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# Status and conservation of the grass snake in Jersey

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# **Status and conservation of the grass snake in Jersey**

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Ms Nina Cornish

***“There is no fundamental distinction to be made between the extinction of a local population and the extinction of a species other than that the species becomes extinct with the extinction of the last local population”***

(Andrewartha and Birch, 1954, p. 665)

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## **Author's declaration**

All chapters of this thesis were written by Robert J. Ward. Comments and editorial input were provided by the supervisors Richard A. Griffiths, John W. Wilkinson, Nina Cornish and Jim J. Groombridge as listed below. Chapter 5 also received comments and editorial input from additional researchers within the University of Kent and from external institutions. All research within this thesis was approved by the University of Kent, School of Anthropology and Conservation Research Ethics Committee.

**Chapter 1.** The chapter was written by R.J. Ward and received editorial suggestions from R.A. Griffiths and J.J. Groombridge.

**Chapter 2.** R.J. Ward, R.A. Griffiths, J.W. Wilkinson and N. Cornish conceived the idea. R.J. Ward designed the study, conducted all data collection and analyses, and wrote the manuscript with editorial input from the other co-authors. It has been submitted to Scientific Reports with permission of all co-authors and accepted for publication with some modifications.

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**Chapter 5.** R.J. Ward, R.A. Griffiths, J.J. Groombridge, J.W. Wilkinson and N. Cornish conceived the idea. R.J. Ward conducted data collection in Jersey, conducted all analyses and wrote the manuscript with input from the other co-

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## Abstract

Global biodiversity losses are being driven by anthropogenic pressures; the most pervasive of which is habitat loss resulting in fragmentation and population isolation. These issues are prevalent throughout Europe due to high intensity agriculture and increasing human population densities. Limitations imposed by resources and the secretive lifestyles of many species hinder the ability of conservationists to undertake status assessments and identify conservation actions. This thesis investigates the threats to an isolated population of grass snakes *Natrix helvetica* on the island of Jersey, providing recommendations for conservation management and recovery, whilst testing the suitability of tools for monitoring cryptic species. Grass snakes were historically widespread throughout Jersey; however, anthropogenic influences have restricted their distribution to the west and southwest. Furthermore, recent monitoring efforts have detected few individuals and their status is unknown.

Intensive surveys to locate individuals combined with occupancy and N-mixture (abundance) models identified continued occupancy of semi-natural sites in the island's west and southwest, but also highlighted poor detectability of the species unless utilising a large survey effort. Therefore, a large amount of effort is required to determine absence of snakes, and declines in the population cannot be detected with reasonable power. Occupancy models were more reliable than N-mixture models, particularly due to the risks of closure violation when estimating abundance. Nonetheless, N-mixture models estimated an abundance of 48 snakes (95% CI: 23–1279) across the study sites. Radio-tracking also provided evidence for low detection rates. Additionally snakes demonstrated small ranges (mean: 2.48 ha  $\pm$  3.54 SD), site fidelity, preferences for ranges close to paths and

compost heaps, but avoided crossing roads. Snakes were positively associated with structurally complex habitats including rough grassland, dense scrub and gorse at multiple spatial scales, but negatively with open and wooded habitats.

Species distribution modelling indicated similar habitat preferences to radio-tracking and poor suitability of agricultural habitats. Areas close to amphibian prey populations were also suitable whereas those with high road densities were not. A fifth of Jersey contained priority conservation areas, however almost 90% of these areas do not receive statutory protection. Those in the west and southwest should be prioritised for protection due to their proximity to extant subpopulations.

Mitochondrial genes identified the population to belong to a western lineage of grass snakes *Natrix helvetica helvetica*, with a probable natural colonisation prior to separation from northwest France. Within Jersey, microsatellite markers identified three subpopulations, with significant differentiation between snakes in the south and west. This coincides with a dense urban area, through which connectivity needs improvement.

The Jersey grass snake population can be classified as regionally Vulnerable (D2) under IUCN guidelines. The study illustrates how nature reserves are important for maintaining isolated subpopulations and the potential avenues by which statutory protection, sympathetic management practices and efforts to improve inter-reserve connectivity can contribute to conservation objectives.

**Keywords:** Channel Islands, conservation genetics, crypsis, insular, Jersey, monitoring, *Natrix*, occupancy, radio-tracking, snake, species distribution modelling

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## Chapter 1. Introduction

### 1.1. Biodiversity in crisis

#### 1.1.1. Anthropocene

The importance of effective conservation measures has never been greater as we are currently experiencing accelerated extinction rates, defining the sixth mass extinction event (Pimm *et al.*, 1995; Ceballos *et al.*, 2017). These global declines in abundance and species diversity are primarily anthropogenic, with secondary stochastic events causing extinction (Frankham *et al.*, 2002). Hence, the current period in which humans have had continued negative impacts on the environment has been termed the Anthropocene (Crutzen, 2006; Dirzo *et al.*, 2014). The degree of losses has far-reaching consequences on ecosystem function and consequently, upon human wellbeing and survival (Dirzo *et al.*, 2014; Ceballos *et al.*, 2017). Prevalent threats include habitat loss, climate change, fires, wildlife use and trade, pollution, invasive species and emerging diseases (Sala *et al.*, 2000; Hilton-Taylor *et al.*, 2009); affecting vertebrates (Ceballos and Ehrlich, 2002; Alroy, 2015; Regan *et al.*, 2015), invertebrates (Collen *et al.*, 2012) and plants (Hahs *et al.*, 2009).

#### 1.1.2. Policies and priorities

Efforts to halt biodiversity loss have resulted in a number of multilateral environmental agreements (MEAs) such as the Convention on Biological Diversity (Balmford *et al.*, 2005). Signatories to these agreements make efforts to reduce biodiversity loss, and may implement local or regional legislation to accomplish this. Due to limited resources, setting conservation priorities is an essential step in



## Chapter 1. Introduction

maximising cost-effectiveness (Murdoch *et al.*, 2007). Priority setting occurs at global, regional and local levels for species and ecosystems alike, using available information such as the IUCN Red List to identify priorities (Brooks *et al.*, 2006; Rodrigues *et al.*, 2006). Declines occur across a continuum, from the loss of individuals, to populations, species and ultimately biodiversity. Diagnosing the causes of decline at different scales has far-reaching implications for the conservation of species, genetic diversity and ecosystem function, and is necessary in order to plan and implement appropriate remedial actions. Understanding local threats and adaptations within a population allows for actions at a manageable scale.

Conservation priorities are derived from two main considerations; (i) the irreplaceability and (ii) vulnerability of a population, species or ecosystem (reviewed in Brooks *et al.*, 2006; Arponen, 2012). Irreplaceability refers to rarity, endemism and the presence of genetic diversity not found elsewhere (Frankham *et al.*, 2002). Indeed, a key consideration for conservation management is whether the taxonomy of a study group is known in order to identify management units appropriately (Frankham *et al.*, 2002). The genetic divergence of populations is often driven by isolation (allopatric speciation), and can lead to evolutionarily distinct lineages. These distinct lineages are often prioritised for conservation measures, as done in the EDGE (Evolutionarily Distinct and Globally Endangered) programme (<http://www.edgeofexistence.org>; Safi *et al.*, 2013). Where distinct lineages are not found elsewhere, they are referred to as endemic. These endemics are at greater risk of extinction than non-endemics (Frankham, 1998, but see Elgar and Clode, 2001). Therefore, priority hotspots for conservation often consist of regions with high accumulations of endemic species, such as the island

of Madagascar (Raxworthy and Nussbaum, 2000). In comparison, vulnerability typically refers to the likelihood of a species or ecosystem being lost, as measured by criteria such as changes in range size and species abundance (IUCN, 2012a). Other factors that may influence decision-making include the feasibility and cost of conservation efforts, the return on investment (Murdoch *et al.*, 2007), a species' contribution to the function of the ecosystem, and the complementarity of areas containing priority populations (Terribile *et al.*, 2009).

### **1.1.3. Problems of small population size**

Frankham *et al.* (2002) identified 11 key genetic problems of interest in conservation biology, several of which are associated with fragmentation and small population size. Such isolated populations may require different management strategies to large widespread ones due to their inherent vulnerability. Declines in gene flow and genetic diversity, and the occurrence of inbreeding are of major concern; potentially resulting in deformities, reduced fitness (e.g., Madsen *et al.*, 1996; Gautschi *et al.*, 2002) and increased extinction risk as they lose the ability to adapt to changes in the environment and the arrival of pathogens and parasites (Frankham *et al.*, 2002). Further pressures on small, isolated populations arise in the form of catastrophic events such as volcanic eruptions (e.g., Quito rocket frog *Colostethus jacobuspetersi*; IUCN, 2016) and stochastic changes in demography or the environment (Frankham *et al.*, 2002). Conservation management of small, threatened populations therefore often attempts to buffer them from stochastic events by using management strategies such as captive assurance colonies and translocation (e.g., Harding *et al.*, 2016; Ward *et al.*, 2016).

#### **1.1.4. Islands**

The formation of islands occurs through several processes. Land-bridge islands (also referred to as continental shelf islands) are those that were once part of a larger land mass and have since been separated, often by changes in sea level. Conversely, oceanic islands typically emerge from volcanic processes (Whittaker and Fernández-Palacios, 2007). Key differences in the biogeography of these, and other island types, are the ways in which they are colonised and how the resulting communities form. Prior to isolation, land-bridge islands contain levels of species diversity similar to those present on the mainland to which they are connected. Following separation, relaxation occurs, whereby species are lost until the resources available within the island can support the remaining biodiversity (Wilcox, 1978). In contrast, equilibrium theory may explain species richness in oceanic islands. In this case, following the formation of an island, colonisation slows and rates of extinction increase until equilibrium in the number of species is reached (MacArthur and Wilson, 1967).

Due to their isolation and relative simplicity, islands make excellent systems for the study of ecological and evolutionary processes (Whittaker and Fernández-Palacios, 2007). Moreover, their isolation results in speciation and consequently, they host disproportionate amounts of global biodiversity. However, island populations tend to be more threatened, and experience greater extinction rates than on the mainland (Frankham, 1998; Frankham *et al.*, 2002; Tershy *et al.*, 2015). For example, 81% of reptile extinctions and 47% of Critically Endangered reptiles originate on islands (Tershy *et al.*, 2015). These elevated levels of threat or extinction on islands may be attributed to the genetic effects of small population size described above, further exacerbated by founder effects, genetic drift and

reduced or absent gene flow (Frankham, 1998; Frankham *et al.*, 2002). Additional pressures come in the form of introduced species (e.g., Daltry *et al.*, 2001), stochastic events (e.g., volcanic activity), harvesting, trade and habitat loss. Of these, the most pervasive is the introduction of non-native species and pathogens, as island populations are naive and often lack the ability to adapt to counteract their effects (Tershy *et al.*, 2015).

Many of the processes discussed above for true islands, can also be seen in 'habitat islands'; whereby continued habitat fragmentation across landscapes results in 'island' populations surrounded by a matrix of impermeable habitat. These isolated populations exhibit similar traits, including extinction risk, to those described for island populations (Frankham *et al.*, 2002). Therefore, studies of island systems have wide-ranging implications for the conservation of small, isolated populations elsewhere.

### **1.1.5. Detectability and data deficiency - barriers to conservation**

Data deficiency across most taxa (perhaps with the exception of birds, Butchart and Bird, 2010), limits our ability to estimate extinction risk and generate priorities for conservation actions effectively. Further challenges arise if we also consider the continued discovery and description of new species (Mora *et al.*, 2011), and the taxonomic changes that come with new scientific evidence (reviewed in Morrison *et al.*, 2009). These issues not only influence our ability for monitoring and conservation prioritisation, but also for assessing the responses of species to management actions (Thompson, 2004). A species may be data deficient due to perceived rarity, inaccessibility, or simply a lack of effort.

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The definition of rarity is not a simple one (reviewed in McDonald, 2004), and several definitions may be applied when referring to rare or elusive species. McDonald (2004) defines rare species as those with a low detection probability. This may be due to low abundance, cryptic behaviour, the spatial distribution and density of a population (i.e., clumped or dispersed), definition of a population or an ineffective sampling strategy (McDonald, 2004). Comparatively, Specht et al. (2017) define rare species as those that occupy less than 30% of available sites, and cryptic species as those that are detected less than 30% of the time when they are present. The latter definition of Specht et al. (2017) is aligned with the increasing literature on occupancy modelling, where detection probability is considered as the probability of detecting any individual of a species given that the site being sampled is occupied (MacKenzie *et al.*, 2002).

Evaluating appropriate sampling methods within a pilot study minimises wasted effort by identifying the best tools for maximising species detection. Any resulting improvements in our capability to detect and monitor populations eluding current efforts are beneficial for conservation (Böhm *et al.*, 2013; Dirzo *et al.*, 2014). An increasing range of methods is available for improving species detection. Some of the more novel methods include the use of detection dogs (e.g., Browne *et al.*, 2015), attractant pheromones as already commonly used for monitoring insects (reviewed in Larsson, 2016), environmental DNA (eDNA) (reviewed in Rees *et al.*, 2014), camera traps (reviewed for squamate reptiles in Welbourne *et al.*, 2017) or Light Detection and Ranging (LiDAR) scanners for counting bat and bird roosts (McFarlane *et al.*, 2015; Shazali *et al.*, 2017). These and other emerging technologies should be assessed for their applicability to a study's aims, and contrasted to traditional survey methods early in the design

process. As a result, the efficiency and cost-effectiveness of sampling efforts can be improved, and in some cases, disturbance to target organisms can be reduced.

Very rarely is the detection of a species perfect, and estimates of occurrence and abundance that do not take this into account are prone to biases (Guillera-Aroita *et al.*, 2014). Statistical models that account for imperfect detection are now well-established, including distance-sampling, removal sampling, capture-mark-recapture, occupancy and N-mixture models. Furthermore, new extensions are emerging regularly, allowing for more flexible approaches and the incorporation of population dynamics (Kéry and Royle, 2015). Although these advances have alleviated some of the issues with imperfect detection, cryptic or rare species continue to defy statistical advances and may have low power to detect population trends (Steen, 2010; Miller *et al.*, 2011). Evaluating their suitability for a range of rare or elusive taxa can assist in study design.

### **1.2. Reptile declines and conservation**

Reptiles have received little research attention when compared with other vertebrate groups, particularly when considering their species diversity (Bonnet *et al.*, 2002; Clark and May, 2002). As a result of species discoveries and phylogenies becoming resolved (e.g., Maddock *et al.*, 2017), the number of known reptile species increases by approximately 1.6% each year (Pincheira-Donoso *et al.*, 2013); indeed, almost 10,500 reptile species are now recorded (Uetz *et al.*, 2016). Snakes, lizards and amphisbaenians (the squamates) make up 96.3% of this reptilian diversity, of which 98% are lizards (Sauria) and snakes (Serpentes) (Pincheira-Donoso *et al.*, 2013). These latter lineages occupy most biogeographic regions, with many successfully inhabiting temperate zones (Vitt and Caldwell,

2009; Pincheira-Donoso *et al.*, 2013), and even the Arctic Circle (Andersson, 2003).

Squamate reptiles have strong evolutionary and ecological roles, and heavily influence present-day ecosystems (Pough *et al.*, 2004; Pincheira-Donoso *et al.*, 2013) by acting as predators, prey, seed-dispersers, grazers, and commensal species (Böhm *et al.*, 2013). Snakes in particular are often top predators within an ecosystem; therefore the decline of a snake population may affect or reflect the function of the ecosystem it inhabits (Reading *et al.*, 2010). Compared with other vertebrates, particularly birds and mammals, reptiles tend to occupy narrow niche breadths and distributional ranges. Those with specialist adaptations, sedentary lifestyles and close proximity to urban areas are at high risk of extinction (Reading *et al.*, 2010; Böhm *et al.*, 2013; 2016). Indeed, global reptile declines have been raised as a concern by a number of authors (Gibbons *et al.*, 2000; Winne *et al.*, 2007; Collen *et al.*, 2009; Reading *et al.*, 2010; Böhm *et al.*, 2013; 2016).

Discerning the causes of reptile declines is not always possible, but they are rarely natural (Gibbons *et al.*, 2000). Instead, they may be driven by several anthropogenic factors (Gibbons *et al.*, 2000; Cox and Temple, 2009; Reading *et al.*, 2010; Todd *et al.*, 2010; Böhm *et al.*, 2013). These include climate change (e.g., Araújo *et al.*, 2006; Sinervo *et al.*, 2010), disease and parasitism (Schumacher, 2006), unsustainable wildlife trade and use (e.g., Zhou and Jiang, 2004; Schlaepfer *et al.*, 2005; Andreone *et al.*, 2006; Auliya *et al.*, 2016), invasive species (e.g., Daltry *et al.*, 2001; Schlaepfer *et al.*, 2005) and habitat change (e.g., Driscoll, 2004; Beaupre and Douglas, 2009; Heigl *et al.*, 2017). Recent studies have estimated 15–37% of reptiles to be at risk of extinction, and approximately a fifth to be Data Deficient (Collen *et al.*, 2009; Böhm *et al.*, 2013). Similarly, a fifth of

European reptile species are threatened with extinction and 42% are undergoing declines (Cox and Temple, 2009).

Over half of Data Deficient reptile species, particularly snakes, require further information on population status and trends in order to be assessed (Böhm *et al.*, 2013; Bland and Böhm, 2016). Gathering this information to make assessments is hindered by the resource requirements associated with monitoring, and the biological traits of the species being investigated. Most monitoring efforts are short term; thus, assessments of population dynamics and threats are ambiguous. Furthermore, many reptile species are not easily studied in the field due to their solitary lifestyles, low population densities, fossorial, arboreal or aquatic habitats and inconspicuous behaviour, colouration, patterning and vocalisations (Rodda, 1993; Gibbons *et al.*, 2000; Kéry, 2002; Colli *et al.*, 2016). Snakes typify a number of these traits, resulting in low detection probabilities (Dorcas and Willson, 2009; Steen, 2010; Durso *et al.*, 2011). Therefore, despite a common concern amongst herpetologists that declines of snakes may be occurring globally, there is still little evidence due to few long-term studies and imperfect detection (Dorcas and Willson, 2009; Terribile *et al.*, 2009; Reading *et al.*, 2010; Maritz *et al.*, 2016). Further research is needed to better understand the status of snake populations worldwide (Böhm *et al.*, 2013).

### **1.2.1. Habitat loss and degradation**

As noted above, threat status in reptiles is often driven by a combination of factors (Todd *et al.*, 2010; Clark *et al.*, 2011). However, as with biodiversity as a whole (Sala *et al.*, 2000), the most pervasive is the effect of anthropogenically driven loss and fragmentation of habitat (Gibbons *et al.*, 2000; Collen *et al.*, 2009;



Cox and Temple, 2009; Böhm *et al.*, 2013). Having a relatively low dispersal ability, high trophic position, specialised morphology, limited home range size and physiological constraints such as thermal requirements leads reptiles to be highly sensitive to habitat change (Kearney *et al.*, 2009; Maritz *et al.*, 2016; Todd *et al.*, 2017). These sensitivities are highly variable between species, with differences attributed to assorted ecological and biological constraints (Reed and Shine, 2002; Santos *et al.*, 2006). Thus, the responses of reptile species to habitat change are varied, with positive, as well as negative effects on populations (e.g., Driscoll, 2004).

### **1.2.2. Squamate declines in Europe**

Within Europe, around 145 squamate reptile species are represented across three suborders, of which the Serpentes comprise approximately a third (Cox and Temple, 2009; Sillero *et al.*, 2014). Colubrid snakes (Colubridae) are the second most diverse reptile family in Europe with 28 species, superseded only by lacertid lizards (Lacertidae), which consist of 65 species (Cox and Temple, 2009). A primary concern for these species is that Europe has the most fragmented landscape of all continents, with over 80% of land directly managed in some way (European Environment Agency, 2007; Cox and Temple, 2009). A large contributor has been the intensification of agriculture and subsequent loss of connectivity, resulting in large swathes of unsuitable habitat (Ribeiro *et al.*, 2009; Biaggini and Corti, 2015). Changes in habitat structure through afforestation have also resulted in low-quality habitat for reptiles, as the thermal properties of terrestrial patches are altered through vegetative succession (Jaggi and Baur, 1999; Cox and Temple, 2009; Bonnet *et al.*, 2016; Jofré *et al.*, 2016). Other threats

facing European reptiles include pollution, anthropogenic climate change, overharvesting and deliberate persecution (Cox and Temple, 2009).

### **1.3. Natricine snakes**

The natricines are a widespread group of predominantly semi-aquatic snakes, with many species being common and inhabiting large ranges (Gregory and Isaac, 2004). This group of colubrid snakes have been studied intensively across a number of populations and locations (e.g., Thorpe, 1975; Madsen, 1984; Gregory, 2004; Lind *et al.*, 2005; Meister *et al.*, 2012; Kindler *et al.*, 2013; Gregory and Tuttle, 2016), providing comparative studies for this thesis. The semi-aquatic *Natrix* genus (Laurenti, 1768) has undergone a number of taxonomic changes (Thorpe, 1979; Hille, 1997; Guicking *et al.*, 2006; Fritz *et al.*, 2012; Kindler *et al.*, 2013; 2017). Until recently, it was thought to contain three species (*N. maura* Linnaeus, 1758; *N. tessellata* Laurenti, 1768 and *N. natrix* Linnaeus, 1758) which are found from North Africa, through Europe and across to central Asia (Kreiner, 2007; Kindler *et al.*, 2013). However, contemporary work has suggested elevating *N. n. astreptophora* (Pokrant *et al.*, 2016) and other western lineages (Kindler *et al.*, 2017) to full species status; resulting in five species after the addition of *N. astreptophora* and *N. helvetica* respectively. This proposed taxonomy is used from here on.

#### **1.3.1. The grass snake**

The number of recognised grass snake subspecies has varied between four and 14 (Thorpe, 1979; Kreiner, 2007; Fritz *et al.*, 2012), and further taxonomic revision is needed (Fritz *et al.*, 2012; Kindler *et al.*, 2013). The grass snake (*Natrix helvetica*; Lacépède, 1789) as it is known in Britain, has a widespread distribution

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occupying much of Western Europe (Steward, 1971; Kabisch, 1999; Beebee and Griffiths, 2000; Kindler *et al.*, 2017). The subspecies *N. h. helvetica* (Lacépède, 1789) occupies the western part of the range, encompassing the British Isles, France, Switzerland, Italy and west of the Rhine in Germany (Steward, 1971; Kreiner, 2007). Habitat preferences generally consist of lowland areas containing deciduous woodland, hedgerows, scrub, heathland, marshland and moorland (Smith, 1951; Steward, 1971; Madsen, 1984; Reading, 1997; Stumpel and van der Werf, 2012), with some use of agricultural land (Steward, 1971; Madsen, 1984; Wisler *et al.*, 2008).

The ecological requirements of grass snakes centre around four key resources; (i) hibernation sites, (ii) nesting sites, (iii) prey and (iv) cover with thermoregulation opportunities (Madsen, 1984; van Roon *et al.*, 2006). They are commonly active and primarily diurnal from March through until October, and overwinter in hibernacula underground (Steward, 1971; Luiselli *et al.*, 1997; Mertens, 2008). The annual active cycle (Figure 1.1) may be split in to three phases; (i) lying out and mating, (ii) feeding and egg laying, and (iii) hatching and returning to hibernation. Courtship and mating usually occurs from late March through to May (Steward, 1971), followed by egg-laying between June and August (Madsen, 1984); often in decomposing vegetation such as manure and compost heaps (Steward, 1971; Löwenborg *et al.*, 2010; 2012a).

Grass snakes primarily prey on amphibians, and so often show a strong affinity for freshwater habitats (Madsen, 1983; Brown, 1991; Gleed-Owen, 1994; Reading and Davies, 1996; Luiselli *et al.*, 1997; Beebee and Griffiths, 2000). However, they also predate fish, small mammals, birds, invertebrates and other reptiles (Steward, 1971; Arnold and Burton, 1978; Kabisch, 1999; Gregory and

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Isaac, 2004; Consul *et al.*, 2009; Ahmadzadeh *et al.*, 2011). Both the size and type of prey may shift ontogenetically and seasonally (Gregory and Isaac, 2004; Mertens, 2008).

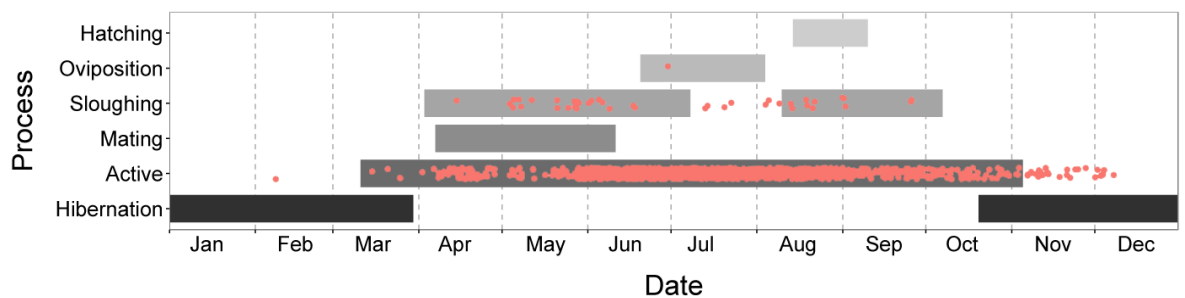


Figure 1.1 Seasonal cycle of grass snakes based on the literature (grey bars) and observed processes from this study in Jersey (red points). Observed data points are combined from repeat site surveys (Chapter 2) radio-tracking (Chapter 3), and public records from 2006 to 2015 (Chapter 4).

Generally, grass snakes are olive, green, or brown in colouration (Figure 1.2), but can be variable (Steward, 1971). This colouration is broken up by two rows of dark markings along the dorsal surface, a distinctive yellow, white or orange collar at the rear of the head which is lost with age in some localities including Jersey (Frazer, 1949), and a symmetrical pair of black crescents posterior to the collar (Steward, 1971). The ventral surface of grass snakes is white or cream in colour with irregular black or grey chequering (Figure 1.2) (Steward, 1971), which can be used in determining individual identification of specimens (Mertens, 2008). Females attain larger body and head sizes than males, with maximum body lengths of approximately 76 cm for males and 91 cm for females (Steward, 1971; Madsen, 1983; Gregory, 2004; Gregory and Isaac, 2004), whilst males reach maturity in approximately three years, and females four to five years (Appleby, 1971; Madsen, 1983).

a



b



Figure 1.2 Colouration and patterning of grass snakes showing (a) the dorsal view of a juvenile grass snake, and (b) the chequered ventral surface of an adult female feigning death. The adult female is in slough and shows a severe eye injury (b). Both images are of *N. helvetica* from Jersey.

### 1.3.2. Grass snake declines

Localised declines in grass snake populations are occurring, as habitat loss and other pressures produce isolated subpopulations. At the northern edge of their range in Sweden, declines of grass snakes are attributed to a reliance upon anthropogenic egg-laying sites such as manure piles (Löwenborg *et al.*, 2010; Hagman *et al.*, 2012). These declines have been driven by a reduction in small-scale farms containing manure heaps, poor hatching success at natural nest sites and a change in law causing manure piles to be contained and subsequently,

inaccessible to snakes (Weatherhead and Madsen, 2009; Hagman *et al.*, 2012; Löwenborg *et al.*, 2012a; 2012b). Elsewhere in Europe, declines of 30% over a century have been noted following drainage of wetlands and associated losses of amphibian prey (Monney and Meyer, 2005; Meister *et al.*, 2010). Furthermore, the population of *N. n. cypriaca* inhabiting Cyprus is heavily threatened due to limited reproduction, small population size, habitat loss, pollution and water extraction (Baier and Wiedl, 2010).

Within Britain, grass snakes are regarded as common, but are thought to be undergoing declines in some areas due to dependencies on, and the distribution of, landscape-level features (Gent and Gibson, 1998). Individual-based long-term studies on grass snakes in Britain and France (from 1997–2009 and 1995–2009 respectively) found the populations within protected areas to be stable (Reading *et al.*, 2010). However, the same study suggested synchronous declines of some other snake species from Europe and Africa. Despite all of the stable populations occurring within protected areas, those undergoing declines occurred both within and outside of protected areas (Reading *et al.*, 2010). Moreover, all three of the populations outside of protected areas declined, suggesting protected areas may buffer some of the effects imposed by habitat loss.

### **1.4. Jersey**

The British Channel Island of Jersey is approximately 117 km<sup>2</sup> and was isolated from northwest France around 7,000 years ago following the rise of the English Channel (Johnston, 1981; Jones, 1993). Anthropogenic pressures have resulted in a severely fragmented and diminished semi-natural landscape, with agriculture accounting for over half of the island's land use (States of Jersey

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Department of the Environment, 2016a). Further pressures arise from one of the highest human population densities in the world (The World Bank, 2015), which, combined with tourism, puts the remaining semi-natural areas of the island under a large amount of pressure to support both biodiversity and the recreational needs of people (Young *et al.*, 2005; Jersey Tourism, 2010; States of Jersey Department of the Environment, 2016b). Jersey's biodiversity is also negatively impacted by the over extraction and pollution of water (e.g., Gibson and Freeman, 1997).

In an effort to abate these threats to biodiversity, the States of Jersey government is a signatory to multiple multilateral environmental agreements (e.g., Convention of Biological Diversity; Bern Convention), resulting in its own biodiversity strategy (States of Jersey Planning and Environment Committee, 2000). These efforts have also resulted in the development of Species Action Plans for a number of species, including the grass snake. Several laws govern the protection and use of the island's natural environment. Freshwater resources are regulated by the Water Pollution (Jersey) Law 2000 and the Water Resources (Jersey) Law 2007. Comparably, site protection, sustainable development and other forms of terrestrial land management come under the Planning and Building (Jersey) Law 2002, which includes the designation of Sites of Special Interest (SSIs). There are currently 19 ecological SSIs designated and several more under consideration. Some of those proposed are among the many semi-natural areas in the island of conservation importance that are currently only assigned non-statutory designations, with limited or no legal protection (e.g., Sites of Importance for Nature Conservation). The largest of these is the Jersey National Park (JNP), which is situated along the coast and encompasses approximately 16.5% of the island and several SSIs (States of Jersey Department of the Environment, 2016a).

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Of great complementarity to the laws governing protection of the landscape, has been the introduction of the Conservation of Wildlife Law (Jersey) 2000. This law affords strong species-specific protection for much of Jersey's flora and fauna, including full protection of all herpetofauna. Specifically, it prevents the killing of protected species, the damage, destruction or obstruction of dens or nest sites, the disturbance of nests or dens that may be occupied, the buying, selling or keeping of those species, and the export of any native reptile or amphibian.

Like many islands, Jersey has a depauperate fauna, hosting only four reptile species; the green lizard *Lacerta bilineata*, the wall lizard *Podarcis muralis*, the slow-worm *Anguis fragilis* and the grass snake *Natrix helvetica*. The rarest of these is undoubtedly the grass snake, which is infrequently encountered and is suggested to be restricted to the west, southwest and possibly southeast of the island with an unclear status (Hall, 2002; McMillan, 2003). Historically, it was considered to be widespread (Poingdestre, 1682) and subsequently common in the northwest and southwest of the island, whilst occurring at low densities elsewhere (Sinel, 1908; Le Sueur, 1976). More recently, studies have suggested that two SSIs; Les Blanchés Banques in the west and Ouaisné Common in the southwest, provide key grass snake habitats (Hall, 2002; McMillan, 2003). Conversely, no grass snakes have been recorded in the northwest since a juvenile was observed at Grosnez pond in 1992 (J.W. Wilkinson, pers. comm.). The most recent attempts to monitor herpetofauna in Jersey under the National Amphibian and Reptile Recording Scheme (NARRS) (Wilkinson *et al.*, 2014) found only four grass snakes in six years, sparking concern over the potential for extinction. The threats to this population are not entirely clear (Hall, 2002; McMillan, 2003), though it is likely a combination of increasing habitat loss and degradation through



development and expanding agriculture, a decline in amphibian prey, reduced connectivity between habitats, and associated consequences of isolation.

Additional pressures may be inflicted from mortality, associated to roads (e.g., Borczyk, 2004) and predators such as corvids (Madsen, 1987) and introduced feral cats *Felis catus* and polecats *Mustela putorius*.

## 1.5. Aims and objectives

In order to assess and improve the conservation status of grass snakes in Jersey, this thesis sets out to answer the following questions:

- Do grass snakes still occur in the west and southwest of Jersey and how many are there? If so, why have recent efforts struggled to detect them and how can monitoring for rare and elusive species be improved?
- What are the resource requirements for this population, and what management actions can be taken to improve its status?
- How effective is the current protected area network in providing grass snake habitat, and which areas should receive prioritisation for monitoring and habitat protection?
- Where did the population originate and how has subsequent isolation, fragmentation and population decline affected its genetic structure? What are the appropriate steps for managing any problems identified?

## 1.6. Thesis structure

The thesis approaches these questions in the following way:

**Chapter 2** applies occupancy and N-mixture models to two years of survey data to infer the occupancy and abundance within specific sites previously known or

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thought to be occupied, predominantly in the west and southwest of the island.

This chapter identifies the drivers of species detection, site occupancy and abundance, and uses this information to inform the design of future monitoring efforts.

**Chapter 3** uses radio-telemetry to assess habitat use at two spatial scales. The resulting data give insights in to habitat requirements, range size and movement ecology. These findings are discussed in the context of species detectability, site management and connectivity.

**Chapter 4** tests the application of species distribution modelling to identify priority areas for monitoring and protection whilst accounting for sampling bias. The study also identifies landscape features influencing distribution, and evaluates the effectiveness of the current protected area network for protecting the grass snake population.

**Chapter 5** investigates the phylogeography of the population in Jersey, and the effects of isolation, fragmentation and decline upon its genetic diversity and structure. The study uncovers barriers in the landscape that may be interrupting dispersal.

**Chapter 6** presents a general discussion and overall summary based on the findings of the previous chapters. Following an assessment of the population's conservation status, recommendations are made for conservation management and research to improve the viability of the population.

## **Chapter 2. Optimising monitoring efforts for secretive snakes: a comparison of occupancy and N-mixture models for assessment of population status**

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## 2.1. Abstract

A fifth of reptiles are Data Deficient; many due to unknown population status. Monitoring snake populations can be demanding due to crypsis and low population densities, with insufficient recaptures for abundance estimation via Capture-Mark-Recapture. Alternatively, binomial N-mixture models enable abundance estimation from count data without individual identification, but have rarely been successfully applied to snake populations. We evaluated the suitability of occupancy and N-mixture methods for monitoring an insular population of grass snakes (*Natrix helvetica*) and identified covariates influencing detection, occupancy and abundance within remaining habitat. Snakes were elusive, with detectability increasing with survey effort (mean:  $0.35 \pm 0.07$  s.e.m.). Site-occupancy was moderate (mean:  $0.70 \pm 0.15$  s.e.m.) and positively related to the length of transect surveyed. Abundance estimates indicate a small threatened population across our study sites (mean: 48, 95% CI: 23–1279) favouring semi-natural heterogeneous habitat. Power analysis indicated that the survey effort required to detect occupancy declines would be prohibitive. Both occupancy and N-mixture models fitted well, but N-mixture models provided little extra information over occupancy models and were at greater risk of closure violation. Therefore, we suggest occupancy models are more appropriate for monitoring snakes and other elusive species, but that trends may go undetected.

**Keywords:** abundance, cryptic, detection, distribution, monitoring, N-mixture, occupancy, snake

## 2.2. Introduction

Monitoring populations is crucial for informing conservation measures. The status of a population and the drivers influencing it are often assessed over time using measures of occupancy or abundance (Yoccoz *et al.*, 2001; MacKenzie *et al.*, 2005; IUCN, 2012a). These measures vary in terms of the quality and quantity of data needed, and the appropriate monitoring strategy for a population is often unclear (Joseph *et al.*, 2006; Pollock, 2006). The choice of monitoring strategy can result in different status assessments (Joseph *et al.*, 2006) and must consider that population changes can occur through subpopulation colonisation and extinction, or a decline across the whole population (Pollock, 2006). Occupancy can be assessed as the proportion of an area containing a species, based on repeated observations of presence or absence at a number of sites. Alternatively, abundance measures can make use of counts and vary in their logistical requirements (Couturier *et al.*, 2013).

Capture-Mark-Recapture (CMR), removal and distance-sampling are common methods for estimating abundance (Fitch, 1999; Beaupre and Douglas, 2009), but are time-consuming and not always applicable (White, 1982; Buckland *et al.*, 2001; Royle, 2004). Indeed, for some snake species, recaptures are often low (Mertens, 1995; Dorcas and Willson, 2009). Alternatively, low-cost methods that integrate imperfect detection into presence-absence (MacKenzie *et al.*, 2002) and simple count methods (e.g., binomial N-mixture, Royle, 2004) are attractive and have been shown to provide reliable estimates of occupancy and abundance respectively without the need for individual identification of animals (MacKenzie *et al.*, 2002; 2005; Guillera-Aroita *et al.*, 2014). Furthermore, as counts are often conducted simultaneously with presence-absence surveys, estimating abundance

## Chapter 2. Optimising monitoring efforts for snakes

from counts may require little additional survey effort. With an appropriate level of survey effort, both methods can be used for rare or cryptic populations where detections are low and other, more intensive methods would be unsuitable (MacKenzie *et al.*, 2002; Royle, 2004; Durso *et al.*, 2011; Couturier *et al.*, 2013).

The design of an optimal monitoring scheme can require a choice between measuring occupancy or abundance, and must consider the economic and logistical costs associated with each one (MacKenzie *et al.*, 2005; Joseph *et al.*, 2006; Pollock, 2006). The two measures are closely related but do not provide the same information (Gaston *et al.*, 2000; Durso *et al.*, 2011; Kéry and Royle, 2015). Occupancy methods may fail to detect changes in population size, and therefore underestimate extinction risk if changes in occupancy and abundance are occurring at different rates (Böhm *et al.*, 2013; but see Joseph *et al.*, 2006). However, they are cost-effective, can aid conservation assessment and be used to monitor cryptic taxa such as snakes (MacKenzie *et al.*, 2005; Durso *et al.*, 2011; Sewell *et al.*, 2012; Bonnet *et al.*, 2016). Alternatively, abundance measures can provide data on population size but tend to require greater resources (Gaston *et al.*, 2000; Pollock, 2006), high species detectability (Kéry *et al.*, 2009; Couturier *et al.*, 2013) and more stringent modelling assumptions (Royle, 2004; MacKenzie *et al.*, 2005).

A fifth of reptiles are considered threatened, and a further fifth Data Deficient, due largely to limited data on population trends (Böhm *et al.*, 2013). Declines have occurred at global (Gibbons *et al.*, 2000) and regional levels (Cox and Temple, 2009), and there is growing concern over potential widespread snake declines (Zhou and Jiang, 2004; Reading *et al.*, 2010) with a poor understanding of the underlying causes (Rodda, 1993; Gibbons *et al.*, 2000; Beaupre and Douglas,

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2009; Reading *et al.*, 2010). Bridging the gap in reptile threat assessment is challenging, with evolutionary and biological traits influencing both extinction risk and our ability to gather appropriate information (Colli *et al.*, 2016; Tonini *et al.*, 2016). Attempts to monitor these populations are often carried out by regional or national organisations, such as those in the UK (Wilkinson *et al.*, 2014) and the Netherlands (Kéry *et al.*, 2009).

Snakes have some of the lowest detection rates among reptiles (Durso *et al.*, 2011) (perhaps with the exception of fossorial taxa such as *Amphisbaenia* (Böhm *et al.*, 2013; Colli *et al.*, 2016)). They can occur at low densities, have wide ranges, cryptic colouration and behaviour, and are often unobservable due to their chosen habitats (Durso *et al.*, 2011). They are therefore particularly difficult to study (Turner, 1977), and previous work has often struggled to attain reliable estimates of snake occupancy, detection and abundance (Rodda, 1993; Dorcas and Willson, 2009; Steen, 2010). This highlights a need to identify the most appropriate tools for monitoring snake populations and optimising detectability (Reading *et al.*, 2010; Durso *et al.*, 2011; Böhm *et al.*, 2013) in order to reduce Data Deficiency in this group.

We test the application of two low-cost approaches to monitoring; occupancy (MacKenzie *et al.*, 2002) and binomial N-mixture models (a count-based method) (Royle, 2004), on two years of survey data of a rare, insular population of grass snakes in Jersey (British Channel Islands) (Le Sueur, 1976; Wilkinson *et al.*, 2014). Previous studies of other grass snake populations have found them to be stable (Reading *et al.*, 2010) or in decline (Hagman *et al.*, 2012). However, the species is known to be wide-ranging (Madsen, 1984) and elusive with low to moderate detectability (Reading *et al.*, 2010; Sewell *et al.*, 2012), making it difficult

## Chapter 2. Optimising monitoring efforts for snakes

to monitor (Steen, 2010). The status of Jersey's grass snake population is unknown, and is currently monitored using an occupancy framework under the National Amphibian and Reptile Recording Scheme (NARRS). However, this citizen science scheme recorded only four grass snakes in Jersey between 2007 and 2012 (Wilkinson *et al.*, 2014) and is likely to have underestimated the species' distribution. Therefore we assessed the ability of the NARRS protocol to detect grass snakes and changes in its population, and provide recommendations for future monitoring efforts.

### **2.3. Materials and methods**

To identify the best strategy for monitoring Jersey's grass snake population and determine current population status, we conducted intensive surveys of remaining habitat over two years. We evaluated the goodness-of-fit and applicability of occupancy and N-mixture models to our data, and identified the factors influencing species detectability. This enabled us to calculate the survey effort required to determine absence from a site, and the number of sites to be surveyed to detect an occupancy decline.

#### **2.3.1. Surveys**

The island of Jersey (49°12'N, 2°8'W) is 117 km<sup>2</sup> and lies 22 km west of Normandy, France. The main pressures to its biodiversity are anthropogenic, with 83% of land-cover modified for human use (States of Jersey Department of the Environment, 2016a). We selected 14 study sites (Figure 2.1) based upon grass snake distribution data from previous monitoring (Wilkinson *et al.*, 2014), the local biological records centre (<http://jerseybiodiversitycentre.org.je/>) and expert opinion. These were largely within remaining semi-natural areas of Jersey National Park in



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the west and southwest of the island where the species has historically persisted (Le Sueur, 1976). Sites comprised a mixture of habitats including dune grassland, coastal plains, heath and scrub along with amenity grassland and semi-urban areas, and were assigned to one of four habitat classes; amenity grassland, dune grassland, rough grassland or scrub (Table 2.2). Sites were deliberately large to meet closure assumptions (mean: 27.9 ha, range: 1.01–75.91 ha, Table 2.2), were delineated by (i) boundaries in land management, (ii) changes in vegetation composition and/or (iii) the presence of significant barriers to movement (e.g., roads), and were considered to contain all necessary resources for a population.

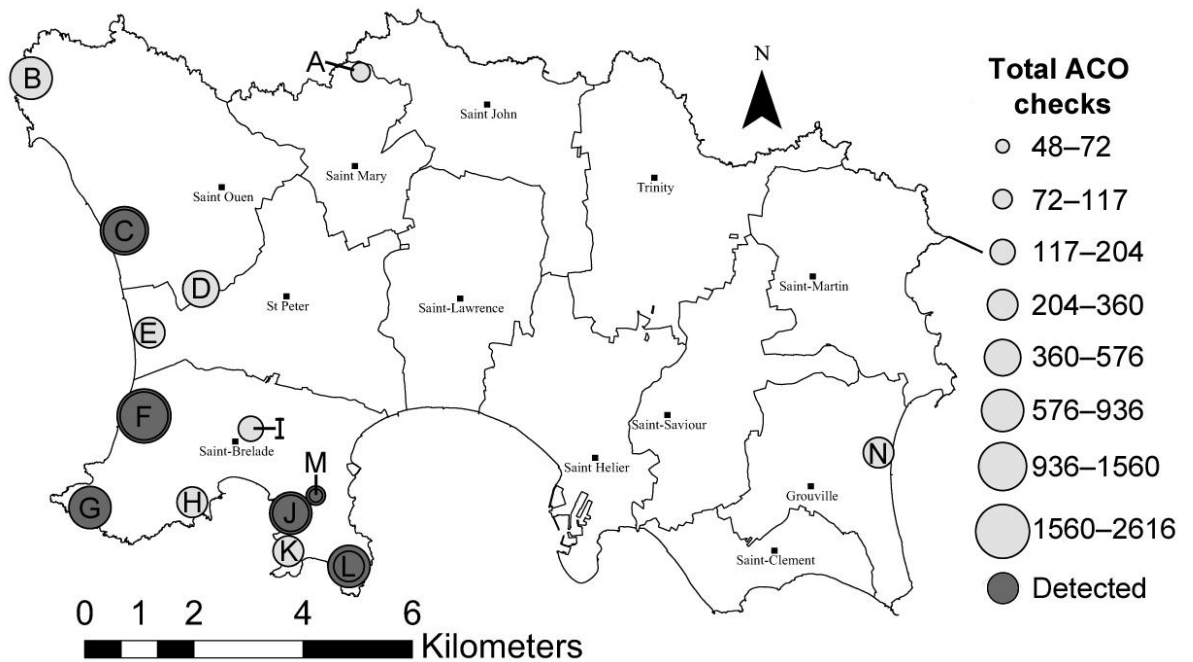


Figure 2.1 Map of Jersey showing study sites (labelled circles), number of artificial cover objects (ACOs) checked in each season (size of circle) and naïve occupancy (dark grey = occupied, light grey = unoccupied). Sites with concentric circles were surveyed in both years. The map was generated in ArcMap v10.2.1 (ESRI, Redlands).

Sites were visited up to eight times (mean:  $6.95 \pm 0.36$  s.e.m., Table 2.2) per season (March–October) in 2014 or 2015 by the same surveyor, and surveyed using a combination of visual encounter and artificial cover object (ACO) methods

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(Sewell *et al.*, 2013). Five of the 14 sites were surveyed in both years. Repeat surveys of a site were at least seven days apart to reduce disturbance and behavioural effects upon the study population. To ensure site closure for statistical modelling, a robust survey design is recommended (Pollock, 1982) where repeat site surveys are carried out in a short time-frame to ensure no change in the population. However, monitoring schemes may conduct surveys over a wide period (Kéry *et al.*, 2009; Wilkinson *et al.*, 2014) and so we tested the applicability of occupancy and N-mixture models on data collected in this way. If violation of the closure assumption occurred through random emigration and immigration, the probability of site occupancy should instead be considered as the probability of site use, or for abundance, the number of individuals associated with, rather than resident within, a site (MacKenzie *et al.*, 2005; Kéry and Royle, 2015).

Transect length and number of ACOs varied between sites (Table 2.2). At each site a comparable mixture of roofing felt, corrugated tin and corrugated bitumen sheets 0.45–0.5 m<sup>2</sup> in surface area were used as ACOs to maximise detection in differing thermal conditions (Sewell *et al.*, 2013). Due to the heterogeneity of the habitats, ACOs were not spaced evenly, but were primarily placed in south-facing habitats away from public disturbance (Wilkinson *et al.*, 2014). We tested the relationship between the number of ACOs and transect length for a site using Kendall's tau rank correlation (Kendall, 1976) as the data were non-normally distributed and comprised small sample sizes. These two measures of survey effort were significantly correlated ( $r_{\tau} = 0.82$ ,  $n = 19$ ,  $p < 0.001$ ), so for analysis we consider only the number of ACOs. Covariates thought to influence occupancy, detection or abundance were recorded based upon suspected and known life-history knowledge (Joppa *et al.*, 2009; Sewell *et al.*,

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2012) (Table 2.1). Data were organised by week to test for effects of survey timing.

Encounters included live individuals, sloughed skins and carcasses. Sloughs and carcasses were removed when found, to avoid duplicated records. All captured live snakes were photographed for individual identification (Mertens, 1995). The ventral patterns were compared across all captures to calculate a minimum population size based on the maximum number of unique individuals identified. This also provided evidence for validations of site-independence and independence between detections within a survey. This study was approved by the University of Kent School of Anthropology and Conservation ethical review committee. All handling and disturbance was conducted under licence (CR 23), issued under the Conservation of Wildlife Law (Jersey) 2000 by the States of Jersey Department of the Environment and in accordance with current guidelines (Association for the Study of Animal Behaviour, 2017).

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Table 2.1 Covariates evaluated as potential predictors of grass snake occupancy ( $\psi$ ), detection ( $p$ ) or abundance ( $\lambda$ ). The level indicates whether the covariate was measured at the site-level (site), or within each survey visit (survey). Continuous covariates were scaled to their mean and one standard deviation.

Covariate	Type	Level	Description
habitat <sup>a</sup>	Factor	Site	Habitat type categorised by dominant habitat class from Phase 1 survey data provided by the Jersey States Department of the Environment: 'Amenity grassland', 'Dune grassland', 'Rough grassland', 'Scrub'
ACOs	Continuous	Site	Number of artificial cover objects (ACOs) surveyed on each site visit
aspect <sup>a</sup>	Factor	Site	Mean aspect azimuth of site calculated using script from Carl Beyerhelm, Coconino National Forest. Available at: <a href="https://geonet.esri.com/thread/47864">https://geonet.esri.com/thread/47864</a> [accessed 31 December, 2015]: 'N', 'NE', 'E', 'SE', 'S', 'SW', 'W', 'NW'
conditions	Factor	Survey	Weather conditions during survey: 'Sunny', 'Sunny / Overcast', 'Overcast'
cloud	Continuous	Survey	Estimated % of cloud cover at start of survey
temperature	Continuous	Survey	Mean daily temperature (°C) from Jersey Meteorological Section of the Department of the Environment Jersey (linear and quadratic)
rain	Continuous	Survey	Daily rainfall (mm) from Jersey Meteorological Section of the Department of the Environment Jersey (linear and quadratic)
week	Continuous	Survey	Calendar week with week 1 adjusted to the first week of March (linear and quadratic)

<sup>a</sup>All land-cover covariates were calculated in ArcMap v10.2.1 (ESRI, Redlands).

