Shrinking body length in snakes in the United Kingdom: ecological phenomenon or sampling error?

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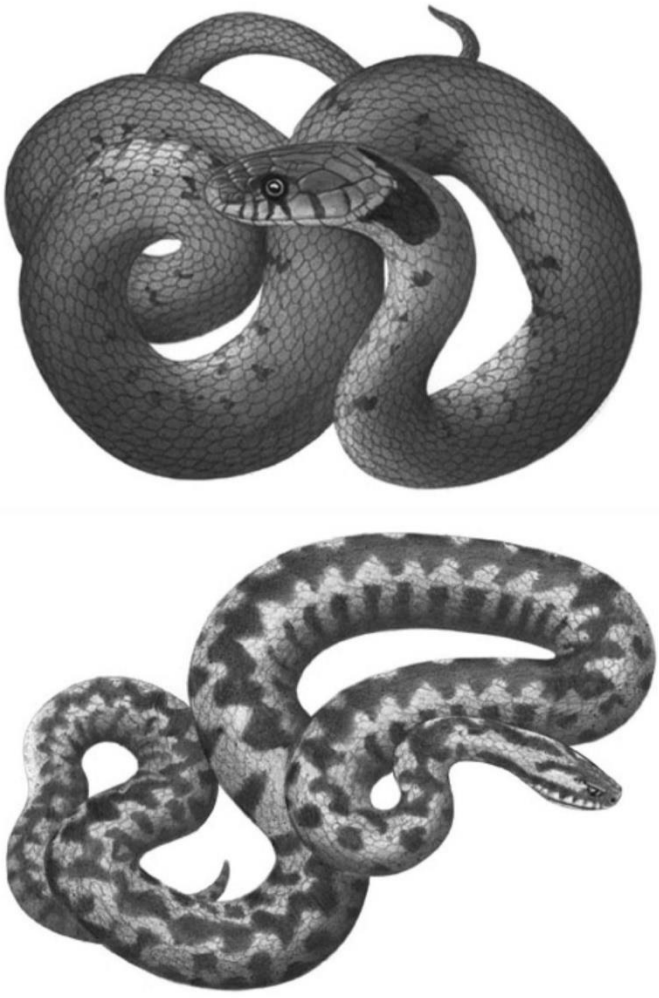

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It's been a long, winding road to reach my destination - and life certainly bowled the odd googly along the way - but to have finally attained this goal has made the blood, sweat, and tears all worthwhile.

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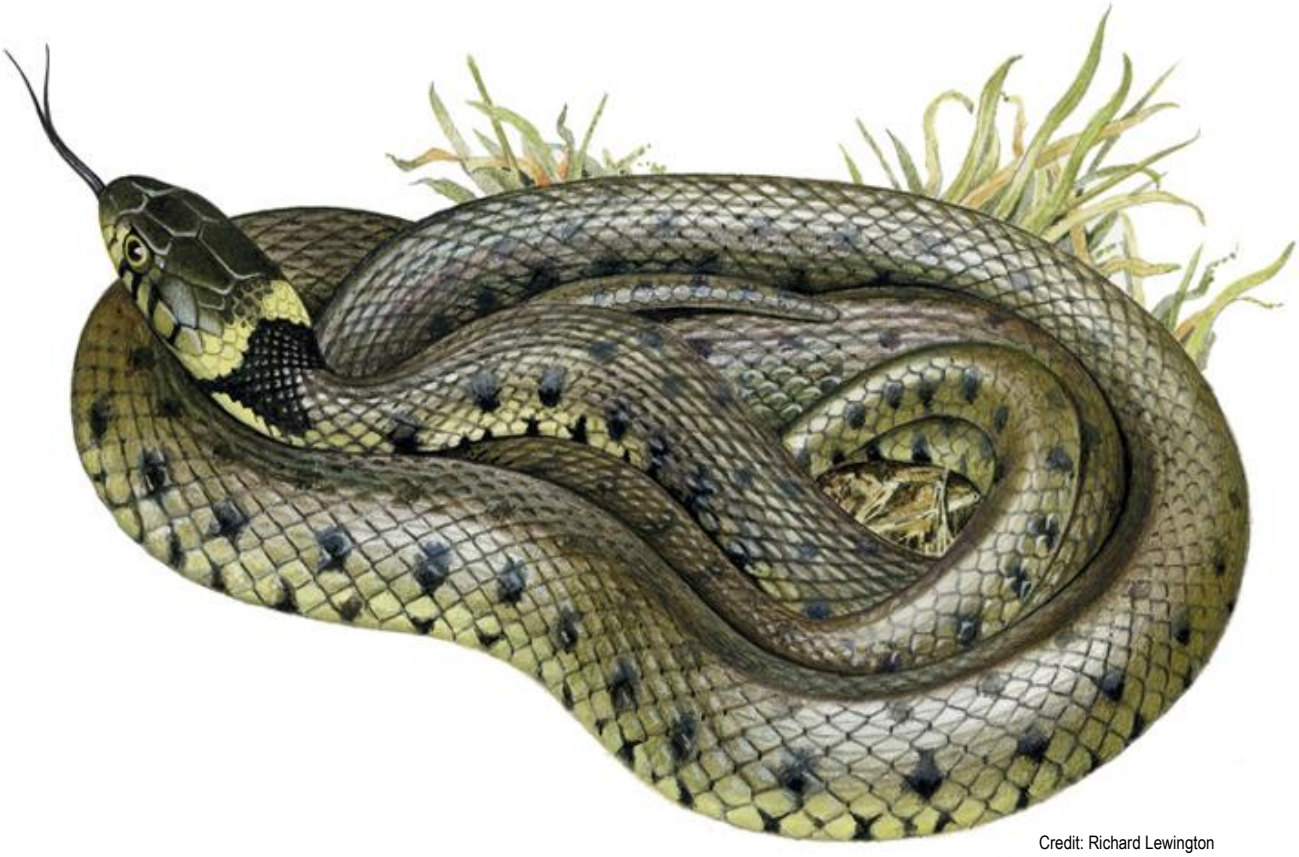

