# Habitat Restoration and Management of Endangered Species on Sydney Sandstone Outcrops

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# Declaration

I hereby certify that the work presented in this thesis is my own (except where specifically acknowledged), and that this thesis has not been used to attain a degree at another university.

# Preface

In accordance with current practice at The University of Sydney, this thesis is presented as a series of stand-alone manuscripts that have either been submitted to international journals for peer review, or have been peer reviewed and accepted for publication. Because they are similarly themed stand-alone manuscripts, there is some unavoidable repetition.

Most of the ideas contained in this thesis are my own. In other cases I contributed substantially to the development of ideas, and the majority of work (including planning, designing, implementing, analysing and writing of manuscripts) was undertaken by me under the guidance of my supervisors. To maintain transparency regarding the level of contribution, I have listed all co-authors as they appear in published articles. Due to joint-authorship, the work presented in most chapters is discussed as "we" instead of "I".

All work was conducted under the approval of the Animal Ethics Committee of The University of Sydney (Animal ethics permit number: L04/12-2008/3/4927) and was approved by New South Wales National Parks and Wildlife Service (NPWS Scientific Licence: SL100472).

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I would like to thank my family for supporting me throughout my candidature. It has been nice to live around the bulk of my family after living away for so long. First, thanks to **Mum** and **Greg** for allowing me to live with them, charging me very reasonable board and listening to me complain when things weren't going right. This probably hasn't been easy for them but they rarely complained and made me feel welcome at all times. Mum generously gave up her time to help me in the field, as did my son **Jaiden**. Thanks also to **Dad** and **Robyn**. My Dad openly encouraged and supported my years as an undergraduate student at the University of New England and helped set me on the path to

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As, I imagine, with any largely field based PhD project, there is a large list of volunteers to thank. There have been so many over the years that I can't recall them all. The ones that stuck in my mind, and thus made it onto this list, either did a really good job or helped me out multiple times or both. First of all Dr **David Vella** and Dr **Michelle Bingley** performed surgeries on my snakes for my radio-telemetry project and did not charge me anything. Their dedication to science and the preservation of endangered broad-headed snakes is worth noting. Similar to Matt Greenlees, **George Madani** assisted me with field work when I was incapacitated and George is another one whose

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## Thesis Summary

Rock outcrops are home to a wide variety of fauna, including many endemic taxa of reptiles and invertebrates that depend upon the thermally distinctive characteristics provided by crevices under thin sun-exposed rocks. Sandstone outcrops in the Sydney basin of southeastern Australia host an assemblage of highly specialised ectotherms, including the endangered broad-headed snake (*Hoplocephalus bungaroides*) and its main prey species the velvet gecko (*Oedura lesueurii*).

My thesis considers a wide range of questions about management, conservation and restoration of this unique habitat, at multiple spatial scales. My first chapter investigates the effects of anthropogenic disturbance to exfoliated rock on thermal regimes in crevices, and on usage of those crevices by reptiles. My second chapter builds upon an ambitious project to restore degraded habitat (areas where surface rocks have been removed) by deploying artificial rocks. The standardised sizes and thicknesses of those artificial rocks enabled me to assess the relative importance of landscape-scale features (such as proximity to cliff edges and forests) in habitat selection by reptiles, without the confounding effects usually introduced by variation in shelter-site attributes. My third chapter quantifies the effects of restoring habitat (i.e., providing artificial rocks) on the growth rates and survival of free-ranging geckos (*O. lesueurii*) to evaluate the often-assumed but rarely-tested claim that habitat restoration provides significant benefits to native fauna. The fourth and fifth chapters consider aspects of *O. lesueurii* life history. I use behavioural methods to explore the importance of predator cues in habitat selection, and genetic methods to identify factors that influence rates of gene flow at a broader

spatial scale. My final chapter describes a two-year radio-telemetric investigation into habitat use by the broad-headed snake (*H. bungaroides*) in the northern part of its geographic range. Previous ecological research on this species has focused almost exclusively on a single population in the extreme south of its known range, precluding any meaningful generalisations about the ecology of this highly endangered sandstone specialist.

In total, my work provides a detailed picture of several facets of reptile ecology on sandstone outcrops in southeastern Australia. It shows how an understanding of habitat use by specialized endemic animals not only can illuminate general issues in ecology, but also can suggests ways in which such systems can most effectively be managed, conserved and (if necessary) restored.

# General Introduction and Thesis Structure

The anthropogenic modification of natural habitats poses a major threat to global biodiversity (Dirzo & Raven 2003, Fischer & Lindenmayer 2007, Gibson et al. 2011). For example, tropical forests disappeared at an estimated rate of 5.8 million hectares per year worldwide between 1990 and 1997 due to anthropogenic activities (Achard et al. 2002). Thus, natural habitats are becoming increasingly reduced, fragmented and homogenized (Huxel & Hastings 1999, Fischer & Lindenmayer 2007). The reduction in extent and connectivity of critical habitat has become so extreme that we are currently seeing rates of extinctions estimated to be between 100 and 1000 times greater than in pre-human times (Pimm et al. 1995, Seabloom et al. 2002).

Many species that become endangered or go extinct do so because they have evolved to occupy specific niches that are being degraded by anthropogenic activities (Munday 2004). The high frequency of ecologically specialized taxa suggests that such specialization often has enhanced population viability over evolutionary time (Julliard et al. 2003, Munday 2004, Bonin 2012). Unfortunately, specialization may reduce a species' capacity to deal with rapid changes in environmental features (Julliard et al. 2003, Munday 2004, Bonin 2012). A suite of life history traits can exacerbate this risk. For example, many such species have relatively low rates of dispersal, have low fecundity or are slow to reach maturation (Gaston 1994, Webb et al. 2002). All of these traits may imperil taxa that are faced with altered, fragmented or changing habitats. If we are to conserve these disappearing taxa, we need a better understanding of their ecology (Cooke

2008). Land managers can use this information to make informed decisions regarding habitat protection and/or restoration.

One system worthy of such detailed investigations is the sandstone outcrop landscape of the Sydney basin, in southeastern coastal Australia. These outcrops include distinctive habitat types, including thin, exfoliated rocks that break away from the parent bedrock and form tight crevices between the exfoliation and the parent rock. Those gaps are a unique, essentially non-renewable habitat type for a variety of species that have evolved to exploit this specific niche. The crevices beneath sun-warmed exfoliations provide warm microhabitats even during winter, enabling a diverse range of ectothermic taxa to function effectively throughout most of the year (Webb & Shine 1998a, Goldsbrough et al. 2003).

Recent research has identified two processes that threaten this system. First, vegetation thickening has increased shading over exfoliated rocks, significantly reducing the availability of crevices with thermal regimes suitable for the rock-dwelling fauna (Webb et al. 2005, Pringle et al. 2009, Pike et al. 2011). Second, surface rock ("bush-rock") has been removed (both legally and illegally) from many outcrops, for use in the gardening and landscaping industry (Schlesinger & Shine 1994, Shine et al. 1998). The threat posed by rock removal stimulated a research program to develop and deploy artificial (fibre-reinforced cement) rocks to replace those stolen by rock thieves (Croak et al. 2008, 2010, 2012). This ambitious habitat-restoration project provided a unique opportunity to assess a variety of basic ecological processes within the rock-dwelling fauna, an opportunity that directly stimulated my doctoral project.

Sun-warmed exfoliated rocks in southeastern Australia support a wide variety of both invertebrate and vertebrate species. Some of these are ecologically generalized taxa, but others are highly specialized: that is, they depend upon this habitat type either seasonally (usually in winter) or year-round (Webb & Shine 1998a, Goldsbrough et al. 2003, 2004). Invertebrate species that rely solely on exfoliated rock as retreat sites include flat cockroaches (*Laxta granicollis*) and flat-rock spiders (*Hemicloea major*), dorso-ventrally flattened animals that can access very tight crevices (Goldsbrough 2003). Perhaps the most abundant vertebrate species that relies on exfoliated rock is Lesueur's velvet gecko (*Oedura lesueurii*) which lives on outcrops year round (Cogger 2000). The endangered broad-headed snake (*Hoplocephalus bungaroides*) lives under exfoliated rock during winter (when these rocks provide the warmest available retreat sites) and disperses to tree hollows in adjacent woodland during the warmer parts of the year (Webb & Shine 1997). *Hoplocephalus bungaroides* often feeds on *O. lesueurii* (particularly during winter when they share the same habitat). Indeed, *O. lesueurii* is a major part of juvenile snakes' diet (Webb & Shine 1998b).

The official classification of *H. bungaroides* as endangered, and its highly specialized ecology, have made it the major focus of the habitat-restoration attempts noted above. Nonetheless, we will only be able to maintain populations of these predators if we also maintain populations of the species (such as velvet geckos) upon which they feed. In practice, the similarity in habitat use by velvet geckos and broad-headed snakes (at least in winter) facilitates simultaneous studies on both taxa. Thus, my thesis describes investigations into the ecology of both these species. I explored the ecology of these species via a range of techniques such as capture-mark-recapture studies in restored areas, genetic methods, and radio-telemetric monitoring to elucidate issues relevant to the conservation and management of *H. bungaroides*. My thesis comprises the following sections:

**PART 1: Habitat Disturbance.** The first part of my thesis consists of a single chapter that investigates the effects of rock disturbance

**Chapter 1:** *Subtle – but easily reversible – anthropogenic disturbance seriously degrades habitat quality for rock-dwelling reptiles.* I investigate the impact of anthropogenic disturbance to exfoliated rocks, by documenting how rock displacement affects the thermal regimes within crevices, and the three dimensional structure of the crevices beneath rocks. I then monitor the subsequent usage of disturbed and undisturbed rocks by reptiles.

**PART 2: Assessment of Outcrop Restoration.** The second part of my thesis consists of two chapters that explore the consequences of restoring rock outcrops with artificial rocks, and the implications of those consequences for evaluating the overall impact and value of rock restoration.

**Chapter 2:** *Habitat selection in a rocky landscape: experimentally decoupling the influence of retreat site attributes from that of landscape features.* The standardized sizes and thicknesses of the artificial rocks enabled me to assess the relative importance of landscape-scale features (such as proximity to cliff edges and forests) in habitat selection by reptiles, without the confounding effects usually introduced by variation in shelter-site attributes. By understanding the role of landscape features, we can better identify the optimal places for rock deployment.

**Chapter 3:** *The benefits of habitat restoration for rock-dwelling geckos* (Oedura lesueurii). Many attempts at habitat restoration are not followed by any rigorous evaluation of the impacts of that work. My third chapter quantifies the effects of habitat restoration (i.e., the provision of artificial rocks) on rates of growth and survival of velvet geckos, and evaluates the often-assumed but rarely-tested claim that habitat restoration provides significant benefits to native fauna.

**PART 3: Velvet Geckos.** The third part of my thesis consists of two chapters that investigate behavioural responses and life history traits of velvet geckos.

**Chapter 4:** *Context-dependent avoidance of predatory centipedes by nocturnal geckos* (Oedura lesueurii). I use behavioural experiments to explore the influence of chemical cues from a predatory invertebrate (centipede, *Scolopendra* spp.) on microhabitat selection by velvet geckos.

**Chapter 5:** *Phylogeography and dispersal in the velvet gecko* (Oedura lesueurii), *and potential implications for conservation of an endangered snake* (Hoplocephalus bungaroides). I use genetic methods to identify factors that influence rates of gene flow of velvet geckos, at a broad spatial scale. Available data on gene flow and dispersal capacity in the species suggest management strategies that may aid habitat restoration attempts.

**PART 4: Broad-headed Snakes.** The fourth part of my thesis consists of a single chapter that investigates the ecology of a previously unstudied, but genetically distinct, northern clade of broad-headed snakes.

**Chapter 6:** *Movements and habitat use of an endangered snake,* Hoplocephalus bungaroides *(Elapidae): implications for conservation.* My final chapter describes a two-year radio-telemetric investigation into habitat use by the broad-headed snake (*H. bungaroides*) in the northern part of its geographic range. Previous ecological research on this species has focused almost exclusively on a single population in the extreme south of its known range, precluding any meaningful generalizations about the ecology of this highly endangered sandstone specialist. I compare differences and similarities in habitat use to the well-studied southern clade and also to congeneric species, and suggest management strategies to conserve northern-clade *H. bungaroides*.

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## PART 1: Habitat Disturbance

*Natural bush-rock on sale at a nursery. This particular nursery is within 10 km of the largest viable population of broad-headed snakes known. Many of these rocks would have provided non-renewable retreat sites for broad-headed snakes and a host of other highly specialised ectothermic fauna endemic to rock outcrop systems in the Sydney basin.*

# **CHAPTER 1:** *SUBTLE – BUT EASILY REVERSIBLE – ANTHROPOGENIC DISTURBANCE SERIOUSLY DEGRADES HABITAT QUALITY FOR ROCK-DWELLING REPTILES*

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*These natural bush-rocks have been flipped over by amateur reptile enthusiasts, revealing the reddish underside of the rock and the natural "scar" where the rock should sit.*

## Abstract

Even apparently subtle disturbance to habitat may have severe long-term consequences if that disturbance alters specific microhabitat features upon which animals depend. For example, in southeastern Australia, the endangered broad-headed snake (*Hoplocephalus bungaroides*) and its prey (velvet geckos *Oedura lesueurii*) shelter in narrow crevices beneath sun-warmed rocks. Humans frequently displace rocks while searching for snakes and lizards, and these reptiles are rarely found under such displaced rocks (even when the rocks superficially appear suitable). We quantified disturbance to rock outcrops and show that most disturbance was subtle (rocks were typically displaced < 30 cm from their original position), but that disturbed rocks harboured fewer reptiles than undisturbed rocks. In a field experiment, we replaced half of the rocks back to their original positions to test whether crevice structure and microclimates differed between disturbed and restored rocks. Crevices beneath displaced rocks were larger and cooler than those beneath restored rocks, and precise repositioning of rocks enhanced usage by reptiles. Both crevice size and temperature influence reptile retreat-site selection; hence, minor displacement of overlying rocks reduces habitat quality by modifying critical crevice attributes. The subtlety of this disturbance suggests that even well-intentioned researchers could damage habitat during field surveys. Conservation of rock outcrop systems requires efforts to reduce rock disturbance, and to educate those searching for animals beneath rocks about the importance of replacing rocks properly. Encouragingly, if rocks are not completely removed, disturbed outcrops can be quickly and easily restored by returning displaced rocks to their original locations.

**Keywords:** habitat disturbance, reptile collection, retreat-site, sandstone, thermal regime

## Introduction

Human activities can imperil biodiversity in many ways, including some pathways that are obvious and some that are not. For example, removing animals from wild populations can directly threaten population viability if offtake levels are too high to sustain; but more subtly, human activities can inflict long-term damage to critical components of the habitat (Webb et al. 2002, Chabanet et al. 2005). Identifying these less obvious effects is difficult, given that the physical presence of humans, or interactions with humans, can modify habitat use or behaviour in some animal species (Frid & Dill 2002), and immune system function in others (Romero & Wikelski 2002, Amo et al. 2006). The challenge we face is to (a) identify whether human activities are having detrimental effects on fauna, (b) understand the causal mechanisms behind such effects, and (c) design solutions to those problems. Our study on rock-dwelling reptiles combines these three approaches; we show that the habitats of these animals are highly vulnerable to disturbance, but that the impacts of that disturbance can readily be reversed.

Many studies have shown that animals are less abundant at sites disturbed by humans than at non-disturbed sites. Understanding the causal mechanisms responsible for this relationship is critical for predicting and reducing longer-term impacts. For example, if animals simply avoid sites frequented by humans, restricting access may allow the system to return to its previous state. In contrast, recovery may be delayed or precluded if human activities have modified the habitat in ways that reduce suitability for wildlife (Goode et al. 2004). In such cases, more intensive management may be necessary. Clearly, we need experimental studies to identify causal mechanisms underlying the observed negative correlation between habitat disturbance and animal abundance.

Reptiles living on rock outcrops provide excellent model systems for investigating this issue, because many species use crevices beneath loose surface rocks as retreat sites. These loose surface rocks are warmer than other available microhabitats, and thus represent optimal habitat (Huey et al. 1989, Webb & Shine1998a). However, loose rocks are also easily removed from the system (Shine et al. 1998) or disturbed in the search for animals (Webb et al. 2002). These activities could permanently degrade habitat because new rocks only form over geological time (Larson et al. 2000). In combination, laboratory studies and field surveys demonstrate that subtle aspects of crevices including thermal regimes (and hence, attributes such as the thickness of the overlying rock, and sun exposure) and three-dimensional geometry (crevice thickness, number of external openings, absolute size) influence habitat selection by reptiles (e.g., Huey et al. 1989, Pringle et al. 2003, Croak et al. 2008). Plausibly, even minor displacement of the overlying rock (as occurs when a person lifts the rock and sets it back down in a slightly different position) might affect attributes such as the degree of closure of the crevice (and thus, potentially, thermal regimes, ingress for predators, etc.) and the detailed geometry of the space enclosed between the overlying rock and the substrate (and thus, the space available for animals to use). Experimental studies in Arizona, USA showed that permanent destruction of rock outcrops using extreme methods employed by some reptile collectors (i.e., using steel pry bars to "remove slabs of exfoliating rock, widen existing cracks, and overturn boulders") reduced reptile diversity and abundance at these sites as compared to controls (Goode et al. 2004, 2005). However, in many areas reptile collectors do not go to such extremes; instead, they simply lift loose rocks and look for sheltering animals. This less obvious disturbance often leaves rocks slightly out of position but not damaged. Because many other taxa also rely on loose surface rocks (e.g., Larson et al. 2000, Goldsbrough et al. 2003) disturbance could affect entire faunal compositions in rocky habitats.

This type of subtle disturbance is common on sandstone rock outcrops in southeastern Australia. These outcrops support many endemic species, notably the endangered broad-headed snake *Hoplocephalus bungaroides*. During winter and spring, nocturnal reptiles shelter beneath sandstone rocks (Webb & Shine 1998a), and they actively select rocks with narrow crevices (Croak et al. 2008) that are exposed to full sun during the afternoon (Webb & Shine 1998a, 1998b, Pringle et al. 2003). In many areas (including National Parks), humans have disturbed rocks while searching for reptiles (for the illegal pet trade, for retention as domestic pets, or simply to see and/or photograph these spectacular animals: Shine et al. 1998, Goldingay & Newell 2000). Often, disturbed rocks have been lifted and replaced a few centimetres away from their original position (Shine et al. 1998, Webb & Shine 2000, Webb et al. 2002). Broad-headed snakes are less abundant on disturbed than on undisturbed outcrops (Goldingay 1998, Webb et al. 2002). Potentially, this difference could result from removal of snakes by collectors (Webb et al. 2002), or because disturbance has altered crevice attributes, thereby degrading habitat quality (Goode et al. 2005). We investigated the effects of subtle rock disturbance on reptiles by addressing three broad questions: (1) do reptiles avoid individual rocks that have been displaced?, (2) does minor displacement of surface rocks affect the thermal, hydric and physical attributes of crevices beneath those rocks, and thus their suitability for reptiles? And if so, (3) does returning rocks to their original positions encourage subsequent use by reptiles, thereby restoring habitat quality?

## Methods

### *Study area*

Our study sites were located on a sandstone plateau in Morton National Park, near Nowra, New South Wales, Australia. In this area, rock outcrops form sandstone cliffs that are bordered by eucalyptus forest. The high cliffs allow sunlight to penetrate to the ground at the cliff edges, and sun-exposed rocks at these sites often shelter reptiles, especially during cooler months of the year (see Webb et al. 2003).

### *Assessment of disturbance to rocks*

Exfoliated sandstone rocks conform to the substrate in precise ways, like pieces of a jigsaw puzzle; rocks fit flush with the underlying substrate and do not wobble or move around when pushed. Lichens and mosses also cover rocks and their surrounds, and rock displacement typically leaves a light-coloured "scar" indicating where the rock originated (Shine et al. 1998, Fig. 1). These two characteristics make it easy to determine where displaced rocks originally lay, to measure how far they were displaced, and to restore them to their original positions.

We assessed the extent and type of rock disturbance at three study sites by grouping disturbance into three categories: (1) displaced, (2) propped up, or (3) overturned. Although we also saw evidence of rocks being thrown over cliff edges (see also Goldingay & Newell 2000, Webb & Shine 2000, Webb et al. 2002), for this study we focused on rocks that were shifted from their original position (laterally displaced) but still available to reptiles (Figs. 1a, b). Rocks that were lifted up and had a portion of the rock elevated above the substrate by another rock or piece of wood were classified as "propped up" (Fig. 1c). "Overturned" rocks were completely flipped over, such that the top of the rock contacted the substrate (Fig. 1d). Although some native animals displace rocks while foraging, during this study we focused on rocks that were too large to be moved by animals other than humans (Webb & Whiting 2006).

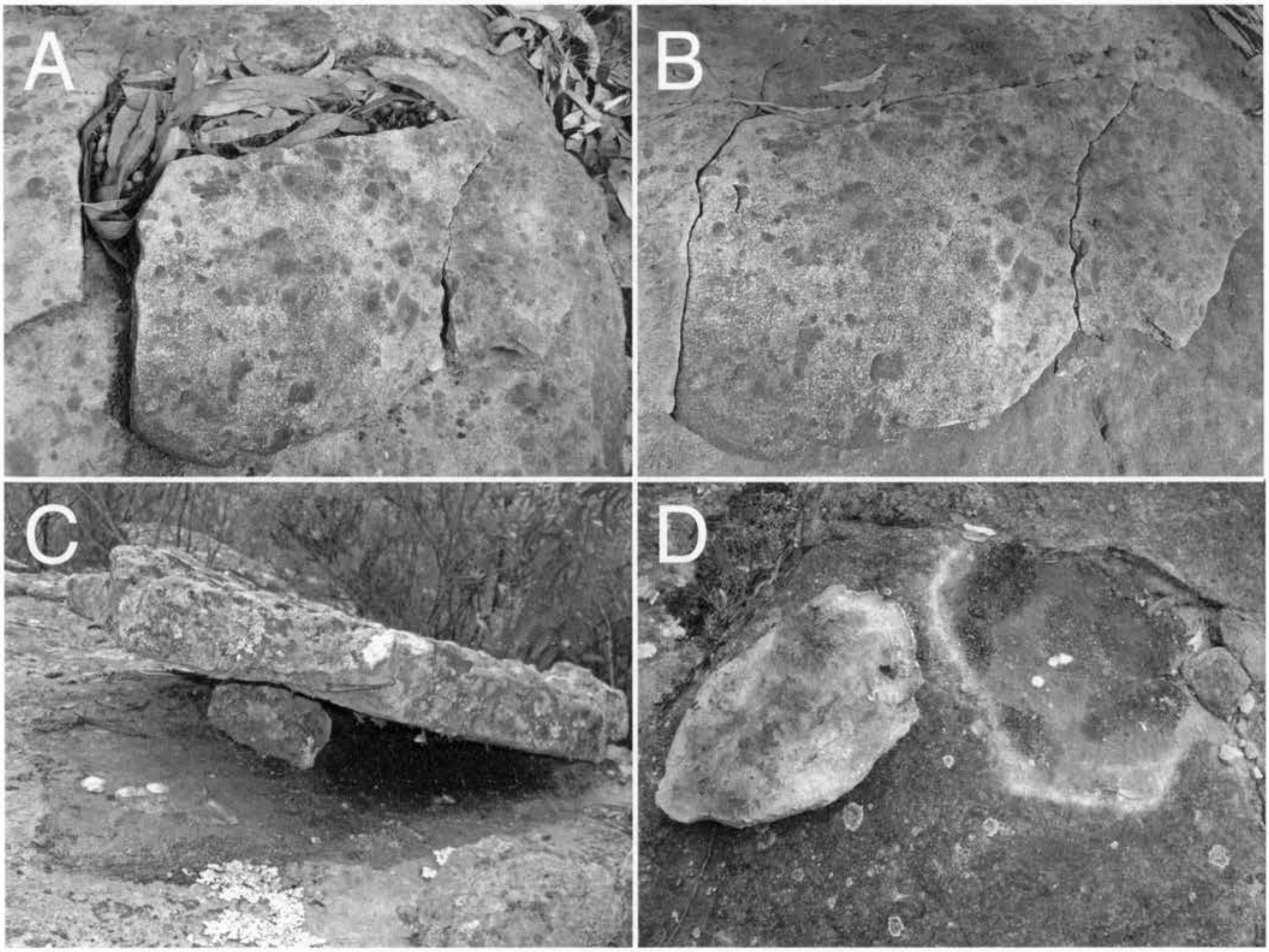

**Figure 1:** *Examples of the types of rock disturbance we observed in the field: (a) a rock that has been displaced from its original position (where it exfoliated from the parent rock); (b) the same rock, but restored to its original position; (c) a rock that has been propped up with another rock, causing much of the rock to be elevated above the substrate; and (d) a rock that has been completely flipped over, such that the bottom of the rock is facing upright. Notice the distinctive "scars" in (c) and (d) that indicate where the rock originally lay.*

We placed each disturbed rock into one of these three categories, and measured the size (length x width) and displacement distance (to 0.5 cm) of a subset of these rocks. The displacement distance was taken as the straight-line distance from a point on the edge of the displaced rock to the same point after we restored the rock to its original position. We compared whether the number of rocks differed between disturbance categories (displaced, propped up, overturned) using a contingency table analysis. To determine whether the type of disturbance was dependent upon rock size we compared the sizes of rocks in each category using ANOVA with disturbance type as the factor and rock area as the dependent variable. We used linear regression to test whether displacement distance was related to rock area.

### *Do reptiles use disturbed rocks?*

*Field observations.*— In early spring 2007 we surveyed three additional study sites once each for reptiles; these sites have regularly been surveyed since 1992 (see Webb et al. 2003, Webb et al. 2008) and periodically are disturbed by reptile collectors (Webb et al. 2002). Disturbance occurred 3–5 months before our surveys. Prior to turning all suitable rocks and looking for reptiles, we classified each rock as disturbed or undisturbed. After identifying reptiles, we restored rocks to their original position (i.e., the configuration from which they exfoliated from the parent rock) and encouraged reptiles to crawl back beneath the rock under which they had been found (for further details see Webb et al. 2003, Webb & Shine 2008). We used a contingency-table analysis to determine whether the abundance of each reptile species was affected by rock disturbance.

*Field experiment.*— Fewer animals might be captured under disturbed rocks because (a) people have removed the animals or (b) reptiles avoid disturbed rocks due to unsuitable thermal or physical properties (as hypothesized by Goode et al. 2005). To distinguish between these two hypotheses, we set up a field experiment along a 300-m stretch of cliffline where disturbance was evident (disturbance occurred several years prior to our study). We found 26 pairs of displaced rocks (paired rocks were < 2 m apart) lying on rock substrates, and returned one randomly chosen rock from each pair to its original position (as described above). We predicted that if reptiles simply avoid disturbed rocks because of unsuitable microhabitat conditions, they would colonise the restored rocks but continue to avoid the displaced rocks. If reptiles have been physically removed from the site, we do not expect to see such a response. We individually numbered the underside of each rock with a paint pen and measured the size (length x width x maximum thickness) and distance it was displaced. We conducted this study in an area behind newly erected locked gates, which prevented additional disturbance during sampling.

We set up the experiment on 8 May 2008 and surveyed the rocks 10 times at two to four week intervals during winter and spring 2008 (22 May to 21 October) and 2009 (9 September to 12 October). During surveys we lifted each rock and captured, individually marked, and released any reptiles sheltering beneath. We used a contingency-table analysis to determine whether the abundance of individual reptile species was dependent upon whether or not the disturbed rocks had been restored. Because rock area can influence reptile usage (Schlesinger & Shine 1994a, Webb & Whiting 2006), we also used a logistic regression to test whether reptile presence was influenced by rock area or disturbance category.

### *Microhabitat characteristics of displaced rocks*

*Crevice structure.*— To determine whether rock displacement changes the three-dimensional structure of the space used by reptiles, we took life-size casts of the crevice beneath nine displaced rocks before and after they were restored. We chose rocks based on three criteria: each rock (1) had been disturbed by humans and was initially out of place, (2) was within the size range commonly used by snakes and lizards (Schlesinger & Shine 1994b, Webb & Shine 1998b), and (3) could readily be returned to its original position. To make a cast, we noted the exact position of the rock, wrapped the rock and substrate in plastic wrap, and coated the bottom with expanding foam that hardens after 24 hours (for details see Croak et al. 2008). We then placed the rock back where it was found, and allowed the foam to expand and harden. We removed the casts after they dried, restored each rock to its original position, and subsequently took an additional cast.

In the laboratory, we systematically measured the thickness of each cast at multiple sites (by pushing a needle through it) to delineate areas of the cast falling into three categories of thickness: 4–11 mm (suitable for reptiles); < 4 mm thick (too small for reptiles to squeeze into), and > 11 mm thick (not used by reptiles in the laboratory or field: Schlesinger & Shine 1994a, Croak et al. 2008). We measured the area ($mm^2$) of each crevice compartment using scaled digital images (Rasband 2001), and then calculated the proportion of each crevice that was suitable for reptiles (i.e., total area suitable for reptiles divided by the total surface area of the crevice). We compared this variable between rock positions (displaced vs. restored) using a paired *t*-test.

*Thermal and hydric regimes.*— Rock displacement also could affect thermal and hydric conditions within crevices (Goode et al. 2005). These are important criteria for retreat-site selection by reptiles; for example, many reptiles actively select hotter rocks (e.g., Huey et al. 1989, Webb & Shine 1998a, 1998b). To determine whether disturbance affects the thermal or hydric regimes of crevices, we placed miniature dataloggers (Thermochron or Hygrochron iButtons) beneath rocks at our experimental site (see above). These were placed in natural indentations under the rocks so that they would not affect rock position. Dataloggers were synchronized and set to record temperatures, or both temperatures and relative humidity, at hourly intervals during a 10-day period in early spring (13 to 22 September 2008).

We compared thermal and hydric regimes between rock types using repeated measures ANCOVAs with rock type as the factor, rock area as the covariate, and temperature or humidity as the dependent variable. We ran these analyses using daily minimum, maximum, mean, and range of temperatures. In addition, we analyzed mean hourly changes in temperature over this 10-day period using the subset of rocks for which we had both thermal and hydric data; for this analysis we averaged hourly temperatures over the entire 10-day period.

## Results

### *Assessment of disturbance to rocks*

Of 85 disturbed rocks that we encountered, most were laterally displaced (88.2%) rather than propped up (9.4%) or overturned (2.4%; $\chi^2 = 115.9$, df = 2, $P < 0.0001$). Mean rock size did not differ among the three types of disturbance ($F_{2,76} = 1.17$, $P = 0.32$). On average, rocks were displaced 28.3 ± 3.4 cm from their original position ($N = 79$, range: 2.0–210.0 cm; Fig. 2), irrespective of their size ($R^2 = 0.01$, $F_{1,77} = 0.51$, $P = 0.48$). Most disturbance was subtle; to a casual observer, the sites likely would appear undisturbed.

*Do reptiles use disturbed rocks?*

*Field observations.*— In 2007 we sampled 190 rocks at three disturbed sites, of which 55 (28.9%; range of 9–48% per site) were disturbed by humans. Disturbed rocks were similar in area to undisturbed rocks (using a subset of rocks for which we had size data; $F_{1,166} = 1.23$, $P = 0.27$). We captured a total of 7 broad-headed snakes, 11 small-eyed snakes (*Cryptophis nigrescens*), and 55 velvet geckos. Broad-headed snakes and velvet geckos showed strong responses to disturbance, and were less likely to be found beneath disturbed rocks than undisturbed rocks (broad-headed snakes $\chi^2 = 3.94$, df = 1, $P < 0.05$; geckos $\chi^2 = 15.50$, df = 1, $P < 0.0001$). We did not find any broad-headed snakes beneath disturbed rocks, but 7% of the undisturbed rocks harboured snakes; velvet geckos used 9% and 38%, respectively. By contrast, small-eyed snakes were found equally under both rock types ($\chi^2 = 0.21$, df = 1, $P = 0.64$). A logistic regression confirmed that rock area did not influence reptile usage ($\chi^2 = 0.87$, df = 1, $P = 0.35$), whereas disturbance significantly lowered the likelihood that reptiles would use a rock as shelter ($\chi^2 = 18.32$, df = 1, $P < 0.001$). Overall, reptiles used 19% of disturbed rocks, and 50% of undisturbed rocks.

