CHAPTER 6

Photographic identification: an alternative to toe clipping for patterned lizards

ABSTRACT

Persistence, reliability and individual discrimination are essential components for animal marking systems used in behavioural ecology studies. Toe-clipping is the most established marking technique for lizards, but current threats to native populations leave some researchers questioning the use of invasive field techniques. In particular, the effects of toe-clipping on juveniles are not well understood. In this study naturally occurring toe loss in the field is analysed to provide an accurate context against which to examine alternative identification methods for patterned lizards. Pattern persistence and individuality are demonstrated in a sample group of *Egernia whitii* (White's skink), an Australian lizard species. A photographic technique for use in identification is developed and the reliability of the method is tested for use in broad scale application by researchers. Field results show that less than 2% of juveniles and only those approaching adult size, appear to survive with toe loss, compared to >15% of the adult population. Pattern markings remained stable from birth to adulthood with minor contrast alterations. There was a significant association between human test subjects with a high level of training and high scores in pattern matching.

INTRODUCTION

Persistence, reliability and individual discrimination are essential components for animal marking systems used in behavioural ecology. Permanently marked populations are valuable for long term research (Hare & Cree 2005, Dodd 1993) in many different ecological and behavioural projects Three frequently used marking systems for lizards and amphibians are toe clipping, pit tags and pattern mapping. The most popular of these is toe clipping (Ferner 1979).

Reptiles and amphibians have been marked by toe clipping at almost every level of ecological research endeavour, including life history (Hare & Cree 2005), population density (Kwiatkowski & Sullivan 2002), diversity and abundance sampling (Hobbs *et al.* 1994), genetic diversity (Garner *et al.* 2003), home range (Rocha 1999), habitat relationships (Shenbrot & Krasnov 1997), community structure (Krasnov & Shenbrot 1998), habitat fragmentation (Funk *et al.* 2005), conservation (Dodd & Cade 1998), competition (Civantos 2000), dispersal (Olsson & Shine 2003), philopatry (Fuller *et al.* 2005), sociality (O'Connor & Shine 2003), growth (Guarino *et al.* 2003), growth modelling (Haigen & Fengxiang 1993), reproduction (Read 1999), physical performance (Le Galliard *et al.* 2004), tail autotomy (McConnachie & Whiting 2003), clinging performance (Bloch & Irschick 2005), trapping methods (Moseby & Read 2001) and trapping design (Hobbs *et al.* 1994). In many studies toe clipping is used solely as a means of individually identifying animals.

Studies which identify individual animals by toe clipping and combine this with estimations of age using skeletochronology (Guarino *et al.* 2003, Driscoll 1999) are far less numerous. Removing toe sections rather than major long bones in these studies avoids sacrificing the entire animal for an analogous result. Equally, studies which individually identify animals by toe clipping and examine compromised health (Reaser & Dexter 1996, Golay & Durrer 1994), performance effects (Bloch & Irschick 2005, Paulissen & Meyer 2000), or a reduction in survival or return rates (McCarthy & Parris 2004) as a consequence of the procedure, are also scant.

The rationale for toe clipping small lizards is that toe clipping is widely accepted as an inexpensive method that is simple, reliable and persistent (Hudson 1996), and a method that appears to conform to the criteria of causing minimal pain, and allowing behaviour and survival to remain unaffected (Ferner 1979). Adherents point to the natural occurrences of toe loss amongst field populations, and argue that high frequencies of this injury would not be found naturally if the loss of toes severely affected survivorship (Hudson 1996).

A limited amount of data concerning lizards is available on locomotor speed (Borges-Landez & Shine 2003, Dodd 1993) and clinging ability (Bloch & Irschick 2004, Paulissen & Myer 2000) before and after toe clipping, but long term consequences appear to be somewhat inconclusive. Data specifically for juveniles, in the vast majority of species, is particularly limited (Hudson 1996, Dodd 1993).

Toes that have been removed can be used in current research or preserved for future use as a source of DNA, but they are frequently discarded by researchers (Gonser & Collura 1996) even though the possibility of extracting DNA is a consideration in the debate on toe clipping (Funk *et al.* 2005). Conversely, blood is also used for DNA analysis, and can be removed from the ventral coccygeal vein (BSAVA 2004) or by using gravity and massage to gently press a small amount of blood through the tail tip after a tiny fleck at the distal tail end has been removed with nail clippers (unpublished data). These alternatives do not involve mutilation, and are less likely to cause suffering or damage, the latter being no more invasive than a scratch.

Over the last decade research has included the development of a coated filter paper for DNA analysis (Burgoyne 1996) on which < 1-2 µl of blood can be stored over extended periods (Fici *et al.* 1998) if DNA analysis is a requirement for the study. This method is suitable for a wide range of wildlife species (Smith & Burgoyne 2004).

Using amphibian data, McCarthy & Parris (2004) have established that with each additional toe missing the chances of recapture decrease. Regardless of whether the animals ultimately fail to survive or move to an alternative location, there is a potential for biased results in research which includes toe clipping. McCarthy & Parris argue that if a marking method influences survival or behaviour it has violated the important assumption that there are no adverse effects, and this could compromise the quality of the data, when the effect is unknown. May (2004) cautions that as a ‘sixth wave of mass extinction looms’ balancing the costs and benefits of present field practices becomes even more significant as we seek knowledge in conservation biology.

Amphibians have different skin than lizards, and different requirements for the preparation and treatment of wounds (Green 2001). Unlike the majority of lizards, much of their time is spent in water. The marked predisposition to infection shown by amphibians following toe clipping (Reaser & Dexter 1996, Golay & Durrer 1994) is not so manifest in lizards whose habitat is much dryer.

The end goal of guidelines for the use of live animals (Australian Code of Practice 2004) is to reduce the negative impacts and thereby reduce morbidity and mortality in laboratory and field populations during animal experimentation. Ethical discussions about the wellbeing of animals and ecosystems studied in scientific research are currently stimulating debate amongst researchers (Minteer & Collins 2005, May 2004, Schilthuizen 2004). The effect of procedures which may have adverse survival effects, such as toe clipping is particularly important when dealing with endangered or vulnerable native wildlife.

Permanently marking smaller lizards poses several challenges. Their size prohibits any but the smallest and lightest of markers, their need to refuge in narrow crevices and continued growth throughout their lives means that externally attaching identification devices increases their risk of direct damage or inability to avoid predators, and regular shedding removes any exterior marking within days or weeks. Passive integrated transponder (PIT) tags are radio-frequency identification tags. Funk *et al.* (2001) describe the smallest PIT tag as being about 10 mm long. Each tag holds a unique code which can be read with a portable scanner. The size of PIT tags available commercially prohibits their use in small species (Funk *et al.* 2005).