Credit: Richard Lewington

ETHICAL STATEMENT

The research herein was conducted under and conforms to the ethical guidelines on wildlife research as provided by the *Association for the Study of Animal Behaviour Guidelines for the Treatment of Animals in Research and Teaching, and the Home Office's Guidance on Operation of the Animal (Scientific Procedures) Act 'Working With Wild Animals'.* This privately funded project was approved by the Research Ethics Committee, School of Anthropology and Conservation, University of Kent, (Canterbury, Kent).

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ABSTRACT

In recent years, studies have been linking climate warming with a decrease in body size across many aquatic and terrestrial taxa including reptiles. The impact that fluctuations in temperature are having on reptile populations is widely recognised, yet surprisingly little is understood about the apparent decrease in reptile body size in contemporary times. In the United Kingdom (UK), grass snakes (*Natrix helvetica*) and adders (*Vipera berus*) are not only in decline, but there is some evidence that body lengths appear to be decreasing too. Whether the 'shrinkage' phenomenon is real or is a result of measurer error or collection bias, remains a controversial topic among herpetologists. Comparative analyses using historic data from preserved specimens and contemporary measurements from field caught snakes found that *N. helvetica* were smaller on average by 14.1%, and *V. berus* by 11.1% than historic counterparts collected between the late 1800s to 1950 in the UK. It was important to establish whether these findings represented a true reflection of trends over time or whether they had been influenced by collection, sampling and / or measurer bias. Visual surveys of model snakes placed in reptile habitat revealed that more larger models were detected than smaller models, and that an experienced observer found more models than two groups (n=9 and n=10) of inexperienced observers. This supports the finding that detectability and collection is biased by snake size. Measuring snakes is notoriously difficult as evidenced by the considerable amount of literature on the topic. Further experiments were conducted to test the biases associated with multiple measurers, multiple measuring methods and repeatability, accuracy, and precision. There was no difference in measurements when they were made by a traditional method (squash box and string) or an image analysis program (Image J). However, experienced measurers varied in how they reported the size measurements of the same snakes using measuring software. Moreover, the position of the snake in the image influenced size measurements highly significantly. Caution must be taken if measurements are obtained using different methods by different measurers or combining data from live and preserved specimens. It is clear, however, that the smaller body size of contemporary *N. helvetica* and *V. berus* and the relation to potential environmental change factors are worthy of further investigation.

Key words: Grass snake (*Natrix [natrix] helvetica*), adder (*Vipera berus*), body size, shrinking length, detectability, measurer bias, sampling bias, digital morphometrics, climate change.

CHAPTER 1: GENERAL INTRODUCTION

The phenomenon of body shrinkage in the animal kingdom is not novel (Dietl, 2013). During the Palaeocene-Eocene Thermal Maximum (PETM) temperatures rose considerably and the fossil record demonstrates that many animal species responded by shrinking in size. The rate at which temperatures rose during PETM was such that species were able to adapt. Over the last 150 years, anthropogenic influences have greatly contributed to global climate change (GCC), the effects of which are taking a huge toll on biodiversity (Sheridan & Bickford, 2011). Studies are increasingly linking climate change with a decrease in body size across many aquatic and terrestrial taxa, including reptiles (Wikelski & Thom, 2000; Gardner *et al*., 2011; Nicholls, 2013; Caruso *et al.,* 2015). It remains unclear whether reduction in body size because of climate warming is a genetic or phenotypically plastic response, although it is possible growth and development rates will evolve in response to climate change. Ohlberger, (2013) bases the prediction on evidence that variation in physiological traits is exhibited in similar species due to past adaption; survival is differentially affected for small vs large individuals; adaptation can occur on time scales during which climate is predicted to change.

The application of generic 'rules' (e.g. Allen, 1877; Bergmann, 1877; Cope, 1896 ; Gloger, 1833) to describe patterns of morphological or phenotypic traits attributable to, in the broadest sense, tropical or temperate species is highly controversial (Ashton & Feldman, 2003). However, discussion over Bergmann's rule in particular has been brought back to the fore in light of burgeoning research on shrinking body size in reptiles. Reptiles tend to be larger in tropical environments and smaller in temperate ones: the complete reverse of Bergmann's rule. In cooler climates, reptiles need a large surface area to body volume ratio for thermoregulatory purposes whereas in tropical climates where temperatures are generally warm consistently, warming up and cooling down quickly is much less of an issue (Pincheira-Donoso & Meiri, 2013). However, distributional shift in response to climate change may have implications for thermoregulation and, consequently, body size in reptiles (Walther *et al*., 2002).

THE SIZE OF SNAKES

Size, as it is defined in this study, relates purely to lengths and not mass. Researchers rarely measure mass in snakes as it can be affected by season, when the snake last ate, breeding condition, and health (Feldman & Meiri, 2013). There have, however, been calls to include mass measurements when conducting biometric snake studies as length data alone may exclude valuable information on life history traits (Feldman & Meiri, 2013).

