Shelter aggregations, social behaviour, and seasonal plasma corticosterone levels in captive and wild Duvaucel's geckos, *Hoplodactylus duvaucelii*

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"Whatever you do will be insignificant,
but it is very important that you do it. "

Mahatma Gandhi (1869 - 1948)

Abstract

The development of effective conservation management strategies is reliant on a thorough understanding of the basic biology and life history of the species of concern. New Zealand's endemic herpetofauna species have undergone severe range contractions since human arrival due to habitat modifications and predation by introduced mammalian pests. Current conservation management practice involves the eradication of such pests as well as the restoration of habitat involving native species reintroductions. Albeit these conservation attempts, detailed information on physiology, ecology and behaviour of most New Zealand's lizards is scarce, including the Duvaucel's gecko (*Hoplodactylus duvaucelii*). In recent years, several *H. duvaucelii* have been translocated to islands within their historical range as part of restoration projects and in 2006/7 a captive breeding-for-release programme has been established. This provided an excellent opportunity to explore some fundamental aspects of *H. duvaucelii's* basic physiology and behavioural ecology. I investigated seasonal plasma corticosterone concentrations of captive and wild geckos in relation to several physiological and ecological factors to enhance our understanding of natural fluctuations of seasonal hormone patterns. Such information can aid in evaluating stress related changes in hormone patterns of individuals and populations, which is particularly relevant for the captive management. I compared plasma steroid hormone patterns of captive geckos from a breeding colony with their wild source population over several seasons to assess whether captive lizards suffered from chronic stress, which could compromise their wellbeing and the fitness of future offspring. Further, I explored several aspects of *H. duvaucelii's* social behaviour, including diurnal shelter aggregations, scent communication and social interactions using experimental and exploratory studies on captive and wild geckos. The hormone study provided evidence that *H. duvaucelii* are suitable for captive breeding as they seem to have adapted well to captivity and did not show a chronic elevation of stress hormone levels. Moreover, my research has shown that *H. duvaucelii* are essentially social lizards that form shelter aggregations year-round. Adults showed a high tolerance of juveniles in their diurnal shelters and geckos were able to detect and discriminate scents from conspecifics in several social contexts. These social traits as well as the year-round occurrence of male-female pairs combined with *H. duvaucelii's* life history traits suggest that this species may possess a social system of high complexity. In conclusion, this dissertation provides a foundation for future research and delivers the first insight into the social behaviour and basic endocrinology of this New Zealand endemic lizard.

Foreword

This dissertation explores some fundamental aspects of the endocrinology and behavioural ecology of *Hoplodactylus duvaucelii,* a New Zealand endemic gecko. Chapter One introduces the reader to the main objectives of this dissertation and provides information on the study species and the central areas this research has focused on. The data Chapters Two to Six are based on manuscripts that were written as independent scientific research papers intended for publication in relevant peer reviewed journals. Each data chapter includes a detailed introduction providing specific background information on the respective research topic. Due to this approach some repetition was inevitable. However, chapters were cross-referenced wherever possible and all references and appendices were combined at the end of this dissertation. The final conclusion Chapter Seven synthesizes all findings and discusses future directions.

The research presented in this dissertation has been designed, implemented, analysed and written by myself. I received significant guidance and advice by my supervisors Dianne Brunton, Uri Shanas and Weihong Ji who provided valuable input on data analyses and essential feedback on the write-up process.

Chapter Two is based on a research paper published in the *Australian Journal of Zoology* (http://www.publish.csiro.au/nid/90/paper/ZO10023.htm, doi: 10.1071/ZO10023) with co-authors Dianne Brunton and John Cockrem. John Cockrem contributed significantly to this study by providing hormone kits and conducting all necessary hormone assays to analyse the plasma samples. Both co-authors provided advice on the data interpretation and manuscript preparation. I have conducted the field work, collected all blood samples used for the hormone analysis, carried out the data analyses and wrote the paper.

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CHAPTER ONE

General Introduction

The ultimate goal of conservation biology is to maintain the world's biodiversity. The development of effective management strategies requires detailed knowledge of the organisms as well as a thorough understanding of the underlying physiological, behavioural and evolutionary processes that shape individuals, populations, species and communities within biological systems (Vitt and Caldwell 2009). On-going threats are largely human induced. Environmental alteration including habitat destruction, pollution, spread of exotic species and diseases and global climate change lead to the inevitable loss of tens of thousands animal and plant species every year (Eldredge 1998, Wilson 1992).

New Zealand's unique endemic herpetofauna is no exception to this scenario. Since human arrival many species have become extinct on the mainland and currently only survive in remnant offshore island habitats. Present conservation management practice in New Zealand involves the eradication of introduced mammalian pest species, particularly rodents, from offshore islands and other conservation areas. This enables the recovery of relict lizard populations, and allows reintroductions of species of conservation concern into their former range. One central tool for species management is the translocation of organisms into restored habitats, whereby animals are either harvested from wild sustainable populations or sourced from captive bred stock (Towns and Ferreira 2001, Blanchard 2002, Collen *et al.* 2009, Sherley *et al.* 2010). Such translocations rely on the availability of viable source populations that can supply sufficient numbers of animals to establish a new self-sustaining population in the target area. Translocations are often entirely experimental, as detailed information on the basic biology, life-histories, and ecological significance of the species of concern is scarce. In addition to the harvest of wild lizards, captive breeding programmes have been established (Collen *et al.* 2009) to supplement existing populations or to (re)introduce populations into restored habitat.

Captive breeding programmes offer an excellent opportunity to study various aspects of an animal's biology. This is particularly useful when there are inherent difficulties in obtaining data from wild living organisms or populations that have an elusive lifestyle and a limited accessibility. *Hoplodactylus duvaucelii* (Dumeril and Bibron 1836), a cryptic nocturnal gecko, is such a range restricted endemic reptile, for which many aspects of its biology are unknown. In recent years small numbers of individuals (19 to 40) have been reintroduced to island habitats within their geographical range as part of ecological restoration projects (Jones 2000, van Winkel *et al.* 2010) and a captive breeding programme has been established.

In this dissertation I investigated fundamental aspects of the physiology and behavioural ecology of *H. duvaucelii*. To address questions concerning the social behaviour and effects of captivity on this New Zealand endemic gecko, I conducted a series of observations, measurements and tests involving a captive breeding colony and a wild population.

Conservation and biology of *Hoplodactylus duvaucelii*

Conservation status and management

Fossil deposits provide evidence that *H. duvaucelii* was once widespread throughout New Zealand (Worthy 1987, Worthy and Holdaway 1995, Worthy 2001). The introduction and rapid spread of small predatory land-mammals, particularly rats, in the past 1000 years since human settlement led to severe range contractions and extinctions of the local herpetofauna on the mainland (Towns and Dougherty 1994). The extant distribution of *H. duvaucelii* is limited to offshore islands, most of which are rat-free, along the east coast of New Zealand's North Island and in the Cook Strait (Towns and Daugherty 1994).

While *H. duvaucelii* populations are range restricted, the local abundance of such populations is often high (van Winkel 2008, Wilson 2010). *H. duvaucelii* have been evaluated by the New Zealand Department of Conservation (DOC) as 'at risk' and are listed under the DOC Threat Classification System as 'relict' with moderate to large populations, but 'conservation dependent' (Hitchmough *et al.* 2010). The status of *H. duvaucelii* in the IUCN Red list of threatened species is 'Lower Risk/least concern', however a revision of this classification is required (IUCN 2010).

Since 1998, 79 *H. duvaucelii* were translocated to three islands. In 1998, 40 adults and sub-adults sourced from North Brother Island were released on Mana Island (Jones 2000) and in 2006 a further 39 adults were translocated from Korapuki Island to Motuora (20) and Tiritiri Matangi Islands (19) (van Winkel *et al.* 2010). In conjunction with the latter two translocations a further 30 geckos (sourced from Korapuki Island) were transferred to a reptile facility at the Massey University Albany Campus, Auckland to set-up a long-term breeding-for-release programme (van Winkel *et al.* 2010).

Biology

Pioneer studies provided valuable insights into the life-history, reproductive biology and feeding ecology of *H. duvaucelii*, revealing traits that are remarkable and highly unusual compared to other gekkonid lizards worldwide. *H. duvaucelii* is New Zealand's largest extant gecko, attaining a total body-size of up to 32 cm (≥160 mm snout-to-vent-length, SVL). This cryptically coloured gecko is extremely long-lived (at least 43 to 50 years, Wilson 2010) and has a low annual reproductive output of estimated 1.12 offspring per female (Cree 1994). Females are viviparous, and give birth to one or two large live young after an extended gestation period of 5 to 8 months or in some instances possibly longer than a year (Barwick 1982, Cree 1994). Juveniles are slow growing and may reach sexual maturity approximately in their 7th year (Barwick 1982). *H. duvaucelii* is a habitat generalist showing terrestrial, arboreal and saxicolous habits (Whitaker 1982).

While the main period of activity is at night, the geckos may occasionally leave their diurnal shelters to sun bask (Whitaker 1968). *H. duvaucelii* actively forage at night on invertebrates, fruit, nectar or honey dew from endemic scale insects *Coelostomidia zealandica* (Hemiptera: Margarodidae) and may occasionally prey on smaller lizards (Barwick 1982, Christmas 1995, Towns 2002). The geckos are active year-round, even in low ambient temperatures (Barwick 1982). Anecdotal reports indicate that *H. duvaucelii* are tolerant of conspecifics and that several individuals may share diurnal retreats (Robb 1980, Christmas 1995). Furthermore, mark-recapture studies provide evidence that *H. duvaucelii* show high long-term site-fidelity (Thompson *et al*. 1992, Christmas 1995, Wilson 2010).

Recent research has predominantly focused on the species' conservation management. Studies have investigated habitat use, population structure and diet of *H. duvaucelii* in the presence and absence of rats (Christmas 1995, Hoare *et al.* 2007), dispersal patterns of individuals post translocation (Jones 2000, Van Winkel 2008) or assessed the viability of source populations, from which geckos where harvested for translocations (van Winkel 2008, Wilson 2010). However, virtually nothing is known about *H. duvaucelii's* behavioural ecology, social structure or mating system. Such knowledge is not only of great importance for effective conservation management and particularity relevant for captive breeding programmes, it may also provide important insights into lizard social systems.

Lizard social systems

Social systems of lizards are diverse and range from simple territoriality over prolonged monogamous relationships to complex sociality with stable family groups. The most basic and widespread system is territoriality, whereby a male typically defends optimal resources (e.g. food, shelter, mates) within a well-defined area, from other males (Brattstrom 1974). The level of territorial behaviour varies seasonally in some lizards and territoriality may cease completely in response to certain ecological factors. This can result in the formation of temporary aggregations. For example, winter aggregations may be formed to withstand less favourable environmental conditions during cooler seasons (Elfstroem and Zucker 1999), and feeding aggregations may occur due to a temporary overabundance of an otherwise limited food-resource (Ruby 1978). When population density is high and resources or dispersal opportunities are limited, individual territories can not be maintained and a hierarchical system may form (Brattstrom 1974). Such environmental constraints may promote long-term group formation and thus mediate increased social interactions which could ultimately provide a pathway for complex social behaviour.

A diverse range of lizard taxa permanently form shelter aggregations but often the temporal stability of such groups is unknown. One Australian scincid lineage (*Egernia*) contains several highly socially organised species, which form stable aggregations comprising of closely related individuals as well as breeding pairs that exhibit long-term social monogamy (Gardner *et al*. 2002, Chapple 2003, Gardner *et al.* 2007). A recent study by Davis *et al*. (2010) provided evidence that the xantusid lizard *Xantusia vigilis* also exhibits kin based sociality and forms stable family groups. The non-territorial Australian skink *Tiliqua rugosa* is typically solitary but males and females form monogamous pair-bonds several weeks before mating (Bull 2000). After mating, these pairs split up, just to reunite with the same partner in the following year (Bull 2000). Several nocturnal gecko species have been reported to form shelter aggregations

(Cooper 1985, Burke 1994, Kearney *et al*. 2001, Todd 2005, Meyer and Mouton 2007, Shah 2003), however, detailed information on the stability of such groups as well as genetic and social relationships among group members remain unknown.

Scope of study and thesis structure

The establishment of a captive breeding programme at Massey University, Albany Campus, in 2006 has offered an exceptional opportunity to study various aspects of *H. duvaucelii*'s biology. However, it also represented a challenge to accommodate wild lizards over the long term in a captive environment without detailed knowledge of their behavioural ecology. The success of the breeding programme requires the best possible conditions to ensure that the wellbeing of the animals and breeding patterns are not compromised.

Motivated by these challenges, I focused on the following objectives: Firstly, I aimed to explore seasonal plasma corticosterone of wild geckos in relation to several ecological and physiological factors to enhance current understanding of natural fluctuations of hormone patterns in *H. duvaucelii*. Knowledge of basic physiological parameters such as seasonal variation of steroid hormone levels (i.e. plasma corticosterone) can be crucial for evaluating stress related changes of hormone patterns in an individual or in a population. In **Chapter Two** I documented seasonal plasma corticosterone levels of wild *H. duvaucelii* and explored potential relationships of hormone levels with sex, body temperature, body condition and female reproductive condition. Further, I compared seasonal hormone data from the captive breeding colony with hormone patterns of the wild (source) population. The information on hormone patterns of the wild population represents not only a reference for future studies, it also allowed me to assess the wellbeing of our captive breeding colony (i.e. whether or not the colony suffered from chronic stress) by determining whether the captive geckos showed a chronic elevation of basal stress hormone levels in comparison to the wild source population. Furthermore, this study highlights the importance of a thorough understanding of the species social structure and behavioural ecology as – particularly in a captive environment – hormone levels of individuals may vary considerably in relation to social factors.

The other major focus of this study was the exploration of aspects of *H. duvaucelii's* behavioural ecology. Inspired by anecdotal reports, which suggest that *H. duvaucelii* form shelter aggregations (Robb 1980, Christmas 1995), I investigated in **Chapter Three** the diurnal spatial distribution patterns of wild geckos across seasons to determine whether *H. duvaucelii* showed a distinct tendency to share shelter sites. Additionally, I examined group composition and gecko body temperatures as well as shelter temperatures to evaluate the possible causation and function of such shelter aggregations. Information on the spatial distribution patterns of wild *H. duvaucelii* is not only of important value for their conservation management, it also adds to our understanding of social systems in lizards in general.

In conjunction with the investigation of aggregative behaviour in wild geckos (Chapter Three) I used captive *H. duvaucelii* to experimentally test in **Chapter Four** whether aggregations are likely to be resource driven i.e. are caused by a limited availability of high quality shelters or could be mediated by social factors such as conspecific attraction based on specific social traits. Preceding this I developed an optimised shelter type based on what structural and environmental shelter traits geckos favoured in choice experiments.

Chemosensory communication is a central aspect of lizard social behaviour and chemosensory abilities may provide clues about the underlying social ecology of a species. In **Chapter Five**, I investigated the ability of *H. duvaucelii* to detect and discriminate between scents from conspecifics. If animals form permanent stable groups, a recognition system would be expected that allows individuals to distinguish between group members and non-group members. The detection and recognition of conspecific scents could assist individual geckos to seek out or avoid conspecifics which could influence group composition or facilitate group cohesion. I used laboratory based scent choice experiments to test whether juvenile and adult *H. duvaucelii* can detect and discriminate between scents from conspecifics in relation to their sex, familiarity or relatedness.

Courtship and specific social interactions between males and females are fundamental components of the mating behaviour in many animal taxa. In **Chapter Six** I documented the repertoire of social behaviours exhibited by male and female geckos during experimentally staged intersexual interactions and investigated the effects of season and familiarity status on these interactions. This allowed me to determine *H. duvaucelii's* courtship behaviours. Based on the observed grouping patterns of wild and captive geckos (Chapter Three and Four), I tested whether male and female *H. duvaucelii* can become familiar with one another and thus adjust their behavioural responses according to their familiarity status. The ability to distinguish between familiar and non-familiar conspecifics, and particularly, a decreased level of agonistic interactions and higher frequency of contact behaviours between familiar pairs could be an indication that heterosexual pairs may form long-term associations.

Finally, in **Chapter Seven** I summarised the main findings of this thesis. Based on the information gained in Chapter Two, Three and Four I discussed implications for *H. duvaucelii's* captive management and provided suggestions for improvement. Further, I discussed my observations on *H. duvaucelii's* aggregation patterns, scent discrimination abilities and social behaviour (Chapters Three to Six) on the background of the species' life-history traits and highlighted *H. duvauceliis* potential as a model species to explore lizard social behaviour. I pointed out directions for further research not only relevant to *H. duvaucelii's* conservation management but also to further unravel the behavioural ecology of this species.

CHAPTER TWO

Seasonal variation in plasma corticosterone concentrations in wild and captive adult Duvaucel’s geckos (*Hoplodactylus duvaucelii*) in New Zealand

Abstract

Most of New Zealand's herpetofauna species are threatened and in decline. Yet, little is known about their basic physiology and endocrinology. This study examined plasma corticosterone (CORT) concentrations in a threatened endemic New Zealand reptile, the Duvaucel's gecko, in relation to season, sex, body temperature, body condition and female reproductive condition (pregnant *vs.* non-pregnant). In addition, seasonal plasma CORT concentrations were compared between captive geckos from a breeding facility and a wild population to determine whether concentrations of CORT were chronically elevated in captive lizards. Plasma CORT concentrations showed significant seasonal variation, but were not related to sex, body temperature, body condition or female reproductive condition. Geckos, which successfully bred in captivity each year, did not exhibit chronically elevated plasma CORT levels but showed an elevation of plasma CORT concentrations in summer compared to wild geckos. Information on basal CORT concentrations in species of conservation concern can be beneficial for improving their management and can aid in identifying and evaluating stress-related changes in hormone patterns of individuals and chronic stress, which is particularly relevant for threatened species breeding programmes.

Introduction

The majority of New Zealand's unique herpetofauna species are classified as threatened or in decline (Hitchmough *et al.* 2007). Many species have been driven to extinction on the mainland since human settlement or only survive in remnant habitat pockets, typically on mammal-free islands (Towns and Daugherty 1994). Most species remain unstudied and basic knowledge of their biology is urgently needed to aid an effective conservation management. In recent years, the New Zealand Department of Conservation (DOC) has developed management strategies to guide in the recovery and conservation of New Zealand's herpetofauna, involving translocations, reintroductions and captive breeding programmes (Newman 1996; Towns and Ferreira 2001; Blanchard 2002; Collen *et al*. 2009). One important goal of translocations and breeding programmes is to increase the number of populations and distribution of threatened or endangered species and thereby reduce a species' risk of extinction. For threatened species, the number of animals available to breeding programmes or translocations is generally very limited. Therefore, it is crucial to ensure that managed populations breed successfully and are self-sustaining. However, such programmes can inflict various degrees of stress on the animals involved. If stress becomes chronic, breeding and survival may be compromised. Thus, the early detection of changes in stress hormone patterns may enable scientists and conservation managers to respond to a disruption by reducing or eliminating the potential stressors before the effects of stress reduce productivity or even become lethal to the animal or its developing young.

Corticosterone (CORT) is a major glucocorticoid in reptiles and amphibians and is released in response to stress-related stimuli (Bentley 1989). This coping mechanism allows the organism to react to internal or external changes through immediate physiological and behavioural adaptations hence increasing an individual's chances of survival (Sapolsky *et al.* 2000). However, prolonged high levels of plasma glucocorticoids, as secreted in response to a chronic stressor, can have adverse effects on the organism, such as inhibited growth and suppression of immune function (Morici *et al.* 1997; French *et al.* 2007), increased susceptibility to parasitemia (Oppliger *et al.* 1998) or inhibited reproductive related behaviours (Moore and Miller 1984). Moreover, chronic maternal stress has been shown to reduce embryonic growth and survival (Cree *et al*. 2003). Elevated CORT levels in gravid females can also affect offspring phenotype (Vercken *et al.* 2007) and alter offspring behaviour, such as activity patterns and thermoregulation (Belliure *et al.* 2004), dispersal strategy (de Fraipont *et al.* 2000), escape behaviour (Meylan and Clobert 2004) and anti-predator behaviour (Robert *et al.* 2009). This has significant implications for translocations and threatened species breeding programmes, and thus conservation management. It is crucial that captive-bred animals for reintroductions have the optimal requisites for successful establishment and are not compromised in phenotype or behaviour. This is particularly important for threatened lizards in New Zealand, for which founder numbers are typically limited and recruitment rates are characteristically low.

Monitoring basal plasma CORT concentrations in reptiles can be a useful conservation management tool. Detecting disruptions in a population of concern may aid in identifying stressors and predicting survival (Romero and Wikelski 2001; Homan *et al*. 2003; Wikelski and Cooke 2006). Therefore, information on basic physiology of a wide range of species, particularly seasonal hormone levels in free-living populations, is needed (Cockrem 2005).

The current study examined plasma CORT concentrations in the Duvaucel's gecko (*Hoplodactylus duvaucelii*), a threatened endemic New Zealand lizard. This largest of New Zealand's gecko species is restricted to offshore islands along the north-east coast of the North Island and the Cook Strait, New Zealand. In recent years Duvaucel's geckos have been (re)introduced in low numbers to several offshore islands (e.g. Mana, Tiritiri Matangi and Motuora) as part of conservation and restoration programmes. In December 2006 thirty individuals from Korapuki Island were transferred to a reptile facility at Massey University, Auckland, to establish a breeding-for-release programme. Although the geckos have successfully bred in captivity each year since their arrival, it is unknown what physiological effects such long-term captivity has on wild caught Duvaucel's geckos and what the consequences will be for their offspring. While suppressed reproduction generally can be linked with stress, unsuppressed reproductive activity does not necessarily denote that an animal is free of stress (see Wingfield and Sapolsky 2003 for review). It is crucial to ensure that the breeding population does not experience chronic stress, which could impair (future) breeding success or negatively impact the fitness of the offspring.

The specific aims of this study were to 1) examine plasma CORT concentrations of Duvaucel's geckos in relation to season and sex, 2) investigate relationships between plasma CORT concentrations and body condition, and between plasma CORT concentrations and body temperature, 3) determine whether plasma CORT concentrations vary with female reproductive condition (pregnant *vs.* non-pregnant) and 4) compare plasma CORT concentrations between wild and captive (wild caught) geckos to determine whether plasma CORT levels of adult geckos in captivity are chronically elevated.

Materials and methods

Study species

Duvaucel's geckos are primarily nocturnal and utilise arboreal and terrestrial habitats (Whitaker 1968; van Winkel 2008). They typically shelter during the day in rock or tree crevices, under boulders or bark, but occasionally emerge to sun bask (Whitaker 1968). Females reproduce either annually or biennially and give birth to one or two live young (Barwick 1982; Cree 1994). Duvaucel's geckos are sexually mature at five to seven years (Barwick 1982) and may live more than 43-50 years (Wilson 2010). Information on the reproductive cycle is very limited. A few studies examining female reproductive tracts provide evidence that the mating season occurs in spring (September-October), when ovulation takes place (Barwick 1982; Christmas 1995). Gestation may last for up to eight months (Barwick 1982) and in some females up to one year (Cree 1994; Christmas 1995). Births occur in late summer / autumn (Barwick 1982) and possibly in spring (Christmas 1995). Although Duvaucel's geckos remain active throughout winter (June – August; Whitaker 1968; Barwick 1982), physical activity and food intake appear to be reduced during the winter months (M. Barry, *pers obs*).

Study sites and data collection

Morphometric data and blood samples were collected from adult wild and captive Duvaucel's geckos from February 25 to 28 (late summer; breeding season), July 10 to 15 (winter; season of reduced activity) and October 10 to 18 in 2008 (spring; mating season). The wild population was sampled on Korapuki Island (Mercury Island Group), an 18 ha scenic reserve and wildlife sanctuary off the northeast coast of Coromandel, New Zealand (36°39.5' S, 175°51' E). Duvaucel's geckos are abundant (van Winkel 2008) and widespread throughout all habitats on the island, which have been broadly characterised by Towns and Atkinson (2004) as manuka-scrub (*Leptospermum scoparium)*, pohutukawa (*Metrosideros excelsa)* and mahoe forest *(Melicytus spp)* and coastal flax-vegetation (*Phormium tenax)*. Korapuki Island became free of introduced mammalian pest-species, following the eradication of kiore (*Rattus exulans*) and rabbits (*Oryctolagus cuniculus)* in the mid-1980s (Towns and Atkinson 2004). Morepork (*Ninox* *novaezealandiae*), and other potential lizard predators, e.g. kingfisher (*Halcyon sancta*) are present on Korapuki; however, tuatara (*Sphenodon punctatus*) are absent.

Samples were collected at night during the period of greatest gecko activity. Duvaucel's geckos typically emerged after dusk from their diurnal retreats and could be readily found travelling through leaf litter or along tree trunks and rock outcrops. In winter a significant proportion of geckos remained in their shelters and had to be extracted by hand. Systematic behavioural observations could not be conducted in the wild due to time constraints and the secretive nature of the animals. The geckos were caught by hand, processed within ten minutes and released at the capture point. All wild geckos were marked individually on the flank using a non-toxic (xylene-free) silver pen to avoid re-sampling during each trip. The marking was temporary only and remained visible until the skin was shed (approx. 4-12 weeks, depending on the time of year).

All captive adult geckos (n = 30) originated from Korapuki Island and were captured in November/December 2006. The geckos (9 males, 21 females) were held in six outdoor enclosures that were furnished with native plants, boulders, leaf-litter and shelters to mimic natural conditions. Each enclosure contained five individuals in two sex ratios (three enclosures with one male and four females and three enclosures with two males and three females). Enclosure sizes ranged from 1.44 m^3 - 1.5m^3 (densities: 3.3-3.5 geckos per m^3). Geckos were fed twice a week (in winter once a week) with live insects or insect larvae and fruit-puree with vitamin/mineral supplements. Water was provided *ad libitum*. The geckos have successfully produced viable offspring each year since being in captivity. At the time of first blood sampling (February 2008) 12 of the 21 captive females were pregnant and gave birth approximately one month later. All captive geckos were regularly handled (morphometric measurements taken monthly) and used as part of a parallel study involving behavioural observations. Handling was limited to ten to fifteen minutes per animal per month and geckos were left undisturbed at least ten days before the blood sampling was carried out. Each gecko was labelled with an individual silver pen marking on its flank to allow easy identification. However, if the skin with the marking was shed individuals could also be identified by their unique colour patterns and tail condition. Markings were renewed during monthly checks if an animal had shed its skin.

Measurements taken from wild and captive geckos included snout-vent length (SVL) and vent-tail-tip length (VTL), measured against the ventral surface to the nearest mm with a ruler. Body mass was determined to the nearest gram with a 100 g Pesola spring-scale.

Additional variables recorded were state of tail (regenerating or original) and sex. Reproductive state of females was determined by abdominal palpation (Cree and Guillette 1995). Reproductive stages of females were categorised as: (1) early-mid pregnancy, (2) late pregnancy, (3) not pregnant: vitellogenic or spent (adapted from Cree and Guillette 1995). Cloacal body temperature (T_b) was measured with a fast reading digital thermometer (Digitech QM-1600) with thermocouple within five minutes of capture, immediately after a blood sample was obtained.

Blood sampling

Blood sampling was carried out at night (October and February: 2100-0300 h; July: 1930–0230 h) when the species is most active, and to avoid possible confounding effects of diel variation in plasma CORT concentrations between day and night-time samples (Girling and Cree 1995; Hanley *et al.* 1998; Jones and Bell 2004). Only adult individuals were sampled, whereby minimum adult size was determined by the smallest SVL found for wild pregnant females (110 mm). All blood samples (up to 200 μl) were collected within five min of capture. Samples were taken from the caudal vein at the tail base using a heparinised syringe and needle. The blood was held on ice for up to 7 h and then centrifuged. Plasma samples were stored in liquid nitrogen (-196 ºC) in the field, and later transferred to a -80 ºC freezer at Massey University. Given the high density of Duvaucel's geckos on Korapuki Island, it was assumed that different individuals were likely to have been sampled each season. Captive geckos were sampled repeatedly, as total sample size was too small to allow sampling of different individuals per sex-category in each season. However, it was only possible to obtain blood samples of sufficient quantity for hormonal analyses from 23 of the 30 captive geckos. Of these 23 individuals, eight geckos were successfully bled in two seasons and one gecko was bled in all three seasons. The remaining 14 individuals represent single independent samples. Of 162 wild individuals captured throughout the study only 74 were bled successfully. Two wild geckos, one male and one female, caught in winter 2008 had to be excluded from the analyses as they may have been disturbed for longer than five minutes by the extraction of another animal that shared the same shelter.

Radioimmunoassay of corticosterone

CORT concentrations in plasma diluted in phosphate buffered saline with gelatine (PBSG) were measured by radioimmunoassay following the method of Cockrem *et al.* (2006). Plasma samples were initially spun for 10 min at 18 000 g to separate lipid from the plasma. Samples were assayed in duplicate. Samples of 10 μl diluted plasma were incubated for 2 h at room temperature (22°-25°C) with iodinated CORT and antiserum from an MP Biomedicals, USA, CORT radioimmunoassay kit. Precipitant solution (MP Biomedicals, USA) was added and each sample vortexed thoroughly, then 50 µl egg white (10g/l dried egg white (Sigma) in PBSG) was added to increase adhesion of the pellet to the tube after centrifugation. The samples were incubated for 15 min at room temperature to separate bound and free CORT, then centrifuged for 15 min, the supernatant aspirated and the pellets were counted on a LKB Wallac 1261 Multigamma counter.

Serial dilutions of gecko plasma in PBSG were parallel to the CORT standard curve, and the recoveries of CORT added to three samples of gecko plasma were 107.5 ± 6.8, 99.2 ± 7.1 and 97.5 ± 7.2%. The sensitivity of the CORT assay was the minimum hormone level that could be consistently distinguished from zero. It was determined as the hormone concentration at the mean ± 2 standard deviations from the zero hormone point on the standard curves. The assay sensitivity expressed as ng CORT/ml plasma, was 0.46 ng/ml. Solutions of CORT in PBSG at concentrations that gave approximately 80, 50 and 20 % binding on the standard curve were used as low, medium and high quality controls in every assay. All samples were measured in one assay, and the intra-assay coefficients of variation were 8.4, 6.0 and 7.2 % for low, medium and high quality controls.

Data analyses

The effects of season and sex on plasma CORT concentrations and body condition were analysed for wild geckos with two-way ANOVAs. Plasma CORT values did not meet the assumptions of normality and homogeneity of variances, thus the analysis was conducted using $\log_{10}$ transformed data, which complied with the statistical assumptions. Significant findings were further analysed with post-hoc tests (Hochberg's GT2 post-hoc test for unequal samples sizes). Generalised estimating equations (GEE, Liang and Zeger 1986) were used to analyse effects of sex and season on plasma CORT concentrations and body condition of captive geckos, whereby gecko ID was used as the subject variable and season as the within-subject variable to account for the multiple sampling of some individuals across seasons. The working correlation matrix was set as independent. Significant results were further investigated with pairwise contrasts under application of sequential Bonferroni corrections.

Plasma CORT concentrations and body condition were compared between captive and wild geckos with Mann-Whitney U tests, separate for each season. Spearman's rank correlations were used to examine associations between plasma CORT concentrations and T_b for wild and captive geckos and to explore the relationship between plasma CORT and body condition in wild geckos each season. Sequential Bonferroni corrections were applied to account for multiple testing in all instances where separate analyses were conducted for each season. For the analysis of the effect of reproductive state on plasma CORT concentrations, we only considered samples from the main seasons of reproductive activity, spring and summer (only one wild female was found in early pregnancy in winter). Both reproductive stages 'early-mid pregnancy' and 'late pregnancy' were combined to 'pregnant' due to a limited availability of samples from females in late pregnancy (wild: n = 1, captive: n = 3). The captive female dataset included three individuals that were sampled in both seasons. To comply with the requirement of independence of data one of the two samples from each female was randomly chosen and excluded; and hormone data from captive and wild females were analysed with Mann-Whitney-U tests with respect to reproductive state. Statistical significance was assumed at $\alpha < 0.05$ and means ± SE were reported. Sequential Bonferroni corrected p –values were reported where multiple tests were applied.

To obtain an estimate of body condition that indicates how light or heavy a gecko is for any given length, a simple ratio index of the cubic root of body mass divided by SVL multiplied by 100 was calculated. Based on data from 251 geckos of all age and size groups collected in February 2008 on Korapuki Island (this study; M. Barry, unpubl. data; van Winkel 2008) the mass-length relationship in Duvaucel's geckos is exponential and becomes linear if the cube root of the mass is regressed on SVL (intercept = -0.14; slope = 0.03, $R^2 = 0.94$, $p < 0.001$, residuals normally distributed). The ratio index was size independent when regressed against SVL (intercept = 2.88; slope = 0.00, $R^2 = 0.05$). As the extent of tail loss influences the body condition index, only geckos with a tail length of at least 75% of the mean original tail length were considered. Therefore, the mean VTL/SVL (vent-tail-tip length / snout-to-vent length) ratio of all geckos (this study) with intact tails was calculated (n = 26, $\bar{x} = 1.15 \pm 0.01$) and geckos with VTL/SVL ratios of 75% or less (≤0.86) were excluded in examining the relationship between plasma CORT and body condition. To avoid a possible confounding effect of female reproductive condition on the body condition index pregnant females were excluded from the analysis. The elimination of pregnant females and short-tailed geckos from the dataset resulted in a sample size of 32 wild males and 18 wild females. The captive dataset was reduced to 3 male samples and 13 female samples.