Pattern mapping is an identification method in which colour patterns and other features are used to identify individuals (Mellor *et al.* 2004). This paper documents a technique used for identifying patterned lizards by their individual markings using *Egernia whitii,* a medium sized Australian lizard species from the Scincidae family. *E. whitii* are polymorphic in pattern, with three pattern morphs represented. Fully patterned individuals display dorsal and lateral patterning, and comprise the most abundant morph (Milton 1990, Donnellan *et al.* 2002, Chapple 2005). Partially patterned individuals retain lateral patterning but lack dorsal patterning, and there is a patternless morph which is extremely uncommon and occurs in distinct regions (Donnellan *et al.* 2002).

On Wedge Island only one individual *E. whitii* has been recorded with a plain back. All others are recorded as fully patterned. Patterns consist of a series of light and dark markings. Back patterns consist of a series of intermittent light dots and dashes along two parallel dark bands running along the full length of the back and fading out gradually in the proximal third of the tail, and lateral patterns composed of linear markings and ocelli which are most distinct along the side of the neck and shoulders and fade to a more dappled effect towards the back half of the body.

These patterns bestow very effective camouflage in habitat composed of dry sclerophyll vegetation which, in the long hot summer months, provides a contrast of sunlight and shade. This species patterning was investigated for persistence and individuality from birth as a potential means of identifying animals during field studies.

There are a number of advantages in using a system where photographs of lizard patterns are used for individual identification. Photographic identification requires minimal handling time, it is non-invasive, and it avoids misidentification. Natural toe loss in the field or laboratory confounds the results of research when toe clipping has occurred, because it delivers erroneous identification of the individuals concerned.

In this study 365 field captures of *E. whitii* are analysed for toe damage to provide an accurate context against which to examine an alternative identification method. Pattern persistence and individuality in a sample group is investigated, a technique for pattern identification is presented, and the reliability of the method is rigorously assessed using a sample of laboratory reared *E. whitii* individuals photographed over developmental time from birth to adult size.

MATERIALS AND METHODS

Naturally occurring toe loss

Egernia whitii from Wedge Island in the Spencer Gulf of South Australia (35°09'S, 136°27'E) were used in a three year field study between March 2001 and March 2004. Wedge Island has been separated from the South Australian mainland for over 9,000 years (Robinson *et al.* 1996), and has a temperate Mediterranean climate with hot, dry summers and cool, wet winters. Much of the original woodland has been cleared but some patches of *Melaleuca lanceolata* (Dryland Tea-tree) woodland persist where the substrate is covered with loose and embedded rocks, which prevented clearing for agricultural use. There are large areas of coastal heath and vegetated sand dunes along with salt marshes in an inland drainage depression in the island’s north-west. Relatively flat cliff-top platforms with embedded, loose and exfoliating rocks exist over approximately ¾ of the island’s perimeter. A more detailed description can be found in Chapter 2.

During a study on Wedge Island between March 2001 and March 2004 north-south sampling transects at 200 metre intervals across the island were conducted, and several areas were sampled intensively. Three hundred and sixty five lizards were captured and processed. A laboratory colony of 98 skinks was retained for experimental trials from the transect captures and the remainder were released at the point of capture.

A description of location, habitat, weather conditions, sampling for DNA and parasites, and social proximity was recorded for each individual skink along with weight, snout to vent length (SVL), tail length, evidence of tail regeneration, hind leg and foot length, head length, head width and head depth. In addition detailed physical descriptions of all naturally occurring toe and nail loss, damage and scarring were completed.

The sample included 68 lizards from a capture-mark-recapture trial held in December 2003 (N=16) over 10 days, and a home range study in March 2004 (N=52) held over 20 days. Each skink was photographed, given a unique paint mark using Liquitex™ acrylic non-toxic artist colour, sex was determined when possible, and standard measurements were recorded. Individuals were remarked with fresh paint at each recapture.

Pattern persistence and individuality

Ten pregnant female *E. whitii* were captured in December 2003, and established in separate glass aquaria at Flinders University Animal Care Unit to await the birth of their young. They were kept in individual glass tanks 300mm deep x 600mm long x 305 mm wide lined with 20-30 mm of sand. At one end there was heat lamp warming a basking rock to 32°C for eight hours a day, and at the other end a concrete paver supported by two pieces of angle plastic formed a permanent refuge.

As the newborn juveniles were born they were maintained together with their mothers and siblings in the same enclosures and three smaller refuges built with smaller pavers were substituted to accommodate the family groups. The tanks were housed in 25°C constant room temperature with photoperiods of 12:12 hour light and dark.

Ultra-violet lights were suspended above the tanks and automatically activated for eight hours during the light hours to aid newborn individuals in calcium absorption. The family groups were fed three different meals a week: crickets, meal worms, and pureed fruit and vegetables with lizard supplement, and for the first few months after birth extra baby crickets were supplied to the juveniles.

Pattern individuality and stability for this species has not previously been documented. If patterns are to be used as a form of individual identity it would be necessary to establish that each individual's pattern was unique, and that despite multiple sheddings, each would remained recognizable from birth to adulthood. Each juvenile in the trial was photographed laterally within the first three days of birth, then fortnightly for two months, and then every two or three months until a SVL of 70mm had been achieved. Detailed measurement and physical condition records were made at the same intervals.

Newborn skinks were photographed for the first time at four different body sites: 1) chins, 2) heads, 3) backs and 4) left and right sides. Juvenile *E. whitii* are small and have a dark skin layer before they first shed, making it difficult to capture a clear image. The neonates resisted being turned over to expose their ventral surfaces, and although their chins are differently patterned between individuals, the white ventral scales reflected light so strongly that using this area for identification was not optimal.

In contrast, while it is easy to capture a clear head photograph, there are few distinguishing features on plan view of the head. Back patterns are very detailed, making the back a complex site to use for identification. The most practical site for pattern identification was lateral patterning. Potentially all but the patternless morph can be identified by lateral patterns, and individuals are asymmetrically patterned, allowing a greater amount of useful identification information.

The most noticeable difference in lateral patterning occurs in the area between the ear and immediately behind the shoulder (Figure 2). The light markings around the ear and neck contrast with darker background scales, and while many of the side markings are ocelli, there are frequently distinctive linear markings in this region of the body. The neck and shoulder areas of left and right lateral patterns were therefore selected for individual identification, and dual photographs at regular intervals of growth became standard practice (Figure 3).

