

A Comparison of Two Populations of Tiger
Snakes, *Notechis scutatus occidentalis*:



The Influence of Phenotypic Plasticity on
Various Life History Traits

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“Not a single one of your ancestors died young. They all copulated at least once.”

Richard Dawkins (b. 1941).

Summary

The phenotype of any living organism reflects not only its genotype, but also direct effects of environmental conditions. Some manifestations of environmental effects may be non-adaptive, such as fluctuating asymmetry. Growing evidence nevertheless suggests that natural selection has fashioned norms of reaction such that organisms will tend to display developmental trajectories that maximise their fitness in the environment which they encounter *via* enhanced growth, survival, and/or reproduction. Over recent decades, the adaptive value of phenotypic plasticity has become a central theme in evolutionary biology. Plasticity may have evolutionary significance either by retarding evolution (by making selection on genetic variants less effective), or by enhancing evolution (as a precursor to adaptive genetic change). Reptiles are excellent models for the study of such theories, notably because they show high degrees of phenotypic plasticity. Many plastic responses have now been documented, using a diversity of taxa (turtles, crocodiles, snakes, lizards) and examining a number of different traits such as morphology, locomotor performance, and general behaviour.

Islands are of special interest to ecologists and evolutionary biologists because of the rapid shifts possible in island taxa with small and discrete populations, living under conditions (and selective pressures) often very different from those experienced by their mainland conspecific.

Tiger snakes (*Notechis scutatus*) are found in all Southern parts of Australia, including Tasmania and on several offshore islands. Detailed ecological studies have shown definite ecological differences between mainland and island populations and also differences between different islands (for instance in body size, adult sex ratio, prey types, venom and population structure). Mainland Tiger snakes usually live in swamps and feed mainly on frogs (such as the population of Herdsman Lake, WA) whilst others survive on waterless islands (such as Carnac Island, WA) where no frogs occur and silver gull chicks

constitute the main prey source of adult snakes. The habitat and prey resources available on the island therefore differ considerably from those on the mainland from whence the snakes originated, giving the opportunity to dissect the relative contribution of genetic divergence and phenotypic plasticity with regard to the Tiger snakes' morphological, behavioural and ecological differences.

Tiger snakes are larger on Carnac Island (CI) than in Herdsman Lake (HL), in body length, body mass, and in absolute and relative head size. A divergence in morphology between adjacent populations exposed to different selective forces can arise in two ways. Firstly, a change in the frequency of genes that code for specific traits, for example, snakes that encounter larger prey will accumulate genetic mutations that code for larger head size. Secondly, through adaptive plasticity, that is, increased frequency of genes that modify growth trajectories of relative head size in response to local conditions. The end result in both cases is a match between environmental pressures and organismal morphology, but the proximate basis is very different. In one case variation in morphology is driven by genes for specific traits, whereas in the other case variation in morphology is driven by genes for developmental plasticity. The notion of adaptive plasticity has been controversial, but my study on snake populations provides evidence for both processes. A higher degree of plasticity in jaw development as well as larger jaws at birth have been selected for within the Carnac Island Tiger snake population because superior swallowing capacities are advantageous as larger than usual prey (silver gull chicks) are the main prey source.

Many phylogenetic lineages of animals have undergone major habitat transitions in the course of their evolutionary history, often stimulating dramatic morphological and physiological changes as adaptations to a novel environment. Although most such traits clearly reflect genetic modifications, phenotypic plasticity nonetheless may have played a significant rôle in the initial transition between habitat types. Presumably, proto-sea-snakes spent

much of their time swimming rather than crawling, thus favouring adaptations for aquatic locomotion. My experiments showed that Tiger snakes raised in an aquatic environment displayed significantly enhanced swimming speeds but reduced crawling speeds compared with their terrestrially-raised siblings. Hence, adaptively plastic responses to local environments may have played a major rôle in facilitating the evolutionary transition from land to water in proteroglyphous snakes.

Despite being identical at birth for a very large array of traits (body size, behaviour, locomotor performances, growth rates), my work exposes a number of significant differences for the relevant traits between field-experienced older animals from both populations. This suggests a potential rôle for phenotypic plasticity (possibly adaptive) in the expression of such traits, such as locomotor performances, defensive behaviour and prey preferences. The multiple systems of island/mainland population of Tiger snakes across Australia may also provide an unique opportunity to understand the rôle of plasticity in the persistence of populations in newly colonised environments.

Declaration

I certify that this thesis does not incorporate, without acknowledgment, any material previously submitted for a degree or diploma in any university, and that to the best of my knowledge and my belief, it does not contain any material previously published or written by another person except where due reference is made in the text.

Fabien Aubret

Organisation of thesis

This thesis is organised into five chapters. The first chapter is a general introduction that considers the relevant theory and the significance of the research, and describes the study species and study sites. Chapter 2 is aimed at precisely describing the morphology, diet and reproductive output of the study species using mostly field data. Chapters 3, 4 and 5 are presented as a series of papers that are either published or under review. These papers are based on experiments conducted throughout my PhD.

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Publications arising from this thesis and statement of candidate contribution

All co-authors have given permission for the work that arose from collaboration to be included in this thesis. For all publications, excluding publication 6, I designed and conducted the experiments, performed the statistical analyses and produced the manuscripts. I benefited from co-authors' advice and assistance with data collection, statistical analyses and writing at various stages of most publications. Publication 6 resulted from the combination of two independent data sets; one collected by Bonnet *et al.* on field-caught adult animals, one collected by myself on neonates in the laboratory. An estimation of the relative contribution to each publication is provided below. The chapter in which each publication appears is provided in brackets.

1. Aubret F (70%), Maumelat S (15%), Bonnet X (10%) and Bradshaw D (5%) 2003. Ecailles et taille des proies. *Pour la science* 311. (**Chapter 2**)
2. Aubret F (100%) (2004). Aquatic locomotion and behaviour in two disjunct populations of Western Australian Tiger snakes, *Notechis ater occidentalis*. *Australian Journal of Zoology* 52: 357-368. (**Chapter 5**).
3. Aubret F (75%), Shine R (20%) and Bonnet X (5%) 2004. Adaptive developmental plasticity in snakes. *Nature* 43: 261-262. (**Chapter 4**).
4. Aubret F (70%), Bonnet X (10%), Maumelat S (10%), Bradshaw D (5%) and Schwaner T (5%) 2004. Diet divergence, jaw size and scale counts in two neighbouring populations of Tiger snakes (*Notechis scutatus*). *Amphibia-Reptilia* 25: 9-17. (**Chapter 2**).
5. Aubret F (60%), Bonnet X (20%), Shine R (15%) and Maumelat S (5%) 2005. Swimming and pregnancy in Tiger snakes, *Notechis scutatus*. *Amphibia-Reptilia*, in press. (**Chapter 5**).
6. Bonnet X (35%), Aubret F (25%), Lourdais O (15%), Ladyman M (15%), Bradshaw D (5%) and Maumelat S (5%) 2005. Do "quiet" places make

animals placid? Island *versus* mainland Tiger snakes. *Ethology*, in press.
(Chapter 3).

7. Aubret F (50%), Bonnet X (40%) and Maumelat S (10%) 2005. Tail amputation, body condition and swimming performances in Tiger snakes, *Notechis scutatus*. *The Journal of Experimental Zoology*, under review. (Chapter 5).
8. Aubret F (70%) and Bonnet X (30%) 2005. State-dependent “decision”: Body reserves, predator avoidance and foraging in immature Tiger snakes. *Oikos*, under review. (Chapter 4).
9. Aubret F (65%), Bonnet X (20%) and Shine R (15%) 2005. A rôle for adaptive plasticity in a major evolutionary transition: early aquatic experience affects locomotor performance of terrestrial snakes. *Evolution*, under review. (Chapter 5).
10. Aubret F (65%), Bonnet X (10%), Maumelat S (10%), Burghardt G (10%) and Bradshaw D (5%) 2005. Feeding preferences in two disjunct populations of Tiger snakes, *Notechis scutatus*. *Behavioral Ecology*, under review. (Chapter 3).
11. Aubret F (65%), Bonnet X (20%), Pearson D (10%) and Shine R (5%) 2005. How can blind Tiger snakes (*Notechis scutatus*) forage successfully? *Functional Ecology*, under review. (Chapter 3).
12. Aubret F (90%), Bonnet X (5%) and Bradshaw D (5%) 2005. Food *versus* risk: foraging decision in young Tiger snakes, *Notechis scutatus*. *The Australian Journal of Zoology*, under review. (Not included).

Chapter 1

Introduction

“Variation, the fuel that feeds evolutionary change, originates at the levels of both the genotype and the phenotype” Stearns (1989)

A. Phenotypic plasticity

Phenotypic plasticity is the property of a given genotype to produce different phenotypes in response to distinct environmental conditions (Stearns 1989). Despite this deceptively-simple definition, the concept of phenotypic plasticity remains very confusing, essentially due to the apparent overlap of two supposedly distinct entities: the genotype and the environment. It is convenient to understand phenotypic plasticity as a general term that covers all types of environmentally-induced phenotypic variations.

An intuitive way of visualising phenotypic plasticity is a norm of reaction:

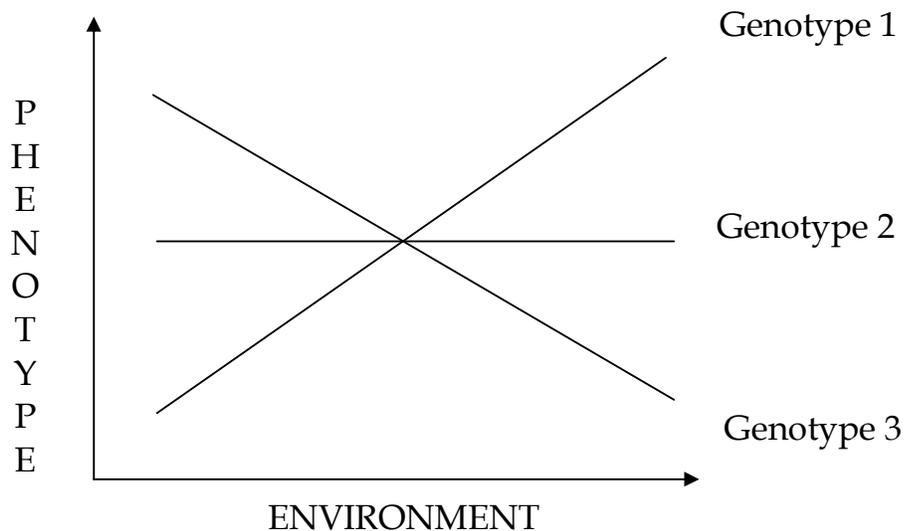


Figure 1: Phenotype as a function of the environment.

The production of a phenotype that varies as a continuous function of the environmental signal in an organism is called a reaction norm (Figure 1; Woltereck 1909). If organisms always produce the same phenotype, regardless of variation in the environment, the relationship is described as environmental canalization (Stearns 1989).

Reaction norms can be inflexible: a characteristic once determined is never changed later in the organism's life. This is the case for phenotypic variation in most life history traits, such as body size or age at maturity. Or they can be flexible: a characteristic can be altered more than once in the development of the same individual (sex changes for instance in a variety of fish, invertebrates, and plants; Charnov 1982; Policansky 1982; Allsop and West 2003).

In some cases, one genotype can produce two or more discrete phenotypes in response to an environmental signal, in which case the situation is called polyphenism. For instance, spring caterpillars of *Nemora arizonaria* feed on oak catkins low in tannin and mimic catkins. Summer caterpillars feed on leaves high in tannins and mimic twigs. The switch between the two morphs is determined by the concentration of tannin in the food (Greene 1989).

Polyphenism may occur in butterflies, rotifers, locusts, and barnacles. An alternation of phenotypes is usually associated with a seasonal alternation of backgrounds (for crypsis) or models (for mimics).

B. Historical overview

The study of phenotypic plasticity can be seen as the modern form of the ancient philosophical debate about the rôles of nature *versus* nurture (Pigliucci 2001). John Locke (1632-1704) suggested that humans are born as blank slates on which the environment writes their character (early representation of the "nurturist" position). On the other hand, Thomas Hobbes (1588-1679) was a pioneer of the application of mechanistic principles to explain human motivation (a forerunner of modern genetic determinism, the "naturist" school).

The first scientific study of genotype/environment interactions began with the introduction of the concept of the reaction norm by Woltereck in 1909. He studied a phenomenon in *Daphnia* known today as cyclomorphosis: when exposed to the presence of a predator, *Daphnia* respond by altering the shape of their body to produce a “helmet” which is effective in reducing predation pressure.

Reaction norms and phenotypic plasticity did not play a prominent role during the neo-Darwinian synthesis of the 1930 s and 1940 s. Between 1930 and 1970, however, many evolutionary biologists commented on the significance of reaction norms. Wright (1932) saw phenotypic plasticity as an agent that uncoupled phenotype from genotype: if organisms were adaptively plastic, they would produce superior phenotypes across a range of environmental conditions and make genetic changes less necessary. On the other hand, Waddington (1953, 1957) suggested that phenotypic plasticity extended the ecological range of a species, exposing it to selection pressures that it would otherwise not encounter and creating the opportunity for genetic assimilation. In other terms, as opposed to Wright’s ideas that plasticity reduces the amount of genetic change in evolution, Waddington saw it as creating the opportunity for more genetic change. Both views were correct but apply to different time scales and different ecological situations.

In 1965, Bradshaw published an influential review that brought research on phenotypic plasticity into the main stage of evolutionary theory. Bradshaw was the first to clearly state two fundamental concepts of plasticity:

–First, plasticity can be a character in its own right, genetically controlled, and it can therefore be under selection.

–Second, plasticity is not a property of an entire genotype and it needs to be studied in reference to specific environments and traits: a given genotype can be plastic for one trait in response to one set of environmental conditions but not to another set, or it can be plastic for some traits but not others in response to the same set of conditions.

Finally in 1985, Via and Lande incorporated the concept of reaction norms within the new framework of evolutionary quantitative genetics. They treated reaction norms as inter-environmental genetic correlations. They considered the expression of one character in an environment as a trait distinct from the expression of the same character in another environment; therefore they could calculate a genetic correlation between the “two” traits and use it as an indicator of the degree to which the environment alters genetic expression.

C. Phenotypic plasticity as an adaptive strategy

One of the most controversial and difficult areas of study of phenotypic plasticity concerns the possibility that plasticity may be an adaptive character directly targeted by natural selection. Simplistically, plasticity may have an evolutionary impact if it affects organismal fitness. But for one thing, the very definition of fitness, and especially its measurement, constitutes one of the recurring nightmares of organismal biologists (see Lande and Arnold 1983; Ollason 1991; Rausher 1992; De Jong 1994; Schluter and Nychka 1994; Burt 1995; Brodie III and Janzen 1996; Bell 1996, 1997). Furthermore, the concept of adaptation and the process of adaptogenesis are among the thorniest concepts in modern evolutionary biology (Lewontin 1978; Gould and Lewontin 1979; Gould and Vrba 1982; Levins and Lewontin 1985; Huberman and Hogg 1986; Gittleman and Kot 1990; Harvey and Purvis 1991; Huynen *et al.* 1996; Reznick and Travis 1996; Seger and Stubblefield 1996; Gavrillets 1997; Gotthard 1998; Orr 1998).

Nonetheless, the putative adaptive value of phenotypic plasticity has recently become a major focus of theoretical and empirical effort by evolutionary biologists (Scheiner 1993; Via *et al.* 1995; Schlichting and Pigliucci 1998; Pigliucci

and Murren 2003). Plastic responses to environmental cues are often assumed to be adaptations that allow organisms to develop phenotypes appropriate to the environments they experience. Yet, despite considerable interest in this topic, there have been few explicit tests of the hypothesis that plasticity is adaptive (Kingsolver 1995, 1996; Schmitt *et al.* 1995; Dudley and Schmitt 1996). To support this hypothesis, it is necessary to demonstrate that the phenotype induced in each relevant environment confers high fitness in that environment relative to alternative phenotypes. Unfortunately, such tests are difficult to perform because plasticity prevents the expression of “inappropriate” phenotypes within each environment. Some authors (Schmitt *et al.* 1999) have successfully used genetic and physiological manipulation to extend the range of phenotypes within environments and thus provide powerful tools for testing the adaptive plasticity hypothesis.

There are cases where reaction norms have no adaptive value: a reaction norm can represent a non-adaptive response to unusual environmental conditions (Schmalhausen 1949). All chemical systems are sensitive to temperature, pressure, pH, and substrate concentration. Any organism not buffered against those environmental variations (during embryogenesis for example) will inevitably be phenotypically plastic.

Gene flow can render a reaction norm maladaptive, for instance when one secondary population is held back by genes evolved for a reaction norm in a different environment within the original population. There can be a phenotypic response to the environment in the second population, but it is a reaction norm determined by genes that have evolved primarily to deal with the original environment (the main population).

Many reaction norms have, however, proved to be adaptive. A change in phenotype that occurs in response to a specific environmental signal and that has a clear functional relationship to the signal may be an adaptation if the relationship results in an improvement in growth, survival, or reproduction

(Stearns 1989). Two main examples of adaptive reaction norms are responses to changes in diet (prey-induced) and in risk (predator- or parasite-induced). For example, the changes in jaw morphology of cichlid fish fed different diets are continuous and can be described as a reversible prey-induced reaction norm (Meyer 1987).

1. Costs and limits of plasticity

There are costs associated with plasticity (De Witt *et al.* 1998):

Maintenance: energetic costs of maintaining sensory and regulatory mechanisms.

Production: excess cost of producing structures plastically (when compared to the same structures produced through fixed genetic responses).

Developmental instability: plasticity may imply reduced canalization of development within each environment, or developmental “imprecision”.

Genetic: deleterious effects of plasticity genes through linkage, pleiotropy, and epistasis with other genes.

and limits to plasticity:

Information reliability: the environmental clues may be unreliable or changing too rapidly.

Lag time: the response may start too late compared with the time schedule of the environmental change, leading to maladaptive plasticity.

Developmental range: plastic genotypes may not be able to express a range of phenotypes equivalent to that typical of a polytypic population of specialists.

Epiphenotype problem: the plastic responses could have evolved very recently and function as an ‘add-on’ to the basic developmental machinery than an integral unit; as such, its performance may be reduced.

2. Evolution of and by phenotypic plasticity (Pigliucci 2001)

The modern view of adaptive plasticity is that phenotypic plasticity can evolve by natural selection as can any trait. Below are summarized the most important modern ideas on phenotypic plasticity (Baldwin 1896; Wright 1932; Goldschmidt 1940; Waddington 1942; Schmalhausen 1949; Matsuda 1982; West-Eberhard 1989; Sultan 1992).

1. Plastic reaction norms may allow a population to persist under temporarily stressful conditions.
2. Plasticity could allow persistence of the population under novel environmental conditions, leaving more time for mutation, recombination, and selection to fine-tune the level of adaptation.
3. Variation among reaction norms in a population may slow down selection (stasis) if the pattern of genotype-environment interactions is such that the reaction norms of different genotypes tend to yield similar phenotypes under the prevailing environmental conditions.
4. Variation among reaction norms in a population may accelerate selection (punctuated evolution) if the environmental range is such that the reaction norms of different genotypes tend to yield highly dissimilar phenotypes.
5. Plasticity may generate phenotypic novelties as a secondary effect of environmental changes, as when a new portion of the reaction norm is exposed to selection.
6. Plasticity at the cellular or molecular level (e.g. enzyme reaction curves) may play a major rôle in the evolution of development, if the internal environment to which cells are exposed changes. This process may have started the evolution of differentiation in multi-cellular organisms.
7. Phenotypic plasticity should probably be considered the default state of organic systems (whole organisms or their components), because of the inherent physical-chemical properties of bio-molecules, which tend to alter their properties when some aspects of their environment change. Any lack of

plasticity (homeostasis) is then to be considered the result of canalizing selection and the derived, presumably adaptive state.

8. Plastic reaction norms can be the target of selection (through their effects on fitness) and yield flexible strategies under the conditions of temporal or spatial heterogeneity.

In conclusion, plasticity can be invoked in explanations of speciation, modifications of life histories, and the appearance of phenotypic novelties. The appearance of phenotypic novelties is a particularly interesting topic as this mechanism could be involved in major evolutionary transitions. One striking example was described by West-Eberhard (1989) and commented upon by Pigliucci (2001):

“This is the case of the behavioural and morphological changes affecting a mutant goat originally described by E. J. Slijper (1942) [...]. The animal was born with very short front legs, but was otherwise normal for all other morphological features. It displayed a remarkable behavioural shift early on by starting to walk in an upright posture. This caused a cascade of morphological alterations during development, which culminated in longer hind legs, a large neck, modified muscle insertions, and an altered shape of the thorax. The point is that if we had found such an animal in the fossil record we would have thought of a complex case of mosaic evolution (Futuyma 1998). Instead, a simple case of behavioural plasticity had catalysed a composite series of morphological reactions through the correlations connecting different aspects of morphological development. It is easy to see how natural selection could rapidly fine-tune such a promising starting point. Perhaps a similar phenomenon guided the very rapid evolution of the cetacean body plan in mammals (Thewissen et al. 1994) or even the origin of terrestrial vertebrates from fish exposed to tidal conditions.”

D. “Reptiles” as models for the study of phenotypic plasticity

The research on phenotypic plasticity in “reptiles” was stimulated by the description of temperature-dependent sex determination in embryos (TSD; e.g. Bull 1980). TSD is widespread in “reptiles” but its adaptive significance remains controversial (Shine 1999). “Reptiles” are excellent models for the study of phenotypic plasticity, notably because they show high degrees of phenotypic plasticity. This is due to their very flexible physiology and the non-fixity of many of their life history traits (for instance growth, age at sexual maturity, reproductive output - Lang 1985; Burger 1989, 1998; Shine and Harlow 1996; Shine *et al.* 1997; Elphick and Shine 1998; Bonnet *et al.* 2001a; Seigel and Ford 2001). An enormous and growing body of publications has documented plastic traits in “reptiles” using a range of taxa (turtles, crocodiles, snakes, lizards) and examined a number of different organismal traits involving physiology (for example evaporative water loss - Kobayashi *et al.* 1983), morphology, locomotor performances, and general behaviour (Bradshaw and Main 1968; Bradshaw 1986; Burger 1989, 1998; Shine and Harlow 1996; Losos *et al.* 2000; Bonnet *et al.* 2001a). For instance, semi-arboreal snakes have shown a propensity to acclimate to higher gravity environment (Conklin *et al.* 1992): acclimated snakes showed higher centrifugal force tolerance, greater increases of heart rate in response to head-up tilt and acceleration, greater increase of blood pressure in response to circulating norepinephrine, lower plasma and whole blood volumes, and greater levels of circulating substances that control blood pressure and flow.

Experimental studies on a wide range of reptiles have shown that the physical conditions experienced by an embryo (mean and variations in incubation temperature - Vinegar *et al.* 1970; Vinegar 1973; Harlow and Grigg 1984; Van Damme *et al.* 1992; Shine *et al.* 1997; Flatt *et al.* 2001; Shine and Elphick 2001; hydric balance - Brown and Shine 2004; Aubret *et al.* 2003, 2005a; Deeming 2004) can substantially affect the size, body condition, behaviour (i.e. anti predator

tactics) or even the physical and physiological performance of the hatchling (Lang 1985; Shine *et al.* 1997; Elphick *et al.* 1998; Shine and Downes 1999; Andrews 2004; Blouin-Demers *et al.* 2004; Deeming 2004; Shine 2004; Aubret *et al.* 2005a).

Regarding plasticity in morphology related to performances, a remarkable study was conducted on *Anolis* lizards by Losos *et al.* (2000). Species of *Anolis* lizards that use broad surface have long legs, which provide enhanced maximal sprint speed. However species that use narrow surfaces have short legs, which permit careful movements. Hatchlings of *Anolis sagrei* were raised in terraria provided with only broad or narrow surfaces. After five months, lizards in the broad treatment had developed relatively longer hindlimbs than lizards in the narrow treatment. By matching phenotype to local conditions, phenotypic plasticity may have played an important rôle in the evolutionary radiation of *Anolis* lizards across habitat types throughout the diverse islands of the Caribbean (Losos *et al.* 1997, 1998, 2000).

E. Study model

Between 6,000 and 10,000 years ago, sea levels were on the rise (Rawlinson 1974; Schwaner 1985). Before this time Tiger snakes presumably formed a continuous population from Queensland through to Western Australia. In southern Australia a broad coastal plain, now submerged, linked the eastern and western populations. When rising sea levels inundated this plain, Tiger snake populations became fragmented. These isolated populations of Tiger snakes are now found in Western Australia, South Australia, Tasmania and on some former mountain tops, which are now offshore islands (Rawlinson 1974).

Tiger snakes belong to the family Elapidae. The current taxonomy of Tiger snakes was not precisely defined until a recent date, but genetic analyses have

brought clarification in the debate (Keogh *et al.* 2005). Below is presented the classification proposed by Wilson and Swan (2003) that describes two species and a number of sub-species. Some authors' views differed slightly (see Wilson and Knowles 1988; Rawlinson 1991; Cogger 2000).

1. *Notechis scutatus scutatus* (Common Tiger snake).
2. *Notechis scutatus occidentalis* (Western Tiger snake).
3. *Notechis ater ater* (Krefft's Tiger snake).
4. *Notechis ater niger* (Peninsula Tiger snake).
5. *Notechis ater seroventyi* (Chappell Island Tiger snake).
6. *Notechis ater humphreysi* (Tasmanian and King Island Tiger snake)

However those classification schemes used body size and colour variation as two of the most important characteristics, but molecular data clearly showed that neither was phylogenetically useful in Tiger snake taxonomy (Keogh *et al.* 2005). Given the extremely small amount of genetic divergence between Tiger snake populations across their range and the extremely short amount of time required for major body size shifts (and presumably colour changes) to evolve (Keogh *et al.* 2005), it can now be soundly stated that Tiger snakes comprise a single polymorphic species, *N. scutatus*, under a phylogenetic species concept (Figure 1).

Tiger snakes display extensive insular body size variation (two-fold difference in body length and five-fold difference in body mass between dwarf and giant populations - see section 2.A.). On the mainland, adult mean total body length is typically 80 to 110 cm, and body mass ranges from 200 to 850 g (Schwaner 1990; Cogger 1992). On Chappell Island however, dependence on a brief glut of fat mutton-bird chicks favours large sizes, commonly exceeding a meter and a half (Schwaner and Sarre 1988; see section 1.E.). Conversely, on Roxby Island off Eyre Peninsula (South Australia) populations of dwarf snakes subsist on small skinks and rarely exceed 0.9 m.

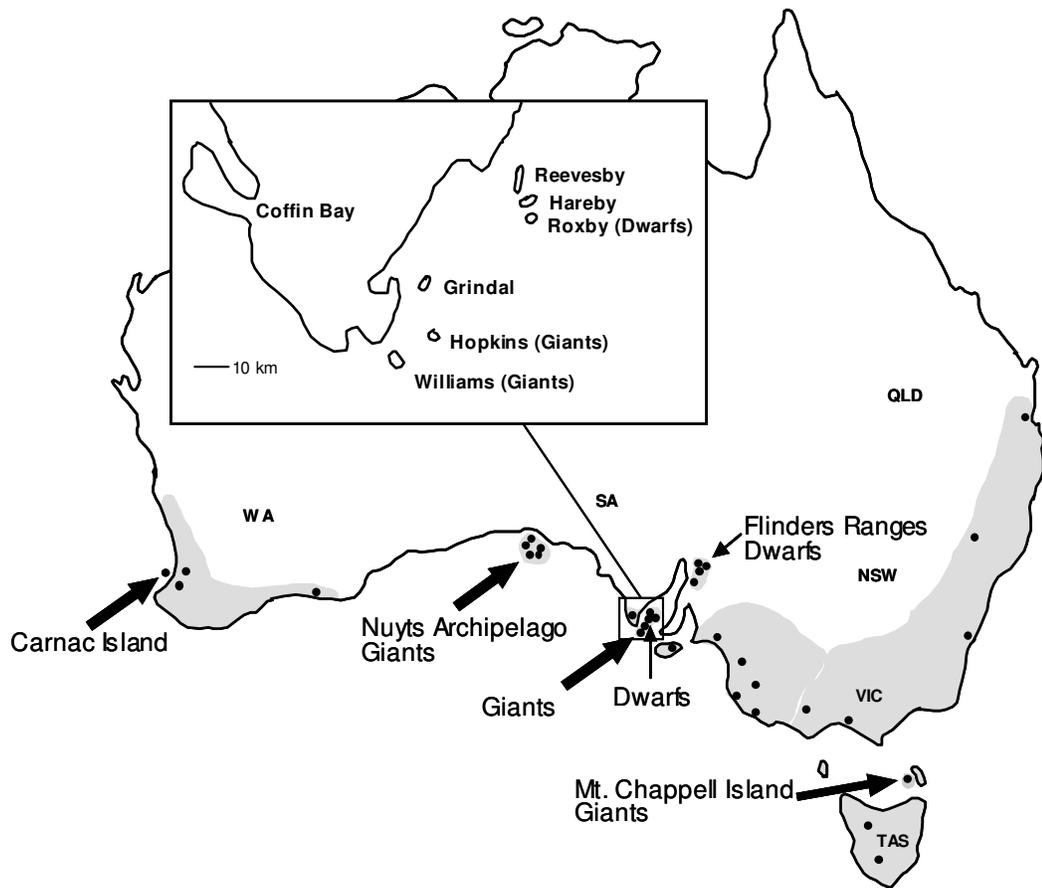


Figure 1: Distribution map of Australian Tiger snakes (Keogh *et al.* 2005). Dots show the populations sampled by Keogh *et al.* (2005). The insert is a blow up of the Sir Joseph Banks Group and the Port Lincoln Island Group in South Australia where islands with dwarfs (Roxby), giants (Hopkins and Williams) or typical mainland sized Tiger snakes (other islands) are found within 30 kilometres of each other.

Molecular studies have revealed a striking example of extremely fast morphological evolution in a large vertebrate. Based on work involving a large mitochondrial DNA data set from individuals across the range and all relevant islands, comprising five mitochondrial genes (cytochrome b, control region, ND4, ND2, 16 s) and 4825 base pairs of data, it seems that island populations

have been separated from their mainland ancestors for no more than 10,000 years (Scott *et al.* 2001; Keogh *et al.* 2005). Yet island dwarfs and giants have evolved five times independently in this time, presumably due to extremely strong selection on available prey items. Secondly, there was surprisingly little molecular divergence, even between widely-separated populations. The maximum overall genetic distance is only 1.4% between Western Australia and eastern populations. Within south-eastern Australia, where populations of island giants, populations of island dwarfs and mainland Tiger snakes all occur, the maximum genetic divergence is only 0.38%. This extraordinary example of fast morphological divergence is best exemplified by the distribution of body sizes and genetic divergence among island populations in the Sir Joseph Banks and the Port Lincoln Island Groups in South Australia. In this region there are islands of giant Tiger snakes that are less than 30km from other island populations with dwarfs and still other islands populated by Tiger snakes of similar adult size to the mainland. The dwarf population on Roxby Island is less than 3km from Tiger snakes of typical mainland size on Hareby Island. The mainland and island populations in this region are extremely closely related (maximum of 0.23% divergence). This strongly suggests that Tiger snakes have been continuous across their range until very recently and support the idea that body size variation has evolved in the last 10,000 years.

F. Scientific relevance

Detailed ecological studies of *Notechis* have shown definite ecological differences between mainland and island populations and also among island populations (Shine 1977, 1978, 1987; Schwaner 1985; Schwaner and Sarre 1990). They suggested that each area (i.e. each island) studied in the past supports a unique population of Tiger snakes in terms of body size, population density, sex ratio, diet, and probably many other features, despite the virtual absence of

genetic variation throughout the whole range of populations from eastern to western Australia (see section 1.E.). Such a situation highlights the potential for comparison between life-history traits of Tiger snake populations. Most notable is the potential to study the effects of phenotypic plasticity on life-history traits over a very wide range of ecological situations.

Some variations in life history traits (notably in body size) have been interpreted by Schwaner (1985) and Shine (1987) as consequences of differences in prey availability. Whether they reflect genetically-based adaptations, or simply direct phenotypic responses to different environmental cues (for instance food supply), remains an open question. For instance, mainland Tiger snakes average 81 cm in snout-vent length *versus* 100 cm in offshore islands of South Australia (Shine 1987). Mainland Tiger snakes are mainly banded whereas island Tiger snakes are predominantly black (presumably to warm up quickly when basking and enhance digestive efficiency of large prey such as large bird chicks). Another important divergence is that mainland Tiger snakes usually live in swamps whilst others survive on waterless islands such as Carnac Island. The diet of mainland Tiger snakes of South Australia (Shine 1987, 1977) remains consistent among the mainland states. Mainland Tiger snakes feed principally on frogs, which constitute from 50-81% of all prey items recorded, whereas the proportion of endotherms is much lower (14-41%). By contrast, island Tiger snakes consume fewer frogs (23%) than endotherms (66%).

The two populations of western Tiger snake that I studied offer an ideal opportunity to investigate marked differences in eco-ethology within a single species. Despite a virtual lack of genetic divergence, field-caught HL and CI Tiger snakes are easily distinguishable. Habitat and prey resources available on Carnac Island differ considerably from those on the mainland whence the snakes originated (Abbott 1978). But in addition to these differing habitats and diet, CI snakes grow larger (Bonnet *et al.* 2002a), have a much lower parasite load (intra gastric - see section 1.G.3.), and display a more placid behaviour (Bonnet *et al.* 2005).

If differences are obvious between field-caught adults from both populations in behaviour and morphology, very little information is available about neonates. Provided that gestation occurred in similar conditions (laboratory) these neonates offer valuable information as they can be considered as “naïve” for a number of traits. Crossed comparison between adults and neonates of both populations was a central tool of my investigations, along with experimental work in controlled environmental conditions (common garden experiments). This allowed direct teasing apart of genetic *versus* environmental influences on a large array of traits (behaviour, locomotion, diet, growth, etc).

G. Study sites

I studied two populations separated by 25km in Western Australia: Carnac Island and Herdsman Lake on the mainland (Figure 1).

1. Carnac Island

Carnac Island is one of the many continental islands forming a dissected chain parallel to the west-coast of Western Australia, from Penguin Island in the south to the Abrolhos archipelago in the north. Carnac Island is one of only two islands in Western Australia that is known to support a population of Tiger snakes (Figure 2), the other being the nearby Garden Island. Carnac Island is a small limestone plateau surrounded by sand beaches (total area of 16 ha), located approximately 12 km off the coast of Fremantle (S 32°07'; E 115°39'). The island was formed some 6,000 years ago by rising sea levels (Seddon 1972). The vegetation of the island was described by Abbott (1978, 1980). The faunal assemblages of Carnac Island have been dynamic and influenced by human activities. Although frogs are the staple diet of Tiger snakes on the mainland

(Shine 1987; Cogger 1992; Bush *et al.* 1995) there are no frogs on Carnac Island, due to the lack of permanent or intermittent water bodies. Hence there are no fresh water sources available except rainfall or dew, and field observations suggest that snakes may suffer from long summer droughts in Perth (Pers. Obs.). A number of snakes seem to migrate to the edges of the island during the drought (February and March) and seek refuge in limestone crevasses that potentially run deep into the island.

