shift from the wild-caught behavioural type with each subsequent generation. There was evidence of transgenerational effects in captivity for some but not all behavioural traits (in both females and males), with three behavioural traits in captive-reared F_5 mice significantly differing from wild-caught mice, however these did not significantly differ from captive-reared F_4 mice (Centre: maximum speed; % Time active and % Time mobile in males only; with behavioural traits indicating an increase in activity and boldness in

captive-reared mice). This result provided some support for the second of my predictions; that with each subsequent generation in captivity the behavioural type would shift away from the wild phenotype. Specifically, captive-reared F_5 mice significantly differed from wild-caught mice in only one behavioural trait (Centre: maximum speed). Captive-reared F_5 male mice significantly differed from wild-caught male mice in only two behavioural traits (% Time active, % Time mobile; with behavioural traits indicating an increase in activity in captive-reared males). Conversely, for four behavioural traits (Distance, % Time freezing, Mean speed, Perimeter: mean speed), captive-reared F_5 mice did not differ from wild-caught mice but significantly differed from captive-reared F_4 mice. Given the limited evidence for transgenerational effects on behavioural type and behavioural traits between captive-reared F_4 and F_5 mice, there are two important factors to consider. First that the captive-reared mice were compared with unrelated wild-caught mice, and that this might have created opportunities for random sources of variance in the 'adaptive baseline'. For instance, environmental factors that changed across time that caused behavioural variation between the initial UNSW founders and behaviour recorded for wild-caught mice used in this present study could have influenced the findings. Second, the experimental captive-reared population used in this study was derived from 3 – 4 previous captive-reared generations. Consequently, behavioural changes may have occurred relatively quickly in these previous generations, making it difficult to detect any additional changes in this study. However, I was able to demonstrate that captive-reared F_5 behavioural traits shifted from the wild-caught behavioural phenotype, indicating that transgenerational effects are likely to occur quickly.

Previous studies have reported transgenerational effects in the captive environment, with these studies focussing on particular behavioural traits rather than a composition of behavioural traits (behavioural type) that an individual would express (*see* Evans *et al.*, 2014; Paproth, 2011; Håkansson and Jensen 2008). For example, a past study investigating the temporal changes in behaviour of house mouse in response to captivity reported a reduction in a single exploratory behaviour (time spent touching tunnels) after two generations (Paproth 2011). The lack of transgenerational effects on all behavioural traits that contributed to a behavioural type observed in the present study may have occurred because some, but not all, behavioural traits had an impact on

individual performance (and potentially fitness) in the captive environment (McPhee 2004). Furthermore, transgenerational effects on behavioural type in the captive environment may have remained undetected simply because such effects require multiple generations to manifest. This could occur if individual traits differ in how quickly they respond to change. Another possibility is that differences in social environment during early development may have masked transgenerational effects, resulting in a reduced ability to detect a shift towards 'captive-like' behavioural traits in subsequent captive generations. Consequently, although an identical captive-environment was used for all individuals, and an acclimation period was used to account for any effects of the prior environment for captive-reared and wild-caught mice, inadvertent differences in social rearing-environment may have occurred for the captive-reared F_4 and F_5 mice. Specifically, captive-reared F_4 were transferred from one captive environment to another, where subtle changes in the environment may have been evident (such as stock-density of females and potentially diet) that may have exerted effects on the behavioural type of captive-reared F_4 individuals either closer or further away from the wild-caught behavioural type. For example, captive-reared F_4 females were group-housed prior to introduction to this study, whereas males and all captive-reared F_5 mice were separated at weaning age. Indeed, solitary housing has been shown to increase exploratory behaviour (a proxy for boldness) in house mouse (Goldsmith *et al.*, 1978). Likewise, early social experience has been shown to influence the expression of stereotypic behaviours in striped mice (*Rhabdomys* sp.), with early weaning (physical separation from the mother and siblings) increasing the incidence of stereotypic behaviours (Jones *et al.*, 2010).

Furthermore, captive-reared F_5 mice had behavioural traits that sat between captive-reared F_4 and wild-caught mice, this suggests that some behavioural traits did not shift away from the wild-caught phenotype. This may indicate a lack of transgenerational effects in the captive environment. While age was not considered in this study (sampling behavioural types was unable to be conducted on same-age populations), age may have had a significant influence on the degree of behavioural change. That is, I may not have observed transgenerational effects in the captive-reared F_5 mice simply because behavioural traits were not fully developed. If we assume animals are held under consistent captive conditions during ontogeny and through to reproductive

maturity, over their lifecycle the behaviour of individuals should adjust to the captive environment. Therefore, I suspect that the captive-reared F_5 behavioural types would change to reflect a behavioural type more similar to captive-reared F_4 mice, primarily due to similar captive environments and similar selective pressures. To substantiate whether behavioural types respond to captivity over an individual lifetime, and are subject to transgenerational effects, (i.e. behavioural type shifts away from a wild-caught phenotype over time and with each subsequent generation maintained in captivity) studies would need to measure behavioural type throughout an individual's lifecycle, and across generations. Developmental plasticity in boldness has previously been documented in swift fox (*Vulpes velox*), with captive-bred adult foxes displaying a higher level of boldness compared with juveniles (Bremner-Harrison *et al.*, 2004). To date, there have been limited efforts to determine how developmental plasticity influences transgenerational effects in the captive environment, but this may be a valuable inclusion in future research (Evans *et al.*, 2014).

2.5.3 Sex differences in behavioural type in captivity

Overall, it was found that the behavioural type did not differ significantly depending on sex, indicating each sex displayed similar behavioural types. This finding did not support my third prediction that each sex would display differing behavioural types. Further, the behavioural type of each sex did not differ in captive environments, but behavioural types were significantly different between wild-caught females and males. I suggest my findings indicate that there is a loss of sex-specific behaviours in captivity. Similarly, another study investigating the temporal changes in behaviour of house mouse resulting from maintenance in captivity also reported no significant differences in exploratory or risk-taking behaviours between each sex, but unlike my study, there was no evidence for sex-specific behavioural differences in their wild-caught founder population (Paproth, 2011).

Sex-specific differences in behavioural type occur because the strength and targets of sexual selection differ between sexes (Yuen *et al.*, 2015; Fresneau *et al.*, 2014; Biro and Stamps 2008; Stamps 2007; Sih *et al.*, 2004). A lack of sex-specific differences in behavioural type in captivity may have occurred because the behavioural types examined in this study were subject to natural rather than sexual selective pressures

(Dammhahn 2012; Coleman and Wilson 1998). Boldness and activity relate to a tendency for risk-taking particularly in novel environments (Coleman and Wilson 1998). Risk-taking may influence mate-selection, as well as other behaviours such as foraging, interactions with predators, conspecifics and the environment, all of which are experienced by both sexes (Coleman and Wilson 1998). As such, testing behaviour in a novel environment may not be appropriate for detecting sex-specific differences of captive-reared animals, as sex-specific behavioural differences in a novel environment may not present an evolutionary advantage, unless there is an increased reproductive advantage in captivity. For example, wild grey mouse lemur (*Microcebus murinus*) males were consistently bolder than wild females, with boldness correlating with fecundity in males but not in females (Dammhahn 2012). Similarly, in wild African striped mice (*Rhabdomys pumilio*) there were consistent differences in activity between females and males across reproductive tactics (group- or solitary-living in females, breeding or non-breeding males; Yuen *et al.*, 2015). To the best of my knowledge, there remains a limited understanding of whether these sex-specific differences in behavioural type would be lost in captivity.

Despite emerging evidence that the sexes show behavioural differences prior to introduction to captivity, most previous studies investigating the effect of captivity on behaviour have ignored the effect of sex-specific differences, and associated differences in sexual selection pressure. Clearly, further investigation is required to determine whether captivity can result in losses of sex-specific behaviours. Such studies could focus on examining and comparing the behaviour of females and males in intra- and inter-sexual selection experiments (Chargé *et al.*, 2014; Slade *et al.*, 2014). If differences between the sexes can be consistently demonstrated, sex-specific management strategies may be required to improve CBPs. In recognition of this possibility, several recent studies have begun to explore whether sexual selection theory can be used to inform management strategies (Chargé *et al.*, 2014; Slade *et al.*, 2014).

2.5.4 Implications for Captive Breeding Programmes

Our findings that captivity can result in the change of behavioural type and loss of sex-specific behaviours have significant implications for CBPs. Knowing how captivity changes behaviours across generations, and whether these changes differ between sexes,

can help managers develop and refine approaches used in captive-breeding and reintroduction programmes.

The comparative approach (comparing captive-reared with wild-caught animals) used in this study allows predictions to be made about how behavioural types displayed in captivity may impact the fitness of individuals following reintroduction (Mathews *et al.*, 2005). Past studies have reported links between behavioural change and post-reintroduction fitness (Bremner-Harrison *et al.*, 2004). For example, evidence for maladaptive behavioural changes has been obtained for swift foxes (*V. velox*). A comparative study in this species revealed that a combination of habituation and directional selection resulted in individuals becoming bolder in captivity, and that the boldest individuals had a reduced probability of survival post release (Bremner-Harrison *et al.*, 2004). However, in the present study, without evaluating the fitness of the captive-reared mice upon reintroduction, it is premature to speculate about implications for reintroduction success.

The evidence for transgenerational effects on behavioural type in the captive environment observed in my study highlights the potential for conservation biologists to manipulate the captive environment to induce phenotypic changes that may improve the fitness of animals following reintroduction. One approach may include providing natural conditions during early development, which may reduce the behavioural changes occurring in captivity (Evans *et al.*, 2014). For example, in Atlantic salmon (*Salmo salar*) exposure of parents to natural conditions resulted in a two-fold increase in offspring survivorship in the wild, thereby mitigating the effects of captivity on descendants following reintroduction (Evans *et al.*, 2014).

For most animal groups the effects of captivity on sex-specific differences in behaviour remain unknown. My findings that captivity potentially may lead to the loss of sex-specific behavioural types provided important insights into the potential impacts of captivity on behavioural phenotypes. Specifically, my results suggest that the sexes may need to be treated differently during the management of captive colonies, or when establishing reintroduction programmes. Gaining further information on sex-specific responses to captivity will assist with the development of effective sex-specific management strategies in captivity. Finally, incorporating knowledge of phenotypic

traits such as behaviour into captive breeding and reintroduction programmes improves the likelihood of minimising unfavourable phenotypic changes (Mathews *et al.*, 2005; Smith and Blumstein 2008; Evans *et al.*, 2014; Courtney Jones *et al.*, 2015).

2.5.5 Conclusions

This study aimed to determine whether behavioural types displayed in a novel environment differed between captive-reared and wild-caught house mouse (*Mus musculus*), to test whether these behavioural types were subject to transgenerational effects in captivity, and whether there were sex differences in behavioural types. Mice reared in a captive environment were found to differ in their boldness and activity behavioural type compared with their wild-caught conspecifics. After one generation, there was evidence for transgenerational effects in captivity on behavioural traits but not behavioural type (with behavioural traits indicating an increase in boldness and activity in captive-reared individuals), and there was no evidence that changes in behavioural type were dependent on sex. Importantly, however behavioural type did differ between wild-caught females and males, suggesting that captivity resulted in the loss of sex specific behaviours. These findings contribute to a small but growing body of evidence that i) captivity can result in a change of behavioural type and the loss of sex-specific behaviours, and ii) phenotypic plasticity might have a significant influence on behavioural types across captive generations. This knowledge may prove to be important for developing methods to improve CBPs and reintroduction programmes.

2.6 Supporting Information

See Appendix A for supporting table.

2.7 References

Anderson MJ and Gorley RN (2007) PERMANOVA+1 for primer: guide to statistical methods. PRIMER-E, Plymouth.

Anthony LL and Blumstein DT (2000) Integrating behaviour into wildlife conservation: the multiple ways that behaviour can reduce Ne. Biological Conservation **95:** 303 – 315

Araki H, Cooper B and Blouin MS (2007) Genetic effects of captive breeding cause a rapid, cumulative fitness decline in the wild. Science **318:**100 – 103

Ariyomo TO, Carter M and Watt PH (2013) Heritability of boldness and aggressiveness in the zebrafish. Behavior Genetics **43:** 161 – 167

Augustsson H, Dahlborn K and Meyerson BJ (2005) Exploration and risk assessment in female wild house mice (*Mus musculus musculus*) and two laboratory strains. Physiology and Behavior **84:** 265 – 277

Augustsson H and Meyerson BJ (2004). Exploration and risk assessment: a comparative study of male house mice (*Mus musculus musculus*) and two laboratory strains. Physiology and Behavior **81:** 685 – 698

Bell AM (2007) Future directions in behavioural syndromes research. Proceedings of the Royal Society of London. Series B: Biological Sciences **274:** 755 – 761

Benson-Amram S, Weldele ML and Holekamp KE (2013) A comparison of innovative problem-solving abilities between wild and captive spotted hyaenas, *Crocuta crocuta*. Animal Behaviour **85:** 349 – 356

Biro PA, and Stamps JA (2008) Are animal personality traits linked to life-history productivity? Trends in Ecolog and Evolution **23:** 361 – 368

Bremner-Harrison S, Prodohl P and Elwood RW (2004) Behavioural trait assessment as a release criterion: boldness predicts early death in a reintroduction programme of captive-bred swift fox (*Vulpes velox*). Animal Conservation **7:** 313 – 320

Brenes JC, Padilla M and Fornaguera J (2009) A detailed analysis of open-field habituation and behavioral and neurochemical antidepressant-like effects in postweaning enriched rats. Behavioural Brain Research **197:** 125 – 137

Bryant EH, Backus VL, Clark ME and Reed DH (1999) Experimental tests of captive breeding for endangered species. Conservation Biology **13:**1487 – 1496

Carducci JP and Jakob EM (2000) Rearing environment affects behaviour of jumping spiders. Animal Behaviour **59:** 39 – 46

Champagnon J, Guillemain M, Elmberg J, Massez G, Cavallo F and Gauthier-Clerc M. (2012) Low survival after release into the wild: assessing “the burden of captivity” on

mallard physiology and behaviour. European Journal of Wildlife Research **58:** 255 – 267

Chargé R, Teplitsky C, Sorci G and Low M (2014) Can sexual selection theory inform genetic management of captive populations? A review. Evolutionary Applications **7:** 1120 – 1133

Christie MR, Marine ML, French RA and Blouin MS (2012) Genetic adaptation to captivity can occur in a single generation. Proceedings of the National Academy of Sciences **109:** 238 – 242

Clarke KR and Gorley RN (2015) PRIMER v7.0.7: User Manual/Tutorial. PRIMER-E, Plymouth.

Coleman K and Wilson DS (1998) Shyness and boldness in pumpkinseed sunfish: individual differences are context-specific. Animal Behaviour **56:** 927 – 936

Conde DA, Flesness N, Colchero F, Jones OR and Scheuerlein A (2011) An emerging role of zoos to conserve biodiversity. Science **331:** 1390 – 1391

Courtney Jones SK, Munn AJ, Penman TD and Byrne PG (2015) Long-term changes in food availability mediate the effects of temperature on growth, development and survival in striped marsh frog larvae: implications for captive breeding programmes. Conservation Physiology **3:** cov029

Dammhahn M (2012) Are personality differences in a small iteroparous mammal maintained by a life-history trade-off? Proceedings of the Royal Society B: Biological Sciences **279:** 2645 – 2651

Dingemanse NJ, Both C, Drent PJ, van Oers K and van Noordwijk AJ (2002) Repeatability and heritability of exploratory behaviour in great tits from the wild. Animal Behaviour **64:** 929 – 938

Drent PJ, van Oers K and van Noordwijk AJ (2003) Realized heritability of personalities in the great tit (*Parus major*). Proceedings of the Royal Society of London. Series B: Biological Sciences **270:** 45 – 51

Evans ML, Wilke NF, O'Reilly PT and Fleming IA (2014) Transgenerational effects of parental rearing environment influence the survivorship of captive-born offspring in the wild. Conservation Letters **7:** 371 – 379

Fischer J and Lindenmayer DB (2000) An assessment of the published results of animal relocations. Biological Conservation **96:** 1 – 11

Fresneau N, Kluen E and Brommer JE (2014) A sex-specific behavioral syndrome in a wild passerine. Behavioral Ecology **25:** 359 – 367

Geiser F and Ferguson C (2001) Intraspecific differences in behaviour and physiology: effects of captive breeding on patterns of torpor in feathertail gliders. Journal of Comparative Physiology B **171:** 569 – 576

Goldsmith JF, Brain PF and Benton D (1978) Effects of the duration of individual or group housing on behavioural and adrenocortical reactivity in male mice. Physiology and Behavior **21:** 757 – 760

Håkansson J and Jensen P (2008) A longitudinal study of antipredator behaviour in four successive generations of two populations of captive red junglefowl. Applied Animal Behaviour Science **114:** 409 – 418

Herborn KA, Macleod R, Miles WTS, Schofield ANB, Alexander L and Arnold KE (2010) Personality in captivity reflects personality in the wild. Animal Behaviour **79:** 835 – 843

Hubrecht R and Kirkwood J (2010) The UFAW handbook on the care and management of laboratory and other research animals. John Wiley and Sons. United States

Jarvie S, Senior AM, Adolph SC, Seddon PJ and Cree A (2015) Captive rearing affects growth but not survival in translocated juvenile tuatara. Journal of Zoology **297:** 184 – 193

Johnson DW, Grorud-Colvert K, Sponaugle S and Semmens BX (2014) Phenotypic variation and selective mortality as major drivers of recruitment variability in fishes. Ecology Letters **17:** 743 – 755

Jones MA, Mason G and Pillay N (2010) Early social experience influences the development of stereotypic behaviour in captive-born striped mice *Rhabdomys*. Applied Animal Behavior Science **123:** 70 – 75

Kokko H (2005) Treat'em mean, keep'em (sometimes) keen: evolution of female preferences for dominant and coercive males. Evolutionary Ecology **19:** 123 – 135

Mason G, Burn CC, Dallaire JA, Kroshko J, McDonald Kinkaid H and Jeschke JM (2013) Plastic animals in cages: behavioural flexibility and responses to captivity. Animal Behaviour **85:** 1113 – 1126

Mathews F, Orros M, McLaren G, Gelling M and Foster R (2005) Keeping fit on the ark: assessing the suitability of captive-bred animals for release. Biological Conservation **121:** 569 – 577

McDougall P, Réale D, Sol D and Reader S (2006) Wildlife conservation and animal temperament: causes and consequences of evolutionary change for captive, reintroduced, and wild populations. Animal Conservation **9:** 39 – 48

McPhee ME (2004) Generations in captivity increases behavioral variance: considerations for captive breeding and reintroduction programs. Biological Conservation **115:** 71 – 77

McPhee ME and McPhee NF (2012) Relaxed selection and environmental change decrease reintroduction success in simulated populations. Animal Conservation **15:** 274 – 282

Moseby KE, Hill BM and Lavery TH (2014) Tailoring release protocols to individual species and sites: one size does not fit all. PLoS ONE **9:** e99753

Nelson AC, Cauceglia JW, Merkley SD, Youngson NA, Oler AJ, Nelson RJ, Cairns BR, Whitelaw E and Potts WK (2013) Reintroducing domesticated wild mice to sociality induces adaptive transgenerational effects on MUP expression. Proceedings of the National Academy of Sciences U.S.A. **110:** 19848 – 19853

Paproth A (2011) Measuring fitness loss in captive breeding programs: implications for threatened species recovery. Masters Thesis. University of Melbourne, Melbourne, Australia.

Philippart JC (1995) Is captive breeding an effective solution for the preservation of endemic species? Biological Conservation **72:** 281 – 295

Réale D, Reader SM, Sol D, McDougall PT and Dingemanse NJ (2007) Integrating animal temperament within ecology and evolution. Biological reviews **82:** 291 – 318

Rosemberg DB, Rico EP, Mussulini BHM, Piano ÂL, Calcagnotto ME, Bonn CD and de Oliveira DL (2011) Differences in spatio-temporal behavior of zebrafish in the open tank paradigm after a short-period confinement into dark and bright environments. PLoS One **6:** e19397

Shier DM and Owings DH (2006) Effects of predator training on behavior and post-release survival of captive prairie dogs (*Cynomys ludovicianus*). Biological Conservation **132:** 126 – 135

Sih A, Bell A Johnson JC (2004) Behavioral syndromes: an ecological and evolutionary overview. Trends in Ecology and Evolution **19:** 372 – 378

Slade B, Parrott ML, Paproth A, Magrath MJL, Gillespie GR and Jessop TS (2014) Assortative mating among animals of captive and wild origin following experimental conservation releases. Biology Letters **10:** 20140656

Smith BR and Blumstein DT (2008) Fitness consequences of personality: a meta-analysis. Behavioural Ecology **19:** 448 – 455

Snyder NFR, Derrickson SR, Beissinger SR, Wiley JW, Smith TB, Toone WD and Miller B (1996) Limitations of captive breeding in endangered species recovery. Conservation Biology **10:** 338 – 348

Stamps JA (2007) Growth-mortality tradeoffs and 'personality traits' in animals. Ecology Letters **10:** 355 – 363

Stoinski TS and Beck BB (2004) Changes in locomotor and foraging skills in captive-born, reintroduced golden lion tamarins (*Leontopithecus rosalia rosalia*). American Journal of Primatology **62:** 1 – 13

Thomas CD, Cameron A, Green RE, Bakkenes M, Beaumont LJ, Collingham YC, Erasmus BFN, de Siqueira MF, Grainger A, Hannah L, Hughes L, Huntley B, van Jaarsveld AS, Midgley GF, Miles L, Ortega-Huerta MA, Townsend Peterson A, Phillips OL and Williams SE (2004) Extinction risk from climate change. Nature **427:** 145 – 148

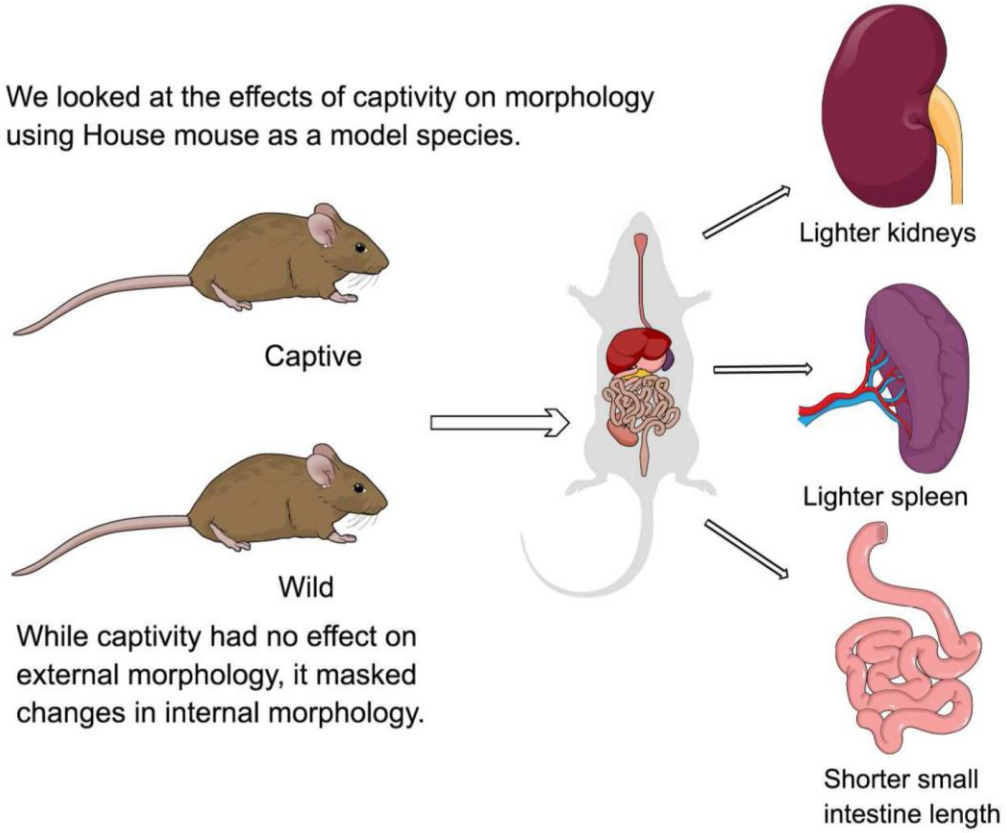
Walf AA and Frye CA (2007) The use of the elevated plus maze as an assay of anxiety-related behavior in rodents. Nature Protocols **2:** 322 – 328

Wilson ADM and Godin J-GJ (2009). Boldness and behavioral syndromes in the bluegill sunfish, *Lepomis macrochirus*. Behavioral Ecology **20:** 231 – 237

Wisely S, Santymire R, Livieri T, Mueting S, and Howard J (2008) Genotypic and phenotypic consequences of reintroduction history in the black-footed ferret (*Mustela nigripes*). Conservation Genetics **9:** 389 – 399

Yuen CH, Pillay N, Heinrichs M, Schoepf I and Schradin C (2015) Personality does not constrain social and behavioural flexibility in African striped mice. Behavioral Ecology and Sociobiology **69:** 1237 – 1249

3 EFFECT OF CAPTIVITY ON MORPHOLOGY IN MICE: NEGLIGIBLE CHANGES IN EXTERNAL MORPHOLOGY MASK SIGNIFICANT CHANGES IN INTERNAL MORPHOLOGY



3.1 Abstract

Captive breeding programmes assist in the recovery of threatened taxa by generating animals for reintroduction and supplementing wild populations. However, morphology differs between captive-reared and wild animals. While captivity is known to cause changes in external morphological traits, captivity can also drive changes in internal morphology. Despite this potential, few studies have explicitly compared morphological differences between captive and wild animals, and even fewer have examined internal morphology. Further, transgenerational effects on the morphology, and potential differences between sexes in response to captivity remain almost completely unknown. I tested whether external and internal morphology differed between captive and wild animals using the house mouse (*Mus musculus*) as a model species. Further, I tested whether morphology was subject to transgenerational effects in captivity, and compared morphology between sexes in animals from both captive and wild environments. I found an absence of changes in external morphology that masked more pronounced internal morphological changes, with captive-reared mice having a heavier caecum, lighter kidneys and spleen and shorter small intestine lengths compared to wild-caught individuals. These internal morphological changes may have major impacts on organismal functioning and viability, including digestive efficiency, and influence immune response and disease resistance, which may reduce survival following reintroduction. There was evidence for transgenerational effects in captivity, however only in internal morphology and only in females. Morphological changes were also evident within the acclimation period, suggesting that phenotypic plasticity contributed to rapid changes in morphology. Finally, morphology significantly differed depending on sex, and sexual dimorphism was maintained in captivity. These findings contribute to a small but growing body of evidence that captivity can result in changes to morphology, and are some of the first to indicate that negligible changes in external morphology can mask significant changes in internal morphology. Implications of these findings for captive breeding and reintroduction programmes are discussed.

Key words: Captive breeding, morphology, reintroduction, phenotypic plasticity, transgenerational effects, conservation biology

3.2 Introduction

Ex-situ conservation, such as captive breeding programmes (henceforth CBPs), assist in the recovery of threatened taxa by providing supplementary animal populations or individuals for reintroduction. However, following reintroduction, released individuals have a low probability of survival (Conde *et al.* 2011; Snyder *et al.* 1996). Causes of reintroduction failure vary, but have been associated with phenotypic change in the physiology and morphology of captive-bred animals (Fischer and Lindenmayer 2000; Hartstone-Rose *et al.* 2014; O'Regan and Kitchener 2005; Paproth 2011; Snyder *et al.* 1996; Tarszisz *et al.* 2014). The ability of an individual to adjust its morphological phenotype in response to abiotic and biotic environmental factors may result from phenotypic plasticity (Miner *et al.* 2005; Schulte-Hostedde and Mastromonaco 2015). Plastic changes in morphology often occur in response to environmental conditions during development and can also result from lagged effects of environmental conditions on the parental generation (Monaghan 2008).

Changes in morphology may also be attributed to selection pressures in captivity differing from those in the natural environment, resulting in selection for morphological phenotypes that maximise individual fitness in the captive environment (Mathews *et al.* 2005; McPhee 2004; Schulte-Hostedde and Mastromonaco 2015). Changes in selection on morphological traits in captivity have been identified to occur in one of two possible ways (McPhee 2004; McPhee and Carlstead 2010). First, captivity could change the direction of selection, with a significant shift in mean expression of morphological traits; however, the variance surrounding the mean remains unchanged. Thus, with increasing generations in captivity there would be a directional change in morphology (McPhee 2004; Schulte-Hostedde and Mastromonaco 2015). Alternatively, captivity may either strengthen or relax selection pressures, allowing for the expression of morphological traits that would be maladaptive in the wild. With a strengthening of selection pressure, trait variation is expected to decline (McPhee 2004). By contrast, with a relaxation of selection pressure, trait variation is expected to increase with increasing generations in captivity (McPhee 2004; McPhee and Carlstead 2010).

Despite the potential for trait change in the captive environment, few studies have examined the effects of captivity on morphology across multiple generations (McPhee 2004; O'Regan and Kitchener 2005). With a change in the strength and targets of selection in captivity, a transgenerational shift in morphology that increases fitness in captivity can be expected (McPhee 2004). Therefore, one would expect morphology to shift away from the wild morphological phenotype with each subsequent generation in captivity. There is emerging evidence that morphological changes can occur quickly. For example, McPhee (2004) tested for directional and relaxed selection in populations of oldfield mice (*Peromyscus polionotus subgriseus*) maintained in captivity for differing periods (2, 14 and 35 generations) and found an increased magnitude of change in cranial and mandibular size and shape with each subsequent generation maintained in captivity. Although the morphological changes became more pronounced as the number of generations increased, these changes were not cumulative or progressive, likely due to relaxed selection pressures in captivity allowing morphological traits to shift in multiple directions. These findings have important implications as they suggest that captivity can impose changes in selective pressures, and, that over multiple generations, these shifts can lead to the morphology of individuals in captive populations differing from individuals in wild populations (McDougall *et al.* 2006; McPhee and Carlstead 2010; O'Regan and Kitchener 2005).

When considering how traits change in captivity, it is also important to consider the effect of sex. Sexual dimorphism typically results from morphological traits being favoured by either intra- or inter-sexual selection (e.g. body size; Hedrick and Temeles 1989; McPherson and Chenoweth 2012). There is a growing body of literature investigating how sexual selection influences morphology within the captive environment (Hartstone-Rose *et al.* 2014; O'Regan and Kitchener 2005). Differences between captive and natural environments, such as reduced competition for resources and artificial selection for animals suited to captivity inadvertently lead to morphological change; in turn, this may lead to changes to or a reduction in sexual dimorphism (McPherson and Chenoweth 2012; O'Regan and Kitchener 2005). Body size is one morphological trait that is known to change; for example, a study investigating the effects of captivity on morphology in American mink (*Mustela vison*) found a reduction in sexual dimorphism in body size and craniometric variation (Lynch

and Hayden 1995). However, empirical evidence for changes to morphology for each sex in captivity is largely limited to a small number of studies in birds and fish (O'Regan and Kitchener 2005).

In captivity, animals face changes in various environmental conditions, but the most pronounced are associated with diet and nutrition (O'Regan and Kitchener 2005), social interactions (Håkansson and Jensen 2005) and degree of cognitive stimulation (Carducci and Jakob 2000). Changes in such factors are known to lead to changes in external morphological traits (Geiser and Ferguson 2001; Håkansson and Jensen 2005; Lema and Nevitt 2006) and skeletal traits (Hartstone-Rose *et al.* 2014; McPhee 2004; Wisely *et al.* 2002). However, captivity can also drive changes in soft tissue morphology (McPhee and Carlstead 2010), with empirical studies beginning to document changes in the size and shape of the brain (Burns *et al.* 2009; Freas *et al.* 2013) and the digestive tract (Champagnon *et al.* 2012; Håkansson and Jensen 2005; O'Regan and Kitchener 2005). Changes in internal morphology are of interest because they are the major interface between an organism and the environment (Courtney Jones *et al.* 2012). Further, internal changes can have major impacts on organismal functioning and viability. For example, captive animals are likely to have little to no exposure to parasites, thus requiring a reduced immune response in captivity (Berzins et al., 2008; Kuhlman and Martin 2010; Martin 2009). Therefore, organs such as the spleen and small intestine that are known to elicit changes in response to parasitism may have reduced in size to maximise their functional capacity in the captive environment (Kristan 2002; Kristan and Hammond 2004). In addition, food provided in captivity is likely to be higher in nutrient and energy density and more freely available compared to natural conditions (Courtney Jones et al. 2015; Diamond and Hammond 1992; Williamson et al. 2014). The changes in resource availability and quality may change the demands placed on an animal's gastrointestinal tract, thus eliciting changes in the small intestine, as well as the kidneys and spleen (Courtney Jones et al., 2012; Cruz et al., 2004; Konarzewski and Diamond 1995; Kristan and Hammond 2006; Kristan & Hammond 2001; Kristan & Hammond 2003).