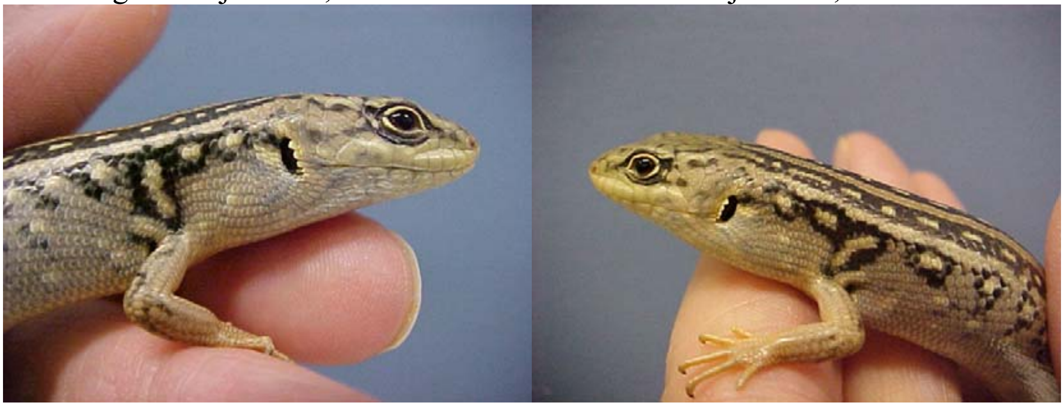
Figure 2: Choice of body areas for identification. Rectangle indicates area used for pattern matching.

Pattern identification processing involved counting the number of ocelli and linear markings between the ear and the shoulder area and noting their size, shape and orientation. Many juveniles had such distinct patterns that identification was easily achieved from the pattern on one side. However for some siblings the left side of one individual's pattern was similar to the left side of another individual's pattern, and matching the right side in conjunction with the left side was an essential criterion for correct identification. Therefore the established procedure for all skinks became matching the left side pattern first, then the right side pattern to add complementary information and complete the match. All photographs for the experiment were taken at intervals as the juveniles grew to mature size, shedding multiple times along the way.

A Sony Mavica 2.0 megapixel digital camera and an O'Rite VC3210 3.1 megapixel digital camera were used for the photography. Both cameras were used on the lowest resolution available for the individual camera, to facilitate downloading of photographs onto limited PC space.

277a: Right side newborn, SVL 39mm.
277a: Left side newborn, SVL 39mm.

277a: Right side juvenile, SVL 50mm.
277a: Left side juvenile, SVL 50mm.



277a: Right side adult, SVL 74mm.
277a: Left side adult, SVL 74mm.

Figure 3: Dual photographs displaying right and left side patterning

Method reliability testing

To assess how robust pattern matching might be for broad-scale application by researchers, a group of 40 human subjects were divided into ‘expert’ and ‘naïve’ groups.

The ‘expert’ volunteer group was drawn from the biology community and included representatives from undergraduate level through to professional and experienced biologists, in widely diverse fields from herpetology to molecular biology and microbiology. The ‘naïve’ volunteer group was drawn from a variety of lay persons including clerical, administrative and information technology staff through to academic staff in fields other than biology. No-one in this group had biological training.

Data generated from these tests were analysed to assess pattern matching ability in terms of level of education, exposure to formal biological training and training in ‘whole organism’ or ‘non-whole organism’ fields.

Six of 23 newborn juveniles in the cohort died during the neo-natal period. Seventeen juveniles reached adult size for inclusion in the recognition trial, and of these ten were randomly selected for testing. Each person taking part in the tests was given an individual pack comprising the same ten adult photographs, three groups of six randomly selected juveniles, a record form for details of education and experience, and a marking grid (see Figure 4) to score matches. The three juvenile groups were labelled juvenile 1 (J1), juvenile 2 (J2), and juvenile 3 (J3) groups.

The J1 group were neonates between 39-47mm SVL ranging in age between a few hours after birth to 20 days old. The J2 group consisted of individuals that were no longer neonates but still in their first year of growth (35-56mm SVL), and this group was between five and ten weeks old. The J3 group comprised a combination of individuals from all three size classes: two juveniles in their first year (33.3%), three juveniles in their second year (50%), and one adult in the third size class (16.7%).

This combination represented a more difficult matching situation than field conditions, because 1) neonates are rarely encountered during field activities, 2) one and two-year old juveniles (SVL 35-69mm) make up only 30% of captures on Wedge Island, and 3) adult identification is much more easily achieved than juvenile identification. All the adult photographs were selected from the third size class (70 – 92 mm SVL) and the skinks in this group ranged between 70-79 mm SVL, within the size class attributed to three years of growth or greater in the field.

Table 1 presents data on size classes and age to maturity on Wedge Island (Chapter 3).

	Juvenile 1	Juvenile 2	Juvenile 3	Adult
1				
2				
3				
4				
5				
6				
7				
8				
9				
10				

Figure 4: Marking grid for pattern identification tests

The test subjects were asked to display photographs from the J3 group in front of them and then to go through the adult photographs one at a time trying to find a match. No juvenile photographs were to be eliminated by marking or turning over, and all the adult photographs were to be turned face side down except when in use. The adult photographs were designated with a number and the juvenile photographs were identified by alphabet letters.

On the marking grid the number of the adult was to be written in the right hand column and the juvenile alphabet code, if a match was found, was to be written in the juvenile column to the left. If no match was found then this was to be indicated. When all entries had been made for the J3 group, they were replaced with the J2 group and then the J1 group until all photographs were recorded. In this way each test subject made 30 matching decisions.

Each of the ten packs followed the same format, differing only in content. Each of the packs was used multiple times. The same ten adult photographs were used in each of the packs, but the 18 juveniles in three groups of six were selected randomly and each pack was different.

To ensure that the test subjects could not predict six matches in each column, it was randomly determined whether a matching juvenile photograph was removed from each group and replaced with one of the seven individuals taken at a comparative age that had not been selected for the trials. The test subjects did not know how many matches they had in any one group. For statistical purposes no elimination or comparison of photos was allowed during the trial, whereas informal learning is possible in field or laboratory conditions when this technique is used.

RESULTS

Naturally occurring toe loss

Concomitant research on 365 *E. whitii* encountered in the field (Chapter 3) identified three age classes found in Table 1.

Table 1: Age or size classes of *E. whitii* on Wedge Island

Years from birth	Size class in mm SVL
1st year: juvenile	39–56
2nd year: juvenile	57-69
3rd year and >: adult	70-92

I examined the effect of age class on toe damage using a Kruskal-Wallis non-parametric AVOVA. This indicated a highly significant effect ($X^2_2 = 33.943$, P = <0.001) and the indices of damage are summarised in Table 2. In their first two years of life all *E. whitii* skinks are still juveniles, but during their third year they grow from sub-adult to sexually mature individuals. One hundred and ten (30.14%) were from the juvenile size classes, and 255 (69.86%) were either sub-adults or mature individuals.

Forty-six skinks (12.6%) had some physical damage recorded. One adult had minor toe damage without toe loss, another had lost one toe nail, but still retained an intact tail and had no other scarring or injuries, one had minor scarring and a soft double end to one toe with no evidence of a nail, and two adults had lost one toe nail each and were described as having minor scarring. The remaining 41 skinks (11.23% of the total field population used in this survey) recorded with damage had one or more missing or partially missing toes.