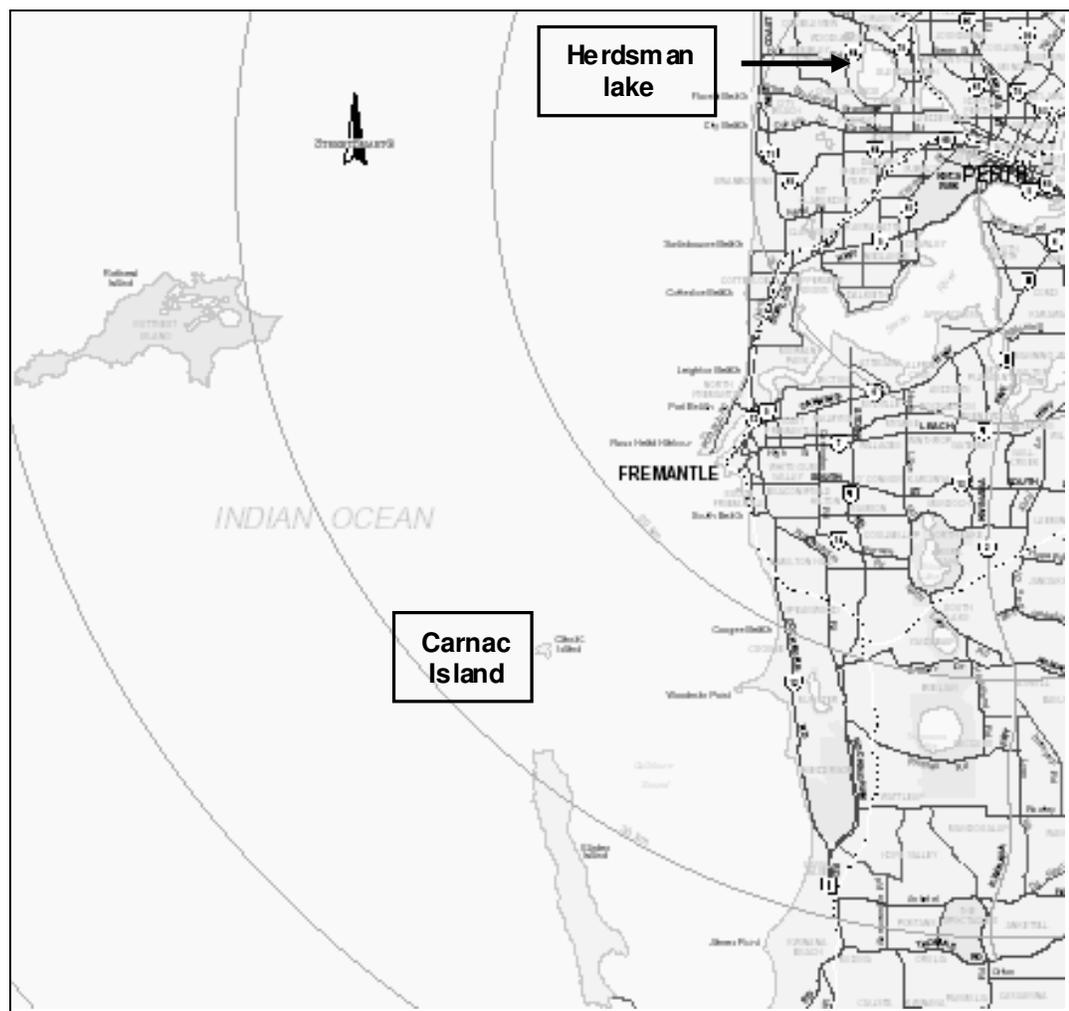


Figure 1: Geographic situation of Carnac Island and Herdsman Lake.

Although several bird species (including pied cormorants *Phalacrocorax varius*, little penguins *Eudyptula minor*, and wedge-tailed shearwaters *Puffinus pacificus*)

nest on Carnac, the silver gull (*Larus novaehollandiae*) is by far the most abundant bird species with an estimated 3000-4000 breeding pairs in 1981 (Lane 1979; Dunlop and Storr 1981). Gull numbers are increasing around Perth in response to the availability of food at domestic rubbish disposal sites (Dunlop and Storr 1981) and thus constitute a significant portion of the island's biomass. Terrestrial vertebrate fauna on Carnac includes the King skink (*Egernia kingii*), the gecko (*Christinus marmoratus*), the small skink (*Morethia obscura*), and the introduced house mouse (*Mus domesticus*). Skinks and mice constitute the major diet components of neonate and juvenile snakes whereas the adults feed mostly on silver gull chicks (83% of the prey; Bonnet *et al.* 1999, 2002) with mice (15%) and lizards (2%) constituting the remainder.

The Carnac Island population of Tiger snakes is estimated to be approximately 300 to 400 adult snakes (Bonnet *et al.* 2002a). Although very small, this island supports a stable population of snakes, and the density recorded is one of the highest reported for sedentary vertebrates (19 to 25 adult snakes per ha; Bonnet *et al.* 2002a). In comparison, Schwaner and Sarre (1988) estimated densities of 4 to 13 snake per ha in other island populations of Tiger snakes in South Australia. In terms of biomass of top predators, the density on Carnac Island represents more than 100 kg of snakes for the 16 ha of the study area using the minimalist estimate of 250 adults weighing on average 400 g each (Bonnet *et al.* 2002a). Carnac Island population is also particular in the fact that some of the snakes are partially or fully blind (Bonnet *et al.* 1999; see section 3.B.).

The origin of Tiger snakes on Carnac Island remains confused. It is possible that the population may be derived, at least in part, from individuals marooned by the rising sea level when Carnac Island was separated from the mainland. Cann (1986) stated that a founder population of some 80 individuals was released on the island around 70 years ago. The Department of Conservation and Land Management (DCLM) indicates that this introduction occurred around 1929. However, it is uncertain whether snakes inhabited the island

before this introduction or not. In either case, CI and HL snakes are undoubtedly closely related to each other.



Figure 2: Adult female Tiger snake basking on Carnac Island.



Figure 3: Checking for reproductive status on a large female Tiger snake on Carnac Island.

2. Herdsman Lake

Around Perth are found a number of fresh water permanent lakes that constitute favourable habitats for Tiger snakes populations (for instance Lake Monger, Lake Joondalup). Herdsman Lake (S 31° 55' 44"; E 115° 48' 19") is located approximately six kilometres north-west of Perth City (Figure 4). This lake is surrounded by houses and is located in a very busy suburb of Perth. A dredged moat encircling the 300 ha lake ensures permanent open water. It is

composed of artificial banks and paths sometimes bordered by trees that create a mosaic of vegetation patches (mostly reeds) and a net of interconnected shallow fresh waters.



Figure 4: A typical view of Herdsman Lake showing Perth central business district in the distance.

The lake is a favourable habitat for numerous frog species (including *Crinia insignifera* and *Litoria moorei*). Long-neck turtles (*Chelodina longicollis*), feral cats (*Felix catus*), house-mice (*Mus domesticus*) also occur. The lake is also an important breeding site for many species of waterbird: Blue-billed Duck (*Oxyura australis*), Musk Duck (*Biziura lobata*), Black Swan (*Cygnus atratus*), Australasian Shoveller (*Anas rhynchos*), Pink-eared Duck (*Malacorhynchus membranaceus*), Great Crested Grebe (*Podiceps cristatus*), Nankeen Night Heron (*Nycticorax caledonicus*), Yellow-billed Spoonbill (*Platalea flavipes*), Swamp Harrier (*Circus approximans*), Short-billed Black-Cockatoo (*Calyptorhynchus latirostris*), White-cheeked Honeyeater (*Phylidonyris nigra*) and Clamorous Reed-warbler (*Acrocephalus stentoreus*). Although the largest snakes in HL could potentially eat the chicks of ground-nesting ducks and grebes (there are no records however), frogs and mice are far the more accessible prey and

constitute their main diet with, respectively, 53% and 47% of all prey items (see section 2.E. and Figure 5).



Figure 5: Foraging behaviour in an adult female Tiger snake from Herdsman Lake. The snake was closely observed for 20 minutes actively browsing the substrate with its snout.

3. Field and laboratory methods

Field work: the following information was recorded for each captured snake: snout-vent length (SVL: from the tip of the snout to the sub-cloacal scale; ± 0.5 cm), body length (BL: from the tip of the snout to the tip of the tail; ± 0.5 cm), body mass (BM: using a digital scale; ± 1 g in adults; ± 0.1 g in small snakes of less than 100g), sex (eversion of the hemipenes), presence and type of prey ingested (palpation), reproductive status (enlarged follicles or embryos are detectable by palpation) and presence of body or head injuries (scars). Head dimensions were measured to the nearest 0.1 mm using a digital calliper. These included jaw length (JL: from the tip of the snout to the quadrato-articular), head width (HW: maximal width above the supra-oculars), skull length (SL: from the tip of the snout to the base of the skull), eye diameter, inter-nostril gap, and fang length. A body condition index (BCI) was calculated for each snake as the residual values of the linear regression of Log BM against Log SVL. The

condition of the tail was recorded (intact, missing part of or all of), ventral scales counted, and sloughing status noted. On capture, the behaviour of the snake was also recorded (basking, coiled, moving, feeding, sexually behaving, etc). Time and weather conditions were also recorded at the time of capture. As a means of permanent identification, each snake was individually scale clipped.

Laboratory work: all experiments were performed in a controlled temperature room within the animal yards (School of Animal Biology, UWA). The temperature was set at 27°C during the day and allowed to drop with outside temperature at night to partially follow natural variation. Basking opportunities were provided using several high pressure Na+ lamps (600 W) for 15 minutes three times a day. Large animals were housed in individual plastic boxes (50 x 40 x 30 cm) containing shelter, water dish and wood shavings as substratum. Neonates were housed similarly, but in smaller plastic boxes (20 x 15 x 5 cm). Upon arrival in the laboratory colony, and every 6 months thereafter, snakes were individually treated against worms (nematodes; see section 1.G.4.) with Panacur (100 mg.kg⁻¹; injected in snake food prior to feeding). All snakes were given a final treatment against worms before being released. All captive animals (adult, juveniles, and neonates) were fed with dead mice, purchased from the Animal Care Unit of UWA, and dead chicks purchased from Altona Hatchery (Forrestfield, Western Australia). Snakes that were temporarily not involved in experiments were kept in three outdoor pits under natural conditions. Pits were two meters in diameter and one meter deep, with a concrete base and mesh lid to prevent escapes. Snakes were provided with several basking rocks, aerial and underground shelters and a large water container (that allowed complete immersion).

4. Parasitism

Elapid snakes in Western Australia are commonly infested by nematodes

Ophidascaris pyrrhus (Ascaridoidea). The highest prevalence of infection and the largest worms are found in the south-west part of the state, and the epidemiological evidence presented suggests that amphibians and reptiles may be intermediate hosts (Jones 1978, 1980).

Jones (1978, 1980) found *O. pyrrhus* in the following species: *Notechis ater*, *Pseudonaja nuchalis*, *P. textilis*, *P. modesta*, *Echiopsis curta* and *Denisonia punctata*.

The worms were found in the oesophagus and stomach, usually threaded through the stomach mucosa so that both ends of the worm protruded into the lumen. Worm numbers varied from one to 35 per snake. *Notechis ater* was the most heavily infected snake species, and sometimes presented concurrent infection by *O. pyrrhus* and *Abbreviata spp.* (Nematoda: Physalopteroidea).

In North American colubrid snakes, frogs are the intermediate hosts for *Ophidascaris labiatapapillosa* (Walton 1937; Ash and Beaver 1962, 1963). Rodents and small marsupials are experimental intermediate hosts for *O. moreliae* from Australian pythons (Sprent 1970). *Notechis ater* feeds mostly on frogs and small mammals (Cogger 1975). It is very likely that frogs would be an intermediate host for *Notechis* in Western Australia. Notably, the Tiger snakes from Carnac Island are exempt of worms where no frogs occur. However, laboratory observations showed that CI snakes could get worms when in contact with infested snakes and materials.

Tiger snakes from Herdsman Lake are commonly infested with nematodes. Fifteen snakes died in captivity and were subsequently dissected (10 from CI and 5 from HL). All of the Herdsman Lake snakes were infested with worms and three out of five CI snakes. Among 8 individuals that were infected, parasite load averaged $1.04 \pm 1.06\%$ of body mass (most worms were located in the stomach). In one case, parasite load reached 3.21% of the snake's body mass. Worms' maximum length was 10 cm for a maximum diameter of 1 mm. In several cases, infestation was generalized, affecting the lungs, liver, fat storages, underneath the skin, trachea, heart, digestive track, and even eggs and embryos. The action of the worms was always destructive and may likely be responsible for the death of the snakes.

Chapter 2

Morphology

Aims - In this Chapter are described the patterns of body size evolution in animals, with special emphasis on island populations. Such patterns potentially apply to a wide range of taxa, including snakes. Tiger snakes are ideally suited to investigate the causes of insular body variations. As previously mentioned in Chapter 1, Tiger snake populations are often isolated (notably on islands), occur in a wide range of ecological conditions (including diet), and display extensive body size variation (potentially *via* phenotypic plasticity). In this chapter I demonstrate that these differences exist between the CI and HL populations of Tiger snakes. I present data on morphology in adults and neonates from both populations, along with a description of their respective reproductive outputs in an attempt to link those traits with their respective diet (quality and quantity of prey). This chapter, along with Chapter 1, provide the background information necessary for the experimental work described in Chapters 3, 4, and 5.

A. Body size evolution in animals and evidence from island populations.

Body size is a conspicuous trait that is strongly associated with other life history traits of an organism such as age at first reproduction, reproductive output, and survival (Tinkle 1962; Ankney and Mac-Innes 1978; Blueweiss *et al.* 1978; Stearns 1983; Charnov 1993; Shine 1994). Body size is also a characteristic that tends to change most dramatically between islands and mainland conspecific populations, and many islands are known to support populations of giant or dwarf forms of their mainland conspecifics (Case 1978). Taking advantage of those systems of island and mainland populations, some authors have attempted to define an optimal body size in animals (Brown *et al.* 1993; Brown 1995). In mammals for example, three statistical approaches (Lomolino 1985; Brown and Maurer 1989; Marquet and Tarper 1998) have been developed to predict optimal body size in a given fauna assemblage. According to those models, mammals with body sizes larger than 100 g on the mainland tend towards dwarfism on islands, whereas those smaller than 100 g tend towards gigantism on islands. Similar patterns were found in fish, molluscs, and insects (Morse *et al.* 1988; Blackburn and Gaston 1994; Marquet and Taper 1998; Maurer 1998; Roy *et al.* 2000) suggesting that such patterns may lead to a common evolutionary theory that could apply to many types of organisms.

Islands are of special interest to ecologists and evolutionary biologists because of the rapid shifts possible in island taxa with small and discrete populations, living under conditions (and selective pressures) often very different from those experienced by their mainland conspecifics (Gorman 1968; Fitch 1976; Case 1978; Dunham *et al.* 1978; Gotelli and Graves 1990; Losos 1995; see sections 1.E. and 1.F.). The most common explanations for shifts in island body size are the relaxation of predation, competition pressures and genetic drift, but the evolution of body size in general and body size shifts specifically is a complex and controversial issue because there are a number of other non-mutually

exclusive explanations for the patterns observed in nature (Case 1978; Barton 1996; Whittaker 1998; Schluter 2001). For example, in the lizard *Anolis* morphological shifts can occur extremely rapidly (Losos *et al.* 1997) and repeatedly (Losos *et al.* 1998) as the lizards adapt to new environments with the help of phenotypically plastic traits (Losos *et al.* 2000). In marine iguanas the differing food energy levels available on different islands are important in determining adult body size (Wikelski and Trillmich 1997) but sexual selection in the form of sexual size dimorphism also is involved in complex ways in this (Wikelski *et al.* 1997) and other species (Madsen and Shine 1992). Head size of island populations of European adders appears to be determined by the size of available prey items (Forsman 1991), while relaxation of predation pressure as opposed to simple retention of an ancestral condition (phylogenetic constraint) seems to have selected for large size in chuckwalla lizards (Petren and Case 1997).

In snakes, three patterns of body size variation were identified that indicate an optimal length of one metre (Boback 2003). Interestingly, those patterns were concordant with those found in mammals despite the fact that snakes and mammals exhibit very different features (ectothermy, gape-limited predators, elongated body shape). Firstly, according to Boback (2003), a distribution of largest body length of 618 snake species had a single mode at 1.0 m. Secondly, there is a positive relationship between the size of the largest member of an island snake assemblage and island area, and a negative relationship between the size of the smallest member of an island snake assemblage and island area (the presumed optimal size for an one-species island being 1.0 m). Thirdly, mainland snake species smaller than 1.0 m become larger on islands, whereas those larger than 1.0 m become smaller on islands. There are a number of factors that can potentially explain body size differences in island snake populations (island area, island age, distance to mainland and latitude). However, the selective response to local prey availability could explain the difference in body size of snake populations on islands relative to mainland populations (Barnett and Schwaner 1985; Forsman 1991; Madsen and Shine

1993). Decreased average prey size on some islands is associated with increased abundance of squamates (relatively smaller prey) and decreased abundance of birds and mammals (relatively large prey - Case 1978; Hasegawa and Moriguchi 1989). Other islands may exhibit increased average prey size because of increased availability of seabird chicks (Schwaner and Sarre 1988; Kohno and Ota 1991; Mori 1994). Most snake populations that became giant are known to consume sea-birds chicks (Boback 2003). For instance, the seasonality of large prey, such as seabird chicks, may have been a major selective pressure for a larger body size in the elapid *Notechis ater* because the larger snake are better able to consume large, fast-growing chicks (Schwaner and Sarre 1988).

The Australian Tiger snakes populations provide an excellent example of body size variation as a result of geographic isolation (see section 1.E and 1.F.; Keogh *et al.* 2005). Adult mainland Tiger snakes typically reach sizes of approximately 78-92 cm snout-vent length (Shine 1987; Schwaner and Sarre 1990). Some offshore islands are also populated by Tiger snakes that reach a similar average adult size to those on the mainland, but other islands are home to populations that exhibit extreme shifts in adult body size relative to their mainland counterparts (see section 1.E. Figure 1). Roxby Island is populated only by dwarfs that reach an average of approximately 70 cm SVL and weigh less than 200 g while Mount Chappell Island is populated by giants that reach an average of approximately 120 cm but can range up to 160 cm SVL and well over 1kg (Schwaner 1985; Shine 1987; Schwaner and Sarre 1988, 1990). Giant Tiger snakes of a similar size also are found on the islands of the Nuyts Archipelago in the Great Australian Bight and on Hopkins and Williams Island in the Port Lincoln and Neptune Island groups in South Australia (Schwaner 1985; Robinson *et al.* 1996). With the exception of one highly isolated population of dwarfs in the Flinders Ranges of South Australia, all mainland Tiger snake populations that have been examined are intermediate in adult body size to the island populations that display extreme body size shifts. A number of hypotheses have been suggested to explain body size shifts in island Tiger snakes. Natural selection acting to optimise snake body size to available prey

size is supported strongly by the compelling correlation between snake size and available prey size (Schwaner 1985; Shine 1987; Schwaner and Sarre 1988, 1990) but other possibilities exist. Island Tiger snakes display no obvious male-male competition and they are the top predator on all the islands on which they occur, and so competition, predation and sexual selection already have been rejected as possible explanations (Schwaner 1985; Schwaner and Sarre 1988, 1990). Phenotypic plasticity was discounted early as a possible explanation of this phenomenon in Tiger snakes as growth rate differences between dwarfs and giants seemed to be genetically based (Barnett and Schwaner 1984; Schwaner 1985) but a recent common garden experiment has demonstrated that both plasticity and genetic history act to influence body size in island Tiger snakes (Aubret *et al.* 2004a).

Example of insular gigantism in a population of Tiger snakes: *Notechis ater seroventyi* from Chappell Island (Schwaner and Sarre 1988).

Chappell Island (325 ha total area), in the Furneaux group of islands, Bass strait, supports a dense population of Tiger snakes reported to attain body size of 1.5 m (Worrell 1957, 1963). Adult snakes survive by eating young mutton birds (*Puffinus tenuirostris*) which, because of a precise breeding cycle and rapid growth of the chicks (Naarding 1980), are available as prey for only a few weeks each year. The population size was estimated at 1414 snakes (density of 13.3 snakes per ha). Food includes rats (*Rattus rattus*), mice (*Mus domesticus*), young of the Cape Barren goose (*Cereopsis novaehollandia*) and the mutton bird (*Puffinus tenuirostris*). Only snakes larger than 70 cm are able to feed on mutton bird chicks. Tiger snakes mature at about 100 cm in snout-vent length on Chappell Island (Barnett and Schwaner 1984). The smallest captured gravid snake measured 103 cm in SVL. Tail injuries were rare (only 59 out of 256 snakes) and reflect the potentially null predation for adults on the island. Snakes are very placid (Worrell 1957) and male-male combats were never observed (Schwaner 1985, Schwaner and Sarre 1988) on Chappell Island, nor on other populations

on off-shores islands studied (Schwaner 1985). Hence, it seems that food might be the only factor that can explain gigantism pattern on Chappell Island. Mutton bird chicks are a saturating resource (density of 36 chicks per snake). The selective advantage of larger body size is increased clutch size and more frequent reproduction (in females), facilitated by greater fat storage capacity for surviving long periods of fasting (due to the seasonality of food intake). Adult snakes on Chappell Island eat an abundant, high-quality resource that requires little searching time to locate and consume.

B. Morphology in adult Tiger snakes

All individuals larger than 66 cm in SVL were considered as being adult, as this length corresponds to the smallest pregnant females found on both CI and at HL. Males were assumed to be sexually mature at a similar body size.

Carnac Island snakes reach larger body size than HL individuals, almost double in BM and 11% more in SVL (Table 1). CI adult snakes are in better body condition compared with HL snakes and have larger heads (Table 1; see sections 2.E. and 4.B.). In order to avoid allometry effects (the proportions head - body size vary with body length in snakes - Queral-Regil and King 1998), I restricted the following analysis to similar-sized animals (SVL < 122 cm; which equals the largest snake captured in HL). Head parameters were adjusted for SVL to compensate for potential differences in the distribution (i.e. more animals in the upper size range in CI than in HL). For a given body size, CI adult snakes head parameters remain significantly larger: in skull length ($F_{1,507}=40.53$; $P<0.001$); jaw length ($F_{1,545}=39.72$; $P<0.001$); head width ($F_{1,513}=5.23$; $P<0.023$); eye diameter ($F_{1,383}=10.07$; $P<0.002$); inter-nostril gap ($F_{1,359}=36.80$; $P<0.001$); fang length ($F_{1,361}=7.67$; $P<0.006$). Tail injuries were very common in HL snakes: 117 out of 207 snakes were injured in HL *versus* only 12 out of 658 CI

snakes (Pearson $\chi^2_{(df=1)} = 371.26$; $P < 0.001$). The exact causes of such injuries remain unclear (see section 5.A.).

Table 1: Morphological comparison between adult snakes from CI and HL.

Mean values \pm Standard Deviations (here after SD) are given. P values were obtained with ANOVAs. * Pregnant females were withdrawn from the analysis. ** animals with partial tails were excluded from the analysis. A body condition index was calculated as the residual values of the linear regression of Log BM against Log SVL.

Adults	Carnac Island	Herdsmen Lake	DI; F	P
Body mass (g)*	452.10 \pm 129.33	243.10 \pm 67.10	1, 650; 468.06	0.001
Snout-vent length (cm)	89.52 \pm 9.29	79.05 \pm 7.45	1, 651; 171.09	0.001
Total length (cm)**	104.20 \pm 11.16	89.05 \pm 7.93	1, 579; 115.70	0.001
Body condition*	0.024 \pm 0.076	-0.079 \pm 0.066	1, 650; 225.80	0.001
Skull length (mm)	27.03 \pm 2.34	24.22 \pm 1.71	1, 551; 187.98	0.001
Jaw length (mm)	33.03 \pm 3.17	29.06 \pm 2.32	1, 600; 205.77	0.001
Head width (mm)	12.76 \pm 1.04	11.79 \pm 1.01	1, 553; 97.70	0.001
Fang length(mm)	4.69 \pm 0.54	4.09 \pm 0.41	1, 385; 104.84	0.001
Eye diameter (mm)	4.74 \pm 0.52	4.31 \pm 0.36	1, 408; 72.53	0.001
Inter nostril gap (mm)	7.99 \pm 0.73	7.00 \pm 0.57	1, 404; 197.77	0.001

Sexual dimorphism - adult males were larger than females in both populations. Carnac Island males reached 486.58 \pm 124.05 g and 92.90 \pm 8.27 cm in SVL *versus* 371.64 \pm 103.02 g and 81.56 \pm 6.31 cm in females (respectively $F_{1,495}=93.99$; $P < 0.001$; $F_{1,495}=226.38$; $P < 0.001$). Females were however in better body condition than males (respectively 0.016 \pm 0.089 *versus* -0.0063 \pm 0.065; $F_{1,495}=9.64$; $P < 0.002$). Similarly, HL males reached 257.21 \pm 68.97 g in BM and 81.31 \pm 7.11 cm in SVL *versus* 207.01 \pm 45.75 g and 73.05 \pm 4.48 cm in SVL in females (respectively $F_{1,150}=19.56$; $P < 0.001$; $F_{1,150}=49.90$; $P < 0.001$). However, females were not in significantly better condition than males (respectively 0.016 \pm 0.080 *versus* -0.0061 \pm 0.054; $F_{1,150}=3.79$; $P = 0.061$) potentially reflecting the lower food supplies at the lake compared with CI.

C. Morphology in neonate Tiger snakes

Data were gathered from 11 pregnant females from Carnac Island and 14 from Herdsman Lake in 2003 (see section 2.E. for details on females housing and parturition). The analysis was based on 156 live neonates from CI (86 males and 70 females) and 184 live neonates from HL (101 males and 83 females).

Because there was very little dimorphism between males and females in CI and HL populations at birth (see below), males and females were pooled for analysis. Any dimorphism would anyway be balanced between both populations as the proportions males / females are similar for CI (52.9% of males out of 121 neonates) and for HL (52.7% of males out of 129 neonates). Results are presented in the table 1.

Table 1: Morphological comparison between CI and HL neonates.

P values were obtained with Nested ANOVAs to account for potential maternal effect, with the factor “litter” nested into the factor “population”. Adjusted mean values \pm SD are given. Body condition is BM relative to SVL.

Traits	Carnac Island	Herdsman Lake	DI; F	P
Body mass (g)	4.82 \pm 1.15	5.03 \pm 0.73	1, 230; 4.03	0.046
Body condition	4.88 \pm 1.15	5.00 \pm 0.73	1, 226; 3.26	0.07
Snout-vent length (cm)	17.56 \pm 1.72	17.70 \pm 1.26	1, 227; 0.72	0.40
Skull length (mm)	12.40 \pm 0.5	12.11 \pm 0.40	1, 227; 15.43	0.001
Jaw length (mm)	13.90 \pm 0.6	13.73 \pm 0.51	1, 226; 6.98	0.009
Head width (mm)	5.65 \pm 0.32	5.61 \pm 0.27	1, 227; 0.93	0.33
Eye diameter (mm)	2.45 \pm 0.20	2.42 \pm 0.15	1, 226; 1.16	0.28
Inter nostril (mm)	3.45 \pm 0.22	3.40 \pm 0.22	1, 225; 3.10	0.08
Ventral scales	160.08 \pm 4.19	155.91 \pm 4.98	1, 44; 13.47	0.007
Infra labials	7.05 \pm 0.49	6.63 \pm 0.64	1, 109; 8.24	0.005
Supra labials	6.28 \pm 0.46	5.71 \pm 0.63	1, 109; 17.22	0.001
Body rows	18.26 \pm 0.88	17.22 \pm 0.53	1, 109; 31.30	0.001

Herdsman Lake neonates were heavier at birth and almost significantly in better condition than CI neonates. CI neonates had higher values for skull and jaw length (see section 4.B. for a discussion) and these differences remain significant if weighed by SVL for jaw length ($F_{1,224}=13.06$; $P<0.0004$) and by head width for skull length ($F_{1,226}=13.62$; $P<0.0003$).

Neonates were not fed during the first month post birth, while body size was recorded one week and one month after birth. Repeated measures ANOVA were conducted with population of origin as factor and the successive measures of body mass as the repeated variable. For body mass, the analysis yielded Wilks' Lambda=0.31; $P<0.0001$; interaction term $F_{2,156}=5.10$; $P<0.0072$: CI neonates lost more weight over the first month than HL neonates (in average 11.9% in HL *versus* 10.7% in CI). However, CI neonates did not grow as much in SVL over the same period: snout-vent length significantly increased over time (Wilks' Lambda=0.30; $P<0.0001$; effect of time $F_{2,154}=186.92$; $P<0.001$) and significantly more in HL neonates (effect of origin $F_{1,77}=156.24$; $P<0.0001$; 5.7% increase in HL *versus* 5.4% in CI neonates).

Sexual dimorphism - CI males and females exhibited similar body mass

($P=0.50$) but males were longer than females in snout-vent length (respectively 18.30 ± 1.94 cm *versus* 17.55 ± 2.14 cm; $F_{1,153}=5.15$; $P<0.025$). Female neonates were in better body condition than males (SVL relative to body mass; $F_{1,152}=6.16$; $P<0.014$). There was no significant difference between sexes in the change of body mass and snout-vent length over the first month (both $P>0.45$). Similar results were found in HL neonates between males and females: males and females exhibited similar body mass ($P=0.95$) at birth but females were shorter in snout-vent length (respectively 17.38 ± 1.35 *versus* 17.85 ± 1.34 cm; $F_{1,181}=5.58$; $P<0.002$). Again, females were in better condition than males (respectively 4.91 ± 1.35 *versus* 4.68 ± 0.86 ; $F_{1,180}=9.85$; $P<0.002$). Finally, the changes of body mass and snout-vent length over the first month were similar between males and females (both $P>0.49$).

Dissections

A number of stillborn animals was obtained. They were kept in a freezer and dissected subsequently. Results are presented in the table 2.

Table 2: Internal composition in CI and HL neonates.

* Relative to SVL. Body condition is BM relative to SVL. Adjusted means \pm SD are presented.

Traits	Carnac Island	Herdsmen Lake	Df; F	P
Body mass (g)	4.52 \pm 1.38	4.18 \pm 0.99	1, 138; 2.18	0.14
Body condition	4.34 \pm 1.39	4.37 \pm 0.99	1, 135; 0.03	0.85
Snout-vent length (cm)	16.89 \pm 1.92	16.23 \pm 1.73	1, 136; 3.75	0.055
Heart mass (g)*	0.039 \pm 0.02	0.038 \pm 0.02	1, 137; 0.13	0.72
Liver mass (g)*	0.19 \pm 0.08	0.14 \pm 0.06	1, 134; 18.36	0.001
Anterior fat body (g)*	0.023 \pm 0.01	0.016 \pm 0.01	1, 131; 7.34	0.008
Posterior fat body (g)*	0.19 \pm 0.08	0.15 \pm 0.09	1, 133; 7.92	0.006
Left kidney (g)*	0.020 \pm 0.01	0.017 \pm 0.01	1, 122; 4.60	0.033
Right kidney (g)*	0.021 \pm 0.01	0.018 \pm 0.01	1, 118; 4.36	0.039
Digestive track (g)*	0.29 \pm 0.12	0.31 \pm 0.16	1, 135; 1.38	0.24
Body skin (g)*	0.91 \pm 0.28	0.93 \pm 0.25	1, 120; 0.15	0.69
Body muscles (g)*	1.56 \pm 0.58	1.63 \pm 0.48	1, 131; 1.49	0.22
Yolk (g)*	0.23 \pm 0.21	0.16 \pm 0.10	1, 64; 1.39	0.24

Yolk presence was observed in 56% of CI neonates (54 out of 97) *versus* in only 27% of HL neonates (12 out of 44) and the difference is significant (Pearson $\chi^2_{(df=1)}=9.80$; $P<0.002$).

In CI neonates, regarding internal morphology, no significant difference was found between sexes for any of the traits measured apart from heart mass relative to SVL (larger in females; 0.045 \pm 0.017 g *versus* 0.038 \pm 0.015 g; $F_{1,79}=5.62$; $P<0.021$). In HL neonates, no sexual dimorphism was found in internal composition for any traits.

D. Reproductive output

All pregnant females were field-caught in early summer 2002 and 2003. They were individually housed in the laboratory, until parturition. Housing conditions were identical both years (2002 and 2003). Parturition occurred between March and May in both years. Neonates were weighed and measured rapidly after birth (standard measurements of the body and the head), sexed and housed individually in the same conditions as their mothers (see Bonnet *et al.* 2005 for details of housing conditions). In many cases, the neonates were still attached to their annexes, allowing the measurement of the matching egg components (remaining yolk, placenta and embryonic annexes). Results are presented in the Table 1.

Table 1: Description of pregnant females and their litters in 2002 and 2003.

Relative Clutch Mass is (clutch mass / post-parturient body mass)×100. Body condition is body mass relative to snout-vent length.

Traits	Carnac Island		Herdsman Lake	
	2002 (N=7)	2003 (N=11)	2002 (N=12)	2003 (N=14)
Snout-vent length (cm)	84.00±4.45	80.81±5.67	72.5±4.87	73.78±2.47
Body mass (g) pre-parturition	428.90±102.72	418.85±82.24	235.48±54.58	270.41±50.30
Body condition pre-parturition	360.76±102.72	384.83±82.24	294.77±54.58	313.28±50.30
Body mass (g) post-parturition	322.56±60.98	282.10±56.14	186.97±45.57	202.19±32.62
Body condition post-parturition	273.72±60.98	258.22±56.14	228.38±45.57	233.51±32.62
Relative clutch mass (%)	27.58±8.70	33.69±14.15	20.47±7.67	26.80±10.41
% of males per litter	72.40±20.70	49.55±9.02	61.20±19.60	54.05±20.21
Total number of males	39	89	51	72
Total number of females	20	82	30	67

1. Litter sex ratio

The percentage of males per litter varied from 2002 to 2003 in both Carnac Island and Herdsman Lake populations. For example, in CI snakes, the sex ratio (all litter pooled; sex ratio = number of males divided by the number of females) was biased towards males in 2002 (1.95) but not as much in 2003 (1.08); the difference between years being close to the conventional level of significance ($\chi^2_{(df=1)}=3.51$; $P<0.061$). In HL snakes, similar results were obtained in 2002 (sex ratio of 1.7) and 2003 (1.07; $\chi^2_{(df=1)}=2.59$; $P=0.11$). In both years however, there was no significant difference between the two populations in sex ratio at birth (both $P>0.70$).

2. Litter description

With both years and both populations pooled, remaining egg mass was strongly correlated with neonates' body mass at birth (Spearman's rank correlations $N=147$; $R=-0.30$; $P<0.001$), reflecting the relationship between the amount of egg yolk assimilated by the embryo and its body mass at birth (Tracy 1982; Sinervo 1990; Aubret *et al.* 2005a). Female snout-vent length was strongly correlated with litter size ($N=44$; $R=0.49$; $P<0.001$) and litter mass ($N=44$; $R=0.55$; $P<0.001$), reflecting trade offs between space available to carry more eggs in females' bodies and litter size: selection for fecundity favours larger body size in most snakes (Bonnet *et al.* 1998). Litter mass and litter size were also strongly linked to each other ($N=44$; $R=0.89$; $P<0.001$).

In order to perform further analyses, I calculated an average body mass and average snout-vent length for each different litter, based on fully developed neonates (live and stillborn). In this case, there was no significant correlation between the average neonate's body mass and litter size ($N=43$; $R=0.05$; $P=0.75$) or litter mass ($N=43$; $R=0.27$; $P=0.08$); or between the average neonate's snout-vent length and litter size ($N=43$; $R=0.08$; $P=0.60$) or litter mass ($N=43$; $R=0.23$;

P=0.12). These results show that, independently of the amount of energy available for the production of eggs, females produce relatively standardised eggs and subsequent neonates.

Table 2 shows that in 2003, HL females managed to convert, on average, 10.86 eggs into 9.21 live neonates (84.8%); whereas from 20.91 eggs, CI females only managed to produce 11.18 live neonates (53.8%). The rates of conversion were significantly different between CI and HL (Repeated measures ANOVA, with origin as factor, and litter size and number of live neonates as the two repeated measures; Wilks' Lambda=0.40; P<0.001; interaction term $F_{1,23}=19.81$; P<0.001). Similarly, the conversion rates of litter mass into live material (neonates) largely differed between HL and CI females (Same design ANOVA; Wilks' Lambda=0.70; P<0.020; interaction term $F_{1,23}=8.20$; P<0.009; respectively 25.0% *versus* 19.5%). In 2003, HL females produced on average half as many young as CI females. Despite almost identical relative clutch mass in 2003 (see section 2.D. Table 1; $F_{1,23}=1.97$; P=0.17), HL females produced heavier babies (section 2.C.) and in better body condition than did CI females.

Table 2: Litter description in 2002 and 2003.

Traits	Carnac Island		Herdsman Lake	
	2002	2003	2002	2003
Litter size	15.00±3.70	20.91±7.24	10.41±3.06	10.86±4.15
Litter mass (g)	91.46±40.24	90.12±35.26	37.57±14.67	53.98±22.22
Non-fecund eggs	2.57±2.29	1.55±1.97	1.58±2.35	0.28±0.61
Fecund eggs	2.14±2.26	1.91±2.88	1.33±1.50	0.14±0.36
Aborted eggs	1.28±1.60	1.27±1.55	0.50±0.79	0.36±0.50
Stillborns	3.00±1.91	4.82±6.69	2.08±1.93	0.86±1.70
Live born	5.14±7.01	11.18±8.01	4.58±4.29	9.21±5.52
Malformed neonates	1.43±1.72	0.64±1.21	1.25±2.52	0.92±1.21

3. Inter-population comparison

Litter size varied with year and origin (Two-ways ANOVA with origin and year as factors, and litter size as variable; interaction term $F_{1,40}=3.30$; $P=0.08$).