All data analyses were performed using PASW Statistics 18 Core System for Windows with PASW Advanced Statistics add-on module (SPSS Inc, 2009).

Results

Effects of sex and season on plasma corticosterone

Seasonal plasma CORT concentrations (means ± SE) in captive and wild Duvaucel's geckos are presented in Table 1. Wild geckos showed a significant seasonal variation in plasma CORT concentrations ($F_{2, 66} = 8.465$, $p = 0.001$). Post-hoc tests revealed that winter values were significantly higher than hormone concentrations in spring ($p = 0.017$) and summer ($p < 0.001$), but spring and summer concentrations did not differ significantly ($p = 0.386$). Plasma CORT concentrations were not significantly affected by sex ($F_{1, 66} = 1.175$, $p = 0.282$). The interaction of sex and season was not significant ($F_{2,66} = 1.674$, $p = 0.195$).

GEE analysis revealed that season significantly accounted for variation in plasma CORT concentrations in captive geckos (Wald $\chi^2 = 15.417$, df = 2, $p < 0.001$), but no significant main effect was found for sex (Wald $\chi^2 = 0.890$, df = 1, $p = 0.345$). However, the interaction between season and sex was highly significant (Wald $\chi^2 = 36.652$, df = 2, $p < 0.001$). Plasma CORT concentrations in captive geckos were lower in spring in comparison to winter and summer values (Table 1). Pairwise contrasts revealed a significant difference in hormone concentrations between spring and summer ($p = 0.002$), but differences between spring and winter values were not significant ($p = 0.062$). There were no significant differences between summer and winter plasma CORT concentrations ($p = 1.000$) in captive geckos. Further analysis of the significant interaction effect between sex and season revealed that captive females had significantly higher plasma CORT concentrations in summer ($p = 0.012$) and winter ($p < 0.001$) in comparison to captive females in spring, but no significant seasonal variation in hormone levels was found for captive males (all comparisons $p > 0.1$). Females and males did not differ significantly in their hormone concentrations in summer ($p = 1.000$) and winter ($p = 0.121$). The single captive male sampled in spring had a more than fourfold higher concentration of plasma CORT in comparison to females in the same season.

Table 1: Plasma corticosterone concentrations (mean ± SE, minima and maxima; ng/ml) in wild and captive adult *H. duvaucelii* sampled across seasons. Asterisk (*) indicates that actual value is displayed. Some captive geckos were repeatedly sampled across months. Different wild geckos were sampled each season.

	Females				Males			
Month	N	$\bar{x}$ ± SE	min	max	N	$\bar{x}$ ± SE	min	max
Wild H. duvaucelii								
Summer	8	1.42 ± 0.39	0.46	3.75	22	1.73 ± 0.26	0.46	5.23
Winter	8	4.84 ± 1.28	1.70	11.95	7	2.85 ± 0.43	1.48	5.02
Spring	14	2.60 ± 0.43	0.52	5.65	13	2.05 ± 0.66	0.48	8.85
Captive H. duvaucelii								
Summer	9	5.77 ± 1.48	0.46	13.21	2	6.40 ± 0.82	5.57	7.22
Winter	10	6.77 ± 0.99	2.88	12.37	6	3.46 ± 1.04	1.53	8.30
Spring	5	1.28 ± 0.31	0.66	2.45	1	5.88*	-	-

Relationship between plasma corticosterone and body temperature

Owing to seasonal variation in temperatures and plasma CORT concentrations, the relationship between T_b and plasma CORT was analysed separately per season, but data from sexes were pooled. Mean T_b were highest in summer (wild geckos: 25.99 ± 0.21 ºC, captive geckos: 23.78 ± 0.71 ºC), moderate in spring (wild geckos: 21.76 ± 0.46 ºC, captive geckos: 20.02 ± 0.49 ºC) and lowest in winter (wild geckos: 19.77 ± 0.34 ºC, captive geckos: 18.27 ± 0.24 ºC). No significant relationship between plasma CORT concentrations and T_b was found for captive geckos (Spearman's rank correlations: summer: $r_s = -0.041$, $n = 11$, $p = 0.904$, winter: $r_s = 0.131$, $n = 16$, $p = 0.628$, spring: $r_s = -0.600$, $n = 6$, $p = 0.208$) or wild geckos (summer: $r_s = -0.232$, $n = 30$, $p = 0.218$, winter: $r_s = -0.420$, $n = 15$, $p = 0.119$, spring: $r_s = 0.412$, $n = 27$, $p = 0.099$).

Relationship between plasma corticosterone and body condition

Body condition indices of Duvaucel's geckos ranged from 2.72 to 3.33 in the wild population and from 3.14 to 3.43 in the captive population and showed little variation among seasons and between sexes (Table 2). Body condition of wild geckos was not significantly affected by season (2-way ANOVA: $F_{2,44} = 0.252$, $p = 0.778$) or sex ($F_{1,44} = 0.531$, $p = 0.400$) and there was no significant interaction between season and sex ($F_{2,44} = 0.818$, $p = 0.448$). Likewise, body condition of captive geckos did not vary with season (GEE: Wald $\chi^2 = 3.977$, df = 2, $p = 0.137$) or sex (Wald $\chi^2 = 0.615$, df = 1, $p = 0.433$). An interaction of sex and season could not be tested due to the lack of samples for captive males in summer and spring. Captive geckos had on average significantly higher body condition indices than wild geckos (Table 2) in summer (Mann-Whitney U test: Z = -2.311, p = 0.042) and winter (Z = -3.561, p < 0.002), and marginally higher indices in spring (Z = -1.942, p = 0.052).

Table 2: Body condition indices (mean ± SE) of wild and captive adult *H. duvaucelii* across seasons (based on a dataset from males and non-pregnant females with VTL (vent-tailtip length) / SVL (snout-vent length) ratios < 0.86).

	Wild				Captive			
	Females		Males		Females		Males	
Month	N	$\bar{x}$ ± SE	N	$\bar{x}$ ± SE	N	$\bar{x}$ ± SE	N	$\bar{x}$ ± SE
Summer	4	3.10 ± 0.04	14	3.10 ± 0.02	4	3.33 ± 0.06	0	-
Winter	5	3.10 ± 0.03	7	3.08 ± 0.04	7	3.27 ± 0.02	3	3.23 ± 0.06
Spring	9	3.04 ± 0.05	11	3.11 ± 0.03	2	3.26 ± 0.08	0	-

Given that plasma CORT concentrations varied with season the relationship between body condition and plasma CORT concentrations was explored separately in wild and captive geckos for each season. For wild geckos, no relationship between body condition and plasma CORT concentrations was found in summer (Spearman's rank correlations: r_s = -0.012, p = 0.961, n = 18), winter (r_s = -0.133, p = 0.681, n = 12) or spring (r_s = -0.465, p = 0.117, n = 20). Likewise, no significant relationship between body condition and plasma CORT concentrations was found for captive geckos in summer (r_s = -0.500, p = 0.391, n = 4) or winter (r_s = -0.164, p = 0.651, n = 10). Sample size of captive geckos was too low for analysis in spring (n = 2).

Plasma corticosterone concentrations in pregnant and non-pregnant females

Plasma CORT concentrations in wild pregnant and non-pregnant females were comparatively low (Fig. 1) and did not differ significantly (Mann-Whitney U test: Z = -0.901, p = 0.401). Plasma CORT concentrations did not differ significantly between captive pregnant females and captive non-pregnant females (Z = -0.945, p = 0.412). Differences in plasma CORT concentrations between captive and wild pregnant females were small (Fig. 1) and not significant (Z = -0.081, p = 0.935). Wild non-pregnant females had slightly lower plasma CORT concentrations than captive non-pregnant females but differences were non-significant (Z = -1.613, p = 0.107).

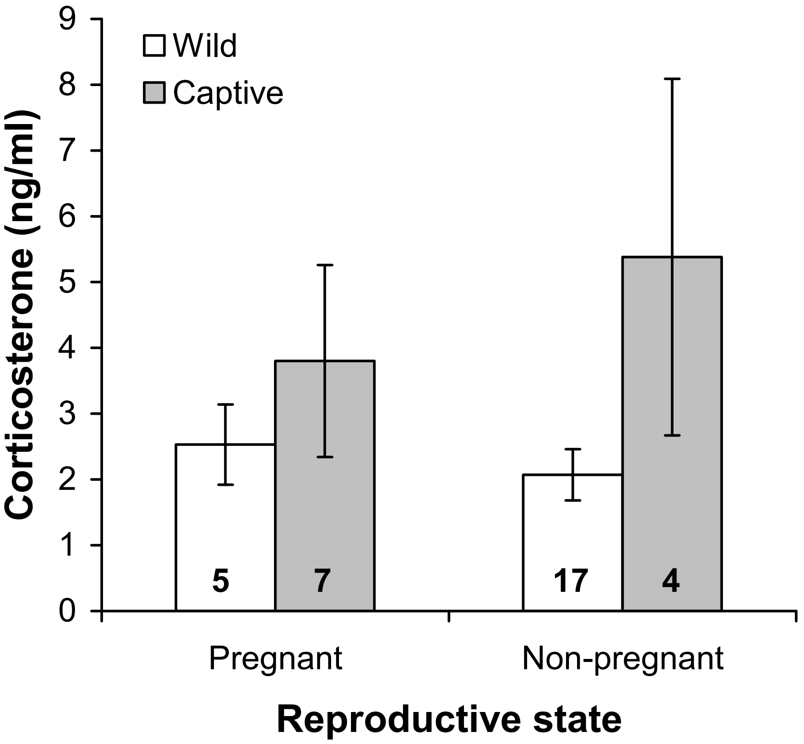


Figure 1: Comparison of plasma corticosterone concentrations (ng/ml) between wild and captive female *H. duvaucelii* sampled over spring and summer per reproductive condition (pregnant and non-pregnant). Bars represent means ± SE; numbers at base are sample sizes.

Comparison of plasma corticosterone concentrations between wild and captive geckos

Data from the sexes were pooled for this analysis. Captive geckos showed significantly higher mean plasma CORT concentrations in summer (Mann-Whitney U test: Z = -2.826, p = 0.015), but not in winter (Z = -1.344, p = 0.179) or in spring (Z = -0.327, p = 0.744) in comparison to wild animals (Fig. 2).

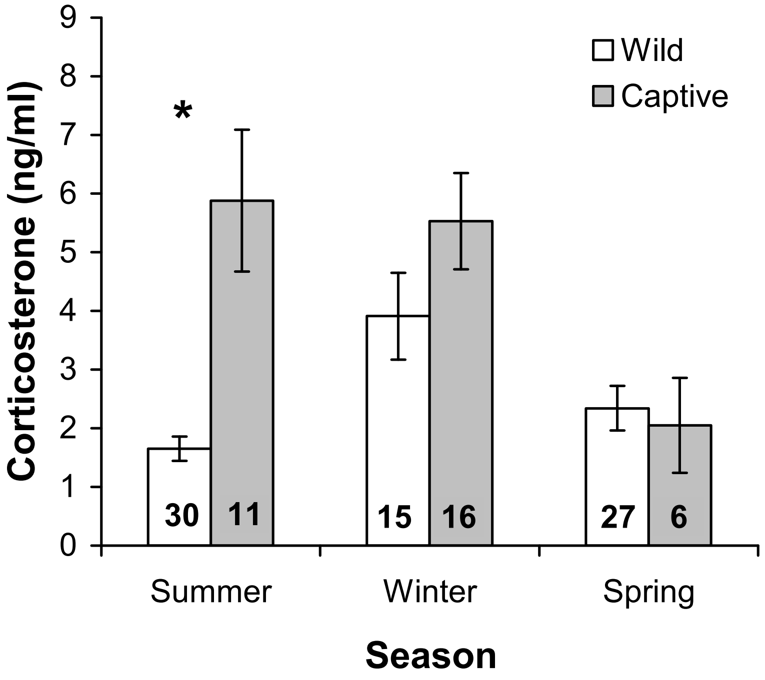


Figure 2: Comparison of plasma corticosterone concentrations (ng/ml) between wild and captive *H. duvaucelii* (sexes combined). (*) Asterisk indicates a significant difference between wild and captive geckos (Mann-Whitney U test). Bars represent means ± SE; numbers at base are sample sizes.

Discussion

This is the first study to examine seasonal variation of plasma CORT concentrations in Duvaucel's geckos. Geckos showed significant seasonal variation in plasma CORT concentrations, but hormone concentrations did not differ between sexes. Associations between plasma CORT concentrations and body temperature or between plasma CORT concentrations and body condition were not detected, and no differences in CORT concentrations between pregnant and non-pregnant females were found. The seasonal comparison between captive and wild geckos revealed that captive geckos had higher plasma CORT concentrations in summer, but no differences were found in other seasons.

Plasma corticosterone in relation to season, sex and female reproductive condition

Plasma CORT concentrations in adult wild Duvaucel's geckos on Korapuki Island varied with season but did not differ between sexes. Wild males and females showed similar seasonal patterns with a peak in winter and lower levels of CORT in spring and summer. It remains to be tested whether the increase in CORT concentrations observed in winter was related to a change in the physiological state of the geckos that could have been triggered by environmental changes, such as low temperatures and low light levels. Dauphin-Villemant *et al.* (1990) observed elevated plasma CORT concentrations during artificial hibernation in female and male common lizards *Lacerta vivipara* and noted that this increase appeared to be related to the physiological state of hibernation. Female *L. vivipara* not in hibernacula sampled at the same time, showed significantly lower plasma CORT concentrations. Duvaucel's geckos generally remain active throughout winter, but activity patterns and food intake are likely to be reduced during the cooler winter season. Geckos in captivity were observed to consume less food in winter and a higher proportion of wild individuals remained in their shelters at night (M. Barry, *pers obs*), which is usually the time of greatest activity. Glucocorticoids play a significant role in regulating intermediary metabolism in reptiles. An elevation in circulating CORT concentrations during winter may aid in mobilising stored energy resources such as lipids and proteins (Guillette *et al.* 1995). Other reptiles however, for example tuatara, *S. punctatus* and Italian wall lizards, *Podarcis sicula* (Tyrrell and Cree 1998; De Falco *et al*. 2004) exhibit comparable low plasma CORT concentrations in winter, which can be explained by a general decrease in activity and maintenance levels. For Duvaucel's geckos, it remains to be determined whether a change in intermediary metabolism has caused the increase in hormone levels or whether other factors were responsible.

Low plasma CORT concentrations were observed for wild Duvaucel's geckos in spring and summer, the seasons of greatest physical and reproductive activity. Correspondingly, female common geckos, *H. maculatus* maintained relatively low and constant CORT levels during late spring and summer (Girling and Cree 1995). Seasonal CORT cycles are variable among amphibian and reptilian taxa (see Moore and Jessop 2003 for review). Some species appear to lack any seasonal pattern (e.g. Turks and Caicos Iguanas, *Cyclura carinata carinata*; Macdonald *et al.* 2007, spotted salamanders, *Ambystoma maculatum;* Homan *et al*. 2003, bearded dragon, *Pogona barbata;* Amey and Whittier 2000) and others exhibit an increase in plasma CORT during seasons of reproductive activity (e.g. female tuatara, *Sphenodon punctatus*; Tyrrell and Cree 1998, Texas horned lizards, *Phrynosoma cornutum*; Wack *et al.* 2008, Side-blotched lizard, *Uta stansburiana*; Wilson and Wingfield 1994). Elevated levels of plasma CORT during periods of mating and breeding may reflect a need to mobilise energy resources during energetically demanding times (Moore and Jessop 2003), e.g. during territorial defence, extensive courtship displays and nest construction. However, New Zealand's Duvaucel's and common geckos do not appear to occupy discrete territories that require defence (Christmas 1995; Todd 2005) nor does mating involve any energetically demanding behaviours such as displays or calls (M. Barry, unpubl. data; Todd 2005). The gestation period is extended in both species and generally lasts up to eight months in Duvaucel's geckos (Barwick 1982) and up to five months (in some populations up to 14 months) in common geckos (Cree and Guillette 1995). Neonates are independent upon birth and no nest construction is necessary. Absence of energetically demanding behaviours may contribute to the low plasma CORT concentrations during spring and summer in both species.

Furthermore, we did not detect any differences in plasma CORT concentrations between pregnant and non-pregnant female Duvaucel's geckos as found in females of other gecko species, e.g. common geckos, *H. maculatus* (Girling and Cree 1995; Cree *et al*. 2003), common house geckos, *Hemidactylus frenatus* and mourning geckos, *Lepidodactylus lugubris* (Hanley *et al*. 1998).

A significant seasonal variation in plasma CORT concentrations was also found for captive adult Duvaucel's geckos. CORT concentrations in captive females were elevated in winter and low in the spring, comparable to the pattern found for wild geckos. However, captive females also showed increased CORT concentrations in summer. Captive males had comparably low levels of CORT in winter, but summer concentrations showed an increase similar to captive females. One captive male, sampled in spring, had more than four times higher plasma CORT concentrations than captive females in the same season. This male shared an enclosure with a dominant male (M. Barry, *pers obs*) and the elevated hormone value could have been related to the (subordinate) social status of the animal (Greenberg *et al.* 1984). Our limited records of hormone concentrations of captive males show that males with a higher dominance status (M. Barry, *pers obs*) and males that did not share an enclosure with another male were characterised by low plasma CORT concentrations: the hormone values of two dominant males sampled in winter were 1.53 ng/ml and 1.87 ng/ml and two single males also sampled in winter showed comparable low concentrations of 2.94 ng/ml and 2.01 ng/ml. In contrast, two subordinate males showed elevated plasma CORT concentrations in summer: 7.22 ng/ml and 5.57 ng/ml. The same two males were resampled in spring and winter (respectively) and their CORT values were 5.88 ng/ml and 4.14 ng/ml, respectively. A third subordinate male, sampled in winter, also showed increased glucocorticoid levels of 8.30 ng/ml. These observations suggest that social rank status could have an effect on plasma CORT levels in captive male Duvaucel's geckos and it may be beneficial to house only one male per cage. It remains to be determined whether dominant and single males show consistently low plasma CORT levels in all seasons. Research is needed to elucidate the effects of social structure on plasma CORT levels not only of male but also of female Duvaucel's geckos (whose social structure was unknown in this study), particularly when confined in a limited space.

Plasma corticosterone in relation to body temperature and body condition

Plasma concentrations of CORT were not significantly related to night-time T_b in male or female Duvaucel's geckos, which corresponds to findings for female common geckos (Girling and Cree 1995). However, common geckos did show a positive association between plasma CORT concentrations and day time body temperatures. This relationship remains to be tested in Duvaucel's geckos.

An association between plasma CORT concentrations and body condition of Duvaucel's geckos was not detected. In other reptiles, plasma CORT concentrations appear to be negatively correlated with body condition (Macdonald *et al.* 2007). However, the relationship is more pronounced when animals have comparatively low body condition indices, for example as seen in populations that suffer from food shortage (brown tree snakes *Boiga irregularis;* Moore *et al.* 2005; Waye and Mason 2008). Romero and Wikelski (2001) found no relationship between basal CORT and body condition in Galapagos Marine Iguanas (*Amblyrhynchus cristatus*), but when body condition fell below a certain threshold due to starvation during an El Niño event, CORT concentrations were highly negatively correlated with body condition, whereby CORT levels increased as body condition decreased. The lack of association between plasma CORT concentrations and body condition in wild Duvaucel's geckos could indicate that the food availability on Korapuki Island was sufficient and even the lightest geckos may not have experienced starvation. Likewise, we did not find a relationship between plasma CORT concentrations and body condition indices for captive geckos. Captive geckos in this study had greater body condition indices in comparison to the wild geckos, presumably reflecting differences in diet composition, feeding frequency and level of exercise. Reptiles in captivity generally tend to be heavier than wild conspecifics of comparable size and often show increased blood plasma lipid levels (Cartland *et al.* 1994) and differences in plasma lipid composition (Cartland-Shaw *et al.* 1998, Lance *et al.* 2001). The nutritional state of the captive Duvaucel's geckos has not been considered in this study, but further research is required to investigate the relationship between plasma CORT and nutritional state in this species.

Comparison of plasma corticosterone between wild and captive geckos

A prolonged elevation (over several seasons) of plasma CORT concentrations was not observed for geckos in captivity and no differences in hormone concentrations were found between captive and wild geckos in two out of three seasons. Furthermore, maximum plasma CORT values of captive geckos (13.21 ng/ml) were similar to the maximum values measured in the wild population (11.95 ng/ml); thus, we conclude that the captive geckos did not suffer from chronic stress.

The elevation of plasma CORT values observed in captive geckos in late summer (February) requires further investigation. Although regular health checks did not reveal any unusual bite marks or tail losses that may have been caused by possible increased aggressive interactions during summer, future research should consider the social structure and individual social status when investigating plasma CORT levels particularly in captive individuals. February lies outside the species' typical mating season (September-October), and is the time when females generally are in their last trimester of pregnancy. However, plasma CORT concentrations were not affected by female reproductive condition (pregnancy *vs.* non-pregnancy) and hormone levels of pregnant females were comparatively low in wild (2.53 ± 0.61 ng/ml) and captive geckos (3.80 ± 1.46 ng/ml).

In summary, our data provide important baseline information on seasonal plasma CORT patterns of a threatened New Zealand lizard. This is (to our knowledge) the first comparison of plasma CORT concentrations between captive and wild geckos aimed at assessing whether a captive breeding population experiences chronic stress. The limited differences in hormone patterns between the captive and wild population indicate that the Duvaucel's geckos may have adapted well to the captive situation. Experimental studies are now required to investigate relationships of population density, social status and nutritional state with plasma CORT in Duvaucel's geckos; aspects directly relevant to the captive management of this species.

CHAPTER THREE

Year-round shelter aggregations of Duvaucel's geckos (*Hoplodactylus duvaucelii*)

Abstract

Little is known about the spatial distribution and social organisation of New Zealand's large cryptic Duvaucel's geckos. Anecdotal reports suggest that small groups of Duvaucel's geckos share shelter sites but detailed information on the extent and potential function of this behaviour is not currently available. This study investigated the distribution patterns of a wild population of Duvaucel's geckos in diurnal retreats over four seasons and tested whether geckos formed aggregations more often than expected by chance. Additionally, the thermal properties of shelters, body temperatures of individuals and group composition were investigated as factors that may affect group formation. Duvaucel's geckos utilised a wide range of natural structures as diurnal shelters and showed a significant tendency to aggregate throughout the year (47-71 % of individuals). Group sizes ranged from two to eight and all sex and age groups participated in shelter aggregations, however aggregations never contained more than one adult male. Moreover, juvenile-only groups were rarely observed. Shelter thermal properties did not appear to have influenced aggregative behaviour. A greater proportion of geckos shared shelters in winter and spring in comparison to autumn and summer; however possible factors influencing these distribution patterns remain speculative. The large proportion of mixed sex groups and sex specific distribution patterns may be related to mating behaviour. Further research is required to explore the possible causation and function of such shelter aggregations. Long-term mark and recapture as well as molecular studies could help to determine the temporal stability of groups and uncover the genetic relationships between group members.

Introduction

Aggregations can occur when individuals are communally attracted to a limited resource (Graves and Duvall 1995, Leu *et al.* 2010). For instance, animals may aggregate in a shelter site that offers preferable habitat features such as optimal thermal attributes and protection from predators (Graves and Duvall 1995). Alternatively, conspecifics may be mutually attracted to each other for physiological (Shah *et al.* 2003, Lancaster *et al.* 2006) or social benefits. Social benefits can include indirect parental care (O'Connor and Shine 2004), enhanced group vigilance (Lanham and Bull 2004) or mating benefits (Chapple and Keogh 2005). Although social attraction may not be the primary motive for aggregative behaviour in many group living species, the close proximity to conspecifics likely facilitates intra-specific interactions and may provide a pathway for complex social behaviour (Graves and Duvall 1995).

Several phylogenetic lineages of squamate reptiles form aggregations. The majority of these aggregations are temporary only, and may serve thermoregulatory (e.g. winter aggregations; Cooper and Garstka 1986, Elfström and Zucker 1999), anti-predatory (Burghard 1977, Aubret and Shine 2009) or reproductive purposes (Graves and Duvall 1995). However, the formation of permanent aggregations is thought to be uncommon among squamate reptiles and stable social groups consisting of closely related individuals have rarely been documented. For example, several species of the Australian scincid genus *Egernia* exhibit such complex sociality (see Chapple 2003 for review). Long-term behavioural and molecular studies have confirmed that these *Egernia* species and also the North-American desert night lizard *Xantusia vigilis* exhibit kin based sociality (Gardner *et al.* 2001, O'Connor and Shine 2004, Davis *et al.* 2010), whereby lizards form aggregations consisting of several family members (i.e. adults and their offspring). While *X. vigilis* only aggregate seasonally (Davis *et al.* 2010), various *Egernia* species, form social groups that are stable over time and in some instances may contain adult breeding pairs that resemble long-term monogamous associations (Gardner *et al.* 2002, O'Connor and Shine 2003, Gardner *et al.* 2007, While *et al.* 2009). Although such complex sociality may be exceptional among lizards, there is a number of species that are tolerant towards conspecifics when sharing retreats that serve as resting shelters. Year-round shelter aggregations have been reported for a range of taxa i.e. Cordylidae (Nieuwoudt *et al.* 2003, Schutz *et al.* 2007), Scincidae (Osterwalder *et al.* 2004, Gardner *et al.* 2007), Phrynosomatidae (Lemos-Espinal *et al.* 1997) and Gekkonidae (Burke 1994, Kearney *et al.* 2001, Todd 2005, Meyer and Mouton 2007).

Several geckos form aggregations in their natural retreats in the wild (Burke 1994, Kearney *et al.* 2001, Todd 2005, Meyer and Mouton 2007) or under laboratory conditions (Shah *et al.* 2003, Lancaster *et al.* 2006, Meyer and Mouton 2007) including *Coleonyx variegatus* (Eublepharidae), *Nephrurus milii, Hoplodactylus maculatus* (Diplodactylidae), *Christinus marmoratus* and *Chondrodactylus bibronii* (Gekkonidae), but little is known about the complexity of their social organisation. While geckos may primarily aggregate for physiological rather than social benefits (Shah *et al.* 2003, Lancaster *et al.* 2006), the occurrence of certain age-sex combinations and evidence of size-assortive grouping in some species (Kearney *et al.* 2001) could indicate that social factors may influence group composition. Furthermore, some geckos have been shown to recognise the scents of conspecifics and to discriminate between their sex (Mason and Gutzke 1990, Cooper and Steele 1997), familiarity (Steele and Cooper 1997) and kin status (Chapter 5), suggesting a potential for complex social organisation.

The social organisation of New Zealand's geckos is largely unknown, but at least two species of the genus *Hoplodactylus* have been reported to aggregate in natural retreats in the wild (Robb 1980, Todd 2005). However, this behaviour has never been systematically studied in New Zealand geckos and little is known about the extent, cause and possible function of these shelter aggregations. This study investigated the seasonal grouping behaviour of a wild population of Duvaucel's geckos (*H. duvaucelii*) in their natural habitat. These large (snout to vent length [SVL] up to 160 mm, Whitaker 1968), long-lived geckos (Thompson *et al.* 1992, Wilson 2010) are habitat generalists showing terrestrial, arboreal and saxicolous habits (Whitaker 1968, Barwick 1982, Towns and Atkinson 2004). Duvaucel's geckos are mainly nocturnal and typically retreat during the daytime into rock crevices as well as other sheltered spaces in the environment, but may occasionally emerge to sun bask (Whitaker 1968). Several individuals may share the same diurnal shelter (Robb 1980, Bell 2009), however it is unclear whether Duvaucel's geckos exhibit a general tendency to form shelter aggregations and which factors may influence group formation. Thus, this study aimed to determine whether wild Duvaucel's geckos tend to form aggregations more often than expected by chance and to test whether aggregation patterns varied with season. Additionally, group composition was examined with respect to sex, age class and body size. Shelter and body temperatures were investigated to assess whether aggregation behaviour was influenced by shelter thermal properties.

Methods

Study species and site

Duvaucel's geckos are active throughout the year (Barwick 1982), but may remain in shelters during cool winter nights (Barry, *pers obs.*). Mating typically occurs in spring (September-November) and females give birth in late summer - autumn (February-May) (Barwick 1982, Gill and Whitaker 1996). Juveniles are slow growing and reach sexual maturity at approximately six to seven years (Barwick 1982). The minimum snout-vent-length (SVL) of juveniles recorded in this study was 50 mm and the maximum size of adults was 133 mm.

The study population is located on Korapuki Island, New Zealand (Mercury Islands, 36°39.5' S, 175°51' E), an 18 ha wildlife sanctuary and scenic reserve, free of introduced mammalian predators. Duvaucel's geckos are abundant and widespread throughout most habitats on the island (Towns and Atkinson 2004). Typical habitats include coastal forest, rock outcrops and boulder beaches. The region is temperate with cool humid winters and hot dry summers. The 2.5 ha study site encompassed coastal forest of regenerating mahoe (*Melicytus ramiflorus*) and karo (*Pittosporum crassifolium*) with few mature pohutukawa (*Metrosideros excelsa*) and ngaio (*Myoporum laetum*) and an open understory. Leaf litter covered the forest floor, which also featured numerous seabird burrows. Large boulders and rock outcrops were scattered throughout the site.

Data collection

Data on gecko retreat site use and aggregation behaviour were collected in late summer 2008 (23 to 25 February), spring 2008 (17 to 21 October), early autumn 2009 (13 – 16 March) and winter 2009 (18 to 20 July). The first data collection period in spring 2008 served as a pilot study during which shelter types were identified based on whether they were used by geckos. The different shelter types were not equally abundant and were defined as: a) cavities under boulders and rocks (including seabird burrows) with soil as the underlying substrate (51 %); b) rock crevices with rock as the underlying substrate (28 %); c) tree cavities (9 %); d) cavities beneath loose material such as leaf litter or twig piles accumulated against boulders or tree roots (8 %); e) cavities beneath or within dense vegetation i.e. dead pampas grass stumps *Cortaderia* spp. or flax bushes (3 %); and f) cavities under loose bark (1 %). Hand searches in subsequent trips focussed on these shelter types and encompassed all potential shelters (large enough to harbour an adult-sized gecko) up to 2 m above ground. Surveys were conducted by experienced field-workers and it was assumed that all potential shelters identified represented suitable retreats for Duvaucel's geckos. All potential shelters were searched during the day (09:00 to 17:00 h) and checked once during each trip. When an individual or group was located, geckos were extracted and cloacal body temperature (Tb) was measured immediately using a fast reading digital thermometer with thermocouple (Digitech QM-1600, precision ± 0.05°C). Other variables recorded were air temperature (Ta) and shelter temperature (Ts). Geckos were measured (snout-to-vent length = SVL, vent-to-tail-tip length = VTL, to the nearest mm), weighed (to the nearest gram) and the size and composition (age/sex class) of each group was recorded. Based on the minimum SVL found for gravid females (110 mm) on Korapuki Island (Barry and van Winkel, *unpubl. data*), geckos ≥ 110 mm were classified as adults and all smaller animals were recorded as juveniles. A group was defined as two or more geckos that shared the same physical crevice and that were in close proximity (≤50 cm) to each other. It was generally easy to detect all geckos within a radius of 50 cm of one another. However some individuals may have been missed due to the high structural complexity of some shelter types, particularly of crevices formed by several large boulders. Sheltering Duvaucel's geckos could easily be identified and distinguished from the sympatric common gecko *H. maculatus,* and were counted with the aid of a torch. Some geckos identified as *H. duvaucelii* could not be extracted and were recorded as 'unknown' but subjectively classed as adults or juveniles according to their body size appearance (i.e. unknown adult = UA, unknown juvenile = UJ).

All sampled geckos were temporarily marked on their flank with a xylene-free silver pen (marks were lost when the skin was shed) to allow identification during each field collection period. After the data collection, all geckos were released back into their original shelters. Crevice widths of shelters with geckos were opportunistically recorded throughout the study (39 of 103 records).