*Field experiment.*— At our experimental restoration site, we did not find any reptiles during initial habitat restoration. During subsequent surveys we recorded two lizard

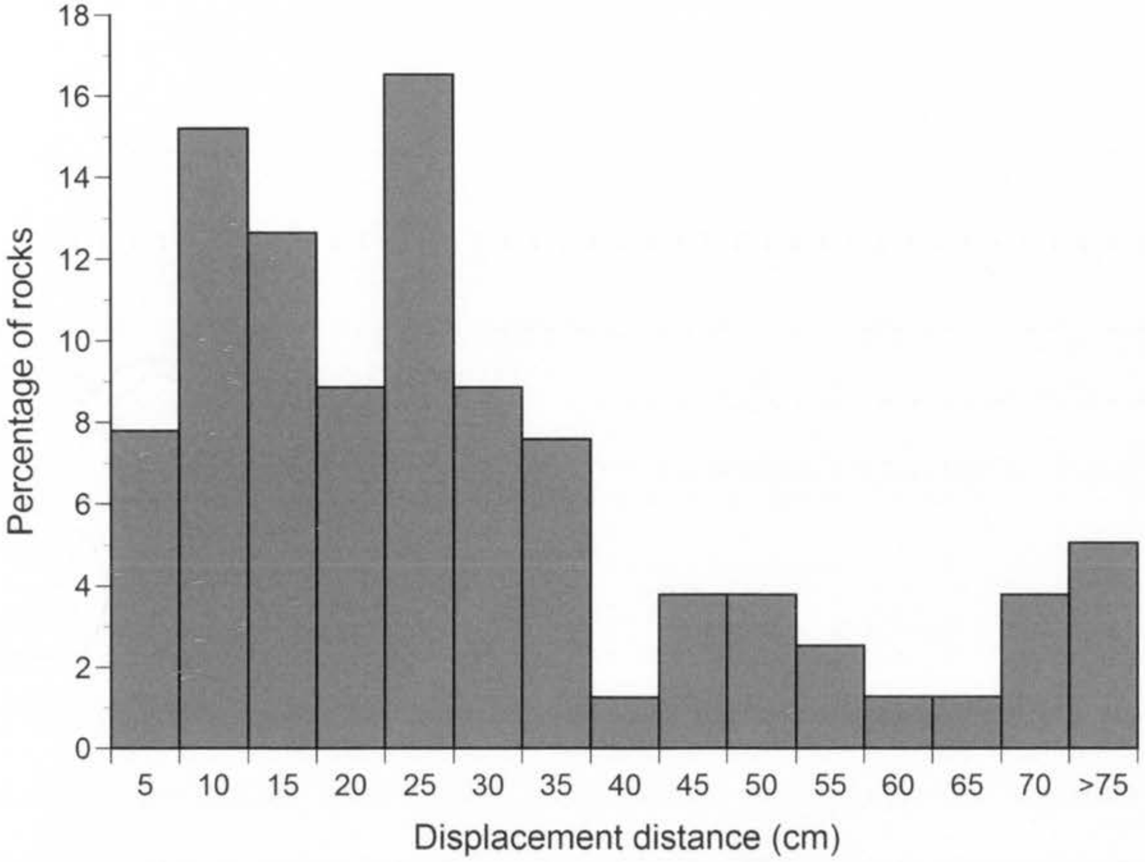

**Figure 2:** *Frequency histogram showing the distance that humans displaced rocks ($N$ = 79). The x-axis represents the upper limit of each distance interval, and the y-axis shows the percentage of rocks within each category. See text for details on how we measured displacement distance.*

species: wall skinks (*Cryptoblepharus pulcher* $N = 17$) and velvet geckos ($N = 4$). Wall skinks were more abundant beneath restored rocks ($N = 16$; $\chi^2 = 13.24$, df = 1, $P < 0.001$), and we found only one individual beneath a displaced rock. All four of the velvet geckos were beneath restored rocks. Overall, lizards were found more frequently beneath restored rocks ($\chi^2 = 17.69$, df = 1, $P < 0.0001$; Fig. 3a). A logistic regression confirmed that lizard presence was unrelated to rock area ($\chi^2 = 0.22$, df = 1, $P = 0.64$), but disturbance lowered the likelihood that lizards would use a rock as shelter ($\chi^2 = 10.36$, df = 1, $P = 0.001$).

*Microhabitat characteristics of displaced rocks*

*Crevice structure.*— The rocks under which we quantified crevice structure were representative of our sample of displaced rocks, both in area ($F_{1,68} = 3.26$, $P = 0.08$) and in displacement distance ($F_{1,56} = 0.06$, $P = 0.81$). Rock position directly influenced the area that reptiles could use as shelter (paired $t_8 = 4.56$, $P = 0.002$), with restoration increasing the area that reptiles prefer by 59% (Fig. 3b).

*Thermal and hydric regimes.*— At the experimental restoration site, we obtained thermal data from 51 rocks (25 displaced, 26 restored) and both thermal and humidity data from 19 of these rocks (10 displaced, 9 restored). The mean thickness of rocks for which we had thermal data did not differ by rock type ($F_{1,50} = 2.27$, $P = 0.14$), although the mean area of restored rocks was larger than displaced rocks (means ± SE of 1437 ± 100 $cm^2$ vs. 1109 ± 84 $cm^2$, respectively; $F_{1,50} = 6.32$, $P = 0.02$). This pattern also occurred in rocks for which we had both humidity and temperature data. Thus, we included rock area as a covariate in our statistical analyses.

Over a 10-day period in early spring, crevices beneath restored rocks ($N = 26$) were warmer than those beneath displaced rocks ($N = 25$). Differences were evident for three of the four thermal variables that we calculated: mean daily minimum temperature (treatment effect, $F_{1,48} = 0.01$, $P = 0.91$, rock type * time interaction, $F_{9,432} = 0.75$, $P <$ 0.0001; Fig. 4a), mean daily maximum temperature ($F_{1,48} = 21.05$, $P < 0.0001$, interaction, $F_{9,432} = 98.13$, $P < 0.0001$; Fig. 4b), mean daily temperature ($F_{1,48} = 8.53$, $P =$ 0.005, interaction, $F_{9,432} = 236.32$, $P < 0.0001$; Fig. 4c), and mean diel range in temperatures ($F_{1,48} = 20.63$, $P < 0.0001$, interaction, $F_{9,432} = 56.50$, $P < 0.0001$; Fig. 4d).

That is, the thermal difference between rock types varied among days, but temperature was usually higher beneath restored rocks (Fig. 4).

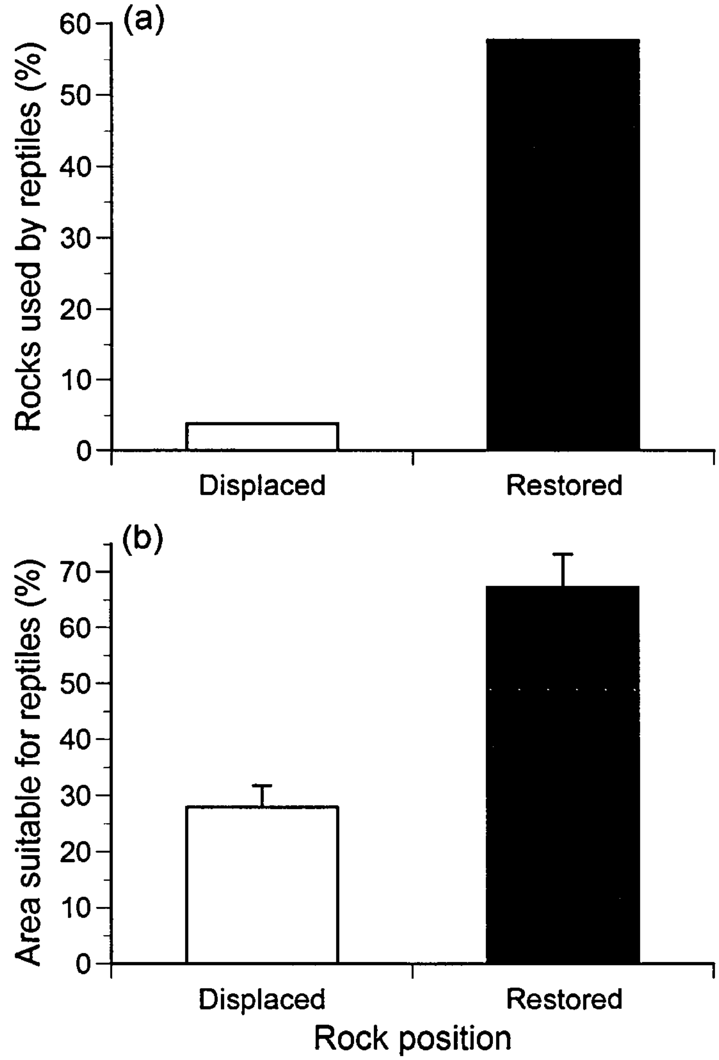

**Figure 3:** *(a) The proportion of displaced and restored rocks (N = 26 of each) used by reptiles from 22 May to 22 September 2008 and on 9 September 2009. All rocks were initially found displaced and we restored half of them to their original position at the start of the experiment. (b) Mean percentage (+ SE) of the crevice area beneath individual rocks (N = 9) suitable for reptiles, measured when the rocks were displaced and after we restored them to their original position. See text for details on how crevices were measured and categorized.*

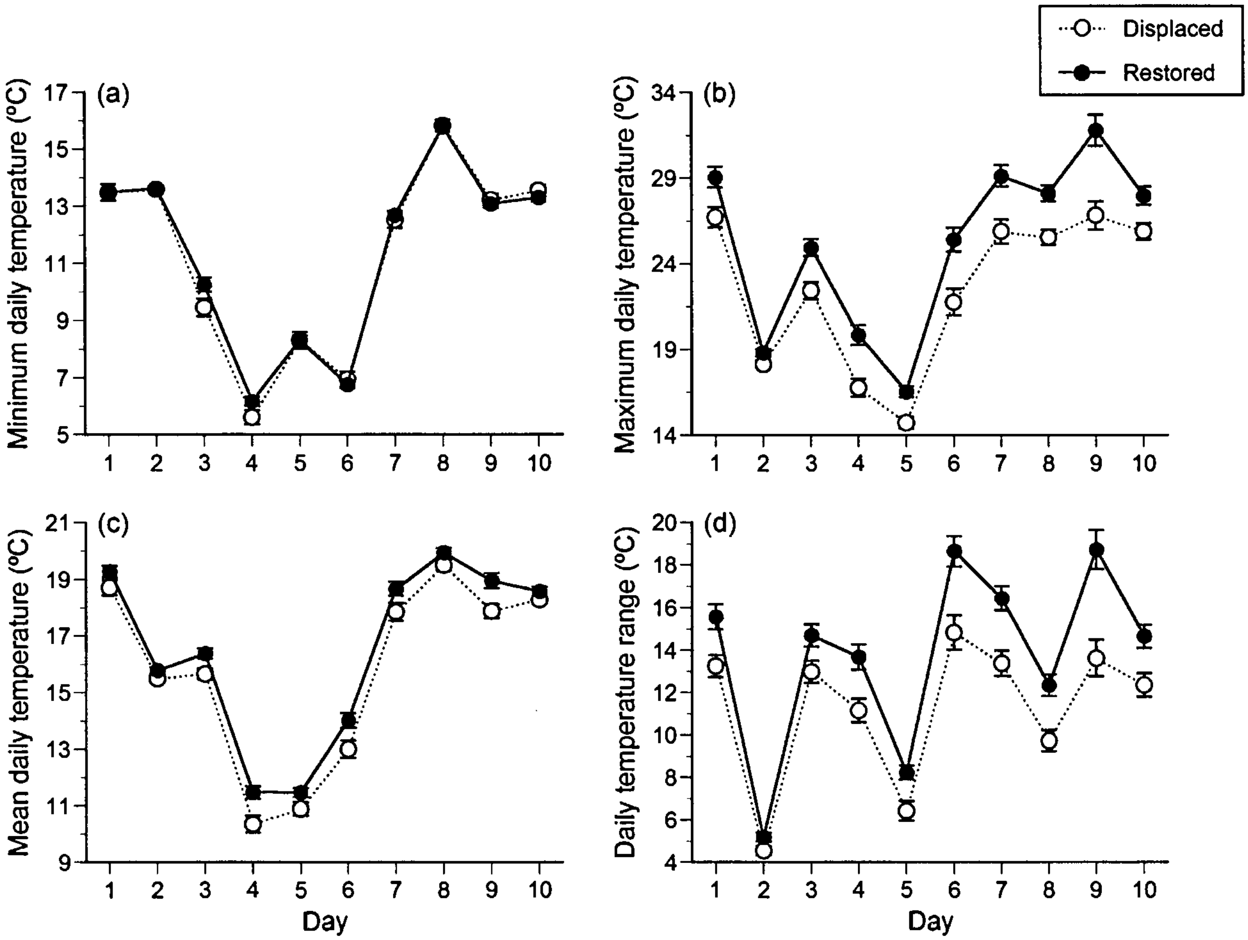

**Figure 4:** *Thermal regimes beneath displaced rocks (N = 25) and those that were restored to their original position (N = 26) over a 10-day period in spring (13 to 22 September 2008). Shown are mean values (± SE) for both rock types for the following daily temperatures: (a) minimum, (b) maximum, (c) mean, and (d) range.*

Like temperature, relative humidity varied significantly throughout the 10-day period (time effect; all $P < 0.0001$), but there was no significant difference between rock types (all $P > 0.31$) or an interaction between rock type and time (all $P > 0.71$). Overall mean daily relative humidity during this period was 70.7 ± 5.1% (absolute range: 8.4–100%) and 68.1 ± 5.3% (absolute range: 14.1–100%) for restored and displaced rocks, respectively.

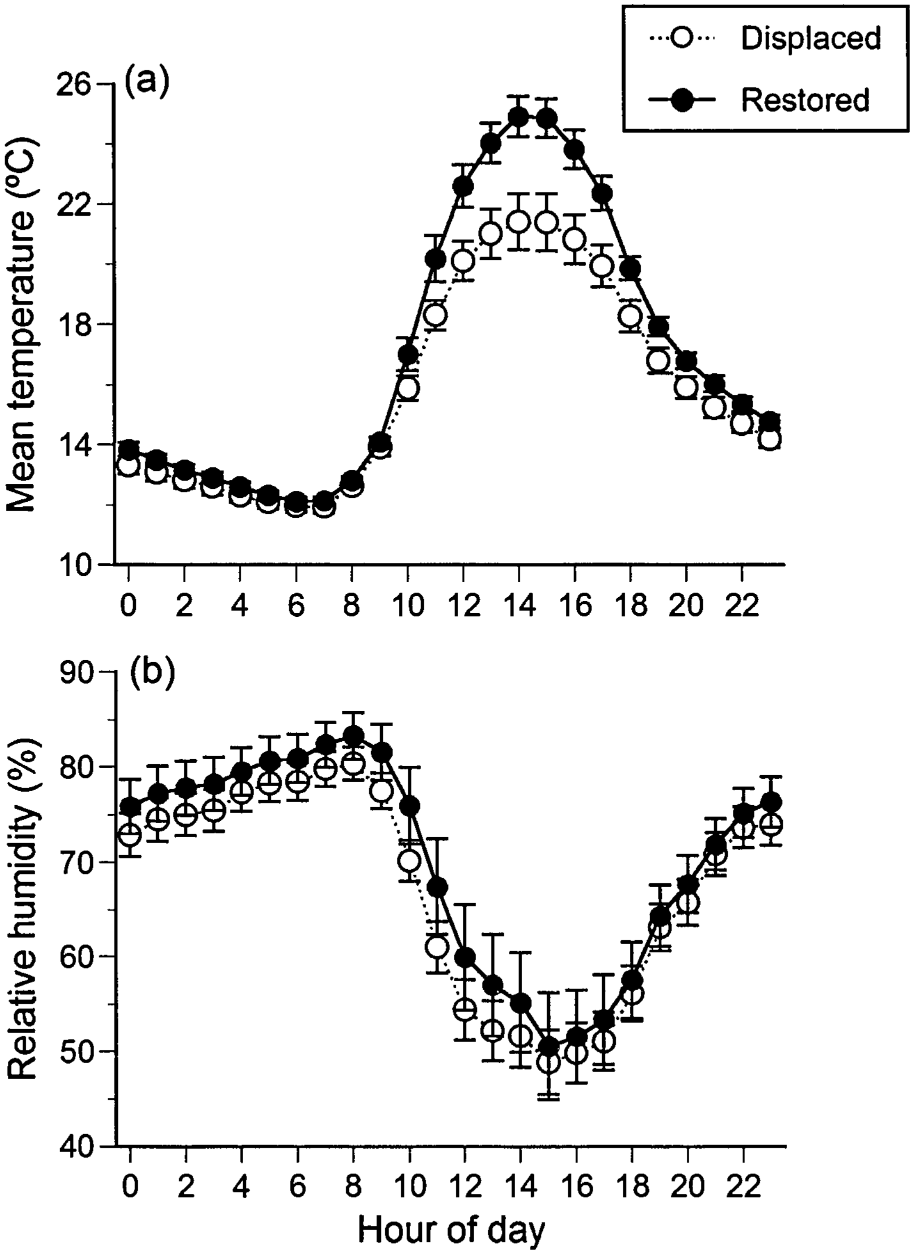

**Figure 5:** *Daily (a) thermal and (b) hydric regimes beneath rocks that were either displaced ($N = 10$) or restored to their original position ($N = 9$). Shown are mean values ± SE for hourly intervals averaged over a 10-day period in spring (13 to 22 September 2008). Temperature data are shown only for those rocks for which we also had humidity data.*

For rocks with both thermal and humidity data ($N = 19$), mean hourly temperatures varied significantly over the 10-day period by rock type ($F_{1,16} = 7.38$, $P = 0.02$), throughout the day ($F_{23,368} = 35.56$, $P < 0.0001$), and between rock type and time ($F_{23,368} = 7.27$, $P < 0.0001$; Fig. 5a). At midday, displaced rocks were cooler than restored rocks (grand means during the hottest part of the day of 21.4 ± 0.85 °C vs. 24.9 ± 0.63 °C; Fig. 5a). Although relative humidity changed throughout the course of the day ($F_{23,368} = 28.37$, $P < 0.0001$), there was no significant difference in hydric conditions between rock types ($F_{1,16} = 0.50$, $P = 0.49$) nor an interaction between rock type and time ($F_{23,368} = 0.79$, $P = 0.75$; Fig. 5b).

Although our sample sizes are limited for the rocks with both thermal and humidity data, those used by reptiles ($N = 7$) were warmer at midday than were unused rocks ($N = 12$; treatment effect, $F_{1,16} = 11.31$, $P = 0.004$; time effect, $F_{23,368} = 45.65$, $P < 0.0001$; rock type * time interaction, $F_{23,368} = 12.06$, $P < 0.0001$). However, mean relative humidity did not differ between used and unused rocks (time effect, $F_{23,368} = 27.25$, $P < 0.0001$; all other effects, $P > 0.83$).

## Discussion

Rock outcrops are characterized by high levels of diversity and endemism (Larson et al. 2000, McMillan & Larson 2002, Goldsbrough et al. 2003, Trager & Mistry 2003, Goode et al. 2005, Porembski 2007, Michael et al. 2008). However, these habitats are also extremely vulnerable to human disturbance. To date, most studies of the effects of such disturbance have focused on permanent physical destruction of outcrops (Goode et al. 2004, 2005), complete removal of loose surface rocks (e.g., Shine et al. 1998), or broad patterns of site-specific disturbance (e.g., Goldingay 1998, Goldingay & Newell 2000, Webb et al. 2002). Our study extends this earlier work by documenting causal links between minor displacement of rocks (on average, < 30 cm shift) and (a) thermal regimes under those rocks, (b) crevice structure under those rocks, and (c) use of rocks by reptiles in the field. The processes that generate loose surface rocks require many thousands of years (Larson et al. 2000); so in practical conservation terms, any degradation (even slight displacement without physical damage) is effectively permanent. Disturbance of this type is widespread; our extensive surveys throughout the geographic range of the broad-headed snake have revealed human disturbance of rocks at virtually every site we have visited, including those that are difficult to access (Shine et al. 1998, unpublished data). Similar types of disturbance have been documented in many other parts of the world, including Africa (Trager & Mistry 2003, Porembski 2007), Canada (McMillan & Larson 2002, McMillan et al. 2003), Mexico (see Goode et al. 2004), and the United States (Goode et al. 2004, 2005), suggesting that the processes we have measured are likely to occur widely.

The explanation behind our results is likely simple: variation in crevice structure directly affects thermal regimes beneath rocks, such that large (thick) crevices are cooler than small, thin crevices (Croak et al. 2008). Displacement enlarges the underlying crevice (Fig. 3b), which may increase airflow into the crevice or decrease heat retention due to lessened contact with the substrate, thereby keeping temperatures cooler (Figs. 4, 5). Although this hypothesis does not explain why mean relative humidity was similar between restored and displaced rocks, hydric variation beneath a given rock was much greater than thermal variation (Fig. 5), suggesting that our sample sizes may have been inadequate to detect a difference in humidity. Because any disturbance to a rock will simultaneously change both the physical structure of the crevice and its thermal attributes, we cannot determine which of these changes actually causes reptiles to avoid disturbed retreat sites. Detailed laboratory studies could potentially disentangle these two factors, and thereby determine the relative importance of crevice structure and thermal regimes for reptile retreat-site selection.

Thermal regimes profoundly influence the physiology and behaviour of ectotherms (Huey 1991, Webb & Shine 1997). By disturbing rocks, humans impose a physiological cost to reptiles that are forced into cooler retreat sites (i.e., the disturbed rocks, cooler rocks left undisturbed, or other nearby microhabitats, which are cooler than rocks: Webb & Shine 1998a, 1998b; Figs. 4, 5). This cost may be particularly high in nocturnal species (such as broad-headed snakes, velvet geckos, and small-eyed snakes) that spend long periods (weeks to months) thermoregulating beneath the same individual rocks (Webb & Shine 1998a, 1998b, Webb 2006, Webb & Whiting 2006). Additionally, retreat sites with large (as opposed to small) crevices might increase the risk of predation (Nemeth 1998, Cooper et al. 1999, Webb & Whiting 2006) or facilitate the build up of debris under the rocks. Many saxicolous species avoid such crevices containing soil or leaf litter (Schlesinger & Shine 1994a, Webb & Shine 1998b, Goldsbrough et al. 2004, unpublished data). Thus, even slight displacement of individual rocks can have far-reaching consequences for habitat quality.

Our findings have direct consequences for the conservation and management of rock outcrops. For example, well-intentioned faunal surveys and educational activities could permanently degrade large areas of habitat unless great care is taken to ensure that rocks are repositioned properly. Disturbance could also increase the error associated with count data collected during subsequent surveys. Also, the number of rocks present on a rock outcrop may tell us little about habitat quality. For example, heavily disturbed areas containing many rocks may not provide enough suitable habitat to allow persistence of rock-dwelling species. Finally, because reptiles avoid rocks with larger crevice spaces (Croak et al. 2008), rocks that do not fit closely to the substrate likely will be ineffective as replacement habitat. Thus, attempts to restore rock-depleted outcrops by placing rocks from adjacent areas onto disturbed rock outcrops (e.g., Goldingay & Newell 2000) are likely to be less effective than restoring displaced rocks to their original positions. Restoring areas where humans have completely removed the loose surface rocks is more difficult because the shelter sites are no longer there. However, artificial rocks with purpose-designed crevices may offer a viable solution to this problem (Croak et al. 2009).

Our study suggests that simple restoration of disturbed rock outcrops, by returning rocks to their original positions, will benefit many saxicolous species, including the broad-headed snake and its lizard prey (e.g., velvet geckos and wall skinks; Webb & Shine 1998c), which can potentially recolonise restored areas from adjacent sites. Returning rocks to their original position rapidly restores habitat and is a cost effective method, but is limited in this system as surface rock is often completely removed by anthropogenic activities. However, this technique may also be effective in aquatic systems where human disturbance is a problem because rocks often provide shelter or nesting sites for aquatic species (e.g., Bishop 1941). To reduce the impact of humans on these fragile ecosystems, wildlife managers need to inform and educate the public, environmental consultants, and scientists about the importance of replacing rocks correctly. This task is necessary if we are to conserve the distinctive fauna of rock outcrops.

## Acknowledgements

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# PART 2: Assessment of Outcrop Restoration

*Artificial rocks deployed in an area stripped of natural bush-rock.*

# **CHAPTER 2:** *HABITAT SELECTION IN A ROCKY LANDSCAPE: EXPERIMENTALLY DECOUPLING THE INFLUENCE OF RETREAT SITE ATTRIBUTES FROM THAT OF LANDSCAPE FEATURES*

Published as: Croak BM, Pike DA, Webb JK, Shine R (2012) Habitat selection in a rocky landscape: experimentally decoupling the influence of retreat site attributes from that of landscape features. PLoS ONE 7: E37982

*Four small-eyed snakes sharing a single artificial rock. Some artificial rocks were used more frequently than others based on their positioning within the landscape.*

## Abstract

Organisms selecting retreat sites may evaluate not only the quality of the specific shelter, but also the proximity of that site to resources in the surrounding area. Distinguishing between habitat selection at these two spatial scales is complicated by co-variation among microhabitat factors (i.e., the attributes of individual retreat sites often correlate with their proximity to landscape features). Disentangling this co-variation may facilitate the restoration or conservation of threatened systems. To experimentally examine the role of landscape attributes in determining retreat site quality for saxicolous ectotherms, we deployed 198 identical artificial rocks in open (sun-exposed) sites on sandstone outcrops in southeastern Australia, and recorded faunal usage of those retreat sites over the next 29 months. Several landscape-scale attributes were associated with occupancy of experimental rocks, but different features were important for different species. For example, endangered broad-headed snakes (*Hoplocephalus bungaroides*) preferred retreat sites close to cliff edges, flat-rock spiders (*Hemicloea major*) preferred small outcrops, and velvet geckos (*Oedura lesueurii*) preferred rocks close to the cliff edge with higher-than-average sun exposure. Standardized retreat sites can provide robust experimental data on the effects of landscape-scale attributes on retreat site selection, revealing interspecific divergences among sympatric taxa that use similar habitats.

## Introduction

Many animals spend long periods (on a diel cycle, and/or seasonally) sheltered within retreat sites and the choice of retreat site may influence organismal fitness (Huey & Pianka 1981, Jones 2001, Kearney 2002). Thus, it is not surprising that both field-survey and experimental studies reveal strongly non-random selection of retreat sites by animals, based on a diverse array of biotic and abiotic cues. For example, the frog *Phrynobatrachus guineensis* breeds in tree hollows and selects nesting sites that contain conspecifics (thereby reducing the chance of predation) and suitable hydric regimes (Rudolf & Rodel 2005). Common brushtail possums (*Trichosurus vulpecula*) living in woodland habitat select tree-hollows high above the ground that provide protection from predators, a buffer against environmental extremes, and favourable temperatures (Issac et al. 2008). Many ectotherms select retreat sites based on thermal regimes (Huey 1991, Webb & Shine 1998a, Kearney & Predavec 2000, Sabo 2003), scent cues from other species (Downes 1999, Clark 2004, Du et al. 2009), and/or the three-dimensional structure of the retreat site itself (Croak 2008).

Most research on retreat site selection has focused on the attributes of individual retreat sites. However, habitat selection by animals also involves criteria that relate to a much larger spatial scale. Many species are restricted to distinctive macrohabitats (e.g., rocky areas, thick forests, and the like) so that to understand habitat selection, we need to gather data at a variety of spatial scales (Rosenzweig 1991, Indermaur et al. 2009). For example, red foxes (*Vulpes vulpes*) create dens at non-random sites at both small spatial scales (i.e., on slopes that provide stable soils) and at large spatial scales (i.e., close to foraging sites and water bodies: Uraguchi & Takahashi 1998). By analogy, people buying homes are influenced not only by the specific features of the house, but also by the resources accessible from that site. Indeed, the latter often may be more important (as suggested by the real-estate agent's adage that the three most important factors in house desirability are "location, location, location").

One important challenge to understanding habitat selection is the effect of co-variation of features across multiple spatial scales. For example, the availability of loose surface rocks (potential retreat sites) often will be higher close to a large rock outcrop, so that a tendency for animals to shelter under rocks found close to an outcrop might reflect either features of the specific shelter (because more choice is available closer to the outcrop), or the proximity of the outcrop itself (and hence, access to resources such as food, water, or escape from predators). Similarly, proximity to woodland might affect both shading levels experienced by a given rock (and thus, thermal regimes within the retreat site) as well as effects of woodland proximity *per se* (such as the distances to resources [e.g., food, water, and nesting sites] restricted to that habitat type). Such co-variation makes it difficult to distinguish criteria for habitat selection at a landscape scale as opposed to the level of the individual retreat site.

To experimentally test the causal role of habitat attributes in retreat-site selection at larger spatial scales, we need to standardize the attributes of individual retreat sites, in order to minimize variance in habitat-selection resulting from faunal preferences at that smaller spatial scale. Artificially-created retreat sites (such as nest boxes provided for bird breeding: Smith & Belthoff 2001) are well suited to this purpose, enabling researchers to investigate faunal responses to habitat-scale factors by creating near-identical retreat sites in a range of locations.

The system we have investigated involves crevice use by nocturnal rock-dwelling animals and is part of a long-term study of this system. Previous research shows that many nocturnal rock-dwelling species utilize both structural and biotic cues to select diurnal retreat sites. These include physical space configurations (Croak 2008), temperatures (Huey et al. 1989, Webb & Shine 1998, Pringle et al. 2003, Webb et al. 2009), moisture levels (Schlesinger & Shine 1994a), and scent cues from predators (Downes & Shine 1998a, Head et al. 2002, Webb et al. 2009), prey (Downes & Shine 1998b, Clark 2004, Du et al. 2009), and conspecifics (Downes & Shine 1998a). At a large spatial scale, GIS studies have documented significant associations between species distributions and climatic variables (Penman et al. 2010) and overall landscape features (e.g., availability of sandstone rocks: Shine et al. 1998). We designed our study to fill the gap between these two spatial levels of analysis, by investigating habitat selection at intermediate spatial scales. To do so, we constructed and deployed artificial rocks that are identical to each other in size and crevice structure (thereby controlling for factors intrinsic to the retreat site). We also quantified the location of the rock in terms of several habitat variables, and tested how faunal use relates to these variables.

## Methods

### *Study sites, rock placement, and sampling*

We placed 198 artificial rocks on flat areas of two sandstone plateaus near Nowra, southeastern New South Wales, Australia. The rocks were created to restore anthropogenically-degraded habitat that supports a unique assemblage of specialized fauna. The rocks were designed to create crevices that were structurally and thermally similar to natural rocks used by these target faunal assemblages (Croak et al. 2008, 2010). We deployed these rocks non-randomly, based on previous studies of the target faunal groups. That is, we placed them on flat ground in open areas close to the outcrop edges (Croak et al. 2008, 2010). These sites consist of relatively small open clearings within eucalypt forest, close to steep cliffs (up to 50 m high) that prevent trees from shading the rocky areas along the cliff edge (Pike et al. 2011a). The area contains an endangered snake species, the broad-headed snake (*Hoplocephalus bungaroides*), its major lizard prey, the velvet gecko (*Oedura lesueurii*), and a wide range of other ectotherms (Goldsbrough 2003, Pike et al. 2011a). Thus, the artificial rocks were placed out non-randomly based on our knowledge of the ecology of these species; that is, we placed artificial rocks on the western/northwestern side of the plateaus and in areas with relatively open canopies (i.e., areas that receive high levels of incident radiation; see Webb & Shine 1998b, Pringle et al. 2003: Fig. 1). Incident radiation determines the thermal regime experienced in the retreat site created between the rocks and the substrate (Pringle et al. 2003). We avoided any sites with soil or leaf litter substrate, or that were shaded by overhanging trees (Pike et al. 2011a). The artificial rocks were identical in size, shape, thickness, and coloration (broadly rectangular, 550 x 385 mm, with each rock ranging in thickness from 27–65 mm (Croak et al. 2008; Fig. 1) and differed only in the number of entrance holes. Half of the artificial rocks (*N* = 99) contained four entrance holes, whereas the other half (*N* = 99) contained two entrance holes; because these variables do not appear to influence reptile use (Croak et al. 2010), we treated all rocks as identical replicates for analyses in the current study. Each artificial rock was constructed and deployed to create similar thermal regimes, crevice structure and aspect exposure, all of which influence the use of these artificial rocks by habitat specific fauna (Croak et al. 2008, 2010). Because we controlled for factors that are known to influence retreat site use in these taxa (crevice configuration – Croak et al. 2008, and aspect, canopy cover and resultant incident radiation; see above), any non-random patterns of retreat site selection should reflect other, previously unrecognized variables.

**Figure 1:** *An artificial rock on site. Artificial rocks were designed to provide crevices with attributes preferred by saxicolous reptiles. All rocks were placed on flat ground to provide crevices 4 to 11 mm high, in areas with open canopies overhead and on the western side of the outcrops to allow relatively high sun exposure (and thus, favourable thermal regimes).*

We deployed the artificial rocks late in the austral winter (August 2007) and monitored their use by reptiles and invertebrates every two weeks from August to November 2007 ($N = 8$ sampling sessions) and on a monthly basis thereafter (from December 2007 to December 2009, $N = 25$ sessions; total $N = 33$ sampling sessions spanning 29 months).

During sampling, we turned all rocks and captured, identified, marked, and released any animals using the crevice formed between the rock and the underlying substrate. All rocks were sampled on all sampling occasions. For analysis, we treated any rock that harboured a given species on any of the 33 sampling trips as used by that species. We conducted analyses of used *versus* unused rocks for each species, and also examined frequency of use within the subset of artificial rocks known to have been used by each taxon.

### *Rock attributes*

For each artificial rock, we measured the following environmental factors: the incident radiation received by each rock ($MJ/m^2$ per day: quantified by taking 180° hemispherical photographs of the forest canopy directly above each artificial rock and importing them into Gap Light Analyzer software (GLA). Incident radiation is calculated from canopy cover determined by GLA and inputted location and day length data: Frazer et al. 2000, Doody et al. 2006); distance to the closest west or northwest facing cliff (m); distance to adjacent woodland (m); distance to the nearest natural rock large enough to house our focal species (m) and the size (length x width; $cm^2$) of that nearest rock; distance to leaf litter (m); distance to nearest rock crevice large enough to house our focal species (m); and the size of the contiguous bare rock outcrop on which the artificial rock was located (outcrop area; length x width, $m^2$). We recorded linear dimensions using a tape measure (to 0.5 cm).

*Data analysis*

We used the statistical package R (2.10.0) for all analyses (R development core team 2009). Because habitat variables are often correlated at different habitat scales, we used Spearman's rank correlation tests to assess whether the habitat variables that we measured were significantly correlated with one another. No variables were significantly correlated (all $P > 0.05$), so we included them all in the analyses (Quinn & Keough 2002). To allow comparison of model parameter estimates, we standardized all variables to a mean of zero and a standard deviation of one. To compare factors that influenced rock usage by each species, we used univariate generalized linear mixed models (GLMM) with the binomial family (link function type=logit) and ranked the models using a corrected Akaike's information criteria ($AIC_c$: Crawley 2009), with site as the random factor. We also investigated factors influencing the relative frequency of use of artificial rocks (among those used at least once by that species, thus omitting data for rocks that were never used) by developing univariate generalized linear models using the Poisson family (link function type=Poisson) and ranking the models using $AIC_c$. For both analyses, we used a model averaging approach to account for model and parameter uncertainty (Crawley 2009). We developed alternative models from all linear combinations of the explanatory variables, ranked these by their $AIC_c$ values and obtained the Akaike weight for each model (Crawley 2009). Magnitude and direction of the effect of a variable were calculated from model-averaged parameter estimates, which we obtained by using the mean of the coefficient estimates of all models weighted by the Akaike weight. We also assessed the relative importance of individual variables for each target species by summing the Akaike weights from all model combinations where the variable occurred, then ranking the variables according to their Akaike weight, with larger values indicating greater importance (Crawley 2009).