### 2.3.1. Statistical analysis - detection, occupancy and abundance

We used a static single-season model whereby each site-year combination was treated as a separate site, and the occupancy or abundance at a site was assumed to be independent for each year. This enabled an effective sample size of 19 site-years for investigating the influences of covariates upon each parameter with greater precision (Fogg *et al.*, 2014; Kéry and Royle, 2015). Within the main text, we refer to site-years simply as sites. Non-independence between site-years could lead to underestimation of model error for occupancy and abundance parameters, so we apply caution in their interpretation. To this single-season

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dataset we applied a set of hierarchical models, whereby an observation model with the detection/non-detection or count data is conditionally related to a state model describing occupancy or abundance (Kéry and Royle, 2015). We assumed that sites had constant occupancy throughout each year and detections between sites were independent (MacKenzie *et al.*, 2002). N-mixture models provide estimates of abundance and rely on similar closure assumptions to occupancy models, but instead incorporate count data, assume that no changes in abundance occur across the sampling period and that detections within a survey are independent of each other (Royle, 2004).

All analyses were conducted in R v3.2.3 (R Core Team, 2015) with packages unmarked v0.11-0 (Fiske and Chandler, 2011; <https://cran.r-project.org/web/packages/unmarked/index.html>) and AICcmodavg v2.1-0. (<https://cran.r-project.org/web/packages/AICcmodavg/index.html>). A set of candidate models were developed for each parameter; occupancy, detection or abundance (Table 2.1; Table S2.1–S2.3). Models held occupancy/abundance and detection as constant, or allowed them to vary by site or observation covariates (detection) or site covariates only (occupancy and abundance). Covariates were incorporated through a logit-link function with only a single covariate for detection or occupancy/abundance for simplicity. Rainfall, temperature and week were incorporated into models as linear and quadratic effects (Kéry *et al.*, 2005).

We discarded models that failed to converge, and those remaining were ranked by Akaike weight ( $w_i$ ) and  $\Delta AICc$  values, as  $\Delta AICc$  is considered more appropriate for small sample sizes than  $\Delta AIC$  (Burnham and Anderson, 2002). The number of observations was set to 132 (total number of site surveys) for detection and 19 (number of site-years) for occupancy and abundance (Kéry and Royle,

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2015) (Table 2.2). Top models were considered as those with  $\Delta\text{AICc} \leq 2$  (Burnham and Anderson, 2002), with goodness-of-fit assessed using Pearson's  $\chi^2$  statistic and 1000 bootstrap simulations (MacKenzie and Bailey, 2004). For abundance, different mixtures (Poisson, Negative Binomial or Zero-Inflated Poisson) were evaluated via model selection (AICc) and goodness-of-fit tests (Kéry *et al.*, 2005). Where more than one top model was selected, model-averaged estimates (Burnham and Anderson, 2002) are reported unless stated otherwise.

With low detectability and limited sampling occasions, infinite (Dennis *et al.*, 2015) or biased (Couturier *et al.*, 2013) estimates of abundance can occur. To avoid this it is important to test the effects of varying upper limits for site-specific abundance until abundance estimates stabilise. In doing so, we found an upper bound of 250 to be sufficient. Bayesian approaches were used to estimate mean site- and total abundance using the *ranef* and empirical best unbiased predictor (*BUP*) functions in unmarked (Fiske and Chandler, 2011). These methods calculate the posterior abundance distribution based on the data and model parameters. Parametric bootstrapping with 1000 simulations was used to identify 95% confidence intervals for total abundance across sites. Snake density was calculated by dividing site abundance by area (ha).

### **2.3.2. Statistical analysis - survey effort**

The minimum number of surveys required to detect a grass snake at an occupied site were calculated for probabilities of 0.80, 0.90 or 0.95 (McArdle, 1990). We used model-averaged detection estimates from our top occupancy models and calculated the minimum number of surveys needed for survey efforts

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of 0–500 ACOs. These were used to evaluate our own survey efforts and to make recommendations for future monitoring.

Currently, NARRS requires volunteers to survey sites four or more times between March and June. Occupancy data are combined across six-year cycles, where any sites surveyed in multiple years are considered independent. Each six-year cycle is then treated as an independent period and occupancy trends are assessed between the different cycles (Wilkinson *et al.*, 2014). Using modified R code from a previous study (Guillera-Arroita and Lahoz-Monfort, 2012) (Methods S2.1), we conducted a one-tailed power analysis with a closed formula to estimate the number of sites required to detect a significant occupancy decline between two independent periods. The probability of both Type I ( $\alpha$ ) and Type II ( $\beta$ ) errors were expected to be under a normal distribution ( $z$ ). The initial ( $\psi_1$ ) and resulting ( $\psi_2 = \psi_1 (1 - R)$ ) occupancy probabilities and detection estimates ( $p$ ) were derived from our model-averaged estimates.

We varied occupancy and detection predictions based on number of ACOs from 0 to 100 at 10 ACO increments, noting that currently the number of ACOs used in Jersey rarely exceeds 10 per site (States of Jersey Department of the Environment, pers. comm.). We assessed the number of sites required to detect declines ( $R$ ) of 50%, 30% and 15% using a significance level of  $\alpha = 0.05$  with either four, six or eight survey visits ( $K$ ). We assumed detection and occupancy were constant across seasons and the same number of surveys were made in each period.

## 2.4. Results

A total of 12,335 ACOs and 613 km of transects were surveyed across the whole study period. We recorded 51 snake observations with an average of one observation every two to three surveys (mean per survey:  $0.39 \pm 0.07$  s.e.m., range: 0–4), and an average of 2.68 ( $\pm 0.84$  s.e.m., range: 0–12) observations per site in a season. Only 8.3% of survey visits resulted in counts  $> 1$ . Three observations were sloughed skins and one a carcass. ACOs proved effective in aiding detection, with 76.5% of observations occurring beneath them. A further 15.7% of detections were of basking individuals and 3.9% of active snakes.

### 2.4.1. Detection and occupancy

Snakes were detected at 11 out of 19 study sites, with no changes in observed occupancy between years (Figure 2.1). Goodness-of-fit tests indicated good model fit, and ranking identified three top detection and two top occupancy models with  $\Delta AICc < 2$  (Table 2.3). Estimates of detectability and occupancy were model-averaged from the top models. Mean detectability (i.e., the probability of detecting grass snakes at a site during a single survey if they were present,  $p$ ) was estimated at  $0.35 (\pm 0.07$  s.e.m.), which was greater than our observed detection rate of  $0.25 (\pm 0.04$  s.e.m) across all surveys (including from potentially unoccupied sites). An estimated mean occupancy ( $\psi$ ) rate of  $0.70 (\pm 0.15$  s.e.m.) was also greater than our naïve occupancy of 0.58. Occupancy and detection estimates were variable between sites. For sites surveyed in both years, estimates of occupancy were fairly stable between years, whereas detection varied with survey effort (Table 2.2).



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Table 2.2 Study sites in Jersey, sampled in 2014 and 2015 showing: year sampled, dominant habitat type, area surveyed in hectares (ha), number of surveys ( $K$ ) and number of ACOs. Model-averaged site occupancy ( $\psi$ ), detection ( $\rho$ ) and abundance ( $\lambda$ ) estimates are shown with 95% confidence intervals in brackets.

Site	Year	Habitat <sup>a</sup>	Area (ha)	$K$	ACOs	$\psi$	$\rho$	$\lambda$
A	2015	SC	8.10	3	39	0.59 (0.24–0.87)	0.26 (0.14–0.42)	0.80 (0–3)
B	2014	SC	75.91	8	90	0.69 (0.37–0.90)	0.35 (0.23–0.48)	0.34 (0–2)
C	2014	DGr	36.99	8	117	0.73 (0.33–0.94)	0.40 (0.28–0.53)	5.05 (2–8)
C	2015	DGr	36.50	6	238	0.82 (0.08–1.00)	0.65 (0.44–0.81)	10.59 (7–15)
D	2014	SC	23.19	8	72	0.66 (0.35–0.87)	0.31 (0.20–0.46)	0.34 (0–2)
E	2014	AGr	29.63	8	34	0.57 (0.21–0.87)	0.25 (0.13–0.41)	0 (0–0)
F	2014	DGr	65.72	8	195	0.80 (0.15–0.99)	0.56 (0.39–0.71)	7.54 (5–11)
F	2015	DGr	65.64	6	436	0.83 (0.00–1.00)	0.91 (0.59–0.98)	8.88 (5–13)
G	2014	SC	38.81	8	81	0.67 (0.36–0.88)	0.33 (0.21–0.47)	1.34 (1–3)
H	2014	RGr	29.53	8	45	0.60 (0.27–0.86)	0.27 (0.15–0.42)	0.32 (0–2)
I	2015	AGr	3.89	4	51	0.61 (0.29–0.86)	0.28 (0.16–0.43)	0 (0–0)
J	2014	SC	10.48	8	60	0.63 (0.32–0.86)	0.29 (0.18–0.44)	1.93 (1–4)
J	2015	SC	10.48	6	110	0.72 (0.35–0.93)	0.38 (0.27–0.52)	3.91 (2–6)
K	2014	SC	15.77	8	42	0.59 (0.25–0.87)	0.26 (0.15–0.42)	0.34 (0–2)
L	2014	SC	37.54	8	90	0.69 (0.37–0.90)	0.35 (0.23–0.48)	1.60 (1–3)
L	2015	SC	3.61	6	46	0.60 (0.26–0.86)	0.27 (0.15–0.42)	1.48 (1–3)
M	2014	RGr	1.01	8	6	0.52 (0.11–0.90)	0.21 (0.10–0.38)	1.88 (1–4)
M	2015	RGr	1.01	5	18	0.54 (0.15–0.89)	0.22 (0.11–0.40)	1.53 (1–3)
N	2014	AGr	36.06	8	36	0.58 (0.22–0.87)	0.25 (0.14–0.41)	0 (0–0)

<sup>a</sup> Habitat classifications; AGr = Amenity grassland, DGr = Dune grassland, RGr = Rough grassland, SC = Scrub.

Top selected models were driven by similar covariates. Detection increased with survey effort (number of ACOs) in all three top models, whereas occupancy

either showed no influence of covariates or increased with survey effort (Table 2.3). In this case, greater survey effort (i.e., a larger number of ACOs) was strongly related to longer transect lengths, and therefore larger survey areas had a higher likelihood of occupancy (Kéry *et al.*, 2005; MacKenzie *et al.*, 2005; Sewell *et al.*, 2012). However, due to few sites with a large survey effort, the predicted relationship becomes uncertain (Figure 2.2). We failed to identify any environmental covariates that reliably described detection.

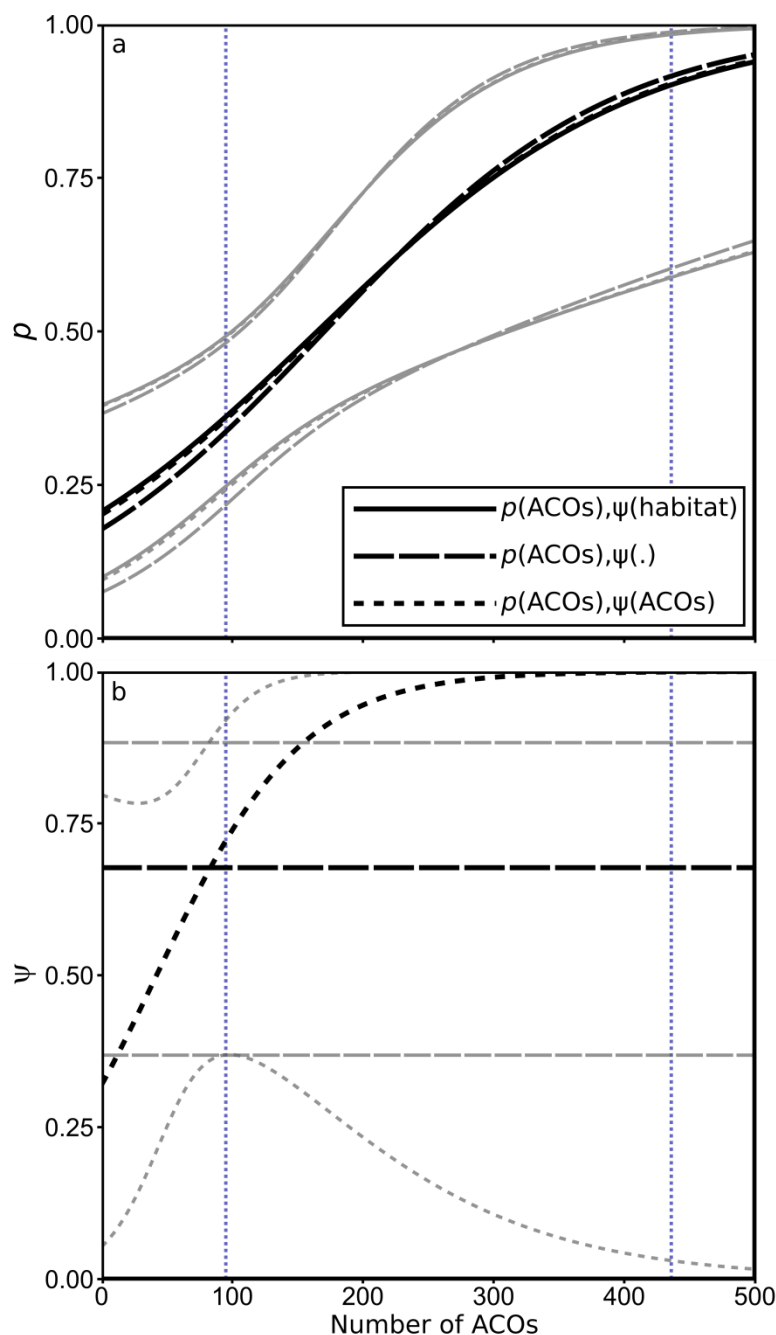


Figure 2.2 Predicted (a) detection and (b) occupancy probabilities based on top models (Table 2.3), with number of ACOs from 0–500. Models shown are  $\rho(\text{ACOs}), \psi(\cdot)$ ;  $\rho(\text{ACOs}), \psi(\text{ACOs})$  and;  $\rho(\text{ACOs}), \psi(\text{habitat})$  (panel a only) as long-dash lines, small-dash lines or solid lines respectively. Grey lines indicate 95% confidence intervals. Blue vertical dotted lines show mean (left) and maximum (right) number of ACOs used in this study.

Table 2.3 Top models of detection ( $p$ ), occupancy ( $\psi$ ) and abundance ( $\lambda$ ) for grass snakes in Jersey. Parameters ( $p$ ,  $\psi$  and  $\lambda$ ) were constant (.) or allowed to vary with covariates. Top models with  $\Delta\text{AICc} < 2$  are displayed in descending order. Due to the small sample size, models are ranked by their AICc and weight ( $w_i$ ). Models are shown for number of observations set to 132 (total number of surveys) for detection and 19 (number of sites) for occupancy and abundance.  $N$  is number of parameters in the model and LL the log-likelihood. The mean prediction and its standard error are shown for each parameter. Goodness-of-fit statistics are also shown.

Model	$N$	AICc	$\Delta\text{AICc}$	$w_i$	Cum. $w_i$	LL	Prediction <sup>a</sup>	Goodness-of-fit		
								$\chi^2$	$p$	$\hat{c}$
<b>detection</b>										
$p(\text{ACOs}), \psi(\text{habitat})$	6	122.11	0.00	0.42	0.42	-54.72	0.36 (0.06)	291.44	0.84	0.84
$p(\text{ACOs}), \psi(\text{ACOs})$	4	122.87	0.76	0.29	0.71	-57.28	0.36 (0.07)	306.91	0.78	0.86
$p(\text{ACOs}), \psi(.)$	3	123.86	1.75	0.18	0.89	-58.83	0.34 (0.07)	339.34	0.53	0.94
<b>occupancy</b>										
$p(\text{ACOs}), \psi(.)$	3	125.27	0.00	0.45	0.45	-58.83	0.72 (0.15)	339.34	0.53	0.94
$p(\text{ACOs}), \psi(\text{ACOs})$	4	125.41	0.14	0.41	0.86	-57.28	0.68 (0.14)	306.91	0.78	0.86
<b>abundance</b>										
$p(.), \lambda(\text{habitat})$	5	185.27	0.00	0.45	0.45	-85.33	AGr 0.00 (0.02) DGr 8.01 (3.76) RGr 1.24 (0.96) SC 1.34 (0.72)	122.37	0.126	1.16

<sup>a</sup>Habitat classifications for abundance model: AGr = Amenity grassland, DGr = Dune grassland, RGr = Rough grassland, SC = Scrub

### **2.4.2. Abundance**

The Poisson distribution was found to give the best overall fit for abundance ( $\lambda$ ) and was subsequently used for estimation (Table S2.4). Based on a single model with  $\Delta\text{AICc} < 2$ , abundance varied with habitat type and was highest in dune grassland habitats (Table 2.3). Rough grassland and scrub also provided suitable habitat but gave lower values of abundance than dune grassland. Amenity grassland had a predicted abundance of zero with no detections occurring in this habitat (Table 2.3).

Site-specific empirical Bayes estimates of abundance were typically low (mean:  $2.52 \pm 0.74$  s.e.m., range: 0–10.6) (Table 2.2). Collectively they provided a total estimated abundance of 48 (95% CI: 23–1279) individuals across the study sites. For sites surveyed in both years, abundance was similar, except at site C where there was almost a doubling in predicted abundance between years. This difference may be due to a 52.6% increase in survey effort in the second year (Table 2.2); however the model supporting this was outside of our top model selection ( $\Delta\text{AICc} = 2.75$ , Table S2.3). During the study, 43 unique individual snakes were identified based on ventral patterns. Therefore, we are able to raise our lower confidence bound of total abundance across sites to 43 individuals. Overall mean snake density was low (mean:  $0.28$  /ha  $\pm 0.12$  s.e.m., range: 0–1.86).

### **2.4.3. Survey effort requirements and recommendations**

The number of survey visits required to have confidence in species absence is highly dependent upon survey effort and associated species detectability (Figure 2.2; Figure 2.3). With our mean survey effort of 95 ACOs per site and a predicted

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detection of 0.36 (95% CI: 0.24–0.49), four (95% CI: 2–6), five (95% CI: 3–8) or seven (95% CI: 4–11) site surveys would be needed for 80, 90 or 95% confidence of absence respectively. In comparison, the current NARRS survey effort of 10 ACOs per site gives a detection estimate of 0.21 (95% CI: 0.10–0.39), and would require seven (95% CI: 3–15), 10 (95% CI: 5–21) or 13 (95% CI: 6–28) surveys for the same three levels of confidence of absence. For the surveys conducted in this study, at the 80% confidence level, three of the 19 sites were not surveyed sufficiently to declare absence with confidence (Table 2.2). This increased to five sites requiring further surveys for 90% confidence and 10 sites for 95% confidence. Snakes were not detected at six of these 10 sites, so we should not declare them absent without further survey effort.

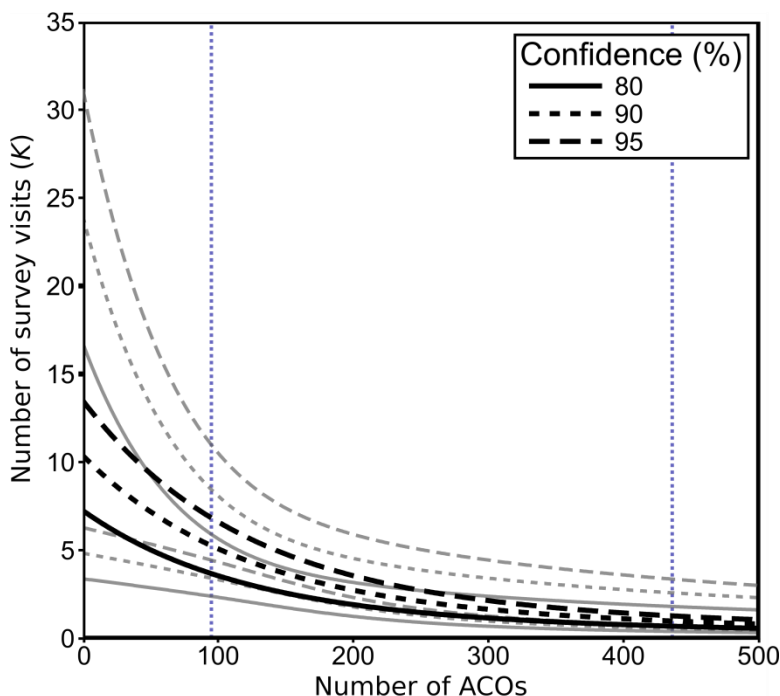


Figure 2.3 Number of survey visits ( $K$ ) required to determine species presence at an occupied site with a given probability, with number of ACOs from 0–500. Grey lines show 95% confidence intervals. Vertical dotted lines show mean (left) and maximum (right) number of ACOs used in this study.

For NARRS to be able to detect an occupancy decline with 80% power, we found the number of survey sites needed to be prohibitively large given the current sampling of  $\leq 50$  sites per six-year cycle. For example, with the current Jersey NARRS effort of ca. 10 ACOs per site and four surveys, 546 sites (95% CI: 183–

2732) are needed within a six-year cycle to detect a 30% decline. By increasing the number of sites surveyed within each survey cycle, the number of ACOs at each survey site or the number of times a site is surveyed, the ability to detect smaller declines improves. To detect any decline with four surveys at  $\leq 50$  sites (mean number of sites needed: 46, 95% CI: 29–107) per sampling period and 80% power, at least 90 ACOs are needed per site, and even then only a 50% decline may be detected. A more achievable level of survey effort would be a design where 114 sites are surveyed within each cycle with 30 ACOs and eight repeat visits, but this would still only permit a 50% decline to be detected (Figure 2.4; Table S2.5).

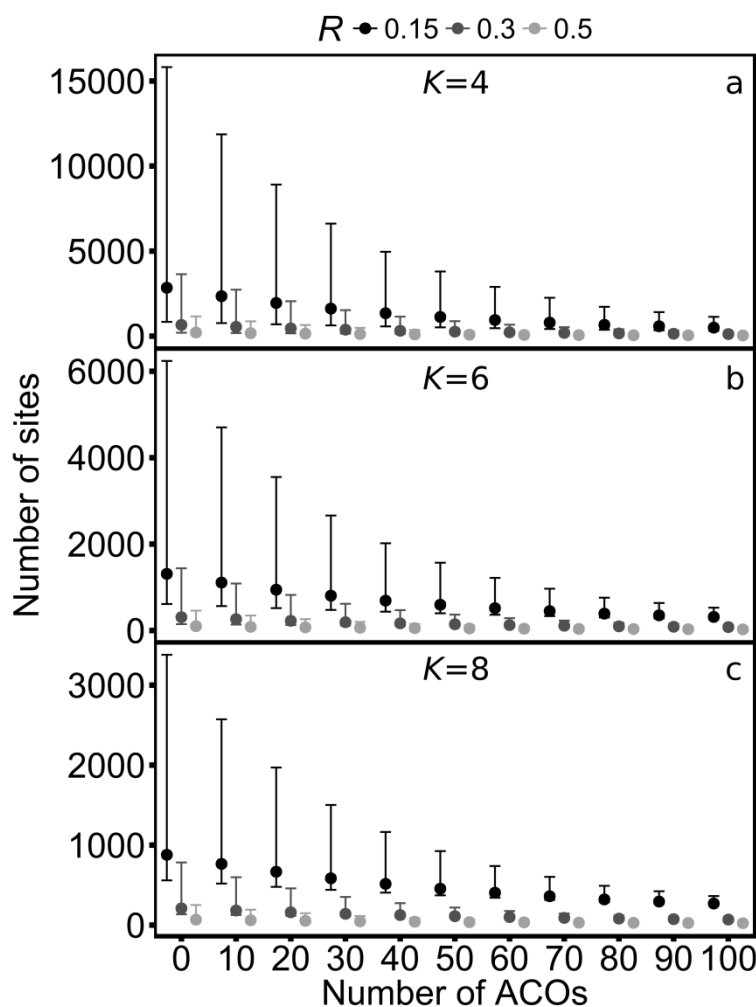


Figure 2.4 Number of survey sites required to detect a decline in occupancy at different levels of survey effort (numbers of ACOs) with varying proportional changes in occupancy ( $R$ ) at a power of 0.8, and (a) two, (b) six or (c) eight survey visits ( $K$ ). The figure displays the number of sites required when alpha is set to 0.05, with bars showing 95% confidence intervals.

## 2.5. Discussion

We applied two commonly used methods for assessing population status in long-term monitoring programmes to a population of cryptic and elusive snakes, and estimated their site occupancy, detectability and local abundance. Our findings suggest that both occupancy and N-mixture models can be used to assess the current status of populations with small sample sizes. However, statistical power to detect occupancy declines will be poor (Guillera-Arroita and Lahoz-Monfort, 2012) and parameter estimates may exhibit wide confidence limits. Unlike some previous studies, we did not encounter problems with N-mixture models yielding confidence limits including zero (Steen *et al.*, 2012) or convergence issues (Couturier *et al.*, 2013). Both modelling approaches showed good fits to the data, although the fit and standard errors associated with N-mixture models were less satisfactory. This was likely caused by differences in sample size, where the abundance model predicted values across four habitat classes rather than a single continuous covariate as in our occupancy models (Table 2.3).

We selected semi-natural sites *a priori* which were likely to have higher rates of occupancy, abundance and therefore detectability than the wider island landscape. This allows our results to be more effective in informing management actions than if sites had been more widely distributed (MacKenzie *et al.*, 2005), but limits our ability to generalise any findings across Jersey due to differences in landscape and survey effort. As we focused on the 'best' sites in the island, we infer that grass snake occupancy and abundance elsewhere in Jersey will be lower than our estimates.

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Our estimates of detection were lower than a previous grass snake study that used fewer ACOs (Sewell *et al.*, 2012), but higher than those from Kéry (2002) that only used visual searches. The top models suggest that detection primarily increases with survey effort (Kéry *et al.*, 2005; Lind *et al.*, 2005; Sewell *et al.*, 2012), despite environmental, demographic and physical factors also expected to have an influence (Bonnet and Naulleau, 1996; Kéry, 2002; Lind *et al.*, 2005; Joppa *et al.*, 2009; Rodda *et al.*, 2012; Gregory and Tuttle, 2016). We did not test for demographic effects due to sample size restrictions, and other influences may have gone undetected due to low abundance and a limited population available for detection (Bailey *et al.*, 2004; Tanadini and Schmidt, 2011). Previous studies of low-density snake populations may have struggled with N-mixture models due to lower individual detection rates than we experienced, driven in part by less-intensive monitoring efforts and high mobility of study species (Steen, 2010; Steen *et al.*, 2012). In these cases, detection may be improved by greater survey intensity, using radio-telemetry to identify optimal placement of ACOs or trap arrays, or through novel means such as detector dogs (Browne *et al.*, 2015).

Detection plays a key role in determining the presence or absence of a species at a site. In Britain, guidelines recommend four to five site surveys with 30 ACOs to achieve a 95% confidence of grass snake absence (Sewell *et al.*, 2012), increasing to seven or more at marginal sites (Sewell *et al.*, 2013). We observed similar requirements for site visit numbers, but only if we consider our sites to be marginal and use larger quantities of ACOs. Furthermore, our results indicated that the current NARRS effort of four site visits per season would be insufficient for assuming grass snake absence from a site with any reasonable confidence. Future analysis of NARRS occupancy data should therefore account for imperfect



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detection to resolve these issues, or the number of surveys at a site should be increased appropriately to limit the possibility of non-detection. With so little semi-natural habitat remaining, it is vital that sufficient effort is used before sites are designated as absent; particularly where development may occur.

In our study we found a greater area occupied than expected based on results from previous monitoring efforts (Wilkinson *et al.*, 2014). This was driven by our improved ability to detect the species due to an intensive survey effort. Simulations have shown occupancy estimates to be fairly unbiased when  $p > 0.3$  and there are five or more surveys (MacKenzie *et al.*, 2002), therefore our occupancy estimates are likely to be unbiased. However, populations with extremely low detection may result in biased occupancy estimates (MacKenzie *et al.*, 2002).

The efficiency of presence-absence over count-based methods can be improved by using a 'removal' design (MacKenzie *et al.*, 2005), whereby some or all sites are surveyed until a single detection occurs or a given number of surveys are completed. This method would have allowed us to reduce our survey effort from 132 surveys and 12,335 ACO checks, to 90 surveys and 6,556 ACO checks. However, this would lower detection estimates, increase occupancy estimates and generally create greater uncertainty. It may also be an unsuitable approach for multi-species monitoring.

A primary aim of monitoring is to detect population changes and subsequently inform management. Our study indicated that there is very poor power to detect occupancy declines, even when improvements are made by increasing the number of survey sites or ACOs. However, increasing the number of surveys carried out at each site may give a more practical and cost-effective

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solution to detecting declines (Table S2.5). Moreover, as our estimates of detection and occupancy were based on surveys carried out in suitable habitat, monitoring carried out over a larger, less suitable landscape with reduced occupancy would likely reduce power further. Consequently, for rare species or those with small population changes, it may not be possible to have sufficient power to detect trends (Pollock, 2006; Couturier *et al.*, 2013).

This is the first study to estimate population size of grass snakes with N-mixture models, although others have been successful in using CMR (e.g., Mertens, 1995). Despite not encompassing the whole island, our estimates suggest a very small population inhabits the remaining suitable habitats. Previous simulation studies found there to be only a small positive bias in mean abundance with similar sample sizes (Royle, 2004). However, wide confidence limits in this, and previous studies (Doré *et al.*, 2011; Steen *et al.*, 2012) will afford little power to detect trends. This uncertainty in our abundance estimates may arise from several factors. These include un-modelled heterogeneity in detection between individuals, risk of temporary emigration (e.g., use of burrows), non-independence of sites sampled in both years and only a single recapture amongst the 43 identified snakes occurring across the whole survey effort. This infers low individual detection, and further supports the unsuitability of CMR for elusive snake populations. Considering these issues, it is likely that our abundance and density estimates are negatively biased, and may be further confounded by having an unknown effective sampling area associated to a transect, unless a site is saturated with survey effort (Kéry *et al.*, 2009). Nevertheless, as a range-restricted insular population, it is probable that a regional classification of Vulnerable according to IUCN category D applies (IUCN, 2012a), and that there is risk of the

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population becoming unviable and extirpated from sites due to low abundance (Soulé, 1980).

To avoid bias in population estimates from all model types, the assumptions of those models must be met. The biological reality is that without controlled experiments, assumption violations are likely to occur. Mobile study organisms such as snakes provide several challenges for monitoring studies (Steen *et al.*, 2012), including the potential to violate closure assumptions by leaving sites or concealing themselves (Bailey *et al.*, 2004). These movements can be considered as temporary emigration, which can be driven by variation in environment, season or lifestage (Couturier *et al.*, 2013). Furthermore, on publicly accessible sites such as ours, seemingly unobtrusive recreational activities can disturb snakes (Weatherhead and Madsen, 2009). Studies on these effects are lacking, however it is likely that they could influence detectability, emigration and survival. For example, massasauga rattlesnakes *Sistrurus c. catenatus* move away or conceal themselves more when disturbed by humans (Prior and Weatherhead, 1994).

To meet site closure and independence assumptions (Bailey *et al.*, 2004; Royle, 2004), sites should be an appropriate size (Kéry *et al.*, 2009; Chandler *et al.*, 2011), a robust survey design should be used where repeat surveys are carried out in a short time-frame (Pollock, 1982; Dorcas and Willson, 2009; Kéry and Royle, 2015), and sites should be sufficiently separated to prevent movement between them within a season (MacKenzie *et al.*, 2002; 2005; Steen, 2010). Within Europe, herpetofauna monitoring schemes rarely use the robust design (Kéry *et al.*, 2009; Sewell *et al.*, 2013), risking closure violation. However, if surveys are conducted in close succession, then independence between them may be lost and seasonal effects upon detection may not be evaluated. Moreover,

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logistical constraints may limit the ability of surveyors to visit a site regularly.

Datasets may therefore require truncation or pooling to meet closure assumptions (MacKenzie *et al.*, 2005; Kéry *et al.*, 2009). A parallel study (Chapter 3) carried out

short-term radio-tracking of 16 adult grass snakes at three study sites in Jersey.

The results indicate that the snakes exhibit site fidelity and small ranges, but within the site may be undetectable as they undergo a form of temporary emigration in which they are concealed within burrows or dense vegetation 84% of the time.

Where datasets are sufficient, models that account for birth, death and temporary emigration may improve abundance estimation for rare and elusive species (Bailey *et al.*, 2004; Chandler *et al.*, 2011; Denes *et al.*, 2015).

Few studies have compared occupancy and N-mixture models (but see Doré *et al.*, 2011), instead focusing on differences between abundance methods (Lind *et al.*, 2005; Couturier *et al.*, 2013; Denes *et al.*, 2015). Due to their comparatively low cost however, presence-absence and count based methods may be the most appropriate for monitoring multiple species, or those with difficult traits such as low detection and high mobility. Of these low-cost options, this study indicates that occupancy frameworks are more appropriate than count based methods for snakes and other elusive and mobile species (Dorcas and Willson, 2009; Zylstra *et al.*, 2010; Durso *et al.*, 2011) due to (i) low frequency of encounters; (ii) low site abundance with little variation in counts (Pollock, 2006; Denes *et al.*, 2015); (iii) unresolved issues in N-mixture modelling such as choice, fit and convergence of different error distributions (Kéry *et al.*, 2005; Joseph *et al.*, 2009); (iv) ability to meet model assumptions; and (v) resource requirements (Gaston *et al.*, 2000; Pollock, 2006; Zylstra *et al.*, 2010). The first two of these issues indicate that in this instance, little extra information was gained by recording counts.

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Monitoring comes in many forms, and may be carried out by academics, citizen scientists, non-governmental organisations, consultant ecologists or other interested parties. Generally all efforts will be to determine population status, but at different scales and with varying resources and intensities. Therefore, broader recommendations can be gleaned from other simulated and real-world studies. These indicate that occupancy measures tend to be more suited to widespread monitoring and rare species than abundance measures. This is particularly true when there is low cost associated with sampling, a small monitoring budget, many sites, a high frequency of sampling, few observations with little variation and low detection probability (Manley *et al.*, 2004; Joseph *et al.*, 2006; Pollock, 2006; Zylstra *et al.*, 2010). Depending on budget, occupancy measures may detect changes in population size or area occupied respectively (Gaston *et al.*, 2000; Joseph *et al.*, 2006), and may be best suited to detecting unrelated losses of subpopulations rather than a single synchronous decline (Pollock, 2006). However, the efficiency of occupancy measures may decrease with increasing scale as more effort is taken to visit each site (Zylstra *et al.*, 2010) unless the removal design is used (MacKenzie *et al.*, 2005). Furthermore, these measures may have limited power to detect changes, and exhibit sensitivity to the number of observations per site and sampling frequency (Pollock, 2006; Zylstra *et al.*, 2010). Abundance measures are better suited when there are fewer sites ( $\leq 150$ ), high detectability, high costs associated with sampling, and observations are variable but well explained by covariates (Joseph *et al.*, 2006; Pollock, 2006; Kéry *et al.*, 2009; Zylstra *et al.*, 2010). Identifying the thresholds at which these methods are no longer feasible for snakes would be a useful step (Steen, 2010), as done for

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birds, which showed abundance surveys to be more cost-effective than occupancy when the species was detected at > 16 sites in a season (Joseph *et al.*, 2006).

In order to make use of scarce resources, monitoring programmes are often designed for multiple widespread species instead of species-specific programmes of umbrella or indicator species (Manley *et al.*, 2004). Generally speaking, the former approach whilst utilising citizen scientists may have greater benefits for biodiversity (Manley *et al.*, 2004) and is the most cost-effective (O'Donnell and Durso, 2014). Attempts to monitor rare and range-restricted populations may require different approaches due to their spatial scale, number of occupied sites and as in this study, a large investment at each visit in order to get observations (Joseph *et al.*, 2006). As an example, the Jersey NARRS scheme is aimed at multiple widespread species and benefits from low costs for visiting sites due to the island's small spatial scale. With sufficient resources, many sites could be sampled making occupancy the appropriate choice. However, as a small island, there are a limited number of sites, volunteers and resources. Even if all available 1 km<sup>2</sup> cells were surveyed ( $n = 140$ ) (Wilkinson *et al.*, 2014) each year (an unlikely feat), NARRS could still only survey a maximum of 840 sites in a six-year cycle, many ACOs would be needed, and few sites are likely to be occupied by grass snakes. This may confound attempts to make reliable assessments of population status and change (Wilkinson *et al.*, 2014) (Table S2.5). Conversely, with such a limited area it may be possible to survey a larger and more representative proportion of the overall landscape than on the mainland. Applying these issues to other hypothetical restricted populations where the costs of monitoring are greater, or fewer resources are available, the most appropriate monitoring strategy may differ. Indeed, where a high intensity of sampling is required for monitoring as with

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many snakes (Lind *et al.*, 2005) and other elusive species, widespread citizen science programmes may not be suitable. Therefore, without specific investment, and noting a lack of power, detecting trends in Jersey's grass snake population is unlikely. To improve the ability of NARRS to monitor Jersey's grass snake population, we recommend that efforts are made to enhance species detection at each survey site through increases in the number of ACOs and the number of survey visits. A robust sampling design (Pollock, 1982) will aid statistical analyses, and these methods will generally provide reliable results for other reptile species (Sewell *et al.*, 2012). Incorporation of a partial or full removal design (MacKenzie *et al.*, 2005) may also be beneficial if the primary aim is to ascertain occupancy status.

In summary, few long-term studies of snake populations have been conducted, with available examples using simple counts or CMR to estimate abundance (Lind *et al.*, 2005; Reading *et al.*, 2010). We recommend the incorporation of detection and the influences of covariates upon simple count data to provide more reliable population assessments, whilst monitoring at larger scales than possible by CMR and other high-intensity methods. At larger scales still, where closure violation is a risk, or monitoring costs are reduced by using a removal design, occupancy provides a suitable method. Further work comparing the accuracy of different parameter estimates would be useful and could be carried out through simulation (Zylstra *et al.*, 2010; Couturier *et al.*, 2013).

For our study population, the remaining semi-natural areas containing structurally diverse habitats in the west and southwest of Jersey have maintained their occupancy status from previous surveys in 2002 (Hall, 2002). This highlights the importance of this region for the locally scarce grass snake population, and

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warrants further study to inform conservation management of these sites. We encourage others to carry out pilot studies and power analysis during development of monitoring schemes, and to test the application of N-mixture models on small populations where conventional CMR methods are unsuitable. Generally however, we recommend the use of occupancy methods for rare and elusive species. Providing data that can enable reliable assessment of snake populations should be prioritised to assess status and investigate potential declines.

### **2.6. Acknowledgements**

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## 2.7. Supplementary Information

Table S2.1 Full set of candidate models with number of observations set to 132 for detection. Models are displayed in descending order and are ranked by their AICc and weight (Wt). LL is log likelihood.

Models	K	AICc	$\Delta$ AICc	AICc Wt	Cum. Wt	LL
$p(\text{ACOs}), \psi(\text{habitat})$	6	122.11	0.00	0.42	0.42	-54.72
$p(\text{ACOs}), \psi(\text{ACOs})$	4	122.87	0.76	0.29	0.71	-57.28
$p(\text{ACOs}), \psi(\cdot)$	3	123.86	1.75	0.18	0.89	-58.83
$p(\text{habitat}), \psi(\cdot)$	5	127.50	5.39	0.03	0.92	-58.51
$p(\text{ACOs}), \psi(\text{aspect})$	8	127.61	5.50	0.03	0.94	-55.22
$p(\text{habitat}), \psi(\text{ACOs})$	6	128.10	5.99	0.02	0.96	-57.71
$p(\cdot), \psi(\text{habitat})$	5	131.24	9.13	0.00	0.97	-60.38
$p(\text{temperature})+(\text{temperature}^2), \psi(\text{habitat})$	7	131.36	9.25	0.00	0.97	-58.23
$p(\text{habitat}), \psi(\text{habitat})$	8	131.41	9.30	0.00	0.98	-57.12
$p(\text{cloud}), \psi(\text{habitat})$	6	132.00	9.89	0.00	0.98	-59.66
$p(\text{temperature})+(\text{temperature}^2), \psi(\text{ACOs})$	5	132.06	9.95	0.00	0.98	-60.79
$p(\cdot), \psi(\text{ACOs})$	3	132.21	10.10	0.00	0.99	-63.01
$p(\text{cloud}), \psi(\text{ACOs})$	4	132.83	10.72	0.00	0.99	-62.26
$p(\text{aspect}), \psi(\cdot)$	7	133.37	11.26	0.00	0.99	-59.23
$p(\text{aspect}), \psi(\text{habitat})$	10	133.75	11.64	0.00	0.99	-55.97
$p(\text{temperature})+(\text{temperature}^2), \psi(\cdot)$	4	133.89	11.78	0.00	0.99	-62.79
$p(\text{aspect}), \psi(\text{ACOs})$	8	133.98	11.87	0.00	0.99	-58.40
$p(\text{rain})+(\text{rain}^2), \psi(\text{habitat})$	7	134.09	11.98	0.00	0.99	-59.59
$p(\cdot), \psi(\cdot)$	2	134.12	12.01	0.00	1.00	-65.01
$p(\text{week})+(\text{week}^2), \psi(\text{habitat})$	7	134.53	12.42	0.00	1.00	-59.81
$p(\text{cloud}), \psi(\cdot)$	3	134.61	12.50	0.00	1.00	-64.21
$p(\text{rain})+(\text{rain}^2), \psi(\text{ACOs})$	5	134.92	12.81	0.00	1.00	-62.22
$p(\text{condition}), \psi(\text{habitat})$	7	135.35	13.24	0.00	1.00	-60.22
$p(\text{week})+(\text{week}^2), \psi(\text{ACOs})$	5	135.40	13.28	0.00	1.00	-62.46
$p(\text{condition}), \psi(\text{ACOs})$	5	136.18	14.07	0.00	1.00	-62.85
$p(\text{rain})+(\text{rain}^2), \psi(\cdot)$	4	136.92	14.81	0.00	1.00	-64.30
$p(\cdot), \psi(\text{aspect})$	7	136.98	14.87	0.00	1.00	-61.04
$p(\text{week})+(\text{week}^2), \psi(\cdot)$	4	137.57	15.46	0.00	1.00	-64.63
$p(\text{cloud}), \psi(\text{aspect})$	8	137.83	15.72	0.00	1.00	-60.33
$p(\text{condition}), \psi(\cdot)$	4	138.03	15.92	0.00	1.00	-64.86
$p(\text{rain})+(\text{rain}^2), \psi(\text{aspect})$	9	139.73	17.62	0.00	1.00	-60.13
$p(\text{week})+(\text{week}^2), \psi(\text{aspect})$	9	139.98	17.87	0.00	1.00	-60.25
$p(\text{condition}), \psi(\text{aspect})$	9	141.24	19.13	0.00	1.00	-60.88

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Table S2.2 Full set of candidate models with number of observations set to 19 for occupancy. Models are displayed in descending order and are ranked by their AICc and weight (Wt). LL is log likelihood.

Models	K	AICc	$\Delta$ AICc	AICc Wt	Cum. Wt	LL
$\rho(\text{ACOs}), \psi(\cdot)$	3	125.27	0.00	0.45	0.45	-58.83
$\rho(\text{ACOs}), \psi(\text{ACOs})$	4	125.41	0.14	0.41	0.86	-57.28
$\rho(\text{ACOs}), \psi(\text{habitat})$	6	128.44	3.17	0.09	0.95	-54.72
$\rho(\text{habitat}), \psi(\cdot)$	5	131.64	6.37	0.02	0.97	-58.51
$\rho(\cdot), \psi(\text{ACOs})$	3	133.62	8.36	0.01	0.98	-63.01
$\rho(\text{habitat}), \psi(\text{ACOs})$	6	134.43	9.16	0.00	0.98	-57.71
$\rho(\cdot), \psi(\cdot)$	2	134.78	9.51	0.00	0.99	-65.01
$\rho(\text{cloud}), \psi(\text{ACOs})$	4	135.38	10.11	0.00	0.99	-62.26
$\rho(\cdot), \psi(\text{habitat})$	5	135.38	10.11	0.00	0.99	-60.38
$\rho(\text{cloud}), \psi(\cdot)$	3	136.02	10.75	0.00	0.99	-64.21
$\rho(\text{temperature})+(\text{temperature}^2), \psi(\text{ACOs})$	5	136.20	10.93	0.00	1.00	-60.79
$\rho(\text{temperature})+(\text{temperature}^2), \psi(\cdot)$	4	136.43	11.16	0.00	1.00	-62.79
$\rho(\text{cloud}), \psi(\text{habitat})$	6	138.33	13.06	0.00	1.00	-59.66
$\rho(\text{rain})+(\text{rain}^2), \psi(\text{ACOs})$	5	139.06	13.79	0.00	1.00	-62.22
$\rho(\text{rain})+(\text{rain}^2), \psi(\cdot)$	4	139.46	14.19	0.00	1.00	-64.30
$\rho(\text{week})+(\text{week}^2), \psi(\text{ACOs})$	5	139.53	14.27	0.00	1.00	-62.46
$\rho(\text{week})+(\text{week}^2), \psi(\cdot)$	4	140.11	14.84	0.00	1.00	-64.63
$\rho(\text{condition}), \psi(\text{ACOs})$	5	140.32	15.05	0.00	1.00	-62.85
$\rho(\text{condition}), \psi(\cdot)$	4	140.57	15.30	0.00	1.00	-64.86
$\rho(\text{temperature})+(\text{temperature}^2), \psi(\text{habitat})$	7	140.64	15.37	0.00	1.00	-58.23
$\rho(\text{ACOs}), \psi(\text{aspect})$	8	140.84	15.57	0.00	1.00	-55.22
$\rho(\text{aspect}), \psi(\cdot)$	7	142.65	17.38	0.00	1.00	-59.23
$\rho(\text{rain})+(\text{rain}^2), \psi(\text{habitat})$	7	143.37	18.10	0.00	1.00	-59.59
$\rho(\text{week})+(\text{week}^2), \psi(\text{habitat})$	7	143.80	18.54	0.00	1.00	-59.81
$\rho(\text{condition}), \psi(\text{habitat})$	7	144.63	19.36	0.00	1.00	-60.22
$\rho(\text{habitat}), \psi(\text{habitat})$	8	144.64	19.37	0.00	1.00	-57.12
$\rho(\cdot), \psi(\text{aspect})$	7	146.26	20.99	0.00	1.00	-61.04
$\rho(\text{aspect}), \psi(\text{ACOs})$	8	147.21	21.94	0.00	1.00	-58.40
$\rho(\text{cloud}), \psi(\text{aspect})$	8	151.06	25.79	0.00	1.00	-60.33
$\rho(\text{rain})+(\text{rain}^2), \psi(\text{aspect})$	9	158.26	32.99	0.00	1.00	-60.13
$\rho(\text{week})+(\text{week}^2), \psi(\text{aspect})$	9	158.50	33.23	0.00	1.00	-60.25
$\rho(\text{aspect}), \psi(\text{habitat})$	10	159.43	34.16	0.00	1.00	-55.97
$\rho(\text{condition}), \psi(\text{aspect})$	9	159.76	34.49	0.00	1.00	-60.88

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Table S2.3 Full set of candidate models with number of observations set to 19 for abundance. Models are displayed in descending order and are ranked by their AICc and weight (Wt). LL is log likelihood.

Models	K	AICc	$\Delta$ AICc	AICc Wt	Cum. Wt	LL
$p(\cdot), \lambda(\text{habitat})$	5	185.27	0.00	0.45	0.45	-85.33
$p(\cdot), \lambda(\text{ACOs})$	3	188.01	2.75	0.11	0.57	-90.21
$p(\text{ACOs}), \lambda(\text{habitat})$	6	188.80	3.53	0.08	0.64	-84.90
$p(\text{ACOs}), \lambda(\text{ACOs})$	4	188.91	3.65	0.07	0.72	-89.03
$p(\text{ACOs}), \lambda(\cdot)$	3	189.58	4.32	0.05	0.77	-90.99
$p(\text{cloud}), \lambda(\text{habitat})$	6	189.65	4.38	0.05	0.82	-85.32
$p(\text{habitat}), \lambda(\cdot)$	5	190.72	5.45	0.03	0.85	-88.05
$p(\text{cloud}), \lambda(\text{ACOs})$	4	191.20	5.93	0.02	0.87	-90.17
$p(\text{temperature})+(\text{temperature}^2), \lambda(\text{ACOs})$	5	191.42	6.15	0.02	0.89	-88.40
$p(\text{rain})+(\text{rain}^2), \lambda(\text{ACOs})$	5	191.76	6.50	0.02	0.91	-88.57
$p(\text{condition}), \lambda(\text{habitat})$	7	191.84	6.57	0.02	0.93	-83.83
$p(\text{habitat}), \lambda(\text{ACOs})$	6	191.90	6.63	0.02	0.94	-86.45
$p(\text{condition}), \lambda(\text{ACOs})$	5	191.91	6.65	0.02	0.96	-88.65
$p(\text{temperature})+(\text{temperature}^2), \lambda(\text{habitat})$	7	192.14	6.87	0.01	0.98	-83.98
$p(\text{rain})+(\text{rain}^2), \lambda(\text{habitat})$	7	192.33	7.07	0.01	0.99	-84.08
$p(\text{week})+(\text{week}^2), \lambda(\text{ACOs})$	5	194.07	8.81	0.01	0.99	-89.73
$p(\text{week})+(\text{week}^2), \lambda(\text{habitat})$	7	194.22	8.95	0.01	1.00	-85.02
$p(\text{habitat}), \lambda(\text{habitat})$	8	199.33	14.07	0.00	1.00	-84.47
$p(\text{aspect}), \lambda(\text{ACOs})$	8	200.70	15.43	0.00	1.00	-85.15
$p(\text{ACOs}), \lambda(\text{aspect})$	8	201.54	16.28	0.00	1.00	-85.57
$p(\cdot), \lambda(\cdot)$	2	203.25	17.99	0.00	1.00	-99.25
$p(\text{temperature})+(\text{temperature}^2), \lambda(\cdot)$	4	205.50	20.23	0.00	1.00	-97.32
$p(\text{cloud}), \lambda(\cdot)$	3	206.07	20.80	0.00	1.00	-99.23
$p(\text{condition}), \lambda(\cdot)$	4	206.13	20.87	0.00	1.00	-97.64
$p(\text{rain})+(\text{rain}^2), \lambda(\cdot)$	4	206.54	21.27	0.00	1.00	-97.84
$p(\cdot), \lambda(\text{aspect})$	7	207.81	22.54	0.00	1.00	-91.81
$p(\text{week})+(\text{week}^2), \lambda(\cdot)$	4	208.01	22.74	0.00	1.00	-98.57
$p(\text{aspect}), \lambda(\cdot)$	7	209.16	23.89	0.00	1.00	-92.49
$p(\text{aspect}), \lambda(\text{habitat})$	10	210.99	25.73	0.00	1.00	-81.75
$p(\text{cloud}), \lambda(\text{aspect})$	8	213.91	28.64	0.00	1.00	-91.75
$p(\text{habitat}), \lambda(\text{aspect})$	10	216.10	30.83	0.00	1.00	-84.30
$p(\text{condition}), \lambda(\text{aspect})$	9	218.05	32.78	0.00	1.00	-90.02
$p(\text{temperature})+(\text{temperature}^2), \lambda(\text{aspect})$	9	218.20	32.93	0.00	1.00	-90.10
$p(\text{week})+(\text{week}^2), \lambda(\text{aspect})$	9	218.93	33.67	0.00	1.00	-90.47
$p(\text{rain})+(\text{rain}^2), \lambda(\text{aspect})$	9	218.94	33.67	0.00	1.00	-90.47

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Methods S2.1 R code for conducting a one-tailed power analysis of occupancy data (modified from Guillera-Arroita and Lahoz-Monfort, 2012).