Critically, the extent of external and internal morphological changes may differ in both direction and magnitude (O'Regan and Kitchener 2005). Specifically, subtle external changes may mask more pronounced internal changes. For example, a study comparing

the morphology of captive-reared mallards (*Anas platyrhynchos*) to wild individuals showed no differences in external morphological traits but lower gizzard weights in captive-reared mallards (Champagnon *et al.* 2012). Despite similar time budgets, captive-reared individuals were unable to reach the body condition of wild individuals following release, resulting in a reduced probability of survival (Champagnon *et al.* 2012). Given the potential fatal consequences of changes in internal morphological traits in the absence of detectable changes in external morphological traits, studies attempting to investigate the influence of captivity on morphological change should aim to quantify changes in both external and internal traits. Such research will provide important insights into the types of traits most susceptible to change, and whether the direction and magnitude of change differ between external and internal traits (McDougall *et al.* 2006; McPhee 2004; McPhee and Carlstead 2010).

While future studies might benefit from focussing on key phenotypic traits critical for post-release fitness, we firstly need to identify what morphological traits might change in captivity. Future studies then can explicitly compare or even manipulate environmental factors in captivity to provide robust inferences about the mechanisms for morphological change in captivity. The overall aim of this study was to provide a holistic assessment and investigate the impact of captivity on morphology using house mouse (*Mus musculus*) as a model species for small mammals. To address this overall aim, three specific aims were proposed: i) to compare the external and internal morphological traits between captive-reared and wild-caught individuals; ii) to examine the effect of captivity on external and internal morphology across generations; and iii) to compare the internal and external morphology of each sex from the captive and wild environments.

3.3 Methods

3.3.1 Ethics permit

This study was conducted under University of Wollongong Animal Ethics Approval AE13/17.

3.3.2 Study species

The house mouse (*Mus musculus*) is a small rodent species distributed globally; the wild-derived strain was used in this study. *Mus musculus* is a good study species to examine the effects of captivity on morphology. The species can be easily maintained in captivity and has a short generation time which permits transgenerational studies to be conducted over relatively short periods (O'Regan and Kitchener 2005). Further, *M. musculus* provides a good model for investigating the effects of captivity on small mammals because this species shares a number of life-history traits in common with other small mammals. These include short generation time, high reproductive value, large litter sizes, iteroparity, sexual dimorphism and early age at maturity (Austad 1997; Glucksmann 1974; Millar and Zammuto 1983; Promislow and Harvey 1990; Stearns 1983; Latham and Mason 2004). For these reasons, *M. musculus* is being increasingly used as a model to address questions related to small mammal captive breeding and reintroduction (O'Regan and Kitchener 2005; Paproth 2011; Slade *et al.* 2014).

3.3.3 Housing and feeding

All individuals (wild-caught and captive-reared) were maintained separately in opaque plastic cages with a metal top (32 x 18 x 12 cm; MB1 Mouse Box, Wiretainers Pty. Ltd., Melbourne, Victoria, Australia). I used wood shavings as cage substrate and all cages were provided with bedding material (shredded paper) and a 6 x 4 cm cardboard tube (toilet paper roll) for cover. Water and food (Vella Stock Feeds Brand Rat and Mouse Nut; The Vella Group, Glendenning, New South Wales, Australia) were available *ad libitum*, determined as 20 grams of food per 100g of body mass was supplied daily (Hubrecht and Kirkwood 2010). Room temperature was maintained at 22 ± 2^{o}C on a reversed 12: 12 lights: dark cycle, with full spectrum UV light provided. Humidity was not controlled, however was monitored daily and recorded as 75 ± 10%. Animals were monitored daily, with cages cleaned once a week by removing the occupant and placing it in a round escape-proof container (54 x 52 cm; Spacepac Industries Pty. Ltd., Wollongong, NSW, Australia) before placement in a new cage.

3.3.4 Captive-reared F_4 *generation*

Eleven virgin adult males and fifteen virgin adult females *M. musculus* were sourced from an existing captive population maintained at the University of New South Wales

(UNSW), Sydney, under Ethics Permit UNSW Reg. No. 12/88A. All mice were third or fourth generation captive-reared mice born between late-2012 and mid-2013. No individuals shared parents or grandparents descended from the original wild-caught founder generation. The original population consisted of 42 females and 45 males captured between March – May 2011 at an agricultural site in the western Sydney area (34°4′36.48″S, 150°34′15.6″E) where March – May temperatures averaged 24.0 – 26.3°C. Prior to relocation to the University of Wollongong, captive-reared F_4 mice were housed at UNSW in a temperature (19 - 25°C) and light controlled room (12: 12 hr reverse light cycle, lights on at 9:00am AEST). Humidity was not controlled but was ~30% (A. Gibson, personal communication, 17 January 2014). Males were housed separately at weaning but female siblings were housed together in groups of up to three individuals. All animals had been provided with food and water *ad libitum*. Mice were monitored daily and thoroughly checked three times a week for body condition, injuries and behaviour.

For this study, captive-reared F_4 individuals were collected late January 2014 and transported to the Ecological Research Centre at the University of Wollongong, Wollongong. Mice were weighed (grams) on digital scales (Mettler-Toledo PJ3600, Mettler-Toledo Ltd., Port Melbourne, Victoria, Australia) upon entry into the individual housing (*see* 3.3.3 Housing and feeding).

Once acclimated to the individual housing, captive-reared F_4 individuals were used to breed the F_5 generation. At the conclusion of the F_5 breeding period, captive-reared F_4 individuals were then re-acclimated to the individual housing for a minimum period of twelve days before quantifying external and internal morphological traits (*see* 3.3.7 External and internal morphological traits).

3.3.5 Captive-reared F_5 *generation*

Pedigree mapping was used to ensure that individuals from the founder generation were paired so that captive-reared F_5 females and males did not share parents or grandparents. Monogamous breeding pairs were held together for one week. Each breeding pair was housed in the same caging used for all wild-caught and captive-reared individuals in this study (*see* 3.3.3 Housing and feeding).

Once mated, the captive-reared F_4 mothers were minimally disturbed, but were closely monitored on a daily basis around the expected due date to check for young. Offspring were housed with their mother until they were weaned at 25 days of age; this was kept uniform across all litters to reduce differences in maternal investment post-pregnancy. At 25 days of age, the captive-reared F_4 mother was removed from the breeding cage, and the litter housed for two days under *ad libitum* conditions, this was done to reduce post-weaning stress on the litter. After two days, the offspring were then housed individually in the same caging used for all wild-caught and captive-reared individuals in this study (*see* 3.3.3 Housing and feeding). The sex of each offspring (henceforth, captive-reared F_5) was determined as the mouse was placed in its individual housing (13 males and 14 females). Captive-reared F_5 mice were individually housed until they reached sexual maturity before quantifying external and internal morphological traits (*see* 3.3.7 External and internal morphological traits).

3.3.6 Wild-caught population

Eight adult males and fifteen adult females *M. musculus* were captured in October – November 2014, at the same agricultural site in the western Sydney area (34°4′36.48″S, 150°34′15.6″E) as the source population of the original wild-caught founder generation (*see* 3.3.4 Captive-reared F_4 generation). Elliott traps (30 x 10 x 8 cm; Sherman Traps Inc., Florida, USA) were set inside and outside sheds and surrounding vegetation. These were checked and emptied daily in the early morning approximately 8.00 am AEST. Elliott traps were baited with honey and peanut butter rolled oat balls.

Once captured, animals were transported to the Ecological Research Centre at the University of Wollongong, Wollongong (34°24′24″S 150°52′46″E) and housed in the same caging as the captive-reared generations (*see* 3.3.3 Housing and feeding). Wild-caught individuals were acclimated to the individual housing for a minimum period of twelve days before quantifying external and internal morphological traits (*see* 3.3.7 External and internal morphological traits).

3.3.7 External and internal morphological traits

Animals were euthanased using CO_2 asphyxiation. Immediately following euthanasia, external body morphological trait measurements and macroscopic dissection of organs

were conducted to study morphometric differences between wild-caught and captive-reared F_4 and F_5 generations. External traits were: body mass (grams), skull length, snout to vent length, tail length and foot length (right hind leg; millimetres). Internal traits were: weights of brain, liver, kidney, heart, lungs, testes/ovaries, spleen, stomach, caecum, small- and large-intestine and the lengths of the small- and large-intestine. Organs were weighed using scales with ± 0.01 g precision (Mettler-Toledo PJ3600, Mettler-Toledo Ltd., Port Melbourne, Victoria, Australia). Where applicable, digestive organs were emptied of their contents and rinsed with a 0.9% saline solution and weighed. The lengths of the small- and large-intestine measured using slide callipers with ± 0.05 mm precision.

3.3.8 Statistical Analysis

3.3.8.1 Multivariate analysis

To examine the effects of rearing environment on the external and internal morphology of mice, I used permutational analysis of variance (PERMANOVA) with 9999 permutations in Primer 7 (PRIMER-E Ltd, Plymouth, UK; Clarke 2015) and PERMANOVA+ B version (Anderson and Gorley 2007). Permutational analyses were selected in favour of parametric analyses for these data sets as they can be used for small and unequal sample sizes when comparing treatments (Drummond and Vowler 2012; Goncalves *et al.* 2015; Little and Seebacher 2014) and for examining transgenerational changes in morphology (Cattano *et al.* 2016).

To control for the effects of body size on morphological traits, I calculated the residuals of a least squares regression of each morphological trait on body size using body mass or snout to vent length where lengths were measured. I then normalised the morphological trait data so that all morphological traits would take values within the same limits (-2 to +2 to cover all entries).

To test whether morphological traits differed between rearing environment and sex, a two-factor PERMANOVA was used on the external and internal morphological traits. In this analysis, the factors were rearing environment (3 levels orthogonal and fixed; wild-caught; captive-reared F_4 and captive-reared F_5) and sex (2 levels orthogonal and

fixed; female and male), with acclimation period (number of days acclimated) included as a covariate. An interaction term between rearing environment and sex was also included to account for any interactive effects of rearing environment and sex on morphology. All analyses used Euclidean similarity measures. Following PERMANOVA, means were compared using pairwise tests in PERMANOVA+ B version (Anderson and Gorley 2007). Similarity percentage (SIMPER) analysis was used to identify the morphological traits that were primarily responsible for the compositional differences in external and internal morphology between captive-reared F_5, captive-reared F_4 and wild-caught animals. Only traits that contributed >10% to compositional changes were used in univariate analyses, as these traits were likely to be primarily responsible for the compositional differences. One individual was excluded from external and internal morphological trait SIMPER analysis due to missing morphometric values.

3.3.8.2 Univariate analyses

To examine the effects of sex on external morphology in mice, four external morphological traits that contributed >10% to compositional changes in external morphology between sexes in SIMPER were analysed using analysis of variance (ANOVA; Table 3.3; *see* 3.6 Supporting Information). To correct p-values for multiple testing, a Bonferroni adjusted alpha level (α= 0.0125) was used. To control for the effects of body size on external morphological traits, I used the residuals of a least squares regression of each morphological trait in analyses. Where individuals were unable to be sampled for analysis of external morphological traits, the degrees of freedom for these respective analyses were adjusted to account for these exclusions. Residuals from ANOVAs were inspected to verify normality and homogeneity of variances. For all morphological data, Tukey's HSD pairwise comparison tests were used for post-hoc comparisons between treatments. Where normality was unable to be met, Kruskal-Wallis tests were used, with post-hoc comparisons made using Wilcoxon tests.

To examine the effects of rearing environment and sex on the internal morphology in mice, internal morphological traits that contributed >10% to compositional changes in internal morphology between rearing environments and sex in SIMPER were analysed

using analysis of covariance (ANCOVA; Table 3.3; *see* 3.6 Supporting Information). To correct p-values for multiple testing, a Bonferroni adjusted alpha level was used (α= 0.0055). For internal morphology, the effects of rearing environment and sex were the fixed effects, and acclimation period (number of days acclimated) was the covariate. An interaction term between rearing environment and sex was also included. To control for the effects of body size on internal morphological traits, I calculated the residuals of a least squares regression of each morphological trait on body mass (or snout to vent length where length was measured). Where individuals were unable to be sampled for specific internal morphological traits, the degrees of freedom for these respective analyses were adjusted to account for these exclusions. Residuals from ANCOVAs were visually inspected to verify normality and homogeneity of variances. As there was no interaction between rearing environment and sex on any internal morphological traits, ANOVAs were then conducted to estimate the effect of rearing environment or sex on internal morphological traits (brain, liver, kidneys, spleen, small intestine length, large intestine, large intestine length, caecum) showing significance in the ANCOVA. Where the assumptions of normality and/or homogeneity of variance were not met, Kruskal-Wallis tests were used, with post-hoc comparisons made using Wilcoxon tests. All morphological data were analysed in the JMP 11.2.0 statistical package.

3.4 Results

3.4.1 Effects of rearing environment and sex on morphology

The rearing environment showed a significant influence of sex on internal morphology (Internal: Pseudo-F_2: 1.926, p= 0.018; Table 3.1). There was no significant interaction between rearing environment and sex on external morphology (External: Pseudo-F_2: 1.997, p= 0.081; Table 3.1). Further, there were no significant interactions between acclimation period, rearing environment and/or sex on external or internal morphology (*see* Table 3.1).

External morphology did not significantly differ between rearing environments or acclimation period (External – Rearing environment: Pseudo-F_2= 1.472, p= 0.135; Acclimation period: Pseudo-F_2= 0.792, p= 0.528; Table 3.1). However, external morphology did significantly differ between sex (External – Sex: Pseudo-F_2= 3.401, p= 0.009; Table 3.1). The internal morphology significantly differed between individuals

from differing rearing environments (Internal – Rearing environment: Pseudo-F_2= 2.853, p= 0.004; Table 3.1), between sex (Internal – Sex: Pseudo-F_2= 6.296, p <0.0001; Table 3.1) and acclimation period (Internal – Acclimation period: Pseudo-F_2= 8.678, p <0.0001; Table 3.1). SIMPER analysis revealed four external and nine internal morphological traits were driving the compositional differences in external and internal morphology between captive-reared F_4, captive-reared F_5 and wild-caught individuals and sex (only morphological traits with >10% contribution were considered; Table 3.3; *see* 3.6 Supporting Information).

Table 3.1 PERMANOVA analyses testing the effects of rearing environment (rearing env.), sex and acclimation period (accl.) on external and internal morphology.

	External				Internal		
	d.f.	MS	Pseudo-F	P(perm)	MS	Pseudo-F	P(perm)
Accl. x Rearing env. x Sex	2	2.582	0.543	0.737	8.991	0.895	0.558
Accl. x Rearing env.	2	2.062	0.434	0.848	12.516	1.246	0.226
Accl. x Sex	1	8.168	1.717	0.153	17.243	1.717	0.080
Accl.	1	3.766	0.792	0.528	87.151	8.678	<0.0001*
Rearing env. x Sex	2	9.499	1.997	0.081	19.343	1.926	0.018*
Rearing env.	2	7.000	1.472	0.135	28.648	2.853	0.004*
Sex	1	16.176	3.401	0.009*	63.232	6.296	<0.0001*
Residual	64	4.756			10.043		

3.4.2 Transgenerational effects on internal morphology in captivity

Transgenerational effects in captivity were defined as morphology shifting away from the wild morphological phenotype with each subsequent generation in captivity. The internal morphology significantly differed between captive-reared F_5 and wild-caught females (t_{25}= 1.805, p= 0.001; Table 3.2). The internal morphology of captive-reared F_5 and captive-reared F_4 females was also found to differ significantly (t_{25}= 1.650, p= 0.007; Table 3.2). There was no significant difference between captive-reared F_4 and wild-caught females (t_{26}= 1.094, p= 0.293; Table 3.2). SIMPER analysis revealed five morphological traits (brain, kidneys, stomach, caecum and ovaries; Supporting Information) were driving compositional differences in internal morphology between captive-reared F_4 and captive-reared F_5 females. No morphological traits contributed >10% to compositional differences in internal morphology between captive-reared F_5 and wild-caught females (*see* 3.6 Supporting Information).

The composition of internal morphology did not significantly differ between captive-reared F_5 and wild-caught males (t_{17} = 1.151, p = 0.223; Table 3.2) or between captive-reared F_5 and captive-reared F_4 males (t_{20} = 1.186, p = 0.219; Table 3.2). Further, there was no significant difference between captive-reared F_4 and wild-caught males (t_{15} = 0.996, p = 0.434; Table 3.2). No internal morphological traits contributed >10% to compositional differences in internal morphology between captive-reared F_4, captive-reared F_5 and wild-caught individuals.

Table 3.2 PERMANOVA pairwise tests comparing external and internal morphology between rearing environments and sex.

Pairwise Tests	t	Den. d.f.	P (perm)
	External morphology		
Female, Male	1.844	64	0.009*
	Internal morphology		
F_5 Female, F_4 Female	1.650	25	0.007*
F_5 Female, Wild Female	1.805	25	0.001*
F_4 Female, Wild Female	1.094	26	0.293
F_5 Male, F_4 Male	1.186	20	0.219
F_5 Male, Wild Male	1.151	17	0.223
F_4 Male, Wild Male	0.996	15	0.434
F_4 Female, F_4 Male	2.026	22	0.002*
F_5 Female, F_5 Male	1.674	23	0.004*
Wild Female, Wild Male	1.588	19	0.012*

3.4.3 Sexual dimorphism in external and internal morphology

The external morphology differed significantly between female and males (t_{64} = 1.884, p = 0.009; Table 3.2), SIMPER analysis revealed body mass, snout to vent, skull and tail lengths were driving compositional differences in external morphology between the sexes. Only body mass differed significantly following Bonferroni adjustment in the ANOVA between females and males in external morphological traits (Table 3.3; *see* 3.6 Supporting Information).

The internal morphology differed significantly between captive-reared F_4 females and males (t_{22} = 2.026, p = 0.002; Table 3.2); SIMPER analysis revealed that large intestine length, kidney and large intestine masses were driving compositional differences in internal morphology between captive-reared F_4 females and males. Captive-reared F_5 female and males differed significantly (t_{23} = 1.674, p = 0.004; Table 3.2) with caecum, brain and stomach mass driving compositional differences in internal morphology between captive-reared F_5 females and males. Wild-caught female and males differed significantly (t_{19}= 1.588, p= 0.012; Table 3.2); SIMPER analysis revealed that liver, spleen and kidney mass were driving compositional differences in internal morphology between wild-caught females and males (*see* 3.6 Supporting Information).

3.4.4 Effects of rearing environment and sex on external and internal morphological traits

There was no significant interaction between rearing environment and sex for any internal morphological traits (Table 3.3). Before the Bonferroni adjustment, five internal morphological traits contributing >10% to compositional differences in internal morphology between rearing environments differed significantly (Table 3.3; *see* 3.6 Supporting Information). Of these five traits, only kidney mass was significant following Bonferroni adjustment in the ANCOVA (Table 3.3). Before the Bonferroni adjustment, four internal morphological traits and one external morphological trait contributing >10% to compositional differences in external and internal morphology differed significantly between sexes (Table 3.3; Supporting Information). Of these traits, body mass, large intestine length, brain and kidney masses were significant following Bonferroni adjustment in the ANCOVA (Table 3.3). Before the Bonferroni adjustment, acclimation period had a significant effect on two internal morphological

traits. Of these traits, liver mass was significant following Bonferroni adjustment in the ANCOVA (Table 3.3).

There was a significant effect of rearing environment on kidney, spleen and caecum mass and small intestine length (Table 3.4). Kidneys and spleen were lighter and the small intestine length shorter in captive-reared F_4 compared to wild-caught individuals. The caecum was heavier in captive-reared F_4 compared to wild-caught individuals, but caecum mass did not differ significantly between captive-reared F_5 and wild-caught individuals.

There was evidence for transgenerational effects in captivity in kidney and spleen masses and small intestine length, with captive-reared F_5 differing significantly from wild-caught individuals (Table 3.5). Body mass, brain, kidney masses and large intestine length differed significantly between males and females (Table 3.3, 3.5).

Table 3.3 Effect of rearing environment and sex on external and internal morphological traits in house mouse. Statistical output from ANOVA for external morphological traits, output from ANCOVA for internal morphological traits.

	Rearing Environment X Sex			Rearing environment			Sex				Acclimation period		
	F	d.f.	p	F	d.f.	p	χ^2	F	d.f.	*p*	F	d.f.	p
External morphological traits													
Body mass							10.296		1	0.001**			
Snout to vent length								3.331	1, 74	0.072			
Foot length								0.289	1, 74	0.592			
Tail length							0.772		1	0.380			
Internal morphological traits													
							χ^2	F	d.f.	p			
Brain	0.324	2, 68	0.724	0.655	2, 68	0.523		11.229	1, 68	0.001**	0.441	1, 68	0.509
Liver	1.262	2, 68	0.289	2.624	2, 68	0.079		4.033	1, 68	0.047*	9.899	1, 68	0.003**
Kidneys	2.346	2, 68	0.104	6.711	2, 68	0.002**		47.262	1, 68	<0.0001**	1.332	1, 68	0.253
Stomach	0.924	2, 68	0.402	0.359	2, 68	0.699		0.842	1, 68	0.362	0.597	1, 68	0.442
Spleen	2.426	2, 68	0.096	5.433	2, 68	0.006*		0.111	1, 68	0.740	4.005	1, 68	0.049*
Small Intestine length	0.286	2, 68	0.752	4.670	2, 68	0.012*		0.611	1, 68	0.437	0.809	1, 68	0.372
Large Intestine	2.592	2, 68	0.082	3.138	2, 68	0.049*		0.708	1, 68	0.403	1.269	1, 68	0.264
Large Intestine length	2.713	2, 68	0.074	2.408	2, 68	0.098		8.644	1, 68	0.004**	1.554	1, 68	0.217
Caecum	0.384	2, 68	0.683	5.355	2, 68	0.007*		0.042	1, 68	0.839	0.016	1, 68	0.900

Footnote: p-values include both unadjusted and adjusted α levels

* Significant (α= 0.05)

** Significant under a Bonferroni adjusted α level

Table 3.4 Effect of rearing environment on internal morphological traits in house mouse. Values are raw values mean ± SE.

	Wild (n= 23)		Captive F_4 (n= 26)		Captive F_5 (n= 27)		χ^2	d.f.	*p*
Kidneys (g)	0.291± 0.020	A	0.296± 0.017	A	0.239± 0.009	B	10.862	2	0.004**
Spleen (g)	0.043± 0.006	A	0.019± 0.002	B	0.018± 0.001	C	24.370	2	<0.0001**
Small intestine length (mm)	349.607± 8.212	A	302.160± 6.967	B	286.555± 3.548	B	31.538	2	<0.0001**
Large intestine (g)	0.204± 0.011	A	0.235± 0.014	A	0.165± 0.005	A	4.702	2	0.095
Caecum (g)	0.090± 0.005	A	0.125± 0.005	B	0.087± 0.005	A	17.454	2	0.0002**

Footnote: p-values include both unadjusted and adjusted α levels
* Significant (α= 0.05)
** Significant under a Bonferroni adjusted α level

Table 3.5 Effect of sex on external and internal morphological traits in house mouse. Values are raw values mean ± SE.

	Female (n= 44)		Male (n= 32)		F	χ^2	d.f.	*p*
Body mass (g)	13.164± 0.380	A	15.788± 0.667	B		10.296	1	0.001**
Brain (g)	0.381± 0.005	A	0.383± 0.007	B	14.039		1	0.0004**
Liver (g)	0.746± 0.026	A	0.862± 0.044	B		5.252	1	0.022*
Kidneys (g)	0.231± 0.007	A	0.334± 0.015	A	41.973		1	<0.0001**
Large Intestine length (mm)	80.568± 1.371	A	88.226± 1.773	B	7.869		1	0.006**

Footnote: p-values include both unadjusted and adjusted α levels
* Significant (α= 0.05)
** Significant under a Bonferroni adjusted α level.

3.5 Discussion

3.5.1 Effects of captivity on morphology

Captive-reared mice had differing internal morphology but not external morphology compared with their wild-caught conspecifics. Differences in morphology between captive and wild environments can be expected due to these environments differing in a multitude of biotic and abiotic factors (Burns *et al.* 2009). The absence of significant changes to external morphology could be explained in one of two possible ways. First differences between captive and natural environments may induce changes in life-history organisation, such as early sexual maturity as a trade-off to potential increased somatic growth of external morphological traits. Indeed, this has been observed in hatchery chinook salmon (*Oncorhynchus tshawytscha*) with egg size decreasing across a 20-year period with no change in female body mass (Heath *et al.*, 2003). Second, external morphological traits may be less plastic; with changes in external morphology occurring more slowly and taking multiple generations to manifest (McPhee 2004; O'Regan and Kitchener 2005). Indeed, in captive black-footed ferrets (*Mustela nigripes*), skull and dental traits were 5 – 6% smaller than wild populations (founder population; museum specimens), and 3 – 10% smaller than wild-caught populations (collected near the founding population), however, these external morphological differences only became apparent after more than 10 years of captive breeding (Wisely *et al.* 2002). The captive-reared individuals used in this study may not have been sufficiently removed from the wild-caught founders (individuals were three to five generations removed) for changes in external morphology to become apparent (McPhee 2004).

In the present study, the absence of changes in external morphology masked more pronounced internal morphological changes. Specifically, captive-reared individuals had lighter kidneys and spleens and shorter small intestine lengths compared to wild-caught individuals. The changes in organ size occurring in captivity could be due to the functional capacity being in excess of the actual demand, and expensive and inefficient to maintain. Subsequently, the size of organs may have altered to deal with this inefficiency (Courtney Jones *et al.*, 2012; Diamond and Hammond 1992; Piersma and Drent 2003). For example, intestine weight may have reduced due to an increased

digestive efficiency; (*see* Bailey *et al.* 1997; Champagnon *et al.* 2012), and kidney and spleen weight reduced with decreased immunological and disease exposure in the captive environment (*see* Bonnet *et al.* 1998; Swallow *et al.* 2005; Tschirren *et al.* 2009; van Oosterhout *et al.* 2007). However, identifying the specific mechanisms that cause morphological changes can be challenging. This is largely because multiple environmental factors can affect internal morphology, and the effects of these factors are likely to be interactive (Courtney Jones *et al.* 2015). Future studies would benefit from explicitly comparing the nutrient and energy content of diets, or even by manipulating these in captivity to provide robust inferences about the mechanisms for morphological change in captivity.

Some degree of phenotypic plasticity in morphological traits is likely to occur in captivity. That is, the morphological phenotype adjusts in response to the differing environmental factors experienced in captivity (Miner *et al.,* 2005). Plasticity in morphology can be demonstrated in this present study by the significant effect of the acclimation period on internal morphology. There are many examples of plastic responses in morphology to changes in environmental conditions, and these plastic responses can be fast, repeatable and reversible (Lema and Nevitt 2006; McWilliams and Karasov 2001; Piersma and Drent 2003; Piersma and Lindström 1997; Starck 1999). However, evidence of morphological plasticity during captivity is yet to be acquired (O'Regan and Kitchener 2005). Further, it is unknown whether the subsequent generations will reflect the same plasticity in internal morphology, or whether transgenerational effects in captivity will result in a shift away from the morphological phenotype adapted to captivity.

3.5.2 Transgenerational effects on morphology

Between captive generations, transgenerational effects in captivity were only apparent in internal morphological traits with captive-reared individuals showing a directional shift away from the wild-caught morphological phenotype. Specifically, captive-reared F_5 individuals had significantly lighter kidneys and spleens and shorter small intestine lengths compared to wild-caught individuals but there were no differences in external morphological traits between captive-reared F_5 and wild-caught individuals. The lack of transgenerational effects on external morphology may indicate that external morphology

may not play a significant role in individual fitness, and thus be slower to display a shift in the captive environment (McPhee 2004). Alternatively, transgenerational changes in internal morphology may occur more quickly compared with external morphology. Previous studies have also reported transgenerational effects of captivity in internal morphological traits within just one generation (Burns *et al.* 2009; Håkansson and Jensen 2005). To the best of my knowledge, this study is the first to show rapid transgenerational changes in the kidneys, spleen and small intestine. The absence of significant changes in other internal and external morphological traits suggests that at least some morphological traits can shift towards a captive morphological phenotype within one generation. With multiple generations, other morphological traits are also likely to display a shift (McPhee 2004; O'Regan and Kitchener 2005).

The transgenerational effects in captivity were only observed in internal morphology and only in females but not in males. Captive-reared F_5 female internal morphology differed significantly from wild-caught and captive-reared F_4 females, displaying a directional shift away from the wild morphological phenotype. Conversely, captive-reared F_5 male internal morphology did not differ significantly from wild-caught or captive-reared F_4 male internal morphology. The lack of evidence of transgenerational effects in male internal morphology may be due to sex-based differences in the magnitude of change in response to captivity (McPhee 2004; O'Regan and Kitchener 2005). A previous study investigating the effects of selective breeding for high activity in house mouse reported females and males having differing rates of morphological change in response to high activity; indicating that trait plasticity differed between the sexes (Swallow *et al.* 2005). Given these findings, changes in internal morphological traits may take multiple generations to manifest in males (McPhee 2004; O'Regan and Kitchener 2005).

3.5.3 Effect of captivity on sexual dimorphism in morphology

Both external and internal morphology were found to differ significantly between females and males, and these sex-based morphological differences occurred in both captive-reared and wild-caught animals. While we can expect sex-based differences in morphology as an outcome of sexual selection favouring different trait values in males and females, we might expect a loss of sexual dimorphism in captivity due to changes in

resource availability and the strengths and targets of sexual selection (Lynch and Hayden 1995; O'Regan and Kitchener 2005). The maintenance of sexual dimorphism in the present study suggests that sexual selection pressures remained unchanged in the captive environment. Alternatively, changes or loss of sexual dimorphism may take multiple generations to manifest, and may not have been observed in my study (McPhee 2004; O'Regan and Kitchener 2005). There is emerging evidence that sexual dimorphism can be maintained in captivity, however, most studies have not investigated whether relaxation or reduction in sexual selective pressures occurs in captivity (McPherson and Chenoweth 2012; O'Regan and Kitchener 2005). As such, to allow for a greater understanding of the effects of captivity on sexual dimorphism, it would be valuable to test for sex-specific differences in various morphological traits across a diversity of taxonomic groups. In recognition of this possibility, several recent studies have explored whether sexual selection theory can be used to inform management strategies (Chargé *et al.* 2014; Slade *et al.* 2014).

3.5.4 Implications for captive breeding programmes and management

Our finding that negligible changes in external morphology masked significant changes to internal morphology have implications for captive breeding programmes. Changes to internal morphology in captivity are known to impact digestive efficiency (*see* Bailey *et al.* 1997; Champagnon *et al.* 2012) and immune responses and disease resistance (*see* Bonnet *et al.* 1998; Swallow *et al.* 2005; Tschirren *et al.* 2009; van Oosterhout *et al.* 2007). Consequently, rapid changes in internal morphology could have severe and unforeseen effects on the viability of small mammals held in captivity, however, this is dependent on what morphological traits change, and whether those changes are maladaptive for natural environments. If the morphological change is shown to be maladaptive, these changes would have significant implications for captive-source populations that are used for reintroduction. While there is currently no information on the effect of internal changes on the post-release viability of small mammals, there is some evidence for these effects in birds (*see* Champagnon *et al.* 2012). Future research on small mammals would benefit from investigating the extent to which internal morphological changes occurring in captivity are maladaptive under natural conditions, and whether these impacts can be mitigated by manipulating the captive environment.