## Results

AIC rankings of the results from our GLMM analyses showed that our seven study species; broad-headed snakes (*Hoplocephalus bungaroides*), small-eyed snakes (*Cryptophis nigrescens*), velvet geckos (*Oedura lesueurii*), red-throated skinks (*Acritoscincus platynotum*), copper-tailed skinks (*Ctenotus taeniolatus*), wall skinks (*Cryptoblepharus pulcher*) and flat-rock spiders (*Hemicloea major*) used rocks non-randomly with respect to intermediate-scale habitat attributes. We focus below on those with high importance based on the sum of Akaike weights (Table 1).

### *Used* versus *unused artificial rocks*

All seven of the species that we studied were recorded often enough for us to conduct robust comparisons between the habitat attributes surrounding used *versus* unused rocks (Table 1). Velvet geckos showed non-random rock use with respect to four variables, red-throated skinks, copper-tailed skinks, small-eyed snakes and broad-headed snakes showed non-random rock use with respect to three variables (Table 1, Fig. 2), wall skinks responded to two variables, and flat-rock spiders responded to a single variable (Table 1, Fig. 2).

Velvet geckos appeared to base retreat-site selection on more habitat variables than did any other species. The geckos chose rocks on large outcrops, close to the cliff edge, far from leaf litter and close to natural rocks (Table 1, Fig. 2B, 2C, 2E, 2F). Of the four species that responded to three variables, three were affected by the distance of artificial rocks from nearby leaf litter. Broad-headed snakes chose rocks far from leaf litter, whereas small-eyed snakes and red-throated skinks chose rocks close to leaf litter (Table 1, Fig. 2E). Small-eyed snakes and broad-headed snakes also both chose rocks close to the cliff (Table 1, Fig. 2C), but differed in other criteria. Broad-headed snakes chose rocks that received higher than average solar radiation (Table 1, Fig. 2A) and small-eyed snakes chose rocks located far from crevices (Table 1, Fig. 2H). Red-throated skinks selected rocks close to woodland and near large natural rocks (Table 1, Fig. 2D, 2G). Copper-tailed skinks showed a preference for artificial rocks close to the cliff and woodland yet far from other rocks (Table 1, Fig. 2C, 2D, 2F). Wall skinks chose artificial rocks far from woodland and leaf litter (Table 1, Fig. 2D, 2E). Finally, flat-rock spiders were most common under artificial rocks located on smaller outcrops (Table 1, Fig. 2B).

### *Frequency of artificial rock usage*

Broad-headed snakes used individual artificial rocks too infrequently for statistical analysis, because this endangered species is too rare to generate suitable sample sizes. However, the remaining six species showed strong patterns.

Wall skinks commonly used artificial rocks that were influenced by five habitat variables: rocks on large outcrops, close to the cliff edge, far from crevices, leaf litter and woodland (Table 2). Three of the remaining five species (velvet geckos, copper-tailed skinks and flat-rock spiders) commonly used artificial rocks that were distinctive in terms of four habitat variables. Rock usage by these species was influenced by distance to the nearest

**Table 1:** *Details of generalized linear mixed models comparing the attributes of used* versus *unused artificial rocks by species*

| | Broad-headed snake | Small-eyed snake | Velvet gecko | Red-throated skink | Wall skink | Copper-tailed skink | Flat-rock spider |
|---|---|---|---|---|---|---|---|
| Number of artificial rocks colonised (%) | 18 (9.09) | 13 (6.57) | 165 (83.33) | 28 (14.14) | 105 (53.03) | 20 (10.1) | 165 (83.33) |
| Radiation ($w_i$) | **0.66 ± 0.45 (0.54)** | -0.10 ± 0.32 (0.28) | 0.10 ± 0.27 (0.28) | -0.24 ± 0.23 (0.40) | 0.22 ± 0.21 (0.39) | 0.22 ± 0.26 (0.33) | 0.19 ± 0.20 (0.35) |
| Outcrop area ($w_i$) | -1.14 ± 1.13 (0.39) | -0.02 ± 0.96 (0.43) | **0.63 ± 0.57 (0.50)** | 0.03 ± 0.26 (0.26) | 0.07 ± 0.17 (0.28) | 0.25 ± 0.36 (0.30) | **-0.29 ± 0.18 (0.60)** |
| Nearest natural rock area ($w_i$) | -0.06 ± 0.02 (0.31) | 0.27 ± 0.28 (0.36) | 0.33 ± 0.26 (0.47) | **0.43 ± 0.19 (0.81)** | -0.22 ± 0.17 (0.45) | -0.30 ± 0.30 (0.38) | 0.11 ± 0.12 (0.29) |
| Distance to west-facing cliff ($w_i$) | **-1.82 ± 0.83 (0.94)** | **-1.10 ± 0.68 (0.72)** | **-0.42 ± 0.26 (0.62)** | -0.16 ± 0.31 (0.30) | -0.13 ± 0.18 (0.32) | **-0.52 ± 0.26 (0.53)** | -0.16 ± 0.22 (0.35) |
| Distance to woodland ($w_i$) | 0.16 ± 0.34 (0.29) | -0.05 ± 0.36 (0.28) | 0.32 ± 0.27 (0.44) | **-0.37 ± 0.26 (0.51)** | **0.27 ±0.17 (0.56)** | **-1.09 ± 0.42 (0.98)** | -0.19 ±0.20 (0.36) |
| Distance to nearest natural rock ($w_i$) | 0.25 ± 0.29 (0.34) | 0.17 ± 0.31 (0.29) | **-0.44 ± 0.26 (0.58)** | -0.05 ± 0.24 (0.26) | 0.03 ± 0.17 (0.26) | **0.58 ± 0.26 (0.81)** | -0.18 ± 0.18 (0.36) |
| Distance to leaf litter ($w_i$) | **0.38 ± 0.26 (0.51)** | **-1.00 ± 0.58 (0.77)** | **0.52 ± 0.33 (0.61)** | **-0.41 ± 0.28 (0.54)** | **0.54 ± 0.20 (0.96)** | -0.43 ± 0.40 (0.41) | -0.07 ± 0.20 (0.28) |
| Distance to nearest crevice ($w_i$) | -0.20 ± 0.37 (0.30) | **0.13 ± 0.42 (0.76)** | 0.01 ± 0.26 (0.27) | -0.26 ± 0.29 (0.36) | -0.12 ± 0.17 (0.31) | -0.53 ± 0.42 (0.36) | 0.22 ± 0.24 (0.36) |

*Sample sizes, average parameter estimates, standard errors of these estimates, and sum of Akaike weights ($w_i$) for explanatory variables derived for all combinations of the generalized linear models comparing the attributes of used artificial rocks* versus *those not known to have been used by seven different wildlife species on sandstone outcrops in southeastern Australia. Boldface values have a high $w_i$ value, indicating high importance.*

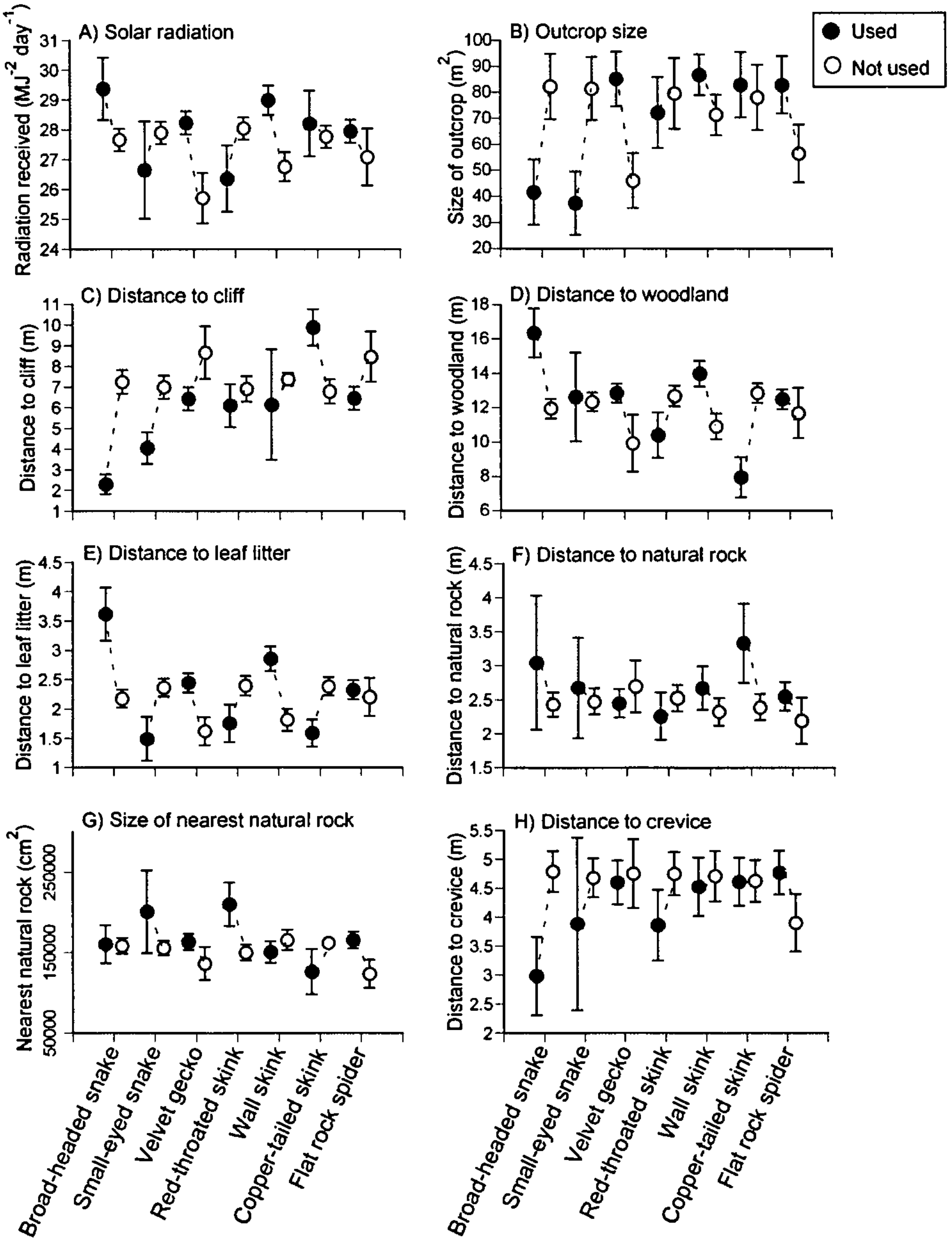

**Figure 2:** *Means and standard errors of habitat variables associated with artificial rocks either used or not used by seven saxicolous wildlife species. "Used" rocks were those where we found the species sheltering in the crevice formed between the artificial rock and the underlying rock substrate. These values are based on measurements of 198 identical artificial rocks deployed across the landscape, and the use of those rocks by fauna over 29 months, from August 2007–December 2009.*

**Table 2:** *Details of generalized linear mixed models predicting the frequency of use of artificial rocks by species*

| | Broad-headed snake | Small-eyed snake | Velvet gecko | Red-throated skink | Wall skink | Copper-tailed skink | Flat-rock spider |
|---|---|---|---|---|---|---|---|
| Radiation ($w_i$) | - | -0.05 ± 0.27 (0.27) | **0.12 ± 0.04 (0.99)** | -0.24 ± 0.23 (0.40) | 0.10 ± 0.09 (0.37) | 0.28 ± 0.21 (0.46) | **-0.17 ± 0.04 (1.00)** |
| Outcrop area ($w_i$) | - | **-1.35 ± 0.94 (0.60)** | **0.11 ± 0.04 (0.95)** | 0.03 ± 0.26 (0.26) | **0.14 ± 0.09 (0.57)** | 0.27 ± 0.33 (0.31) | -0.05 ± 0.04 (0.45) |
| Nearest natural rock area ($w_i$) | - | -0.01 ± 0.26 (0.26) | 0.02 ± 0.03 (0.32) | **0.43 ± 0.19 (0.81)** | -0.00 ± 0.06 (0.25) | -0.18 ± 0.23 (0.33) | 0.03 ± 0.03 (0.38) |
| Distance to west-facing cliff ($w_i$) | - | **-0.73 ± 0.49 (0.60)** | **-0.33 ± 0.05 (1.00)** | -0.24 ± 0.22 (0.36) | **-0.23 ± 0.12 (0.80)** | **-0.60 ± 0.37 (0.73)** | 0.04 ± 0.04 (0.37) |
| Distance to woodland ($w_i$) | - | 0.05 ± 0.27 (0.28) | -0.01 ± 0.04 (0.27) | **-0.37 ± 0.26 (0.51)** | **0.26 ± 0.08 (0.99)** | **-1.06 ± 0.35 (1.00)** | 0.03 ± 0.04 (0.34) |
| Distance to nearest natural rock ($w_i$) | - | -0.06 ± 0.31 (0.26) | **-0.04 ± 0.03 (0.51)** | -0.05 ± 0.24 (0.26) | -0.05 ± 0.07 (0.31) | **0.51 ± 0.19 (0.89)** | **-0.10 ± 0.04 (0.93)** |
| Distance to leaf litter ($w_i$) | - | **-0.89 ± 0.48 (0.79)** | 0.01 ± 0.03 (0.28) | **-0.41 ± 0.29 (0.54)** | **0.20 ± 0.07 (0.95)** | **-0.49 ± 0.35 (0.54)** | **0.06 ± 0.03 (0.63)** |
| Distance to nearest crevice ($w_i$) | - | -0.43 ± 0.46 (0.39) | -0.04 ± 0.04 (0.39) | -0.26 ± 0.29 (0.36) | **0.20 ± 0.07 (0.99)** | -0.23 ± 0.35 (0.32) | **0.08 ± 0.03 (0.78)** |

*Average parameter estimates, standard errors of these estimates, and sum of Akaike weights ($w_i$) for explanatory variables derived for all combinations of the generalized linear models predicting the frequency of usage of artificial rocks by six different wildlife species on sandstone outcrops in southeastern Australia. Rocks never recorded as being used by a given species are omitted from these analyses. Bold values have a high $w_i$ value, indicating high importance.*

natural rock (Table 2); velvet geckos and flat-rock spiders preferred artificial rocks close to natural rocks, whereas copper-tailed skinks showed the opposite preference (Table 2) Velvet geckos and copper-tailed skinks both preferred artificial rocks close to the cliff, but differed in other respects. The geckos were found most often beneath artificial rocks exposed to higher-than-average radiation, and located on large outcrops (Table 2). In contrast, copper-tailed skinks repeatedly used artificial rocks that were close to leaf litter and adjacent woodland (Table 2). As well as preferring artificial rocks that were close to natural ones, flat-rock spiders repeatedly used rocks that received less-than-average radiation exposure and that were located far from leaf litter and crevices (Table 2). Rock use by the remaining two species, small-eyed snakes and red-throated skinks, was influenced by three variables. Both species preferred rocks close to leaf litter, but small-eyed snakes selected rocks on small outcrops close to the cliff edge (Table 2), whereas red-throated skinks used artificial rocks close to woodland and large natural rocks (Table 2).

## Discussion

By standardizing three major aspects of individual retreat sites that influence thermal regimes (rock size and thickness, three-dimensional crevice structure beneath the rock, and canopy openness: Webb & Shine 1998a, 1998b, Pringle et al. 2003, Croak et al. 2008), we showed that landscape-scale features influence habitat selection by most of the rock-dwelling species that we studied (Tables 1 and 2; Fig. 2). Importantly, each of the seven species showed different patterns of spatial association with landscape features. Below, we first consider the nature of (and possible causes for) such patterns, before considering the broader implications of our results.

*Used* versus *unused artificial rocks*

Broad-headed snakes selected artificial rocks exposed to high levels of incident radiation (Table 1, Fig. 2A). This result supports previous work (based on selection of natural rocks by snakes and lizards) that has identified thermal cues as important in diurnal retreat site selection for many nocturnal saxicolous reptile species (Huey et al. 1989, Webb & Shine 1998a, Pringle et al 2003, Sabo 2003). Previous experiments have also shown that thermal cues influence retreat site selection in four species whose spatial distributions were not strongly associated with canopy cover (based on low $AIC_c$ weightings) in the present study (small-eyed snakes, velvet geckos, copper-tailed skinks and flat-rock spiders: Schlesinger & Shine 1994a, 1994b, Webb et al. 2004, Goldsbrough 2006, Pike et al. 2011b). The other species that did not respond to canopy cover in the present study (the red-throated skink) has not been studied experimentally in this respect (however, red-throated skinks prefer shadier habitat, potentially explaining this result: Pike et al. 2011a).

Why was the concordance between previous experiments and our field experiment excellent for one species (broad-headed snakes), and poor for the others? A likely reason is that we deployed all of the artificial rocks in open areas with high levels of incident radiation (canopy openness in our study ranged from 33–85%, canopy openness in nearby areas ranged from 15–75%: Pringle et al. 2003). The range in canopy openness above our artificial rocks was similar to that selected by reptiles in a previous field study at a nearby site (38–75%: Pringle et al. 2003). Thus, the areas where we deployed the artificial rocks largely provided optimal levels of canopy openness (and thus thermal regimes: Pringle et al. 2003), reducing the importance of thermal cues (and hence, elevating the relative importance of non-thermal cues) for retreat site selection. The relative importance of thermal *versus* other cues presumably differs among species, so that some taxa responded to temperature during our field trials whereas others did not.

Other macrohabitat correlates of faunal distribution are more difficult to interpret, and do not relate as closely to the parameters manipulated in previous experimental studies. The preference of copper-tailed skinks and red-throated skinks to use artificial rocks close to woodland (Table 1, Fig. 2D) may reflect substrate attributes, because these lizards actively select rock-on-soil habitats (enabling burrow construction beneath the rocks) for nocturnal retreats (Goldsbrough et al. 2006), and soil depths typically are greater close to the woodland than on large open exposed areas of plateau. More puzzlingly, copper-tailed skinks also preferred rocks that were far from other rocks, and wall skinks preferred rocks that were far from leaf litter (Table 1, Fig. 2F, 2G). The latter effect may reflect the predation risk posed by large invertebrates, such as centipedes and spiders (Bauer 1990, Pike et al. 2010); wall skinks are the smallest reptile species on these rock outcrops (mean snout-vent length 40 mm: Wilson & Swan 2003), which may render them especially vulnerable to invertebrate predation (Pike et al. 2010).

*Frequency of use of artificial rocks*

Six species used individual artificial rocks frequently enough to allow comparisons within the subset of used artificial rocks (Table 2). Repeated use of individual artificial rocks by some taxa appears to be thermally driven. For example, the frequency of rock usage by two species (velvet geckos and flat-rock spiders) was influenced by the amount of radiation received (Table 2), but other variables appear to influence these species differently. The tendency for velvet geckos to reuse artificial rocks located close to woodland may indicate a preference for proximity to foraging sites, and the reuse of artificial rocks located close to other rocks may reflect territoriality (these lizards often use two or three adjacent rocks as shelter and foraging sites: Schlesinger & Shine 1994b, Downes & Shine 1998a). Interestingly, wall skinks showed an almost opposite trend to velvet geckos by repeatedly using artificial rocks located further from woodland, leaf litter and crevices (Table 2). As noted above, the small size of wall skinks may render them vulnerable to large invertebrate (centipedes, scorpions: Bauer 1990, Pike et al. 2010) and vertebrate (small-eyed snakes: Cogger 2000) predators that forage in these areas. Flat-rock spiders repeatedly used artificial rocks located close to other rocks, far from leaf litter and far from crevices (Table 2). These spiders are sedentary cannibalistic predators (Goldsbrough et al. 2004), and are vulnerable to larger invertebrates (such as huntsman spiders, *Sparassidae* spp., and large centipedes) that forage in these areas. Small-eyed snakes are ecological generalists (Cogger 2000) and in our study, showed few strong landscape-scale preferences in terms of which rocks they used repeatedly (Table 2). These snakes may readily shelter under any rocks that provide broadly suitable crevice dimensions, thermal regimes and foraging opportunities (leaf litter).

In addition to suggesting novel hypotheses about cues for macrohabitat selection in our study species, our results have direct implications for conservation and management of this system. Given the highly endangered status of the broad-headed snake (Hersey 1980, Shine & Fitzgerald 1989), the macrohabitat correlates of its retreat site selection are of particular interest. A trend for these snakes to be most abundant in sites close to steep cliffs has long been noted (Krefft 1869, Shine & Fitzgerald 1989), but has been attributed to the role of these cliffs in creating canopy gaps that allow solar radiation to warm the rocks (Webb & Shine 1998a, 1998b, Pringle et al. 2003). Laboratory experiments and experimental field studies also have demonstrated that thermal regimes beneath rocks influence rock selection by the snakes (Pringle et al. 2003, Webb et al. 2004, Pike et al. 2011a). However, our data suggest that proximity to cliffs also has a different and more direct effect on broad-headed snakes, perhaps by facilitating escape because these snakes readily escape over the cliff edge when we attempt to capture them (B. Croak, personal observation).

More generally, our results can guide attempts at habitat restoration in such a system by identifying how alternative manipulations are likely to affect target species. To enhance habitat suitability for the endangered broad-headed snake, for example, special effort should be given to creating suitable retreat sites close to cliff edges (for the snakes) and in areas exposed to high levels of solar radiation (for velvet geckos, a major prey species for the snake: Webb & Shine 1998c). Future work could usefully explore the functional significance of macrohabitat-scale factors for the fitness (e.g., growth, survival) of individual reptiles, and hence clarify why the species that we studied differ so profoundly in the landscape features that predict their spatial distribution (Tables 1 and 2, Fig. 2). Integrating information on criteria for habitat selection at a range of spatial scales can substantially improve our understanding of the determinants of spatial distribution of these animals. Ongoing landscape modification such as bush-rock removal (eliminating a non-renewable critical habitat), alteration of forest cover and climate change (and potentially, their interactions) threaten this rock dwelling faunal assemblage (Webb & Shine 1998b, Pringle et al. 2003, Croak et al. 2008, Penman 2010, Pike et al. 2011a), creating a special urgency in understanding how artificial retreat sites can be used to mitigate these effects.

Retreat site selection by fauna is of great interest in many systems (Martin 2001, Dunlap & Oliveri 2002, Scharf & Ovadia 2005). Future research could benefit by standardizing attributes of retreat sites that are important determinants of faunal use. For example, artificial retreat sites often are used to assist in the capture of elusive animals (Isaia et al. 2006, Lettink & Patrick 2006, Bell 2009), restoration of degraded systems (Webb & Shine 2000, Souter et al. 2004, Croak et al. 2010) and increased productivity of animal populations harvested for human consumption or use (Briones-Fourzan et al. 2007). By standardizing retreat sites to account for factors that influence faunal use, and monitoring their subsequent usage by animals in the field, we may discover less obvious, but perhaps equally important, determinants of retreat site selection. In turn, a better understanding of the factors affecting the spatial distribution of animals across the landscape can facilitate management and conservation.

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# **CHAPTER 3:** *THE BENEFITS OF HABITAT RESTORATION FOR ROCK-DWELLING GECKOS* (Oedura lesueurii)

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*A velvet gecko using an artificial rock as a retreat site.*

## Abstract

Worldwide, efforts to restore habitat quality are rarely matched by efforts to evaluate the effects of those restoration attempts. Simply documenting usage of the newly created habitats by biota is not enough, because such areas may serve as sink populations. We need to monitor viability (growth, survival, reproduction) of individuals that colonise the newly created habitat, compared to conspecifics in non-restored areas. We recorded and analysed rates of growth and survival in velvet geckos (*Oedura lesueurii*) in anthropogenically-degraded rock outcrops in southeastern Australia, over a 2-year period following habitat restoration (addition of artificial rocks, to replace those taken for landscaping use). Gecko growth rates were unaffected by habitat restoration, but restoring sites with artificial rocks increased the numbers of lizards (both adults and juveniles). The apparent survival rates of adult male lizards (as estimated using MARK) were not significantly affected by habitat restoration, but juvenile geckos (and perhaps adult females) experienced higher survival at restored sites. Juvenile geckos living under artificial rocks had significantly higher apparent survival rates than did conspecifics living under nearby natural rocks. Habitat restoration has had measurable conservation benefits on these rocky outcrops. Quantifying those benefits enables decisions about habitat restoration to be based upon evidence rather than wishful thinking or untested intuition.

**Keywords:** capture-mark-recapture, anthropogenic degradation, restoration monitoring, rock outcrop, saxicolous reptiles

## Introduction

The current scale of habitat degradation means that simply preserving existing areas of relatively unspoiled habitat is no longer enough. To conserve many systems that are currently under threat, we need to actively restore degraded habitats (Sinclair *et al.* 1995; Miller & Hobbs 2007; Kouki *et al.* 2011). Ideally, efforts at habitat restoration should be based on a detailed knowledge of habitat requirements for the biota of interest; and the effects of those manipulations on the biota need to be monitored carefully, over biologically meaningful time periods (Block *et al.* 2001; Lovett *et al.* 2007; Miller & Hobbs 2007). Attempts at habitat restoration often fail because of unsuspected factors such as responses of other (non-desired) species (Bice & Zampatti 2011), or low vagility (and hence, low colonisation rates) of the target species (Dubey *et al.* 2012). Even if the "at risk" species colonise the restored habitat, however, the net impact of our manipulations on those species may not be positive. The newly restored habitat may simply attract animals out of the few patches of less-degraded habitat, and potentially expose them to new risks such as disease, parasites, predation or costs of increased social conflict (Nicol *et al.* 2004; Klein *et al.* 2007; Skwarska *et al.* 2009; Bice & Zampatti 2011). For example, some birds use artificial nesting boxes in preference to natural tree-hollows, but may be at greater risk of predation as a result (Skwarska *et al.* 2009). Analogously, urban birds and other wildlife enthusiastically consume the supplemental food offered by householders, tourists and the like, but may thereby experience severe alterations in behaviour, foraging ability and nutritional balance (Orams 2002; Newsome & Rodger 2008).

The possibility of inadvertent collateral damage from efforts to conserve species places a premium on careful monitoring of the consequences of any conservation-oriented habitat manipulations (Block *et al.* 2001; Lovett *et al.* 2007; Miller & Hobbs 2007). Unfortunately, more effort typically is devoted to manipulating habitats than to monitoring the effects of the implemented changes (Block *et al.* 2001; Ruez-Jaen & Aide 2005). In particular, we need to quantify not only the rate at which animals colonise the newly created habitat, but also the consequences of that colonisation for individual viability. Ideally, the restored habitat should provide conditions that enable animals to achieve rates of survival, growth and reproduction at least as high as is obtainable in patches of the original (non-degraded) habitat (Block *et al.* 2001). Such data are difficult to obtain in the field, especially from vagile animals.

Our long-term research on rock-dwelling reptiles in southeastern Australia provides an ideal opportunity to evaluate the impacts of habitat restoration. Sandstone outcrops in this region provide thermally distinctive microenvironments that support a unique reptile fauna, including the endangered broad-headed snake (*Hoplocephalus bungaroides* Schlegel, 1837) and its major prey, the velvet gecko (*Oedura lesueurii* Dumeril & Bibron 1836: Webb & Shine 1998; Pringle, Webb & Shine 2003). Illegal removal of exfoliated rocks, which are popular for garden landscaping, has denuded many areas of the rock crevices required by these animals (Schlesinger & Shine 1994a; Shine *et al.* 1998). We restored sites in a bush rock denuded area with artificial rocks enabling us to examine the impacts of rock-replenishment not only on gecko numbers, but also on the viability of individual geckos. The strong philopatry of these lizards (Webb, Pike & Shine 2008) facilitates direct comparisons between individuals that rely upon artificial versus natural rocks on the same or adjacent sites.

## Methods

The velvet gecko inhabits rocky outcrops in Australia from southeastern New South Wales to southeastern Queensland (Cogger 2000). This small (up to 65 mm snout-vent length [SVL]) nocturnal lizard shelters by day in crevices beneath small rocks and under exfoliated bedrock (Schlesinger & Shine 1994a; Webb & Shine 1998; Croak *et al.* 2008, 2010). At dusk, the lizards emerge from shelters to forage in leaf litter (Cogger 2000). Adult male velvet geckos are territorial and defend their retreat sites from other males and juveniles (Downes & Shine 1998). Velvet geckos form the main prey base for the highly endangered broad-headed snake (Webb & Shine 1998).

### *Study sites*

We conducted a two-year mark recapture project (2009–2010) on velvet geckos at 12 sites in the Dharawal Conservation area (see Field Methods below, Fig. 1). The sites were rock outcrops (on average 50 m x 50 m in surface area) separated by mixed eucalyptus forest dominated by scribbly gum *Eucalyptus haemastoma*, Sydney peppermint *E. pipperita* and red bloodwood *E. gummifera*.

### *Field Methods*

We deployed 50 artificial rocks made from fibre-reinforced cement (each measuring 512 mm long x 352 mm wide x 46 mm thick) that provide crevices similar (both structurally and thermally) to those under natural rocks (Croak *et al.* 2008, 2010) on flat ground and

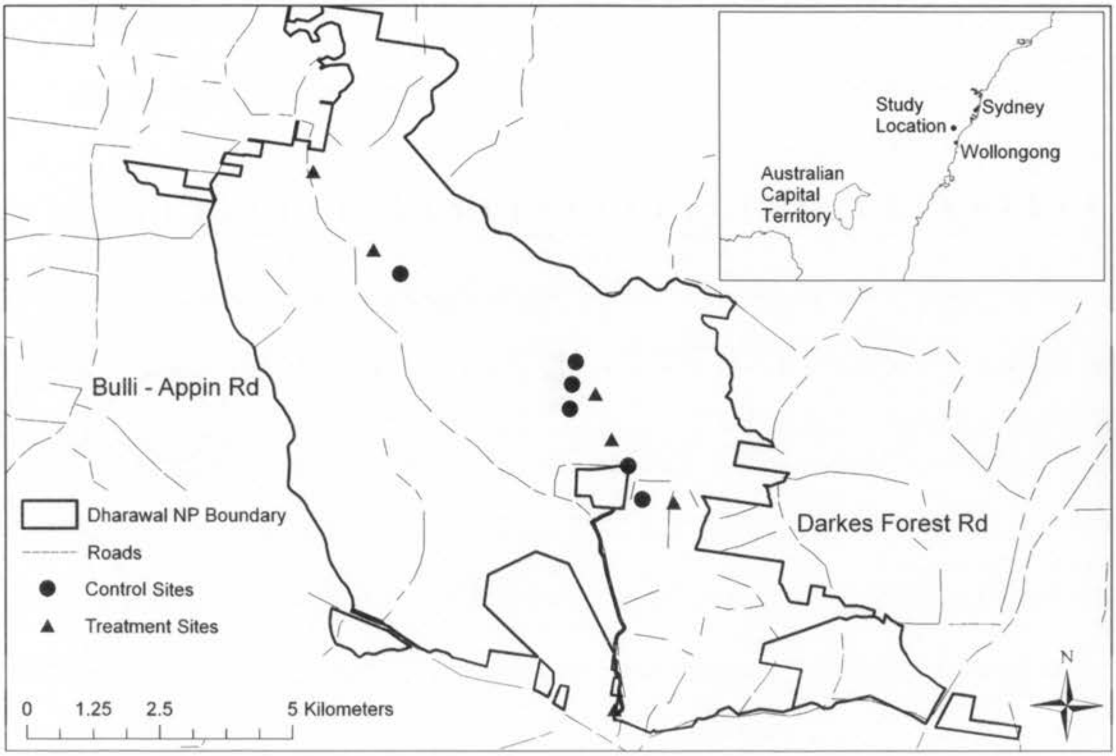

**Figure 1:** *Location of study sites within Dharawal National Park, New South Wales, Australia, an area affected by surface rock removal. Triangles are treatment sites where we deployed artificial rocks to restore habitat and enhance retreat site availability. Circles are control sites that we monitored to gauge the effects of our habitat restoration attempt.*

in open areas at each of six treatment sites throughout the area (see Croak *et al.* 2008, 2010 for details on construction and deployment criteria). Each habitat-restored (treatment) site was paired with a nearby unrestored (control) site that contained a similar number of natural rocks as was present on the control site. Although we matched sites as closely as possible, control sites had a slightly higher average number of natural rocks than did treatment sites (Fig. 2a). On average 500 m separated treatment and control sites (range 500 m to 13 km); we never recorded geckos moving from one site to another. We monitored faunal usage by sampling all rocks (both natural and artificial) at all (i.e. both treatment and control) sites on a monthly basis from the beginning of 2009 until the end of 2010. We sampled the sites by turning all rocks by hand and capturing any animals found beneath. We gave any velvet geckos encountered a unique toe-clip sequence for individual recognition. We also recorded the sex, SVL (mm) and tail length (mm) of the lizards. We then carefully returned rocks to their original positions, and allowed the lizards to retreat beneath them.

### *Statistical Analyses*

#### *Captures*

We classified animals as adults based upon the minimum size for reproductive adult males (SVL $\geq$ 50 mm) and females (SVL $\geq$ 56 mm) with males and females differentiated by the presence of enlarged male gonads and cloacal spurs as reported by Webb, Pike & Shine (2008). We used an analysis of variance (ANOVA) with site type as a factor and numbers of male, female and juvenile geckos as the dependant variables, to compare numbers of individual geckos captured at control and treatment sites. We used analysis of covariance (ANCOVA) with rock number as the covariate, site type as a factor and numbers of male, female and juvenile geckos as dependant variables to assess gecko numbers at the different site types relative to the total number of rocks available.

#### *Survival*

We used the Cormack-Jolly-Seber method (Cormack 1989) to estimate recapture ($p$) and survival ($\varphi$) probabilities from the mark-recapture data at control versus treatment (restoration) sites using the software package Program MARK v 6.1 (White & Burnham 1999). We included site (restoration vs. control) as a factor (group) in the input file. Because survival probabilities of *O. lesueurii* can vary ontogenetically (Webb 2006), we included gecko age (adult vs. juvenile) and sex as factors in the input file. We classified animals as described above. Prior to data analysis in MARK, we formulated a series of a priori candidate models to determine whether survival and recapture probabilities varied over time, among treatment groups (control vs. restoration), between age classes, or as a function of interactions between these factors. To determine whether the most general model in our candidate model set ($\varphi$ t $p$ t) provided an adequate fit to the data (i.e. met the mark-recapture assumptions), we used the bootstrap GOF procedure in MARK (Cooch & White 2011). Based on 500 bootstrap replicates, there was no significant deviation from the mark-recapture assumptions for our general model ($P = 0.06$).