# Function 'calcSFormula' to calculate the number of sites needed to detect an occupancy decline at a given power

```
calcSFormula <- function(K1,K2,p1,p2,psi1,R,alpha,pow)
{
  psi2 <- psi1*(1-R)
  pp1 <- 1-(1-p1)^K1
  pp2 <- 1-(1-p2)^K2
  F1 <- (1-pp1)/(pp1-K1*p1*(1-p1)^(K1-1))
  F2 <- (1-pp2)/(pp2-K2*p2*(1-p2)^(K2-1))
  f1 <- psi1*(1-psi1+F1)
  f2 <- psi2*(1-psi2+F2)
  S <- (f1+f2)*((qnorm(1-alpha)+qnorm(pow))/(psi1-psi2))^2
  return(ceiling(S))
}

# Set the parameters (values given are examples)
K<- 4 # number of replicates
p <- 0.364 # detection probability
psi1 <- 0.50 # initial occupancy probability
R <- 0.5 # proportional change in occupancy. Positive value for a decrease, negative value
for an increase.
alpha <- 0.05 # significance level
pow <- 0.8 # target power level

# Perform the calculation
(SS <- calcSFormula(K1 = K, K2 = K, p1 = p, p2 = p, psi1, R, alpha, pow))
```

## Chapter 2. Optimising monitoring efforts for snakes

Table S2.4 Evaluation of binomial mixture models (Poi = Poisson, NB = negative binomial, ZIP = zero-inflated Poisson) for grass snake abundance with number of observations set to 19. Displayed are model selection criteria (AICc), goodness-of-fit (GoF) statistics from 1000 simulations, and empirical estimates of abundance ( $\hat{N}_{total}$ ) with 95% confidence intervals calculated through 1000 parametric bootstrapping simulations. Best models according to AICc are in bold.

Models	Distribution	AICc	Goodness of fit			$\hat{N}_{total}$ (95% CI)
			$\chi^2$	$P$	$\hat{c}$	
Covariate						
	<b>Poi</b>	<b>185.27</b>	<b>122.37</b>	<b>0.13</b>	<b>1.16</b>	<b>47.88 (23–1279)</b>
	NB	188.34	189.79	0.70	0.00	81.41 (24–518)
	ZIP	189.65	150.37	0.38	1.00	73.88 (20–2748)
Null						
	Poi	203.25	213.84	0.00	1.65	29.78 (17–82)
	<b>NB</b>	<b>194.63</b>	<b>483.16</b>	<b>0.46</b>	<b>0.00</b>	<b>205.88 (18–814)</b>
	ZIP	200.09	218.84	0.03	1.35	37.23 (14–1400)

Table S2.5 Number of sites to be surveyed in any six-year Jersey NARRS cycle to detect an occupancy decline at a power of 0.8. Results are shown for differing numbers of surveys ( $K$ ), survey effort (numbers of ACOs) and their associated detection ( $p$ ) and occupancy ( $\psi$ ) probabilities from model-averaged predictions (Figure 2.2). Detection ( $p$ ) and the number of sites needed to detect a decline are shown with 95% confidence intervals. Figures shown in bold indicate numbers of sites  $\leq 50$  (the number surveyed in the previous six-year Jersey NARRS cycle (Wilkinson *et al.*, 2014)).

ACOs	$p$	$\psi$	$K = 4$			$K = 6$			$K = 8$		
			$R = 0.15$	$R = 0.3$	$R = 0.5$	$R = 0.15$	$R = 0.3$	$R = 0.5$	$R = 0.15$	$R = 0.3$	$R = 0.5$
0	0.199 (0.092–0.379)	0.505	2845 (840–15817)	660 <sup>†</sup> (200–3641)	212 <sup>†</sup> (65–1158)	1311 <sup>†</sup> (613–6239)	308 <sup>†</sup> (147–1440)	100 <sup>†</sup> ( <b>49</b> –459)	879 (559–3380)	209 <sup>†</sup> (135–783)	68 <sup>†</sup> ( <b>45</b> –251)
10*	0.213 (0.103–0.388)	0.524	2348 (767–11865)	546 <sup>†</sup> (183–2732)	175 <sup>†</sup> (60–870)	1109 <sup>†</sup> (565–4701)	262 <sup>†</sup> (137–1087)	85 <sup>†</sup> ( <b>45</b> –347)	765 <sup>†</sup> (519–2573)	182 <sup>†</sup> (126–598)	60 <sup>†</sup> ( <b>42</b> –192)
20	0.227 (0.115–0.398)	0.545	1950 (695–8911)	455 <sup>†</sup> (166–2054)	146 <sup>†</sup> (55–654)	944 <sup>†</sup> (518–3552)	224 <sup>†</sup> (126–823)	73 <sup>†</sup> ( <b>42</b> –263)	667 <sup>†</sup> (478–1970)	160 <sup>†</sup> (117–459)	53 <sup>†</sup> ( <b>39</b> –148)
30	0.242 (0.129–0.407)	0.566	1619 (632–6616)	379 <sup>†</sup> (152–1527)	122 <sup>†</sup> ( <b>50</b> –487)	806 <sup>†</sup> (476–2661)	192 <sup>†</sup> (116–618)	63 <sup>†</sup> ( <b>39</b> –198)	585 <sup>†</sup> (441–1502)	141 <sup>†</sup> (108–352)	47 <sup>†</sup> ( <b>36</b> –114)
40	0.258 (0.144–0.418)	0.587	1347 (572–4960)	316 <sup>†</sup> (138–1146)	102 <sup>†</sup> ( <b>46</b> –366)	691 <sup>†</sup> (436–2019)	166 <sup>†</sup> (107–471)	55 <sup>†</sup> ( <b>36</b> –151)	515 <sup>†</sup> (406–1164)	125 <sup>†</sup> (100–274)	42 <sup>†</sup> ( <b>34</b> –89)
50	0.274 (0.159–0.429)	0.609	1128 (516–3800)	266 <sup>†</sup> (125–880)	86 <sup>†</sup> ( <b>42</b> –281)	596 <sup>†</sup> (398–1568)	144 <sup>†</sup> (98–367)	48 <sup>†</sup> ( <b>33</b> –119)	455 <sup>†</sup> (372–925)	111 <sup>†</sup> (92–219)	37 <sup>†</sup> ( <b>31</b> –72)
60	0.291 (0.176–0.441)	0.630	947 (467–2897)	224 <sup>†</sup> (114–672)	73 <sup>†</sup> ( <b>38</b> –216)	517 <sup>†</sup> (364–1218)	126 <sup>†</sup> (90–286)	42 <sup>†</sup> ( <b>31</b> –93)	405 <sup>†</sup> (342–738)	100 <sup>†</sup> (85–176)	34 <sup>†</sup> ( <b>29</b> –58)
70	0.309 (0.193–0.454)	0.651	797 <sup>†</sup> (421–2255)	190 <sup>†</sup> (103–525)	62 <sup>†</sup> ( <b>35</b> –169)	450 <sup>†</sup> (333–967)	110 <sup>†</sup> (83–229)	37 <sup>†</sup> ( <b>28</b> –75)	361 <sup>†</sup> (315–603)	90 <sup>†</sup> (79–145)	30 <sup>†</sup> ( <b>27</b> –48)
80	0.329 (0.213–0.469)	0.673	666 <sup>†</sup> (376–1724)	160 <sup>†</sup> (93–403)	53 <sup>†</sup> ( <b>32</b> –130)	390 <sup>†</sup> (302–759)	96 <sup>†</sup> (76–181)	33 <sup>†</sup> ( <b>26</b> –59)	321 <sup>†</sup> (288–491)	81 <sup>†</sup> (73–119)	28 <sup>†</sup> ( <b>25</b> –40)
90	0.345 (0.229–0.483)	0.690	581 <sup>†</sup> (343–1412) <sup>†</sup>	140 <sup>†</sup> (86–331)	46 <sup>†</sup> ( <b>29</b> –107)	351 <sup>†</sup> (280–636)	87 <sup>†</sup> (71–153)	30 <sup>†</sup> ( <b>25</b> –51)	294 <sup>†</sup> (268–423)	74 <sup>†</sup> (68–104)	26 <sup>†</sup> ( <b>24</b> –35)
100	0.364 (0.248–0.499)	0.707	501 <sup>†</sup> (312–1136)	122 <sup>†</sup> (78–268)	41 <sup>†</sup> ( <b>27</b> –87)	314 <sup>†</sup> (259–528)	79 <sup>†</sup> (66–128)	27 <sup>†</sup> ( <b>23</b> –43)	269 <sup>†</sup> (249–363)	69 <sup>†</sup> (64–90)	24 <sup>†</sup> ( <b>22</b> –31)

\*Number of ACOs used in NARRS surveys 2007–2012 rarely exceeded 10 per site; <sup>†</sup>Mean number of sites  $\leq 840$ ; the maximum available in a six-year survey cycle (140 sites\*6 years).

## **Chapter 3. Habitat use and coexistence between grass snakes and people in a fragmented island landscape**

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### 3.1. Abstract

As habitats become further fragmented and eroded, nature reserves play increasingly important roles as wildlife refuges. However, reserves are often used for recreational activities, with management having to consider the needs of both humans and wildlife. We utilised short-term radio-telemetry of 16 grass snakes *Natrix helvetica* to study their habitat use at two spatial scales within reserves in a fragmented landscape on the island of Jersey. Our aims were to investigate how these reserves support vulnerable snake populations in the presence of human activity, and to inform site management. Structurally complex habitats, specifically rough grassland and dense scrub, were preferred over open and woodland habitats. Areas close to paths and compost heaps frequently occurred in home ranges, providing basking and potential nesting opportunities respectively. Snakes displayed site fidelity, and were not observed to cross tarmacked roads. Although snakes clearly moved through areas subject to public pressure, they were rarely directly observed. Indeed, on over 83% of fixes, snakes were not visible to the observer even when tracked to the nearest metre. Thus, coexistence between snakes and people may rely on a high degree of crypsis and avoidance of the public. Reserve managers should consider unobservable species diversity in their plans. Maintaining areas of complex vegetation structure and intervening habitat that may otherwise appear to be of low value in addition to key habitat features (freshwater ponds for prey, hibernacula and suitable oviposition sites) should improve site suitability for grass snakes.

**Keywords:** compositional analysis, distance-based analysis, fragmentation, habitat use, island, radio-tracking, snake



### 3.2. Introduction

The loss, degradation, and fragmentation of habitat are the greatest threats to biodiversity (Wilson, 1992; Wilcove *et al.*, 1998). Within islands the effects of fragmentation may be amplified, with reduced opportunities for dispersal and immigration due to isolation (Richman *et al.*, 1988; Fattorini, 2010). Island populations can respond differently to threats and environmental change to those on the mainland (Stamps and Buechner, 1985), and may be at greater risk of extinction or extirpation than their mainland counterparts due to small population size and anthropogenic pressures (Diamond, 1984).

Basic life-history information is often lacking for cryptic species, and anthropogenic disturbance can further reduce detectability through impacts on behaviour (e.g., Parent and Weatherhead, 2000). Alternative methods for investigating the ecology of elusive species such as snakes (Durso *et al.*, 2011) are therefore required. Radio-telemetry is one such tool, providing detailed information of direct relevance to studies of ecology and conservation (Reinert, 1993; Plummer and Ferner, 2012). Snakes typically exhibit negative responses to anthropogenic habitat change (Filippi and Luiselli, 2000; Gibbons *et al.*, 2000) and interact with their environment at different spatial scales (Row and Blouin-Demers, 2006a). It is therefore possible to assess snake conservation status based on analyses of their habitat use via radio-tracking (Reinert, 1993; DeGregorio *et al.*, 2011).

Grass snakes *Natrix helvetica* can have large seasonal home ranges (e.g. 30 ha in Mertens, 2008; 120.5 ha in Wisler *et al.*, 2008), with movements influenced by season, temperature, reproductive state, sloughing, hibernation and the distribution of resources (Madsen, 1984; Eckstein, 1993; Mertens, 1994; 2008;

## Chapter 3. Grass snake habitat use and telemetry

Wisler *et al.*, 2008; Pittoors, 2009). Theoretically, habitat fragmentation will have a greater impact on species with larger home ranges. However, this is difficult to identify when the species is rare or cryptic.

Within Jersey, British Channel Islands, the landscape is heavily fragmented with approximately 17% of semi-natural habitats remaining, largely within Jersey's National Park (States of Jersey Department of the Environment, 2016a). Furthermore, with one of the highest human population densities globally (The World Bank, 2015) and over 100,000 recreational users expected to access the island's green spaces each year (Jersey Tourism, 2010; States of Jersey Department of the Environment, 2016b), the anthropogenic pressures on these areas are considerable. The grass snake population here is considered threatened, but monitoring efforts are hampered by low detection rates (Chapter 2). Local declines are attributed to habitat loss, fragmentation and absence of key habitat features (Le Sueur, 1976). Given low detection rates, the present study used radio-telemetry (White and Garrott, 1990; Wisler *et al.*, 2008) to determine how (1) small patches of fragmented habitat were used by a wide-ranging species on an island; (2) key habitat features were used by the species in the face of regular disturbance.

### 3.3. Materials and methods

#### 3.3.1. Study sites and sampling

This study was conducted within Jersey National Park, Channel Islands (49°12'N, 2°8'W) at three publicly accessible sites (Figure S3.1): (1) Les Mielles Nature Reserve (**LM** - 53.9 ha) (49°13'N, 2°13'W); (2) Ouaisné Common Site of Special Interest (SSI) (**OU** - 10.4 ha) (49°10'N, 2°10'W); and (3) Les Blanchés

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Banques SSI (**BB** – 117.2 ha) (49°11'N, 2°13'W). These contained semi-natural dune and grassland habitats (Table 3.1) with site boundaries defined by roads or management limits. Each site was surveyed six to eight times a year between March–October 2014 and 2015 using combined visual and artificial cover object (ACO) surveys (Sewell *et al.*, 2013; Chapter 2). Snakes were captured by hand, sexed based on morphological characters (Smith, 1951), weighed and measured. Gravid females were identified by gentle palpation for eggs, however this was sometimes difficult to determine and some individuals were considered to have uncertain reproductive status.

#### **3.3.2. Radio-telemetry**

Radio-telemetry of snakes typically uses implanted tags which allow for a long battery life (Reinert and Cundall, 1982). However, there are associated risks including tag expulsion (Pearson and Shine, 2002), infection, impaired movement and mortality (Brown, 1991; Weatherhead and Blouin-Demers, 2004; Lentini *et al.*, 2011). Due to the rarity of snakes on Jersey, these risks were unacceptable and so we opted for an external tag attachment (e.g., Tozetti and Martins, 2007) with the trade-off that the tracking duration may be reduced.

Captured snakes were weighed to ensure external tag attachments were less than 5% of their body weight (Plummer and Ferner, 2012), and examined to determine whether they were sloughing and in good health. Those not meeting these weight and health criteria were not tracked. We prioritised snakes that were post-slough for tag attachment to maximise the tracking period. Prior to tag attachment, males ( $n = 3$ ) and females ( $n = 13$ ) weighed an average of 62.0 g ( $\pm$

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29.59 SD) and 147.4 g ( $\pm$  108.18 SD), and had snout-vent lengths of 436.3 mm ( $\pm$  56.58 SD) and 522.7 mm ( $\pm$  98.88 SD) respectively.

Tag attachment was similar to previous studies (Tozetti and Martins, 2007; Pettersson, 2014), but modified to obtain a longer tracking duration (Methods S3.1; Figure S3.2). Snakes were released at the point of capture and observed until out of view. They were located (each tracking location is subsequently referred to as a fix) multiple times (mean:  $3.13 \pm 1.31$  SD) per day (07:00–20:00 h) via direct tracking to provide details on short-term movements. Tag frequencies were ~ 173 MHz and snakes were located with a Sika receiver and flexible Yagi antenna (Biotrack Ltd, Wareham, Dorset, UK) until the tag was sloughed, lost by other means or removed. Snakes were located to an accuracy of 1 m<sup>2</sup> unless barriers were present, in which case triangulation was used. At each fix, coordinates were recorded in Jersey Transverse Mercator (JTM) format with a global positioning system (GPS) (Model Dakota 20, Garmin International, Inc., Olathe, Kansas, U.S.A.) along with habitat covariates, and notes on behaviour and position (Table 3.2). This study was conducted under licence CR23 (Conservation of Wildlife Law 2000) administered by the States of Jersey Department of the Environment and approved by the School of Anthropology and Conservation Ethical Review Committee.

### **3.3.3. Tracking duration and movements**

Total tracking duration for each snake was calculated as the elapsed time between the release and final tag loss or removal. For two snakes we excluded a period where the animal was not tracked (Table 3.3). Estimated hourly and daily movement distances (Carbone *et al.*, 2005) were calculated using straight line

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distances between fixes. Daily movement was measured as the sum of linear distances between the first fix of a day to the first of the following day. Mean daily movement was calculated for each snake as the total distance covered divided by the duration in days. We excluded days with only one fix for hourly movement rates, and days without monitoring the following day for daily rates.

### **3.3.4. Habitat use**

As habitat selection can be variable across different spatial scales (Aebischer *et al.*, 1993; Row and Blouin-Demers, 2006a), we investigated habitat use compared to availability within (1) a site, and (2) an individual's range (Johnson, 1980; Aebischer *et al.*, 1993). This method has often been applied to snakes (e.g., DeGregorio *et al.*, 2011; Hofer and Wisler, 2011) and we believe this is an appropriate way of identifying key habitats relevant to the conservation of the population. Range sizes were calculated for each snake with package `adehabitatHR` v0.4.14 (Calenge, 2006) in R v3.2.3 (R Core Team, 2015) as 100% minimum convex polygons (MCP) incorporating all fixes.

Habitat use was assessed using two methods; (1) compositional analysis (Aebischer *et al.*, 1993), and (2) distance-based methods (Conner and Plowman, 2001). All habitat data were derived from aerial imaging and Phase 1 maps (based on guidelines from the Joint Nature Conservation Committee, 2010) provided by the States of Jersey Department of the Environment. Habitat types and features were ground-truthed from field notes and adjusted accordingly. ArcMap v10.2.1 (ESRI, Redlands) was used to classify each site by up to 11 discrete habitat types, and to produce a set of distance layers where each cell contained the straight-line

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distances to features of interest (Table 3.1). All final geographic layers were produced at a five metre cell resolution.

### **3.3.4.1. Habitat composition**

The proportions of available and used habitat types were compared using compositional analysis in R package *adehabitatHS* v0.3.12 (Calenge, 2006) to identify whether snakes were found in habitat types more than, less than, or equal to habitat availability (Aebischer *et al.*, 1993). Habitats that were infrequently used (< 3%) by a snake were removed to avoid calculation errors (Harvey and Weatherhead, 2006) (Table 3.1; Table S3.1). Where habitats were unused by individuals, zeros were replaced with 0.01 to avoid errors (Aebischer *et al.*, 1993). Overall significance of habitat selection was tested using a Wilks' Lambda test, and habitats were ranked based on the number of positive comparisons between habitat types.

To explore variation between snakes in habitat use, we used eigenanalysis of selection ratios (Calenge, 2006; Calenge and Dufour, 2006) at both site and range scales. This approach was used to (1) compare habitat use in individual snakes with that generated randomly, and (2) determine whether the habitat types were being used in proportion to their availability. The resulting graphical output was visually inspected to identify any groupings, and to investigate underlying drivers of heterogeneity in habitat selection among individuals (Calenge and Dufour, 2006).

### **3.3.4.2. Distance to features**

A distance-based analysis of habitat use (Conner and Plowman, 2001; Conner *et al.*, 2003) was used to test for influences of key habitat features (compost heaps and water bodies) and areas of high anthropogenic disturbance

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(roads and paths) on habitat selection (Table 3.1). We refer to these as 'features'. For each snake ( $i$ ), we generated 1000 random points across its site in ArcMap. The distance between each random point and the nearest representative of each feature was then measured, and the mean of these measurements ( $r_i$ ) represented random selection of each feature for each snake. Similarly, we calculated the distances between the fixes from each snake ( $i$ ) and each feature to generate a set of mean used distances ( $u_i$ ). The distance ratio between these random and used distances was calculated as  $d_i = u_i/r_i$  for each feature, where a  $d_i$  of one represented random selection (Conner *et al.*, 2003). These methods were repeated at the range scale, resulting in a set of distance ratios for each snake at both site and range scales for each feature.

The mean distance ratios from all snakes ( $n = 16$ ), and for those only from site LM ( $n = 11$ ) were compiled (Table 3.5); referred to as  $\mathbf{p}_{all}$  and  $\mathbf{p}_{LM}$  respectively. A distance-based analysis of compost heap influence was tested only at site LM due to the absence of this feature elsewhere. A MANOVA was used to test for habitat selection, considered as the set of distance ratios ( $d_i$ ) in  $\mathbf{p}_{all}$  or  $\mathbf{p}_{LM}$  differing significantly from a corresponding set of 1s. Paired t-tests were used to identify preference ( $d_i < 1$ ) or avoidance ( $d_i > 1$ ) of each feature separately by comparing each snake's  $d_i$  against a value of one. Pairwise t-tests enabled comparisons and ranking of values within  $\mathbf{p}_{LM}$  (Conner *et al.*, 2003).

#### 3.3.5. Data analysis

Statistical analyses were conducted in R v3.2.3 (R Core Team, 2015) with each snake as the unit of study. The effects of site, sex and status (Table 3.2) upon movement rates and range size were tested by incorporating them as factors

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within ANOVAs. As range (R) may be influenced by tracking duration (td), we used an adjusted range ( $R_{adj}$ ) within ANOVAs calculated as  $R_{adj} = R/td$ . All ANOVAs were non-significant (Table S3.2), suggesting no differences between sites, sex and status. All data were therefore pooled for all analyses.

Table 3.1 Habitat covariates mapped at study sites incorporated into analyses of habitat selection.

Habitat covariate	Description
<i>Habitat composition</i>	
Amenity grassland	Inclusive of gardens, golf courses, parkland and other sources of improved grassland
Arable <sup>a</sup>	Arable / agricultural land
Bare ground	Inclusive of tracks, open sand, and other non-vegetated areas
Bracken	Continuous bracken cover
Buildings <sup>a</sup>	Any permanent anthropogenic structure in the landscape
Dense scrub	Inclusive of blackthorn, bramble, gorse, willow, heathland and other scrub vegetation
Rough grassland	Inclusive of coastal, dune, marram semi- and un-improved grassland
Ruderal <sup>a</sup>	Tall ruderal vegetation
Swimming pool <sup>a</sup>	Residential swimming pools
Wetland <sup>a</sup>	Natural and semi-natural water bodies inclusive of swamp
Woodland <sup>a</sup>	Inclusive of planted and semi-natural coniferous, mixed and broadleaved woodland
<i>Distance-based analysis</i>	
Distance to road	Any paved road surface
Distance to path	Any path or track used by the public
Distance to wetland	Any freshwater body which may host prey
Distance to compost heap <sup>b</sup>	Any vegetation heap which may be suitable for oviposition

<sup>a</sup>Habitat covariates excluded from analyses due to low (< 3%) or no use. <sup>b</sup>Les Mielles Nature Reserve (LM) only.



## Chapter 3. Grass snake habitat use and telemetry

Table 3.2 Position and behaviour assessment of radio-tracked snakes

Variable	Details
<i>Position</i>	defined as: (a) visible (i) moving in open (ii) basking - settled with at least part of the body exposed to the sun (b) concealed (iii) under artificial cover object (ACO) (iv) underground - radio signal clearly indicated individual is underground, or is observed underground (v) unknown (concealed by cover and / or underground)
<i>Behaviour / Status</i>	
Reproductive	Male / Female - status unknown / Non-gravid female / Gravid female
Sloughing	Yes / No

### 3.4. Results

Of 37 snakes captured, 23 were radio-tagged. Success of tag attachment was variable, with 30.4% of tags developing electronic faults or becoming rapidly displaced during sloughing or movements (Figure S3.3), leaving 16 snakes in all further results (n=3 males, n=13 females). As it was not always possible to differentiate between tag loss or stationary behaviour, we excluded periods when there was uncertainty. Snakes were tracked for 4–75 days (mean:  $24.41 \pm 22.21$  SD) with a total of 1046 fixes (Table 3.3).

Table 3.3 Summary of tracking results. ID's denote each snake and its site (BB, LM or OU). N is the number of fixes. Bursts are the number of separate tracking periods. Ranges are given as 100% minimum convex polygons. Distance represents the summed straight line distances across the duration.

ID	Sex	N	Start (Date, time)	End (Date, time)	Duration ( <u>Days</u> hours:minutes)	Bursts	Range (ha)	Distance (m)
BB01	F	23	06/06/2014 13:30	14/06/2014 09:08	<u>7</u> 19:38	1	0.24	288.38
BB02	F	10	20/04/2015 11:48	28/04/2015 14:40	<u>8</u> 02:52	1	0.04	86.96
LM01	F	65	28/05/2015 13:48	09/06/2015 16:47	<u>12</u> 02:59	1	0.41	440.50
LM02	F	102	28/05/2015 18:45	29/06/2015 09:40	<u>26</u> 19:40	2	0.92	801.11
LM03	F	27	29/05/2015 12:26	03/06/2015 16:05	<u>5</u> 03:39	1	0.54	218.48
LM04	M	83	03/06/2015 12:02	21/06/2015 16:37	<u>18</u> 04:35	1	4.90	750.25
LM05	F	42	17/06/2015 12:37	29/06/2015 08:50	<u>11</u> 20:13	1	0.52	348.83
LM06	F	139	17/06/2015 18:28	24/07/2015 18:01	<u>36</u> 23:33	1	7.54	1501.42
LM07	F	211	18/06/2015 13:55	14/10/2015 09:50	<u>74</u> 14:49	2	13.38	4411.91
LM09	F	20	19/06/2015 18:03	25/06/2015 11:00	<u>5</u> 16:57	1	1.67	614.61
LM10	F	87	12/07/2015 17:21	09/08/2015 11:00	<u>27</u> 17:39	1	1.99	939.93
LM11	M	102	20/07/2015 14:55	27/08/2015 09:25	<u>37</u> 18:30	1	3.63	1690.68
LM12	M	50	23/09/2015 13:57	04/12/2015 16:15	<u>72</u> 02:18	1	1.35	843.15
OU01	F	11	21/05/2014 13:40	25/05/2014 15:00	<u>4</u> 01:20	1	0.44	240.96
OU02	F	28	18/08/2015 17:30	29/08/2015 09:32	<u>10</u> 16:02	1	0.33	321.01
OU03	F	46	18/08/2015 19:45	18/09/2015 16:20	<u>30</u> 20:35	1	1.83	943.98

### 3.4.1. Movement and range

Snakes moved an average total distance of 902.6 m (SD: 1040.02, range: 86.96–4411.91) across the study period, with distance correlated to tracking duration ( $r_{\tau} = 0.78$ ,  $n = 16$ ,  $P < 0.001$ ). Movement rates were typically low with occasional large movements (Figure 3.1), moving an average of 42.9 m per day (SD: 59.99, range: 0.00–452.43) and 4.2 m an hour (SD: 8.98, range: 0.00–90.21). Range sizes were variable (mean: 2.48 ha  $\pm$  3.54 SD, range: 0.04–13.38) (Table 3.3) and correlated with tracking duration ( $r_{\tau} = 0.52$ ,  $n = 16$ ,  $P < 0.01$ ).

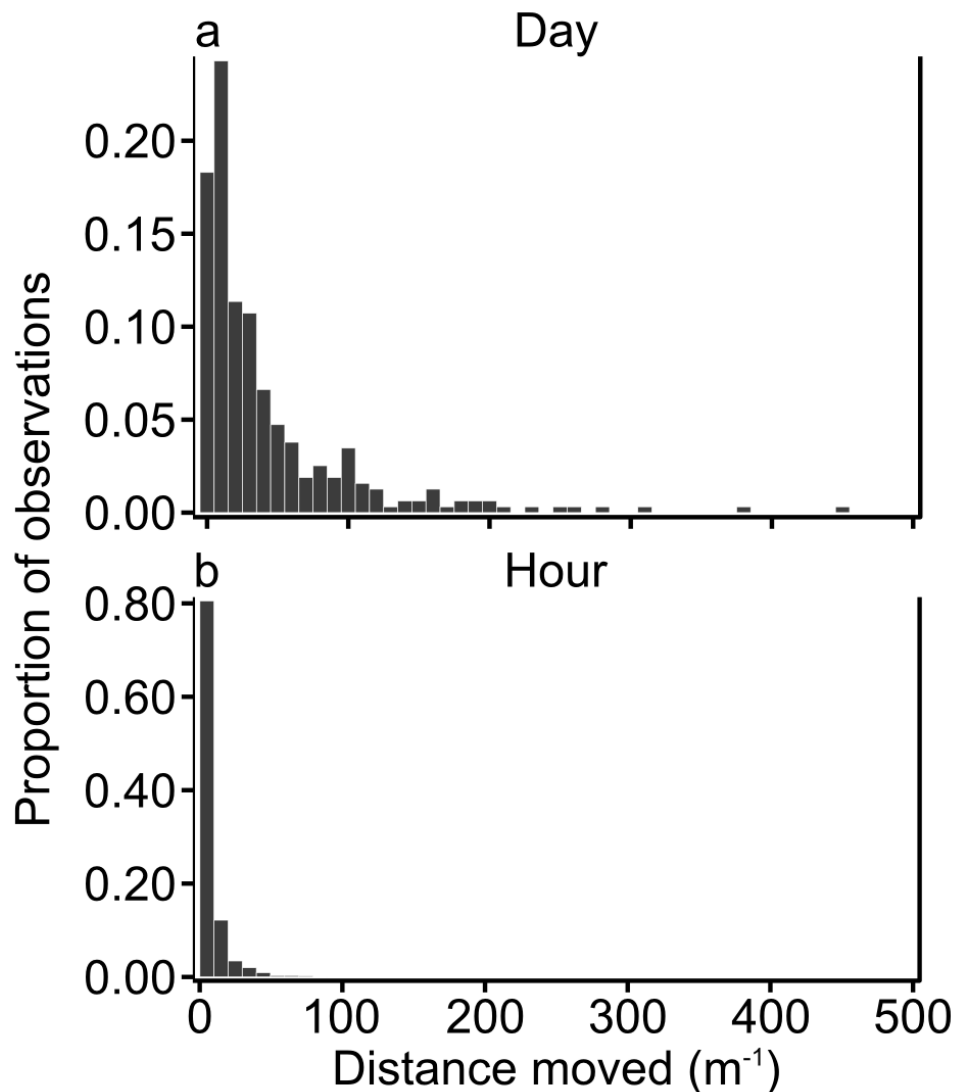


Figure 3.1 Distances moved per day (a) ( $n = 317$ ) and per hour (b) ( $n = 961$ ) for 16 snakes.

### 3.4.2. Habitat use

Compositional analysis at the site-level showed significant habitat selection (weighted mean  $\Lambda_{16,5} = 0.38$ ,  $P = 0.018$ ), with preferences for scrub and grassland habitats over bare ground (Table 3.4). At the range-level however there was no significant difference between used and available habitats (weighted mean  $\Lambda_{16,5} = 0.30$ ,  $P = 0.366$ ) but similar habitat rankings (Table 3.4). At this level, only rough grassland was significantly preferred over bare ground.

Habitat selection was explained by two factors describing 77.9% of the information at the site level, three factors explaining 94.2% at the range-level, and suggested variation in habitat selection amongst snakes (Figure S3.4) (Calenge and Dufour, 2006). Therefore averaging of selection-ratios may not be appropriate. At both levels, most snakes favoured dense scrub and rough grassland habitats, with five snakes strongly preferring bracken at the site-level and three at the range-level. Bare ground was used comparatively rarely. This variation in habitat use among animals remains unexplained, and may be due to any combination of variability in tracking period, sex, lifestage, site or other factors.

Snakes were significantly influenced by their distance to features at the site-level ( $V = 0.96$ ,  $F_{4,17} = 104.08$ ,  $P < 0.001$ ) but not within ranges ( $V = 0.05$ ,  $F_{4,17} = 0.24$ ,  $P > 0.05$ ), preferring areas closer to paths when considering all snakes ( $t_{15} = 2.28$ ,  $P = 0.04$ ) or only those at LM ( $t_{10} = 11.00$ ,  $P < 0.001$ ). Snakes at LM also selected ranges that were closer to compost heaps than available throughout the site ( $t_{10} = 5.57$ ,  $P < 0.001$ ), but within their ranges, did not use areas in close proximity to compost heaps more than expected at random ( $t_{10} = -0.68$ ,  $P = 0.51$ ). No other evidence of selection was found (Table 3.5; Figure S3.5). Pairwise t-tests

### Chapter 3. Grass snake habitat use and telemetry

of site-level data for LM revealed that snakes were most influenced by their proximity to paths, compared to roads ( $P = 0.026$ ) and water ( $P < 0.001$ ).

Therefore, snakes prefer ranges close to paths and compost heaps, but other features have little influence on their habitat selection.

Table 3.4 Ranking of habitats from compositional analyses at the site- and range- levels for 16 snakes. Signs indicate positive (+) and negative (-) associations between habitat types. A triple symbol represents a significant difference in preference ( $\alpha = 0.05$ ) between habitats.

		Available habitat					
		Dense scrub	Rough grassland	Amenity grassland	Bracken	Bare ground	Rank
Used habitat (site-level)	Dense scrub	0	+	+	+	+++	1
	Rough grassland	-	0	+	+	+++	2
	Amenity grassland	-	-	0	+	+++	3
	Bracken	-	-	-	0	+	4
	Bare ground	---	---	---	-	0	5
Used habitat (range-level)	Dense scrub	0	-	+	+	+	2
	Rough grassland	+	0	+	+	+++	1
	Amenity grassland	-	-	0	-	+	4
	Bracken	-	-	+	0	+	3
	Bare ground	-	---	-	-	0	5

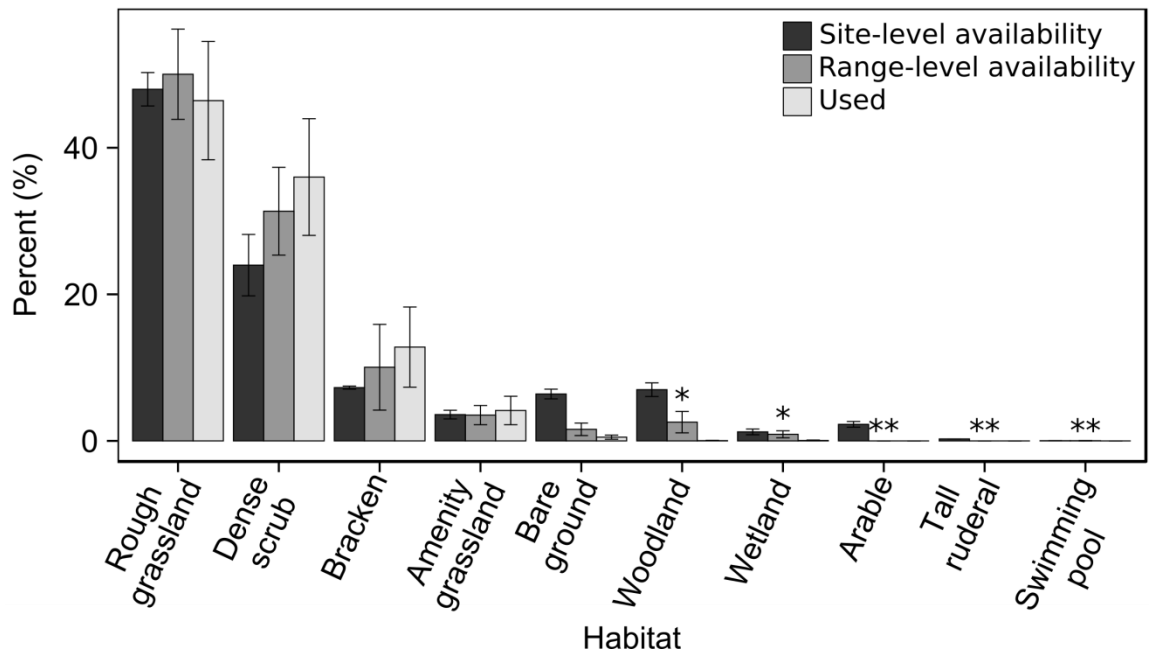


Figure 3.2 Comparisons of used (light grey) and available habitats within (i) sites (dark grey) and (ii) 100% MCP ranges (mid-grey). Values are displayed as mean percentage across all sites  $\pm$  1 SE ( $n = 16$  snakes). Categories are displayed in order of use, from most to least. Habitats excluded from analyses due to low (< 3%) (\*) or no (\*\*) use are displayed to show their contribution.

Table 3.5 Ratio ( $\rho$ ) of mean distances between snake locations and habitat features, to mean distance between random locations and habitat features. Ratios ( $\rho$ ) <1 indicate preference and those >1 indicate avoidance. Significance of habitat selection ( $P$ ) is shown for each feature with those below the nominal value ( $P < 0.05$ ) displayed in bold.

Analysis	Level	Feature	$\rho$	$P$
All sites	Site	Road	0.98	0.59
		Path	<b>0.43</b>	<b>0.04</b>
		Water	1.01	0.92
		Compost	–	–
	Range	Road	1.01	0.59
		Path	0.96	0.92
		Water	1.02	0.19
		Compost	–	–
LM only	Site	Road	1.07	0.70
		Path	<b>0.36</b>	<b>&lt;0.001</b>
		Water	1.06	0.66
		Compost	<b>0.57</b>	<b>&lt;0.001</b>
	Range	Road	0.94	0.73
		Path	1.04	0.56
		Water	1.00	0.84
		Compost	0.97	0.51

#### **3.4.2.1. Position and activity**

Despite locating individuals to the nearest metre, snakes were unobservable 83.5% of the time. This was due to concealment amongst vegetation and other features (77.5%), or within underground burrows (6.0%). Snakes also used artificial cover objects in 8.1% of fixes. It was therefore rarely possible to determine behaviour, with stationary individuals recorded in 7.0% of fixes and active individuals a further 2.2%.

### **3.5. Discussion**

Priorities for habitat management are complex, with conflict between the needs of humans and biodiversity (Young *et al.*, 2005). Nature reserves often act as habitat patches in otherwise fragmented landscapes. Habitat types such as scrub and rough grassland may provide vital habitats for biodiversity, yet be seemingly unattractive and of little value to recreational users (Gobster *et al.*, 2007; Qiu *et al.*, 2013). For reptiles, these 'unattractive' habitats often provide opportunities for thermoregulation and should be incorporated in management plans (Bonnet *et al.*, 2016).

Here, we investigated habitat selection and movements of a wide-ranging snake species within habitat patches on a largely urbanised and agricultural island. We identified complex vegetation structure and the availability of basking and nesting opportunities as important contributors to habitat use, providing considerations for reserve management. Furthermore, our findings highlight issues in species detectability with impacts for monitoring and research.

### **3.5.1. Movement and range**

Despite mostly small movements, snakes covered large distances and ranges, similar to their mainland counterparts (Brown, 1991; Mertens, 2008; Wisler *et al.*, 2008). Moreover, annual movement distances and ranges are likely to be larger than those estimated here. This mobility highlights the need to consider habitat connectivity and patch size. Although active snakes are rarely observed by recreational users of the sites, snakes regularly moved through areas with high footfall. Nevertheless, no road crossings were observed, although both live and dead snakes have been reported on Jersey's roads. Brown (1991) only recorded a single grass snake to cross roads, but studies of other snake species have noted their negative impacts (Andrews and Gibbons, 2005; Roe *et al.*, 2006).

To estimate true annual home-range sizes, a longer tracking period would be needed (Madsen, 1984; Reading and Jofré, 2009), requiring tag implantation. We avoided this due to the associated risks, but future studies could benefit from its use. We are therefore cautious in generalising our habitat use data as it may not represent overall habitat use over longer periods. Nevertheless, the large amount of data we accrued could not have been attained otherwise, and we believe the short-term data obtained remains informative about habitat use during the snake's active period when the sites are regularly used by the public (Figure S3.3).

### **3.5.2. Habitat use**

The preference for complex habitat structure and avoidance of open and wooded areas, reflects previous findings obtained from mainland populations (Madsen, 1984; Mertens, 2008; Pittoors, 2009; Pettersson, 2014). We found no use of agricultural habitats, whereas elsewhere they have provided opportunities



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for cover and movement between preferred habitats (Madsen, 1984; Wisler *et al.*, 2008). However, homogeneous habitats are generally unsuitable (Reading and Jofré, 2009).

That snakes preferred areas close to paths reinforces the importance of heterogeneous edge habitat (Madsen, 1984; Wisler *et al.*, 2008). These areas provide easily navigable corridors, with edges providing basking opportunities and protective cover (Madsen, 1984; Mertens, 2008; Wisler *et al.*, 2008; Reading and Jofré, 2009; Pettersson, 2014). Moreover, areas close to paths are often managed, reducing succession and maintaining thermally preferable areas. 'Disturbed' recreational areas containing paths may therefore improve basking opportunities. However, there is likely to be a threshold where disturbance outweighs the thermal benefits. More research into these effects would enable better guidance for reserve design.

Neither analysis indicated water bodies as influencing habitat selection. This is unexpected given that grass snakes are often associated with wetlands, feeding on fish and amphibians (Gregory and Isaac, 2004; Mertens, 2008). However, although amphibian populations have declined in Jersey and are currently in recovery (Wilkinson, 2007; Ward *et al.*, 2016), terrestrial amphibians and other vertebrate prey were available throughout our study sites (Gregory and Isaac, 2004; Pittoors, 2009). Whether Jersey's grass snakes are restricted by prey availability is therefore unknown, but we noted snakes to have consumed toads *Bufo spinosus*, small mammals and a green lizard *Lacerta bilineata* (R. Ward, unpublished data). This indicates a wide trophic niche, requiring further investigation to identify the role of prey in habitat selection.

Hibernation and nesting sites can also be limiting features for grass snakes. The availability of nesting sites is of concern and may depend on anthropogenic features such as compost heaps (Madsen, 1984; Wisler *et al.*, 2008; Löwenborg *et al.*, 2012a). We identified the first known oviposition site in Jersey in three decades (Chapon, 1986); a compost heap. This was confirmed through observation and tracking of a gravid female that spent several consecutive days at the heap. On the day of oviposition, the snake did not stray from the heap and was observed in the afternoon to have considerably decreased in body condition before moving elsewhere (R. Ward pers. obs.). Despite the relationship between distribution and compost heaps, all other known compost heaps were investigated for eggs but none were found. Further efforts to identify and protect nesting habitat are therefore warranted.

### **3.5.3. Detectability of snakes**

Snakes in this population have a low detection probability (Chapter 2) which is further confirmed by poor visibility during tracking. Monitoring efforts should acknowledge this limited availability for detection. Low detection and use of cover and burrows has also been noted by others (Wisler *et al.*, 2008; Pittoors, 2009). Indeed Pittoors (2009) noted snakes to be visible only 16.0% of the time; a value remarkably similar to ours at 16.5%. Despite the apparent preference for paths, disturbance within the reserves created by recreational users and their dogs may influence the behaviour and reduce detectability of snakes (Parent and Weatherhead, 2000). Burrows may provide retreat sites, hibernacula and opportunities for movement through otherwise unsuitable habitats, as well as containing prey (Wilkinson, 2007).

#### **3.5.4. Limitations**

Our study was limited by sample size associated with a small population (Chapter 2), size restrictions of study animals and issues of detectability at different lifestages (e.g., Gregory and Tuttle, 2016). Nevertheless, the sample size was larger than most previous telemetry studies of this species (e.g., Madsen, 1984; Mertens, 2008; Reading and Jofré, 2009) and may represent about half of the known individuals in Jersey (Chapter 2). Radio-telemetry data have the potential to be autocorrelated. We did not modify our data to account for this as the short intervals between sampling should provide more accurate and biologically representative measures of range size, movement and habitat utilisation (Aebischer *et al.*, 1993; Rooney *et al.*, 1998; Row and Blouin-Demers, 2006b).

#### **3.5.5. Conclusions**

Grass snakes can clearly survive in isolated habitat patches despite high levels of fragmentation and public use. With ongoing anthropogenic pressures throughout Jersey and Europe, the effects of fragmentation upon biodiversity warrant greater attention, and without ensuring site connectivity, the future of this, and other insular populations is uncertain (Újvári *et al.*, 2002; Jansen *et al.*, 2008; Hofer and Wisler, 2011). For many species, green spaces such as nature reserves may provide the only remaining suitable habitat; therefore, adequate site protection is vital. A greater effort is needed to demonstrate the value of these spaces to elusive and uncharismatic species and to promote them through sympathetic management. Combining habitat augmentation and public education may be the best avenue to achieve this (Bonnet *et al.*, 2016).

### **3.6. Acknowledgements**

We are grateful to landowners for granting access, volunteers for assisting with data collection and to Nigel Hand and Darryn Nash for advice on radio-telemetry. This work was supported by the States of Jersey Department of the Environment, Jersey Countryside Enhancement Scheme, Durrell Institute of Conservation and Ecology, ARG-UK Research Fund, British Herpetological Society Student Grant Scheme and Durrell Conservation Awards.

### 3.7. Supplementary Information

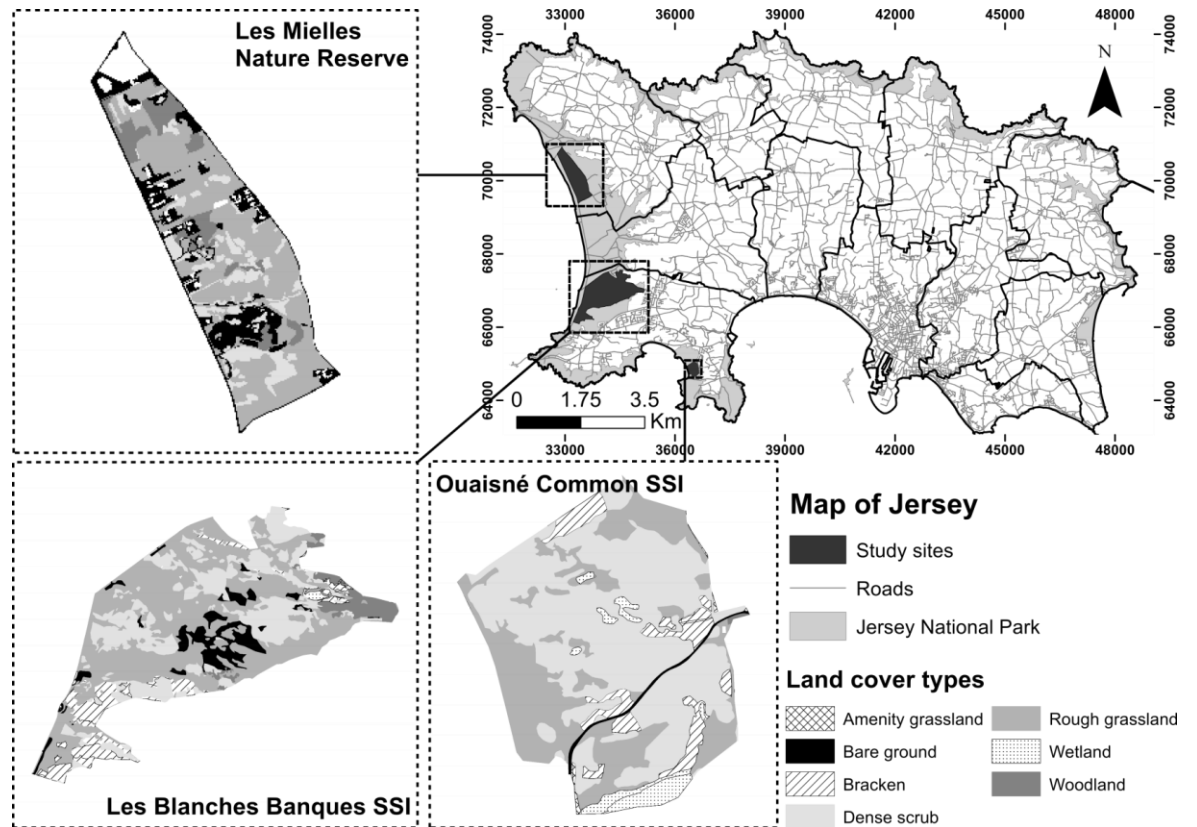


Figure S3.1 Map of Jersey with parish boundaries (black lines), the road network (grey lines), and the Jersey National Park (grey). Tracking sites are shown in dark grey, with close-ups of each site showing land cover types.