Data analysis

Following the approach by Cooper *et al.* (1985) and Burke (1994), I compared the observed distribution of geckos in all shelters with an expected random distribution. The numbers of shelters yielding 0, 1, …n geckos (n = maximum number of geckos encountered in a shelter per season) were compared to Poisson generated expected values to determine whether geckos distributed themselves randomly in their diurnal shelters. This method assumes that all shelters included in the analysis represented suitable retreats for Duvaucel's geckos. Expected values were calculated per season based on a mean number of 0.063 geckos per shelter (across all seasons) and based on the maximum number of geckos found within any shelter in each season. The expected frequencies for shelters with group size categories of three or more geckos were pooled. Chi-square goodness-of-fit exact tests (SPSS Exact Test module, SPSS Inc. 2009) were used to compare expected and observed frequencies, as expected frequencies of some group size categories were below five (despite pooling larger group categories). The observed proportion of aggregating geckos in the pilot study (47.2 %, 17 of 36 geckos) was used as a reference (i.e. expected proportion) in the analysis of subsequent seasons. The proportions of aggregating geckos in October 2008, March and June 2009 were compared to the expected proportion by binomial exact tests to determine whether aggregation patterns varied across seasons.

Only captured geckos of known gender and size were used in the group composition analysis. Contingency table analyses (Fisher's exact tests) were used to examine whether juveniles, females and males differed in their tendency to aggregate. The low sample size of geckos in each sex group precluded a meaningful statistical analysis of body size (SVL) with respect to aggregation status for each season, thus data were pooled across seasons for analyses. Because the population was repeatedly sampled across seasons, I cannot exclude the possibility that some individuals were recaptured in successive field trips. Therefore, statistical tests assuming the independence of data could not be applied. Instead, I presented means and 95 % confidence intervals of SVL measurements (normally distributed) of females, males and juveniles with respect to aggregation status and considered non-overlapping 95% confidence intervals as an indication for significant differences between groups. Seasonal body temperatures (Tb, pooled for groups), shelter temperatures (Ts) and air temperatures (Ta) were compared using Wilcoxon signed-rank tests. Seasonal temperature profiles of aggregating (pooled) and solitary geckos and their shelters were compared using Mann-Whitney U tests. An alpha level of < 0.05 was used as criterion for statistical significance. Sequential Bonferroni corrections (Holm 1979) were applied to adjust for multiple testing across seasons and corrected p–values were reported. The uncorrected p-value was also listed when the sequential Bonferroni correction increased the p-value above the significance level of 0.05. Means ± SE were reported (unless otherwise stated). All data analyses were performed using PASW Statistics 18 Core System for Windows with PASW exact module for small sample sizes (SPSS Inc, 2009).

Results

Duvaucel's geckos were located in 3.1 – 4.5 % of all shelters that were checked throughout the study (Table 1). Geckos were mainly encountered in rock crevices, cavities under boulders (including sea bird burrows), ngaio tree cavities or under loose bark of old pohutukawa trees (Fig. 1). Few lizards, mostly juveniles, sheltered within the stumps of dead pampas grass bushes (vegetation) (Fig. 1).

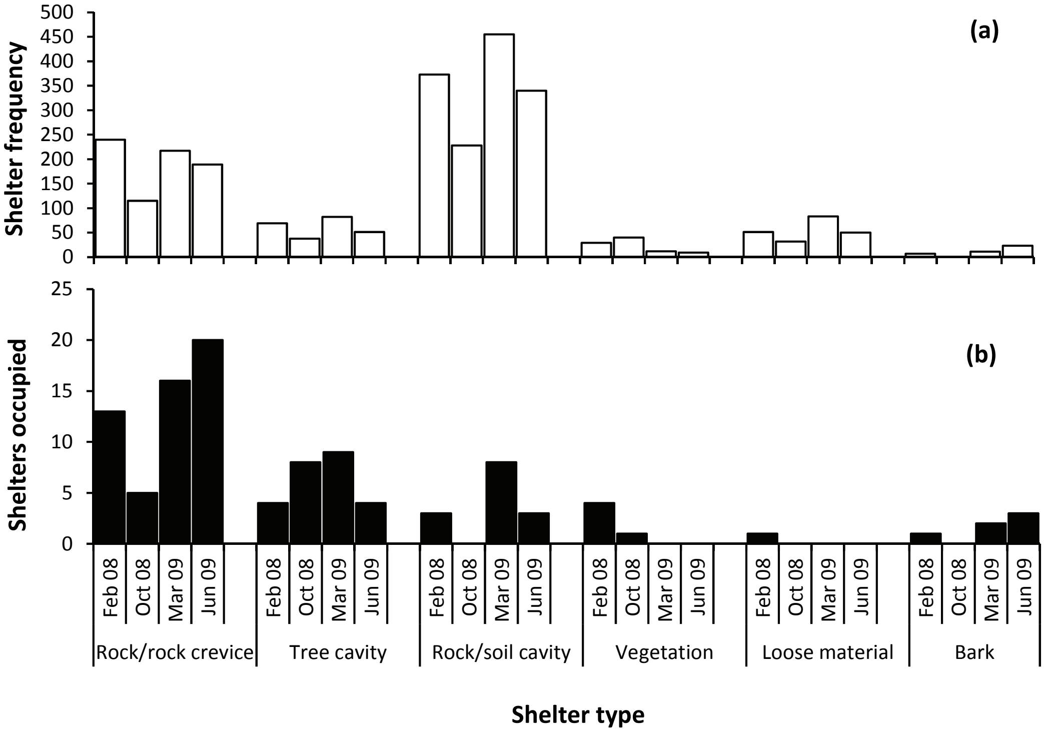


Figure 1: Number of shelters searched (a) and occupancy (b) of different shelter types by Duvaucel's geckos across seasons.

Crevice widths of shelters with geckos varied from 1.5 to 30 cm; however geckos generally used relatively narrow crevices of approximately 2 to 5 cm width (4.8 ± 0.78 cm). Particularly large gecko aggregations were found in old ngaio tree cavities (maximum group size = 8) that were infested with scale insects *Coelostomidia zealandica* or in rock crevices between large boulders (maximum group size = 7). Aggregations were generally loose and animals were rarely in physical contact with one another. However, large groups sheltering in retreats with limited surface area, particularly in hollow tree branches, were tightly packed.

Table 1: Comparisons of observed and expected frequencies of shelters harbouring different group sizes of geckos (Chi-square goodness-of-fit exact tests: all p < 0.001, group sizes of ≥ three were pooled). Expected shelter frequencies were derived from Poisson probabilities based on an average of 0.063 geckos per shelter (across all months). Plus (+) and minus (-) signs indicate the direction the observed frequencies deviate from the expected frequencies. Frequencies of all age/sex class combinations and solitary geckos are listed (F = female, M = male, J = juvenile, UA = unknown adult, UJ = unknown juvenile). Groups of which all individuals were captured and identified are underlined.

Month (season)	Group size category	Combinations	Observed frequencies	Expected frequencies	
February 2008	0	-	747	725.80	+
(late summer)	1	3F, 3M, 3J, 9UA, UJ	19	45.73	-
	2	FJ, MJ, MUA, JUJ	4	1.44	+
	3	FMJ, FFM, UAUAUJ	3	0.03	+
			773	773.00	
October 2008	0	-	441	427.22	+
(spring)	1	3M, 2J, 3UA, UJ	9	26.91	-
	2	FF, FJ	2	0.85	+
	3	-	0	0.02	+
	4	FMJUA	1	0.00	
	5	-	0	0.00	
	6	FMJJJJ	1	0.00	
	7	-	0	0.00	
	8	FFFMJJJJ	1	0.00	
			455	455.00	
March 2009	0	-	825	807.49	+
(early autumn)	1	F, 9M, 5J, 9UA, 2UJ	26	50.87	-
	2	3FM, FJ, MUA, UAUA	6	1.60	+
	3	FMJ	1	0.03	+
	4	FFJJ, MJJJ	2	0.00	
			860	860.00	
June 2009	0	-	632	621.58	+
(winter)	1	5F, 3M, 2J, 8UA, UJ	19	39.16	-
	2	FM, 2MUA, UAUA, UJUJ	5	1.23	+
	3	FFM, FJUJ	2	0.03	+
	4	FFUAUJ, FJUAUA	2	0.00	
	5	-	0	0.00	
	6	FFFMJJ	1	0.00	
	7	UAUAUAUJUJUJUJ	1	0.00	
			662	662.00	

Do Duvaucel's geckos aggregate?

Duvaucel's geckos sheltered solitarily and in aggregations. Group size ranged from 2 to 8 (Table 1) with an overall average of 3.1 ± 0.3 geckos per group (summer 2008: 2.4 ± 0.2, n = 7; spring 2008: 4.4 ± 1.2, n = 5; autumn 2009: 2.6 ± 0.3, n = 9; and winter 2009: 3.4 ± 0.5, n = 11). In all seasons, shelters were occupied by solitary individuals less often than expected and harboured groups more often than expected by chance (Table 1), indicating a clumped distribution of geckos (summer 2008: χ^2 = 314.825, d.f. = 3, $p < 0.001$; spring 2008: χ^2 = 505.199, d.f. = 3, $p < 0.001$; autumn 2009: χ^2 = 281.799, d.f. = 3, $p < 0.001$; winter 2009: χ^2 = 1378.902, d.f. = 3, $p < 0.001$, Chi-square goodness-of-fit exact tests). While a significantly greater proportion of geckos was found in aggregations in spring (22 of 31, 71%, p = 0.012, Binomial test, BT) and winter (37 of 56, 57%, p = 0.009, BT), aggregation patterns in early autumn did not differ (23 of 49, 47%, p = 0.542, BT) from the expected proportion of 47.2%. All age-sex classes participated in aggregations. Females, males and juveniles did not differ significantly in their tendency to aggregate in any season (summer 2008: p = 1.0, spring 2008: p = 0.291, autumn 2009: p = 0.105, summer 2009: p = 1.0, Fisher's exact tests, FET). However, a larger proportion of all identified females and juveniles occurred in aggregations rather than solitarily, but only approximately half of the males were found in aggregations (Table 2).

Table 2: Proportions of female, male and juvenile Duvaucel's geckos aggregating in each month. Numbers after the slash (/) refer to the total number of all identified individuals in each age/sex category. Percentages of aggregating geckos are given in parentheses to facilitate interpretation.

	Proportion aggregating					
Month	Females		Males		Juveniles	
Summer 2008	4/7	(57.1%)	4/7	(57.1%)	4/7	(57.1%)
Spring 2008	8/8	(100%)	3/6	(50.0%)	10/12	(83.3%)
Autumn 2009	7/8	(87.5%)	6/15	(40.0%)	7/12	(58.3%)
Winter 2009	10/15	(66.7%)	5/8	(62.5%)	4/6	(66.7%)

Group composition

Males in aggregations were significantly larger than solitary males (Table 3). Body sizes of females and juveniles in contrast, did not differ in relation to aggregation status (Table 3).

Table 3: Comparisons of body size (snout–to-vent length, SVL) between solitary and aggregating female, male and juvenile Duvaucel's geckos. Presented are N (number of individuals), minimum and maximum SVL (Min, Max) and 95% confidence interval (95% CI). Asterisks (*) indicate that two groups are significantly different based on non-overlapping confidence limits.

Age/sex	Status	N	Min SVL (mm)	Max SVL (mm)	Mean SVL (mm)	95% CI
Females	solitary	10	111.00	127.50	121.25	116.77 - 125.73
	aggregating	28	110.50	128.00	119.32	117.32 - 121.32
Males	solitary	18	111.00	126.00	120.11	117.80 - 122.42*
	aggregating	17	114.00	133.00	125.28	122.63 - 127.92*
Juveniles	solitary	12	52.00	106.00	82.96	69.48 - 96.44
	aggregating	25	50.00	107.00	77.36	69.72 - 85.01

The majority of groups were comprised of a mix of juveniles and adults, but adult-only groups were also encountered frequently (Table 4). Juvenile-only aggregations were rarely seen: A pair of juveniles was found in a stump of dead pampas grass in February 2008 and another pair of juveniles was encountered in a narrow rock crevice in June 2009 (Table 1). However, in both instances, not all juveniles could be captured, and thus were not listed in Table 4. Overall, mixed sex groups (with or without juveniles) occurred 5.5 times as often as female only groups (Table 4). Adult males never occurred together in any aggregation (Table 4).

Table 4: Frequencies of groups (all group members identified) for each age and sex composition category. Asterisks (*) indicate that groups can in include juveniles.

Composition	Summer 2008	Spring 2008	Autumn 2009	Winter 2009	Total
Adults - juveniles	3	3	4	1	11
Juveniles only	0	0	0	0	0
Adults only	1	1	3	2	7
Males - females*	2	2	4	3	11
Males only*	0	0	0	0	0
Females only*	0	1	1	0	2

Temperature profiles

Average Ts of shelters harbouring gecko aggregations did not differ significantly from Ts of shelters with solitary geckos in any season (Table 5, all p > 0.1, Mann-Whitney U tests, MWT). Correspondingly, no differences were found in average Tb between aggregating and solitary geckos (Table 5, all p > 0.1, MWT).

Table 5: Mean ± SE shelter and body temperatures (°C) of aggregating (based on pooled values of all group members) and solitary geckos. N denotes the number of groups and the number of solitary geckos.

Parameter/ Season	Aggregating			Solitary		
	N	Mean	± SE	*N*	Mean	± SE
Shelter temperature						
Summer 2008	4	23.3	± 0.3	9	22.5	± 0.4
Spring 2008	4	19.0	± 0.2	5	17.9	± 0.2
Autumn 2009	7	21.7	± 0.5	15	21.7	± 0.3
Winter 2009	3	15.2	± 0.1	10	14.8	± 0.3
Body temperature						
Summer 2008	4	25.0	± 0.5	9	23.4	± 0.6
Spring 2008	4	20.4	± 0.6	5	19.4	± 0.9
Autumn 2009	7	22.1	± 0.4	15	21.9	± 0.3
Winter 2009	3	15.3	± 0.5	10	15.3	± 0.4

Diurnal body temperatures (Tb) of geckos in shelters ranged from 12.8 °C in winter to 26.6 °C in summer. Similarly, lowest shelter temperatures (Ts) and air temperatures (Ta) were observed in winter (13.0 °C and 13.2 °C, respectively) and maximum values were obtained in summer (24.3 °C and 26.6 °C, respectively). While average seasonal Ts were always lower than average Ta and Tb, there was little difference between Tb and Ta (Figure 2). Ts differed significantly than Ta in summer 2008 (Z = -3.061, p = 0.002, WSRT), spring 2008 (Z = -2.524, p = 0.024, WSRT), but not in autumn 2009 (Z = -0.967, p = 0.347, WSRT) or winter 2009 (Z = -2.121, p = 0.068, WSRT, before sequential Bonferroni correction: p = 0.034). Ts were significantly lower than Tb in summer 2008 (Z = -3.182, p < 0.001, WSRT), but not in spring 2008 (Z = -1.960, p = 0.055, WSRT), autumn 2009 (Z = -1.057, p = 0.301, WSRT) or winter 2009 (Z = -0.664, p = 0.530, WSRT). Tb and Ta did not differ significantly in any season (all p > 0.1, WSRT).

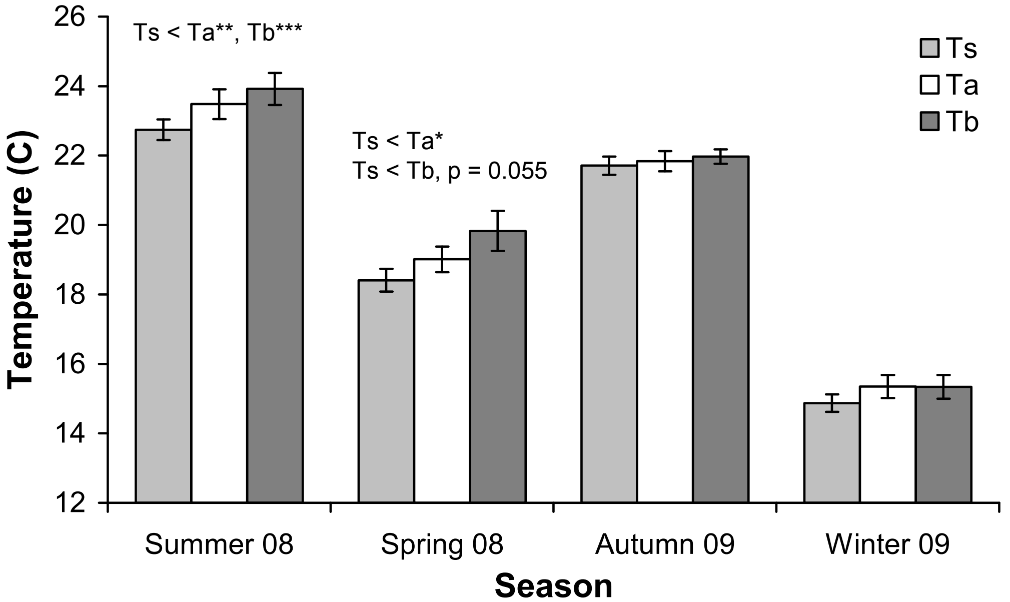


Figure 2: Comparison of shelter temperatures (Ts), air temperatures (Ta), and body temperatures (Tb) of sheltering Duvaucel's geckos in summer (n = 13), spring (n = 9), autumn (n = 22) and winter (n = 13). Temperature data were pooled per gecko group. Asterisks (*** $p < 0.001$, ** $p < 0.01$, ** $p < 0.05$) indicate significant differences between temperature profiles. Bars represent means ± SE.

Discussion

Duvaucel's geckos showed a clumped distribution and formed diurnal aggregations in their shelter sites throughout the year. It is unlikely that aggregations occurred because of a limited availability of shelters, as unoccupied suitable shelters were abundant in the study area. Moreover, geckos utilised a wide range of natural structures as retreats and thus were not restricted to a particular type of shelter. Higher proportions of geckos aggregated in winter and in spring. These were also the seasons in which the largest aggregations were recorded. The increased number of geckos in aggregations during winter could reflect an attraction of geckos to shelters with preferred thermal properties or because geckos gained some physiological benefits by aggregating with conspecifics (see Shah *et al.* 2003, Lancaster *et al.* 2006). However, I did not observe any temperature differences between shelters with gecko aggregations and shelters with solitary geckos in any season. Likewise, body temperatures of aggregating geckos did not differ from those of solitary geckos. Moreover, gecko body temperatures were generally warmer than shelter temperatures and were similar to air temperatures, suggesting that geckos regulated their body temperature by direct sun-basking (Whitaker 1968), which has also been observed in other nocturnal New Zealand gecko species (Werner and Whitaker 1978). Most shelters within the study site were located under the forest canopy and in shaded areas, which may explain why shelter temperatures were generally cooler than air temperatures. The cooler shelter temperatures may have helped the geckos to maintain constant body temperatures, particularly in the warmer seasons. However, body temperatures of both, solitary and aggregating geckos, largely resembled air temperatures, which may indicate that shelter thermal properties did not play a significant role in the formation of groups. Furthermore, group members were rarely found in close physical contact, suggesting that Duvaucel's geckos were unlikely to gain thermoregulatory benefits by aggregating with conspecifics. In contrast, Shah *et al.* (2003) found that thick tailed geckos (*N. milii*) typically position themselves in close physical contact with other group members and thereby gain control over thermal exchange rates i.e. their body temperature increases or decreases less rapidly in comparison to solitary individuals. Alternatively, Lancaster *et al.* (2006) suggested that banded geckos (*C. variegatus*) gain a physiological benefit from aggregating with conspecifics by increasing the humidity in retreat sites, which in turn decreases rates of evaporative water loss in grouping individuals. In contrast to Duvaucel's geckos, banded geckos inhabit hot dry desserts and may experience a considerable variation in their shelter climate due to extreme fluctuations of temperature and humidity levels. Duvaucel's geckos live in a temperate climate and, in this study, utilised shelters within the forest where humidity and temperature levels are not extreme. The largest gecko aggregations were recorded in winter and spring, when levels of respiration are typically high. Therefore it seems unlikely that humidity and evaporative water loss influenced the distribution of Duvaucel's geckos in diurnal shelters. However, it could be possible that the availability of easy accessible basking sites in the vicinity of a shelter may account for the non-random distribution of geckos in the field. Such basking sites could be areas where sun-rays break through the forest canopy for longer periods of the day. Since the aforementioned variables were not measured in the present study, the relationships with humidity levels and basking site availability remain to be tested.

An alternative explanation to aggregation patterns of Duvaucel's geckos may lie in their feeding habits. For example, larger groups of geckos occupied the cavities of hollow branches of old ngaio trees in winter and spring. These trees were all heavily infested with *Coelostomidia zealandica,* small sap-feeding scale insects that exude droplets of a sugar-rich liquid (honey dew) year-round. Geckos are known to feed on honey dew and large feeding congregations have been observed on infested trees at night on Korapuki Island (Towns 2002). Hence, geckos could be attracted by these food sources and may choose shelter sites close by. Furthermore, whether the seasonal availability of some food sources such as seasonal fruits or nectar influences the distribution of Duvaucel's geckos in shelter sites requires further study.

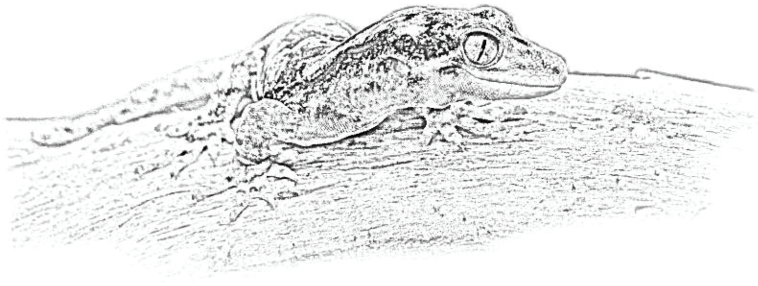
Although no statistical evidence was found that females, males and juveniles differed in their tendency to form aggregations, a greater proportion of females (57 to 100 %) and juveniles (57 to 83 %) aggregated in comparison to males (40 to 62.5 %) throughout the study period. I detected a sex ratio of approximately 1:1 (M:F) based on all identified lizards captured in the course of this study. This confirms the results of a night-time spot-light survey carried out in February 2008 on Korapuki Island (van Winkel and Barry, *unpubl. data*) and corresponds to sex ratios reported for adult Duvaucel's geckos on other offshore islands (Christmas 1995, Wilson 2010). The sex ratio of the groups however, ranged from 1:1 to 1:3, illustrating that never more than one adult male was found within the same group. Mixed sex groups with or without juveniles occurred year-round and groups with 1:1 sex ratios were more common than groups consisting of one male and several females. This could indicate that male and female Duvaucel's geckos may stay in pairs most of the time. Male Duvaucel's geckos that did aggregate were larger than solitary males; this has also been observed in shelter sharing Bibron's geckos *C. bibronii* (Meyer and Mouton 2007). If these sex-specific grouping patterns were related to mating, then larger males may have been guarding females and potentially have excluded smaller males from associating with females (Cuadrado 2001). Christmas (1995) noted that neither male nor female Duvaucel's geckos appeared to be territorial and further suggested that (pairs of) geckos may defend shelter sites. My data indicate that females and males accept other females within their daytime shelters but males do not tolerate other adult males. Dominance and aggressiveness during male-male encounters are known to be positively related to body size in many squamates (e.g. broad-headed skink *Eumeces laticeps,* Cooper and Vitt 1987, copperheads *Agkistrodon contortrix*, Schuett 1997, common chameleons *Chamaeleo chamaeleon,* Cuadrado 2002). By guarding females, the larger Duvaucel's gecko males could prevent smaller males from mating opportunities and thereby gain mating advantages themselves (Censky 1997, Cuadrado 2002). However, little is known about the species mating system and further research is required to determine whether these male – female associations are stable over time, particularly during the night when geckos leave their resting sites to forage.

Juvenile Duvaucel's geckos were found in close association with adults of both sexes throughout the study, suggesting a high tolerance of juveniles by adults. Large groups contained juveniles of several size classes and in some instances groups included pairs of juveniles that resembled each other in size, which could indicate that pairs of siblings may stay together. Juvenile-only groups were rarely observed in this study, although, solitary juveniles were relatively common. However, the large proportion of adult-juvenile associations could be evidence that juvenile dispersal is limited and that offspring may remain with their parents. The tolerance exhibited by adults towards juvenile conspecifics (potentially kin) may provide an advantage for juvenile survival. Juveniles that share shelters with their parents could benefit from indirect parental care such as defence of shelter sites from other conspecifics, which may reduce the risk of infanticide by unrelated individuals (see O'Connor and Shine 2004, Langkilde *et al.* 2007). Another benefit of remaining in close association with parents may be increased predator vigilance (Lanham and Bull 2006). Whether the tolerance of juveniles by adults and the presence of several size classes of young individuals in Duvaucel's gecko aggregations is evidence for kin sociality requires further research. Exploring juvenile dispersal patterns could help to determine whether juveniles remain with their parents. Little is known about the temporal stability of these aggregations or shelter site fidelity. Molecular methods could assist in determining the genetic structure within a group and elucidate whether group members, particularly females and juvenile represent kin. Moreover it could help to determine possible sex specific dispersal patterns.

In conclusion, my findings suggest that Duvaucel's geckos may possess a social system of high complexity. Further research determining group stability, genetic relationships of group members as well as the degree of juvenile dispersal could help to clarify this. To uncover the causation and function of such shelter aggregations, it could help to explore whether social factors such as relatedness and familiarity influence shelter choices of individuals (see Chapter 4). Information about the spatial organisation and social structure of gecko communities does not only advance our limited knowledge of lizard social systems, it may also be of benefit to conservation practitioners when choosing individuals for captive breeding programmes and translocations. Furthermore, it could be of great importance for assessing abundance and carrying capacities.

CHAPTER FOUR

An experimental investigation of shelter-trait preferences and aggregation behaviour in Duvaucel's geckos (*Hoplodactylus duvaucelii*)



Abstract

Wild Duvaucel's geckos (*Hoplodactylus duvaucelii*) tend to aggregate in small groups in their diurnal shelters throughout the year. However, it is unknown whether these aggregations are resource driven or are mediated by social factors. I offered adult and juvenile geckos a suite of different shelter types and determined their preferences for specific shelter traits through choice experiments. I then offered small groups of geckos an excess of optimised shelters to explore whether geckos aggregated with conspecifics in relation to social factors such as familiarity, relatedness or sex and season (mating *vs.* non-mating season). Geckos selected shelters based on size, degree of crevice concealment and substrate, but showed no preference for crevice width or the shelter's overlaying material. Geckos preferred a warm rather than a cool shelter environment but humidity levels played no role in shelter selection. Up to 44 % of all geckos aggregated throughout the study, but aggregation behaviour was not found to be mediated by familiarity or relatedness between individuals. Geckos of all sex and age classes participated in aggregations. Aggregation patterns of mixed-sex adult groups did not vary between the mating and non-mating season. Among adults, mixed-sex groups occurred more frequently than same-sex groups and males generally avoided each other.

Introduction

Structural and thermal features play an important role for nocturnal lizards when selecting suitable day-time retreat sites (Schlesinger and Shine 1994, Kearney and Predavec 2000). For non-burrowing lizards, suitable shelters that provide optimal environmental conditions and sufficient protection from predators may not be abundant enough to accommodate all individuals in a population. Therefore, lizards may share shelters if their tolerance level towards conspecifics is high and the risks associated with sharing a shelter are low. The occurrence of shelter aggregations can be mediated by communal attraction to a limited resource (Nieuwoudt *et al.* 2003) as well as by conspecific attraction, whereby animals aggregate because they gain some benefit from the close proximity to conspecifics. These benefits can include increased vigilance (Lanham and Bull 2004), mating advantages (Chapple and Keogh 2005) or protection from infanticide by indirect parental care (O'Connor and Shine 2004, Langkilde *et al.* 2007). Individuals may also gain a physiological benefit by sharing a shelter with conspecifics, such as thermoregulatory advantages (Shah *et al.* 2003) or control over rates of evaporative water loss (Lancaster *et al.* 2005). Shelter aggregations may only occur temporarily as seen in winter aggregations (Boykin and Zucker 1993, Elfstroem and Zucker 1999) or breeding aggregations (reviewed in Graves and Duvall 1995), or are formed on a regular basis. The latter is generally considered to be uncommon among lizards. However, there is growing evidence that the formation of permanent shelter aggregations is more widespread across taxa than previously thought (Lemos-Espinal *et al*. 1997, Kearney *et al.* 2001, Duffield and Bull 2002, Nieuwoudt *et al.* 2003, Todd 2005, Chapple and Keogh 2006, Meyer and Mouton 2007, Schutz *et al.* 2007, Chapter 3). Aggregation behaviour and social organisation have been studied intensely in few scincid and cordylid lizards, mainly *Egernia spp.* (e.g. Duffield and Bull 2002, Osterwalder *et al.* 2004, Langkilde *et al.* 2007) and *Cordylus spp.* (e.g. Nieuwoudt *et al*. 2002, Visagie *et* al. 2002, Visagie *et al.* 2005). Although it has been shown that various nocturnal gecko species form shelter aggregations in the wild (Burke 1994, Kearney *et al.* 2001, Todd 2005, Meyer and Mouton 2007, Chapter 3) and under laboratory conditions (Cooper *et al.* 1985, Shah 2003, Lancaster *et al.* 2006, Meyer and Mouton 2007), the social structure and stability of these groups is poorly understood.

This study experimentally investigated aggregation behaviour of Duvaucel's geckos (*Hoplodactylus duvaucelii*), large cryptic geckos from New Zealand. Wild Duvaucel's geckos tend to share diurnal shelters year-round and groups often consist of several adults and young (Chapter 3). To date, it is unknown whether Duvaucel's geckos share shelters for social reasons or whether other factors such as physiological benefits play a role in group formation. Thus, I conducted controlled laboratory experiments to explore the possible mechanisms behind the group formations observed in wild Duvaucel's geckos (Chapter 3). The objectives of this study were to investigate what shelter traits Duvaucel's geckos prefer and to determine whether social aspects such as familiarity, relatedness or sex and season mediate aggregation behaviour. First, I developed an optimised and easy to replicate shelter based on the lizards' preferences for specific shelter traits. I then offered groups of three geckos an excess of identical optimised shelters, to minimise the potential effects of limited shelter availability and varying shelter quality on aggregation behaviour, and examined aggregation patterns with respect to social variables.

Methods

Study species and animal maintenance

Duvaucel's geckos (*H. duvaucelii*) are nocturnal lizards with terrestrial and arboreal habits (Whitaker 1968, Barwick 1982). The geckos are endemic to New Zealand and although once wide spread (Towns and Daugherty 1994), their current distribution is restricted to offshore islands along the north-east coast of New Zealand's North Island and in the Cook Strait. Single individuals or small groups typically shelter during the day in crevices beneath boulders, rocks, bark or in tree cavities and may occasionally emerge to sun bask (Whitaker 1968, Chapter 3). Duvaucel's geckos are long-lived (at least 43-50 years in the wild, Wilson 2010), viviparous and their annual reproductive output is extremely low among geckos (approximately 1.12 offspring per female, Cree 1994). Mating occurs in spring (September to November), followed by an extended gestation period, (5 to 8 months or longer, Barwick 1982, Cree 1994, Christmas 1995) and females typically give birth between late summer to autumn (February – May). Juveniles are slow growing and may reach sexual maturity in their seventh year (Barwick 1982). For this study, I used 29 adult (20 females and 9 males) and 20 juvenile Duvaucel's geckos from a captive breeding facility at Massey University, Albany Campus in Auckland. The adult geckos were wild born (snout-to-vent-length [SVL] range: 113 to 127 mm, weight:

45 to 70 g) and collected in November 2006 from Korapuki Island (Mercury Islands, New Zealand). Adult geckos were housed in groups of four or five in six naturalistic outdoor enclosures. Juveniles (SVL range: 58 to 68.5 mm, weight: 5.7 to 10.9 g) were the progeny of the wild captured adults and were born in captivity in March 2008. Throughout the study, juveniles were held in groups of four to six in naturalistic indoor vivaria under a light source, with a 12:12 L:D cycle to imitate natural light conditions. All lizards were fed live insects or tenebrionid larvae and fruit puree mix with vitamin/mineral supplements twice a week (once a week in winter). Water was provided *ad libitum*.