To explore the influence of rock type (artificial vs. natural) on gecko survival and recapture independent of site type, we assigned each individual gecko to one of three groups: geckos that only used artificial rocks, geckos that only used natural rocks and those which used both. Because survival varies with age and sex, we analysed each age group separately. Thus, we used the Cormack-Jolly-Seber method (Cormack 1989) to estimate recapture ($p$) and survival ($\varphi$) probabilities from the mark-recapture data collected from individual juvenile geckos using "only natural", "only artificial" or "both rock types" (thus three factors, = groups). We estimated recapture ($p$) and survival ($\varphi$) probabilities for individual male velvet geckos that used only natural versus only artificial rocks (we omitted the "both rock types" category for male geckos because most of these lizards used only a single rock type). We omitted female geckos from our analysis, due to low sample sizes ($N = 11$ individuals using only natural rocks). Prior to data analysis in MARK, we formulated a series of a priori candidate models to determine whether survival and recapture probabilities varied over time, among treatment groups, or interactions between these factors. To determine whether the most general model in our candidate model sets [$\varphi$ (t) $p$ (t)] met the mark-recapture assumptions, we used the bootstrap GOF procedure in MARK (Cooch & White 2011). Based on 500 bootstrap replicates, we found no significant deviation from the mark-recapture assumptions for our general models ($P = 0.07$ for juveniles, $P = 0.09$ for males).

For all analyses, we used the Akaike Information Criterion (AIC) to select the most parsimonious model (Burnham &Anderson 1998). We adjusted the AIC values for over-dispersion using the variance inflation factor $c$, which we calculated from the GOF statistics (Cooch & White 2011). The AIC value adjusted for over-dispersion and finite sample size is termed the corrected quasi-likelihood AIC (denoted as QAICc). We ranked models by comparing $\Delta$AICc (the difference between the lowest AIC and AIC from the set of models), and we used normalised AIC weights to evaluate the relative strength of evidence of models (Burnham & Anderson 1998).

### *Growth rates*

We used ANCOVA to compare growth rates of velvet geckos captured under artificial versus natural rocks. This analysis included rock type as a factor, mean SVL (average of initial and final length) as a covariate (to allow for ontogenetic changes in growth rate), and growth rate (mm day$^{-1}$) as the dependent variable.

## Results

### *Captures*

Our control sites had an average of 26 natural rocks and our treatment sites had an average of 18 natural rocks and 50 artificial rocks (Fig. 2a). On average, we captured 27 individual geckos under natural rocks at each control site, 7 individual geckos under natural rocks at each treatment site and 60 individuals under artificial rocks at each treatment site (Fig. 2b). Over the two-year period, we captured 563 individual velvet geckos: 312 juveniles, 80 females and 171 males. Treatment sites had more male ($F_{1,9}$ = 5.25, $P = 0.04$, Adjusted $R^2 = 0.30$) and female geckos ($F_{1,9}$ = 9.94, $P = 0.01$, Adjusted $R^2$ = 0.47) than did control sites. Although numbers of juveniles increased to a similar degree as in adults, high variances meant that the increase in juvenile numbers in habitat-restored sites was not statistically significant ($F_{1,9}$ = 1.93, $P = 0.20$, Adjusted $R^2 = 0.08$; Fig. 3a).

When we controlled for the number of rocks at each site, there were no significant differences between control and habitat-restored sites in the numbers of male, female or juvenile geckos (males, slope: $F_{1,7} = 0.47$, $P = 0.52$, effect of site type: $F_{1,8} = 1.71$, $P$ = 0.23, effect of rock number: $F_{1,8} = 0.32$, P = 0.59 females: slope: $F_{1,7} = 0.44$, $P = 0.53$, effect of site type: $F_{1,8} = 2.04$, $P = 0.19$, effect of rock number: $F_{1,8} = 0.06$, $P = 0.70$ Juveniles: slope: $F_{1,7} = 0.02$, $P = 0.90$, effect of site type: $F_{1,8} = 1.93$, $P = 0.2$, effect of rock number: $F_{1,8} = 0.93$, $P = 0.36$).

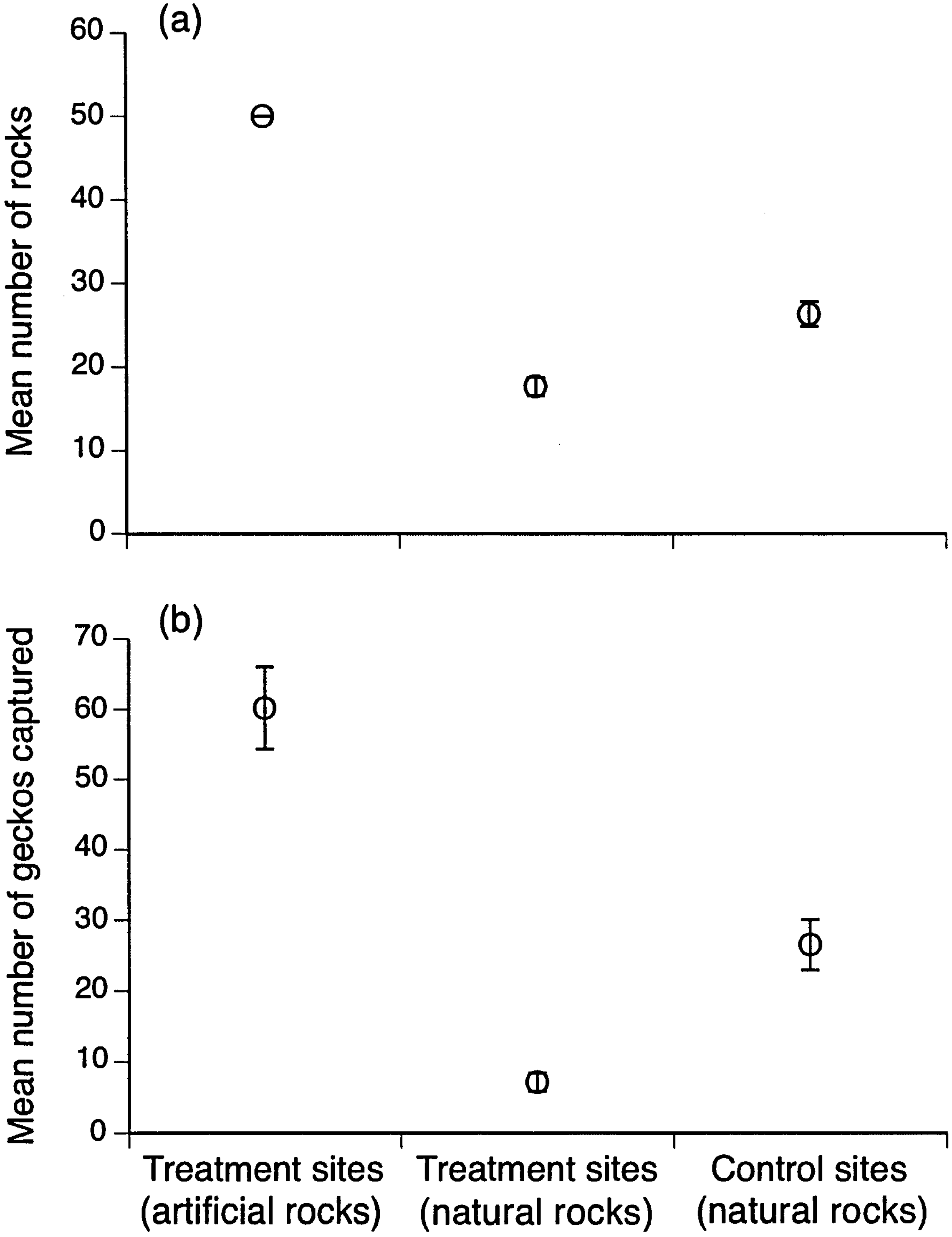

**Figure 2:** *(a) Mean and associated standard errors of the numbers of natural and artificial rocks at sites augmented with artificial rocks (treatment sites) and of natural rocks at unrestored (control) sites. (b) The mean and associated standard errors of the abundance of velvet geckos captured under artificial and natural rocks at treatment and control sites.*

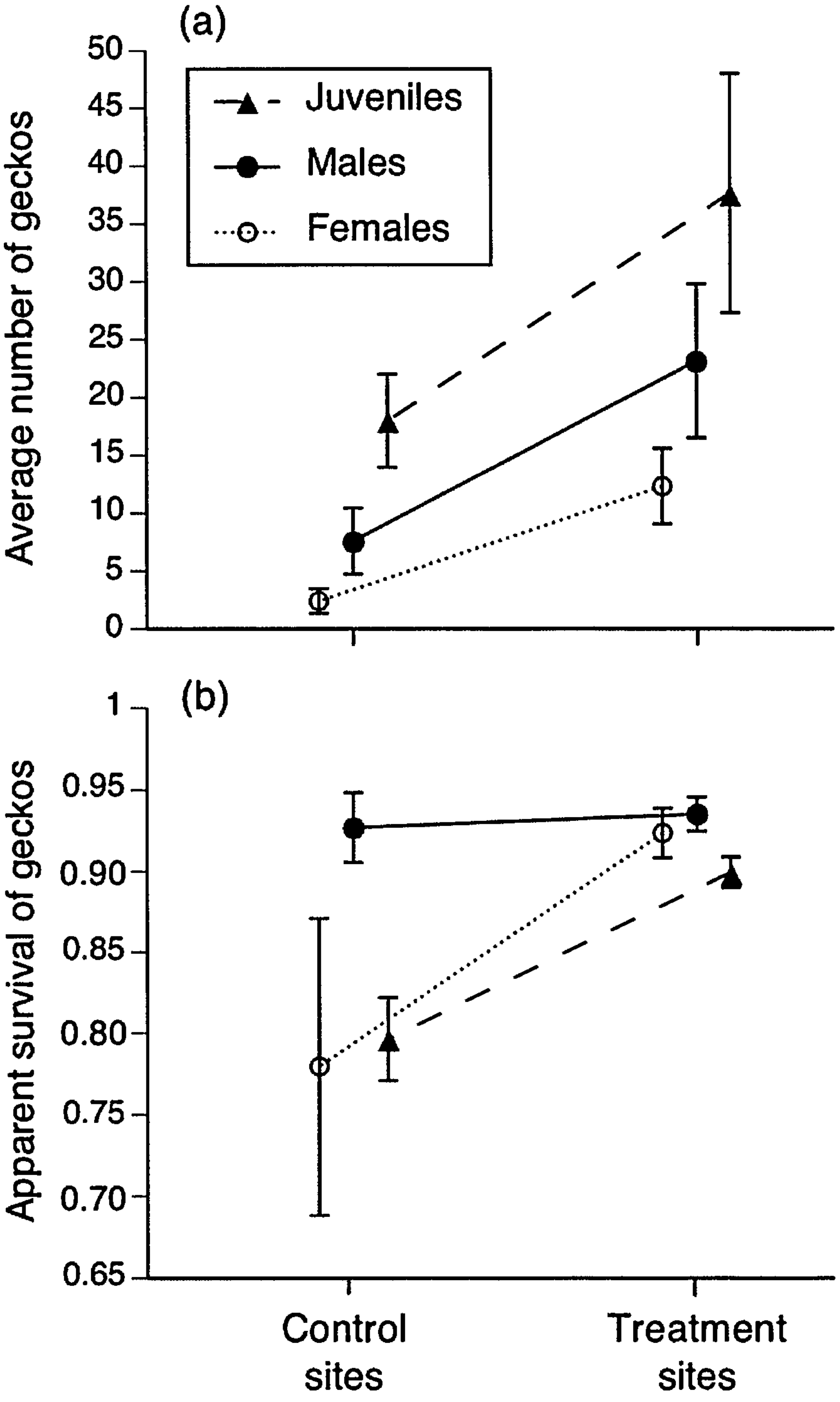

**Figure 3:** *(a) The average number of male, female and juvenile geckos captured at treatment and control sites over the course of the study with associated error bars. (b) Survival estimates and associated standard errors of male, female and juvenile velvet geckos captured at either restored or unrestored sites. Estimates were derived from the most parsimonious* ($\varphi(g)$, $p(t)$) *Cormack-Jolly-Seber model.*

### *Survival*

### *Survival at restored vs. unrestored sites*

The model φ (g), *p* (t) was highest ranked in the candidate model set and had most support (ΔQAICc = 0.00, QAICc weight = 0.9981; Table 1). In this model, survival was group dependant and the probability of recapture was time dependant. From this model, survival rates for juveniles were higher at restored sites (φ = 0.90, SE = 0.01) than at unrestored sites (φ = 0.80, SE = 0.03; Fig. 3b). Survival rates of males were similar at restored (φ = 0.93, SE = 0.01) and control sites (φ = 0.92, SE = 0.02; Fig. 3b). Survival rates of adult females showed a non-significant trend for increased survival at treatment sites (restored sites φ = 0.92, SE = 0.01; control sites φ = 0.78, SE = 0.09; Fig. 3b).

**Table 1:** Candidate models used to determine whether survival (φ) and recapture probability (*p*) of geckos was influenced by treatment (restoration versus control), sex, or interactions between age and treatment

| Model | QAICc | ΔQAICc | QAIC weight | Model likelihood | *n* | QDeviance |
|---|---|---|---|---|---|---|
| φ(g), *p*(t) | 3362.22 | 0.00 | 0.9981 | 1.0000 | 25 | 1849.72 |
| φ(c/r), *p*(t) | 3374.74 | 12.53 | 0.0019 | 0.0019 | 21 | 1870.55 |
| φ(.), *p*(t) | 3391.01 | 28.79 | 0.0000 | 0.0000 | 20 | 1888.88 |
| φ(g), *p*(g x t) | 3395.13 | 32.91 | 0.0000 | 0.0000 | 111 | 1690.49 |
| φ(t), *p*(t) | 3398.27 | 36.06 | 0.0000 | 0.0000 | 35 | 1864.79 |

The letters g, c, r, and t refer to group, control site, restoration site and time respectively. The term "." denotes constant survival. The most parsimonious model was φ(g), p(t), where survival was group dependent and the probability of recapture was time dependant. Models are ordered according to the adjusted Akaike's information criterion (QAICc), with model parsimony increasing with decreasing QAICc weighting. Models with ΔQAICc <2.0 have the greatest statistical support.

### *Survival of juvenile velvet geckos under artificial and natural rocks*

The model φ (t), *p* (g) was highest ranked in the candidate model set and had the most support (ΔQAICc = 0.00, QAICc weight = 0.9893; Table 2). In this model, survival was

**Table 2:** Candidate models used to determine whether survival ($\varphi$) and recapture probability ($p$) of juvenile velvet geckos was influenced by their choice of shelter site (only artificial rocks, only natural rocks or a combination of both rock types)

| Model | QAICc | ΔQAICc | QAIC weight | Model likelihood | $n$ | QDeviance |
|---|---|---|---|---|---|---|
| $\varphi$(t), $p$(g) | 1522.69 | 0.00 | 0.9893 | 1.0000 | 19 | 655.39 |
| $\varphi$(g), $p$(t) | 1533.18 | 10.49 | 0.0052 | 0.0053 | 19 | 665.88 |
| $\varphi$(t), $p$(g x t) | 1534.54 | 11.85 | 0.0026 | 0.0027 | 61 | 572.24 |
| $\varphi$(t), $p$(t) | 1535.26 | 12.57 | 0.0018 | 0.0019 | 31 | 642.06 |
| $\varphi$(g), $p$(g x t) | 1536.97 | 14.28 | 0.0008 | 0.0008 | 49 | 603.10 |

The letters g and t refer to group and time respectively. The most parsimonious model was $\varphi$(t), $p$(g), where survival was time dependent and the probability of recapture was group (artificial, natural or both) dependant. Models are ordered according to the adjusted Akaike's information criterion (QAICc), with model parsimony increasing with decreasing QAICc weighting. Models with ΔQAICc <2.0 have the greatest statistical support.

time dependant, and the probability of recapture was group dependant; that is, juveniles using artificial rocks were more likely to be recaptured ($p$ = 0.49, SE = 0.02; Fig. 4) than were individuals using natural rocks ($p$ = 0.26, SE = 0.04; Fig. 4) or both rock types ($p$ = 0.22, SE = 0.06; Fig. 4).

### *Survival of male velvet geckos under artificial and natural rocks*

The model $\varphi$(.), $p$ (g x t) was highest ranked in the candidate model set and had the most support (ΔQAICc = 0.00, QAICc weight = 0.720; Table 3). In this model survival was constant, with male geckos showing similar rates of survival under both artificial and natural rock types ($\varphi$ = 0.89, SE = 0.01). Probability of recapture was group x time dependant, indicating that recapture probability differed between groups over time.

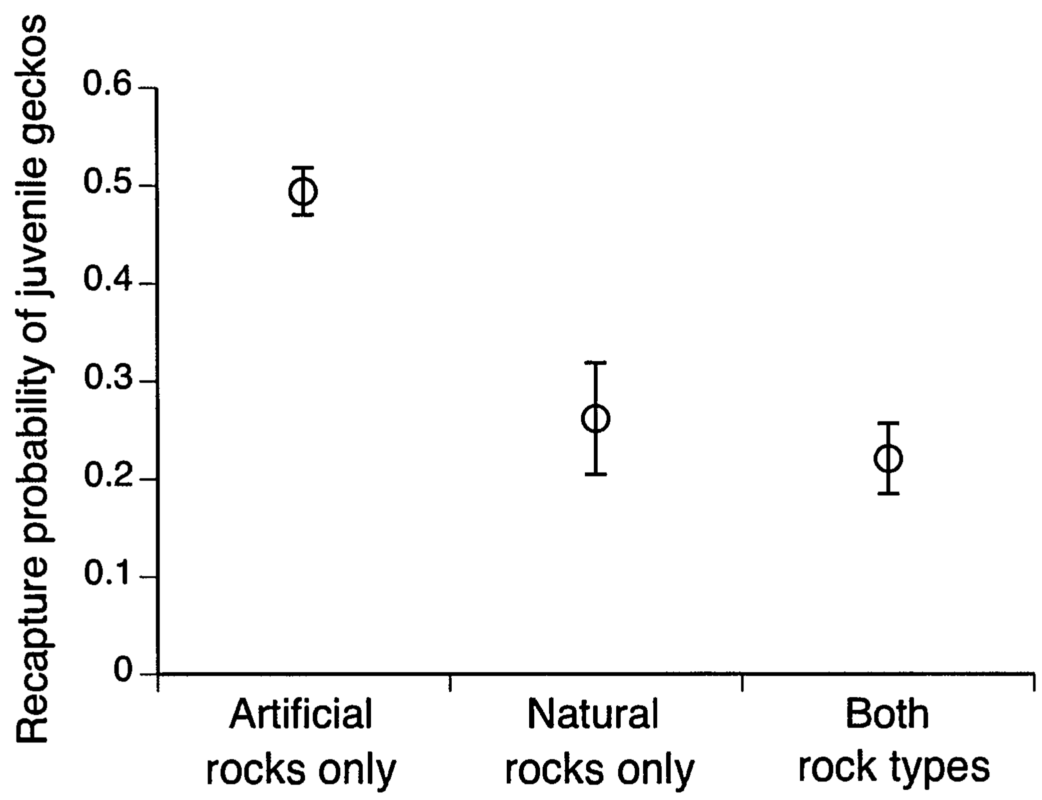

**Figure 4:** *Recapture probabilities and associated standard errors of juvenile velvet geckos captured under artificial, natural or both rock types. Estimates were derived from the most parsimonious (φ(t), p(g)) Cormack-Jolly-Seber model.*

**Table 3:** Candidate models used to determine whether survival (φ) and recapture probability (*p*) of male velvet geckos was influenced by their choice of shelter site (only artificial rocks or only natural rocks)

| Model | QAICc | ΔQAICc | QAIC weight | Model likelihood | *n* | QDeviance |
|---|---|---|---|---|---|---|
| φ(.), *p*(g x t) | 1098.41 | 0.00 | 0.7200 | 1.0000 | 32 | 635.74 |
| φ(g), *p*(g x t) | 1100.69 | 2.28 | 0.2306 | 0.3204 | 33 | 635.68 |
| φ(.), *p*(t) | 1104.60 | 6.18 | 0.0327 | 0.0454 | 17 | 675.62 |
| φ(g), *p*(t) | 1106.00 | 7.57 | 0.0163 | 0.0227 | 18 | 674.84 |
| φ(t), *p*(g x t) | 1536.97 | 15.84 | 0.0003 | 0.0004 | 46 | 617.84 |

The letters g and t refer to group and time respectively. The term . denotes constant survival. The most parsimonious model was φ(c), *p*(g x t), where survival was constant between animals using natural and artificial rocks, and the probability of recapture was dependant on group (natural or artificial) and time. Models are ordered according to the adjusted Akaike's information criterion (QAICc), with model parsimony increasing with decreasing QAICc weighting. Models with ΔQAICc <2.0 have the greatest statistical support.

*Growth rates*

Of 563 individuals captured during this study, we caught 281 individuals more than once. Growth rates of these recaptured geckos were not significantly different (slopes: $F_{1,277}$ = 0.58, $P = 0.45$, main effect: $F_{1,278}$ = 0.84, $P = 0.36$; Fig. 5) between artificial and natural rocks.

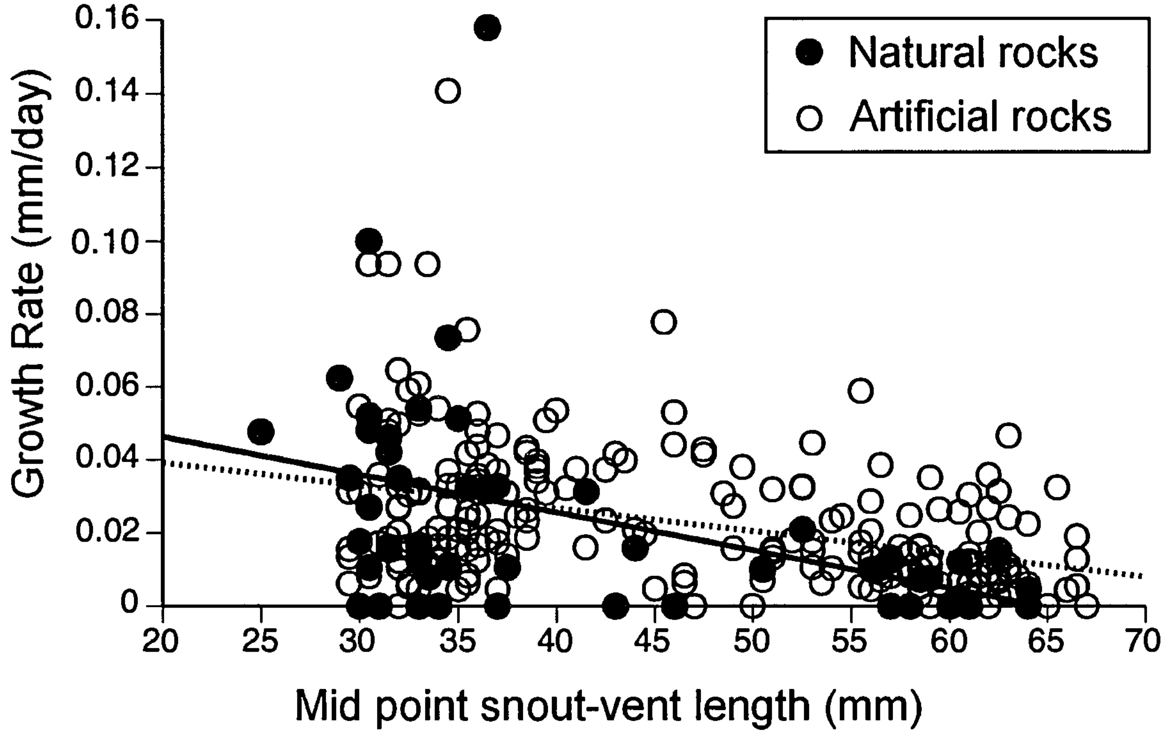

Fig. 5. Growth rates versus body size (mean snout-vent length over the interval between successive captures) of velvet geckos captured under artificial versus natural rocks.

## Discussion

Our results show that velvet geckos used artificial rocks placed on degraded rock outcrops. The addition of artificial rocks to degraded sites enhanced gecko abundance, and on average, we captured more than twice as many geckos at habitat-restored sites than at control sites (means of 67 vs. 27 lizards, respectively; Fig. 3a). More importantly, individuals that used artificial rocks did not incur any survival or growth costs from using such sites. Apparent survival rates of juveniles were higher at restored sites than at control sites, but rates of apparent survival of adults were not significantly affected by habitat restoration. Growth rates of free-living geckos were not affected by habitat restoration, suggesting that competition for resources did not increase in the restored sites.

The effects of habitat restoration on velvet geckos will depend upon the social system of this species. For example, resident adult males actively repel other adult males (and juveniles) from retreat sites (Schlesinger & Shine 1994b). This territorial exclusion may limit the numbers of geckos at a site, even if unoccupied shelter-sites are available (as in many other species in which adult males vigorously exclude potential rivals: e.g. Le Boeuf 1974; Shine *et al.* 1981; Emlen & Nijhout 1999). Nonetheless, habitat restoration increased the number of adult male geckos on a site (Fig. 3a). The increased numbers and stable survival that we observed for male geckos (Fig. 3a vs. 3b) suggest that adding additional cover items allowed more of the local male lizards to remain in our study area for longer periods (and hence, become more accessible to capture). As previous studies have shown, adult geckos spend much of the year in deep crevices in the bedrock where they are inaccessible to investigators (Pike, Webb & Shine 2010). Thus, the primary impact on male geckos of adding artificial cover items was to modify their seasonal patterns of habitat use rather than to increase their survival rates (which were already very high, even in the absence of habitat restoration; Fig. 3b).

In contrast, habitat restoration increased the numbers of juvenile geckos by increasing their apparent survival (Figs 3a, 3b). After dispersing from communal nest sites, juveniles settle under rocks, and use one or two rocks as shelter sites for much of the year (Webb 2006). Notably, apparent survival was higher at restored sites, but was unaffected by the type of rock that a gecko sheltered under (Table 2). Hence, adding rocks translated into increasing shelter site availability for this age class. The same may well have been true of adult female geckos in our study. Their numbers increased, and their survival rates tended to be higher in habitat-restored sites (the trend was strong, although the effect fell short of statistical significance because of low sample size: see Fig. 3a). Because juveniles and adult female geckos are less territorial than adult males (Schlesinger & Shine 1994b), their numbers are likely to be limited by resource (food, shelter) availability as well as by social interactions. Low rates of juvenile survival in unrestored habitats mean that there is considerable opportunity for habitat restoration to elevate survival rates for this age class.

In summary, our data show that habitat restoration increases gecko abundance, at least partly because juvenile survival depends upon shelter-site availability. Lizards in these higher-density populations in rock-restored sites exhibit growth rates as high as their conspecifics living under natural rocks in control sites. Thus, replacing stolen "bush-rocks" with artificial rocks is an effective conservation tool for this system. The population viability of velvet geckos is not a major conservation issue, because these lizards are abundant over a broad area (albeit, as a series of genetically distinctive sub-populations: Dubey *et al.* 2012). However, these lizards are the main prey of juvenile broad-headed snakes, an endangered species endemic to the Sydney region (Webb & Shine 1998). Broad-headed snakes have become endangered due to habitat degradation (especially, rock theft and forest overgrowth: Shine *et al.* 1998; Pringle, Webb & Shine 2003; Pike, Webb & Shine 2011), and have disappeared from much of their former range (Shine & Fitzgerald 1989). Maintaining abundant stocks of velvet geckos is clearly crucial to conserving broad-headed snakes.

Ecosystems worldwide are affected by anthropogenic degradation through activities such as deforestation, urbanisation, farming and mining (Cooke & Johnson 2002; Lal 2008; McKinney 2008; Bradshaw 2012). The protection of remaining "intact" systems coupled with habitat restoration is needed to conserve threatened flora and fauna and evolutionary processes at work in those systems (Sinclair *et al.* 1995; Knowlton & Graham 2011; Perez *et al.* 2012). Thus habitat restoration has been attempted in a variety of systems (Webb & Shine 2000; Croak et al 2010; Roni, Hansen & Beechie; Hugel 2012; Jones & Kress 2012). However, we have surprisingly little information on the impacts of restoration activities on biota and in many cases we do not even have counts of abundance through a sufficiently long time period that we can assess overall impacts; nor data from adjacent control (undisturbed) areas to use for comparison (Block *et al.* 2001; Lovett *et al.* 2007; Miller & Hobbs 2007). Even when such data exist, a simple increase in numbers of some target species in rehabilitated habitat is not necessarily indicative of success. In the worst case, our provision of "attractive" habitat within an otherwise degraded mosaic may simply lure animals out of the surrounding matrix into areas where their high densities render them liable to threatening processes such as disease and parasite transmission, social stress, or predation (Sugden & Beyersbergen 1986; Reitsma 1992; Gustavo *et al.* 2006). To ensure that restored habitats are not simply "sinks" for surrounding areas, we need to monitor vital processes – such as rates of growth and survival – in the restored habitat patches as well as in "natural" (unrestored) areas. Our studies on velvet geckos are encouraging as they confirm that we can provide a real and measurable benefit to wild populations by reversing the habitat-degradation processes that threaten so many populations of this species. However, more work is needed to assess the responses of this system as a whole to our restoration attempts.

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# PART 3: Velvet Geckos

*The velvet gecko* (Oedura lesueurii).

# **CHAPTER 4:** *CONTEXT-DEPENDENT AVOIDANCE OF PREDATORY CENTIPEDES BY NOCTURNAL GECKOS* (Oedura lesueurii)

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*An example of a predatory centipede* (Cormocephalus *spp.*) *used in this study. This species predates velvet geckos and other lizard species that use bush-rock exfoliations as retreat sites.*

## Abstract

Most research on the antipredator behaviour of vertebrate prey has focused on their responses to vertebrate predators. Nonetheless, invertebrates are abundant predators in terrestrial ecosystems, and some invertebrates (e.g., centipedes, spiders) attack and consume vertebrates. We used a combination of field data and laboratory experiments to determine whether a predatory centipede (Scolopendridae: *Cormocephalus* sp.) influences habitat selection by the nocturnal rock-dwelling velvet gecko, *Oedura lesueurii*. In the field, centipedes and geckos were syntopic, and sheltered beneath rocks with similar physical dimensions, thermal regimes, and degree of sun exposure. Nonetheless, geckos rarely shared rocks with centipedes in the field. In laboratory trials, both geckos and centipedes selected shelters with narrow rather than wide crevices. The presence of a centipede modified habitat selection by juvenile geckos, which preferred wide crevices without centipedes to narrow crevices containing centipedes. In contrast, adult geckos continued to select narrow crevices even if these contained centipedes. When we added centipedes to narrow crevices beneath small and large rocks occupied by geckos, both juvenile and adult geckos exited the crevice (especially if it was under a small rather than large rock). Our results show that centipedes influence habitat selection by velvet geckos, and demonstrate that antipredator behaviours of geckos are both size- and context-dependent. Ontogenetic shifts in behaviour and habitat selection by vertebrates in response to invertebrate predators may be widespread.

**Keywords:** centipedes, *Cormocephalus* sp., predation, rocks, saxicolous, Scolopendridae, shelter-site

## Introduction

Predation is a major selective force that has shaped the behaviour and ecology of animals (Sih 1987, Lima & Dill 1990). For example, predators or the risk of predation can influence foraging time (Huey & Pianka 1981, Bouskila 1995), nest-site selection (Spencer 2002), and choice of shelter sites (Resetarits & Wilbur 1991, Downes & Shine 1998a, 1998b, Stapley 2003, 2004). In many animals, the risk of predation also decreases with increasing body size because as prey size increases there are fewer predators that are physically able to consume them (Polis et al. 1989). This size-dependence in vulnerability to predators can lead to ontogenetic shifts in antipredator behaviour. For example, juvenile bluegill sunfish (*Lepomis macrochirus*) avoid larger fish predators by using structurally complex littoral zones, but larger adult sunfish inhabit open water (Werner et al. 1983). Such size-dependent shifts in predator avoidance in response to vertebrate predators are well studied, but relatively little is known about the responses of vertebrates to invertebrate predators.

In terrestrial ecosystems, the most commonly studied predators occupy higher trophic levels than their prey (Clark 1982, Bauer 1990). Unsurprisingly, most studies on vertebrate predator-prey interactions have focused on vertebrate predators (Lima & Dill 1990). For example, mammals, birds, and other reptiles are regarded as the most important predators of lizards (Whitaker 1973; reviewed in Bauer 1990). Nonetheless, invertebrates are abundant predators in most ecosystems (e.g., Lewis 1965, Wise & Chen 1999, Scheu et al. 2003), and larger species (e.g., centipedes, spiders, mantids) attack and consume small vertebrates (e.g., Humphreys 1976, McCormick & Polis 1982, Carpenter & Gillingham 1984, Bauer 1990, Kehr & Schnack 1991, Anthony et al. 2006, Metcalfe &

Peterson 2007). Hence, invertebrates may influence antipredator behaviour and habitat selection of small vertebrates.

In this study, we investigated whether predatory centipedes influence habitat selection by velvet geckos, *Oedura lesueurii*. Velvet geckos and centipedes are nocturnal, and spend the day sheltering in crevices beneath loose rocks. Given published reports of centipedes consuming lizards (reviewed in Bauer 1990), we hypothesized that velvet geckos would avoid rocks occupied by centipedes. Juvenile geckos are smaller than adults, and may be more vulnerable to centipede predation. Hence, we predicted that juveniles would show stronger antipredator responses to centipedes than adults. We used a combination of field data and laboratory experiments to investigate whether juvenile and adult geckos: (1) select shelter sites in the field with similar physical and abiotic characteristics to those used by centipedes; (2) share rocks with centipedes in the field; (3) modify their choice of shelter-site in the presence of centipedes; and (4) actively avoid centipedes entering their crevices.