It is reported that snakes with more ventral scales will not only produce bigger neonates, but those neonates will typically become larger than those born with fewer ventral scales (Fox, 1948; Head & Polly, 2007; Lee *et al*., 2016, Lindell *et al.,* 1993; Lourdais *et al.,* 2004). Pleomerism describes the relationship between larger body size and higher vertebral count and is a phenomenon that has predominantly been associated with fish (Lindsey, 1975). Snake pleomerism was first described by Fitch (1940) during his observations of garter snakes (*Thanmnophi*s sp.). Klauber (1956) later identified that smaller crotalid species and subspecies had fewer ventral scales and vertebrae than larger ones: a pattern also evident in European viperids (Saint Girons, 1978). More recent research, however, indicates pleomerism is widespread in snakes and depends on the number of somites produced at tailbud stage during embryogenesis (Head & Polly, 2007). Unlike other amniotes, snake somitogenesis may be unconstrained by the normal parameters that dictate the number of vertebrae that will develop in the neonate (Head & Polly, 2007). Indeed, its occurrence has been examined more recently in nightsnakes (*Hypsiglena,*) and the positive relationship between greater number of vertebrae and greater body size in this taxon has been confirmed (Lee *et al*., 2016).

Temperature contributes to pleomerism in ectotherms (Lee *et al*., 2016) and a number of studies have shown that climatic variation has an impact on embryogenesis in some snakes (Fox, 1948; Lee *et al*., 2016; Lourdais *et al.,* 2004). A study of gravid *Thamnophis elegans atratus* where one group of females was housed in a cool room and the other in a warm room during gestation found that cool room females not only gave birth to fewer young, but the young had significantly fewer ventral scales to those born in the warm room (Fox, 1948). In *V. berus*, Lindell *et al.,* (1993) found that the number of ventral scales correlate with the number of vertebrae and that snakes with a higher ventral scale count (and subsequently vertebrae) were not only larger at birth, but also at sexual maturity. In viperids, it has been argued females maintain reasonably stable thermoregulation throughout gestation, but there is some doubt whether their developing embryos can be completely shielded from thermal differentials year-to-year (Lourdais *et al*., 2004). For example, neonate aspic vipers (*Vipera aspis*) that had been exposed to higher temperatures in the early stages of embryogenesis had more ventral scales and subsequently a larger body size (Lourdais *et al*., 2004). This correlation has also been demonstrated in studies where the temperature has been manipulated whilst captive rearing other ectotherms such as *Batrachoseps* salamanders (Jockush,1997) and Chinese giant salamanders (*Andrias davidianus*) (Zhang *et al*., 2014) as well as snakes such as Cape coral cobras, *Aspidelaps lubricus* (Reichling & Gutzke, 1996). Raising the temperature of the substrate used for egg incubation

in *A. lubricus* resulted in larger hatchlings higher ventral counts. However, when the same experiment was repeated with *Aspidelaps scutatus*, found no significant difference in neonate size indicating that the event could be species-specific (Reichling & Gutzke, 1996). Moreover, in female snakes, there is further evidence that there may be a positive correlation between mothers with higher ventral scale counts and higher scale counts in their young (Lee *et al.,* 2016). A comprehensive review of pleomerism in the families Colubridae, Elapidae and Viperidae sought to determine whether ecological factors, body shape or family affiliation would explain deviations from the norm (Lindell, 1994). In comparison to all other non-fossorial species, burrowing snakes have the fewest number of vertebrae than any other species occupying other environments, and stout species have less vertebrae than longer, slender species (Lindell, 1994). These findings were recently further supported by Tingle *et al*., (2017) who confirmed that burrowing snake species – which are as a rule considerably smaller than most terrestrial or arboreal snake species - also have shorter spinalis-muscle tendon units in line with a having a lower vertebrae count. Moreover, constricting elapid and colubrid species from Lindell's research were found to have more vertebrae than non-constrictors indicative of selection targeting *per se* (Jayne, 1982; Lindell, 1994), findings that were again supported by Tinkle *et al*., (2017).

The common viewpoint on growth in snakes is that age and body size are positively related given that snakes continue to grow for almost their entire lives. The link between age and size in reptiles has been described as a Von Bertalanffy curve (Halliday & Verrell, 1988), and indeed reptilian growth rates over time predominantly follow this description, with growth rate gradually slowing as the animal ages. It is important to bear in mind, however, that the link between age and size differs between time and space so variations can be significant between populations (Forsmann, 1991).

Snakes exhibit a significant, phenotypically plastic response to both prey size and prey availability. Body size in snake populations tends to be small where prey availability is scant or where prey items are small. Snakes are gape-limited predators, and this aspect of plasticity has been tested multiple times in snakes and some interesting morphological variations have been observed. Northern water snakes (*Nerodia sipedon*) that feed more regularly on larger fish, develop longer jaws (Queral-Regil & King, 1998). In Sweden, *V. berus* that preyed on larger field voles (*Microtus agrestris*) grew faster and were larger than those that fed on smaller voles (Forsmann, 1991). Additionally, faster growth and larger heads are linked to increased survivorship in male *V. berus*. A faster growth rate in young snakes reduces their vulnerability to predators and enables them to reach sexual maturity sooner,

while larger head size increases feeding opportunities as snakes can ingest bigger prey items (Forsman, 1994). A comparative study between an island population of small *N. helvetica* (Hallands Vadero, Sweden) and a mainland population (Mayrd, Sweden) where the snakes are larger, found that captive reared hatchlings from both populations resulted in equivalent growth rates and body size attainment (Madsen & Shine, 1993).