Shelter choice experiments

Five sets of shelter-choice experiments were carried out between 4 June and 5 August 2008 (winter) in an unheated room at ambient temperature, humidity and light levels. Room temperature (8 - 16 °C; mean ± standard deviation [SD]: 11.12 ± 2.25 °C) and absolute humidity (6.1 - 12.5 mg/m^3; 8.3 ± 1.83 mg/m^3) were recorded daily at 08:25 h with a HOBO® data logger (Model H08-004-02). Forty randomly selected geckos (20 juveniles and 20 adults) were given a choice of two to four different shelter types per experiment, whereby up to six trials were run simultaneously. Each gecko was tested only once per experiment. Test arenas were white plastic containers (620×420×370 mm) or Perspex® enclosures (800×510×520 mm) with meshed lids and a viewing window on one side, lined with absorbent paper. Three sides of the Perspex® enclosures were covered with white paper to prevent lizards in adjacent test arenas from seeing each other. Basic shelters were inverted rectangular plastic trays (240×125×26 mm), which were modified with respect to the shelter trait that was tested (Figure 1). An 'entrance' was created by cutting out the rim on one short side of the rectangular tray (medium opening, Figure 1, 1a-d). In five successive trials geckos could select between the following shelter traits:

Shelter size and crevice width (Figure 1, 1a-1d): Individuals were given a choice of four shelter types with varying surface area and crevice width (i.e. shelter height): a) large and narrow: juveniles: 120×125×10 mm; adults: 240×125×22 mm, b) small and narrow: juveniles: 65×125×10 mm; adults: 120×125×22 mm, c) large and wide: juveniles: 120×125×34mm; adults: 240×125×34 mm, d) small and wide: juveniles: 65×125×34 mm; adults: 120×125×34 mm). The minimum shelter crevice width was determined by that height in which the "thickest" gecko (with the greatest dorso-ventral width) could just fit under the shelter, but was still able to move in and out without lifting the shelter. Crevice width was increased by placing shelters on thin Corflute® strips. The minimum shelter size (surface area) was large enough to cover the largest gecko entirely.

Entrance size (degree of concealment, Figure 1, 2a-2c): Geckos were given a choice of three shelters with different sized openings: a) small (50 mm, short side), b) medium (125 mm, short side) and c) large (240 mm, long side).

Overlaying material (Figure 1, 3a-3b): Shelter covers were a) 'plastic' (inverted plastic trays) or b) 'stone' (granite tiles fitted on walls of thin Corflute® strips). The two shelter types had equal crevice sizes and heights as well as openings.

Substrate (Figure 1, 4a-c): Equally sized shelters were placed over a) 'stone' (granite tiles), b) 'soil' (sieved black soil) or c) 'paper' (white absorbent paper).

Temperature and humidity (Figure 1, 5a-5d): Four equally sized shelters were placed on top of granite tiles. Two of these shelters were heated and two shelters remained cold (ambient temperature). To heat shelters, a heat pad (Hagen®, 8 Watts) was placed underneath the test arena below the shelter. One of the 'cool' and one of the 'warm' shelters were moistened using an ultrasonic fog generator (Exoterra®) immersed in a water-filled plastic container, that produced a fine mist and increased the humidity level in the adjacent shelters. The water container with the fog generator was placed between the two 'humid' shelters opposite the shelter-entrance side. Temperature and humidity levels of shelters were monitored with HOBO® data loggers, which were placed in all four shelters of one randomly chosen test arena. The average temperatures and absolute humidity levels of the four different shelter climate conditions recorded daily at 08:00 h were: a) warm-dry (mean Temp ± SD: 28.27 ± 3.89 °C; mean absolute humidity ± SD: 11.63 ± 4.47 mg/m^3); b) warm-humid (27.65 ± 4.06 °C; 15.62 ± 5.20 mg/m^3); c) cool-dry (14.82 ± 3.16 °C; 8.75 ± 5.20 mg/m^3); and d) cool-humid (14.23 ± 3.43 °C; 11.96 ± 3.03 mg/m^3).

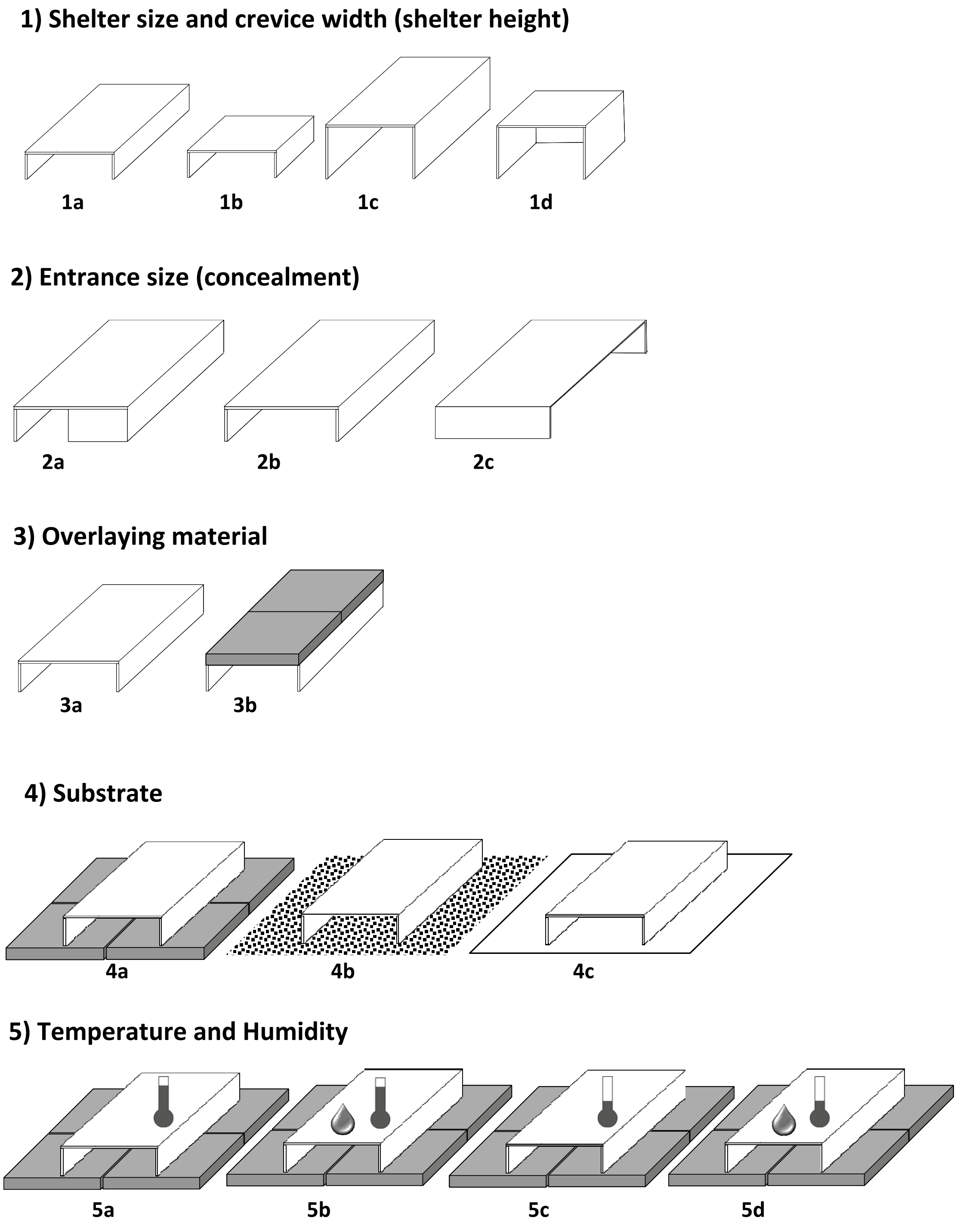


Figure 1: Schematic diagram of shelter types used in choice experiments. Geckos could select between two to four different shelter types in each of the five subsequent experiments. Shelter traits altered were 1) shelter size and crevice width (shelter height), choices were: 1a) large surface area and narrow crevice, 1b) small surface area and narrow crevice, 1c) large surface area and wide crevice, 1d) small surface area and wide crevice, 2) entrance size, choices were: 2a) small entrance, 2b) medium entrance, 2c) large entrance, 3) overlaying material, choices were: 3a) plastic, 3b) stone tile, 4) substrate, choices were: 4a) stone tile, 4b) soil, 4c) paper, 5) temperature and humidity (shelter climate), choices were: 5a) warm and dry, 5b) warm and humid, 5c) cool and dry, 5d) cool and humid. See text for detailed description of shelter types.

Test shelters were arranged adjacent with the entrances facing the same direction (three shelter choices) or placed in opposite corners with entrances facing towards the centre of the arena (two or four shelter choices). Positions of the different shelter types were randomly assigned across trials. In trials that involved the ‘humid’ treatment, the two humid shelters (warm and cool) were placed opposite both ‘dry’ shelters (warm and cool). This was necessary because the fog generator produced a fine mist that moistened areas within 25 cm, which eliminated the option of placing a ‘dry’ shelter next to a ‘humid’ one. However, positions of wet and dry shelters as well as positions of warm and cool shelters were randomly interchanged.

Geckos were transferred from their home enclosures into the test arenas in the evening before the data collection (between 17:00 and 18:00 h). This allowed the geckos time to explore their new environment overnight, and to choose their shelters before sunrise. Positions of geckos were recorded the next morning between 08:00 and 09:00 h when the lizards typically had retreated under a shelter. After data collection, geckos were returned to their original enclosures. All test arenas, shelters and tiles were thoroughly cleaned with 95% ethanol and rinsed with water to remove scent traces. Once dry, test arenas were lined with new absorbent paper. Soil used in trials for substrate preference was discarded after each trial and fresh soil was laid out. The final optimised shelter type incorporated all traits that were preferred by the test geckos. If geckos did not favour one particular shelter type, either a combination of the traits or the easiest option to replicate, was chosen.

Aggregation experiment

Three sets of aggregation experiments were conducted between 7 August 2008 and 10 February 2009. For each trial, a group of three geckos were placed in the centre of a test arena with six identical optimised shelters in the evening before data collection (between 16:00 and 18:00 h) to allow acclimatisation. Shelter positions of geckos were recorded the next morning between 08:00 and 10:00 h. The test arena was a rectangular Perspex® container (as described above), lined with filter paper and covered with a meshed lid. Since some geckos had not retreated to a shelter during morning checks in shelter choice experiments (presumably due to low ambient light conditions), a light source was placed on top of the lid and set to a 12:12 L:D photo cycle, controlled by a timer (lights on at 07:00 h) to encourage geckos to retreat. The six optimised shelters (see Table 1) were placed side-by-side on top of granite tiles with all openings facing towards the viewing window. The room temperature was set at 24 °C (controlled by a thermostat) and the tiles under each shelter were moistened at trial start with a water mister. Shelter temperatures and humidity levels were monitored with a HOBO® data logger that was randomly assigned to one of the six shelters (mean Temperature ± SD at 8:00 h: 20.58 ± 1.38 °C, min-max: 18.28 – 24.40 °C, mean absolute humidity ± SD: 12.36 ± 2.33 mg/m^3, min-max: 8.40 – 19.00 mg/m^3). Individual geckos that were combined in a trial were taken from different enclosures and had never encountered one another during their time in captivity (>15 months), unless otherwise stated. The focal animal was randomly chosen from the study population and had the choice of grouping with two randomly selected conspecifics (test geckos). Each focal animal was only used once per experiment and no gecko was combined with the same individual more than once throughout the entire study. Each gecko was labelled with a non-toxic (xylene-free) silver-pen marking on their flank to allow individual identification. The following experimental treatments and group combinations were employed: (1) *Sex and Season:* Adult focal geckos (4 males, 9 females) were placed in test arenas, each with an adult male and a female conspecific. Trials were carried out during the non-mating season (winter, 7 to 17 August 2008) and during the mating season (spring, 24 September to 21 October 2008). (2) *Relatedness:* Focal geckos (juveniles) were either placed in a test arena with a sibling and an unrelated juvenile (10 trials) or with their mother and an unrelated female (9 trials). Trials were carried out 8 December 2008 to 7 January 2009 (summer). (3) *Familiarity:* Eighteen adult focal geckos (9 males, 9 females) were placed in the test arenas with a familiar and unfamiliar adult conspecific of the opposite sex. Additionally, nine adult focal geckos (females) were placed in a test arena with a familiar and unfamiliar conspecific of the same sex. Familiarity status was based on whether or not geckos shared the same outdoor enclosure during the 24 months period before the trial was carried out. Trials were carried out 8 January 2009 to 10 February 2009 (summer, non- mating season).

All experimental treatments and group combinations are summarised in Table 2. The unequal sample sizes were due to the limited availability of study animals with specific ‘social traits’.

Data analyses

Preliminary contingency table analysis (Fisher’s exact tests) revealed no differences in shelter trait selection behaviour between adult and juveniles in any experiment (all $p > 0.1$). Therefore both groups were combined in the analysis for shelter preference. Selection frequencies of the different shelter types were compared using binomial tests (two shelter types) or chi-square goodness-of-fit tests (three or four shelter types) assuming that each shelter was equally attractive. Binomial tests were computed following a significant result of a chi-square test to compare pairs of shelter traits. Sequential Bonferroni corrections (Holm 1979) were applied to correct for multiple comparisons. If a shelter type was not visited by any gecko, then it was assumed that this shelter type was avoided, and thus was excluded from the analysis to reduce the number of comparisons. Trials in which geckos failed to retreat under any shelter were excluded from analyses.

To determine whether focal geckos aggregated, the number of partners of each focal gecko per trial was compared with random expected frequencies. Expected frequencies were derived from calculating Poisson probabilities based on the mean observed frequencies of partners per focal animal for the entire study ($\bar{x} = 0.274$) and by multiplying the probabilities with the number of trials per experiment.

As focal geckos never shared shelters with more than one test gecko, frequency categories with one or two partners were pooled for analyses. Expected and observed frequencies were compared with chi-square goodness-of-fit exact tests to test the null hypothesis that shelter aggregations involving focal and test geckos occurred at random.

Given that only a small proportion of the focal geckos aggregated across all experiments, a statistical analysis of the influence of social factors on grouping behaviour of focal geckos was not possible. Therefore all occurring aggregations (including between non-focal geckos) were described and summarised in relation to each treatment category. Contingency table analysis (Fisher's exact test) was used to examine whether the aggregation tendency of mixed-sex adult groups varied between mating and non-mating season. All data analyses were performed using PASW Statistics 18 Core System for Windows with PASW Exact tests add-on module (SPSS Inc, 2009) and statistical significance was considered at alpha < 0.05. Sequential Bonferroni corrected p-values were reported (where correction was applied).

Results

Shelter preference

Duvaucel's geckos were selective in most of the shelter traits tested (Fig. 2). Geckos preferred shelters with a large surface area and avoided small ones, but did not show any significant preference for crevice width (large and wide *vs.* large and narrow: p = 0.281, n = 31, binomial test, bt).

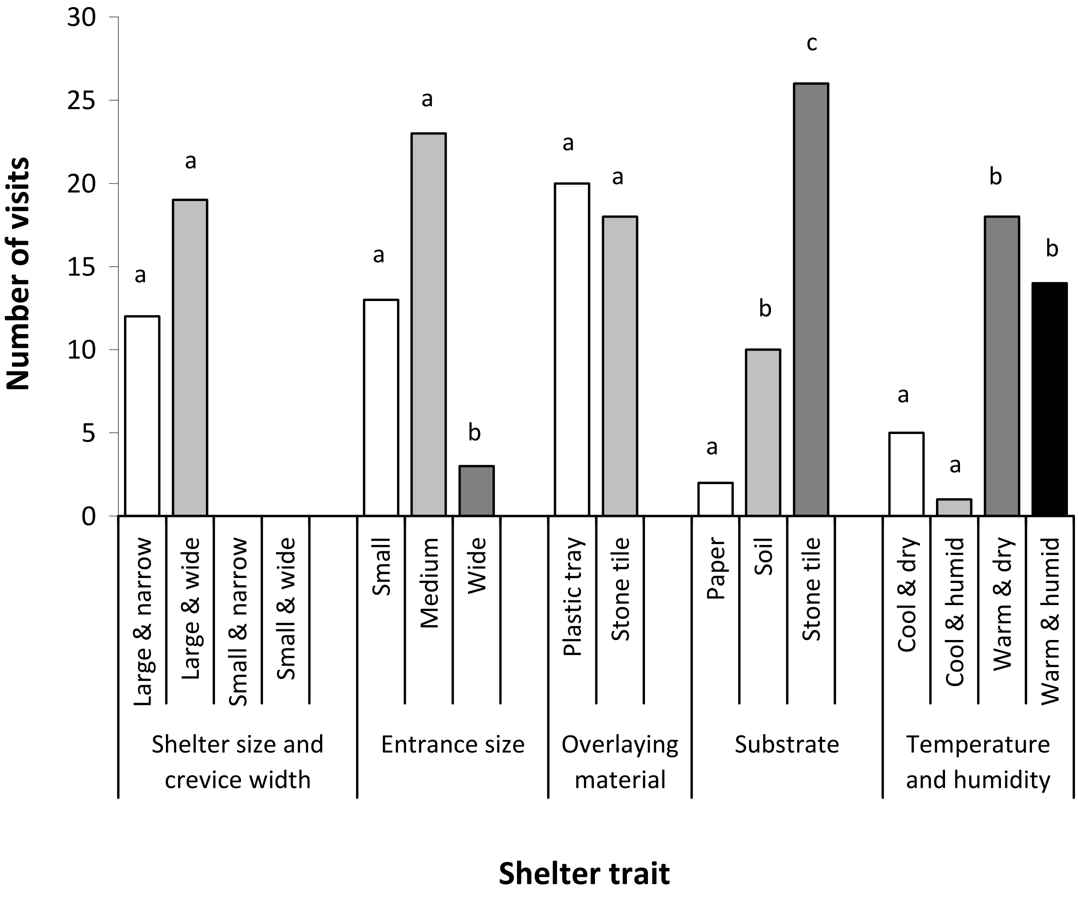


Figure 2: Shelter trait preferences of Duvaucel's geckos (*Hoplodactylus duvaucelii*). Bars represent the number of visits per shelter type. Significant differences from binomial tests ($p < 0.05$, after sequential Bonferroni corrections are labeled with different letters per experiment). Forty geckos were tested in each trial, lower sample sizes are due to some individuals not selecting a retreat.

Geckos differentiated between three shelter-concealment types ($\chi^2 = 15.385$, df = 2, $p < 0.001$, n = 39, Chi-square test), whereby shelters with wide openings had least attention (wide *vs.* medium: $p < 0.001$; wide *vs.* small: $p < 0.042$, bt). Although more geckos rested under shelters with medium openings, shelter occupancy rate did not differ significantly between shelters with small and medium sized openings ($p = 0.132$, bt). Overlaying material did not affect the shelter choice by the geckos (plastic tray *vs.* stone tile: $p = 0.871$, n = 38, bt). Geckos were highly selective of the substrate the shelter was placed on ($\chi^2 = 23.579$, df = 2, $p < 0.001$, n = 38, Chi-square test), with stone being the most preferred substrate (stone *vs.* paper: $p < 0.001$, soil *vs.* stone: $p = 0.022$, bt) and paper the least (soil *vs.* paper: $p = 0.039$, bt). Geckos were also highly selective with respect to the shelter microclimate ($\chi^2 = 19.474$, df = 3, $p < 0.001$, n = 38, Chi-square test). Warm shelters were clearly preferred over cool shelters (warm dry *vs.* cool dry: $p = 0.033$; warm humid *vs.* cool and humid: $p = 0.004$, binomial tests). However, geckos did not differentiate between humid and dry shelters (cool humid *vs.* cool dry: $p = 0.219$; warm humid *vs.* warm dry: $p = 0.597$, binomial tests). Sequential Bonferroni corrections did not change statistical significance in any test. The attributes of an optimised easy to replicate shelter are summarised in Table 1.

Table 1: Shelter traits preferred by Duvaucel's geckos in shelter choice experiments, and attributes of optimised shelters used in subsequent aggregation experiments. Two traits listed under 'preferred shelter traits' indicates that both traits were equally preferred and either an intermediate form of both traits or the easier to replicate option were incorporated in the final optimised shelter type.

Category	Preferred shelter traits	Optimised shelter traits	Measurement
Shelter size and crevice width	Large and narrow Large and wide	Large and medium	240×125×26 mm
Shelter entrance size (degree of concealment)	Small Medium	Medium	125 mm
Overlaying material	Plastic tray Stone tile	Plastic tray	-
Substrate	Stone tile	Stone tile	-
Temperature and humidity	Warm and dry Warm and humid	Warm and humid	*20.58 ± 1.38 °C *12.36 ± 2.33 mg/m^3

* $\bar{x}$ ± SD

Aggregation experiment

Between 10 and 40 % of all focal animals shared a shelter with one other gecko, however, groups of three were never observed. If all aggregations were considered (including between non-focal geckos) then the proportion of trials with shelter sharing geckos ranged from 10 to 66.7 %. Chi-square tests did not reveal any significant differences between the observed and the expected proportion (Table 2), suggesting that the observed aggregation patterns were due to chance alone. However, due to the limited sample size, the power of the statistical analysis may have been compromised.

Table 2: Comparisons (Chi-square exact tests) of observed and expected Poisson frequencies of the number of partners of focal geckos in each experiment. Observed and expected frequencies of one and two partners were pooled for analyses. Number of focal geckos (*n*) and chi-square test statistic are given.

Experimental context	Number of partners	Observed frequency	Expected frequency	χ^2	*df*	*p*
Season and sex						
Mating season	0	11	9.9	0.504	1	0.547
n = 13	1	2	3.1			
	2	0				
Non-mating season	0	9	9.9	0.553	1	0.745
n = 13	1	4	3.1			
	2	0				
Relatedness						
Juveniles and females	0	6	6.8	0.430	1	0.696
n = 9	1	3	2.2			
	2	0				
Juveniles only	0	6	7.6	1.406	1	0.266
n = 10	1	4	2.4			
	2	0				
Familiarity						
Females and males	0	12	13.7	0.860	1	0.406
n = 18	1	6	4.3			
	2	0				
Females only	0	9	7.6	1.075	1	0.469
n = 10	1	1	2.4			
	2	0				

Social assortment

Season and sex: Two focal geckos (15 %) formed mixed-sex groups in the mating season (spring) and three mixed-sex pairs and one male-only pair involving focal geckos (31 %) were found in non-mating season (winter). Aggregation frequency of focal geckos did not differ significantly between seasons ($p = 0.645$, Fisher's exact test). Additional groups were formed by non-focal test geckos in the mating (two mixed-sex and one female-only groups) and non-mating season (two mixed-sex groups, Table 3).

Relatedness: Four focal juveniles (40 %) aggregated with other juvenile test geckos: Two shared a shelter with their sibling and two grouped with an unrelated juvenile. Three focal juveniles (33 %) were found sharing a shelter with an adult female. One of those focal juveniles sheltered with its mother and the other two juveniles each sheltered with an unrelated female. Additionally, non-focal geckos formed three female-only pairs.

Familiarity: Six focal geckos (33 %) aggregated in the mixed sex trial. Of these, five focal geckos sheltered with an unfamiliar conspecific and two focal gecko associated with a familiar conspecific. Only one pair (10 %) aggregated in the female-only trial.

Compositions of all groups with respect to social factors (including groups involving non-focal geckos) are summarised in Table 3.

Table 3: Summary of aggregation experiments (M = adult male, F = adult female, J = juvenile). Listed is the number of trials in each experimental treatment (i.e. number of focal geckos). The total number of groups includes associations between non-focal geckos. Group composition refers to the age/sex combination of groups and social assortment refers to the group composition with respect to social traits (sex, relatedness, familiarity). The last column displays the proportion of all aggregating geckos per experiment.

Experimental treatment	Trials	Combination	Focal geckos grouping	Total number of groups	Group composition	Social assortment of groups	% of geckos aggregating
Sex & season							
Mating season:							
Same sex + opposite sex	13	F-FM, M-FM	2	5	4xMF, 1xFF	4/5 opposite sex, 1/5 same sex	26%
Non-mating season:							
Same sex + opposite sex	13	F-FM, M-FM	4	5	4xMF, 1xMM	4/5 opposite sex, 1/5 same sex	26%
Relatedness							
Related + unrelated	9	J-FF	3	6	3xJF, 3xFF	1/6 related, 5/6 unrelated	44%
	10	J-JJ	4	4	4xJJ	2/4 related, 2/4 unrelated	27%
Familiarity							
Familiar + unfamiliar	18	F-MM, M-FF	6	6	6xFM	2/6 familiar, 4/6 unfamilair	22%
	10	F-FF	1	1	1 x FF	1/1 unfamiliar	7%

Discussion

Shelter preference

Duvaucel's geckos selected their diurnal retreats based on shelter size, degree of crevice concealment, the substrate beneath the shelter and the temperature regime. Crevice width, shelter material and humidity levels did not appear to influence shelter choices.

Shelter dimensions, particularly shelter size, crevice width and the degree of crevice concealment can be crucial traits when selecting a retreat that provides safety from native avian (e.g. *Ninox novaeseelandiae, Halcyon sancta)* or reptilian (*Sphenodon punctatus)* predators. Both adult and juvenile geckos clearly preferred larger shelters with small or medium entrance sizes over less protected ones with wide entrances. Surprisingly, Duvaucel's geckos did not appear to select the narrowest crevices; in fact more individuals chose to retreat in shelters with a wider crevice height (34 mm). In contrast, the saxicolous nocturnal geckos *Oedura lesuerii* and *Nephrurus milii* strongly preferred narrow crevices (Schlesinger and Shine 1994, Shah *et al.* 2004). Unlike New Zealand's lizards, these Australian geckos evolved with a diverse array of native predators, including small mammals and snakes, which could selectively have influenced their retreat site selection behaviour. Lizards in New Zealand probably evolved in the absence of terrestrial snakes and mammals, with the possible exception of a small mouse-like mammal from the Miocene (Worthy *et al.* 2006). Thus, Duvaucel's geckos may not have exhibited a strong preference for the narrowest spaces as their native predators typically have only limited access to the sheltering geckos due to their comparable large body sizes and hunting techniques. However, the tolerance threshold for crevice width was not determined in this study and it would be worthwhile to explore whether geckos avoid shelters with wider crevices than tested here. Wild Duvaucel's geckos were observed sheltering in crevices of varying height (1.5 to 30 cm), but the majority of geckos showed an overall preference for relatively narrow, but not tight-fitting shelters (approx. 5 cm, Chapter 3). The retreat choice of Duvaucel's geckos may be sufficient to protect these large lizards from potential avian or reptilian predators, but illustrates their susceptibility to predation by small introduced mammals such as rats (*Rattus spp*.), pests that are widespread in New Zealand's extant ecosystems.

Furthermore, crevice dimensions and type of substrate may also have an effect on the thermal properties of a shelter. Duvaucel's geckos preferred warmer diurnal shelters, similar to other lizards (Downes and Shine 1998, Shah *et al.* 2004, Goldsbrough *et al.* 2006). A warmer shelter climate likely facilitates thermoregulation and higher body temperatures may not only be beneficial for digestion and other physiological processes, but may also enhance locomotory performance (Huey 1982, Kearney and Prevadec 2000, Aguilar and Cruz 2010). However, shelters used by geckos in the field were generally cooler than their body temperatures (Chapter 3), suggesting that the geckos actively thermo regulated, probably by direct sun basking (Whitaker 1968). Thus, the experimental setting in this present study may not have resembled the true range of thermal conditions of most natural shelters (see Chapter 3), but rather represented an optimised scenario. For example, if the geckos do not have to leave their shelters to thermo regulate, they may be less exposed to predators and hence would gain a direct fitness benefit from warmer shelter temperatures. Such warm shelters may be relatively rare within the forest, but could be more abundant in other parts of the gecko habitat, for example within boulder beaches. It is also possible that wild Duvaucel's geckos select shelters with particular thermal properties, such as shelters that support a relatively constant thermal environment. However, this remains to be tested.

Aggregation and social factors

The proportions of aggregating geckos in this study ranged from 7 to 44 % across trials and shelter aggregations occurred despite an excess of available optimised shelters. However, there was no evidence that social factors, such as familiarly or relatedness, increased the geckos' tendency to aggregate. A considerably larger proportion of Duvaucel's geckos aggregated in the field (47 to 71 %, Chapter 3) in comparison to this standardised laboratory setting, suggesting that environmental factors such as the distribution of resources may have influenced the distribution patterns of wild geckos in shelters to some extent. A direct comparison of aggregation frequency between wild and captive geckos should be made with caution: wild geckos have free choice to share shelters with a range of conspecifics, but captive geckos were randomly combined. Factors such as social rank status or personality traits, e.g. the degree of sociability, may well influence decisions on whether an animal chooses to seek out or avoid conspecifics (Cote and Clobert 2007). The close proximity of conspecifics in aggregations likely promotes social interactions, which may affect the social assortment of a group, i.e. some individuals could either be attracted to or repelled from other conspecifics based on specific social traits (e.g. Schlesinger and Shine 1994). The results of the present study show that Duvaucel's geckos were generally tolerant of their conspecifics and did not avoid them when an excess of suitable shelters was available. Similar proportions of adults and juveniles shared shelters (up to 33 % of adults and 40 % of juveniles were aggregating) and there was no indication that juveniles avoided shelters with adult females, which was also observed in the field study (Chapter 3). Same sex pairs were formed less often than mixed sex groups. Males typically avoided sheltering with other males, which corresponds to the spatial distribution patterns of male Duvaucel's geckos in shelter sites in the wild (Chapter 3). These results confirm the sex specific distribution patterns reported for other gekkonids (Schlesinger and Shine 1994, Kearney *et al.* 2001, Lancaster *et al.* 2006, Meyer and Mouton 2007).

On a single occasion, two males occurred under the same shelter. However, this likely reflects an agonistic association as shelters in the area had been notably disturbed, presumably by these males fighting. The distribution of males in shelter sites in the present study could have been influenced by the social status of the males, whereby more dominant males may have excluded subordinates (see Downes and Shine 1998). Overall, male-female pairs were the most common combination. In three sets of mixed-sex trials, geckos formed groups in 16 instances, of which 14 groups were male-female pairs. This is comparable with the wild population, where male-female pairings were observed year-round (Chapter 3). Hence, it seems plausible that conspecific attraction plays a role in the formation of heterosexual groups. A higher frequency of male-female associations has been reported for several lizard species that form shelter aggregations (e.g. Lemos-Espinal *et al.* 1997, Nieuwoudt *et al.* 2003, Osterwalder *et al.* 2004, Lancaster *et al.* 2006), suggesting that males may guard females and exclude other males to increase their mating opportunities. This could explain why no more than one male was found in a group that included a female in this study as well as in the field study (Chapter 3). The fact that female-male pairs were also formed during non-mating seasons requires further study and may help to elucidate the social mating system of this species. For example several *Egernia* species form stable social groups consisting of one or several breeding pairs that exhibit high levels of social and genetic monogamy (e.g. Gardner *et al.* 2002, Chapple and Keogh 2005).

The results of this study do not explain why Duvaucel's geckos, particularly females and juveniles form shelter aggregations in the field. Social factors such as familiarity and relatedness did not encourage geckos to aggregate in the present study, yet geckos of all age groups formed shelter aggregations in the field year-round (Chapter 3). Shelter sites were abundant in the field and it seemed less likely that geckos aggregated for physiological benefits (Chapter 3). Perhaps limited dispersal and a high level of social tolerance towards conspecifics, particularly among females and juveniles, may be responsible for the observed distribution patterns of wild geckos. Little is known about habitat use and dispersal patterns in Duvaucel's geckos but there is evidence that geckos exhibit long-term site fidelity (Thompson *et al.* 1992, Wilson 2010).

In conclusion, the results of the present study did not provide evidence that social factors such as familiarity and relatedness influence aggregation behaviour. However, the observed sex specific grouping patterns indicate that social status and mating benefits could play a role in the spatial distribution of geckos in diurnal shelter sites and could affect the composition of aggregations. Research exploring the stability of groups in wild populations as well as the social and genetic relationships between group members, could aid in the understanding of the social organisation and mating system of this cryptic lizard. Moreover, such research would significantly contribute to our limited knowledge of reptilian social systems.

CHAPTER FIVE

Chemosensory discrimination of conspecifics by *Hoplodactylus duvaucelii*

Abstract

Little is known about the behavioural ecology of the Duvaucel's gecko (*Hoplodactylus duvaucelii*), a large cryptic lizard endemic to New Zealand. Since chemical communication is a fundamental part of social behaviour in gekkonid lizards, exploring chemoreceptive behaviours and scent discrimination abilities in various social contexts could provide clues about the social organisation of this species. I investigated the behavioural responses of adult and juvenile *H. duvaucelii* to chemical cues from conspecifics in relation to the sex, familiarity status and relatedness of the scent donor. In scent choice experiments, geckos were simultaneously offered three filter papers, two marked with body odours from conspecifics and one unscented control. Average tongue flick rate and amount of time spent at filter papers served as measures to assess the geckos' ability to detect scent cues and discriminate between scent stimuli. Other visible behaviours exhibited at filter papers were also recorded. It was assumed that a scent had been detected when geckos elicited a stronger response towards the scent stimuli than unscented controls and a differential response towards two scent stimuli was considered as discrimination between those scents. Juvenile and adult geckos detected scents from conspecifics in most cases. Some individuals (of all age and sex classes) performed potential marking behaviours at scented and unscented filter papers by rubbing their snout or dragging their cloaca over the substrate. Males discriminated between same and opposite sex conspecifics. There was also evidence that males discriminated between familiar and unfamiliar males, but the difference between responses towards both scent stimuli was non-significant ($p = 0.06$). Females did not vary their investigative responses towards scents in relation to the sex, familiarity or relatedness status of the scent donor. However, females exhibited most marking behaviours at scents from males in comparison to female scents and unscented controls, indicating a potential ability for sex recognition. Juveniles discriminated between siblings and non-siblings as well as mothers and unrelated females. These findings expand our knowledge of chemical discrimination abilities in gekkonid lizards and show that *H. duvaucelii* may use scent communication in a wide range of social contexts. Further study is now needed to explore the adaptive function of these discrimination abilities.