There was a significant effect of year: litter size averaged 12.10 ± 3.92 in 2002 *versus* 15.28 ± 7.55 in 2003 ($F_{1,40}=4.45$; $P<0.042$); and a significant effect of origin: 10.65 ± 3.62 eggs in HL females *versus* 18.61 ± 6.67 eggs in CI females ($F_{1,40}=23.62$; $P<0.0001$).

The percentage of fertile eggs per litter did not vary with year or origin (all $P>0.18$), nor did the occurrence of malformed neonates in litters (all $P>0.20$), stillborns (all $P>0.46$), or aborted eggs (all $P>0.12$). However, the proportion of live neonates per litter increased from 2002 to 2003 with $37.41\pm 37.11\%$ in 2002 *versus* $64.32\pm 35.18\%$ in 2003 ($F_{1,40}=6.15$; $P<0.017$) but was not significantly different between populations ($60.35\pm 39.23\%$ in HL *versus* $41.66\pm 34.40\%$ in CI; $F_{1,40}=3.26$; $P<0.08$). The analysis revealed a decrease from 2002 to 2003 in respect to the proportions of non-fertile eggs, with $18.62\pm 23.09\%$ in 2002 *versus* $6.24\pm 10.15\%$ in 2003 ($F_{1,40}=5.26$; $P<0.027$). Hence it seems that the proportions of live neonates increased from 2002 to 2003 because the rate of fertilisation increased within litters. More eggs were fertilised, increasing the number of live young produced. Admittedly however, the fertile or non fertile status of eggs was determined by sight (presence of an embryo visible to the bare eye), hence some fertilised eggs with microscopic embryos may have been classified as non fecund. This potential source of error applies both years however and thus cannot fully invalidate the results.

E. Diet divergence, jaw size and scale counts

Chapter 3

Anti-predator and predator
behaviours

Aims - In the previous Chapter I have shown that the populations of Tiger snakes from CI and HL live in disparate environments for a number of variables, including diet. CI also provides a novel environment as CI is a predator-free environment for adult Tiger snakes.

Variations at both genetic and phenotypic levels play an important rôle in the response to food and food-related stimuli. The knowledge of such variations is crucial to understanding how populations adapt to changing environments, notably to different prey types - in this case Silver gull chicks instead of frogs. The exposure to a novel prey and the resultant blindness through aggressive defense of gull chicks by adults provided the opportunity to experimentally address the foraging aptitude of these blind snakes in order to understand how they can survive despite the loss of one of their primary senses. In addition, the absence of predators on CI offers the opportunity to assess the rôle of genetics and plasticity in the expression of defensive behaviour by comparing the responses to threat in "naïve" neonates from both populations in the laboratory with the results obtained on field-experienced adult snakes from both locations. This novel environment provides the opportunity to reveal micro-evolutionary steps (genetically-fixed prey preferences may arise) and a rôle for behavioural plasticity in adjusting towards new types of prey and predatory threat.

A. Feeding preferences

[Aubret F, Burghardt GM, Maumelat S, Bonnet X, and Bradshaw SD. Feeding preferences in two disjunct populations of Tiger snakes, *Notechis scutatus* (Elapidae). Behavioral Ecology, under review.]

Comparisons of conspecific populations can be effective in elucidating selective pressures and adaptive causes of differentiation in animal behavior because confounding variables are not as prevalent, and small evolutionary steps can be revealed (Foster and Endler 1999). Comparisons between conspecific populations are also useful in understanding the evolutionary significance of phenotypic plasticity (e.g. Sclichting and Pigliucci 1995; Via *et al.* 1995; Wagner and Altenberg 1996; Aubret *et al.* 2004a). Knowledge of genetic-environment interactions in behavior within natural populations is crucial to our understanding of how populations adapt to changing circumstances. However, measuring such interactions within natural populations has proven difficult (Plomin and Hershberger 1991).

Dietary selection is a major arena for the operation of behavioral plasticity, and it is known that both genetic and phenotypic variation play important rôles in moulding responses to food and food-related cues (Falciglia and Norton 1994; Segal and Topolski 1995). The rôle of early experiences can be particularly profound and influence chemoreceptive and feeding responses in birds (Marchetti and Price 1989), fish (Croy and Hughes 1991), insects (Kral 1998), as well as reptiles (Burghardt 1993; Mori 1996; Burghardt and Krause 1999; Krause and Burghardt 2001). Intraspecific variations, on the other hand, may reflect micro-evolutionary processes related to geographical differences in available prey, predators, or interspecific competition for resources (Burghardt 1970a; Arnold 1981 a, b, c; Burghardt and Schwartz 1999; Cooper *et al.* 2000). The quantitative genetics of the initial responses of diet-naïve or prey-naïve snakes is well established (Brodie and Garland 1993a) but, given the demonstration of experience and prey type interactions (e.g. Burghardt *et al.* 2000), the micro-evolutionary consequences of selection on older animals may underestimate

both the rate of microevolution (Resnick *et al.* 1997) and the rôle of experience and ontogenetic processes in evolutionary events (Burghardt *et al.* 2000).

Geographical separation of conspecific populations of predatory snakes offers opportunities to investigate the evolutionary mechanisms acting on dietary preferences. Notably, interspecific differences in chemosensory preferences in neonatal snakes may reflect species-typical diets (Burghardt 1969, 1993) or the retention of ancestral diet-specific preferences (Burghardt 1967; Coss 1999). Responses to prey chemical cues at birth are heritable in several species of snakes (reviews in Arnold 1981a; Brodie and Garland 1993a), and are often modifiable with feeding experience (Burghardt 1993; Burghardt *et al.* 2000). In order to better understand the relative importance of genes, experience, and ontogenetic processes on feeding preferences, we studied two nearby, but disjunct, populations of Tiger snakes, *Notechis scutatus*, from Western Australia. As described below, the two populations appear genetically very similar, but live in differing environments with different prey available and different diets (confirmed by field studies; Aubret *et al.* 2004b). The potential for behavioral plasticity in those two populations has been revealed (Aubret 2004; Aubret *et al.* 2004a; Bonnet *et al.* 2005).

Snakes have a well developed chemoreceptive organ, the vomeronasal organ, situated in the roof of the oral cavity (Halpern 1992). Snakes transport chemicals (vomodors) from the environment to this organ *via* the tongue as it flicks (Halpern 1992). Snakes will often emit more tongue flicks and even attack items such as cotton swabs containing aqueous chemicals from the species' typical prey. The term "preference" has been applied to situations in which chemical or other cues from one prey are responded to relatively more than another (Burghardt 1967).

We investigated the prey preferences of both populations using prey-naïve animals, juveniles reared on a controlled diet, and wild-caught adults. We attempted to answer the following questions. Are feeding preferences similar at birth in the two populations? Are ancestral preferences still present in the

isolated population? What is the rôle of experience in the expression of feeding preferences, especially toward new types of prey?

Methods

Species

Highly venomous Australian Tiger snakes, *Notechis scutatus* (Elapidae), are found in all southerly parts of Australia, including Tasmania. Mainland Tiger snakes feed principally on frogs, which constitute 50-81% of all prey items recorded, whereas the proportion of endotherms (small mammals) is much lower (14-47%; Shine 1977, 1987; Aubret *et al.* 2004b). By contrast, Tiger snakes on islands typically eat far fewer frogs (23%) and more endotherms (66%).

Study sites

We studied two populations separated by 25 km in Western Australia: Carnac Island (CI) and Herdsman Lake (HL).

Carnac Island is a small limestone plateau surrounded by sand beaches (total area of 16 ha), located approximately 12 km off the coast of Fremantle (S 32°07'; E 115°39'). The island was formed some 6,000 years ago by rising sea levels (Seddon 1972). The vegetation of the island was described by Abbott (1978, 1980). There are no fresh water sources available except rainfall or dew and no frogs occur on Carnac Island. Although several bird species (including pied cormorants *Phalacrocorax varius*, little penguins *Eudyptula minor*, and wedge-tailed shearwaters *Puffinus pacificus*) nest on Carnac, the silver gull (*Larus novaehollandiae*) is by far the most abundant bird species (Lane 1979). Large lizards (*Egernia kingii*) are common, and smaller skinks (*Morethia obscura*) also occur on Carnac Island (Bush *et al.* 1995). The only mammals are house-mice (*Mus musculus*) and sea lions (*Neophoca cinerea*) (Abbott 1978; Young 1981). Skinks and mice constitute the major diet components of neonate and juvenile snakes whereas the adults feed mostly on silver gull chicks (83% of the prey; Bonnet *et al.* 2002a; Aubret *et al.* 2004b) with mice (15%) and lizards (2%)

constituting the remainder. There is one record of a wedge-tailed shearwater chick being eaten. However, field observations suggest that the proportion of *P. pacificus* in the diet of adults may be under-estimated, essentially because few research trips in the field coincided with the nesting period of this bird.

Herdsmen Lake (S 31° 55' 44"; E 115° 48' 19") is a nature reserve in Perth. A dredged moat encircling the 300 ha lake ensures permanent open water. It is composed of artificial banks and paths sometimes bordered by trees that create a mosaic of vegetation patches (mostly reeds) and a net of interconnected shallow fresh waters. The lake constitutes a favorable habitat for several frog species (*Litoria moorei*, *Litoria adelaidensis*, and *Crinia insignifera*). Long-neck turtles (*Chelodina longicollis*), domestic and feral cats, and house-mice (*Mus musculus*) also occur in HL. HL Tiger snakes do not feed on sea-gull chicks as this bird does not nest in the area. Although the largest snakes in HL could potentially eat the chicks of ground nesting ducks and grebes, there are no records of this occurring. Frogs and mice are far more accessible prey and constitute 53% and 47% respectively, of all prey items (Aubret *et al.* 2004b).

Recent mtDNA genetic studies on snakes from these two populations and others (years 1999 and 2000) found little divergence (less than 0.3%) in a matrix of genetic distances (5 mitochondrial genes, 4825 base pairs) between CI and other Western Australian mainland Tiger snakes, including the HL population (Scott *et al.* 2001; Keogh *et al.* 2005). CI and HL populations are nevertheless morphologically distinguishable (Bonnet *et al.* 2002a; Aubret *et al.* 2004b), suggesting that Tiger snakes exhibit phenotypic plasticity; or alternatively, that the portion of mitochondrial DNA sampled does not detect subtle differences between populations. For instance, CI adults average 11% longer in SVL and 100% heavier in mass compared with HL individuals. CI snakes also have more mid body scale rows, more infra and supra labials, and possess larger jaws relative to body size. These characters are possibly related the larger prey diet of CI snakes (see Aubret *et al.* 2004a, b). The origin of Tiger snakes on Carnac Island remains unclear. It is possible that the population may be derived, at

least in part, from individuals marooned by the rising sea level 5000-7000 years ago when Carnac was separated from the mainland. However, Cann (1986) stated that a founder population of some 80 mainland individuals was released on the island around 70 years ago. Department of Conservation and Land Management (DCLM) records indicate that this introduction occurred around 1929. In either case, CI and HL snakes are undoubtedly closely related to each other.

Feeding preferences in field-caught adult Tiger snakes

Subjects - A total of 55 adult Tiger snakes were captured in the wild; 16 males and 11 females from CI; 17 males and 11 females from HL. Snout-vent length (SVL) was recorded to the nearest 0.5 cm. Body mass (BM) was recorded to the nearest 1 g with an electronic scale. A body condition index (BCI) was calculated for each snake, using the residual values of the linear least-squares regression of Log body mass against Log SVL. Such an index provides accurate estimates of body reserves (fat, muscle) in snakes (Bonnet and Naulleau 1994). These morphological data are presented in Table 1.

Maintenance - Snakes were individually housed in plastic boxes (50 x 40 x 30 cm), with shelter, and paper toweling and water provided *ad libitum*. Because mice are the only natural prey shared by HL and CI snakes, all snakes were offered dead mice approximately every 3 weeks. At least 15 days lapsed between feeding and testing. Feeding tests took place between October 2001 and January 2002. The experimental room was set at 27.5°C by day and 20°C by night. All tests were performed at approximately 27°C, as the average body temperature chosen by both HL and CI snakes in captivity is 26.8±0.7°C (Ladyman and Bradshaw 2003).

Experimental design - The testing environment was a 90 x 40 x 50 cm high glass aquarium. A sliding door partitioned the aquarium into two equal

compartments, and a wooden board covered the top. All sides were covered with paper to avoid disturbance to the snake, and a small window (5 x 5 cm) in the paper allowed for observations. Snake subject, prey type, and time of the experiments were always balanced.

The snake was placed in one side of the aquarium. In the centre of the other compartment a live prey animal was placed under a steel strainer (10 cm diameter, 7 cm deep, mesh size of 1 mm) and taped to the floor. Each strainer was associated with one prey type and prior to the experiment the body of each prey was gently rubbed onto the whole surface of its strainer.

Following each experiment, the whole aquarium was cleaned with soap and bleach to remove all odor cues, rinsed and then allowed to dry before the next trial. Prey species were always kept in a separate room so as not to introduce mixed odors to the experimental room or accustom the snakes to ambient odors (Burghardt and Layne 1995).

After introduction into the empty compartment, snakes were allowed to habituate for approximately 15 minutes. The sliding door was then lifted with a remotely operated rope and pulley system. As soon as the snake's head entered the test compartment containing the prey, data recording commenced for a 10 minute period. We recorded the time spent by the snake orienting tongue flicks toward the strainer and the number of tongue flicks (TF) directly contacting the strainer.

Experiment 1. Adult single prey tests - Five prey types (mammal, reptiles (2), amphibian, and bird) were used for the experiment: lab mice (*Mus musculus*; Body Mass (BM)=30 g approximately), blue-tongue skinks (*Tiliqua occipitalis*; BM=41 g), common skinks (*Ctenotus fallens*; BM=15 g), frogs (*Litoria moorei*; BM=16 g), and domestic chicks (*Gallus domesticus*; BM=35 g approximately). Blue-tongue skinks do not occur on either CI or HL, but are closely related to *Egernia kingii*, which is abundant on CI. The common skink *Ctenotus fallens* is commonly found on HL but not on CI, however a closely-related species (*Morethia obscurus*) does occur on CI. Due to the protected status of the Silver gull, it was not possible to use a live Silver gull chick for the experiment, so we

used domestic chicks as a substitute. Adult CI snakes readily feed on domestic chicks in captivity (personal observation). A clean empty strainer served as control.

Experiment 2. Adult choice tests - We used a similar experimental design as in the single-prey tests, but this time used two strainers, placed at equal radial distance from the first compartment. Different prey items were placed under each strainer. At this stage of the study, preliminary results showed that adults from both populations were most reactive toward three prey types (see Results). Thus only three pairings of stimuli were tested: mouse *versus* frog, mouse *versus* chick, and chick *versus* frog. We recorded which strainer/prey a snake first approached, the number of approaches to each strainer/prey, and the time spent tongue-flicking at them. Number of TF were not recorded as this was investigated in experiment 1.

Feeding preferences in neonates and juveniles

Subjects and maintenance - Between the 17 March 2002 and 18 May 2002, 13 wild-caught females gave birth in the laboratory (9 from HL and 4 from CI). Litter size averaged 5.8 ± 3.7 in HL females and 7.7 ± 7.5 in CI females. All females were maintained individually in plastic boxes (50 cm x 40 cm x 30 cm) with paper towel, water dish and shelter in a controlled temperature room, at 27.5°C by day and 20°C by night. A heat source provided basking opportunity four times per day, 15 minutes each (High pressure Na+ lamp; 600 Watts). Food (dead mice) was offered approximately every three weeks and water provided *ad libitum*. Neonates were weighed, sexed and measured soon after birth (Table 1) and housed in individual plastic boxes (20x10x5 cm) with water dish, shelter, and paper towel substrate.

Experimental design - Individuals were never allowed to eat the prey stimulus and the order of presentation of food items was with equal frequency and in

random order among individuals as test order and prior experience may determine subsequent behavior (Burghardt 1969; Arnold 1992; Burghardt and Schwartz 1999). Each prey stimulus was presented hooked at the end of a 30 cm long chopstick, the observer moving very little to minimize disturbance. Each prey stimulus consisted of a piece of flesh and skin of either mouse (*Mus musculus*), frog (*Litoria moorei*), skink (*Ctenotus fallens*), wedge-tailed shearwater (*Puffinus pacificus*), chicken (*Gallus domesticus*), or silver gull (*Larus novaehollandiae*). No animals were specifically killed for this study. Food portions were similarly sized (approximately 10 mm x 5 mm) and at ambient temperature. The control stimulus was an equivalent sized piece of white plastic. Each stimulus was slowly introduced 1 cm in front of the snake's snout, without making any direct contact. The first tongue flick directed at the stimulus began a 60 second trial where we recorded the number of tongue flicks elicited. If the snake attacked the piece of food, the latency of the attack was recorded and the test ended.

Experiment 3. Neonate single prey tests - Tests started when neonates were 3 weeks old, allowing for behavioral maturation and depletion of yolk sacs. Snakes were tested on a 7-day schedule. A total of 528 tests were performed: 209 on 31 CI neonates and 319 on 51 HL neonates. Testing order of litters, individuals, and prey samples were balanced throughout the experiment.

Experiment 4. Mouse fed juvenile single prey tests - 15 CI neonates were kept in the laboratory and only fed baby mice until they were 8 months of age (208±1.78 days of age). They averaged 17.38±5.61 g in BM and 27.96±2.70 cm in SVL when tested using the same prey samples as in Experiment 3, with the exception of *P. pacificus* which was not available at this time.

Analyses

+1 was added to all Tongue Flick scores (TFS) and Time scores and both were LN transformed prior to analysis. In tests where bites were observed (experiments 3 and 4), we used the method described by Cooper and Burghardt (1990) as TFAS (Tongue flick - attack score) to account for shortened trials in TFS. Wilcoxon matched signed rank tests were used in the choice experiments in adults (experiment 2). With multiple measures (different prey items and control) on same individuals within each population, we used Friedman ANOVAs (experiment 1). In neonates, maternal effects were controlled by using Nested ANOVAs with “litter” nested within “population” (experiment 3 and 4). TFAS at 6 weeks and 8 months on the same individuals were compared using Wilcoxon matched pairs tests (experiment 4).

The experimental design balanced test order and individual adults and juveniles. Each individual was tested for each prey item and the control; thus scores are not independent of each other. Furthermore, the level of responsiveness to the experiment differed between the two populations (scores for control were typically higher in CI individuals in adults as well as in neonates – see results). Hence, in order to compare the scores (TFS, Time scores, TFAS) between the two populations, we performed repeated measures ANOVA with “origin” as factor, and two repeated measures: “LN score (control)” and “LN score for each different prey items”. The level of significance given by the interaction term “origin” x “scores” indicated if the two populations differed in the score for a given prey item, independently of their level of responsiveness.

Results

Experiment 1. Adult single prey tests - The number of tongue-flicks directed at the strainer was strongly correlated with the time spent by snakes tongue flicking on the strainer for each of the 5 prey items as well as the control (Spearman’s ranks correlations, $35 < N < 55$; $0.96 < r < 0.98$; All $P < 0.001$).

Within each population

Results of TF scores within each population of Tiger snakes are presented in Figure 1 and time scores in Figure 2. We found a significant effect of prey item on TFS in CI snakes (Friedman $\chi^2_{(27,5)}=56.36$; $P<0.001$) and in HL snakes ($\chi^2_{(28,5)}=32.07$; $P<0.001$) as well as in time scores in CI snakes ($\chi^2_{(27,5)}=53.57$; $P<0.001$) and HL snakes ($\chi^2_{(28,5)}=24.68$; $P<0.001$). Results were unchanged when control scores were withdrawn from analysis.

Between the two populations

The level of responsiveness differed between the two populations: control scores in TFS ($F_{1,53}=8.22$; $P<0.001$; mean= 13.37 ± 11.45 in CI *versus* mean= 6.21 ± 6.46 in HL) and Time scores ($F_{1,53}=6.46$; $P<0.014$; mean= 16.15 ± 11.62 in CI *versus* mean= 9.14 ± 8.64 in HL)

There were no significant differences between the two populations in average interest shown towards the various prey items (Repeated measures ANOVA with Origin and Stimulus as factors, and Ln(TFS) for control and each of the prey items as the second repeated measure) with *M. musculus* (TFS: Wilks' Lambda=0.75; $P<0.001$; interaction term $F_{1,53}=1.46$; $P=0.24$; Time scores: Wilks' Lambda=0.77; $P<0.001$; $F_{1,53}=2.54$; $P=0.12$); *L. moorei* (TFS: Wilks' Lambda=0.88; $P<0.035$; $F_{1,53}=0.09$; $P=0.77$; Time scores: Wilks' Lambda=0.90; $P=0.067$; $F_{1,53}=0.88$; $P=0.36$); *G. domesticus* (TFS: Wilks' Lambda=0.86; $P<0.023$; $F_{1,53}=0.05$; $P=0.83$; Time scores: Wilks' Lambda=0.91; $P=0.072$; $F_{1,53}=0.17$; $P=0.69$); *T. occipitalis* (TFS: Wilks' Lambda=0.82; $P<0.006$; $F_{1,53}=0.45$; $P=0.51$; Time scores: Wilks' Lambda=0.87; $P=0.024$; $F_{1,53}=0.70$; $P=0.41$); *C. fallens* (TFS: Wilks' Lambda=0.82; $P<0.006$; $F_{1,53}=0.23$; $P=0.63$; Time scores: Wilks' Lambda=0.87; $P=0.026$; $F_{1,53}=0.42$; $P=0.52$).

A few snakes attempted to bite the prey under the strainer. Of the 7 bites recorded, 3 were induced by *M. musculus* and 4 by *L. moorei*. All were by 6 different HL snakes, except for one CI snake that attempted a bite at *M. musculus*.

Experiment 2. Adult choice tests - In HL as well as CI snakes, there was no significant preference in any pair of prey in time spent tongue flicking at each prey and number of visits to each prey (Table 2).

Experiment 3. Neonate single prey tests

Within each population

Within CI neonates, there was a significant effect of prey on TFAS (Nested ANOVA with “Items” as factors, “Litter” variable nested into “Items”; LN (TFAS) as variable; $F_{6,178}=11.13$; $P<0.001$; see Figure 3). However, the statistical significance was lost when control scores were removed from analysis ($F_{5,152}=1.73$; $P=0.13$) suggesting that there were no significant differences among the prey types tested. Within HL neonates, we also observed an overall difference (same design ANOVA; $F_{6,264}=14.68$; $P<0.001$) but the statistical significance was again lost when control scores were removed from the analysis ($F_{5,222}=2.02$; $P=0.08$).

Between populations

The TFAS for the control significantly differed between the two populations (Nested ANOVA with LN (TFAS) as variable, “litter” nested into origin; $F_{1,68}=4.12$; $P<0.046$). CI neonates scored an average of 18.84 ± 13.97 TFAS versus 10.45 ± 10.76 in HL neonates.

If we compare the TFAS between CI and HL neonates (Repeated measures Nested ANOVA with Population as factors, “litter” nested into population; LN (TFAS) of “control”) and each of the various prey items as the second repeated measures, interaction term yields for *M. musculus* ($F_{1,68}=0.69$; $P=0.41$; Figure 3); *L. moorei* ($F_{1,68}=0.79$; $P=0.38$); *C. fallens* ($F_{1,68}=0.64$; $P<0.43$); *P. pacificus* ($F_{1,68}=0.68$; $P=0.41$); *G. domesticus* ($F_{1,68}=0.76$; $P=0.39$); and *L. novaehollandiae* ($F_{1,33}=6.34$; $P<0.017$).

Bites on prey

No attacks were directed at the control stimuli. All prey samples elicited some attacks by animals from both populations. In CI neonates, we did not find any significant difference in proportions of bites between prey items (Pearson

$\chi^2_{(Df=5)}=8.69$; $P<0.12$; Figure 4), as well as within HL neonates ($\chi^2_{(Df=5)}=2.79$; $P=0.73$).

Between the two populations (see Figure 4), the frequency of bites were not different for any items presented, such as *M. musculus*, *L. moorei*, *C. fallens*, and *L. novaehollandiae* (Yates corrected χ^2 tests; all $P>0.17$). We found no overall difference in attack latency between CI and HL neonates (all tests pooled; Nested ANOVA with “litter” nested into “origin”; respectively 37.60 ± 24.06 s versus 24.18 ± 19.46 s; $F_{1,47}=0.19$; $P=0.67$).

Experiment 4. Mouse fed juvenile single prey tests - At 8 months of age, we found a significant difference in TFAS between the different items in CI neonates (Nested ANOVA with “litter” nested into “Prey item” and LN(TFAS) as variable; $F_{5,72}=15.37$; $P<0.001$) and the difference remained significant when TFAS with control were withdrawn from analysis $F_{4,59}=7.03$; $P<0.001$; Figure 5).

TFAS at 6 weeks and 8 months were compared using Wilcoxon matched pair tests (see Figure 5). Statistical analysis led for *Gallus*: $N=13$; $Z=1.57$; $P=0.12$; Control: $N=15$; $Z=0.51$; $P=0.61$; for *Litoria*: $N=15$; $Z=1.81$; $P=0.074$; for *Larus*: $N=11$; $Z=0.87$; $P=0.39$; for *Mus*: $N=15$; $Z=2.67$; $P<0.008$; and for *Ctenotus*: $N=14$; $Z=0.97$; $P=0.33$.

Occurrence of bites

Given that 15 young snakes were only fed mice throughout the experimental period, the comparison of the frequencies of bites to prey samples at 3 weeks and 8 months of age should reveal whether the initial responses of naïve neonates is influenced by dietary experience. Indeed, the proportion of *M. musculus* samples bitten significantly increased from 20% to 80% (Yates corrected $\text{Chi}^2_{(df=1)}=8.53$; $P<0.004$).

Frequency of bites on *L. moorei*, *G. domesticus*, or *L. novaehollandiae* did not change significantly throughout ontogeny (All $P>0.10$). Surprisingly however,

frequencies of bites on *C. fallens* s also significantly increased from 0% to 43% (Yates corrected $\chi^2_{(df=1)}=5.30$; $P<0.021$; See Burghardt *et al.* 2000).

Discussion

Animals can behaviorally adapt to changed habitat and prey resources by adapting genetically, by individual phenotypic plasticity, or by an interaction process. In widespread species, local adaptation is possible (Brodie and Garland 1993; Burghardt 1993; Burghardt and Schwartz 1999). However, gene flow may be considerable between contiguous populations and thus counteract selection for differences between population (Krause and Burghardt 2001).

Between island and mainland populations however, gene flow may be considered as nil, unless the particular animals are capable of flying or swimming. Therefore it is likely that genetic differences arise as two populations become geographically isolated. Where prey types differ for instance, genetically-based preferences may arise (Arnold 1981a; Arnold 1992; Brodie and Garland 1993; Burghardt 1993; Cooper *et al.* 2000).

Despite the fact that diets vary tremendously between both habitats, little divergence was found between the two populations of adult Tiger snakes in the scores to different prey. Notably, adults from both locations exhibited similar interest to the various prey items offered. Unexpectedly, there were marked preferences for *M. musculus*, *L. moorei*, and *G. domesticus* in both populations. These results appear counter-intuitive as early dietary experience has proven to influence prey preferences in some, although not all, species of snakes (Burghardt 1967, 1969; Mori 1996; Burghardt and Krause 1999; Krause and Burghardt 2001).

Neonates responded to all prey stimuli more than to control stimuli, although there was no significant difference among the prey samples offered within CI and within HL in TFAS or in rates of bites. Moreover, the rate of biting was similar between the two populations for the prey sampled, as well as the TFAS for most prey samples. However, CI neonates displayed significantly greater

TFAS toward silver gull samples than HL neonates. In spite of the fact that these two populations (HL and CI) are genetically very close to one another, this last result suggests that some degree of genetic and behavioral divergence exists. Previous work identified divergence for morphological traits that are genetically fixed between HL and CI populations (i. e. scalation - Aubret *et al.* 2004b).

The common mouse (*M. Musculus*) was one of the items preferred by adults and is commonly eaten by snakes in both populations. However frogs do not and have never occurred on CI, as there are no fresh-water bodies on the island. Nevertheless, CI snakes (adults and neonates) responded to *L. moorei* stimulus (a typical prey of HL snakes). Such behavior is probably sustained by genetic bases. Several studies showed an apparent decoupling in prey preference between two geographically-separated populations of snakes (Burghardt 1967, 1969; Burghardt *et al.* 2000; Tanaka *et al.* 2001). This may reveal ancestral genetically-fixed responses to a particular prey that is no longer consumed naturally. This is highlighted in our study, with CI snakes readily recognizing frogs as a potential prey.

A recent study has shown that in many aspects, Carnac Island represents a very favorable environment for Tiger snakes (Bonnet *et al.* 2002a): food is abundant and snakes grow to be larger than in HL. This fits the prediction that behavioral characters are sometimes retained for long periods under relaxed selection (see Coss 1999 for review). Interestingly, it also shows that genetically-based preferences are sustained throughout ontogeny, even if the stimulus (i. e. *L. moorei* on CI) is never encountered. This may provide a selective advantage in variable environments and may account for the very large area of distribution of Tiger snakes in Australia, and their success in many situations where uncommon prey occur and common prey are no longer available (typically offshore islands; Shine 1977, 1987).

On the other hand, shearwater and silver gull (and the domestic chicken) are bird species that are never consumed by HL snakes, as they do not nest around this lake. Despite this, HL neonates displayed interest in these prey items. For instance, the three bird species were bitten at similar rates by HL neonates as

for CI neonates. Such behavioral flexibility may also be an advantage in variable environments, especially when populations have become isolated on islands with the rise in sea level occurred in the past. Indeed, several Tiger snakes island populations have been described where nesting marine birds are a major component of the diet (Schwaner 1985; Shine 1977, 1987).

Although snakes from both populations responded similarly as both neonates and adults (no influence of dietary experience was observed), the potential for behavioral plasticity was revealed by comparing the feeding preferences of naïve neonates and laboratory-reared juveniles. Previous work highlighted the potential for behavioral plasticity in these two populations (Bonnet *et al.* 2005). CI neonates raised exclusively on *M. musculus* tended to increase their response to *M. musculus* stimuli even though the response at birth was already very high in both TFAS and bite rate. That is, a strong preference for *M. musculus* arose as a result of a specific diet. Unexpectedly however, skink samples triggered significantly more bites at 8 months of age than at 3 weeks of age, although TFAS were similar. These results highlight the importance in behavioral studies of comparing captive-trained animals and field-experienced animals, as this study proves that results may diverge.

This study supports the importance of studying recently separated populations with differing available resources as a means to explore the microevolutionary and phenotypic plasticity responses that populations use to adapt to environments in the early stages of genetic isolation. Firstly, this study provides a clear example of relaxed selection on prey preference traits in a snake invading a novel environment (Coss 1999) through the retention of ancestral behavioral characters. Secondly, this study also provides an example of rapid selection for responsiveness towards Silver gulls in CI neonates. This result is extremely interesting considering that Silver gulls are the main prey of adult snakes on CI, although never actually eaten by neonates. A recent study suggested that CI snakes have been selected at two levels in the process of colonizing CI (Aubret *et al.* 2004a). Silver gull chicks are larger than the prey usually consumed by mainland conspecifics (Aubret *et al.* 2004a, b). CI snakes (neonates and adults) not only have relatively larger heads (independent of

body size) as compared with HL snakes, but they are also capable of displaying greater plasticity in head size development than HL individuals. Both adaptations supposedly enable the ingestion of large prey and hence constitute a selective advantage in snakes that display them (Forsman 1991; Shine 1991; Forsman and Lindell 1993; Forsman and shine 1997). The present study therefore relates a striking example of directional evolution in Tiger snakes: a taste for a novel prey was selected concurrently with the means of ingesting it.

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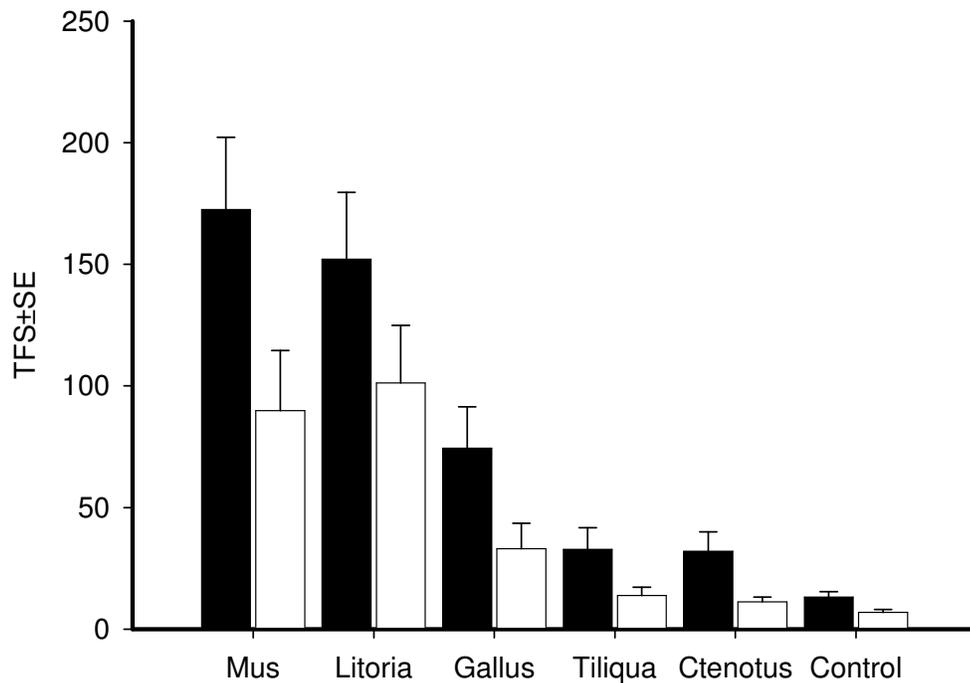


Figure 1: Graph represents the Tongue Flick score (mean \pm SE) in response to prey stimuli in 27 Carnac Island (CI; black charts) and 28 Herdsman Lake (HL; white charts) adult Tiger snakes.

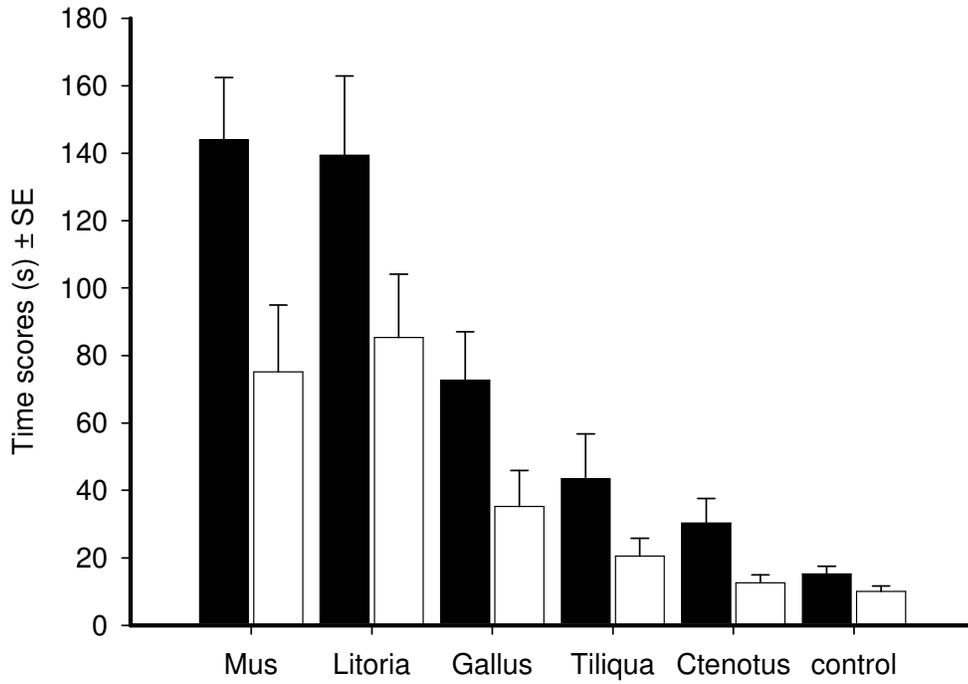


Figure 2: Graph represents the Time scores (mean \pm SE) in response to prey stimuli in 27 CI (black charts) and 28 HL (white charts) adult Tiger snakes.