## Methods

### *Study species*

Velvet geckos are small (to ca. 65 mm snout-vent length [SVL]) nocturnal lizards that inhabit rocky outcrops in southeastern Australia (Cogger 2000). By day, velvet geckos shelter beneath small sandstone rocks (Schlesinger & Shine 1994), and at night they actively forage for small invertebrates (Cogger 2000). In the field, velvet geckos select thin rocks in sun-exposed locations; these rocks allow the animals to achieve high body temperatures during the cooler months of the year (Webb & Shine 1998a, 1998b, Webb et al. 2008). The centipede species that we studied (Scolopendridae: Scolopendrinae, *Cormocephalus* sp.; ca. 50–80 mm in total length) inhabits forest edges and rock outcrops within the geographic range of velvet geckos. Like most centipedes in this family, they are nocturnal, have poor vision, actively forage, and are indiscriminate and voracious predators (Summers & Uetz 1979, Lewis 1981, Bauer 1990). Our study was prompted by observations of centipedes feeding on a velvet gecko and a copper-tailed skink (*Ctenotus taeniolatus*) beneath rocks. We studied velvet geckos and centipedes on sandstone rock outcrops in Morton National Park, southeastern New South Wales, Australia.

### *Selection of shelter-sites in the field*

To determine whether centipedes and velvet geckos occupy similar shelter-sites, we sampled rocks at two study sites during May, August, and October 2007 (see Webb et al. 2008 for site descriptions). During each sample, we carefully turned all loose surface rocks (that we could physically lift) on exposed rock outcrops near cliff edges. When we found a gecko or centipede, we numbered the underside of the rock with a paint pen, marked its location with a GPS unit (Garmin 12XL), recorded the length, width, and minimum and maximum thickness (to nearest mm) of the rock, and scored the substrate below (rock, soil or both). Sampling effort was equal across time (i.e., we lifted the same individual rocks during each sampling event), and because we were most interested in broad patterns of retreat-site selection (rather than site or temporal differences), we combined all data for analysis. This resulted in each individual rock being scored as used by geckos, centipedes, both, or neither.

We placed miniature thermal dataloggers (Thermochron iButtons) beneath rocks used by geckos ($N = 45$) and centipedes ($N = 16$) to quantify thermal regimes. Dataloggers recorded temperatures at hourly intervals over three consecutive days during cloudless weather in May 2007, when both species were using rocks. To test for differences in thermal regimes between species we used a repeated-measures ANOVA with time as the repeated measure and temperature over one randomly-selected 24-h period as the dependent variable.

We also used hemispherical photography and gap light analysis (Frazer et al. 1999) to quantify the canopy cover above the rock, and estimate the incident radiation reaching the rock. To do this, we mounted a Nikon Coolpix 995 digital camera fitted with a Nikon FC-E8 fisheye lens to a small tripod, positioned the tripod over the rock, oriented the camera to magnetic north, pointed the lens upward, and took the photo using the camera self timer (Doody et al. 2006). We imported these photos into Gap Light Analyzer software (Version 2.0), which estimates canopy openness (%) and the amount of solar radiation (in $\text{mols}\cdot\text{m}^{-2}\cdot\text{d}^{-1}$) reaching the rock (Frazer et al. 1999). These variables are calculated after manually accounting for site location (latitude/longitude, elevation) and day length (i.e., sunrise/sunset times and number of sunshine hours; daily values were obtained from a nearby weather station and averaged monthly over the entire calendar year). We compared canopy openness and incident radiation intensity between species (geckos, centipedes) using ANOVAs.

Finally, we used our field observations on rock usage to test whether geckos and centipedes share the same shelter site as expected under the null hypothesis of no avoidance. We first calculated the probability of finding a gecko or centipede alone (i.e., the number of rocks used by a gecko or centipede as a proportion of the total number of rocks lifted), and multiplied these values together to obtain the expected probability of both occurring together. We then converted this probability to the number of rocks that both species would be expected to share if they are not avoiding one another (based on the number of rocks we sampled). We compared this expected value to the actual observed value using a chi-squared test.

*Animal husbandry*

We collected 44 juvenile (28–32 mm SVL), 37 adult male, and 36 adult female geckos (50–61 mm SVL), and 40 centipedes (50–80 mm long) from our study sites in August 2008 and 2009. Animals were taken to the University of Sydney and housed in separate constant temperature rooms (19.5–20.5 °C) with a 12:12 h light:dark cycle. Geckos were housed individually in ventilated paper-lined plastic cages (200 x 140 x 55 mm) containing a water dish, plastic shelter, and transparent lid. Cages were placed on timer-controlled heating racks (12:12 h on:off) that created a thermal gradient within each cage from 24.0–39.5 °C. We fed lizards crickets dusted with vitamins two to three times each week and water was provided *ad libitum*. Individual geckos were used once in each experiment, except for the trials testing whether geckos actively avoid centipedes, where we accounted for repeat use of individuals in the analysis. In trials using adults, approximately equal numbers of males and females were used to eliminate any potential sex bias.

Centipedes were housed communally (2–4 per cage) in plastic cages (310 x 220 x 100 mm) similar to those used for geckos, but containing moist soil, leaf litter, and woody debris. The cages were misted with water once every second day. Centipedes were fed crickets dusted with vitamin powder two to three times each week. When experiments were completed, we released each animal at its capture location.

### *Crevice selection in the absence of predators*

Geckos and centipedes shelter in crevices formed beneath overlying surface rocks, and the size and shape of these crevices can influence reptile retreat-site selection (Schlesinger & Shine 1994, Croak et al. 2008). To establish whether geckos ($N = 14$ juveniles, 20 adults) and centipedes ($N = 12$) prefer the same type of crevice, we offered them the choice between a shelter-site with a narrow versus a wide crevice. Laboratory experiments were carried out inside a constant-temperature room (25 °C) with a 12:12 h lighting cycle. Each test arena consisted of a plastic tub (645 x 400 x 275 mm) containing two concrete pavers (230 x 190 x 40 mm), one at each end. One paver was raised 6 mm off the substrate to form a narrow crevice, and the other was raised 21 mm above the substrate to form a wide crevice; the location of each was randomized within a tub. At 1700 h, we placed the test subject into the centre of each tub and secured the clear lid. The next morning we scored which crevice the test subject was using as a diurnal shelter site. After each trial, all tubs, concrete pavers, and spacers were washed in a detergent solution and air-dried to eliminate the possibility that chemicals might affect subsequent trials (Downes & Shine 1998a).

*Crevice selection in the presence of predators*

To determine whether velvet geckos modify shelter-site selection in the presence of centipedes, we used the same procedures outlined above, except that we placed a velvet gecko and a centipede together into the test arena at 1700 h. The following morning we scored the locations of the centipede and the gecko (*N* = 18 juveniles, 32 adults). To control for the possibility that a gecko's behaviour might be influenced by the presence of a novel object inside the crevice, we also ran trials in which a plastic centipede was placed inside the thin crevice (*N* = 12 juveniles, 16 adults); live centipedes were not used in these trials. We examined the influence of stimulus type (no stimulus, plastic centipede, live centipede) and life stage (juvenile, adult) on shelter-site selection (narrow or wide crevice) using logistic regression.

*Do geckos actively avoid centipedes?*

To determine whether geckos respond to a live centipede entering their shelter site, we placed a single gecko (juvenile or adult) inside a clean plastic cage (310 x 220 x 100 mm) containing a concrete paver elevated 6 mm above the floor of the cage. The geckos were allowed to settle into the cage overnight, and all were inside the crevice the next morning. We randomly allocated geckos to one of three treatment groups: live centipede (*N* = 11 juveniles, 11 adults), plastic centipede (*N* = 12 juveniles, 21 adults), and a disturbance control (*N* = 12 juveniles, 20 adults). For each trial, we opened the lid of the cage and recorded the geckos' behaviour during the next 5 min. For the live centipede trials, we allowed a centipede (> 60 mm long) to crawl inside the gecko's crevice. Centipedes that exited the crevice were encouraged to crawl back inside. Because the presence of a novel object inside of the crevice might confound any response due to the live centipede, we also used a plastic centipede as a control. We slowly pushed a 70 mm long plastic centipede (similar in size, shape, and colour to the live centipedes) inside the crevice and left it there. Before re-using plastic centipedes we soaked them in hot water for 1 h, washed them thoroughly with detergent, and allowed them to air dry. For the control trials, we simply lifted the lid of the gecko's cage and left it open for 5 min. For each trial, we scored whether or not the gecko exited the crevice (= entire body exposed), and if so, the time taken for the gecko to exit the crevice (s). If a gecko did not leave the shelter, the time was recorded as 300 s.

We ran this experiment using both small (180 x 180 mm) and large (230 x 190 mm) shelter-sites, which allowed us to determine whether rock size influenced the likelihood of a gecko abandoning a shelter site or the time taken to abandon a shelter site. We exposed each individual gecko to both shelter sites in a randomized order, and tested for differences in propensity to abandon the retreat using a contingency table analysis (using trials with a live centipede only), and whether time to abandonment differed between juveniles and adults using a repeated-measures ANOVA with each individual treated as a repeated measure. This is the only experiment in which individual geckos were used more than once.

## Results

### *Selection of shelter-sites in the field*

We found 35 centipedes and 80 geckos (60 juveniles, 20 adults) under 112 individual rocks in the field. The rocks used by geckos ($N = 70$) and centipedes ($N = 25$) had similar physical dimensions (all $P > 0.05$; Table 1). Most gecko and centipede rocks were located on rock substrates (93% and 79%, respectively, $\chi^2 = 1.73$, df = 1, $P = 0.19$), and the remaining rocks had substrates composed of both rock and soil.

**Table 1:** *Comparison of mean rock sizes (± SE) used by velvet geckos* (Oedura lesueurii, *N = 70) and centipedes* (Cormocephalus *sp., N = 25), from field surveys in southeastern Australia. Also shown are the results of one-way ANOVAs comparing the attributes of rocks (length, width, area, minimum thickness, maximum thickness) that form the upper surface of crevices within which these two taxa shelter.*

| Rock variable | Gecko | Centipede | $F$-value $_{(1,93)}$ | $P$-value |
|---|---|---|---|---|
| Length (mm) | 454.8 ± 18.84 | 469.6 ± 28.26 | 0.17 | 0.68 |
| Width (mm) | 312.5 ± 13.44 | 316.2 ± 22.02 | 0.02 | 0.89 |
| Area (mm$^2$) | 155,352.0 ± 12,629.80 | 159,775.60 ± 18,756.21 | 0.03 | 0.85 |
| Minimum thickness (mm) | 39.2 ± 4.13 | 31.9 ± 4.28 | 2.89 | 0.09 |
| Maximum thickness (mm) | 75.4 ± 5.40 | 58.2 ± 31.92 | 1.00 | 0.32 |

Daily thermal regimes differed beneath rocks used by geckos and those used by centipedes throughout the course of the day (rock type: $F_{1,59} = 3.61$, $P = 0.06$; time: $F_{95,5605} = 556.39$, $P < 0.0001$; rock type * time interaction: $F_{95,5605} = 1.54$, $P < 0.0006$; Fig. 1). Rocks used by geckos were similar in temperature to those used by centipedes, except during the warmest part of the day, when gecko rocks were on average 1.9 °C warmer.

Rocks used by geckos and centipedes overlapped considerably in canopy openness (range in canopy openness: 39.5–71.4% and 42.8–84.1%, respectively), but on average the rocks used by geckos had more open canopies ($F_{1,71} = 5.96$, $P = 0.02$; Fig. 2a). A similar trend was evident for solar radiation exposure (geckos, range: 8.3–14.4 mols·m$^{-2}$·d$^{-1}$; centipedes, 8.5–14.1 mols·m$^{-2}$·d$^{-1}$, $F_{1,70} = 5.35$, $P = 0.02$; Fig. 2b). The similarity in these two results reflects the high correlation between canopy openness and incident radiation ($r = 0.72$, $P < 0.0001$); more open canopies allow more incident radiation to penetrate through to the rock.

On three occasions we found geckos (two juveniles and one adult) sharing the same rock with a centipede; in one of these instances the juvenile was found dead. Under the null hypothesis of no avoidance, we would expect to observe both species sharing 20 individual rocks. Thus, geckos and centipedes shared rocks less often than would be expected by chance ($\chi^2 = 14.73$, df = 1, $P < 0.001$). We also observed geckos and centipedes using the same rocks on four occasions, but in different months. Together, these results suggest that centipedes and geckos use shelter sites that overlap in both physical attributes and thermal regimes, but that either one or both of these species avoids the other under field conditions.

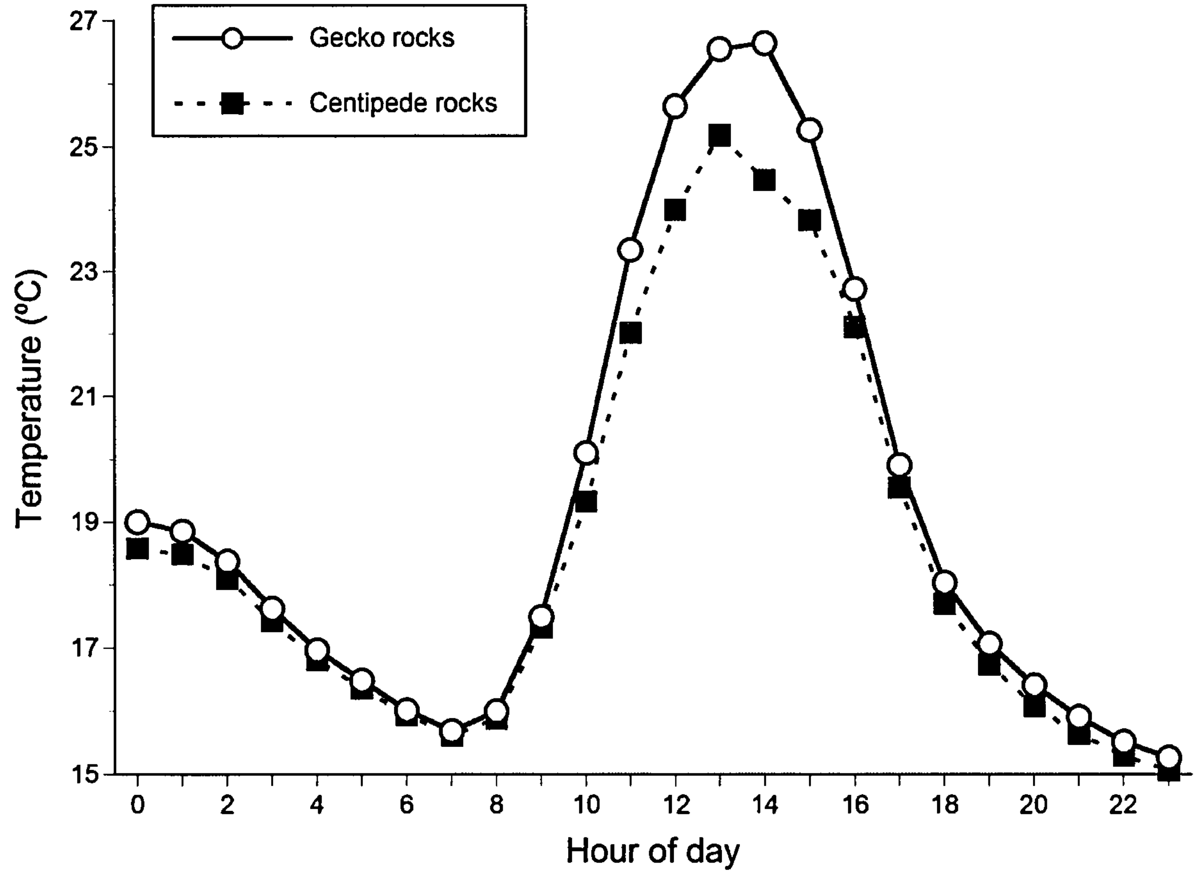

**Figure 1:** *Mean temperatures beneath rocks used as shelter-sites by geckos* (Oedura lesueurii, *N = 45) and by centipedes* (Cormocephalus *sp., N = 16) during a sunny day in May 2007. Rocks used by geckos averaged 1.9 °C warmer during midday than those used by centipedes. Error bars were too small to be visible in most cases and are not shown.*

### *Crevice selection in the absence of predators*

When given the choice between a wide versus a narrow crevice, most centipedes chose narrow crevices as diurnal shelter-sites ($N = 11$ of 12, $\chi^2 = 8.33$, df = 1, $P = 0.004$). In the absence of predators, velvet geckos also selected narrow crevices (juvenile geckos: $N =$ 14 of 14, $\chi^2 = 14.0$, df = 1, $P = 0.002$; adult geckos: $N = 16$ of 20, $\chi^2 = 7.20$, df = 1, $P =$ 0.007). The preference for narrow crevices was equally strong in centipedes, juvenile geckos, and adult geckos ($\chi^2 = 3.51$, df = 2, $P = 0.17$).

### *Crevice selection in the presence of predators*

In crevice selection trials involving a gecko plus a centipede, all centipedes selected the narrow crevice as shelter. Thus, geckos could: (1) share the narrow crevice with the predator, (2) avoid the predator by using the wide crevice, or (3) avoid the predator by clinging to the side or roof of the cage (centipedes could not climb the arena sides). A logistic regression revealed a significant interaction between "predator" type (live centipede, plastic centipede, or no predator) and lizard life stage (juvenile or adult; $\chi^2 =$ 9.22, df = 2, $P < 0.01$). When alone or exposed to the plastic centipede only, most or all geckos selected the narrow crevice, as before (Fig. 3). In contrast, in trials with a live centipede, fewer juvenile geckos used narrow crevices than did adults (Fig. 3). Juveniles also were less likely to share a thin crevice with a centipede than were adults ($\chi^2 = 6.38$, df = 1, $P = 0.01$; Fig. 3). In these trials, three of 32 adult geckos (9.4%) and two of 18 juvenile geckos (11.1%) did not use retreat sites, and were clinging to the arena walls the following morning.

### *Do geckos actively avoid centipedes?*

All juvenile and adult geckos remained inside their crevices when we opened the cages (disturbance control) or slowly inserted the plastic centipede into the crevice (visual control). In trials involving live centipedes, all of the juvenile and adult geckos abandoned the small shelter site during the 5 min observation period. Due to the consistency of these responses, we only analysed whether juveniles and adults sheltering beneath the large paver differed in their response to live centipedes. Most juveniles (64%) and adults (85%) abandoned their crevice in trials involving the large shelter site, but there was no difference with life stage (Fisher's exact test, $P = 0.59$). All geckos that remained inside the crevice moved to the side of the crevice away from the centipede; often portions of the gecko were visible at the edge of the crevice, with the animal moving around the outside of the paver. Geckos were never visible at the edge of the crevice during control trials.

Juvenile and adult geckos remained within shelter sites for similar amounts of time after a centipede entered (repeated-measures ANOVA with gecko size [juvenile, adult] and paver size [small, large] as factors, with square-root transformed time as the dependent variable: $F_{1,20} = 0.58$, $P = 0.45$). However, both adult and juvenile geckos abandoned the small shelter site more quickly than the large shelter site ($F_{1,20} = 9.27$, $P = 0.01$; interaction term, $P = 0.46$). On average, geckos abandoned small shelter sites 90 s sooner than large shelter sites.

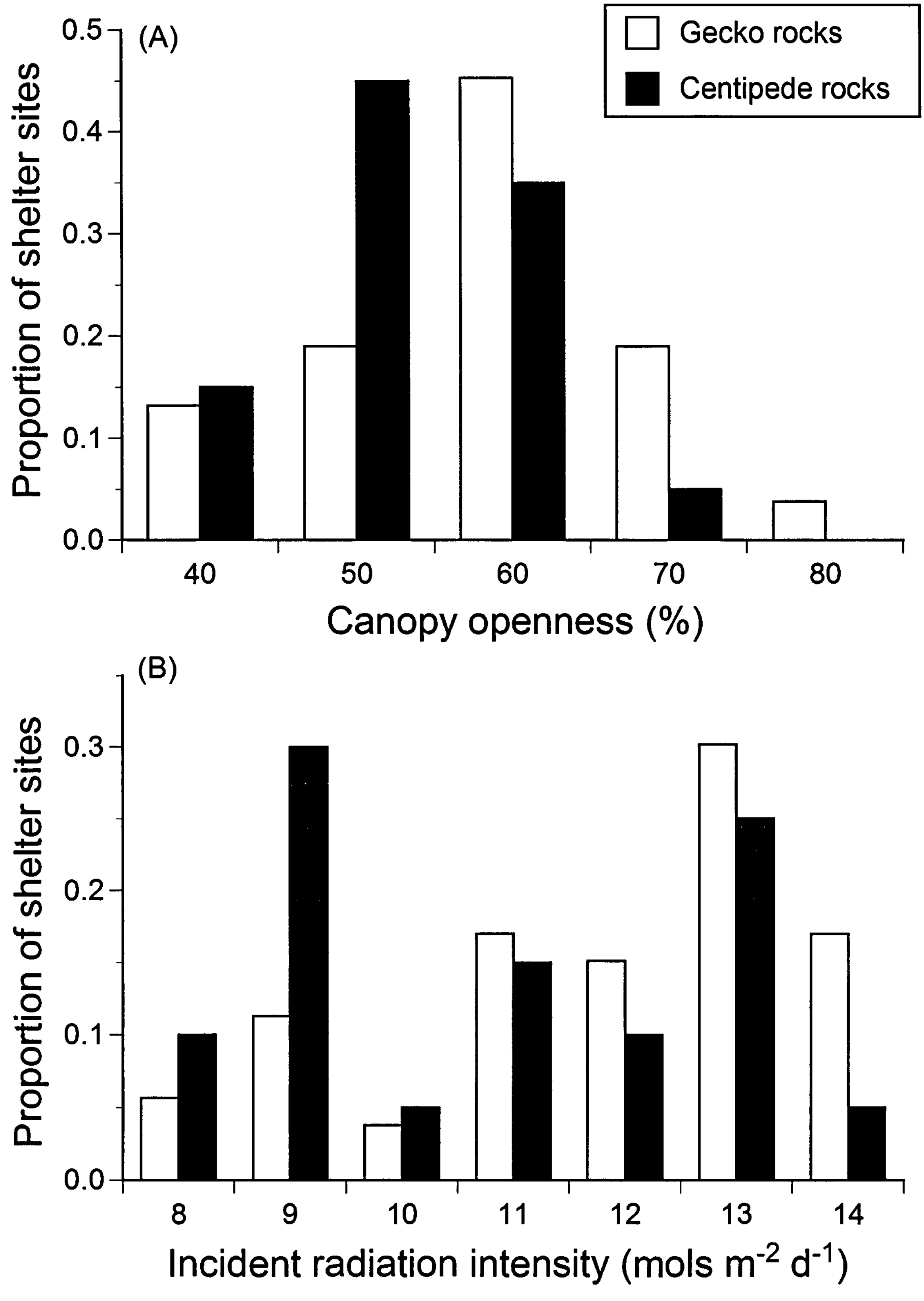

**Figure 2:** *Frequency distributions of (A) canopy openness above (%), and (B) incident radiation (mols·m$^{-2}$·d$^{-1}$) reaching the rocks used by juvenile geckos* (Oedura lesueurii) *and centipedes* (Cormocephalus *sp.*) *in Morton National Park, NSW, Australia.*

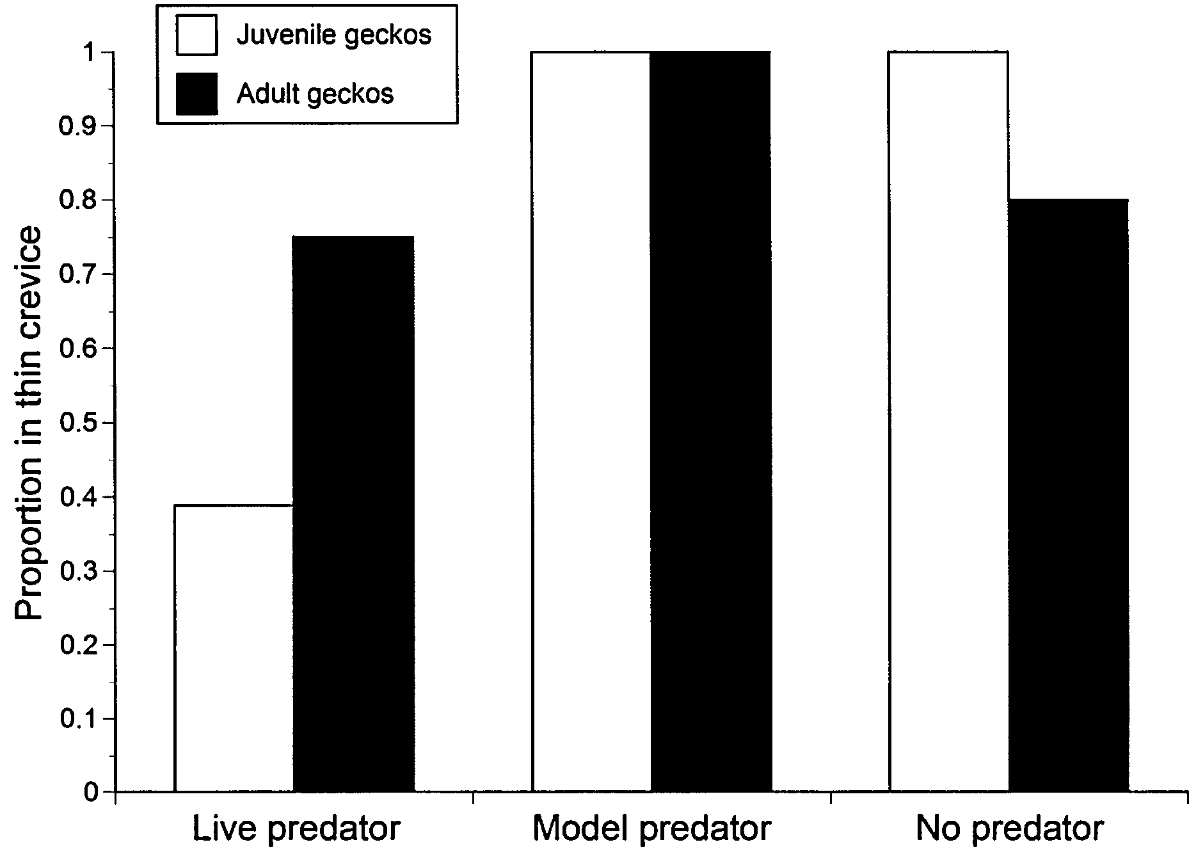

**Figure 3:** *Proportion of juvenile and adult geckos* (Oedura lesueurii) *choosing a narrow crevice (6 mm high) as a diurnal shelter site when tested with a live centipede (N =18 juveniles, 32 adults), a model (plastic) centipede (N =12 juveniles, 16 adults), or without a centipede (N =14 juveniles, 20 adults). In all trials involving a live centipede, the centipede was inside the narrow crevice the following morning; plastic centipedes were placed inside the narrow crevice.*

## Discussion

Our field data show that centipedes and geckos used rocks with similar structural features (size and thickness; Table 1), daily thermal regimes (Fig. 1), degree of canopy openness (Fig. 2a), and exposure to solar radiation (Fig. 2b). Subtle differences in habitat usage were also apparent; for example, rocks used by geckos were slightly warmer than centipede rocks (Fig. 1), possibly reflecting differences in desiccation rates or thermal tolerances (Lewis 1981). Despite this high degree of microhabitat overlap, geckos and centipedes rarely shared rocks at our field sites. This pattern is likely the result of juvenile geckos avoiding rocks used by centipedes. In laboratory trials, both adult and juvenile geckos selected narrow crevices and avoided wide crevices. However, the presence of centipedes induced juvenile (but not adult) geckos to modify their behaviour and use wide crevices (Fig. 3). In combination with field studies showing that velvet geckos select rocks with narrow crevices (Croak et al. 2008), these results suggest that the presence of invertebrate predators modifies habitat selection of juvenile geckos. Similar ontogenetic shifts in antipredator behaviour occur in other lizard species, and may well be widespread. For example, juvenile water skinks (*Eulamprus quoyii*) avoid sheltering under refuges previously used by funnel web spiders, whereas adult water skinks do not avoid such sites (Head et al. 2002). Such size-dependent shifts in antipredator behaviour presumably reflect differences in the vulnerability of adult and juvenile lizards to invertebrate predators. The larger, wider tails of adult velvet geckos may be more effective at directing predatory strikes away from the body than are the smaller tails of juveniles (e.g., Daniels 1986). In velvet geckos (and many other lizard species), the tail is readily autotomized during predatory attacks, and facilitates escape from predators (Daniels 1986).

Our laboratory trials also revealed that a gecko's response to centipedes was influenced by the size of the shelter-site. When centipedes entered a small shelter site, the resident gecko invariably abandoned the crevice; but when the shelter was large the geckos moved around the outside of the crevice to avoid contact with the centipede. Presumably, a gecko's response to an intruding centipede will depend on the costs and benefits associated with staying or leaving the retreat site (Lima & Dill 1990), which in turn will depend on factors such as the size of the overlying rock (and hence, crevice), and its proximity to other suitable shelter sites. The obvious cost for a gecko sharing a shelter site with a centipede is the risk of predation (as we witnessed in the field), though reports of such predation events are rare (Greer 1989, Bauer 1990). The risk of predation is likely to be higher under small rocks than large rocks, simply because a centipede is more likely to come in contact with a gecko. Centipedes rely on tactile and chemical senses to locate prey, and because their vision is poor, they cannot pursue fast moving prey (Lewis 1981). The dangers associated with remaining close to a centipede must be balanced against the costs of leaving the shelter-site. Geckos leaving a shelter must travel over exposed areas to find a new shelter-site, potentially increasing their vulnerability to predation or increasing the likelihood of caudal autotomy (e.g., Huey & Pianka 1981, Downes & Shine 2001, Webb & Whiting 2005). Thermally optimal shelter-sites also may be in short supply, generating competition for shelter-sites (Pringle et al. 2003). Thus, even if a gecko finds another thermally suitable rock, it is likely to be occupied by another gecko (or a predatory snake), and the non-resident must then engage in a territorial dispute with the resident before it can settle under the new rock (e.g., Kondo et al. 2007). Because juvenile geckos often share rocks with other juveniles, whereas adult males do not (at least under laboratory conditions; Schlesinger & Shine 1994, Downes & Shine 1998b), the costs of leaving rocks and engaging in territorial disputes with residents may be higher for adults than for juveniles.

In many predator-prey systems, the risk posed by a predator depends not only on the overlap in habitat use between predator and prey, but also on ontogenetic shifts in risk that arise as a result of the potential prey item's life stage and/or body size (Lima & Dill 1990). Variation in the magnitude of such risks can in turn influence antipredator behaviours. In the present study, the greater vulnerability of smaller lizards appears to have favoured a stronger avoidance of predatory centipedes than is shown by adult conspecifics. The small size and reduced locomotor capacities of juvenile vertebrates often may render them more vulnerable to invertebrate predators than are larger members of the same species, suggesting that ontogenetic shifts in the nature of avoidance responses to predatory invertebrates may be widespread (Kehr & Schnack 1991, Resetartis & Wilbur 1991, Hickerson et al. 2004, Anthony et al. 2006).

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# **CHAPTER 5:** *PHYLOGEOGRAPHY AND DISPERSAL IN THE VELVET GECKO* (Oedura lesueurii)*, AND POTENTIAL IMPLICATIONS FOR CONSERVATION OF AN ENDANGERED SNAKE* (Hoplocephalus bungaroides)

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**Sylvain Dubey & Benjamin Croak contributed equally to this work and are listed as first co-authors*

*Typical habitat of velvet geckos and broad-headed snakes.*

## Abstract

To conserve critically endangered predators, we also need to conserve the prey species upon which they depend. Velvet geckos (*Oedura lesueurii*) are a primary prey for the endangered broad-headed snake (*Hoplocephalus bungaroides*), which is restricted to sandstone habitats in southeastern Australia. We sequenced the ND2 gene from 179 velvet geckos, to clarify the lizards' phylogeographic history and landscape genetics. We also analysed 260 records from a long-term (3-year) capture-mark-recapture program at three sites, to evaluate dispersal rates of geckos as a function of locality, sex and body size. The genetic analyses revealed three ancient lineages in the north, south and centre of the species' current range. Estimates of gene flow suggest low dispersal rates, constrained by the availability of contiguous rocky habitat. Mark-recapture records confirm that these lizards are highly sedentary, with most animals moving < 30 m from their original capture site even over multi-year periods. The low vagility of these lizards suggests that they will be slow to colonise vacant habitat patches; and hence, efforts to restore degraded habitats for broad-headed snakes may need to include translocation of lizards.

**Keywords:** Australia, phylogeography, dispersal, reptile, landscape genetics, conservation

## Introduction

To conserve an endangered species, we need to provide suitable habitat, shelter, prey items, and other resources (see: Finnoff & Tschirhart 2003, Hanson et al. 2010, Pike et al. 2011a, 2011b). Prey availability may be one of the most critical issues, especially for predators with specialized diets (Webb & Shine 1998, Boyles & Storm 2007). If management plans for endangered species include the restoration of habitat, we need to know if the endangered taxon itself is vagile enough to locate and colonise the newly-available sites. Evaluating the likelihood that significant prey species also will colonise restored areas is also important; if they do not do so (perhaps because of poor dispersal capacity), otherwise-suitable habitat may be unable to support populations of the endangered taxon.

The broad-headed snake (*Hoplocephalus bungaroides*, Elapidae) is a small elapid snake restricted to rocky areas (sandstone plateaus) within a 200 km radius of Sydney, in southeastern Australia (Webb & Shine 1998). These snakes were abundant at the time of European colonisation 200 years ago, but have now disappeared from most of its former range (Krefft 1869, Shine et al. 1998). The threatening processes include habitat degradation and fragmentation resulting from the removal and destruction of critical shelter sites (especially, exfoliated rock that forms thermally-suitable retreat sites during the coldest parts of the year: Shine et al. 1998), forest overgrowth (Pringle et al. 2003, Pike et al. 2011a, 2011b) and illegal collection of animals for the pet trade (Webb et al. 2002). Efforts at habitat restoration have produced encouraging results, with the snakes and their lizard prey rapidly colonising sites by themselves where artificial rocks have replaced stolen natural rocks (Croak et al. 2010) and where trimming of vegetation has allowed increased sunlight penetration (Pike et al. 2011a, 2011b). However, these studies have focused on sites very close to extant populations of snakes and their prey; the prospectus for successful colonisation of more distant sites remains unclear.