Introduction

Geckos have highly specialised nasal chemosensory organs (Schwenk 1993, Rehorek *et al*. 2000). Their vomeronasal and olfactory systems are well developed (Schwenk 1993, Dial and Schwenk 1996) and facilitate the detection of various chemical signals. The ability of many reptiles to recognise scent cues from predators (Dial *et al.* 1989, Robert and Thompson 2007), prey or food items (Cooper 1995, Hoare *et al.* 2007), conspecifics (Cooper *et al*. 1996, Bull *et al*. 2000, Cooper and Perez-Mellado 2002) and self (Graves and Halpern 1991) may have important implications for their fitness; and not least for their social behaviour. Chemical communication in social interactions is mediated by semiochemicals such as pheromones (Nordlund 1981, Mason *et al.* 1998, Houck 2009) that can carry unique signatures (Mason and Gutzke 1990, Alberts 1993) and generally serve as intra-specific signals. An animal may detect and recognise a conspecific signal and may or may not react to it according to its needs, physiological state or environmental conditions.

The ability to recognise social, physiological or genetic traits of a conspecific based on scent cues may be widespread among lizards. Several studies have shown that a range of species can detect chemical signals from conspecifics and were able to discriminate between scent cues from males and females (Cooper *et al*. 1996, Cooper and Perez-Mellado 2002, Gonzalo *et al*. 2004), familiar and unfamiliar (Cooper 1996, Aragon *et al.* 2001, Font and Desfilis 2002), or closely and less closely related individuals (Main and Bull 1996, Lena and de Fraipont 1998, Olsson *et al.* 2003, Head *et al.* 2008). However, only a few studies have examined chemoreceptive abilities in social contexts for gekkonid lizards, and most research has focussed on eublepharid geckos. For example, leopard geckos of either sex (*Eublepharis macularius*, Eublepharidae) are capable of sex recognition (Mason and Gutzke 1990, Cooper and Steele 1997) and males can discriminate between familiar and unfamiliar females (Steele and Cooper 1997) based on scent cues alone. Moreover, *E. macularius* can discriminate between their own scent cues and same-sex conspecifics (Brillet 1990, Steele and Cooper 1997), and *Coleonyx variegatus*, Euplepharidae, also appear to recognise their own scents (Carpenter and Duvall 1995). Hoare *et al.* (2007) found evidence that chemosensory behaviour is conserved in apparently visually oriented diurnal geckos (*Naultinus manukanus*, Diplodactylidae), which responded to a suite of chemical stimuli including predator and conspecific scent cues. However, detailed information on scent discrimination abilities of other New Zealand geckos is lacking.

Gekkotans are a large group comprising of 970+ species (Kluge 2001, Pianka and Vitt 2003). Given this great diversity, social systems - and hence chemosensory abilities - are likely to vary. For nocturnal species that form shelter aggregations (Cooper *et al*. 1985, Kearney *et al.* 2001, Hare and Hoare 2005, Meyer and Mouton 2007), or for those that lack territoriality and tend to be gregarious (Todd 2005), the ability to discriminate between individuals or groups of individuals may be advantageous. For example, scent discrimination may be beneficial for maintaining stable relationships between group members (Bull *et al.* 2000, O'Connor and Shine 2003) or for minimising aggression between potential competitors (Lopez and Martin 2002).

This study investigated the behavioural responses of *Hoplodactylus duvaucelii,* Diplodactylidae, a viviparous gecko endemic to New Zealand, to scents (body odours) from conspecifics in several social contexts. The aim of this study was to assess the chemosensory ability of *H. duvaucelli* to discriminate between chemical cues of conspecifics in relation to their sex, relatedness and familiarity status. Chemoreceptive behaviour in New Zealand geckos is largely unstudied and information on scent recognition abilities may yield important insights into life history strategies and social organisation of these lizards. Wild *H. duvaucelii* share diurnal shelter sites and may form aggregations consisting of individuals of varying age and sex (Chapter 3). Although it is unknown whether these aggregations are stable and are formed by the same individuals over time, there is substantial evidence that wild *H. duvaucelii* show high long-term site fidelity (Thompson *et al*. 1992, Jones 2000, Wilson 2010). Therefore it may be possible that some individuals repeatedly encounter each other over time. A recognition system that allows discrimination between group members, neighbours or kin, may be beneficial for social cohesiveness or for the detection of potential competitors. Moreover, chemical signposts advertising the presence of a male or a female may be advantageous for increasing mating opportunities. I conducted scent choice experiments and used lingual sampling rate (tongue flicking) and visit duration at scent cues as measures to: a) determine whether male, female and juvenile *H. duvaucelii* are able to detect scents from conspecifics, and b) explore whether geckos can discriminate between conspecific scents with respect to the sex, familiarity or relatedness status of the scent donor.

Materials and Methods

H. duvaucelii are large geckos attaining snout-to-vent lengths (SVL) of up to 160 mm (Whitaker 1968). Their main activity is at night, but geckos occasionally leave their diurnal retreats to sun-bask (Whitaker 1968, Barry *pers. obs.*). Mating occurs in the austral spring (September to November) and females give birth to one or two live young after a gestation period of at least six to eight months or longer (Barwick 1982, Cree 1994). Juveniles are slow growing and may mature in their seventh year (Barwick 1982) and the life expectancy may exceed 43 to 50 years (Wilson, 2010). Adult and juvenile *H. duvaucelii* from a captive breeding facility (Massey University Albany Campus, Auckland) were used in scent choice experiments. Twenty-one adult females and nine adult males were collected in November 2006 from Korapuki Island (Mercury Island group). The juveniles used in this study were the progeny of wild adults and were born in captivity in 2007 (14 juveniles) and 2008 (22 juveniles). Adult geckos were held in mixed-sex groups of five individuals in naturalistic outdoor enclosures (1.44 m^3 - 1.5 m^3). All juveniles, which at the time of study were less than fourteen months old, were kept in groups of four to six in naturalistic indoor vivaria, under a light source (L:D cycle 12:12). Geckos were fed live insects and fruit-puree with vitamin/mineral supplements twice a week (in winter once a week) and water was available *ad libitum*.

General experimental design

Geckos were transferred from their home enclosures to a temperature-controlled room (18 to 20 °C) two days before they participated in an experiment. This was done to allow acclimatisation and to collect chemical cues from those individuals that acted as scent donors. During the scent collection period geckos were individually housed in plastic containers (25×45×17 cm) with meshed lids, equipped with paper towel lining, a shelter (plastic tray) and a water dish. A light source was placed on top of the container (12:12 L:D cycle) to simulate natural light conditions. Equally sized absorbent filter paper circles (55 mm in diameter) were placed under each shelter to collect body odours from scent donors over a two day period. However, in mother-offspring scent choice experiments, larger filter-paper circles (110 mm diameter) were used. Plastic containers (45×62×37 cm) lined with absorbent paper, equipped with a meshed lid and a transparent Perspex® window were used as test arenas for scent choice experiments. A plastic sheet with three circular outlines (diameter 4 cm larger than the filter papers) drawn in a line at equidistance to each other served as a template on which scent stimuli (two scented filter-papers and one unscented filter paper that served as a control) were presented. Filter papers bearing scent stimuli and the unscented control paper were placed in random order at trial start in the centre of each circle outline and fixed underneath with tape. The focal animal was moved into the test arena ten minutes prior to the trial and placed in the far end of the arena under a shelter. An infrared camera was placed < 1 m in front of the Perspex® screen and trials were video-recorded for three hours over night. The next morning, geckos were either returned to their original enclosures or to the scent collection containers, if they were scent donors for another trial. After every trial, test-arenas and plastic sheets were thoroughly cleaned with water and detergent and wiped with 95 % ethanol to remove scent traces and new paper lining was added. All equipment and filter papers were handled using disposable latex gloves.

Four sets of scent-choice experiments were carried out from April 2007 to May 2009. All experiments and behaviour video-recordings were conducted using ambient light levels (all lights switched off and window blinds closed) between 1700 h and 2400 h. Individuals were used as focal animals only once per trial. Chemical cues of the same gecko were presented up to three times to different focal animals per experiment. However, no scent – focal animal combination was used twice across all experiments to assure that focal animals were scent naïve to unfamiliar conspecific scents.

Experiment 1: Opposite sex

Eighteen scent choice trials were conducted from 12 to 23 September 2008, the beginning of the mating season, to determine whether adult *H. duvaucelii* (9 males, 9 females) respond to and discriminate between scent cues from unfamiliar conspecifics of the same and opposite sex. Each focal animal was given a choice of: a) scent of an unfamiliar conspecific of the same sex, b) scent of an unfamiliar conspecific of the opposite sex, and c) an unscented control.

Experiment 2: Familiarity

Scent choice trials were carried out from 21 April to 8 May 2009 to determine whether adult female and male *H. duvaucelii* are able to discriminate between scents from familiar and unfamiliar individuals of the same sex. Trials were conducted outside of the species mating or breeding season and no females were gravid at the time of study. Eighteen adult females and six males were offered three filter papers with: a) scent from a familiar conspecific of the same sex, b) scent from an unfamiliar conspecific of the same sex, and c) an unscented control. Geckos were defined as “familiar” or “unfamiliar” based on whether or not they shared the same outdoor enclosure. It is possible that some animals may have encountered each other in the wild prior to their capture in 2006, as they all originated from the same island. However, at the time of this experiment the geckos had shared enclosures for over 28 months and therefore it was assumed that current relations would be stronger than potential associations prior to captivity.

Experiment 3: Siblings

Two sets of scent choice trials were conducted from 6 to 29 May 2008 to determine whether juvenile *H. duvaucelii* (n = 20) respond to scent cues from other juveniles of the same age and are able to discriminate between kin and non-kin. In the first trial (Trial 1), ten juveniles (born 2008, approx. 3 months old) were offered three filter papers with: a) scent from an unfamiliar sibling (same litter, housed separately), b) scent from an unfamiliar juvenile (non-sibling, housed separately) and c) an unscented control. Focal juveniles used in this experiment were separated from their siblings (and mothers) within 24 h of birth and kept in groups of four to six with other unrelated juveniles of the same age prior to the experiment. Unfamiliar non-siblings (scent donors) were housed in separate enclosures and had never encountered focal juveniles prior to the experiment. Although juveniles from the same litter were separated shortly after birth it is possible that siblings may have formed a bond in the first hours of birth (learned association). Thus, a second trial (Trial 2) was carried out, in which ten focal juveniles were presented: a) scent from an unfamiliar half-sibling (same mother, different litter, housed separately), b) scent from an unfamiliar juvenile (non-sibling, housed separately), and c) an unscented control. For these experiments a mix of yearlings, born 2007, and 2008 juveniles were used. However, all scents presented to focal juveniles came from scent donors of the same age.

Experiment 4: Mother – offspring

Twenty-three scent choice trials were conducted from 13 to 27 April 2007 to determine whether juveniles discriminate between scents from their own mother and an unrelated adult female (non-mother), and whether females discriminate between scents from their own offspring and a juvenile (non-offspring) of the same age. Eight females and 14 juveniles from eight litters (born March 2007) were used in this experiment. Four females and their offspring (n = 7) were separated within 1-12 h of birth and juveniles were housed indoors without adults in groups of four to six same aged juveniles. The remaining juveniles (n = 7) were left with their mothers and each family unit (mother and her offspring) was housed indoors in an individual container for approximately one month until 2 days prior to the experiment.

Female – juvenile trials: Four females were offered filter papers with: a) scent from an unfamiliar offspring (separated after birth), b) scent from an unfamiliar juvenile (non-offspring, housed separately), and c) an unscented control. A further four females were presented: a) scent from a familiar offspring (housed together), b) scent from an unfamiliar juvenile (non-offspring, housed separate), and c) an unscented control.

Juvenile - only trials: Seven focal juveniles were offered: a) scent from their unfamiliar mother (separated after birth), b) scent from an unfamiliar female (non-mother, housed separately) and c) an unscented control. A further seven juveniles were presented: a) scent from their familiar mother (housed together), b) scent from an unfamiliar female (non-mother, housed separate), and c) unscented control.

Data analyses

Response variables measured from videotapes were: rate of tongue-flicks emitted to filter-papers and visit duration (s). A visit was scored if a focal animal placed its snout on or within 2 cm of a filter-paper circle. Tongue flicking indicates sampling for vomodors by vomerolfaction (Cooper and Burghardt 1990) in squamate reptiles and is commonly used in experiments for scent recognition as a measure for detection and discrimination abilities. As geckos are also able to retrieve specific information from volatile components of scent marks in absence of tongue-flicking through olfaction (Dial *et al.* 1989, Schwenk 1993, Dial and Schwenk 1996), visit time served as another measure of scent detection and discrimination ability assuming that investigation time is linked to the scent evaluation process. Lizards are likely able to detect highly volatile compounds of the different scent cues at distance, similarly to the mixture of chemicals an animal would encounter in a natural setting. However, it was assumed that geckos had to examine volatile and non-volatile compounds at close range to retrieve detailed information about the scent donor.

All other conspicuous behaviours (Table 1) exhibited by geckos at the filter papers were recorded throughout the experiment (3 h).

Average duration and tongue flick rate of all visits within 30 min of the first filter paper encounter were compared between scent treatments using non-parametric Wilcoxon signed ranks tests. To determine whether the geckos detected scents i.e. responded stronger to scent stimuli than controls, one-tailed tests were used to compare scented with unscented filter papers. A differential response between two scent stimuli was considered as discrimination between those scents and responses were compared with two-tailed tests. Trials where focal animals failed to visit all filter papers within 30 min of the first encounter and trials that were not recorded for the full length of time (3 h) due to a camera malfunction were excluded from the analyses (12 out of 94). Medians, 25th and 75th percentiles and minima / maxima were reported. Significance was set at $p<0.05$. All analyses were performed using PASW Statistics 18 Core System for Windows (SPSS Inc, 2009).

Results

Experiment 1: Opposite sex

Females (n = 8) spent more time at scent stimuli from other females (Figure 1b) in comparison to the unscented controls (Z = -2.100, p = 0.020, 1-tailed test, Wilcoxon signed ranks test, WSRT), and emitted (non-significantly) more tongue flicks per visit at female scents (Figure 1a) than unscented controls (Z = -1.572, p = 0.078, 1-tailed test, WSRT). The chemosensory response by females to male scent stimuli was not significantly stronger than towards the unscented controls (tongue flicks: Z = -0.845, p = 0.234, visit time: Z = -1.260, p = 0.125, 1-tailed tests, WSRT, Figures 1a, b). Furthermore, female tongue-flick-rate (Z = -0.141, p = 0.938, WSRT) and visit time (Z = -0.420, p = 0.742, WSRT) at male and female scents was similar and did not differ significantly. Males (n = 8) showed a significantly stronger response to scent stimuli from females (tongue flicks: Z = -2.521, p = 0.004, visit time: Z = -2.100, p = 0.020, 1-tailed tests, WSRT, Figures 1c, d) and males in comparison to the unscented control paper (tongue flicks: Z = -2.375, p = 0.008, visit time: Z = -1.820, p = 0.039, 1 tailed tests, WSRT*,* Figure 1c, d).

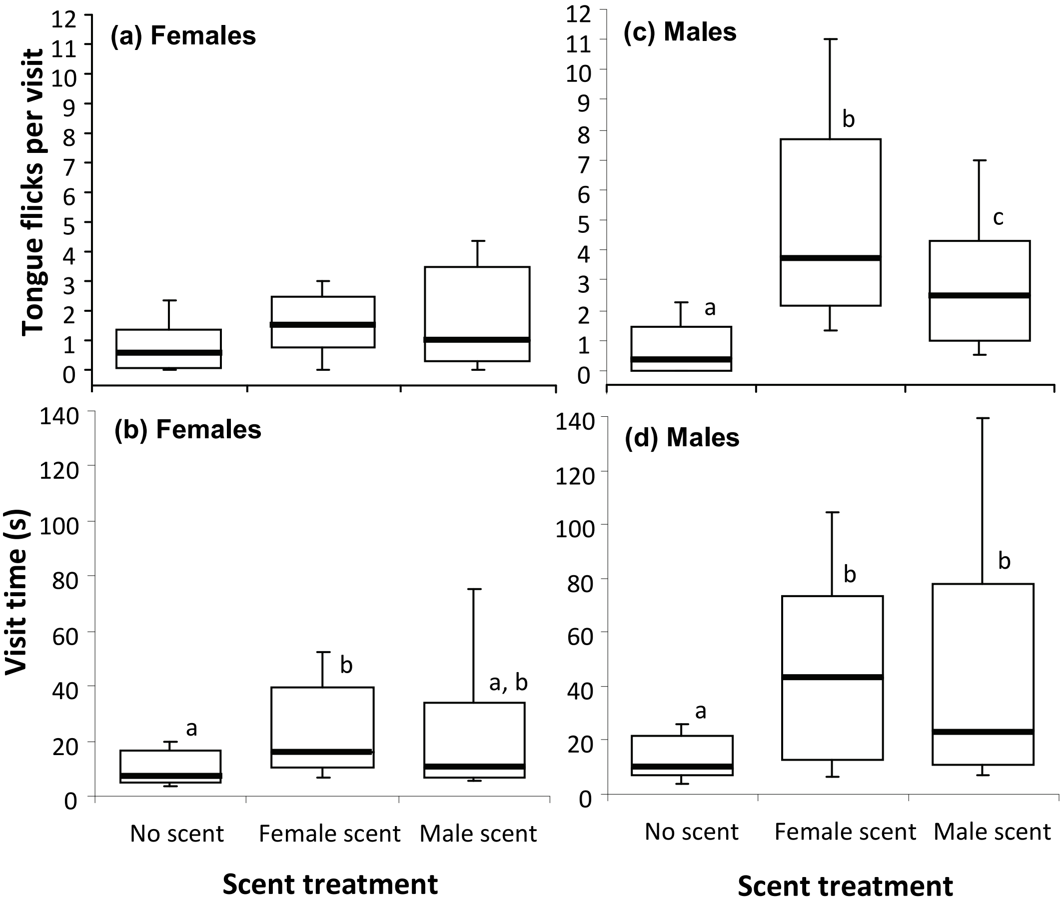


Figure 1: Tongue flick rate and visit time of female (a, b) and male (c, d) Duvaucel's geckos in response to scents from opposite and same-sex conspecifics and unscented controls. Whiskers denote maximum/minimum values, boxes represent 25th and 75th percentiles and bold bars are median values. Different letters denote significant differences between scent treatments (Wilcoxon signed ranks tests).

Males elicited a significantly higher tongue flick rate at female scents in comparison to male scent stimuli (Z = -2.240, p = 0.023, WSRT, Figure 1c), but the visit time did not differ between the sexes (Z = -0.280, p = 0.844, WSRT).

Four of eight females (50 %) and two of eight males (25 %) showed additional behaviours at filter papers with scent stimuli and unscented controls (Table 1a). Geckos of both sexes rubbed their snout on the substrate or dragged their cloaca over the substrate and one female scratched a filter paper.

Table 1: Number of geckos exhibiting additional behaviours at filter-papers with scent stimuli and unscented controls in trials testing for (a) discrimination of sex, (b) familiarity status and (c) kin. The total number of visits (per 3 h) during which the behaviour occurred is displayed in parenthesis.

(a)

Behavioural acts	Unscented control	Female scent	Male scent
Females (n = 4)			
Snout rub	1 (1)	-	3 (6)
Cloaca drag	-	-	1 (1)
Scratching	-	-	1 (1)
Behaviours combined	**1 (1)**	-	**4 (8)**
Males (n = 2)			
Snout rub	-	-	2 (2)
Cloaca drag	1 (1)	-	-
Behaviours combined	**1 (1)**	**-**	**2 (2)**

(b)

Behavioural acts	Unscented control	Familiar conspecific scent	Unfamiliar conspecific scent
Females (n = 4)			
Snout rub	1 (1)	1 (1)	2 (2)
Males (n = 1)			
Snout rub	-	1 (1)	1 (1)

(c)

Behavioural acts	Unscented control	Sibling scent	Unrelated juvenile scent
Juveniles (n = 3)			
Snout rub	1 (1)	2 (10)	2 (3)

Experiment 2: Familiarity

One of 17 females failed to visit all three filter papers within 30 min of the first filter paper visit. Females (n = 16) showed a weak tendency of more tongue flicks and longer visits at scents from familiar (tongue flicks: Z = -1.477, p = 0.074, visit time: Z = -1.189, p = 0.126, 1-tailed tests, WSRT) and unfamiliar females (tongue flicks: Z = -1.850, p = 0.032, visit time: Z = -1.551, p = 0.065, 1–tailed tests, WSRT) in comparison to unscented controls (Figure 2a, b). Female responses to scent stimuli from familiar and unfamiliar females were similar (Figure 2a, b) and did not differ significantly (tongue flicks: Z = -

0.512, p = 0.629, visit time: Z = -0.625, p = 0.552, WSRT). Males (n = 6) responded stronger to scent stimuli from unfamiliar males in comparison to unscented controls (tongue flicks: z = -1.782, p = 0.047, visit time: Z = -2.201, p = 0.016, Fig. 2c, d). The responses by males to scents from familiar males did not differ significantly from the responses to unscented controls (tongue flicks: Z = -0.674, p = 0.313, visit time: Z = -0.943, p = 0.219, 1-tailed tests, WSRT). Males elicited (non-significantly) more tongue flicks (Z = -1.997, p = 0.063) and spent non-significantly more time (Z = -1.992, p = 0.063) at filter-papers with scents from unfamiliar males in comparison to papers with scents from familiar males (Figure 2c, d). Four of 17 females (23.5 %) and one of six males (17 %) performed snout-rubbing behaviour at filter-papers (Table 1b).

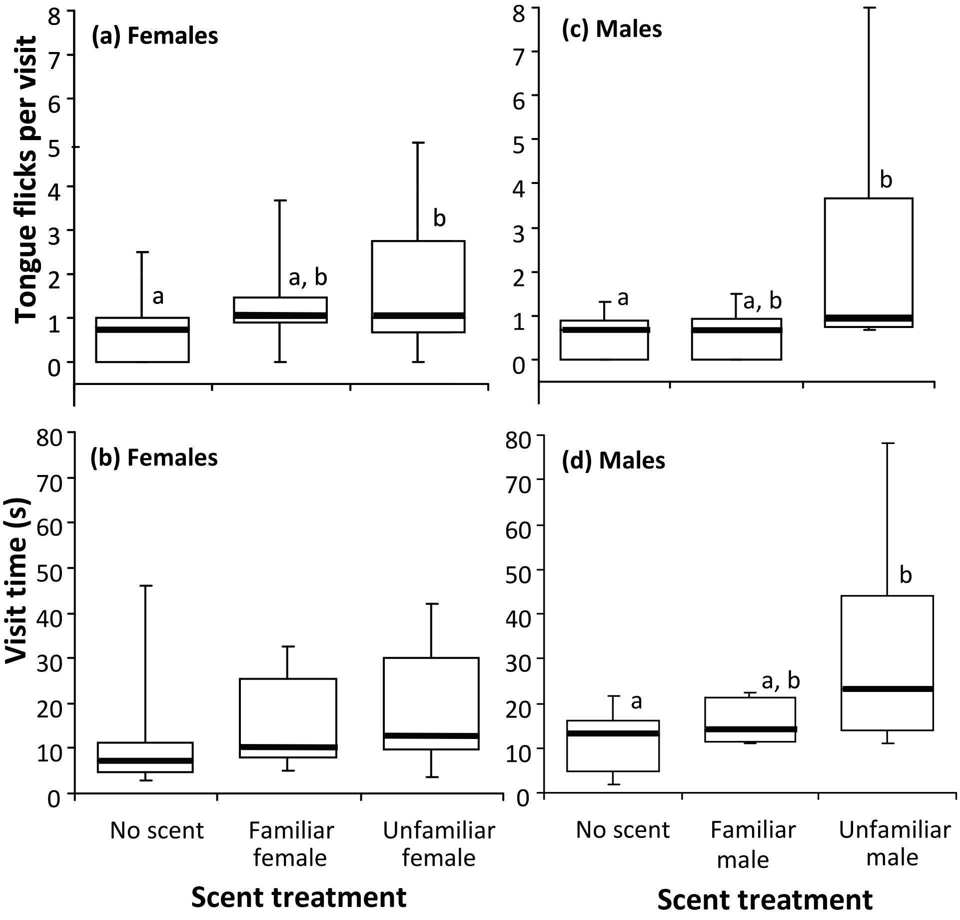


Figure 2: Tongue flick rate and visit time of female (a, b) and male (c, d) Duvaucel's geckos in response to scents from familiar and unfamiliar same-sex conspecifics and unscented controls. Whiskers denote maximum/minimum values, boxes represent 25th and 75th percentiles and bold bars are median values. Different letters denote significant differences between scent treatments (Wilcoxon signed ranks tests).

Experiment 3: Siblings

Two of 20 juveniles failed to visit all three filter papers within 30 min of the first filter paper encounter. In trial 1, juveniles (n = 10) elicited a greater number of tongue flicks in response to filter papers with odours from siblings (median: 1.5, range: 1-10, Z = -2.703, p = 0.002, 1-tailed test, WSRT) and non-siblings (median: 1.0, range: 0-7, Z = -2.201, p = 0.014, 1-tailed tests, WSRT) in comparison to unscented controls (median: 0.0, range: 0-2). Visit time did not differ significantly between unscented controls (median: 21 s, range: 4-36 s) and scent from siblings (median: 21.3 s, range: 7-151 s, Z = -0.561, p = 0.305, 1-tailed tests, WSRT) or scent from non-siblings (median: 26.3 s, range: 10-140 s, Z = -1.478, p = 0.080, 1-tailed tests, WSRT).

In trial 2, juveniles (n = 8) showed a significantly higher tongue flick rate to half-sibling scents (median: 3.0, range: 1-7, Z = -2.521, p = 0.004, 1-tailed test, WSRT) but not in response to non-sibling scents (median: 0.3, range: 0-6.3, Z = -0.524, p = 0.344, 1 –tailed test, WSRT) in comparison to unscented controls (median: 0.2, range: 0-2.3). Juveniles spent significantly more time at filter papers with scent from half-siblings (median: 39.7 s, range: 14.7-114 s, Z = -2.240, p = 0.012, 1-tailed, WSRT), but not at scents from non-siblings (median: 13.1s, range: 7-59.5s, Z = 0.000, p = 0.512, 1-tailed tests, WSRT) in comparison to unscented controls (median: 16.6 s, range: 4.3-62.3 s). The tendency of increased tongue flick rates in response to scents from related juveniles in comparison to unrelated juveniles was significant in trial 1 (sibling *vs.* non-sibling: Z = -2.019, p = 0.047, WSRT) and marginally significant in trial 2 (half-siblings *vs.* non-sibling: Z = -1.820, p = 0.078, WSRT). Visit time did not differ significantly for either trial between the two scent conditions (sibling *vs.* non-sibling: Z = -0.119, p = 0.093 and half-sibling *vs.* non-sibling: Z = -1.680, p = 0.109, WSRT).

Given that juveniles showed the same trend in their responses to scent stimuli in trial 1 and 2 (i.e. regardless whether they originated from the same litter or not), both trials were combined to increase the sample size and thus enhance statistical power. Juveniles tongue-flicked significantly more towards scent stimuli than towards the unscented control paper (related juveniles: Z = -3.682, p < 0.001, unrelated juveniles: Z = -2.105, p = 0.017, 1-tailed tests, WSRT, Figure 3a). The tongue-flick rate of juveniles (n = 18) was significantly greater at filter papers with scent from related juveniles (sibling or half sibling) in comparison to papers bearing scent from unrelated juveniles (Z = -2.748, p = 0.004, WSRT, Figure 3a). Average visit time was also greater in response to scents from related juveniles in comparison to unscented controls (Z = -2.135, p = 0.016, 1-tailed test, WSRT). However, visit time did not differ significantly between scents from unrelated juveniles and unscented controls (Z = -1.154, p = 0.132, 1-tailed test, WSRT) or between scents from unrelated and related juveniles (Z = -1.420, p = 0.163, WSRT, Figure 3b). Three of 18 juveniles (17 %) performed snout rubbing behaviour at filter papers (Table 1c).

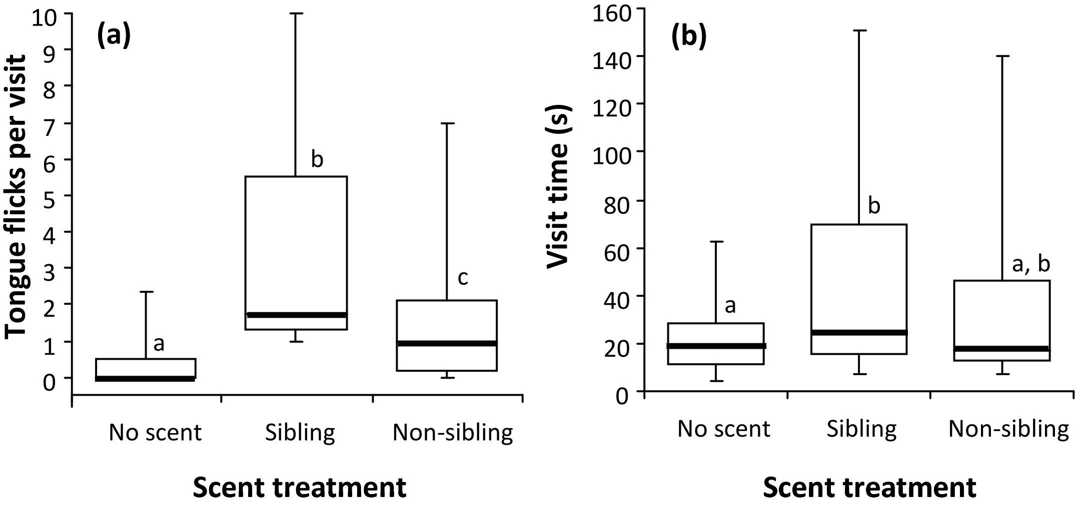


Figure 3: Tongue flick rate (a) and visit time (b) of juvenile Duvaucel's geckos in response to scents from related and unrelated juveniles and unscented controls. Based on a combined data set of trial 1 (siblings) and trial 2 (half-siblings). Whiskers denote maximum/minimum values, boxes represent 25th and 75th percentiles and bold bars are median values. Different letters denote significant differences between scent treatments (Wilcoxon signed ranks tests).

Experiment 4: Mother – offspring

The reduced sample size precluded statistical analysis for each rearing condition (offspring kept with mother and offspring separated after birth): thus both rearing conditions were combined for the following analysis. Females (n = 6) responded stronger to scented filter papers in comparison to unscented controls (offspring vs. unscented control, tongue flicks: Z = -2.023, p = 0.031, visit time: Z = -1.992, p = 0.031, unrelated juvenile vs. unscented control, tongue flicks: -1.826, p = 0.063, visit time: Z = -2.201, p = 0.016, all tests 1-tailed, WSRT, Figure 4a, b). Female tongue flick rate (Z = -0.944, p = 0.438, WSRT) and visit time (Z = -0.105, p = 1.000, WSRT) at scents from own offspring and unrelated juveniles did not differ significantly. Juveniles (n = 9) elicited more tongue flicks (Z = -2.032, p = 0.010, 1-tailed test, WSRT) and remained longer (Z = -2.429, p = 0.006, 1-tailed test, WSRT) at scent stimuli from their mothers in comparison to unscented controls (Figure 4c, d). Juveniles also spent more time (Z = -2.192, p = 0.014, 1–tailed test, WSRT) but did not elicit more tongue flicks (Z = -0.862, p = 0.227, 1–tailed test, WSRT) at scents from unrelated females in comparison to unscented controls

(Figure 4c, d). Tongue flick rate was significantly greater (Z = -2.395, p = 0.020, WSRT) at scents from mothers in comparison to scents from unrelated females but visit time did not differ significantly (Z = 0.534, p = 0.629, WSRT) in response to both scent stimuli. Females and juveniles did not exhibit any additional behaviours at the filter papers in this experiment.