IMPLICATIONS FOR REPRODUCTION AND SURVIVORSHIP

Behaviour varies dependent on body size, and in snakes, can influence many functional traits including reproductive strategy, predator avoidance tactics and prey selection. Mayer *et al*., (2016) found a relationship between larger body size and increased boldness in hatchling keelback snakes (*Tropidonophis mairii*) indicating that behaviour as well as morphology may increase the survival advantage associated with larger offspring.

In endotherms, maternal transfer of antibodies, hormones and antioxidants can alter immunity in the progeny. Very little is known about how reptilian maternal health and traits may impact offspring immune function but developing an understanding in this area is becoming increasingly important given the numerous threats reptiles face today. The extent to which immunomodulatory elements are passed on to offspring are dependent on factors such as maternal health status and whether the mother is well fed. In a first of its kind, a study on maternal body size, transfer and immune function in neonates was conducted by Brown & Shine (2016). They found a strong link between maternal body size and leukocyte differentials in neonates. It is plausible that this provides neonates from larger mothers a fitness advantage, which in turn may have significant ecological and evolutionary implications (Brown & Shine, 2016). These findings are highly relevant, given the increased emergence of pathogens affecting wildlife globally.

EMERGING FUNGAL PATHOGENS AND IMPACT ON SNAKES

Of particular concern is the nascency of fungal diseases within the past few decades and the high virulence associated with them (Fisher *et al.,* 2012). While it is clear that climate change has played a key role in the emergence and spread of plant-infecting fungal disease (Anderson, *et al.,* 2004), the relationship between warming trends and the emergence of fungal pathogens within wildlife populations is less evident and has been cause for much debate (Fisher *et al.,* 2012). In the case of the disease chytridiomycosis, and more specifically *Batrachochytrium dendrobatidis*, the link between its spread and climate change has divided opinion in scientific

circles with some proponents strongly supporting global warming as a driver of spread (Pounds *et al.,* 2006); conversely, others question the validity of multi-decadal correlations as evidence for such a causal relationship when there are potentially other, more intrinsic factors to consider (Rohr *et al.,* 2008). It is undeniable, however, that chytridiomycosis (*B. dendrobatidis and B. salamandrivorans*) has been responsible for devastating population decline worldwide in amphibians (Anderson *et al.,* 2004; Franklinos *et al.,* 2017; Pounds et al., 2006).

In the UK, Walmsley *et al*. (2007) report that climate change has not only facilitated the spread of invasive species which act as vectors for disease, it has also enabled diseases to propagate in areas they previously could not. Moreover, Garner *et al*. (2005) estimated that chytridiomycosis has been present in the UK since 2005.

Now, the emerging fungal pathogen *Ophidiomyces ophiodiicola* (snake fungal disease, SFD), which was previously restricted to North America (Lorch *et al.,* 2016), has been reported to have been present in Europe since at least 2010 – possibly longer – and it is also present in the UK (Franklinos *et al.,* 2017) Similarly to chytridiomycosis, the spread of *O. ophiodiicola* has been associated with climate change, habitat degradation, underlying poor health and inbreeding depression (Franklinos *et al*., 2017). SFD has already been associated with declines in timber rattlesnakes (*Crotalus horridus*) and massasaugas (*Sistrurus catenatus*) in the United States (Lorch, *et al.,* 2016). In the UK, Franklinos *et al.* (2017) used PCR targeting to reveal SFD in both *N. helvetica* and *V. berus*. Alarmingly, in some cases SFD infection has proven severe enough to be fatal in *N. helvetica*. Therefore, the implications of this emerging disease for native UK snakes are potentially severe and cannot be ignored (Franklinos *et al.,* 2017).

IMPORTANCE OF MUSEUM SPECIMENS AND THEIR USES IN RESEARCH

Museum specimens and the unique data they yield can prove highly valuable to researchers investigating growth (Pyke & Erhlich, 2010; Wandeler *et al.,* 2007). Such biological repositories – many of which archive vast collections covering considerable geographic breadth – can enable the researcher to study species that may currently occur in areas that are logistically difficult to access (Burrell *et al.,* 2015). Moreover, they can be a vital resource for researchers studying the aetiology of disease and infection (Yates *et al*., 2002).

Historic specimens offer considerable research potential in many study areas including evolutionary, environmental and ecological changes (Walther *et al.,* 2002); biodiversity distribution, loss and occurrence (Fisher & Shaffer, 1996; Laughlin, 2003; Selander & Johnston, 1967); morphological and biological changes over time (Ricklefs, 1980; Olsson *et al.,* 1996; Reed, 2001); epidemiological events including identification of pathogen vectors and sources (Yates *et al.,* 2002); and the impacts of global climate change on species, ecosystems and environments (Pyke & Erhlich, 2010; Suarez & Tsutsui. 2004; Winker, 2004). Certainly, in terms of ecological and environmental research, the use of biological collections is well established and there have been numerous studies for which historical data has proven invaluable (Suarez & Tsutsui, 2004).