## Chapter 3. Grass snake habitat use and telemetry

### Methods S3.1 External radio-tag attachment methodology

The process required snakes to be held firmly, with the anterior end held within a snake tube or cloth bag to minimise movement; particularly when no assistance was available. The site of attachment was a dorso-lateral position posterior to the cloaca to avoid interference with passing of faeces, mating, and egg deposition (Pettersson, 2014). The area was cleaned with acetone and allowed to dry. Surgical tape (approx. 20 x 30 mm) (3M™ Blendtherm™, 3M United Kingdom PLC, Bracknell, Berkshire, UK) was then placed around the tail's circumference to provide a secure attachment (Figure S3.2). A 1.3g Pico-Pip tag (Biotrack Ltd, Wareham, Dorset, UK, lifespan ~eight weeks) was then attached with cyanoacrylate glue and cyanoacrylate accelerator spray to increase speed of curing (Bond it, Elland, West Yorkshire, UK). Once dry, duct tape (UniBond, Henkel, Düsseldorf, Germany) was placed on top of the tag. To avoid snagging, subsequent injuries and tag displacement, the profile of the tag was smoothed and the edges of the duct tape did not extend beyond the base layer of surgical tape. For long tagging durations to succeed, we carried out visual checks on the quality of tag attachment. If necessary, individuals were recaptured and tape was repaired or reapplied.

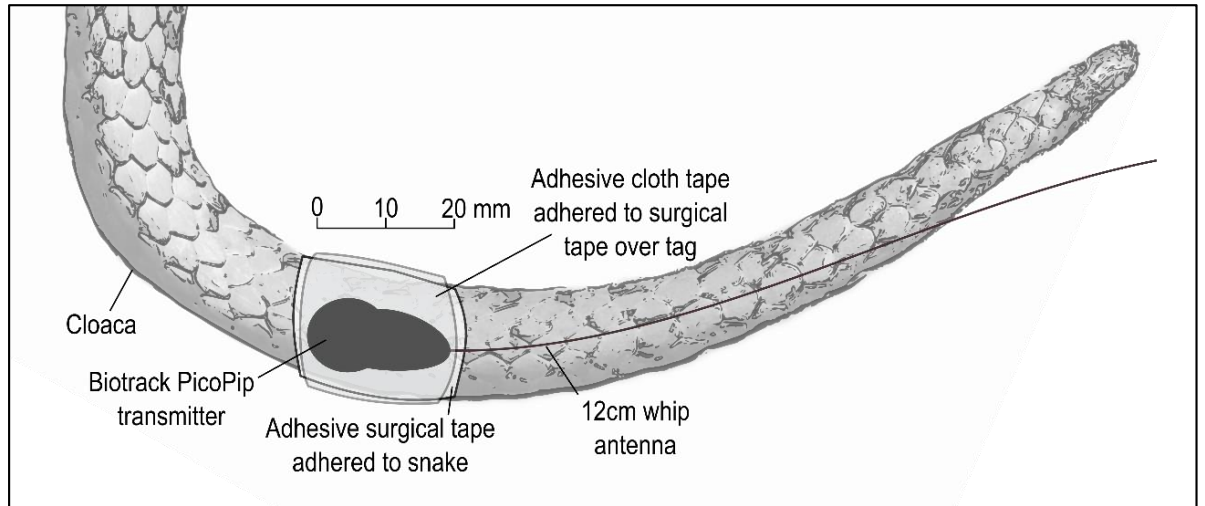


Figure S3.2 Illustration of external radio-tag attachment on tail. Tags were positioned dorso-laterally posterior of the cloaca.

Table S3.1 Availability and use of habitats for 16 individual snakes (ID), with the two letter ID prefix identifying the site they were tracked at (BB, LM or OU). Habitat availability is shown within each site (SA) and each individual's range (RA) as number of 5 m cells containing that habitat type. Used (U) habitats are shown as number of relocations in an available habitat. \*Wetland and woodland were not analysed due to their low use.

ID	Amenity Grassland			Bare Ground			Bracken			Dense Scrub			Rough Grassland			Wetland*			Woodland*		
	SA	RA	U	SA	RA	U	SA	RA	U	SA	RA	U	SA	RA	U	SA	RA	U	SA	RA	U
BB01	15	0	0	3579	0	0	4201	0	0	13485	48	6	23101	45	17	208	0	0	2217	0	0
BB02	15	0	0	3579	0	0	4201	17	8	13485	1	2	23101	0	0	208	0	0	2217	0	0
LM01	1085	9	9	1607	0	0	1423	6	19	3016	57	16	11119	87	21	106	0	0	1955	0	0
LM02	1085	31	4	1607	0	0	1423	0	0	3016	61	42	11119	277	56	106	0	0	1955	0	0
LM03	1085	29	0	1607	1	0	1423	0	0	3016	39	0	11119	142	27	106	0	0	1955	0	0
LM04	1085	6	0	1607	53	1	1423	313	16	3016	482	16	11119	1065	51	106	1	0	1955	38	0
LM05	1085	2	0	1607	0	0	1423	0	0	3016	96	37	11119	114	5	106	0	0	1955	0	0
LM06	1085	14	5	1607	75	1	1423	15	0	3016	784	37	11119	2026	95	106	1	0	1955	88	1
LM07	1085	53	4	1607	112	0	1423	535	3	3016	1242	48	11119	3283	153	106	6	2	1955	91	0
LM09	1085	102	5	1607	84	0	1423	31	1	3016	20	4	11119	358	10	106	0	0	1955	16	0
LM10	1085	37	16	1607	0	2	1423	0	0	3016	93	13	11119	661	57	106	0	0	1955	0	0
LM11	1085	64	0	1607	38	0	1423	30	7	3016	144	24	11119	826	71	106	0	0	1955	322	0
LM12	1085	0	0	1607	6	2	1423	36	12	3016	81	0	11119	366	36	106	0	0	1955	49	0
OU01	12	0	0	46	0	0	337	0	0	2353	116	9	1243	51	2	184	8	0	6	0	0
OU02	12	0	0	46	0	0	337	28	11	2353	87	15	1243	10	2	184	5	0	6	0	0
OU03	12	19	0	46	0	0	337	246	0	2353	605	68	1243	104	2	184	42	0	6	0	0
Mean	750.1	22.9	2.7	1560.8	23.1	0.4	1566.6	78.6	4.8	4200.3	247.3	21.1	10765.0	588.4	37.8	133.4	3.9	0.1	1622.3	37.8	0.1



### Chapter 3. Grass snake habitat use and telemetry

Table S3.2 Outputs from ANOVAs (Analysis of Variance) to test group differences between tracked snakes.

	Reproductive state		Sex		Sloughing status		Site	
	$F_{3,12}$	$P$	$F_{1,14}$	$P$	$F_{1,14}$	$P$	$F_{2,13}$	$P$
Mean distance per day	2.25	0.14	0.34	0.57	0.001	0.98	0.72	0.51
Mean distance per hour	1.34	0.31	0.88	0.48	0.12	0.73	1.31	0.30
Range (adjusted)	1.75	0.21	0.45	0.52	1.4	0.26	1.35	0.29

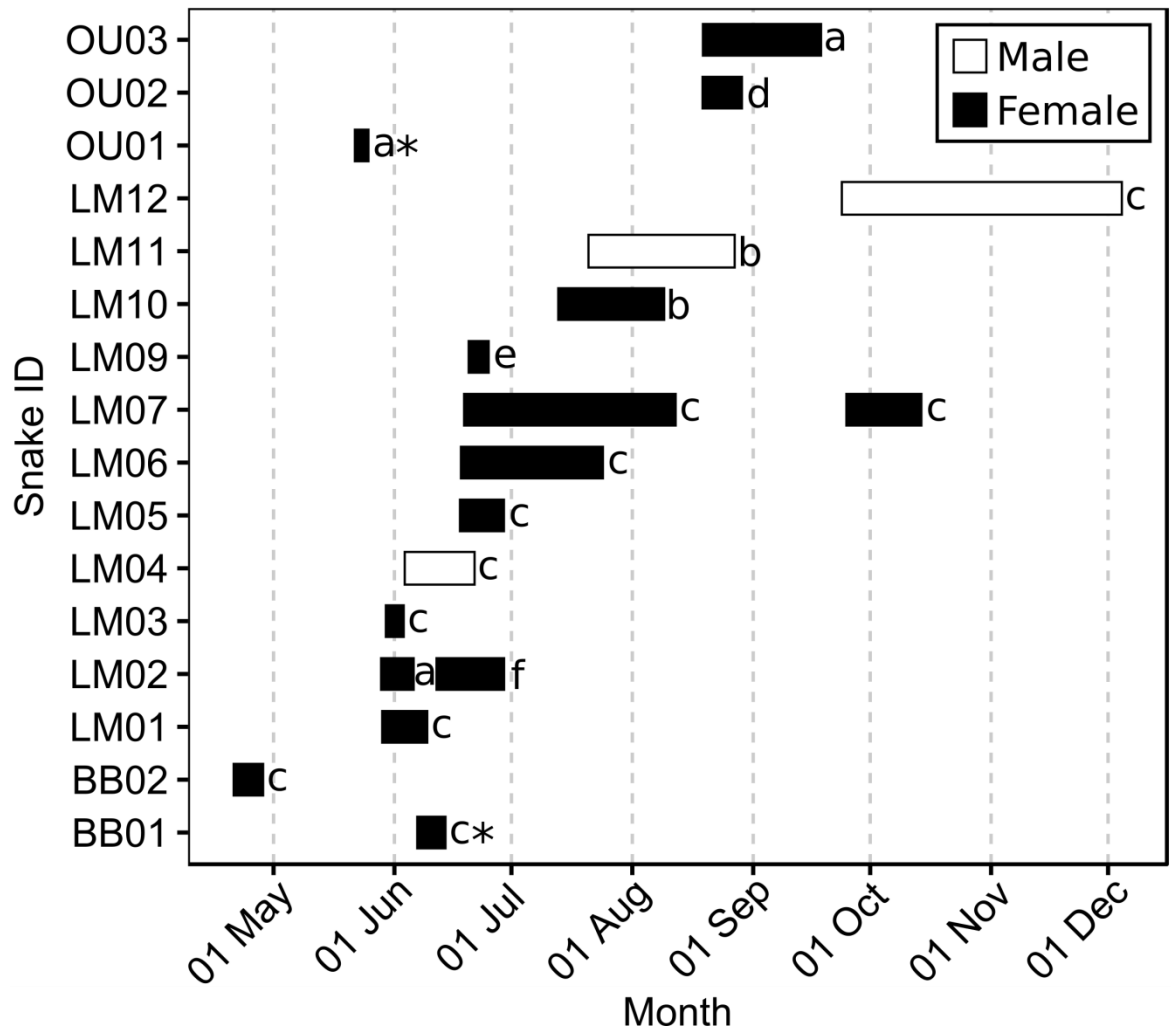


Figure S3.3 Timing and duration of tag attachments. Sex is shown by colour (white=male, black=female). Two individuals were tracked in 2014 (\*), all others were tracked in 2015. Causes of tracking ending are indicated as (a) sloughed, (b) tag removed, (c) other loss of tag, (d) tag failure, (e) unknown and (f) died.

Chapter 3. Grass snake habitat use and telemetry

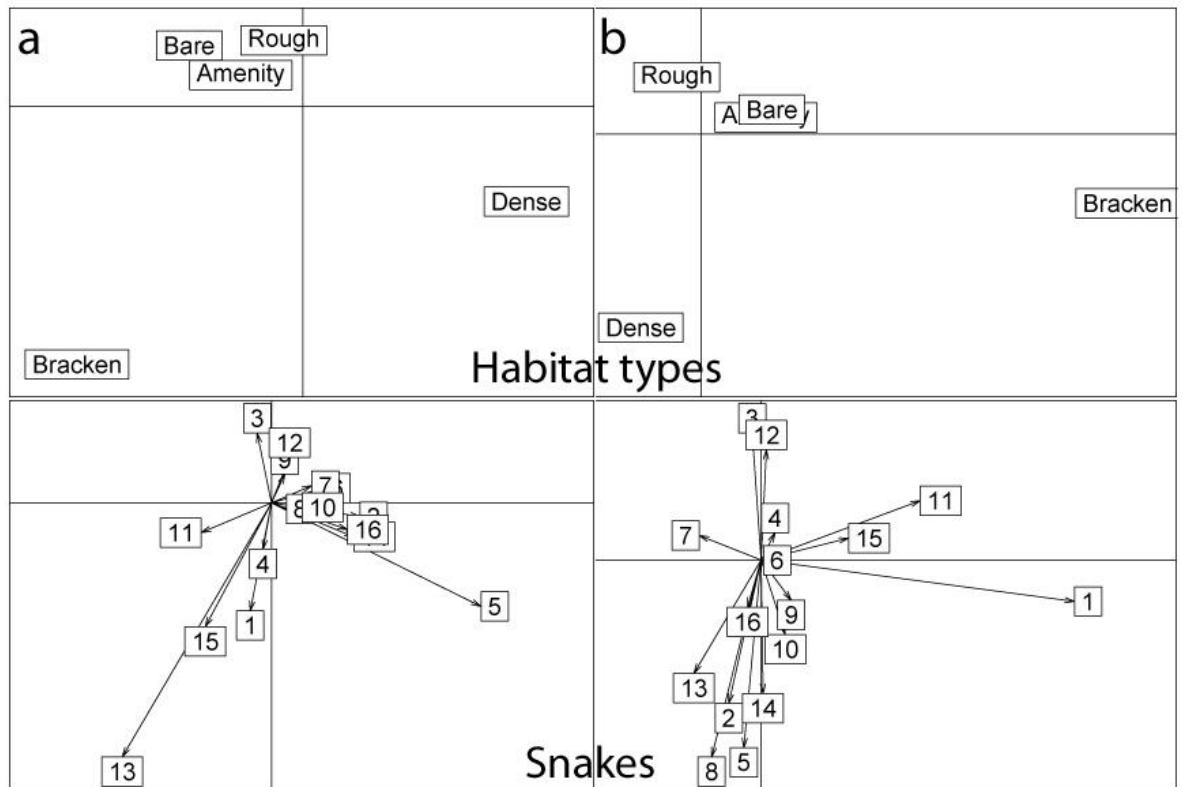


Figure S3.4 Eigenanalysis of selection ratios showing differential site- (a) and range-level (b) habitat use (top) by 16 snakes (bottom) at all sites. Positions are shown on the first two factorial axes.

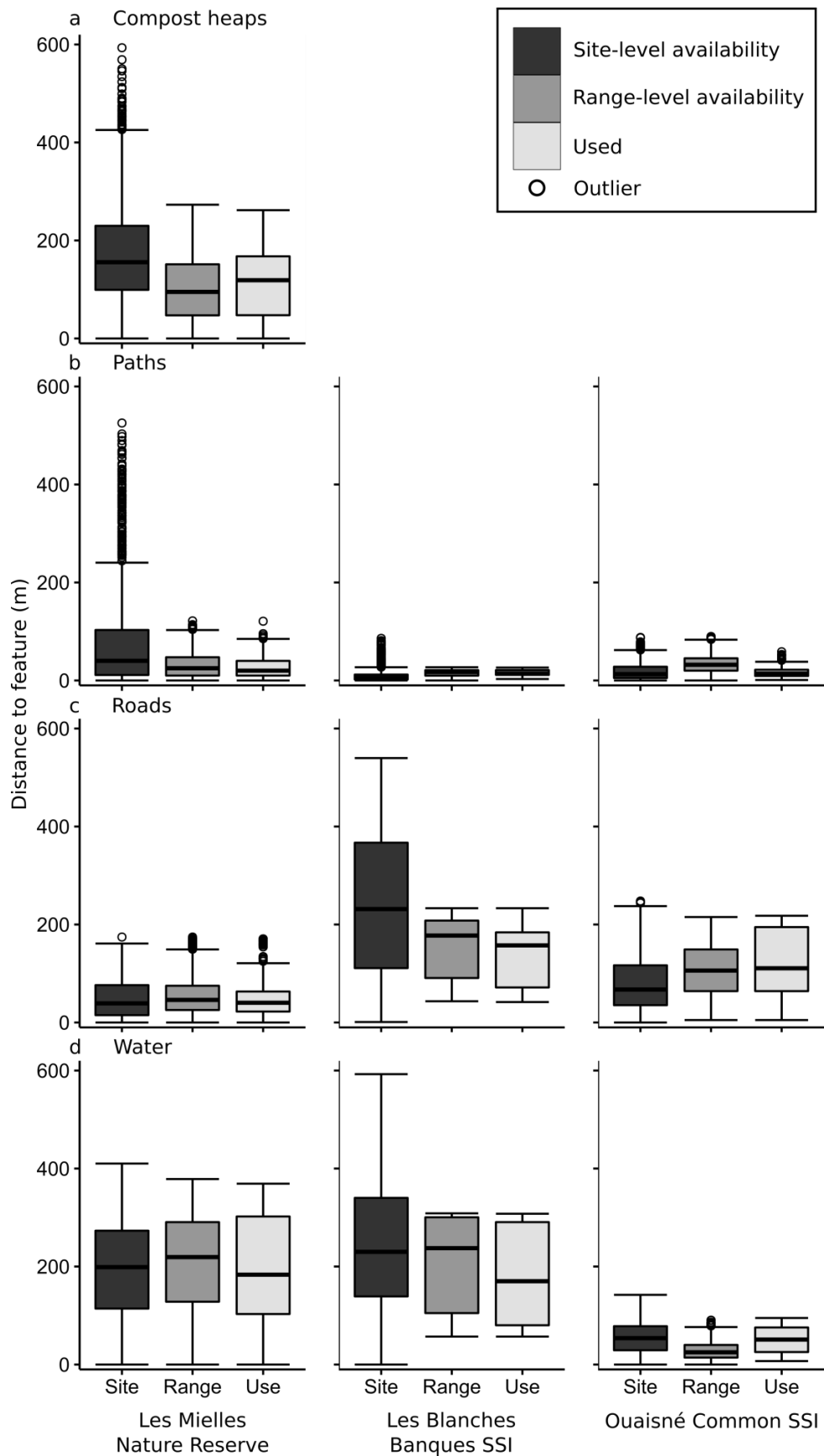


Figure S3.5 Boxplots showing distances to (a) compost heaps, (b) paths, (c) roads and (d) water from 1000 random points generated at the site- and range- level for each study site, and distances from snake tracking locations (Use). Outliers are shown as circles.

## **Chapter 4. Priority conservation areas for the grass snake in Jersey**

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#### 4.1. Abstract

The loss, degradation and fragmentation of habitat is a key driver of biodiversity declines. Remaining fragments provide refuges for biodiversity, but do not always receive adequate protection. Identifying simple methods for predicting priority areas for monitoring and protection contributes to status assessment and management actions, subsequently improving the viability of, and connectivity between, populations. These measures can improve the efficiency of conservation prioritisation for rare and elusive species which otherwise require significant resources for assessment. Here we use species distribution modelling to identify priority habitats for monitoring and protection for the grass snake *Natrix helvetica* on the island of Jersey, whilst presenting one of the first studies to account for sampling bias and model complexity when modelling a snake's distribution. Snake distribution was skewed towards the west of the island, with positive associations to structurally complex habitats and proximity to prey populations, but negative associations with high road densities and agricultural habitats. A binary threshold selecting the most suitable areas in the island identified almost 20% of the island as having conservation priority. Of these areas, 38% were situated within the Jersey National Park, but only 11% were in areas with legal protection. Improving the coverage of the protected area network, particularly in the west and southwest of the island in areas with non-statutory designations, would be of great benefit to grass snakes and biodiversity in general. A multi-species approach will assist further in identifying priority areas for biodiversity.

**Keywords:** Ecological Niche Modelling (ENM), entropy, fragmentation, Maxent, *Natrix helvetica*, presence-background, species distribution model (SDM)

## 4.2. Introduction

Assessing the status of rare and elusive species is a demanding task and may be constrained by low detectability, insufficient sampling effort and limited records (Bland and Böhm, 2016; Galante *et al.*, 2017; Chapter 2). Having a well-informed understanding of a species' distribution and the features within its environment that enable or limit survival is important for framing conservation objectives (Lyet *et al.*, 2013). These include invasive species modelling (e.g., Rose and Todd, 2014), prioritising areas for monitoring, protection or management (e.g., de Pous *et al.*, 2011; Lyet *et al.*, 2013) and informing IUCN status assessments (IUCN Standards and Petitions Subcommittee, 2017).

Current methods for modelling species distributions include maximum likelihood-based and information theoretical approaches. At a minimum, these require occurrence records and background information on which to base predictions (Phillips *et al.*, 2006). Available occurrence data such as those from museums and herbaria (Newbold, 2010), biological recording repositories (e.g., Pocock *et al.*, 2015) and focal studies are rarely unbiased spatially and temporally (Phillips *et al.*, 2009; Peterson *et al.*, 2011; Guillera-Arroita *et al.*, 2015). For rare species, it may be necessary to collate occurrences from several of these sources. The inclusion of absence records gives more robust predictions (Guillera-Arroita *et al.*, 2015); however, presence-background (also termed presence-only) models are often used because establishing true absence is difficult (e.g., Chapter 2). Additional biotic and abiotic data such as environmental measurements, landcover maps and the distribution of heterospecifics provide background information to inform the model, leading to predictions of suitability across a landscape.

## Chapter 4. Priority areas for grass snake conservation

Species distribution models have proved to be useful tools for assessing the distribution and conservation status of elusive snake species (e.g., Santos *et al.*, 2006; Bombi *et al.*, 2009). Snakes are primarily threatened by habitat loss and degradation (Gibbons *et al.*, 2000; Ribeiro *et al.*, 2009), and the presence of suitable habitat for dispersal can be key to their survival (Bonnet *et al.*, 1999). Consequently, isolation heightens their extinction risk due to reductions in range, population size and gene flow (Frankham *et al.*, 2002). The expansion and intensification of agriculture and road networks are barriers to dispersal (Mader, 1984), and can result in high snake mortality (Roe *et al.*, 2006; Weatherhead and Madsen, 2009). An understanding of a species' distribution can mitigate some of these effects through spatial conservation planning. Specifically, ensuring habitat patches are of appropriate size and are well-connected can assist in the persistence of a metapopulation.

Within Jersey, British Channel Islands, terrestrial fauna persist in a highly anthropogenic landscape populated by over 100,000 people; giving it one of the highest human population densities in the world (The World Bank, 2015). As a result, 53% of the island is used for agriculture, 23% is urbanised and heavy fragmentation arises from a road network covering over 800 km. The remaining semi-natural areas comprise a mixture of terrestrial habitats including mixed woodland, coastal sand dunes, wet meadows, maritime heath, marshland and freshwater (States of Jersey Department of the Environment, 2016a).

As with many islands, Jersey hosts an impoverished native fauna (Le Sueur, 1976). One of Jersey's largest remaining native vertebrates, and its only snake species, is the grass snake *Natrix helvetica*. Present knowledge suggests that it is restricted to semi-natural areas in the west and southwest of the island; however,



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this prediction is hindered by low detection and the mobile nature of the species (Chapter 2; Chapter 3). Moreover, habitat suitability may vary seasonally depending on selection of specific resources.

In this study we use grass snake occurrence data from several sources to make predictions of landscape suitability on the island of Jersey with a commonly used machine-learning method; Maxent (<https://www.cs.princeton.edu/~schapire/maxent/>; Phillips *et al.*, 2006).

Specifically, we highlight areas of suitability in a closed island system, and identify variables influencing the species' distribution. We also assess the existing protected areas and the newly established Jersey National Park (JNP) in their ability to provide habitat for the grass snake population. Our main aim is to identify priority areas for conservation measures consisting of survey targets for potentially undetected populations, informing areas for management and legal protection and considerations for development. To produce an accurate model, we allow model complexity to vary and test the effects of two bias-treatment approaches; (1) systematic sampling of occurrence records and (2) the use of a bias file that accounts for sampling bias (Phillips *et al.*, 2009; Kramer-Schadt *et al.*, 2013; Fourcade *et al.*, 2014).

### 4.3. Methods

#### 4.3.1. Study area

The island of Jersey is 117 km<sup>2</sup> and lies approximately 22 km from mainland France. It contains 19 ecological Sites of Special Interest (SSI) that are protected under the Planning and Building (Jersey) Law 2002 (States of Jersey Department of the Environment, 2016a), with several more proposed. In addition, the island

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contains several non-statutory conservation areas including Environmentally Sensitive Areas, Sites of Importance for Nature Conservation and the JNP which itself contains 1,925 hectares of coastal habitats (States of Jersey, 2011; States of Jersey Department of the Environment, 2016a).

### **4.3.2. Study species**

Grass snakes are wide-ranging with requirements for key features in the landscape (Madsen, 1984; Meister *et al.*, 2010) and a preference for amphibian prey (Gregory and Isaac, 2004). They can be found in a variety of lowland habitats including rough grassland, heathland, moorland and agricultural land (Steward, 1971; Madsen, 1984; Wisler *et al.*, 2008; Chapter 3). Across their European range their status is unclear (Reading *et al.*, 2010), but localised declines are suspected (Hagman *et al.*, 2012). Within Jersey, grass snakes are considered rare with a low abundance and a restricted range (Le Sueur, 1976; Hall, 2002; Chapter 2).

### **4.3.3. Species distribution modelling**

To model the distribution of grass snakes in Jersey, we used Maxent v3.3.3 ([http://biodiversityinformatics.amnh.org/open\\_source/maxent/](http://biodiversityinformatics.amnh.org/open_source/maxent/)) (Phillips *et al.*, 2006). This is a popular programme which employs a machine learning approach, using Maximum Entropy to predict a species' distribution from a combination of presence and environmental data (Phillips *et al.*, 2006), referred to as Presence-Background (Guillera-Arroita *et al.*, 2015). Maxent compares presences against background points (pseudo-absences), producing suitability predictions across the landscape of interest (Phillips *et al.*, 2006; Phillips and Dudík, 2008). It also has low sensitivity to small sample sizes and is therefore useful for predicting the distributions of rare and elusive species (Pearson *et al.*, 2007; Wisz *et al.*, 2008).

## Chapter 4. Priority areas for grass snake conservation

### **4.3.3.1. Occurrence data**

Grass snake occurrences were collated from long-term monitoring efforts (Wilkinson *et al.*, 2014), intensive surveys (Chapter 2), radio-tracking (Chapter 3), public sightings collected during a public media campaign termed 'Think Grass Snake' which was designed to encourage public reporting of grass snake and slow-worm *Anguis fragilis* sightings (2014–2016, <http://www.ThinkGrassSnake.Je>), and data provided by the Jersey Biodiversity Centre (<http://jerseybiodiversitycentre.org.je/>) and the States of Jersey Department of the Environment. Commonly, slow-worms or escaped pet snakes (e.g., corn snakes *Pantherophis guttatus*) may be misidentified as grass snakes in Jersey (R. Ward pers. obs.). Therefore, to ensure identifications were accurate, their reliability were ranked as unconfirmed, reliable, or confirmed based on the following attributes: the wildlife knowledge of the species identifier (e.g., professional ecologist, amateur ecologist, no specialist knowledge), the description of the animal, the location of the sighting and photographic or video evidence. This resulted in the exclusion of multiple records that we attributed to other species. Remaining records were filtered so that only those that had (i) a geographic accuracy of  $\leq 100$  m, (ii) reliable species identification (classed as 'reliable' or 'confirmed' during validation) and (iii) been recorded from 1990 onwards were included in further analyses. Duplicate locations were also removed, resulting in 1019 occurrences (Figure S4.1).

### **4.3.3.2. Reducing sampling bias**

Occurrence data are often biased by sampling effort, leading to spatial autocorrelation and subsequent poor-quality models (Kramer-Schadt *et al.*, 2013; Fourcade *et al.*, 2014). This occurs as model accuracy is inflated (Veloz, 2009),

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thereby producing biologically inaccurate estimates and erroneous inferences regarding predictors of distribution (Kühn, 2007; Kramer-Schadt *et al.*, 2013; Merow *et al.*, 2013). There may also be an observer bias, with recorders only reporting those species they are interested in (Kramer-Schadt *et al.*, 2013; Strien *et al.*, 2013).

The majority of grass snake occurrences were from the west and southwest of the island where intensive surveying and research has taken place (Chapter 2; Chapter 3; Figure S4.1). We tested two methods to account for this sampling bias; (i) spatial filtering (SF) (Kramer-Schadt *et al.*, 2013; Fourcade *et al.*, 2014) and (ii) a bias file (BF) (Phillips *et al.*, 2009). Spatial filtering reduces the density and number of occurrences by ensuring occurrences are spaced a minimum distance from one another; thereby reducing the effect of spatially clustered records on Maxent predictions. This was carried out at two scales with the *Create Random Points* tool in ArcMap v10.5 (ESRI, Redlands) to identify an optimum filtering resolution; 100 m or 500 m (referred to as SF<sub>100</sub> and SF<sub>500</sub> from here on). We chose these levels to reflect the  $\leq 100$  m geographic accuracy of the data and a maximum distance for daily movement of the species respectively (Mertens, 2008; Chapter 3). During filtering we kept the most occurrences possible after 10 runs of the *Create Random Points* tool, giving 97 occurrences at 100 m spacing and 34 at 500 m spacing (Table 4.2) for inclusion within the Maxent models.

For the second method, we generated a bias file (Kramer-Schadt *et al.*, 2013) in R v3.3.3 (R Core Team, 2017) to reflect the bias in our occurrence data. Our sampling locations (ACOs) from chapter 2 were used as point data to produce a raster layer representing our survey effort with the kernel density estimation function (*kde2d*, MASS package v7.3-45, <https://cran.r->

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[project.org/web/packages/MASS/index.html](https://project.org/web/packages/MASS/index.html)) and published code (Methods S4.1).

Each combination of spatial filtering and the presence or absence of a bias file was tested resulting in four model scenarios: SF at 100 m only (SF<sub>100</sub>BF<sub>no</sub>), SF at 500 m only (SF<sub>500</sub>BF<sub>no</sub>), SF at 100 m with BF (SF<sub>100</sub>BF<sub>yes</sub>) and SF at 500 m with BF (SF<sub>500</sub>BF<sub>yes</sub>) (Fourcade *et al.*, 2014).

### **4.3.3.3. Predictor variables**

Maxent models often utilise bioclimatic variables to infer species distributions. Furthermore, environmental factors are expected to be key drivers of reptile distribution due to their thermal requirements. However, we excluded these variables as they had limited variation across the island (Table S4.1) and hypothesised that habitat availability has a greater influence. Instead, we focused on the influences of 14 quantitative predictors related to habitat, prey and anthropogenic variables to improve model-fitting (Table 4.1). Landcover classifications were derived from Phase 1 habitat survey data (Joint Nature Conservation Committee, 2010) provided by the States of Jersey Department of the Environment, and subsequently reclassified to provide 20 broad classes (Table S4.2). Furthermore, we calculated variables associated with freshwater due to the species' preference for these habitats (Steward, 1971) and derived topographic variables from a digital elevation model sourced from the Earth Observing System Data and Information System (EOSDIS) (2009).

To account for anthropogenic influences, a set of variables were extracted from landcover and government census data provided by the States of Jersey Department of the Environment. Variables involving density were calculated with the *point density* or *line density* tools for point or linear features respectively in ArcMap, at a radius of 500 m from features (e.g., buildings, roads). However,

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population density was calculated per enumeration district. Additionally, we calculated three landscape fragmentation metrics across the island (Table 4.1) with a sliding window analysis in Fragstats v4.2 (McGarigal *et al.*, 2012). We used user-defined tiles of 1 ha (i.e., the landscape was divided into 1 ha parcels measuring 100 x 100 m for analysis) from our modified landcover map, and the 8-neighbour rule where all cells bounding a cell were used for metric calculations. These metrics were (i) patch density, (ii) contagion index and (iii) patch richness; where a patch was defined as a continuous set of cells containing the same discrete habitat classification.

Patch density was calculated as the number of patches per 100 ha. The contagion index (Li and Reynolds, 1993) is a measure of how a landscape is configured, and is dependent on the probability of different landcover types occurring adjacent to one another. Higher levels of contagion are associated with few, clustered landcover types, whereas lower values suggest a variety of landcover types and that cells of the same landcover type are dispersed. See Li and Reynolds (1993) or the Fragstats documentation (available from <http://www.umass.edu/landeco/research/fragstats/fragstats.html>) for further details on its calculation. Additionally, patch richness was calculated as the number of different landcover types per hectare. All predictor variables were prepared and projected in the Jersey Transverse Mercator coordinate system in ArcMap, at a resolution of 100 m (1 ha cells) to fit with the lowest accuracy of our occurrence records (Engler *et al.*, 2004).

### **4.3.3.4. Multi-collinearity**

Overfitting and poor model performance can occur due to high model complexity and correlations between predictor variables. We tested for correlation

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between variables using the *vifcor* and *vifstep* functions in R package *usdm* v1.1-18 (<https://cran.r-project.org/web/packages/usdm/index.html>) (Naimi, 2015), with values compared from a maximum of 10,000 corresponding cells from each layer. All 14 variables had Pearson's correlation values  $\leq 0.7$  and VIF values  $< 3$  (Zuur *et al.*, 2010) and were kept for further analyses (Table 4.1). This set of variables is referred to as our 'full' set, as in addition, we compared the outcomes of using a 'reduced' variable set consisting of nine of the same variables after removing those with little contribution to permutation importance. The two sets (full and reduced) were compared in order to assess whether over-fitting may be occurring through greater model complexity when using the full set (e.g., Struebig *et al.*, 2015).

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Table 4.1 Spatial variables used to predict the relative probability of occurrence for grass snakes in Jersey. Variance inflation values (VIF) are shown. Correlation tests were not applied to landcover classifications. Variables included in the 'reduced' set are indicated with an asterisk (\*).

Variable	VIF	Source <sup>a</sup>
<b>Landscape features</b>		
<i>Landcover classifications*</i>	NA	DoE
<i>Distance to water*</i> (Euclidean, metres. Calculated from landcover map)	1.25	DoE
<i>Pond density*</i> (Ponds per km <sup>2</sup> )	1.20	DoE
<i>Slope</i> (Calculated from Digital Elevation Model)	1.15	EOSDIS, 2009
<i>Aspect*</i> (Calculated from Digital Elevation Model)	1.02	EOSDIS, 2009
<i>Flow accumulation</i> (Calculated from Digital Elevation Model)	1.04	EOSDIS, 2009
<b>Anthropogenic</b>		
<i>Human population density*</i> (People per km <sup>2</sup> , per enumeration district)	2.16	DoE
<i>Distance to roads</i> (Euclidean, metres)	1.52	DoE
<i>Distance to buildings</i> (Euclidean, metres)	1.42	DoE
<i>Road density*</i> (Roads per km <sup>2</sup> )	2.73	DoE
<b>Fragmentation</b>		
<i>Patch density</i> (Number of habitat patches per 100 ha)	1.47	DoE
<i>Contagion Index*</i> (Patch aggregation %)	1.26	DoE
<i>Patch richness*</i> (Number of patch types per ha)	1.14	DoE
<b>Prey distribution</b>		
<i>Distance to toads*</i> (Euclidean, metres)	1.16	DoE; Toadwatch

<sup>a</sup> Data sources are States of Jersey Department of the Environment (DoE), Jersey Toadwatch (Wilkinson and Starnes, 2016) and the Earth Observing System Data and Information System (EOSDIS) (2009).

### 4.3.3.5. Regularisation

To further address the risks of over-fitting and complexity, we investigated the effects of regularisation (Merow *et al.*, 2013). Regularisation can be used to control over-parameterisation and improve the generalisation of predictions by smoothing the response curves. Higher levels of regularisation will result in a smoother and more regular model. Default regularisation values are based on a test set (Phillips and Dudík, 2008) which may not be suitable for all species (Warren and Seifert, 2011). Therefore, to tune the model appropriately we tested



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regularisation multiplier values between 0.5 and 6.0, at intervals of 0.5 for all four model scenarios and for both variable sets (full and reduced). This entails multiplying the default regularisation parameters by our given values, with the optimum value determined based on threshold-independent and threshold-dependent measures described below (Pearson *et al.*, 2007; Merow *et al.*, 2013; Boria *et al.*, 2014; Radosavljevic and Anderson, 2014).

Threshold-independent measures were based on the receiver operating characteristics (ROC) area under the curve (AUC), which can be used to rank models by testing their ability to discriminate between presences and the background (Phillips *et al.*, 2006). The first of these was the AUC value from the test data ( $AUC_{\text{test}}$ ), with higher values expected to represent better fit. However this approach does not assess overfitting (Radosavljevic and Anderson, 2014); therefore we also used the difference ( $AUC_{\text{diff}}$ ) between training AUC ( $AUC_{\text{train}}$ ) and testing AUC ( $AUC_{\text{test}}$ ) values, calculated as

$$AUC_{\text{diff}} = AUC_{\text{train}} - AUC_{\text{test}}$$

where lower values should represent more reliable models (Warren and Seifert, 2011). Threshold-dependent measures comprised training presence omission rates, with lower omission rates indicating better models (Phillips *et al.*, 2006). Specifically, we used average values from 20 runs for minimum training presence training omission (also known as lowest presence threshold - LPT) and 10th percentile training presence training omission (T10) (Pearson *et al.*, 2007; Boria *et al.*, 2014; Radosavljevic and Anderson, 2014).

The best performing levels of regularisation and variable complexity were used for subsequent selection between the four model scenarios. Of these four

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scenarios, we considered the best scenario to be that which showed optimum performance based on the threshold-independent and dependent measures described above, and as indicated by qualitative visual interrogation of maps for biological plausibility (i.e., those that fit biological expectations and expert knowledge of the landscape) (Franklin, 2009). Additional discrimination between SF<sub>100</sub> scenarios was carried out using information theoretical approaches (AIC and BIC) (Burnham and Anderson, 2002; Warren and Seifert, 2011). This was not possible for SF<sub>500</sub> scenarios due to the low number of occurrence points. The best performing scenario was used as a final prediction of habitat suitability across Jersey. From this best scenario, the influence of predictor variables was assessed from permutation importance and by inspecting the outputs from jackknife tests, to identify which variables contributed the greatest training gain with no other variable contributions and which, when left out resulted in the greatest decrease in training gain.

### **4.3.3.6. Model settings**

All models were run with a random test percentage of 25% using the subsample method and a maximum of 10,000 background points. Models were trained with up to 5,000 iterations and we ran 20 replicates, using the mean of the raw outputs for further investigation and model selection. All other settings were left as the defaults. We used the raw output as it does not rely on post-processing assumptions and is therefore favoured, providing estimates of the proportional contribution of each cell to the overall distribution, with all cells used during training summing to one across the landscape (Phillips *et al.*, 2006; Merow *et al.*, 2013). For ease of interpretation, raw outputs were converted to cumulative outputs using the *cumulative* function (bossMaps package v0.1.0, <https://cran.r->

[project.org/web/packages/bossMaps/](https://r-project.org/web/packages/bossMaps/)) in R, and are subsequently displayed in all maps. This cumulative output consists of values between 0 and 100, where each cell in the landscape has a value calculated as the sum of all other cells that contain less or equal probabilities multiplied by 100. A value of 100 is therefore the cell predicted to have the greatest suitability for the species, and 0 the least.

#### **4.3.3.7. Identifying key habitat**

Due to the threatened status of the grass snake population in Jersey, identifying primary areas for habitat protection, management and monitoring is an important step in affording long-term security to the population. To do so, we applied binary thresholds to our final map prediction to highlight priority areas for conservation managers. First we applied a subjective fixed threshold of 10% to incorporate all possible areas of suitability (referred to as suitable areas). This liberal threshold excludes the bottom 10% of cells with the lowest values as unsuitable. Subsequently, we used an objective threshold that maximises the sum of sensitivity (percentage of correctly classified presences) and specificity (percentage of correctly classified absences), termed maxSSS (Liu *et al.*, 2005; Peterson *et al.*, 2011), to identify priority areas for conservation focus (e.g., Araújo and Williams, 2000; Peterson *et al.*, 2011). We then evaluated the proportion of these areas encompassed within current protected areas and the JNP in ArcMap with the *Zonal Statistics* tool. Based on previous telemetry studies, we consider patches smaller than 40 ha and / or isolated by 1 km of unsuitable habitat to be inadequate for long-term population viability (Brown, 1991; Zuiderwijk *et al.*, 1998; Wisler *et al.*, 2008; Chapter 3) and discuss priorities for site protection in this context.

## 4.4. Results

### 4.4.1. Model complexity

Models with full and reduced variable sets showed similar performance based on threshold-independent measures, but models with the full variable set generally performed better than the reduced variable set when assessed with threshold-dependent measures. This indicates better model performance when supplied with a greater selection of variables, and so the full model set was therefore favoured; however, this was more evident for LPT and at higher levels of regularisation (Table 4.2; Figure S4.2).  $AUC_{diff}$  was improved by increased regularisation, whereas there was little effect on  $AUC_{test}$  unless considering the  $SF_{500}BF_{yes}$  scenario. The  $SF_{100}BF_{no}$  scenario gave the best overall performance across a range of regularisation values, but a value of 4.5 appears to be most appropriate (Figure S4.2). Therefore, we continue with the use of the full variable set due to better performance and a greater variety of predictors of suitability. Additionally, we use a regularisation value of 4.5 as it reduces the risk of underestimating the species' distribution whilst still maintaining good model fit.

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Table 4.2 Summary of model evaluation for each model scenario. Values given are the mean values across all regularisation tests. Full and reduced variable sets are shown, containing 14 and nine variables respectively.

Variable set	Scenario	No. samples		AUC		Omission	
		Training	Test	test	diff	LPT	T10
Full	SF <sub>100</sub> BF <sub>no</sub>	73	24	0.86	0.04	0.04	0.17
	SF <sub>100</sub> BF <sub>yes</sub>	73	24	0.82	0.07	0.05	0.21
	SF <sub>500</sub> BF <sub>no</sub>	26	8	0.71	0.15	0.13	0.27
	SF <sub>500</sub> BF <sub>yes</sub>	26	8	0.63	0.18	0.17	0.33
Reduced	SF <sub>100</sub> BF <sub>no</sub>	73	24	0.87	0.03	0.18	0.19
	SF <sub>100</sub> BF <sub>yes</sub>	73	24	0.82	0.06	0.21	0.23
	SF <sub>500</sub> BF <sub>no</sub>	26	8	0.73	0.12	0.23	0.21
	SF <sub>500</sub> BF <sub>yes</sub>	26	8	0.66	0.14	0.30	0.37

### 4.4.2. Bias correction and scenario selection

With a regularisation value of 4.5 and the full variable set, all models performed well when evaluated using  $AUC_{\text{test}}$  (mean:  $0.77 \pm 0.08$  SD, range: 0.68–0.86), and showed low  $AUC_{\text{diff}}$  values (mean:  $0.07 \pm 0.03$  SD, range: 0.04–0.11). Omission rates were generally low but increased with bias correction, with lower omission rates for LPT (mean:  $0.06 \pm 0.03$  SD, range: 0.04–0.09) than for T10 (mean:  $0.20 \pm 0.03$  SD, range: 0.18–0.24). Overall, threshold-independent and dependent measures indicated SF<sub>100</sub> scenarios to perform better than SF<sub>500</sub> scenarios, producing higher  $AUC_{\text{test}}$  rankings, lower  $AUC_{\text{diff}}$  values and lower omission rates (Table 4.2; Table 4.3; Figure S4.2). Furthermore, visual inspection of the maps (Figure S4.3) also suggested unrealistic predictions from SF<sub>500</sub> scenarios; therefore we disregard them. Of the remaining two SF<sub>100</sub> scenarios, threshold-dependent, threshold-independent and information criteria approaches indicated better performance within the SF<sub>100</sub>BF<sub>no</sub> scenario (Table 4.3). Therefore we use this scenario for all further analysis and interpretation (Figure 4.1).

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Table 4.3 Assessment of the four scenarios at a regularisation of 4.5 with all (n=14) variables included using threshold-independent (AUC), threshold-dependent (omission) and information theoretical (information criterion) measures. Assessment of the SF<sub>500</sub> scenarios was not possible with information criterion approaches.

Scenario	No. samples		AUC		Omission		Information criterion	
	Training	Test	test	diff	LPT	T10	AIC	BIC
SF <sub>100</sub> BF <sub>no</sub>	73	24	0.86 ± 0.04	0.04	0.04	0.18	1622.07	1805.34
SF <sub>100</sub> BF <sub>yes</sub>	73	24	0.82 ± 0.03	0.05	0.04	0.18	1690.22	1822.57
SF <sub>500</sub> BF <sub>no</sub>	26	8	0.72 ± 0.08	0.11	0.09	0.19	—	—
SF <sub>500</sub> BF <sub>yes</sub>	26	8	0.68 ± 0.07	0.08	0.08	0.24	—	—

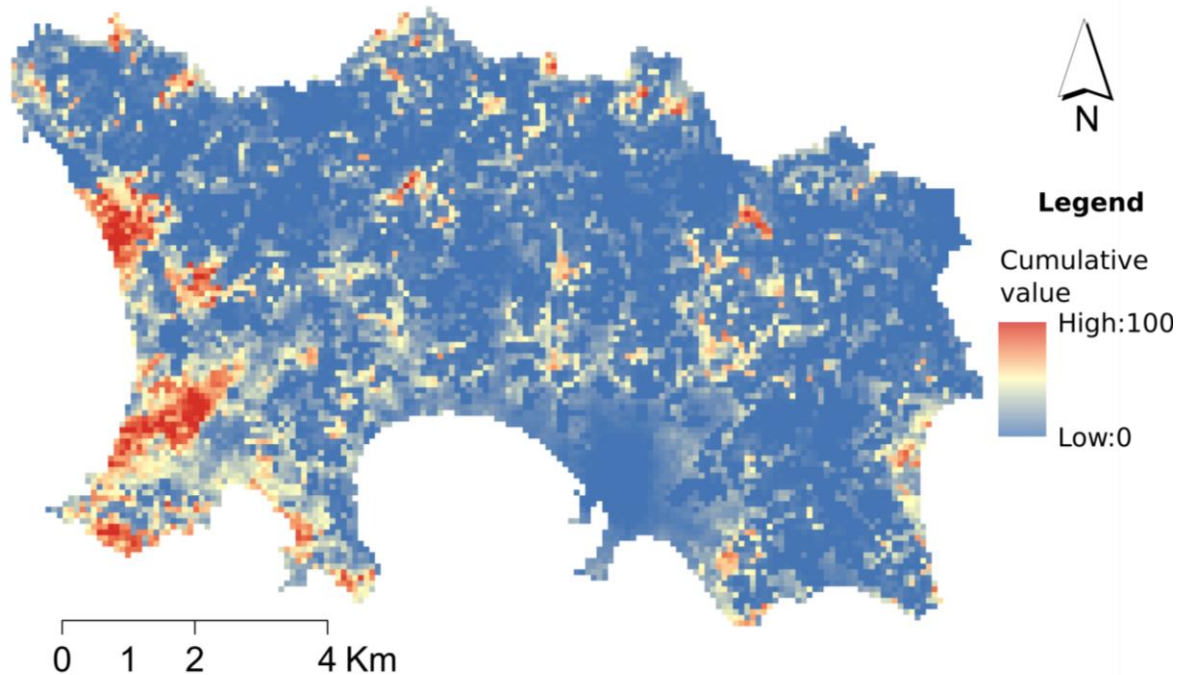


Figure 4.1 Predicted suitability for grass snakes in Jersey with scenario BF<sub>100</sub>SF<sub>no</sub> in Maxent v3.3.3. Cumulative output is displayed; higher values have greater prediction of suitability.

### 4.4.3. Variable importance

Landcover gave the largest permutation importance (53.9), percent contribution (62.3%) (Table S4.3) and the greatest increase in training gain by itself in addition to the greatest loss of training gain when excluded from the variable set (Figure S4.4). This was followed by distance to toad populations (permutation importance = 14.7) and road density (permutation importance = 10.5) (Table S4.3). Inspection of the response curves and lambda coefficients indicated

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rough grassland and dense scrub habitats to be positively associated with grass snake distribution, marsh somewhat positive and arable negative. Areas closer to toad populations were likely to be more suitable, and areas with higher road densities were less favourable (Figure 4.2).