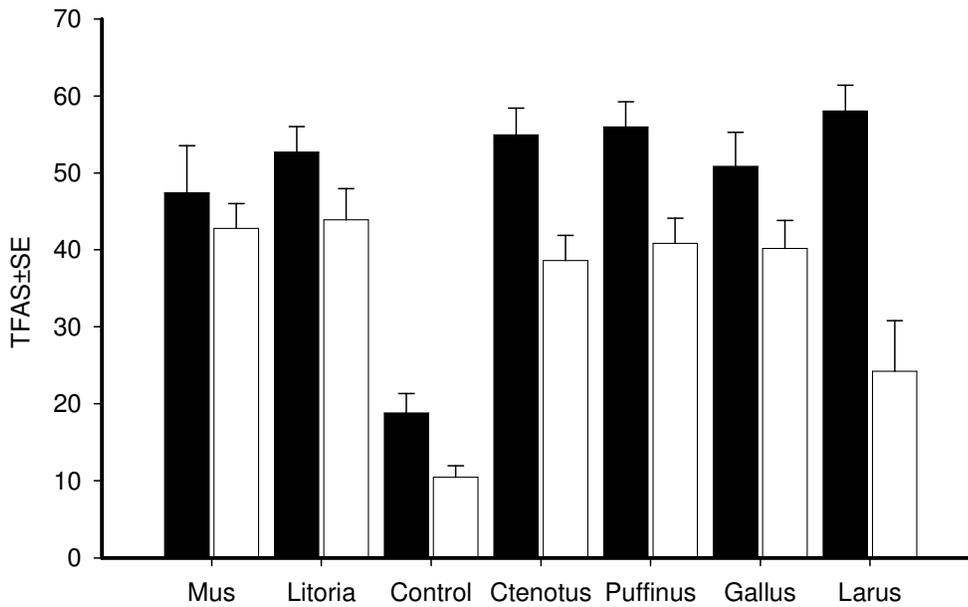


Figure 3: Graph represent TFAS \pm SE in neonate Tiger snakes from CI (N=31; black charts) and HL (N=51; white charts).

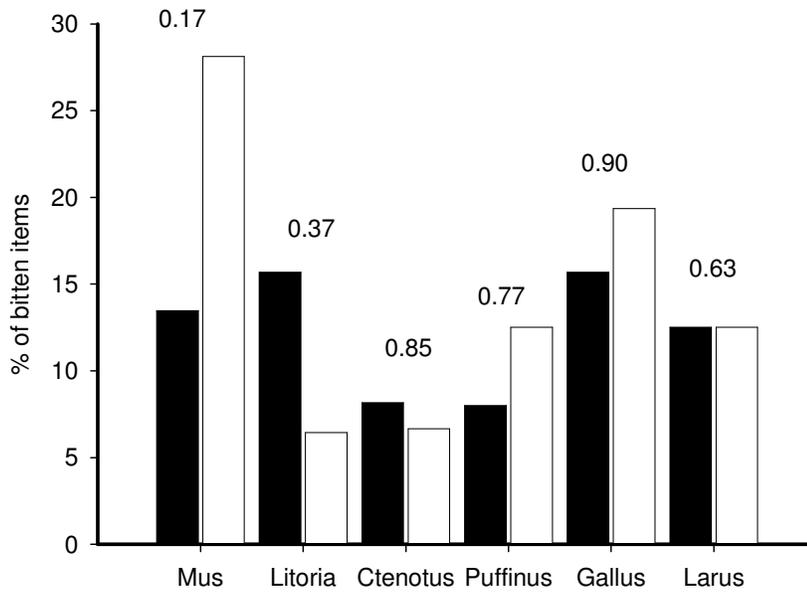


Figure 4: Graph represents the proportions of bitten items in CI (black charts) and HL (white charts) neonates at 3 weeks of age. Twenty-six attacks were recorded in CI neonates and 33 in HL. P values obtained with Yates corrected χ^2 tests for each sample between the two populations are given.

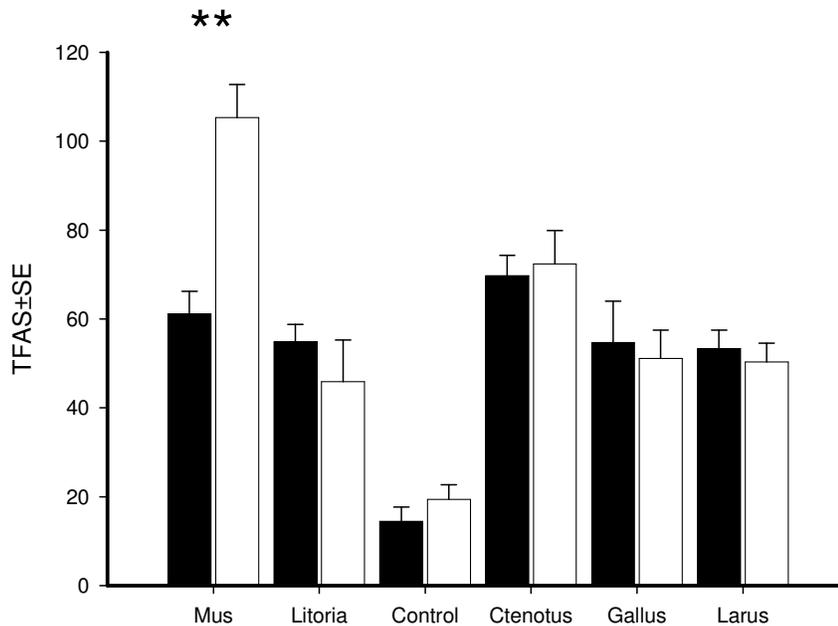


Figure 5: Graph represents the comparisons of TFAS \pm SE at 3 weeks (black charts) and 8 months (white charts) of age in 15 CI neonates. The difference was significant with *M. musculus* (Wilcoxon matched pairs tests: N=15; Z=2.67; P<0.008) but not for any other items (all P>0.07).

Table 1: Morphological comparison at birth and as adults between the two populations.

Nested ANOVA were used in neonates to account for potential maternal effect, with factor “Mother” nested into “Origin”, and the relevant trait as the dependent variable.

Traits	Carnac Island	Herdsmen Lake	Df; F	P
<i>Adults</i>				
Body mass (g)	376.02±139.05	226.03±102.38	1, 53; 20.86	0.001
Snout-vent length (cm)	84.50±12.27	75.89±13.28	1, 53; 6.22	0.016
Body condition	0.044±0.06	-0.043±0.06	1, 53; 28.23	0.001
<i>Neonates</i>				
Body mass (g)	6.70±0.82	4.25±0.86	1, 82 ; 168.76	0.001
Snout-vent length (cm)	20.52±1.19	16.98±1.34	1, 82; 150.34	0.001
Body condition	0.0085±0.05	0.0052±0.04	1, 82; 1.70	0.20

Table 2: Time scores and number of approaches of each prey in the choice tests.

Values are means \pm SD. Probabilities values are given by Wilcoxon matched pair tests.

Carnac Island	<i>G. domesticus</i>	<i>M. musculus</i>	<i>L. moorei</i>	P
Test				
<u><i>G. domesticus</i> versus <i>M. musculus</i></u>	N=25			
Time (s)	48.96 \pm 60.63	36.96 \pm 51.92	-	Z=0.22; P=0.83
N approaches	1.32 \pm 0.90	1.40 \pm 1.22	-	Z=0.94; P=0.36
<u><i>L. moorei</i> versus <i>G. domesticus</i></u>	N=25			
Time (s)	34.24 \pm 38.85	-	45.60 \pm 50.98	Z=0.57; P=0.56
N approaches	1.12 \pm 1.09	-	1.32 \pm 0.80	Z=1.32; P=0.18
<u><i>L. moorei</i> versus <i>M. musculus</i></u>	N=26			
Time (s)	-	44.77 \pm 46.79	45.54 \pm 59.93	Z=0.42; P=0.67
N approaches	-	1.73 \pm 1.31	1.34 \pm 1.26	Z=0.02; P=0.98
Hersdman Lake				
Test				
<u><i>G. domesticus</i> versus <i>M. musculus</i></u>	N=23			
Time (s)	21.93 \pm 15.01	15.00 \pm 14.43	-	Z=0.76; P=0.44
N approaches	2.36 \pm 1.45	1.44 \pm 1.81	-	Z=0.06; P=0.95
<u><i>L. moorei</i> versus <i>G. domesticus</i></u>	N=19			
Time (s)	22.25 \pm 27.33	-	12.64 \pm 12.08	Z=0.15; P=0.87
N approaches	1.25 \pm 0.71	-	1.73 \pm 1.55	Z=0.28; P=0.77
<u><i>L. moorei</i> versus <i>M. musculus</i></u>	N=26			
Time (s)	-	42.85 \pm 54.48	13.54 \pm 24.75	Z= 0.79; P=0.43
N approaches	-	2.00 \pm 1.35	0.84 \pm 0.80	Z=1.04; P=0.29

B. How can blind snakes survive?

[Fabien Aubret, Xavier Bonnet, David Pearson and Richard Shine. 2004. How can blind Tiger snakes (*Notechis scutatus*) forage successfully? *Functional Ecology*, under review]

Predators locate and capture their prey based on a diverse array of sensory modalities, but vision is one of the most important such modalities for many taxa (Land and Nilsson 2002; Oakley 2003). This sense is used during day-to-day activities such as moving about, selecting habitats and shelters, acquiring resources, reproducing, and detecting predators (Salvini-Plawen and Mayr 1977; Land and Nilsson 2002). Although individuals from visually-oriented species do not rely entirely on vision, this sense nonetheless is critical for their survival. For example, seals are “blind” when they hunt in deep and dark waters (Dehnhardt *et al.* 1998), but rely upon vision to escape predators, to find mates, and to move on land. Hence, a sudden loss of vision (due to either disease or injury) will prove fatal almost immediately (Brown *et al.* 1984; Gauthier 1991). In natural situations, the deleterious effects of sudden blindness are so strong that very few populations are likely to contain accidentally blind individuals; indeed, the phenomenon mostly has been observed only briefly (during epizootics) with blind animals dying quickly (Martin 1981; Brown *et al.* 1984; Gauthier 1991; Callait 1992; Langon 1996). Two studies of snakes on islands provide dramatic counter-examples to these generalisations. First, Wharton (1969) reported the occurrence and good health of congenitally blind adult pit-vipers (*Agkistrodon piscivorus*) on Seahorse Key in Florida. Second, Bonnet *et al.* (1999a, 2002) reported that on a small island off the coast of southwestern Australia, approximately five percent of adult Tiger snakes (*Notechis scutatus*) are blind, due to injuries inflicted by silver gulls that defend their chicks (see <http://www.monalisa-prod.com/> for scenes of interactions between gulls and snakes). Remarkably, these blinded snakes exhibit survival rates, growth rates and body condition comparable to vision-

intact snakes, suggesting that blindness does not affect their frequency of feeding (Bonnet *et al.* 1999).

How can this be true? In the present paper, we pose and test two alternative hypotheses that might explain the continued foraging success of snakes after they have lost their sight. First, vision may be unimportant for foraging success. For example, the snakes might forage mostly at night, or on prey sequestered in burrows and located by chemical trails left on the substrate. Indeed, many scolecophidian snakes possess rudimentary eyes and manage to forage successfully in this way throughout their lives (Webb and Shine 1993). However, most "advanced" (caenophidian) snakes, including elapids, use vision to identify and target prey (Naulleau 1965; Herzog and Burghardt 1974; Drummond 1985; Teather 1991; Mullin and Cooper 1998; Cooper *et al.* 2000; Shine and Sun 2003; Shine *et al.* 2004a). It is difficult to imagine effective foraging by an animal that uses vision in its day-to-day life then is suddenly deprived of visual information. Nonetheless, an animal may be able to develop enhanced acuity in other senses to compensate for loss of vision (Cohen *et al.* 1997; Lessard *et al.* 1998). For instance, fish blinded by murky water exhibit sensory compensation under predatory threat (Hartman and Abrahams 2000). The second alternative hypothesis is that vision is indeed critical for capturing some types of prey, but not others. It is known that a predator's efficiency (including search distance, search speed, pursuit dynamics) may affect prey selection (Bakker 1983; Endler 1991; Emerson *et al.* 1994; Garland and Losos 1994). Thus, blinded snakes may be able to survive by feeding on sessile prey that are unable to escape. Fast moving prey on the other hand would quickly detect and avoid the predator. The Tiger snakes of Carnac Island provide a unique opportunity to test predictions from these two hypotheses, and hence to clarify the impact of sudden loss of vision on foraging abilities in a natural ecological context.

Methods

Laboratory studies

We captured adult snakes from two nearby populations (roughly 25 km in a straight line) of Tiger Snakes in Western Australia situated respectively on Carnac Island (CI) and Herdsman Lake (HL). Carnac Island is a small limestone plateau surrounded by sand beaches (total area of 16 ha), approximately 12 km off the coast of Fremantle (S 32°07'; E 115°39'). Carnac Island hosts a large population of Tiger snakes (about 400 snakes). Eight percent of the adults are half blind (one eye destroyed), and 4.5% are totally blind (Bonnet *et al.* 1999, 2002a). On Carnac Island, the snakes feed on lizards, mice and bird chicks (Bonnet *et al.* 1999). Herdsman Lake (S 31° 55' 44"; E 115° 48' 19") is a nature reserve in Perth. A dredged moat encircling the 300 ha lake ensures permanent open water. Frogs and mice are abundant, and constitute the main diet of snakes in this population (respectively, 53% and 47% of all prey items recorded: 19 records - Aubret *et al.* 2004b).

Two adult snakes from Carnac Island (one male, one female) and five adults from Herdsman Lake (two males, three females) were captured between 13 Dec 2001 and 3 Sept 2002. Another three adult snakes from Carnac Island, born in captivity in March 2002, were used also. All these snakes were long-term captives (average 296±188 days, range 90 to 603 days) and were feeding regularly prior to experiments that took place between 28 Oct 2002 and 27 Feb 2004.

All snakes were maintained in a room maintained at 27°C by day and 20°C by night. They were housed individually in transparent plastic boxes (50x40x30 cm) with water dish, shelter, and paper towel as substrate. A heat source provided basking opportunities four times a day (4 x 15 minutes/day, 600 watt Na⁺ lamp). Water was available at all times and food was offered once a week (previously euthanased laboratory mice supplied by the UWA animal care unit). A body condition index was calculated for each snake as the residual values of the linear regression of Log BM against Log SVL.

Experimental procedure - To simulate the effect of loss of vision on foraging efficiency, we ran tests before and after rendering snakes blind by covering

their eyes. To render an animal blind, we affixed a rectangular piece of black tape across its head, covering both eyes and left in place until all “blinded” tests had been completed. Because snake eyes are covered by transparent scales, the application and removal of tape was painless. Tape was reapplied immediately after snakes shed their skin. The duration of time for which the snakes were deprived of vision before testing averaged 11 days (range 4 to 25 days). The animals adjusted quickly to blindness, easily finding their usual shelter, water dish, and basking spot. Importantly, they kept feeding on dead mice (see results). We used mean scores per treatment (control *versus* blinded) per individual for analysis.

Experiment 1 - effect of blindness on acceptance of dead mice by snakes

Dead mice were offered to the snakes weekly throughout the experiment, so we can compare the rate of prey acceptance before and after snakes were rendered blind by tape application. Dead mice were placed in front of the snake's snout and left for 24 hours. In nine adults, we recorded if the mouse offered was eaten.

Experiment 2 - effect of blindness on hunting ability of adult snakes

A total of 58 trials was conducted on 10 individuals. A live mouse was introduced into the snake's shelter-box, limiting disturbance to the snake as much as possible, after which we recorded timing of subsequent events with a stopwatch. Mouse food was always available in the snake cage. The snake and mouse were left together for 30 minutes, after which the trial stopped if the snake was not searching for prey. We recorded the time elapsed before the first strike (and its location on the mouse body, and whether or not venom was injected), and time to paralysis of the prey, and to death of the prey (cessation of breathing). We counted the total number of missed *versus* successful bites over the trial; and noted whether the mouse was held after a successful bite or released. For simplicity in analysis and interpretation, we only use data from trials where the snake killed the prey. Data on some variables were unavailable

for some trials, because of difficulties in seeing encounters without disturbing either the snake or mouse.

Field study

During fieldwork (1997-2003) on Carnac Island (Bonnet *et al.* 2002a), snakes were captured by hand and each animal was individually marked (scale-clipped), and carefully examined to determine its visual ability. We scored the snake as “normal” when both eyes were intact, “half-blind” when one eye was destroyed, and “blind” when both eyes were destroyed (Bonnet *et al.* 1999). We palpated the stomach of each snake for the presence of prey. Faeces were also examined for the presence of feather, hairs or reptile scales (from sea-gull chicks, mice, and lizards respectively). Gull chicks are taken soon after they hatch, so are sessile prey. In contrast, lizards and mice are mobile prey.

Results

Laboratory studies

Captive adult snakes from CI and HL did not differ significantly from each other in mean snout-vent length (respectively 74.00 ± 21.98 cm *versus* 80.80 ± 6.04 cm, Kruskal-Wallis ANOVA: $H_{1,10} = 0.27$; $P = 0.60$), body mass (392.28 ± 353.47 g *versus* 330.86 ± 63.35 g; $H_{1,10} = 0.27$; $P = 0.60$), or body condition (0.040 ± 0.075 *versus* -0.040 ± 0.06 ; $H_{1,10} = 1.84$; $P = 0.17$).

Experiment 1 - effect of blindness on acceptance of dead mice by adult snakes

The proportion of trials in which mice were taken did not differ between the island and mainland snakes either during the control tests (Kruskal-Wallis ANOVA $H_{1,9} = 1.26$; $P = 0.26$) or during the “blind” tests ($H_{1,9} = 1.35$; $P = 0.24$). Hence we pooled data for CI and HL snakes and conducted the analysis on a total of 91 control tests and 61 “blind” tests in 9 different snakes. Prey size was similar in control tests (22.49 ± 9.95 g) and “blind” tests (23.77 ± 0.73 g; t-test for

dependant sample; $N=9$; $t=-1.29$; $P=0.55$). There was no difference in the rate of acceptance of dead mice between the control and the “blind” tests ($86.4\pm 14.0\%$ *versus* $93.1\pm 10.5\%$; t-test for dependant samples; $N=9$; $t=1.26$; $P=0.24$).

Experiment 2 - effect of blindness on hunting ability of adult snakes

Kruskal-Wallis ANOVAs did not detect any significant differences between CI and HL snakes either in the control tests (all $P>0.17$) or in the “blind” tests (all $P>0.13$) for any of the variables recorded (see Table 1). Therefore, we pooled data for snakes from the two localities. Artificial blindness severely impaired the hunting ability of the snakes, especially their capacity to locate, strike and kill their prey (Table 1). Compared to control trials, blind snakes delayed the initial strike, missed their target more often, and thus took longer to consume the prey item (Figure 1).



Figure 1: Blind-folded adult Tiger snake feeding on a mouse.

Field study

Blind snakes and half-blind snakes preyed almost exclusively on sea-gull chicks (sessile prey) whereas snakes with normal vision regularly consumed lizards and mice (mobile prey) ($\chi^2=12.78$, $df=2$, $P<0.002$, $N=257$; Table 2). There is also an ontogenic shift in diet in this population (adult snakes consume mostly sea-

gull chicks; Bonnet *et al.* 1999) so we repeated this analysis after restricting the data to adult snakes only; the results were unchanged ($\chi^2=6.82$, $df=2$, $P=0.033$, $N=236$).

Discussion

Our data strongly falsify one of the hypotheses proposed in the Introduction to this paper, and support the other. Although loss of vision did not reduce the snakes' willingness to feed, blindness strongly compromised the animals' ability to locate and effectively strike prey. Thus, in a "normal" situation where vision was important in detecting and capturing prey, there would likely be a strong fitness decrement to impaired vision. On Carnac Island, however, the availability of an abundant sessile food source (seagull chicks) enabled snakes to forego hard-to-capture prey items (mice, lizards) and limit their diet to more-easily-captured prey.

Blindness and prey targeting ability

A sudden loss of vision severely impaired the hunting abilities of Tiger snakes and especially, their capacity to target and kill mobile prey. The delay necessary to kill mobile prey dramatically increased when snakes were "blinded". For example, the time taken to initiate a first strike increased 20-fold, and the time to successful envenomation increased 28-fold. These results support other studies showing a decreased prey-targeting ability by snakes after their eyes were covered (Naulleau 1965; Chiszar *et al.* 1981; Kardong 1992; Haverly and Kardong 1996). Observations using normal snakes, as well as congenitally totally or unilaterally blind snakes, have demonstrated that prey movements detected by visual as well as infrared senses are important to elicit hunting behaviour, and for snakes to localise and capture their prey (Drummond 1985; Kardong and Mackessy 1991; Haverly and Kardong 1996; Lindberg *et al.* 2000; Grace *et al.* 2001). Wild mice or lizards presumably would pose an even more challenging targets than did the naive laboratory rodents

used in our study (Amo *et al.* 2003); in the field, mobile prey likely would evade the strike of blind snakes.

Blindness and post-strike feeding behaviour

Despite their reduced striking abilities, blinded snakes were able to inject a lethal dose of venom and to swallow their prey. Interestingly, there was no significant difference in the percentage of prey held after a bite in control *versus* blinded animals. Similarly, a lack of visual and infrared senses decreases the capacity of rattlesnakes to find and kill prey, but does not alter their ability to relocate and swallow prey after a lethal strike has been delivered (Haverly and Kardong 1996). Tiger snakes that held their prey after the initial strike did so over a shorter time when they were blinded, perhaps because the initial strike was less precisely targeted and the mice managed to escape more often. Nonetheless, any prey item that was seized was almost certain to die and be consumed. The only major foraging obstacles for blind Tiger snakes are to locate and to strike their prey.

Immobile prey items are mostly found (or relocated after a strike) using chemosensory cues (Naulleau 1965; Chiszar *et al.* 1981 1990; Furry *et al.* 1991; Haverly and Kardong 1996). On Carnac Island, gull chicks are very abundant (Dunlop and Storr 1981) and remain virtually immobile even if a snake tongue-flicks them (pers. obs.; <http://www.monalisa-prod.com/>). Hence, although the entire pre-strike feeding sequence is prolonged in blind snakes (at least in the laboratory), the situation still allows snakes to feed effectively on this abundant and immobile food source. Further work is required to clarify the (chemical?) cues by which blind snakes locate gull chicks.

Feeding preferences

We found one lizard and one mouse as prey of blind snakes on Carnac Island (Table 3), suggesting that blind snakes were still prepared to consume such items if they could capture them. In our laboratory experiments, blind Tiger snakes fed readily on mice. Thus, the scarcity of mice and lizards in the diet of blind snakes in the field suggests that mobile prey escape capture, rather than

any active selection against such items. Tiger snakes in captivity are renowned for their willingness to take dead (even, decomposing) prey (Fearn 1993) and also do so in the field. On Carnac Island, we collected two maggot-covered prey items from the stomachs of two different snakes. Similar scavenging behaviour has been recorded in other species of snake that usually hunt mobile prey, and olfaction seemed to be the overriding sensory modality used for carrion detection (see DeVault *et al.* 2002 for a review). These observations reinforce the notion that prey movement is not an obligatory trigger for prey recognition, even for snake species that usually hunt mobile prey.

Our laboratory data suggest that the only consequence of imprecise prey targeting by blind Tiger snakes feeding on seagull chicks would be a delay between prey location and prey swallowing. Even if prey movement is important in stimulating Tiger snake feeding behaviour under normal circumstances, a switch to chemical modalities for prey location and to a diet of sessile chicks (Lankford 1989) allows blind Tiger snakes to feed and survive at close-to-normal rates. Such a shift may extend to other behaviors also: for example, blind Tiger snakes tongue-flick intensively when they detect the presence of human observers (pers. obs.).

In conclusion, our results clarify the mechanisms underlying the extraordinary survival of the blind Tiger snakes of Carnac Island. Blindness substantially impedes a snake's ability to locate and capture mobile prey, but in a habitat where sessile prey are readily available (such as occurs at Carnac Island), this apparently major impediment does not translate into any major fitness decrement. The extraordinary flexibility of Tiger snakes allows them to compensate for loss of this major sensory modality by modifying foraging tactics and thus, diet composition.

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Table 1: The effects of blindness on hunting ability.

Ten captive adult Tiger snakes were tested over 58 trials with live mice. For each individual snake, mean values were calculated for each trait over on average 3.5 ± 0.85 control tests and 2.3 ± 0.82 “blind” tests. Wilcoxon matched-paired tests were run using those means per individuals. “Strike time” = time elapsed from prey introduction to the first strike delivered by the snake (either missed or successful); “Envenomation time” = time elapsed from prey introduction to the first successful strike; “paralysed prey” = time elapsed until the prey stopped moving; and “dead prey” until respiratory movements are no longer visible. “Death time” = time elapsed between the first successful bite and the death of the prey. “Holding time” = duration for which the prey was held in the snake’s mouth.

Traits	Control	“Blind”	N; Z	P
Prey body mass (g)	27.86±2.42	29.03±3.21	10; 1.07	0.29
Strike time (s)	12.65±8.44	256.27±527.33	10; 2.60	0.009
Envenomation time (s)	16.54±10.54	467.10±705.55	10; 2.00	0.009
Paralysed prey (s)	128.56±28.57	360.40±263.43	6; 2.20	0.028
Dead prey (s)	145.10±25.28	871.17±1202.82	7; 2.37	0.018
Number of missed strikes	1.10±0.68	3.90±3.65	10; 2.19	0.028
Number of successful strikes	2.58±1.41	1.86±0.80	10; 1.58	0.11
Death time (s)	117.81±27.82	176.27±89.82	7; 1.52	0.13
Holding time (s)	80.08±37.12	37.19±34.58	6; 1.99	0.046
% Prey held	41.66±30.68	48.33±41.90	10; 0.42	0.67

Table 2: Prey items recorded from alimentary tracts.

Data were gathered on free-ranging Tiger snakes on Carnac Island (Western Australia), with special reference to whether the prey were sessile (sea-gull chicks eaten soon after hatchling) or mobile (lizards and mice). Data on visual status indicate whether both eyes of the snakes were intact (“Normal”), one eye was destroyed (“half-blind”), or both eyes were destroyed (“blind”). The numbers in italics indicate analyses restricted to adult snakes.

Visual Status	Sessile Preys	Mobile Preys	Total
Normal snakes	156 (72%)	60 (28%)	216
	<i>156 (80%)</i>	<i>39 (20%)</i>	<i>195</i>
Half-blind snakes	21 (95%)	1 (5%)	22
Fully blind snakes	18 (95%)	1 (5%)	19

C. Do quiet places make animals placid?

[Bonnet X, Aubret F, Lourdais O, Ladyman M, Bradshaw D and Maumelat S 2004. Do “quiet” places make animals placid? Island *versus* mainland Tiger snakes. *Ethology*, in press.]

Various alternative anti-predator tactics contribute to an animal’s self-preservation, such as size, speed, fortification, crypsis, or aggressive displays for example. It is intuitive that distasteful, highly toxic species, or species that possess potent venom (e.g. snails, nudibranchs, insects, spiders, amphibians), should be advantaged if they can clearly advertise that they are dangerous or unpalatable (Goodale and Sneddon 1977; Brodie and Brodie 1980; Gittleman and Harvey 1980; Sillén-Tullberg 1990). Threatening coloration is often based on bands of contrasting colours (Smith 1975; Brodie and Janzen 1995), while anti-predator displays include raised postures, indicating willingness in some species to deliver their venom should a threat persist (Whitaker *et al.* 2000).

All of these anti-predator tactics serve as a warning to predators of the potential risk associated with the coveted prey and the same tactics have arisen independently many times across different lineages. Within squamate reptiles, venom has become highly derived and very toxic and almost all venomous snakes engage in an array of anti-predator repertoires to advertise their toxic disposition. In snakes, intimidation is a major and varied behavioural trait. Many species produce a loud hissing sound by blowing air through the glottis (e.g. *Bitis*), and make other noises to indicate their agitation, such as scraping their body scales against each other (*Cerastes*) or rattling their tail (*Crotalus* - Fenton and Licht 1990). Others have evolved bright and contrasted colours (*Micrurus*, *Elapsoide* - Brattstrom 1955; Jackson *et al.* 1976; Greene 1988; Brodie 1993a, b). The great majority of large species also raise a portion of their body off the ground and flatten the neck (Greene 1979; Chippaux 2001; Rasmussen *et al.* 1995; Whitaker *et al.* 2000). Intuition suggests that this makes them appear larger and more intimidating to a potential aggressor and warns of their willingness to strike. Widely recognised among “Cobras” (i.e. *Naja*, *Ophiophagus*, *Hemachatus*, *Boulengerina*) and shield-nose snakes (*Aspidelaps*), this stance is also observed in highly venomous Colubrids (i.e. *Dispholidus*, *Thelotornis*, *Toxicodryas*) and in many Australasian large elapids (i.e. *Notechis*, *Pseudechis*, *Pseudonaja*). As well as giving a warning signal when threatened, all of these species can bite and inject lethal doses of venom (Sutherland 1992; Chippaux 2001).

Besides the variety of anti-predator tactics used by different snake species, important geographic variations in behaviours have been documented among different populations of the same species (Burghardt 1970a; Ayres and Arnold 1983; Brodie and Garland 1993; Ford and Burghardt 1993; Burghardt and Schwartz 1999 for a review). Experience during development also influences snakes anti-predator behaviours (Burghardt 1971; Hampton and Gillingham 1989; Herzog 1990). Overall, anti-predator behaviours exhibited by snakes are likely to be the result of complex interactions between genetic and environmental factors (Brodie and Garland 1993; Burghardt and Schwartz 1999).

One of the assumptions for the evolution of anti-predator displays is that they should be more developed in ecological situations where the probability of predation or harassment is high (Alcock 1993). In these scenarios there are strong selective pressures favouring the evolution of such traits. In contrast, we may expect that the absence of predation will lead to a progressive reduction, either rapidly (phenotypic plasticity) or more slowly (through natural selection if exhibiting anti-predator behaviours is costly and hence possibly counter-selected) of the expression of anti-predator behaviours. In this paper we report data that support such a hypothesis. It should be noted that a lack of predators (hence the use of the term “quiet place”) does not necessarily mean a lack of stimulation in general; for instance, non-threatening species may well be detected without triggering an anti-predator response.

We took advantage of a contrasted ecological situation to examine the propensity of snakes to display defensive when threatened: do snakes that live in a very quiet place, lacking predators, respond less to threatening situations than snakes that live in a much more hazardous place? Notably, we hypothesise that neonates (naïve snakes) will respond in an intermediate manner when compared with adults that experienced either hazardous or safe environments whilst growing. A complementary hypothesis is that anti-predator behaviour should respond over time to some degree (i.e. intensity of defensive displays) to a changing environment.

Methods

Study species and Study sites

Tiger snakes (*Notechis scutatus occidentalis*) are large and highly venomous elapids (Broad *et al.* 1979; Cogger 1992). We compared the anti-predator behaviour of snakes belonging to two neighbouring populations in the southwest of Western Australia: Herdsman Lake (31° 55' S; 115° 48' E) and Carnac Island (32° 07' S; 115° 39' E). Herdsman Lake (HL) is a 300 ha perennial freshwater swamp Nature Reserve within the Perth metropolitan area. Carnac Island (CI) is a small island (also a Nature Reserve) situated five nautical miles

off the coast of Perth. CI snakes may have been introduced to the island as little as 80 years ago (Cann 1986; archives of Department of Conservation and Land Management in Western Australia). It is also possible that the population may be (partly?) comprised of individuals that have immigrated from the mainland or nearby Garden Island (situated 1.1 nautical miles to the south) and/or have been marooned by the rising sea level 5000-7000 years ago. Whatever the case, a virtual absence of divergence between CI and HL populations (less than 0.3% in a matrix of genetic [5 mitochondrial genes, 4825 base pairs] distances between CI and other Western Australia mainland, including HL, Tiger snakes; Scott *et al.* 2001; Keogh *et al.* 2005; Keogh pers. com.) makes them a suitable system for a comparative study of anti-predator behaviour.

Adult CI snakes have no natural or feral predators and do not suffer at all from human harassment. The presence of human beings on CI is exceptional (Category 'A' reserve with restricted access to the general public). In contrast, Herdsman Lake is an intensively managed recreation reserve utilised by the general public every day. The reserve is dissected by many pathways and boardwalks utilised by walkers, cyclists and joggers alike, all of whom impact the Tiger snake population either directly or indirectly. HL snakes also suffer predation from birds such as the Swamp Harrier (*Circus approximans*), Australian Little Eagle (*Hieraaetus morphnoides*) and the introduced Kookaburra (*Dacelo gigas*), as well as cats and dogs (pers. obs.).

The present investigation is based on results gathered from adults and neonates both in natural conditions and raised in captivity. This allows a greater range of comparisons (Arnold and Bennett 1984) than is possible from experiments conducted on neonates only (i.e. Mori and Burghardt 2000). Although the anti-predator behaviour of most snakes' species is logistically very difficult to study in the field (Shine *et al.* 2002), population density is very high in both sites (HL and CI; Bonnet *et al.* 1999, 2002a; Aubret *et al.* 2004b) enabling behavioural observations on a reasonable number of individuals in standardised situations.

Anti-predator behaviours displayed by the snakes

Despite their venom toxicity, individuals generally attempt to flee when threatened (Whitaker and Shine 1999; this study). However, snakes that are continually harassed (i.e. during manipulations) will embark on an impressive display of swaying and neck flattening that culminates in repeated strikes with an abundant release of venom. Components of this anti-predator display are easy to quantify (see below). These behaviours were progressive and typical of those normally observed in this species (Johnson 1975).

Experimental design

In November – December 1999 and 2000 we collected data on 42 CI snakes and 32 HL snakes immediately in the field to ensure that snakes did not become accustomed to capture (Fitch 1975; Greene 1988). The snakes were located by sight when basking under the sun and were caught by hand. The time elapsed between snake localisation and the onset of the test was less than 30 seconds. Investigations were conducted between 07:10 to 13:00hrs. We chose not to over-disturb the snakes before behavioural tests (that lasted 3 minutes on average from capture to completion: see below); consequently we did not record the snakes's body temperatures. However, complex effects of temperature on anti-predator tactics have been documented in snakes (Hertz *et al.* 1982; Arnold and Bennett 1984; Bennett 1984; Hailey Davies 1986; Goode and Duvall 1989; Schieffelin and De Queiroz 1991; Keogh and DeSerto 1994; Passek and Gillingham 1997; Brodie and Russel 1999; Whitaker and Shine 1999; Cooper 2000; Shine *et al.* 2000, 2002; Mori and Burghardt 2001; Webb *et al.* 2001). Hence, we were careful to conduct experiments only in moderate ambient temperatures (air temperature fluctuating between 15°C to 25°C, usually 20°C) when Tiger snakes are normally active and visible (during basking, foraging, and mating). Nevertheless, we do acknowledge a potential effect of difference in body temperature amongst the snakes tested. For instance, although snakes rapidly reach their preferred body temperature during basking and avoid overheating by ceasing basking (Peterson *et al.* 1993), it is possible that the later snakes tested were warmed because they may have required less time to reach

such preferred body temperature compared with the snakes caught earlier during the day. Importantly, the potential effect of various body temperatures on anti-predator behaviours was relatively equally distributed between CI and HL individuals as we collected the data over the same time in the two study sites (on average at 8:50h am [7:10 – 11:00] on CI *versus* 9.32h am [8:30 – 13:00] on HL, the lag between the two sites is due to the fact that CI is a completely open [hence sunny] area compare to the relatively forested [hence more shady] HL area). Regardless, such time-lag cannot explain our results, as all CI snakes were all docile, whilst all HL snakes displayed marked anti-predator behaviours and very often flattened their neck (see results). Hence even the late CI snakes (presumably “warm”) remained docile, whereas even the early HL snakes (presumably “cool”) displayed vigorously.

Following experimental tests, we recorded sex, snout-vent length (SVL), and the body mass of each individual. Each individual was marked by scale clipping and then released (no individual is represented more than once in our data set). In every case, the first reaction of the snake to our presence was to try to escape. For our purposes it was thus necessary to place the snake in a critical situation to elicit anti-predator behaviours (Prior and Weatherhead 1994).

Experiment 1: Harassment in an Enclosure

Immediately after capture, each snake was placed in a small portable enclosure that remained open at the top (L-40 cm, W-36 cm, H-30 cm, the walls being totally opaque). Within the enclosure a small shelter was made available, under which the tested individual could easily seek refuge (L-22 cm, W-17 cm, H-6 cm). When placed into the box, snakes had several alternatives: 1) to seek refuge, 2) to remain immobile, 3) to attempt escape, 4) to display anti-predator behaviour (flattening of the neck and raising part of their body in a “cobra” posture), 5) to try to strike at the observer, 6) or a combination of these. Each snake was left in the enclosure for one minute with observers visible, but not intrusive (standing approximately 10 cm from the portable enclosure and as motionless as possible). Two observers were assigned to a specific rôle: one observer (always the same) handled the snakes whilst the other observer

recorded the data. As the behaviour exhibited by the snakes was obvious and easy to quantify, no conflict arose in their identification between the observers. When individuals escaped they were immediately placed back in the enclosure. For a further 30 seconds, snakes were placed under the shelter and were harassed by moving a stick 20 cm in front of the opening. The stick had a padded end making it an obvious target for the snake and allowing bites without injury. We recorded the number of strike(s) and bites(s) delivered to the stick over the 30-second period (flat-neck displays were invisible). A snake under a “safe” place (i.e. a shelter) will not try to escape or to bite unless the level of stress forces it to take major risks such as striking the aggressor and leaving the shelter. We interpreted escape attempt and/or strikes as strong (i.e. desperate) reactions compare to the relative immobility of snakes that remained under the shelter.