Table 2: Number and percentage of skinks with toe loss

Size class in mm SVL	Number in sample group	Number of skinks with toe loss	% of size class	% of total population
35 - 56	59	0	0	0
57 – 69	51	2	3.9	0.5
70 – 92	255	39	15.3	10.7
Totals	**365**	**41**		**11.2%**

The number of toes lost by individual skinks varied between one and seven (Table 3). Skinks with five toes missing included a juvenile with multiple toe abnormalities, and a young adult with a missing foot and lower leg.

Table 3: Individual toe losses and percentage representation of the sample of skinks with toe damage

Number of toes missing	Number of skinks affected	Percent	Cumulative percent
1	17	41.5	41.5
2	14	34.1	75.6
3	6	14.6	90.2
5	2	4.9	95.1
6	1	2.4	97.6
7	1	2.4	100.0
Total	**41**	**100.0**	

I combined the two juvenile classes, and the sub-adult and adult classes for further analysis. From a total of 110 juveniles, 1.81% had naturally occurring toe loss, and from a population of 255 adult individuals, 15.29% showed toe loss.

Only six (14.6%) of the 41 damaged skinks retained full tails. The remaining 35 (85.4%) had some degree of tail loss or regeneration indicating other prior damage. Two skinks with toe loss also had forked tails, and the smaller of the juveniles (64mm SVL) had multiple toe deformities and losses, and was described as very weak and thin.

Figures 4 and 5 respectively show the number of individuals with toe loss for age, and the mean of naturally occurring toe loss for every 10mm SVL of body growth.

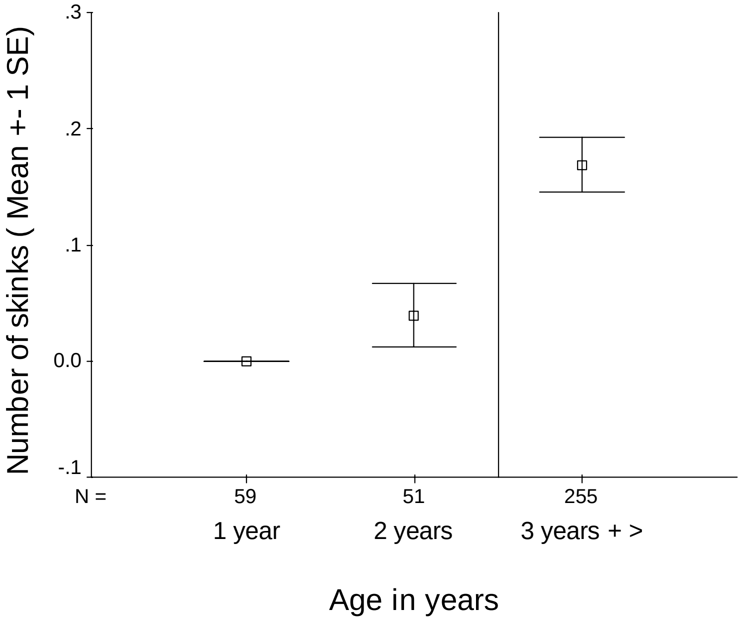


Figure 4: Mean number of individuals with toe loss for each age category (1-2 years = juvenile, 3 years + = adult). The vertical line divides juvenile from adult groups.

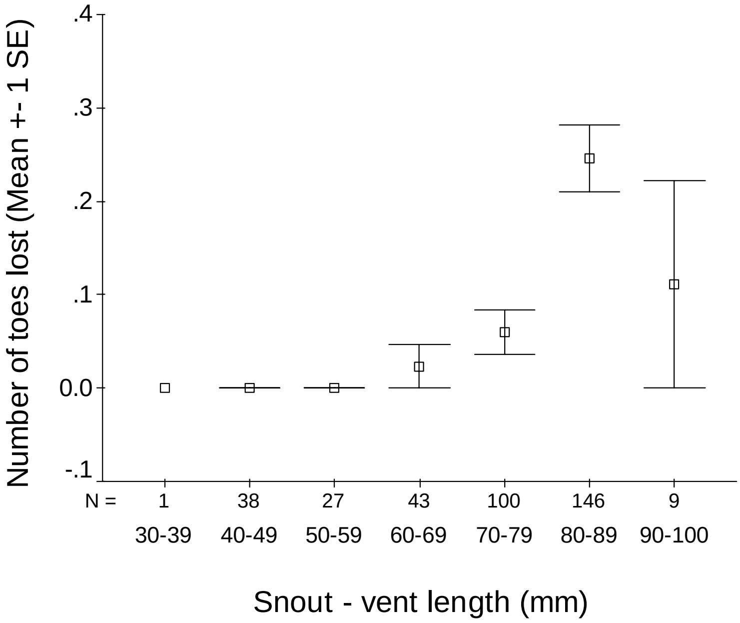


Figure 5: Mean number of individuals with toe loss for each size category, grouped in 10 mm intervals.

Wedge Island field captures (N= 365) and their laboratory born progeny (N = 26) were used as the data base for Discriminant Analysis (N = 391) to determine the sex of the skinks with toe loss. Only skinks with a snout to vent length ≥ 70mm were used in the analysis (N = 280), and the reliability of the data was recorded in percentages.

The number of missing toes amongst individuals was analysed to identify any relationship between sex and severity of injury. There were 19 skinks with a reliability prediction of sex 95% or greater (females = 9, males = 10). A Mann-Whitney U-test showed a Asymp. Sig. (2-tailed) score of P = 0.894. As there was no significant difference in the number of toes lost between females and males in this group, 28 skinks with a reliability prediction of 80% or greater were also tested (Females = 13, males = 15). However, the results were similar (Asymp. sig.2-tailed, P = 0.800). Because of this result male and female data were combined for the remaining tests.

The position of toe loss injury was analysed to identify whether front (N = 11 cases of loss) or back toes (N= 26) were statistically more likely to be lost. A Chi-Square test showed a significant difference (P = 0.014) between the number of front feet and hind feet injuries. However when the number of toes lost in front or hind feet was compared using a Mann-Whitney U-test, the Asymp. Sig. (2-tailed) was P = 0.473. While hind feet were more likely to be injured, there is no significant difference in the number of toes lost per injury (Figure 6).