Moreover, as scientific interest in conservation genetics grows and techniques in the field develop, biological collections provide a sound platform upon which researchers can compare historical and contemporary genetic diversity (Wandeler *et al.,* 2007). Such studies provide considerable insight into not only evolutionary processes but also temporal gene frequency changes (i.e. micro-evolutionary processes), ergo underpinning the usefulness of museum specimens as tools to detect selection either through environmental causes or molecular signatures (Wandeler *et al.,* 2007). For example, Hartley *et al.* (2006) investigated insecticide resistance in blowflies (*Lucilia cuprina*) comparing sequence data from historic blowfly specimens dating back over 75 years with that of contemporary blowflies. Unlike contemporary specimens, historic specimens had not been exposed to organophosphate insecticide. It was established that *L. cuprina's* rapidly evolved resistance to organophosphate insecticide was due to pre-existing mutant alleles in the historic gene pool. The methods used by Hartley *et al.* (2006) have enabled further research into invertebrate responses to environmental change using *post hoc* genetic analysis. Assaying candidate genes is becoming simpler and the number of genetic markers is increasing (Wandeler *et al.,* 2007). The difficulties that have traditionally been associated with the analysis of museum specimen DNA – e.g. highly fragmented and short strings – have now been overcome through technological advancement. Next generation high-throughput (HT) DNA sequencers have greatly

facilitated the identification of multilocus genes and, as such, the usefulness of museum specimens in research is increasing (Burrell *et al*., 2015; Pyke & Ehrlich 2010; Winker, 2004).

PRESERVATION AND EFFECTS ON SPECIMENS

Surprisingly, the effects of preservation and preservation techniques on reptiles has been relatively understudied even though the use of museum specimens as sample populations is well recognised (Natusch, 2012; Reed, 2001; Vervust *et al.,* 2009). Furthermore, not all preservatives preserve in the same way, yet many researchers have traditionally dismissed the potential effects on analyses as negligible (Vervust *et al*., 2009). This is particularly problematic in the case of older specimens where information is frequently lacking on how specimens have been handled, fixed and preserved. Similarly, the effects of preservation differ dependent on the organism that is being preserved. Fluid preservation has various negative impacts including altering phenotypic traits such as pattern and colour. For example, after storage in alcohol for over five years, Mexican garter snakes (*Thamnophis rufipunctatus*) lost yellow and red pigmentation, and dorsal patterning became aberrant to the extent the specimens no longer resembled the live phenotype (Smith,1955). Preservation can induce a change in weight and loss of structural integrity in invertebrates (Mills *et al.,* 1982), and in amphibians not only is distortion more significant in smaller specimens than larger ones (Lee, 1982), it can change skin morphology. A study found that one preservation method increased dermal pustularity in *Gastrophyrne* and *Hypopachus* frog specimens, while another smoothed out the dermis in the same genera (Nelson, 1971). The two most significant factors to consider for morphological and biometric studies using preserved specimens are syneresis (the shrinkage of cellular contents) which can cause tissue distortion and consequently size decreases, and the time it takes for a specimen to attain equilibrium in the preservative.

Shrinkage has been well documented in fish, and not only are researchers encouraged to apply corrective factors for shrinkage due to preservation, but also shrinkage for rigor mortis (Shetter, 1936). Specimen shrinking is a finite event and essentially halts once the specimen has reached an osmotic equilibrium with the preservative (Vervust *et al*., 2009). The time it takes for this equilibrium to establish differs between taxa. Significant shrinkage of Black guillemot (*Cepphus grylle*) wings was recorded up to twelve months following preservation whereas puffin (*Fratercula arctica*) wings were stable after two months (Ewins, 1985; Harris, 1980). A study on cane toads (*Chaunus marinus* [*Bufo marinus*]) found that most changes occurred within six months of preservation (Lee,

1982). In snakes, six to eight months is proposed as sufficient for specimens to stabilise (Reed, 2001), however the extent of shrinkage in snakes varies considerably (Klauber, 1943; Reed, 2001; Natusch & Shine, 2012).

Shrinkage in preserved snakes was perhaps first established as a potential source of error by Klauber (1943). Following length measurements of three snake species preserved in an unspecified alcohol, Klauber noted a mean shrinkage factor of around 3%. Other studies measuring preserved snake lengths have varied dramatically in the mean shrinkage factor obtained. For example, Reed (2001) recorded a mean of 6-7% shrinkage factor across 41 species (*Boidea*, *Colubridae*, *Elapidae* and *Viperidae*). Natusch (2012) reported a mean 22% shrinkage factor when examining preserved specimen length for two python species (*Morelia viridis* and *Leiopython albertisii*).

Clearly, the physical and chemical changes preservation arrests in tissue decay are unequal across processes and across taxa. Dependent on study type, degree of precision required, magnitude of inconsistency, and how analyses are performed, not accounting for preservation type and its effects on specimens may confound comparative studies where preserved specimens are proxies for living organisms (Natusch & Shine, 2012; Vervust *et al.,* 2009). The effects of fixation and preservation remain remarkably understudied even though it is well documented that the process can alter morphology, phenotypic traits, and biometrics (Bernal & Clavijo, 2009; Martinez *et al.,* 2013; Vervust *et al.,* 2009). Calculating a simple corrective factor per taxon, therefore, is difficult (Simmons, 2014) and further study is needed to fully understand the different interactions between fixation, preservation, and specimens. Until such time researchers should approach such studies with caution. For the purpose of the comparative analyses conducted herein however, the correction factor of 6.5% based on Reed's mean (6-7%) has been used.