Experiment 2: Harassment whilst restrained

Immediately after experiment 1 (thus, for each individual, experiment 2 immediately followed experiment 1), the same snakes were removed from the enclosure and were placed on the ground in an open area. They were gently immobilised by placing one foot lightly across the body, approximately one third of the body length from the head. The pressure was moderate and the snake was able to move laterally and vertically, but was not able to escape restraint. Although harmless to the snake, such stimulus was intensive enough to elicit bites. As a further stimulus to provoke an anti-predator display we presented the snake (still underfoot) with the stick consistently and continually over a 30-second period. As well as striking and biting the stick, other observed anti-predator behaviours included biting the boot under which they were restrained (in the analyses we pooled together bites on the boot and bites on the stick), flattening the head and raising the fore-body off the ground.

No snakes were injured, and all individuals were immediately released at the exact place of capture. In the course of this study, the perturbation caused to the snakes was always brief, and many individuals were seen basking in the sun very close to the place of capture a few hours, to a few days, following the

experiments. The Animal Ethics Committee of UWA approved all procedures (Project 01/100/177) and the Department of Conservation and Land Management (WA) issued fauna collection permits (Permit #CE000347#).

Anti-predator behaviours of neonates

To better identify the determinants (i.e. genetic factors in interaction with learning processes, genetic factors being involved in learning itself; Burghardt and Schwartz 1999) of anti-predator behaviours exhibited by two different populations we compared the anti-predator behaviours of naïve individuals: newborns. For this purpose, we captured 13 pregnant females (5 from CI and 8 from HL) that were kept in the laboratory until parturition in a controlled temperature room (27°C by day and 20°C by night). They were housed in individual cages (50x40x30 cm transparent plastic boxes) with water dish, shelter, and paper towel paper as substrate. A heat source provided basking opportunity four times a day (4 x 15 minutes/day, High pressure Na⁺ lamp; 600 Watts). Water was available at all times and food offered approximately every 3 weeks. We obtained 80 neonates (mean, S.D.: 6±5 per litter) for behavioural tests. Neonates were weighed soon after birth with an electronic scale (body mass; precision ± 0.1 g), measured in snout-vent length and total length (precision ± 0.5 cm) and sexed by eversion of the hemipenes. They were housed in individual plastic boxes (20x10x5 cm) for the duration of the experiment, under the same conditions as the females.

Tests were conducted no more than 12 hours after birth. Each neonate was restrained in the middle of the body using a stick wrapped with foam to prevent injury to the snakes. We moved a pen (13 cm long and 2 cm of diameter, white in colour) toward the snake's head, trying to elicit a bite by moving the pen a few cm in front of it. The total number of bites elicited was scored for a 30 seconds period (the same time period as for the "harassment whilst restrained" inflicted on adults in natural situations on CI and HL). We did not perform the whole series of tests on neonates so as to avoid excessive stress as neonates are sensitive and may become anorexic in captivity.

However, we also counted the number of flat-neck displays after 3 months of

captivity at the completion of this experiment (see below). All behavioural tests on the neonates were carried out in the laboratory at 27°C; the average body temperature chosen by both HL and CI snakes in captivity being 26.8±0.7°C (Ladyman and Bradshaw 2003).

Changes in neonate anti-predator behaviours over time

We examined if the snakes are able to modify their anti-predator behaviour over time and in face of different environments (Herzog and Burghardt 1986, 1988a; Herzog *et al.* 1989). Among the 80 neonates, 58 were kept in the laboratory in individual cages (food and water was provided) and tested 10 days, one month and 3 months after birth. The neonates were regularly manipulated for measurements, being disturbed several times a week. To evaluate the influence of captivity, we captured in the field 10 neonates from unknown mothers three months after the parturition period and kept them in the laboratory for behavioural tests. Those 10 snakes had never seen any human before capture, and the absence of scars on their body suggest they had not been injured by any potential predator. As above, the total number of bites elicited over 30 seconds was scored, and behavioural tests were carried out in the laboratory at 27°C. All of these 10 young snakes came from CI, because 3-months old wild HL snakes escaped capture. We acknowledge that the lack of testing of such wild-caught young HL animals led to an incomplete design for this specific comparison (Herzog 1990). Nonetheless, results (see below) suggest that this did not invalidate entirely an assessment of the changes in neonatal anti-predator behaviours over time.

Does captivity influence the anti-predator behaviours of adults?

The anti-predator tactics adopted by adults may vary also in response to different situations. We recorded strike number as an index of the anti-predator attitude of adult CI and HL snakes caught in the field and kept in the laboratory, either in individual cages (under the same housing conditions as were the pregnant females) or in outdoor enclosures. Four HL, eight CI and

another 7 CI snakes were housed in three identical pits within the enclosures. Pits were two metres in diameter and one metre deep with sand substratum, and featured three large shelters (breeze blocks), a large water dish and one underground shelter. Hence, shade or cool were always available to the snakes. Direct sunlight provided basking opportunities for several hours a day. Captivity represented a radical change of environment for all snakes with a smaller, defined space available and continual disturbance during husbandry procedures (on average the number of snake/human encounters was 6 per month, for events such as weighing etc.). Overall, 55 adult snakes (15 from CI and 40 from HL) were maintained in captivity for prolonged periods. After at least 2 months of captivity (128 ± 91 days in average) we simply recorded the number of strikes elicited in 30 seconds at an ambient temperature of 27°C.

Statistics

Some variables were normally distributed (i.e. SVL; all Shapiro-Wilk tests were non-significant) while others were not (i.e. wild adult CI snakes did not display any “flat-neck” leading to a suite of null scores). Consequently, we used both parametric (ANOVAS) and non-parametric tests (Mann-Whitney U-test). Our sample sizes were not always large, thus we used the adjusted z-score (Siegel 1956). Slight variations in sample size occurred, for instance because we decided to restrain the snake under the boot only after the eighth CI specimen tested. Although Mann-Whitney U test is often considered as the most powerful (or sensitive) nonparametric alternative to the *t*-test for independent samples, we also used the Wald-Wolfowitz runs test to better assess the hypothesis that the two populations differ in some respect (i.e. not just with respect to the mean, but also with respect to the general shape of the distribution). When using Mann-Whitney U or Wald-Wolfowitz runs tests led to similar results only the former were presented. For the analyses based on the neonates kept in captivity, we used a mixed model ANOVA procedure with the mother as a random factor to take into account possible maternal pseudo-replication effects on neonates’ behaviour. Changes in anti-predator behaviours over time on the same individuals (captive neonates) were analysed through

repeated measures analyses of variance with snake's origin (CI or HL) as between-subjects factors and the number of strikes as the dependent variable (Greenhouse and Geisser 1959; O'Brien and Kaiser 1985; Forsman 1996). Mean values are presented with their SD; P values were two-tailed. Statistics were carried out with Statistica 6.0 software (Statsoft 2000).

Results

Body size: comparisons between the two populations

Body size of all snakes tested is given in the Table 1. On average, CI snakes were larger than HL ones (ANOVA with provenance as the factor and snout-vent length as the dependent variable: $F_{1,72}=33.3$; $P<0.001$) and heavier when the effect of size was taken into account (ANCOVA with SVL as a covariate: $F_{1,69}=11.34$; $P=0.001$; Table 1). Males attained larger body size than females from the same populations, but there was no difference in the degree of sex dimorphism between the two populations (two factor ANOVA with provenance and sex as the factors, and snout-vent length as the dependent variable; effect of provenance: $F_{1,70}=29.1$; $P<0.001$; effect of sex: $F_{1,70}=31.4$; $P<0.001$; interaction: $F_{1,70}=1.3$; $P=0.26$).

Influence of body size and sex on anti-predator behaviours

Despite the size difference between HL and CI snakes, the size of both females and males of the two populations largely overlapped; hence it was possible to compare CI snakes and HL snakes in a straightforward way. A generalised mixed model ANOVA with behaviour as the dependent variable, sex (fixed factor), SVL (covariate) and population (CI *versus* HL as a random factor) failed to detect any effect of sex or SVL (all $P>0.40$) without interaction between them (all $P>0.05$). When sex and population were considered separately, we found no effect of body size or sex on the anti-predator traits we observed (all ANOVA with sex as the factor, correlation with size as the independent variable, or ANCOVA to take both into account, gave P values > 0.05 ; Figure 1).

The correlations between these variables were almost always positive however, suggesting that if a larger sample had been used, we may have found a positive influence of size on anti-predator behaviour. The mean sample size required to have obtained a “significant” effect (with $\alpha < 0.05$ and $\beta < 0.1$) was 324 ± 359 (range 31-1046; N=8 correlation analyses), suggesting that the effect of body size, if any, was probably slight.

Anti-predator behaviour when the “predator” is close to the snake

When snakes were placed into the box and observed, but not disturbed (Figure 2), HL snakes spent more time in the open than did CI snakes which hid under the shelter more often (Mann-Whitney U test: adjusted $Z = -3.88$; N=42 and 32; $P < 0.001$; Figure 2-A). HL individuals also attempted to escape more frequently than CI snakes, despite our obvious presence (4.1 ± 3.2 versus 1.1 ± 1.7 ; Mann-Whitney U test: adjusted $Z = -4.37$; N=42 and 32; $P < 0.001$; Figure 2-B). HL snakes struck spontaneously more often than CI snakes (Mann-Whitney U test: adjusted $Z = -2.08$; N=42 and 32; $P = 0.038$; Figure 2-C). Seventy one percent of HL snakes also displayed a flattened neck but CI snakes never showed flattened neck during the tests (Mann-Whitney U test performed using a score for each individual, not percentages: adjusted $Z = -6.43$; N=42 and 32; $P < 0.001$; Figure 2-D). Using the frequency of display per unit time out of the shelter, since flat neck displays were invisible when snakes were under the shelter, did not change the result as CI snakes never displayed with a flattened neck.

Anti-predator behaviour when the snake is under shelter and harassed

When placed under the cover of the small shelter in the box (Figure 3-a), all snakes tended to remain sheltered. However, HL snakes were more prone to bite the stick moving in front of them (Mann-Whitney U test: adjusted $Z = -4.82$; N=41 and 29; $P < 0.001$); and, despite their relatively smaller size (that provides them more room in the shelter), they came out of the shelter more often when compared to CI snakes (only one CI snake [2%] came out of the shelter versus 17 HL snakes [59%]; Mann-Whitney U test: adjusted $Z = -5.18$; N=41 and 29; $P < 0.001$).

Anti-predator behaviour when snakes are restrained

When restrained under the boot (Figure 3-b and 4), HL snakes bit the boot and the stick they were harassed with more often than CI snakes (Mann-Whitney U test: adjusted $Z=-5.40$; $N=34$ and 32 ; $P<0.001$). Similarly, they often displayed a flattened neck whilst CI snakes did not (Mann-Whitney U test: adjusted $Z=-2.49$; $N=25$ and 22 ; $P=0.01$; Figure 4).

Anti-predator behaviour of neonates

Compared to HL snakes, CI neonates were larger (Mixed model ANOVAs with maternal identity as a random factor to take into account maternal effect and neonate SVL as the dependent variable; $F_{1,11}=15.27$; $P<0.001$) but were not in better body condition (same design mixed model ANCOVA with neonate SVL as a covariate and neonate body mass as the dependent variable; $F_{1,11}=3.02$; $P=0.11$; Table 1). However, neonates from both sites were not different in terms of number of strikes elicited by harassment (Mixed model ANOVA: $F_{1,10}=0.20$; $P=0.67$; Figure 5). Neonates responded particularly intensively to harassment with a high number of strikes toward the aggressor compared with adults (Figure 5).

Changes over time in the anti-predator behaviour of neonates

From birth to three months of age, the young snakes tended to respond more intensively (repeated measures over time: Wilks $\lambda=0.714$; $F_{4,53}=5.30$; $P<0.001$; specific effect of time: $F_{3,168}=8.65$; $P<0.001$; Figure 5). Although the exact trajectory exhibited by CI and HL neonates over the three months of experiment differed, with a regular and progressive increase in CI neonates and a more irregular increase for HL snakes (interaction between time and origin of the snake: $F_{3,168}=3.84$; $P<0.011$); the overall anti-predator behaviour of CI snakes was not less intense compared to HL snakes, a weak reverse trend being observed instead (effect of snake origin: $F_{1,56}=4.46$; $P<0.042$; Figure 5). A mixed model ANOVA with maternal identity as a random factor to take into account

maternal effect and neonate anti-predator behaviour as the dependent variable suggested that the HL and CI snakes responded with the same intensity after three months of captivity in terms of number of defensive strikes ($F_{1,6}=0.88$; $P=0.39$). Similarly, flat-neck displays were observed in the same proportion among HL and CI snakes (38.7% HL *versus* 23.1% CI snakes flattened their neck; $\chi^2=1.60$; $df=1$; $P=0.21$). In stark contrast, the 3 months old CI snakes caught in the field ($N=10$) were very placid compared to the same age snakes maintained in captivity and were reluctant to strike (mixed model ANOVA with mother identity as a random factor and snake provenance [lab *versus* wild] as the fixed factor: $F_{1,16}=18.81$; $P<0.001$; Figure 5). Only one 3-month-old wild snake among ten flattened its neck, whilst 32% of the captive snakes did (however the difference between captive *versus* wild snakes did not reach statistical significance: $\chi^2=1.95$; $df=1$; $P=0.16$).

Effect of captivity on adults

CI adult snakes maintained in captivity were larger than HL snakes (mean SVLs were respectively 84.8 ± 6.35 cm and 76.7 ± 6.32 ; $F_{1,53}=17.83$; $P=0.001$). The number of strikes observed in snakes kept in the laboratory in individual cages did not differ from the number recorded in snakes kept in outdoor enclosures (8.08 ± 8.30 *versus* 5.63 ± 6.57 respectively; $F_{1,53}=1.24$; $P=0.27$). The number of strikes elicited in response to harassment was greater in HL snakes compared with CI snakes (8.13 ± 8.16 *versus* 4.87 ± 6.29 respectively) but this difference did not reach statistical significance ($F_{1,53}=1.95$; $P=0.17$). A comparison with the results gathered in the field suggest that after a prolonged period of captivity, CI snakes tended to increase the intensity of their anti-predator behaviours whilst HL snakes showed a decrease (comparing responses recorded in the field *versus* in captivity: Wald-Wolfowitz runs test z -adjusted= 2.84 ; $P<0.005$ for CI snakes; Wald-Wolfowitz runs test z -adjusted= 1.22 ; $P=0.18$ for HL snakes); in addition the variance increased in both groups. Notably, although 7 captive CI adults among the 15 tested remained very placid (no strike), five (33%) became “very” defensive and delivered 11-16 strikes. In comparison, only one CI snake (3%) struck 11 times (the maximum observed) among the 34 individuals tested

in the field. Only 1 HL snake (3%) among the 32 tested in the field did not strike; but 11 (28%) among the 40 tested in captivity refused to strike.

Discussion

Our data reveal that even small degrees of spatial separation (<25 km) can lead to a marked divergence in the intensity of the anti-predator behaviours - from placidity to vigorous defence - exhibited by adults from two separate populations of Tiger snakes. Two alternative and non-exclusive hypotheses may explain such divergence. Firstly, a genetic difference may exist in the mechanisms that control the intensity of anti-predator behaviours, for example CI snakes may have lost the ability to flatten their neck (Mori and Bughardt 2000). Secondly, phenotypic flexibility influenced by environmental conditioning may exist in these same mechanisms (Burger 1989, 1990; Alcock 1993; Webb *et al.* 2001). During the current investigation, and despite strong divergence in the intensity of the anti-predatory defences, we nonetheless observed strong similarities between mainland and island populations.

Notably, we observed the same anti-predator repertoire in CI and HL snakes, from the systematic tendency to attempt escape prior to initial capture, to anti-predator displays after a sufficient threat was inflicted. Although the flat-neck display was not recorded in the field in CI snakes in the present investigation, we have observed it on later occasions in the field (2%; N=208 captures) and in captivity (so they must not have genetically lost the ability to flatten their neck as suggested above). Overall, our results revealed very strong inter-population and age-classes differences in the degree of the expression of anti-predator behaviours, rather than clear-cut differences in the anti-predator repertoire. Flight responses often take precedence over all other activities (Dixon 1998). It is therefore not surprising that this was a shared response between CI and HL populations (Whitaker and Shine 1999). Only when animals were faced with inescapable threats, did they revert to anti-predator strategies (Greene 1988; Prior and Weatherhead 1994). The results gathered on HL snakes and on all

neonates were also not surprising. The majority of individuals displayed warning signals before striking and biting behaviours commenced: as the intensity of our threat increased, the anti-predator attitude response intensified (Arnold and Bennett 1984; Prior and Weatherhead 1994; Whitaker *et al.* 2000; Shine *et al.* 2002). Most striking, however, was the contrasted response of CI Tiger snakes that were extremely placid. This is counter-intuitive to what was expected for of a large and dangerously venomous animal. On Carnac Island, snakes (at least the adults) have no natural or feral predators. Therefore, the CI snakes have little cause to ever become defensive (Maddox 1993; Kitchener 1996). Alternatively, diseases or parasite load may make the snakes drowsy and result in the observed docility of CI snakes. The body condition of a snake is an accurate indicator of its foraging success (Bonnet *et al.* 2001b; Lourdais *et al.* 2002), and sick snakes are anorexic. CI snakes, however, were in better condition than HL, making it unlikely that the population differences in behaviour were due to weakness or illness in the CI snakes. In addition, although HL snakes carry significant parasite loads, CI snakes are virtually parasite-free, probably due to the absence of secondary hosts on the waterless island (personal observations). Another explanation for this docility may be that CI snakes have become habituated to mild agitation, such as the continuous stimulus of the thousands of seabirds that inhabit the island; such agitation may raise the threshold to above that which launches anti-predator attacks (Herzog *et al.* 1989). In stark contrast, snakes inhabiting Herdsman Lake are confronted by many threatening situations (e.g. raptors, Kookaburra, cats, dogs...) mostly caused by humans (HL is crossed by many pathways and regularly managed, the basking zones being mown). Human/snake encounters often result in the snake being injured or killed (Herzog and Burghardt 1988b; Whitaker 1999; Whitaker and Shine 1999). Perhaps snakes accurately adjust their anti-predator behaviours to the degree of threats they experience, or to other characteristics of their habitat, such as quality and availability of shelters (i.e. the thick and dark bushes that cover CI provide safe harbours; Dickman 1992). If this hypothesis is valid we may expect that either neonates and/or adults are able to modify rapidly the intensity of their anti-predator attitude

when confronted with different environments. Data gathered during the captivity experiments support this notion.

As generally observed in snakes (Arnold and Bennett 1984), neonates from both populations exhibited intensive anti-predator behaviours with an increase in such intensity over time in captivity. However, young snakes (3 months old) caught in the field on CI were remarkably docile compared with snakes of the same age held in captivity. We do not know if the snakes that were born on CI became docile or were already very quiet at birth. Indeed, the mothers of the captive neonates were caught before parturition and may have been stressed and somehow influenced (i.e. through hormonal cues) the behaviour of their offspring during pregnancy. The influence of maternal (or incubation) environment on neonate behaviour and mobility has been documented in different taxa (Burghardt 1970b; Burger 1989, 1990, 1991, 1998; Bernardo 1991, 1996; Sorci and Clobert 1997; Sood *et al.* 2001; Webb *et al.* 2001). Whatever the case, our results show that changing environmental conditions (either intra-uterine or after birth) led to marked differences in anti-predator behaviour. This also applies to adult Tiger snakes maintained in captivity; CI and HL snakes tended to respond the same way when they were kept under similar environment. Our results suggest that some of the very placid adult CI snakes became more defensive in face of previously inexperienced harassment whilst some of the very defensive HL became more placid perhaps due to habituation. The strong similarities between CI and HL neonates and a virtual lack of genetic variance between the two populations suggest that our results probably reflect nurtured differences. However, because genetic studies sample only a very small fraction of the genome, lack of difference for the fractions sampled cannot be used to argue that there is no difference in the specific genetic basis of the anti-predatory defences. It remains possible that selection has acted on the behavioural response to threat (Garland 1988) and we do not claim any certainty in the proximate mechanisms involved in the population divergences. Although our study is based on two populations only, our captive experiments nonetheless show that the anti-predator behaviour of Tiger snakes is flexible, as documented in other snake species (Herzog and Burghardt 1986, 1988a; Herzog

et al. 1989; Burger 1989, 1990; Webb *et al.* 2001). In addition, such flexibility was expressed as expected within the CI/HL context: placid Tiger snakes inhabit a relatively quiet place whilst very responsive Tiger snakes live in a more hazardous place (such tendency is experimentally partly reversible). The CI/HL system provides an exceptional opportunity to examine the adaptive value of phenotypic plasticity in anti-predator behaviours *versus* the adaptive value of a selected behavioural trait. More generally, Tiger snakes have colonised very diverse habitats, both on the mainland and on islands (Schwaner 1991). Such diversity in the habitats is probably associated with divergence in predation threat (i.e. many islands lack predators for Tiger snakes). In the future, it would be interesting to better assess the respective influence of possible genetic divergences (for instance, although the two neighbouring populations, CI *versus* HL, were genetically-indistinguishable, they may nonetheless diverge in un-sampled alleles, for example those that are involved in anti-predator behaviour) and the influence of phenotypic plasticity on the anti-predator behaviours of Tiger snakes. This will allow us to assess more fully the long standing (i.e. Darwin already suggested that islands may well have a taming effect on animals; 1845) and largely admitted (Stamps and Buechner 1985; Shine *et al.* 2002), yet untested, hypothesis that quiet places make animals placid.

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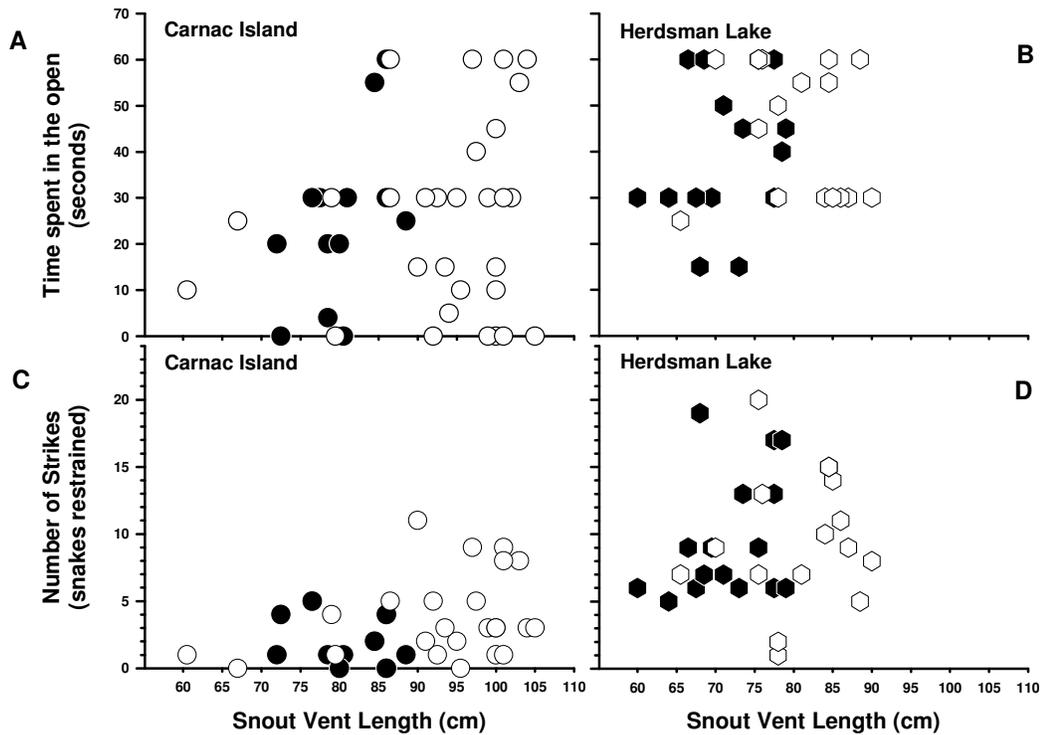


Figure 1: Body size does not influence the time that confined snakes spent exposed in a portable enclosure fitted with a small shelter (A, B; values equal to zero mean that the snake hid itself immediately), nor does it influence the number of strikes delivered towards the observer whilst snakes were restrained underfoot (C, D). This relationship was consistent when data were pooled or when sex (black symbols represent females, open symbols represent males) and population (circles represent Carnac Island snakes; hexagons represent Herdsman lake snakes) were considered separately. Black circles=females from Carnac Island; open circles=males from Carnac Island; black hexagons=females from Herdsman Lake; open hexagons=males from Herdsman Lake.

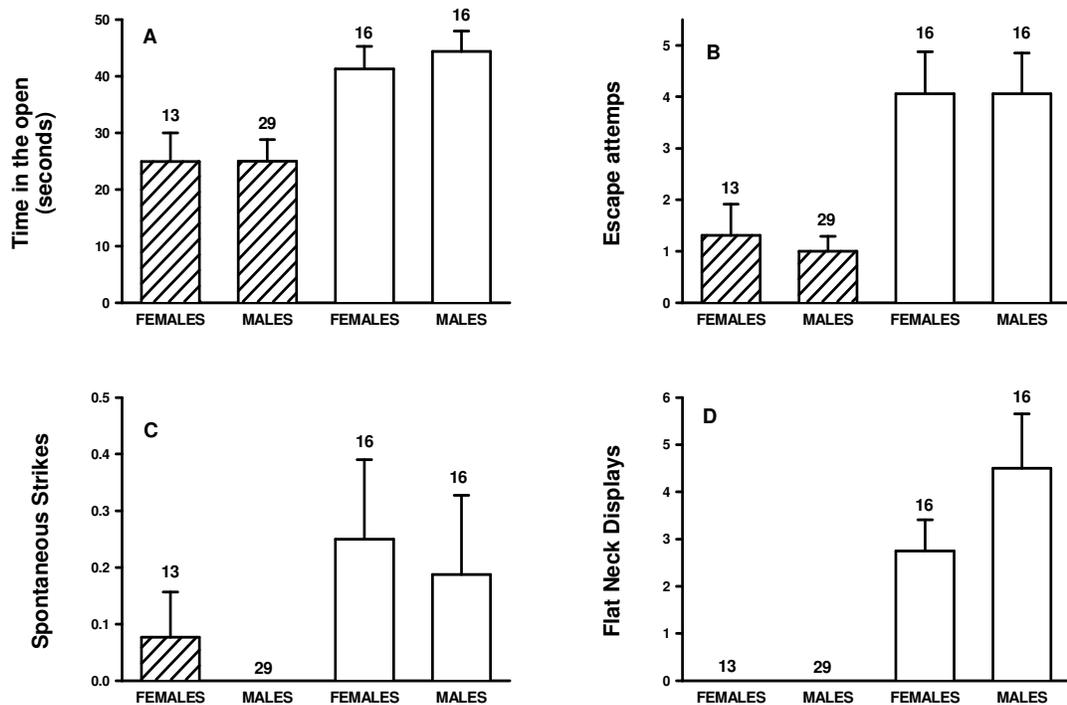


Figure 2: Tiger snakes from Carnac Island (hatched bars) and Herdsman Lake (open bars) exhibit very different anti-predator behaviours. The four graphs represent the frequency that each response type was selected in individuals from both populations. CI snakes sort refuge under the shelter more often (A); attempted escape less often (B); extremely rarely struck at observers (C, note the scale on the “y” axis); and they did not flatten their neck (D). Data are expressed as means \pm SE; the numbers indicate sample size.

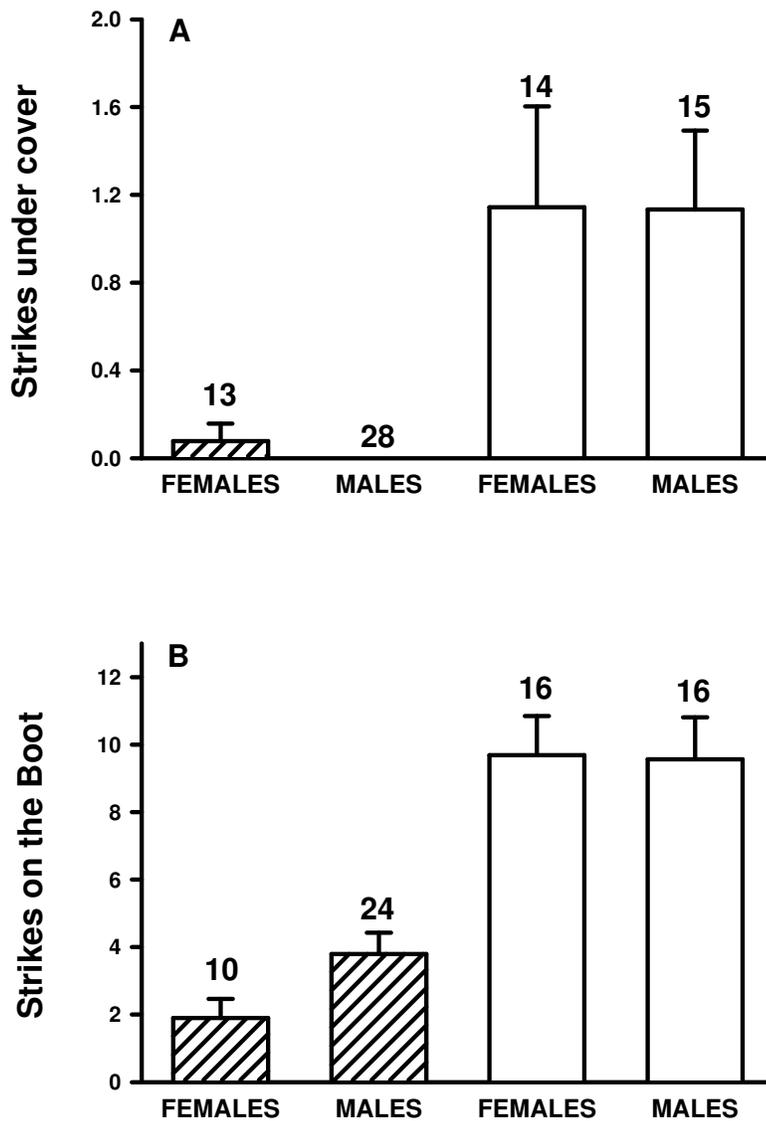


Figure 3: When snakes were placed under the shelter and harassed with a stick moved continuously in front of them for 30 seconds, CI snakes very rarely struck or bit the stick, compared with HL snakes (A); similarly, when restrained under foot and harassed for 30 seconds, anti-predator behaviours (bites on the stick and on the boot pooled) were less frequent than in HL snakes (B). CI snakes are represented by hatched bars while HL snakes are represented by open bars. Data are expressed as means \pm SE; the numbers indicate sample size.

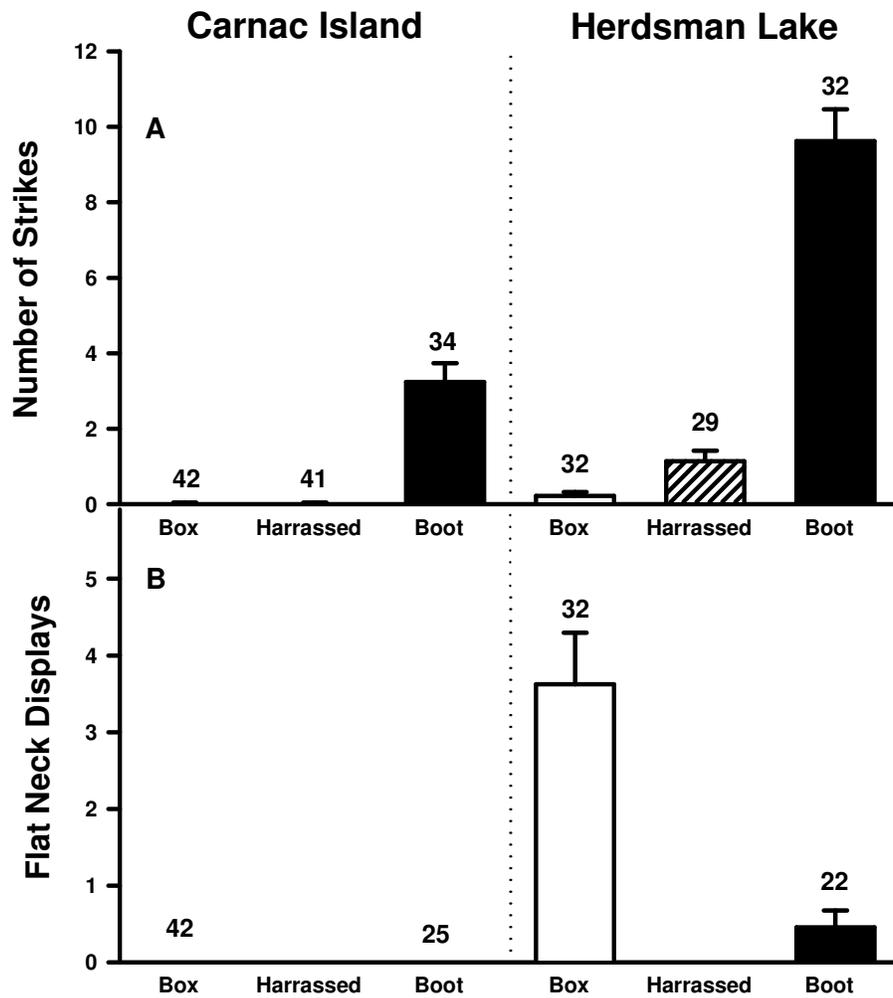


Figure 4: Anti-predator behaviours were increasingly frequent as the threat to the snakes increased (A). The threat to snakes increased as each was placed in the portable enclosure (=box), harassed with a stick when under shelter, and finally restrained underfoot. Notably the number of spontaneous strikes was very low, but many snakes bit repeatedly when kept under foot and harassed. As the number of bites increased, the number of threatening displays (flat neck) decreased (B). Note that flat neck displays remained invisible to the observer when the snakes were under the shelter. Data are expressed as means \pm SE; the numbers indicate sample size.

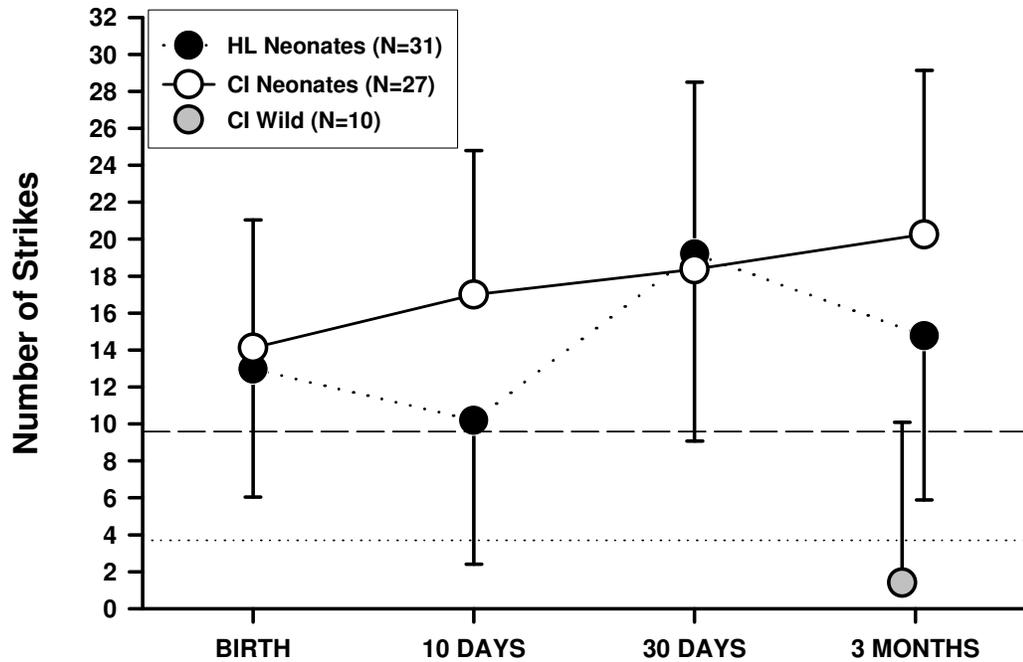


Figure 5: Neonates from CI (open circles) and from HL (black circles) responded with similar intensity (number of strikes) when harassed (see text). In captivity Tiger snakes tended to respond more intensively at three months of age than at birth. In strong contrast, same age snakes caught in the field (grey circle) were very docile. The two lines provide the average value observed in adults, from HL (dashed) and CI (dotted) respectively. Each symbol represents a mean value along with the SD. The grey symbol was artificially and slightly moved back to avoid overlapping of the error bars.