For relatively isolated habitat patches to be colonised, both the snakes and their prey must be able to reach them. Landscape-genetic analyses have confirmed that broad-headed snakes often move between adjacent outcrops (distance between outcrops: 0.9 to 10.7 km), and thus are likely to rapidly find any restored habitat patches (Dubey et al. 2011). The probability of colonisation by the snakes' prey species has not been studied, and is the subject of the present paper. Broad-headed snakes consume a diversity of vertebrate prey taxa, but the most important taxon (especially during cooler months of the year, while the snakes are restricted to rock outcrops) is the velvet gecko (*Oedura lesueurii*, Diplodactylidae: Webb & Shine 1998). Indeed, velvet geckos comprised 70% of prey items consumed by juvenile *H. bungaroides* (Webb & Shine 1998). Like *H. bungaroides*, *O. lesueurii* is restricted to rock outcrops (Schlesinger & Shine 1994, Webb et al. 2008). The predator-prey interaction between these two taxa presumably has been a long-running one, because geckos from populations sympatric with this snake species are reported to display a suite of antipredator tactics not seen in conspecific geckos from populations allopatric to broad-headed snakes (Downes & Adams 2001; but see Webb et al. 2009 for data that challenge this conclusion). Local coadaptation is likely only when gene flow is restricted between populations (e.g., Riechert 1993, Downes & Adams 2001, Pafilis et al. 2009), allowing the evolution of spatial heterogeneity in relevant traits.

To evaluate the history of this predator-prey interaction, we need to know the timeline not only for the predator's evolution (Dubey et al. 2011) but also for the prey's evolution (current study). Because *O. lesueurii* is an important prey species for *H. bungaroides*, we also need to evaluate the potential for *O. lesueurii* to colonise newly restored areas of rocky habitat. We can clarify this issue with a study of landscape genetics (for example; what are the spatial scales of current and historical rates of gene flow?) and direct measures of dispersal, based on mark-recapture fieldwork.

## Methods

### *Tissue collection*

We collected tissue samples from 179 geckos representing 20 populations in southeastern Australia (see Fig. 1 and Table 1), by turning rocks and capturing animals by hand. Tissues were collected by toe-clipping, or from voluntary tail autotomy. Tissue samples were placed in 100% ethanol, transported to the laboratory and stored below 0 °C prior to processing.

### *DNA extraction and PCR amplification*

We placed tissues in 200 mL of 5% Chelex containing 0.2 mg/mL of proteinase K, incubated them overnight at 56 °C, boiled them at 100 °C for 10 min, and centrifuged them at 13,300 g for 10 min. The supernatant, containing purified DNA, was then removed and stored at -20 °C.

Double-stranded DNA amplifications of NADH dehydrogenase 2 (ND2) were performed with the primer pairs AT4882 (5'caacatgacaaaaattrgcccc 3'; see [36])/ND2R2 (5' ratctaggaggccttakc 3'; specifically designed for this study). Amplification conditions included a hot start denaturation of 95 °C for 3 min, followed by 35 cycles of 95 °C for 1 min, 55 °C annealing temperature for 1 min, 72 °C for 1 minute 45 seconds. We then performed a final extension of 72 °C for 7 min and visualized the sequence reactions on a 3730 xl DNA Analyzer (Applied Biosystems, CA, USA).

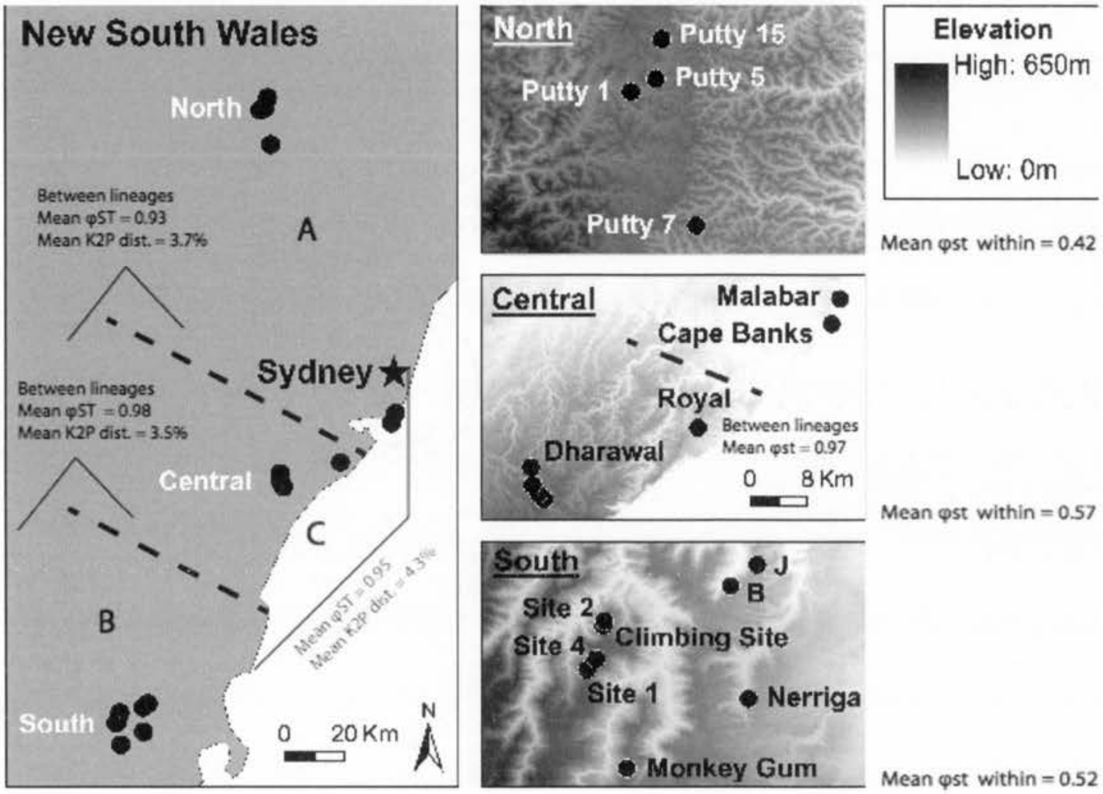

**Figure 1:** *Geographical distribution of the gecko* (Oedura lesueurii) *tissue samples used for genetic analyses, with mean $\phi_{ST}$ values and K2P distance between lineages (A, B, and C).*

*Phylogenetic analyses*

We aligned sequences using BioEdit (Hall 1999) and assessed them by eye. A sequence of *Crenadactylus ocellatus* ([GenBank:AY369016]; the basal species of the Diplodactylidae according to Oliver & Sanders 2009) was used to root the tree.

Additional sequences of Diplodactylidae were included in the analyses: *Pseudothecadactylus lindneri* [GenBank:AY369024], *Rhacodactylus chahoua* [GenBank:DQ533741], *Oedura marmorata* [GenBank:AY369015], *Diplodactylus taenicauda* [GenBank:AY369006], *Diplodactylus intermedius* [GenBank:AY369001], and *Strophurus williamsi* [GenBank:AY369007].

**Table 1:** *Number of tissue samples of the gecko* Oedura lesueurii, *and the longitude, latitude, and elevation, length and width of the collecting site, the number of samples and of haplotypes, and the nucleotide diversity at that site.*

| Sites | Long. | Lat. | Elevation (m) | Length (m) | Width (m) | # samples | # hapl. | Nuc div. |
|---|---|---|---|---|---|---|---|---|
| Dharawal Site 6_ | 150.8739 | -34.2025 | 334 | 93 | 44 | 13 | 2 | 0.000403 |
| Dharawal Site 12 | 150.8873 | -34.2213 | 439 | 71 | 60 | 11 | 2 | 0.00026 |
| Dharawal Site 13 | 150.8807 | -34.2139 | 382 | 73 | 60 | 7 | 2 | 0.000408 |
| Dharawal Site 15 | 150.8709 | -34.1801 | 268 | 234 | 191 | 7 | 2 | 0.00084 |
| Dharawal Site 18 | 150.8726 | -34.2027 | 335 | 105 | 76 | 4 | 2 | 0.000714 |
| Climb_Morton | 150.3770 | -34.9505 | 393 | 340 | 20 | 11 | 2 | 0.000779 |
| Nerrigera_Morton | 150.4695 | -34.9973 | 201 | 800 | 50 | 12 | 2 | 0.000716 |
| B_Morton | 150.4583 | -34.9248 | 234 | 172 | 25 | 10 | 1 | 0 |
| J_Morton | 150.4752 | -34.9109 | 211 | 187 | 15 | 11 | 1 | 0 |
| MonkeyGum | 150.3919 | -35.0420 | 367 | 5000 | 10 | 11 | 2 | 0.00026 |
| Yarramunmun site 1 | 150.3666 | -34.9788 | 395 | 1100 | 50 | 12 | 2 | 0.000584 |
| Yarramunmun site 4 | 150.3724 | -34.9719 | 392 | 700 | 50 | 10 | 1 | 0 |
| Yarramunmun site 2 | 150.3777 | -34.9475 | 395 | 1200 | 50 | 11 | 2 | 0.000781 |
| Putty_site 7 | 150.7251 | -33.2068 | 271 | 216 | 97 | 4 | 2 | 0.001433 |
| Putty_site 15 | 150.6998 | -33.0697 | 266 | 77 | 30 | 5 | 3 | 0.002873 |
| Putty_site 5 | 150.6954 | -33.0987 | 325 | 178 | 40 | 9 | 3 | 0.007716 |
| Putty_site 1 | 150.6769 | -33.1084 | 294 | 241 | 42 | 8 | 5 | 0.012807 |
| Malabar | 151.2602 | -33.9666 | 15 | 350 | 80 | 4 | 3 | 0.002933 |
| Cape Banks | 151.2496 | -33.9983 | 15 | 950 | 60 | 11 | 1 | 0 |
| Royal NP | 151.0816 | -34.1297 | 117 | 400 | 10 | 8 | 3 | 0.004823 |

We performed ML heuristic searches and bootstrap analyses (1000 replicates) with phyml (Guindon & Gascuel 2003) and we selected the model of DNA substitution using jModelTest 0.1.1 (Guindon & Gascuel 2003, Posada 2008). The HKY + G model (Hasegawa et al. 1985) best fitted the dataset with a Bayesian Information Criterion (BIC;

Schwarz 1978). Finally, we used Paup* 4.0b10 (Swofford 2001) to perform maximum parsimony (MP) analyses using 100 random additions of sequences followed by tree bisection and reconnection branch swapping, and retaining at most 100 trees at each replicate. We estimated branch support using 1000 bootstrap replicates with the same heuristic settings.

### *Population and landscape genetic analyses*

We estimated population structure between all sites sampled by calculating $\phi_{ST}$, taking into account haplotype frequencies and the genetic distance between haplotypes, in Arlequin 3.0 (Excoffier et al. 2005). We used the Kimura two-parameter genetic distance (K2P; Kimura 1980) as our genetic model.

We performed Mantel and partial Mantel tests (Mantel 1967) using the software FSTAT Version 2.9.3.2 (Goudet 2002), with genetic distance as the dependent variable. The independent variables were the number of intervening rivers (River; i.e., the number of rivers crossing the straight distance between two locations) and roads (Walking track; Dirt road; Paved road; All roads) between sites, the minimum elevation between sites, the mean elevation of sites minus the minimum elevation between sites, the straight-line distance and true distance between site (i.e., by calculating the surface length of a line connecting each pair of sites while incorporating an underlying digital elevation model at a resolution of 25 m; implemented using the 3D Analyst Tool in ArcMap 9.3, 9). *P*-values were calculated after 10,000 randomizations. The level of significance for our tests was set at $\alpha = 0.0028$ (Bonferroni correction; i.e., 0.05 / 18 = 0.0028, where 18 represents the number of tests performed). Based on the results of the Mantel and partial Mantel tests, we selected the best model using Akaike's information criterion (AIC; Akaike 1973; based on the variance of the residuals). We compared each candidate model based on its AIC scores and weights. The best-supported models are those with high Akaike weights, and that deviate from the best model by less than two units (i.e., $\Delta$AIC < 2; Burnham & Anderson 1998).

We used the program SAMOVA 1.0 (Dupanloup et al. 2002) to characterize population structure and to define groups of populations using genetic criteria. Given an *a priori* number of clusters (*K*), the software uses a simulated annealing procedure to define the cluster composition in which populations within a cluster are as genetically homogeneous as possible ($F_{SC}$ minimised) and clusters are maximally differentiated from each other ($F_{CT}$ maximised). The analysis was run for $K = 2$ to $K = 19$ and the significance of fixation indices was tested by 1023 permutations.

### *Molecular dating*

We performed dating analyses using BEAST 1.6.2 (Drummond et al. 2006) with an uncorrelated lognormal relaxed clock and a coalescent tree prior. The coefficient of variation frequency histogram was not abutting against zero, meaning that there was among branch rate heterogeneity within our data (Drummond et al. 2007). Consequently, as suggested by Drummond et al. (2007), we used a relaxed molecular clock.

We used two secondary calibration points from a robust phylogeny focusing on Australasian geckos (Oliver & Sanders 2009): (1) The oldest split within the Diplodactylidae (i.e., between *Crenadactylus ocellatus* and the other members of the family: 66.2 Mya [95% HPD: 46.6–87.0]) and (2) the split between *Pseudothecadactylus* and the New Caledonian *Rhacodactylus chahoua* and the remaining members of the Diplodactylidae (60.3 Mya [95% HPD: 41.5–79.2]).

The analysis was performed with two independent chains and 20 million generations; chains were sampled every 1000 generations with a burn-in of 2 million generations. Additional simulations were run with the same dataset and the same models, but strictly based on a rate of divergence of 1.3% derived from numerous studies as, for example, Zamudio & Greene's (1997) study on snake mtDNA and from Macey et al.'s (1999; see also Daniels et al. 2004, Torres-Carvajal et al. 2009, Dubey & Shine 2010a) work on lizards.

### *Dispersal distances of free-ranging geckos*

We conducted mark-recapture surveys on velvet geckos by turning rocks and measuring, individually marking (by toe-clipping) and releasing any geckos found. These studies were conducted in and around Morton National Park (Morton) on a monthly basis between March 2007 and October 2009, and in Dharawal Conservation area (Dharawal) and Yengo and Wollemi National Park (collectively, Putty) from March 2008 until November 2010. We classified geckos as adult males if they were > 40 mm snout-vent length (SVL) with overt hemipenial bulges; adult females if they were > 40 mm SVL and without such bulges; and juveniles if they were < 40 mm SVL. We determined the distance between rocks used by individual *O. lesueurii* using GPS co-ordinates imported into ArcGIS 10.0 (ESRI 2011).

## Results

### *Phylogenetic analyses and molecular dating*

The 179 samples of *O. lesueurii* showed 29 haplotypes (H1–H29, [GenBank:JQ779339–JQ779366]) of 710bp. The complete dataset included 369 variable sites of which 237 were parsimony-informative. As the two phylogenetic methods showed similar arrangements of the main branches, Fig. 2 only shows the relationship between haplotypes for the ML analyses (see Supplementary Material 1 for the MP tree). Three main lineages are present within the study area, the first (A) including populations from the north and central areas (Putty, Malabar, and Cape Banks; ML and MP analyses show bootstrap support of 96% and 90% respectively), the second (B) restricted to populations from the south (Morton; ML and MP analyses show bootstrap support of 100% and 99%), and the third (C) strictly central populations (Dharawal and Royal NP sites; ML and MP analyses show support of 84 and 77). The mean K2P distance between the lineages was 3.7, 3.5, and 4.3% for A-C, B-C, and A-B respectively.

Dating analyses based on the secondary calibration points revealed a first divergence within *O. lesueurii* about 5.68 million years ago (Mya; 95% HPD: 2.73–10.76), with a split between haplotypes within lineages occurring 2.94 Mya (95% HPD: 1.21–5.18), 1.07 (95% HPD: 0.28–0.94), and 1.58 (95% HPD: 0.50–2.96) for A, B, and C respectively. Dating analyses based on a standard divergence rate of 1.3% (derived from numerous previous studies; see Methods section) gave similar results, with a first divergence within the species about 5.00 Mya (95% HPD: 2.88–8.06), with a split between haplotypes within lineages occurring 2.36 Mya (95% HPD: 1.30–3.96), 0.83

Mya (95% HPD: 0.30–1.83), and 1.25 Mya (95% HPD: 0.56–2.53) for A, B, and C respectively.

*Population and landscape genetic analyses*

Overall, the $\phi_{ST}$ between populations varied from 0 to 1.0 (see Supplementary Material 2), with a mean value of 0.81. The mean of the pairwise $\phi_{ST}$ value within each lineage was 0.62, 0.52, and 0.24 for A, B, and C respectively.

Based on the Mantel and partial Mantel tests, the observed genetic structure ($\phi_{ST}$) in *Oedura lesueurii* populations was best predicted by a combination of distance and minimum elevation between sites (AIC value = -257.78; AIC weight = 0.27; $R^2 = 57.87$; straight-line distance, partial corr.: 0.56; minimum elevation, partial corr.: -0.51; Table 2). The second-best model included the number of rivers, the minimum elevation and the distance between sites (AIC value = -257.72; AIC weight = 0.26; $R^2 = 58.85$; rivers, partial corr.: -0.44; minimum elevation, partial corr.: -0.61; straight-line distance, partial corr.: 0.15). Three more models deviated from the best model by less than two units (i.e., $\Delta$AIC < 2), and all these models include the minimum elevation and the straight-line distance between sites as explanatory variables, further indicating the importance of these parameters. Because the true distance between sites was less informative than the straight-line distance (straight-line distance, $R^2 = 31.48$; true distance, $R^2 = 20.78$), we used the latter variable in our analyses (see Table 2).

The nucleotide diversity at a site tended to increase with latitude (i.e., was higher at more northern sites; $F_{1,19} = 13.88$, $P = 0.002$; Fig. 3), and we did not detect any significant

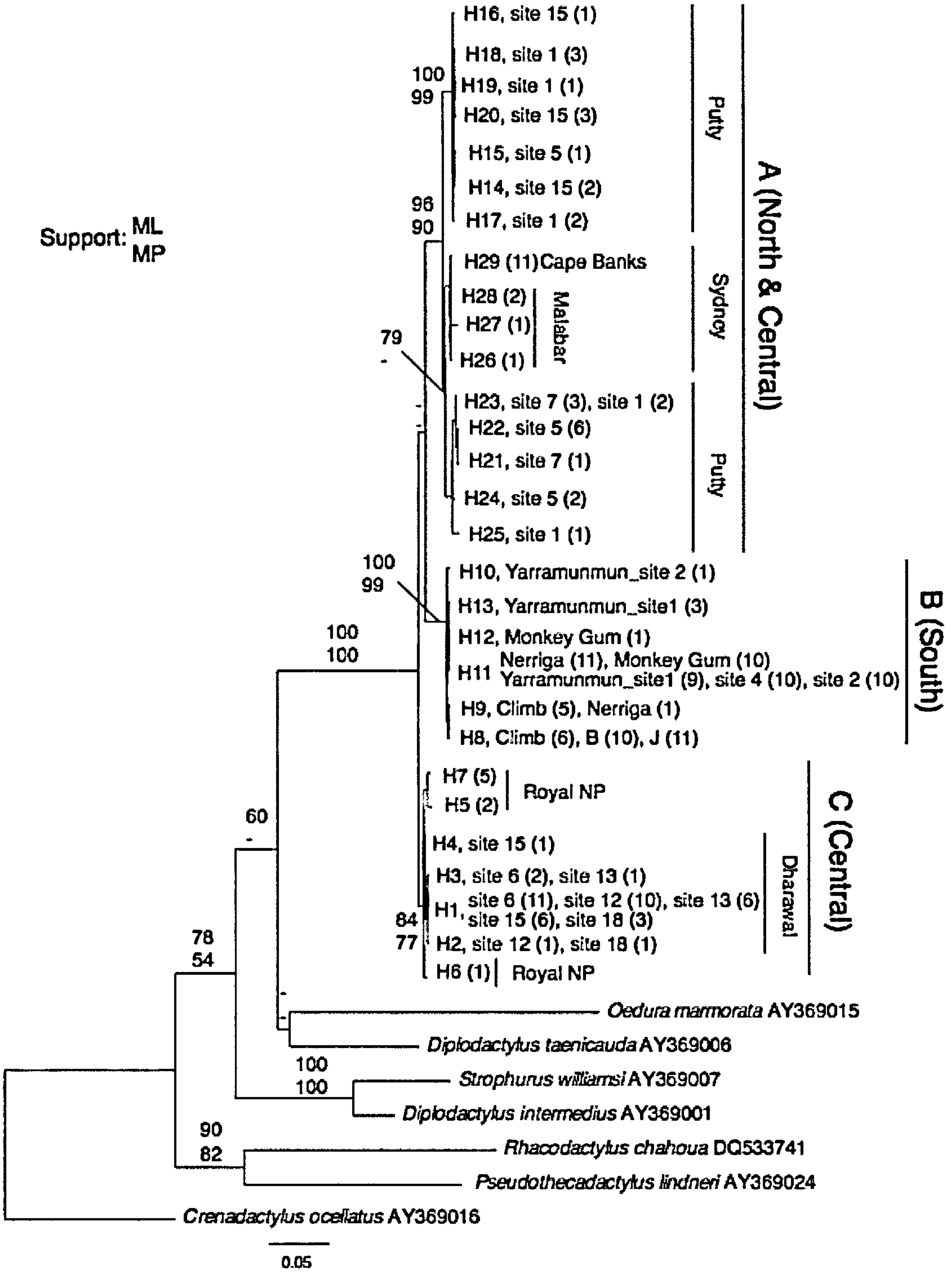

**Figure 2:** *Phylogeny of the ND2 fragment of the gecko* Oedura lesueurii *in southeastern Australia, analysed using a maximum likelihood procedure. Values in branches are indices of support for the major branches for maximum likelihood (ML) and maximum parsimony (MP) analyses (percentage of 1000 replications for ML and MP).*

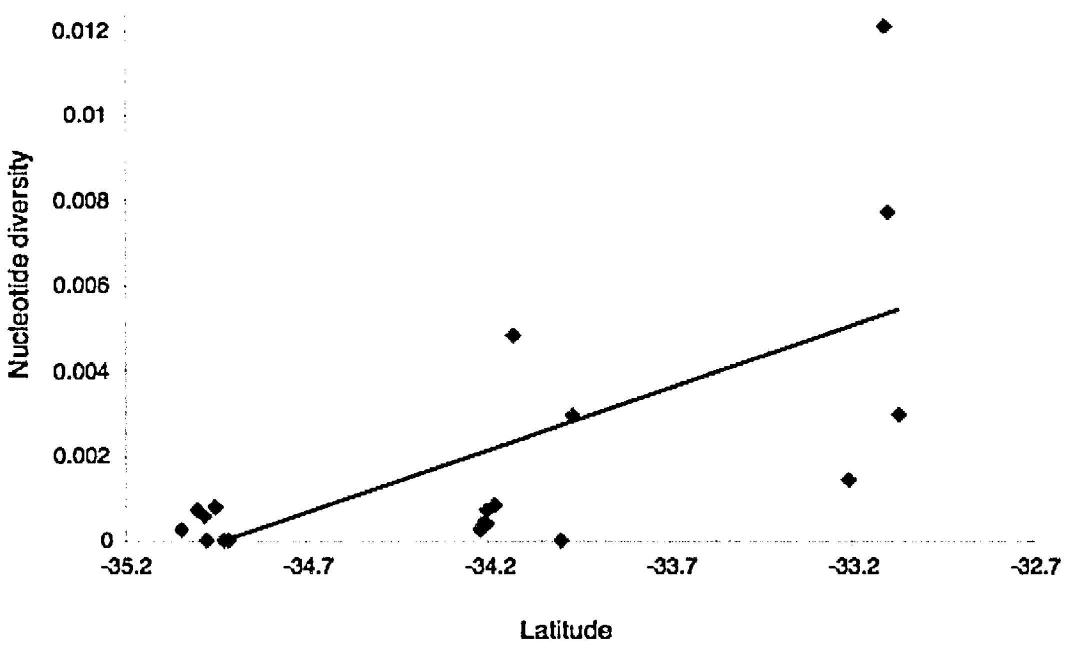

**Figure 3:** *Relationship between the latitude of a site, and the nucleotide diversity of the gecko* Oedura lesueurii *within that site.*

relationships between nucleotide diversity and the number of samples, the elevation, or the longitude of sites.

The SAMOVA revealed high $F_{CT}$ (among population groups) values for all the groups and small $F_{SC}$ (within population group) values in cases of 9 to 19 groups, indicating very high population structure. For example, at $K = 9$ the majority of variation (94.32%) is among groups, although 0.03% of variation at the level of among populations within groups still represents highly significant population structuring in the remaining population groups ($P < 0.001$). At $K = 2$, the two clusters identified were the populations of lineage B (Morton) vs. lineages A (Putty, Malabar, and Cape Banks) and C (Dharawal and Royal National Park), and at $K = 3$, the three clusters were the populations of lineage A, B, and C.

**Table 2:** *Results of Mantel and partial Mantel test of landscape genetics of the gecko* Oedura lesueurii, *with a listing of variables included in the models (number of rivers [River] and number of roads [Walking track; Dirt road; Paved road; All roads] between sites, minimum elevation between sites [min. elevation], mean elevation of sites minus the minimum elevation between sites [Mean elevation - min. elevation], straight-line distance [Distance] and true distance between sites [True distance]), the number of parameters per model, $R^2$ (total variance explained by the model), coefficient of correlation, P-value of parameters (The level of significance for our tests was set at $\alpha = 0.0028$ (Bonferroni correction; i.e., 0.05 / 18 = 0.0028, where 18 represents the number of tests performed), AIC, ΔAIC, and AIC weight. In grey, best model (bold) and models with a ΔAIC < 2. Only Mantel and partial Mantel tests (total of 18) with the 10 best AIC values are shown.*

| Variable | K | $R^2$ | coeff corr. | *P*-value | AIC | ΔAIC | AIC w |
|---|---|---|---|---|---|---|---|
| **Distance & min. elevation** | **3** | **57.87** | | | **-257.78** | **/** | **0.2686** |
| **Distance** | | | **0.56** | **0.0001*** | | | |
| **Min. elevation** | | | **-0.51** | **0.0001*** | | | |
| River & Min. elevation & Distance | 4 | 58.85 | | | -257.72 | 0.06 | 0.2606 |
| River | | | -0.44 | 0.0001* | | | |
| Min. elevation | | | -0.61 | 0.0001* | | | |
| Distance | | | 0.15 | 0.0362 | | | |
| River & Dirt road & Distance & Min. Elevation | 5 | 59.13 | | | -256.29 | 1.49 | 0.1275 |
| River | | | -0.44 | 0.0001* | | | |
| Dirt road | | | 0.19 | 0.0085 | | | |
| Distance | | | 0.37 | 0.0001* | | | |
| Min. elevation | | | -0.47 | 0.0001* | | | |
| Min. elevation & Dirt road & Distance | 4 | 58.08 | | | -256.20 | 1.58 | 0.1219 |
| Min. elevation | | | -0.75 | 0.0001* | | | |
| Dirt road | | | 0.15 | 0.0465 | | | |
| Distance | | | 0.06 | 0.3982 | | | |
| Distance & mean - min. elevation & min. elevation | 4 | 57.87 | | | -255.79 | 1.99 | 0.0993 |
| Min. elevation | | | -0.75 | 0.0001* | | | |
| Mean - min. elevation | | | 0.05 | 0.4932 | | | |
| Distance | | | 0.14 | 0.0516 | | | |
| Min. elevation | 2 | 55.59 | -0.75 | 0.0001* | -255.44 | 2.34 | 0.0834 |
| Min. elevation & mean - min. elevation | | 55.84 | | | -253.91 | 3.87 | 0.0388 |
| Min. elevation | 3 | | -0.75 | 0.0001* | | | |
| Mean - min. elevation | | | 0.05 | 0.4870 | | | |
| Distance & mean elevation - min. elevation | 3 | 39.71 | | | -228.22 | 29.56 | 0.0000 |
| Distance | | | 0.56 | 0.0001* | | | |
| Mean elevation - min. elevation | | | 0.29 | 0.0002* | | | |
| Distance & River | 3 | 36.65 | | | -224.13 | 33.65 | 0.0000 |
| Distance | | | 0.56 | 0.0001* | | | |
| River | | | -0.23 | 0.0019* | | | |
| Distance & Dirt road | 3 | 31.49 | | | -217.67 | 40.11 | 0.0000 |
| Distance | | | 0.56 | 0.0001* | | | |
| Dirt road | | | 0.01 | 0.9289 | | | |

*Dispersal distances of free-ranging geckos*

In total, we obtained records of the distances moved by 260 geckos, over time periods ranging from 24 to 928 days between recaptures (average time between recaptures = 203.1 days). We used ANOVA to compare gecko movements among regions and between sexes and age classes over time. There were no significant interaction effects, so we only describe main effects. *Oedura lesueurii* was highly sedentary. Marked lizards did not tend to move further away from their initial capture site with increasing time ($F_{2,252}$ = 2.08, $P$ = 0.15), indicating that they have fixed home ranges. There was no difference in mean movement distances between male, female or juvenile geckos ($F_{2,252}$ = 1.84, $P$ = 0.16), and the average distances moved were less than 30 m (Fig. 4). The maximum

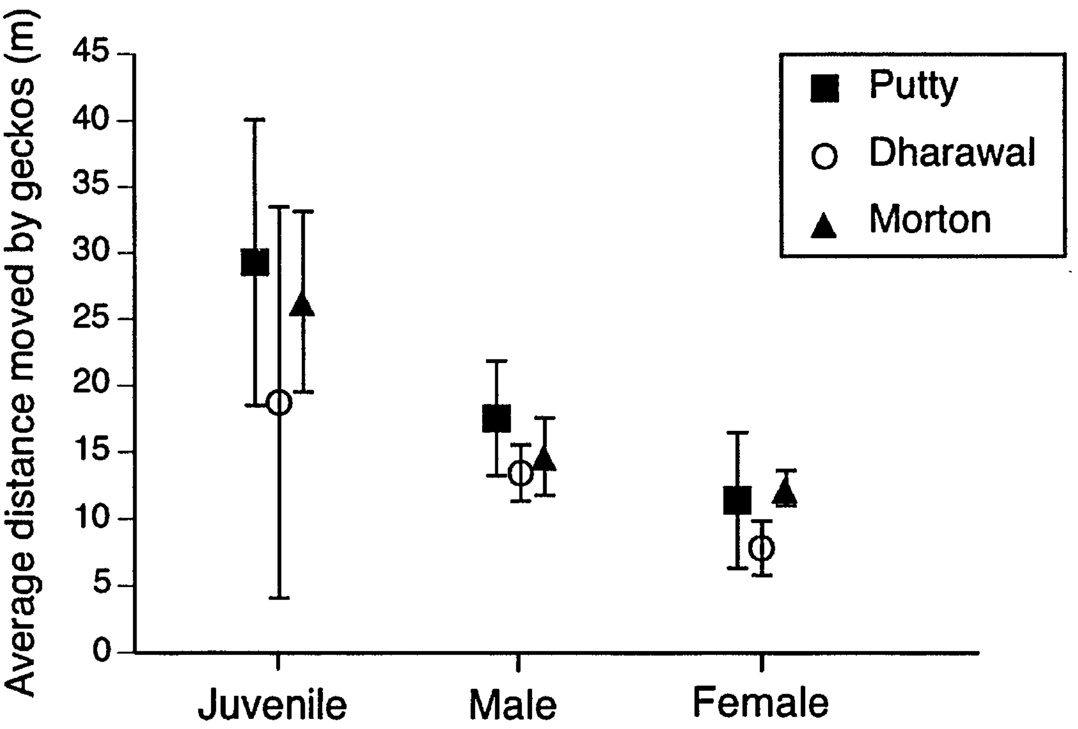

**Figure 4:** *Average distances moved by juvenile, male and female geckos* Oedura lesueurii *at sites in the Putty region (Yengo and Wollemi National Parks), Dharawal State Conservation area and Morton National Park. Mean recapture intervals differed among samples, but averaged 203 days. The graph shows mean values and associated standard errors.*

dispersal distances recorded were 1.648 km for an adult male, 1.442 km for an adult female, and 1.577 km for a juvenile.

## Discussion

Our study revealed ancient genetic divergences within *Oedura lesueurii* from southeastern Australia, beginning in the Miocene-Pliocene (5.68–5.0 Mya) and resulting in three geographically well-defined lineages (North, Central, and South; Figs 1,2). Similarly, our population genetics analyses showed a strong spatial structure among our 20 populations as well as within lineages, with a lack of haplotype sharing between populations separated by only 3.7 km ($\phi_{ST} = 0.70$). In addition, our landscape genetic analyses identified distance as the major barrier to gene flow ($\phi_{ST}$) between populations. In contrary, an absence of areas with low elevation between sites (for example; the absence of deep valleys separating populations) favoured dispersal. In this case, areas of high elevation between sites reflects continuous favourable habitat (for example; rocky outcrops). Similarly, gene flow in the broad-headed snake *H. bungaroides* mostly occurs along sandstone plateaux rather than across the densely forested valleys that separate plateaux (Dubey et al. 2011). Consistent with these genetic analyses, our field data (5 years of mark-recapture studies) revealed that *O. lesueurii* are sedentary. Marked individuals typically remained within close proximity (tens of metres) to their original capture site for years, consistent with earlier reports that some females return to their natal sites to lay eggs (Webb et al. 2008).

Previous phylogenetic studies on southeastern Australian reptiles (for example; Dubey & Shine 2010b, Sumner et al. 2010) have revealed similar ancient splits between populations.

In both of these taxa, a southern lineage (restricted to Morton NP) differs significantly from conspecifics in the Sydney area. Sumner et al. (2010) suggested that the break between the southern and northern clade of *H. bungaroides* occurs in a geologically distinctive area where volcanic soils cover the sandstone plateaux (Branagan & Packham 2000), acting as a barrier to gene flow. The same may be true of other sandstone specialist species such as *O. lesueurii.* The strong genetic structure observed in this study is consistent with general patterns observed in various taxa distributed in eastern Australia (Byrne et al. 2011) and could be attributed to the ancestral position of the mesic biome (which is dominating in eastern Australia), and hence allowed localized endemism from long-term persistence of populations through multiple climatic cycles; Byrne et al. 2011). Finally, the observed gradient of genetic diversity in *O. lesueurii* through the study area (decreasing diversity with increasing latitude) may be the result of harsher historical conditions in the southern part of the range (Last Glacial Maximum; Markgraf et al. 1995). The species reaches its current southern distributional limit close to our study sites in Morton NP (Wilson & Swan 2010).