MEASURER ERROR

With an elongate and elastic body that can contort, contract, and extend, measuring a snake is not an easy task. There are many considerations that influence which method will be most suitable as different species may require different techniques. Moreover, the physical state of the snake will influence the measurements recorded (Setser, 2007; Rivas *et al*., 2008; Natusch & Shine, 2012). Equally, a variety of methods to take snake measurements have conventionally been used such as the squash box (Bertram & Larsen, 2004), running a soft tape along the body (Rivas *et al.,* 2008), or physically straightening the snake along a ruler (Cundall *et al*., 2016). For methods that focus on restraint (e.g. the squash box), there are different techniques for obtaining the actual

measurement. This traditionally has been a piece of string run along the ventral midline and then measuring the string or tracing the ventral midline with a pen and measuring the resulting line. Methods such as stretching a snake along a fixed rule are not only notoriously unreliable in terms of precision, but they are also associated with welfare issues. Snakes struggle and resist being straightened – it is a remarkably unnatural state to stretch a snake into – and if care is not taken, it can result in serious injury for the snake (Astley *et al*., 2017). Furthermore, the very act of stretching a snake introduces bias as the natural elasticity may misrepresent natural range measurements. Researchers would be prudent to disinfect all restraining and measuring equipment between each use, particularly with SFD present in the UK.

Anaesthesia, although unsuitable for field work, has also been explored as a method to gain measurements that are more precise than conventional methods (Setser, 2007). Anaesthesia may yield more precise measurements, but arguably not more accurate ones when it comes to a snake's overall length: measuring an anaesthetised snake that is relaxed with no muscle tone will not, according to Reed (2001), reflect the length of a conscious (i.e. living) animal. Moreover, anaesthesia also presents a welfare issue as the long-term effects or effects of repeated exposure are unknown, and mortality may be a risk (Blouin-Demers *et al*., 2000).

Measurer experience not only influences measurement error, but measuring different morphological characteristics yields variation in error too. A study on skeletal characteristics of passerine birds found that measurement error was relatively low when determining tibiotarsus length but very high for femur proximal end width lengths (Yezerinac *et al*., 1992). This is not an unusual finding. In snakes, SVL measurements, which are considered one of the most important characteristics in snake morphometrics, have proven very unreliable not only intra-measurer but also inter-measurer (Houston & Shine, 1994; Cundall *et al.,* 2016).

Over the past decade, digital image analysis has been gaining momentum as a very useful tool to take accurate and precise morphometric measurements. The technology is user-friendly, easily calibrated, and freely available for download. Using digital analysis software removes certain biases associated with measuring and measurement error, but it is not without its own limitations and issues. It is an area garnering interest in research fields, and one that is potentially valuable in a range of disciplines of which measurements are a feature.

SAMPLING BIASES

Cover-seeking behaviour is well evidenced in snakes and the use of cover boards in herpetological surveys is a widely used technique. There is a correlation between the use of refugia by small-bodied snakes and snakes in earlier life stages (neonates, juveniles, and sub adults of both large and small species). This behavioural trait is a confounding variable to visual surveying. In the case of *V. berus*, juveniles appear to seek refuges irrespective of the cost of thermoregulation. This may be due to risk of predation outweighing the need to bask in an exposed area. It is also possible that smaller size enables more efficient thermoregulation - albeit on a cooler site - than larger counterparts (Herczeg *et al.,* 2007). A study on three small north American snake species (*Storeria dekayi*, *Storeria occipitomaculata* and *Thamnophis sirtalis*) found that *S. dekayi* and *S. occipitomaculata* were exclusively caught under cover regardless of life stage. Larger *T. sirtalis* individuals were caught in the open while smaller individuals were collected under coverboards (Halliday & Blouin-Demers, 2015). This finding was further supported by Gregory & Tuttle (2016) who found that refugia use was not only preferential for smaller *Thamnophis* individuals, but also smaller *N. helvetica*. Samples of snakes may therefore be biased towards smaller individuals if the majority of snakes are captured under refugia. Likewise, smaller snakes may be less easily detected when basking in the open than larger snakes. It can be important, therefore, that sampling and observer detectability biases are controlled for.

CLIMATE CHANGE AND ITS POTENTIAL IMPACT ON SNAKES

The impact of climate change on *N. helvetica* and *V. berus* in the UK is difficult to predict. However, it is recognised that temperature variation disrupts phenological events, and this has a negative effect on reptiles. For example, the mating cycle in *V. berus* is a well-orchestrated event the brevity of which is likely due to environmental adaptions. Climate plays an important role in spermatogenetic development in *V. berus* with milder winters promoting earlier spermatogenesis. (Nilson, 1980). As winters become warmer in the UK, it is feasible that *V. berus* will emerge earlier, extending the mating period. A longer mating period reduces the feeding period, and a low food intake in males can delay or even stop spermatogenesis, reducing their ability to mate the following year (Nilson, 1980). Moreover, milder winters can disrupt hibernation patterns resulting in reptiles (and amphibians) expending valuable energy resources and emerging in poor body condition in spring. Interestingly, in *N. helvetica*, egg incubation temperature has a direct affect not only on the size of the developing hatchling, but also on

colouration such as nuchal spots in *N. helvetica* (Hagman *et al.,* 2015). Nuchal spot colouration has been linked to not only fitness (Hagman *et al.,* 2015), but also aposematism whereby brighter nuchal spots seemingly deter avian predators (Madsen, 1987).