Table 1: Body sizes (SVL) and body condition (body mass adjusted to body size).

We used body mass as a dependent variable and SVL as a covariate in ANCOVAs of adult (females *versus* males) and neonate Tiger snakes from Carnac Island and Herdsman Lake. Mean values are expressed \pm SD.

Population	Sex (N)	Snout-vent length (cm) [range]	Adjusted body mass (g)
Adults			
CARNAC	Females (13)	80.2 \pm 5.1 [72.0 - 88.5]	337.6 \pm 21.3
CARNAC	Males (29)	[93.5 \pm 10.6] [65.5 - 105.0]	382.3 \pm 15.3
HERDSMAN	Females (16)	[71.7 \pm 5.7] [60.5 - 79.0]	279.4 \pm 20.2
HERDSMAN	Males (16)	[80.6 \pm 6.9] [65.5 - 90.0]	325.9 \pm 20.0
Neonates			
CARNAC	(32)	20.4 \pm 1.2 17.5 - 22.5	5.8 \pm 0.6
HERDSMAN	(48)	17.15 \pm 1.2 14.0 - 19.5	5.3 \pm 0.7

Chapter 4

Influence of diet on phenotype
and behaviour

Aims - In the previous Chapter I demonstrated that Tiger snakes invading a novel environment (Carnac Island) had adapted by developing a taste for a novel prey item. Can this novel diet be responsible for body size differences observed between CI and HL populations? In this chapter, I firstly investigate the effects of food intake on anti-predatory behaviour in relation to growth rates in juvenile Tiger snakes. Then, I investigate if the larger prey consumed by CI Tiger snakes has resulted in the selection of greater swallowing capacities, with important theoretical implications about the rôle of adaptive plasticity in the initial stages of colonisation of novel environments.

A. Plasticity in foraging behaviour

[Aubret F and Bonnet X 2004. Multiple state-dependent “decisions”: body reserves and eye opacity influence foraging in immature Tiger snakes. *Oikos*, under review.]

Whatever the scale of observation, life history traits are the phenotypic expression of the interactions between the genes and their environment. In such a broad context, state-dependent regulations of main functions (growth, reproduction etc) occupy a central place (MacNamara and Houston 1996; Gerhart and Kirschner 1997). Notably, animals are assumed to make optimal behavioural “decisions” depending upon their own physiological state (i.e. amount of body reserves) in interaction with the environment (i.e. climatic conditions - Houston and MacNamara 1999).

Recent studies have demonstrated that animals can use multiple external cues, such as visual and chemical, in an additive manner to assess predator threat (Smith and Belk 2001). This ability to respond in a gradual (optimal) way offers strong support to the threat-sensitivity hypothesis (Helfman 1989). However, to our knowledge, the other side of this concept has not yet been explored: different internal (physiological) variables may also have additive effects on anti-predator behaviors. For example, we may imagine that a potential prey can assess its own body size and its own relative musculature in an additive way to modulate its anti-predator response. Large body size along with well-developed musculature may improve fleeing speed and/or self-defensive capacities. Considering a given predatory threat, a large and strong animal may be less prone to flee, increasing the time available for foraging, compared with a small and weak individual that may be more inclined to flee.

State-dependent “decisions” of immature individuals have received little attention in vertebrates (most attention has been devoted to the processes that regulate reproduction - Bongaarts 1980; Wade and Schneider 1992; Alcock 1993; Houston and McNamara 1999). Juveniles are under strong selection to maximise survival until they reach maturity. Intensive foraging activity accelerates growth rate and facilitates earlier maturation (Congdon and Van

Loben Sels 1993), but exposes individuals to predation (Anderson 1986; Werner 1986; Lima and Dill 1990; Magnhagen 1990). As immature individuals are not involved in reproduction, we may expect that the balance between the risks and the benefits associated with energy acquisition would be one of the main factors regulating their behavioural “decisions” (Werner 1986). Despite its apparent simplicity (free from the complications associated with reproduction), the investigation of the foraging/ predation-risk trade-off in juveniles is logistically difficult. For instance, although documented in several aquatic insects and fishes (Magnhagen 1988; Pierce 1988; Lima and Dill 1990; Smith and Belk 2001), little information is available on most taxa, particularly terrestrial species. Juveniles are very secretive and even intensive long-term studies usually miss the vast majority of them (Madsen *et al.* 1999). Secretive species may nonetheless offer opportunities to study state-dependent foraging “decisions”. If food acquisition entails a marked shift in predator exposure (i.e. when animals leave their shelter to hunt), non-foraging individuals should remain hidden to minimise predation while foraging individuals would be at risk. In snakes, sight can be considered as a physiological state that varies during sloughing, independently from another physiological state: body reserves. Sight is important to detect predators, while body reserves enable individuals to withstand food shortages and to sustain growth. Hence we may expect that these two internal variables will strongly influence foraging decisions in juvenile snakes. Such a situation, where two physiological variables generate multiple physiological states, provides an opportunity to test the notion that animals can use additive internal cues to modulate their behavioural decisions. As in other ectotherms, a snake’s metabolism and energy acquisition rate are positively correlated with body temperature (Lillywhite 1987). In temperate or cool climates, metabolic increase generally requires prolonged exposure to solar radiation, a very conspicuous behaviour with a concomitant increase in predation risk (Huey and Slatkin 1976). In snakes, foraging (and the prolonged digestion that follows) and skin-sloughing (ecdysis) are two major processes directly related to growth that generate predation risks. Many snakes feed on large prey and must bask in the sun for most of the long digestion period (Slip

and Shine 1988; Sievert 1989). The presence of large prey in the stomach also impedes movement and therefore reduces escape ability (Garland and Arnold 1983; Shine and Shetty 2001a). Growth also requires periodic skin sloughing with long periods of sun basking (Lillywhite 1987). Importantly, skin sloughing is accompanied by a deterioration of sight. The detachment of the superficial layer of the skin renders the eyes completely opaque for several days (Cliburn 1976). Digestion and sloughing both increase vulnerability to predation through prolonged sun exposure. Moreover, digestion reduces locomotor ability whilst sloughing degrades the ability to detect predators. Therefore, both processes may represent a cumulative risk if they occur simultaneously. As growth can be sustained by body reserves (Ji *et al.* 1997), we may further expect that individuals in good body condition will take fewer risks than individuals in poor condition. Theoretically, a young snake with large body reserves may “decide” not to feed whilst sloughing. By contrast, a young snake with fewer body reserves may decide to feed and slough simultaneously in order to avoid a cessation of growth. To test such hypothetical multiple-state dependent “decisions” we experimentally addressed two simple questions: does skin sloughing represent an additional risk to digestion in young snakes, and do immature snakes adjust their foraging decision according to their body reserves and visual status?

Methods

We studied immature Tiger snakes (*Notechis scutatus occidentalis*), a semi-aquatic species that generally matures at a size of 65 cm (snout-vent length; Bonnet *et al.* 2002). The present investigation is based on two subsets of animals caught on Carnac Island (total area of 16 ha) located approximately 12 km off the coast of Fremantle (S 32°07'; E 115°39') in Western Australia. Forty-four neonates were used to investigate the effects of eye opacity and of the presence of prey in the stomach on anti-predator performance independently of energy stores. Another twenty-four neonates were raised in captivity under two contrasting

diets (high *versus* low food availability) to test the combined effects of food abundance and sloughing on foraging “decision”.

Neonates were obtained from 18 pregnant females caught in the field (December 2002 and January 2003), and transported in individual calico bags to the laboratory at the University of Western Australia. Pregnant females were housed individually in plastic boxes (50 x 40 x 30 cm) with paper towel as a substrate, water dish and shelter. Water was available *ad libitum*. The cages were regularly inspected and cleaned with bleach. All the snakes, adult females and neonates, were fed with previously-euthanased laboratory mice supplied by the UWA animal care unit. A heat source provided basking opportunity four times per day (4 x 15 minutes/day, High pressure Na⁺ lamp; 600 Watts). Parturition occurred from late March to May 2003. Neonates were weighed and measured soon after birth. They were housed individually in plastic boxes (20 x 10 x 5 cm) under the same conditions as the females. The relative humidity was not kept constant, and varied from 60% to 90% depending upon ambient conditions. After parturition, females were fed and released at their exact capture location. After completion of the experiment, the 9 month-old neonates were released at the site of capture of their mother. The Animal Ethics Committee of UWA approved all procedures (Project 01/100/177) and the Department of Conservation and Land Management (WA) issued fauna collection permits (Permit #CE000347). All the tests below were performed at a room temperature of 27°C.

Influence of eye opacity and stomach-content on anti-predator performance

We randomly selected 44 neonates (4 per litter among 11 litters), and allocated them to one of the four experimental groups to control for a potential maternal effect among treatments. The snakes were tested shortly after birth. Diet (dead laboratory mice) and conditions were identical. They were randomly allocated into four groups:

1. *Vision altered*: a small piece of semi-transparent tape was placed on the eyes five minutes before testing. This mimicked the opacity due to sloughing. Such manipulation did not harm the snakes as a transparent scale covers the eyes.

The snakes were tested after completion of digestion. We tested that the tape fitting mimicked the effect of natural eye opacity on seven snakes not involved in the current experiments. The seven snakes (SVL=26.64±1.43 cm; BM=16.07±3.35 g) were tested twice: during the phase of eye opacity, and soon after the completion of sloughing (Table 1). Although tape fitting and natural skin sloughing impaired the snake's ability to detect and escape predators in the same manner (Table 1 and results), we preferred to use tape fitting rather than natural eye's opacity to control for the complex physiological and behavioural modifications associated with sloughing (Lillywhite 1987). Notably, many snakes tend to be more defensive and are more prone to strike defensively when sloughing; perhaps due to a change in body temperature, to a behavioural shift linked to their diminished escape ability, or to a combination of these and other factors (Lillywhite 1987). This enabled us to focus on the vision factor more accurately, and to combine the effects of tape fitting to the presence of prey in the stomach independently from foraging decisions (see Results).

2. *Prey in the stomach*: one day prior to testing, neonates were force-fed a small mouse (approximately 25% of the snake's body mass, thus below the maximal relative prey size [$>50\%$] observed in the field; unpublished). A 24-hour delay prior to testing limited the potential effect of stress and ensured that snakes had undertaken digestion (i.e. no regurgitation occurred). Tape was applied to the eyes but removed before testing in order to standardise the levels of stress.
3. *Accumulation of handicaps*: we combined force-feeding and tape fitting.
4. *Control group*: In this group, the snakes had completed digestion before testing and, as in group 2; tape was applied to the eyes but removed before testing.

All snakes were then subjected to a set of tests to assess their anti-predator characteristics:

- **Sprint swimming speed**: neonates were dropped at one end of a swimming track (84 cm long by 12 cm wide). They immediately started to swim toward the other end. We recorded the time taken to cross the pool with a digital stop

watch. This test was done twice for each neonate and the faster time was used for analysis.

- **Terrestrial crawling speed:** a one-meter long crawling track was set up using a mix of sand and wood shavings as substratum. Neonates from each group were released at one end of the track. We recorded the time to reach the other end. Full speed was kept by stimulating the snakes' tails with a small stick.
- **Ability to reach a shelter:** the neonates were put under a small cup (10 cm in diameter) on an empty table 10 cm away from an obvious shelter. They were allowed to relax for 5 minutes. The cup was then lifted and the time taken to reach the shelter was recorded.
- **Fleeing distance/defensive strike:** The same design as above was used. This time however, a "predator attack" was simulated immediately after the cup was lifted as the experimenter slowly brought a glove toward the snake. The distance from the glove at which the snake started to flee was recorded.
- **Number of defensive strikes:** to elicit a defensive strike, it was necessary to force a snake into a situation without alternative options. Neonates were placed on the laboratory bench. Whilst crawling, they were suddenly restrained in the middle of the body using a stick wrapped with foam to prevent injury to the snakes. Whilst still restrained, a plastic cylinder was brought close to the snake's head (3 cm) to elicit a bite. The delay preceding the first bite was recorded, and the number of bites elicited was recorded for 30 seconds.

Influence of body reserves and eye opacity on foraging "decision"

The 24 neonates involved in the diet-experiment were first kept under similar conditions until all neonates regularly accepted food (euthanased laboratory mice). The 24 snakes were then randomly separated in two groups: 13 individuals were subjected over 6 months to a high food diet (HF: approximately 16% of their body mass/week), and 11 individuals to a low food diet (LF: approximately 9% of their body mass/week). The LF diet provided enough energy and materials to sustain a positive growth rate, the high food

diet allowed a higher growth rate with a concomitant elevated rate of energy storage (see results). Snakes were offered neonate mice (each weighing 1.0–2.5 g) once per week. LF animals were offered one mouse per week while the HF animals were systematically fed until repletion. Digestion was completed within 5–7 days as indicated by defecation and gentle stomach palpation. An interval of one week between meals was therefore sufficient to ensure that all the snakes were tested with an empty stomach removing any possible effect of satiation (full stomach) on their foraging decision. The prey was held close to the snake's snout to ensure meal identification by tongue flicking, and was then deposited on the substrate and left overnight. To remove the satiation effect on foraging decision, we scored the first prey as being "accepted" *versus* "refused".

Analyses

For comparisons among treatment groups, we used ANOVA when the data were normally distributed; otherwise, we used the non-parametric Median test. Although less powerful, this test is particularly useful when many cases fall at either extreme of the scale. For example, in the "fleeing distance / defensive strike" experiment, 13 neonates among 44 refused to bite. For crawling speed, ANOVA showed no significant effect ($P=0.083$); consequently we performed a series of simple ANOVA tests to compare all possible pairs of means. This actually overestimates the statistical significance of mean differences. However, the only significant effect was found when comparing the control group with the "prey in the stomach and vision altered" group ($P<0.03$, all the other P values being > 0.30).

Results

Influence of eye opacity and stomach-content on anti-predator performance

The four groups of snakes were indistinguishable in terms of size and body condition (Table 2). The alteration of their vision and the presence of prey in the stomach strongly decreased their ability to escape from predators (Figure 1

and Table 3). As expected, the deterioration of sight mostly degraded the performances in tasks where sight is essential (i.e. ability to detect a predator, Tables 1 and 3) while the presence of prey in the stomach reduced crawling speed and suppressed the flight response. Importantly, the combination of both “handicaps” often resulted in a cumulative decrease in neonate performances, notably in crawling speed, fleeing distance, and ability to reach a shelter.

Influence of body reserves and eye opacity on foraging “decision”

At the start of the experiment, the two groups of snakes were similar in size (25.85±4.09 cm *versus* 27.59±5.27 cm in high diet and low diet respectively; $F_{1,22}=0.83$; $P=0.37$; Levene's test for homogeneity of variances 0.24), body mass (12.27±7.22 g *versus* 13.69±7.19 g in high diet and low diet respectively; $F_{1,22}=0.23$; $P=0.63$; Levene's test for homogeneity of variances 0.52), and body condition (size-adjusted body mass, 13.56±1.74 g *versus* 12.41±0.64 g in high diet and low diet respectively; $F_{1,21}=1.74$; $P=0.20$).

During the experiment and excluding sloughing periods from the analysis, the 24 snakes accepted most of their meals (88.6±9.7%; $N=643$ trials) without significant difference in the acceptance rate between the two treatments (mixed model ANOVA with individual acceptance rate as the dependent variable, diet as the factor and maternal identity as a random factor; $F_{1,10}=0.60$; $P=0.44$; $P=0.10$ in Levene's test for homogeneity of variances). Consequently, the snakes increased in size (from 26.65±0.95 cm to 35.25±1.29 cm on average) and mass (from 12.93±1.45 g to 28.15±2.93 g). As expected, the snakes on the high diet grew more rapidly than those on a low diet (repeated measures ANOVA over time with body mass as the dependent variable and diet as the factor; Wilks' $\lambda=0.30$; $F_{5,18}=9.09$; $P<0.001$; specific effect of time $F_{4,88}=53.05$; $P<0.001$; interaction $F_{4,88}=20.74$; $P<0.001$; $P>0.22$ in all Levene's test for homogeneity of variances), and consequently they became heavier (34.96±3.69 g *versus* 20.11±3.45 g). Importantly, the snakes on the high diet stored larger body reserves (repeated measures ANOVA over time with body condition [residuals from the log body mass/log snout-vent length regression] as the dependent

variables and diet as the factor; Wilks' $\lambda=0.51$; $F_{5,18}=3.50$; $P<0.022$; specific effect of time $F_{4,88}=12.63$; $P<0.001$; interaction $F_{4,88}=4.38$; $P<0.003$; $P>0.14$ in all Levene's test for homogeneity of variances) and they became relatively heavier at the end of the experiment (size-adjusted body masses were 29.34 ± 1.59 g *versus* 25.73 ± 1.71 g).

In the course of the experiment, 64 sloughing events occurred. Due to different growth rates, HF snakes shed their skin more often ($N=43$; 3.53 ± 0.88 times per snake on average) compared to LF snakes ($N=21$; 2.00 ± 0.89 times per snake) (Mann-Whitney U-test= 17.50 ; $Z=3.13$; $P<0.002$). During the phase of eye opacity, the HF snakes refused their food at a rate of 78.2%. In contrast, the LF snakes accepted their meal most of the time during eye opacity: 86.1% (Figure 2). The difference between the acceptance rates was highly significant (Figure 2; mixed model ANOVA with individual acceptance rate as the dependent variable, diet as the factor and maternal identity as a random factor; $F_{1,10}=25.96$; $P<0.001$; $P=0.12$ in Levene's test for homogeneity of variances). We also observed an anecdotal, but somewhat surprising fact: two LF neonates ate their shed skin after sloughing.

Discussion

“La faim chasse le loup du bois” (XIV°)

It is a common observation that animals in strong negative energy balance (i.e. after prolonged starvation, food shortage, or body reserves exhaustion) often take important risks in order to forage (Lima and Dill 1990; Houston and MacNamara 1999 and references therein). Underlying proximate mechanisms have been partly elucidated. After depletion of fat reserves, protein catabolism acts as a powerful stimulus on foraging “decision” that overrules other functions (Robin *et al.* 1998). Ultimate explanations are straightforward: without rapid food intake, starving animals in negative energy balance are likely to die, and this may decrease their lifetime reproductive success (Houston and MacNamara 1999). For instance, long-lived animals neglect their offspring

when they reach a low body condition that may jeopardise their own survival and return to their foraging sites (Chaurand and Weimerskirch 1994).

In stark contrast, our study animals were not starving as they all showed an increase in size and mass; hence, we stress that our results are relevant to the foraging “decision” of animals in positive energy balance. The possible consequences of their foraging “decision” are related to growth rate, not to a mortality risk associated with emaciation. In addition, where previous studies explored the effect of a single current physiological variable (i.e. starvation) on foraging “decision”, our results provide a link between two independent current physiological variables (eye opacity and body reserves) and a third expected physiological status post-foraging (empty/full stomach) on foraging “decision”. The presence of prey in the stomach will provide energy for growth, but will also decrease predator escape abilities worsening the effect of eye opacity. Hence only the less-fed snakes accepted the cumulative risks of eye opacity combined with future locomotor impediment. That is, the snakes can somehow evaluate the cumulative impacts of sloughing and of an eventual digestion locomotor impairment (that both generate predation exposure) on their body reserves turn over which in turn influence their growth rate. As small snakes face greater predation risks than larger snakes, a related hypothesis is that the risk of predation in slowly-growing snakes could theoretically outweigh the risks associated with sloughing and eating at the same time. Whatever the case, snakes with limited food access (slowly growing and/or with relatively little body reserves) may accept both risks in order to accelerate growth rate. Overall, our results suggest that immature snakes are able to integrate complex effects over different time-scales. As growth rate (hence size) and body reserves strongly influence survival and reproduction in adult snakes (Bonnet *et al.* 2002), they also provide empirical evidence on the interaction between “foraging decision” and condition on the organism’s ability to survive and reproduce (recently, Houston and MacNamara [1999] emphasised the need for such information to give biological realism to current decision-making models).

The physiological mechanisms that underlie the behavioural shifts we observed are unknown. The two groups of snakes were tested with empty stomachs, however, removing a possible effect of satiation. Also, skin sloughing did not provoke an obligatory anorexia as slow-growing snakes were always hungry. Because well-fed snakes can utilise their body reserves for growth (Ji *et al.* 1997), temporarily postponing energy intake probably did not stop their growth. In contrast, less-fed snakes gave a high priority to growth, even at the cost of combining eye opacity with the presence of prey in the stomach.

Our study lacks field observations, and some of our results may be questionable. Tiger snakes, as many species from temperate areas, are diurnal. They spend most of their time sheltered, invisible to predators (Bonnet *et al.* 2002). Snakes are mostly at risk when they travel or bask in the sun, and birds are their main predators in our study area as in most other parts of Australia (Bonnet *et al.* 1999). When threatened, snakes flee, and only when there is no other option left do they adopt a defensive posture and eventually strike (Bonnet *et al.* 2005). Although indirect, our measurements assessed all these traits and hence they should be relevant to natural conditions.

In the future it would be interesting to identify the signals that modulate feeding “decision”. Notably we didn’t take into account the size of the meal. Relatively large prey would potentially bring high predation costs *via* an increase in basking time and a marked reduction of locomotor performance (Garland and Arnold 1983). On the other hand, a relatively small meal may not be refused because the potential predation costs may be negligible. Thus, further complex regulation of the feeding “decision” may be expected. Most snakes feed infrequently, eat large prey, can store large body reserves, and exhibit a temporary reduction of sight during ecdysis. This unique set of characteristics makes them suitable organisms for unravelling complex interactions between physiological status and the environment (i.e. predation and food availability) on foraging “decision”.

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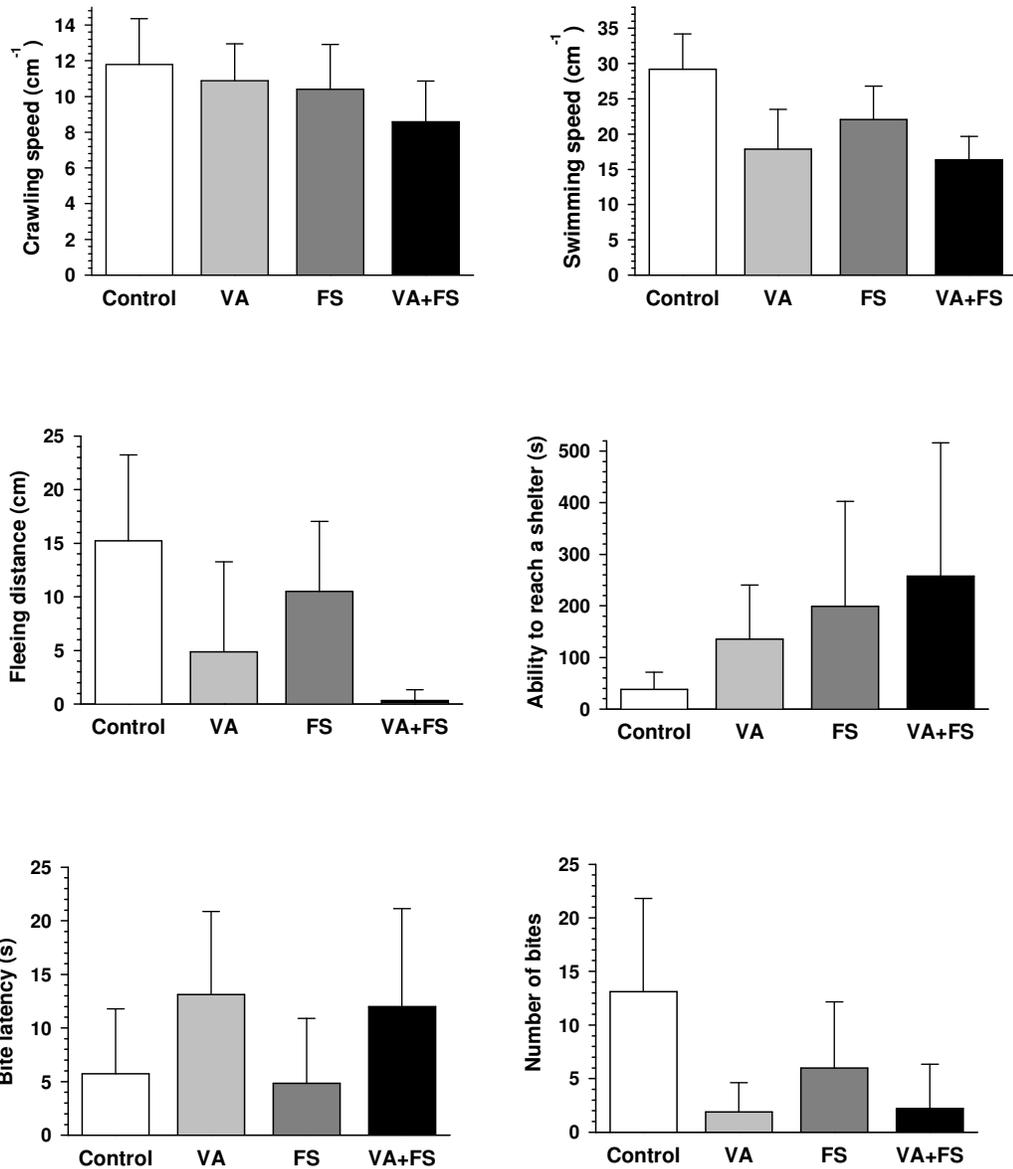


Figure 1: Influence of eye opacity and presence of prey in the stomach on anti-predator performance in neonate Tiger snakes. Vision altered (VA): a small piece of semi-transparent tape covered both eyes to mimic temporary opacity due to skin sloughing. Full stomach (FS): neonates were force-fed a small mouse one day prior to the tests. Accumulation (VA+FS): the snakes accumulated the handicaps of altered vision and a full stomach. Sample size was 11 in each group. The graphs (mean values \pm SD) on the top provide results on locomotor performances on land and in water (Tiger snakes are semi-aquatic); the two graphs in the middle provide results on fleeing ability; the two graphs on the bottom provide results on defensive strikes.

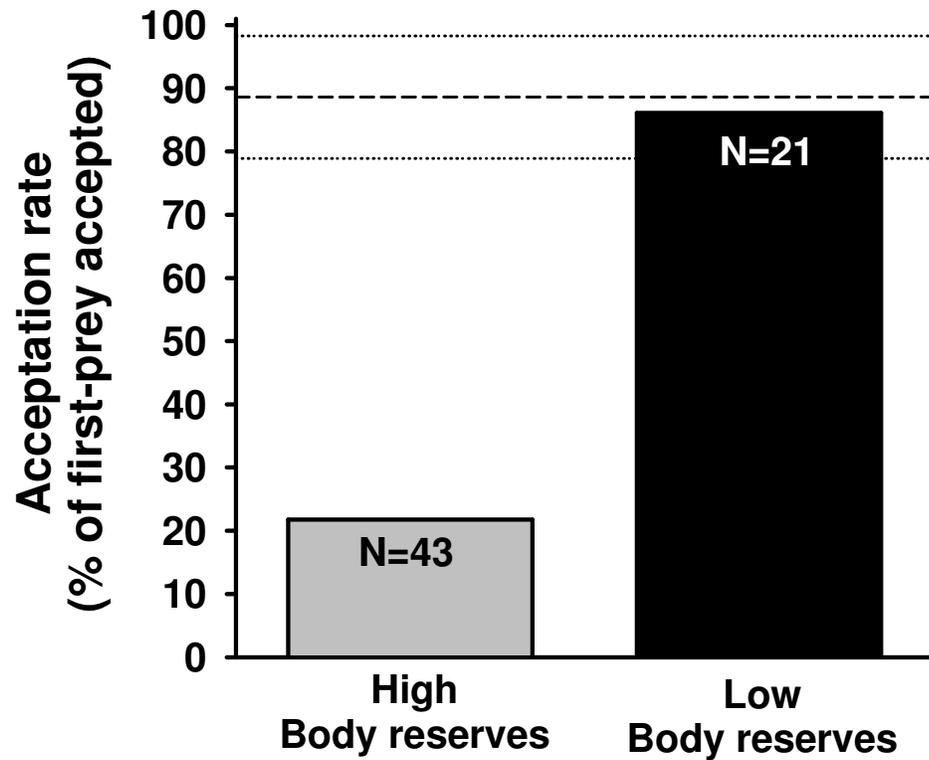


Figure 2: Influence of body reserves on foraging decision of snakes during the phase of temporary sight alteration (eye opacity during skin sloughing). Two groups of snakes were kept in captivity and fed contrasted diets over 6 months. Each group was fed once a week with standard mice. Over the long term, the snakes from the high diet group (N=13 snakes) ate more than the snakes from the low diet group (N=21 snakes) and consequently were in higher body condition (see text). Apart from sloughing periods, all the snakes accepted their meal most of the time (88.6%; N=643 trials; short-dashed line \pm 1 SD; upper and lower dotted lines), without difference between the groups. However, during the phase of eye opacity specifically, the snakes with high body reserves often refused their meal (grey bar) while the snakes with lower body reserve “decided” to eat most of the time (black bar). The number of sloughing events, during which a meal was proposed, are indicated inside the bars. All the snakes were tested with stomach empty (see text).

Table 1: Effect of natural eye opacity during skin sloughing.

Seven snakes were tested for their ability to detect a predator, to escape and to defend themselves. Each snake was tested twice: during the phase of eye opacity, and soon after the completion of sloughing. The last column indicates the *p* value obtained using a T-test for dependent samples. Mean values \pm SD are given.

	Opaque eyes	Normal eyes	<i>p</i>
Fleeing distance (cm)	0.71 \pm 0.95	13.57 \pm 9.01	0.014
Ability to reach a shelter (s)	204.55 \pm 115.21	46.10 \pm 26.19	0.010
Bite latency (s)	9.09 \pm 4.76	2.12 \pm 2.25	0.008

Table 2: Characteristics of the 44 neonates used to test the effect of eye opacity and presence of prey in the stomach on anti-predator performance.

Data were log-transformed before analysis to meet the normality assumption. Mean values (\pm SD) are given without log transformation. Body condition is expressed as size-adjusted body mass. Comparisons were performed using ANOVA (group as the factor) or ANCOVA (with snout-vent length as the covariate) for body condition. The assumption of homogeneity of variance was never violated.

	Body mass (g)	Snout-vent length (cm)	Body condition (g)
Control group	5.26 \pm 0.72	18.41 \pm 0.86	5.27 \pm 0.17
Vision altered	5.09 \pm 0.68	17.96 \pm 0.71	5.09 \pm 0.17
Prey in digestion	5.06 \pm 0.54	17.95 \pm 0.72	5.06 \pm 0.17
Accumulation of handicaps	5.02 \pm 0.47	18.11 \pm 1.30	5.01 \pm 0.17
Df	3, 40	3, 40	3, 39
F	0.31	0.66	0.12
P	0.81	0.42	0.95
Homogeneity of variance	0.32	0.77	0.32

Table 3: Comparisons of anti-predator performance of the four groups of snakes from figure 1.

ANOVA and non-parametric Median tests were used depending upon the normality in the distribution of the data. The last column indicates cases when, using post-hoc tests, a cumulative effect of eye opacity and presence of prey in the stomach was detected.

Trait	Distribution	Test	P	Cumulative effect
Crawling speed (cm.s ⁻¹)	Normal	F _{3,32} =2.45	P=0.080	P<0.05
Swimming speed (cm.s ⁻¹)	Not normal	χ ² =14.90; df=3	P=0.002	NS
Fleeing distance (cm)	Not normal	χ ² =15.49; df=3	P=0.001	P<0.05
Ability to reach a shelter (s)	Normal	F _{3, 32} =5.36	P=0.004	P<0.05
Bite latency (s)	Not normal	χ ² =10.80; df=3	P=0.013	NS
Number of bites	Not normal	χ ² =9.33; df=3	P=0.025	NS

B. Prey size, jaw size, and developmental plasticity

See next page

Chapter 5

Locomotion, environment and
life history

Aims - I have so far focused my investigations primarily on the influence of differing diet on plasticity in behaviour and growth rates. Another major difference between mainland habitat and insular ecosystems such as Carnac Island is the absence of fresh permanent water bodies. This situation can be challenging for a semi-aquatic species such as the Tiger snake that normally feeds on frogs. Locomotor performances in animals are indeed critical to fitness. In this Chapter I identify two potential sources of alteration in swimming performances: pregnancy and tail injuries. I also specifically assess the potential for plasticity in swimming performances by comparing the swimming performances of neonates and field-experienced adult Tiger snakes from CI and HL. A final experiment directly measured the potential for plasticity in locomotor performances in young Tiger snakes and the rôle of phenotypic plasticity in a major evolutionary transition from an aquatic to a terrestrial habitat.

A. Tail loss, body condition and swimming performances

[Aubret F, Bonnet X and Maumelat S 2004. Tail loss, body condition and swimming performances in Tiger snakes, *notechis ater occidentalis*. The Journal of Experimental Zoology, under review]

Many animal species rely on locomotor performances for the acquisition of resources and to escape predation (Huey *et al.* 1984; Seigel *et al.* 1987; Losos and Sinervo 1989; Domenici and Blake 1991; Garland and Losos 1994; Ojanguren and Braña 2000; Srygley and Kingsolver 2000; Voelker 2001). Partial or total absence of propulsive structures, caused by either genetic or accidental factors, should compromise both resource acquisition and survival. In order to appreciate the physical contribution of the propulsive structures *per se*, we need to measure the influence of phenotypic variations independently from the genetic quality of the individuals, impeding the use of natural variations solely. In natural conditions, this restricts the investigations to fortuitous deteriorations of locomotor structures.

Assessing the impact of accidental degradation of the propulsive structures, both in controlled conditions to quantify its effect on locomotor performances, and in natural conditions to replace such impact in a realistic context, is a complex task however, both for ethical and logistical reasons. The use of artificial loads to handicap individuals during locomotion (Srygley and Kingsolver 2000) deals with the mass of the body scaled by size or with the energy budget rather than with the characteristics of the propulsive structures *per se*. Amputation would be a straightforward way to modify propulsive structures independently from other causes (i.e. interactions between genetic and environmental factors during development), but is not acceptable. Natural amputation caused by predation or due to accidents provides a valuable alternative. Unfortunately, very few populations are likely to contain a sufficient number of amputee individuals to perform standardised analyses,

presumably due to very low survival rates associated with a decrease in locomotor performances.

Both substantial natural and accidental variations in the propulsive structures occur in some taxa, notably in limbless tetrapods (Gans 1973, 1975, 1983; Cundall 1987). The number of vertebrae (trunk and tail) and the length of the tail relative to the body are variable among species, among populations within species, and among individuals within a population (Arnold and Bennett 1988; Jayne and Bennett 1989; Lindell 1994; Kelley *et al.* 1997; Shine 2000; Shine and Shetty 2001a). Both of these traits influence locomotor performances (Arnold and Bennett 1988; Jayne and Bennett 1989; Garland 1994; Kelley *et al.* 1997).

Another important source of variation is generated by tail injuries (Jayne and Bennett 1989), which potentially offers a substitute to experimental amputation. However, the effect of relative tail loss has seldom been examined in legless tetrapods, and never studied for aquatic locomotion. A study suggested that natural tail loss had no effect on terrestrial locomotion in a natural population whereas experimental tail removal suggested that tail length was relatively unimportant until over half of the tail was lost (Jayne and Bennett 1989). Overall, the exact influence of tail integrity on locomotion is still unclear, particularly for swimming performance.

A swimming snake moves forward by accelerating portions of the surrounding water; the reaction to this effect generates the propulsive forces. Sea snakes are well adapted for underwater locomotion as they possess a paddle-shaped tail that increases the transfer of propulsive forces (Gans 1973, 1975). However, many other highly aquatic species (i.e. genus *Natrix*, *Cerberus*, *Myron*, *Nerodia*, *Acrochordus*) escape predation under water, forage on fishes, but nonetheless possess a thin and elongated tail (Boulenger 1913; Wilson and Swan 1903). Moreover, many terrestrial snakes are able to swim efficiently using their thin and elongated tail (Gans 1973, 1975), sometimes exhibiting higher maximal swimming speed compared to sea snakes (Shine and Shetty 2001b). As the paddle-shaped tail of sea-snakes is thought to be derived from a typical elongated tail of terrestrial snakes (elapids), and also because all fresh-water aquatic snakes possess a thin and elongated tail, we need more information

about the rôle of a more “primitive” elongated and thin tail on swimming performances.