Overall, the diversification of Australian geckos is ancient and may have originated from a Gondwanan vicariance (e.g., about 70 Mya for the diplodactyloids: Oliver & Sanders 2009). In this respect the geckos differ from most other squamates, which colonised Australia from Asia more recently (see Hugall et al. 2008, Sanders et al. 2008, Skinner et al. 2008, Oliver & Sanders 2009). Similarly, Australian geckos show relatively ancient intraspecific diversification (see Oliver et al. 2007, Oliver et al. 2010, Oliver & Bauer 2011, Dubey & Shine 2011, this study). The diversification of at least one of the gecko's major predators (the broad-headed snake *H. bungaroides*) is much more recent, as the split between the genera *Hoplocephalus* and *Paroplocephalus* occurred less than 3 Mya (Sanders et al. 2008), and the oldest split between *H. bungaroides* lineages about 0.8 Mya (Sumner et al. 2010). Consequently, *O. lesueurii* was established across much of its current range in southeastern Australia long before the evolutionary origin of *H. bungaroides*. Our results support the plausibility of the conditions required for natural selection to produce adaptive local differentiation in geckos: that is, genetic variation among populations and low gene flow between them (Endler 1986, Lynch & Walsh 1998, Urban 2010).

From a conservation perspective, the low dispersal rates of *O. lesueurii* have two main implications. The first is that this gecko will be slow to recolonise any local areas from which it is extirpated (perhaps by chance abiotic events, predators, or human disturbance). Thus, habitat suitability for the endangered broad-headed snake may be spatially heterogeneous as a result of relatively ancient local events that reduced gecko numbers. Second, the low dispersal rates of the geckos need to be considered in any management plan that includes the restoration of degraded habitat previously hosting *H. bungaroides*. The poor dispersal capacity of *O. lesueurii* (unlike *H. bungaroides* itself; Dubey et al. 2011) likely will delay or prevent natural recolonisation of geckos in restored areas, unless those areas are very close to extant populations. Consequently, we may need to reintroduce *O. lesueurii* to such areas in order to guarantee successful habitat restoration for *H. bungaroides*.

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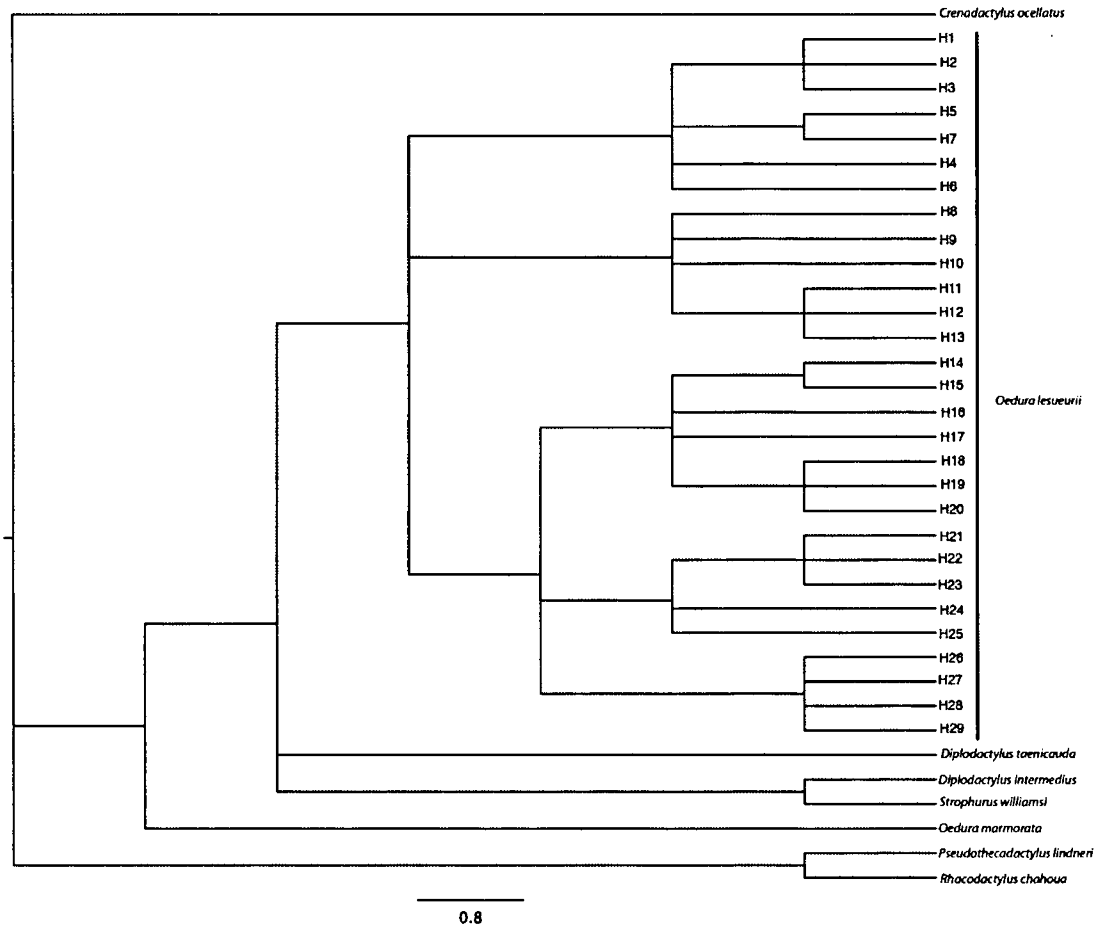
# Supplementary Material 1



# Supplementary Material 2

| Population name | |
|---|---|
| 1 | Dharawal Site 6 |
| 2 | Dharawal Site 12 |
| 3 | Dharawal Site 13 |
| 4 | Dharawal Site 15 |
| 5 | Dharawal Site 18 |
| 6 | Climb_Morton |
| 7 | Nemgera_Morton |
| 8 | B_Morton |
| 9 | J_Morton |
| 10 | MonkeyGum |
| 11 | Yarramunmun site 1 |
| 12 | Yarramunmun site 4 |
| 13 | Yarramunmun site 2 |
| 14 | Putty_site 7 |
| 15 | Putty_site 15 |
| 16 | Putty_site 5 |
| 17 | Putty_site 1 |
| 18 | Malabar |
| 19 | Cape Banks |
| 20 | Royal NP |

Population pairwise ΦSTs

| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 0 | | | | | | | | | | | | | | | | | | |
| 2 | 0.0487 | 0 | | | | | | | | | | | | | | | | | |
| 3 | -0.12292 | 0.01289 | 0 | | | | | | | | | | | | | | | | |
| 4 | 0.05717 | 0.03178 | 0.00014 | 0 | | | | | | | | | | | | | | | |
| 5 | 0.09203 | -0.07677 | 0.03464 | -0.00769 | 0 | | | | | | | | | | | | | | |
| 6 | 0.98266 | 0.98427 | 0.98068 | 0.97573 | 0.97704 | 0 | | | | | | | | | | | | | |
| 7 | 0.98302 | 0.98464 | 0.98135 | 0.97659 | 0.97811 | 0.77283 | 0 | | | | | | | | | | | | |
| 8 | 0.99291 | 0.99577 | 0.99496 | 0.98978 | 0.9945 | 0.38433 | 0.85784 | 0 | | | | | | | | | | | |
| 9 | 0.99323 | 0.99598 | 0.99527 | 0.99041 | 0.99492 | 0.4 | 0.86385 | 0 | 0 | | | | | | | | | | |
| 10 | 0.98963 | 0.992 | 0.99031 | 0.9854 | 0.98883 | 0.85749 | -0.00346 | 0.95439 | 0.95659 | 0 | | | | | | | | | |
| 11 | 0.98507 | 0.98687 | 0.98409 | 0.9794 | 0.98141 | 0.82502 | 0.09111 | 0.90093 | 0.90528 | 0.12915 | 0 | | | | | | | | |
| 12 | 0.99291 | 0.99577 | 0.99496 | 0.98978 | 0.9945 | 0.88386 | -0.01617 | 1 | 1 | -0.00917 | 0.15691 | 0 | | | | | | | |
| 13 | 0.98234 | 0.98397 | 0.98032 | 0.97527 | 0.9766 | 0.78592 | -0.02946 | 0.8633 | 0.8694 | 0.0002 | 0.08868 | -0.00917 | 0 | | | | | | |
| 14 | 0.98405 | 0.98607 | 0.98041 | 0.97304 | 0.97215 | 0.97857 | 0.97968 | 0.99159 | 0.99223 | 0.98755 | 0.98187 | 0.99159 | 0.97825 | 0 | | | | | |
| 15 | 0.96303 | 0.96284 | 0.95028 | 0.94325 | 0.93374 | 0.96574 | 0.9673 | 0.97552 | 0.97712 | 0.97322 | 0.96901 | 0.97552 | 0.96527 | 0.88693 | 0 | | | | |
| 16 | 0.9057 | 0.89924 | 0.87363 | 0.86732 | 0.84351 | 0.91026 | 0.91345 | 0.9145 | 0.91893 | 0.91564 | 0.9142 | 0.9145 | 0.90905 | 0.10653 | 0.68306 | 0 | | | |
| 17 | 0.85051 | 0.83928 | 0.80073 | 0.79481 | 0.75595 | 0.86664 | 0.87119 | 0.86786 | 0.87445 | 0.87145 | 0.87141 | 0.86786 | 0.86487 | 0.44358 | 0.14542 | 0.34559 | 0 | | |
| 18 | 0.97185 | 0.97236 | 0.96091 | 0.95255 | 0.94356 | 0.96328 | 0.96449 | 0.97711 | 0.97884 | 0.97295 | 0.96681 | 0.97662 | 0.96196 | 0.83845 | 0.83115 | 0.59135 | 0.4771 | 0 | |
| 19 | 0.99381 | 0.99633 | 0.99568 | 0.99125 | 0.99536 | 0.9896 | 0.98983 | 1 | 1 | 0.99648 | 0.99159 | 1 | 0.98941 | 0.97667 | 0.93927 | 0.76225 | 0.65061 | 0.70421 | 0 |
| 20 | 0.75756 | 0.74654 | 0.68915 | 0.65156 | 0.62319 | 0.92805 | 0.93129 | 0.9367 | 0.94024 | 0.93665 | 0.93422 | 0.93741 | 0.92763 | 0.90875 | 0.89236 | 0.83728 | 0.78222 | 0.88457 | 0.94639 |

ΦST p-values

| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | * | | | | | | | | | | | | | | | | | | |
| 2 | 0.36036+0.0606 | * | | | | | | | | | | | | | | | | | |
| 3 | 0.99099+0.0030 | 0.48649+0.0562 | * | | | | | | | | | | | | | | | | |
| 4 | 0.27928+0.0370 | 0.33333+0.0451 | 0.53153+0.0345 | * | | | | | | | | | | | | | | | |
| 5 | 0.19820+0.0353 | 0.99099+0.0030 | 0.33333+0.0333 | 0.68468+0.0279 | * | | | | | | | | | | | | | | |
| 6 | 0.00000+0.0000 | 0.00000+0.0000 | 0.00000+0.0000 | 0.00000+0.0000 | 0.00000+0.0000 | * | | | | | | | | | | | | | |
| 7 | 0.00000+0.0000 | 0.00000+0.0000 | 0.00000+0.0000 | 0.00000+0.0000 | 0.00000+0.0000 | 0.00000+0.0000 | * | | | | | | | | | | | | |
| 8 | 0.00000+0.0000 | 0.00000+0.0000 | 0.00000+0.0000 | 0.00000+0.0000 | 0.00901+0.0091 | 0.03604+0.0148 | 0.00000+0.0000 | * | | | | | | | | | | | |
| 9 | 0.00000+0.0000 | 0.00000+0.0000 | 0.00000+0.0000 | 0.00000+0.0000 | 0.00000+0.0000 | 0.00000+0.0000 | 0.00000+0.0000 | 0.99099+0.0030 | * | | | | | | | | | | |
| 10 | 0.00000+0.0000 | 0.00000+0.0000 | 0.00000+0.0000 | 0.00000+0.0000 | 0.00000+0.0000 | 0.00000+0.0000 | 0.72072+0.0470 | 0.00000+0.0000 | 0.00000+0.0000 | * | | | | | | | | | |
| 11 | 0.00000+0.0000 | 0.00000+0.0000 | 0.00000+0.0000 | 0.00000+0.0000 | 0.00000+0.0000 | 0.00000+0.0000 | 0.14414+0.0337 | 0.00000+0.0000 | 0.00000+0.0000 | 0.17117+0.0550 | * | | | | | | | | |
| 12 | 0.00000+0.0000 | 0.00000+0.0000 | 0.00000+0.0000 | 0.00000+0.0000 | 0.00901+0.0091 | 0.00000+0.0000 | 0.99099+0.0030 | 0.00000+0.0000 | 0.00000+0.0000 | 0.99099+0.0030 | 0.18919+0.0344 | * | | | | | | | |
| 13 | 0.00000+0.0000 | 0.00000+0.0000 | 0.00000+0.0000 | 0.00000+0.0000 | 0.00000+0.0000 | 0.00000+0.0000 | 0.99099+0.0030 | 0.00000+0.0000 | 0.00000+0.0000 | 0.45946+0.0656 | 0.14414+0.0337 | 0.99099+0.0030 | * | | | | | | |
| 14 | 0.00000+0.0000 | 0.00901+0.0091 | 0.00000+0.0000 | 0.00000+0.0000 | 0.02703+0.0139 | 0.00000+0.0000 | 0.00000+0.0000 | 0.00000+0.0000 | 0.00000+0.0000 | 0.00000+0.0000 | 0.00000+0.0000 | 0.00901+0.0091 | 0.00000+0.0000 | * | | | | | |
| 15 | 0.00000+0.0000 | 0.00000+0.0000 | 0.00000+0.0000 | 0.00000+0.0000 | 0.00000+0.0000 | 0.00000+0.0000 | 0.00000+0.0000 | 0.00000+0.0000 | 0.00000+0.0000 | 0.00901+0.0091 | 0.00000+0.0000 | 0.00000+0.0000 | 0.00000+0.0000 | 0.00901+0.0091 | * | | | | |
| 16 | 0.00000+0.0000 | 0.00000+0.0000 | 0.00000+0.0000 | 0.00000+0.0000 | 0.00000+0.0000 | 0.00000+0.0000 | 0.00000+0.0000 | 0.00000+0.0000 | 0.00000+0.0000 | 0.00000+0.0000 | 0.00000+0.0000 | 0.00000+0.0000 | 0.00000+0.0000 | 0.15315+0.0333 | 0.00000+0.0000 | * | | | |
| 17 | 0.00000+0.0000 | 0.00000+0.0000 | 0.00000+0.0000 | 0.00000+0.0000 | 0.00000+0.0000 | 0.00000+0.0000 | 0.00000+0.0000 | 0.00000+0.0000 | 0.00000+0.0000 | 0.00000+0.0000 | 0.00000+0.0000 | 0.00000+0.0000 | 0.00000+0.0000 | 0.00901+0.0091 | 0.11712+0.0385 | 0.00901+0.0091 | * | | |
| 18 | 0.00000+0.0000 | 0.00000+0.0000 | 0.01802+0.0121 | 0.00901+0.0091 | 0.01802+0.0182 | 0.00000+0.0000 | 0.00000+0.0000 | 0.00000+0.0000 | 0.00901+0.0091 | 0.00000+0.0000 | 0.00000+0.0000 | 0.00000+0.0000 | 0.00000+0.0000 | 0.00901+0.0091 | 0.00901+0.0091 | 0.00000+0.0000 | 0.00000+0.0000 | * | |
| 19 | 0.00000+0.0000 | 0.00000+0.0000 | 0.00000+0.0000 | 0.00000+0.0000 | 0.00000+0.0000 | 0.00000+0.0000 | 0.00000+0.0000 | 0.00000+0.0000 | 0.00000+0.0000 | 0.00000+0.0000 | 0.00000+0.0000 | 0.00000+0.0000 | 0.00000+0.0000 | 0.00000+0.0000 | 0.00000+0.0000 | 0.00000+0.0000 | 0.00000+0.0000 | 0.00000+0.0000 | * |
| 20 | 0.00000+0.0000 | 0.00000+0.0000 | 0.00000+0.0000 | 0.00000+0.0000 | 0.00000+0.0000 | 0.00000+0.0000 | 0.00000+0.0000 | 0.00000+0.0000 | 0.00000+0.0000 | 0.00000+0.0000 | 0.00000+0.0000 | 0.00000+0.0000 | 0.00000+0.0000 | 0.00000+0.0000 | 0.00000+0.0000 | 0.00000+0.0000 | 0.00000+0.0000 | 0.00000+0.0000 | 0.00000+0.0000 |

Significance level (after Bonferroni correction) = 0.0002

# PART 4: Broad-headed Snakes

*The broad-headed snake* (Hoplocephalus bungaroides) *with offspring*.

# **CHAPTER 6:** *MOVEMENTS AND HABITAT USE OF AN ENDANGERED SNAKE,* Hoplocepalus bungaroides *(ELAPIDAE): IMPLICATIONS FOR CONSERVATION*

Submitted as: Croak BM, Crowther MS, Webb JK, Shine R (2012) Movements and habitat use of an endangered snake, *Hoplocephalus bungaroides* (Elapidae): implications for conservation. Biological Conservation.

*A radio-tracked broad-headed snake basking on the outside of a tree. Snakes were observed basking in the open multiple times, a previously unrecorded behaviour.*

## Abstract

A detailed understanding of how extensively animals move through the landscape, and the habitat features upon which they rely, can identify conservation priorities and thus inform management planning. For many endangered species, information on habitat use either is sparse, or is based upon studies from a small part of the species' range. The broad-headed snake (*Hoplocephalus bungaroides*) is restricted to a specialized habitat (sandstone outcrops and nearby forests) within a small geographic range in southeastern Australia. Previous research on this endangered taxon was done at a single site in the extreme south of the species' geographic range. We captured and radio-tracked 9 adult broad-headed snakes at sites in the northern part of the species' distribution, to evaluate the generality of results from prior studies, and to identify critical habitat components for this northern population. Snakes spent most of winter beneath sun-warmed rocks then shifted to tree hollows in summer. Thermal regimes within retreat sites support the hypothesis that this shift is thermally driven. Intervals between successive movements were longer than in the southern snakes but dispersal distances per move and home ranges were similar. Our snakes showed non-random preferences both in terms of macrohabitat (e.g., avoidance of some vegetation types) and microhabitat (e.g., frequent use of hollow-bearing trees). Despite many consistencies, the ecology of this species differs enough between southern and northern extremes of its range that managers need to incorporate information on local features to most effectively conserve this threatened reptile.

**Keywords:** radio-telemetry, endangered, significant evolutionary unit, *Hoplocephalus bungaroides*, thermal regimes

## Introduction

Human-induced fragmentation of landscapes and habitats can lead to a reduction in biodiversity (Dirzo & Raven 2003, Gibson et al. 2011). Although species that are able to exploit a variety of habitats may be relatively insensitive to habitat disturbance (Bonin 2012), species that have evolved behaviours or physical traits that facilitate reliance on specialized habitat use may find altered habitats difficult or impossible to occupy (Julliard et al. 2003, Munday 2004, Bonin 2012). For such species, disturbance of critical habitat can lead to endangerment or extinction (Bonin 2012). Life history traits also influence a species' ability to tolerate degradation of preferred habitat type; for example, taxa with small population sizes and low rates of reproduction and dispersal may be at particular risk (Gaston 1994, Webb et al. 2002a).

To conserve highly specialized animals, we need detailed information on habitat use, dispersal and movement patterns (Cooke 2008). Unfortunately, such data often are laborious to collect, especially for endangered species – both because they are rare, and because research methods must not inflict additional stress (Caughley & Gunn 1995). As a result, our knowledge on many endangered taxa is based on studies that have been performed at only a single site (where researchers can most easily obtain and study animals: Hucke-Gaete 2003, Mills et al. 2004). Often, such sites are atypical of conditions that pertain over most of the species' range (Hucke-Gaete 2003, Mills et al. 2004). Indeed, a disproportionate reliance on studies on a small and unrepresentative series of populations is a general problem in ecological research: much of what we know about even widely-distributed lineages is based upon multiple studies on a small number of populations (e.g., gartersnakes in Manitoba: Shine 2012). This is especially worrying for endangered-species research, because logistics may make studies elsewhere almost impossible.

One such species is the broad-headed snake (*Hoplocephalus bungaroides*), an elapid species that has drastically declined since European settlement of Australia (Krefft 1869, Shine & Fitzgerald 1989, Shine et al. 1998, Webb et al. 2002b). Broad-headed snakes rely on specific habitat attributes; they shelter beneath thin, sun-exposed exfoliated rocks on sandstone rock outcrops with western or north-western aspects (Webb & Shine 1997a). These retreat sites allow snakes to thermoregulate during winter and spring. *Hoplocephalus bungaroides* also exhibit other life history traits that render them vulnerable to disturbance e.g., dependence on high rates of adult survival, infrequent breeding (every 3 to 4 years), low fecundity (3 to 4 offspring per litter), late maturity (up to 6 years), low rates of dispersal and a small geographic range. All of these traits contribute to the endangered status of *H. bungaroides* (Webb & Shine 1998b). Also, the habitat of *H. bungaroides* has become fragmented, and subject to vegetation overgrowth (Webb & Shine 1997b, Pringle et al. 2003, Pike et al. 2011) and removal of shelter-sites (exfoliated rock) for landscaping and gardening (Shine et al. 1998, Webb and Shine 1998a, 2000, Croak et al. 2008).

To date, most research on *H. bungaroides* has been conducted on a single population in the extreme south of the species' range (Webb & Shine 1997a, 1998a, 1998b, 2000, 2008). Genetic data show that this intensively-studied population belongs to a genetically distinct clade, with another isolated, evolutionarily significant unit identified in the north of the species range. Those two clades diverged approximately 800,000 years ago (Sumner et al. 2010). Vegetation, temperatures and potential prey species differ between the northern and southern parts of the species' range (Cogger 2000), and may be similar to those experienced by con-generic *Hoplocephalus stephensii*, a species that shows some similarity in niche utilisation to *H. bungaroides*. In the current paper, we describe habitat use and movements of snakes from the previously unstudied northern clade.

## Methods

### *Study species*

*H. bungaroides* are medium-sized (to 90 cm: Cogger 2000), brightly coloured ambush predators (Webb et al. 2003). During winter the snakes live in thermally suitable crevices that form between thin, exfoliated rock and parent bedrock that is exposed to afternoon sun (Webb & Shine 1997a). During the warmer parts of the year, these exposed rock exfoliations become too hot and snakes move into tree hollows in adjacent woodlands (Webb & Shine 1997b). This habitat specificity means that *H. bungaroides* are restricted to areas that provide access both to sun exposed rock-on-rock exfoliations, and to suitable areas of surrounding forest (Webb & Shine 1997a, 1997b).

### *Study sites*

Yengo and Wollemi National Parks are 100 km northwest of Sydney. We radio-tracked snakes at one study site inside Wollemi National Park (NP), and at two study sites inside Yengo NP. All sites were approximately 2 km apart, and consisted of exposed Hawkesbury sandstone outcrops surrounded by open eucalypt woodland dominated by Sydney peppermint (*Eucalyptus urceolaris*), narrow-leafed stringy-bark (*Eucalyptus sparsifolia*), yellow bloodwood (*Corymbia eximia*), red bloodwood (*Corymbia gummifera*), grey gum (*Eucalyptus punctata*), and scribbly gum (*Eucalyptus haemastoma*).

### *Capturing snakes*

To track *H. bungaroides* in the spring/summer period of 2010/2011 and 2011/2012 we captured nine snakes during late winter of 2010 and 2011. Snakes are accessible at this time of year because they shelter beneath thin rock exfoliations that are easily lifted and replaced. We captured seven snakes in Wollemi NP and two snakes in Yengo NP. We tracked two of the snakes caught in Wollemi NP and the two snakes caught in Yengo NP over the spring/summer of 2010/2011 (20/10/2010 to 07/02/2011). We tracked one of the snakes from Yengo NP, one of the snakes from Wollemi NP and an additional snake captured in Wollemi NP during the winter of 2011 from 10/05/2011 to 11/08/2011. We tracked the remaining four snakes in Wollemi NP over the spring/summer of 2011/2012 (16/11/2011 to 16/01/2012: see Table 1). We captured all snakes by hand and placed them in cotton bags for transportation to the laboratory. We housed snakes individually in plastic containers (31 x 22 cm, 10 cm high, containing a shelter and water dish) in a 12:12 h light:dark regime and constant temperature of 19 °C. We placed a heat mat under one end of the enclosure to allow snakes to thermoregulate. We fed the snakes fortnightly on frozen-then-thawed laboratory mice. We transported snakes to an approved veterinarian as per animal ethics protocol L04/12-2008/3/4927 for surgical implantation of transmitters (BD-2T, Holohil Systems, Carp, Ontario, Canada). We recaptured snakes prior to signal failure so that we could surgically remove the transmitters.

### *Surgical methods*

All surgeries were carried out by a qualified veterinarian. Each snake was examined and weighed, then pre-medicated with morphine 1 mg. $kg^{-1}$ intramuscularly 10 min prior to induction. Snakes were induced with alfaxan 10 mg. $kg^{-1}$ (intramuscular, or injected into the tail vein). Once the snake was anesthetized, a mask made from a 10 ml syringe was placed over the snake's head (held in place with transpore tape) to provide a mixture of isoflourane and oxygen for anesthesia. Transmitters were cold-sterilized in a solution of F10 and water, and scales/skin were prepared using chlorhexidine scrub followed by an iodine spray. The transmitter aerial was trimmed to fit within the snake's body.

**Table 1:** *Home ranges of radio-tracked broad-headed snakes,* Hoplocephalus bungaroides, *at sites in the extreme north of the species' range. "Season" shows season and year: for example, "S10/11" = spring and summer of 2010–2011.*

| ID | Site | Sex | SVL (mm) | Mass (g) | Season | Home Range (ha) |
|---|---|---|---|---|---|---|
| Snake 1 | Y | M | 550 | 50.5 | S10/11 | 9.43 |
| Snake 3 | Y | F | 565 | 52.0 | S10/11 | 6.36 |
| Snake 4 | W | F | 565 | 51.5 | S10/11 | 9.89 |
| Snake 5 | W | F | 670 | 62.0 | S10/11 | 1.39 |
| Snake 3 | W | F | 565 | 52.0 | W 11 | 0.09 |
| Snake 5 | W | F | 670 | 62.0 | W 11 | 0.83 |
| Snake 7 | W | M | 555 | 51.0 | W 11 | 0.57 |
| Snake 6 | W | F | 672 | 63.5 | S11/12 | 1.22 |
| Snake 9 | W | F | 554 | 50.0 | S11/12 | 0.01 |
| Snake 11 | W | M | 570 | 52.0 | S11/12 | 2.43 |
| Snake 12 | W | M | 650 | 62.0 | S11/12 | 0.24 |

*SVL = snout to vent length; M = male; F = female; W = Wollemi National Park; Y = Yengo National Park; S10/11 = spring/summer tracking period of 2010–2011; S11/12 = spring/summer tracking period of 2011–2012; W11 = winter tracking period of 2011. Home ranges were estimated using the minimum convex polygon method in ARC GIS 9.3.*

A scalpel was used to make a small incision 20 mm above the vent, and then alligator forceps were used to blunt-dissect against the body wall up to a point two-thirds of the way up the snake's body. A second incision was made over the tip of the alligator forceps and the transmitter antenna was grasped with the forceps and pulled through the coelom such that the aerial sat flat within the body cavity. The transmitter body was then introduced into the coelom. Both incisions were closed with 3-0 premilene non-absorbable suture material. A mixture of 41% warm water, 9% saline and 50% Hartmann's fluids were then injected subcutaneously at a dose of 3% body mass. To remove transmitters, the above procedure was reversed. No adverse effects were noted from surgery, and we released all snakes within one week after surgery.

### *Tracking snakes*

We tracked snakes twice per week during spring and summer in 2010–2011 (October to February) and 2011–2012 (November to February). We tracked snakes once per week during winter 2011. We used a hand-held UHF tracking receiver (Australis 26K, Titley Scientific, QLD, Australia) fitted with a Yagi antenna, and recorded location data using a hand-held global positioning system (GPS) device (GPSMAP 76, Garmin International, Olathe, KS, USA). We quantified attributes of trees used by snakes as retreat sites in spring/summer, plus five randomly chosen nearby trees (see analysis of microhabitat use by snakes). We also quantified the thermal regime of rocks used by snakes in winter (see seasonal shifts in thermal regimes within retreat sites).

### *Analyses of snake movements*

We used ARC GIS 9.3 (Esri, Redlands, CA, USA) to calculate the total distances moved by snakes throughout the study (m), the mean distance per move (movement < 1 m) and the time interval between moves (moves. day$^{-1}$). We also calculated moves per tracking day; that is, the number of movements divided by the number of radio-tracking days. We used a two-factor analysis of variance (2-way ANOVA) to test the effect of year and sex on these variables.

### *Analyses of snake home ranges*

We imported GPS points of snake retreat sites into ARC GIS 9.3 and estimated home range sizes using the minimum convex polygon method (Harris et al. 1990, White & Garrott 1990), to allow comparison with Webb & Shine (1997a). We imported layers (on vegetation types, elevation, waterways and roads and access points) to facilitate visual interpretation of habitat types.

### *Analyses of macrohabitat use by snakes*

Using Student's two sample *t*-tests, we compared tree characteristics (number of hollows, tree diameter at breast height [DBH; mm]) and tree height (m) in the vegetation structure types most often used by snakes ("Hawkesbury–Hornsby plateau exposed woodland" and "Mellong sandmass dry woodland"; see home ranges in Results section) to those in a widespread adjacent but non-used vegetation type ("Hawkesbury sheltered dry forest"; see home ranges in Results section).

### *Analyses of microhabitat use by snakes*

We compared the following characteristics of trees used by snakes to those of five nearby (unused) trees: species, alive or dead, DBH (mm), tree height (m), the number of visible hollows large enough to accommodate snakes, and height above-ground of the lowest hollow (m).

We used a generalized mixed effects model (GLMM: McCulloch & Searle 2001) in the R package 'nlme' (Pinheiro et al. 2011) with a binomial distribution to compare the characteristics of used trees to nearby trees, with individual snake being the random variable. GLMMs account for the non-independence of multiple measurements from each snake in resource selection models (Gillies et al. 2006). To assess which models best fitted the data, we ranked models, using all combinations of the variables, by the AICc. Any model with a $\Delta$AICc $< 4$ was considered a good fit to the data (Burnham & Anderson 2002).

Some arboreal retreat sites were inaccessible to us, but where feasible we measured thermal regimes inside used and unused hollows by attaching thermal data loggers (iButtons, Maxim Integrated, Sunnyvale, CA, USA) to lengths of wire, and inserting these as far as possible (typically, 10–50 cm) into hollows. We compared thermal data collected from used *versus* unused hollows with a repeated-measures ANOVA, with time of day as the repeated measure, and year and "used or not" as factors. We also compared maximum temperatures experienced within hollows of used versus unused trees using a Student's paired *t*-test.

*Seasonal shifts in thermal regimes within retreat sites*

Our radio-tracked snakes consistently used crevices beneath sun-warned rocks as winter retreat sites, and hollows within trees as spring/summer retreat sites. Thus, we compared thermal regimes under rocks with those in tree hollows (in both summer and winter), to compare the conditions that are available to snakes inside these types of shelter-sites at different times of year. We used thermal data loggers to measure temperatures under five rocks and four tree hollows (see above) used by *H. bungaroides* throughout 2010, 2011 and early 2012. We used one-way ANOVA to compare the total number of hours over both summers that 17 tree hollows and 16 rocks exceeded 32 °C, the VTMax (Voluntary Maximum Temperature) for *H. bungaroides* (Webb & Shine 1998b). The CTMax (Critical Maximum Temperature) has not been determined for *H. bungaroides*, so our analyses of this parameter were based on an estimate for the closely-related tiger snake, *Notechis scutatus* (38.0 °C: Greer 1997, Keogh et al. 2000).

*Spatial ecology in the north versus the south of the species' range*

We compared home range size, mean distance per move and total distance moved of snakes tracked in the north to those of snakes tracked in the south during a study conducted over the spring/summer of 1992–93, 1993–94 and 1994–95 (Webb & Shine 1997a).

## Results

*Analyses of snake movements*

We found no significant effect of sex or year on total distances moved by our radio-tracked snakes (sex: $F_{1,5} = 1.64$, $P = 0.26$; year: $F_{1,5} = 2.71$, $P = 0.16$; Fig. 1a), nor on mean distance per move (sex: $F_{1,5} = 3.59$, $P = 0.12$; year: $F_{1,5} = 0.37$, $P = 0.57$; Fig. 1b), interval between successive moves (sex: $F_{1,5} = 0.24$, $P = 0.65$; year: $F_{1,5} = 0.10$, $P = 0.77$; Fig. 1c) or moves per tracking day (sex: $F_{1,5} = 0.05$, $P = 0.83$; year: $F_{1,5} = 0.44$, $P = 0.54$; Fig 1d). No interactions between year and sex were statistically significant (i.e., all $P > 0.05$) in any of the above analyses.