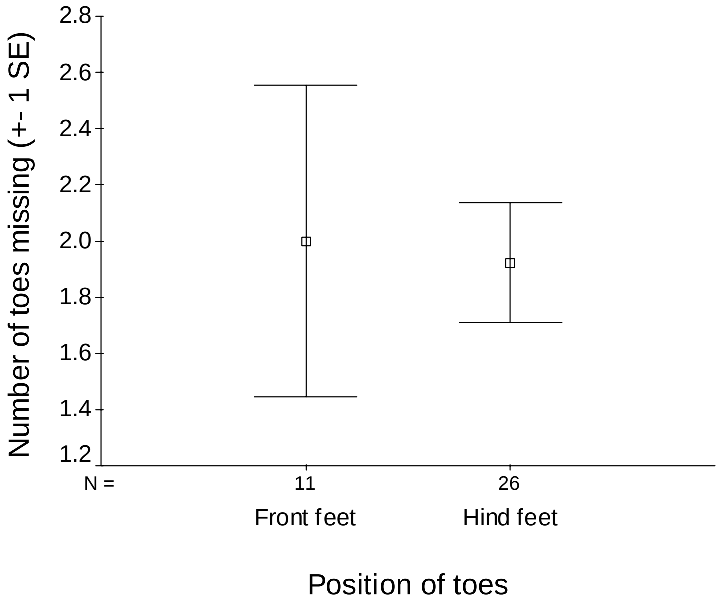


Figure 6: The mean number of missing toes per injury in front and hind feet.

Pattern persistence, reliability and photographic technique

Individual photographs tracking successive developmental stages in each laboratory born skink meant that I was able to track all individuals over time.

As the newborn skinks grew in size, the contrast between light and dark markings changed and some of the lighter markings became less distinct towards the ventral surface. The overall pattern however remained similar. Each of the 17 laboratory born skinks (100%) maintained an individual pattern which persisted from birth, through multiple sheddings, to adulthood.

Method reliability testing

The J3 group was nearest in age to the adults and more easily matched than the others. In contrast the J1 group consisted only of neonates, and the J2 group was composed only of juveniles less than 1 year of age, but older than neonates. J1 and J2 sizes are less frequently captured in the field, and were expected to be associated with the greatest degree of difficulty in pattern matching.

A boxplot contrasting scores for the expert and naïve groups showed that 50% of the expert group had a score of nine or more from a total of 10 for the J3 group (Figure 7).

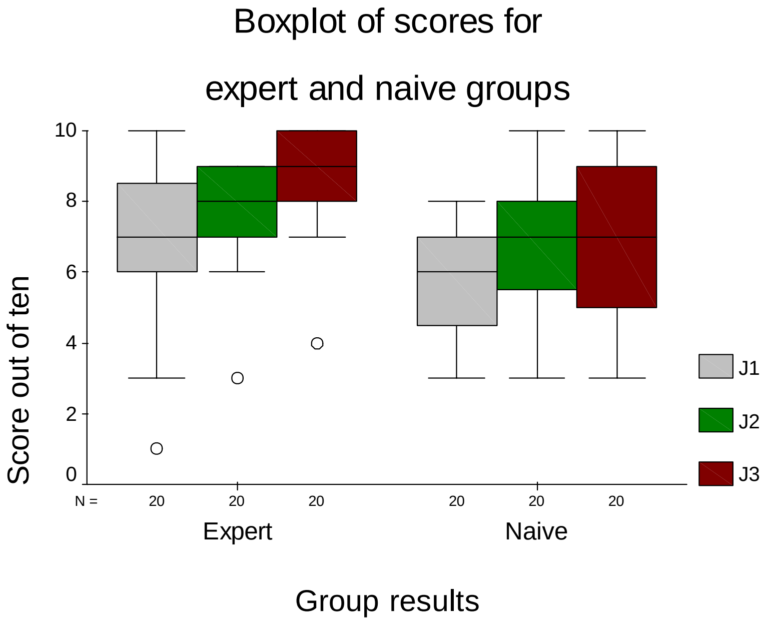


Figure 7: Naïve and Biologist results showing median and inter-quartile ranges of matching scores for each group.

We explored this by further analysis of the expert and naïve groups. The groups were divided into those scoring 10 and those scoring 9 or less, with each section further classified by educational qualifications (Table 4).

Table 4: Test scores achieved by subjects with different educational levels

Group	Score	BSc (Hons) or below	PhD in progress or above
J3	10	0	9
J3	≤9	10	1
J2	10	3	0
J2	≤9	7	10
J1	10	0	2
J1	≤9	10	8

Fisher's Exact test, 2-sided significance (P = < 0.001) confirmed that there was a significant association between human test subjects with a score of 10 for matches in the J3 group and PhD training or completion. A Mann-Whitney U-test was used to analyse J1 and J2 results because of the low scores. There was no significant difference between scores in the J2 group, although the expert group did tend to do better. However the J1 group also confirmed a significant association between human test subjects with PhD training or completion and high scores (W = 318.5, P = 0.012).

The J3 group most closely resembled the composition of field captures with the adult component deleted. Neonates (J1) usually remain hidden during their first 4 weeks of life. Juveniles in their first year of life (J2) make up a minority (16%) of field captures leaving 84% of captures in the second (J3), and third years of life. Nine out of ten volunteers with a perfect score of ten for the J3 group had reached a training level of PhD in progress or completion.

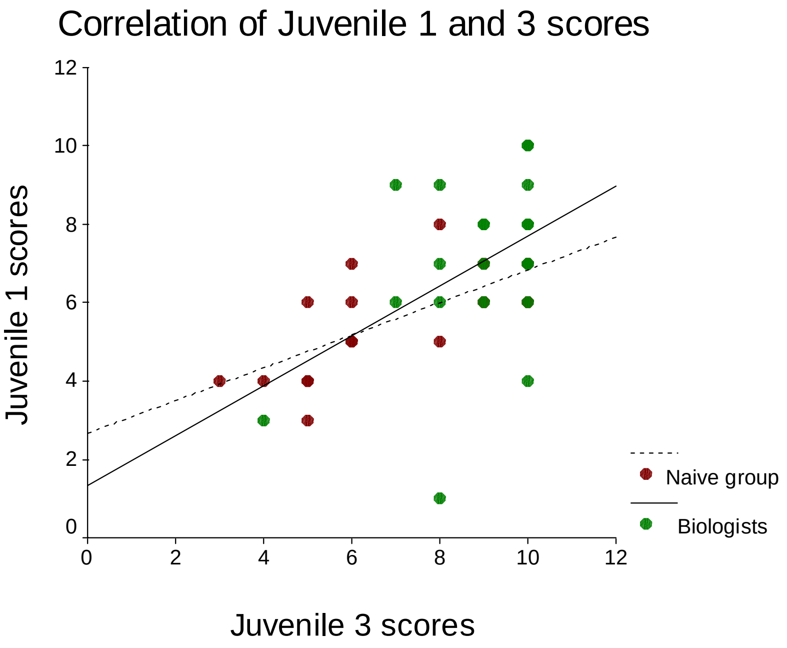


Figure 8: Naïve and Biologist group correlation of J1 and J3 pattern matching.