In terms of body size, larger male *N. helvetica* fare considerably better than their smaller counterparts in mating balls and tail wrestling and are often able to mate multiple times whereas smaller males may not even mate at all. The ability to successfully ward off mating rivals is particularly important for *N. helvetica* as, once mated, females are no longer courted. Furthermore, larger female *N. helvetica* attract more males, possibly because pheromone production is lower in smaller females, or that simply larger females are easier to find (Luiselli, 1996).

Climate change is predicted to considerably impact the British landscape, introducing new threats such as SFD (Franklinos *et al.,* 2017), and degrading fragile habitats to the detriment of many species (Walmsley *et al.,* 2007). While vagile and generalist species may be able to adapt or move rapidly, it is probable that niche specialists and species with limited dispersal ability will not (Sutherland *et al.,* 2008). The main causes of decline in British snakes are predominantly linked to anthropogenic factors such as habitat loss and fragmentation, disturbance, persecution, pollution, and invasive species further exacerbated by climate change (Beebee *et al*., 2009). Moreover, with snake populations becoming increasingly small and isolated, inbreeding depression is a significant issue (Beebee *et al*., 2009). There is an urgent requirement to revise land management techniques if snake populations are to be protected in the UK. Some habitat management strategies – including high-density conservation grazing – may not be effective for reptiles (Reading & Jofré, 2016). There needs to be a shift to enable species to disperse via improved ecological corridors. This can be achieved by creating, restoring, and protecting new habitats (Hopkins *et al*., 2007).

SPECIES ACCOUNTS

THE GRASS SNAKE *(NATRIX [NATRIX] HELVETICA)*

A semi-aquatic, natricine colubrid, the grass snake (Fig. 1. *Natrix [natrix] helvetica*) is the UK's largest snake with females attaining a greater size than males upon maturity (800-1000 mm and 700-800 mm respectively), (Inns, 2009), but smaller mature individuals are not unusual (Gregory, 2004). *N. helvetica* is characterised by distinctive yellow nuchal spots although orange, white, brown, and cream variants are not uncommon (Hagman *et al.,* 2015;). These spots – or collar – make the snake easily recognisable in the field. *N. helvetica* are oviparous with clutches of 10 to 40 eggs, which are laid in June through to July. With a particularly strong presence in the south and southeast of England, the species is encountered predominantly in riparian habitat although occurrences have been reported in open woodland and grasslands. *N. helvetica* feeds mainly on amphibians but it will predate fish and occasionally nestling birds and small mammals (Gregory & Tuttle, 2016). Populations are in decline due to loss of habitat and reductions in prey availability. It is a UKBAP (Biodiversity Action Plan) species.

FIG. 1: Grass snake (*Natrix helvetica*)

THE ADDER (*VIPERA BERUS*)

The adder *(*Fig. 2. *Vipera berus)*, easily distinguishable by its bold 'zig-zig' dorsal pattern, is a small viperid. It is the UK's only venomous snake species. It has a wide yet patchy distribution across the country with abundance lower in the north although it is widely – but patchily – distributed in Scotland. The species inhabits a diverse array of habitats including (but not limited to) chalk downlands, moors and heathlands. Female *V. berus a*re larger than males (500-700 mm and 400-500 mm respectively) and give birth, on average, to around 10 young from July to early September. *V. berus* feed mainly on small mammals but will infrequently take lizards, nestling birds or small frogs (Inns, 2009). Populations are in decline due to habitat loss as well as human persecution as the snake is still often killed on sight. It is also a UKBAP species.

FIG. 2: Adder (*Vipera berus*)

STUDY OBJECTIVES

1. Comparing historic data from preserved specimens to contemporary data from live snakes, determine whether *N. helvetica* and *V. berus* have decreased in average body length from the late 1800s to present day, and identify if there is a difference between genders (pages 19-34).
2. Using models, determine the influence of size, morphology and posture on the detectability of *N. helvetica* in the field (pages 35-49).
3. Investigate measurer bias and differences between measuring techniques using measurements from and images of live *N. helvetica* and *V. berus* (pages 50-69).
    i. Compare *N. helvetica* and *V. berus* measurements taken using the string and squash box technique vs measurements obtained using software (Image J) to determine whether measurement method affects recorded results (pages 57-58, 61-62, 67).
    ii. Explore whether different software packages give different measurements for the same *N. helvetica* images (n=10) (pages 62-64, 67).
    iii. Examine if experienced users obtain different measurements using the same software (pages 57-59, 67).
    iv. Determine the level of variation between repeated measurements by the same user using the same measuring method (pages 62-64, 67).
    v. Identify if different positions of the same *N. helvetica* in a squash box will give different measurements (pages 65-67).

CHAPTER 2: ARE SNAKES GETTING SMALLER IN THE UK?

SUMMARY

Comparing preserved specimens with living organisms can offer invaluable insights into evolutionary and ecological processes and phenomena. Preservation can distort specimens, however, and studies analysing historic morphological characteristics infrequently consider the possible differences between preserved and live specimens or the differences between historic and contemporary datasets. A comparison between the body lengths of *N. helvetica* and *V. berus* collected between 1880-1950 with live individuals measured between 2007-2016 was conducted. After accounting for biases due to shrinkage caused by preservation and the potential differential representation of younger life stages in recent data, it was found that live, contemporary snakes were considerably smaller than museum counterparts on all counts. These trends may be due to sampling and collection bias, measurement method, measurer error, climate change or a combination of these factors.