*Analyses of macrohabitat use by snakes*

During summer, our radio-tracked snakes remained within two specific macrohabitat types: the "Hawkesbury–Hornsby plateau exposed woodland" and "Mellong sandmass dry woodland" (Fig. 2) where they used hollows in a variety of tree species, most notably red bloodwood, yellow bloodwood, grey gum, scribbly gum, narrow-leaved stringy bark and stags (standing dead trees). The snakes avoided the adjacent "Hawkesbury sheltered forest" (Fig. 2) which contained thinner trees (based on DBH, $t = 5.44$, df = 270, $P < 0.001$; Fig. 3a) with fewer hollows per tree ($t = 8.16$, df = 218, $P < 0.001$; Fig. 3b), but

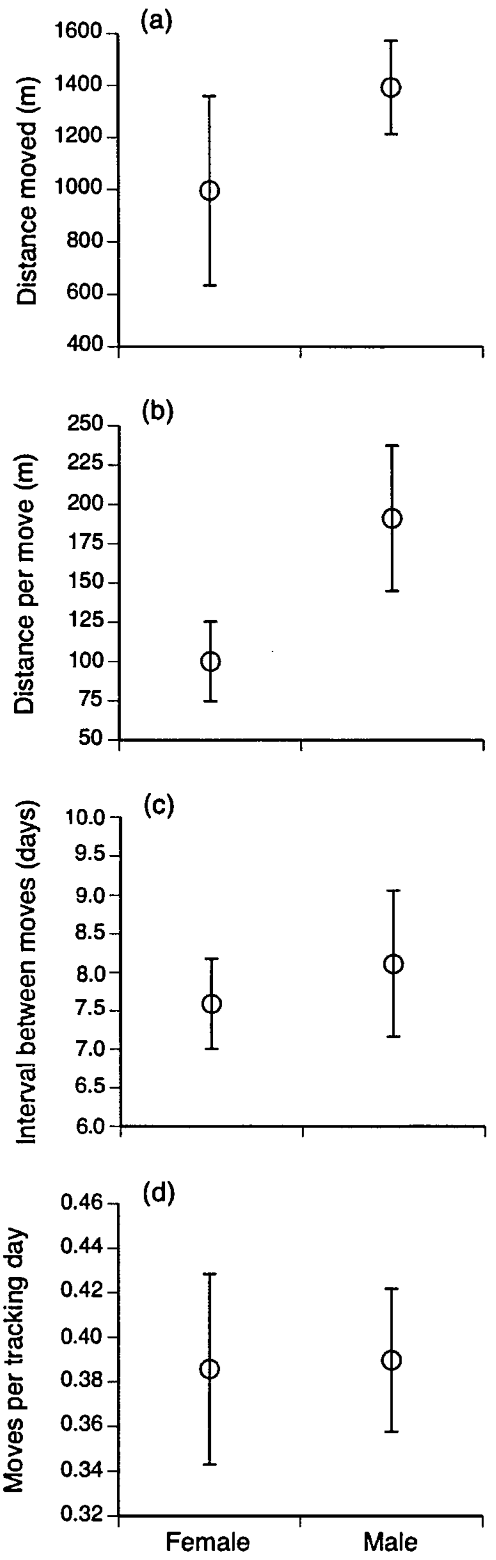


**Figure 1:** *(a) Mean total distance (in metres) moved by male and female broad-headed snakes over the entire period they were tracked. (b) Mean distance moved in metres per location shift by male and female broad-headed snakes. (c) Mean number of days between location shifts by male and female broad-headed snakes. (d) The number of moves per tracking day for male and female broad-headed snakes. Graphs show mean values and associated standard errors. All data collected over the Australian spring and summer seasons of 2010/2011 and 2011/2012.*

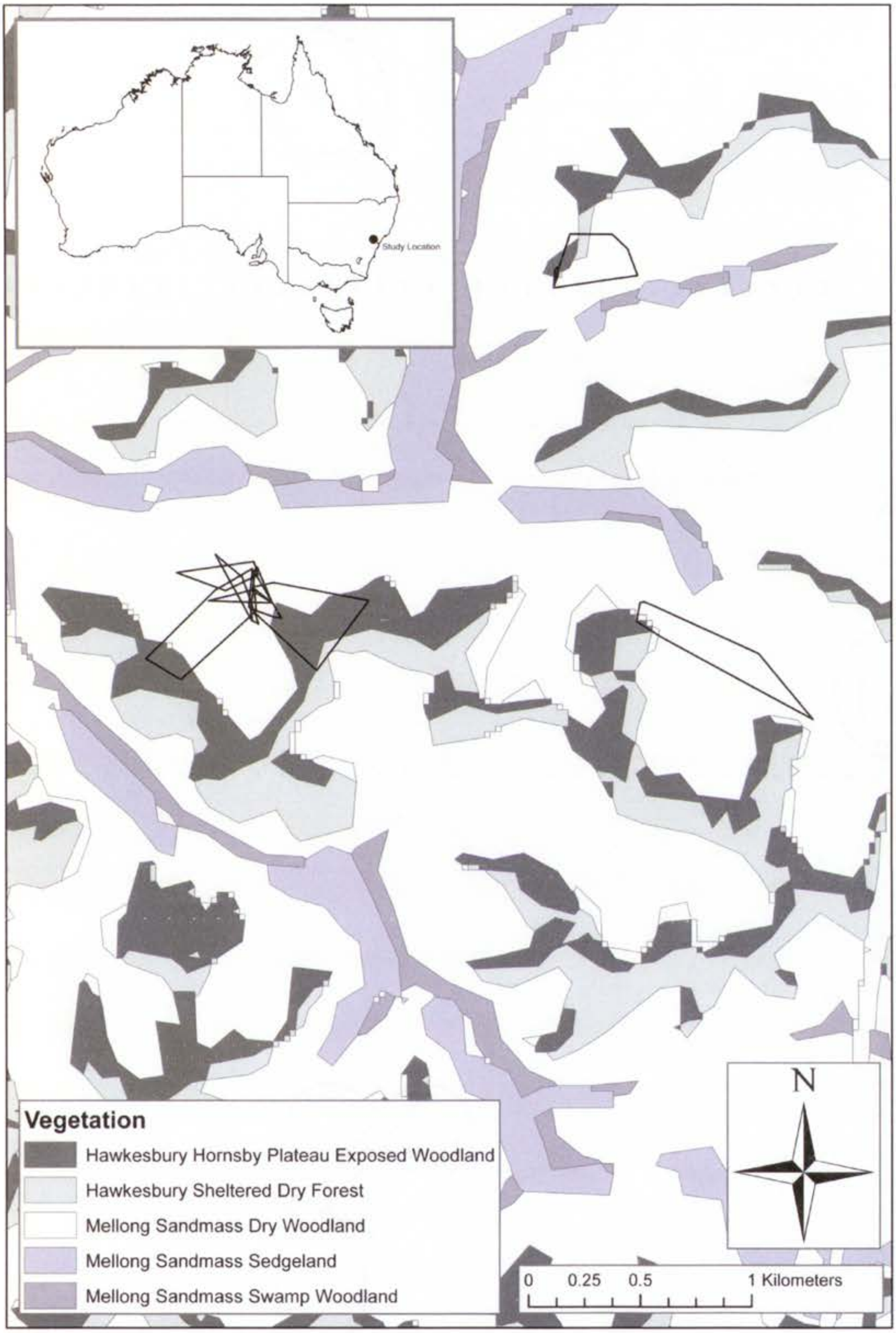


**Figure 2:** *Summer home ranges of five adult female and three adult male broad-headed snakes that we radio-tracked. The home range boundaries were estimated using the minimum convex polygon method, with vegetation layer overlaid. All snakes that were tracked remained within "Mellong sandmass dry woodland" and "Hawkesbury–Hornsby plateau exposed woodland" and avoided all other vegetation types.*

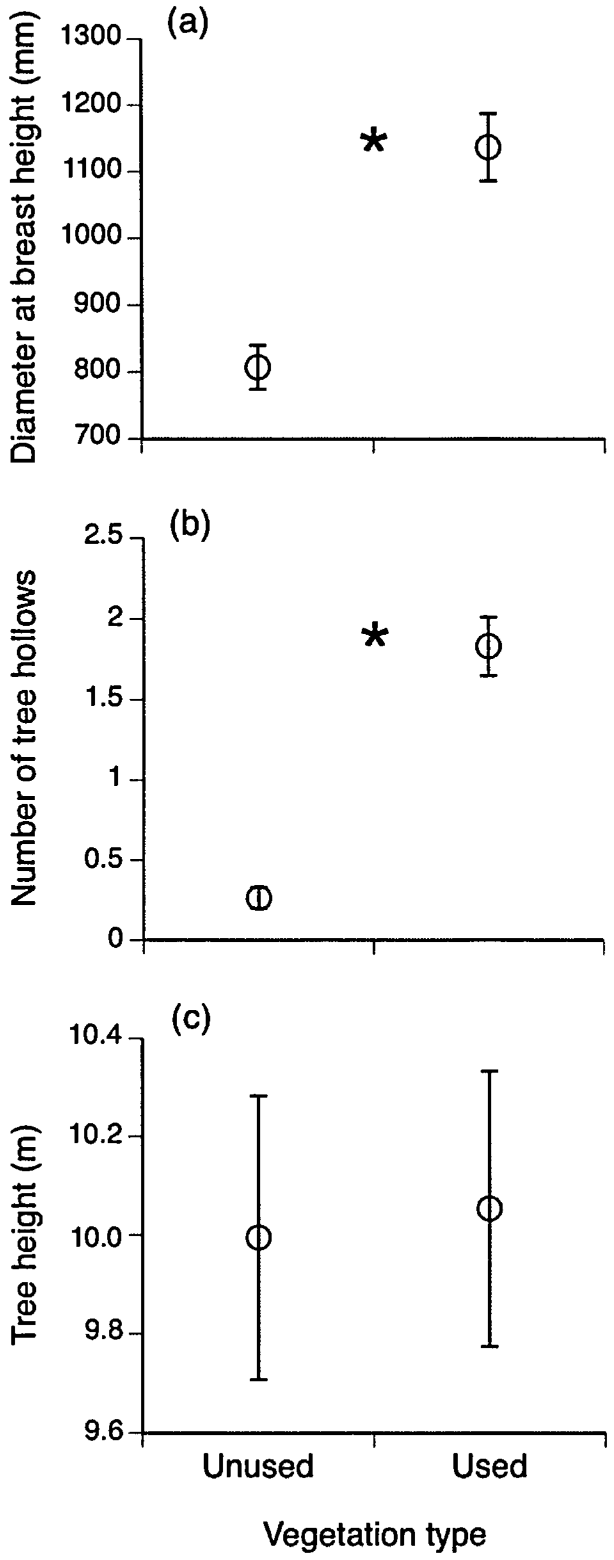


**Figure 3:** *Characteristics of trees in vegetation types that were either used by radio-tracked broad-headed snakes ("Mellong sandmass dry woodland" and "Hawkesbury–Hornsby plateau exposed woodland") or were not used by our snakes ("Hawkesbury sheltered dry forest"). In the preferred macrohabitat types, tree diameter at breast height (a) was greater and trees had more hollows (b). Mean tree height (c) did not differ between used and unused vegetation types. Graphs show mean values and associated standard errors. * indicates a statistically significant difference.*

which were similar in mean height ($t = 0.146$, df = 247, $P = 0.884$; Fig. 3c) to those in the preferred habitat types.

### *Analyses of microhabitat use by snakes*

A comparison between used and adjacent unused trees (all within the same habitat type) showed that snakes sheltered within a non-random subset of trees with respect to several variables. Our analysis of all combinations of variables produced 65 models, with 7 in the 95% confidence set ($\Sigma w_i = 0.95$). Only 4 models had a $\Delta$AICc < 4. Compared to availability, snakes selectively used dead trees that were wider at the diameter at breast height, shorter and had many hollows relatively close to ground level (Table 2). The species of tree appeared to be less important than these structural features, with tree species not appearing in any of the highly-ranked models.

We found no significant difference in mean temperatures between used versus unused tree hollows ($F_{1,36} = 0.10$, $P = 0.75$), and no significant thermal difference between years ($F_{1,36} = 0.65$, $P = 0.43$; interaction NS also). Temperatures within a tree hollow shifted with time of day ($F_{11,26} = 19.31$, $P < 0.001$; Fig. 4), with a significant interaction between time of day and year ($F_{11,26} = 7.63$, $P < 0.001$: the summer of 2011–2012 was cooler than that of 2010–2011; Fig. 4). Maximum temperatures were higher in unused tree hollows than in hollows of used trees ($t = 2.25$, df = 15.9, $P = 0.02$).

### *Seasonal shifts in thermal regimes within retreat sites*

Mean temperatures under rocks differed from those inside tree hollows ($F_{1,13} = 22.01$, $P < 0.001$; Fig. 5) and were higher in summer than in winter ($F_{1,13} = 442.40$, $P < 0.001$; Fig. 5). Temperatures also differed with time of day ($F_{1,13} = 106.932$, $P = 0.001$; Fig. 5), but

**Table 2:** *Coefficients of the four best generalized mixed models and standard errors, with AICc values, change in AICc values (ΔAICc) and Akaike weight ($w_i$).*

| Intercept | Alive or Dead | DBH | Hollow Height | # Hollows | Tree Height | AICc | ΔAICc | $w_i$ |
|---|---|---|---|---|---|---|---|---|
| -4.114 | + | 0.002 ± 0.001 | - | 0.415 ± 0.145 | -0.198 ± 0.102 | 94.499 | 0 | 0.425 |
| -4.223 | + | 0.002 ± 0.001 | -0.086 ± 0.124 | 0.451 ± 0.154 | -0.185 ± 0.104 | 96.169 | 1.670 | 0.184 |
| -5.425 | + | 0.001 ± 0.001 | - | 0.327 ± 0.134 | - | 96.672 | 2.173 | 0.143 |
| -5.495 | + | 0.002 ± 0.001 | -0.134 ± 0.123 | 0.394 ± 0.148 | - | 96.934 | 2.435 | 0.126 |

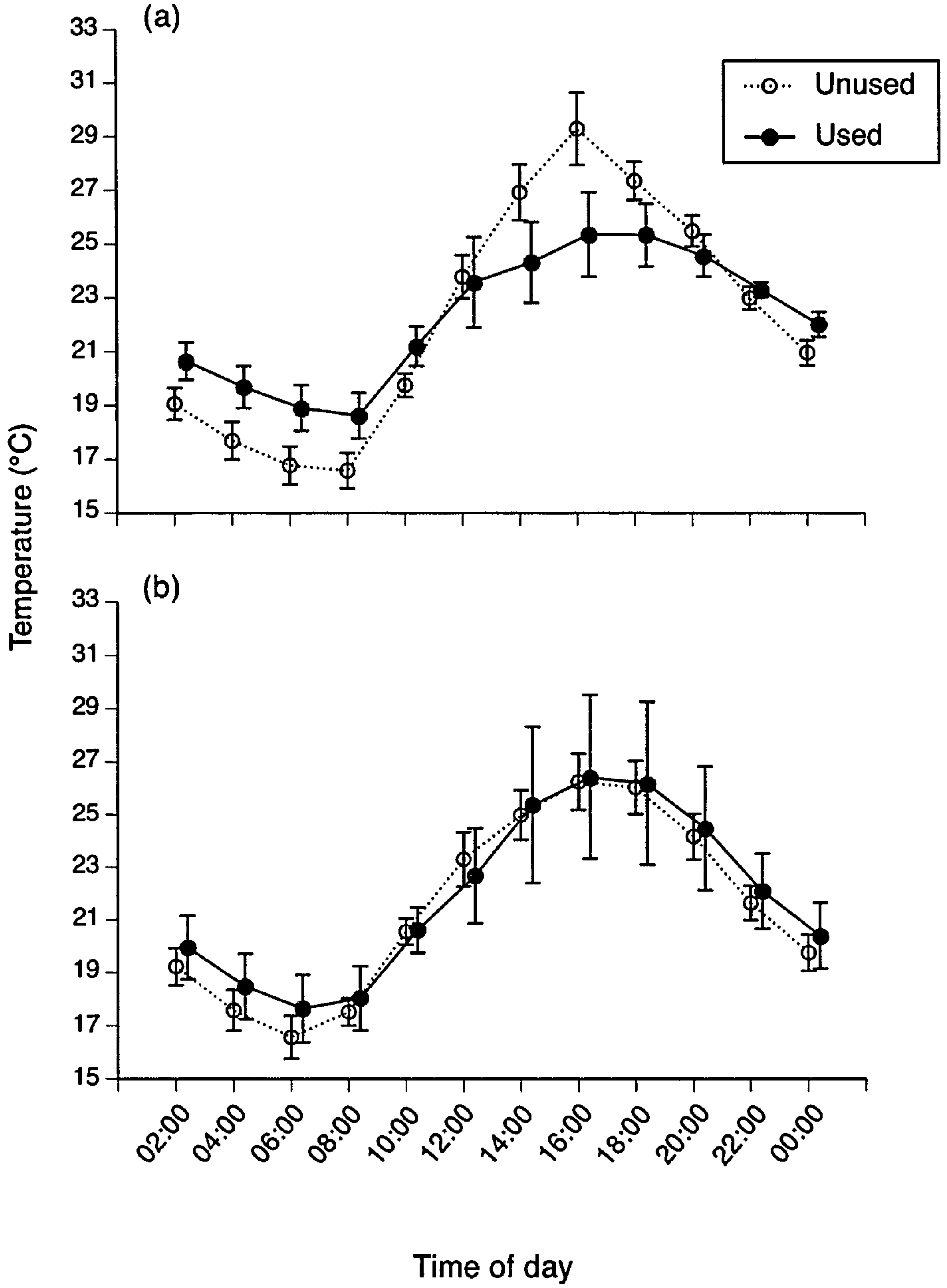


**Figure 4:** *Thermal regimes in retreat sites and potentially available retreat sites. Panel (a) shows the temperature cycle on a hot day in the summer of 2010/2011 and (b) shows the same cycle on a hot day in the (cooler) summer of 2011/2012. Graphs show mean values and associated standard errors.*

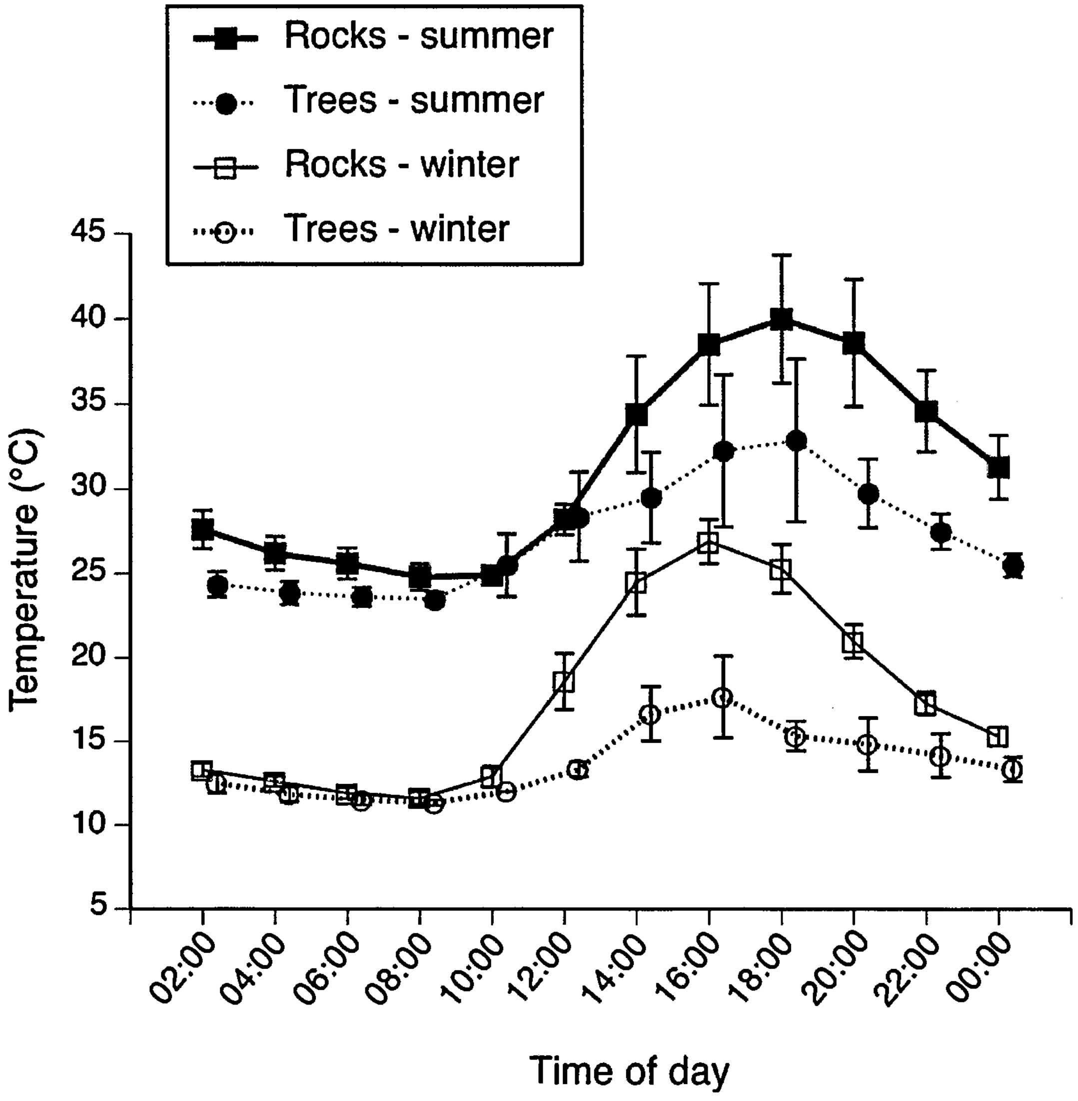


**Figure 5:** *Mean maximum ambient temperatures experienced under rocks (winter habitat for broad-headed snakes) and in tree hollows (summer habitat for broad-headed snakes) during winter and summer. By shifting habitat between winter and summer, these snakes experience relatively stable thermal regimes and avoid extreme temperatures.*

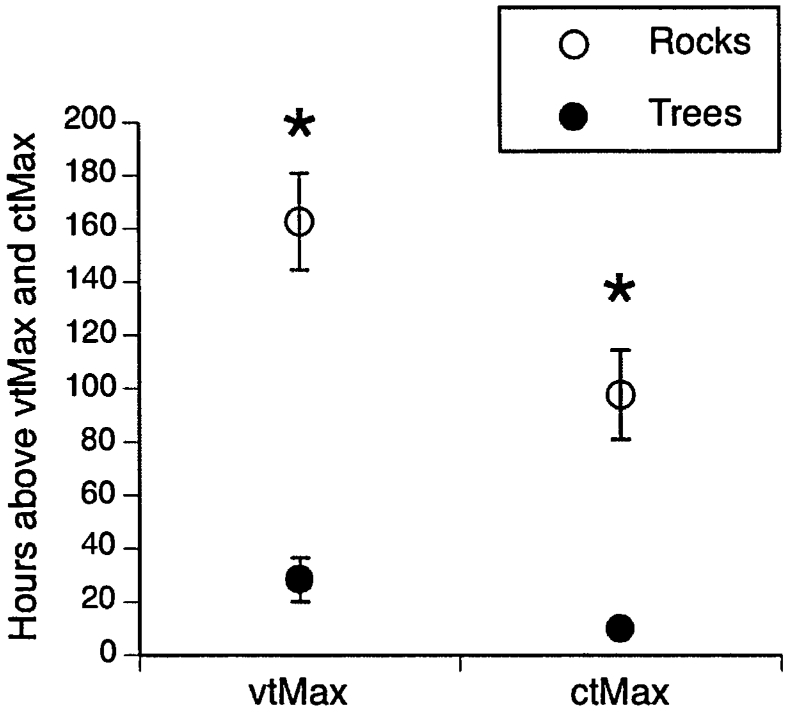


**Figure 6:** *Thermal extremes in snake retreat sites during the summer (1 December to 28 February) of 2010/2011. The graph shows the total number of hours at which retreat site temperatures were above the voluntary thermal maximum (VTMax, 32.5 °C) and estimated critical thermal maximum (CTMax, 38.0 °C) of broad-headed snakes. Data are shown separately for two habitat types used by those snakes: those used in summer (tree hollows) and in winter (under rocks). Rocks often exceeded both VTMax and CTMax in summer, whereas tree hollows exceeded these limits only rarely. * indicates a statistically significant difference.*

with no significant interaction between time of day and habitat type or time of day and season (all $P > 0.05$). The number of hours during summer that CTMax ($F_{1,33} = 20.12$, $P < 0.001$) and VTMax ($F_{1,33} = 32.19$, $P < 0.001$) were exceeded was higher under rocks than in tree hollows, with rocks often exceeding those thermal limits (Fig. 6).

*Spatial ecology in the north versus the south of the species' range*

Home range size did not differ significantly between northern and southern populations of *H. bungaroides* ($F_{1,33} = 0.035$, $P = 0.852$), nor did mean distance per move ($F_{1,33} = 0.813$, $P = 0.375$), nor total distances moved ($F_{1,33} = 0.518$, $P = 0.476$).

## Discussion

Our data reveal many similarities between *H. bungaroides* in southern populations (as previously studied) and those in the north of the range (the subject of our current work). For example, our radio-tracked snakes showed the same seasonal shift in habitat types, from rock crevices in winter to tree hollows in summer. Our data on thermal regimes within those habitat types support Webb and Shine's (1997a) hypothesis that these seasonal shifts are driven by temperature. By moving between these habitat types seasonally, the snakes had access to relatively consistent thermal conditions inside refuges year-round (Fig. 5). Trees (summer habitat) are too cool to allow snakes to thermoregulate in winter, whereas sun-exposed rock crevices (winter habitat) attain lethally high temperatures in summer (see Figs 5 and 6). During the entire summer of 2010/2011, ambient temperatures in crevices (both under randomly-chosen rocks, and those actually used by *H. bungaroides* as winter retreat sites) exceeded the broad-headed snake VTMax for an average of 162.6 hours, and exceeded the snakes' estimated CTMax for an average of 97.6 hours. In contrast, the temperatures that we measured inside randomly-chosen and used tree hollows exceeded VTMax for an average of only 28.3 hours, and exceeded CTMax for 9.8 hours (Fig. 6). Trees that were used by snakes never exceeded CTMax, and exceeded VTMax for only 9 hours throughout the summer. The hollows used by snakes were cooler then nearby unused hollows during the hottest parts of the day (see Fig. 4). We observed snakes basking outside tree hollows, and signal directions suggested that snakes moved up and down hollow limbs and trunks depending on ambient temperature. That mobility suggested active temperature selection by snakes within tree-hollows, similar to southern populations of the same species (Webb & Shine 1997b) and the more northerly-distributed congener, Stephen's banded snake (*H. stephensii*: Fitzgerald et al. 2005). The smaller size of rocks precludes this kind of active behavioural thermoregulation, so the snakes' shift to tree hollows in the warmer months of the year may reflect the advantages of greater accessible spatial thermal heterogeneity, as well as lower mean temperatures (Webb & Shine 1997b).

In terms of spatial ecology, the snakes we tracked in the extreme north of the species' range were similar to conspecifics in southern populations. As well as the thermally-driven shifts in habitat noted above, mean home range sizes were similar for snakes in both regions (3.3 ha versus 3.0 ha: Webb & Shine 1997a), as were mean movement distances between successive movements (134 m versus 159 m: Webb & Shine 1997a), the active selection of trees with many hollows and relatively large DBH (Webb & Shine 1997b), and fidelity for specific trees (Webb & Shine 1997b). Like the southern snakes (J. K. Webb, unpublished data), our radio-tracked northern snakes sometimes were encountered under bushes and in hollow logs on the forest floor.

However, our data also identify some points of difference between snakes from the northern versus southern clades. In summer, southern conspecifics used trees on top of plateaus and below cliffs (Webb & Shine 1997a) whereas snakes in our northern study areas moved into shallow valleys away from rock outcrops, and occupied specific vegetation types while avoiding others. No such selectivity at this macrohabitat level has been recorded for the southern population, perhaps reflecting more homogeneous forest-habitat types in that region. The northern snakes showed several points of similarity with *H. stephensii*, an arboreal congeneric species that occurs north of the range occupied by *H. bungaroides* (Cogger 2000, Fitzgerald et al. 2005). Our study sites were close to the southern limit of *H. stephensii* distribution, and thus experience climatic conditions more similar to those encountered by southern *H. stephensii* populations than southern *H.*

*bungaroides* populations. Thermal regimes may drive similarities such as occasional rock use by *H. stephensii* (Wilson & Swan 2010), as well as similar periods of sequestration inside retreat sites (mean of 7.9 days for *H. bungaroides* versus 8 days for *H. stephensii*: Fitzgerald et al. 2002a). The southern *H. bungaroides* studied by Webb and Shine (1997a) moved much more frequently (mean of 2.9 days: Webb & Shine 1997a), suggesting that higher ambient temperatures may restrict the frequency of movement. Some specific trees were used by different individual snakes (both simultaneously and at different times) in our study, and snakes also used leaf litter as retreat sites. Both of these patterns were noted for *H. stephensii* (Fitzgerald et al. 2002b), but rarely for southern *H. bungaroides* (Webb & Shine 1997a, 1997b). In combination, these trends suggest that suitable arboreal shelter-sites may be more limiting in the north, such that in hot weather snakes may re-use a limited set of tree hollows, or else abandon arboreal sites for the cooler leaf litter.

The snakes that we radio-tracked used different species of trees for shelter than did the previously-studied southern population of *H. bungaroides* or *H. stephensii*, but this difference is most parsimoniously attributed to geographic differences in forest composition. In support of that inference, tree species seemed to have less effect than tree structure in determining frequency of use by snakes. The four generalized mixed models with best fit to our data (Table 2) suggested that snakes prefer trees that were dead, with a large DBH but were also fairly short with many hollows close to the ground. In contrast, the tallest available trees were preferred by both southern-clade *H. bungaroides* and more northern *H. stephensii* (Webb & Shine 1997b, Fitzgerald et al. 2002b). That difference in tree height may explain an otherwise-puzzling discrepancy between our study and the earlier work: that is, we saw snakes basking in exposed positions on trees a total of 9 times (including 5 different snakes), whereas this behaviour was rarely observed in the studies on other *Hoplocephalus* (Webb & Shine 1998b, Fitzgerald et al. 2002b). Overt basking may be more likely on shorter trees (because they tend to be mostly in shade due to adjacent taller trees); and also, basking may be easier to observe if the snakes are closer to the ground (and thus, to the observer).

During our study, three trees were used by more than one snake, with up to four individual snakes using the same tree throughout both summers and the winter tracking period. Two of these trees were inhabited by two snakes simultaneously, a phenomenon also observed (albeit rarely) in *H. stephensii* (Fitzgerald et al. 2002b) but not in southern clade *H. bungaroides*. Northern clade *H. bungaroides* showed strong site fidelity, as also reported in southern clade *H. bungaroides* and *H. stephensii*. We observed five snakes return to the same trees, with one snake using the same tree on four occasions throughout the summer of 2010/2011. Such re-use suggests that trees with suitable hollows are a limiting resource for snakes in this system, however more work is needed to investigate this.

Broadly, the habitats used by our northern-clade *H. bungaroides* were similar to those used by southern-clade conspecifics in winter. The reliance on hollow-bearing trees during summer is shared not only by the two *H. bungaroides* clades, but also by two congeneric arboreal taxa (*H. stephensii* and *H. bitorquatus*, the pale-headed snake: Fitzgerald et al. 2002a, 2002b). Although detailed studies are lacking, arboreality and tree-hollow use also are likely to be important for the most closely related outgroup taxa to *Hoplocephalus* – *Tropidechis carinatus* (the rough-scaled snake) and *Paroplocephalus atriceps* (the Lake Cronin snake: Keogh et al. 2000, 2003, Sanders et al. 2008).

Phylogenetic reconstructions suggest that *H. bitorquatus* most closely resembles the ancestral *Hoplocephalus* species (Keogh et al. 2003); hence, year-round arboreality may be an ancestral trait for this lineage. The expansion of the southernmost taxon (*H. bungaroides*) into cooler areas rendered local tree-hollows too cool for foraging in winter, plausibly stimulating a behavioural shift towards rock-crevice use during cooler times of year.

Our results have direct implications for conservation and management of the genetically distinct northern clade of *H. bungaroides*. First, the population that we studied relies upon both rock outcrops (in winter) and nearby forests (in summer), so management needs to conserve that combination of habitat types in close proximity. That requirement is similar to that for southern conspecifics, whereas rock crevices appear to play only a minor role in the ecology of the other *Hoplocephalus* species (Fitzgerald et al. 2002b). In terms of conserving rock-outcrop habitats, attention needs to focus not only on human disturbance to local areas (especially, rock theft for landscaping, and illegal collection of animals for the pet trade: Shine et al. 1998, Webb et al. 2002b) but also on broader landscape-scale processes. Analyses of historical photographs, and long-term field studies, have shown that vegetation overgrowth imperils *H. bungaroides* at the southern study sites (Pringle et al. 2003). Removal of shading vegetation significantly enhanced habitat quality for *H. bungaroides* in this area (Pike et al. 2011). We have no equivalent data for the northern-clade populations, but they may well be under similar threats (e.g., illegal rock collection is rife: B. Croak, unpublished data). Thus, management should prioritize retention of existing surface rock, and mitigation of processes that facilitate vegetation overgrowth (Shine & Fitzgerald 1989, Shine et al. 1998, Pringle et al. 2003, Croak et al. 2008, Pike et al. 2011).

Because *H. bungaroides* show such a profound seasonal shift in habitat use, we also need to maintain large forest blocks that contain hollow-bearing trees, in areas adjacent to sandstone outcrops (Webb & Shine 1997b, Fitzgerald et al. 2005). For the northern population, that forested area should lie within vegetation types such as "Hawkesbury–Hornsby plateau exposed woodland" and "Mellong sandmass dry woodland", rather than other locally occurring forest types. The preferred macrohabitats may be distinctive because they contain a relatively high number of trees with large hollows suitable for retreat site use by *H. bungaroides*. The same types of hollows are used by many other arboreal taxa, emphasizing the importance of this critical habitat for a wide variety of species (Gibbons & Lindenmayer 2002, Gibbons et al. 2002). Thus, forestry management plans should aim to conserve tree hollows. In Australia, managed landscapes generally support less than half the number of hollow-bearing trees as occur in natural stands (Gibbons & Lindenmayer 2002). This issue may be especially critical for the northern populations of *H. bungaroides*, because their frequent re-use of the same tree hollows, and use of those hollows by multiple animals, suggests that such trees may be a limiting resource in this system.

The spatial extent of reserves to protect northern-clade *H. bungaroides* is likely to be similar to that needed for their southern-clade conspecifics. Movement patterns of northern *H. bungaroides* were broadly similar to those of their southern conspecifics. Rates of gene flow also are likely to be similar in the two clades (Dubey et al. 2011), with a complex metapopulation structure that includes unidirectional gene flow from source to peripheral sink populations (Dubey et al. 2011). Identification of source and sink populations through genetic investigation within the northern range of this endangered species would facilitate effective conservation and/or habitat restoration (Croak et al. 2010, 2012, Dubey et al. 2011).

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