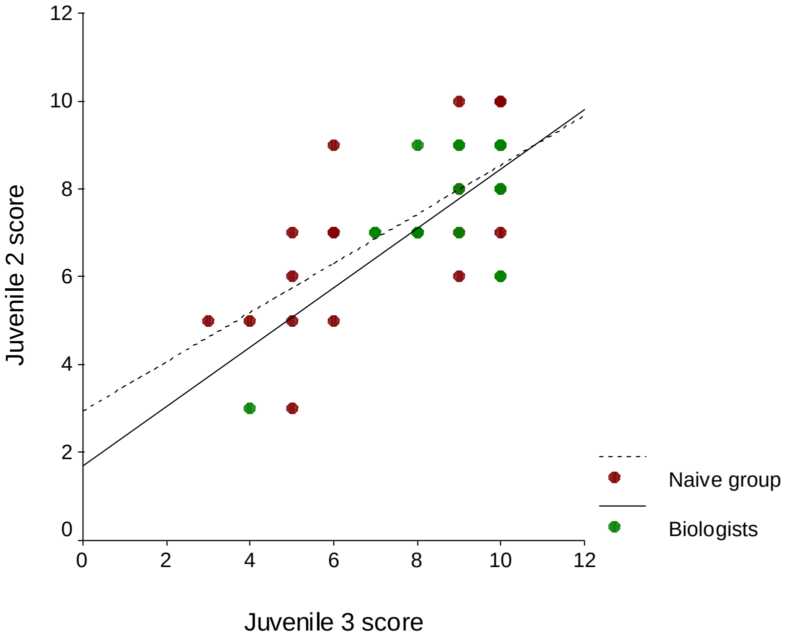


Figure 9: Pattern matching accuracy correlating J2 and J3 skinks with group scores, with linear regression lines fitted.

Scatter plots showed that matching difficulty met expectations (Figures 8 and 9). Those who scored well for the J3 photographs also scored better in the J2 and J1 sections.

We found a significant difference between experts and non-experts in matching the J1 group (Mann-Whitney U test, Z = -2.515, P = 0.012) and the J3 group (Mann-Whitney U test, Z = -2.314, P = 0.021) but not for J2 (Mann-Whitney U test, Z = -1.457, P = 0.145).

DISCUSSION

Toe loss

The only potential predator of *E. whitii* on Wedge Island are raptors. Tails provide an obvious 'target' on fleeing skinks and *E. whitii* are one of the many skink species utilising voluntary caudal autotomy, a mechanism which is thought to provide an effective distraction to pursuing predators.

Although toe loss has also been speculatively attributed to predation (Hudson 1996) because it occurs more frequently on rear toes than front toes (Bellamy 2006, Hudson 1996), animals kept in the predator free environment of the laboratory also suffered toe loss. This problem presented as multiple shedding difficulties. Skin shedding from the feet and toes appeared to become dehydrated in the dry atmosphere of the laboratory, and tended to form constricting bands around the toes. When the skin was removed in the early stages of this event, the toes underneath were swollen and red, but in severe cases the toe was missing or dry, blackened and decaying.

Hazell *et al.* (1995) reported similar findings in a group of *Tiliqua scincoides.* Failure to shed (dysecdysis) in combination with a contaminating organism led to subsequent necrosis of bone and tissue below the constriction and multiple toe losses. In their study, and in my experience, progressive necrosis in some individuals can lead to the loss of whole feet.

The loss of whole feet is also reported in the field (Hudson 1996, Bellamy 2006). Hazell *et al.* (1995) investigated the causative organism in their study and reported fungal invasions of *Trichophyton terrestre* at all affected sites in all the skinks examined. If *T. terrestre* is able to invade pre-existing lesions and play a further role in the development of progressive digital necrosis as suggested, the fewer opportunities it gains through toe clipping, the better.

There are other factors that may be implicated in toe loss. Toe nail loss may be a predisposing factor. Four skinks were recorded with toe nail loss in the field and this was a common injury in the laboratory. Toe nail loss may occur when the nail catches in rough surfaces and the skink pulls vigorously to free its foot, as observed during filming in the laboratory. It is possible that when the toe pad is no longer protected by a nail, it becomes vulnerable to further damage. Toe nail losses were recorded in the laboratory in all age groups after the first few weeks of life, both in shedding and non-shedding individuals.

Among different species toe loss is greater in one sex over the other (Hudson 1996). This study found that toe loss was more frequent in rear feet, but that there were no significant differences in injury rate between the sexes. Male, female and juvenile *E. whitii* display aggression within and between groups (Bruyn 1994). Sibling rivalry is particularly extreme in this species as six of 23 neonates (26%) born in the laboratory were constantly harassed by siblings and failed to survive the first four weeks of life despite the provision of ample food and protection from predation (unpublished).

In *E. whitii*, an unequal size difference between skinks does not usually results in conflict, as the smaller skink flees. Similar sized *E. whitii* individuals engaging in aggressive or assertive behaviour seek to display their size, strength and motivation by several different behaviours including circling snout to tail with arched backs (Bruyn 1994). Bruyn (1994) reports that tail biting is the most common outcome in these encounters, but it is possible that hind legs and toes are sometimes damaged during agonism. Aggressive chasing taking place between unfamiliar individuals of unequal sizes when they confront is usually followed by biting, and limbs and toes could be damaged in this way.

The limited reporting of separate juvenile results reduces our capacity to assess the effect of toe clipping on them. Hudson (1996) documents that in a sample group of 145 *Pseudemoia pagenstecheri* juveniles, only three had toe loss. In the Wedge Island population of *E. whitii* juveniles less than one year of age with toe loss were not present. One of the two-year old juveniles with toe loss was described as thin and weak. It appeared to have congenital abnormalities affecting size and growth of toes and its subsequent death after release and before reproductive age appeared inevitable. The only healthy juvenile recorded in the sample population had lost only part of one toe and was 2mm short of the sub-adult/adult age class.

One hypothesis used to explain the lack of juveniles with toe loss in the population is that toe losses accumulate with age. However we should not ignore the possibility that juveniles with toe damage are less likely to survive. Lizards begin reproducing before they attain maximum body size, although the rate of growth declines with age (Shine & Charnov 1992). The greatest percentage of *E. whitii* affected by toe loss on Wedge Island is found in the 84-92mm SVL. These individuals are the largest in the population.

The positive relationship between body size and dominance in lizard studies (Martín & Forsman 1999, Olsson & Shine 1996, Olsson 1992, Cooper & Vitt 1987, Tokarz 1985, Brackin 1978, Ruby 1978) gives rise to the conjecture that that mature skinks, having attained a large body size, are likely to have gained and maintained a high-quality group territory, where they can remain dominant despite incurring toe injuries.

The corollary of this may be that the vulnerable position of smaller skinks renders them less able to survive because of their toe loss. Chapple (2005) reports litters of one to four offspring and data from lizards on Wedge Island concurs (Chapter 3). Because agonism occurs amongst siblings shortly after birth (unpublished data), a small number of healthy juveniles suffering toe loss from aggressive encounters should be present in the population if they are able to survive and develop to adulthood. The lack of juveniles with toe loss in the field highlights the need to further investigate the consequences of routine toe clipping, as it may diminish their survival.