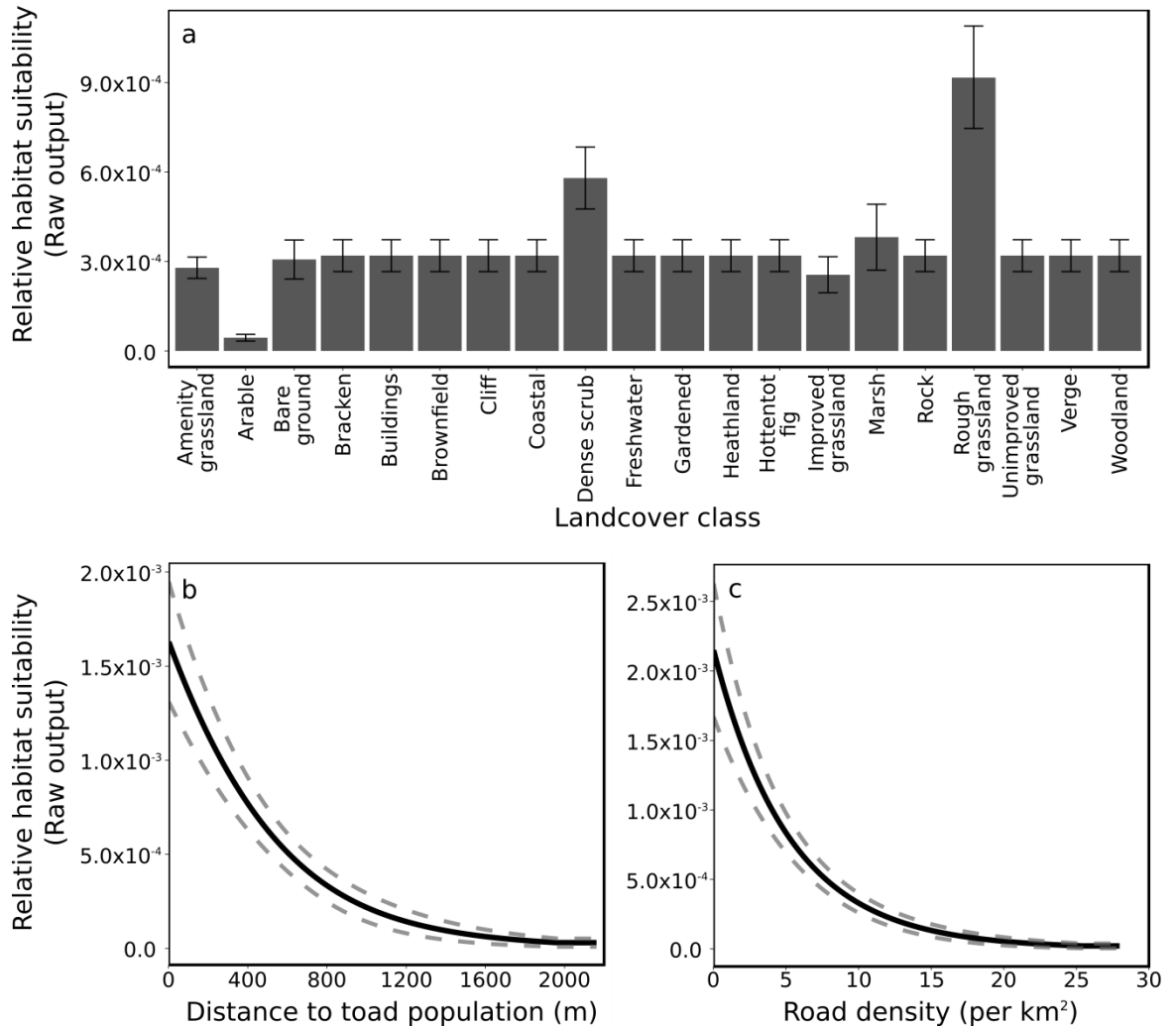


Figure 4.2 Mean responses  $\pm$  1 SD (shown as error bars (a) or dashed lines (b and c)) of grass snakes from 20 Maxent runs for landcover classifications (a), distance to toads (b) and road density (c). Figures show responses for models with all other variables held at their average value for model scenario SF<sub>100</sub>BF<sub>no</sub>. Values shown are from the raw output of Maxent v3.3.3, giving the probability that a randomly selected species occurrence would occur in a cell with the attributes shown on the x-axis when all probabilities from training data sum to one across Jersey.

#### **4.4.4. Priority conservation areas**

Binary predictions of suitable habitat resulted in 40.4% (47.74 km<sup>2</sup>) of 1 ha cells being identified as suitable (above the 10% threshold), and 19.8% (23.42 km<sup>2</sup>) as priority when applying maxSSS (Figure 4.3). Priority habitats were skewed towards the west and southwest of the island with a patchy distribution elsewhere. Comparatively, suitable areas were spread across the island with noticeable absences around the largest urban area. When comparing these suitable areas to the current protected area network, we found that only 11.2% of priority habitats currently receive legal protection. Of these SSIs, Les Blanchés Banques gave the largest contribution towards priority areas (5.3%) (Figure 4.3). This may be unsurprising given its size and relatively good condition. When also considering the addition of habitats that were classed as suitable, the SSIs still only contain 15.3% of the total predicted suitable and priority areas. The JNP provides greater coverage, encompassing 37.6% of priority habitats, including the majority of the aforementioned SSIs, and a further 19.3% of habitat classed as suitable (Figure 4.3). Conversely, 45.7% of the JNP contained priority habitats, and 69.2% of it was suitable. This highlights the importance of Jersey's semi-natural areas and their long-term management to meet conservation aims. The west, southwest and east coast showed the greatest potential for conservation by providing priority habitats of a size suitable for maintaining a viable grass snake population, although those adjacent to Grouville Marsh SSI were heavily isolated. Elsewhere, priority areas tended to consist of small, isolated patches which would not be viable for long-term persistence of a grass snake population.



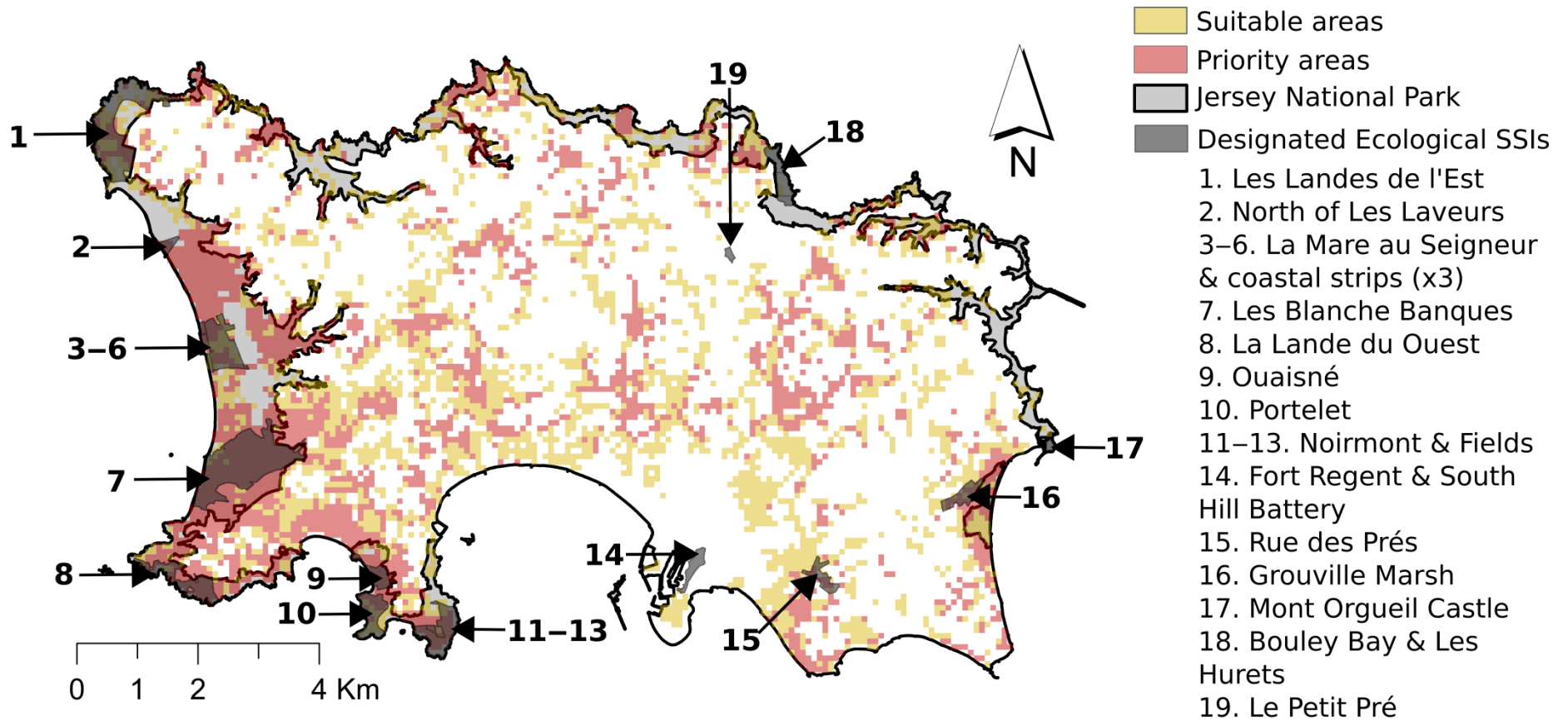


Figure 4.3 Predicted suitability and priority areas for grass snakes in Jersey based on a 10% error threshold (suitable areas - yellow) and maximum sensitivity specificity (priority areas - red) with the SF<sub>100</sub>BF<sub>no</sub> scenario. Currently designated ecological Sites of Special Interest are shown in dark grey and the Jersey National Park in light grey. The former are labelled for reference within the text.

## 4.5. Discussion

Here we provide the first prediction of habitat suitability for Jersey's threatened grass snake population using occurrence data from a range of sources, and evaluate landcover, prey and anthropogenic variables as predictors of distribution. We also provide one of the first studies to comprehensively account for biases and model complexity within snake occurrence data; specifically, by testing the use of spatial filtering and a bias file (Kramer-Schadt *et al.*, 2013), whilst additionally accounting for model over-fitting by comparing two levels of variable complexity and testing a range of regularisation values. Our results identified landcover type as the most important driver of suitability, along with close proximity to toad populations or their associated habitats. In contrast, areas of high road density showed negative effects on the species' distribution. We demonstrate that spatial filtering at a resolution of 100 metres was sufficient to counter the effects of sampling bias, whereas increasing filtering to 500 metres reduced model performance. In order to avoid over-fitting, it was necessary to use a regularisation multiplier of 4.5.

The habitat associations identified by our model match those seen in previous surveys and radio-tracking work (Chapter 2; Chapter 3). This may not be surprising given they are based on the same data, but considering these preferences are still acknowledged at a coarser scale after occurrences have been filtered, their importance is further highlighted. Indeed, the preference for dense scrub, heathland, and rough grassland has been found in previous studies elsewhere in Europe (Madsen, 1984; Mertens, 2008) and these may be considered key habitats for the species. Negative associations with arable land

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have also been noted elsewhere (e.g., Reading and Jofré, 2009), but their suitability remains unclear (Madsen, 1984; Wisler *et al.*, 2008). Following landcover, we also found preferences for areas close to toad *Bufo spinosus* populations. These populations in Jersey have been well mapped across the island as part of the Jersey Toadwatch scheme (Wilkinson and Starnes, 2016), which highlighted the importance of man-made ponds on the island. These same ponds not only support toad populations, but other grass snake prey species including palmate newts *Lissotriton helveticus* and freshwater fish (Gregory and Isaac, 2004; Mertens, 2008; R. Ward unpublished data). They are therefore likely to be an important source of prey, particularly in spring during the amphibian breeding season. The establishment of further wildlife ponds can only be beneficial for the grass snake and its favoured prey, building in resistance to stochastic changes in prey populations.

The effects of roads upon snakes have been well studied (reviewed in Weatherhead and Madsen, 2009) and are a source of mortality (Roe *et al.*, 2006; Meek, 2015) as well as barriers to dispersal and gene flow (Andrews and Gibbons, 2005; DiLeo *et al.*, 2010). The ability of grass snakes to disperse widely through suboptimal landscapes should put them at lower risk of extinction (Meister *et al.*, 2010). However, within an urbanised island there are limited areas to disperse to, and the same is true of mainland populations where fragmentation may be similarly severe. Our model predicts a negative influence of road density upon grass snake distribution. Similarly, genetic work has indicated that a subpopulation in the south of the island shows isolation from subpopulations along the west coast (Chapter 5). Our model shows there to be some discontinuity in habitat suitability between these areas, coinciding with an urban area containing two major roads

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between Les Blanchés Banques and Ouaisné (Figure 4.3). Therefore, focusing connectivity efforts on this region will be beneficial for gene flow and overall population health. Where continuous wildlife corridors are unfeasible, providing a mosaic of suitable habitat patches can enable snake dispersal (e.g., Lacki *et al.*, 2005). Otherwise, with the exception of tunnels (e.g., Shine and Mason, 2001), there are limited options to mitigate the negative effects of such a vast road network. Dispersal requirements could be reduced by providing appropriate habitat within continuous patches (Weatherhead and Madsen, 2009); however, this will not resolve issues related to poor gene flow.

Model predictions of priority areas largely resemble the known species range within the west and southwest of the island (Chapter 2). It is unlikely that the range of the grass snake will extend far beyond these coastal semi-natural areas; however, our analyses have also indicated the potential suitability of several areas lacking in contemporary records, including areas in the centre of the island, and along the coast in the north, west and southeast. Many of these potentially suitable priority areas are isolated due to a matrix of agriculture, roads and urban areas, which may preclude their use. Nonetheless, these areas require further investigation to determine the presence or absence of the species with confidence. In addition, several potential range extensions in the west and southwest of the island require confirmation.

Although 23.42 km<sup>2</sup> of the island was identified as containing priority habitats, the legal protection afforded to these areas were limited. Due to the isolation of many priority areas, those occurring within the remit of the JNP and non-statutory nature reserves are likely to provide the best opportunity for maintaining and enhancing population connectivity and overall metapopulation persistence.

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Greater legal protection should be afforded to these areas that this, and other work (Chapter 2), have highlighted as key sites for the species. As a relatively generalist species, such improvements in protection of its habitat will also provide benefits to a wealth of the island's biodiversity. However, there are several considerations to overcome including the provision of strong evidence for site protection to policy makers, and inter-connectivity between protected areas. It is important to note that although we applied binary thresholds to a continuous output to identify conservation priorities, suitability is not binary and occurs along a continuum. We provide Figure 4.1 as an alternative for highlighting priority areas; however, binary thresholds can be beneficial when clear targets are set, such as in the identification of key areas for threatened species (Peterson *et al.*, 2011; Guillera-Aroita *et al.*, 2015).

The effects of spatial filtering and bias files on model performance have been the focus of several recent studies. Spatial filtering has been found to reduce model performance (Kramer-Schadt *et al.*, 2013), which may be caused by a reduction in the number of occurrence records used to model distributions. We observed a similar effect, and it is likely that the spatial scale and the distribution of occurrences may affect how spatial filtering influences performance. Where occurrences may naturally be clustered, rather than due to sampling bias, bias files may be a more appropriate method for treating the data (Dorman *et al.*, 2013; Kramer-Schadt *et al.*, 2013). Despite accounting for sampling bias, limited species detectability is an inherent issue which may affect our attempts to predict a species' distribution. This limitation is exacerbated in small or rare populations, whereby they may cluster (Dormann *et al.*, 2013) or conversely, be widely dispersed. Observer bias may also greatly affect the quality of our predictions

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(e.g., Kadmon *et al.*, 2004). For instance, comparatively inaccessible areas such as agricultural field margins may provide suitable reptile habitat (Biaggini and Corti, 2015) but go un-surveyed. In this study we also assume that occurrence records from secondary sources have been correctly validated. The inclusion of inaccurate records may affect model predictions, but only if invalid records were spatial outliers and gave unrepresentative associations with predictor variables. Similar effects may occur through the inclusion of records over a long time period, whereby the variables the original record was associated with have changed at the time of variable measurement for the study. Both of these issues may be dealt with by removing spatial and temporal outliers.

Aside from working at a coarser resolution than the original data (a form of spatial filtering), previous snake distribution modelling studies have rarely accounted for the effects of sampling bias and imperfect detection. Distribution modelling of rare and elusive species such as those undertaken on Lataste's viper *Vipera latestei* (Santos *et al.*, 2006), the Southern smooth snake *Coronella girondica* (Bombi *et al.*, 2009), the Cyprus whip snake *Hierophis cypriensis* (Baier *et al.*, 2014) and Orsini's viper *Vipera ursinii* (Lyet *et al.*, 2013) may risk making misleading predictions and conservation recommendations if these effects are not considered. Instead, they have often relied on using presence-background data only, which can reduce some issues surrounding imperfect detection, but does not specifically account for sampling bias or completely resolve non-detection concerns. This is particularly concerning given detection issues in a number of snake species (Durso *et al.*, 2011; Steen *et al.*, 2012). Despite this, our study suggests that spatial filtering may be a quick and effective method for accounting for some of these issues. Occupancy-detection data (e.g., Chapter 2) may yield

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improved predictions of a species' true distribution (Guillera-Arroita *et al.*, 2015). However, due to the intensive survey effort required for rare and elusive species, this is not a feasible approach for landscape-wide predictions related to this (Chapter 2), or other species with low detection. Instead, some generalisation will be required based on incomplete sampling. Additionally, studies often rely on the default regularisation values developed by Phillips and Dudík (2008; but see Silva-Rocha *et al.*, 2015), but the calculation of these included only a single snake species. Therefore, we advise caution in accepting default parameters and strongly recommend others to consider these issues when developing species distribution models.

This work provides a base for future assessments of fragmentation and connectivity issues, and for informing island planning. Our findings provide benefits for spatial planning, assigning land protection, connectivity analyses and informing future research as a benchmark within the island. Further afield, our results highlight the methods available for dealing with sampling bias as well as considerations for improvements. By combining our findings with that of other species, it will be possible to conduct an island-wide assessment of priority areas for conservation. Further work testing the effects of distribution modelling at multiple scales may reveal greater details of the drivers of habitat selection. By incorporating other anthropogenic variables such as the effects of recreational use of outdoor spaces and traffic density across the road networks, it may be possible to refine models at greater resolution and give better insights in to connectivity, behavioural impacts and human-wildlife coexistence. In summary, we have highlighted the importance of providing protection to semi-natural habitats in the west and southwest of Jersey, and also identified priority areas for exploratory

## Chapter 4. Priority areas for grass snake conservation

monitoring and management practices to improve connectivity between isolated populations. In addition, we have demonstrated the importance of bias correction and model tuning in order to produce an accurate distribution model.

### **4.6. Acknowledgements**

We wish to thank the Jersey Biodiversity Centre and the States of Jersey Department of the Environment for sharing their records. We are also grateful to members of the public who have reported sightings both during the Think Grass Snake campaign and prior to its inception. Geographic Information Systems layers were kindly provided by the States of Jersey Department of the Environment, who provided assistance and support throughout the study. This research was funded by the States of Jersey Department of the Environment, the Jersey Countryside Enhancement Scheme, British Herpetological Society and Jersey Ecology Fund, with equipment support from Harsco Jersey.



## 4.7. Supplementary Information

Table S4.1 Variation in BIOCLIM climatic variables across Jersey.

Layer	Description	Mean $\pm$ SD	Range
Bio1	Annual Mean Temperature	117.27 $\pm$ 1.66	114–121
Bio2	Mean Diurnal Range (Mean of monthly (max temp - min temp))	63.1 $\pm$ 0.3	63–64
Bio3	Isothermality (BIO2/BIO7) (* 100)	31.69 $\pm$ 0.46	31–32
Bio4	Temperature Seasonality (standard deviation *100)	4835.56 $\pm$ 32.9	4755–4941
Bio5	Max Temperature of Warmest Month	223.31 $\pm$ 2.05	219–229
Bio6	Min Temperature of Coldest Month	27.97 $\pm$ 1.25	25–31
Bio7	Temperature Annual Range (BIO5-BIO6)	194.78 $\pm$ 1.19	192–199
Bio8	Mean Temperature of Wettest Quarter	68.49 $\pm$ 1.4	66–72
Bio9	Mean Temperature of Driest Quarter	172.94 $\pm$ 5.95	161–183
Bio10	Mean Temperature of Warmest Quarter	179.72 $\pm$ 1.93	176–185
Bio11	Mean Temperature of Coldest Quarter	57.1 $\pm$ 1.24	55–60
Bio12	Annual Precipitation	848.95 $\pm$ 14.19	811–877
Bio13	Precipitation of Wettest Month	107.07 $\pm$ 2.09	101–111
Bio14	Precipitation of Driest Month	43.03 $\pm$ 0.7	42–45
Bio15	Precipitation Seasonality (Coefficient of Variation)	30 $\pm$ 0	30–30
Bio16	Precipitation of Wettest Quarter	306.07 $\pm$ 5.58	291–317
Bio17	Precipitation of Driest Quarter	142.49 $\pm$ 2.29	137–147
Bio18	Precipitation of Warmest Quarter	142.61 $\pm$ 2.34	137–152
Bio19	Precipitation of Coldest Quarter	274.69 $\pm$ 5.02	263–285

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Table S4.2 Landcover classifications used in analysis and their original descriptions in the file provided by the States of Jersey Department of the Environment.

New class	Description(s) in original file
Amenity Grassland	Amenity Grassland; Parkland (amenity + scattered trees)
Arable	Arable Land; Arable Land short term ley
Bare Ground	Bare Ground; Open Dune; Roads; Sand / Mud
Bracken	Continuous Bracken, open; Continuous Bracken, scrub underlay
Brownfield site	Brownfield site
Buildings	Buildings
Cliff	Hard Cliff; Soft Cliff
Coastal	Brackish Pool ; Saltmarsh; Strand-line vegetation
Dense scrub	Dense Scrub, Blackthorn; Dense Scrub, Bramble; Dense Scrub, Gorse; Dense Scrub, other; Dense Scrub, Sallow; Dune Dwarf scrub
Freshwater	Running Water; Standing Water
Gardened	Gardened
Heathland	Coastal Heathland; Dune Heath
Hottentot Fig	Hottentot Fig
Improved Grassland	Grassland, improved; Grassland, semi-improved
Marsh	Marshy Grassland, Oenanthe dominated; Marshy Grassland, semi-improved; Marshy-Grassland, unimproved; Swamp
Rock	Quarry; Rock; Shingle
Rough Grassland	Coastal Grassland; Coastal Grassland, Molinia; Coastal Grassland, species rich short turf; Dune Grassland; Dune Marram dominated; Dune Slack; Tall Ruderal
Unimproved Grassland	Grassland, unimproved
Verge	Verge
Woodland	Woodland, Planted Broadleaved; Woodland, Planted Coniferous; Woodland, Planted Mixed; Woodland, Plantation (orchard etc); Woodland, Semi-natural, Broadleaved

## Chapter 4. Priority areas for grass snake conservation

Table S4.3 Relative contributions of predictor variables in the distribution of grass snakes in Jersey based on scenario  $SF_{100}BF_{no}$ , a full set of variables and a regularisation of 4.5. Variables are shown in descending order of percent contribution.

Variable	Percent contribution	Permutation importance
Landcover	62.3	53.9
Distance to toads	7.7	14.7
Aspect	7.1	4.7
Road density	5.4	10.5
Building density	3.6	4.7
Distance to roads	3.1	0.9
Distance to water	2.6	1.9
Pond density	1.9	1.9
Contagion	1.8	2.3
Patch richness	1.8	2.8
Population density	1.4	0.2
Slope	1.0	1.1
Flow accumulation	0.2	0.0
Patch density	0.1	0.3

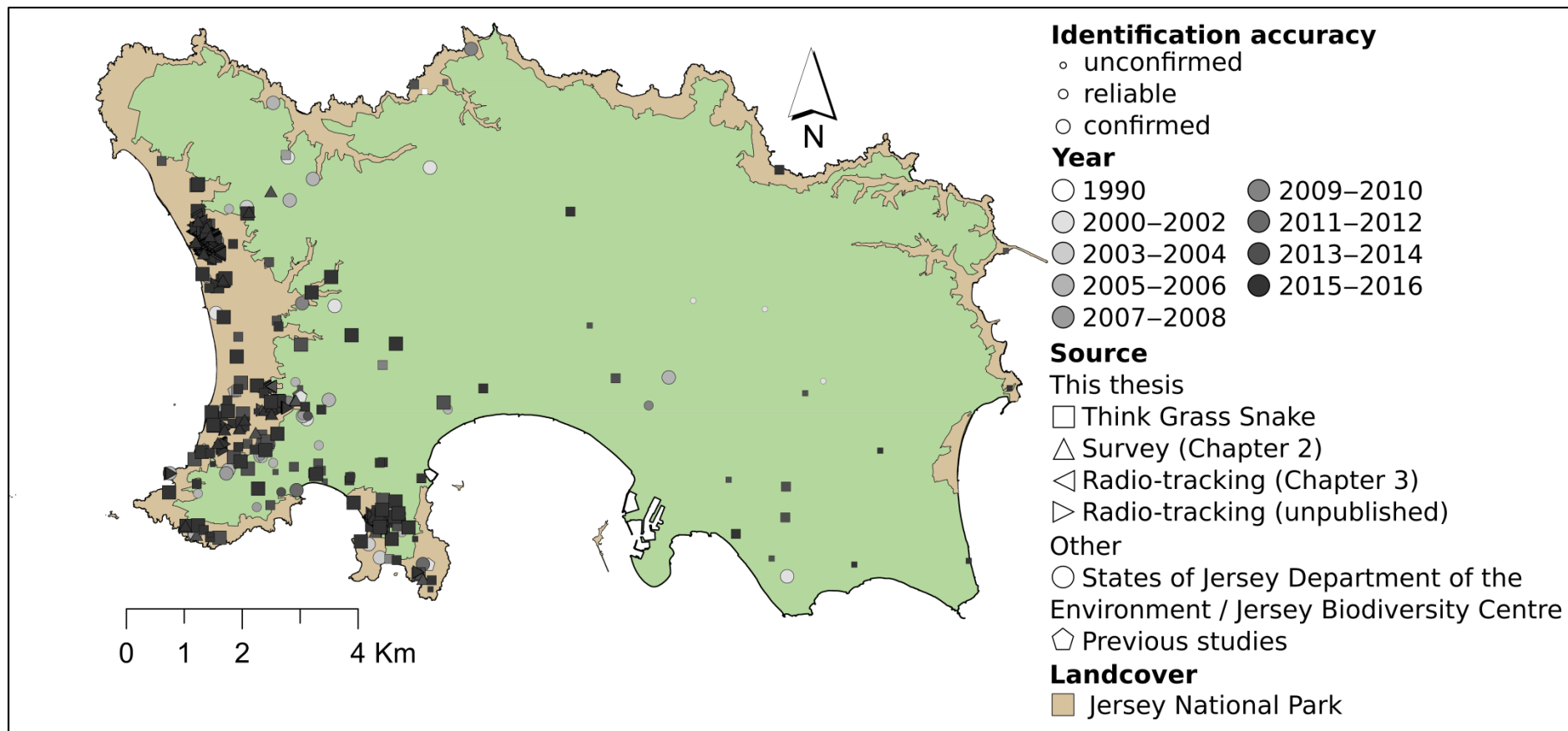


Figure S4.1 Distribution of occurrence points prior to removal of unreliable locations and filtering. Identification accuracy is defined by size, the year reported is shown by colour and the data source is shown by shape.

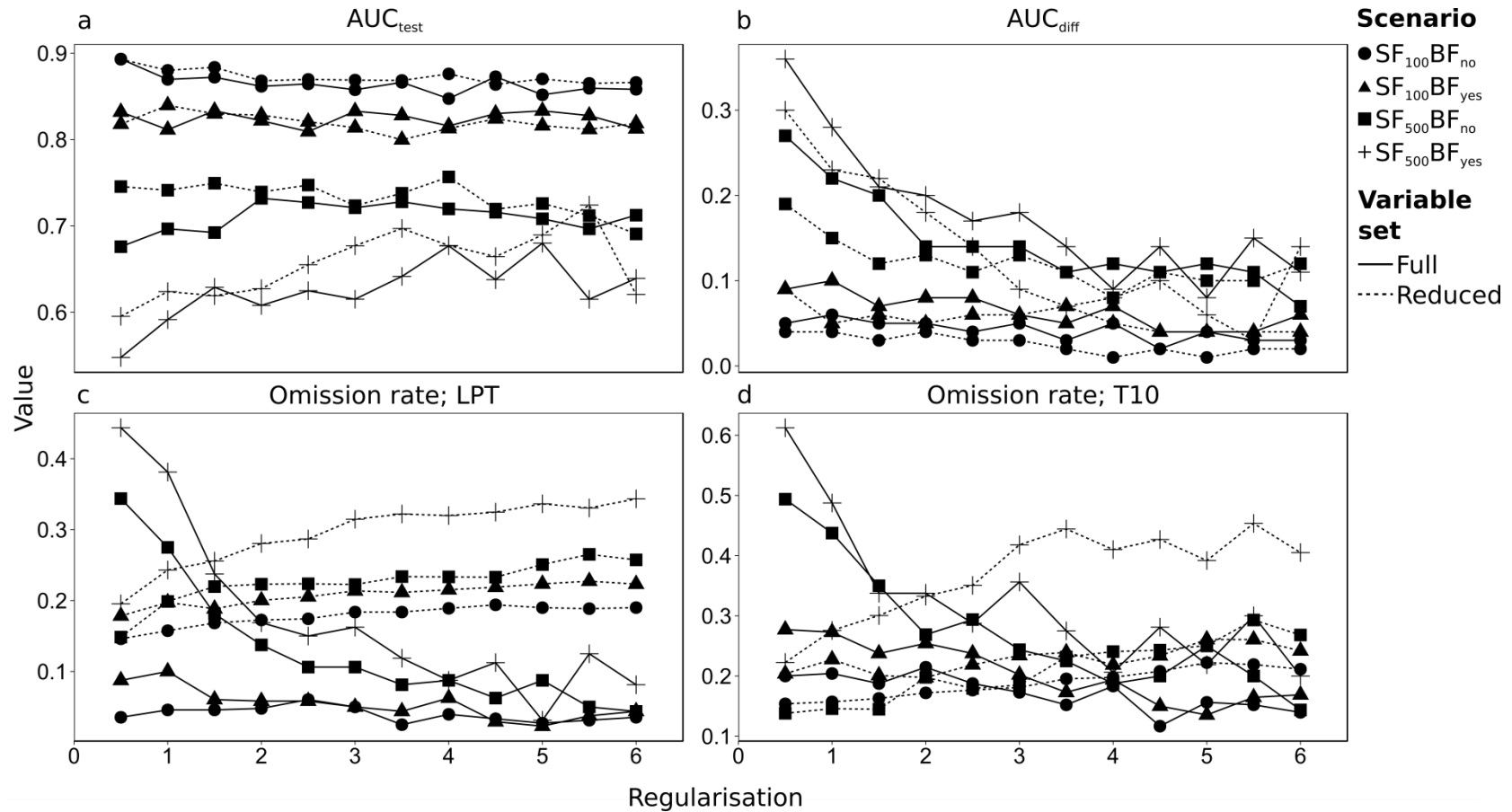


Figure S4.2 Results of regularisation tests for four different bias correction scenarios and two levels of variable complexity. Models were evaluated with threshold independent measures; (a) the AUC value from the test data ( $AUC_{test}$ ) and (b) the difference between training AUC and testing AUC values ( $AUC_{diff}$ ), and threshold dependent measures; (c) lowest presence threshold (LPT) and (d) 10th percentile training presence training omission (T10).

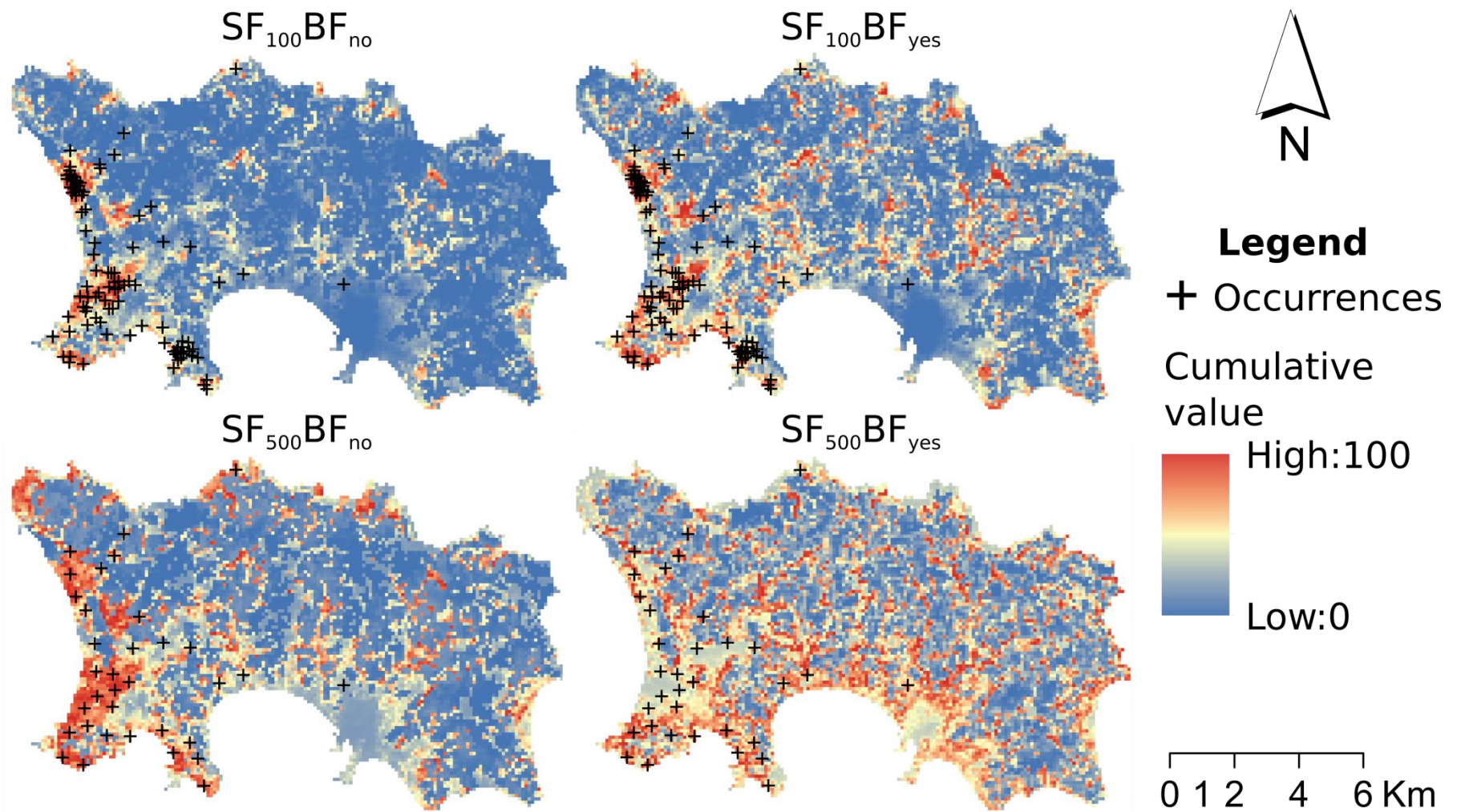


Figure S4.3 Cumulative Maxent (v3.3.3) outputs from four bias correction scenarios with a regularisation value of 4.5. Occurrence points used are shown as crosses, and warmer colours indicate greater suitability for grass snakes.

## Chapter 4. Priority areas for grass snake conservation

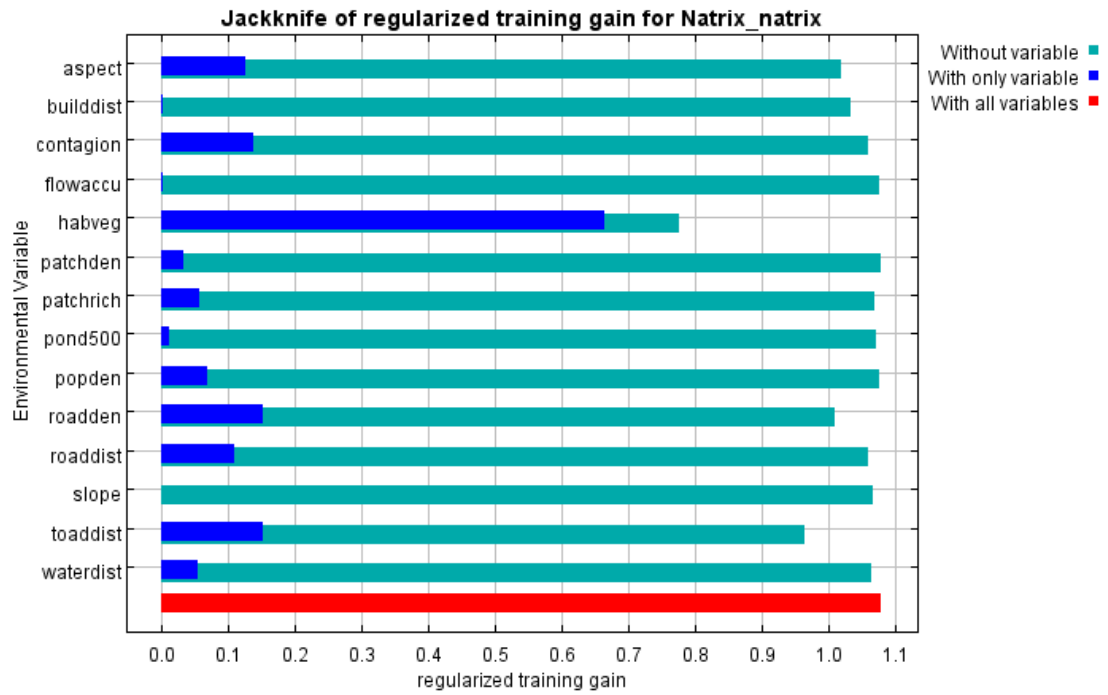


Figure S4.4 Jackknife of regularised training gain for variable importance to grass snakes for model scenario SF<sub>100</sub>BF<sub>no</sub> and a regularisation multiplier of 4.5.

## Chapter 4. Priority areas for grass snake conservation

Methods S4.1 R code used to create the bias file.

```
#### Creating a bias file for Species Distribution Modelling in R ####
```

```
# Modified from code provided by Scott Rinnan, with survey points used to represent sample effort
```

```
# https://scottrinnan.wordpress.com/2015/08/31/how-to-construct-a-bias-file-with-r-for-use-in-maxent-modeling/
```

```
####1: SETUP ####
```

```
####1.1 Install packages ####
```

```
install.packages("raster")
```

```
install.packages("MASS")
```

```
install.packages("magrittr")
```

```
install.packages("maptools")
```

```
####1.2 Library packages ####
```

```
library(raster) # spatial data manipulation
```

```
library(MASS) # for 2D kernel density function
```

```
library(magrittr) # for piping functionality, i.e., %>%
```

```
library(maptools) # reading shapefiles (needs rgeos)
```

```
####1.3 Set working directory####
```

```
setwd("C:/SDM/")
```

```
getwd()
```

```
####1.4 Read in CSV containing point data of our survey effort####
```

```
Ref<-read.csv("Ref.csv", header=T)
```

```
head(Ref)
```

```
####2 Turn data into spatial grid###
```

```
####2.1 Concatenate XY coordinates ####
```

```
coordinates(Ref)<-c("long", "lat") #set spatial coordinates
```



## Chapter 4. Priority areas for grass snake conservation

```
plot(Ref) # plot to check

####2.2 Set projection of data (recorded in WGS1984) ####

proj4string(Ref) #Check projection of effort points

crs.WGS<-CRS("+init=epsg:4326") # create a datum for WGS1984

proj4string(Ref)<-crs.WGS #convert projection of effort points to WGS1984

proj4string(Ref) # check projection

####2.3 Project data to Jersey Transverse Mercator (JTM) ####

crs.JTM<-CRS("+init=epsg:3109") #create a datum for Jersey Transverse Mercator (JTM)

RefJTM <- spTransform(Ref, CRS("+init=epsg:3109")) #convert projection of effort points to JTM

proj4string(RefJTM) # check projection

plot(RefJTM, col="blue") #plot to check projection

####2.2 Convert to raster at 100m resolution####

dem<-raster("C:/SDM/input/DEM") #read in a raster at appropriate resolution (100 m). In this case
Digital Elevation Model of Jersey

plot(dem) #plot dem raster to check

plot(RefJTM, add=T, col="green") #overlay plot of effort points

ref.ras<-rasterize(RefJTM, dem, 1, getCover=TRUE) #rasterize survey effort at the resolution of
dem raster (100 m)

crs(ref.ras)<-"+init=epsg:3109" #convert raster projection of effort points to JTM

plot(ref.ras, col="blue") #check it plots well

extent(ref.ras) == extent(dem) #check extents of dem and effort raster match

res(ref.ras) == res(dem) #check resolution of dem and effort raster match (should be 100 m)

#Resample to same resolution as dem

ras.new = resample(ref.ras, dem,"bilinear") #resample effort raster to same grid as dem raster

extent(ras.new)== extent(dem) #check extents of dem and new resampled effort raster match
```

## Chapter 4. Priority areas for grass snake conservation

```
plot(dem)

plot(ras.new, add=TRUE, col='red')

crs(ras.new)<-"+init=epsg:3109"

####3 Create kernel density estimate using kde2d function from MASS ####

# Read in a shapefile with the extent our final layer should match. In this case one of the
enumeration districts in Jersey

Jersey<-readShapePoly("C:/SDM/input/EnumerationDistricts.shp", proj4string=crs.JTM)

occur.jsy<-mask(ras.new,Jersey)%>%crop(.,Jersey) # Crop effort raster to the extent of Jersey (if
needed)

extent(occur.jsy) # check extent of new cropped effort raster 'occur.jsy'

presences<-which(values(occur.jsy)==1) # create 'presences' object from effort raster, consisting of
raster cells with effort in them

pres.locs<-coordinates(occur.jsy)[presences,] # create 'pres.locs' object containing the coordinate
data also

# Calculate 2d kernel density layer #

dens <- kde2d (pres.locs [,1], pres.locs [,2], n = c (nrow (occur.jsy), ncol (occur.jsy)), lims = c
(31300.41, 49200.41, 62958.16, 74158.16)) #use lims argument to set extent to match other SDM
layers

extent(dens) # check extent of new density layer

dens.ras <- raster (dens) # create density raster from density file

crs (dens.ras) <- "+init=epsg:3109" #make sure projection is JTM

plot (dem) # plot digital elevation model

plot (dens.ras, add=T) # add density raster layer on top to check they match

extent (dens.ras) == extent (dem) # check extents - they don't match

# Resample the density raster to the extent and resolution of the dem raster #
```

## Chapter 4. Priority areas for grass snake conservation

```
densras.new = resample (dens.ras, dem, "bilinear")

res(densras.new) #check resolution of new raster

extent (densras.new) #check extent of new raster

crs (densras.new) <- "+init=epsg:3109" #make sure projection is JTM

plot (densras.new) # plot new resampled density raster to check 'fit'

####4 Save bias layer for use in MaxEnt ####

writeRaster (densras.new, "C:/SDM/Ref bias file1.tif", overwrite=TRUE)

### END ###
```

Following this, the resulting .tif file was modified in ArcMap v10.5 using the raster calculator to add 1E-10 to each cell in order to avoid '0' values in any cell. Otherwise MaxEnt would not include un-sampled areas of the island in the model.

## **Chapter 5. Conservation genetics of an island grass snake population**

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## 5.1. Abstract

Island populations are inherently more prone to reduced genetic diversity and subsequent extinction risk compared to mainland populations due to their size and isolation. Conservation managers must consider the genetic consequences of isolation on islands, and if necessary, identify the ancestral origins and close relatives of such populations in case genetic restoration or reintroduction is required. In this study we used two mitochondrial DNA markers, cytochrome *b* and ND4, and 10 microsatellite loci to examine the phylogeography and conservation genetics of an island population of grass snakes, *Natrix helvetica*, on Jersey (Channel Islands, UK). We investigate the genetic consequences of isolation and small population size on the Jersey population (n=58), and make comparisons with the neighbouring mainland population from northwest France (n=12). All samples formed part of a previously described clade containing *Natrix helvetica helvetica*. Within this we observed a single unique haplotype in the Jersey population, and a second haplotype shared between Jersey and northwest France suggesting a shared origin. Samples from northwest France formed a single genetic cluster containing higher (though non-significant) levels of genetic diversity than those in Jersey, which comprised three genetically differentiated subpopulations. Of these, a subpopulation on a southern peninsula was significantly differentiated from the two subpopulations on the west coast of Jersey. This separation appears to be driven by anthropogenic barriers in the landscape; therefore, work to improve habitat connectivity between remaining subpopulations is advisable to prevent reductions in sub-population genetic diversity and promote admixture.

**Keywords:** conservation genetics, isolation, microsatellite, mitochondrial, *Natrix helvetica*, phylogeography, snake, structure

## 5.2. Introduction

Population isolation and a reduction in population size can lead to loss of gene flow, inbreeding and genetic drift (Lande, 1980; Frankham, 1998). Within reptiles, these effects are primarily driven by habitat loss and fragmentation (Gibbons *et al.*, 2000; Collen *et al.*, 2009). Subsequently, a decrease in effective population size can lead to loss of population viability and ultimately, extinction (King, 2009). Ensuring gene flow between remnant populations therefore serves as an important step in maintaining population viability.

Compared to mainland populations, island populations are at greater risk of deleterious impacts associated with small population size, such as low genetic diversity and increased extinction risk (Frankham, 1998). Within snakes, isolation and small population size has resulted in low levels of genetic diversity and reduced fitness in a number of species (King, 2009). In some instances (e.g., Madsen *et al.*, 1996; Daltry *et al.*, 2001; Gautschi *et al.*, 2002), the effects of inbreeding depression and genetic bottlenecks can result in individuals with poor development, fecundity and survival (Charlesworth and Charlesworth, 1987). Therefore, ensuring the long-term viability of such populations requires strategies which identify and address population genetic issues.

Isolation and resulting genetic differentiation among snake populations can occur over varying spatial scales, and may depend on species or landscape characteristics (e.g., King, 2009; Meister *et al.*, 2012). Differentiated populations can be of conservation interest (Crandall *et al.*, 2000), containing genetic variation

not found elsewhere (Wilson *et al.*, 2009). However, failure to apply the relevant tools to identify these divergent populations of conservation interest can result in a lack of legal protection and their loss through inappropriate genetic management or extinction processes (e.g., Russello *et al.*, 2005).

The *Natrix* genus is currently undergoing taxonomic revision (Fritz *et al.*, 2012; Kindler *et al.*, 2013; 2017; Pokrant *et al.*, 2016) which may result in new conservation priorities within the taxon. A threatened and poorly studied grass snake *Natrix helvetica* (formerly *Natrix natrix*; Kindler *et al.*, 2017) population occurs on the 117 km<sup>2</sup> island of Jersey (British Channel Islands), which has been isolated from mainland France for approximately 7,000 years (Jones, 1993). It has been suggested that this population is *N. astreptophora* due to phenotypic traits (Frazer, 1949), or more plausibly that it is an allopatric population of *N. helvetica* based on morphological analyses (Thorpe, 1979; 1984); however until now no molecular work has been carried out to corroborate this.

The grass snake has undergone a decline in its distribution and abundance in Jersey following urbanisation and widespread agricultural conversion of the landscape, which now covers over half of the island (States of Jersey Department of the Environment, 2016a). As a result, it now occurs in small fragmented subpopulations predominantly restricted to the west and southwest of the island (Chapter 2). In comparison, grass snakes are widespread in northwest France but may be declining in density due to habitat fragmentation and conversion of wetland habitats, with subsequent losses of prey (Barrioz *et al.*, 2015). In both instances, agriculture dominates the landscape; however, studies of grass snakes in an agricultural landscape in Switzerland could not attribute population differentiation to impaired dispersal due to agriculture, but instead to isolation by

distance (Meister *et al.*, 2010; 2012). The large home ranges of grass snakes may aid them in traversing suboptimal habitats (e.g., up to 120 ha in Wisler *et al.*, 2008), but radio-tracking in Jersey indicated a maximum range of 13.4 ha and avoidance of crossing roads (Chapter 3). Despite the potential impacts on population viability, the influences of anthropogenically modified habitats upon grass snake habitat selection and gene flow are not fully understood (Wisler *et al.*, 2008; Reading and Jofré, 2009; Meister *et al.*, 2012).

In this study we use a combination of two mitochondrial (mtDNA) markers (cytochrome *b* (cyt *b*) and ND4) and 10 microsatellite loci to (1) establish the ancestral origins of the grass snake on Jersey, (2) detect patterns of genetic structure, and (3) compare levels of genetic diversity between populations in Jersey and northwest France. We use our results to identify and discuss requirements and opportunities for conservation management.

### **5.3. Methods**

#### **5.3.1. Study species**

Taxonomy of the grass snake is currently undergoing revision, and we follow the suggestions of recent publications recognising three grass snake species: (*N. astreptophora* Seoane, 1884; *N. natrix* Linnaeus, 1758; *N. helvetica* Lacépède, 1789) with lineages and subspecies (*sicula*, *lanzai*, *helvetica*, *corsa* and *cetti*) from Western Europe treated as subordinates to *Natrix helvetica* (Fritz *et al.*, 2012; Kindler *et al.*, 2013; 2017; Pokrant *et al.*, 2016). Although widespread throughout Europe (Steward, 1971), the grass snake is suspected to be undergoing localised declines (e.g., Monney and Meyer, 2005; Hagman *et al.*, 2012). It currently receives full protection in Jersey under the Conservation of Wildlife Law (Jersey)



2000; however, the species is threatened due to a restricted range and small population size (Chapter 2).

### **5.3.2. Study populations**

We focus on a grass snake population on the island of Jersey, and make comparisons to a population from the closest regions of northwest France (Normandy and Bretagne), which are separated from Jersey by a minimum of 22 km of sea. Within Jersey, three sampling locations were considered *a priori* covering a total area of around 14.6 km<sup>2</sup>; (i) St Ouen (ii) and Les Blanches Banques dune system on the west coast, and (iii) a southern headland. These areas are from here on referred to as St Ouen, Dunes and South (Figure 5.1), and are dominated by dune grassland, maritime heath and scrub. We hypothesise that individuals within each of these sampling areas form separate subpopulations capable of mixing freely, whereas barriers between them including roads, urban areas and agriculture may limit dispersal. Jersey subpopulations were separated by distances of 3.5–6.3 km (between subpopulation centroids), and individuals within predefined subpopulations were separated by maximum distances of 1.49–1.6 km.

### **5.3.3. Sampling, sequencing and genotyping**

### **5.3.4. Sample collection**

Grass snakes in Jersey (n=58) were sampled between 2014 and 2016 during surveys (Chapter 2), or from carcasses and sloughs reported by the public via the Think Grass Snake campaign ([www.ThinkGrassSnake.Je](http://www.ThinkGrassSnake.Je)). DNA was sampled by taking up to three ventral scale clips with a sharp pair of scissors (e.g., Wüster *et al.*, 2008; Meister *et al.*, 2012), a buccal swab (e.g., Lanci *et al.*, 2012), collection

## Chapter 5. Grass snake conservation genetics in Jersey

of sloughed skin or by sampling carcasses for muscle tissue. Genetic material from northwest France (n=12; 10 sloughs and two tissue samples from road-killed carcasses) were provided by the Société Herpétologique de France. Handling and sampling of Jersey specimens was conducted under a Conservation of Wildlife Law (Jersey) 2000 licence (no. CR 23), issued by the States of Jersey Department of the Environment. This study was also approved by the School of Anthropology and Conservation Research and Ethics Committee of the University of Kent.