Transgenerational changes in internal morphology during captivity are also likely to have significant implications for captive-bred animals following release (*see* (O'Regan and Kitchener 2005; Slade *et al.* 2014). A recent study comparing the morphology of third-generation captive-bred house mouse, *M. musculus*, to wild conspecifics following release found significant differences in body mass between third-generation captive-bred and wild-caught individuals. Further, 83% of offspring post-release were of same-source parentage, suggesting that captive conditions cause transgenerational effects on traits (such as body size) that are important to mating preference (Slade *et al.* 2014).

While some degree of phenotypic plasticity in morphological traits can be expected, evidence of whether the morphological changes occurring in captivity are plastic responses are yet to be examined (McWilliams and Karasov 2001; O'Regan and Kitchener 2005; Piersma and Drent 2003; Piersma and Lindström 1997; Starck 1999). If morphological traits are shown to be plastic, this presents an opportunity for strategic management of morphological phenotypes. That is, the captive phenotypic traits may be altered to better suit the wild environment; but tailoring methods (such as pre-release exposure) may be required to increase likelihood of survival following release (Moseby *et al.* 2014). For example, post-release survival of pheasants (*Phasianus colchicus*) was higher in pheasants that had exposure to more natural diets prior to release. One of the mechanisms to explain this increased survivorship was the development of gut morphology (changing intestine and caecum lengths) to suit a natural diet (Whiteside *et al.* 2015).

3.5.5 Conclusions

In conclusion, this study aimed to investigate whether morphology differed between captive-reared and wild-caught individuals, to determine whether morphological changes in captive-reared individuals were subject to transgenerational effects, and whether the sexes responded differently to the captive-rearing environment. The absence of changes to external morphology masked more pronounced and potentially fatal internal morphological changes. Between captive generations, there was evidence for transgenerational effects in captivity; however, this was only observed in internal morphology, and only in females. Morphology adjusted within the acclimation period, suggesting that morphological traits may be plastic. It was found that morphology

significantly differed depending on sex, and that sex-based morphological differences were maintained in the captive rearing environment. By identifying the consequences of morphological changes in captivity, we begin to gain insights for developing and refining methodologies to minimise unfavourable phenotypic changes in captivity. In turn, this knowledge may be used to improve captive breeding and reintroduction programmes (McDougall *et al.* 2006). Overall, my findings suggest that subtle external changes may mask more pronounced internal changes, and that phenotypic plasticity may have a significant influence on morphology across captive generations and between sexes. This knowledge may prove to be important for developing methods to improve CBPs and reintroduction programmes.

3.6 Supporting Information

See Appendix B for supporting tables.

3.7 References

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

Anderson MJ and Gorley RN (2007) PERMANOVA+1 for primer: guide to statistical methods. PRIMER-E, Plymouth.

Austad SN (1997) Comparative aging and life histories in mammals. Experimental Gerontology **32:** 23 – 38

Bailey TA, Mensah-Brown EP, Samour JH, Naldo J, Lawrence P and Garner A. (1997) Comparative morphology of the alimentary tract and its glandular derivatives of captive bustards. Journal of Anatomy **191:** 387 – 398

Berzins LL, Tilman-Schindel E, and Burness G (2008) Sex-specific effects of handling time on an index of immune activity in zebra finches. Physiological and Biochemical Zoology **81:** 383 – 387

Bonnet X, Shine R, Naulleau G and Vacher-Vallas M (1998) Sexual dimorphism in snakes: different reproductive roles favour different body plans. Proceedings of the Royal Society of London. Series B: Biological Sciences **265:** 179 – 183

Burns JG, Saravanan A and Helen Rodd F (2009) Rearing environment affects the brain size of guppies: lab-reared guppies have smaller brains than wild-caught guppies. Ethology **115:** 122 – 133

Carducci JP and Jakob EM (2000) Rearing environment affects behaviour of jumping spiders. Animal Behaviour **59:** 39 – 46

Cattano C, Giomi F and Milazzo M (2016) Effects of ocean acidification on embryonic respiration and development of a temperate wrasse living along a natural CO_2 gradient. Conservation Physiology **4:** cov073.

Champagnon J, Guillemain M, Elmberg J, Massez G, Cavallo F and Gauthier-Clerc M. (2012) Low survival after release into the wild: assessing "the burden of captivity" on mallard physiology and behaviour. European Journal of Wildlife Research **58:** 255 – 267

Chargé R, Teplitsky C, Sorci G and Low M (2014) Can sexual selection theory inform genetic management of captive populations? A review. Evolutionary Applications **7:** 1120 – 1133

Clarke KR and Gorley RN (2015) PRIMER v7.0.7: User Manual/Tutorial. PRIMER-E, Plymouth.

Conde DA, Flesness N, Colchero F, Jones OR and Scheuerlein A (2011) An emerging role of zoos to conserve biodiversity. Science **331:** 1390-1391

Courtney Jones S, Cowieson A, Williamson S and Munn A (2012) No effect of short-term exposure to high-fibre diets on the gastrointestinal morphology of layer hens (*Gallus gallus domesticus*): body reserves are used to manage energy deficits in favour of phenotypic plasticity. Journal of Animal Physiology and Nutrition **97:** 868 – 877

Courtney Jones SK, Munn AJ, Penman TD and Byrne PG (2015) Long-term changes in food availability mediate the effects of temperature on growth, development and survival in striped marsh frog larvae: implications for captive breeding programmes. Conservation Physiology **3:** cov029

Cruz-Neto A and Bozinovic F (2004) The relationship between diet quality and basal metabolic rate in endotherms: insights from intraspecific analysis. Physiological and Biochemical Zoology **77:** 877 – 889

Diamond J and Hammond K (1992) The matches, achieved by natural selection, between biological capacities and their natural loads. Experientia **48:** 551 – 557

Drummond GB and Vowler SL (2012) Different tests for a difference: how do we do research? Advances in Physiology Education **36:** 3 – 5

Evans ML, Wilke NF, O'Reilly PT and Fleming IA (2014) Transgenerational effects of parental rearing environment influence the survivorship of captive-born offspring in the wild. Conservation Letters **7:** 371 – 379

Fischer J and Lindenmayer DB (2000) An assessment of the published results of animal relocations. Biological Conservation **96:** 1 – 11

Freas CA, Bingman K, Ladage LD and Pravosudov VV (2013) Untangling elevation-related differences in the hippocampus in food-caching mountain chickadees: the effect of a uniform captive environment. Brain, Behavior and Evolution **82:** 199 – 209

Geiser F and Ferguson C (2001) Intraspecific differences in behaviour and physiology: effects of captive breeding on patterns of torpor in feathertail gliders. Journal of Comparative Physiology B **171:** 569 – 576

Glucksmann A (1974) Sexual dimorphism in mammals. Biological Reviews **49:** 423 – 475

Goncalves IB, Ahnesjö I and Kvarnemo C (2015) Embryo oxygenation in pipefish brood pouches: novel insights. Journal of Experimental Biology **218:** 1639 – 1646

Håkansson J and Jensen P (2005) Behavioural and morphological variation between captive populations of red junglefowl (*Gallus gallus*) – possible implications for conservation. Biological Conservation **122:** 431 - 439

Hartstone-Rose A, Selvey H, Villari JR, Atwell M and Schmidt T (2014) The three-dimensional morphological effects of captivity. PLoS ONE **9:** e113437

Heath DD, Heath JW, Bryden CA, Johnson RM and Fox CW (2003) Rapid evolution of egg size in captive salmon. Science **299:** 1738 – 1740

Hedrick AV and Temeles EJ (1989) The evolution of sexual dimorphism in animals: hypotheses and tests. Trends in Ecology and Evolution **4:** 136 – 138

Hubrecht R and Kirkwood J (2010) The UFAW handbook on the care and management of laboratory and other research animals. John Wiley and Sons. United States.

Konarzewski M and Diamond J (1995) Evolution of basal metabolic rate and organ masses in laboratory mice. Evolution **49:** 1239 – 1248

Kristan D and Hammond K (2006) Effects of three simultaneous demands on glucose transport, resting metabolism and morphology of laboratory mice. Journal of Comparative Physiology B **176:** 139 – 151

Kristan DM (2002) Effects of intestinal nematodes during lactation: consequences for host morphology, physiology and offspring mass. Journal of Experimental Biology **205:** 3955 – 3965

Kristan DM and Hammond KA (2001) Parasite infection and caloric restriction induce physiological and morphological plasticity. American Journal of Physiology-Regulatory, Integrative and Comparative Physiology **281:** R502 – R510

Kristan DM and Hammond KA (2003) Physiological and morphological responses to simultaneous cold exposure and parasite infection by wild-derived house mice. Functional Ecology **17:** 464 – 471

Kristan DM and Hammond KA (2004) Morphological plasticity varies with duration of infection: evidence from lactating and virgin wild-derived house mice (*Mus musculus*) infected with an intestinal parasite (*Heligmosomoides polygyrus*; Nematoda). Journal of Experimental Biology **207:** 2351 – 2360

Kuhlman JR and Martin LB (2010). Captivity affects immune redistribution to skin in a wild bird. Functional Ecology **24:** 830 – 837

Latham N and Mason G (2004) From house mouse to mouse house: the behavioural biology of free-living *Mus musculus* and its implications in the laboratory. Applied Animal Behaviour Science **86:** 261 – 289

Lema SC and Nevitt GA (2006) Testing an ecophysiological mechanism of morphological plasticity in pupfish and its relevance to conservation efforts for endangered Devils Hole pupfish. Journal of Experimental Biology **209:** 3499 – 3509

Little AG and Seebacher F (2014) Thyroid hormone regulates cardiac performance during cold acclimation in zebrafish (*Danio rerio*). Journal of Experimental Biology 217**:** 718 – 725

Lynch JM and Hayden TJ (1995) Genetic influences on cranial form: variation among ranch and feral American mink *Mustela vison* (Mammalia: Mustelidae). Biological Journal of the Linnean Society **55:** 293 - 307

Martin LB (2009) Stress and immunity in wild vertebrates: timing is everything. General and comparative endocrinology **163:** 70 – 76

Mathews F, Orros M, McLaren G, Gelling M and Foster R (2005) Keeping fit on the ark: assessing the suitability of captive-bred animals for release. Biological Conservation **121:** 569 – 577

McDougall P, Réale D, Sol D and Reader S (2006) Wildlife conservation and animal temperament: causes and consequences of evolutionary change for captive, reintroduced, and wild populations. Animal Conservation **9:** 39 – 48

McPhee ME (2004) Morphological change in wild and captive oldfield mice *Peromyscus polionotus subgriseus*. Journal of Mammalogy **85:**1130 – 1137

McPhee ME and Carlstead K (2010) The importance of maintaining natural behaviors in captive mammals. In: Kleiman DG, Thompson KV, and Baer CK, editors. Wild mammals in captivity: principles and techniques for zoo management. (pp. 303-312) University of Chicago Press, Chicago

McPherson FJ and Chenoweth PJ (2012) Mammalian sexual dimorphism. Animal Reproduction Science **131:** 109 – 122

McWilliams SR and Karasov WH (2001) Phenotypic flexibility in digestive system structure and function in migratory birds and its ecological significance. Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology **128:** 577 – 591

Millar JS and Zammuto RM (1983) Life histories of mammals: an analysis of life tables. Ecology **64:** 631 – 635

Miner BG, Sultan SE, Morgan SG, Padilla DK and Relyea RA (2005) Ecological consequences of phenotypic plasticity. Trends in Ecology and Evolution **20:** 685 – 692

Monaghan P (2008) Early growth conditions, phenotypic development and environmental change. Philosophical Transactions of the Royal Society of London B: Biological Sciences **363:** 1635 – 1645

Moseby KE, Hill BM and Lavery TH (2014) Tailoring release protocols to individual species and sites: one size does not fit all. PLoS ONE **9:** e99753

O'Regan HJ and Kitchener AC (2005) The effects of captivity on the morphology of captive, domesticated and feral mammals. Mammal Review **35:** 215 – 230

Paproth A (2011) Measuring fitness loss in captive breeding programs: implications for threatened species recovery. Masters Thesis. University of Melbourne, Melbourne, Australia.

Philippart JC (1995) Is captive breeding an effective solution for the preservation of endemic species? Biological Conservation **72:** 281 – 295.

Piersma T and Drent J (2003) Phenotypic flexibility and the evolution of organismal design. Trends in Ecology and Evolution **18:** 228 – 233

Promislow DEL and Harvey PH (1990) Living fast and dying young: a comparative analysis of life-history variation among mammals. Journal of Zoology **220:** 417 – 437

Schulte-Hostedde AI and Mastromonaco GF (2015) Integrating evolution in the management of captive zoo populations. Evolutionary Applications **8:** 413 – 422

Seddon PJ, Armstrong DP and Maloney RF (2007) Developing the Science of Reintroduction Biology. Conservation Biology **21:** 303 – 312

Slade B, Parrott ML, Paproth A, Magrath MJL, Gillespie GR and Jessop TS (2014) Assortative mating among animals of captive and wild origin following experimental conservation releases. Biology Letters **10:** 20140656

Snyder NFR, Derrickson SR, Beissinger SR, Wiley JW, Smith TB, Toone WD and Miller B (1996) Limitations of captive breeding in endangered species recovery. Conservation Biology **10:** 338 – 348

Starck JM (1999) Phenotypic flexibility of the avian gizzard: rapid, reversible and repeated changes of organ size in response to changes in dietary fibre content. Journal of Experimental Biology **202:** 3171 – 3179

Stearns SC (1983) The influence of size and phylogeny on patterns of covariation among life-history traits in the mammals. Oikos **41:** 173 - 187

Swallow JG, Rhodes JS and Garland T (2005) Phenotypic and evolutionary plasticity of organ masses in response to voluntary exercise in house mice. Integrative and Comparative Biology **45:** 426-437.

Tarszisz E, Dickman CR and Munn AJ (2014) Physiology in conservation translocations. Conservation Physiology **2:** cou054.

Tschirren B, Rutstein AN, Postma E, Mariette M and Griffith SC (2009) Short- and long-term consequences of early developmental conditions: a case study on wild and domesticated zebra finches. Journal of Evolutionary Biology **22:** 387 – 395

van Oosterhout C, Smith AM, Hänfling B, Ramnarine IW, Mohammed RS and Cable J (2007) The guppy as a conservation model: implications of parasitism and inbreeding for reintroduction success. Conservation Biology **21:** 1573 – 1583

Whiteside MA, Sage R and Madden JR (2015) Diet complexity in early life affects survival in released pheasants by altering foraging efficiency, food choice, handling skills and gut morphology. Journal of Animal Ecology **84:** 1480 – 1489

Williamson SA, Courtney Jones SK and Munn AJ (2014) Is gastrointestinal plasticity in king quail (*Coturnix chinensis*) elicited by diet-fibre or diet-energy dilution? Journal of Experimental Biology **217:** 1839 – 1842

Wisely SM, Ososky JJ and Buskirk SW (2002) Morphological changes to black-footed ferrets (*Mustela nigripes*) resulting from captivity. Canadian Journal of Zoology **80:** 1562 – 1568

4 WHAT ROLE DOES HERITABILITY PLAY IN TRANSGENERATIONAL EFFECTS IN CAPTIVITY? IMPLICATIONS FOR MANAGING CAPTIVE POPULATIONS.

4.1 Abstract

Animals maintained in captivity exhibit rapid changes in phenotypic traits, which may be maladaptive for natural environments. Further, the phenotype can shift away from the wild phenotype via transgenerational effects, with the environment experienced by parents influencing the phenotype and fitness of offspring. There is emerging evidence that controlling transgenerational effects could help mitigate the effects of captivity, improving the success of captively bred animals post release. However, controlling transgenerational effects requires a better understanding of the mechanisms driving transgenerational changes. To better understand the genetic mechanisms underpinning transgenerational effects in captivity I investigated the heritability of behavioural and morphological phenotypes using mid parent- and single parent-offspring regressions in a population of captive-reared house mouse (*Mus musculus*) known to exhibit transgenerational effects. Specifically, I measured the heritability of boldness and activity behavioural types as well as internal morphology. Slopes for boldness and activity were all positive, indicating a low to moderate degree of heritability. The slopes for internal morphology were undetectable. Importantly, none of the heritability estimates were statistically significant due to the large surrounding errors. However, the large error surrounding the heritability estimates may also suggest there is variability in phenotypic traits between litters and individuals. This might suggest that the potential for genetic change in captivity varies considerably between traits. Continued investigation of the potential for traits to evolve in captivity is needed to better inform captive breeding and reintroduction programmes.

Key words: Captivity, heritability, transgenerational effects, phenotype, natural selection, transgenerational plasticity

4.2 Introduction

Captive breeding programmes breed and raise threatened species in the captive environment with the goal of reintroducing animals back into the natural environment (Conde *et al.* 2011). However, there is increasing evidence that post-release captively bred and reared animals have significantly lower fitness than wild animals (Araki *et al.* 2007; Christie *et al.* 2012; Williams and Hoffman 2009). This may be due to differences in selective pressures experienced in captivity, resulting in selection for phenotypes that maximise individual fitness in the captive environment, but not necessarily in the wild (McDougall *et al.* 2006; Schulte-Hostedde and Mastromonaco 2015; Snyder *et al.* 1996).

Animals maintained in captivity may exhibit rapid changes in phenotypic traits, such as behaviour and morphology, that may be maladaptive for natural environments (see DeWitt *et al.* 1998; Johnsson *et al.* 2014; Mathews *et al.* 2005; McPhee 2004b; Philippart 1995). Further, the phenotype can shift away from the wild phenotype with each subsequent generation in captivity, impacting the performance of captive individuals as well as the post release fitness (Araki *et al.* 2009; McPhee 2004b; McPhee and McPhee 2012). This occurs via transgenerational effects, with the environment experienced by the parent influencing the phenotype and fitness of offspring (Salinas *et al.* 2013). The transgenerational effects on phenotypes in captivity may result from transgenerational plasticity or genetic changes such as heritable genetic mutations (Chakravarti *et al.* 2016; Evans *et al.* 2014; Richards *et al.* 2010; Martos *et al.* 2015). If transgenerational effects result from transgenerational plasticity, environmental factors that the parental generation experiences will trigger particular trait expressions in offspring. Thus, the parental-environment could be manipulated to regulate fitness-determining traits in offspring (Shama *et al.* 2014; Evans *et al.* 2014). If genetic change results in transgenerational effects within captivity, environmental factors that change the strength or direction of selection pressures could be manipulated to drive artificial selection for favourable phenotypic changes. There is emerging evidence that understanding and controlling transgenerational effects may be able to mitigate the effects of captivity that influence the success of offspring in the wild (Clarke *et al.* 2016; Evans *et al.* 2014). For example, exposing captive-reared Atlantic

salmon (*Salmo salar*) to natural river environments resulted in a two-fold increase in survivorship compared to offspring of captive parents (Evans *et al.* 2014).

To begin to understand the mechanisms underpinning transgenerational effects in captivity, an important first step is to estimate the amount of variation in a phenotypic trait that is explained by genetic variation (i.e. heritability; Falconer *et al.* 1996). Heritability estimates are one method to indicate the genetic variation, and the heritability of traits, in turn, can illuminate the evolutionary potential for a phenotypic trait (such as a behavioural or morphological trait) to respond to selection pressure imposed by the captive environment (Falconer *et al.* 1996; Réale and Festa-Bianchet 2000; Rodriguez-Clark 2004; Richards *et al.* 2010). For example, a heritable trait may be explained by additive genetic variance, indicating an evolutionary potential for a phenotypic trait, suggesting transgenerational effects may be caused by genetic change (Houle 1992; Rodriguez-Clark 2004). If animals maintained in captivity for multiple generations display high heritability, we expect to see changes in the genetic variation of phenotypic traits (e.g. morphology and behaviour) due to changes in the strength and direction of selective pressures (McPhee 2004b; McPhee and McPhee 2012). However, previous studies investigating transgenerational effects have not examined the heritability of phenotypic traits (Clarke *et al.* 2016; Evans *et al.* 2014).

Genetic determination of behaviour has been used to explain the existence and maintenance of consistent individual differences in behaviour (Edenbrow and Croft 2013). Recent studies have demonstrated that behaviour has a genetic basis, with animal personality defined as individual behavioural differences consistent across time and context (Dingemanse *et al.* 2002; Drent *et al.* 2003; Sih *et al.* 2004; Van Oers *et al.* 2005). However, behaviour is also known to display high levels of plasticity in response to environmental change (Wong and Candolin 2015). Thus, the proximate mechanisms driving behavioural differences between captive and wild animals may be due to a combination of genetic change and transgenerational plasticity occurring in captivity. To know whether the environment should be manipulated to trigger transgenerational plasticity or create selective pressures for genetic change, it is valuable to have an understanding of trait heritability.

Behavioural differences between captive and wild individuals are well documented, with consistent differences reported in particular configurations of behavioural traits (hereafter behavioural type; Bell 2007) such as boldness and activity (Herborn *et al.* 2010; Mathews *et al.* 2005). Boldness and activity behaviours relate to risk-taking, exploring novel environments and may affect performance and fitness (Coleman and Wilson 1998). Further, these behaviours can be used to predict the probability of an individual surviving and reproducing following reintroduction (Coleman and Wilson 1998; Herborn *et al.* 2010; Wilson and Godin 2009). Previous work has shown boldness and activity to be highly heritable in captivity; however, single trait heritability approaches have been used to measure heritability, rather than a multitude of behavioural traits (behavioural type). As a result, heritability estimates for single behavioural traits may not be ecologically relevant (Blows and Hoffmann 2005; see Ariyomo *et al.* 2013; Dingemanse *et al.* 2002; Drent *et al.* 2003).

Behaviour and morphology can be interlinked with morphological traits having a direct influence upon behavioural traits (Price and Schluter 1991; Sih *et al.* 2015). Furthermore, behavioural type can be state dependent, with morphological traits such as body size influencing individual differences in behaviour (Sih *et al.* 2015; Stamps 2007). If specific behaviours are shown to strongly interlink with morphological traits that show rapid adaptation in captivity, this may be a key factor causing the reduced fitness of individuals post release. As such, it is also important to determine the heritability of morphological traits of captive-reared animals. There is emerging evidence that changes to morphology can occur quickly in captivity (McPhee 2004b; O'Regan and Kitchener 2005), and morphological traits are likely to be heritable. Previous work has shown morphological traits, such as body mass, and wing and tarsus length in birds, to be highly heritable (Keller *et al.* 2001; Réale *et al.* 1999). However, studies examining the heritability of internal morphological traits such as gastrointestinal tract length, brain size and size and shape of reproductive organs, are lacking, and these traits may be critical state variables driving behavioural variation (Dall *et al.* 2004; Sih *et al.* 2015).

The overall aim of this study was to investigate the heritability of multiple behavioural and morphological traits in a population of the house mouse (*Mus musculus*) in which I have previously shown transgenerational changes in both behavioural morphological

traits in captivity (*see* Chapters 2 and 3; Courtney Jones *et al.* 2017). To address this aim, I measured the broad sense heritability of boldness and activity behavioural types displayed in a novel environment along with internal morphological traits in the same population of captive-reared individuals using parent-offspring regressions.

4.3 Methods

4.3.1 Ethics permit

This study was conducted under University of Wollongong Animal Ethics Approval AE13/17.

4.3.2 Study species

The wild-derived strain of the house mouse (*Mus musculus*), a small rodent species, was used in this study. Mice are an ideal model organism for studies of transgenerational effects on phenotypes in captivity (Dew-Budd *et al.* 2016). Because house mouse are easily maintained in captivity, studies can occur over short time periods (O'Regan and Kitchener 2005). *Mus musculus* also share a number of life-history traits in common with other small mammals. These traits include short generation time, large litter sizes, iteroparity, polygamous mating strategies, sexual dimorphism and early age at maturity (Austad 1997; Glucksmann 1974; Latham and Mason 2004; Millar and Zammuto 1983; Promislow and Harvey 1990; Stearns 1983). For these reasons, *M. musculus* is becoming a model species to address questions related to small mammal captive breeding and reintroduction (O'Regan and Kitchener 2005; Paproth 2011; Slade *et al.* 2014).

4.3.3 Housing

All individuals (captive-reared F_4 and F_5) were maintained separately in opaque plastic cages (32 x 18 x 12 cm; MB1 Mouse Box, Wiretainers Pty Ltd., Melbourne, Australia) with a metal top. Wood shavings were used as cage substrate and all cages were provided with bedding material (shredded paper) and a 6 x 4 cm cardboard tube (toilet paper roll) for shelter. Water and food (Vella Stock Feeds Brand Rat and Mouse Nut; The Vella Group, Glendenning, New South Wales, Australia) were available *ad libitum*, determined as 20 grams of food per 100g of body mass was supplied daily (Hubrecht and Kirkwood 2010). Room temperature was maintained at 22 ± 2°C on a 12: 12 hr

reverse light: dark cycle, with full spectrum UV light provided. Housing conditions were based on conditions supplied to the original wild-caught founder generation and average temperatures in the field during the study period. Humidity was not controlled, but was monitored daily and recorded as 75 ± 10% (mean ± SD). Animals were monitored daily, with cages cleaned once a week by removing the occupant and placing them in a round escape-proof container (54 x 52 cm; Spacepac Industries Pty. Ltd., Wollongong, NSW, Australia) then placing them in a new cage.

4.3.4 Captive-reared parent generation (hereafter captive-reared parents)

Eleven sexually mature virgin male and fifteen sexually mature virgin female *M. musculus* were sourced from a captive population maintained at the University of New South Wales (UNSW), Sydney under Ethics Permit UNSW Reg. No. 12/88A. All individuals were third or fourth generation captive-maintained mice born between late-2012 and mid-2013. All captive-reared parent females and males had unrelated parents and grandparents from multiple litters. The mice were descendants of an original wild-caught founder generation, consisting of 42 females and 45 males captured between March and May 2011 at an agricultural site in the western Sydney area (34°4′36.48″S, 150°34′15.6″E).

Prior to this study, the captive-reared parent mice were housed in a temperature (19 – 25°C) and light controlled room (12: 12 hr reverse light cycle, lights on at 9:00 AM AEST). Humidity was not controlled but was ~70% (A. Gibson, personal communication, 17 January 2014). Males were housed separately at weaning to avoid aggression and physical injury but female siblings were housed together in groups of up to three individuals. All animals were provided with food and water *ad libitum*.

For this study, captive-reared parents were collected from UNSW (17 January 2014) and transported to the Ecological Research Centre at the University of Wollongong, Wollongong (34°24′24″S 150°52′46″E). Mice were weighed (g) on digital scales (Mettler-Toledo PJ3600, Mettler-Toledo Ltd., Port Melbourne, Australia) and then housed individually (*see* 4.3.3 Housing). Captive-reared parents entered the behavioural characterisation assay (*see* 4.3.6 Behavioural Characterisation) before breeding for the captive-reared offspring generation. Due to the restrictions in processing mice through the behavioural characterisation, mice were housed in

individual housing for a maximum of 21 days (male: average 11 ± 2 days; female: average 16 ± 5 days) prior to behavioural characterisation. At the conclusion of the breeding period, captive-reared parents were then re-acclimated to the individual housing for a minimum period of twelve days before quantifying internal morphological traits (*see* 4.3.8 Internal morphological traits).

4.3.5 Captive-reared offspring generation (hereafter offspring)

Pedigree mapping was used to ensure unrelated individuals from the captive-reared parents were paired so that captive-reared offspring had unrelated parents and grandparents. I paired a total of 48 breeding pairs between February and April 2014, with 6 breeding pairs resulting in litters. Sample sizes were comparable with other heritability studies (Dingemanse *et al.* 2002; Dor and Lotem 2009; Van Oers *et al.* 2005). Each monogamous breeding pair was held together for one week in standard caging (*see* 4.3.3 Housing). Water and food were available *ad libitum*, and temperature and light: dark cycles were uniform to those provided for the F_4 acclimation period.

Once mated, the captive-reared parent females were monitored to check for young. Mice were checked once a day, commencing ten days following the male being removed, with the monitoring period lasting an average of 10 ± 2 days. Offspring were housed with their mother until they were weaned at 25 days of age; weaning age was kept uniform across all litters to reduce differences in maternal investment post-pregnancy. At 25 days of age, the captive-reared parent mother was removed from the breeding cage, and the litter was then housed for two days under *ad libitum* conditions to reduce post-weaning stress on the litter following removal of the mother. Offspring were then housed individually in standard caging (*see* 4.3.3 Housing). Captive-reared offspring (13 males and 14 females) were individually housed until they reached sexual maturity before behavioural characterisation and quantifying internal morphological traits (*see* 4.3.8 Internal morphological traits).

4.3.6 Behavioural characterisation

Behavioural characterisation occurred at sexual maturity for all captive-reared parents and offspring. To ensure no effects of mating on behavioural characterisation, both

captive-reared parent and offspring behavioural characterisations were conducted when individuals were virgins.

Behavioural characterisations for captive-reared parents and offspring (successful breeding pairs = 6; captive-reared parents = 12; captive-reared offspring = 27) were conducted in late Australian Spring/early Summer and in late Autumn/early Winter 2014. As behavioural analyses were unable to be run simultaneously for all individuals, I assumed acclimation period would account for any confounding effects induced by season. To determine how individuals displayed behavioural traits along the bold/shy and active/inactive axes of variation, 14 behavioural traits were used. These 14 behavioural traits have previously been measured as boldness or activity based on methodologies used in the following empirical studies: Augustsson *et al.* 2005; Augustsson and Meyerson 2004; and McPhee 2004a. For the full ethogram *see* Table 4.1.

4.3.7 OFT procedure and apparatus

I used an Open Field Test (OFT) to determine the boldness and activity behavioural types individuals would display in a novel environment which can be used to predict the probability that individuals survive and reproduce following reintroduction (Herborn *et al.* 2010; Wilson and Godin 2009). The OFT arena was constructed from an opaque plastic tank with an arena size of 90 x 60 cm with 60 cm high walls (Spacepac Industries Pty. Ltd., Wollongong, NSW, Australia). Two PVC tunnels (6 x 4 cm) were placed in the central part of the arena at opposite ends (located 10 cm from the arena walls) to simulate shelter. Above each arena (n = 4), a video camera (PRO-735 Camera, Swann Systems, Melbourne, Australia) was placed to record the entire OFT trial. Recorded videos were stored on a Digital Video Recorder (DVR8-4100, Swann Systems, Melbourne, Australia) and behaviour was analysed using ANY-maze® software (Stoelting Co., U.S.A). The location and behaviours (duration) of the mice for the entire duration of the OFT were recorded. Trials were conducted at the same time of day and were conducted in the dark half of the light cycle. At the conclusion of the OFT observation period, the mouse was removed from the OFT arena and the OFT arena and shelters were thoroughly cleaned using 70% ethanol to remove any traces of animal scent. Individual mice were transferred to the OFT arena and were placed in the

estimated central point of the OFT arena. Following an acclimation period (2 min), behaviour was recorded for 20 min (1200 s). Fourteen behavioural traits were measured (Table 4.1).

Table 4.1 Ethogram of behaviours measured in open field test in captive-reared house mouse.