Natural toe loss in the field or laboratory can confound the results of research when toe clipping has occurred, by delivering erroneous identification of some of the individuals marked in this way. This would lead to misidentification rates of about 15% for adult *E. whitii*. Importantly, natural toe loss will be scored as a false positive during identification, so any toe loss will always corrupt the data. Statistics from Wedge Island demonstrate that toe clipping this species should proceed with great caution.

Photographic identification

For *E. whitii* my results show that photographic technique can be an important component of identification. Allowances must be made for 1) left and right lateral views for each individual skink, 2) ensuring the front leg does not obscure the pattern during photography, 3) looking for contrast in patterns, not colours, 4) adapting to different angles, magnitude and lighting for each photograph.

Depending on the experimental design, photographic identification can be used in conjunction with non-invasive marking methods such as paint marking, location, sex, snout to vent length, tail regeneration, or plain or patterned backs. In a field trial of this method, the use of paint-marking, measurements and observations eliminated many of the possible matches. Sixty-eight lizards were captured and released, but only two identifications required the use of photographs after paint marking, measurements of body length ±2mm, and tail length and regeneration records were taken into account.

Extrapolating from these statistics, and continuing the use of non-invasive supplementary information and techniques in intensive surveys, an average of three individuals per 100 lizards might require a search of photographic records, therefore 3 x 5, or 15 lizards in a group of 500. In this scenario the majority of the 500 photographs would be of lizards that are either too small (animals do not shrink in size) or too large (given standard growth rates) to feasibly fit the criteria, so the majority of these photographs could be set aside, leaving a small number to process.

However, animals that have dispersed great distances from the original capture site, animals that have evaded capture for a period of time, and populations that exceed an expeditious size, are likely to impose time constraints on human identification. The use of human skills to match photographs will conceivably be replaced in the future, in the laboratory, and in the field, by software identification programs. Technological advances will forseeably allow ‘on the spot’ identification without the use of invasive procedures or stress inducing prolonged handling.

Sophisticated software identification programs (e.g. Mandrake™, Unisys™, VeriLook™) that are currently commercially available provide matching accuracy (Adler & Maclean 2004), and are capable of handling considerably greater numbers of individuals. They would have broad applicability to individual identification in many species of lizards and amphibians. A presentation of these programs is outside the scope and funding of the present study.

There is no doubt that the field practice of toe clipping, which May (2004) describes as ‘casual barbarity’, will be subjected to greater scrutiny in the future. The development of a PIT tag with a diameter size of 1mm, capable of holding a six to ten digit number, obtainable in bulk at a low cost, pre-packaged with a sterile applicator with a fine bore cannula, which can be inserted beneath the skin or scales with minimal pain and trauma to the reptile, would be universally welcome. Until this is achieved the adoption of a variety of alternative methods, each chosen to suit the species studied, may be the ultimate solution to the current conservation dilemma.

REFERENCES

Adler A. & Maclean J. (2004) Performance comparison of human and automatic face recognition. Biometrics Consortium Conference **2004** Available**:** www.site.uottawa.ca/~**adler**/ publications/publications.

Australian code of practice for the care and use of animals for scientific purposes (7th Edition 2004). Australian Government, National Health and Medical Research Council.

Bloch N. & Irschick D.J. (2004) Toe clipping dramatically reduces clinging performance in a pad-bearing lizard (*Anolis carolinensis*). *Journal of Herpetology* **39**, 288-293.

Brackin M.F. (1978) The relation of rank to physiological state in *Cnemidophorus sexlineatus* dominance hierarchies. *Herpetologica* **34**, 185-191.

Bruyn M.J. (1994) The social organisation of *Egernia whitii* in Tasmania. Honours Thesis, University of Tasmania, Hobart, Tasmania.

BSAVA Manual of Exotic Pets, 4th Edition; Exotic Animal Medicine for the Veterinary Technician; Compendium June 2004 - Diagnostic Techniques and Sample Collection in Reptiles

Burgoyne L. (1996) *Solid Medium and Method for DNA Storage*. US 5,496,562.

Chapple D.G. (2005) Life history and reproductive ecology of White's skink, *Egernia whitii*. *Australian Journal of Zoology* **53**, 353-360.

Civantos E. (2000) Home-range ecology, aggressive behaviour, and survival in juvenile lizards, *Psammodromus algirus*. *Canadian J. Zoology*. **78**, 1681-1685.

Cogger H.G. (2000) *Reptiles and amphibians of Australia.* Reed New Holland, Sydney, NSW, Australia.

Cooper W.E. & Vitt L.J. (1987) Deferred agonistic behaviour in a long-lived scincid lizard *Eumeces laticeps*. *Oecologica* **72**, 321-326.

Dodd C.K. (1993) The effects of toeclipping on sprint performance of the lizard *Cnemidophorus sexlineatus*. *Journal of Herpetology* **27**, 209-213.

Dodd, Jr. C.K. & Cade B.S. (1998) Movement patterns and the conservation of amphibians breeding in small, temporary wetlands. *Conservation Biology* **12**, 331-339.

Donnellan S.C., Hutchinson M.N., Dempsey P. & Osbourne W. (2002) Systematics of the *Egernia whitii* species group (Lacertilia: Scincidae) in south-eastern Australia. *Australian Journal of Zoology* **50**, 439-459.

Driscoll D.A. (1999) Skeletochronological assessment of age structure and population stability for two threatened frog species. *Australian Journal of* Ecology **24**, 182-189.

Ferner J.W. (1979) *A review of marking techniques for amphibians and reptiles*. SSAR Herpetological Circular No. 9.

Fici D.A., Mahan S., Williamson J., Houranieh R., Mitchell S. & Awdeh Z. (1998) Sequence Based Typing of Blood Spots on Filter Paper After Extended Storage. *Human Immunology*, **59**, 141.

Fuller S.J., Bull C.M., Murray K. & Spencer R.J. (2005) Clustering of related individuals in a population of the Australian lizards, *Egernia frerei*. *Molecular Ecology* **14**, 1207-1213.

Funk W.C., Donnelly M.A. & Lips K.R. (2005) Alternative views of amphibian toe-clipping. *Nature* **433**, 193.

Funk W.C., Greene A.E., Corn P.S. & Allendorf F.W. (2005) High dispersal in a frog species suggests that it is vulnerable to habitat fragmentation. *Biology Letters* (*Royal society of London*) **1**, 13-16.

Garner T.W.J., Angelone S. & Pearman P.B. (2003) Genetic depletion in Swiss populations of *Rana latastei*: conservation implications. *Biological Conservation* **114**, 371-376.