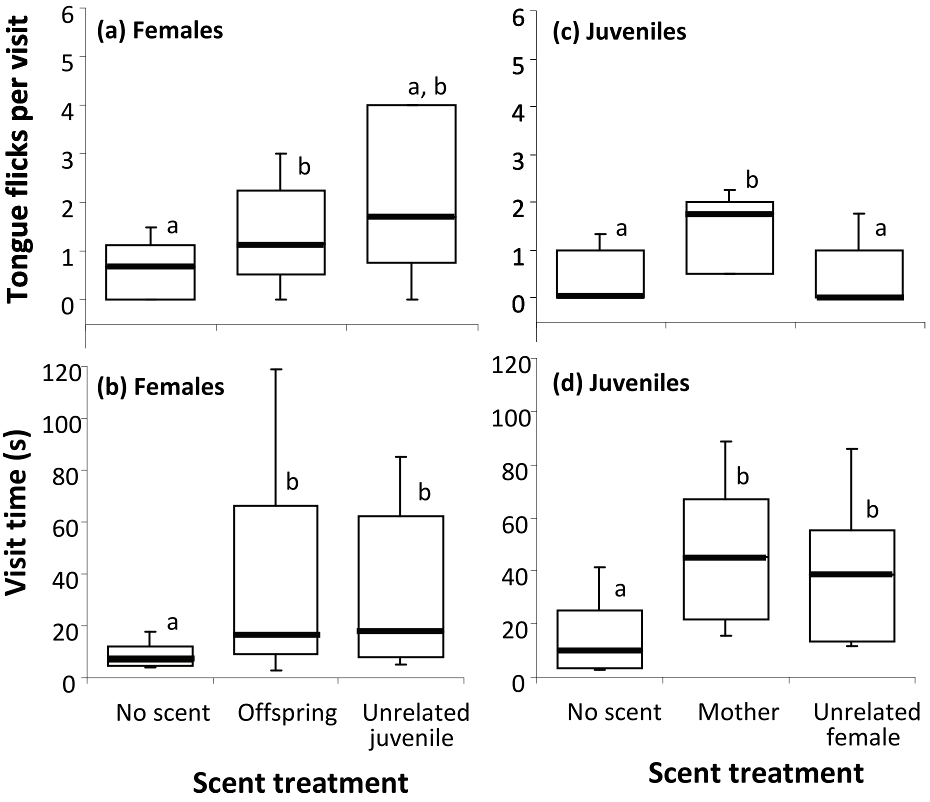


Figure 4: Tongue flick rate and visit time of adult female (a, b) and juvenile (c, d) Duvaucel's geckos in response to unscented controls and scents from genetic mothers and unrelated females as well as offspring and unrelated juveniles, respectively. Whiskers denote maximum/minimum values, boxes represent 25th and 75th percentiles and bold bars are median values. Different letters denote significant differences between scent treatments (Wilcoxon signed ranks tests).

Discussion

Scent cues from conspecifics elicited a greater response in *H. duvaucelii* than the unscented control in most cases. A higher tongue-flick rate and longer visit time at scented filter papers indicated that scents were detected and examined. Furthermore, responses to conspecific scent stimuli varied in several social contexts, suggesting that geckos were able to discriminate between scents based on specific traits of the scent donor. In addition to these investigative behaviours, which are linked to olfaction and vomerolfaction and thus information retrieval, some individuals scratched, rubbed their snout or dragged their cloaca over filter-papers, with a greater tendency to perform these acts over the scented filter papers. The latter two behaviours are likely to represent scent marking whereby geckos deposit pheromones on the substrate, indicating their presence to conspecifics (Mason 1992, Cooper and Steele 1997, Mason and Parker 2010).

Responses to scents from same sex and opposite sex conspecifics

Males clearly detected scents from male and female conspecifics and responded with increased chemosensory behaviours and longer visits to scent cues than to unscented controls. Tongue flick rates were significantly increased in response to female scents than to male scents, suggesting that males are able to discriminate between the sexes of conspecifics based on scent cues alone. The detection of female scent cues could aid males in locating potential mating partners. A male may remain in close vicinity to a female's scent mark thereby increasing the chance of an encounter and hence, a potential mating opportunity. Alternatively, males could use scent to follow a female by scent trailing, a behaviour observed in other lizards (Cooper and Vitt 1986*,* Olsson and Shine 1998). Recognising scent marks from conspecific males could allow a male to prepare for a confrontation with a potential competitor or to avoid an encounter. In addition, two males displayed marking behaviours by rubbing their snout or dragging their cloaca over unscented and male scented filter papers.

Scent marking is typically linked with territorial behaviour in many animals, but may also be associated with dominance in non-territorial species (Wyat 2003). For example, territorial male leopard geckos *Eublepharis macularius* exhibited aggressive (i.e. stilting) displays in the presence of male scents and frequently marked scented and unscented substrates (Cooper and Steele 1997). Male geckos in the present study did not mark filter papers bearing female scent cues, similarly to male *E. macularius,* which showed a stronger tendency to over-mark male scents and mark unmarked surfaces rather than female scent stimuli (Cooper and Steele 1997). However, in contrast to male *E. macularius,* male *H. duvaucelii* neither marked surfaces as intensely nor did they exhibit any aggressive displays in response to male scents. This could indicate that male *H. duvaucelii* are relatively tolerant towards the presence of other males in their home ranges. Similarly, Christmas (1995) suggests that *H. duvaucelii* do not occupy discrete territories, but may defend shelter sites (from other males).

Females, on the contrary, showed no different investigative response to scents from males in comparison to female scents. In fact, I found no variation in female tongue flick rate or visit time between scents from males and unscented filter papers. However, scents from other females elicited significantly stronger responses in females than unscented controls. Interestingly, 50 % of all females showed additional behavioural responses to scents, specifically marking behaviours (snout rubbing and cloaca dragging), or in one instance scratching, mostly towards male scents. This implies that females retrieved information about the sex of the scent-bearer via olfaction and were potentially able to discriminate between the sexes, although this requires further investigation. Similarly, female *E. macularius* did not increase their tongue flick rate in response to male scents, but almost exclusively chin - rubbed male scent stimuli (Cooper and Steele 1997). By marking areas where a male scent was detected, females may advertise their presence to a (likely) returning male, and thus facilitate mate attraction. However, the motivation of a female to respond to a male signal may be dependent on her receptivity (Head *et al.* 2005).

Responses to scents from familiar and unfamiliar same sex conspecifics

Female *H. duvaucelii* detected scents from unfamiliar and familiar females and marginally increased their tongue flick rate towards these scents in comparison to the unscented controls. Females did not appear to discriminate between scents from familiar and unfamiliar females and their investigative responses were similar at both scent conditions. A few females (4 of 17) snout rubbed scented and unscented filter papers but no preference was evident. This lack of differential response to the scent stimuli may indicate that females either are unable to discriminate between chemical cues from other females in relation to their familiarity status or do not have a motive to alter their behaviour. Females are usually tolerant of other females, as they share shelter sites in the wild (Chapter 3), and may not perceive the presence of unfamiliar females as threatening and thus behavioural responses may only be very subtle. Similarly, Steele and Cooper (1997) detected no differential behavioural responses by female *E. macularius* in cages marked with their own scent in comparison to cages marked with scents from other females. The authors note that discrimination abilities may remain undetected in the absence of overt behavioural responses.

Natural shelter aggregations containing several *H. duvaucelii* females are not uncommon (Chapter 3). It has been shown that *H. duvaucelii* exhibit strong site fidelity (Thompson *et al.* 1992, Wilson 2010), thus females may become familiar with each other if they gather in the same diurnal retreats over time. Further research could determine whether females use cues other than scent to discriminate between familiar and unfamiliar conspecific females. Furthermore, it would be worthwhile to determine whether females show a different response to odours during the mating season as odour production and scent profiles may seasonally vary (see Buesching *et al.* 2002).

Males exhibited no differential exploratory response towards scents from familiar males and unscented controls but showed an increased response to scents from unfamiliar males. Although males spent more time and tongue flicked on average more often at scents from unfamiliar males in comparison to unscented controls as well as to familiar male scents, the latter differences were only marginally significant. Only one of six males exhibited marking behaviour by rubbing its snout over both filter-papers with male scents. Although the statistical power may be compromised due to the limited number of males available for this experiment, the observed trend may indicate that male *H. duvaucelii* have the ability to distinguish between familiar and non-familiar males. In contrast to females, male *H. duvaucelii* are less tolerant towards same sex conspecifics and engage in prolonged aggressive interactions when paired (Barry, unpubl. data). Furthermore, wild males tend to avoid each other and typically do not share the same diurnal retreats (Chapter 3). Thus, the recognition of males that have been encountered before may be beneficial for decreasing the costs of aggressive interactions, as decisions can be made based on the outcome of a previous agonistic encounter (sensu ‘dear enemy hypothesis’ Fisher 1954, Lopez and Martin 2002). These discrimination abilities are beneficial in any season.

Responses to related and unrelated individuals

Juvenile *H. duvaucelii* discriminated between scents from their siblings and unrelated juveniles. Their investigative behaviours were greatest at scents from siblings regardless whether juveniles originated from the same or a different litter. This suggests that discrimination is based on genetic cues and not due to learned association with scents from siblings, which potentially could have been established prior, during or shortly after birth. Kin recognition in reptiles, based solely on genetic relatedness, has only been demonstrated for the scincid lizard *Egernia striolata* (Bull *et al.* 2001), which discriminated between unfamiliar closely and more distantly related juveniles. However, the adaptive function remains speculative. Such kin discrimination may be facilitated by major histocompatibility complex (MHC) genes, which have been suggested to influence individual scent signatures of vertebrates (e.g. Olsen *et al.* 1998, Willse *et al.* 2006). Olsson *et al.* (2003) have shown that Swedish sand lizards (*Lacerta agilis*) preferentially associated with odours from potential mates that were most distantly related (i.e. that shared the least alleles at the MHC class 1 loci).

Sibling recognition based on genetic similarities may be beneficial in populations where young do not disperse far after birth and an encounter or re-encounter, in case of the same litter, is likely. Such discrimination could enable related individuals to avoid inbreeding at later life stages (Waldman *et al*. 1992, Gerlach and Lysiak 2006) or could aid in reducing aggression between siblings (Walls and Roudebush 1991). However, it is yet to be determined whether the ability of juvenile *H. duvaucelii* to discriminate between siblings and non-sibling persists in adults. Sibling recognition based on learned associations, i.e. post-natal determination rather than genetic similarities *per se,* has been reported for juvenile *Lacerta vivipara* (Lena and de Fraipont 1998) and hatchling *Iguana iguana* (Werner *et al.* 1987). Learned association may be beneficial in species that form stable family groups or where young juveniles remain together after birth temporarily, which could aid in predator avoidance, as seen in iguanas (Werner *et al.* 1987). Young *H. duvaucelii* are independent immediately after birth and parental care has not been reported. Juvenile dispersal is not known, however, pairs of young have been found sheltering together, often in association with one or more females (Chapter 3). Whether or not such juvenile associations are comprised of siblings, is unclear. Three of 18 juveniles exhibited snout rubbing behaviour at scented and unscented filter papers, whereby two juveniles snout rubbed particularly intensely at scents from siblings. These potential scent marking behaviours (Mason and Parker 2010) may indicate that juveniles advertise their presence, perhaps to facilitate attraction or avoidance of kin, but this requires further investigation.

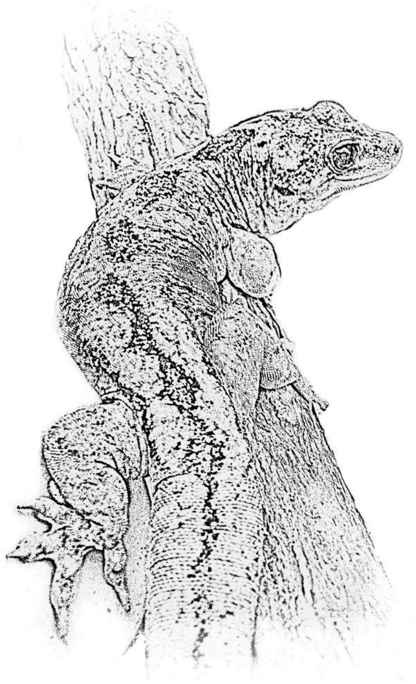
Juvenile *H. duvaucelii* detected scents from adult females and females detected scents from juveniles by showing an increased investigative response towards both scent stimuli in comparison to unscented controls. While females did not appear to discriminate between scents from offspring and non-offspring and showed a similar chemoreceptive response at both scent conditions, juveniles tongue flicked significantly more to scents from mothers in comparison to scents from unfamiliar females. However, juvenile tongue flick rate did not differ between unrelated females and unscented controls. The increased chemosensory response by juveniles at scents from mothers could be evidence for kin recognition. But, it remains to be determined if the recognition of mothers by juveniles is based on familiarity (post-natal determination) or phenotype matching (pre-natal determination), as I have used a mixture of familiar and non-familiar mothers in my study. Due to a camera malfunction the sample size of females was reduced to six, thus an analysis in relation to rearing condition i.e. females kept together with offspring or separated after birth, was not possible. Four of the six remaining focal females were housed together with their offspring. Hence the scent from unfamiliar unrelated juveniles could have elicited a response in females because of its novelty. The familiar scent from their own offspring, in contrast, could have caused females to investigate scents more intensely due to recognised genetic similarities. Consequently, the lack of a differential response by females to scents from juveniles may be related to the fact that females investigated either scent more intensely for different reasons. Thus, the ability of offspring recognition by females remains to be tested with unfamiliar offspring (separated after birth) and unfamiliar unrelated juveniles.

The data indicate that, at least, juveniles possess the ability to recognise scent from their mothers. Mother-offspring recognition has also been reported for other lizards, such as *Lacerta vivipara* (Lena and de Fraipont 1998), *Tiliqua rugosa* and *Egernia stokesii* (Main and Bull 1996), species where mothers and offspring have overlapping home ranges and where an encounter is likely (Bull and Baghurst 1998, Duffield and Bull 2002). It is possible that *H. duvaucelli* juveniles remain within the home range of their mothers and thus reciprocal kin recognition may be advantageous for their fitness and survival. For instance, the ability of juveniles to detect scent cues from their mothers may aid in locating suitable habitat and shelters, whereas scent recognition of progeny could facilitate tolerance by females towards their own offspring i.e. by reduced aggression (O'Connor and Shine 2004) or cannibalism (Gibbons *et al.* 2003). Other benefits of kin recognition could be reduced kin competition and inbreeding avoidance, which may promote dispersal (Lena *et al.* 1998) and avoidance of kin (Lena and de Fraipont 1998).

In conclusion, this study provides evidence that *H. duvaucelii* are capable of discriminating conspecifics in a variety of contexts, based on scent cues alone. Particularly, the ability of juveniles to detect scent marks and recognise kin could have implications for kin-based social coherence and habitat selection. To unravel the potential function of genetic sibling discrimination and possible recognition of mothers by juvenile *H. duvaucelii,* further research is needed to examine juvenile dispersal patterns in wild populations. Furthermore, experimental studies could help to determine whether juveniles are attracted to or repelled by scent from genetically closely related individuals. In this study I tested kin recognition between siblings and mother-offspring pairs with juveniles less than 14 months old and it remains to be determined how long this discriminatory ability persists.

CHAPTER SIX

Intersexual interactions and courtship in Duvaucel's geckos - does familiarity play a role?



Abstract

The social behaviour of New Zealand's endemic gecko fauna is poorly understood. Here, I staged interactions between heterosexual pairs of Duvaucel's geckos during the mating and non-mating season to describe the repertoire and use of social behaviours, and to determine the species courtship behaviour. Additionally, I tested the hypothesis that males and females adjust their interactions according to their familiarity status. The repertoire of behaviours was similar in both sexes, but the use of several behaviours differed between sexes and in relation to season. Males used more tactile and chemosensory behaviours than females, and females used tail displays more often than males. Females were investigative in the non-mating season, but during the mating season they were largely passive and appeared to avoid males. In contrast, males approached females more often in the mating season and courted them. Few courtship specific behaviours were observed: the male courtship grip and the female courtship pose. Prior to a mating attempt males used chemosensory and tactile behaviours to investigate and potentially stimulate females. If a female rejected a male and retreated into a shelter, males added a visual component to their courtship, which could potentially be of greater informative value for females. Overall, there were less agonistic and more amicable interactions between familiar pairs. Unfamiliar males were avoided by females during the non-mating season, which indicated that males and females were able to distinguish between familiar and unfamiliar opposite sex conspecifics. This study provides a foundation for future research and delivers a first insight into the social behaviour and courtship of this elusive New Zealand reptile.

Introduction

Duvaucel's geckos (*Hoplodactylus duvaucelii*) are large, New Zealand endemic lizards (Whitaker 1968) inhabiting offshore islands along the north-east coast of the North Island and within Cook Strait (Towns and Daugherty 1994). Due to their current restricted distribution (Towns and Daugherty 1994), limited accessibility and secretive nature, the social behaviour of these geckos is poorly known. Duvaucel's geckos are nocturnal habitat generalists and show terrestrial as well as arboreal habits (Whitaker 1968, Towns *et al.* 1985). They typically rest during the day in rock crevices, tree cavities or seabird burrows, but may occasionally leave their shelters to sun bask (Whitaker 1968). Diurnal retreats are often shared by male-female pairs or small groups of up to 8 individuals, which may consist of several females, young of varying size but not more than one adult male (Chapter 3). Male-female shelter associations are not restricted to the spring mating season and occur throughout the year (Chapter 3), however it is unknown whether these associations are stable over time. The sharing of shelters and aggregation of individuals in a limited space likely facilitates complex intra-specific social interactions. Mark-recapture studies of Duvaucel's geckos provide evidence of long-term site fidelity (Thompson *et al.* 1992, Wilson 2010). Thus, it is plausible that Duvaucel's geckos utilise the same diurnal retreats repeatedly over time and therefore have a high probability of re-encountering the same individuals during subsequent visits. Close proximity and repeated encounters between individuals may promote social familiarisation (Cheetham *et al.* 2008) and can influence the extent and type of social interactions (Guffey *et al.* 1998, Husak 2004, Tokarz 2007). For example, male *Lacerta monticola* show a higher social tolerance towards familiar male conspecifics (Aragon *et al.* 2007), and the ability of the gregarious *Egernia stokesii* skinks to discriminate between conspecific group members and non-group members may be important for maintaining a cohesive social group structure (Bull *et al.* 2000). Males of different taxonomic groups such as the lizard *Holbrookia propinqua,* the skink *Eumeces laticeps* and gecko *Eublepharis macularius* (Cooper 1985, Cooper 1996, Steele and Cooper 1997) have the ability to discriminate between familiar and unfamiliar females, which may allow these males to adjust their courtship and mating effort, and hence, maximise their reproductive success. For example, a male may concentrate his mating effort only on novel (not previously inseminated females) instead of investing time and energy on courting females that already have been mated with. Furthermore, in monogamous species recognition of familiar mates and mate scent trailing may help males to find and remain with their partner (Bull *et al.* 1998, Olsson and Shine 1998).

In the present study I investigated social interactions between male-female pairs of Duvaucel's geckos during staged encounters in the mating and non-mating season. The study aim was to explore the repertoire and use of social behaviours during intersexual interactions in relation to sex and season to determine the species courtship behaviour. Secondly, I investigated whether social interactions were influenced by the familiarity status of heterosexual pairs. I hypothesised that male Duvaucel's geckos increase the intensity of sexually motivated behaviours towards unfamiliar in comparison to familiar (i.e. potentially previously inseminated) females in the mating season to maximise their reproductive success. I further hypothesised that familiar pairs engage in more amicable and fewer agonistic or aggressive interactions in comparison to unfamiliar pairs in the non-mating season. Fewer agonistic or aggressive interactions between familiar pairs in the non-mating season could be an indication of whether males and females form long-term associations.

Materials and methods

Study animals and their maintenance

Duvaucel's geckos are active throughout the year (Whitaker 1968; Barwick 1982). Mating occurs in the austral spring (September to November) and females typically give birth to one or two live young in late summer/autumn (February to May) in the following year (Barwick 1982; Cree 1994, Barry *unpubl. data*). Juveniles reach sexual maturity at approximately seven years of age (Barwick 1982) and life expectancy may exceed 43-50 years (Wilson 2010).

Thirty adult Duvaucel's geckos (9 males, mean SVL ± SE, 122.2 ± 1.2 mm, range: 117.5 - 128.5 mm and 21 females, 121.0 ± 0.8 mm, range: 114.5 – 125.5 mm) were captured in December 2006 from Korapuki Island (Mercury Islands, 36°39.5' S, 175°51' E), northeast of New Zealand's Coromandel Peninsula, and transferred to a captive breeding facility at Massey University, Auckland. The lizards were housed in groups of five (sex ratio M:F 1:4 and 2:3) in six outdoor enclosures (1.44 m^3 - 1.5 m^3) with naturalistic habitats. Geckos were fed live insects and fruit puree mixed with vitamin and mineral supplements up to twice a week (depending on the season). Water was available *ad libitum.*

Experimental design

In winter (11 June to 18 August 2007, non-mating season) and spring (1 September to 31 October 2007, mating season) thirty-six encounters between pairs of male and female geckos were staged. Each male participated in four sets of nine trials and was randomly assigned a familiar and a non-familiar female in two subsequent trials in the mating and non-mating seasons, respectively. This resulted in four experimental treatments: a) male and familiar female in the mating season, b) male and unfamiliar female in the mating season, c) male and familiar female in the non-mating season, and d) male and unfamiliar female in the non-mating season. Females participated in trials once per season, and all male-female combinations in this study were unique. Familiarity status of a pair was assigned based on whether or not the geckos shared the same enclosure in the five month period prior to this study.

A Perspex® enclosure (total size: 800 × 510 × 520 mm) with two chambers served as the test arena. Each chamber was lined with absorbent paper and equipped with an opaque plastic tube (300 mm, diameter: 50 mm) which served as a shelter. One male and one female gecko were placed in each chamber of the test arena up to 10 h before the trial commenced to allow acclimatisation. The chambers were separated by an opaque plastic divider which prevented the lizards from seeing each other. Water was provided *ad libitum*. The air temperature of the experimental room (controlled by a thermostat) was monitored throughout the study with a HOBO® data logger (Model H08-004-02, mean ± SE air temperature recorded at 21:30 h in winter: 17.7 ± 0.2 °C, range: 16 to 19.4 °C, spring: 17.7 ± 0.2 °C, range: 16.4 to 19.8 °C). Between 18:00 and 19:00 h the divider was removed and the room light was switched off. All geckos had retreated in their shelters at trial start. Social interactions were recorded for a period of six hours using an infrared camera with infrared light source, which was placed < 1 m in front of the test arena and connected to a VCR device (Xpose, model QV3053). The next morning lizards were returned to their home cages or to a temporary holding container and the test arena and shelters were thoroughly cleaned with ethanol and water and fresh paper lining was laid out prior to the next trial. Trials with familiar and unfamiliar pairs were alternated.

Video analysis

All occurrence sampling and scan sampling (Altman 1974) were used to analyse the 216 h of behavioural data. Variables recorded were: start and end time of each interaction (hh:mm:ss), the sex of the gecko that initiated an interaction, the type of behaviours

(Table 1) performed by each individual during an interaction and their sequence. Additionally, the duration pairs spent *in copulo* was recorded. The start of an interaction was recorded, when one gecko either oriented itself towards the other individual and performed a visual display or when one gecko approached the other and the gecko being approached exhibited a visible behavioural response. An interaction was also recorded when one lizard approached the other directly and came within 5 cm of the other individual, but no visible behavioural response was elicited by the other lizard. The end of an interaction was recorded when one of the lizards moved > 5 cm away from the other without being followed.

The following response variables were measured: 1) total number of interactions, number of amicable, agonistic and aggressive interactions, 2) average duration of the first two interactions, 3) number of approaches by females and males, number of amicable, agonistic and aggressive approaches per sex. An approach included all behavioural acts exhibited by the gecko that initiated an interaction until the opponent exhibited a visual response. An interaction or approach was categorised as 'aggressive' if it included a biting event or a charge. An interaction or approach was defined as 'agonistic' if behaviours included a visual display but no aggressive act, and 'amicable' if it lacked any agonistic or aggressive component. Additionally, I conducted scan sampling every 10 min (36 scans per 6 h) and recorded for each individual whether it was a) exploring, b) resting, c) sheltering alone, d) sheltering with the other lizard or e) interacting to assess activity budgets of geckos in relation to sex, season and familiarity status.

Statistical analyses

The unconditional probability that a behavioural act was performed by a female or male during an interaction was calculated. This was defined as the number of interactions in which the male or female elicited a specific behaviour divided by the total number of interactions between that pair. To determine whether specific behaviours were more or less likely to be used by females or males and whether the likelihood of a behaviour being performed varied with season, I compared the probabilities between sexes and seasons using Wilcoxon-signed ranks tests. For this analysis, data from trials with familiar and unfamiliar pairs were averaged per sex to reduce the number of statistical comparisons.

A generalised estimating equations (GEE) approach (Liang and Zeger 1986), which accounts for the repeated nature of the data, was used to test whether familiarity, season and their interaction account for variability in the behavioural response variables and activity budgets. The distribution of each response variable was examined and the appropriate model type and link function was specified. The optimal correlation structures were determined based on the smallest QIC (quasi-likelihood under the independence model criterion). A robust estimator for the variance of the response variables was used to calculate standard errors and confidence intervals. A significant interaction term (familiarity status x season) was further investigated by comparing the estimated marginal means of the response variable between the familiar and unfamiliar group in either season using two least significant difference (LSD) tests. No Bonferroni adjustment was applied as these tests represented planned comparisons (conditional upon a significant overall test result) and no other comparisons were of interest for this study.

To determine whether there were differences in approach frequencies and activity patterns between males and females (regardless of familiarity status), the response variables were compared between sexes using Wilcoxon signed ranks tests. This analysis was based on averaged data from familiar and unfamiliar treatments.

Thirty-one of the 36 male–female pairs engaged in interactions. Five trials were excluded from the analyses as no interactions were observed. In four of these trials neither geckos left their shelter during the six hour observation period and in one trial only the male left its shelter briefly to defecate.

Medians, means and maximum / minimum values were reported for probability data. Estimated marginal means and standard errors were reported to summarize variables analysed with the GEE procedures. Statistical significance was considered at $\alpha < 0.05$. Effect sizes (ES) were reported for Wilcoxon signed ranks tests (Corder and Foreman 2009). All analyses were conducted using PASW statistics v. 18 (SPSS Inc 2009)

Results

General description of social behaviours

In 31 trials a total of 246 interactions were observed (mean: 7.9 ± 0.7, range: 2 – 16 interactions per pair per six hours). Twenty-six different behavioural acts were identified during social interactions between male-female pairs of Duvaucel's geckos (Table 1). Geckos typically used display behaviours such as **tail wave** (in 50 % of all interactions) or **head raised** (24 %) when interacting as well as contact behaviours (**snout touch**, 43 %, and **touch,** 28.5 %). Aggressive acts such as **short bite**s (34 %) were also frequently observed, whereas **snap** (14 %) or **attack**s (14 %) were less common. All other behaviours listed in Table 1 occurred infrequently (<10 %).

Males and females showed a similar repertoire of behaviours during dyadic interactions (Table 1). Sex specific courtship displays were limited to the female **courtship pose,** which was exclusively displayed by females following the male **courtship grip** in the mating season**.** The **courtship grip** was solely performed by males before and during **copulation** (Table 1). The likelihood that behaviours such as **snout touch, snap, bite short, attack, back arch and tail-wave** were used by geckos during an interaction varied significantly between females and males either in one or in both seasons (Table 1, Appendix 1). Likewise, the probability that specific aggressive and agonistic behaviours were performed (i.e. **snap, bite short, legs extended** and **tail-wave)** varied with season for both sexes (Table 1, Appendix 1).

Intersexual interactions frequently involved aggressive acts such as short bites or threat displays, but encounters rarely escalated and never resulted in one gecko chasing another. No lizard experienced injuries such as broken skin or tail loss during the experimental trials. At the end of an interaction one gecko typically walked slowly away (**withdrawal slow**, 80 %) or both geckos simultaneously moved in different directions (13 %). Geckos rarely fled from another (**withdrawal fast**, 7 %) and if so, the fleeing gecko (females only) always settled in close vicinity (<10 cm) of the opponent. In one instance a male attempted to force copulation with a rejecting female, which led to a two minute struggle during which the male repeatedly bit the female on neck and back, then grasped her and attempted to insert his everted hemipenis into her cloaca. The female eventually freed herself from his grip by thrashing her tail and using her hind feet to push him off. The female immediately sought shelter and was not further pursued by the male.

Table 1: Ethogram of behaviours and average probabilities (mean, median) of behavioural acts performed during interactions by pairs of male (M) and female (F) Duvaucel's geckos in the mating (spring) and non-mating season (winter). '-' indicates that the behaviour was not observed. ^{a} denotes a significant difference ($p < 0.05$) between the seasons for each sex. ^{b} denotes a significant difference ($p < 0.05$) between males and females within a season (Wilcoxon signed ranks tests).

Variable	Sex	Season	Mean	Median	Range	Description	Context	Possible function	Reported for other gekkonids
Snout touch/ Tongue flick	F	Spring	0.15	0.11	(0-0.33)	Gecko briefly touches with snout or tongue the snout / body surface of opponent	Occurs throughout interaction, snout-to-snout touch: during initial phase of interaction	Information retrieval, snout to snout touch: greeting?	e.g. *Coleonyx variegatus* (Greenberg 1943), *H. pacificus* (Rieppel 1976), *Hemidactylus mabouia* (Regalado 2003a)
		Winter	0.14	0.13	(0.03-0.25)				
	M	Spring	0.28	0.28	(0.13-0.50) ^{b}				
		Winter	0.31	0.30	(0.06-0.48) ^{b}				
Touch/ sit on	F	Spring	0.17	0.11	(0-0.50)	Gecko steps with feet on / walks over / sits on or rests tail on opponent	During advanced phase of interaction, opponent often calm and stops tail shake; male sits on female during copulation	Appeasement? Contact behaviour	*H. pacificus* (Rieppel 1976), *Gonatodes vittatus* (Demeter and Marcellini 1981), *Sphaerodactylus vincenti vincenti* (Marcum et al 2008)
		Winter	0.19	0.19	(0-0.42				
	M	Spring	0.12	0.12	(0-0.36)				
		Winter	0.15	0.11	(0.04-0.50)				
Nudge*	F	Spring	0.02	0.00	(0-0.08)	Gecko pushes with nose against body of opponent	Occurred infrequently	Contact behaviour?	*C. variegatus* (Greenberg 1943)
		Winter	-	-	-				
	M	Spring	0.01	0.00	(0-0.04)				
		Winter	-	-	-				
Snap	F	Spring	0.13	0.10	(0-0.31)	Gecko rapidly moves head towards opponent, opens snout and fast snaps jaws shut, without physical contact	Following approach or in response to approach, snout touch or bite by opponent	Threat, aggressive	
		Winter	0.01	0.00	(0-0.05)				
	M	Spring	0.03	0.00	(0-0.17)				
		Winter	0.12	0.12	(0-0.27) ^{a,b}				
Bite short	F	Spring	0.22	0.21	(0-0.42)	Gecko rapidly moves head towards opponent, grasps it with its jaws and releases it immediately (less than 1 s)	Frequently performed during interactions	Aggressive, courtship stimulation	e.g. *C. variegatus* (Greenberg 1943), *H. pacificus* (Rieppel 1976), *G. vittatus* (Demeter and Marcellini 1981)
		Winter	0.03	0.00	(0-0.10) ^{a}				
	M	Spring	0.25	0.25	(0-0.61)				
		Winter	0.24	0.16	(0-0.67) ^{b}				
Bite long	F	Spring	0.02	0.00	(0-0.13)	Gecko rapidly moves head towards opponent, grasps it with its jaws and holds on to it (up to 10 s)	During agonistic interactions, also performed by males during mating attempt	Aggressive, courtship stimulation	e.g. *C. variegatus* (Greenberg 1943), *H. mabouia* (Regalado 2003a)
		Winter	-	-	-				
	M	Spring	0.09	0.04	(0-0.25)				
		Winter	-	-	-				
Grip*	F	Spring	-	-	-	Gecko grasps body part of opponent with its jaws but without force	Occurred infrequently	Contact behaviour?	*Lygodactylus pictatus keniensis* (Greer 1967)
		Winter	-	-	-				
	M	Spring	0.01	0.00	(0-0.04)				
		Winter	0.01	0.00	(0-0.05)				

Table 1 continued.