Behavioural trait	Behavioural measure description	Functional category/ Base component for behavioural type
Distance (m)	Total distance covered in OFT	Activity
Meandering (°/m)	Absolute turn angle/Total distance travelled	Boldness
Mean speed (m/s)	Average speed during OFT	Activity
Maximum speed (m/s)	Maximum speed reached during OFT	Activity/Boldness
% Time mobile	% Total time spent mobile (Animal is in motion)	Activity
% Time active	% Total time spent active (Animal is mobile or performing some other behaviour)	Activity
% Time freezing	% Total time spent freezing (Animal is not moving, may be performing some other behaviour)	Boldness
Jumping: total number	Total count of jumps in OFT	Boldness
In tunnel: total time (s)	Total time spent in the tunnels (May include or exclude tail)	Boldness
% Centre: total time spent	% Time spent in the centre of the arena	Boldness
Centre: mean speed (m/s)	Average speed in centre zone of OFT	Activity
Centre: maximum speed (m/s)	Maximum speed in centre zone of OFT	Activity/Boldness
Perimeter: mean speed (m/s)	Average speed in perimeter zone of OFT	Activity
Perimeter: maximum speed (m/s)	Maximum speed in perimeter zone of OFT	Activity/Boldness

4.3.8 Internal morphological traits

Animals were euthanased using CO_2 asphyxiation. Immediately following euthanasia, macroscopic dissections of organs were conducted to determine heritability of morphological traits between captive-reared parents and offspring. Body morphological traits included body mass (g), snout to vent length (mm) and internal organ morphological traits: brain, liver, kidney, heart, lungs, testes/ovaries, spleen, stomach, caecum, small- and large-intestine (mass and length). Organs were weighed using scales with ± 0.01 g precision (Mettler-Toledo PJ3600, Mettler-Toledo International Inc., U.S.A). Where applicable, digestive organs were emptied of contents and rinsed with a 0.9% saline solution and weighed. The lengths of the small- and large-intestine were measured using slide callipers with ± 0.05 mm precision.

4.3.9 Statistical analysis

I measured the broad sense heritability (h^2) of behaviour and morphology of captive-reared individuals using parent-offspring regressions (Falconer *et al.* 1996; Lynch and Walsh 1998). To control for the effects of body size on behavioural and morphological traits, I calculated the residuals of a least squares regression of each trait on body size using body mass or snout to vent length where length was measured. To reduce the number of analyses performed, the internal morphology and the measure of activity and boldness for behavioural type were determined using Principal Components Analysis (PCA), with the 14 behavioural traits measured assigned as base components of either the active or bold behavioural type and 18 morphological traits measured were assigned as base components of internal morphology (see Table 4.1, 4.2 and 4.3 for assigning of behavioural and morphological traits). This generated one main principal component for each behavioural type, which were used in all subsequent analyses and hereafter referred to as ‘activity’ or ‘boldness’ behavioural types and ‘internal morphology’ (see Table 4.2 and 4.3 for PC1 loadings). Where individuals were unable to be sampled for analysis of morphological traits, the degrees of freedom for these respective analyses were adjusted to account for these exclusions.

The resemblance of offspring to their captive-reared parents was calculated from mid parent-offspring, single parent-offspring regressions of mean values of boldness,

activity and internal morphology. For offspring data regressed against the mother or father separately, the data estimated a slope equal to half of the heritability estimate. Thus, slopes and associated standard errors for single parent-offspring and single parent-single sex offspring were multiplied by two to give h^2 estimates (Falconer *et al.* 1996; Réale *et al.* 1999).

Because behavioural types and internal morphologies were not correlated between captive-reared parents (Boldness: $r^2_5 = 0.29$, $F = 0.6396$, $p = 0.4687$; Activity: $r^2_5 = 0.55$, $F = 0.4869$, $p = 0.5237$; Internal: $r^2_5 = 0.41$, $F = 2.0575$, $p = 0.2469$), I did not need to correct estimates for assortative mating. For this study, I ended up with a total sample size of 6 pairings, 6 litters and 27 offspring tested for broad sense heritability of behaviour and morphology (from 6 individual mothers and 6 individual fathers). The number of offspring per litter (litter size) varied between 3 and 6 (mean = 4.5). To minimise sampling error of the heritability estimates, weighted least-square regressions were used (Lynch and Walsh 1998). Weighting factored in unequal sample sizes in the number of offspring per litter by the square root of the number of offspring per litter for each litter (Sokal and Rohlf 1995). Sample sizes used in this present study were comparable with other studies of this nature (Dingemanse *et al.* 2002; Dor and Lotem 2009; Van Oers *et al.* 2005). As negative heritability estimates were possible with the experimental design employed, I considered negative estimates equal to zero (Robinson *et al.* 1955). Data were analysed using JMP 11.2.0 statistical package.

4.4 Results

There were no significant differences in boldness and activity behavioural types between parental males and females (Boldness: $t = 0.883$, $p = 0.399$, d.f. = 9.54; Activity: $t = 1.412$, $p = 0.195$, d.f. = 8.14). Further, there were significant differences in internal morphology between parental males and females ($t = 4.199$, $p = 0.003$, d.f. = 7.63).

The slopes of boldness behavioural type derived from parent-offspring regressions were all positive, and heritability estimates were 0.46 ± 0.20, 0.54 ± 0.50 and 0.74 ± 0.30 for mid parent-offspring, and father-offspring and mother-offspring regressions, respectively (Table 4.4). Slopes of activity behavioural type derived from parent-offspring resemblances were all positive, and heritability estimates were 0.19 ± 0.16,

0.32 ± 0.38 and 0.24 ± 0.24 for mid parent-offspring, and father-offspring and mother-offspring regressions, respectively (Table 4.4).

The slopes of internal morphology derived from parent-offspring regressions were all negative, heritability estimates were -0.07 ± 0.23, -0.24 ± 0.34 and -0.82 ± 0.74 for mid parent-offspring, and father-offspring and mother-offspring regressions, respectively (Table 4.4). I considered negative heritability estimates of internal morphology to be equal to zero, and therefore undetectable.

All parent-offspring regressions for behavioural types and internal morphology were not statistically significant.

Table 4.2 Principal components analysis, eigenvalues, % variance and loading values for bold and active behavioural types in captive-reared house mouse

Behavioural type	Eigenvalue	% Variance	Loadings of variables
Boldness	3.2772	40.965	Meandering: 0.90600 % Time freezing: 0.77554 Jump: number of presses: 0.25763 In tunnel: time pressed: 0.46355 % Centre: Total time spent: 0.37159 Maximum speed: -0.87551 Centre: maximum speed: -0.38566 Perimeter: maximum speed: -0.72134
Activity	5.6664	62.960	Distance: 0.97350 Mean speed: 0.97341 Maximum speed: 0.78829 % Time active: 0.58182 % Time mobile: 0.58314 Centre: mean speed: 0.96063 Centre: maximum speed: 0.47198 Perimeter: mean speed: 0.95830 Perimeter: maximum speed: 0.63819

Table 4.3 Principal components analysis, eigenvalues, % variance and loading values for internal morphology in captive-reared house mouse, *Mus musculus*.

Morphology	Eigenvalue	% Variance	Loadings of variables
Internal morphology	3.2219	24.784	Liver: 0.82407 Kidneys: -0.53162 Heart: 0.13755 Lungs: 0.35028 Spleen: 0.3731 Brain: 0.21204 Stomach: 0.67308 Small intestine: 0.44215 Large intestine: 0.7335 Caecum: 0.51557 Ovaries/Testes: -0.22482 Small Intestine length: 0.50797 Large Intestine length: 0.41652

Table 4.4 Heritability estimates of bold and active behavioural types and internal morphology of captive-reared house mouse. (Abbreviations: N_f, total number of families tested; N_{off}, total number of offspring tested; h^2± SE, heritability score ± standard error; d.f., degrees of freedom; F, F ratio)

Method	N_f	N_{off}	h^2± SE	*d.f.*	*F*	*p-value*
Boldness						
Mid parent-offspring	6	27	0.46± 0.20	1, 5	5.0785	0.0873
Father-offspring	6	27	0.54± 0.50	1, 5	1.1517	0.3436
Mother-offspring	6	27	0.74± 0.30	1, 5	5.8749	0.0725
Activity						
Mid parent-offspring	6	27	0.19± 0.16	1, 5	1.3730	0.3063
Father-offspring	6	27	0.32± 0.38	1, 5	0.7225	0.4432
Mother-offspring	6	27	0.24± 0.24	1, 5	1.0283	0.3679
Internal morphology						
Mid parent-offspring	5	27	-0.07± 0.23*	1, 4	0.1000	0.7725
Father-offspring	5	27	-0.34± 0.17*	1, 4	0.5066	0.5280
Mother-offspring	6	27	-0.82± 0.37*	1, 5	1.2168	0.3319

* I considered negative heritability estimates of internal morphology equal to zero. See results for true output

4.5 Discussion

To understand and potentially harness transgenerational effects that can influence the outcomes of captive breeding programmes, it is first necessary to elucidate the heritability of phenotypic traits displaying transgenerational changes in captivity. This study investigated heritability of behaviour and morphology in a population of captive-reared house mouse (*Mus musculus*) using mid parent- and single parent-offspring regressions. It was found that slopes for boldness and activity behavioural types derived from parent-offspring regressions were all positive. For boldness behavioural type, heritability estimates ranged from 0.46 to 0.74, and for activity behavioural type heritability estimates ranged from 0.19 to 0.32, suggesting a low to moderate degree of heritability. Slopes for internal morphology were found to be undetectable. These findings suggest that the transgenerational effects previously demonstrated in my study population could have resulted from genetic changes (i.e. animals adapting to captivity), but may also likely have resulted from transgenerational plasticity (*see* Chapter 2; Courtney Jones *et al.* 2017).

The heritability estimates for boldness and activity behavioural types ranged from low to moderate, consistent with other studies, suggesting that behaviour and morphology may be heritable (Ariyomo *et al.* 2013; Dingemanse *et al.* 2002; Dor and Lotem 2009; Drent *et al.* 2003). Furthermore, my heritability estimates imply that transgenerational effects previously demonstrated may have had a genetic basis (*see* Chapter 2; Courtney Jones *et al.* 2017), suggesting that there may be a small level of genetic change occurring in captivity (Houle 1992; Rodriguez-Clark 2004). The captive-reared parents and offspring used in this study were derived from 3 – 4 previous captive-reared generations. Consequently, transgenerational effects, and associated genetic change, may have occurred relatively quickly in previous generations, indicating that, following the introduction of animals into captivity, transgenerational effects occur quickly. Indeed, genetic adaptations can occur within one generation as a result of selection pressures changing in captivity (Christie *et al.* 2012). Additionally, there can be a rapid change in genetic variation, with animals being brought into captivity likely to experience genetic bottlenecks (Briscoe *et al.* 1992). Given these possibilities, future studies investigating the mechanisms of transgenerational effects would benefit from quantifying trait change across multiple generations.

Heritability estimates were unable to be detected for internal morphological traits. While heritability estimates are sensitive to environmental variation (Van Oers *et al.* 2005; Weigensberg and Roff 1996), phenotypic traits may also be strongly influenced by, and exhibit, plasticity in response to environmental change (Monaghan 2008; Wong and Candolin 2015). In the study population, transgenerational effects in captivity were apparent in internal morphological traits (*see* Chapter 3). This may indicate that the transgenerational effects detected may have resulted from transgenerational plasticity rather than genetic change. For example, internal morphology may be able to rapidly adjust via phenotypic plasticity to improve organismal functioning and viability in captivity, and these plastic changes are then transferred onto offspring via transgenerational plasticity (McPhee 2004b; Miner *et al.* 2005). Thus, I suspect that some internal morphological traits, such as the gastrointestinal tract, may exhibit lower or undetectable heritability estimates which enable traits to be more plastic in response to changes in environmental conditions. However, the relative importance of transgenerational plasticity in allowing phenotypic traits, particularly internal morphology, to rapidly adjust to environmental changes are largely unknown, and this is an area that requires research attention (Chevin *et al.* 2010).

It is important to note that the experimental approach used in this study lacked the power to detect differences, and that none of the heritability estimates were statistically significant. Such a finding is not uncommon in studies testing for trait heritability (Dor and Lotem 2009; Réale *et al.* 1999; Rodriguez-Clark 2004; Van Oers *et al.* 2005). High error in heritability estimates may be caused by multiple phenotypic traits rather than a single trait being examined, causing the pattern of heritability to become less clear with additional traits (Blows and Hoffmann 2005; Rodriguez-Clark 2004; Weigensberg and Roff 1996). Further, the large errors surrounding heritability estimates may also suggest there is variability in phenotypic traits between litters and individuals, even when maintained in the same captive environment (Rodriguez-Clark 2004). This has been observed previously, with within-individual variation in exploratory behaviour in the great tit (*Parus major*) potentially attributed to undetected differences in rearing conditions, resulting in changes in fledging weight (Dingemanse *et al.* 2002). It is important to recognise, however, that the lack of significance may also be the outcome of the small sample sizes used, resulting in high standard errors for the heritability

estimates (Réale *et al.* 1999). Indeed, other studies with significant heritability estimates had proportionally larger sample populations (e.g. Dingemanse *et al.* 2002; Drent *et al.* 2003; Rodriguez-Clark 2004). While my study would have benefited from a larger population size, the heritability estimates were consistent with other studies (Lewis and Thomas 2001; O'Regan and Kitchener 2005). My findings indicate that future studies investigating the mechanisms of transgenerational effects in captivity will require substantially larger numbers of breeding pairs and offspring (e.g. breeding pairs: n > 20; offspring n > 40; e.g. Ariyomo *et al.* 2013; Drent *et al.* 2003; van Oers *et al.* 2004).

I suggest that the transgenerational effects that occurred in this population of mice may have resulted from the individual and combinatory effects of genetic change and transgenerational plasticity. However, it is challenging to determine the mechanistic basis of these transgenerational effects, and these may differ depending on what phenotypic trait is examined (Nadeau 2009; Nelson and Nadeau 2010). Laboratory experiments or captive breeding experiments can be used to control environmental conditions to allow identification of the mechanisms driving transgenerational effects (Chakravarti *et al.* 2016). Ultimately, however, qualitative genetic and epigenetic techniques, combined with common garden experiments or cross-fostering experiments, may be required to elucidate the source of transgenerational effects in captivity (Dor and Lotem 2009). Previous studies have attributed transgenerational effects to environmental changes and transgenerational plasticity to the rapid changes that occurred (Chakravarti *et al.* 2016; Evans *et al.* 2014). Indeed, transgenerational plasticity may play a key role in facilitating rapid adaptation to a captive environment (Bonduriansky *et al.* 2012). However, rapid selection under captive conditions can also occur within one generation, and may influence offspring performance in captivity (Christie *et al.* 2012). Further, previous studies have reported a decrease in genetic variation and changes to heritability for populations of invertebrates brought in from the wild and maintained in captivity for multiple generations, and this may be attributed to a reduction in environmental variability in captive conditions (Briscoe *et al.* 1992; Rodriguez-Clark 2004).

4.5.1 Implications for captive breeding management

Evidence suggests that with each subsequent generation in captivity, behavioural and morphological traits shift away from the wild phenotype, towards an optimal mean trait value for captive conditions (Evans *et al.* 2014; McPhee 2004a, b; McPhee and Carlstead 2010; McPhee and McPhee 2012). The captive environment presents an ideal opportunity to control environmental conditions and identify whether transgenerational effects occur, which phenotypic traits are susceptible to change, and what mechanisms are driving transgenerational effects. Furthermore, model species such as *M. musculus* provide a suitable model system for examining the phenotypic changes in captivity and provide valuable information for applying to endangered species captive breeding (Fischer and Lindenmayer 2000). In turn, this knowledge can be potentially harnessed to enhance the resilience of organisms following reintroduction into natural environments (Chakravarti *et al.* 2016; Evans *et al.* 2014).

Our finding of low to moderate levels of heritability suggests that transgenerational effects occurring in my study population could have resulted from genetic change (i.e. animals adapting to captivity), but are also likely to have resulted from transgenerational plasticity. Furthermore, the low heritability estimates in my study indicate that transgenerational effects occurred via non-genetic mechanisms such as transgenerational plasticity, or that the offspring displayed developmental plasticity, adjusting their behavioural and morphological phenotypes in response to the environmental conditions experienced in captivity. Nevertheless, certain phenotypic trait changes may be heritable and demonstrate an evolutionary potential, allowing for trait modification and adaptation to captive conditions. In consideration of this knowledge, future studies in captive breeding research might benefit from examining how patterns of heritability are influenced in captivity across multiple generations. If heritable changes are found to be commonplace, it may be necessary to apply control measures to regulate adaptations to captivity. Controlling the evolution of captive animals may require manipulating environmental conditions in captivity to create more heterogeneous or more naturalised environments (Ashley *et al.* 2003). This may result in variability in heritability within species across various environments (or indeed the same environment) (Rodriguez-Clark 2004) and may present an opportunity to maintain genetic variation by inducing varying heritabilities; populations may exhibit a spread of

trait values across the mean, potentially allowing populations to persist following release (McPhee and Carlstead 2010; McPhee and McPhee 2012). However, the impacts of variable captive conditions on heritability of phenotypic traits are yet to be examined.

4.5.2 Conclusions

To begin to understand the mechanisms underpinning transgenerational effects in captivity I investigated heritability of behaviour and morphology in a population of captive-reared house mouse (*Mus musculus*) using mid parent- and single parent-offspring regressions. It was found that slopes for boldness and activity behavioural types derived from parent-offspring regressions were all positive, with low to moderate trait heritability. The slopes for internal morphology derived from parent-offspring regressions were undetectable. None of the heritability estimates were statistically significant due to the large surrounding errors. These large errors indicate variability in phenotypic traits between litters and individuals. Alternatively, this might indicate the potential for genetic change in captivity to vary considerably between traits and that some but not all phenotypic traits may be heritable, highlighting the potential for rapid adaptation to captive conditions. However, continued investigation of the mechanisms underpinning transgenerational effects in captivity is needed. By identifying mechanisms that drive transgenerational effects in captivity, wildlife managers will be better placed to develop and implement strategies for manipulating the viability of captive populations.

4.6 References

Araki H, Cooper B and Blouin MS (2007) Genetic effects of captive breeding cause a rapid, cumulative fitness decline in the wild. Science **318:**100 – 103

Araki H, Cooper B and Blouin MS (2009) Carry-over effect of captive breeding reduces reproductive fitness of wild-born descendants in the wild. Biology Letters **5:** 621 – 624

Ariyomo TO, Carter M and Watt PH (2013) Heritability of boldness and aggressiveness in the zebrafish. Behavior Genetics **43:** 161 – 167

Ashley MV, Willson MF, Pergams ORW, O'Dowd DJ, Gende SM and Brown JS (2003) Evolutionarily enlightened management. Biological Conservation **111:** 115 – 123

Augustsson, H., K. Dahlborn, and B. J. Meyerson. 2005. Exploration and risk assessment in female wild house mice (*Mus musculus musculus*) and two laboratory strains. Physiology and Behavior **84:**265-277

Augustsson, H., and B. J. Meyerson. 2004. Exploration and risk assessment: a comparative study of male house mice (*Mus musculus musculus*) and two laboratory strains. Physiology and Behavior **81:**685-698

Austad, S. N. 1997. Comparative aging and life histories in mammals. Experimental Gerontology **32:**23-38

Bell, A. M. 2007. Future directions in behavioural syndromes research. Proceedings of the Royal Society of London B: Biological Sciences **274:**755-761

Blows, M. W., and A. A. Hoffmann. 2005. A reassessment of genetic limits to evolutionary change. Ecology **86:**1371-1384

Bonduriansky, R., A. J. Crean, and T. Day. 2012. The implications of nongenetic inheritance for evolution in changing environments. Evolutionary Applications **5:**192-201

Briscoe DA, Malpica JM, Robertson A, Smith GJ, Frankham R, Banks RG and Barker JSF (1992) Rapid loss of genetic variation in large captive populations of *Drosophila* flies: implications for the genetic management of captive populations. Conservation Biology **6:** 416 – 425

Chakravarti LJ, Jarrold MD, Gibbin EM, Christen F, Massamba-N'Siala G, Blier PU and Calosi P (2016) Can trans-generational experiments be used to enhance species resilience to ocean warming and acidification? Evolutionary Applications **9:** 1133 – 1146

Chevin L-M, Lande R and Mace GM (2010) Adaptation, plasticity, and extinction in a changing environment: towards a predictive theory. PLoS Biol **8:**e1000357

Christie MR, Marine ML, French RA and Blouin MS (2012) Genetic adaptation to captivity can occur in a single generation. Proceedings of the National Academy of Sciences **109:** 238 – 242

Clarke, C. N., D. J. Fraser, and C. F. Purchase. 2016. Lifelong and carry-over effects of early captive exposure in a recovery program for Atlantic salmon (*Salmo salar*). Animal Conservation **19:**350-359

Coleman K and Wilson DS (1998) Shyness and boldness in pumpkinseed sunfish: individual differences are context-specific. Animal Behaviour **56:** 927 – 936

Conde DA, Flesness N, Colchero F, Jones OR and Scheuerlein A (2011) An emerging role of zoos to conserve biodiversity. Science **331:** 1390 – 1391

Courtney Jones SK, Munn AJ and Byrne PG (2017) Effects of captivity on house mice behaviour in a novel environment: implications for conservation practices. Applied Animal Behaviour Science **189:** 98 – 106

Dall SRX, Houston AI and McNamara JM (2004) The behavioural ecology of personality: consistent individual differences from an adaptive perspective. Ecology Letters **7:**734 – 739

Dew-Budd K, Jarnigan J and Reed LK (2016) Genetic and sex-specific transgenerational effects of a high fat diet in *Drosophila melanogaster*. PLoS ONE **11:** e0160857

DeWitt TJ, Sih A and Wilson DS (1998) Costs and limits of phenotypic plasticity. Trends in Ecology and Evolution **13:** 77 – 81

Dingemanse NJ, Both C, Drent PJ, van Oers K and van Noordwijk AJ (2002) Repeatability and heritability of exploratory behaviour in great tits from the wild. Animal Behaviour **64:** 929 – 938

Dor R and Lotem A (2009) Heritability of nestling begging intensity in the house sparrow (*Passer domesticus*). Evolution **63:** 738 – 748

Drent PJ, van Oers K and van Noordwijk AJ (2003) Realized heritability of personalities in the great tit (*Parus major*). Proceedings of the Royal Society of London. Series B: Biological Sciences **270:** 45 – 51

Edenbrow M and Croft DP (2013) Environmental and genetic effects shape the development of personality traits in the mangrove killifish *Kryptolebias marmoratus*. Oikos **122:** 667 – 681

Evans ML, Wilke NF, O'Reilly PT and Fleming IA (2014) Transgenerational effects of parental rearing environment influence the survivorship of captive-born offspring in the wild. Conservation Letters **7:** 371 – 379

Falconer DS, Mackay TF, Frankham R. 1996. Introduction to quantitative genetics (4th edn). Trends in Genetics **12**:280.

Fischer J and Lindenmayer DB (2000) An assessment of the published results of animal relocations. Biological Conservation **96:** 1 – 11

Frankham R (2008) Genetic adaptation to captivity in species conservation programs. Molecular Ecology **17:** 325 – 333

Glucksmann A (1974) Sexual dimorphism in mammals. Biological Reviews **49:** 423 – 475

Herborn KA, Macleod R, Miles WTS, Schofield ANB, Alexander L and Arnold KE (2010) Personality in captivity reflects personality in the wild. Animal Behaviour **79:** 835 – 843

Houle D (1992) Comparing evolvability and variability of quantitative traits. Genetics **130:** 195 – 204

Johnsson J, Brockmark S and Näslund J (2014) Environmental effects on behavioural development consequences for fitness of captive-reared fishes in the wild. Journal of Fish Biology **85:** 1946 – 1971

Keller LF, Grant PR, Grant BR and Petren K (2001) Heritability of morphological traits in Darwin's finches: misidentified paternity and maternal effects. Heredity **87:** 325 – 336

Latham N and Mason G (2004) From house mouse to mouse house: the behavioural biology of free-living *Mus musculus* and its implications in the laboratory. Applied Animal Behaviour Science **86:** 261 – 289

Lewis OT and Thomas CD (2001) Adaptations to captivity in the butterfly *Pieris brassicae* (L.) and the implications for ex situ conservation. Journal of Insect Conservation **5:** 55 – 63

Lynch M and Walsh B (1998) Genetics and analysis of quantitative traits. Sinauer Sunderland, MA.

Martos SN, Tang WY and Wang Z (2015) Elusive inheritance: transgenerational effects and epigenetic inheritance in human environmental disease. Progress in Biophysics and Molecular Biology, **118:** 44 – 54

Mathews F, Orros M, McLaren G, Gelling M and Foster R (2005) Keeping fit on the ark: assessing the suitability of captive-bred animals for release. Biological Conservation **121:** 569 – 577

McDougall P, Réale D, Sol D and Reader S (2006) Wildlife conservation and animal temperament: causes and consequences of evolutionary change for captive, reintroduced, and wild populations. Animal Conservation **9:** 39 – 48

McPhee ME (2004a) Generations in captivity increases behavioral variance: considerations for captive breeding and reintroduction programs. Biological Conservation **115:** 71 – 77

McPhee ME (2004b) Morphological change in wild and captive oldfield mice *Peromyscus polionotus subgriseus*. Journal of Mammalogy **85:**1130 – 1137

McPhee ME and Carlstead K (2010) The importance of maintaining natural behaviors in captive mammals. In: Kleiman DG, Thompson KV, and Baer CK, editors. Wild

mammals in captivity: principles and techniques for zoo management. (pp. 303-312) University of Chicago Press, Chicago

McPhee ME and McPhee NF (2012) Relaxed selection and environmental change decrease reintroduction success in simulated populations. Animal Conservation **15:** 274 – 282

Millar JS and Zammuto RM (1983) Life histories of mammals: an analysis of life tables. Ecology **64:** 631 – 635

Miner BG, Sultan SE, Morgan SG, Padilla DK and Relyea RA (2005) Ecological consequences of phenotypic plasticity. Trends in Ecology and Evolution **20:** 685 – 692

Monaghan P (2008) Early growth conditions, phenotypic development and environmental change. Philosophical Transactions of the Royal Society of London B: Biological Sciences **363:** 1635 – 1645

Nadeau JH (2009) Transgenerational genetic effects on phenotypic variation and disease risk. Human Molecular Genetics **18:** R202 – R210

Nelson VR and Nadeau JH (2010) Transgenerational genetic effects. Epigenomics **2:** 797 – 806

O'Regan HJ and Kitchener AC (2005) The effects of captivity on the morphology of captive, domesticated and feral mammals. Mammal Review **35:** 215 – 230

Paproth A (2011) Measuring fitness loss in captive breeding programs: implications for threatened species recovery. Masters Thesis. University of Melbourne, Melbourne, Australia.

Philippart JC (1995) Is captive breeding an effective solution for the preservation of endemic species? Biological Conservation **72:** 281 – 295.

Price T and Schluter D (1991) On the low heritability of life-history traits. Evolution **45:** 853 – 861

Promislow DEL and Harvey PH (1990) Living fast and dying young: a comparative analysis of life-history variation among mammals. Journal of Zoology **220:** 417 – 437

Réale D and Festa-Bianchet M (2000) Quantitative genetics of life-history traits in a long-lived wild mammal. Heredity **85:** 593 – 603

Réale D, Festa-Bianchet M and Jorgenson JT (1999) Heritability of body mass varies with age and season in wild bighorn sheep. Heredity **83:** 526 – 532.

Richards CL, Bossdorf O and Pigliucci M (2010) What role does heritable epigenetic variation play in phenotypic evolution? BioScience **60:** 232 - 237

Robinson H, Comstock R and Harvey P (1955) Genetic variances in open pollinated varieties of corn. Genetics **40:** 45 – 60

Rodriguez-Clark KM (2004) Effect of captivity on genetic variance for five traits in the large milkweed bug (*Oncopeltus fasciatus*). Heredity **93:** 51 – 61

Salinas S, Brown Simon C, Mangel M and Munch Stephan B (2013) Non-genetic inheritance and changing environments. Non-Genetic Inheritance **1:** 38 – 50

Schulte-Hostedde AI and Mastromonaco GF (2015) Integrating evolution in the management of captive zoo populations. Evolutionary Applications **8:** 413 – 422

Shama LN, Strobel A, Mark FC and Wegner KM (2014) Transgenerational plasticity in marine sticklebacks: maternal effects mediate impacts of a warming ocean. Functional Ecology **28:** 1482 – 1493

Sih A, Bell A Johnson JC (2004) Behavioral syndromes: an ecological and evolutionary overview. Trends in Ecology and Evolution **19:** 372 – 378

Sih A, Mathot KJ, Moirón M, Montiglio P-O, Wolf M and Dingemanse DJ (2015) Animal personality and state–behaviour feedbacks: a review and guide for empiricists. Trends in Ecology and Evolution **30:** 50 – 60

Slade B, Parrott ML, Paproth A, Magrath MJL, Gillespie GR and Jessop TS (2014) Assortative mating among animals of captive and wild origin following experimental conservation releases. Biology Letters **10:** 20140656

Snyder NFR, Derrickson SR, Beissinger SR, Wiley JW, Smith TB, Toone WD and Miller B (1996) Limitations of captive breeding in endangered species recovery. Conservation Biology **10:** 338 – 348

Sokal RR and Rohlf FJ (1995) Biometry: the principles and practice of statistics in biological research. W.H. Freeman, New York

Stamps JA (2007) Growth-mortality tradeoffs and 'personality traits' in animals. Ecology Letters **10:** 355 – 363

Stearns SC (1983) The influence of size and phylogeny on patterns of covariation among life-history traits in the mammals. Oikos **41:** 173 – 187

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

van Oers K, Drent PJ, de Goede P and van Noordwijk AJ (2004) Realized heritability and repeatability of risk-taking behaviour in relation to avian personalities. Proceedings of the Royal Society of London B: Biological Sciences **271:** 65 – 73

Weigensberg I and Roff DA (1996) Natural heritabilities: can they be reliably estimated in the laboratory? Evolution **50:** 2149 – 2157

Williams SE and Hoffman EA (2009) Minimizing genetic adaptation in captive breeding programs: a review. Biological Conservation **142:** 2388 – 2400

Wilson ADM and Godin J-GJ (2009). Boldness and behavioral syndromes in the bluegill sunfish, *Lepomis macrochirus*. Behavioral Ecology **20:** 231 – 237

Wong BBM and Candolin U (2015) Behavioral responses to changing environments. Behavioral Ecology **26:** 665 – 673

5 LONG-TERM CHANGES IN FOOD AVAILABILITY MEDIATE THE EFFECTS OF TEMPERATURE ON GROWTH, DEVELOPMENT AND SURVIVAL IN STRIPED MARSH FROG LARVAE: IMPLICATIONS FOR CAPTIVE BREEDING PROGRAMMES

5.1 Abstract

Food availability and temperature are known to trigger phenotypic change, however, the interactive effects between these factors are only beginning to be considered. The aim of this study was to examine the independent and interactive effects of long-term stochastic food availability and water temperature on larval survivorship, growth and development of the striped marsh frog, *Limnodynastes peronii*. Larval *L. peronii* were reared under either constant or stochastic food availability conditions and in three different water temperatures (18, 22 and 26°C) and effects on survival, growth and development were quantified. Over the experimental period, larval growth rate was highest, and survivorship was lowest, in the warmest temperatures. However, changes in food availability mediated the effects of temperature, with slower larval growth and higher survivorship in stochastic food availability treatments in warmer water temperatures. Tadpoles in the stochastic food availability treatments did not reach metamorphosis during the experimental period, suggesting that developmental stasis may have been induced by food restriction. Overall, these results demonstrate that changes in food availability alter the effects of water temperature on survival, growth and development. From an applied perspective, understanding how environmental factors interact to cause phenotypic change may assist with amphibian conservation by improving the number of tadpoles generated in captive breeding programmes.