The consequence of tail loss on locomotor ability in undulating vertebrates may be explained by three main hypotheses (Jayne and Bennett 1989): 1) the entire length of the tail functions similarly to the trunk, contributing actively to production of propulsive forces. Any shortening of the tail would theoretically decrease locomotor speed. 2) An inactive portion of the tail could passively interact with the undulating body to allow more favourable transfer of propulsive forces, perhaps by favourably altering the waveform of a more anterior region. The loss of the tip of the tail (which is not actively involved in the production of thrust) alters waveform and reduces swimming performance in tadpoles (Wassersug and Hoff 1985). 3) Part of, or the whole tail, could be dragged passively by the snake. The mass of the inactive portion would decrease the speed of locomotion because of increased inertia and frictional resistance. Loss of part, or all, of this inactive portion should improve performance. Although hypothesis 1 may apply to sea-snakes with a paddle-shaped tail; hypothesis 2 may be particularly relevant for aquatic lateral undulation in snakes that possess a slender and elongated tail that presumably can not push efficiently against water.

In this article, we report results on the influence of relative tail loss in a semi-aquatic snake species, the Tiger snake (elapid, *Notechis scutatus occidentalis*). If a massive tail loss entails a major locomotor impediment, it may also degrade foraging efficiency either during capture of the prey and/or during prospecting trips. In natural conditions, reduced foraging ability translates into lower than average body condition (Bonnet *et al.* 2002a), and eventually to a smaller than average body size (due to a reduction in growth rate). Reduced locomotor performances may also increase predation risks (mainly from birds; Greene 1997), although anti-predator tactics of the highly venomous Tiger snakes rely on threatening displays rather than on speed (Bonnet *et al.* 2005). We used a large population of Tiger snakes living in a swamp area, where tail loss is frequent (>50% of individuals) and often very severe (i.e. total loss of the tail). We took advantage of this situation to examine the effect of relative tail loss on

snake body size, body condition, and swimming ability. Our study reports the first data relating to the effects of accidental tail loss on individuals monitored both in natural and controlled conditions.

Methods

Study species

Tiger snakes are large and highly venomous snakes widely distributed in southern Australia (Cogger 1992). Many populations live in the vicinity of water bodies (swamps, lakes, rivers) and feed largely on frogs (Shine 1987; Cogger 1992; Bush *et al.* 1995; Wilson and Swan 2003; Aubret *et al.* 2004b). Tiger snakes can nuzzle under water beneath rocks to find their prey (tadpoles); and often flee into water to escape predation (Mirtshin and Bailey 1990). Overall, aquatic locomotion is likely to be a major component of the day-to-day activities in many populations. A large population of Tiger snakes (*Notechis ater occidentalis*; Cogger 1992) occurs at Herdsman Lake (HL) nature reserve (Perth, WA: S 31° 55' 44"; E 115° 48' 19"). A dredged moat encircling the 300 ha lake ensures permanent open water. It is composed of artificial banks and paths sometimes bordered by trees that create a mosaic of vegetation patches (mostly reeds) and a net of interconnected channels and shallow fresh waters. The diet of HL Tiger snakes, mostly composed of frogs and mice, is also typical of mainland populations (Shine 1977, 1987; Bush *et al.* 1995; Aubret *et al.* 2004b). Snakes are often spotted in the water, slowly swimming across the channels or immobile on reeds.

Morphology

Between September 1999 and June 2002, 203 adult or sub-adult Tiger snakes were captured by hand. Each snake was sexed by eversion of the hemipenes, and individually marked by scale clipping. Two individuals with total tail loss were sexed as females (e.g. presence of follicles during recaptures). Snout-vent length (SVL; length from the tip of snout to the cloacae) and total body length

(from the tip of the snout to the end of the tail) were recorded to the nearest 0.5 cm. Body mass was recorded to the nearest 1 g with a portable electronic scale (see Bonnet *et al.* 1999, 2002a for details). Body condition (size corrected body mass) is an accurate indicator of foraging success in snakes (Madsen and Shine 2000; Bonnet *et al.* 2001b, 2002b). As ectothermic organisms, snakes convert a large proportion of their food into new tissues whilst their low energy requirements avoid a rapid exhaustion of their body reserves (Pough 1980). Consequently, subtle variations in food intake among individuals translate into measurable variations of body mass and body condition (Lourdais *et al.* 2002, 2003), and allow indirect assessment of foraging success during the weeks preceding measurements. In addition, the simple morphology of snakes enables abdomen palpation in order to discard individuals with growing follicles (or developing embryos), and/or with large food items in the stomach. Inclusion of these individuals in the analyses would introduce highly biased data to the calculation of body condition (Bonnet *et al.* 2003). Dissections have shown that most of the variance in body condition index is explained by variations in the main body reserves in snakes (Bonnet 1996). We also measured jaw length, head width and eye diameter with a digital calliper (± 0.01 mm), and counted the number of ventral scales and dorsal scale rows, all these traits may reveal subtle genetic differences among individuals (Aubret *et al.* 2004b).

Tail integrity

Any loss of the tail is easily recognisable because the tip of the tail is characterised by a terminal conical scale. We adopted two different methods for analyses, depending upon the question addressed. To determine the occurrence and influence of tail loss *per se* we simply considered all individuals with any loss of the tail, even moderate (i.e. only the very tip of the tail missing), *versus* intact snakes. To examine the impact of relative tail length (tail loss severity) on other traits (body condition, swimming performances, etc) we estimated tail loss by comparing the actual damaged tail length with the expected normal tail length based on the respective regressions between SVL

and tail length based on 64 intact females ($r^2=0.93$; $F_{1, 62}=791.8$; $P<0.001$) and 93 intact males ($r^2=0.95$; $F_{1, 91}=1777.8$; $P<0.001$).

Neonates exhibited a normal tail (with the exception of a few malformations). Tail loss therefore, occurs after birth, possibly due to parasitism (we observed several snakes with a necrosis of the tail), or to predation (the abundant long neck turtles at HL may grasp the tail of the snakes). The percentage of tail missing was not correlated with body size (excluding neonates, $r=-0.14$; $N=118$; $P=0.12$). As we could not define the exact source of tail loss, we considered that tail loss was accidental whatever the exact environmental cause, diseases or injuries, possibly in combination with other unknown factors.

Swimming trials

We randomly selected a sub-sample of 60 snakes from the 203 snakes captured for the swimming trials. We covered the range of variation in tail loss observed in the field with 27 individuals with their entire tail (19 females and 8 males) and 33 snakes with a moderately to extremely damaged tail (25 females and 8 males).

The snakes were held in the laboratory before trials. Each individual was housed in an individual plastic cage between tests (40 x 40 x 30 cm; with paper towel as substrate, shelter, and water was provided *ad libitum*). The temperature of the room was set at 27°C during the day and 20°C during the night. Therefore, all experiments were performed at 27°C. The average body temperature chosen by HL snakes in captivity is $26.8\pm 0.7^\circ\text{C}$ (Ladyman and Bradshaw 2003). Food (dead mice) was offered every 3 weeks to the snakes. All snakes had completely digested their prey when they participated in the tests, as the burden of a prey may have effects on locomotor performances in general (Garland and Arnold 1983; Huey *et al.* 1984; Ford and Shuttlesworth 1986), particularly on swimming performances (Shine and Shetty 2001b).

To estimate swimming performances we used a procedure that has been validated for snakes, including Tiger snakes (Shine and Shetty 2001a, b; Shine *et al.* 2003; Aubret 2004; Aubret *et al.* 2005b). We used a rectangular raceway lined with black plastic (370 cm long, 64 cm wide and 100 cm high). The 64 cm width

was sufficient to allow normal swimming waves of the largest snake involved in the study (i.e. the amplitude of the waveform was always less than 64 cm). Water depth (40 cm) was constant in all experiments, and prevented the largest snake from touching the bottom. The different tests were separated by several days. Although video taping is best suited for studies on locomotor performances, this option was not available. Snakes were hand-timed with stopwatches (precision 0.1 s). However, special attention was paid to overcome accuracy issues: the following parameters were recorded independently by two observers located at each end of the pool (mean values were then calculated).

Burst speed: the snakes were picked up by the head, and dropped at one end of the raceway. This way of handling was threatening to the snake, comparable to a situation where the snake must escape predation, and this provoked a rapid burst towards the other end. The time taken to swim across a 300 cm section of the pool was recorded.

Total distance swum, routine speed and endurance: using a hook, the snakes were removed from their cage and slowly lifted above water at one end of the pool. Gently dropped from approximately 15 cm above the water surface, the snake started to swim instantaneously towards the other end, presumably in an attempt to flee. All snakes swam in a stereotypical manner: fast swimming along the length of pool after release and then turning back at the end of the raceway and continuing to swim to the original start position. This pattern of lapping the raceway continued. Thus, recording the number of crossings (length and width of the raceway) provided an accurate measurement of the total distance swum by each snake during the trial. During a 5 minute test (a prolonged time period for a snake in continuous movement), we recorded the total time spent swimming by the snakes (*versus* immobile) and the total distance travelled by each snake. For unknown reasons (one possibility is the stress or fear caused by the test), several snakes did not swim normally: these snakes began shaking once in the water which prevented efficient locomotion. Consequently, for several snakes, it was not possible to record the entire set of swimming tests, yielding some variations in the sample size (i.e. for 53 of 60

snakes the burst speed was accurately recorded). At least one week elapsed between the two swimming tests (burst *versus* 5 minutes test) to ensure that each snake had recovered from the first trial.

Data analyses

Our study mixed morphological (body mass, body size, etc), and physiological/behavioural (swimming speed, stamina, etc) variables that exhibited different distribution patterns. ANOVA or ANCOVA was used in the majority of analyses that compared tail-damaged groups and the interaction with sex. We tested the normality of the distributions and the homogeneity of the variances/covariances in the different groups (Shapiro *et al.* 1968). The distribution of SVL, body mass, and swimming performance data did not deviate significantly from normality in our sample (all P values ranging from 0.063 to 0.405). In contrast, the distribution of the relative tail loss was not always normally distributed (P<0.001; N= 203 all snakes pooled together; P=0.001; N=85 considering intact snakes only; P=0.092; N=118 considering injured snakes only). We repeated the analyses using non-parametric methods when necessary (Olejnik and Algina 1987). The use of parametric or non-parametric tests did not yield conflicting results, probably because the F-test is robust to deviations from normality in the absence of extreme outliers (Lindman 1974), and because the homogeneity of variances assumption is not crucial for ANOVA (Glass and Hopkins 1996).

As the snakes used in the current study were all adults, the range in body size was limited and SVL did not significantly influence swimming performances (see results). Consequently, similar results were obtained calculating swimming speed in absolute speed (cm.s⁻¹) or in relative units (body lengths travelled s⁻¹). Body condition was calculated using Log-transformed body mass and Log-transformed body length.

Results

Variations of tail length in intact snakes

For a given body size, the length of intact tail varied in both sexes (Figure 1). Although part of this variation may be due to measurement error (i.e. SVL is notoriously difficult to measure with precision in snakes, usually ± 0.5 cm), our data revealed the existence of substantial natural variations in relative tail length. Such variations were nonetheless considerably lower compared to those provoked by injuries (see below).

Occurrence and severity of tail loss

The occurrence of tail loss was extremely high in our study population, involving 118 snakes among 203 (58%). Many snakes lost a large proportion of their tail and several individuals had almost no tail left (Figure 1). Considering injured *versus* intact snakes, both sexes were affected with the same probability, ($\chi^2=0.83$; $df=1$; $P=0.77$; $N=203$). Similarly, tail loss severity was similar between the sexes (logistic regression with tail loss as the dependent variable and sex as the factor, $\chi^2=2.78$; $df=1$; $P=0.10$; $N=118$).

Does tail loss correlate with body size and body condition?

Neither the occurrence of tail loss nor the severity of the tail loss correlated with the main morphological characteristics of the snakes (Table 1, 2). Notably, intact snakes were not larger, heavier or in better condition compared to injured snakes (Table 1). This suggests that extreme tail loss did not decrease their foraging success and the associated growth and/or body reserves storage rates. Similar results were found restricting the analyses to the 60 snakes selected for swimming trials. We found a significant sexual dimorphism in SVL, body mass and body condition (Table 1), as observed in other populations of this species (Bonnet *et al.* 2002a). However, we did not find any interaction between sex and relative tail length on the main morphological characteristics of the snakes (Table 1). Focusing on tail loss severity, and therefore considering only those snakes with a damaged tail, we found no correlation between the degree of tail loss and the other morphological parameters measured (Table 2). A power analysis shows that the required sample sizes to obtain a significant effect with

both an α error of 5% and a β error of 10% were all very large, suggesting that the absence of effect was not due to the low power of our analyses (Table 2).

Swimming performances and tail loss

Pooling intact and injured snakes, we did not find any significant correlations between SVL or body condition in any of the swimming traits measured ($44 < N < 59$; all $r < 0.20$; all $P > 0.18$). Restricting the analysis to injured snakes did not change any of the above and following results.

Burst swimming speed, however, was negatively correlated with relative tail loss ($r = -0.31$; $P < 0.026$; $N = 53$; Figure 2). Relative tail loss did not influence the mean routine swimming speed ($r = -0.12$; $P = 0.39$; $N = 49$) or the mean distance swum ($r = -0.05$; $P = 0.72$; $N = 49$).

Our data allowed us to compare the endurance of the snakes over time by calculating the consecutive swimming speeds over the successive lengths of the pool travelled (see Figure 3). We divided the snakes into three discrete categories (Marti 1990): 1) intact/moderate tail loss corresponded to an average of $6.3 \pm 11.2\%$ of the tail missing; 2) strong tail loss to an average of $33.3 \pm 6.3\%$ missing; and 3) extreme tail loss to an average of $63.9 \pm 14.9\%$ missing. Repeated measures ANOVA with the speed calculated over laps as the dependent variable and the three categories of snakes as the factor revealed a significant effect (Wilks' $\lambda = 0.172$; $F_{20, 28} = 1.98$; $P < 0.047$). A closer analysis showed a very strong effect of time (snakes became exhausted very rapidly; $F_{9, 207} = 15.42$; $P < 0.001$; Figure 3), a weak effect of tail integrity ($F_{2, 23} = 4.69$; $P < 0.020$), and no interaction between these two factors ($F_{18, 207} = 0.61$; $P = 0.89$). The mean swimming speed over the 10 laps was $32.58 \pm 1.69 \text{ cm}\cdot\text{s}^{-1}$ for the intact/moderately damaged snakes, $37.15 \pm 1.98 \text{ cm}\cdot\text{s}^{-1}$ for the snakes with a strong tail loss, and $28.29 \pm 2.12 \text{ cm}\cdot\text{s}^{-1}$ for the snakes with an extreme tail loss.

Discussion

The occurrence and the severity of tail loss were extremely high in our study population, to a degree not yet documented for any snake species. The resulting level of variation in relative tail length (0-100% of the tail missing) provided a unique opportunity to combine field and laboratory data, and to explore the effects of relative tail loss on body size, body condition and on locomotor performances. These traits are directly relevant to survival in snakes (Jayne and Bennett 1990; Bonnet *et al.* 2002b), and in many animal species in general (Domenici and Blake 1991; Srygley and Kingsolver 2000; Arnold 2003). Demographic parameters of large snakes (delayed maturity, low breeding frequency) are associated with a low turn over of the adults (Seigel and Ford 1987). A low survival of tail-damaged snakes would have automatically removed them from the population, but they outnumber intact adults. Moreover, tail loss did not affect body condition, a direct indicator of foraging success (Bonnet *et al.* 2001b, 2002b) which in turn strongly influences survival (Bonnet *et al.* 2000a; Shine *et al.* 2001). Finally, tail loss was not related to body size, a deleterious effect of tail loss would have been a decreased growth rate, restricting tail-damaged snakes to small body sizes. Hence, even in the absence of long-term re-capture data to perform *ad hoc* analyses, our data suggest that tail loss did not jeopardise survival. Probably because Tiger snakes do not rely on burst swimming speed to catch their prey, tail-damaged snakes (even strongly), are presumably still able to capture their prey at a normal rate. We acknowledge that in the absence of radio-tracking data, we can not estimate the amount of time Tiger snakes spend swimming *versus* crawling during foraging activity. Their main prey, frogs (Aubret *et al.* 2004b), can be found either in the water or on the banks; and for example, tail-damaged snakes may forage more on land rather than in the water. However, such uncertainty does not alter our main conclusions. Tail-loss seemingly did not impede foraging success, did not entail a depletion of body reserves, and did not increase mortality. Nevertheless, two biases should be considered as potential errors in correlative studies such as the present one (Jayne and Bennett 1989). First, there may be

interactions between an individual's locomotor speed and the probability of body damages (i.e. slow animals are more likely to get injured). Secondly, interactions between body injuries and consequent health and survival: tail lost may decrease hunting performances and body condition. In this study however, tail-damaged and intact snakes were indistinguishable in terms of morphology, body size, body condition, and routine swimming speed. Moreover, tail integrity had only a weak effect on maximal swimming speed and endurance; and there are no avian or mammalian predators that specifically target the tail of the snakes (they target the head and the body; Greene 1997). Hence, our data enable us to discard the above caveats and suggest that tail loss had a negligible ecological impact on HL snakes. Interestingly, laboratory measurements showed that extreme tail loss decreased only burst swimming speed. The terminal part of the tail is probably important for an optimal fast-undulation of the whole body during burst swimming (Wassersug and Hoff 1985), but seemingly not for routine swimming speed. Although determining the exact contribution of the different segments of the tail during fast/routine swimming would require complementary data on the mechanics of locomotion, our study suggests that an elongated tail is not necessarily advantageous for all swimming activities, especially whilst foraging. This observation bears on the evolution of tail morphology in snakes during the transition from land to water. Sea-snakes move slowly but efficiently under water (Shine and Shetty 2001b), and they exhibit a relatively short tail compared to terrestrial snakes. For instance, the length of the tail represents less than 13.5% of SVL in *Laticauda* (sea-snake) but more than 15.5% in Tiger snakes. Our data suggest that the shortening (and flattening) of the tail of the proto-sea snakes (that presumably exhibited the general morphology of Tiger snakes - Heatwole 1999) did not preclude an improvement of the routine swimming efficiency, although it may have entailed a slight loss of burst swimming ability. Sea snakes spend a considerable amount of time swimming slowly during foraging activity (Shetty and Shine 2002; Shine *et al.* 2004b). A metabolically efficient routine swimming speed is probably more advantageous than a high burst swimming speed under such circumstances.

Despite our ignorance about the factors causing tail loss, Tiger snakes from HL provide ample opportunities to study the ecological importance of a thin and elongated tail in snakes. The HL snake population also illustrates the tremendous flexibility of Tiger snakes in the face of major injuries (and/or disease). At a short distance from the HL population, another large population of Tiger snakes inhabits a small island (Carnac Island) where approximately 7% of the population suffer from accidental blindness caused by sea-bird attacks. In both cases, blind or tail-damaged Tiger snakes survive well, forage and reproduce normally (Bonnet *et al.* 1999, 2002a; unpublished data). In both cases, drastic phenotypic variations are “accidental” but they do not entail detectable ecological consequences, questioning the notion that the observed range of “normal” phenotypes in a given population should necessarily reflect optimal values (Gans 1979).

We wish to thank the region Poitou-charentes (France), the Conseil général des Deux-Sèvres, and the University of Western Australia for funding. Special thanks to Prof Don Bradshaw and Dr. Dale Roberts. Renée Firman and Radika Ly-Yours were helpful with the English. We are also grateful to Wally Gibb, David Pearson, and Zoé Lechat for logistic assistance in the field. We thank Conservation and Land Management (WA) for the issuing of licenses and for continuous support in the course of the study (Permit #SF004604). The UWA Animal Ethics Committee approved all procedures.

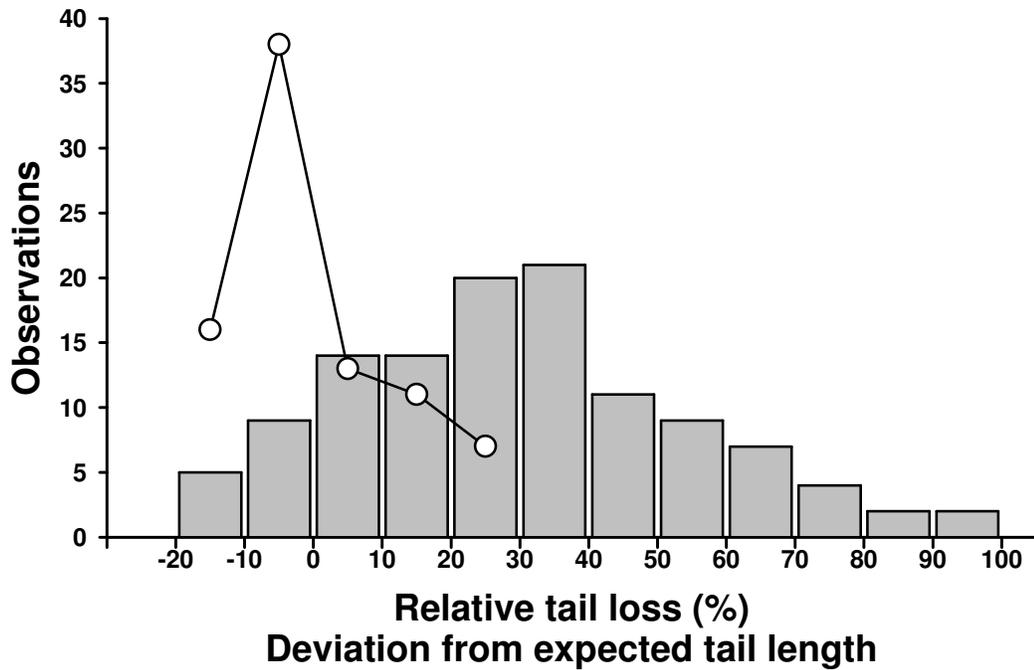


Figure 1: Variations in relative tail length in HL Tiger snakes.

Data on the x axis are expressed as percentage of tail loss relative to the expected tail length in intact snakes (hence positive values imply that the tail was naturally relatively short, 100% was those individuals where the tail was absent). The open circles provide the distribution data for intact snakes, centred on zero as expected, and with a relatively moderate range of deviations from the expected tail length. The grey bars provide the distribution for the snakes with part of the tail accidentally lost (see text). Snakes with a moderately damaged tail (less than 1/3 of the tail missing; N=48) exhibit relative tail length contained within natural variations observed in intact snakes, although significantly biased toward a short tail. Many snakes had extreme tail loss (>1/3 of the tail missing; N=41); some specimens had most, or all, of their tail missing (>2/3 of the tail missing; N=29).

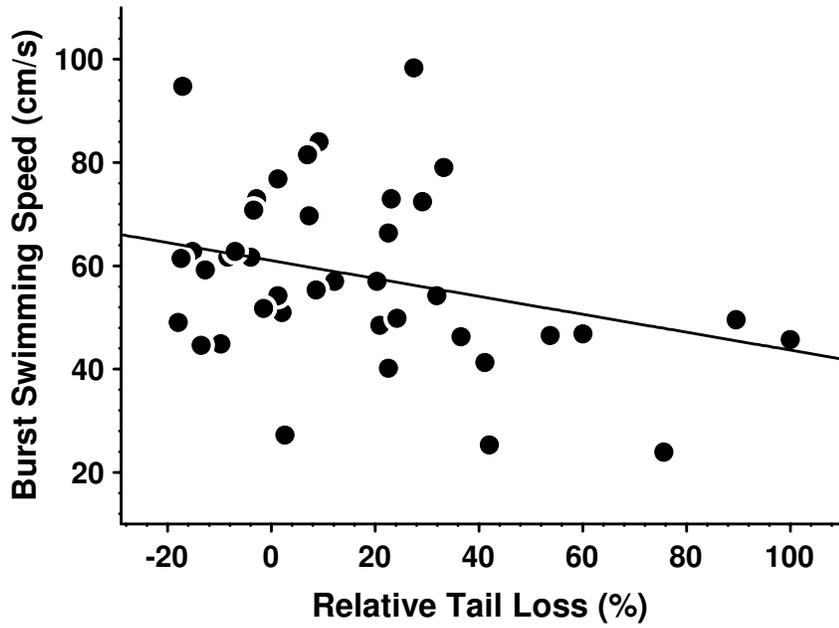


Figure 2. Correlation between relative tail loss and burst swimming speed in Tiger snakes.

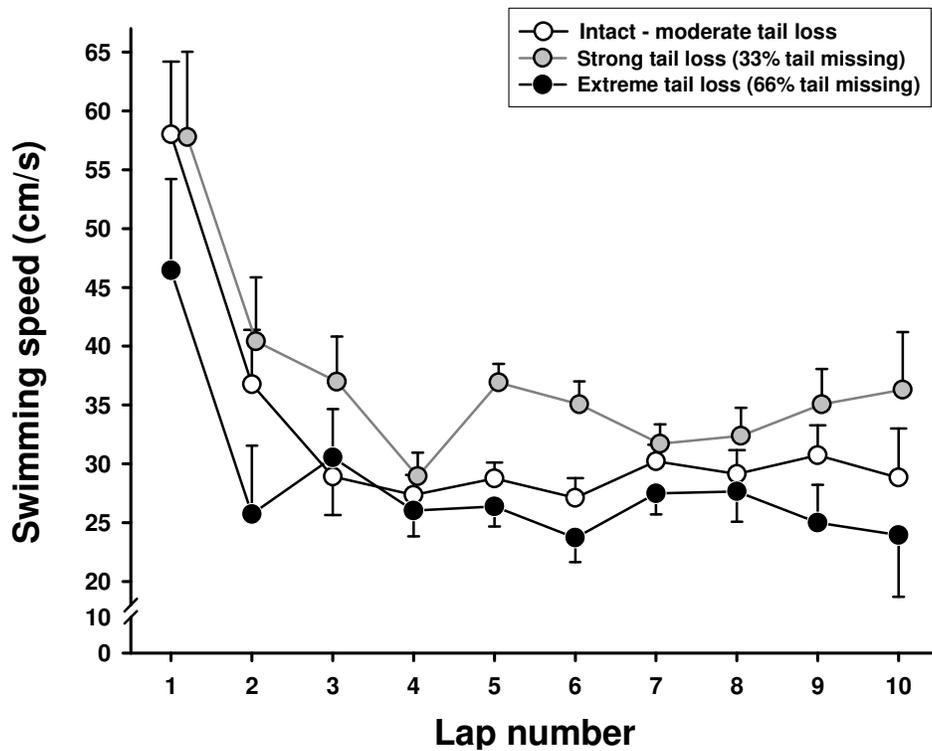


Figure 3. Comparisons of successive laps swimming speed in HL Tiger snakes. Means are given with their SE.

Table 1: Effect of tail loss and sex on the main morphological traits of HL Tiger snakes.

Tail loss: intact (N=85) *versus* damaged tail (N=118). This table presents results from ANOVAs (ANCOVAs with Log SVL as the co-variable for body condition analyses). Body masses were Log transformed prior to analyses. Significant results are indicated in bold.

Trait (dependent variable)	Source	df	MS	F	P
Snout-vent length	Tail loss	1, 200	232	3.85	0.051
	Sex	1, 200	2920	48.43	0.001
	Sex * Tail loss	1, 200	34	0.56	0.456
Body mass	Tail loss	1, 180	0.021	0.95	0.332
	Sex	1, 180	0.157	6.94	0.001
	Sex * Tail loss	1, 180	0.001	0.01	0.912
Body condition	Tail loss	1, 179	0.003	0.57	0.451
	Sex	1, 179	0.154	33.86	0.001
	Sex * Tail loss	1, 179	0.003	0.73	0.393

Table 2: Correlations between relative tail loss as the dependent variable and the main morphological traits of HL Tiger snakes.

Body mass was log transformed prior to analyses. Required N indicates the sample size required to obtain a P value <0.05 with both an α error of 5% and a β error of 10%.

Trait	r	F	df	P	Required N
Snout-vent length	-0.14	2.42	1, 116	0.12	532
Body mass	-0.08	0.71	1, 105	0.40	1637
Body condition	-0.16	1.46	1, 104	0.24	406
Jaw length	-0.14	1.63	1, 84	0.21	532
Head width	-0.00	0.00	1, 84	0.99	>10000

B. Effect of pregnancy on swimming performances

[Aubret F, Bonnet X, Shine R and Maumelat S 2004. Swimming and pregnancy in Tiger snakes, *Notechis scutatus*. Amphibia Reptilia, in press]

Reduced locomotor ability may increase susceptibility to predation and hence may represent a proximate mechanism by which “costs” of reproduction are expressed (Shine 1980). In squamate reptiles, many examples of such effects have been documented, where non-gravid females and/or males showed higher survival rates than gravid females (Shine 1980; Andren 1982, 1985; Madsen 1987). For instance, pregnancy may entail a reduction in locomotor performances in lizards and snakes (Shine 1980, 2003; Bauwens and Thoen 1981; Garland and Else 1987; Seigel *et al.* 1987), including decreased burst speed by 12 to 30%, and endurance by 52 to 55%. Because fleeing from predators or foraging ability depends on speed and/or stamina, reduced locomotor performance resulting from carrying offspring may result in increased risk of predation and/or decreased energy intake compared to non-gravid females.

However, less clear is the effect of pregnancy on aquatic locomotion. Many snakes inhabit aquatic environments such as rivers, lakes, swamps, mangroves, or oceans (Voris and Jayne 1979; Voris and Karns 1996; Jayne *et al.* 1988; Shine and Shetty 2001a) and may rely on water for feeding, and/or as an escape route from predators (such as sharks for sea kraits; Weatherhead and Robertson 1992; Heatwole 1999). Reproductive investment in aquatic snakes may be constrained by the important locomotory rôle of the posterior part of the body during swimming, and to a larger degree than during crawling on land (Jayne 1985; Gans 1986; Shine 1988). Indeed, clutches of aquatic snakes are located more anterior in the body, and overall reproductive investment is reduced compared to terrestrial species (Shine 1988). If such modifications are genuinely related to a reduction of the “costs” associated with pregnancy in swimming snakes, then it may be possible to record an impairment in swimming performances in pregnant snakes compared to non pregnant

individuals, either in terms of stamina and/or swimming speed. To date however, no study has specifically evaluated the effect of pregnancy in swimming snakes; therefore, it is important to determine if pregnancy alters aquatic locomotion in snakes as it may do in terrestrial locomotion. We studied the effect of pregnancy on swimming performance of Tiger snakes (*Notechis scutatus*), a viviparous species that contains populations that exhibit aquatic habits to escape predators or to forage on amphibians (Mirtschin and Davis 1992).

Twenty-two adult females (Table 1) were caught on Carnac Island (S 32°07', E 115°39') in Western Australia in January and February 2002; including 13 pregnant and 9 non-reproductive females (see Bonnet *et al.* 1999 and Aubret *et al.* 2004b for details on study site). Females were measured in snout-vent length (SVL), body length (BL) to the nearest 0.5 cm and weighed to the nearest 0.1 g (Body mass; BM). Reproductive status was determined by gentle abdominal palpation and eggs/embryos counted (January-February correspond to mid-pregnancy, unpublished data). All females were maintained under similar conditions. They were housed individually in plastic boxes (50x40x30 cm) with towel paper, water dish and shelter in a controlled temperature room, at 27.5°C by day and 20°C by night. A heat source provided basking opportunity four times 15 minutes per day (High pressure Na+ lamp; 600 Watts). Food (dead adult mice previously euthanased) was offered approximately every three weeks and water provided *ad libitum*. Although all pregnant females carried eggs/embryos at the time of the experiments, a significant proportion “reabsorbed” (i.e. the eggs were no longer detectable) them long after the end of our experiment: only 7 out of the 13 pregnant females gave birth between two and three months after completion of the experiments. All were then released at their exact capture location. Post reproduction BM was recorded after ventral palpation confirmed that all oviductal items had been expelled. Litter size included fully formed neonates, aborted embryos, and non-developed eggs. Litter mass was determined by weighing all those items. Relative litter mass (=RCM) was calculated by dividing litter mass by post-parturition

maternal mass. All snakes were tested with an empty stomach (Garland and Arnold 1983).

A rectangular swimming pool 370 cm long, 64 cm width, and 100 cm high was lined with black plastic. The 64 cm width was adjusted to allow normal swimming waves of the largest snake involved in the study. Water depth (40 cm) prevented the largest snake from touching the bottom. Two observers (located at each end of the pool) recorded the different parameters. Each of the following tests was separated by two days and performed in the same order for each individual, as follows:

Free swimming trial - snakes were gently manipulated (i.e. slowly lifted up with a hook from their cage) and smoothly dropped above the water level at one end of the pool. All snakes immediately swam instantaneously toward the other end (Shine and Shetty 2001a), a behaviour commonly observed in many “terrestrial” snake species (XB, pers. obs.). During a 5-minute trial, we recorded the total time spent swimming *versus* resting and the distance travelled by each snake (cumulative number of lengths of the pool). No snakes swam in random trajectories in the pool, but all showed stereotypical patterns: fast swimming along the length of pool and turns at each ends. Thus, the total distance swum by each snake was measured relatively accurately. The distance swum and the average speed were expressed either in absolute terms ($\text{cm}\cdot\text{s}^{-1}$) or relative to the snake body size ($\text{BL}\cdot\text{s}^{-1}$). We also recorded the occurrence of shaking (instability in swimming *versus* fluid and regular movements, Jayne 1985) and the occurrence of underwater swimming.

2. Forced swimming trial - the 5 minute trial was repeated but when snakes became tired, they were kept swimming by gently touching their tail with a stick each time they stopped. We recorded the cumulative time periods during which the snakes displayed unstable swimming (shaking), and underwater swimming.

3. Sprint swimming speed - maximum swimming speed was recorded by placing each snake in a situation where they were made to flee under stress: snakes were picked up by the head and suddenly dropped in the water at one

end of the pool. For accuracy, two observers independently recorded the time taken to cross the pool and a mean value was used in the analyses.

4. Voluntary apnea - we used an opaque PVC tube (40 cm long with a diameter of 8 cm), closed at one end. The diameter of the tube was large enough to allow easy movement of the snake inside it. As we suspected that snake's stress level may influence the time spent underwater, we attempted to minimise stress to the snakes prior to testing. Snakes were removed from their cage, and the open end of the PVC tube was presented to them. In each case, the snake voluntarily entered the tube (i.e. snakes normally feel safe in confined environments). Time recording started when the tube was fully immersed in the pool and ceased when snakes chose to move to the surface. To our knowledge, this study provides the first report of such apnea durations, a trait that may well play a rôle in natural conditions. When pursued by a predator, a snake that escaped by taking refuge underwater must remain hidden as long as possible. All experiments were performed at 27°C, close to the average body temperature chosen by Tiger snakes in captivity ($26.8 \pm 0.7^\circ\text{C}$; Ladyman and Bradshaw 2003). Times were recorded using digital stopwatches (0.1 s). All data were log transformed prior to analysis.

The results of this study are summarized in table 1.

Body size and swimming performance: because of the potentially confounding effect of pregnancy we restricted these analyses to non-reproductive females. Average swimming speed was negatively correlated with both BL and BM (respectively $N=6$; $r=-0.91$; $P<0.02$ and $r=-0.89$; $P<0.02$). In contrast, apnea duration was positively correlated with BL and BM (respectively $N=7$; $r=0.76$; $P<0.052$ and $r=0.82$; $P<0.033$). For all other traits, there was no significant correlation (all $r<0.68$; all $P>0.092$).

Pregnancy and swimming performances: litter mass ($N=6$, $r= -0.88$; $P< 0.024$) and relative litter mass negatively affected average swimming speed ($N=6$; $r=-0.83$; $P<0.041$). Apnea duration was positively correlated with litter mass ($N=7$; $r=0.82$; $P<0.031$) and with relative litter mass ($N=7$; $r=0.93$; $P<0.015$). Pregnant females spent less time swimming and consequently travelled shorter distances

during the free-swimming experiments than did the non-pregnant females (Table 1). However, pregnancy did not reduce significantly average or sprint swimming speed (Table 1). We did not find any significant difference between pregnant and non-pregnant females for the other swimming performance traits (Table 1).