Golay N. & Durrer H. (1994) Inflammation due to toe clipping in natterjack toads (*Bufo calamita*). *Amphibia-Reptilia* **15**, 81-96.

Gonser R.A. & Collura R.V. (1996) Waste not, want not: toe clips as a source of DNA. *Journal of Herpetology* **30**, 445-447.

Green D.E. (2001) *Toe-clipping of frogs and toads*. Standard operating procedure ARMI SOP No.1 National Wildlife Health Center.

Guarino F.M., Lunardi S., Carlomagno M. & Mazzotti S. (2003) A skeletochronological study of growth, longevity, and age at sexual maturity in a population of *Rana latastei* (Amphibia, Anura). *Journal of Bioscience 28*, 775-782.

Haigen X. & Fengxiang Y. (1993) Age classification and growth model of *Phrynocephalus przewalskii*. *Ecological Modelling* **70**, 127-135.

Hare K.M. & Cree A. (2005) Natural history of *Hoplodactulus stephensi* (Reptilia: Gekkonidae) on Stephens Island, Cook Strait, New Zealand. *New Zealand Journal of Ecology* **29**, 137-142.

Hazell S.L., Eamens G.J. & Perry R.A. (1985) Progressive digital necrosis in the Eastern Blue-tongued skink, *Tiliqua Scincoides*. *Journal of Wildlife Diseases* **21**: 186-188.

Hobbs T.J., Morton S.R., Masters P. & Jones K.R. (1994) Influence of pit-trap design on sampling of reptiles in arid spinifex grasslands. *Wildlife Research* **21**, 483-490.

Hudson S. (1996) Natural toe loss in southeastern Australian skinks: implications for marking lizards by toe-clipping. *Journal of Herpetology*, **30**, 106-110.

Krasnov B.R. & Shenbrot G.I. (1998) Structure of communities of ground-dwelling animals at the junction of two phytogeographic zones. *Journal of Biogeography* **25**, 1115-1131.

Kwiatkowski M.A. & Sullivan B.K. (2002) Mating system structure and population density in a polygynous lizard, *Sauromalus obesus* (= ater). *Behavioural Ecology* **13**, 201-208.

Le Galliard J-F., Clobert J. & Ferriere R. (2004) Physical performance and Darwinian fitness in lizards. *Nature* **432**, 502-505.

Mandrake: Facial recognition systems. Available: http://tash.gn.apc.org/face_rec.htm

Martín J. & Forsman A. (1999) Social costs and development of nuptial coloration in *Psammodromus algirus* lizards: an experiment. *Behavioural Ecology* **10**, 396-400.

May R.M. (2004) Ethics and amphibians. *Nature* **431**, 403.

McCarthy M.A. & Parris K.M. (2004) Clarifying the effects of toe clipping on frogs with Bayesian statistics. *Journal of Applied Ecology* **41**, 780-786.

Mellor D.J., Beausoleil N.J. & Stafford K.J. (2004) *Marking amphibians, reptiles and marine mammals: animal welfare, practicalities and public perceptions in New Zealand.* New Zealand Department of conservation.

McConnachie S. & Whiting J.M. (2003) Costs associated with tail autotomy in an ambush foraging lizard, *Cordylus melanotus melanotus*. *African Zoology* **38**, 57-65.

Milton D.R. (1990) Genetic evidence for sympatric differentiation between two colour morphs of the skink *Egernia whitii*. *Australian Journal of Zoology* **38**, 117-130.

Minteer B.A. & Collins J.P. (2005) "Why we need an "ecological ethics". *Frontiers in Ecology and the Environment* **3**, 332-337.

Moesby K.E. & Read J.L. (2001) Factors affecting pitfall capture rates of small ground vertebrates in arid South Australia. II. Optimum pitfall trapping effort. *Wildlife Research.* **28**, 61-71.

O'Connor D. & Shine R. (2003) Lizards in nuclear families: a novel reptilian social system in *Egernia saxitilis* (Scincidae). *Molecular Ecology* **12**, 743-752.

Olsson M. (1992) Contest success in relation to size and residency in male sand lizards, *Lacerta agilis*. *Animal Behaviour* **44**, 386-388.

Olsson M. & Shine R. (1996) Does reproductive success increase with age or with size in species with indeterminate growth? *Oecologica*, **105**, 175-178.

Olsson M. & Shine R. (2003) Female-biased natal and breeding dispersal in an alpine lizard, *Niveoscincus microlepidotus*. *Biological Journal of the Linnean Society* **79**, 277-283.

Parris K.M. & McCarthy M.A. (2001) Identifying effects of toe clipping on anuran return rates: the importance of statistical power. *Amphibia-Reptilia* **22**, 275-289.

Paulissen M.A. & Myer H.A. (2000) The effect of toe-clipping on the Gecko *Hemidactylus turcicus*. *Journal of Herpetology* **34**, 282-285.

Read J.L. (1999) Longevity, reproductive effort and movements of three sympatric Australian arid-zone geckos. *Australian Journal of Zoology* **47**, 307-316.

Reaser J.K. & Dexter R.E. (1996) *Rana pretiosa* (spotted frog). Toe clipping effects. *Herpetological Review* **27**, 195-196.

Robinson T., Canty P., Mooney T. & Ruddock P. (1996) *South Australia's offshore Islands.* Commonwealth of Australia and Department of Environment and Natural Resources, South Australia.

Rocha C.F.D. (1999) Home range of the tropidurid lizard *Liolaemus lutzae*: sexual and body size differences. *Revista Brasilia Biologia* **59**, 125-130.

Ruby D.E. (1978) Seasonal changes in the territorial behaviour of the Iguanid lizard *Sceloporus jarrovi*. *Copeia*, 1978, 430-438.

Schilthuizen M. (2004) Don't touch my toes. Sciencenow Available: http://sciencenow.sciencemag.org/cgi/content/full/2004/812/4

Shenbrot G. & Krasnov B. (1997) Habitat relationships of the lizard fauna in the Ramon erosion cirque, Negev Highlands (Israel). *Journal of Zoology London* **241**, 429-440.

Shine R. & Charnov E.L. (1992) Patterns of survival, growth and maturation in snakes and lizards. *American Naturalist* **139**, 1257-1269.

Smith L.M. & Burgoyne L.A. (2004) Collecting, archiving and processing DNA from wildlife samples using FTA® databasing paper. *BMC Ecology* Available: http://www.biomedcentral.com/1472-6785/4/4

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

Unisys & Australian passports. Available: http://www.unisys.com/public_sector/clients/featured_case_studies/asstralian_pa

VeriLook – *Face identification technology*. Fulcrum Strategic Partners, Inc. Available: http://www.fulcrumspi.com/verilook.htm

Williamson I. & Bull C.M. (1996) Population ecology of the Australian frog *Crinia signifera*: adults and juveniles. *Wildlife Research* **23**, 249-266.