Key words: Phenotypic plasticity, food availability, temperature, metamorphosis, growth, development, morphology, survival

5.2 Introduction

Phenotypic plasticity is the ability of an organism to change its phenotype in response to varying abiotic and biotic environmental factors (Miner *et al.*, 2005). When faced with dynamic environmental conditions, some organisms can readily respond by changing phenotypes, allowing for a range of optimal phenotypes to be produced in response to multiple environments (DeWitt *et al.*, 1998). If the optimal phenotype can change with environmental conditions, this presents an adaptive advantage that can improve organismal fitness (De Jong, 2005; Reed *et al.*, 2010). Phenotypic plasticity has evolved in an array of organismal traits, but two traits that appear to be particularly plastic are growth (somatic growth) and development (ontogenic change) (Pfennig *et al.*, 2010; Relyea, 2001). Plastic responses in growth and development can be triggered by various environmental factors. One environmental factor known to trigger plastic responses across a variety of taxa is food availability (*see* Enriquez-Urzelai *et al.*, 2013; Monaghan, 2008; Rosen *et al.*, 2014; Munn *et al.*, 2010). Empirical studies suggest that changes in food availability have long-term consequences for various life-history traits due to a reduction in the amount of energy that can be allocated to somatic growth (Enriquez-Urzelai *et al.*, 2013; Inatsuchi *et al.*, 2010; Yoneda *et al.*, 2005).

Several theoretical models have considered how insufficient energy intake under conditions of reduced food availability might influence organismal growth and development and ultimately the probability of surviving and reproducing. The 'metabolic down regulation model' predicts that food deprivation induces an overall metabolic depression that may occur as a physiological adaptation to reduce metabolic costs, via the down-regulation of metabolic rates, limiting processes such as growth and development (Keys *et al.*, 1950; Rosen *et al.*, 2013, 2014). There is some empirical evidence to support this prediction. For example, in periods of stochastic food availability, coral reef fish exhibit longer time to metamorphosis and smaller size at maturity (McLeod *et al.*, 2013) (*see* Enriquez-Urzelai *et al.*, 2013; Inatsuchi, Yamato and Yusa, 2010; Nicieza *et al.*, 1997; Yoneda and Wright, 2005). The 'general optimisation model', a mathematical formalisation of the Wilbur-Collins model (Wilbur *et al.*, 1973), also predicts slower growth rate and longer developmental periods in response to poor growth conditions. However, this model proposes that developmental thresholds (such as minimum size) need to be attained prior to life-history transition

(Day and Rowe, 2002). This model predicts that to meet minimum size thresholds for metamorphosis, individuals should extend the larval period (Day and Rowe, 2002).

The 'Wilbur-Collins model', developed explicitly for species that experience metamorphosis, also proposes that there should be a minimum threshold size at which developmental transitions occur (Day and Rowe, 2002; Wilbur *et al.*, 1973). However, this model hypothesises a trade-off between growth and development. The model predicts that under conditions of stochastic food availability, larval development is increased to evade the resource-poor environment, and growth rate is slowed, resulting in a smaller size at metamorphosis (Wilbur and Collins, 1973). This negative relationship between growth and metamorphosis has been reported in three Spadefoot toad species (Genus: *Scaphiopus*) (Morey and Reznick, 2000). In low food availability conditions larvae underwent earlier development to evade the resource-poor environment; however, a minimum threshold size had to be met before development could be expedited. Alternatively, if the minimum threshold size was not met, larvae entered a developmental stasis (Morey and Reznick, 2000).

The effect of food availability on growth and development has also been explored using the stochastic dynamic programming (SDP) approach, which has been developed to determine an optimal strategy to maximise a particular fitness trait (Tenhumberg *et al.*, 2000). Using the SDP approach, Tenhumberg *et al.* (2000) developed a SDP model for a syphrid fly system to determine optimal size and age at maturity when exposed to stochastic food availability, but considered the timing of food availability during development, a novel inclusion largely ignored in other models of growth and development. In this SDP model, it is predicted that exposure to stochastic food availability throughout development would result in larvae pupating earlier and at a smaller size. Exposure to stochastic food availability in the early phase of development would result in syphrid pupa pupating later without altering size at pupation. By contrast, exposure to stochastic food availability conditions during the late phase of development would alter weight at pupation, not developmental time (Tenhumberg *et al.*, 2000), providing support for the notion that timing of changes to food availability can control growth and development (*see* Enriquez-Urzelai *et al.*, 2013; Inatsuchi *et al.*, 2010; Morey and Reznick, 2000; Morey *et al.*, 2004; Nicieza and Metcalfe, 1997; Yoneda and Wright, 2005).

Importantly, while the aforementioned models examine the effects of food availability on growth and development, multiple environmental factors can affect these life-history traits, and the effect of these factors are rarely independent. For instance, in temporal water bodies, plastic responses in growth and development may not only be triggered by food availability, but also by temperature (Leips *et al.*, 1994; Sanuy *et al.*, 2008). The 'temperature-size rule (TSR)' predicts that ectothermic species reared under cold temperatures display slow growth rates and a prolonged larval period, resulting in a larger size at metamorphosis (Kozłowski *et al.*, 2004). The TSR has been widely tested and there is now considerable empirical evidence to suggest that this rule applies to the vast majority of ectothermic animals (Angilletta *et al.*, 2004; Walters *et al.*, 2006).

While the independent effects of food availability and temperature on growth and development in larval species is well established (Inatsuchi *et al.*, 2010), the interactive effect of these factors on growth, development, and survival to maturity is only just beginning to be considered (Álvarez and Nicieza, 2002a). One of the few models considering the interaction between environmental factors is the 'fixed-rate model' (Travis, 1984). The model postulates while food availability may regulate specific life-history traits such as larval growth and size at metamorphosis, the developmental rate becomes fixed at a certain point (Travis, 1984; Rose, 2005). However, the length of larval period can be regulated by other environmental factors such as temperature (Álvarez *et al.*, 2002b). There is some experimental evidence for interactive effects. For example, in a study investigating the interactive effects of diet type and temperature on larvae of the Iberian painted frog (*Discoglossus galganoi*), it was found that larval period was extended with cooler temperatures, however, size at metamorphosis was regulated by the interaction between temperature and diet type (Álvarez and Nicieza, 2002b). More specifically, when exposed to plant- or animal-based diets, size at metamorphosis varied inversely to temperature, and although diet did not influence size at 12°C, exposure to the animal-based diet resulted in bigger metamorphs at 17 and 22°C (Álvarez and Nicieza, 2002b).

To date, few studies have investigated how food availability, and interactions between food availability and temperature, influence growth and development in ectotherms. Nevertheless, evidence is emerging to suggest that such interactions can alter developmental trajectories. Newman (1998) conducted a dietary experiment using

Spadefoot toad tadpoles (*Scaphiopus couchii*) and demonstrated that abrupt change in food level during development had significant effects on an individual's age and size at metamorphosis. However, the magnitude and direction of these effects differed depending on environmental temperature and tadpole density. More recently, in a study of coral reef fish, McLeod *et al.* (2013) manipulated food availability by increasing time lags between feeding, at increasing temperatures. Overall, lower feeding regimens decreased survivorship to adulthood and longer time to metamorphosis was observed. However, this study noted that predictable time lags between food supply may not be symptomatic of natural food supplies (McLeod *et al.*, 2013), indicating the importance of investigating the influence of stochastic food availability. Further, changes in food availability occurring throughout the entire developmental period has received limited empirical attention (*see* Leips and Travis, 1994). Using an SDP model approach Tenhumberg *et al.* (2000), considered the effects of timing of changes to food availability on the optimal size at maturity in the syphrid fly system, however, further empirical evidence of the effects of timing of changes on growth and development in other species remains limited (*see* Bull *et al.*, 1996; Tenhumberg *et al.*, 2000). Empirical testing of the interaction between long-term changes in food availability and water temperature is now needed to broaden my understanding of how interactions between environmental conditions shape plastic growth and development responses in ectotherms.

5.2.1 Implications for amphibian conservation

Knowledge of how interactions between food availability and temperature influence larval growth, development and survivorship may also be of value to amphibian conservation. Globally, amphibians are declining faster than any other vertebrate group and for threatened species the recommended recovery action is captive breeding and reintroduction (Stuart *et al.*, 2004, Gascon *et al.*, 2007). While captive breeding programmes have been established for various amphibian species (Gascon *et al.*, 2007, Stuart *et al.*, 2004), many programmes have been constrained by an inability to consistently generate large numbers of healthy individuals. The ability to generate large numbers of individuals is critical for three main reasons. First, it allows the captive population to be maintained at a size that avoids problems associated with inbreeding and/or natural attrition. Second, it supplies large numbers of individuals for release,

which in various species is a predictor of reintroduction success (Armstrong *et al.*, 2008; Tarszisz *et al.*, 2014). Third, it reduces the cost of captive breeding, making recovery programmes more financially viable (Canessa *et al.*, 2014; Tarszisz *et al.*, 2014). In recognition of the need to improve the productivity of amphibian captive breeding programmes, empirical studies have begun to investigate how anuran growth, development and survivorship are influenced by various abiotic factors, including nutrition (Ogilvy *et al.*, 2012; Cothran *et al.*, 2015; Dugas *et al.*, 2013), pH (Mantellato *et al.*, 2013), salinity (Christy and Dickman, 2002), food availability (Gillespie, 2002) and temperature (Browne *et al.*, 2003). Surprising, however, there remains a limited understanding of how interactive effects between abiotic factors influence anuran life history traits. Testing for such effects in common model species can be a valuable first step towards identifying optimal rearing environments for threatened species with analogous life histories. For example, by studying the growth and development of the common frog *Geocrinia rosea*, Mantellato *et al.* (2013), expedited the establishment of *ex-situ* breeding programmes for two rare and threatened species: *G. alba* and *G. vitellina*.

The aim of this study was to investigate the independent and interactive effects of long term exposure to stochastic food availability and water temperature on larval survivorship, growth and development of the striped marsh frog, *Limnodynastes peronii*. To evaluate the effects of food availability and temperature, a 2 X 3 factorial experiment was performed. The "food availability" factor had two levels, *ad libitum* food supply (constant availability) and stochastic food supply (stochastic availability) (Tenhumberg *et al.*, 2000) and the "temperature" factor had three levels: 18, 22 and 26°C. The following hypotheses were tested i) stochastic food availability would decrease larval survivorship, growth and development ii) increased water temperature would increase larval survivorship, growth and development and iii) indicative of an interaction between these environmental factors, water temperature would mediate the effects of food availability on survival, growth and development; with decreased food availability having less of an effect at lower water temperatures due to a lowered metabolic rate, thus requiring less energy to be extracted from the external environment.

5.3 Methods

5.3.1 Ethics information

This study was conducted under approval from the University of Wollongong Animal Ethics Committee (Permit Number AE12/23) and the NSW Office of Environment and Heritage (Parks Permit SL101104).

5.3.2 Study species

The striped marsh frog (*Limnodynastes peronii*) is a common Australia frog species with a wide distribution along the east coast, extending from cool temperate regions in Victoria to the tropical regions of northern Queensland (Wilson, 2001). Larval *L. peronii* are found in various aquatic environments that experience a broad range of nutritional and temperature conditions, making *L. peronii* an ideal model species to examine the effects of food availability in combination with temperature variation on larval survivorship, growth and development (Niehaus *et al.*, 2006). The breeding season of *L. peronii* varies depending on geographical location. Within cool-temperate zones including the Greater Illawarra where the present study was conducted, the breeding season is predominantly late Winter through till early Summer (Wilson, 2001). Eggs clutches are laid in an aquatic foam nest and the number of eggs per clutch ranges between 150 – 2000 (Schell *et al.*, 2002).

5.3.3 Clutch collection and tadpole acclimation

Six egg clutches were collected from 25th to 30th January 2013 from a breeding site in the Greater Illawarra region of south-eastern New South Wales (34°26′S 150°51′E). Clutches were collected by hand and stored in separate polyethylene tubs (600 x 350 x 250 mm) filled with twenty litres of Reverse Osmosis water (RO water) and transported to the Ecological Research Centre at the University of Wollongong, Wollongong (34°24′24″S 150°52′46″E). Clutches were maintained in these tubs under natural light conditions at approximately 25 ± 2°C for a ten day acclimation period. This period was imposed to ensure that tadpoles were viable before being entered into the experiment. To ensure no build-up of nitrogenous waste in tubs during the acclimation period, one third of the water was replaced every fifth day, resulting in two water changes during the acclimation period. Tadpoles hatched from eggs two to three days after collection,

and once tadpoles had hatched, egg jelly was removed from the tubs and tadpoles were fed *ad libitum* every second day with fish flakes (75% Flora, 25% San, sera GmbH, Heinsberg, Germany).

Approximately ten days after hatching, tadpoles were entered into experimental housing. Tadpoles were acclimated to the experimental housing for a period of 24 hours (*see* 5.3.4 Experimental Design), and any individuals that died during the 24 hours were replaced with individuals maintained under identical conditions in order to maintain sample sizes. Tadpoles were fasted during this acclimation period and were only provided with food at the time they were entered into experimental treatments. Upon entry into the experimental treatments, tadpoles (n = 48; split between two replicate rearing tanks per clutch per treatment) were photographed, so that baseline body size measurements could be made at a later date (*see* 5.3.4 Experimental Design). Measurements were not made on back-up replicates, which were euthanased after use. Once tadpoles were entered into the experimental treatment, no further replacement of individual tadpoles occurred.

5.3.4 Experimental Design

To examine the effect of temperature and food availability on larval survival, growth and development, a 3 X 2 factorial design was used. The experiment involved three rearing temperatures (18, 22 and 26°C) and two feeding regimes (constant and stochastic food availabilities), resulting in six experimental treatments referred to as 1) Constant 18°C, 2) Constant 22°C, 3) Constant 26°C, 4) Stochastic 18°C, 5) Stochastic 22°C and 6) Stochastic 26°C. A split clutch design was used with tadpoles from each clutch being randomly allocated to an experimental treatment (i.e. 48 tadpoles per clutch in each treatment split between two replicate plastic rearing tanks, and a total of 288 tadpoles per treatment). Split clutch designs provide effect controls for both clutch effects and parental genetic effects. The experimental period lasted 14 weeks and during this time tadpoles were monitored daily. This experimental period was selected because the larval period in populations of *L. peronii* in southern NSW typically lasts two to three months (Anstis, 2013). Furthermore, a past experimental study in *L. peronii* reported that time to metamorphosis under conditions of constant food (*ad libitum* lettuce) and temperature (24°C) ranged between 36 and 55 days (Kraft *et al.*, 2005). The

experiment commenced on 12 February 2013 and was terminated on 24 May 2013. During the experimental period, tadpoles were housed in plastic rearing tanks (250 x 150 x 110mm). The plastic rearing tanks were rafted within the polyethylene tubs (600 x 350 x 250 mm) and a Jäger 100W aquarium water heater (Eheim, Germany) was placed in the polyethylene tub to set the experimental treatment temperature.

A total of 24 tadpoles were housed in each plastic rearing tank at any one time (two replicates of n = 24 tadpoles per clutch per treatment); each plastic rearing tank had 2.5 litres of RO water resulting in one tadpole per 105 mL of water. To account for changes to tadpoles per volume of water as a result of tadpole mortality or metamorphosis and to reduce any potential density-dependent effects on growth and development (Miner *et al.*, 2005); 105 mL of water per tadpole was removed to maintain a fixed number of tadpoles per volume of water. These water volume adjustments were carried out on a weekly basis. Experimental tubs were kept in a temperature and light controlled room maintained at 12 ± 2°C ambient temperature and a 12: 12 light: dark regime. To control water salinity, which can have a significant impact on tadpole growth, development and survivorship (*see* Chinathamby *et al.*, 2006; Kearney *et al.*, 2012), Aquasonic Ocean Nature sea salt was added to the RO water (0.14g/L). To prevent water fouling, partial water changes (≈ 40%) were made once per week.

The three water temperature treatments: (18°C, 22°C and 26°C) in which tadpoles were reared were selected because they reflected the average lower and upper estimates of temperatures that *L. peronii* tadpoles experience in NSW systems during the period between December and April, which is when peak development and metamorphosis typically occurs in this region (Wilson, 2001). It is of note that *L. peronii* tadpoles in NSW have the capacity to overwinter, and metamorphose from October to November (Anstis, 2013). However, I did not simulate temperatures experienced during this period because, in an effort to make my findings relevant to amphibian CBPs, I was focussed on identifying conditions that promoted rapid larval development without compromising tadpole survival. To ensure temperatures were maintained at treatment temperatures throughout the entire experimental period, water temperatures were monitored on a weekly basis using a calibrated digital thermometer probe (Traceable Snap-In Module with Probe, Thomas Scientific). All treatment temperatures remained with a range of ± 2°C. To minimise any room effects or tub effects, the temperature of

polyethylene tubs were randomly assigned, and plastic tanks were rotated within the polyethylene tub on a daily basis.

Tadpoles were exposed to one of two feeding regimes: constant food availability or stochastic food availability. Constant food availability treatments supplied food *ad libitum* (i.e. no food restrictions applied) throughout the entire experimental period. The stochastic food availability treatment had randomly allocated fasting periods of up to three days where no fresh food was provided. At the start of the fasting period, any uneaten food was removed using a siphon, leaving tadpoles with access to faecal material only. On days where tadpoles had access to food, food was provided *ad libitum* (i.e. no food restrictions applied). *Ad libitum* quantities of food were adjusted throughout the experimental period to account for increased tadpole body size (increased quantities of food) and changes in tadpole density (reduced quantities of food). Food consisted of a mixture of frozen endive (*Cichorium endivia*) and commercial Algae sinking fish pellets (Australian Pet Supplies Feedwell Fishfood, Smithfield, NSW, Australia). To ensure that each plastic tank was treated in the same way, constant food availability plastic tanks also had water siphoned and replaced and this occurred simultaneously to the beginning of fasting periods for stochastic food availability treatments. This process also assisted in aerating the water.

5.3.5 Effects of food availability and water temperature on survival, development and growth

Survivorship of individual tadpoles in each experimental treatment was monitored on a weekly basis throughout the 14-week experimental period. In addition, for each experimental treatment, the number of tadpoles reaching metamorphosis, and the time taken to reach metamorphosis were recorded. Metamorphosis was defined as the time taken for the emergence of at least one forelimb (Gosner Stage 42; Gosner, 1960).

The effects of food availability and water temperature on tadpole size were determined by measuring individual snout to vent length (mm). Measurements were made from digital images taken on a weekly basis using a standardised overhead digital camera (Canon Powershot D20 12.1 MP CMOS Waterproof Digital Camera). To measure snout to vent length, each plastic tank had ≈ 40% water removed (coinciding with the partial water change), allowing for enough water to cover the tadpoles but restrict movement

within the water column. Snout to vent length measurements were made using Image J Image Processing Software (Open Source, version 1.42q), calibrated using a standardised scale present in each photograph. Due to tadpoles being housed in groups during the experimental period, tank means, using eight randomly selected tadpoles per tank, were used to preserve data independence. A sub-sample of eight randomly selected tadpoles was assumed to account for any size variation occurring within each plastic replicate tank (Capellan *et al.,* 2007).

Within 12 hours of the emergence of at least one forelimb (Gosner Stage 42; Gosner, 1960), metamorphs were removed from the experimental treatment container, photographed and maintained in separate plastic container with an RO water soaked sponge (3.0 cm^2) until the time of tail reabsorption. Prior to tail reabsorption, commercial fish pellets were provided *ad libitum* and after tail reabsorption pinhead crickets (*Acheta domestica*) were provided *ad libitum.* Containers housing metamorphs were kept in a temperature and light controlled room at 22 ± 2°C ambient temperature under a 12: 12 light: dark regime. Metamorphs were measured within two days of tail reabsorption. Vernier callipers were used to measure snout to vent length (mm).

5.3.6 Statistical Analysis

5.3.6.1 Effects of food availability and temperature on tadpole survivorship

To examine the effects of food availability and water temperature on tadpole survivorship over the 14-week experimental period, a Cox-proportional hazard model (Andersen *et al.*, 1982) was used to determine differences in survivorship distribution; this was displayed as a Kaplan-Meier survival curve. For survival analysis, censorship was applied to death occurring as a result of handling, tadpoles that survived (without metamorphosing) over the experimental period and tadpoles that metamorphosed before the conclusion of the experimental period. To account for any potential clutch effects, clutch ID was included in the model as a random factor. Survival analysis was conducted in R v3.1.0 statistical package (R Developer Core Team, 2014) in conjunction with the *survival* package (Therneau, 2014).

Survivorship at week 14 was further examined using a Generalised Linear Mixed Effects Model (GLMM). In this model food availability and water temperature were

fixed effects and clutch ID was a random effect. The model also included an interaction term between food availability and temperature. Data were analysed in R v3.1.0 statistical package (R Developer Core Team, 2014) in conjunction with the *survival* package (Therneau, 2014).

5.3.6.2 Effects of food availability and temperature on tadpole size

To examine the effects of food availability and water temperature on tadpole size over the 14-week experimental period, a general additive mixed model (GAMM) was used (Lin *et al.*, 1999). The additive model was used because it allows a non-linear growth trajectory in response to experimental treatment (Zuur *et al.*, 2009). Comparisons were made on a weekly basis (weeks 0 – 9) to examine the additive and interactive effects of food availability and water temperature on tadpole size. To account for any potential clutch effects, clutch ID was included in the model as a random intercept. Tadpole size was measured as snout to vent length (SVL). Week 0 was used to provide the baseline snout to vent measurements and weeks 1 – 9 provided the size measurements in response to the experimental treatments. Weeks 10 – 14 were not included in the GAMM because complete tadpole mortality occurred in several replicates during this period. Data were analysed in R v3.1.0 statistical package (R Developer Core Team, 2014) using statistical package gamm4 (Wood, 2014).

5.3.6.3 Effects of food availability and water temperature on development

Over the experimental period, only tadpoles from constant food availability treatments metamorphosed; so all measures relating to metamorphosis were restricted to the constant feeding treatments. To determine the effect of water temperature on the time to metamorphosis and post-metamorphic size, generalised linear mixed models (GLMM) were used. In these models, water temperature was the fixed effect and clutch ID was a random effect to account for potential clutch effects. All post-metamorphic data were analysed using R v3.1.0 statistical package (R Developer Core Team, 2014) using statistical package: nlme (Pinheiro *et al.*, 2014).

5.4 Results

5.4.1 Effects of food availability and temperature on tadpole survivorship

Survival of *L. peronii* tadpoles over the 14-week experimental period was significantly different between water temperatures (Cox-proportional hazard test: z = 6.105, p <0.0001), but not between food availability treatment groups (Cox-proportional hazard test: z = 0.311, p = 0.760), There was no significant interaction between food availability and water temperature (Cox-proportional hazard test: z = -0.843, p = 0.400; Table 5.2; Fig. 5.1). Survival in all treatment groups was high (>90%) up until week 5. After this time, tadpoles began dying in all treatment groups. By experimental week 14, survivorship was lowest in the two warmest Constant food treatments (22 and 26°C treatments) and was highest in the two coolest Stochastic food treatments (18 and 22°C treatments). Survivorship was intermediate in the Constant 18°C and Stochastic 26°C treatments. Clutch had a significant effect on survival (GLMM: z = 7.531, p <0.001; Table 5.1; 5.3; Fig. 2). While there was no overall significant difference between constant and stochastic food availability treatments on survivorship to week 14 (GLMM: z = 1.664, p = 0.0961; Table 5.1; 5.3; Fig. 5.2), there was a significant influence of food availability treatment on water temperature treatments, with tadpoles exposed to stochastic food availability having higher survivorship to week 14 in water temperatures of 22°C and 26°C compared to tadpoles exposed to constant food availability (GLMM: z = -10.758, p <0.001, z = -12.943, p <0.001 respectively; Table 5.1; 5.3; Fig. 5.2).

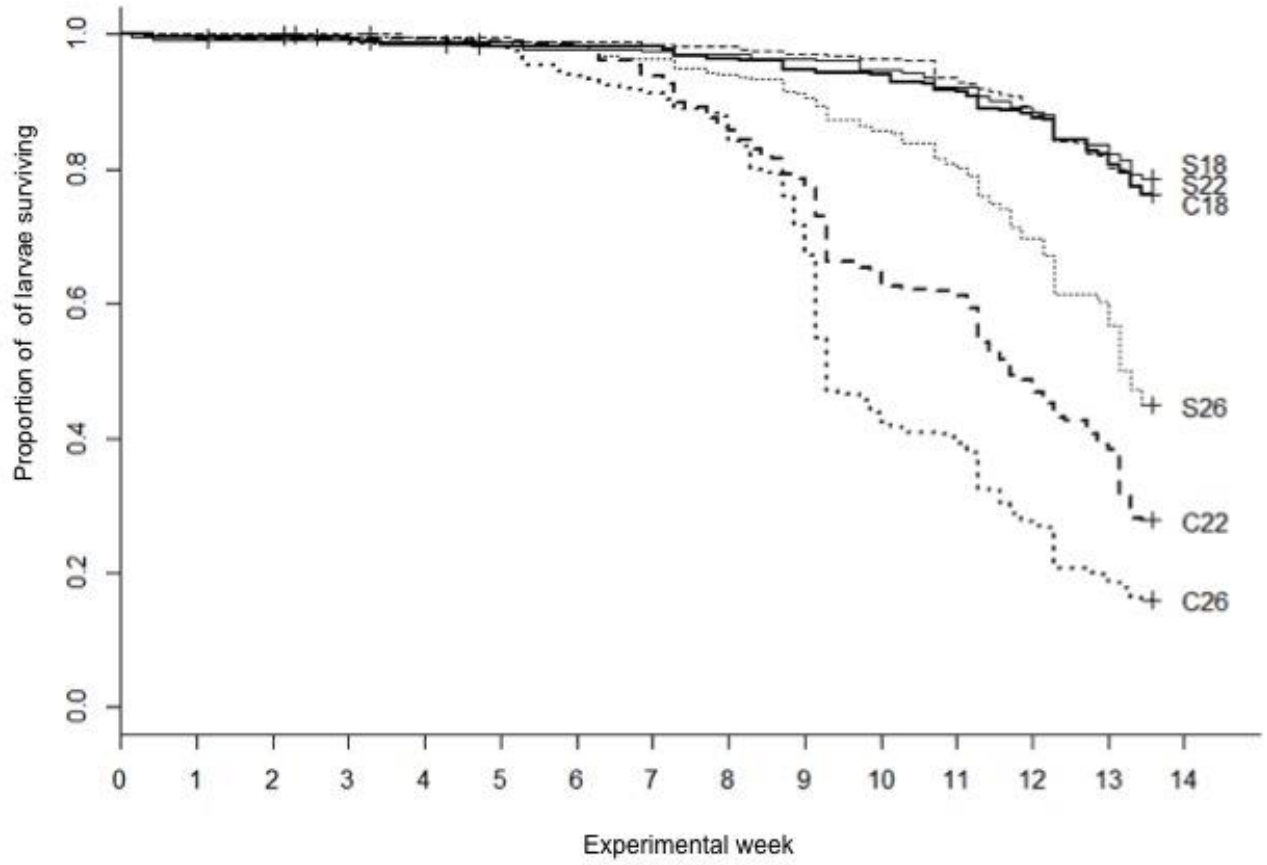


Figure 5.1 Proportion of *L. peronii* tadpoles surviving over 14-week experimental period under six experimental treatments: Constant food at 18°C (C18), Stochastic food at 18°C (S18), Constant food at 22°C (C22), Stochastic food at 22°C (S22), Constant food at 26°C (C26) and Stochastic food at 26°C (S26) (+ indicates a censored event).

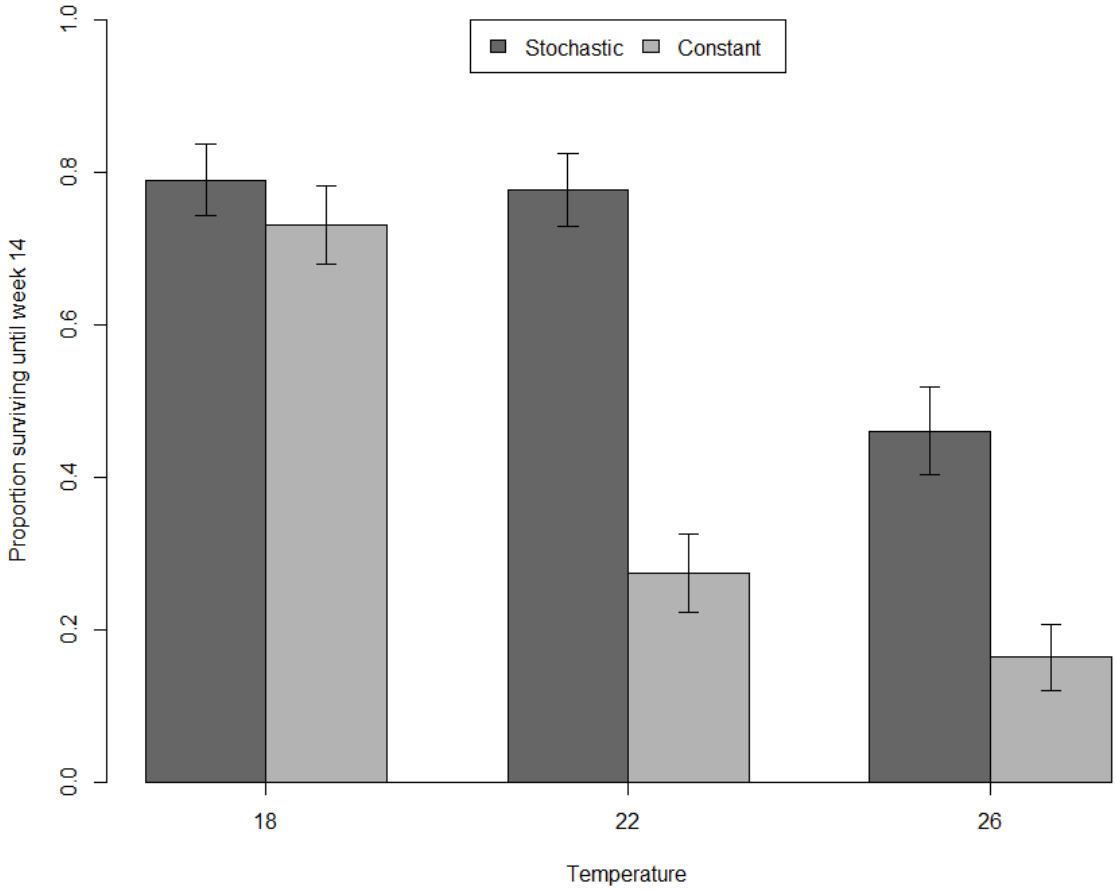


Figure 5.2 Effect of food availability and temperature on proportion of striped marsh frog *L. peronii* tadpoles surviving to week 14. Stochastic food availability treatments represented by dark grey bar graphs and Constant food availability treatments represented by light grey bar graphs. Values represent mean ± SE.

Table 5.1 Effect of food availability and water temperature on percentage of tadpoles surviving to week 14 in the striped marsh frog *L. peronii*. Values represent mean ± SE. Statistical outputs are from a GLMM model (see Table 5.3).

Treatment Food availability	Temperature (°C)	Sample size	% Survival at Week 14
Constant	18	288	73.2± 5.2
	22	289	27.5± 6.9
	26	289	16.1± 5.7
Stochastic	18	287	78.9± 5.9
	22	288	77.8± 4.7
	26	289	46.1± 9.1

Table 5.2 Output from Cox-proportional hazard model testing the effects of food availability and water temperature on proportion of tadpoles surviving a 14-week experimental period in the striped marsh frog *L. peronii*.

	Coef	exp(coef)	SE(coef)	robust SE	z	p-value
Stochastic vs Constant	0.5937	1.811	0.5798	1.9113	0.311	0.760
Temperature	0.2392	1.27	0.0146	0.0392	6.105	<0.001
Stochastic: Temperature	-0.0742	0.929	0.0246	0.088	-0.843	0.400

Table 5.3 Output from General Linear Mixed Effects model testing the effects of food availability and water temperature on proportion of *L, peronii* tadpoles surviving to week 14.