Variable	Sex	Season	Mean	Median	Range	Description	Context	Possible function	Reported for other gekkonids
Charge / charge nudge	F	Spring	-	-	-	Rapid lunge towards the opponent, no physical contact or push with snout	During initial phase of interaction, in response to approach	Threat, aggressive	*G. vittatus* (Demeter and Marcellini 1981), *H. mabouia* (Regalado 2003a)
		Winter	0.01	0.00	(0-0.05)				
	M	Spring	0.06	0.00	(0-0.25)				
		Winter	-	-	-				
Charge snap*	F	Spring	0.02	0.00	(0-0.17)	Rapid lunge towards the opponent followed by a snap, no physical contact	In response to approach	Threat, aggressive	
		Winter	-	-	-				
	M	Spring	-	-	-				
		Winter	0.02	0.00	(0-0.05)				
Attack	F	Spring	0.08	0.08	(0-0.25)	Rapid lunge towards the opponent followed by a bite	Following approach or in response to approach or bite by opponent	Aggressive	*S. vincenti vincenti* (Marcum *et al.* 2008)
		Winter	-	-	-				
	M	Spring	0.14	0.08	(0-0.50)				
		Winter	0.11	0.11	(0-0.23) [b]				
Head raised	F	Spring	0.10	0.11	(0-0.21)	Head lifted off substrate, neck extended	During agonistic interactions or information retrieval	Vigilance?	*C. variegatus* (Greenberg 1943)
		Winter	0.14	0.15	(0.03-0.22)				
	M	Spring	0.13	0.14	(0-0.31)				
		Winter	0.10	0.10	(0.06-0.15)				
Head angled	F	Spring	-	-	-	Head lifted of substrate, snout slightly pointed downwards	During agonistic interactions, often in combination with back arch	Threat?	*H. pacificus* (Rieppel 1976), *S. vincenti vincenti* (Marcum *et al.* 2008)
		Winter	0.01	0.00	(0-0.10)				
	M	Spring	0.05	0.00	(0-0.25)				
		Winter	0.01	0.00	(0-0.05)				
Legs extended / Body raised	F	Spring	0.02	0.00	(0-0.08)	Simultaneous extension of forelimbs or forelimbs and hind limbs, body lifted off ground	Often combined with back arch	Threat, courtship	*H. mabouia* (Regalado 2003a)
		Winter	0.05	0.00	(0-0.27)				
	M	Spring	0.11	0.10	(0-0.25)				
		Winter	0.02	0.00	(0-0.13) [a]				
Back arch	F	Spring	0.01	0.00	(0-0.06)	The spine is curved upward and the ventral side is lifted off the substrate resulting in an arch like posture	Often combined with leg extension	Threat. Courtship	*e.g. H. pacificus* (Rieppel 1976), *G. vittatus* (Demeter and Marcellini 1981), *H. mabouia* (Regalado 2003a)
		Winter	0.02	0.00	(0-0.13)				
	M	Spring	0.20	0.15	(0-0.61) [b]				
		Winter	0.02	0.00	(0-0.15)				

Table 1 continued.

Variable	Sex	Season	Mean	Median	Range	Description	Context	Possible function	Reported for other gekkonids
Tail wave	F	Spring	0.47	0.50	(0.17-1.00)	Tail rests on ground and 2/3 of distal tail end is moved repeatedly from side to side	In response to approach, snout touch or threat display by opponent, after being bitten	Threat display, state of arousal, submission	*H. mabouia* (Regalado 2003a), *S. nicholsi* (Regalado 2003b)
		Winter	0.43	0.42	(0.29-0.68)				
	M	Spring	0.23	0.19	(0-0.71)				
		Winter	0.04	0.00	(0-0.13) [a,b]				
Tail trash	F	Spring	0.08	0.06	(0-0.25)	Entire tail is lifted off ground and moved repeatedly in a wide motion laterally or upward	In response to attack or bite, rejection behaviour by females in response to male mating attempt	Threat display, state of high arousal	*S. vincenti vincenti* (Marcum *et al.* 2008), *H. mabouia* (Regalado 2003a), *S. nicholsi* (Regalado 2003b)
		Winter	0.01	0.00	(0-0.06)				
	M	Spring	0.01	0.00	(0-0.05)				
		Winter	-	-	-				
Tail vibrate	F	Spring	0.01	0.00	(0-0.08)	1/5 of distal tail end is lifted off the substrate and shaken rapidly, low amplitude	Performed by males prior to a mating attempt, in females infrequent	State of arousal, courtship	similar in male *Eublepharis macularius* (Brillet 1991)
		Winter	-	-	-				
	M	Spring	0.10	0.10	(0-0.25)				
		Winter	-	-	-				
Jerky*	F	Spring	-	-	-	Undirected abrupt movements of the body	Infrequently observed	?	
		Winter	-	-	-				
	M	Spring	0.04	0.00	(0-0.25)				
		Winter	0.01	0.00	(0-0.06)				
Snout open*	F	Spring	0.03	0.02	(0-0.13)	Wide opening of snout	In response to bite or threat	Threat and/or vocalisation?	
		Winter	-	-	-				
	M	Spring	0.01	0.00	(0-0.08)				
		Winter	-	-	-				
Flinch	F	Spring	0.06	0.00	(0-0.25)	Jerky movement with head away from opponent	In response to snout-to-snout touch or snap		
		Winter	0.04	0.00	(0-0.14)				
	M	Spring	0.05	0.00	(0-0.29)				
		Winter	0.02	0.00	(0-0.13)				

Table 1 continued.

Variable	Sex	Season	Mean	Median	Range	Description	Context	Possible function	Reported for other gekkonids
Cloaca lick*	F	Spring	-	-	-	Male licks his own vent / genitals	In mating season, typically after copulation	Cleaning / maintenance	*L. pictatus keniensis* (Greer 1967), *H. mabouia* (Regalado 2003a)
		Winter	-	-	-				
	M	Spring	0.12	0.06	(0-0.38)				
		Winter	-	-	-				
Courtship bite*	F	Spring	-	-	-	Rapid repeated bite/nibble in nape or head of female	Prior to a mating attempt and during copulation	Courtship / mating	similar: e.g. *C. variegatus* (Greenberg 1943), *L. pictatus keniensis* (Greer 1967), *H. mabouia* (Regalado 2003a)
		Winter	-	-	-				
	M	Spring	0.05	0.02	(0-0.13)				
		Winter	-	-	-				
Courtship pose*	F	Spring	0.03	0.00	(0-0.13)	Rigid posture by female with head held high and front limbs extended	In response to courtship bite, during copulation	Courtship / mating	*Paroedura pictus* (Brillet 1991), without leg extension: *H. mabouia* (Regalado 2003a), *S. nicholsi* (Regalado 2003b)
		Winter	-	-	-				
	M	Spring	-	-	-				
		Winter	-	-	-				
Copulation*	F	Spring	-	-	-	Period between intromission and extraction of the hemipenis from the cloaca of a female	Mating	Mating	e.g. *C. variegatus* (Greenberg 1943), *E. macularius* (Brillet 1991), *H. mabouia* (Regalado 2003a)
		Winter	-	-	-				
	M	Spring	0.03	0.00	(0-0.13)				
		Winter	-	-	-				
Withdrawal fast	F	Spring	0.06	0.00	(0-0.33)	Jump away or run away from opponent	In response to aggressive act	Submissive?	*H. mabouia* (Regalado 2003a), *S. nicholsi* (Regalado 2003b)
		Winter	0.05	0.20	(0-0.21)				
	M	Spring	-	-	-				
		Winter	-	-	-				
Withdrawal slow	F	Spring	0.25	0.24	(0-0.50)	Slow walk away from opponent	In response to aggressive or non-aggressive act		*H. mabouia* (Regalado 2003a), *S. nicholsi* (Regalado 2003b)
		Winter	0.55	0.52	(0.35-0.81) ^{a}				
	M	Spring	0.58	0.56	(0.31-1.00) ^{b}				
		Winter	0.28	0.26	(0.13-0.50) $^{a, b}$				

*no statistical test was computed to compare groups when the probability of a behaviour occurring during an interaction is less than 5% in all four groups, or when the behaviour was observed in one group only

Probability values based on 8 samples per group; Wilcoxon signed ranks tests: seasonal comparison based on 7 valid pairs, within season comparison based on 8 valid pairs

Courtship and mating

In spring, nine unsuccessful mating attempts and four matings (copulations) were observed. All mating attempts featured aggressive elements and none occurred in winter. Prior to a mating attempt, males repeatedly exhibited tactile and chemosensory behaviours, by **tongue flicking** or **snout-touching** the female's head, dorsal surface and cloacal area. Males also frequently attacked or bit females on the neck, front axillae and tail. Five males exhibited a tail-tip vibration (**tail vibrate**, Table 1) while interacting with a female. Females always responded with a tail display (i.e**. tail wave** or **tail thrash**) and biting. Despite the females' defensive response, males continued their advances, and eventually initiated the mating with a **courtship grip** (Table 1). Females willing to mate (n = 3) calmed down i.e. ceased tail displays and adopted a stiff posture (**courtship pose**) by holding their heads high and slightly lifting the proximal end of their tails. The males then moved onto the female's back and inserted one hemipenis into the female's cloaca. Throughout **copulation,** males held on to the females' nape with the **courtship grip** and females maintained the **courtship pose** (both tails entwined). Copulations lasted between 5 min 16 s and 16 min 28 s (12:22 ± 02:31, mm:ss). However, it is likely that one mating was interrupted and therefore only lasted 5 minutes. Twenty two minutes after this mating the male copulated again with the female and this time the copulation lasted more than twice as long. All other males copulated only once during the observation period. If the potentially interrupted mating was not considered then the average copulation time was 14:44 ± 01:11, mm:ss. After copulation, the males released their grip and moved off the female slowly, thereby carefully extracting the hemipenis from the females' cloaca. The hemipenes of the males remained everted for up to six minutes after copulation and the males repeatedly licked their genitals. **Cloaca lick** (Table 1) was also exhibited by males that did not copulate during the observation period in the mating season. Females were never observed to lick their own cloaca.

Rejecting females thrashed their tails, bit back and retreated into a shelter. Five rejected males adopted a **back arch** posture with **legs extended** (Table 1) and laterally positioned themselves in front of the shelter entrance with the female. Males remained several seconds in this position and then repeatedly tried to courtship bite or to grasp the females on their snout or neck and drag them out of the shelter. Females either retreated deeper into the shelter or attacked males. In response, males often adopted the arched posture again but eventually desisted and moved away. One rejecting female left her shelter and exhibited a back arch in response to a male, which resulted in his immediate (but slow) withdrawal.

Effect of familiarity on mating behaviour

Seven of the nine males either attempted to and/or successfully mated with a female during at least one trial. One male copulated twice with the same female, two males copulated once with a female (one after an unsuccessful mating attempt) and three males attempted twice (unsuccessfully) to mate with a female during the six hour observation period. Two males did not pursue the female further after one unsuccessful mating attempt. In three out of seven trials (43 %) males attempted to mate with familiar females and in five out of eight trials (63 %) males engaged in mating activities with unfamiliar females (Table 2). However, there were no statistically significant differences in the overall mating effort (number of mating attempts and copulations) between familiar and unfamiliar pairs (Z = -0.556, p = 0.688, Wilcoxon signed ranks test, WSRT, based on 7 valid comparisons).

Table 2: Total number of mating attempts and copulations by male Duvaucel's geckos with familiar and unfamiliar females during the six-hour observation period in the mating season. The number of trials is shown in parenthesis.

	Familiar female (7 trials)	Unfamiliar female (8 trials)
Mating attempt (unsuccessful)	3 (2)	6 (4)
Copulation	2 (2)	2 (1)
Mating attempt and copulation combined (overall mating effort)	5 (3)	8 (5)

Effect of season and familiarity on interactions

Thirty-eight percent of all interactions between female and male Duvaucel's geckos were amicable, 20 % were agonistic involving displays and 42 % were aggressive and included at least one biting event. The average duration of the first two interactions was longer in the mating season (mean ± SE [mm:ss], familiar pair: 08:04 ± 01:34, unfamiliar pair: 13:25 ± 05:26) than in the non-mating season (familiar pair: 01:42 ± 00:28, unfamiliar pair: 01:42 ± 00:34) and GEE analysis revealed a significant main effect of season but no effect of familiarity status or familiarity status x season on the interaction length (Table 3).

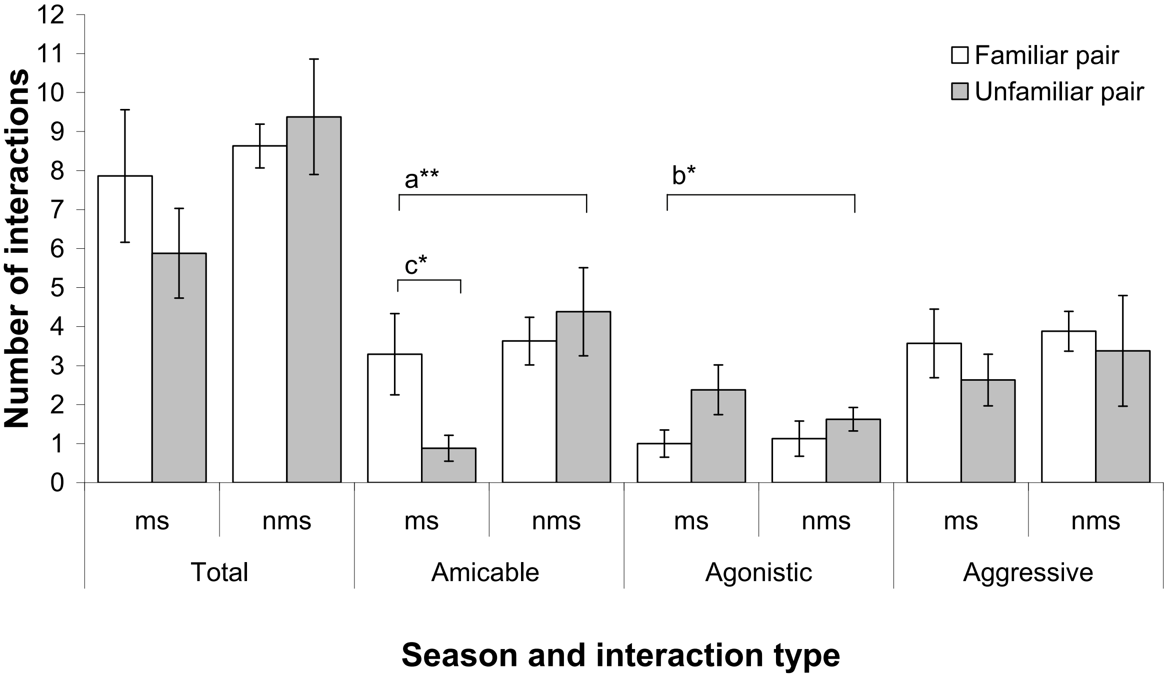


Figure 1: Number of interactions (estimated marginal means ± SE from GEE models) of familiar and unfamiliar male-female pairs of Duvaucel's geckos during the six hour observation period in the mating season (ms) and non-mating season (nms). 'a' indicates a significant main effect of season, 'b' indicates a significant main effect of status and 'c' indicates a significant difference between familiar and unfamiliar pairs within a season revealed by a LSD test following a significant interaction effect of status x season in the GEE models. Asterisks denote the significance level of the difference $*p<0.05$, $** p<0.01$.

The total number of interactions was not significantly affected by familiarity status and season, but GEE analysis revealed a significant seasonal main effect and significant interaction effect of familiarity status x season on the number of amicable interactions (Table 3). LSD tests showed that unfamiliar gecko pairs engaged in significantly fewer amicable interactions ($p = 0.028$) in the mating season than familiar pairs did, but amicable interactions in the non-mating season were not significantly ($p = 0.560$) affected by the familiarity status of a pair (Fig. 1). There was a weak tendency for unfamiliar pairs to engage in more agonistic interactions than familiar pairs (Fig. 1), which was confirmed by a significant main effect of familiarity status on the number of agonistic interactions (Table 3). The number of aggressive interactions did not vary significantly with season or familiarity status (Table 3).

Table 3: Results of GEE analyses of the effects of season, familiarity status and season x familiarity status on the average duration (s) of the first two interactions and the number of interactions between (n = 31) male-female pairs of Duvaucel's geckos per six hour observation period. Significant effects are in bold font.

	Intercept (df = 1)		Season (df = 1)		Familiarity status (df = 1)		Season x familiarity status (df = 1)	
Interactions	χ^2	P	χ^2	P	χ^2	P	χ^2	P
Total	593.258	<0.001	2.756	0.097	0.377	0.539	1.227	0.268
Amicable	43.357	0.001	**8.666**	**0.003**	**3.828**	**0.050**	**6.786**	**0.009**
Agonistic	5.559	0.018	0.177	0.674	**3.918**	**0.048**	0.638	0.425
Aggressive	72.436	<0.001	0.347	0.556	0.623	0.430	0.090	0.764
Average duration (s)	1269.685	<0.001	**34.001**	**<0.001**	0.656	0.418	0.689	0.407

Effect of sex, season and familiarity on approaches

In the mating season, 71 % of all interactions were initiated by males and 29 % by females. This difference was statistically significant (Z = -2.375, p = 0.016, ES = 0.84, Wilcoxon signed rank test, WSRT). In the non-mating season approximately half of all interactions were initiated by males (44 %) and half by females (56 %) and no significant difference was found (Z = - 0.845, p = 0.422, WSRT). The majority of all approaches were amicable (79 % females, 77 % males). In the mating season males initiated interactions with females significantly more often with an amicable approach than vice versa (Z = -2.375, p = 0.016, ES = 0.84, Appendix 2). In the non-mating season in contrast, females approached males significantly more often in an amicable manner than males approached females (Z = -2.388, p = 0.016, ES = 0.84, Appendix 2). Eighteen percent of all approaches by females were agonistic and involved displays and 3 % were aggressive. Males showed little agonistic behaviour (4 %) when approaching a female but 18 % of all approaches by males involved an aggressive act. However, the number of agonistic and aggressive approaches did not differ significantly between males and females (Appendix 2) in either season (mating season – agonistic approaches: Z = -1.633, p = 0.188, aggressive approaches: Z = -1.000, 0.625; non-mating season – agonistic approaches: Z = -1.552, p = 0.172, aggressive approaches: Z = -2.032, p = 0.063, WSRT). While females used aggressive acts (slow approach followed by snap or short bite) only when approaching males in the mating season, males approached females aggressively (slow approach followed by snap, short or long bite) in both seasons. Five males attacked females in the non-mating season upon encounter.

Table 4: Results of GEE analyses of the effects of season, familiarity status and season x familiarity status on the number of approaches by male and female Duvaucel's geckos per six hour observation period. Significant effects are in bold font.

	Intercept (df = 1)		Season (df = 1)		Familiarity status (df = 1)		Season x familiarity status (df = 1)	
Approaches	χ^2	P	χ^2	P	χ^2	P	χ^2	P
Females								
Total	56.125	<0.001	8.523	**0.004**	0.015	0.904	0.254	0.614
Amicable	24.833	<0.001	14.811	**<0.001**	0.072	0.789	0.585	0.444
Agonistic	4.115	0.043	0.270	0.604	0.019	0.890	0.486	0.486
Aggressive	-	-	-	-	-	-	-	-
Males								
Total	388.886	<0.001	0.827	0.363	0.511	0.475	0.542	0.462
Amicable	127.976	<0.001	6.345	**0.012**	0.256	0.613	1.254	0.263
Agonistic	28.267	<0.001	0.085	0.770	0.028	0.867	0.365	0.546
Aggressive	3.179	0.075	6.025	**0.014**	0.176	0.675	0.008	0.928

The total number of approaches by males was similar across seasons (Fig. 2) and was not significantly affected by the familiarity status of the female partner or by season (Table 4). Likewise the number of agonistic approaches by males was similar across all conditions and did not differ significantly (Table 4). There was a significant main effect of season on the number of amicable approaches by males (Table 4), which were slightly higher in the mating season in comparison to the non-mating season (Fig. 2). Males showed an increased tendency to initiate interactions aggressively in the non-mating season in comparison to the mating season which was confirmed by a significant seasonal main effect (Table 4).

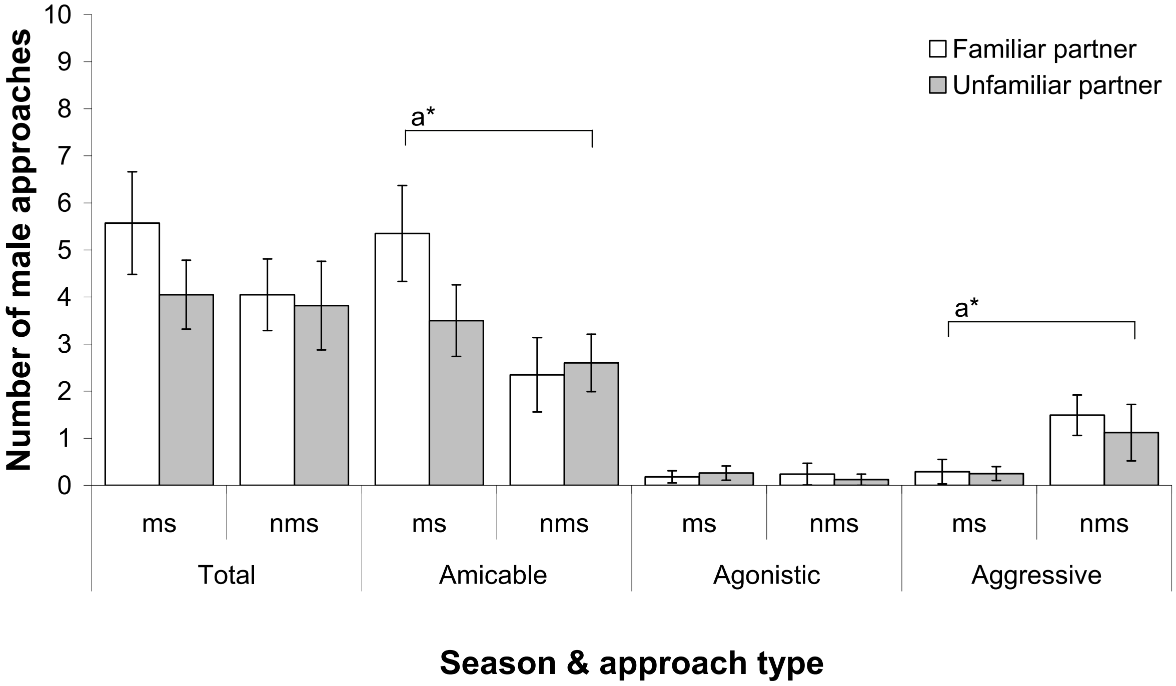


Figure 2: Number of times (estimated marginal means and standard errors from GEE models) male Duvaucel's geckos approached familiar and unfamiliar females during the six hour observation period in the mating season (ms) and non-mating season (nms). '**a**' indicates a significant main effect of season in the GEE model. Asterisks denote the significance level of the difference *p<0.05.

Approach rates of females were similar in the familiar and unfamiliar treatment (Fig. 3) and were not significantly affected by the familiarity status of the male partner in any approach category (Table 4). Females initiated interactions with males significantly less often in the mating season in comparison to the non-mating season (Table 4, Fig. 3), which was mainly due to a significantly decreased rate of amicable approaches in the mating season (Table 4, Fig. 3). The number of agonistic approaches by females was not significantly different between seasons. Aggressive approaches were infrequent and were only performed by two females in the mating season and by one female in the non-mating season (Fig. 3), thus no statistical analysis was carried out.

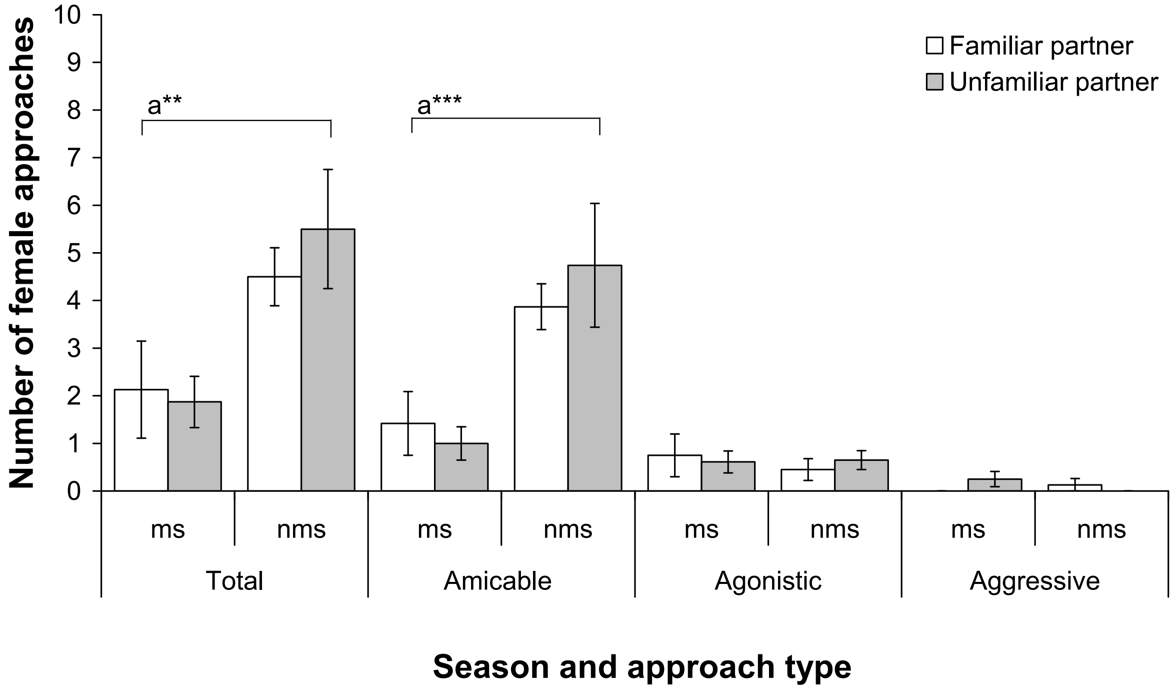


Figure 3: Number of times (estimated marginal means and standard errors from GEE models) female Duvaucel's geckos approached familiar and unfamiliar males during the six hour observation period in the mating season (ms) and non-mating season (nms). '**a**' indicates a significant main effect of season in the GEE model. Asterisks denote the significance level of the difference **p<0.01, *** p<0.001.

Effect of sex, season and familiarity on activity budgets

Interactions were infrequent and geckos were inactive a large proportion of time and rested inside or outside their shelters (Fig. 4). Activity budgets of females varied greatly between the non-mating and mating seasons (Fig. 4) and there was a significant main effect of season on the time females spent exploring and resting in the test arena (Table 5). The presence of familiar or unfamiliar males did not significantly affect the time females spent resting and exploring the test arena in the non-mating season or the mating season (Table 5). However, GEE analysis revealed a significant main effect of season and familiarity status and a significant interaction effect of season x familiarity status (Table 5) on the proportion of time females spent alone in a shelter. LSD tests showed that in the non-mating season, females spent significantly more time (p = 0.035) alone in a shelter in the presence of unfamiliar males than when paired with familiar males (Table 4), but no significant difference was apparent in the mating season (p = 0.929).

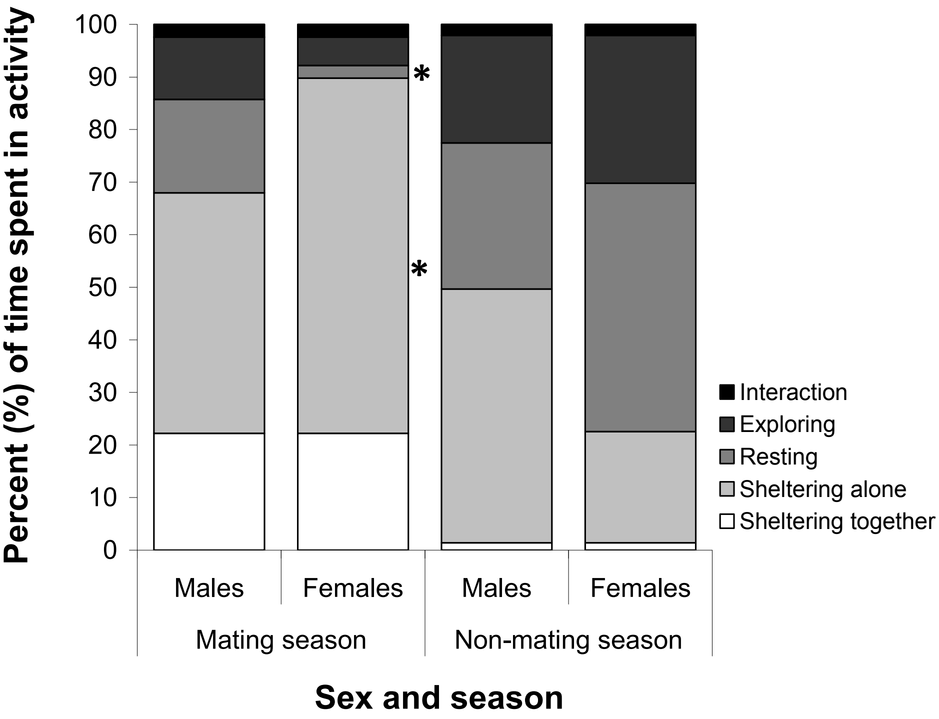


Figure 4: Activity budgets of male and female Duvaucel's geckos over a six hour period in the mating season and the non-mating season. Numbers in bars are percent of time geckos spent in each activity per season. An Asterisk * indicates a significant difference (Wilcoxon signed ranks test, $p < 0.05$) of an activity between males and females within the same season.

In males, the familiarity status of a female partner had no effect on activity budgets (Table 5). Males spent more time exploring the test arena in the non-mating season than in the mating season, which was confirmed by a significant main effect of season (Table 5). The time males spent resting or sheltering alone was not significantly affected by season or familiarity status. The proportion of time that males and females sheltered together was significantly greater in the mating season, but no effect of familiarity status was found (Table 5). As geckos spent only a small proportion of time interacting (Fig. 4), no statistical analysis of this variable was carried out.

The amount of time geckos explored the test arena did not differ significantly between males and females in the mating ($Z = -1.560$, $p = 0.148$, WSRT) or non-mating season ($Z = -1.544$, $p = 0.133$, WSRT). Females rested significantly less outside their shelters ($Z = -2.252$, $p = 0.031$, ES = 0.80, WSRT) and spent significantly more time sheltering alone ($Z = -2.111$, $p = 0.039$, ES = 0.75, WSRT) in the mating season in comparison to males (Appendix 2, Fig. 4). In the non-mating season, no significant differences were found between males and females in the proportion of time spent resting (resting: $Z = -1.122$, $p = 0.297$) or sheltering alone ($Z = -1.820$, $p = 0.078$).

Table 5: Results of GEE analyses of the effects of season, familiarity status and season x familiarity status on the activities of male and female Duvaucel's geckos per six hour observation period. Presented are estimated marginal means and standard errors from GEE models of counts from 10 min-scan samplings.

									Mating Season				Non-mating Season			
	Intercept		Season		Status		Season x status		Familiar		Unfamiliar		Familiar		Unfamiliar	
Activity	χ^2	P	χ^2	P	χ^2	P	χ^2	P	Mean	(SE)	Mean	(SE)	Mean	(SE)	Mean	(SE)
Females																
Exploring	66.701	<0.001	36.331	**<0.001**	0.524	0.469	1.402	0.236	1.27	(0.52)	2.39	(1.05)	11.63	(2.19)	9.41	(1.58)
Resting	58.185	<0.001	55.347	**<0.001**	0.109	0.741	0.187	0.666	0.86	(0.51)	0.87	(0.37)	19.47	(1.98)	14.67	(1.99)
Sheltering alone	530.458	<0.001	30.639	**<0.001**	5.263	**0.022**	6.472	**0.011**	24.59	(3.97)	24.15	(2.96)	3.79	(1.05)	11.42	(3.45)
Males																
Exploring	167.920	<0.001	4.798	**0.028**	0.039	0.844	1.967	0.161	4.71	(0.96)	3.95	(0.94)	6.33	(1.33)	8.03	(1.33)
Resting	64.854	<0.001	1.037	0.308	0.348	0.555	0.008	0.928	5.56	(2.19)	6.40	(3.11)	9.25	(3.04)	10.22	(2.83)
Sheltering alone	522.174	<0.001	0.002	0.964	0.145	0.703	0.272	0.602	16.81	(3.39)	17.03	(3.00)	18.38	(3.74)	15.91	(3.69)
Male and female sheltering together	4.719	0.030	25.960	**<0.001**	1.035	0.309	0.811	0.368	8.29	(3.77)	7.75	(2.82)	0.75	(0.58)	0.25	(0.15)
Male and female interacting	-		-		-		-		0.86	(0.51)	0.88	(0.37)	0.63	(0.35)	0.88	(0.28)

Discussion

Social interactions between adult male and female Duvaucel's geckos occurred at close range and frequently involved physical contact in the form of chemosensory and tactile behaviours, including short bites. Most components of visual display behaviour during intersexual interactions observed in this study have been reported for geckos from other genera, indicating that the basic elements of display behaviour are highly conserved among gekkotans. The behavioural repertoire was largely consistent between males and females, but the use of several behaviours varied considerably between sexes as well as in relation to season.

Geckos readily snout-touched and tongue-flicked their partner during an interaction, thereby likely procuring chemical cues via olfaction and vomerolfaction (Greenberg 1943, Cooper and Burghardt 1990, Schwenk 1995, Regalado 2003a). In contrast to females, male Duvaucel's geckos were more likely to perform these chemoreceptive behaviours during an interaction. Chemical communication is an important component of lizard social behaviour and cues from skin semiochemicals, pheromones and gland secretions can yield information about social (Bull *et al.* 2000), physiological (Head *et al.* 2005, Martin and Lopez 2007) or genotypic (Main and Bull 1996, Lena and de Fraipont 1998, Lopez *et al.* 2002) traits of a conspecific, allowing the individual to assess the relative risk or benefit of a potential interaction.