Individual identification of snakes via their ventral patterns ensured snakes were not sampled twice in Jersey (Carlström and Edelstam, 1946; Chapter 2), and samples from northwest France were sufficiently geographically distant to avoid replication. Scale clips and muscle were stored in 100% ethanol, sloughs were stored dry in individual envelopes and swabs were kept within their original tubes.

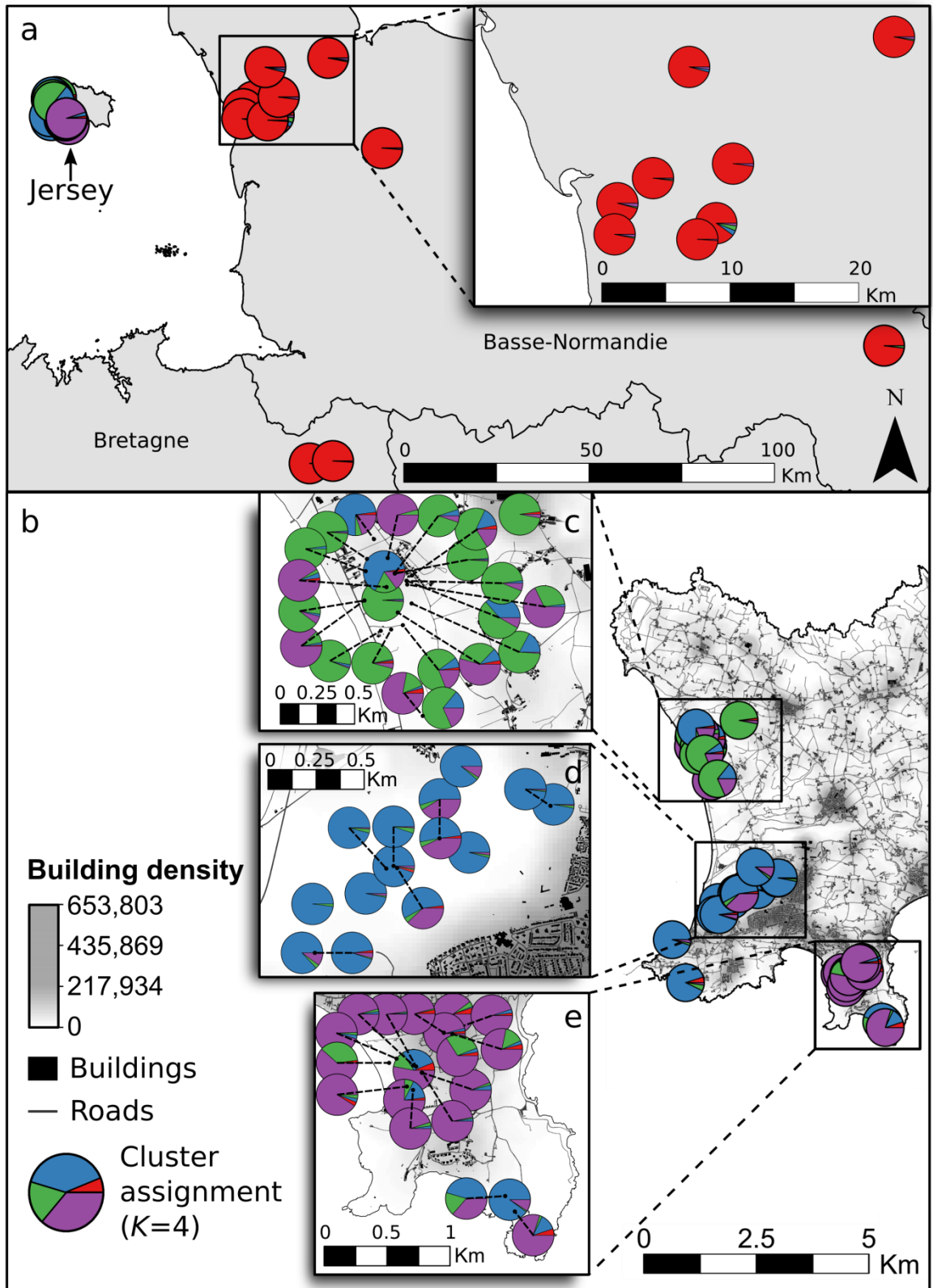


Figure 5.1 Map of sampling locations in (a) northwest France and (b) Jersey. Further details for the three Jersey subpopulations are shown; (c) St Ouen, (d) Dunes and (e) South. Cluster assignment probabilities are shown as a pie charts for each sample when  $K=4$ . Colours follow those shown in Figure 5.4. The density of buildings in Jersey (b–e) is shown as the m<sup>2</sup> covered by building footprints per km<sup>2</sup>, calculated at a radius of 250 m in ArcMap v10.5 (ESRI, Redlands).

### 5.3.5. DNA extraction

Extraction of DNA was carried out via ammonium acetate precipitation (Bruford *et al.*, 1998). Briefly, tissue was digested in 250 µl Digsol buffer (20 mM EDTA, 50mM Tris, 120 mM NaCl, 1% SDS, pH 8.0) with 10 µl Proteinase K (10mg/ml) at 37.5°C overnight. For sloughed skins, the amounts of buffer and Proteinase K were doubled. Samples were then precipitated with 4M ammonium acetate followed by 100% ethanol. Pellets were rinsed with 70% ethanol twice before air-drying for 30 minutes and then suspended in 100 µl of sterile water. DNA was stored at -20°C for long-term preservation.

### 5.3.6. Mitochondrial samples

We selected two mtDNA genes (partial ND4 and *cyt b*) (Guicking *et al.*, 2006; Fritz *et al.*, 2012; Kindler *et al.*, 2013; 2017), that have been widely used in previous phylogeographic studies of the *Natrix* genus to allow for comparison across the wider species range and inclusion in our analyses. Cytochrome *b* was amplified using the primers L14724NAT (Guicking *et al.*, 2002) and Thrsnr2 (Burbrink *et al.*, 2000). When these primers did not yield Polymerase Chain Reaction (PCR) products or performed poorly, we amplified overlapping fragments using L14724NAT and *Natrix\_Cytb\_Rev2*, which gave a fragment of up to 551 bp (Kindler *et al.*, 2013). The partial ND4 gene and the flanking DNA that codes for tRNA-His, tRNA-Ser and tRNA-Leu (referred to from here on as the tRNAs) was initially tested with ND4ab and tRNA-leu primers (Arèvalo *et al.*, 1994) (Table S5.1 and Table S5.2). However, these performed poorly so a combination of ND4\_CDF and ND4\_CDR giving a fragment of up to 607 bp was used instead (Table S5.1 and Table S5.2). PCR was carried out in a total volume of 10 µl containing 3.6 µl

sterile water, 5.0  $\mu\text{l}$  of MyTaq™ Red Mix (Bioline), 0.2  $\mu\text{l}$  (10 pmol) of each primer (Eurofins Genomics) and 1  $\mu\text{l}$  of DNA at 10  $\mu\text{l}/\text{ng}$ . Challenging samples were additionally treated with ExoSap-IT Express (Affymetrix UK Ltd, High Wycombe, Bucks, HP10 0HH, UK). PCR products were checked for successful amplification on an agarose gel and sequenced on an ABI3730XL DNA Analyzer (Applied Biosystems Inc, Foster City, CA 94404, USA). This resulted in sequences 571–607 bp (ND4 + tRNAs) and 862–1116 bp (cyt *b*) long.

### 5.3.7. Microsatellites

We first tested a subset of samples for successful amplification of 19 microsatellite marker sets that have previously been used for grass snakes; Natnat01, Natnat05, Natnat06, Natnat08, Natnat09, Natnat11 (Meister *et al.*, 2009),  $\mu\text{Nt}3$ ,  $\mu\text{Nt}5$ ,  $\mu\text{Nt}7$ ,  $\mu\text{Nt}8\text{new}$  (Gautschi *et al.*, 2000; Meister *et al.*, 2009), Hb30 (Burns and Houlden, 1999), TbuA09 (Sloss *et al.*, 2012), 3TS (Garner *et al.*, 2002), Eob $\mu$ 1, Eob $\mu$ 13 (Blouin-Demers and Gibbs, 2003), Ns $\mu$ 2, Ns $\mu$ 3 (Prosser *et al.*, 1999; Hille *et al.*, 2002), Ts2 and Ts3 (McCracken *et al.*, 1999; Hille *et al.*, 2002). Of these, 14 amplified well and were polymorphic; they were therefore included in final multiplexes and used for genotyping all samples (Table S5.3).

Samples were multiplexed in a total volume of 2  $\mu\text{l}$  reaction mix containing 1  $\mu\text{l}$  Multiplex PCR Master Mix (Qiagen), 0.04  $\mu\text{l}$  of each primer at 10 pmol (Eurofins Genomics) and 0.68  $\mu\text{l}$  (multiplexes 1 and 2), 0.6  $\mu\text{l}$  (multiplex 3) or 0.76  $\mu\text{l}$  (multiplex 4) double-distilled H<sub>2</sub>O with ~10 ng genomic DNA. Each forward primer was fluorescently labelled at the 5'-end (Table S5.3). PCR conditions were 15 minutes of initial denaturation at 95°C, 35 cycles of 30 seconds at 94°C, 90 seconds at 60°C (for multiplexes 1 and 2), 57°C (for multiplex 3) or 54°C (multiplex

4) followed by 1 minute at 72°C and a final extension step of 30 minutes at 60°C (Table S5.3). PCR products were analysed on an ABI3730 DNA Analyser (Applied Biosystems, MA, USA) with a red ROX-500 ROX™ ladder. We scored alleles in GeneMapper v3.7 (Applied Biosystems, CA, USA) and re-analysed any questionable samples up to eight times to account for allelic dropout (Taberlet *et al.*, 1996).

### **5.3.8. Phylogenetic analyses**

#### **5.3.8.1. Alignment and visualisation**

Mitochondrial sequence data were visualised and edited in FinchTV v1.4.0 (Geospiza Inc. Seattle, Washington) before being exported to JalView v2.10.1 (Waterhouse *et al.*, 2009) for alignment and error checking. Sequences were aligned in Mega v7.0 (Kumar *et al.*, 2016) with published homologous sequences downloaded from GenBank (Table S5.4) that were representative of the clades described by Kindler *et al.* (2013). In total we generated an alignment of 302 sequences (Table S5.4) and included *Natrix maura*, *Natrix tessellata* and *Nerodia sipedon* as outgroups. Sequences from both genes were concatenated in SequenceMatrix v1.8 (Vaidya *et al.*, 2011).

#### **5.3.8.2. Phylogenetic Tree reconstruction**

The number of sequences was reduced prior to tree estimation to increase speed of analysis by removing sequences with 99% similarity using the Kitten Sequence Dereplicator v1.2.0 (available from <http://www.dnabaser.com/download/nextgen-fasta-dereplicator/index.html>) which utilises CD-HIT (Li and Godzik, 2006; Fu *et al.*, 2012). This process resulted in sequences from 180 individual snakes (including outgroups) for tree construction,

representing an almost range-wide distribution of grass snakes (Table S5.4; Figure S5.1).

Phylogenetic trees were constructed using Bayesian and maximum likelihood methods. Evolutionary models for each gene partition were identified using PartitionFinder v2.1.1 (Lanfear *et al.*, 2016) and Bayesian information criteria (BIC); treating the merged tRNAs as a single partition. The best partitioning scheme was one with maximum partitioning, where each codon for each gene/merged tRNAs was treated as a separate partition (Table S5.5).

#### **5.3.8.3. Bayesian inference**

Phylogenetic inference was calculated using Bayesian methods implemented in MrBayes v3.2.3 (Ronquist *et al.*, 2012) on the CIPRES Science Gateway v3.3 (Miller *et al.*, 2010). We conducted two parallel runs with four chains each, under different models of evolution for each partition (Table S5.5). Each chain was allowed to run for 10 million generations and every 100th generation was sampled. The heating parameter ( $\lambda$ ) was set to 0.05 to improve convergence as previous runs with default settings ( $\lambda = 0.20$ ) yielded low levels of chain swapping. We used a burn-in of 2.5 million generations to generate the final 50% majority rule. Convergence was assessed based on potential scale reduction factor values, posterior probabilities, the standard deviations of split frequencies and by interrogating the effective sample size in Tracer v1.6 (Rambaut *et al.*, 2014).

#### **5.3.8.4. Maximum likelihood**

Maximum Likelihood methods were also used to infer phylogenetic relationships in RaxML-HPC v8.2.9 (Stamatakis, 2006) on the CIPRES Science Gateway v3.3 (Miller *et al.*, 2010). We employed the GTR+G substitution model for all partitions with 10 independent maximum likelihood searches and rapid

bootstrapping with 1000 replicates. The resulting ML and Bayesian trees were visualised in FIGTREE v1.4.3 (Rambaut, 2016).

#### **5.3.8.5. Haplotype network**

To study the relationships between samples within the *N. h. helvetica* clade (Figure 5.2; clade E in Kindler *et al.*, 2013) we used a median-joining algorithm (Bandelt *et al.*, 1999) in Network v5.0.0.1 ([www.fluxus-engineering.com](http://www.fluxus-engineering.com)). The number of available sequences varied between genes; therefore, we analysed each gene separately (Table S5.4) and trimmed the sequence data to remove regions containing excess missing data. This resulted in 112 *cyt b* sequences trimmed to 866 bp and 169 ND4 + tRNAs trimmed to 579 bp from our sampling areas (Jersey and northwest France), Great Britain and elsewhere in mainland Europe (Figure S5.1). Haplotypes were mapped in ArcMap v10.5 (ESRI, Redlands).

### **5.3.9. Genetic analysis**

#### **5.3.9.1. Data checking**

Genotype data were tested for null alleles in CERVUS v3.0.7 (Kalinowski *et al.*, 2007). Deviations from Hardy-Weinberg equilibrium (HWE) and linkage disequilibrium with Bonferroni corrections were calculated in GenePop v4.6 (Rousset, 2008) for each population (Jersey and northwest France). Loci that exhibited null alleles or were outside of HWE across both populations were excluded from further tests.

#### **5.3.9.2. Population structure and differentiation**

To investigate population subdivision, we implemented a Bayesian clustering approach in STRUCTURE v2.3.4 (Pritchard *et al.*, 2000) to identify the most likely number of genetic clusters ( $K$ ) between populations and within Jersey. At both



spatial scales, we implemented 10 repeated runs with a burn-in of 100,000,  $10^6$  Monte Carlo Markov Chain (MCMC) replicates, and varied  $K$  between one and eight. This was carried out with an admixture model and correlated allele frequencies (Falush *et al.*, 2003). The most plausible value of  $K$  was ascertained using  $\Delta K$  (Evanno *et al.*, 2005) in STRUCTURE HARVESTER v0.6.94 (Earl and vonHoldt, 2012). The resulting assignment probabilities were then plotted in CLUMPAK (Kopelman *et al.*, 2015). This process was repeated with and without prior sampling location information to inform the assignment probabilities in STRUCTURE in the case of weak inference (Hubisz *et al.*, 2009).

As the  $\Delta K$  approach cannot assess the suitability of  $K=1$ , we assessed the likelihood of  $K=1$  using the mean likelihood of the data ( $\text{LnPr}(K)$ ) and a model-based approach; Discriminant Analysis of Principal Components (DAPC) (Jombart *et al.*, 2010). DAPC was calculated with the *dapc* function in R v3.2.3 (R Core Team, 2015) with package *adeigenet* v2.0.1 (Jombart, 2008). In this case, the most appropriate number of clusters was assessed using BIC, and cross-validation with an 80% training set was used to identify the most appropriate number of principal components (PCs) to retain based on the lowest Mean Squared Error.

The most likely number of genetic clusters from the STRUCTURE analysis was used for subsequent tests of genetic differentiation and diversity, treating each cluster as a population. Pairwise differentiation ( $F_{ST}$ ) between clusters was calculated in Genalex v6.5 (Peakall and Smouse, 2006; 2012) using Analysis of Molecular Variance (AMOVA) (Excoffier *et al.*, 1992).

### **5.3.9.3. Genetic diversity**

For each cluster we calculated measures of genetic diversity. We used GenAlEx v6.5 (Peakall and Smouse, 2006; 2012) to calculate expected ( $H_E$ ) and

observed ( $H_O$ ) heterozygosity. Allelic richness ( $A_R$ ) (sampled from 20 alleles) was calculated with the *allele.rich* function in R package PopGenReport v3.0.0 (Adamack and Gruber, 2014), and the level of inbreeding ( $F_{IS}$ ) was calculated in FStat v2.9.3.2 (Goudet, 1995; 2001). Differences in  $H_E$ ,  $H_O$ ,  $F_{IS}$  and  $A_R$  between clusters were tested with Kruskal-Wallis rank sum tests (Kruskal and Wallis, 1952) in R with the *kruskal.test* function from the stats v3.3.3 package.

## 5.4. Results

We sampled a total of 70 individuals comprising 12 from northwest France and 58 from Jersey (Table S5.4). Of those in Jersey, 23 were from St Ouen, 14 from Dunes and 19 from the South subpopulation. A further two individuals were sampled in the southwest of the island in close proximity to the Dunes subpopulation (Figure 5.1). In some instances it was only possible to sequence one mitochondrial gene for a sample due to low sample quality or limited DNA quantity.

### 5.4.1. Phylogenetic reconstruction

Topologies from both tree-building methods were largely congruent with each other and to those described in previous work (e.g., Kindler *et al.*, 2013). Sequences in terminal nodes were also placed in agreement with existing phylogenetic reconstructions. The majority of nodes received strong support (maximum likelihood bootstrap support  $\geq 90\%$ , posterior probabilities  $\geq 0.90$ ); with posterior probabilities generally indicating greater support than maximum likelihood bootstrap values (Figure 5.2). Deeper nodes were less well supported, highlighting the uncertainty in relationships between *Natrix astreptophora* and the two recently separated species; *N. natrix* and *N. helvetica*. All sequences from

Jersey and northwest France clustered within the *N. h. helvetica* clade (clade E, Kindler *et al.*, 2013), supporting the hypothesis that the Jersey population is allopatric to those in mainland Europe (Thorpe, 1984) and not *N. astreptophora* as suggested by Frazer (1949).

#### 5.4.2. Haplotype network

Within this clade (E), we noted nine haplotypes from ND4 + tRNAs and eight from *cyt b* (Figure 5.3; Table S5.4). All individuals from Jersey and northwest France shared a single haplotype based on ND4 + tRNAs. Moreover, we observed a difference between samples from Jersey and northwest France compared to those from Great Britain, consisting of a single transversion in the ND4 + tRNAs. Elsewhere, ND4 + tRNAs sequence data identified one shared haplotype between samples from Great Britain and mainland Europe, with the exception of northwest France. Five unique haplotypes were observed amongst the mainland Europe samples (excluding northwest France) and two from Great Britain. Using *cyt b* sequence data we identified one shared haplotype between all localities. Three other haplotypes were observed from three individual British samples, three from four individuals from mainland Europe, and only a single unique haplotype from one individual in Jersey (Figure 5.3). Cytochrome *b* therefore showed little differentiation of the Jersey population. Overall, these results suggest that the Jersey population is more closely related to samples from northwest France than to others in the clade. However, all sequences were reasonably consistent, with only eight (ND4 + tRNAs) or seven (*cyt b*) mutations in the partial gene respectively.

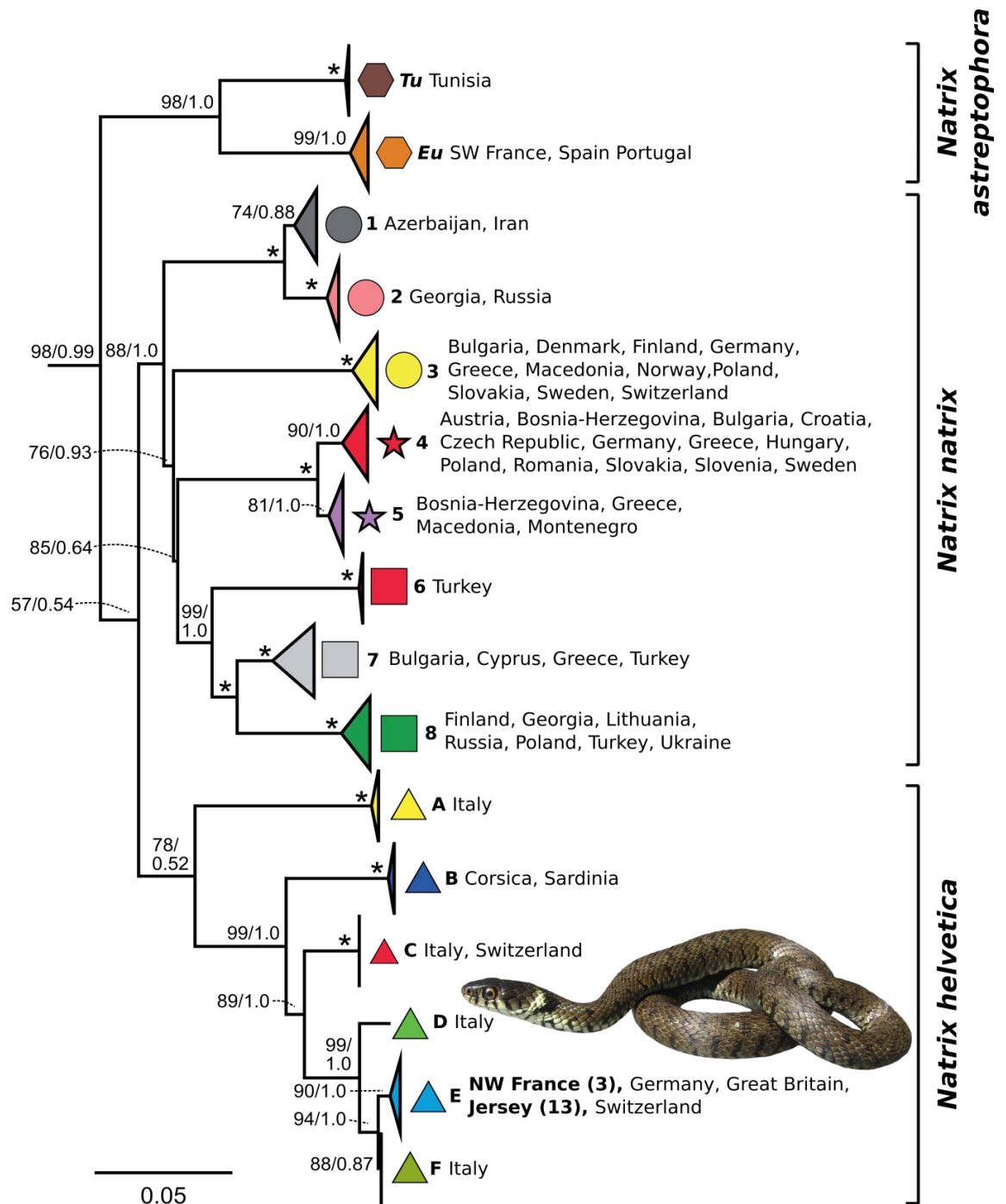


Figure 5.2 Phylogenetic tree of 177 grass snake samples constructed using Maximum Likelihood analyses with a total of 1984 bp (cytochrome *b*, ND4 and tRNAs). For clarity, outgroups ( $n=3$ ) have been removed and the terminal clades collapsed. Branch support is indicated based on 1000 Maximum Likelihood bootstrap replicates and Bayesian posterior probabilities, with asterisks (\*) indicating nodes fully supported by both methods. Clade colours, shapes and labels correspond to the clades described by Kindler *et al.* (2013; 2014; 2017) and presented in Figure S5.1. The distribution range of each clade is shown (from Kindler *et al.*, 2013; 2014), and populations sampled in this study are shown in bold with the number of samples used in tree construction shown in brackets. Inset: *N. helvetica helvetica* from Jersey, Channel Islands (photo: R. Ward).

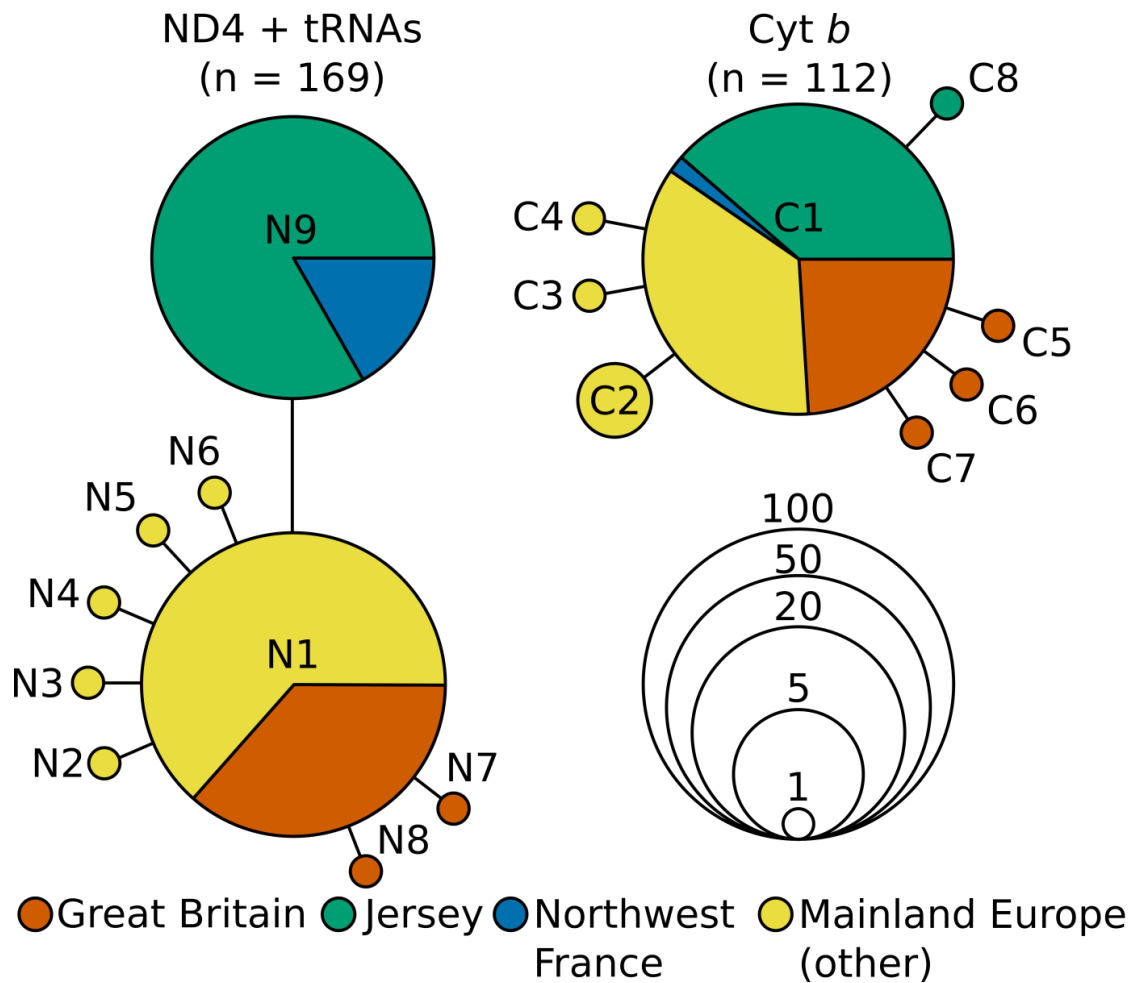


Figure 5.3 Phylogenetic mtDNA network reconstruction of samples from clade E (Figure 5.2; Figure S5.1) based on 579 bp ND4 + tRNAs (left) and 866 bp *cyt b* (right). Constructed using a median-joining algorithm in Network v5.0.0.1 ([www.fluxus-engineering.com](http://www.fluxus-engineering.com)). Colours correspond to sampling locations. Circle size corresponds to the haplotype frequency. All connecting lines represent a single character difference. Codes in or adjacent to nodes refer to haplotypes as listed in Table S5.4.

### 5.4.3. Population genetics

#### 5.4.3.1. Genotyping

A total of 70 individuals were genotyped at 14 loci. Four loci (Natnat09, Hb30, Eobμ1 and μNt5) showed evidence of having null alleles with frequencies above 0.25 (range: 0.25–0.41, Table S5.6), and were excluded from further analyses. Deviation from Hardy-Weinberg equilibrium ( $p < 0.05$ ) was also observed at several loci (Table S5.6), but this was only consistent across both Jersey and France for μNt5 and so no other loci were excluded (Selkoe and Toonen, 2006).

Across the remaining 10 loci there were 59 alleles, with between three and 10 alleles per locus (mean:  $5.90 \pm 2.13$  SD). No linkage disequilibrium was observed between loci following a Bonferroni correction.

#### **5.4.3.2. Population structure**

Bayesian structure analysis indicated two ( $\Delta K = 38.71$ ,  $\ln K = -1403.56$ ) or four genetic clusters without prior location information ( $\Delta K = 72.15$ ,  $\ln K = -1314.84$ ) (Figure S5.2), and two clusters with prior location information ( $\Delta K = 27.45$ ,  $\ln K = -1389.92$ ). Both the mean likelihood of  $K$  and results from DAPC indicated  $K=1$  to be improbable (Figure S5.2 and Figure S5.3); therefore, due to stronger support we continue with interpretation using four clusters. The most distinct cluster originates in northwest France, and a further three clusters are apparent in the Jersey population (Figure 5.4). The presence of these three clusters is further supported when excluding the data from northwest France (Figure 5.4). In this case, Bayesian clustering analysis identified three ( $\Delta K = 6.17$ ,  $\ln K = -1058.30$ ) or five clusters without priors ( $\Delta K = 7.41$ ,  $\ln K = -1153.34$ ) and three clusters with prior sampling location information ( $\Delta K = 14.85$ ,  $\ln K = -1000.28$ ) within the island (Figure S5.2). Again we exclude the possibility of  $K=1$  due to the lack of support shown by the mean likelihood and DAPC (Figure S5.2 and Figure S5.3). Due to the weak signal shown by Bayesian clustering analysis without prior sampling location information, we base our interpretations on  $K=3$  due to the additional support shown when including prior information.

The overall pattern of assignment probabilities in Jersey was highly congruent when including or excluding samples from northwest France (Figure 5.4). Assignment probabilities are therefore mapped based on the four clusters predicted with all samples (Figure 5.1). These clusters follow our *a priori*

assumptions of population structure, whereby samples in northwest France and Jersey are differentiated, and that the three subpopulations in Jersey form their own clusters. However, admixed individuals in Jersey were common, and several individuals showed strong assignment to clusters that differed from their *a priori* sampling locations. Two outlying individuals in proximity to the southwest tip of the island clustered with the Dunes subpopulation.

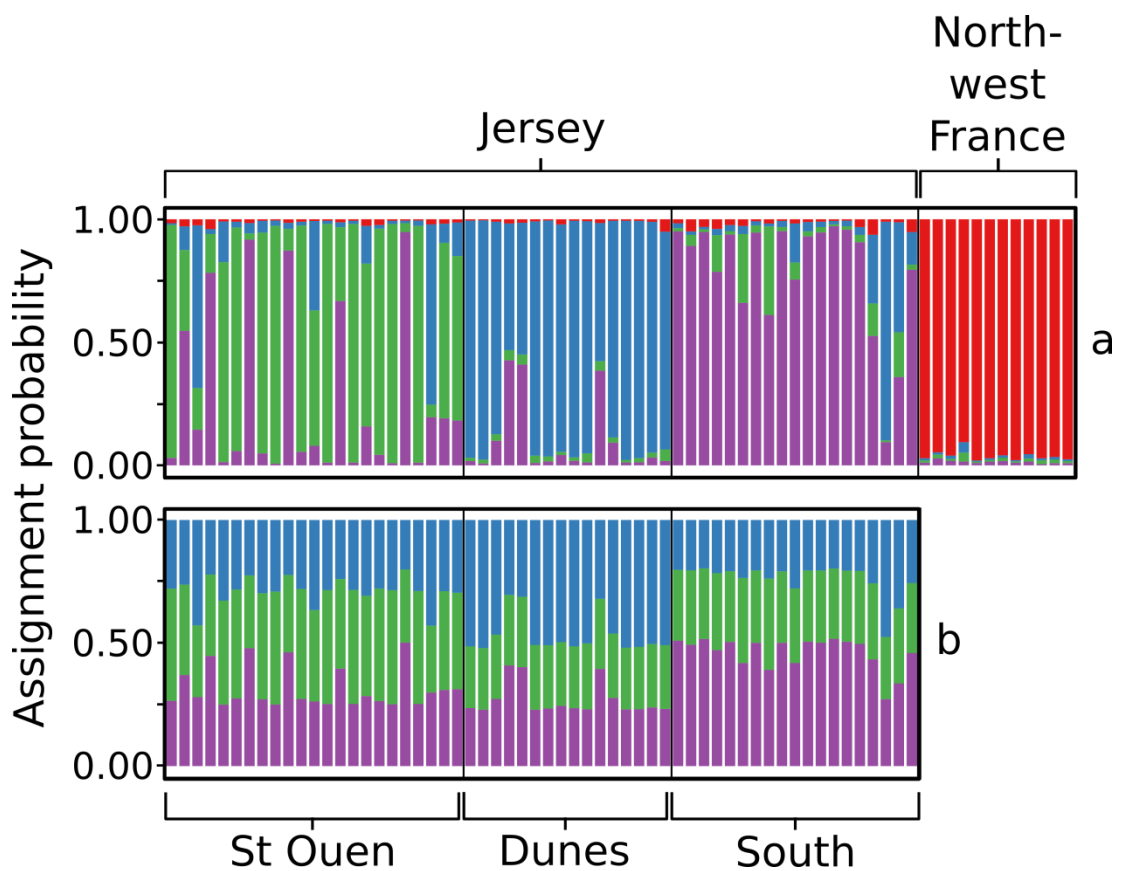


Figure 5.4 Structure output (a) between populations ( $n=70$ ) and (b) within Jersey ( $n=58$ ) inferred from microsatellite data from Jersey and northwest France without prior sampling locations. Each bar represents each snake's assignment probability to a particular cluster ( $K$ ), with  $K$  set to four (a) or three (b).

Analysis of Molecular Variance (AMOVA) found that 9.5% of genetic variation was between clusters, 8.0% was among individuals and 82.5% was within individuals; equating to a significant differentiation between clusters with an  $F_{ST}$  of 0.095 ( $p = 0.001$ ). Significant pairwise distances were observed in all cases except

between St Ouen and Dunes, with values ranging between 0.07 (St Ouen and Dunes) and 0.171 (St Ouen and northwest France) (Table 5.1).

Table 5.1 Genetic differentiation between clusters identified by STRUCTURE. Values above the diagonal are the probability based on 999 permutations. Below the diagonal are  $F_{ST}$  values based on Analysis of Molecular Variance (AMOVA). Measures were calculated with 10 microsatellite loci (Table 5.2).

		Jersey			Northwest France
		St Ouen	Dunes	South	
	St Ouen	–	0.217	0.001*	0.001*
Jersey	Dunes	0.007	–	0.002*	0.001*
	South	0.077	0.055	–	0.001*
	Northwest France	0.171	0.165	0.130	–

\* Significant values ( $p < 0.008$  after Bonferroni correction)

#### 5.4.3.3. Geographic trends in genetic diversity

The three clusters in Jersey showed fewer alleles per locus and total alleles (St Ouen: 35, Dunes: 32, South: 28) than the French cluster (45) (Table 5.2). Kruskal-Wallis tests revealed no significant differences ( $p > 0.05$ ) in genetic diversity between clusters for measures of  $A_R$ ,  $F_{IS}$ ,  $H_O$  and  $H_E$  (Table 5.3). However, clusters in Jersey showed lower genetic diversity than the French cluster. Tests comparing a pooled set of all Jersey samples to those in France revealed similar patterns (not shown), suggesting this reduction in diversity is not related to sample size or area.



Table 5.2 Summary table of genetic diversity for four genetic clusters. Number of samples successfully genotyped ( $n$ ), number of alleles per locus ( $N_a$ ), allelic richness ( $A_R$ ), fixation index ( $F_{IS}$ ), mean observed ( $H_O$ ) and expected ( $H_E$ ) heterozygosity.

		Natnat05	$\mu Nt8new$	$\mu Nt7$	Natnat11	Natnat06	TbuA09	3TS	Eob $\mu$ 13	Natnat08	Natnat01	Mean (SD)
St Ouen ( $n$ 23)	$N_a$	3	5	3	3	2	3	4	5	4	3	3.50 (0.97)
	$A_R$	1.779	4.412	2.871	2.000	1.651	2.871	3.785	3.522	3.755	2.998	2.964 (0.930)
	$F_{IS}$	-0.011	0.066	0.014	-0.073	-0.024	0.389	-0.407	0.100	-0.260	0.131	-0.007 (0.216)
	$H_O$	0.304	0.652	0.391	0.565	0.174	0.435	0.739	0.609	0.652	0.696	0.522 (0.187)
	$H_E$	0.264	0.734	0.443	0.481	0.159	0.615	0.508	0.621	0.669	0.575	0.507 (0.180)
Dunes ( $n$ 15–16)	$N_a$	2	5	3	2	2	4	4	3	4	3	3.20 (1.03)
	$A_R$	1.994	3.770	1.984	2.531	1.906	3.530	2.530	3.692	2.960	2.000	2.690 (0.751)
	$F_{IS}$	-0.200	-0.006	-0.154	-0.245	-0.071	0.382	-0.311	0.023	-0.020	-0.098	-0.070 (0.192)
	$H_O$	0.125	0.688	0.563	0.500	0.067	0.313	0.867	0.333	0.625	0.375	0.445 (0.251)
	$H_E$	0.117	0.688	0.506	0.492	0.064	0.627	0.638	0.460	0.654	0.646	0.489 (0.224)
South ( $n$ 18–19)	$N_a$	2	5	2	2	2	4	3	2	3	3	2.80 (1.03)
	$A_R$	1.992	3.772	1.994	2.000	2.000	3.725	2.999	1.998	2.851	2.982	2.631 (0.732)
	$F_{IS}$	0.151	0.107	-0.214	-0.079	-0.333	0.390	-0.260	0.581	-0.194	0.217	0.037 (0.303)
	$H_O$	0.211	0.579	0.389	0.500	0.632	0.389	0.789	0.158	0.684	0.474	0.480 (0.201)
	$H_E$	0.266	0.602	0.313	0.424	0.465	0.613	0.647	0.301	0.542	0.539	0.471 (0.140)
Northwest France ( $n$ 10–12)	$N_a$	6	5	3	3	4	7	3	2	6	6	4.50 (1.72)
	$A_R$	4.956	4.225	2.665	2.665	3.611	5.627	2.895	2.000	5.222	5.673	3.954 (1.367)
	$F_{IS}$	0.224	0.514	-0.386	-0.085	0.020	0.069	0.450	0.138	0.043	0.131	0.112 (0.256)
	$H_O$	0.500	0.333	0.667	0.583	0.455	0.667	0.333	0.455	0.750	0.700	0.544 (0.151)
	$H_E$	0.611	0.642	0.469	0.517	0.442	0.684	0.569	0.500	0.750	0.760	0.595 (0.114)

Table 5.3 Results of Kruskal-Wallis tests for significance of differentiation in genetic diversity between genetic clusters, based on 10 loci. Genetic diversity measures shown are allelic richness ( $A_R$ ), fixation ( $F_{IS}$ ), observed ( $H_O$ ) and expected heterozygosity ( $H_E$ ).

Measure	$\chi^2$	df	$p$
$A_R$	6.712	3	0.082
$F_{IS}$	4.006	3	0.261
$H_O$	1.541	3	0.673
$H_E$	2.860	3	0.414

## 5.5. Discussion

We were able to sample the majority of the known grass snake population in Jersey to make an assessment of their origin and genetic health. Our results give support to a natural provenance and a probable period of isolation of ca. 7,000 years. Despite subsequent declines, the population has maintained a level of genetic diversity that is only slightly depauperate compared to its mainland counterparts. However, we have identified issues of population fragmentation within the island which align with the distribution of urban areas. This result has implications for long-term viability of the population, with a need for improvements in landscape connectivity. Conservation managers should give greater consideration to the influences of urbanisation and road networks upon dispersal and gene flow, and work towards strategies to resolve these issues.

### 5.5.1. Phylogeography

The *Natrix* genus has been the focus of several recent phylogeographic studies; clarifying some of the debate over species and subspecies delimitations within it (Fritz *et al.*, 2012; Kindler *et al.*, 2013; 2017; Pokrant *et al.*, 2016). Both the previous studies and ours indicate grass snake populations in northwest Europe to be within the same clade containing *Natrix helvetica helvetica* (Kindler *et al.*, 2017) which expanded from southern France post-glaciation (Thorpe, 1984;

Fritz *et al.*, 2012; Kindler *et al.*, 2013). The current range for individuals in this clade appears to extend throughout France eastward in to Switzerland and western Germany where it has a contact zone with *Natrix natrix* in the Rhine region (Kindler *et al.*, 2013; 2017), and north to Jersey and Great Britain (Figure S5.1; Table S5.4).

Aside from Jersey there are a number of island *Natrix* populations; however their taxonomic status is unresolved (Fritz *et al.*, 2012; Kindler *et al.*, 2013). Of comparison to Jersey is the island of Gotland, which emerged approximately 10,300 years ago and was since colonised naturally or by human assistance (Kindler *et al.*, 2014). Grass snakes there are phenotypically divergent (Nilson and Andrén, 1981) but are not considered unique (Kindler *et al.*, 2014). The divergence of other island grass snake populations has typically taken place over much longer periods (e.g., 4.3–4.4 million years ago for *N. h. corsa* and *N. h. cetti*, Fritz *et al.*, 2012). Jersey's snake population experienced a much more recent isolation event, and so the effects of drift have not yet resulted in high levels of divergence. Nonetheless, the Jersey population can be considered as a management unit of conservation interest as it showed genetic differentiation from grass snakes in northwest France and may exhibit local adaptations. Furthermore, many snake species exhibit high levels of genetic diversity that are not recognised at species or sub-species level (Burbrink and Castoe, 2009).

The most parsimonious explanation for the colonisation of Jersey is that grass snakes were present prior to separation from the European continent. Otherwise, colonisation and subsequent migration from external populations may only have occurred through rafting or swimming (reviewed in Baker, 2015), or human-mediated introductions (Michaelides *et al.*, 2015). We would expect

colonisation from these latter routes to have resulted in stronger evidence of founder effects. Furthermore, our study suggests a shared origin with populations from northwest France. The question therefore remains as to how the population has maintained genetic diversity.

### 5.5.2. Population genetic structure

Bayesian clustering analysis indicated differentiation between all three Jersey subpopulations, with some admixture between them (Figure 5.1, Figure 5.4). In contrast,  $F_{ST}$  indicated significant differentiation of the South subpopulation from both subpopulations in the west. The small but significant levels of genetic differentiation between these subpopulations were detected over distances of only 6.3 km between the South and St Ouen subpopulations (pairwise  $F_{ST} = 0.077$ ), and 3.5 km between the Dunes and South subpopulations (pairwise  $F_{ST} = 0.055$ ). Significant differentiation over such a small distance suggests a high level of isolation between the clusters in the west and south of Jersey, which can be attributed to a densely urbanised area with several roads prone to heavy traffic (Figure 5.1), limiting dispersal. Similar effects have been recorded for black rat snakes *Pantherophis obsoletus* (Prior *et al.*, 1997) and timber rattlesnakes *Crotalus horridus* (Clark *et al.*, 2010), whereby genetic differentiation between communal hibernacula has occurred over short distances (<4 km apart) due to roads acting as barriers. Moreover, the effects of urban barriers to gene flow have been noted in multiple taxa, including invertebrates (Keller and Largiader, 2003), amphibians (Reh and Seitz, 1990) and mammals (Kuehn *et al.*, 2006). However differentiation can also occur due to natural barriers (e.g., rivers, elevation, changes in habitat structure), as a function of distance (e.g., Meister *et al.*, 2012) or may remain unexplained (e.g., Lukoschek and Shine, 2012).

The ability of snake species to disperse and maintain gene flow over suboptimal landscapes is associated with their morphology, ecology and the configuration of the landscape (King and Lawson, 2001; Meister *et al.*, 2010; 2012). Sedentary, ambush predators such as vipers often have low dispersal capabilities and can subsequently exhibit genetic differentiation over small spatial scales (e.g., Clark *et al.*, 2010). Similarly, habitat specialists such as the smooth snake *Coronella austriaca*, have been shown to exhibit isolation by distance over less than 6 km (Pernetta *et al.*, 2011). Species with similar traits are therefore at greater risk of experiencing negative consequences of anthropogenic modification and fragmentation.

Within Jersey, populations of the wall lizard *Podarcis muralis* (Michaelides *et al.*, 2015) and toad *Bufo spinosus* (Wilkinson *et al.*, 2007) have a disjunct distribution with limited gene flow, which may be exacerbated by their dispersal capabilities. Comparatively, grass snakes are known to be wide ranging (Madsen, 1984). Indeed, in suboptimal habitats they may utilise larger home ranges in order to meet resource requirements (e.g., Wisler *et al.*, 2008), and can be considered fairly generalist (Meister *et al.*, 2012) as they can utilise a variety of habitats (Steward, 1971) and prey (reviewed in Gregory and Isaac, 2004). Although a recent study found Jersey's grass snakes to use small ranges (Chapter 3), previous studies have shown that remnant grass snake subpopulations can maintain gene flow across agricultural landscapes of 90 km<sup>2</sup> (Meister *et al.*, 2010; 2012). The dominance of agricultural land within Jersey may therefore allow dispersal of grass snakes (Wisler *et al.*, 2008) provided there are suitable 'stepping stones' of suitable habitat types and key resources (Meister *et al.*, 2010). Indeed, the clustering of two outlying samples from the southwest with the Dunes

subpopulation, and evidence of admixture between subpopulations, suggests individuals are able to disperse through the intervening matrix on occasion. Such movements may be aided by private gardens, nature reserves and field margins.

### **5.5.3. Genetic diversity**

With increasing isolation, we expect reductions in genetic diversity and population fitness (e.g., Schwaner, 1990). Studies of critically endangered populations of the dice snake *Natrix tessellata* have found low microsatellite DNA heterozygosity, which may be due to a small population size and the founder effect (Gautschi *et al.*, 2002; Guicking *et al.*, 2004). Similarly, island populations of the wall lizard in Jersey and the nearby island of Chausey showed lower genetic diversity than mainland populations (Michaelides *et al.*, 2015). However, although we observed a reduction in genetic diversity in Jersey compared to northwest France, these differences were not significant and may have occurred as a consequence of recent declines in population size. Evidence from eastern massasauga rattlesnakes *Sistrurus c. catenatus* also suggests that snakes may have the ability to survive long periods as small isolated populations, with few negative genetic consequences (Chiucchi and Gibbs, 2010). Furthermore, some island populations of garter snakes *Thamnophis* spp. have been shown to have similar levels of protein diversity to mainland counterparts (reviewed in Dessauer *et al.*, 1987). It is also possible that due to low detection and high mobility (Chapter 2; Chapter 3), current estimates of population size and distribution within Jersey are too conservative and that there is a larger population present which is maintaining genetic diversity.

Despite the population's isolation and apparent small size, we found no evidence of inbreeding. This observation may suggest that the population has not experienced strong founder effects, and is yet to display the effects of small population size. However, natricines rarely display strong evidence of inbreeding (reviewed in King, 2009) unless they have experienced severe bottleneck events or small population size (Gautschi *et al.*, 2000; 2002; Guicking *et al.*, 2004). Nonetheless, small and isolated snake populations that do not display evidence of inbreeding may still suffer from reduced fitness (Gibbs and Chiucchi, 2012).

#### **5.5.4. Conservation management**

From a management perspective, this study indicates that work within the island to improve connectivity between the southern subpopulation and those on the west coast would be beneficial to improve admixture. Furthermore, the presence of two clusters in the west suggests linkages between them require evaluation. These improvements may be achieved through statutory protection of dispersal routes, habitat management to improve suitability of those routes, and by encouraging private landowners to manage their land in a way that is beneficial to wildlife (e.g., through encouragement of prey species via garden ponds). Although currently we see no need for other interventions, this may change should the population and its genetic diversity continue to decrease. Then, consideration should be given to genetic restoration, using individuals from northwest France which show genetic similarities. These strategies have been shown to be effective in an isolated population of European adders *Vipera berus* with low genetic diversity (Madsen *et al.*, 1999).

### **5.5.5. Recommendations**

In summary, conservation efforts should focus on protection of the three subpopulations in Jersey and the sites they inhabit, and on improving connectivity between them. A greater understanding of survival and landscape permeability would be useful in improving landscape connectivity. Particularly, attention should be paid to the effects of urbanisation and roads on the genetic structuring of populations and to testing novel methods to improve permeability through these areas. Continued genetic sampling within the population will provide a basis for further analyses. Due to their tolerance for suboptimal habitats, efforts to construct wildlife corridors for entire communities should make use of data regarding dispersal and gene flow of more sedentary organisms. Otherwise, the resolution at which connecting fragments are proposed may not resolve issues of isolation.

### **5.6. Acknowledgements**

We are grateful to the Société Herpétologique de France and its members Mickaël Barrioz and Franck Paysant for providing samples from France which were used in this study. We are also grateful to members of the Durrell Institute of Conservation and Ecology conservation genetics lab for their advice and support. This study was supported by funding from the States of Jersey Department of the Environment, the Joint Nature Conservation Committee (JNCC), Royal Bank of Canada (RBC) Green Grants and The Channel Islands Insurance Corporation.