	Estimate	Std. Error	z value	Pr (>|z|)
(Intercept)	1.0547	0.2439	4.324	<0.001
Stochastic vs Constant	0.3362	0.202	1.664	0.0961
Temperature 22 vs 18	-2.0841	0.1937	-10.758	<0.001
Temperature 26 vs 18	-2.7671	0.2138	-12.943	<0.001
Stochastic Diet: Temperature 22 vs 18	2.0122	0.284	7.085	<0.001
Stochastic Diet: Temperature 26 vs 18	1.1995	0.2858	4.196	<0.001

5.4.2 Effects of food availability and temperature on tadpole size

In week 0, there were no significant differences in baseline body size (Table 5.4). In weeks 1 – 9, there were significant differences in body size between treatment groups. In week 1, body size was largest in tadpoles from treatments with the warmest water temperatures (22°C and 26°C), irrespective of whether food availability was Constant or Stochastic. However, in week 2, tadpole body size was larger in warmest water temperatures (22°C and 26°C) and constant food availability treatments (Table 5.4). In weeks 2 to 9, body size was largest in treatments with constant food availability, irrespective of the treatment temperature (Table 5.4). At week 9, a decrease in size with increasing water temperature was evident. An interaction between food availability and water temperature occurred at weeks 3 and 9. Between weeks 0 and 9 there was no effect of clutch ID on tadpole size.

5.4.3 Effects of food availability and temperature on development

Over the 14-week experimental period, only 2.02% of tadpoles (35/1730) reached metamorphosis, and all were from treatments with constant food availability. Of the 35 individuals that successfully metamorphosed, there was no significant effect of temperature or clutch ID on mean time to metamorphosis (days) (Table 5.5; 5.6) and no effect on post-metamorphic body size (Table 5.5; 5.6).

Table 5.4 Effect of food availability and water temperature on tadpole size across experimental period: weeks 0 – 9 (weeks 10 – 14 excluded due to incomplete sample sizes due to mortality). Positive values (+) indicate a significant ($p < 0.05$) increase in size with increasing water temperature and negative (–) a significant ($p < 0.05$) decrease in size with decreasing water temperature. In the case of diet (–) indicates the tadpoles with the stochastic diet were significantly ($p < 0.05$) smaller than the constant diet. In the interaction term, (x) indicates a significant interaction between food availability and water temperature occurring. Significance values were derived from the GAMM analysis.

Week	Water Temperature	Food Availability	Food Availability *Water Temperature
0			
1	+		
2	+	-	
3		-	x
4		-	
5		-	
6		-	
7		-	
8		-	
9	-	-	x

Table 5.5 Effect of food availability and temperature on % tadpoles reaching metamorphosis, time to metamorphosis and post-metamorphic size (SVL), in the striped marsh frog *L. peronii*. Values represent mean ± SE.

Treatment		% Metamorphosed*		Time to metamorphosis (days)	Post-metamorphic size:
Food availability	Water Temperature (°C)				Snout to vent length (mm)
Constant	18	5.2%	(15/288)	62.1± 4.9	15.3± 1.9
	22	2.8%	(8/289)	76.4± 4.6	15.7± 1.5
	26	4.2%	(12/289)	59.4± 3.5	14.5± 0.7
Stochastic	18		(0/287)	-	-
	22		(0/289)	-	-
	26		(0/289)	-	-

* Total number of tadpoles reaching metamorphosis in each treatment reported in parentheses.

Note: no data are presented for the stochastic treatments because no tadpoles in these treatments reached metamorphosis. Sample sizes for post-metamorphic size are n=35.

Table 5.6 Output from Generalised Linear Mixed Effects Models testing the effect of water temperature on time to metamorphosis and post-metamorphic size in the striped marsh frog *L. peronii*.

Time to metamorphosis				
	Estimate	Std. Error	t value	Pr (>|t|)
(Intercept)	4.08631	0.06403	63.82	<2e-16
factor(Temperature)22	0.18496	0.11979	1.544	0.133
factor(Temperature)26	-0.03484	0.09844	-0.354	0.726
Post-metamorphic size				
(Intercept)	2.73954	0.03124	87.687	<2e-16
22 vs 18	0.02102	0.05069	0.415	0.681
26 vs 18	-0.05044	0.03899	-1.294	0.206

5.5 Discussion

The aim of this study was to investigate the independent and interactive effects of food availability and water temperature on larval growth, development and survival in the striped marsh frog, *L. peronii.* Variation in food availability was found to impact larval size and development, with smaller larval size and slower developmental rates in stochastic food availability treatments. Furthermore, changes in food availability mediated the effects of increasing water temperature on survivorship. Specifically, tadpoles were smaller and had higher survivorship to week 14, in stochastic food availability compared to constant food availability in warmer water temperature treatments, rejecting my first hypothesis that stochastic food availability would decrease larval survivorship, growth and development. Interestingly, clutch identity did not have a significant effect on any of my measures of tadpole growth and development, but clutch identity did have a significant effect on tadpole survivorship. Given that my clutches were collected over a period of five days, it is possible that embryos from different clutches were exposed to different environmental conditions (e.g. pre-treatment temperatures) that subsequently affected their probability of survival. Alternatively, survivorship may have been affected by variable maternal provisioning (Dziminski *et al.,* 2006), differences in parental compatibility (Dziminski *et al.,* 2008), or differences in parental genetic quality (Sheldon *et al.,* 2003). Such clutch effects have previously been reported in anurans (Dziminski *et al.,* 2006; Dziminski *et al.,* 2008; Sheldon *et al.,* 2003) and underscore the importance of considering the effects of clutch identity in experimental studies aimed at investigating the influence or rearing environment on anuran life history traits.

The reported effects of food availability on larval growth and development support the predictions of the 'general optimisation model' (Day and Rowe, 2002), which predicts slower growth and longer developmental periods in stochastic food availability conditions. However, the prediction of a trade-off between growth and development as described in the 'Wilbur-Collins model', was unable to be tested due to tadpoles not reaching metamorphosis in stochastic food availability conditions (Wilbur and Collins, 1973). Such trade-offs may not have been observed in this study due to the long-term exposure to stochastic food availability conditions, whereby the threshold sizes or developmental stages were unable to be met. It is probable that the smaller larval size in stochastic food availability conditions resulted in tadpoles being unable to reach this threshold size (to increase developmental rate) within the experimental period. When the experiment was terminated, tadpoles in the stochastic food treatment were still displaying positive growth. Therefore, if the experiment had continued it is possible that tadpoles under these conditions would have reached the minimum size required for metamorphosis, and these individuals would have metamorphosed later and at a smaller size (*see* Lind *et al.*, 2008). Such a result would have provided support for the Wilbur Collins Model.

Exposure to stochastic food availability conditions throughout development impeded the ability of tadpoles to reach metamorphosis, contrary to the predictions of the 'stochastic dynamic programming model' which predicts that larvae respond by pupating earlier and at a smaller size when exposed to changes in food availability throughout development (Tenhumberg *et al.*, 2000). This inability of *L. peronii* to reach metamorphosis in stochastic food availability conditions suggests that a lack of constant food supply may induce developmental stasis. Developmental stasis may occur due to the inability to reach the threshold size or developmental stage required to increase developmental rates (Wilbur and Collins, 1973). Induced developmental stasis due to changes in food supply has previously been observed in Spadefoot toad species (Morey and Reznick, 2000). Spadefoot toad larvae accelerated development in response to restricted food supply, however, individuals that had not met the minimum threshold size for development entered developmental stasis (Denver *et al.*, 1998; Morey and Reznick, 2000). An alternative reason why tadpoles didn't reach metamorphosis is that tadpoles didn't have enough time to reach the developmental threshold required to

metamorphose. Tadpoles were still growing when the experiment was terminated, so metamorphosis might have been reached if the experimental period was extended. For this reason, future studies investigating the effect of stochastic food availability on larval growth and development should maintain tadpoles under treatment conditions until either growth rates plateau, or tadpoles reach metamorphosis.

According to the 'TSR rule' (Kozłowski *et al.*, 2004) growth rate is expected to increase with increasing water temperature because temperature regulates metabolism, growth and differentiation in ectothermic species (Álvarez and Nicieza, 2002b; McLeod *et al.*, 2013). In support of the TSR and the second hypothesis, this study shows that *L. peronii* display increased growth with increased water temperature in constant food availability conditions. However, under conditions of stochastic food availability, the effects of water temperature were reduced, suggesting that food availability may restrict the overall energy available for somatic growth processes, thereby preventing significant differences in growth at different water temperatures (Enriquez-Urzelai *et al.*, 2013; Inatsuchi *et al.*, 2010; Yoneda and Wright, 2005).

The slowed growth and developmental rates under conditions of stochastic food availability may have resulted from an overall metabolic down-regulation, as predicted by the 'metabolic down-regulation model' (Keys *et al.*, 1950). While the metabolic rate of larvae was not quantified in this study, lack of food (food restriction) is expected to decrease metabolic costs by limiting growth and development (Hulbert *et al.*, 2007; Wang *et al.*, 2006). The 'fixed-rate model' predicts a slower growth rate under conditions of stochastic food availability, with temperature regulating the developmental rate, and cooler temperatures extending the length of the larval period (Travis, 1984). Based on the 'fixed-rate model', it would be expected that larvae reared in warmer waters would reach metamorphosis earlier, but exhibit a slower growth rate in stochastic food availability conditions. However, long-term exposure to stochastic food availability may reduce energy available for development, preventing metamorphosis, regardless of increased water temperature. Interestingly, there were no differences in time to metamorphosis or post-metamorphic size between water temperature treatments under constant food availability conditions, which may suggest that the extremes in water temperatures used in the present study may not have differed enough to cause differences in developmental rate. However, this does not seem likely

because temperature differences similar to those imposed in this study (18-26°C) have been shown to have drastic effects on the growth and development of various temperate breeding anuran species (*see* Álvarez and Nicieza, 2002a; Álvarez and Nicieza, 2002b; Blouin *et al.*, 2000; Browne *et al.*, 2003; Walsh *et al.*, 2008).

As mortality in the juvenile life stages of amphibians is typically high (Canessa *et al.*, 2014), it can be difficult to make generalisations about the effects of experimental treatments on growth, development and survivorship. For example, in this present study it is uncertain what component of the stochastic food availability treatment was important; i.e. whether duration of the fasting periods, intervals between fasting, duration of periods with high food availability or the differences total feeding rate between stochastic and constant food availability treatments were driving the observed differences in growth, development and survivorship. However, experimental studies can still be useful for making inferences about treatment effects (e.g. Kearney *et al.*, 2012; Kearney *et al.*, 2014). It was found that survivorship decreased in the warmest temperature treatments and stochastic food availability conditions buffered against mortality losses at higher temperatures. Warmer waters may have compromised survival, due to decreased oxygen-availability (Blaustein *et al.*, 2010; O'Connor *et al.*, 2007), oxidative stress due to higher metabolic rate (Hulbert *et al.*, 2007), the build-up of microbes from decomposing food (McWilliams, 2008) or nitrogenous waste products (Morey and Reznick, 2004) or changes in the intensity of competition (Álvarez and Nicieza, 2002b; Blaustein *et al.*, 2010; Enriquez-Urzelai *et al.*, 2013; McLeod *et al.*, 2013). A previous study examining the effects of long-term changes to food availability and water temperature on coral fish species also reported low survivorship in warmer waters and suggested that survival may have been compromised due to starvation. Consequently, when exposed to high food availability conditions, coral fish survivorship increased (McLeod *et al.*, 2013). Conversely in this study, the long-term stochastic food availability treatment reduced the effects of the warmest water temperatures, with higher survivorship in stochastic food availability treatments. Slower growth rate as a result of stochastic food availability conditions may reduce the effects of water temperature on survivorship in larval *L. peronii*. As a result, growth conditions may influence the risk of mortality (Enriquez-Urzelai *et al.*, 2013).

A number of studies have investigated the impacts of changes in food availability (or

quality) interacting with other environmental factors, and have observed induced changes in growth and development. These include interactions between food quality and temperature (Álvarez and Nicieza, 2002b), predation risk and food availability (Nicieza, 2000); and pond desiccation and food availability (Enriquez-Urzelai *et al.*, 2013). The interactions between environmental factors can be varied, with the interactive effects also being difficult to predict (Álvarez and Nicieza, 2002b). For example, in a study investigating the effects of water temperature and food quality on growth and development in Iberian Painted Frogs (*Discoglossus galganoi*), it was found that water temperature had persistent effects on development and metamorphic traits, with larvae metamorphosing later and at larger body size when reared at lower temperatures. However, the effects of food quality on growth and development were largely dependent on water temperature, with larvae fed carbohydrate-rich diets being smaller at metamorphosis compared to larvae fed protein-rich diets, but not at all water temperatures (Álvarez and Nicieza, 2002b). Conversely, Enriquez-Urzelai *et al.* (2013) investigated the interactive effects between food availability and desiccation on the Painted Frog (*Discoglossus pictus*), observing size and weight at metamorphosis were determined by food availability, but not by the water desiccation regime. The results of the present study strongly suggest that environmental differences in food availability and water temperature, and their interaction, cause differences in growth, development and survivorship. In this experiment, it was determined that water temperature was more important than food availability for survivorship, growth and development, a pattern that has also has been described in larval coral fish species (McLeod *et al.*, 2013).

5.5.1 Implications for amphibian conservation

Our finding that changes in food availability mediate the effects of temperature on *L. peronii* growth, development and survivorship has implications for amphibian conservation. Similar to *L. peronii*, many threatened anurans are temperate-zone pond-breeding species in which larvae experience marked fluctuations in temperature and food availability over extended developmental periods (i.e. >2 months). Captive breeding programmes attempting to breed such species generally rear tadpoles under constant environmental conditions, but my findings suggest that managers might benefit from manipulating both food availability and temperature. Specifically, providing

individuals with stochastic food availability at warmer temperatures may improve individual survivorship, and the likelihood of generating large numbers of tadpoles. However, captive breeding practitioners and managers should assess the practicality of using stochastic treatments; for example, providing threshold feeding rates to determine what combination produces the fastest growth and development, and highest survivorship, may be a more practical approach.

The capacity for most CBPs to test multiple aspects that potentially influence the quality or quantity of animal produced is often constrained by a limited number of animals or resources. However, I suggest that CBPs could adopt a more experimentally informed approach to improve outcomes. For example, if the mechanism for phenotypic change (e.g. manipulating food availability or water temperature) can be identified, it provides a tool to manipulate phenotypic traits, and generate phenotypes or animals that are more suitable for reintroduction. In this case, using closely related model species to guide the management of captive breeding for endangered species, as explored in this present study, is valuable. In addition, replicating natural conditions during early development may lead to animals being able to display phenotypic plasticity, with phenotypes produced that match the local conditions (Norberg et al., 2001; Monaghan 2008). Overall I suggest that using a more experimental approach could benefit the recovery of a target species by improving the sustainability of a captive 'insurance' population, while minimising expenses associated with establishing and maintaining colonies (Canessa *et al.*, 2014). Furthermore, generating large numbers of individuals for release could improve the success of reintroduction programmes by ensuring the release of large groups, which could overcome problems associated with high dispersal, demographic stochasticity, or low reproduction and/or survival at low population densities (Allee effects) (Armstrong and Seddon, 2007; Fischer and Lindenmayer, 2000). For these reasons, I propose that broadening our knowledge of the effects of interactions between environmental factors on anuran growth, development and survivorship might improve the success of amphibian threatened species management (Carey, 2005; Muths *et al.*, 2014).

5.5.2 Conclusions

In conclusion, the aim of this study was to use a manipulative laboratory experiment to examine the independent and interactive effects of long-term stochastic food availability

and water temperature on larval *L. peronii* survivorship, growth and development. Larval growth rate was highest, and survivorship was lowest, at the warmest temperatures. However, changes in food availability mediated the effects of temperature, with slower larval growth and higher survivorship in stochastic food availability treatments at warmer water temperatures. These findings contribute to a small but growing body of evidence that interactions between environmental factors can influence anuran growth, development and survivorship. Such advances have the potential to improve the output of amphibian captive breeding programmes and aid amphibian conservation.

5.6 References

Álvarez D and Nicieza A (2002a) Effects of induced variation in anuran larval development on postmetamorphic energy reserves and locomotion. Oecologia **131:** 186 – 195

Álvarez D and Nicieza AG (2002b) Effects of temperature and food quality on anuran larval growth and metamorphosis. Functional Ecology **16:** 640 – 648

Andersen PK and Gill RD (1982) Cox's regression model for counting processes: a large sample study. The Annals of Statistics **1:** 1100 – 1120

Angilletta MJ, Steury TD and Sears MW (2004) Temperature, growth rate, and body size in ectotherms: fitting pieces of a life-history puzzle. Integrative and Comparative Biology **44:** 498 – 509

Anstis M (2013) Tadpoles and frogs of Australia. New Holland Publishers, Chatswood, NSW

Armstrong DP Seddon PJ (2008) Directions in reintroduction biology. Trends in Ecology and Evolution **23:** 20 – 25

Blaustein AR, Walls SC, Bancroft BA, Lawler JJ, Searle CL and Gervasi SS (2010) Direct and indirect effects of climate change on amphibian populations. Diversity **2:** 281 – 313

Blouin MS and Brown ST (2000) Effects of temperature-induced variation in anuran larval growth rate on head width and leg length at metamorphosis. Oecologia **125:** 358 – 361

Browne R and Edwards D (2003) The effect of temperature on the growth and development of the endangered green and golden bell frog *Litoria aurea*. Journal of Thermal Biology **28:** 295 – 299

Bull CD, Metcalfe NB and Mangel M (1996) Seasonal matching of foraging to anticipated energy requirements in anorexic juvenile salmon. Proceedings of the Royal Society of London. Series B: Biological Sciences **263:** 13 – 18

Canessa S, Hunter D, McFadden M, Marantelli G and McCarthy MA (2014) Optimal release strategies for cost-effective reintroductions. Journal of Applied Ecology **51:** 1107 – 1115

Capellan E and Nicieza A (2007) Non-equivalence of growth arrest induced by predation risk or food limitation: context-dependent compensatory growth in anuran tadpoles. Journal of Animal Ecology **76:** 1026 – 1035

Carey C (2005) How physiological methods and concepts can be useful in conservation biology. Integrative and Comparative Biology **45:** 4 – 11

Chinathamby K, Reina RD, Bailey PCE and Lees BK (2006) Effects of salinity on the survival, growth and development of tadpoles of the brown tree frog, *Litoria ewingii*. Australian Journal of Zoology **54:** 97 – 105

Christy MT and Dickman CR (2002) Effects of salinity on tadpoles of the green and golden bell frog (*Litoria aurea*). Amphibia-Reptilia **23:** 1 – 11

Cooke SJ (2009) Conservation physiology: opportunities and challenges. Comparative Biochemistry and Physiology Part A: Molecular and Integrative Physiology **153:** S56

Cothran RD, Gervasi SS, Murray C, French BJ, Bradley PW, Urbina J, Blaustein AR and Relyea RA (2015) Carotenoids and amphibians: effects on life history and susceptibility to the infectious pathogen, *Batrachochytrium dendrobatidis*. Conservation Physiology **3:** cov005

Day T and Rowe L (2002) Developmental thresholds and the evolution of reaction norms for age and size at life-history transitions. American Naturalist **159:** 338 – 350

De Jong G (2005) Evolution of phenotypic plasticity: patterns of plasticity and the emergence of ecotypes. New Phytologist **166:** 101 – 118

Denver RJ, Mirhadi N and Phillips M (1998) Adaptive plasticity in amphibian metamorphosis: response of *Scaphiopus hammondii* tadpoles to habitat desiccation. Ecology **79:** 1859 – 1872

DeWitt TJ, Sih A and Wilson DS (1998) Costs and limits of phenotypic plasticity. Trends in Ecology and Evolution **13:** 77 – 81

Dugas MB, Yeager J and Richards-Zawacki CL (2013) Carotenoid supplementation enhances reproductive success in captive strawberry poison frogs (*Oophaga pumilio*). Zoo biology **32:** 655 – 658

Dziminski MA and Roberts JD (2006) Fitness consequences of variable maternal provisioning in quacking frogs (*Crinia georgiana*). Journal of Evolutionary Biology **19:** 144 – 155

Dziminski MA, Roberts JD and Simmons LW (2008) Fitness consequences of parental compability in the frog *Crinia georgiana*. Evolution **62:** 879 – 886

Enriquez-Urzelai U, San Sebastián O, Garriga N and Llorente G (2013) Food availability determines the response to pond desiccation in anuran tadpoles. Oecologia **173:** 117 – 127

Franklin C (2008) Climate change and conservation physiology. Comparative Biochemistry and Physiology Part A: Molecular and Integrative Physiology **150:** S167

Franklin CE (2009) Conservation physiology: assessing and forecasting the responses of organisms to environmental change. Comparative Biochemistry and Physiology Part A: Molecular and Integrative Physiology **153:** S56

Gascon C, Collins JP, Moore RD, Church DR, McKay JE and Mendelson JR III (eds) (2007) Amphibian conservation action plan. IUCN/SSC Amphibian Specialist Group. Gland, Switzerland and Cambridge, UK.

Germano JM and Bishop PJ (2009) Suitability of amphibians and reptiles for translocation. Conservation Biology **23:** 7 – 15

Gillespie GR (2002) Impacts of sediment loads, tadpole density, and food type on the growth and development of tadpoles of the spotted tree frog *Litoria spenceri*: an in-stream experiment. Biological Conservation **106:** 141 – 150

Gosner KL (1960) A simplified table for staging anuran embryos and larvae with notes on identification. Herpetologica **16:** 183 – 190

Griffiths RA and Pavajeau L (2008) Captive breeding, reintroduction, and the conservation of Amphibians. Conservation Biology **22:** 852 – 861

Hulbert AJ, Pamplona R, Buffenstein R and Buttemer WA (2007) Life and death: metabolic rate, membrane composition, and life span of animals. Physiological reviews **87:** 1175 – 1213

Inatsuchi A, Yamato S and Yusa Y (2010) Effects of temperature and food availability on growth and reproduction in the neustonic pedunculate barnacle *Lepas anserifera*. Marine Biology **157:** 899 – 905

Kearney BD, Byrne PG and Reina RD (2012) Larval tolerance to salinity in three species of Australian anuran: an indication of saline specialisation in *Litoria aurea*. PLoS ONE **7:**e43427

Kearney BD, Pell RJ, Byrne PG and Reina RD (2014) Anuran larval developmental plasticity and survival in response to variable salinity of ecologically relevant timing and magnitude. Journal of Experimental Zoology Part A: Ecological Genetics and Physiology **321:** 541 – 549

Keys A, Brožek J, Henschel A, Mickelsen O and Taylor HL (1950) The biology of human starvation. University of Minnesota Press, Minneapolis, MN.

Kozłowski J, Czarnołęski M and Dańko M (2004) Can optimal resource allocation models explain why ectotherms grow larger in cold? Integrative and Comparative Biology **44:** 480 – 493

Kraft PG, Wilson RS and Franklin CE (2005) Predator-mediated phenotypic plasticity in tadpoles of the striped marsh frog, *Limnodynastes peronii*. Austral Ecology **30:** 558 – 563

Leips J and Travis J (1994) Metamorphic responses to changing food levels in two species of hylid frogs. Ecology **75:** 1345 – 1356

Lema SC and Nevitt GA (2006) Testing an ecophysiological mechanism of morphological plasticity in pupfish and its relevance to conservation efforts for endangered Devils Hole pupfish. Journal of Experimental Biology **209:** 3499 – 3509

Lin X and Zhang D (1999) Inference in generalized additive mixed models by using smoothing splines. Journal of the Royal Statistical Society: Series B (Statistical Methodology) **61:** 381 – 400

Lind MI, Persbo F and Johansson F (2008) Pool desiccation and developmental thresholds in the common frog, *Rana temporaria*. Proceedings of the Royal Society B: Biological Sciences **275:** 1073 – 1080

Mantellato L, Gaikhorst G, Kruger R, Vitali S and Robertson H (2013) Growth and development of captive *Geocrinia rosea* (Myobatrachidae): a rare species analogue. Zoo biology **32:** 374 – 380

McLeod IM, Rummer JL, Clark TD, Jones GP, McCormick MI, Wenger AS and Munday PL (2013) Climate change and the performance of larval coral reef fishes: the interaction between temperature and food availability. Conservation Physiology **1:** cot024

McWilliams DA (2008) Nutrition recommendations for some captive amphibian species (Anura and Caudata). The Canadian Association of Zoos and Aquariums Nutrition Advisory and Research Group. (GARN-NARG)

Miner BG, Sultan SE, Morgan SG, Padilla DK and Relyea RA (2005) Ecological consequences of phenotypic plasticity. Trends in Ecology and Evolution **20:** 685 – 692

Monaghan P (2008) Early growth conditions, phenotypic development and environmental change. Philosophical Transactions of the Royal Society B: Biological Sciences **363:** 1635 – 1645

Morey S and Reznick D (2000) A comparative analysis of plasticity in larval development in three species of Spadefoot toads. Ecology **81:** 1736 – 1749

Morey SR and Reznick DN (2004) The relationship between habitat permanence and larval development in California Spadefoot toads: field and laboratory comparisons of developmental plasticity. Oikos **104:** 172 – 190

Munn A, Kern P and McAllan B (2010) Coping with chaos: unpredictable food supplies intensify torpor use in an arid-zone marsupial, the fat-tailed dunnart (*Sminthopsis crassicaudata*). Naturwissenschaften **97:** 601 – 605

Muths E, Bailey LL and Watry MK (2014) Animal reintroductions: an innovative assessment of survival. Biological Conservation **172:** 200 – 208

Newman RA (1998) Ecological constraints on Amphibian metamorphosis: interactions of temperature and larval density with responses to changing food level. Oecologia **115:** 9 – 16

Nicieza AG (2000) Interacting effects of predation risk and food availability on larval anuran behaviour and development. Oecologia **123:** 497 – 505

Nicieza AG and Metcalfe NB (1997) Growth compensation in juvenile Atlantic salmon: responses to depressed temperature and food availability. Ecology **78:** 2385 – 2400

Niehaus AC, Wilson RS and Franklin CE (2006) Short- and long-term consequences of thermal variation in the larval environment of anurans. Journal of Animal Ecology **75:** 686 – 692

Norberg J, Swaney DP, Dushoff J, Lin J, Casagrandi R and Levin SA (2001) Phenotypic diversity and ecosystem functioning in changing environments: a theoretical framework. Proceedings of the National Academy of Sciences **98:** 11376 – 11381

O'Connor MI, Bruno JF, Gaines SD, Halpern BS, Lester SE, Kinlan BP and Weiss JM (2007) Temperature control of larval dispersal and the implications for marine ecology, evolution, and conservation. Proceedings of the National Academy of Sciences **104:** 1266 – 1271

Ogilvy V, Preziosi RF and Fidgett AL (2012) A brighter future for frogs? The influence of carotenoids on the health, development and reproductive success of the red-eye tree frog. Animal Conservation **15:** 480 – 488

Pfennig DW, Wund MA, Snell-Rood EC, Cruickshank T, Schlichting CD and Moczek AP (2010) Phenotypic plasticity's impacts on diversification and speciation. Trends in Ecology and Evolution **25:** 459 – 467

Pinheiro J, Bates D, DebRoy S and Sarkar D (2014) Nlme: linear and nonlinear mixed effects models. R package version 3.1–117. *URL: http://cran.r-project. org/web/packages/nlme/index*

Quinn GP and Keough MJ (2002) Experimental design and data analysis for biologists. Cambridge University Press.

R Developer Core Team (2014). R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna Austria

Reed TE, Waples RS, Schindler DE, Hard JJ and Kinnison MT (2010) Phenotypic plasticity and population viability: the importance of environmental predictability. Proceedings of the Royal Society B: Biological Sciences **277:** 3391 – 3400

Relyea RA (2001) Morphological and behavioral plasticity of larval anurans in response to different predators. Ecology **82:** 523 – 540

Rose CS (2005) Integrating ecology and developmental biology to explain the timing of frog metamorphosis. Trends in Ecology and Evolution **20:** 129 – 135

Rosen DAS, Volpov BL and Trites AW (2014) Short-term episodes of imposed fasting have a greater effect on young northern fur seals (*Callorhinus ursinus*) in summer than in winter. Conservation Physiology **2:** cou021

Sanuy D, Oromí N and Galofré A (2008) Effects of temperature on embryonic and larval development and growth in the natterjack toad (*Bufo calamita*) in a semi-arid zone. Animal Biodiversity Conservation **31:** 41 – 46

Schell C and Burgin S (2002) Swimming against the current: the brown striped marsh frog *Limnodynastes peronii* success story. Australian Zoologist **32:** 401 – 405

Sheldon BC, Arponen H, Laurila A, Crochet PA and Merilä J (2003) Sire coloration influences offspring survival under predation risk in the moorfrog. *Journal of* Evolutionary Biology **16:** 1288 – 1295

Stuart SN, Chanson JS, Cox NA, Young BE, Rodrigues AS, Fischman DL and Waller RW (2004) Status and trends of amphibian declines and extinctions worldwide. Science **306:** 1783 – 1786

Tarszisz E, Dickman CR and Munn AJ (2014) Physiology in conservation translocations. Conservation Physiology **2:** cou054

Tenhumberg B, Tyre AJ and Roitberg B (2000) Stochastic variation in food availability influences weight and age at maturity. Journal of Theoretical Biology **202:** 257 – 272

Therneau TM and Grambsch, PM (2000). Modeling survival data: extending the cox model. Springer, New York

Therneau T (2014) A package for survival analysis in s. 2014, r package version 2.37-7 http://CRAN.R-project.org/package=survival

Travis J (1984) Anuran size at metamorphosis: experimental test of a model based on intraspecific competition. Ecology **65:** 1155 – 1160

Wake DB and Vredenburg VT (2008) Are we in the midst of the sixth mass extinction? A view from the world of amphibians. Proceedings of the National Academy of Sciences **105:** 11466 – 11473

Walsh PT, Downie JR and Monaghan P (2008) Temperature-mediated morphology changes during metamorphic climax in the African clawed frog, *Xenopus laevis*. Journal of Thermal Biology **33:** 244 – 249

Walters RJ and Hassall M (2006) The temperature-size rule in ectotherms: may a general explanation exist after all? The American Naturalist **167:** 510 – 523

Wang T, Hung CCY and Randall DJ (2006) The comparative physiology of food deprivation: from feast to famine. Annual Review of Physiology **68:** 223 – 251

Wikelski M and Cooke SJ (2006) Conservation physiology. Trends in Ecology and Evolution **21:** 38 – 46

Wilbur HM and Collins JP (1973) Ecological aspects of amphibian metamorphosis: nonnormal distributions of competitive ability reflect selection for facultative metamorphosis. Science **182:** 1305 – 1314

Wilson RS (2001) Geographic variation in thermal sensitivity of jumping performance in the frog *Limnodynastes peronii*. Journal of Experimental Biology **204:** 4227 – 4236

Wood S (2014). Gamm4: generalized additive mixed models using mgcv and lme4. Version 0.2-2. R package

Yoneda M and Wright PJ (2005) Effect of temperature and food availability on reproductive investment of first-time spawning male Atlantic cod, *Gadus morhua*. ICES Journal of Marine Science: Journal du Conseil **62:** 1387 – 1393

Zuur A, Ieno EN, Walker N, Saveliev AA and Smith GM (2009) Mixed effects models and extensions in ecology with R. Springer Science and Business Media.

6 GENERAL DISCUSSION, CONCLUSIONS AND RECOMMENDATIONS

6.1 Assessment of captive breeding programmes

Captive breeding programmes assist in recovery of threatened taxa by generating animals for reintroduction and supplementing wild populations, with captive breeding being the primary recovery action for many threatened species. However, only 13% of reported reintroductions with a captive source population have resulted in the successful establishment of self-sustaining viable populations following reintroduction (Fischer and Lindenmayer 2000). To address this low success, the central focus of many captive breeding programmes has been to identify and counter adverse genetic changes that occur in captivity, including loss of genetic diversity, inbreeding depression and genetic adaptations to captivity, all of which can compromise population viability, and the success of reintroduction programmes (Frankham 2008; Williams and Hoffman 2009). However, the causes of reintroduction failure vary and have been associated with phenotypic change of captive-bred animals (Snyder *et al.*, 1996; Fischer and Lindenmayer 2000; O'Regan and Kitchener 2005; Tarszisz *et al.*, 2014). The ability of an individual to adjust its phenotype in response to abiotic and biotic environmental factors in captivity may result from phenotypic plasticity (Miner *et al.*, 2005; Schulte-Hostedde and Mastromonaco 2015). These plastic changes in an animal's phenotypes can occur in response to environmental challenges, often during development, but may also result from lagged effects of environmental conditions on the parental generation (Monaghan 2008). Ultimately, a range of factors will determine the success of reintroductions. For example, reintroduction with captive source populations success may be improved through pre-release screening for suitable traits (e.g. Mathews *et al.*, 2005), or pre-release training to reinforce appropriate behaviours (e.g. Shier and Owings, 2006). However, our understanding of phenotypic trait change in captivity remains limited and may be a key factor in the poor success of reintroductions due to the reduced fitness of captive reared individuals following reintroduction. Despite this possibility, few studies have explicitly examined the effect captivity on phenotypic traits.