Several studies have demonstrated that male lizards, including Duvaucel's geckos (Chapter 5), are able to discriminate between chemical stimuli from hetero- and consexual conspecifics (Mason and Gutzke 1990, Cooper and Trauth 1992, Cooper and Steele 1997). Furthermore, chemoreceptive behaviours can aid males in detecting female receptivity (Cooper and Perez-Mellado 2002, Head *et al.* 2005) or in recognising individual competitors from scent cues (Carazo *et al.* 2008). Male Duvaucel's geckos are generally less tolerant of same-sex conspecifics, in contrast to females, and either avoid them (Chapter 3 & 4) or engage in combat (Barry, unpubl. data). It may be of greater importance for a male (rather than a female) to identify the sex, social status or physiological condition of the conspecific enabling it to decide on the course of the interaction i.e. whether to retreat, fight or engage in mating activities. Furthermore, it may be possible that males are able to retrieve information about the level of genetic relatedness of the female, which could influence their decision to mate. For example, Olsson *et al.* (2003) have demonstrated that female Swedish sand lizards (*Lacerta agilis)* preferred odours from more distantly related males, and that large males preferentially associated with less related females in the field. Hence, the thorough chemosensory investigation of females by male Duvaucel's geckos could be associated with inbreeding avoidance.

Males were more likely to perform aggressive acts such as snaps, short bites or attacks in comparison to females, although the differences were more pronounced during the non-mating season. Females typically performed tail displays during interactions and were less likely to show aggressive behaviours, particularly during the non-mating season. However, females showed an increased probability of biting their male opponent during the mating season, which seemed to be a response to the increased importunacy of the males. Females clearly avoided males during the mating season and spent over 90 % of the six hour observation period in a shelter. Females also approached males significantly less often in the mating season in comparison to the non-mating season. Males on the other hand, approached females more often in the mating season and spent about 20 % of the observation period together with them in their shelters. Some male lizards e.g. *Chamaeleo chamaeleon* (Cuadrado 2001), *Aspidoscelis costata* (Zaldívar-Rae and Drummond 2007), *Eumeces laticeps* (Cooper and Vitt 1997) are known to guard females before and after copulation to increase their mating success, which could explain the observed behaviour in this study. In this study, Duvaucel's geckos showed a relatively long copulation time of up to 16 min and 28 s. The duration of copulations reported for other gekkotans ranged from several seconds to three quarters of an hour (< 1min: *Sphaerodactylus elegans*, Regalado 1997, < 2 min: *Hemidactylus mabouia*, Regalado 2003a, *Eublepharis macularius*, Brillet 1991, < 13 min: *Paroedura pictus*, Brillet 1991, ~20 min: *Sphaerodactylus nicholsi*, Regalado 2003b, and ~ 40 min: *Lygodactylus picturatus keniensis*, Greer 1967). A prolonged copulation could be related to sperm competition (Olsson and Madsen 1998). Duvaucel's geckos have relatively large testis sizes (Todd 2008), which may facilitate prolonged sperm transfer, a feature observed in snakes (Olsson and Madsen 1998). These traits imply a promiscuous mating strategy (Anderson 1994, Harcourt 1995, Olsson 1995), which has also been suggested for the sympatric gregarious common gecko (*H. maculatus*, Todd 2008).

Female Duvaucel's geckos rarely performed visual displays such as back arching or leg extensions during an intersexual interaction in any season. Likewise, males seldom displayed these postures in the non-mating season. However in the mating season, males were five times more likely to display leg extensions and ten times more likely to perform a back arch during an interaction in comparison to the non-mating season. These often laterally presented postures are typical threat displays performed by geckos during agonistic intra-specific interactions (e.g. Marcellini 1977, Demeter and Marcellini 1981, Regalado 2003a) allowing the opponent to assess the quality of the sender. The increased likelihood of male Duvaucel's geckos performing these, otherwise rarely used, displays in the mating season, suggests that they play a role in the species courtship behaviour.

The use of conspicuous postures, movements or colours during courtship is common among diurnally active gecko species i.e. *L. picturatus keniensis, Gonatodes vittatus, Phelsuma astriata semicarinata* (Greer 1967, Demeter and Marcellini 1981, Murphy and Myers 1996), but males of nocturnal gekkonids e.g. *Coleonyx variegatus, Hemidactylus mabouia, Hoplodactylus pacificus* generally show little or no visual display behaviour prior to a mating attempt (Greenberg 1943, Rieppel 1976, Regalado 2003a). Marcellini (1977) noted that courtship displays of diurnal geckos largely resemble visual threat displays performed during consexual encounters and it has been suggested that lizard courtship displays may play a role in sex recognition (Martins 1993) as well as female attraction (reviewed in Tokarz 1995). Captive Duvaucel's geckos of either sex were observed to perform back arching and leg extensions during agonistic encounters with same sex conspecifics (Barry, unpubl. data) and in response to an approach by humans (Barry, *pers obs*). The extent and type of display and courtship behaviour exhibited by a species is largely dependent upon its ecological environment (Bosch and Zandee 2001, Ord *et al.* 2007) and diel activity patterns (Marcellini 1977). Although Duvaucel's geckos are primarily nocturnal and typically forage at night (Barwick 1981), they are not entirely inactive during the day and leave their retreats to sun-bask (Whitaker 1967, Barry *pers obs* on captive geckos). Several male-female pairs from the captive population were seen *in copulo* during daylight hours (e.g. 20 September 2007 at 15:00 h, 2 September 2008 at 15:15 h and 24 September 2008 at 16:54 h), which illustrates that mating activities too are not restricted to the night-time and implies that this species may be diurno-nocturnal, showing habits similarly to those of the closely related New Zealand gecko *H. maculatus* (Werner and Whitaker 1978, Todd 2005). It is also possible that some displays produce sound and therefore have a visual as well as auditory component, so can be detected at both day and night.

My observations of male-female interactions during the mating season in the present study suggest that male Duvaucel's geckos may adjust their courtship strategy according to the situation. Initially, males approached females and engaged in tactile and chemosensory behaviours, then applied the courtship grip and eventually copulated. The frequent use of bites prior to copulation may ensure dominance of males over females and may act as stimulation to induce mating (receptivity?) in females (Carpenter 1960). However, if females rejected males and retreated into a shelter, males seemed to change their strategy and performed conspicuous visual displays.

Although female lizards generally play a rather passive role during courtship (Marcellini 1977), their cooperation is required in order to mate (Greenberg 1943). Furthermore, the willingness of a female to mate may not only depend on whether she is receptive or not. For example, male morphological traits i.e. body size (Cooper and Vitt 1993, Censky 1997) can also influence a female's decision to mate. If tactile stimulation during courtship is unsuccessful, male Duvaucel's geckos may add a visual component, which could be of higher informative value for a female. By adopting postures such as a back arch and limb extension, males appear larger, thereby signalling their quality. When females retreated into a shelter, males could follow and join the female but were not able to copulate with them as the space was too narrow to achieve a necessary position. Wild Duvaucel's geckos typically shelter in relatively narrow rock crevices or tree cavities during the day (Chapter 3), therefore if mating activities are not restricted to the night time (when geckos have left their retreats) the use of a strong signal in form of an assertive display could be a common aspect of male courtship to lure females out of their retreats. However, in this study the mating attempts by males performing displays never resulted in copulation and further research is required to investigate this hypothesis.

The role of familiarity

Familiar gecko pairs engaged in slightly less agonistic interactions than unfamiliar pairs throughout the study period. Additionally, familiar pairs were involved in more amicable interactions during the mating season than unfamiliar pairs. Females that were paired with an unfamiliar male spent more time alone in a shelter than females with a familiar male partner in the non-mating season. The number of aggressive interactions did not differ between familiar and unfamiliar pairs in any season and the familiarity status of a pair neither had an effect on the frequency of interactions and approaches nor the length of initial interactions.

The differences in the frequency of agonistic and amicable interactions suggest that Duvaucel's geckos are able to distinguish between cage mates (i.e. residents) and non-cage mates (i.e. non-residents). Moreover, the overall reduction of agonistic interactions and more frequent engagement in amicable interactions between familiar pairs, and, particularly, the avoidance of unfamiliar males by females in the non-mating season could indicate that at least some males and females may have established associations that lasted beyond the mating season. However, the overall differences in interactions between familiar and unfamiliar pairs found in this study were only subtle, which could be related to the fact that the study animals were randomly assembled in the cages prior to the study, regardless of their spatial distribution in the field at the time of capture. While male and female geckos from the same outdoor enclosure likely became familiar with each other during the five month period prior to this study, the composition of the groups may not necessarily reflect the choice of partners the geckos would make in the wild. Thus, not all males and females within the same enclosure may have formed potential associations. Comparing the social interactions between male-female pairs that share diurnal retreats under natural conditions with individuals from different locations could help to confirm whether opposite sex conspecifics form long lasting bonds. The absence of differences in the frequency of aggressive interactions between familiar and unfamiliar pairs suggests that male and female Duvaucel's geckos perceive each other as non-threatening. Therefore, a behavioural adaption such as the 'dear enemy phenomenon', (*sensu* Fisher 1956), which was observed in interactions between neighbouring and strange adult male lizards of a territorial species (*Crotaphytus collaris* (Husak and Fox 2003), may not be necessary. Furthermore, male and female Duvaucel's geckos are generally tolerant of each other, and males often share shelter sites with one or several females and juveniles in the wild throughout the year (Chapter 3).

There was only limited evidence that male Duvaucel's geckos exhibited a higher mating effort when paired with unfamiliar females (63 %) in comparison when paired with familiar females (43 %). While males may have previously mated with the (resident) females from their home cages during the mating season before trial start, they had no prior mating opportunity with females from other cages during that period, and thus some males consequently may have intensified their mating effort with these unfamiliar females to maximise their mating success (Cooper 1985). The lizards *Holbrookia propinqua* (Cooper 1985) and *Anolis sagrei* (Tokarz 1992), in contrast, exhibited a strong courtship / mating preference for unfamiliar females. Further experimental study could help to clarify whether such a "Coolidge effect" (Dewsbury 1981, Wilson *et al.*

1963) is also found in Duvaucel's geckos. The results of the present study suggest that males showed little differences in sexually motivated behaviours towards familiar and unfamiliar females, particularly with respect to male mate guarding behaviour i.e. the length of time males stayed in a shelter together with a female. It may be more important to guard females equally, regardless of their familiarity status, to decrease the potential risk of a female mating with other male competitors.

Conclusions

This study offers the first insight into the repertoire and use of behaviours exhibited by male and female Duvaucel's geckos during dyadic intersexual interactions. Females were inquisitive in the non-mating season but largely passive in the mating season and appeared to avoid males. In the mating season, males increased their approach rate and courted females. The use of courtship specific displays was not evident, other than the male courtship grip and the female courtship pose. Males appeared to adjust their courtship effort according to the situation: While they initially used tactile stimuli to motivate females to mate, they added a visual component upon female rejection to potentially increase the signal. Males also appeared to mate-guard females during the mating season but not during the non-mating season. My data indicates that males and females can become familiar with each other and subsequently modulate their interactions in response to a resident or non-resident individual. While males engaged in slightly more mating attempts with unfamiliar females they guarded all females equally, regardless of familiarity status. The trends observed in this study provide a foundation for further research into the behavioural ecology of Duvaucel's geckos; and a larger sample size could confirm trends that were close to significance in this study. Acoustic signals have not been examined in the present study but should be considered in future research as Duvaucel's gecko are known to produce sounds in response to human disturbance (Rob 1980, Barry *pers obs*) and may use vocalisations during social interactions.

CHAPTER SEVEN

Conclusions and future directions

Overview

In this dissertation I investigated aspects of the basic physiology and behavioural ecology of Duvaucel's geckos (*Hoplodactylus duvaucelii*). Exploratory studies served as the means to document seasonal hormone levels and diurnal spatial distribution patterns of wild and captive geckos. The results provided information directly relevant for *H. duvaucelii's* captive conservation management. Experimental studies were conducted to test hypotheses regarding *H. duvaucelii's* social behaviour and social capabilities. These studies did not only uncover several aspects of the behavioural ecology of this large nocturnal New Zealand gecko but also advanced our knowledge on social systems of geckos in general.

Implications for captive management

Information on life history traits, social relationships and habitat requirements of a species is essential to provide optimal living conditions for individuals used in captive breeding programmes. Moreover, understanding the dynamics of basic physiological parameters such as seasonal steroid hormone levels (i.e. plasma corticosterone), and the impact of extrinsic or intrinsic factors on these, may be critical for assessing animal welfare and thus ensuring a successful long-term captive management.

In Chapter Two, I presented the first broad overview of seasonal plasma corticosterone concentrations in wild *H. duvaucelii* to date. Hormone levels of wild geckos varied across seasons, with higher values in winter than summer and spring. Sex, body temperature, body condition and female reproductive condition did not appear to affect gecko plasma corticosterone levels. A comparison of hormone data between wild and captive (wild born) geckos has revealed that captive individuals did not suffer from chronic stress as their plasma corticosterone levels showed no prolonged elevation in comparison to the wild population. Moreover, pregnant captive females, which are most vital to the breeding programme, exhibited low stress hormone levels, similar to their wild counterparts. However, I detected an elevation of steroid hormone levels in captive compared to wild geckos during summer and observed a trend for higher plasma corticosterone concentrations of apparently subordinate male geckos in contrast to dominant or single males. These findings highlight the need for further research into the causation and function of hormone fluctuations, such as effects of density and social structure on hormone patterns. For example, male Duvaucel's geckos appeared to avoid other male conspecifics under natural and experimental conditions (Chapters Three and Four). Thus the presence of two males per cage might represent a social stressor that could influence social dynamics and hormone levels (not only of males) and consequently may affect breeding patterns. These are important aspects to consider for captive breeding.

Dominance status is characteristically linked to the body size in male lizards (Cooper and Vitt 1987, Cuadrado 2001). In Chapter Three I have shown that large males associated themselves closely with females and juveniles in diurnal shelter aggregations but smaller males typically sheltered alone. Hence, large (dominant) males may prevent smaller (subordinate) males from associating with females, and thereby decrease the mating opportunities of these subordinate males. With regard to *H. duvaucelii's* captive breeding management, it may be beneficial to house females with one rather than several males to prevent social interference between males. This may also provide equal mating opportunities for all males within the breeding programme. As a result of this study, we decreased the number of males per cage from two to one.

In conclusion, wild Duvaucel's geckos have adapted well to long-term captivity and have proven suitable for the breeding programme, which is supported by the lack of chronic stress and successful breeding. However, the long-term monitoring of stress hormone levels in conjunction with environmental, physiological and social variables could aid in detecting changes in hormone patterns over time. This may help to identify and mitigate potential stressors as well as to further improve living conditions. Furthermore, the development of a less invasive technique for monitoring steroid hormone levels could simplify the sample collection and thus make this method more broadly applicable for captive management.

Behavioural ecology and sociobiology

Life history traits of organisms are thought to be shaped by their physical and ecological environment. The evolution of sociality, however, may be facilitated by both aspects (Lacey and Sherman 1997, Hatchwell and Komdeur 2000, Covas and Griessner 2007). For example, stable and rich environments with relatively low predation pressure should favour organisms with a larger body size, a slow development and delayed reproduction, fewer but larger offspring and a long life expectancy (Pianka 1970). Likewise, the occurrence of viviparity in reptiles, a trait shared by all but one native New Zealand lizard species, is seen as an adaptation to a life in cool climate habitats (Shine 1985). The selective forces behind the evolution of complex sociality in some lizards (see Chapple 2003 for review) still remain poorly understood. However, hypotheses predict that factors such as high population density, lack of dispersal opportunities and limited habitat availability (Duffield and Bull 2002, Schutz *et al.* 2007) combined with late maturity, longevity and delayed juvenile dispersal may promote social group living in lizards and hence could mediate social complexity in lizard societies (Duffield and Bull 2002, Chapple 2003, Davis *et al.* 2010). Duvaucel's geckos, which often occur in great abundance in their extant island habitats show life history traits in accordance with these criteria.

Some features of sociality found in mammals and birds are not exhibited by lizards, for instance direct parental care (Shine 1988). However, lizard social systems show remarkable convergences to avian and mammalian social systems, which as well are thought to have evolved on the basis of habitat saturation, high survival and delayed juvenile dispersal (Arnold and Owens 1998, Hatchwell and Komdeur 2000, Covas and Greissner 2007). Genetic relationships between group members have important implications for group structure and social cohesiveness in various systems (Armitage and Johns 1982, Gaspari *et al*. 2007, Hatchwell 2009) and also appear to be a key factor in the social systems of several group living lizards (Gardner *et al.* 2007, While *et al*. 2009, Davis *et al.* 2010).

My research has shown that *H. duvaucelii* are essentially social lizards, forming aggregations in diurnal shelter sites throughout the year. Furthermore, *H. duvaucelii* are long-lived, show delayed maturity and are viviparous, hence have life history traits, which are thought to favour a social life style and are characteristically found in other social lizard species (see Chapple 2003). Adults of both sexes exhibited a high tolerance towards juveniles and shelter aggregations often comprised of individuals of varying age and size. Juveniles demonstrated the ability to discriminate between scent cues from siblings and non-siblings and also between mothers and unrelated females (Chapter 5). The fact that adult *H. duvaucelii* exhibit long-term site fidelity (Thompson *et al*. 1992, Wilson 2010) and that juveniles in the wild population were often observed in close association with adults and rarely sheltered alone (Chapter 3), suggests that juveniles may remain within the range of their parents. Furthermore, the ability to distinguish between kin and non-kin (Chapter 5) implies a social system where there is a need to differentiate between family and non-family members as for instance in family based social groups. Interestingly, I did not find evidence that the distribution patterns of geckos in shelters were influenced by kin relationships in a standardized laboratory experiment (Chapter 4). However, the potential occurrence of family groups in the wild population may not be attributed to kin attraction *per se*, but could rather be a result of limited or delayed juvenile dispersal and the tolerance of genetically closely related juveniles by adults (see Gardner *et al.* 2001).

Chemosensory communication is a central feature of *H. duvaucelii* social behaviour. This was demonstrated in their ability to detect and discriminate between conspecific scents as well as their use of marking behaviours and frequent over-marking of conspecific scents by juveniles and adults. Chemical signal exchange may facilitate relationships with kin (Bull *et al.* 2001, While *et al.* 2009) and other group members (Bull *et al.* 2000) and therefore could enhance group cohesion. Stable social groups and kin based sociality have never been reported for gekkonid lizards. Given *H. duvauceli's* life history traits, aggregation patterns and scent discrimination abilities, strong convergences to several social species of the Australian scincid lineage *Egernia* are apparent. This represents an exciting prospect to test the hypothesis that a gekkonid lizard exhibits kin based sociality and forms family groups. Information on juvenile dispersal and genetic relationships of group members could help to clarify this.

Apart from the frequent presence of juveniles within aggregations, the majority of groups observed in the field were mixed sex groups. Groups with 1:1 adult male:female ratios were more common than groups featuring 1:2 or 1:3 sex ratios, i.e. one male sharing a shelter with several adult females. Male-female associations occurred year-round, suggesting that males and females may remain in pairs most of the time. Likewise, male-female pairs were the most common combination that shared shelters in the laboratory experiment (Chapter 4), regardless of season. Information on the temporal stability of wild male-female associations is needed to determine whether they represent socially monogamous pairs. However, a large testis size (Todd 2008), the tendency of prolonged copulation time (Chapter 6) and the apparent guarding of females by males in the mating season (Chapter 6) implies a potential for sperm competition, which is rather typical for a promiscuous mating strategy (Anderson 1994, Harcourt 1995, Olsson 1995). A polygynandrous mating system has also been suggested for the congeneric common gecko (*H. maculatus*), a gregarious species that shows a courtship pattern with high levels of promiscuity of both sexes (Todd 2005). In contrast to *H. duvaucelii*, *H. maculatus* forms large shelter aggregations (up to 94 individuals) that may comprise of several males and females (Hare and Hoare 2005, Lettink and Cree 2007). Exploring the permanency of male-female associations under natural conditions as well as genetic relationships of offspring could shed light onto *H. duvaucelii's* social and genetic mating system.

Finally, I explored male-female interactions to document *H. duvaucelii's* behavioural repertoire and courtship behaviour (Chapter 6). Intersexual interactions typically involved frequent physical contact and chemosensory behaviours, illustrating the importance of chemical information exchange during interactions. Display behaviours were limited to threat and submissive gestures and a courtship posture by females that indicated willingness to mate. As seen in other nocturnal gekkonids, courtship specific displays were rare (Marcellini 1977). However, male *H. duvaucelii* seemed to alter their courtship strategy according to a female's response: Initially, male courtship was limited to a chemosensory investigation and tactile stimulation of the female and, if the female was willing, followed by copulation. If, however, the female rejected the male and retreated into a shelter, the male performed a conspicuous display in the form of a back arch and leg extension. Subsequent to this display, the male would try to initiate a mating again. The male display, which is a typical threat posture used in agonistic interactions, could be of higher informative value for females as it emphasises the male's size and may convey information about its quality. Female mate choice, based on visual traits (e.g. male size or coloration), is thought to be rare in lizards (Olsson and Madsen 1995) and has only been reported for a few diurnally active lizard species (Cooper and Vitt 1993, Tokarz 1995). It would be worthwhile to investigate whether female *H. duvaucelii* exhibit male mate choice based on visual traits such as body size. The occurrence of different male courtship tactics and female rejection behaviour may be evidence for this.

This study advances our knowledge about lizard social systems and provides the first in-depth documentation of the behavioural ecology of a New Zealand gecko. Furthermore, the information gained on the spatial distribution and shelter preferences of wild geckos is advantageous for *H. duvaucelii's* conservation management. Information on spatial distribution could aid in assessing abundance and carrying capacities and knowledge on the social structure of gecko aggregations is beneficial when selecting individuals for translocations. Moreover, understanding micro-habitat preferences and requirements is vital when choosing potential future release sites.

The combination of life history traits, social behaviours and conspecific discrimination abilities suggests a social system of high complexity and highlights *H. duvaucelii's* potential to serve as a model species to further explore lizard social behaviour. To date detailed information on genetic and social relationships of group living lizards only exist for several species of the Australian scincid genus *Egernia* (While *et al.* 2009, Chapple 2003) and one species of xantusid lizard (Davis *et al.* 2010), however apart from anecdotal records, little is known about social systems in other taxa. Information on social systems of a diverse array of phylogenetic lineages including gekkonids could offer valuable insights into the evolution of sociality in squamate reptiles. This study provides a foundation for future research and delivers a first insight into the social behaviour of *H. duvaucelii*, an elusive New Zealand reptile.

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Appendices

Appendix 1

Appendix 1: Comparison (Wilcoxon signed ranks tests) of probability values of behavioural acts between females and males within each season and across seasons for each sex. Z-values, significance (P) and effect sizes (ES) are reported. Significant effects are in bold letters.

	Mating season *vs* non-mating season (N = 7)						Males *vs* females (N = 8)					
	Females			Males			Spring			Winter		
Behaviour	*Z*	*P*	ES	*Z*	*P*	ES	*Z*	*P*	ES	*Z*	*P*	ES
Snout touch/ tongue flick	-0.338	0.813	(0.1)	-0.338	0.813	(0.1)	-2.103	**0.039**	(0.7)	-2.100	**0.039**	(0.7)
Touch / sit on	-0.169	0.866	(0.1)	-0.676	0.499	(0.3)	-0.280	0.779	(0.1)	-0.734	0.463	(0.3)
Nudge*	-	-		-	-		-	-		-	-	
Snap	-2.023	0.063	-0.8	-2.201	**0.031**	(0.8)	-1.572	0.156	(0.6)	-2.366	**0.016**	(0.8)
Bite short	-2.201	**0.031**	(0.8)	-0.169	0.938	(0.1)	-0.946	0.406	(0.3)	-2.201	**0.031**	(0.8)
Bite long	-1.342	0.500	(0.5)	-1.841	0.125	(0.7)	-1.787	0.094	(0.6)	-	-	
Grip	-	-		-	-		-	-		-	-	
Charge / charge nudge	0.000	1.000	(0)	-1.414	0.500	(0.5)	-1.414	0.500	(0.5)	-1.000	1.000	(0.4)
Charge snap	-1.000	1.000	(0.4)	-1.342	0.500	(0.5)	-1.000	1.000	(0.4)	-1.633	0.250	(0.6)
Attack	-1.826	0.125	(0.7)	-0.524	0.688	(0.2)	-0.351	0.766	(0.1)	-2.201	**0.031**	(0.8)
Head raised	-1.014	0.375	(0.4)	-0.847	0.438	(0.3)	-0.594	0.641	(0.2)	-1.363	0.219	(0.5)
Head angled	-1.000	1.000	(0.4)	-1.069	0.500	(0.4)	-1.342	0.500	(0.5)	-0.447	1.000	(0.2)
Legs extended	-0.535	0.750	(0.2)	-2.214	**0.031**	(0.8)	-2.032	0.063	(0.7)	-1.342	0.500	(0)
Back arch	-0.447	1.000	(0.2)	-1.992	0.063	(0.8)	-2.371	**0.016**	(0.8)	0.000	1.000	(0)
Tail wave	-0.507	0.688	(0.2)	-2.032	**0.047**	(0.8)	-1.540	0.148	(0.5)	-2.521	**0.008**	(0.9)
Tail thrash	-1.826	0.125	(0.7)	-1.000	1.000	(0.4)	-2.032	0.063	(0.7)	-1.000	1.000	(0.4)
Tail vibrate	-1.000	1.000	(0.4)	-2.023	0.063	(0.8)	-1.992	0.063	(0.7)	-	-	
Jerky*	-	-		-	-		-	-		-	-	
Snout open*	-	-		-	-		-	-		-	-	
Flinch	0.000	1.000	(0)	-1.069	0.285	(0.4)	-0.135	0.893	(0)	-0.921	0.357	(0.3)
Cloaca lick*	-	-		-	-		-	-		-	-	
Courtship bite*	-	-		-	-		-	-		-	-	
Courtship pose*	-	-		-	-		-	-		-	-	
Copulation*	-	-		-	-		-	-		-	-	
Withdrawal fast	-0.730	0.625	(0.3)	-	-		-1.604	0.250	(0.6)	-1.826	0.125	(0.6)
Withdrawal slow	-2.197	**0.031**	(0.8)	-2.366	**0.016**	(0.9)	-2.197	**0.031**	(0.8)	-2.240	**0.023**	(0.8)

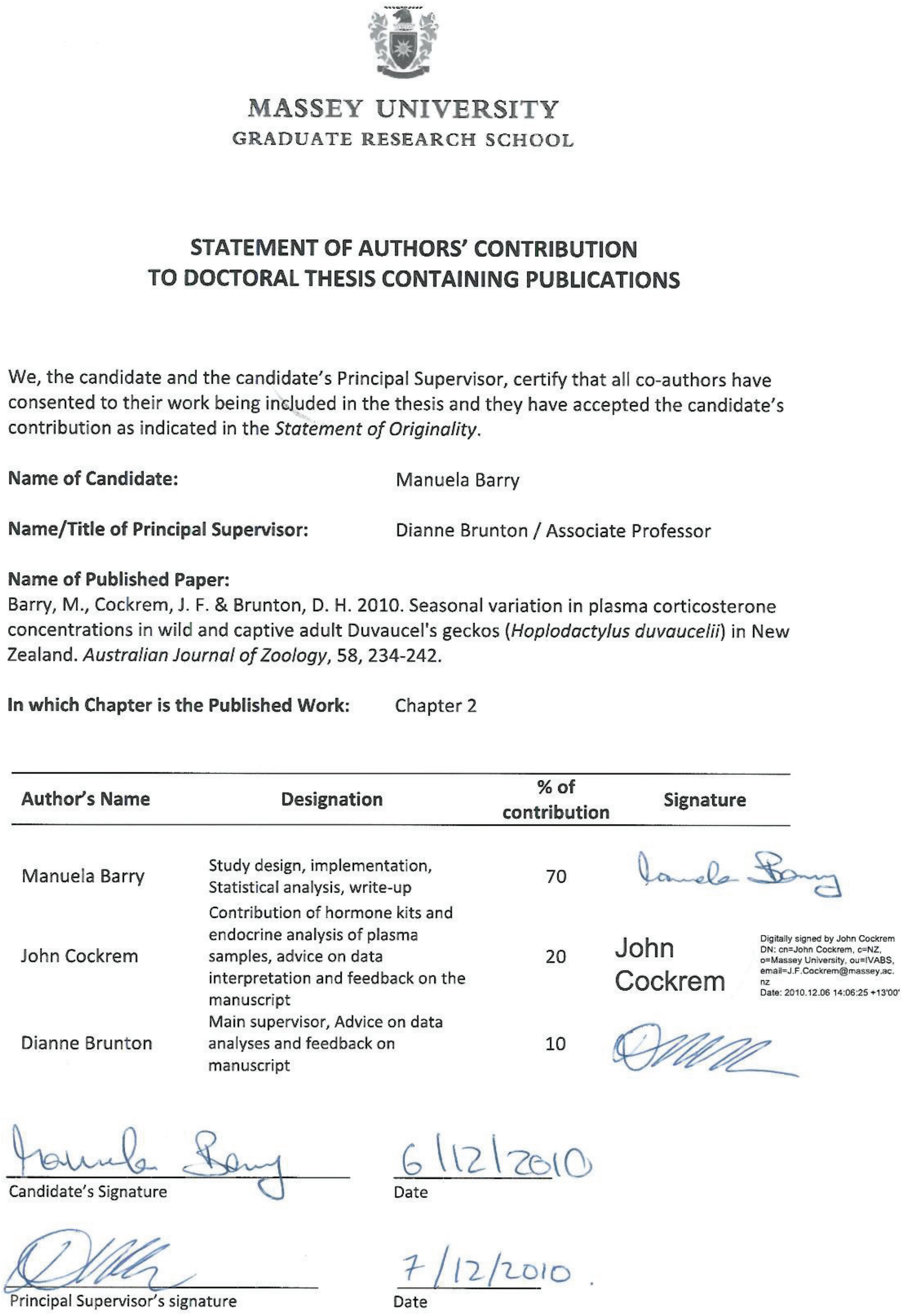
*no statistical comparison was computed when the probability of a behaviour occurring was less than 5% in all four groups, or when the behaviour was observed in one group only

Appendix 2

Appendix 2: Mean/median counts from 10 min-scan samplings (averaged for familiar and unfamiliar treatments) of activities performed by male and female Duvaucel's geckos during the six hour observation period in the mating and non-mating season. Mean/median frequencies of approaches of males and females for each season (averaged for familiar and unfamiliar treatments).

	Mating Season						Non-mating Season					
	Males			Females			Males			Females		
Variable	Mean	Median	(Range)	Mean	Median	(Range)	Mean	Median	(Range)	Mean	Median	(Range)
Activity												
Exploring	4.00	3.75	(0-8)	1.88	1.25	(0-5)	7.38	6.75	(3.5-14)	10.13	10.25	(3-15.5)
Resting	6.13	3.00	(1.5-24)	1.00	0.75	(0-3)	17.38	19.50	(2-28)	7.63	5.75	(1.5-17)
Sheltering (alone)	17.56	16.50	(6.5-34)	24.88	25.75	(8.5-34)	10.00	8.50	(2-18.5)	17.00	15.50	(10-27)
Approaches												
Total	4.94	4.75	(3-7)	2.25	1.75	(0.5-6)	4.25	4.00	(1-9)	5.06	4.75	(3.5-8)
Amicable	4.19	4.25	(2-6)	1.19	0.75	(0-3.5)	2.25	2.25	(0.5-5.5)	4.31	3.50	(3-8)
Agonistic	0.19	0.00	(0-0.5)	0.69	0.75	(0-2)	0.19	0.00	(0-1)	0.63	0.50	(0-1.5)
Aggressive	0.25	0.00	(0-1)	0.13	0.00	(0-0.5)	1.31	1.00	(0-4)	0.06	0.00	(0-0.5)

Appendix 3



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STATEMENT OF AUTHORS' CONTRIBUTION TO DOCTORAL THESIS CONTAINING PUBLICATIONS

We, the candidate and the candidate's Principal Supervisor, certify that all co-authors have consented to their work being included in the thesis and they have accepted the candidate's contribution as indicated in the *Statement of Originality*.

Name of Candidate: Manuela Barry

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In which Chapter is the Published Work: Chapter 2

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John Cockrem	Contribution of hormone kits and endocrine analysis of plasma samples, advice on data interpretation and feedback on the manuscript	20	John Cockrem Digitally signed by John Cockrem DN: cn=John Cockrem, c=NZ, o=Massey University, ou=IVABS, email=J.F.Cockrem@massey.ac.nz Date: 2010.12.06 14:06:25 +13'00'
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