## 5.7. Supplementary Information

Table S5.1 Primers used for mitochondrial sequencing.

mtDNA fragment	Primer	Direction	Primer sequence (5' to 3')	Reference
ND4 + tRNAs	ND4ab	Forward	CACCTATGACTACCAAAAAGCTCATGTAGAAGC	Arévalo <i>et al.</i> , 1994
ND4 + tRNAs	tRNA_leu	Reverse	CATTACTTTTACTTGGATTTGCACCA	Arévalo <i>et al.</i> , 1994
ND4 + tRNAs	ND4_CDF	Forward	CAACAAACAGACTTAAAATCCCT	This study
ND4 + tRNAs	ND4_CDR	Reverse	GAGTTAGCAAGTCTTATTGCA	This study
Cyt <i>b</i>	L14724NAT	Forward	GACCTGCGGTCCGAAAAACCA	Guicking <i>et al.</i> , 2002
Cyt <i>b</i>	Thrsnr2 / H16064	Reverse	CTTTGGTTTACAAGAACAATGCTTTA	Burbrink <i>et al.</i> , 2000
Cyt <i>b</i>	Natrix_Cytb_Rev2	Reverse	AGGGCAAAGAATCGGGTT	Kindler <i>et al.</i> , 2013

Table S5.2 Primer combinations and PCR conditions for mitochondrial sequencing.

mtDNA fragment	Primer combinations	Thermocycling conditions						bp range
		ID	C	D	A	E	FE	
ND4 + tRNAs	ND4ab, tRNA-leu	94°C, 5 min	35	94°C, 45 s	55°C, 45 s	72°C, 60 s	72°C, 10 min	—
ND4 + tRNAs	ND4_CDF, ND4_CDR	94°C, 5 min	35	94°C, 45 s	55°C, 45 s	72°C, 60 s	72°C, 10 min	527–535 (ND4) 38–80 (tRNAs)
Cyt <i>b</i>	L14724NAT, Thrsnr2	94°C, 5 min	40	94°C, 45 s	55°C, 45 s	72°C, 60 s	72°C, 10 min	460–866
Cyt <i>b</i>	L14724NAT, Natrix_Cytb_Rev2	94°C, 5 min	40	94°C, 45 s	54°C, 45 s	72°C, 40 s	72°C, 10 min	443–551

Abbreviations: ID = initial denaturation, C = number of cycles, D = denaturation, A = annealing, E = extension, FE = final extension

Table S5.3 Multiplex and microsatellite marker details. Primer sequences are shown with fluorescent labelling in square brackets [ ]. Tm = Annealing temperature. Loci marked with an asterisk (\*) were excluded from analyses due to evidence of null alleles.

Multiplex	Locus	Primer sequences	Tm (°C)	Repeat motif	Range (bp)	Original reference
1	$\mu$ Nt5*	F: [6-FAM] TGCTTTTCGGATTTGACATTC R: CTGCATTTGAAGCGTGGTAG	60	Di	76–94	Gautschi <i>et al.</i> , 2000
1	$\mu$ Nt8new	F: [ATTO-550] GTATCGTCCTTCCAGACAAG R: GCAAATCAAATAAATCTCACTGG	60	Di	91–129	Gautschi <i>et al.</i> , 2000
1	Natnat05	F: [6-FAM] TCTGCACTGGGGATAGGAAG R: GTCCCTTTTTTCAGTGCTGTTG	60	Di	158–178	Meister <i>et al.</i> , 2009
1	Natnat09*	F: [6-FAM] TGTAATAACTGTACCATTTTTGG R: TGACTGGGCAACAGAAAAGC	60	Di	96–134	Meister <i>et al.</i> , 2009
2	Natnat01	F: [ATTO-550] GATAAAGGCAACGGCAACTG R: CCAGCAGTTAATGTAAACAGAGG	60	Di	154–184	Meister <i>et al.</i> , 2009
2	Natnat06	F: [6-FAM] AATGGCATTCTCTCCAGCTC R: ACCCATATCCGTATCCATATCC	60	Di	163–185	Meister <i>et al.</i> , 2009
2	Natnat08	F: [HEX] GATAAAGGCAACGGCAACTG R: CCAGCAGTTAATGTAAACAGAGG	60	Di	178–202	Meister <i>et al.</i> , 2009
2	Natnat11	F: [HEX] GGCTGTTTTCCAGTGAAGC R: GGTCTGGGGAAAAAGAAAGG	60	Di	106–116	Meister <i>et al.</i> , 2009
3	Hb30*	F: [HEX] CCCACTGGCTCATTTCAGT R: CCACATTTGCATCGGAGTG	57	Di	253–269	Burns and Houlden, 1999
3	$\mu$ Nt7	F: [HEX] TTTGAAAGGAGAATGAATCGTG R: CGCGAGGAATCAGAATGAAC	57	Di	172–186	Gautschi <i>et al.</i> , 2000
3	TbuA09	F: [ATTO-550] CATCTCAACCAAGTCGCTTC R: GGATGTTGTGGGGTGTTTTT	57	Di	108–142	Sloss <i>et al.</i> , 2012
4	Eob $\mu$ 1*	F: [ATTO-550] ATCAGTAGGAGTGAGAGCAACT R: CTGCATACTCTTCCAGAACC	54	Di	128–140	Blouin-Demers and Gibbs, 2003
4	Eob $\mu$ 13	F: [HEX] TGATCTGAGTCTCTTTCTGG R: CAATTCAAATCCATTGGTTT	54	Di	148–160	Blouin-Demers and Gibbs, 2003
4	3TS	F: [ATTO-550] GGTCACCTAAATACAACGAAATTGGTTAGCT R: CGGACAGCTCTGGCTCCCTTG	54	Tetra	178–236	Garner <i>et al.</i> , 2002

Table S5.4 Grass snake samples studied and details of the analysis carried out on each sample. Mitochondrial clades and assigned haplotypes are also shown. GenBank accession numbers are given where available. Taxonomy follows Kindler et al. (2017).

Taxon	Location	Analysis	mtDNA Clade*	Haplotype		Voucher <sup>†</sup>	Accession numbers <sup>†</sup>		Reference
				ND4	Cyt <i>b</i>		ND4+tRNAs	Cyt <i>b</i>	
<b>Samples from this study</b>									
<i>Natrix h. helvetica</i>	Jersey: Creepy Valley	a, c	E	N9	—	CV01	CV01	—	This study
<i>Natrix h. helvetica</i>	Jersey: Creepy Valley	a, c	E	—	C1	CV02	—	CV02	This study
<i>Natrix h. helvetica</i>	Jersey: La Lande du Ouest	a, b, c	E	N9	C1	GL01X	GL01X	GL01X	This study
<i>Natrix h. helvetica</i>	Jersey: Grantez	a, c	E	N9	—	GR01X	GR01X	—	This study
<i>Natrix h. helvetica</i>	Jersey: La Route de Noirmont	a, c	E	N9	—	RN01X	RN01X	—	This study
<i>Natrix h. helvetica</i>	Jersey: Les Blanches Banques	a	—	—	—	BB08X	—	—	This study
<i>Natrix h. helvetica</i>	Jersey: Les Blanches Banques	a	—	—	—	BB10X	—	—	This study
<i>Natrix h. helvetica</i>	Jersey: Les Blanches Banques	a, b, c	E	N9	C1	BB01	BB01	BB01	This study
<i>Natrix h. helvetica</i>	Jersey: Les Blanches Banques	a, c	E	N9	C1	BB01X	BB01X	BB01X	This study
<i>Natrix h. helvetica</i>	Jersey: Les Blanches Banques	a, c	E	N9	C1	BB02X	BB02X	BB02X	This study
<i>Natrix h. helvetica</i>	Jersey: Les Blanches Banques	a, c	E	N9	C1	BB05X	BB05X	BB05X	This study
<i>Natrix h. helvetica</i>	Jersey: Les Blanches Banques	a, b, c	E	N9	C1	BB07X	BB07X	BB07X	This study
<i>Natrix h. helvetica</i>	Jersey: Les Blanches Banques	a, b, c	E	N9	C1	BB11X	BB11X	BB11X	This study
<i>Natrix h. helvetica</i>	Jersey: Les Blanches Banques	a, c	E	N9	C1	BB12X	BB12X	BB12X	This study
<i>Natrix h. helvetica</i>	Jersey: Les Blanches Banques	a, b, c	E	N9	C8	BB03	BB03	BB03	This study
<i>Natrix h. helvetica</i>	Jersey: Les Blanches Banques	a, c	E	N9	—	BB04	BB04	—	This study
<i>Natrix h. helvetica</i>	Jersey: Les Blanches Banques	a, c	E	N9	—	LO01	LO01	—	This study

Table S5.4 (continued)			mtDNA	Haplotype		Accession numbers <sup>†</sup>			Reference
Taxon	Location	Analysis	Clade*	ND4	Cyt <i>b</i>	Voucher <sup>†</sup>	ND4+tRNAs	Cyt <i>b</i>	
<i>Natrix h. helvetica</i>	Jersey: Les Mielles NR	a, b, c	E	N9	C1	LM01	LM01	LM01	This study
<i>Natrix h. helvetica</i>	Jersey: Les Mielles NR	a, c	E	N9	C1	LM02	LM02	LM02	This study
<i>Natrix h. helvetica</i>	Jersey: Les Mielles NR	a, b, c	E	N9	C1	LM03	LM03	LM03	This study
<i>Natrix h. helvetica</i>	Jersey: Les Mielles NR	a, b, c	E	N9	C1	LM04	LM04	LM04	This study
<i>Natrix h. helvetica</i>	Jersey: Les Mielles NR	a, c	E	N9	C1	LM05X	LM05X	LM05X	This study
<i>Natrix h. helvetica</i>	Jersey: Les Mielles NR	a, c	E	N9	C1	LM07X	LM07X	LM07X	This study
<i>Natrix h. helvetica</i>	Jersey: Les Mielles NR	a, c	E	N9	C1	LM10X	LM10X	LM10X	This study
<i>Natrix h. helvetica</i>	Jersey: Les Mielles NR	a, b, c	E	N9	C1	LM11X	LM11X	LM11X	This study
<i>Natrix h. helvetica</i>	Jersey: Les Mielles NR	a, c	E	N9	C1	LM13X	LM13X	LM13X	This study
<i>Natrix h. helvetica</i>	Jersey: Les Mielles NR	a, c	E	N9	C1	LM16X	LM16X	LM16X	This study
<i>Natrix h. helvetica</i>	Jersey: Les Mielles NR	a, c	E	N9	C1	LM17X	LM17X	LM17X	This study
<i>Natrix h. helvetica</i>	Jersey: Les Mielles NR	a, c	E	N9	C1	LM19X	LM19X	LM19X	This study
<i>Natrix h. helvetica</i>	Jersey: Les Mielles NR	a, c	E	N9	C1	LMDX	LMDX	LMDX	This study
<i>Natrix h. helvetica</i>	Jersey: Les Mielles NR	a, c	E	N9	—	LM02X	LM02X	—	This study
<i>Natrix h. helvetica</i>	Jersey: Les Mielles NR	a, c	E	N9	—	LM03X	LM03X	—	This study
<i>Natrix h. helvetica</i>	Jersey: Les Mielles NR	a, c	E	N9	—	LM06X	LM06X	—	This study
<i>Natrix h. helvetica</i>	Jersey: Les Mielles NR	a, c	E	N9	—	LM09X	LM09X	—	This study
<i>Natrix h. helvetica</i>	Jersey: Les Mielles NR	a, c	E	N9	—	LM14X	LM14X	—	This study
<i>Natrix h. helvetica</i>	Jersey: Les Mielles NR	a, c	E	N9	—	LM15X	LM15X	—	This study
<i>Natrix h. helvetica</i>	Jersey: Les Mielles NR	a, c	E	N9	—	LM21X	LM21X	—	This study
<i>Natrix h. helvetica</i>	Jersey: Les Mielles NR	a, c	E	N9	—	LM22X	LM22X	—	This study
<i>Natrix h. helvetica</i>	Jersey: L'Oelliere	a, c	E	N9	C1	LOU01X	LOU01X	LOU01X	This study
<i>Natrix h. helvetica</i>	Jersey: Ouaisné	a, b, c	E	N9	C1	OU01Y	OU01Y	OU01Y	This study
<i>Natrix h. helvetica</i>	Jersey: Ouaisné	a, c	E	N9	C1	OU02	OU02	OU02	This study
<i>Natrix h. helvetica</i>	Jersey: Ouaisné	a, c	E	N9	C1	OU02X	OU02X	OU02X	This study
<i>Natrix h. helvetica</i>	Jersey: Ouaisné	a, c	E	N9	C1	OU03	OU03	OU03	This study
<i>Natrix h. helvetica</i>	Jersey: Ouaisné	a, c	E	N9	C1	OU03X	OU03X	OU03X	This study
<i>Natrix h. helvetica</i>	Jersey: Ouaisné	a, c	E	N9	C1	OU04X	OU04X	OU04X	This study
<i>Natrix h. helvetica</i>	Jersey: Ouaisné	a, b, c	E	N9	C1	OU05X	OU05X	OU05X	This study
<i>Natrix h. helvetica</i>	Jersey: Ouaisné	a, b, c	E	N9	C1	OU06X	OU06X	OU06X	This study
<i>Natrix h. helvetica</i>	Jersey: Ouaisné	a, c	E	N9	C1	OU07X	OU07X	OU07X	This study
<i>Natrix h. helvetica</i>	Jersey: Ouaisné	a, b, c	E	N9	—	OU04	OU04	—	This study
<i>Natrix h. helvetica</i>	Jersey: Noirmont	a, c	E	N9	C1	NO01	NO01	NO01	This study
<i>Natrix h. helvetica</i>	Jersey: Noirmont	a, c	E	N9	C1	NO02	NO02	NO02	This study
<i>Natrix h. helvetica</i>	Jersey: Noirmont	a, c	E	N9	—	NO01X	NO01X	—	This study
<i>Natrix h. helvetica</i>	Jersey: St Ouen's Pond	a, c	E	N9	C1	SO01	SO01	SO01	This study

<b>Table S5.4 (continued)</b>			mtDNA	Haplotype		Accession numbers <sup>†</sup>			Reference
Taxon	Location	Analysis	Clade*	ND4	Cyt <i>b</i>	Voucher <sup>†</sup>	ND4+tRNAs	Cyt <i>b</i>	
<i>Natrix h. helvetica</i>	Jersey: Woodbine Corner	a, c	E	N9	C1	WB01	WB01	WB01	This study
<i>Natrix h. helvetica</i>	Jersey: Woodbine Corner	a, c	E	N9	C1	WB01X	WB01X	WB01X	This study
<i>Natrix h. helvetica</i>	Jersey: Woodbine Corner	a, c	E	N9	C1	WB02	WB02	WB02	This study
<i>Natrix h. helvetica</i>	Jersey: Woodbine Corner	a, c	E	N9	C1	WB02X	WB02X	WB02X	This study
<i>Natrix h. helvetica</i>	Jersey: Woodbine Corner	a, c	E	N9	C1	WB03	WB03	WB03	This study
<i>Natrix h. helvetica</i>	France: Bressolettes	a, c	E	N9	—	MB10	MB10	—	This study
<i>Natrix h. helvetica</i>	France: Carentan	a	—	—	—	MB07	—	—	This study
<i>Natrix h. helvetica</i>	France: Cne. de Saint Brice en Cogles	a, b, c	E	N9	C1	FP01	FP01	FP01	This study
<i>Natrix h. helvetica</i>	France: Cne. de Saint Germain en Cogles	a, c	E	N9	C1	FP02	FP02	FP02	This study
<i>Natrix h. helvetica</i>	France: Créances	a, c	E	N9	—	MB02	MB02	—	This study
<i>Natrix h. helvetica</i>	France: La Feuillie	a, c	E	N9	—	MB05	MB05	—	This study
<i>Natrix h. helvetica</i>	France: Lessay	a, b, c	E	N9	—	MB01	MB01	—	This study
<i>Natrix h. helvetica</i>	France: Lithaire	a, b, c	E	N9	—	MB09	MB09	—	This study
<i>Natrix h. helvetica</i>	France: Millières	a, c	E	N9	—	MB04	MB04	—	This study
<i>Natrix h. helvetica</i>	France: Pirou	a, c	E	N9	—	MB03	MB03	—	This study
<i>Natrix h. helvetica</i>	France: Saint-Lô	a, c	E	N9	—	MB08	MB08	—	This study
<i>Natrix h. helvetica</i>	France: Saint-Patrice- de- Clajds	a, c	E	N9	—	MB06	MB06	—	This study
<b>Samples from other regions</b>									
<i>Natrix h. helvetica</i>	Great Britain: Buckinghamshire: Chesham	b, c	E	N1	—	MTD T 14130	LT839092	LT839229	Kindler <i>et al.</i> , 2017
<i>Natrix h. helvetica</i>	Great Britain: Denbighshire	c	E	N1	—	MTD T 14144	LT839092	—	Kindler <i>et al.</i> , 2017
<i>Natrix h. helvetica</i>	Great Britain: Dorset: Bovington	c	E	N1	—	MTD T 14131	LT839097	—	Kindler <i>et al.</i> , 2017
<i>Natrix h. helvetica</i>	Great Britain: Dorset	c	E	N1	C1	MTD T 14165	LT839092	LT839229	Kindler <i>et al.</i> , 2017
<i>Natrix h. helvetica</i>	Great Britain: Dorset	b, c	E	N1	C1	MTD T 14166	LT839092	LT839229	Kindler <i>et al.</i> , 2017
<i>Natrix h. helvetica</i>	Great Britain: Dorset: Christchurch	b, c	E	N1	—	MTD T 14132	LT839092	LT839229	Kindler <i>et al.</i> , 2017
<i>Natrix h. helvetica</i>	Great Britain: Essex: Loughton	c	E	N1	C1	MTD T 14117	LT839092	LT839229	Kindler <i>et al.</i> , 2017
<i>Natrix h. helvetica</i>	Great Britain: Essex: Loughton	c	E	N1	C1	MTD T 14118	LT839092	LT839229	Kindler <i>et al.</i> , 2017
<i>Natrix h. helvetica</i>	Great Britain: Essex: Loughton	c	E	N1	C1	MTD T 14119	LT839092	LT839229	Kindler <i>et al.</i> , 2017

Table S5.4 (continued)			mtDNA	Haplotype		Accession numbers <sup>†</sup>			Reference
Taxon	Location	Analysis	Clade*	ND4	Cyt <i>b</i>	Voucher <sup>†</sup>	ND4+tRNAs	Cyt <i>b</i>	
<i>Natrix h. helvetica</i>	Great Britain: Essex: Loughton	c	E	N1	C1	MTD T 14120	LT839092	LT839229	Kindler <i>et al.</i> , 2017
<i>Natrix h. helvetica</i>	Great Britain: Essex: Loughton	c	E	N1	C1	MTD T 14121	LT839092	LT839229	Kindler <i>et al.</i> , 2017
<i>Natrix h. helvetica</i>	Great Britain: Essex: Loughton	c	E	N1	C1	MTD T 14122	LT839092	LT839229	Kindler <i>et al.</i> , 2017
<i>Natrix h. helvetica</i>	Great Britain: Gloucestershire: Milkwall	b, c	E	N1	C1	MTD T 14136	LT839092	LT839229	Kindler <i>et al.</i> , 2017
<i>Natrix h. helvetica</i>	Great Britain: Herefordshire: Tedstone Wafre	c	E	N1	—	MTD T 14137	LT839092	—	Kindler <i>et al.</i> , 2017
<i>Natrix h. helvetica</i>	Great Britain: Herefordshire: 4 km E Ledbury	c	E	N1	C1	MTD T 14124	LT839092	LT839229	Kindler <i>et al.</i> , 2017
<i>Natrix h. helvetica</i>	Great Britain: Herefordshire: Abbey Dore	c	E	N1	C1	MTD T 14139	LT839092	LT839229	Kindler <i>et al.</i> , 2017
<i>Natrix h. helvetica</i>	Great Britain: Herefordshire: Ledbury	c	E	N8	C1	MTD T 14123	LT839093	LT839229	Kindler <i>et al.</i> , 2017
<i>Natrix h. helvetica</i>	Great Britain: Hertfordshire: Hatfield	b, c	E	N1	C5	MTD T 14167	LT839092	MF767286	Kindler <i>et al.</i> , 2017; Von Plettenberg Laing <i>et al.</i> , in prep.
<i>Natrix h. helvetica</i>	Great Britain: Kent: Hildenborough	c	E	N1	C1	MTD T 14133	LT839092	LT839229	Kindler <i>et al.</i> , 2017
<i>Natrix h. helvetica</i>	Great Britain: Norfolk: Cirby Cane	a, c	E	N1	C1	MTD T 14116	LT839092	LT839229	Kindler <i>et al.</i> , 2017
<i>Natrix h. helvetica</i>	Great Britain: Northamptonshire	c	E	N1	—	MTD T 14141	LT839092	—	Kindler <i>et al.</i> , 2017
<i>Natrix h. helvetica</i>	Great Britain: Nottinghamshire: Jacksdale	c	E	N1	C1	MTD T 14140	LT839092	LT839229	Kindler <i>et al.</i> , 2017
<i>Natrix h. helvetica</i>	Great Britain: Pembrokeshire: Talbenny	b, c	E	N1	—	MTD T 14113	LT839092	—	Kindler <i>et al.</i> , 2017
<i>Natrix h. helvetica</i>	Great Britain: Scotland	c	E	N1	C1	MTD T 14127	LT839092	LT839229	Kindler <i>et al.</i> , 2017
<i>Natrix h. helvetica</i>	Great Britain: Scotland: near Scotland	c	E	N1	C1	MTD T 14126	LT839092	LT839229	Kindler <i>et al.</i> , 2017
<i>Natrix h. helvetica</i>	Great Britain: Scotland: near Scotland	c	E	N1	C1	MTD T 14128	LT839092	LT839229	Kindler <i>et al.</i> , 2017

Table S5.4 (continued)			mtDNA	Haplotype		Accession numbers <sup>†</sup>			Reference
Taxon	Location	Analysis	Clade*	ND4	Cyt <i>b</i>	Voucher <sup>†</sup>	ND4+tRNAs	Cyt <i>b</i>	
<i>Natrix h. helvetica</i>	Great Britain: Staffordshire: between Wombourn and Sedgley	c	E	N1	C1	MTD T 14142	LT839092	LT839229	Kindler <i>et al.</i> , 2017
<i>Natrix h. helvetica</i>	Great Britain: Suffolk: Martlesham	c	E	N1	C1	MTD T 14163	LT839092	LT839229	Kindler <i>et al.</i> , 2017
<i>Natrix h. helvetica</i>	Great Britain: Suffolk: Martlesham	c	E	N1	C1	MTD T 14164	LT839092	LT839229	Kindler <i>et al.</i> , 2017
<i>Natrix h. helvetica</i>	Great Britain: Surrey: Milford	c	E	N1	C1	MTD T 14134	LT839092	LT839229	Kindler <i>et al.</i> , 2017
<i>Natrix h. helvetica</i>	Great Britain: Sussex: Heathfield	b, c	E	N1	C6	MTD T 14135	LT839092	LT839229	Kindler <i>et al.</i> , 2017
<i>Natrix h. helvetica</i>	Great Britain: Warwickshire: Oxhill	c	E	N1	C1	MTD T 14143	LT839092	LT839229	Kindler <i>et al.</i> , 2017
<i>Natrix h. helvetica</i>	Great Britain: Yorkshire: Bradford	b, c	E	N7	C1	MTD T 14129	LT839092	LT839229	Kindler <i>et al.</i> , 2017
<i>Natrix h. helvetica</i>	Great Britain: Kent: Isle of Sheppey	c	E	N1	C1	LSUMZ41506	AY873710	AY866544	Guicking <i>et al.</i> , 2006
<i>Natrix h. helvetica</i>	Great Britain: Norfolk: Norwich: Brandon	b, c	E	N1	C7	e6116x11B	KC570253	KC570297	Kindler <i>et al.</i> , 2013
<i>Natrix h. helvetica</i>	Great Britain: North Wales: Gwaith Powder	b, c	E	N1	—	MTD T 9982	HF679626	—	Kindler <i>et al.</i> , 2013
<i>Natrix h. helvetica</i>	Great Britain: North Wales: Gwaith Powder	b, c	E	N1	—	MTD T 9983	HF679627	—	Kindler <i>et al.</i> , 2013
<i>Natrix n. natrix</i>	Austria: Lower Austria: Bergern	b	4	—	—	MTD T 9912	HF679668	HF679976	Kindler <i>et al.</i> , 2013
<i>Natrix n. natrix</i>	Austria: Vienna: Donauinsel	b	4	—	—	MTD T 9903	HF679670	HF679979	Kindler <i>et al.</i> , 2013
<i>Natrix n. persa</i>	Azerbaijan: Calilabad	b	1	—	—	MTD T 8956	HF679821	HF680120	Kindler <i>et al.</i> , 2013
<i>Natrix n. persa</i>	Azerbaijan: Qazimammad	b	1	—	—	MTD T 8957	—	HF680122	Kindler <i>et al.</i> , 2013
<i>Natrix n. persa</i>	Bosnia: and Herzegovina: Hutovo Blato	b	5	—	—	MTD T 8644	HF679822	HF680123	Kindler <i>et al.</i> , 2013
<i>Natrix n. persa</i>	Bosnia: and Herzegovina: Hutovo Blato	b	5	—	—	MTD T 8645	HF679823	HF680124	Kindler <i>et al.</i> , 2013
<i>Natrix n. persa</i>	Bosnia: and Herzegovina: Maglič Mountain: Prijedor	b	4	—	—	MTD T 8976	HF679824	HF680125	Kindler <i>et al.</i> , 2013
<i>Natrix n. persa</i>	Bulgaria: Levunovo	b	3	—	—	MTD D 18923	HF679828	HF680129	Kindler <i>et al.</i> , 2013

Table S5.4 (continued)			mtDNA	Haplotype		Accession numbers <sup>†</sup>			Reference
Taxon	Location	Analysis	Clade*	ND4	Cyt <i>b</i>	Voucher <sup>†</sup>	ND4+tRNAs	Cyt <i>b</i>	
<i>Natrix n. persa</i>	Bulgaria: Malko Tarnovo District	b	7	—	—	CAS 219930	AY873717	AY866542	Guicking <i>et al.</i> , 2006
<i>Natrix n. persa</i>	Bulgaria: Mičurin	b	4	—	—	MTD D 29984	HF679831	HF680132	Kindler <i>et al.</i> , 2013
<i>Natrix n. persa</i>	Bulgaria: Pejo Javorov	b	3	—	—	MTD T 9012	HF679832	HF680134	Kindler <i>et al.</i> , 2013
<i>Natrix n. persa</i>	Bulgaria: Ropotamo	b	7	—	—	MTD T 9318	HF679834	HF680136	Kindler <i>et al.</i> , 2013
<i>Natrix h. helvetica</i>	Croatia: Istria: Rovinj	b	4	—	—	MTD D 32031	HF679608	HF679928	Kindler <i>et al.</i> , 2013
<i>Natrix h. helvetica</i>	Croatia: Krk	b	4	—	—	MTD D 20790	HF679610	HF679930	Kindler <i>et al.</i> , 2013
<i>Natrix n. cypriaca</i>	Cyprus: Larnaka: Paralimni	b	7	—	—	ZFMK 76753	HF679604	HF679922	Kindler <i>et al.</i> , 2013
<i>Natrix n. cypriaca</i>	Cyprus: Xyliatos Dam	b	7	—	—	ZFMK 54318	—	HF679923	Kindler <i>et al.</i> , 2013
<i>Natrix n. natrix</i>	Czech: Chřiby Mountains: Halenkovice	b	4	—	—	MTD T 9886	HF679678	HF679985	Kindler <i>et al.</i> , 2013
<i>Natrix n. natrix</i>	Denmark: Funen: NNW Svendborg	b	3	—	—	MTD T 9653	HF679698	HF680001	Kindler <i>et al.</i> , 2013
<i>Natrix n. natrix</i>	Denmark: Langeland: S Tranekær	b	3	—	—	MTD T 9651	HF679705	HF680008	Kindler <i>et al.</i> , 2013
<i>Natrix natrix</i>	Finland: Åland	b	3	—	—	MTD T 11583	LL999849	LL999899	Kindler <i>et al.</i> , 2014
<i>Natrix natrix</i>	Finland: Åland: Finström	b	3	—	—	MTD T 11589	LL999852	LL999902	Kindler <i>et al.</i> , 2014
<i>Natrix natrix</i>	Finland: Southern Finland: Hanko	b	8	—	—	MTD T 11585	LL999856	LL999906	Kindler <i>et al.</i> , 2014
<i>Natrix natrix</i>	Finland: Western Finland: near Kaarina	b	8	—	—	MTD T 11590	LL999857	LL999907	Kindler <i>et al.</i> , 2014
<i>Natrix n. persa</i>	Former Yugoslav Republic of Macedonia: Jakubica Mountains	b	3	—	—	MTD T 9911	HF679844	HF680143	Kindler <i>et al.</i> , 2013
<i>Natrix n. persa</i>	Former Yugoslav Republic of Macedonia: National Park Galičica	b	5	—	—	MTD T 9877	HF679845	HF680144	Kindler <i>et al.</i> , 2013
<i>Natrix n. persa</i>	Former Yugoslav Republic of Macedonia: Porodin	b	5	—	—	MTD T 9909	HF679846	HF680145	Kindler <i>et al.</i> , 2013
<i>Natrix h. helvetica</i>	France: Alpes-de-Haute-Provence: Clumanc	c	E	N3	—	BEV.1690	LN994781	—	Pokrant <i>et al.</i> , 2016
<i>Natrix h. helvetica</i>	France: Ardèche: 10 km N Tournon-sur-Rhône	b, c	E	N1	C1	ZFMK 54711	HF679617	HF679934	Kindler <i>et al.</i> , 2013
<i>Natrix astreptophora</i>	France: Argelès-sur-Mer	b	Eu	—	—	ZFMK 89090	HF679590	HF679907	Kindler <i>et al.</i> , 2013



Table S5.4 (continued)			mtDNA	Haplotype		Accession numbers <sup>†</sup>			Reference
Taxon	Location	Analysis	Clade*	ND4	Cyt <i>b</i>	Voucher <sup>†</sup>	ND4+tRNAs	Cyt <i>b</i>	
<i>Natrix h. helvetica</i>	France: Aveyron: Peyrusse-le-Roc	c	E	N1	—	BEV.6430	LN994782	—	Pokrant <i>et al.</i> , 2016
<i>Natrix h. helvetica</i>	France: Bouches-du-Rhône: between Fos-sur-Mer and Port-Saint-Louis-du-Rhône	c	E	N1	—	BEV.9397	LN994783	—	Pokrant <i>et al.</i> , 2016
<i>Natrix h. helvetica</i>	France: Bouches-du-Rhône: Camargue	c	E	N1	—	ZFMK 54712	HF679614	—	Kindler <i>et al.</i> , 2013
<i>Natrix h. helvetica</i>	France: Bouches-du-Rhône: Camargue: Clos de Lange	c	E	N1	—	BEV.6425	LN994785	—	Pokrant <i>et al.</i> , 2016
<i>Natrix h. helvetica</i>	France: Bouches-du-Rhône: Camargue: Marais du Grenouillet	c	E	N1	—	BEV.284	LN994784	—	Pokrant <i>et al.</i> , 2016
<i>Natrix h. helvetica</i>	France: Bouches-du-Rhône: Camargue: Salin de Badon	c	E	N1	C1	ZMH R07572	LN994786	LN994826	Pokrant <i>et al.</i> , 2016
<i>Natrix h. helvetica</i>	France: Bouches-du-Rhône: Camargue: Salin de Badon	c	E	N1	C1	ZMH R07573	LN994787	LN994827	Pokrant <i>et al.</i> , 2016
<i>Natrix h. helvetica</i>	France: Bouches-du-Rhône: Camargue: Tour du Valat	c	E	N1	C1	ZMH R06950	LN994788	LN994828	Pokrant <i>et al.</i> , 2016
<i>Natrix h. helvetica</i>	France: Bouches-du-Rhône: Camargue: Tour du Valat	c	E	N1	C1	ZMH R06951	LN994789	LN994829	Pokrant <i>et al.</i> , 2016
<i>Natrix h. helvetica</i>	France: Bouches-du-Rhône: Camargue: Tour du Valat	c	E	N1	C1	ZMH R06952	LN994790	LN994830	Pokrant <i>et al.</i> , 2016
<i>Natrix h. helvetica</i>	France: Bouches-du-Rhône: Camargue: Tour du Valat	c	E	N1	C1	ZMH R07062	LN994791	LN994831	Pokrant <i>et al.</i> , 2016
<i>Natrix h. helvetica</i>	France: Bouches-du-Rhône: Camargue: Tour du Valat	c	E	N1	—	ZMH R06997	LN994792	—	Pokrant <i>et al.</i> , 2016
<i>Natrix h. helvetica</i>	France: Bouches-du-Rhône: Saint-Martin-de-Crau: Mas de Pernes	c	E	N1	—	BEV.9401	LN994793	—	Pokrant <i>et al.</i> , 2016
<i>Natrix h. helvetica</i>	France: Bouches-du-Rhône: St. Martin de Crau	b, c	E	N1	C1	ZFMK 54710	HF679618	HF679935	Kindler <i>et al.</i> , 2013
<i>Natrix h. helvetica</i>	France: Cher: St. Amand-Montront	b, c	E	N1	C1	E11102x27F	KC570252	KC570296	Kindler <i>et al.</i> , 2013
<i>Natrix h. corsa</i>	France: Corsica: Étang de Loto	b	B	—	—	MTD T 9902	HF679601	—	Kindler <i>et al.</i> , 2013

<b>Table S5.4 (continued)</b>			mtDNA	Haplotype		Accession numbers <sup>†</sup>			Reference
Taxon	Location	Analysis	Clade*	ND4	Cyt <i>b</i>	Voucher <sup>†</sup>	ND4+tRNAs	Cyt <i>b</i>	
<i>Natrix h. corsa</i>	France: Corsica: Gulf of Sagone	b	B	—	—	MTD D 39091	HF679602	HF679921	Kindler <i>et al.</i> , 2013
<i>Natrix h. corsa</i>	France: Corsica: Santa Giulia	b	B	—	—	MTD D 42489	HE584627	HE584628	Fritz <i>et al.</i> , 2012
<i>Natrix h. helvetica</i>	France: Deux-Sèvres: near Niort	c	E	N1	C1	MTD T 11950	LN994794	LN994832	Pokrant <i>et al.</i> , 2016
<i>Natrix h. helvetica</i>	France: Gard: Saint-Hippolyte-du-Fort	c	E	N6	—	BEV.9400	LN994795	—	Pokrant <i>et al.</i> , 2016
<i>Natrix h. helvetica</i>	France: Gironde: near Léognan: Minoy	c	E	N1	C1	BEV.T9260	LN994796	LN994833	Pokrant <i>et al.</i> , 2016
<i>Natrix h. helvetica</i>	France: Gironde: near Léognan: Minoy	c	E	N1	C1	BEV.T9261	LN994797	LN994834	Pokrant <i>et al.</i> , 2016
<i>Natrix h. helvetica</i>	France: Hérault: Avène dam	c	E	N1	C1	BEV.T9264	LN994798	LN994835	Pokrant <i>et al.</i> , 2016
<i>Natrix h. helvetica</i>	France: Hérault: between Le Bosc and Loiras	c	E	N1	—	BEV.9049	LN994799	—	Pokrant <i>et al.</i> , 2016
<i>Natrix h. helvetica</i>	France: Hérault: Lansargues	c	E	N1	—	BEV.9029	LN994800	—	Pokrant <i>et al.</i> , 2016
<i>Natrix h. helvetica</i>	France: Hérault: Lattes	c	E	N1	C1	BEV.T9266	LN994801	LN994836	Pokrant <i>et al.</i> , 2016
<i>Natrix h. helvetica</i>	France: Hérault: Le Mas Blanc	c	E	N1	C1	BEV.T9259	LN994803	LN994838	Pokrant <i>et al.</i> , 2016
<i>Natrix h. helvetica</i>	France: Hérault: Le Mas Blanc	c	E	N5	C1	BEV.T9258	LN994802	LN994837	Pokrant <i>et al.</i> , 2016
<i>Natrix h. helvetica</i>	France: Hérault: Massif du Caroux	c	E	N1	—	BEV.8499	LN994804	—	Pokrant <i>et al.</i> , 2016
<i>Natrix h. helvetica</i>	France: Hérault: Saint-Pierre-de-la-Fage	c	E	N1	—	BEV.847	LN994805	—	Pokrant <i>et al.</i> , 2016
<i>Natrix h. helvetica</i>	France: Isère: Clelles	c	E	N1	—	BEV.9293	LN994806	—	Pokrant <i>et al.</i> , 2016
<i>Natrix h. helvetica</i>	France: Loire: Sainte-Foy-Saint-Sulpice	c	E	N1	—	BEV.8324	LN994807	—	Pokrant <i>et al.</i> , 2016
<i>Natrix h. helvetica</i>	France: N La Londe les Maures	c	E	—	C1	MTD D 40712	—	HF679933	Kindler <i>et al.</i> , 2013
<i>Natrix h. helvetica</i>	France: Puy-de-Dôme: Massif Central: Monaux	b, c	E	N1	—	MTD T 10092	HF679616	—	Kindler <i>et al.</i> , 2013

Table S5.4 (continued)									
Taxon	Location	Analysis	mtDNA Clade*	Haplotype		Voucher†	Accession numbers†		Reference
				ND4	Cyt <i>b</i>		ND4+tRNAs	Cyt <i>b</i>	
<i>Natrix h. helvetica</i>	France: Pyrénées-Atlantiques: St. Jean de Luz	c	E	N1	—	MTD T 11999	LN994808	—	Pokrant <i>et al.</i> , 2016
<i>Natrix astreptophora</i>	France: Pyrénées-Orientales: Millas	b	Eu	—	—	MTD T 13082	LN994771	LN994817	Pokrant <i>et al.</i> , 2016
<i>Natrix h. helvetica</i>	France: Saône-et-Loire: Anost	b, c	E	N1	C1	ZFMK 61095	HF679613	HF679931	Kindler <i>et al.</i> , 2013
<i>Natrix h. helvetica</i>	France: Saône-et-Loire: Charolles	b, c	E	N1	C4	ZFMK 64930	HF679615	HF679932	Kindler <i>et al.</i> , 2013
<i>Natrix h. helvetica x natrix</i>	France: Strasbourg	b, c	E	N1	—	MTD T 10091	HF679628	—	Kindler <i>et al.</i> , 2013
<i>Natrix h. helvetica</i>	France: Vaucluse: Saint-Saturnin-lès-Apt	c	E	N1	—	BEV.11530	LN994809	—	Pokrant <i>et al.</i> , 2016
<i>Natrix h. helvetica</i>	France: Vicinity of Paris	b, c	E	N1	C1	—	AY873736	AY866537	Guicking <i>et al.</i> , 2006
<i>Natrix n. persa</i>	Georgia: Borshomi	b	8	—	—	ZFMK 73721	HF679850	HF680149	Kindler <i>et al.</i> , 2013
<i>Natrix n. persa</i>	Georgia: Kumisi	b	2	—	—	MTD T 9337	HF679851	HF680151	Kindler <i>et al.</i> , 2013
<i>Natrix n. persa</i>	Georgia: Sakdrioni	b	2	—	—	MTD T 9338	HF679856	HF680156	Kindler <i>et al.</i> , 2013
<i>Natrix h. helvetica</i>	Germany: Baden-Württemberg: between Leonberg and Gerlingen	b	3	—	—	ZFMK 78777	—	HF680013	Kindler <i>et al.</i> , 2013
<i>Natrix h. helvetica x natrix</i>	Germany: Baden-Württemberg: Lake Constance: Radolfzell	b	3	—	—	—	AY487792	AY487727	Guicking <i>et al.</i> , 2006
<i>Natrix n. natrix</i>	Germany: Baden-Württemberg: Oberweissach	b	3	—	—	MTD D 39068	HF679711	HF680014	Kindler <i>et al.</i> , 2013
<i>Natrix n. natrix</i>	Germany: Bavaria: Passau	b	4	—	—	ZFMK 56016	HF679713	HF680016	Kindler <i>et al.</i> , 2013
<i>Natrix n. natrix</i>	Germany: Brandenburg: Cumlosen	b	4	—	—	MTD D 29503	HF679715	HF680017	Kindler <i>et al.</i> , 2013
<i>Natrix n. natrix</i>	Germany: Brandenburg: Hosena	b	3	—	—	MTD D 45304	HF679717	HF680020	Kindler <i>et al.</i> , 2013
<i>Natrix n. natrix</i>	Germany: Brandenburg: Perleberg	b	3	—	—	MTD D 29504	HF679718	HF680021	Kindler <i>et al.</i> , 2013
<i>Natrix n. natrix</i>	Germany: Brandenburg: Senftenberg	b	3	—	—	ZFMK 76358	HF679719	HF680022	Kindler <i>et al.</i> , 2013

Table S5.4 (continued)			mtDNA	Haplotype		Accession numbers <sup>†</sup>			Reference
Taxon	Location	Analysis	Clade*	ND4	Cyt <i>b</i>	Voucher <sup>†</sup>	ND4+tRNAs	Cyt <i>b</i>	
<i>Natrix natrix</i>	Germany: Mecklenburg-Western Pomerania: Usedom	b	3	—	—	MTD T 11483	LL999880	LL999930	Kindler <i>et al.</i> , 2014
<i>Natrix h. helvetica x natrix</i>	Germany: Hesse: Herborn	c	E	N1	C1	ZFMK 89403	HF679630	HF679940	Kindler <i>et al.</i> , 2013
<i>Natrix h. helvetica x natrix</i>	Germany: Hesse: Hofheim/Lorsbuch	b, c	E	N1	C2	MTD D 35776	HF679631	HF679941	Kindler <i>et al.</i> , 2013
<i>Natrix h. helvetica x natrix</i>	Germany: Hesse: Kelkheim/Taunus	c	E	N4	C2	—	AY873711	AY866538	Guicking <i>et al.</i> , 2006
<i>Natrix n. natrix</i>	Germany: Hesse: Steinau	b	4	—	—	ZFMK 82930	HF679721	HF680024	Kindler <i>et al.</i> , 2013
<i>Natrix n. natrix</i>	Germany: Lower Saxony: Lachendorf	b	3	—	—	ZFMK 86134	HF679722	HF680025	Kindler <i>et al.</i> , 2013
<i>Natrix n. natrix</i>	Germany: Lower Saxony: Leiferde	b	3	—	—	ZFMK 89088	HF679723	HF680026	Kindler <i>et al.</i> , 2013
<i>Natrix natrix</i>	Germany: Mecklenburg-Western Pomerania: 3 km E Wesenberg	b	3	—	—	MTD T 11466	LL999860	LL999910	Kindler <i>et al.</i> , 2014
<i>Natrix natrix</i>	Germany: Mecklenburg-Western Pomerania: 3 km E Wesenberg	b	3	—	—	MTD T 11474	LL999868	LL999918	Kindler <i>et al.</i> , 2014
<i>Natrix natrix</i>	Germany: Mecklenburg-Western Pomerania: 3 km E Wesenberg	b	3	—	—	MTD T 11477	LL999870	LL999920	Kindler <i>et al.</i> , 2014
<i>Natrix h. helvetica x natrix</i>	Germany: North Rhine-Westphalia: Bad Honnef	b, c	E	N1	C1	ZFMK 68432	HF679633	HF679942	Kindler <i>et al.</i> , 2013
<i>Natrix h. helvetica x natrix</i>	Germany: North Rhine-Westphalia: Bad Honnef	c	E	N1	—	ZFMK 68431	HF679632	—	Kindler <i>et al.</i> , 2013
<i>Natrix h. helvetica x natrix</i>	Germany: North Rhine-Westphalia: Bad Münstereifel	c	E	N1	C1	ZFMK 92193	HF679634	HF679943	Kindler <i>et al.</i> , 2013
<i>Natrix h. helvetica x natrix</i>	Germany: North Rhine-Westphalia: between Bergisch-Gladbach and Herrenstrunden	c	E	N1	—	ZFMK 75088	HF679635	—	Kindler <i>et al.</i> , 2013

Table S5.4 (continued)			mtDNA	Haplotype		Accession numbers <sup>†</sup>			Reference
Taxon	Location	Analysis	Clade*	ND4	Cyt <i>b</i>	Voucher <sup>†</sup>	ND4+tRNAs	Cyt <i>b</i>	
<i>Natrix h. helvetica x natrix</i>	Germany: North Rhine-Westphalia: between Hürtgenwald and Gey	c	E	N1	C1	ZFMK 70420	HF679636	HF679944	Kindler <i>et al.</i> , 2013
<i>Natrix h. helvetica x natrix</i>	Germany: North Rhine-Westphalia: Bonn	b, c	E	N1	C1	ZFMK 92537	HF679640	HF679947	Kindler <i>et al.</i> , 2013
<i>Natrix h. helvetica x natrix</i>	Germany: North Rhine-Westphalia: Bonn	b, c	E	N1	C1	ZFMK 92536	HF679639	HF679946	Kindler <i>et al.</i> , 2013
<i>Natrix h. helvetica x natrix</i>	Germany: North Rhine-Westphalia: Bonn	c	E	N1	C1	ZFMK 68433	HF679637	HF679945	Kindler <i>et al.</i> , 2013
<i>Natrix h. helvetica x natrix</i>	Germany: North Rhine-Westphalia: Bonn	c	E	N1	—	ZFMK 92228	HF679638	—	Kindler <i>et al.</i> , 2013
<i>Natrix h. helvetica x natrix</i>	Germany: North Rhine-Westphalia: Bonn (Kottenforst)	c	E	N1	C1	ZFMK 89393	HF679641	HF679948	Kindler <i>et al.</i> , 2013
<i>Natrix h. helvetica x natrix</i>	Germany: North Rhine-Westphalia: Drove	c	E	N1	—	ZFMK 86786	HF679642	—	Kindler <i>et al.</i> , 2013
<i>Natrix h. helvetica x natrix</i>	Germany: North Rhine-Westphalia: Königswinter, Ittenbach	c	E	N1	C1	ZFMK 82773	HF679643	HF679949	Kindler <i>et al.</i> , 2013
<i>Natrix h. helvetica x natrix</i>	Germany: North Rhine-Westphalia: Nideggen/Eifel	b, c	E	N1	C1	ZFMK 83771	HF679644	HF679950	Kindler <i>et al.</i> , 2013
<i>Natrix h. helvetica x natrix</i>	Germany: North Rhine-Westphalia: Sankt Augustin-Hangelar	b, c	E	N1	C1	ZFMK 89086	HF679645	HF679951	Kindler <i>et al.</i> , 2013
<i>Natrix h. helvetica x natrix</i>	Germany: North Rhine-Westphalia: Wuppertal: Morsbachtal	b, c	E	N1	C1	ZFMK 73645	HF679646	HF679952	Kindler <i>et al.</i> , 2013
<i>Natrix h. helvetica x natrix</i>	Germany: North Rhine-Westphalia: Wuppertal: Vorwerk	c	E	N1	C1	ZFMK 73646	HF679647	HF679953	Kindler <i>et al.</i> , 2013
<i>Natrix h. helvetica x natrix</i>	Germany: Rhineland-Palatinate: Horhausen	b, c	E	N1	C1	ZFMK 80876	HF679648	HF679954	Kindler <i>et al.</i> , 2013
<i>Natrix h. helvetica x natrix</i>	Germany: Rhineland-Palatinate: Lieser	b, c	E	N1	C1	MTD D 35933	HF679649	HF679955	Kindler <i>et al.</i> , 2013

Table S5.4 (continued)			mtDNA	Haplotype		Accession numbers <sup>†</sup>			Reference
Taxon	Location	Analysis	Clade*	ND4	Cyt <i>b</i>	Voucher <sup>†</sup>	ND4+tRNAs	Cyt <i>b</i>	
<i>Natrix h. helvetica x natrix</i>	Germany: Rhineland-Palatinate: Maria Laach	b, c	E	N1	C1	ZFMK 83703	HF679650	HF679956	Kindler <i>et al.</i> , 2013
<i>Natrix h. helvetica x natrix</i>	Germany: Rhineland-Palatinate: Weltersburg	b, c	E	N1	—	ZFMK 80875	HF679651	—	Kindler <i>et al.</i> , 2013
<i>Natrix h. helvetica x natrix</i>	Germany: Saarland: Hirschberg/Neunkirchen	c	E	—	C3	ZFMK 54321	—	HF679957	Kindler <i>et al.</i> , 2013
<i>Natrix n. natrix</i>	Germany: Saxony: Bad Dübén	b	3	—	—	MTD D 39712	HF679725	HF680028	Kindler <i>et al.</i> , 2013
<i>Natrix n. natrix</i>	Germany: Saxony: Bad Gottleuba	b	4	—	—	MTD D 39331	HF679726	HF680029	Kindler <i>et al.</i> , 2013
<i>Natrix n. natrix</i>	Germany: Saxony: Bärnsdorf	b	4	—	—	MTD D 47231	HF679727	HF680030	Kindler <i>et al.</i> , 2013
<i>Natrix n. natrix</i>	Germany: Saxony: Biehla	b	3	—	—	MTD T 3183	HF679728	HF680031	Kindler <i>et al.</i> , 2013
<i>Natrix n. natrix</i>	Germany: Saxony: Borna	b	4	—	—	MTD D 31066	HF679729	HF680032	Kindler <i>et al.</i> , 2013
<i>Natrix n. natrix</i>	Germany: Saxony: Chemnitz	b	3	—	—	MTD D 47317	HF679730	HF680033	Kindler <i>et al.</i> , 2013
<i>Natrix n. natrix</i>	Germany: Saxony: Dahlen	b	3	—	—	MTD D 46238	HF679732	HF680036	Kindler <i>et al.</i> , 2013
<i>Natrix n. natrix</i>	Germany: Saxony: Dorfhain	b	4	—	—	MTD D 47638	HF679733	HF680037	Kindler <i>et al.</i> , 2013
<i>Natrix n. natrix</i>	Germany: Saxony: Dresden	b	3	—	—	MTD D 45527	—	HF680038	Kindler <i>et al.</i> , 2013
<i>Natrix n. natrix</i>	Germany: Saxony: Dresden	b	4	—	—	MTD D 47639	HF679734	HF680039	Kindler <i>et al.</i> , 2013
<i>Natrix n. natrix</i>	Germany: Saxony: Dresden	b	3	—	—	MTD D 47728	HF679735	—	Kindler <i>et al.</i> , 2013
<i>Natrix n. natrix</i>	Germany: Saxony: Hartmannsgrün	b	3	—	—	MTD D 47002	—	HF680044	Kindler <i>et al.</i> , 2013
<i>Natrix n. natrix</i>	Germany: Saxony: Kreischa-Lockmittel	b	4	—	—	MTD D 40611	HF679741	HF680046	Kindler <i>et al.</i> , 2013
<i>Natrix n. natrix</i>	Germany: Saxony: Langebrück	b	4	—	—	MTD D 48178	HF679742	HF680047	Kindler <i>et al.</i> , 2013
<i>Natrix n. natrix</i>	Germany: Saxony: Linz	b	4	—	—	MWLK 76/01	HF679744	HF680050	Kindler <i>et al.</i> , 2013
<i>Natrix n. natrix</i>	Germany: Saxony: Oberschöna	b	4	—	—	MTD D 47429	HF679749	HF680055	Kindler <i>et al.</i> , 2013
<i>Natrix n. natrix</i>	Germany: Saxony: Oppach	b	3	—	—	MTD D 32501	HF679750	HF680056	Kindler <i>et al.</i> , 2013
<i>Natrix n. natrix</i>	Germany: Saxony: Ottendorf-Okrilla	b	3	—	—	MTD D 42679	HF679751	HF680057	Kindler <i>et al.</i> , 2013
<i>Natrix n. natrix</i>	Germany: Saxony: Waldenburg	b	3	—	—	MTD D 42680	HF679754	HF680061	Kindler <i>et al.</i> , 2013