6.2 Research framework

In this thesis, I aimed to outline how an animal's phenotype may contribute to the success or failure of CBPs, and, in turn, reintroduction success, with a specific focus on changes to behaviour, morphology, and growth and development that occur in captivity. Further, I explored how manipulating environmental conditions in captivity can be used to promote phenotypic plasticity, and the potential for inducing the expression of favourable phenotypic traits in populations of captive-bred species. I used a mammalian and an amphibian species as models for determining the effect of captivity on phenotypes and specifically looked at developmental, morphological and behavioural phenotypes. Model species provided a suitable alternative to examining phenotypic changes in captivity and provided valuable information for applying to endangered species captive breeding programmes. First, using house mouse (*Mus musculus*) as a model species, I sought to determine whether i) behaviour and morphology in captive-reared and wild-caught animals differed, ii) there were transgenerational effects on behaviour and morphology, and iii) there were differences between sexes in response to captivity (Chapters 2 and 3). I then examined the heritability of multiple phenotypic traits (behavioural and morphological) using house mouse (*Mus musculus*) maintained in captivity to illuminate how the captive phenotype can shift away from the wild phenotype with each subsequent generation in captivity, via transgenerational effects (Chapter 4). Finally, I examined how independent and interactive effects of long-term stochastic environmental conditions in captivity, specifically food availability and water temperature, influence larval growth, development and survivorship of the striped marsh frog (*Limnodynastes peronii*), and how manipulating the captive environment can alter developmental trajectories and improve the output of amphibian captive breeding programmes (Chapter 5). In each chapter, I discussed the findings and how they contribute to the current knowledge of captive breeding programmes and reintroductions, and considered the wider implications and future directions of my findings for the role of phenotypic variation in captive breeding programmes.

6.3 The effects of captivity on phenotypic traits

Captive-reared mice differed in their behavioural and morphological phenotypes compared to wild-caught mice (Chapter 2 and 3). These findings support the wealth of literature reporting that captivity can alter phenotypic traits in a variety of taxa (Snyder

et al., 1996; O'Regan and Kitchener 2005; McDougall *et al.*, 2006; Table 1.1). The differences in phenotypic traits between captive-reared and wild populations were expected due to the inherent differences in a multitude of biotic and abiotic factors, and associated differences in selection pressures between captive and wild environments (Burns *et al.*, 2009; Mason *et al.*, 2013). Specifically, for behaviour, captive-reared mice were found to differ in their boldness and activity behavioural type compared to wild-caught mice, with behavioural traits indicating an increase in activity and boldness in captive-reared mice. Although behavioural type in captivity did not differ depending on sex, behavioural types did differ between wild-caught females and males, suggesting there is a loss of sex-specific behaviours in captivity. (Chapter 2). For morphology, captive-reared mice did not differ in external morphology, however this masked more pronounced and potentially detrimental internal morphological changes. Specifically, captive-reared individuals had lighter kidneys and spleens and shorter small intestine lengths compared to wild-caught individuals. Furthermore, sex-based morphological differences were maintained in the captive rearing environment. Finally, internal morphology adjusted within the acclimation period, suggesting that internal morphological traits were plastic (Chapter 3). My results suggest that captivity can result in phenotypic changes in behavioural and morphological traits. Further, while sexual dimorphism can be maintained, there may be a loss of sex-specific behaviours in captivity. In addition, phenotypic plasticity may also have a significant influence on phenotypic change in response to captivity. By identifying phenotypic traits that change in captivity, we stand to gain valuable knowledge for developing and refining methodologies to minimise unfavourable phenotypic changes in captivity. In turn, this knowledge may be used to improve captive-breeding and reintroduction programmes (McDougall *et al.*, 2006).

My findings that captivity can result in phenotypic change have significant implications for CBPs. Firstly, identifying the specific phenotypic traits that change in captivity, and whether these phenotypic changes differed between sexes, can help managers develop and refine approaches used in captive-breeding and reintroduction programmes (McDougall *et al.*, 2006). However, without evaluating the fitness of the captive-reared mice upon reintroduction, any implications for reintroduction success or post-release fitness should be considered with caution. Past studies have shown that the 'captive'

phenotype can remain unchanged, or move toward or away from the 'wild' phenotype. However, in general, we might expect the phenotypic traits to show adaptations to captivity (Mason *et al.*, 2013). If a phenotype in captivity shifts from an adaptive 'wild' phenotype, it is valuable to determine the ongoing impact of these phenotypic changes on individual fitness, particularly if these phenotypic changes have consequences for the viability of captive populations, and/or impact the probability of reintroduction success. However, predicting what phenotypic traits will be affected in captivity, and the magnitude and direction of change in any given trait can be challenging. By making holistic assessments of phenotypic trait change in captivity, future studies then can explicitly compare or even manipulate environmental factors in captivity to provide robust inferences about the mechanisms for phenotypic change in captivity. Indeed, while I found negligible changes in the external morphology of captive-reared mice, this masked significant change in internal morphology. Consequently, rapid and untracked changes in internal morphology could have severe and unforeseen effects on the viability of animals held in captivity by influencing key physiological traits, such as digestive efficiency (Bailey *et al.*, 1997; Champagnon *et al.*, 2012) and immune responses and disease resistance (Bonnet *et al.*, 1998; Swallow *et al.*, 2005; van Oosterhout *et al.*, 2007; Tschirren *et al.*, 2009).

The comparative approach (comparing captive-reared with wild-caught animals) used in this study allowed for predictions to be made about how phenotypic traits, such as behaviour or morphology in captivity, may impact fitness of individuals following reintroduction (Bremner-Harrison *et al.*, 2004; Mathews *et al.*, 2005; Champagnon *et al.*, 2012). Although I was unable to evaluate post-reintroduction success, studies have reported links between behavioural and morphological changes occurring in captivity and post-reintroduction fitness (Bremner-Harrison *et al.*, 2004; Champagnon *et al.*, 2012). Importantly, there is currently limited information on the effect of internal changes on the post-release viability of captive-reared animals, although there is some evidence of these effects in birds (Champagnon *et al.*, 2012). In the present study, without evaluating the fitness of the captive-reared mice upon reintroduction, any implications for reintroduction success should be considered with caution. Future research on captive animals would benefit from investigating how phenotypic changes, such as changes in internal morphology, are maladaptive under natural conditions, and

whether these impacts can be mitigated by manipulating the captive environment. In my study, wild mice displayed phenotypic plasticity within the acclimation period in internal morphological traits, however there were no significant changes in external morphology, suggesting that external morphology traits may be less plastic, with changes in external morphology occurring more slowly, and taking multiple generations to manifest (McPhee 2004a, b; O'Regan and Kitchener 2005). Furthermore, evidence of other phenotypic traits displaying fast, repeatable and reversible changes in captivity is limited (Piersma and Lindström 1997; Starck 1999; McWilliams and Karasov 2001; Piersma and Drent 2003; Bremner-Harrison *et al.*, 2004). But if phenotypic traits can be shown to be plastic, phenotypic traits may be able to be altered to suit the wild environment prior to release (*see* Chapter 5); approaches such as pre-release exposure, which increase likelihood of survival following release, may be required for phenotypic plasticity to occur (Moseby *et al.*, 2014). Alternatively, if traits are shown to have limited or no plasticity, we can apply the criterion for selecting animals with phenotypic traits suitable for reintroduction (Bremner-Harrison *et al.*, 2004).

For most animal groups the effects of captivity on sex-specific behaviour and sexual dimorphism remain unknown. Since sexual selection favours different trait values in males and females, I expected a loss or change in sexual dimorphism in captivity due to changes in the strengths and targets of sexual selection, and reduced resource competition (Lynch and Hayden 1995; O'Regan and Kitchener 2005). However, sexual dimorphism in both external and internal morphology were maintained in captive-reared mice. The maintenance of sexual dimorphism suggests that the intensity and direction of sexual selection on morphological traits may remain unchanged in the captive environment. Alternatively, changes or loss of sexual dimorphism may take multiple generations to manifest, and may have remained undetected (McPhee 2004b).

Conversely, my findings that captivity potentially leads to the loss of sex-specific behaviour have provided important insights into the possible impacts of captivity on behavioural phenotypes. Specifically, this suggests that the sexes may need to be treated differently during the management of captive colonies, or when establishing reintroduction programmes. Gaining further information on sex-specific responses to captivity will determine whether the development of effective sex-specific management strategies in captivity is required. In recognition of this possibility, several recent

studies have started to explore whether sexual selection theory can be used to inform management strategies (Chargé *et al.*, 2014; Slade *et al.*, 2014).

6.4 Multiple generations in captivity: effect on phenotypic traits

There was evidence of transgenerational effects on behaviour and morphology in captivity (Chapter 2 and 3). These findings contribute to the literature reporting that animals maintained in captivity for multiple generations typically display a consistent directional shift in phenotypic traits away from the wild phenotype towards an optimal mean trait value for captive conditions, presumably through transgenerational effects (Snyder *et al.*, 1996; McPhee 2004a, b; O'Regan and Kitchener 2005; McDougall *et al.*, 2006; McPhee and Carlstead 2010; McPhee and McPhee 2012; Evans *et al.*, 2014) (Table 1.1). It is important to consider how selection in captivity operates on phenotypic traits across generations because it is not uncommon for multiple generations to be maintained in captivity prior to reintroduction. Specifically, three behavioural traits displayed a shift away from wild behaviours (Chapter 2) and evidence of transgenerational effects in captivity was observed in internal morphology, and only in females (Chapter 3). While these transgenerational changes in phenotypic traits are likely to increase fitness within the captive environment (McPhee 2004a, b; McPhee and McPhee 2012; Mason *et al.*, 2013; Johnson *et al.*, 2014), transgenerational changes in captivity are also likely to have significant implications for captive-bred animals following release (O'Regan and Kitchener 2005; Slade *et al.*, 2014). For example, transgenerational changes on phenotypic traits may include loss of anti-predator responses, reduced exploratory behaviour (Håkansson and Jensen 2005; Håkansson and Jensen 2008) and shifts in body mass important for mating preference (Slade *et al.*, 2014). The evidence of transgenerational effects on phenotypic traits in captivity observed in my study highlights the importance of manipulating the captive environment to reduce phenotypic changes occurring across generations in captivity. In turn, this may improve the fitness of animals following reintroduction. Approaches could include providing exposure to natural conditions during early development, which may reduce the phenotypic changes occurring in captivity (Evans *et al.*, 2014). Despite potential for trait change in the captive environment, few studies have examined the effects of captivity on phenotypic traits across multiple generations. Furthermore,

understanding the mechanisms underpinning transgenerational change in captivity is critical.

To begin to understand and identify the mechanisms driving transgenerational effects, I used broad sense heritability to estimate the amount of variation in a phenotypic trait explained by genetic variation (Falconer *et al.*, 1996) (Chapter 4). The heritability estimates for boldness and activity behavioural types and external morphology ranged from low to moderate; and an estimate for internal morphology was unable to be determined. However, all heritability estimates were not considered statistically significant, likely due to small sample sizes resulting from low breeding success. These findings were consistent with other studies suggesting that behaviour and morphology may be heritable (Dingemanse *et al.*, 2002; Drent *et al.*, 2003; Dor and Lotem 2009; Ariyomo *et al.*, 2013). However, my findings suggest that some but not all phenotypic traits may be heritable and this may allow for rapid adaptation to captive conditions. Heritability of phenotypic traits can illuminate the evolutionary potential for a phenotypic trait (such as behaviour or morphology) to respond to selection pressures imposed by the captive environment (Falconer *et al.*, 1996; Réale and Festa-Bianchet 2000; Richards *et al.*, 2010). For other traits that did not display a shift in response to captive conditions (Chapter 2 and 3), this may indicate strong influence by environmental conditions and may display a high degree of plasticity. The low to moderate degrees of heritability suggest that the individual and combinatory effects of genetic change and transgenerational plasticity may allow transgenerational effects to occur in captivity (Houle 1992; Rodriguez-Clark 2004). Overall, my findings demonstrate that an understanding of the mechanisms that drive transgenerational effects can be potentially harnessed to enhance the resilience of organisms following reintroduction into natural environments (Evans *et al.*, 2014; Chakravarti *et al.*, 2016). This may include developing adaptive control measures to address genetic change in captivity. For example, using adaptive control measures could include manipulating environmental conditions in captivity to allow for more heterogeneous conditions or indeed more naturalised wild environments to reduce genetic adaptations to captivity (Ashley *et al.*, 2003), or by exposing parental generations to wild environments to mediate the effects of captivity for future generations (Evans *et al.*, 2014; Chakravarti *et al.*, 2016).

6.5 Manipulating the rearing environment

The evidence of the effects of captivity on phenotypic traits observed in my study highlighted the importance of manipulating the captive environment to reduce phenotypic changes occurring in captivity. Further, identifying the phenotypic traits that change, and the specific mechanisms (i.e. the abiotic and biotic factors) associated with phenotypic change in captivity, can help managers develop and refine approaches used in captive-breeding and reintroduction programmes. Throughout my thesis, I have suggested that increasing the level of phenotypic variation within a captive population could improve the likelihood for success upon release (Chapter 1.2). Increasing phenotypic variation may be possible via multiple phenotypes (polyphenism), or phenotypic plasticity, being expressed by a population with the outcome of either a sub-population acquiring phenotypes suitable for the wild (Post *et al.*, 1997; Kussell and Leibler 2005), or individuals able to change their phenotype in response to the environment conditions experienced in the wild (Miner *et al.*, 2005). To promote polyphenism or phenotypic plasticity, in this thesis I have suggested that CBPs could adopt a more experimentally informed approach to improve outcomes (Chapter 5). For example, if the mechanism for phenotypic change (e.g. manipulating specific factors within the captive environment) can be identified, it provides a tool to manipulate species' phenotypic traits, and generate phenotypes or animals that are more suitable for reintroduction.

In my manipulative laboratory experiment in which I examined the independent and interactive effects of environmental conditions in captivity (specifically long-term stochastic food availability and water temperature), I observed changes in larval *L. peronii* growth, development and survivorship (Chapter 5). Specifically, larval growth rate was highest, and survivorship was lowest, in warmer water temperatures. However, these phenotypic changes in response to water temperature were mediated by food availability. Tadpoles were smaller and had higher survivorship to week 14, in stochastic food availability compared to constant food availability in warmer water temperature treatments. My findings contribute to a small but growing body of evidence that interactions between environmental factors can influence phenotypic traits such as growth, development and survivorship (Álvarez and Nicieza 2002a; Álvarez and Nicieza 2002b; McLeod *et al.*, 2013). By identifying how environmental conditions in

captivity influence phenotypic traits, we have the potential to improve the output of CBPs and gain valuable knowledge for developing and refining methodologies to minimise unfavourable phenotypic changes in captivity.

My findings suggest that all CBP rearing methodologies may benefit from featuring challenging abiotic and biotic characteristics of the animal's original environment within captive settings. Indeed, there are examples of CBPs aiming to produce animals for reintroductions by exposing potential founders to the characteristics of the proposed recipient release environment (e.g. Evans *et al.*, 2014; Munkwitz *et al.*, 2005; Moseby *et al.* 2014; Whiteside *et al.* 2015). These challenges, dependent on the recommended CBP approach, should be either provided continuously or stochastically throughout development. Challenges may include exposure to key threatening processes (Fischer and Lindenmayer 2000), environmental heterogeneity (West-Eberhard 1989), parasitism (Summers *et al.*, 2003) or seasonal changes such as food availability. For example, captive breeding programmes attempting to breed species, such as amphibians, generally rear populations under constant environmental conditions, but my findings suggest that managers might benefit from rearing sub-populations under various environmental conditions in captivity to increase the level of phenotypic variation within the captive population.

Providing variable captive conditions such as stochastic food availability and water temperatures may improve average levels of survivorship, and generate larger numbers of individuals for reintroduction. This could benefit the recovery of a target species by improving the sustainability of a captive 'insurance' population, while minimising expenses associated with establishing and maintaining colonies (Canessa *et al.*, 2014; Canessa *et al.*, 2016). Furthermore, generating large numbers of individuals for release could improve the success of reintroduction programmes by overcoming problems associated with high dispersal, demographic stochasticity, or low reproduction and/or survival at low population sizes (Fischer and Lindenmayer 2000; Armstrong and Seddon 2008). For these reasons, I propose that broadening our knowledge of the interactive effects between environmental factors in captivity and their impact on phenotypic traits such as growth, development and survivorship might improve the success of threatened species recovery programs (Carey 2005; Muths *et al.*, 2014).

6.6 Future research priorities

There are several future research priorities that have been highlighted within this thesis. With respect to phenotypic changes occurring in captivity, it would be valuable to determine the ongoing impact of these phenotypic changes on individual fitness, particularly if these changes have consequences for the viability of captive populations, and/or affect the probability of reintroduction success. To this end, future research might benefit from investigating whether phenotypic changes occurring in captivity are maladaptive under natural conditions. This could be achieved by comprehensively evaluating the fitness of individuals pre- and post-reintroduction (Bremner-Harrison *et al.*, 2004; McDougall *et al.*, 2006; Smith and Blumstein 2008; Moseby *et al.*, 2014). In particular, there is a need to further explore the effects of captivity on phenotypic traits, and to understand mechanisms that might be used to either curb phenotypic change in captivity, or direct change to achieve beneficial outcomes. It would also be pertinent to substantiate whether phenotypic traits are plastic; are developmentally plastic in captivity over the duration of an individual's lifetime; and are subject to transgenerational effects, (i.e. shift away from wild-caught phenotypes over time, and with each subsequent generation maintained in captivity). In this regard, studies would need to measure phenotypic change throughout an individual's lifecycle, as well as across multiple generations.

To curb phenotypic change in captivity, further work is necessary to uncover the mechanisms of plasticity that cause phenotypic change. One potential approach could involve manipulating the developmental environment, either by rearing animals in a fluctuating environment, or by providing challenging conditions that simulate the challenges experienced in the natural habitat, to determine whether the developmental environment can produce an environment-specific phenotype (Norberg *et al.*, 2001). Developmental plasticity in behavioural phenotypes, such as boldness, has previously been documented in swift foxes (*Vulpes velox*), with captive-bred adult foxes displaying a higher level of boldness compared with juveniles, indicating that behaviours may be plastic if exposed to variable conditions in captivity (Norberg *et al.*, 2001; Bremner-Harrison *et al.*, 2004; Monaghan 2008). Alternatively, animals exposed to natural conditions during early development may be able to display phenotypic plasticity, with phenotypes produced that match the local conditions (Norberg *et al.*, 2001; Monaghan

2008). If a mechanism for phenotypic change can be isolated, it provides a tool to manipulate species' phenotypic traits, and generate phenotypes that are more suitable for reintroduction.

It is also important to determine how phenotypic plasticity influences transgenerational effects for populations maintained in captivity over multiple generations (Evans *et al.*, 2014). Future studies would benefit from examining trait change across multiple generations, starting at introduction to captivity and across multiple generations to investigate the mechanisms of transgenerational effects in captivity. Ultimately, quantitative genetic and epigenetic techniques are required to elucidate the source of transgenerational effects in captivity. This could include common garden experiments and/or cross-fostering experiments coupled with an assessment of genetic and epigenetic variation and changes (Dor and Lotem 2009). Laboratory experiments or captive breeding experiments can be used to control and manipulate environmental conditions to enable transgenerational effects to occur, but also allow the identification of the specific mechanisms that are driving transgenerational effects (Chakravarti *et al.*, 2016).

Further investigation is required to determine whether captivity can result in losses of sex-specific behaviours and changes to sexual dimorphism. It would be valuable to test for sex-specific differences in various morphological traits across a diversity of taxonomic groups. Such studies could focus on examining and comparing the behaviour and morphology of females and males in intra- and inter-sexual selection experiments, not only between captive-reared individuals, but also between wild individuals (Chargé *et al.*, 2014; Slade *et al.*, 2014). If differences between the sexes can be consistently demonstrated, sex-specific management strategies may be required to improve the efficiency of CBPs.

Finally, future studies investigating the effect of captivity on phenotypic traits would benefit from examining an array of individual and interactive effects of environmental conditions experienced in captivity on species that provide models for endangered species (Fischer and Lindenmayer 2000). This approach will enable generalisations to be made about the impacts of environmental conditions across a diversity of taxa, and provide a deeper understanding of the specific mechanisms driving phenotypic change.

By identifying how environmental conditions in captivity influence phenotypic traits, we have the potential to improve the output of CBPs, the overall long-term productivity of captive populations and maximise the ability of animals to respond to environmental change upon release. For example, by refining methodologies to minimise unfavourable phenotypic changes in captivity, phenotypic traits may be able to be altered to suit the wild environment prior to release.

6.7 Conclusions

My thesis has shown that captivity can have significant impacts on an animal's phenotype. These findings have implications for conservation because rapid plastic changes in captivity are likely to have direct impacts on the success or failure of captive breeding and reintroduction programmes. For example, captive-reared mice were found to differ in behaviour and morphology compared to wild-caught mice. However, phenotypic changes were also evident within the acclimation period, suggesting that some traits may be plastic. Further, while sexual dimorphism was maintained, there was a loss of sex-specific behaviours in captivity. Animals maintained in captivity for multiple generations displayed a consistent directional shift in phenotypic traits, away from the wild phenotype, towards an optimal mean trait value for captivity through transgenerational effects. Of these transgenerational changes, some but not all appeared to display some degree of heritability, which may allow for rapid adaptation to captive conditions. Other traits are likely to display a high degree of plasticity. Further, I have demonstrated that manipulating the independent and interactive effects of environmental conditions in captivity can influence phenotypic traits such as growth, development and survivorship.

By identifying how environmental conditions in captivity influence phenotypic traits, we have the potential to improve the output of CBPs, gain valuable knowledge for developing and refining methodologies to minimise unfavourable phenotypic changes in captivity and contribute to the success of captive-based reintroduction programmes globally (Mathews *et al.*, 2005; Smith and Blumstein 2008; Evans *et al.*, 2014).

6.8 References

Alvarez D and Nicieza A (2002a) Effects of induced variation in anuran larval development on postmetamorphic energy reserves and locomotion. Oecologia **131:** 186 – 195

Álvarez D and Nicieza AG (2002b) Effects of temperature and food quality on anuran larval growth and metamorphosis. Functional Ecology **16:** 640 – 648

Ariyomo TO, Carter M and Watt PH (2013) Heritability of boldness and aggressiveness in the zebrafish. Behavior Genetics **43:** 161 – 167

Armstrong DP Seddon PJ (2008) Directions in reintroduction biology. Trends in Ecology and Evolution **23:** 20 – 25

Ashley MV, Willson MF, Pergams ORW, O'Dowd DJ, Gende SM and Brown JS (2003) Evolutionarily enlightened management. Biological Conservation **111:** 115 – 123

Bailey TA, Mensah-Brown EP, Samour JH, Naldo J, Lawrence P and Garner A. (1997) Comparative morphology of the alimentary tract and its glandular derivatives of captive bustards. Journal of Anatomy **191:** 387 – 398

Bonnet X, Shine R, Naulleau G and Vacher-Vallas M (1998) Sexual dimorphism in snakes: different reproductive roles favour different body plans. Proceedings of the Royal Society of London. Series B: Biological Sciences **265:** 179 – 183

Bremner-Harrison S, Prodohl P and Elwood RW (2004) Behavioural trait assessment as a release criterion: boldness predicts early death in a reintroduction programme of captive-bred swift fox (*Vulpes velox*). Animal Conservation **7:** 313 – 320.

Burns JG, Saravanan A and Helen Rodd F (2009) Rearing environment affects the brain size of guppies: lab-reared guppies have smaller brains than wild-caught guppies. Ethology **115:** 122 – 133

Canessa S, Guillera-Arroita G, Lahoz-Monfort JJ, Southwell DM, Armstrong DP, Chadès I, Lacy RC and Converse SJ (2016) Adaptive management for improving

species conservation across the captive-wild spectrum. Biological Conservation **199**: 123 – 131

Canessa S, Hunter D, McFadden M, Marantelli G and McCarthy MA (2014) Optimal release strategies for cost-effective reintroductions. Journal of Applied Ecology **51:** 1107 – 1115

Carey C (2005) How physiological methods and concepts can be useful in Conservation Biology. Integrative and Comparative Biology **45:** 4 – 11

Chakravarti LJ, Jarrold MD, Gibbin EM, Christen F, Massamba-N'Siala G, Blier PU and Calosi P (2016) Can trans-generational experiments be used to enhance species resilience to ocean warming and acidification? Evolutionary Applications **9:** 1133 – 1146

Champagnon J, Guillemain M, Elmberg J, Massez G, Cavallo F and Gauthier-Clerc M. (2012) Low survival after release into the wild: assessing "the burden of captivity" on mallard physiology and behaviour. European Journal of Wildlife Research **58:** 255 – 267

Chargé R, Teplitsky C, Sorci G and Low M (2014) Can sexual selection theory inform genetic management of captive populations? A review. Evolutionary Applications **7:** 1120 – 1133

Dingemanse NJ, Both C, Drent PJ, van Oers K and van Noordwijk AJ (2002) Repeatability and heritability of exploratory behaviour in great tits from the wild. Animal Behaviour **64:** 929 – 938

Dor R and Lotem A (2009) Heritability of nestling begging intensity in the house sparrow (*Passer domesticus*). Evolution **63:** 738 – 748

Drent PJ, van Oers K and van Noordwijk AJ (2003) Realized heritability of personalities in the great tit (*Parus major*). Proceedings of the Royal Society of London. Series B: Biological Sciences **270:** 45 – 51

Evans ML, Wilke NF, O'Reilly PT and Fleming IA (2014) Transgenerational effects of parental rearing environment influence the survivorship of captive-born offspring in the wild. Conservation Letters **7:** 371 – 379

Falconer DS, Mackay TF, Frankham R. 1996. Introduction to quantitative genetics (4th edn). Trends in Genetics **12**:280.

Fischer J and Lindenmayer DB (2000) An assessment of the published results of animal relocations. Biological Conservation **96:** 1 – 11

Frankham R (2008) Genetic adaptation to captivity in species conservation programs. Molecular Ecology **17:** 325 – 333

Håkansson J and Jensen P (2005) Behavioural and morphological variation between captive populations of red junglefowl (*Gallus gallus*) – possible implications for conservation. Biological Conservation **122:** 431 - 439

Håkansson J and Jensen P (2008) A longitudinal study of antipredator behaviour in four successive generations of two populations of captive red junglefowl. Applied Animal Behaviour Science **114:** 409 – 418

Houle D (1992) Comparing evolvability and variability of quantitative traits. Genetics **130:** 195 – 204

Johnson DW, Grorud-Colvert K, Sponaugle S and Semmens BX (2014) Phenotypic variation and selective mortality as major drivers of recruitment variability in fishes. Ecology Letters **17:** 743 – 755

Kussell E and Leibler S (2005) Phenotypic diversity, population growth, and information in fluctuating environments. Science **309:** 2075 – 2078

Lynch JM and Hayden TJ (1995) Genetic influences on cranial form: variation among ranch and feral American mink *Mustela vison* (Mammalia: Mustelidae). Biological Journal of the Linnean Society **55:** 293 - 307

Mason G, Burn CC, Dallaire JA, Kroshko J, McDonald Kinkaid H and Jeschke JM (2013) Plastic animals in cages: behavioural flexibility and responses to captivity. Animal Behaviour **85:** 1113 – 1126

Mathews F, Orros M, McLaren G, Gelling M and Foster R (2005) Keeping fit on the ark: assessing the suitability of captive-bred animals for release. Biological Conservation **121:** 569 – 577

McDougall P, Réale D, Sol D and Reader S (2006) Wildlife conservation and animal temperament: causes and consequences of evolutionary change for captive, reintroduced, and wild populations. Animal Conservation **9:** 39 – 48

McLeod IM, Rummer JL, Clark TD, Jones GP, McCormick MI, Wenger AS and Munday PL (2013) Climate change and the performance of larval coral reef fishes: the interaction between temperature and food availability. Conservation Physiology **1:** cot024

McPhee ME (2004a) Generations in captivity increases behavioral variance: considerations for captive breeding and reintroduction programs. Biological Conservation **115:** 71 – 77

McPhee ME (2004b) Morphological change in wild and captive oldfield mice *Peromyscus polionotus subgriseus*. Journal of Mammalogy **85:**1130 – 1137

McPhee ME and Carlstead K (2010) The importance of maintaining natural behaviors in captive mammals. In: Kleiman DG, Thompson KV, and Baer CK, editors. Wild mammals in captivity: principles and techniques for zoo management. (pp. 303-312) University of Chicago Press, Chicago

McPhee ME and McPhee NF (2012) Relaxed selection and environmental change decrease reintroduction success in simulated populations. Animal Conservation **15:** 274 – 282

McWilliams SR and Karasov WH (2001) Phenotypic flexibility in digestive system structure and function in migratory birds and its ecological significance. Comparative

Biochemistry and Physiology Part A: Molecular & Integrative Physiology **128:** 577 – 591

Miner BG, Sultan SE, Morgan SG, Padilla DK and Relyea RA (2005) Ecological consequences of phenotypic plasticity. Trends in Ecology and Evolution **20:** 685 – 692

Monaghan P (2008) Early growth conditions, phenotypic development and environmental change. Philosophical Transactions of the Royal Society of London B: Biological Sciences **363:** 1635 – 1645

Moseby KE, Hill BM and Lavery TH (2014) Tailoring release protocols to individual species and sites: one size does not fit all. PLoS ONE **9:** e99753

Muths E, Bailey LL and Watry MK (2014) Animal reintroductions: an innovative assessment of survival. Biological Conservation **172:** 200 – 208

Norberg J, Swaney DP, Dushoff J, Lin J, Casagrandi R and Levin SA (2001) Phenotypic diversity and ecosystem functioning in changing environments: a theoretical framework. Proceedings of the National Academy of Sciences **98:** 11376 – 11381

O'Regan HJ and Kitchener AC (2005) The effects of captivity on the morphology of captive, domesticated and feral mammals. Mammal Review **35:** 215 – 230

Piersma T and Drent J (2003) Phenotypic flexibility and the evolution of organismal design. Trends in Ecology and Evolution **18:** 228 – 233

Piersma T and Lindström Å (1997) Rapid reversible changes in organ size as a component of adaptive behaviour. Trends in Ecology and Evolution **12:** 134 – 138

Post E, Stenseth NC, Langvatn R and Fromentin J-M (1997) Global climate change and phenotypic variation among red deer cohorts. Proceedings of the Royal Society of London. Series B: Biological Sciences **264:** 1317 – 1324

Réale D and Festa-Bianchet M (2000) Quantitative genetics of life-history traits in a long-lived wild mammal. Heredity **85:** 593 – 603

Richards CL, Bossdorf O and Pigliucci M (2010) What role does heritable epigenetic variation play in phenotypic evolution? BioScience **60:** 232 - 237

Rodriguez-Clark KM (2004) Effect of captivity on genetic variance for five traits in the large milkweed bug (*Oncopeltus fasciatus*). Heredity **93:** 51 – 61

Schulte-Hostedde AI and Mastromonaco GF (2015) Integrating evolution in the management of captive zoo populations. Evolutionary Applications **8:** 413 – 422

Slade B, Parrott ML, Paproth A, Magrath MJL, Gillespie GR and Jessop TS (2014) Assortative mating among animals of captive and wild origin following experimental conservation releases. Biology Letters **10:** 20140656

Smith BR and Blumstein DT (2008) Fitness consequences of personality: a meta-analysis. Behavioural Ecology **19:** 448 – 455

Snyder NFR, Derrickson SR, Beissinger SR, Wiley JW, Smith TB, Toone WD and Miller B (1996) Limitations of captive breeding in endangered species recovery. Conservation Biology **10:** 338 – 348

Starck JM (1999) Phenotypic flexibility of the avian gizzard: rapid, reversible and repeated changes of organ size in response to changes in dietary fibre content. Journal of Experimental Biology **202:** 3171 – 3179

Summers K, McKeon S, Sellars J, Keusenkothen M, Morris J, Gloeckner D, Pressley C, Price B and Snow H (2003) Parasitic exploitation as an engine of diversity. Biological Reviews **78:** 639 – 675

Swallow JG, Rhodes JS and Garland T (2005) Phenotypic and evolutionary plasticity of organ masses in response to voluntary exercise in house mice. Integrative and Comparative Biology **45:** 426-437.