A decrease in locomotor performance due to pregnancy has been reported for terrestrial locomotion in reptiles (Shine 1980; Bauwens and Thoen 1981; Garland 1985; Seigel *et al.* 1987). Our study provides the first example of a similar analysis applied to swimming snakes. Activity levels strongly decreased (35% in absolute terms, 40% relative to body length) in pregnant females during the free-swimming tests leading to a marked reduction in the total distance swum. Pregnancy had no effect on apnea duration and underwater swimming (close to significance for this later trait), although it may well be that with larger sample sizes, significant effects might be seen. It is possible that buoyancy changes due to the litter might affect these variables (Jayne 1985). Alternatively, the pregnant females may have tolerated less physiological stress (i.e. anoxia when underwater) to protect their developing, and perhaps fragile, embryos. Overall, the differences observed between pregnant and non-pregnant females may be related either to the physical burden of the litter, or to physiological and behavioural modifications of the maternal organism in response to the physiological requirements of the embryos; or to a combination of both kinds of factors (Sinervo *et al.* 1991; Olsson *et al.* 2000; Shine 2003). Surprisingly, burst and average swimming speed remained unaffected by pregnancy. This last result is counterintuitive and opposes a common finding in snakes and lizards where pregnancy decreases terrestrial burst speed (Shine 1980, 2003; Bauwens and Thoen 1981; Garland and Else 1987; Seigel *et al.* 1987; Sinervo *et al.* 1991; Olsson *et al.* 2000). This result also supports the notion that physiological/behavioural modifications played a rôle in the decrease in time spent swimming by pregnant females (Sinervo *et al.* 1991; Olsson *et al.* 2000). High variances recorded in most traits, and relatively small sample size, make it difficult to interpret some of our results. Hence, the evolutionary significance of our results and their potential impact on female survivorship remain unclear.

Further studies are needed to test the hypothesis that a reduction in reproductive investment or a shift in location of the litter (clutch) more anteriorly in the body would improve swimming performances. This could well be the case, as in our study litter mass and RCM were both negatively correlated with average swimming speed. Apnea duration was nonetheless positively correlated with litter mass, highlighting the importance of measuring various traits simultaneously when we attempt to interpret complex phenomena.

We thank the region Poitou-charentes (France), the Conseil Général des Deux-Sèvres, and the University of Western Australia for funding. We are also very grateful to D. Bradshaw and W. Gibb. Z. Lechat helped with animal handling. R. Avery and an anonymous referee provided useful comments. The Animal Ethics Committee of UWA approved all procedures and the Department of Conservation and Land Management (WA) issued fauna collection permits.

Table 1: Body size and swimming performances in pregnant and non-reproductive female Tiger snakes.

Comparisons between the groups of females were performed using ANOVAs with reproductive status as the factor and the trait under focus as the dependent variable. Snakes body size or body mass influenced several traits (see text), therefore we use ANCOVAs with SVL* or BM** as a covariate.

Means and adjusted means (to SVL*; to BM**) are expressed \pm SD. Df: degree of freedom. F: F value from ANOVAs and ANCOVAs.

Traits	Non pregnant (N=9)	Pregnant (N=13)	Df; F	P
Morphological traits				
Body mass (g)	278.6 \pm 90.8	410.8 \pm 78.3	1, 20; 13.5	0.002
Snout-vent length (cm)	76.9 \pm 8.3	83.9 \pm 4.2	1, 20; 6.5	0.019
Body length (cm)	91.1 \pm 9.8	98.2 \pm 4.5	1, 20; 5.2	0.033
Body condition (g)*	316.2 \pm 90.8	373.3 \pm 78.3	1, 19; 6.7	0.018
Free swimming trial				
Time spent swimming (s)	561.3 \pm 35.9	365.4 \pm 167.7	1, 18; 11.7	0.003
Distance swum (m)	66.1 \pm 17.9	43.3 \pm 24.4	1, 18; 6.1	0.023
Distance swum (BL)	73.3 \pm 21.6	44.0 \pm 25.8	1, 18; 8.2	0.010
Average speed (cm.s ⁻¹)**	22.5 \pm 5.4	24.4 \pm 5.1	1, 17; 0.1	0.71
Average speed (BL.s ⁻¹)	0.3 \pm 0.1	0.2 \pm 0.1	1, 18; 0.5	0.49
Forced swimming trial				
Shaking time (s)	16.6 \pm 18.8	21.6 \pm 19.3	1, 20; 0.2	0.63
Underwater swimming (s)**	42.5 \pm 60.3	15.5 \pm 11.7	1, 19; 4.2	0.053
Sprint swimming speed				
Sprint speed (cm.s ⁻¹)	60.3 \pm 16.5	60.7 \pm 10.4	1, 20; 0.1	0.84
Sprint speed (BL.s ⁻¹)	0.7 \pm 0.2	0.6 \pm 0.1	1, 20; 0.3	0.57
Voluntary apnea				
Apnea duration (s)**	194.5 \pm 220.5	76.94 \pm 77.2	1, 19; 1.2	0.29

C. Aquatic locomotion, behaviour and natural experience

D. A rôle for adaptive plasticity in a major evolutionary transition

[Aubret F, Bonnet X and Shine R 2005. A rôle for adaptive plasticity in a major evolutionary transition: early aquatic experience affects locomotor performance of terrestrial snakes. Evolution, under review]

The phenotype of any living organism reflects not only its genotype, but also direct effects of environmental conditions. Some manifestations of environmental effects may be non-adaptive (such as fluctuating asymmetry: Whitlock 1996; Shykoff and Møller 1999). Growing evidence nevertheless suggests that natural selection has fashioned norms of reaction such that organisms will tend to display developmental trajectories that maximise their fitness in the environment which they encounter *via* enhanced growth, survival, and/or reproduction (Bull 1980; Werner 1986; Stearns 1989; Schlichting and Pigliucci 1998; Losos *et al.* 2000; Agrawal 2001). Over recent decades, the adaptive value of phenotypic plasticity has become a central theme in evolutionary biology (Dudley and Schmitt 1996; Pigliucci 2001).

Studies of phenotypic plasticity typically have focused on relatively short-term effects on organismal fitness, usually involving the effects of environmental factors that vary over relatively small spatial and temporal scales (e.g. Losos 1990; Denver *et al.* 1998; Aubret *et al.* 2004a). However, the phenomenon of phenotypic plasticity may also clarify evolutionary transitions at a far grander scale, including major phylogenetic shifts in important traits (Pigliucci and Murren 2003; West-Eberhard 2003). For example, phenotypically plastic responses of reptilian embryos to incubation temperature may have comprised major selective pressures for the evolutionary transition from oviparity (egg-laying) to viviparity (live-bearing) in more than 100 separate phylogenetic lineages of lizards and snakes (Shine 1985, 2004). That is, maternal retention of developing embryos enhanced fitness because the retained embryos were

thereby kept warmer, and developed into more viable offspring (Mell 1929; Sergeev 1940; Shine 1983, 1985, 2004). Might the same be true for other major evolutionary transitions, such as those between aquatic and terrestrial habitats? Many kinds of organisms have undergone such shifts in the course of their evolutionary history, and we can imagine that in the initial phases of any such transition, the organisms were exposed to environmental conditions that differed in important ways from the ones characteristic of their usual (ancestral) habitat type. Plausibly, then, exposure to the novel habitat may have directly induced changes to phenotypic traits, in ways that enhanced the animal's ability to function in the novel environment (Ehrlich 1989; Holway and Suarez 1999; Baldwin 1896; Yeh and Price 2004). If so, adaptive plasticity may have been a significant contributor to enabling such transitions to occur.

To test this hypothesis, we need to identify a lineage of animals that has displayed multiple evolutionary transitions between major habitat types - preferably, habitat types that impose different, and conflicting, selective pressures on phenotypic traits. Then, we can take a species that is believed to resemble the ancestral forms close to the base of the evolutionary radiation into the environment, expose young individuals of that species to the novel habitat type, and see whether or not direct exposure early in life significantly enhances the organism's ability to function in their new environment. Perhaps the most direct measure of such functioning would be to measure locomotor ability. Modification of locomotor performances due to exposure to different habitat types has been documented in several types of vertebrates (Steinhauss 1933; Jones *et al.* 1977; Bennett and Huey 1990; Losos 1990; Parichy and Kaplan 1995; Losos *et al.* 2000; Nelson *et al.* 2003; Aubret 2004).

We have conducted an experimental study of this kind, using proteroglyphous (elapid) snakes. Although debate continues as to whether the earliest snakes were aquatic or terrestrial (e.g. Cope 1869; Nopsca 1923; McDowell 1972; Wiens and Slingluff 2002; Caprette *et al.* 2004), all authorities agree that modern snakes display multiple phylogenetic shifts from terrestrial to aquatic habitats (e.g. Heatwole 1999). The most successful group in this respect have been the front-

fanged land snakes (Elapidae), which include at least four transitions from terrestrial to aquatic life: once giving rise to laticaudid sea-snakes, once to hydrophiid sea-snakes, once to the water cobra *Boulengerina annulata*, and once to the aquatic coral snake *Micrurus surinamensis* (Keogh 1998; Heatwole 1999). The most speciose group of aquatic snakes are the "true" (hydrophiid) sea-snakes, with at least 57 species in tropical oceans (Keogh 1998). Molecular data unequivocally indicate that these animals arose relatively recently (perhaps only 5 mybp) from a small clade of terrestrial viviparous Australian snakes (the "Notechis group": Keogh 1998).

Thus, members of the *Notechis* group provide the best available evidence as to the characteristics of the terrestrial taxa that gave rise to modern sea-snakes. Of the 20 genera of Australian Elapids, *Notechis* itself is the most widely distributed taxon, and occurs on many oceanic islands and coastal swamps along the southern Australian coast. This situation may well resemble those in which the initial evolutionary transition to aquatic habitats occurred; indeed, isolated populations of Tiger snakes sometimes forage mostly in the water, even on the mainland (e.g. *N. scutatus kreftii*: Mirtschin and Davis 1992). Moreover, *Notechis* have proven to be plastic in many traits (Aubret 2004; Aubret *et al.* 2004a; Bonnet *et al.* 2005), as are many snakes (Bonnet *et al.* 2001a; Burger 1989, 1998; Shine *et al.* 1997). Thus, the Australian Tiger snake (*Notechis scutatus*) provides an ideal model species with which to examine the direct effects of early exposure to aquatic habitats. We focused on locomotor performance as a fitness-relevant measure of the animal's ability to function both in the ancestral habitat (land) and the novel habitat (water).

Methods

Study species

The Australian Tiger snake, *Notechis scutatus*, is a highly venomous elapid species that is widely distributed throughout southern and eastern Australia (Cogger 1992). Mean adult body sizes vary geographically, but generally

average 1 to 2 m (Shine 1987; Schwaner and Sarre 1988). Tiger snakes forage both on land and underwater, taking a diverse range of prey ranging from frogs to nestling birds (Shine 1987; Aubret *et al.* 2004b). Some populations forage mostly on aquatic prey (tadpoles, fishes: Mirtschin and Davis 1992) whereas others live in areas without permanent freshwater bodies and thus, depend entirely upon terrestrial foraging (Bonnet *et al.* 1999, 2002a; Aubret *et al.* 2004b). A previous comparison between two populations of Tiger snakes from Western Australia suggested that plasticity may play a rôle in matching locomotor performance to local conditions (Aubret 2004). Neonatal snakes from a swamp-dwelling mainland population were similar to neonates from a more arid island population in terms of swimming speed, stamina, apnea capacities and swimming behaviour. However, adult mainland snakes had greater apnea capacities and faster burst swimming speed than did island snakes. Mainland adult snakes also spontaneously swam underwater for a longer period of time, and a higher proportion filled their lungs whilst surface swimming compared with to island specimens (Aubret 2004).

Experimental procedure

The study animals were neonates, born to ten pregnant female snakes captured in Herdsman Lake (HL; S 31° 55' 44"; E 115° 48' 19"), a nature reserve near the city of Perth, Western Australia. This perennial lake contains abundant frogs, and these constitute the major dietary items for HL Tiger snakes (Aubret *et al.* 2004b). At this site, Tiger snakes are found both in aquatic and terrestrial habits (Aubret 2004).

Neonates were measured < 24 h after parturition. Body mass (BM) was measured with a digital scale (± 0.01 g); body length (BL) and snout-vent length (SVL) were measured to the nearest 5 mm. Neonates were individually housed in plastic boxes (15 cm x 10 cm x 5 cm), with a water dish, shelter and paper towel as substratum. All snakes were fed dead baby mice once a week. When the snakes were two months old, we randomly selected two neonates per litter,

and allocated these 20 animals to two treatments: terrestrial *versus* aquatic rearing conditions. The two groups were kept in these conditions for five months.

Aquatic environment - The 10 neonates were housed in two plastic boxes (50 cm x 50 cm x 40 cm) filled with 10 cm deep water. A basking rock was available on one side of the enclosure. A shelter (a rock covered by a flower pot) was provided on the other (colder) side of the enclosure, and could be accessed only by diving underwater, through a hole in the side of the flower pot.

Terrestrial environment - The 10 neonates were housed in identical enclosures (2), but with a mixture of sand and sawdust as substratum and a small water dish (too small to allow swimming).

In both treatment groups, heating was provided (three periods of 15 minutes per day) by a Na⁺ high pressure lamp (600 W), directed towards the basking rock. The young snakes basked every day, and to do so they had to crawl or swim from the shelter to the other side of the cage. The room temperature was maintained at 23°C by day and 15°C by night.

Locomotor performance records

We measured locomotor traits twice for each snake: at the beginning of the experiment and after five months spent in the respective habitats. To compare traits between the two groups, ANOVAs and repeated measures ANOVAs were applied to log-transformed data. As commonly occurs during captive raising of very young animals, some animals died (3 “aquatic” and 2 “terrestrial” snakes) for unknown reasons. Hence, our sample sizes changed slightly throughout the course of the experiment.

We measured a series of traits that were chosen to reflect different facets of a snake's relative functioning in aquatic *versus* terrestrial habitats, as follows:

Burst swimming speed - To estimate swimming performances we used a procedure that has been validated for snakes, including Tiger snakes (Shine and Shetty 2001a; Shine *et al.* 2003; Aubret 2004; Aubret *et al.* 2005b). Snakes were dropped in the water at one end of a linear swimming track (glass aquarium; recording section of 170 cm). Using a stopwatch (precision 0.1 s), we recorded the time taken to reach the other end of the pool. Maximum speed was maintained by stimulating the snake's tail with a paintbrush. This test was done twice in a row and the fastest performance retained for analysis.

Burst crawling speed - Neonates were released at one end of a terrestrial raceway using sawdust as substratum (recording section of 100 cm). As above, the snakes were stimulated to keep moving by gently touching their tails with an artist's paintbrush.

Non-forced swimming trials - Snakes were dropped from 5 cm above the water level into a circular track (average diameter 94 cm) containing 15 cm deep water. Immediately upon release, every animal began to swim around the track; they were not touched or disturbed for the next 60 seconds. Over this period, we recorded the number of laps completed, and the time spent swimming *versus* resting (percentage of activity).

Apnea scores - We used an opaque PVC tube 10 cm in length and 2.5 cm in diameter, closed at one end. The diameter of the tube was large enough to allow easy movement of the snake inside it. Snakes were removed from their cage, and the open end of the tube was presented to them. As soon as the snake voluntarily entered the tube (snakes normally feel secure in confined environments), the tube was fully immersed in a pool of water. We recorded the time that the snake remained underwater before surfacing to breathe as the *free apnea score*. The following day, the test was repeated except that this time, we attempted to mimic a predator by moving above the water and touching the snake underwater with a stick each time it was about to reach the surface. This stimulus encouraged the animal to prolong the duration of its time underwater,

presumably until its need to breathe overcame the perceived risk of predation. The time taken to surface was recorded as the *forced apnea score*.

Effect of a full stomach on locomotor performance

After completion of the last series of tests (i.e. after 5 months exposure to the respective treatments), snakes were fed as usual and then tested the following day for burst crawling speed. The next day, forced apnea scores were recorded also. A week later, snakes were fed and burst swimming speed was recorded the following day.

Results

Initial body size (body mass, snout-vent length, body length or body condition; all $P > 0.47$) and locomotor traits were similar between the two treatment groups (burst crawling, burst swimming and apnea performances, nor in any of the variables recorded along the circular swimming track; all $P > 0.10$ – see Table 1). Below, we describe the changes over time of each trait from the beginning to the end of the experiment (Table 1).

Feeding rates and body sizes

The two treatment groups were fed similar amounts of food throughout the experiment (on average $0.07 \text{ g} \pm 0.01 \text{ g}$ of food per day; Wilks' $\lambda = 0.75$, $P = 0.22$; effect of treatment $F_{1,17} = 0.37$; $P = 0.55$). Consequently, both treatment groups exhibited similar body mass ($F_{1,16} = 0.05$; $P = 0.82$), total length ($F_{1,16} = 0.67$; $P = 0.42$), snout-vent length ($F_{1,16} = 0.90$; $P = 0.36$), and body condition (body mass relative to snout-vent length - $F_{1,15} = 0.56$; $P = 0.46$) at the end of the experiment.

Burst swimming speeds

When re-tested after five months, the snakes raised in water displayed enhanced swimming skills compare to their siblings raised on land, being 26% faster in burst swimming speed in absolute terms (cm per sec - $F_{1,15} = 31.73$;

$P < 0.0001$) and 21% faster relative to body length (body length per s; $F_{1,15} = 48.58$; $P < 0.0001$ – see Figure 2 top).

Burst crawling speeds

When tested on the runway, snakes kept in terrestrial conditions crawled more rapidly than did their siblings raised in an aquatic environment (Figure 2 lower panel). This difference between the groups was significant both for absolute crawling speed (by 36%; $F_{1,13} = 10.88$; $P < 0.006$) and speed relative to body length (by 31%; $F_{1,13} = 7.82$; $P < 0.016$).

Non-forced swimming trials

Neither the total distance swum around the circular track (cm; $F_{1,13} = 2.18$; $P = 0.16$; BL swum; $F_{1,13} = 2.76$; $P = 0.12$) nor the percentage of activity in the water differed significantly between the two groups ($F_{1,13} = 0.21$; $P = 0.65$). Nevertheless, non-forced swimming speed was significantly higher (by 87%) in the aquatic group in absolute terms ($F_{1,13} = 7.46$; $P < 0.017$) as well as relative to body length (by 77%; $F_{1,13} = 8.98$; $P < 0.011$).

Apnea scores

Free apnea scores were similar between the two groups at the end of the experiment ($F_{1,13} = 0.13$; $P = 0.73$). However, the aquatic group displayed 119% higher forced apnea scores (See Figure 3; $F_{1,13} = 15.62$; $P < 0.002$).

Effect of a full stomach on locomotor performance

On both occasions where experiments involved prey in the snakes' stomachs, there was no significant difference in prey mass between the aquatic and terrestrial groups (on average 31% of snake body mass; both $P > 0.44$). The presence of prey in the stomach significantly decreased burst crawling speed (terrestrial locomotion, cm per sec), and did so to a greater extent in the aquatic group than in the terrestrial group (Wilks' $\lambda = 0.088$; $P = 0.001$; interaction $F_{1,13} = 48.85$; $P < 0.001$). Similar results were obtained with burst crawling speed relative to body length (see Figure 2 top panel). In contrast, having prey in the

stomach affected burst swimming speed more in the terrestrial group than in the aquatic group (in cm per s; Wilks' $\lambda=0.15$; $P<0.001$; interaction $F_{1,15}=9.71$; $P<0.007$; in body lengths per s; Wilks' $\lambda=0.21$; $P<0.001$; interaction $F_{1,15}=9.71$; $P<0.007$; Figure 2 bottom). Lastly, having prey in the stomach strongly reduced apnea scores in both treatments. The effect was greater in the aquatic group (71% reduction) than the terrestrial group (42%), because all recently-fed snakes showed similar, low durations of forced apnea whereas the duration of forced apnea in unfed animals was much greater in the aquatic than the terrestrial animals (Wilks' $\lambda=0.43$; $P<0.007$; interaction $F_{1,13}=10.13$; $P<0.007$). Thus, the advantages afforded by exposure to aquatic habitats were evident for unfed but not fed snakes.



Figure 1: Swimming Tiger snake in Herdsman Lake.

Discussion

The habitat in which a young Tiger snake spent the first few months of its life profoundly affected its locomotor abilities. The magnitude of locomotor effects was surprisingly high. A clear trade-off was apparent between locomotor abilities in the two habitat types: improved swimming/diving abilities entailed a decrease in terrestrial performances, and *vice versa*. This pattern is consistent with the notion that aquatic and terrestrial environments impose very different constraints on the optimal morpho-functional attributes of locomotion (Jayne 1982, 1985; Cundall 1987), to the extent that muscles structures that optimize swimming in snakes differ from those that optimise terrestrial movement (Jayne 1982). Hence, we would expect to see trade-offs between these traits whilst the snakes were developing (Jayne 1982). Importantly, our animals were reared in two discrete environments (aquatic or terrestrial) so no compromise was offered. Most natural environments provide a more variable situation, favouring a combination of crawling and swimming (as is the case in Herdsman Lake). Presumably, such a situation favours (and induces?) phenotypes that strike a balance that minimize costs and maximize benefits in both environments. Nonetheless, such compromises may be impossible because swimming and crawling in snakes make such conflicting demands on morphology (Gans 1975; Jayne 1985; Cundall 1987).

Our study also suggests that not only does adaptive plasticity enhance organismal fitness by modifying mean locomotor speeds in each environment (as previously reported in other systems: e.g. Losos *et al.* 2000) but it can also lower some costs associated with day-to-day activities. Having food in the stomach impairs locomotion in snakes both in terrestrial locomotion (Garland and Arnold 1983; Ford and Shuttlesworth 1986) and in aquatic locomotion (Shine and Shetty 2001a). In keeping with this conclusion, snakes that contained freshly-ingested prey items in our study were slower in terms of crawling speed as well as swimming speed. However, the degree of impairment differed between treatment groups, suggesting that the "costs" of a

full stomach are manifested differently depending upon the kind of environment that the young animals had experienced prior to testing.

Flexibility of this kind is likely to confer strong fitness benefits to Tiger snakes in nature. We do not know whether locomotor speed affects survival or growth rates in Tiger snakes, although such a link has been documented in a field study of another snake species (Jayne and Bennett 1990). There is massive spatial and temporal heterogeneity of habitat types - especially, the proportion of an area that is underwater - across much of the range occupied by Tiger snakes. On a spatial scale, the swampy habitats occupied by this species consist of complex mosaics of water and land; given the relatively small home ranges of these animals (Shine 1979; Bonnet, unpubl. data), animals in adjacent home ranges may well differ substantially in the proportion of their time spent either on land or in the water. On a temporal scale, the stochastic precipitation schedules characteristic of many Australian habitats mean that a high proportion of waterbodies are ephemeral, varying enormously in the extent of inundation from one year to the next (Flannery 2002). For example, Lake George near Canberra in the southern highlands of New South Wales has long been renowned for its high Tiger snake densities (e.g. Worrell 1972). Its area commonly varies between 130 and 155 sq. km in area but over the last 200 years the lake has been almost completely dry on six known occasions, sometimes for decades (Department of Land and Water Conservation NSW 1999). Thus, a Tiger snake born into such a population might encounter either dry conditions or very wet ones. Genetically-based adaptive processes cannot track such rapid, stochastic fluctuations. However, adaptive plasticity is ideally suited to this challenge (Robinson and Dukas 1999). By facultatively adjusting locomotor performance to the conditions experienced during early life, each cohort of young Tiger snakes can be well-matched to the habitats that they encounter.

In turn, selection for adaptive plasticity in response to spatially and temporally heterogeneous precipitation regimes may have played a significant rôle in pre-adapting Tiger snakes or related taxa to invasion of the oceans. The initial

phases of such a transition presumably occurred in coastal swamps, with some populations exploiting the abundant food resources available underwater in such habitats. By facilitating aquatic locomotion during foraging bouts (and perhaps for predator evasion also), adaptive plasticity of locomotor traits provided an opportunity for selection to operate on genetically-coded traits (i. e. genetic assimilation – Pigliucci and Murren 2003) that ultimately enabled far more effective occupancy of oceanic habitats (Heatwole 1999; Shine and Shetty 2001a; Shine *et al.* 2003).

In conclusion, the plasticity in locomotor performances exhibited by immature Tiger snakes is to our knowledge the most dramatic example yet recorded for locomotor performances in vertebrates. Such plasticity may well provide advantages in terms of organismal fitness. Our study also suggests that plasticity may lower locomotor “costs” associated with feeding. Lastly, because our study organism is closely related to ancestral taxa that gave rise to the hydrophiid sea-snakes, we infer that similar plasticity may well have played an important rôle in pre-adapting these animals to the invasion of a new habitat type that posed novel locomotor challenges. Analogous work on other taxa in similarly critical phylogenetic positions should be feasible, and could help to answer the more general question of whether adaptive plasticity has played a major rôle in facilitating other major evolutionary radiations (Pigliucci and Murren 2003; West-Eberhard 2003).

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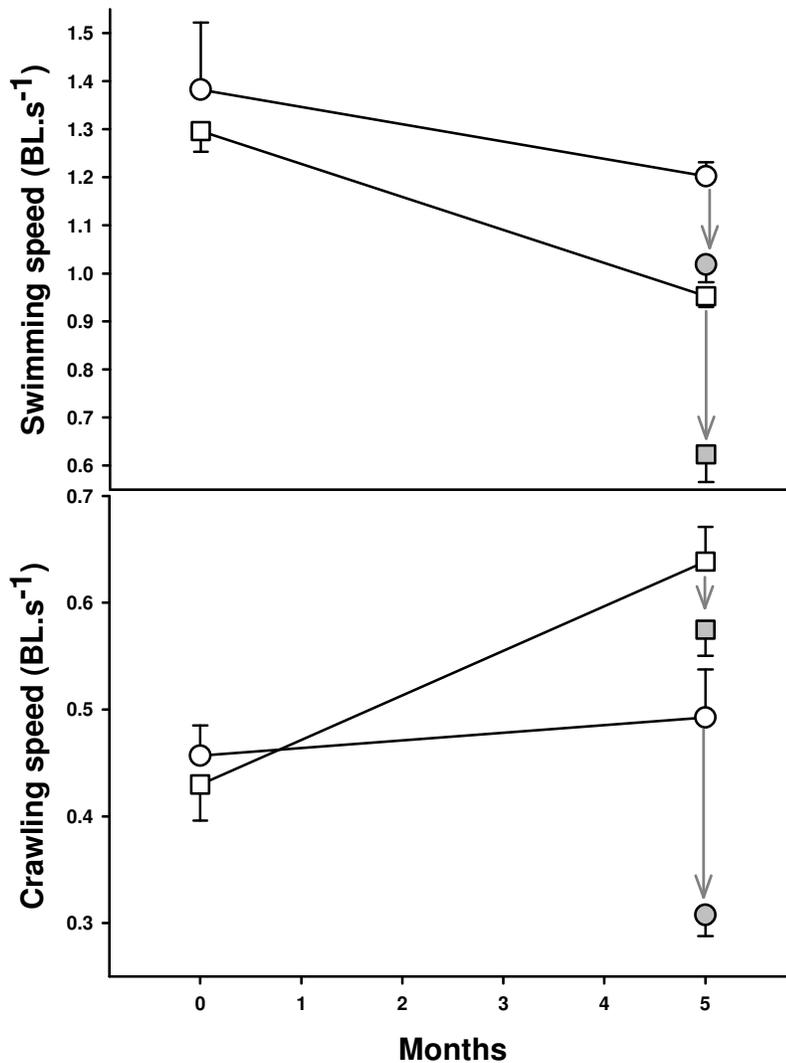


Figure 2: Twenty young Tiger snakes were reared either in aquatic (white circles) or in terrestrial environments (white squares). After 5 months of treatment, the aquatic reared snakes proved faster in burst swimming (top figure; ANOVA $F_{1,15} = 48.58$, $P < 0.001$) than their siblings raised in a terrestrial environment. In contrast, the latter snakes crawled more rapidly on land (bottom figure; $F_{1,13} = 7.82$, $P < 0.016$).

Having prey in the stomach significantly decreased burst swimming speed, but to a lesser extent in the aquatic group (by 15% *versus* 35% in the terrestrial group; grey plots; Wilks $\lambda=0.21$; $P<0.001$; interaction $F_{1,15}=9.71$; $P<0.007$); and crawling speed but to a lesser extent in the terrestrial group (by 10% *versus* 38% in the aquatic group; Wilks $\lambda=0.08$; $P=0.001$; interaction $F_{1,13}=48.85$; $P<0.001$). Means values + SE are plotted.

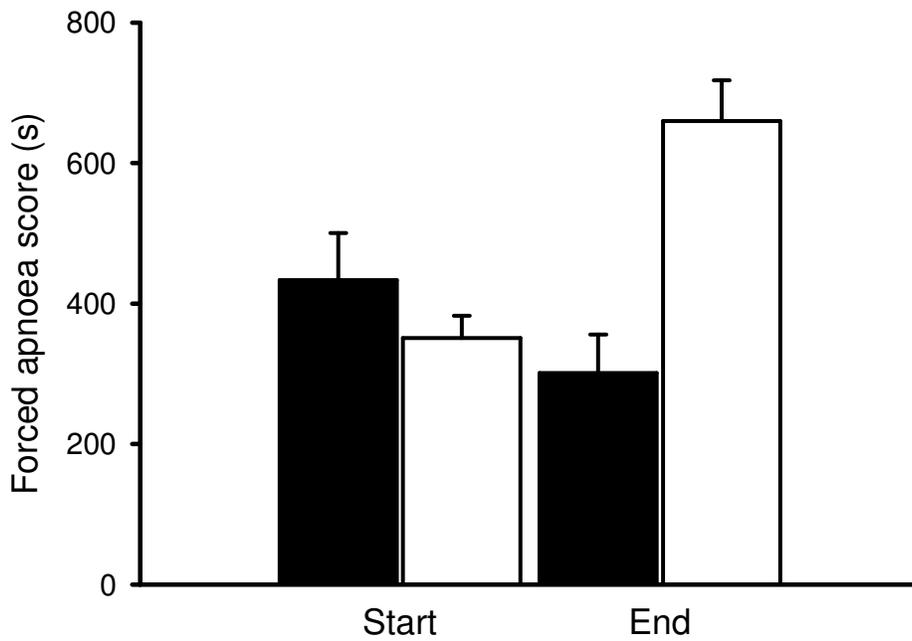


Figure 3: The effect of living environment (aquatic *versus* terrestrial) on forced apnoea scores in young Tiger snakes. The aquatic group (white bars) developed significantly higher forced apnoea scores over the course of the experiment than did their terrestrial siblings (black bars; $F_{1,13}=15.62$; $P<0.002$).

Table 1: Body size and locomotor performances in juvenile Tiger snakes at the start and end of the experiment.

BL=Body Length. Mean values \pm SD are given.

Traits recorded	Aquatic		Terrestrial	
	Start	End	Start	End
Body mass (g)	7.29 \pm 1.23	13.95 \pm 3.02	7.18 \pm 1.09	14.40 \pm 3.27
Snout-vent length (cm)	20.50 \pm 1.22	24.69 \pm 1.56	20.20 \pm 1.62	25.55 \pm 2.10
Body length (cm)	23.97 \pm 1.81	28.93 \pm 1.84	23.98 \pm 2.13	29.85 \pm 2.66
Body condition	7.18 \pm 1.24	14.53 \pm 3.02	7.29 \pm 1.10	13.82 \pm 3.27
<i>Circular track</i>				
Burst crawling speed (cm/s)	9.83 \pm 1.31	14.26 \pm 4.08	9.28 \pm 2.51	19.47 \pm 2.51
Burst crawling speed (BL/s)	0.46 \pm 0.08	0.49 \pm 0.12	0.43 \pm 0.11	0.64 \pm 0.09
Free apnea score (s)	173.10 \pm 73.50	389.68 \pm 155.52	228.66 \pm 70.26	381.05 \pm 268.70
Forced apnea score (s)	339.32 \pm 100.72	659.72 \pm 154.28	418.78 \pm 211.82	301.13 \pm 154.66
Burst swimming speed (cm/s)	33.68 \pm 12.40	34.71 \pm 1.97	31.23 \pm 5.38	28.67 \pm 2.30
Burst swimming speed (BL/s)	1.39 \pm 0.44	1.20 \pm 0.08	1.29 \pm 0.14	0.95 \pm 0.07
Distance swum (cm)	333.70 \pm 155.80	224.93 \pm 107.56	338.40 \pm 80.80	135.13 \pm 43.05
Distance swum (BL)	13.78 \pm 6.32	7.90 \pm 4.05	14.10 \pm 3.01	4.43 \pm 1.45
Activity time (%)	86.67 \pm 10.56	54.99 \pm 24.28	88.80 \pm 11.79	54.55 \pm 18.86
Average swimming speed (cm/s)	6.28 \pm 2.56	7.93 \pm 3.68	6.40 \pm 1.47	4.48 \pm 1.84
Average swimming speed (BL/s)	0.26 \pm 0.10	0.28 \pm 0.13	0.27 \pm 0.05	0.15 \pm 0.06

General conclusion

This PhD study described two disjunct populations of Tiger snakes, and assessed their potential as a model for the study of phenotypic plasticity and especially the rôle of adaptive plasticity in evolutionary processes (see below). Experimental work combined with field data demonstrated the potential for plasticity in many traits, including locomotor performances (swimming, crawling, diving), body and head size, feeding preferences, hunting strategies, etc. Future work would ideally investigate under which circumstances and to what extent such plasticity may genuinely improve organismal fitness.

Although of great interest, the comparative analyses were based on only two populations, rendering some of the conclusions questionable. Nevertheless, this piece of work opens the way for comparative and correlative approaches to the study of Tiger snake populations on a larger scale, where the study of multiple systems of mainland/island populations would greatly increase the power of the findings.

Does adaptive plasticity play a significant rôle in initial colonisation of novel habitat?

Successful invasions by animals into new environments are often associated with altered behaviours and other form of phenotypic plasticity (Ehrlich 1989; Holway and Suarez 1999). These plastic responses may allow a population to persist under temporarily stressful conditions (population establishment) and allow survival of the population under novel environmental conditions (population persistence), leaving more time for mutation, recombination, and selection to fine-tune the level of adaptation (Baldwin 1896; Wright 1932; Goldschmidt 1940; Waddington 1942; Schmalhausen 1949; Matsuda 1982; West-Eberhard 1989; Sultan 1992; Robinson and Ducas 1999). Phenotypic plasticity may also extend the ecological range of a species, exposing it to selection pressures that it would otherwise not encounter and creating the opportunity for genetic assimilation (Waddington 1953, 1957). In the absence of plastic responses, population growth may be low or negative during the early

generations of colonization, before adaptive evolution is possible, making extinction a more likely outcome.

Once the population is established, heritable differences accumulate by natural selection, so that the adaptive phenotype initially achieved by plasticity becomes genetic (Baldwin 1896): if some genotypes show plasticity in the same direction favoured by selection, then plasticity facilitate natural selection and a directional shift in the population mean (Waddington 1961; Robinson and Dukas 1999; Price *et al.* 2003; Schlichting and Pigliucci 1995; West-Eberhard 1989, 2003). These genotypes will then constitute “bridges” from one generation to the next, allowing the time for favourable genetic variants to appear by mutation or recombination. These ideas relate plasticity to Waddington’s concept of genetic assimilation (1942, 1952, 1953).

An interesting component of the concept is that over time the constancy of selection favours hard-wiring the trait. Does plasticity decrease? If so, why does this happen? Is a plastic gene more expensive to keep in the system? Behera (1994) and Mayley (1997) have suggested a possible rôle for costs of plasticity in such process: costs of plasticity may lead to genetic assimilation even under moderately heterogenous environmental conditions. Mayley’s models led to the conclusions that (1) in the absence of costs of plasticity, genetic assimilation was not favoured; (2) when the costs of plasticity are high, selection settles for canalized solutions (genetic assimilation).

While the theoretical concepts of how phenotypic plasticity may be crucial to many successful invasions are well established (see Pigliucci and Murren 2003 for a review), natural evidence are very scarce with only one study directly evaluating the rôle of plasticity (Yeh and Price 2004). It is crucial to now consider some empirical studies of the potential rôle of genetic assimilation in evolution. Especially, little has been done that explicitly incorporates the

transitional rôle of phenotypic plasticity leading to macroevolution level biological change.

Phenotypic plasticity for example may have played an important rôle in the evolutionary radiation of *Anolis* lizards across habitat types throughout the diverse islands of the Caribbean (Losos *et al.* 1994, 2000). If indeed phenotypic plasticity followed by genetic assimilation was the mechanism of radiation of these *Anolis* lizards, then they could provide an excellent example of the rôle of genetic assimilation at the macroevolution scale (Pigliucci and Murren 2003).

The Tiger snakes populations, with multiple replicates of island/mainland systems where the age of isolation is known offer the opportunity to test such theories in the wild. There are multiple populations of dwarfs and giants and the age of the islands and thus the age of the Tiger snake populations are known from detailed sea level studies. Importantly those systems offer a dynamic view of evolution, as the isolation date of the island snakes ranges from a few decades to 10,000 years. This situation is unique in the fact that it may allow further research to unravel selective mechanisms over a significant time scale, and understand the rôle of hard wired genes and genes that confers plasticity in the adaptation processes to new environmental factors.

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