Table S5.4 (continued)									
Taxon	Location	Analysis	mtDNA Clade*	Haplotype		Voucher <sup>†</sup>	Accession numbers <sup>†</sup>		Reference
				ND4	Cyt <i>b</i>		ND4+tRNAs	Cyt <i>b</i>	
<i>Natrix n. natrix</i>	Germany: Saxony: Weixdorf	b	4	—	—	MTD D 47258	HF679755	HF680062	Kindler <i>et al.</i> , 2013
<i>Natrix n. natrix</i>	Germany: Schleswig-Holstein: Kiel: Landwehr	b	3	—	—	ZFMK 62405	HF679759	HF680066	Kindler <i>et al.</i> , 2013
<i>Natrix n. natrix</i>	Germany: Schleswig-Holstein: Probstei: Hagener Moor	b	3	—	—	ZFMK 92535	HF679761	HF680068	Kindler <i>et al.</i> , 2013
<i>Natrix n. schweizeri</i>	Greece: Cyclades: Milos	b	3	—	—	MTD D 19183	—	HF680193	Kindler <i>et al.</i> , 2013
<i>Natrix n. schweizeri</i>	Greece: Cyclades: Milos	b	3	—	—	ZFMK 85407	HF679895	—	Kindler <i>et al.</i> , 2013
<i>Natrix n. persa</i>	Greece: Cyclades: Paros	b	4	—	—	MTD D 25827	HF679861	—	Kindler <i>et al.</i> , 2013
<i>Natrix n. persa</i>	Greece: Dadía	b	7	—	—	MTD T 8646	HF679862	HF680161	Kindler <i>et al.</i> , 2013
<i>Natrix n. persa</i>	Greece: Little Prespa Lake near Florina	b	5	—	—	ZFMK 54702	HF679864	HF680163	Kindler <i>et al.</i> , 2013
<i>Natrix n. persa</i>	Greece: Peloponnesus: Pylos	b	5	—	—	ZFMK 86043	HF679872	HF680170	Kindler <i>et al.</i> , 2013
<i>Natrix n. persa</i>	Greece: Samos: Agios Konstantinos	b	7	—	—	ZFMK 65117	—	HF680172	Kindler <i>et al.</i> , 2013
<i>Natrix n. persa</i>	Greece: Skiros	b	3	—	—	MTD T 9907	HF679874	HF680173	Kindler <i>et al.</i> , 2013
<i>Natrix n. natrix x persa</i>	Hungary: Barcs	b	4	—	—	ZFMK 65686	HF679818	HF680116	Kindler <i>et al.</i> , 2013
<i>Natrix n. natrix</i>	Hungary: between Sarród and Fertóujlak	b	4	—	—	ZFMK 88062	HF679764	HF680070	Kindler <i>et al.</i> , 2013
<i>Natrix n. natrix x persa</i>	Hungary: Villány	b	4	—	—	MTD T 7594	HF679819	HF680118	Kindler <i>et al.</i> , 2013
<i>Natrix n. persa</i>	Iran: Kermanshah Province	b	1	—	—	—	AY487800	AY487756	Guicking <i>et al.</i> , 2004
<i>Natrix n. persa</i>	Iran: Nowshahr	b	1	—	—	MTD T 8954	HF679880	HF680176	Kindler <i>et al.</i> , 2013
<i>Natrix h. lanzai</i>	Italy: Apulia: Torre San Gennaro (Brindisi)	b	D	—	—	—	AY873715	AY487733	Guicking <i>et al.</i> , 2006
<i>Natrix h. lanzai</i>	Italy: Lazio: Monti della Tolfa	b	F	—	—	MZUF 31620	HF679654	HF679959	Kindler <i>et al.</i> , 2013
<i>Natrix h. cetti</i>	Italy: Sardinia: Limbara Mountains	b	B	—	—	ZFMK 60737	HF679600	HF679920	Kindler <i>et al.</i> , 2013
<i>Natrix h. sicula</i>	Italy: Sicily: Lago di Pergusa	b	A	—	—	MZUF 24175	—	HF680199	Kindler <i>et al.</i> , 2013
<i>Natrix h. sicula</i>	Italy: Sicily: Siracusa: Vendicari	b	A	—	—	MTD T 8464	HF679902	HF680200	Kindler <i>et al.</i> , 2013

Table S5.4 (continued)			mtDNA	Haplotype		Accession numbers <sup>†</sup>			Reference
Taxon	Location	Analysis	Clade*	ND4	Cyt b	Voucher <sup>†</sup>	ND4+tRNAs	Cyt b	
<i>Natrix h. helvetica</i>	Italy: Trentino: Vela	b	C	—	—	MTD T 9656	HF679619	HF679936	Kindler <i>et al.</i> , 2013
<i>Natrix h. lanzai</i>	Italy: Tuscany: Chiaveretto	b	F	—	—	ZFMK 64931	HF679655	HF679960	Kindler <i>et al.</i> , 2013
<i>Natrix h. helvetica</i>	Italy: Venezia: NW Asiago	b	C	—	—	e6116x3I	KC570255	KC570299	Kindler <i>et al.</i> , 2013
<i>Natrix n. natrix</i>	Lithuania: Marcinkonys	b	8	—	—	MTD T 8967	HF679772	HF680078	Kindler <i>et al.</i> , 2013
<i>Natrix n. natrix</i>	Lithuania: Trasininkas	b	8	—	—	MTD T 8968	HF679773	HF680079	Kindler <i>et al.</i> , 2013
<i>Natrix n. persa</i>	Montenegro: Sasovići, Zelenica, Boka kotorska	b	5	—	—	MTD T 9875	HF679882	HF680178	Kindler <i>et al.</i> , 2013
<i>Natrix n. persa</i>	Montenegro: Skutari Lake: Limljani	b	5	—	—	ZFMK 92205	HF679883	HF680179	Kindler <i>et al.</i> , 2013
<i>Natrix natrix</i>	Norway: Akershus: Ski	b	3	—	—	MTD T 11568	LL999882	LL999932	Kindler <i>et al.</i> , 2014
<i>Natrix natrix</i>	Norway: Holmestrand: Hallingsrud	b	3	—	—	MTD T 11566	LL999885	LL999935	Kindler <i>et al.</i> , 2014
<i>Natrix n. natrix</i>	Poland: Mazovia: Kampinos National Park	b	4	—	—	MTD T 9968	HF679780	HF680084	Kindler <i>et al.</i> , 2013
<i>Natrix n. natrix</i>	Poland: Podlachia: Białowieża National Park	b	8	—	—	MTD T 9976	HF679786	HF680088	Kindler <i>et al.</i> , 2013
<i>Natrix natrix</i>	Poland: Usedom	b	3	—	—	MTD T 11486	LL999889	LL999939	Kindler <i>et al.</i> , 2014
<i>Natrix astreptophora</i>	Portugal: S Setúbal: Torre	b	Eu	—	—	ZFMK 87516	HF679591	HF679908	Kindler <i>et al.</i> , 2013
<i>Natrix astreptophora</i>	Portugal: Serra de Sintra	b	Eu	—	—	ZFMK 91113	HF679592	HF679909	Kindler <i>et al.</i> , 2013
<i>Natrix n. persa</i>	Romania: Chilia Veche	b	4	—	—	MTD T 9889	HF679886	—	Kindler <i>et al.</i> , 2013
<i>Natrix n. scutata</i>	Russia: Bryansk	b	8	—	—	ZFMK 91040	HF679896	HF680194	Kindler <i>et al.</i> , 2013
<i>Natrix n. persa</i>	Russia: Dagestan: Samur	b	2	—	—	ZFMK 62940	—	HF680184	Kindler <i>et al.</i> , 2013
<i>Natrix n. persa</i>	Russia: Dagestan: Tatayurt	b	8	—	—	ZFMK 62936	HF679888	HF680185	Kindler <i>et al.</i> , 2013
<i>Natrix natrix</i>	Russia: Krasnodar: Otdalyonnyy	b	8	—	—	ZFMK 60732	HF679588	HF679904	Kindler <i>et al.</i> , 2013
<i>Natrix n. scutata</i>	Russia: Tula District	b	8	—	—	CAS 175878	AY873724	AF471059	Lawson <i>et al.</i> , 2005
<i>Natrix n. natrix</i>	Slovakia: Červený Kláštor	b	4	—	—	MTD T 8648	HF679799	HF680100	Kindler <i>et al.</i> , 2013
<i>Natrix n. natrix</i>	Slovakia: Devín	b	4	—	—	MTD T 9014	HF679800	HF680101	Kindler <i>et al.</i> , 2013
<i>Natrix n. natrix</i>	Slovakia: Tatra Mountains: Žiar	b	3	—	—	MTD T 9015	HF679812	HF680110	Kindler <i>et al.</i> , 2013
<i>Natrix h. helvetica</i>	Slovenia: Ljubljana	b	4	—	—	ZFMK 65382	HF679620	HF679937	Kindler <i>et al.</i> , 2013
<i>Natrix n. natrix x persa</i>	Slovenia: Zalec	b	4	—	—	—	AY873720	AY487738	Guicking <i>et al.</i> , 2006
<i>Natrix astreptophora</i>	Spain: Andalusia: Sevilla	b	Eu	—	—	E21023x10S	KC570233	KC570278	Kindler <i>et al.</i> , 2013



Table S5.4 (continued)			mtDNA	Haplotype		Accession numbers <sup>†</sup>			Reference
Taxon	Location	Analysis	Clade*	ND4	Cyt <i>b</i>	Voucher <sup>†</sup>	ND4+tRNAs	Cyt <i>b</i>	
<i>Natrix astreptophora</i>	Spain: Catalonia: Blanes	b	Eu	—	—	ZFMK 90573	HF679595	HF679915	Kindler <i>et al.</i> , 2013
<i>Natrix natrix</i> <i>gotlandica</i>	Sweden: Gotland	b	4	—	—	ZFMK 38356	HF679606	HF679927	Kindler <i>et al.</i> , 2013
<i>Natrix natrix</i>	Sweden: Närke: Klockhammar	b	3	—	—	MTD T 11582	LL999890	LL999940	Kindler <i>et al.</i> , 2014
<i>Natrix natrix</i>	Sweden: Öland: Halltorps Hage	b	3	—	—	MTD T 10920	LL999891	LL999941	Kindler <i>et al.</i> , 2014
<i>Natrix h. helvetica</i>	Switzerland: Astano (Ticino)	b	C	—	—	—	AY487795	AY487751	Guicking <i>et al.</i> , 2006
<i>Natrix h. helvetica</i>	Switzerland: between Neuchâtel, Bern and Biel: Grosses Moos	c	E	N1	—	MTD T 10084	HF679621	—	Kindler <i>et al.</i> , 2013
<i>Natrix h. helvetica</i>	Switzerland: between Neuchâtel, Bern and Biel: Grosses Moos	c	E	N1	—	MTD T 10095	HF679622	—	Kindler <i>et al.</i> , 2013
<i>Natrix h. helvetica</i>	Switzerland: near Bern	b, c	E	N1	C1	MTD T 10083	HF679623	HF679938	Kindler <i>et al.</i> , 2013
<i>Natrix h. helvetica</i>	Switzerland: near Meirigen: Gadmental	c	E	N2	—	MTD T 10086	HF679624	—	Kindler <i>et al.</i> , 2013
<i>Natrix h. helvetica</i>	Switzerland: near Meirigen: Gadmental	c	E	—	C1	MTD T 10085	—	HF679939	Kindler <i>et al.</i> , 2013
<i>Natrix h. helvetica x</i> <i>natrix</i>	Switzerland: St. Gallen: Altenrhein	b	3	—	—	MTD D 30567	HF679653	HF679958	Kindler <i>et al.</i> , 2013
<i>Natrix h. helvetica</i>	Switzerland: Vinzel	c	E	N1	—	MTD T 10079	HF679625	—	Kindler <i>et al.</i> , 2013
<i>Natrix natrix</i>	Tunisia	b	Tu	—	—	E10118x2T	KC570246	KC570290	Kindler <i>et al.</i> , 2013
<i>Natrix astreptophora</i>	Tunisia: Cap Serrat	b	Tu	—	—	ZFMK 67196	HF679598	HF679918	Kindler <i>et al.</i> , 2013
<i>Natrix n. persa</i>	Turkey: Aegean Region: Dalyan	b	7	—	—	ZFMK 82946	HF679889	HF680187	Kindler <i>et al.</i> , 2013
<i>Natrix n. persa</i>	Turkey: Aegean Region: Selçuk	b	7	—	—	MTD D 25230	HF679893	HF680189	Kindler <i>et al.</i> , 2013
<i>Natrix n. persa</i>	Turkey: Black Sea Region: Borçka	b	8	—	—	ZFMK 71145	HF679894	HF680192	Kindler <i>et al.</i> , 2013
<i>Natrix n. syriaca</i>	Turkey: Mediterranean Region: Burmaz (near Botaş)	b	6	—	—	ZFMK 71176	HF679903	HF680201	Kindler <i>et al.</i> , 2013
<i>Natrix n. syriaca</i>	Turkey: Mediterranean Region: SW Osmaniye	b	6	—	—	e6116x10K	KC570263	KC570308	Kindler <i>et al.</i> , 2013
<i>Natrix n. natrix</i>	Ukraine: Crimea: Luchyste	b	8	—	—	MTD D 42725	HF679815	HF680113	Kindler <i>et al.</i> , 2013

<b>Table S5.4 (continued)</b>			mtDNA	Haplotype		Accession numbers <sup>†</sup>			Reference
Taxon	Location	Analysis	Clade*	ND4	Cyt <i>b</i>	Voucher <sup>†</sup>	ND4+tRNAs	Cyt <i>b</i>	
<i>Natrix n. natrix</i>	Ukraine: Oblast Herson: Hola Prystan': Herois'ke	b	8	—	—	MTD D 42724	HF679816	HF680114	Kindler <i>et al.</i> , 2013
<b>Outgroups</b>									
<i>Natrix tessellata</i>	Armenia : Geolazar	b	—	—	—	ROM 23418	AY873734	AY866531	Guicking <i>et al.</i> , 2006
<i>Natrix maura</i>	Spain : Southern Spain	b	—	—	—	MNCN 12016	AY873708	AY866530	Guicking <i>et al.</i> , 2006
<i>Nerodia sipedon</i>	USA : Tennessee	b	—	—	—	—	JF964960	JF964960	GenBank

\*Clades according to the work of Kindler et al. (2013; 2017).

<sup>†</sup>Samples from this study have not yet been assigned voucher codes or accession numbers, and are displayed with their sample ID's instead.

Analysis types: <sup>a</sup> Samples genotyped, <sup>b</sup> samples included in phylogenetic tree and <sup>c</sup> samples included in haplotype network

Museum acronyms are as follows: BEV – Laboratoire de Biogéographie et Ecologie des Vertébrés, Centre d'Ecologie Fonctionnelle & Evolutive, Montpellier; CAS – California Academy of Sciences, San Francisco, California; LSUMZ – Louisiana Museum of Natural History, Baton Rouge, Louisiana; MNCN – Museo Nacional de Ciencias Naturales, Madrid; MTD D – Museum of Zoology, Senckenberg Dresden (Herpetological Collection); MTD T – Museum of Zoology, Senckenberg Dresden (Tissue Collection); MWLK – Museum der Westlausitz, Kamenz; MZUF – Museo di Storia Naturale dell'Università di Firenze, Sezione di Zoologia "La Specola"; ROM – Royal Ontario Museum, Department of Natural History, Toronto, Ontario; ZFMK – Zoologisches Forschungsmuseum Alexander Koenig, Bonn; ZMH – Zoological Museum Hamburg.

## Chapter 5. Grass snake conservation genetics in Jersey

Table S5.5 Details of best partitioning scheme used in MrBayes calculated using PartitionFinder v2.1.1. Branch lengths were linked.

Gene	Partition	Evolutionary model
Cyt <i>b</i>	1-1117:	
	Codon position 1	HKY+I+G
	Codon position 2	HKY+I
	Codon position 3	GTR+G
ND4	1118-1813:	
	Codon position 1	HKY+I+G
	Codon position 2	HKY+I
	Codon position 3	GTR+G
tRNAs	1814-1984	HKY+I+G

Table S5.6 Number of individuals successfully genotyped ( $n$ ) for 14 loci, number of alleles per locus ( $N_a$ ), null allele frequencies ( $F_{NULL}$ ) for the whole dataset and Hardy-Weinberg P-values ( $P_{HWE}$ ) for each population. Loci excluded from further analyses are marked with an asterisk (\*).

Locus	$n$	$N_a$	$F_{NULL}$	$P_{HWE}$	
				Jersey	Northwest France
Natnat05	70	7	0.048	1.000	0.527
$\mu$ Nt8new	70	8	0.128	0.323	0.006
$\mu$ Nt7	69	3	-0.045	0.007	0.638
Natnat11	69	4	-0.021	1.000	0.521
Natnat06	68	4	0.135	1.000	0.404
TbuA09	69	10	0.217	0.000	0.664
3TS	69	5	-0.094	0.006	0.101
Eob $\mu$ 13	68	5	0.128	0.082	1.000
Natnat08	70	6	0.028	0.097	0.752
Natnat01	68	7	0.082	0.347	0.097
Natnat09*	70	9	0.328	0.000	1.000
Hb30*	69	5	0.258	0.126	0.058
Eob $\mu$ 1*	70	4	0.254	0.000	0.089
$\mu$ Nt5*	59	4	0.409	0.000	0.001

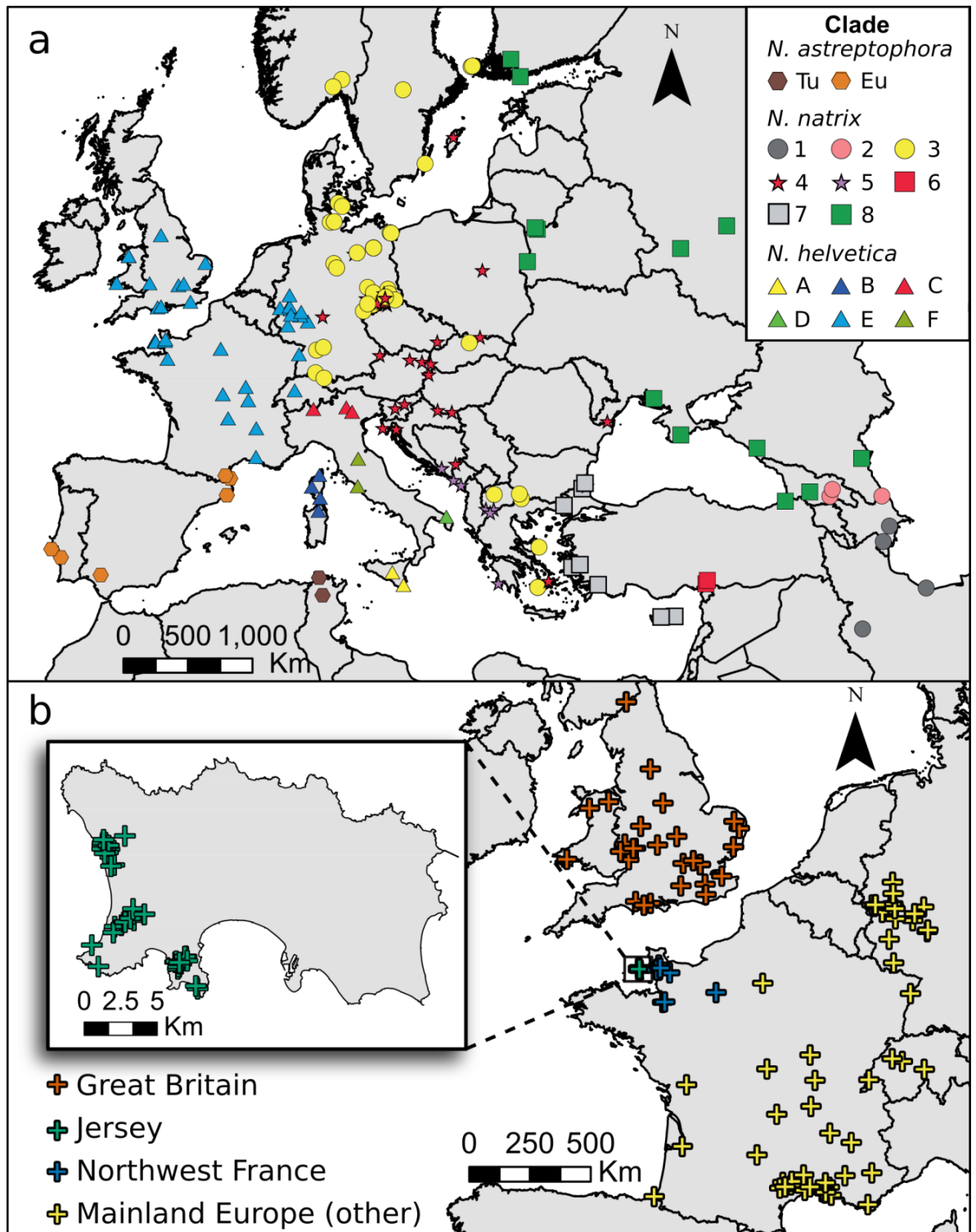


Figure S5.1 Maps showing (a) samples used in phylogenetic tree estimation with colours and shapes according to Kindler *et al.* (2013) and (b) *N. h. helvetica* samples from clade E (Figure 5.2; Table S5.4; Kindler *et al.*, 2013) used in the haplotype network. Colours in (b) indicate their sampling location; Great Britain (orange), Jersey (green), northwest France (blue), or elsewhere in mainland Europe (yellow).

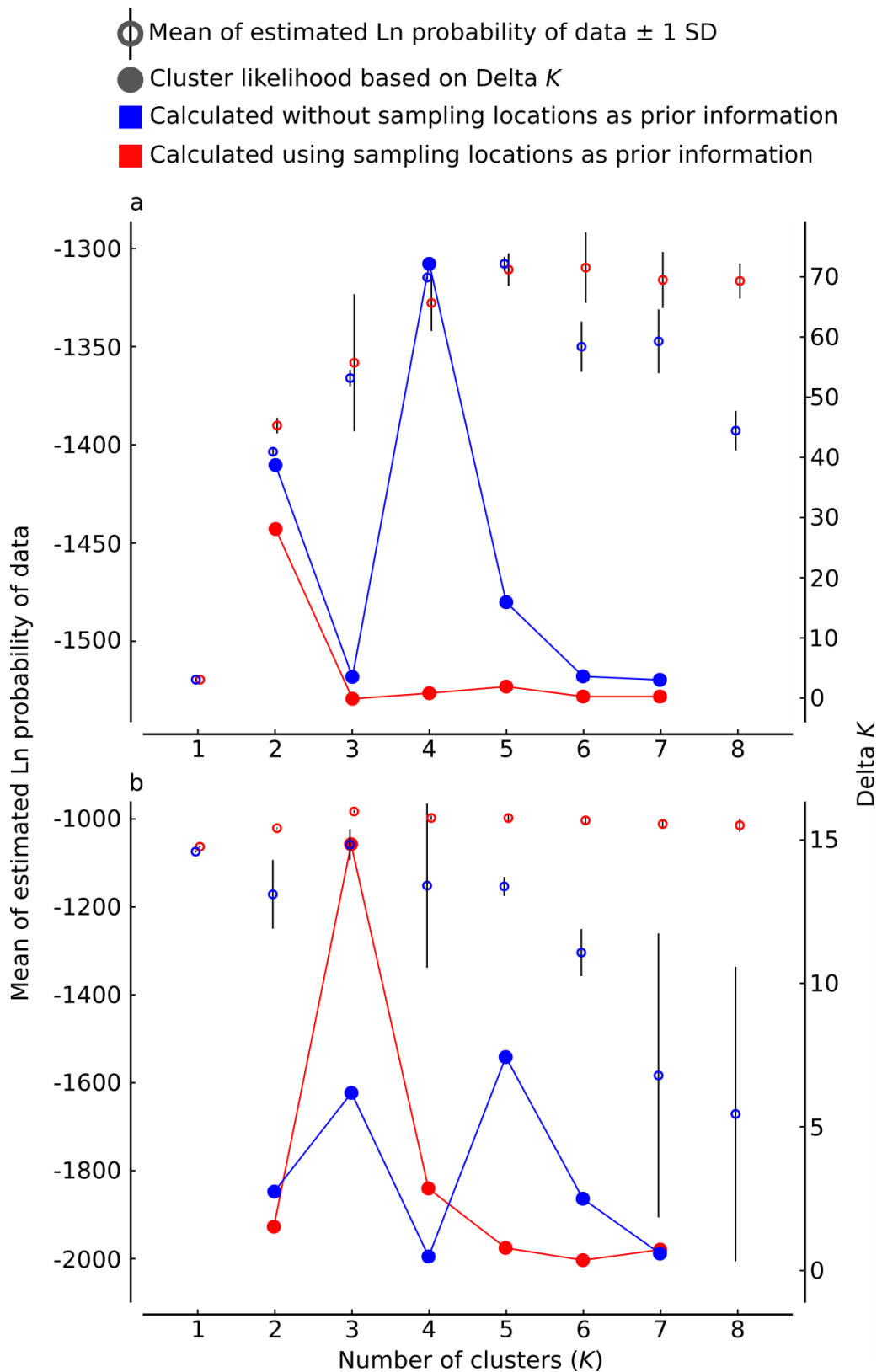


Figure S5.2 Likelihood of clusters ( $K$ ) between one and eight identified by Bayesian clustering analysis in STRUCTURE v2.3.4 (a) between populations and (b) within Jersey. Clusters are scored based on mean likelihoods (hollow circles with black lines showing  $\pm$  1 SD; values on left axis; circles are offset to show overlap) and  $\Delta K$  values, calculated as  $\Delta K = \text{mean}(|L'(K)|)/\text{sd}(L(K))$  (solid circles and lines; values on right axis).

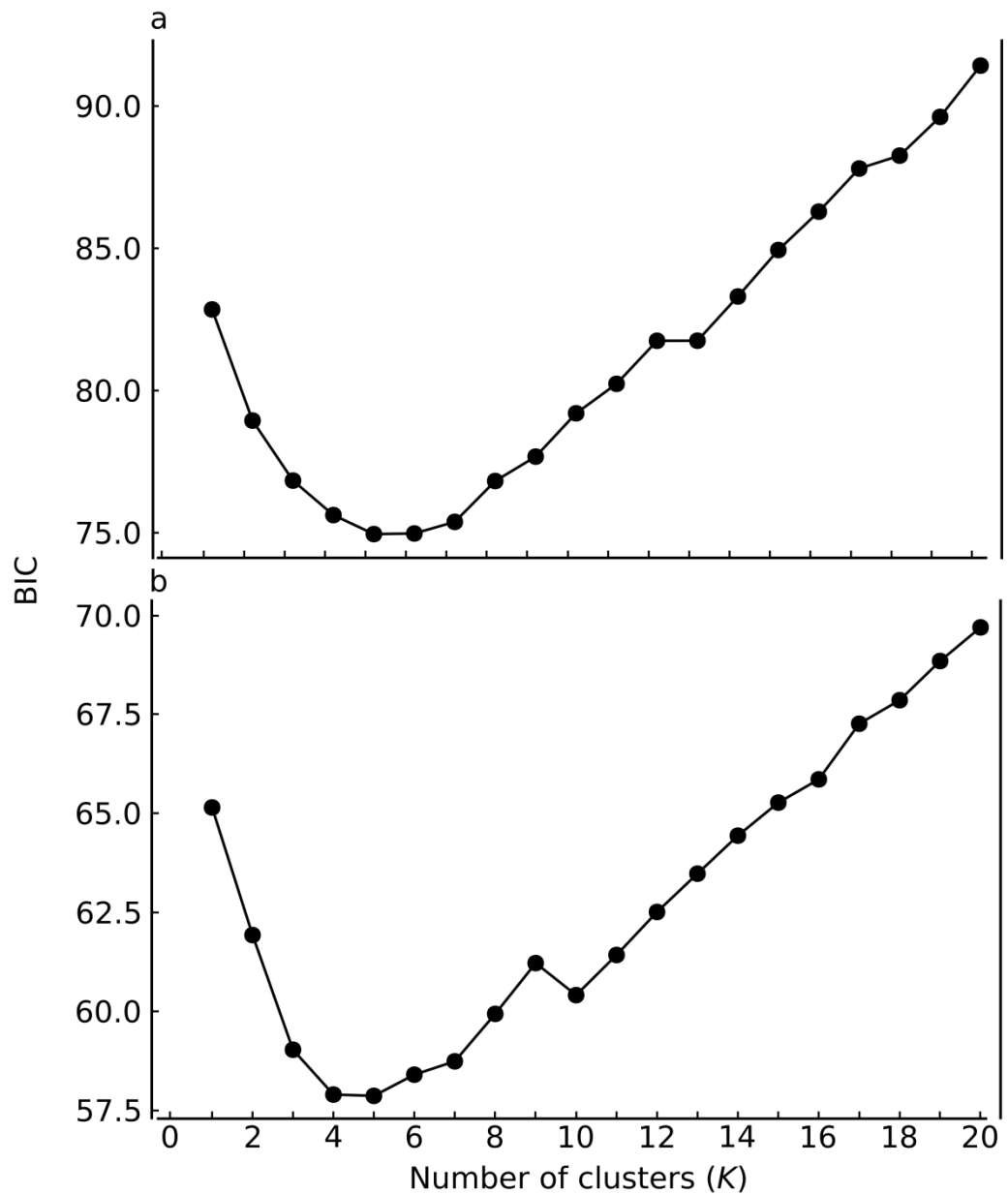


Figure S5.3 Most likely number of clusters as indicated by Discriminant Analysis of Principal Components (DAPC) between populations (a) and within Jersey (b). Assessment is based on Bayesian Information Criterion (BIC).

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Assessing the conservation status of species enables the prioritisation and implementation of measures to reduce extinction risk. Rare and cryptic species often elude monitoring attempts, resulting in a paucity of data for assessments. Habitat loss and fragmentation are widely regarded as the primary drivers of biodiversity loss for a variety of taxa (Gibbons *et al.*, 2000; Sala *et al.*, 2000). The pressures upon grass snakes and in many instances, biodiversity in general within Jersey, have been hypothesised to comprise loss of nesting sites, prey declines, habitat loss and fragmentation, small population size and introduced predators (Gent and Gibson, 1998; Hall, 2002; McMillan, 2003; States of Jersey Department of the Environment, 2006). This thesis set out to inform the appropriate status assessment and conservation management of the population of grass snakes in Jersey. Several methods were applied to fill knowledge gaps; the use and suitability of occupancy and N-mixture models for assessing distribution and abundance (Chapter 2), radio-tracking for identifying habitat associations and dispersal capabilities (Chapter 3), species distribution modelling for identifying priority areas for protection and monitoring (Chapter 4), and molecular approaches to infer the population's origins, genetic structure and consequences of isolation (Chapter 5).

### 6.1. Assessing the distribution and abundance of elusive species

A number of tools are commonly used for monitoring a species' distribution and abundance. A lack of basic distribution and abundance data for many species results in data deficient or ill-informed assessments (Bland and Böhm, 2016). The suitability of methods for data collection and analysis are influenced by spatial scale and data requirements. Survey guidelines are readily available for British

herpetofauna (e.g., Reading, 1996; Gent and Gibson, 1998; JNCC, 2004; Sewell *et al.*, 2013), but variations in survey design and aims, species ecology and detectability, season and location (Reading, 1997; Sewell *et al.*, 2013; Griffiths *et al.*, 2015; Gregory and Tuttle, 2016), can result in imprecise estimates of occupancy or abundance (Kéry, 2002; Guillera-Arroita, 2017). Indeed, efforts to monitor grass snake populations in Jersey as part of widespread herpetofauna monitoring have experienced low success (Wilkinson *et al.*, 2014). Monitoring programmes should establish achievable aims and carefully consider the ecology of their study species. Their efficiency can be improved by conducting pilot studies (Loos *et al.*, 2015). Chapter 2 serves as a pilot study to provide estimates of detectability and inform survey design for future monitoring of the grass snake population. It suggests that species-specific monitoring may be most appropriate for elusive species, as multi-species monitoring designs often fail to provide suitable detection rates (e.g., Wilkinson *et al.*, 2014). Furthermore, efforts to ascertain abundance estimates are intensive and unsuitable for widespread use within elusive snake species (Steen *et al.*, 2012; Chapter 2). Occupancy models provide a more effective alternative, and the development and implementation of flexible sampling strategies and analyses provide a promising avenue for improving the effectiveness of monitoring efforts (e.g., Specht *et al.*, 2017). However, the power to detect trends is likely to be poor (Couturier *et al.*, 2013; Chapter 2) and consideration should be given to the use of a robust survey design (Pollock, 1982) to provide appropriate data for modelling.

Whether grass snakes in Jersey are rare, cryptic or both depends to some extent on scale and survey effort. Based on the definitions of Specht *et al.* (2017), where cryptic species have a detection probability of 0.3 or less, and rare species



occupy 30% or fewer sites, the results of this study (Chapter 2) suggest that Jersey's grass snakes are neither cryptic nor rare. However, as detection was dependent upon survey effort, surveys with a less intensive sampling strategy would classify the population as cryptic. Moreover, this study purposefully selected sites with a high occupancy probability. Island-wide sampling would result in a reduced occupancy probability, and almost certainly classify the species as rare. Based on the findings of this thesis, grass snakes in Jersey are threatened and should be considered as a management unit for conservation given their differentiation from the mainland population and function within the ecosystem (Chapter 5). Three subpopulations are evident within Jersey, with two occurring in the west and a third isolated in the south. Until connectivity between them is improved, each should be managed separately whilst ensuring the provision of appropriate resources in the landscape. Given their limited occurrence, the area of occupancy (AOO) is less than 20 km<sup>2</sup> and the population is therefore regionally Vulnerable (D2) under IUCN guidelines (IUCN, 2012a; 2012b).

Improving species detection is an important step for monitoring elusive species. As noted elsewhere (Reading, 1996; 1997; Gent and Gibson, 1998), artificial cover objects (ACOs) were an effective survey tool for grass snake monitoring, but are needed in high numbers for confident assumptions of site occupancy in Jersey (Chapter 2). Further work investigating ACO preferences across different target species and environmental conditions will improve detectability and therefore efficiency of surveys for grass snakes and other taxa. This work also highlights a need to carry out large numbers of repeat surveys before declaring a site unoccupied (Kéry, 2002; Sewell *et al.*, 2012; Chapter 2). Because of this, guidelines for consultant ecologists should be put in place to

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ensure grass snakes are not assumed to be absent from a site due to insufficient investment in survey effort.

Priority areas for monitoring and protection can be identified through the complementary use of species distribution modelling (e.g., de Pous *et al.*, 2011). Chapter 4 indicates the potential for species distribution modelling to identify these areas within Jersey. Their occupancy status can then be assessed using the methods outlined in chapter 2. Prior to this study, population data stemmed from two short-term studies (Hall, 2002; McMillan, 2003), opportunistic records and a volunteer monitoring scheme (Wilkinson *et al.*, 2014). Based on the findings of chapters 2 and 4, grass snakes have maintained a similar distribution to that found by Hall (2002) within the west and southwest of the island. This continued occupancy in many of the nature reserves highlights their importance to the snake population and mirrors some findings of Reading *et al.* (2010), where many snake populations within protected areas remained stable.

Previous indications that snakes occurred on the north coast and in the southeast (Hall, 2002) may no longer hold true as there was little evidence of their occupancy in these areas. The findings from all chapters would suggest that any snakes persisting in these areas are almost certainly genetically isolated from the known population due to a diverse road network, agriculture and urbanisation. Survey efforts to determine absence from these areas of uncertainty with confidence are underway. Should they be inhabited, DNA sampling from these sites will further elucidate patterns of subpopulation isolation within the island, and provide evidence for management decisions. Until evidence is available to suggest otherwise, it is assumed that the grass snake distribution does not extend east

beyond the A12 main road (La Grande route de Saint-Pierre / La Route de Beaumont).

Site abundance was low, but may be confounded by poor detection and high mobility. In contrast to the study by Hall (2002) which found only a single snake at Les Mielles Nature Reserve, chapters 2 and 3 indicate a higher snake abundance than most other sites and regular site use. Comparatively, Hall (2002) found a relatively high abundance of snakes at the eastern end of Les Blanchés Banques dune system in Creepy Valley. Although grass snakes were found during surveys and reported by the public in this area, changes in habitat during the intervening period may have reduced the suitability of the area. Where vegetative succession has occurred, opportunities for thermoregulation may have been lost (Shoemaker *et al.*, 2009; Bonnet *et al.*, 2016).

Due to the pressures of development within the island and elsewhere, and its impact upon biodiversity, ensuring proper statutory protection of important sites for conservation is paramount. Chapter 4 demonstrates the need for further legal protection of suitable grass snake habitats. Les Mielles Nature Reserve on the west coast and Woodbine corner in the south are key grass snake sites (sites C and M in Chapter 2), so upgrading them from non-statutory nature reserve designations to Special Sites of Interest under the Planning and Building (Jersey) Law 2002 would be a logical and worthwhile step.

## **6.2. Identifying resource requirements**

The availability of suitable habitat and other resources (e.g., prey) directly influence population size and fitness. Resource selection can occur at multiple spatial scales (e.g., Row and Blouin-Demers, 2006a). N-mixture models (Chapter

2), radio-tracking (Chapter 3) and species distribution modelling (Chapter 4) all identified similar habitat associations for grass snakes in Jersey, despite being carried out at different spatial scales. Specifically, heterogeneous habitats dominated by rough grassland, scrub, bracken and heathland were preferred. Conversely, woodland, open habitats (e.g., amenity grassland) and agricultural areas were avoided. These positive and negative habitat associations are highly similar to the findings of previous studies (Madsen, 1984; Mertens, 2008) and have clear implications for habitat management. However, the avoidance of agricultural areas is in contrast to the findings of several studies (Madsen, 1984; Wisler *et al.*, 2008; Meister *et al.*, 2010) and requires further investigation given their dominance in the landscape.

The overall health of a snake population is unavoidably linked to prey availability, as a poor food supply can lead to declines in body condition and population abundance (Beaupre and Douglas, 2009). Amphibian declines in Jersey appear to have ceased following conservation efforts (e.g., Ward *et al.*, 2016). Chapter 4 demonstrated a positive relationship between snake distribution and proximity to toad populations. Although the specific dietary composition of grass snakes was not analysed within this thesis, preliminary investigation indicates them to be a generalist, with evidence of predation upon toads *Bufo spinosus*, palmate newts *Lissotriton helveticus*, small mammals and green lizards *Lacerta bilineata* (McMillan, 2003; R. Ward unpublished data). This varied diet may be important in buffering the effects of stochastic changes in prey availability caused by pollution events (e.g., Gibson and Freeman, 1997) or unsuitable weather (e.g., Wilkinson, 2007). However, the drivers of this variation in prey selection are unclear, and may be due to other factors which require investigation.

### 6.3. Human-dominated landscapes

Jersey's landscape has been shaped by the influence of urbanisation and agriculture. Anthropogenic influences on the grass snake population were challenging to quantify in this study, with radio-tracking (Chapter 2) revealing no road crossings. Species distribution modelling (Chapter 4) suggested agricultural land and areas of high road density to be unsuitable components of the landscape. However, microsatellite markers supported previous findings (Wisler *et al.*, 2008; Meister *et al.*, 2010) that grass snakes may travel through suboptimal landscapes (Chapter 5). Instead, urban areas and major roads probably serve as barriers to dispersal. Existing protected areas are primarily clustered in the west and southwest of the island. Improving the connectivity between these fragments is likely to be a cost-effective approach for retaining viability in a number of species, however it is recommended to use a spatial planning tool such as Marxan (Ball *et al.*, 2009) to ensure efficiency.

An extremely high human population density combined with a strong tourism industry (Jersey Tourism, 2010) has resulted in heavy recreational use of green spaces in Jersey. The negative effects of recreational use of green spaces are well-known, and can induce behavioural changes in animals as well as degrade the quality of the habitat (e.g., McMillan and Larson, 2002; Reed and Merenlender, 2008). The coastal areas inhabited by grass snakes are primarily comprised of nature reserves that border amenity grassland (e.g., golf courses), urban areas or agriculture. The reserves vary in their historical use, statutory protection, ownership and biological diversity. No formal assessments have been made of the pressures imposed upon these reserves by recreational users. However, usage is primarily by dog-walkers, hikers, naturalists, runners and equestrians. As a

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minimum, this is likely to have behavioural effects on wildlife (e.g., Parent and Weatherhead, 2000) and can occasionally result in the death (accidental or otherwise) of snakes (R. Ward pers. obs.). However, as discussed in chapter 3, recreational impacts are not all necessarily negative. Indeed, footfall in scarcely visited parts of Les Mielles Nature Reserve serves to maintain habitat heterogeneity and improve availability of basking areas for reptiles. Further consideration should be given to the benefits and disadvantages of public and domestic animal access on these sites. Users of these sites should be treated as local stakeholders, and could provide a voice for maintaining or improving the protected status of these reserves. Moreover, without public support, the pressure for property developments may lead to further habitat loss and fragmentation. Future study of reserve use would be a useful step for informing reserve design and management.

Urban areas may provide something of a paradox, whereby barriers to movement such as roads and buildings limit dispersal, but some gardens provide hunting, nesting and possibly hibernation grounds. Indeed, we received public reports of grass snakes in 15 different private gardens between 2014 and 2016, with snakes reported in multiple years in some gardens. Proper engagement of landowners and other stakeholders can continue to encourage appropriate habitat management and reporting of sightings. This also includes golf courses, which contribute a large area within the known grass snake distribution. By encouraging the provision of key features (i.e., ponds for amphibians, compost or manure heaps for nesting, well-drained banks or deep stone walls for hibernation) on private lands, the permeability of the landscape may improve as well as contribute to the growth and survival of the snake population. Similar efforts are underway

across the nature reserves to improve nest site availability. Monitoring of these sites will provide valuable data on nest site preferences. Further management to improve habitat suitability can be an effective tool in augmenting snake populations, even in sites heavily used by the public (e.g., Bonnet *et al.*, 2016).

### **6.4. Living in isolation**

The isolation and size of the island predisposes any species unable to migrate or exchange genes with mainland populations, to a heightened risk of extinction (e.g., MacArthur and Wilson, 1967; Richman *et al.*, 1988). Chapter 5 indicates that snakes colonised the island naturally, with the population becoming isolated as the land-bridge was inundated by the rising sea. Divergence between the snake populations in Jersey and mainland France has since occurred (Thorpe, 1984; Chapter 5), but drift and selection pressures have not yet resulted in speciation as the time period has been relatively short. This and previous studies did not record deleterious genetic effects or significant losses of genetic diversity in Jersey's snake (Chapter 5) and toad populations (Wilkinson, 2007) respectively, suggesting isolation has had little negative effect. This fits the hypothesis of natural colonisation prior to the island's separation giving a sufficient population size to avoid founder effects. These findings negate the need for any form of genetic intervention (e.g., genetic restoration, Madsen *et al.*, 1999) aside from improving connectivity between the subpopulations in the west and south of the island.

### **6.5. Limitations**

At the inception of this study, the status of the grass snake population was unknown. The lack of success in recent monitoring efforts (Wilkinson *et al.*, 2014)

meant that a degree of flexibility was built in to the study design in expectation of few grass snake encounters. Intensive survey effort resulted in sufficient sample sizes, but still produced statistical challenges. Moreover, to maximise detection, surveys were biased towards areas where snakes had most recently been confirmed (Hall, 2002; McMillan, 2003). This limits any generalisations across the whole island, but few public sightings occurred through the 'Think Grass Snake' campaign away from our survey areas.

Low levels of species detection and biased sampling could cause grass snakes utilising agricultural habitats such as field margins to go undetected. Moreover, the dominance of agricultural habitats in many parts of the island may mask the presence of suitable habitats that occur at smaller spatial scales. In future, the use of widespread occupancy surveys that record absences and thus account for imperfect detection, will allow for more accurate predictions of distribution across the whole island (Kéry *et al.*, 2013; Guillera-Aroita *et al.*, 2015). Similarly, the unknown status of the population resulted in the use of external radio-tag attachments due to concerns associated with implantation risks. In turn, this resulted in short radio-tracking periods. Given the number of adult snakes found (Chapter 2), future long-term radio-tracking should make use of implanted tags to gain a more complete view of resource requirements and dispersal ability.

### **6.6. Summary and recommendations for the future**

Considering the theory of relaxation in island populations (Wilcox, 1978; Richman *et al.*, 1988), it is likely that Jersey has already lost a number of species that were unable to sustain populations given limited island resources and associated pressures. The remaining biodiversity, although depauperate, is worthy



of attention. Care must be given in maintaining the persisting ecological relationships, as further losses will degrade ecosystem function. The anthropogenic influence on the island expedites a need for long-term management and protection, incurring a burden on those responsible. Efficiency of conservation measures can be gained through multi-species approaches to habitat protection and management.

This, and concurrent work, has provided a number of novel contributions to assessing and improving the conservation status of grass snakes in Jersey, addressing a number of aims outlined in the Species Action Plan (States of Jersey Department of the Environment, 2006). The development and implementation of a public media campaign ('Think Grass Snake') has encouraged reporting of sightings and raised awareness. Estimation of the species' distribution and abundance has led to identification of priority areas for protection, and contributes data for informing planning decisions. Multi-scale assessment of habitat requirements allows for better reserve design and habitat management. Furthermore, new nest sites have also been built, barriers to gene flow have been identified which provides focal areas for improving connectivity, and information on dietary preferences has revealed that the population is guarded from stochastic changes in single prey species. Combined, they culminate in an assessment suggesting a threatened status, and provide scope for further actions to improve the population's viability.

Continued efforts to understand and conserve grass snakes in Jersey should utilise the information laid out in this thesis for the benefit of the snake population. Future monitoring could benefit from use of greater survey effort to ensure snakes that are present are detected. Engaging landowners to build ponds and compost

heaps will help in improving connectivity. Similarly, reserves and wildlife corridors should be designed and managed with consideration given to the needs of grass snakes. The area of Red Houses in St Brelade is a priority for improving connectivity. Details of nest preferences are still lacking, and should be a priority for monitoring. Improved protection of grass snake sites will help to ensure the future of the population. Long-term radio-tracking with implanted radio-tags would improve our understanding of dispersal capabilities and resource requirements. Continued DNA sampling in un-sampled areas will identify further barriers and patterns of dispersal. Finally, attention should be given to the recent emergence of snake fungal disease *Ophidiomyces ophiodiicola*, which is capable of infecting grass snakes (Lorch *et al.*, 2016; Franklinos *et al.*, 2017). Screening of the Jersey population is recommended, and actions should be taken to minimise the risk of it arriving on the island if not already present.

### **6.7. Conclusion**

Locating, studying and assessing the conservation needs of rare and elusive species is an ongoing challenge, but is necessary in order to effectively prioritise those of conservation interest. Existing data and pilot studies provide major contributions towards developing a well-informed study design. The varied traits including detection of different species may require different methodological approaches in order to collect sufficient data on them. With appropriate sampling methods in place, previously unobtainable information can be collected whilst having minimal impact on what may be a rare and threatened study organism. Indeed, using the grass snake as a case study, this thesis highlights the importance of taking a multi-faceted approach at varying spatial scales, to gathering data on elusive organisms and making the most of that data. In

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particular, combining ecological monitoring in the field with sophisticated modelling and molecular approaches can address knowledge gaps and inform status assessments, where single veins of evidence may only provide a partial or even misleading perspective. This is particularly pertinent given that species declines are rarely governed by simple processes.

The persistence of anthropogenic threats to biodiversity has led to a continual need for conservation assessment and intervention. Therefore, being able to utilise all available data and account for biases within it, is pertinent for efficient and effective conservation. Throughout this thesis I have highlighted the suitability and pitfalls of various methods that permit the use of constrained datasets. Moreover, I have shown that population fragmentation can occur over small spatial scales, despite the wide-ranging nature of the focal species. This implies that a wealth of taxa are constrained by barriers in the landscape and will increasingly occur in disjointed patches. The value of nature reserves or similar features in the landscape, and efforts to maintain and improve habitat connectivity between them therefore, cannot be overstated.

Islands offer an often simplified perspective of processes and issues occurring elsewhere. Specifically, they generally host a depauperate fauna contributing to a simplified ecosystem, are often devoid of external influences due to their isolation, and from a conservation standpoint exhibit fewer political obstacles. This latter point requires some discussion as species rarely conform to political boundaries, and as such, their conservation requires a coordinated approach across governments and other stakeholders, which rarely happens. Islands however are often self-governing with few agencies to unify towards a common goal, and in part due to limited biodiversity, can give greater conservation

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focus to the species present. Not only does this allow for fine-scale monitoring due to the size of an island and the simplicity of its ecosystem, but for more cohesive conservation management of that system. Combined, this allows threat processes to be identified and addressed at an earlier stage than is likely to occur at a larger spatial scale. Therefore, I conclude by emphasising the potential for island-based studies in evaluating and developing solutions to widespread threat processes.

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