Tarszisz E, Dickman CR and Munn AJ (2014) Physiology in conservation translocations. Conservation Physiology **2:** cou054

Tschirren B, Rutstein AN, Postma E, Mariette M and Griffith SC (2009) Short- and long-term consequences of early developmental conditions: a case study on wild and domesticated zebra finches. Journal of Evolutionary Biology **22:** 387 – 395

van Oosterhout C, Smith AM, Hänfling B, Ramnarine IW, Mohammed RS and Cable J (2007) The guppy as a conservation model: implications of parasitism and inbreeding for reintroduction success. Conservation Biology **21:** 1573 – 1583

West-Eberhard MJ (1989) Phenotypic plasticity and the origins of diversity. Annual Review of Ecology and Systematics **20:** 249 – 278

Williams SE and Hoffman EA (2009) Minimizing genetic adaptation in captive breeding programs: a review. Biological Conservation **142:** 2388 – 2400

APPENDIX A: CHAPTER 2 ASSOCIATED SUPPLEMENTARY DATA

Table A.1 Behavioural traits that contributed most to similarity in behavioural type composition between rearing environment (captive-reared F_4, captive-reared F_5 and wild-caught individuals) and sex (female and male; based on the SIMPER procedure using normalised data of least squares regression for each behavioural trait on body mass)

Table A.1 Behavioural traits that contributed most to similarity in behavioural type composition between rearing environment (captive-reared F_4, captive-reared F_5 and wild-caught individuals) and sex (female and male; based on the SIMPER procedure using normalised data of least squares regression for each behavioural trait on body mass).

Wild-caught Male - Average squared distance = 17.87					
Behavioural trait	Av. Value	Av. Sq. Dist	Sq. Dist/SD	% Contribution	Cumulative %
Mean speed	-0.628	0.207	0.46	1.16	1.16
Distance	-0.627	0.207	0.46	1.16	2.32
Perimeter: mean speed	-0.63	0.255	0.45	1.42	3.74
Centre: mean speed	-0.538	0.306	0.5	1.71	5.45
Meandering	0.506	0.335	0.59	1.87	7.32
In tunnel: time pressed	0.0649	0.637	0.39	3.56	10.89
Perimeter: maximum speed	0.153	0.735	0.4	4.11	15
% Time freezing	1.25	0.952	0.46	5.33	20.33
Jump: number of presses	0.24	1.24	0.49	6.96	27.29
% Centre: total time spent	-0.48	1.85	0.58	10.33	37.62
% Time active	-1.59	1.93	0.53	10.82	48.43
% Time mobile	-1.47	2.23	0.49	12.5	60.93
Maximum speed	0.595	2.61	0.43	14.6	75.53
Captive-reared F_4 Male - Average squared distance = 6.03					
	Av. Value	Av. Sq. Dist	Sq. Dist/SD	% Contribution	Cumulative %
Perimeter: maximum speed	-0.117	0.0574	0.48	0.95	0.95
In tunnel: time pressed	-0.276	0.0695	0.51	1.15	2.11
% Time mobile	0.507	0.257	0.54	4.27	6.37
% Time active	0.503	0.332	0.54	5.51	11.88
Centre: maximum speed	-0.261	0.34	0.46	5.64	17.52
Jump: number of presses	-0.191	0.35	0.42	5.81	23.33

Maximum speed	-0.219	0.388	0.38	6.44	29.77
% Time freezing	-0.575	0.446	0.57	7.4	37.17
Perimeter: mean speed	0.359	0.465	0.42	7.71	44.88
% Centre: total time spent	-0.0656	0.511	0.55	8.49	53.36
Meandering	-0.48	0.572	0.47	9.49	62.86
Mean speed	0.385	0.645	0.41	10.7	73.55
Captive-reared F_5 Male - Average squared distance = 7.79					
Av. Value		Av. Sq. Dist	Sq. Dist/SD	% Contribution	Cumulative %
Perimeter: maximum speed	-0.273	0.0647	0.47	0.83	0.83
Centre: mean speed	-0.325	0.181	0.45	2.33	3.16
Mean speed	-0.255	0.199	0.51	2.56	5.71
Distance	-0.256	0.2	0.51	2.56	8.28
Perimeter: mean speed	-0.217	0.275	0.5	3.53	11.81
Meandering	0.104	0.34	0.52	4.36	16.17
Maximum speed	-0.35	0.353	0.36	4.52	20.7
Centre: max speed	-0.116	0.489	0.5	6.28	26.98
% Centre: total time spent	0.134	0.658	0.46	8.44	35.41
Jump: number of presses	-0.158	0.669	0.52	8.59	44
% Time freezing	0.143	0.703	0.51	9.02	53.02
% Time mobile	0.191	0.793	0.39	10.17	63.2
% Time active	0.211	0.89	0.4	11.42	74.61
Wild-caught Female - Average squared distance = 12.12					
Av. Value		Av. Sq. Dist	Sq. Dist/SD	% Contribution	Cumulative %
Perimeter: maximum speed	0.0261	0.126	0.43	1.04	1.04
Maximum speed	0.186	0.167	0.43	1.37	2.41
Centre: maximum speed	0.462	0.275	0.5	2.27	4.69
% Time active	0.0255	0.311	0.42	2.57	7.25
Perimeter: mean speed	-0.126	0.49	0.33	4.04	11.3

Mean speed	0.0506	0.678	0.36	5.6	16.89
Distance	0.0504	0.679	0.36	5.6	22.49
Meandering	-0.282	0.745	0.49	6.15	28.64
% Time mobile	-0.0831	0.761	0.35	6.28	34.92
% Time freezing	0.206	0.829	0.46	6.83	41.75
Centre: mean speed	0.269	1.07	0.42	8.81	50.57
% Centre: total time spent	-0.337	1.77	0.49	14.64	65.2
Jump: number of presses	0.618	1.82	0.45	14.99	80.2
Captive-reared F_4 Female - Average squared distance = 26.07					
Av. Value		Av. Sq. Dist	Sq. Dist/SD	% Contribution	Cumulative %
In tunnel: time pressed	-0.186	0.225	0.46	0.86	0.86
% Centre: total time spent	-0.0694	0.516	0.49	1.98	2.84
Centre: maximum speed	-0.472	0.557	0.5	2.14	4.98
% Time mobile	0.322	0.621	0.39	2.38	7.36
% Time active	0.311	0.778	0.38	2.98	10.34
Jump: number of presses	-0.255	1.1	0.49	4.23	14.57
% Time freezing	-0.555	1.17	0.5	4.47	19.04
Maximum speed	0.421	2.29	0.35	8.8	27.84
Centre: mean speed	0.381	2.43	0.44	9.33	37.18
Meandering	0.0543	2.98	0.48	11.44	48.62
Distance	0.535	3.01	0.43	11.54	60.16
Mean speed	0.535	3.01	0.43	11.55	71.72
Captive-reared F_5 Female - Average squared distance = 5.07					
Av. Value		Av. Sq. Dist	Sq. Dist/SD	% Contribution	Cumulative %
Mean speed	-0.335	0.117	0.42	2.3	2.3
Distance	-0.333	0.117	0.42	2.32	4.62
Centre: mean speed	-0.386	0.122	0.41	2.4	7.02
Perimeter: maximum speed	-0.268	0.171	0.32	3.37	10.38

Maximum speed	-0.494	0.202	0.48	3.98	14.36	
Perimeter: mean speed	-0.197	0.249	0.51	4.91	19.27	
Meandering	0.236	0.301	0.46	5.94	25.21	
Jump: number of presses	-0.23	0.404	0.54	7.96	33.17	
% Time mobile	0.0082	0.433	0.41	8.53	41.71	
In tunnel: time pressed	0.0782	0.454	0.51	8.96	50.67	
% Time freezing	-0.0181	0.457	0.52	9.01	59.68	
% Time active	-0.0426	0.545	0.4	10.74	70.42	

Wild-caught Male and Captive-reared F_4 Male - Average squared distance = 39.71

	Wild-caught Male	Captive-reared F_4 Male				
	Av. Value	Av. Value	Av. Sq. Dist	Sq. Dist/SD	% Contribution	Cumulative %
% Time active	-1.59	0.503	6.38	1.04	16.08	16.08
% Time mobile	-1.47	0.507	6.1	0.83	15.37	31.45
Centre: maximum speed	0.695	-0.261	5.05	0.53	12.72	44.17
% Time freezing	1.25	-0.575	4.56	1.1	11.48	55.64
Maximum speed	0.595	-0.219	3.3	0.49	8.31	63.95
% Centre: total time spent	-0.48	-0.0656	2.25	1.07	5.67	69.62
Centre: mean speed	-0.538	0.379	1.97	0.59	4.96	74.58

Wild-caught Male and Captive-reared F_5 Male - Average squared distance = 33.09

	Wild-caught Male	Captive-reared F_5 Male				
	Av. Value	Av. Value	Av. Sq. Dist	Sq. Dist/SD	% Contribution	Cum.%
% Time active	-1.59	0.211	5.77	1	17.43	17.43
% Time mobile	-1.47	0.191	5.45	0.79	16.48	33.9
Centre: maximum speed	0.695	-0.116	4.93	0.54	14.91	48.81
Maximum speed	0.595	-0.35	3.5	0.49	10.58	59.39
% Time freezing	1.25	0.143	2.7	0.89	8.16	67.55
% Centre: total time spent	-0.48	0.134	2.6	0.97	7.85	75.41

Captive-reared F_4 Male and Captive-reared F_5 Male - Average squared distance = 15.90

	Captive-reared F_4 Male	Captive-reared F_5 Male				
	Av. Value	Av. Value	Av. Sq. Dist	Sq. Dist/SD	% Contribution	Cumulative %
In tunnel: time pressed	-0.276	0.385	2.33	0.39	14.63	14.63
% Time freezing	-0.575	0.143	1.57	0.74	9.87	24.5
Centre: mean speed	0.379	-0.325	1.52	0.54	9.57	34.07
% Time active	0.503	0.211	1.21	0.52	7.6	41.67
Distance	0.385	-0.256	1.18	0.55	7.45	49.12
Mean speed	0.385	-0.255	1.18	0.55	7.42	56.54
Meandering	-0.48	0.104	1.17	0.7	7.39	63.93
% Centre: total time spent	-0.0656	0.134	1.11	0.75	6.99	70.92

Wild-caught Male and Wild-caught Female - Average squared distance = 35.44

	Wild-caught Male	Wild-caught Female				
	Av. Value	Av. Value	Av. Sq. Dist	Sq. Dist/SD	% Contribution	Cumulative %
% Time active	-1.59	0.0255	4.6	0.95	12.98	12.98
% Time mobile	-1.47	-0.0831	4.59	0.75	12.96	25.94
Centre: maximum speed	0.695	0.462	4.14	0.59	11.67	37.61
% Centre: total time spent	-0.48	-0.337	3.29	0.82	9.29	46.9
Jump: number of presses	0.24	0.618	2.93	0.64	8.26	55.16
In tunnel: time pressed	0.0649	-0.0527	2.81	0.37	7.93	63.09
% Time freezing	1.25	0.206	2.69	0.79	7.59	70.68

Captive-reared F_4 Male and Captive-reared F_4 Female - Average squared distance = 31.07

	Captive-reared F_4 Male	Captive-reared F_4 Female				
Behavioural trait	Av. Value	Av. Value	Av. Sq. Dist	Sq. Dist/SD	% Contribution	Cumulative %
Perimeter: maximum speed	-0.117	0.465	4.32	0.28	13.91	13.91
Meandering	-0.48	0.0543	3.59	0.73	11.56	25.47

Distance	0.385	0.535	3.42	0.57	11.01	36.48
Mean speed	0.385	0.535	3.42	0.57	11.01	47.49
Perimeter: mean speed	0.359	0.572	3.42	0.55	11.01	58.5
Centre: mean speed	0.379	0.381	3.13	0.65	10.08	68.57
Maximum speed	-0.219	0.421	2.9	0.42	9.35	77.92

Wild-caught Female and Captive-reared F_4 Female - Average squared distance = 39.53

	Wild-caught Female	Captive-reared F_4 Female				
	Av. Value	Av. Value	Av. Sq. Dist	Sq. Dist/SD	% Contribution	Cumulative %
Perimeter: maximum speed	0.0261	0.465	4.24	0.29	10.73	10.73
Perimeter: mean speed	-0.126	0.572	3.9	0.5	9.86	20.59
Mean speed	0.0506	0.535	3.68	0.53	9.31	29.9
Distance	0.0504	0.535	3.68	0.53	9.3	39.2
Meandering	-0.282	0.0543	3.59	0.74	9.09	48.29
Jump: number of presses	0.618	-0.255	3.49	0.65	8.82	57.11
Centre: mean speed	0.269	0.381	3.28	0.63	8.3	65.41
In tunnel: time pressed	-0.0527	-0.186	2.47	0.31	6.24	71.66

Captive-reared F_5 Male and Captive-reared F_5 Female - Average squared distance = 12.43

	Captive-reared F_5 Male	Captive-reared F_5 Female				
	Av. Value	Av. Value	Av. Sq. Dist	Sq. Dist/SD	% Contribution	Cumulative %
In tunnel: time pressed	0.385	0.0782	2.34	0.44	18.84	18.84
% Time active	0.211	-0.0426	1.39	0.63	11.19	30.03
% Centre: total time spent	0.134	0.637	1.39	0.74	11.18	41.21
Centre: maximum speed	-0.116	-0.0734	1.32	0.73	10.58	51.79
% Time mobile	0.191	0.0082	1.17	0.61	9.39	61.18
% Time freezing	0.143	-0.0181	1.1	0.76	8.84	70.02

Wild-caught Female and Captive-reared F_5 Female - Average squared distance = 19.60						
	Wild-caught Female	Captive-reared F_5 Female				
	Av. Value	Av. Value	Av. Sq. Dist	Sq. Dist/SD	% Contribution	Cumulative %
% Centre: total time spent	-0.337	0.637	3.14	0.81	16	16
Jump: number of presses	0.618	-0.23	2.79	0.57	14.23	30.23
In tunnel: time pressed	-0.0527	0.0782	2.68	0.36	13.67	43.9
Centre: mean speed	0.269	-0.386	1.54	0.53	7.85	51.75
Centre: maximum speed	0.462	-0.0734	1.41	0.96	7.17	58.92
% Time freezing	0.206	-0.0181	1.25	0.74	6.36	65.29
Meandering	-0.282	0.236	1.24	0.77	6.34	71.63
Captive-reared F_4 Female and Captive-reared F_5 Female - Average squared distance = 34.38						
	Captive-reared F_4 Female	Captive-reared F_5 Female				
	Av. Value	Av. Value	Av. Sq. Dist	Sq. Dist/SD	% Contribution	Cumulative %
Perimeter: maximum speed	0.465	-0.268	4.63	0.29	13.46	13.46
Perimeter: mean speed	0.572	-0.197	3.77	0.48	10.98	24.43
Mean speed	0.535	-0.335	3.68	0.48	10.69	35.13
Distance	0.535	-0.333	3.67	0.48	10.68	45.8
Maximum speed	0.421	-0.494	3.17	0.42	9.21	55.01
Meandering	0.0543	0.236	3.1	0.77	9.01	64.02
Centre: mean speed	0.381	-0.386	2.97	0.5	8.65	72.67

APPENDIX B: CHAPTER 3. ASSOCIATED SUPPLEMENTARY DATA

Table B.1 External morphological traits that contributed most to similarity in external morphology between sexes (female and male; based on the SIMPER procedure using normalised data of least squares regression for each behavioural trait on body mass).

Table B.2 Internal morphological traits that contributed most to similarity in internal morphology between rearing environment (captive-reared F_4, captive-reared F_5 and wild-caught individuals) and sex (female and male; based on the SIMPER procedure using normalised data of least squares regression for each behavioural trait on body mass).

Table B.1 External morphological traits that contributed most to similarity in external morphology between sexes (female and male; based on the SIMPER procedure using normalised data of least squares regression for each behavioural trait on body mass).

Female – Average squared distance= 5.45					
Morphological trait	Av. Value	Av. Sq. Dist.	Sq. Dist. /SD	% Contribution	Cumulative %
Body mass	-0.309	0.555	0.48	10.17	10.17
Skull length	0.0873	0.88	0.48	16.13	26.3
Snout to vent length	0.166	0.926	0.46	16.98	43.29
Tail length	-0.0918	1.35	0.19	24.73	68.01
Foot length (right hind)	0.105	1.74	0.16	31.99	100

Male – Average squared distance= 4.07					
Morphological trait	Av. Value	Av. Sq. Dist.	Sq. Dist. /SD	% Contribution	Cumulative %
Foot length (right hind)	-0.132	0.0197	0.43	0.48	0.48
Tail length	0.123	0.533	0.36	13.08	13.56
Snout to vent length	-0.242	1.05	0.47	25.88	39.44
Skull length	-0.0727	1.15	0.39	28.13	67.57
Body mass	0.438	1.32	0.43	32.43	100

Female and Male – Average squared distance= 10.12						
	Female	Male				
	Av. Value	Av. Value	Av. Sq. Dist.	Sq. Dist. /SD	% Contribution	Cumulative %
Body mass	-0.309	0.438	2.38	0.64	23.5	23.5
Snout to vent length	0.166	-0.242	2.09	0.68	20.66	44.15
Skull length	0.0873	-0.0727	1.99	0.65	19.7	63.86
Tail length	-0.0918	0.123	1.88	0.25	18.57	82.42

Table B.2 Internal morphological traits that contributed most to similarity in internal morphology between rearing environment (captive-reared F_4, captive-reared F_5 and wild-caught individuals) and sex (female and male; based on the SIMPER procedure using normalised data of least squares regression for each behavioural trait on body mass).

Captive-reared F_4 Female – Average squared distance = 9.74					
Morphological trait	Av. Value	Av. Sq. Dist.	Sq. Dist./SD	Contribution %	Cumulative %
Lungs	-0.28	0.285	0.52	2.93	2.93
Spleen	-0.411	0.366	0.49	3.76	6.69
Large intestine	0.0486	0.381	0.48	3.91	10.6
Heart	0.205	0.525	0.46	5.39	15.99
Liver	-0.00285	0.589	0.51	6.05	22.04
Kidneys	-1.06	0.597	0.48	6.13	28.17
Small intestine	0.0747	0.671	0.49	6.9	35.06
Small Intestine length	-0.746	0.786	0.51	8.08	43.14
Caecum	0.671	0.817	0.52	8.39	51.53
Large Intestine length	-0.405	0.939	0.52	9.64	61.17
Brain	0.45	1.01	0.42	10.37	71.54
Captive-reared F_5 Female – Average squared distance = 5.43					
Morphological trait	Av. Value	Av. Sq. Dist.	Sq. Dist./SD	Contribution %	Cumulative %
Spleen	-0.0906	0.078	0.51	1.43	1.43
Liver	0.163	0.0886	0.46	1.63	3.07
Large intestine	-0.0211	0.0986	0.52	1.81	4.88
Small Intestine length	-0.203	0.122	0.49	2.25	7.13
Kidneys	0.0955	0.14	0.53	2.58	9.72
Small intestine	-0.0922	0.278	0.43	5.12	14.83
Ovaries/testes	-0.0772	0.321	0.45	5.91	20.74
Lungs	0.045	0.332	0.46	6.1	26.84
Large Intestine length	-0.253	0.601	0.46	11.05	37.9

Caecum	-0.03	0.632	0.46	11.62	49.52
Stomach	0.241	0.781	0.53	14.38	63.9
Heart	0.0158	0.847	0.47	15.58	79.48

Wild-caught Female – Average squared distance = 11.85

Morphological trait	Av. Value	Av. Sq. Dist.	Sq. Dist./SD	Contribution %	Cumulative %
Caecum	-0.596	0.286	0.4	2.42	2.42
Ovaries/testes	-0.996	0.288	0.5	2.43	4.84
Kidneys	-0.505	0.335	0.33	2.83	7.67
Small Intestine length	0.864	0.572	0.43	4.83	12.5
Brain	0.35	0.617	0.52	5.21	17.71
Heart	0.188	0.669	0.51	5.65	23.36
Spleen	0.623	0.934	0.33	7.88	31.24
Stomach	-0.13	1.09	0.49	9.24	40.48
Lungs	0.383	1.1	0.48	9.25	49.73
Large Intestine length	-0.123	1.19	0.44	10	59.73
Small intestine	0.549	1.33	0.35	11.25	70.99

Captive-reared F_4 Male – Average squared distance = 14.66

Morphological trait	Av. Value	Av. Sq. Dist.	Sq. Dist./SD	Contribution %	Cumulative %
Spleen	-0.887	0.292	0.49	1.99	1.99
Ovaries/testes	-0.216	0.339	0.43	2.31	4.3
Liver	-0.496	0.349	0.48	2.38	6.69
Small intestine	-1.25	0.505	0.52	3.45	10.13
Stomach	-0.3	0.577	0.59	3.94	14.07
Kidneys	0.508	0.644	0.44	4.4	18.47
Brain	-0.524	0.702	0.48	4.79	23.26
Small Intestine length	-0.422	0.709	0.5	4.83	28.09
Large Intestine length	1.06	0.907	0.48	6.19	34.28
Caecum	0.485	1.33	0.46	9.1	43.38

Heart	-0.354	2.4	0.4	16.38	59.77
Lungs	-0.131	2.79	0.38	19.06	78.83

Captive-reared F_5 Male – Average squared distance = 7.25

Morphological trait	Av. Value	Av. Sq. Dist.	Sq. Dist./SD	Contribution %	Cumulative %
Spleen	-0.111	0.217	0.48	2.99	2.99
Small Intestine length	-0.263	0.223	0.44	3.08	6.07
Kidneys	0.758	0.373	0.54	5.15	11.22
Liver	-0.462	0.382	0.41	5.28	16.5
Heart	-0.316	0.399	0.57	5.51	22.01
Lungs	-0.476	0.416	0.49	5.74	27.75
Small intestine	-0.179	0.434	0.54	5.98	33.73
Large intestine	-0.257	0.521	0.53	7.19	40.92
Ovaries/testes	0.865	0.685	0.46	9.46	50.37
Stomach	-0.144	0.815	0.5	11.24	61.62
Brain	-0.649	0.833	0.51	11.5	73.11

Wild-caught Male – Average squared distance = 19.87

Morphological trait	Av. Value	Av. Sq. Dist.	Sq. Dist./SD	Contribution %	Cumulative %
Large Intestine length	0.293	0.362	0.49	1.82	1.82
Large intestine	-0.717	0.465	0.45	2.34	4.16
Ovaries/testes	0.653	0.74	0.49	3.73	7.89
Small intestine	0.846	0.864	0.52	4.35	12.24
Brain	-0.132	0.948	0.53	4.77	17.01
Caecum	-0.36	1.28	0.55	6.45	23.46
Lungs	0.666	1.45	0.58	7.3	30.77
Kidneys	0.903	1.63	0.48	8.2	38.96
Small Intestine length	1.09	1.72	0.51	8.63	47.6
Stomach	0.115	1.8	0.45	9.04	56.63
Heart	0.193	2.27	0.56	11.41	68.04

Liver	0.399	2.89	0.5	14.55	82.59	

Captive-reared F_4 Female and Captive-reared F_5 Female – Average squared distance = 16.69

	Captive-reared F_4 Female	Captive-reared F_5 Female				
	Av. Value	Av. Value	Av. Sq. Dist.	Sq. Dist./SD	Contribution %	Cumulative %
Brain	0.45	0.195	2.04	0.75	12.24	12.24
Kidneys	-1.06	0.0955	2.03	1	12.14	24.38
Stomach	0.168	0.241	1.95	0.72	11.65	36.03
Caecum	0.671	-0.03	1.84	0.77	11.02	47.06
Ovaries/testes	0.115	-0.0772	1.71	0.58	10.22	57.28
Large Intestine length	-0.405	-0.253	1.46	0.74	8.73	66.01
Heart	0.205	0.0158	1.31	0.77	7.86	73.86

Captive-reared F_4 Female and Wild-caught Female - Average squared distance = 27.95

	Captive-reared F_4 Female	Wild-caught Female				
	Av. Value	Av. Value	Av. Sq. Dist.	Sq. Dist./SD	Contribution %	Cumulative %
Small Intestine length	-0.746	0.864	3.86	0.93	13.82	13.82
Ovaries/testes	0.115	-0.996	2.87	0.61	10.29	24.1
Caecum	0.671	-0.596	2.63	0.79	9.43	33.53
Liver	-0.00285	0.369	2.54	0.6	9.07	42.6
Stomach	0.168	-0.13	2.32	0.73	8.32	50.92
Spleen	-0.411	0.623	2.28	0.52	8.17	59.09
Small intestine	0.0747	0.549	2.1	0.48	7.5	66.59
Large Intestine length	-0.405	-0.123	2.06	0.71	7.38	73.97

Captive-reared F_4 Female and Captive-reared F_4 Male – Average squared distance = 31.23

	Captive-reared F_4 Female	Captive-reared F_4 Male				
	Av. Value	Av. Value	Av. Sq. Dist.	Sq. Dist./SD	Contribution %	Cumulative %
Large Intestine length	-0.405	1.06	3.84	0.96	12.3	12.3
Kidneys	-1.06	0.508	3.6	1.04	11.53	23.83
Large intestine	0.0486	0.649	3.51	0.41	11.24	35.07

Heart	0.205	-0.354	2.96	0.77	9.49	44.56
Small intestine	0.0747	-1.25	2.84	0.93	9.09	53.65
Lungs	-0.28	-0.131	2.8	0.45	8.97	62.62
Brain	0.45	-0.524	2.52	0.81	8.08	70.7

Captive-reared F_5 Female and Captive-reared F_5 Male – Average squared distance = 14.81

	Captive-reared F_5 Female	Captive-reared F_5 Male				
	Av. Value	Av. Value	Av. Sq. Dist.	Sq. Dist./SD	Contribution %	Cumulative %
Brain	0.195	-0.649	2.52	0.81	17	17
Ovaries/testes	-0.0772	0.865	1.82	1.16	12.27	29.27
Caecum	-0.03	-0.206	1.64	0.66	11.08	40.35
Stomach	0.241	-0.144	1.63	0.72	10.98	51.33
Large Intestine length	-0.253	-0.116	1.35	0.7	9.12	60.44
Heart	0.0158	-0.316	1.27	0.65	8.54	68.99
Lungs	0.045	-0.476	0.963	0.74	6.5	75.49

Captive-reared F_4 Male and Captive-reared F_5 Male – Average squared distance = 25.73

	Captive-reared F_4 Male	Captive-reared F_5 Male				
	Av. Value	Av. Value	Av. Sq. Dist.	Sq. Dist./SD	Contribution %	Cumulative %
Large intestine	0.649	-0.257	4.1	0.43	15.91	15.91
Lungs	-0.131	-0.476	3.02	0.44	11.72	27.64
Large Intestine length	1.06	-0.116	2.98	0.89	11.57	39.2
Caecum	0.485	-0.206	2.7	0.78	10.5	49.71
Heart	-0.354	-0.316	2.53	0.54	9.84	59.54
Ovaries/testes	-0.216	0.865	2.11	0.97	8.18	67.73
Small intestine	-1.25	-0.179	2	0.93	7.79	75.52

Wild-caught Female & Wild-caught Male – Average squared distance = 34.82

	Wild-caught Female	Wild-caught Male				
	Av. Value	Av. Value	Av. Sq. Dist.	Sq. Dist./SD	Contribution %	Cumulative %
Liver	0.369	0.399	4.38	0.71	12.58	12.58

Spleen	0.623	1.05	4.08	0.79	11.73	24.3
Kidneys	-0.505	0.903	3.72	0.75	10.69	34.99
Ovaries/testes	-0.996	0.653	3.64	0.94	10.45	45.44
Stomach	-0.13	0.115	2.65	0.69	7.62	53.06
Heart	0.188	0.193	2.61	0.91	7.49	60.55
Large intestine	0.143	-0.717	2.51	0.51	7.21	67.75
Lungs	0.383	0.666	2.37	0.84	6.82	74.57

Captive-reared F_4 Male and Wild-caught Male - Average squared distance = 47.16

	Captive-reared F_4 Male	Wild-caught Male				
	Av. Value	Av. Value	Av. Sq. Dist.	Sq. Dist./SD	Contribution %	Cumulative %
Spleen	-0.887	1.05	7.05	0.81	14.94	14.94
Small intestine	-1.25	0.846	5.61	1.08	11.89	26.83
Large intestine	0.649	-0.717	5.07	0.46	10.75	37.58
Heart	-0.354	0.193	4.44	0.75	9.42	47
Small Intestine length	-0.422	1.09	4.43	0.8	9.38	56.38
Lungs	-0.131	0.666	4.42	0.8	9.37	65.75
Liver	-0.496	0.399	3.64	1.07	7.73	73.48

Captive-reared F_5 Male & Wild-caught Male – Average squared distance = 31.42

	Captive-reared F_5 Male	Wild-caught Male				
	Av. Value	Av. Value	Av. Sq. Dist.	Sq. Dist./SD	Contribution %	Cumulative %
Spleen	-0.111	1.05	4.58	0.75	14.58	14.58
Liver	-0.462	0.399	3.62	1.01	11.53	26.11
Small Intestine length	-0.263	1.09	3.54	0.79	11.26	37.38
Lungs	-0.476	0.666	2.96	0.81	9.41	46.79
Heart	-0.316	0.193	2.61	0.79	8.31	55.1
Stomach	-0.144	0.115	2.39	0.69	7.61	62.71
Small intestine	-0.179	0.846	2.21	0.75	7.02	69.73
Caecum	-0.206	-0.36	2.17	0.69	6.91	76.64

APPENDIX C: PUBLISHED MANUSCRIPTS

Courtney Jones SK, Munn AJ and Byrne PG (2017) Effects of captivity on house mice behaviour in a novel environment: Implications for conservation practices. Applied Animal Behaviour Science **189:** 98 – 106

Courtney Jones SK, Munn AJ, Penman TD and Byrne PG (2015) Long-term changes in food availability mediate the effects of temperature on growth, development and survival in striped marsh frog larvae: implications for captive breeding programmes. Conservation Physiology **3:** cov029.

Williamson SA, Courtney Jones SK and Munn AJ (2014) Is gastrointestinal plasticity in king quail (*Coturnix chinensis*) elicited by diet-fibre or diet-energy dilution? Journal of Experimental Biology **217:**1839 – 1842

Courtney Jones SK and Munn AJ (2013) Caecal abnormality in a layer hen (*Gallus gallus forma domestica*) not accompanied by deficits in digestive performance or egg productivity. Animal Science Journal **84:** 97 – 100

Courtney Jones S, Cowieson A, Williamson S and Munn A (2013) No effect of short-term exposure to high-fibre diets on the gastrointestinal morphology of layer hens (*Gallus gallus domesticus*): body reserves are used to manage energy deficits in favour of phenotypic plasticity. Journal of Animal Physiology and Animal Nutrition **97:** 868 – 877