INTRODUCTION

Gaining significant traction in the literature is support that warmer environments promote earlier attainment of adulthood in reptiles, in turn resulting in smaller body size (Sheridan & Bickford, 2011; Nicholls, 2013;). Other studies report the trend is not linked directly to climate change, but rather the 'resource rule' where growth levels are directly attributable to food availability (Pincheira-Donoso & Meiri, 2013). More recently it was found that older and subsequently larger Montpellier snakes (*Malpolon monspessulanus*) are dying in relation with increasing temperatures rather than reducing body size at the population level (López-Caldéron *et al*., 2016). Alternatively, such patterns may be due to measurer error rather than a naturally occurring phenomenon, and that snakes are not 'shrinking' at all (Luiselli, 2005).

The difficulty associated with obtaining accurate biometrics, especially for snakes, has long been an issue for researchers. There are different techniques to measure snakes, but surprisingly little study has been conducted to test the accuracy of these methods (Setser, 2007). The natural elasticity of a snake's body confounds accurate measurement with inter- and intra- measurer error as well as individual measurer ability (or lack thereof) further compounding the issue (Cundall *et al*., 2016). Moreover, these biases are not restricted to live specimens. Natusch (2012) revealed considerable differences in body length measurements for preserved snakes indicating that

researchers must operate with caution when extrapolating data drawn from historic records to contemporary, living organisms. What is known, regardless of whether snakes are decreasing in size or not, is smaller body size is related reproductive success, survivorship, and population dynamics in reptiles (Walther *et al.,* 2002).

Little work on body size decrease in native herpetofauna has been conducted in the UK. A study on common toads (*Bufo bufo)* spanning over twenty years supported a link between the UK's increasingly mild winters and a decrease in female common toad size (Reading, 2007; Vogelsang & Hans Franses, 2005). It is not implausible that milder winters are also impacting on *N. helvetica* and *V. berus* body size in the UK.

A preliminary study comparing historic and contemporary measurements for *N. helvetica* and *V. berus* found live snakes were smaller than preserved, historic equivalents (Bennie, 2013). Accessing a much larger contemporary dataset, the current study compared measurements from both species for both genders to determine not only whether snakes were smaller as suggested by Bennie (2013), but also to determine if decreases were occurring over time. The study also aimed to identify any trends indicating differences between gender over time. There have been no published studies on body length decrease in *N. helvetica* and *V. berus* in the UK to date.

METHODOLOGY

OVERVIEW

Measurements from historic *N. helvetica* and *V. berus* specimens held at London's Natural History Museum (NHM) were compared with measurements from contemporary individuals caught in the field. All snakes had been collected in the UK. Historic specimens had been submitted to the museum by numerous collectors. The earliest specimens dated back to the late 1830s and the most recent to the late 1990s. Sampling method as well as the technique used to record historic data was unknown. Contemporary data were recorded by three independent researchers with earliest measurements taken at the beginning of 2007 and the latest recorded in late 2016. All contemporary data had been obtained using squash box and string method (Quinn & Jones, 1974) except for some *V. berus* entries which had been digitally measured in the image processing software Image J (Browne, 2014). All contemporary data were obtained from snakes captured opportunistically as well as under refugia (natural and artificial). Unlike historic measurements which had been recorded from snakes nationwide, contemporary data originated from Kent and Norfolk only. Comparative analyses were performed on historic data subsets using two-way ANOVA as well as the same subsets corrected for the shrinkage associated with preservation.

HISTORIC DATA

The NHM dataset was in a raw state with all measurements recorded made by the late Dr. Peter Stafford c. the early 2000s. There was no indication how specimens were collected, preserved, or measured. There were 87 *N. helvetica* entries (male n=25, female n=37, unknown n=25) and 137 *V. berus* entries (male n=55, female n=73, unknown n=9) in total. Dates of collection of the specimens measured spanned over 102 years for *N. helvetica* (1896-1997) and 137 years for *V. berus* (1837-1974).

Plotting the number of snakes collected historically in decade intervals provided a clearer picture of which data were the most suitable for comparative analysis (Figs. 1 & 2). Based on frequency distribution for each species, subsets were extracted from the historic data for measurements recorded between 1880-1950. This timeframe ensured that historic data were distant from the contemporary measurement timescale (2007-2016), did not introduce a time overlap bias and yielded an adequate sample size. There were 30 entries in the *N. helvetica* subset (males: n=9, females n=21) and 102 for *V. berus* (males: n=45, females n=57).

The data were further arranged into gender and measurement type: snout-to-vent-length (SVL), tail length, and total length. Only SVL and tail lengths were physically measured. All total lengths for individuals were simply recorded by adding the corresponding SVL and tail length together. Specimens for both species had been collected from all over the UK including Wales, Scotland and the Isle of Wight (Appendix 2.I.a & b). Entries for Jersey (*N. helvetica*) were also included. Jersey measurements were included because sample size for male *N. helvetica* would have been too small to analyse without them once data had been organised in Excel. Any entries with damaged tails, unknown gender or unknown year of collection were removed.

Only adult snakes were analysed because including smaller juvenile measurements collected using contemporary methods (i.e. using artificial cover objects) risked biasing results. *N. helvetica* were identified as immature if total length was 220 mm or smaller. This cut-off point was derived from Inns (2009) whereby juveniles measure, on average, 160-200 mm. The extra 20 mm accounted for any exceptionally large juveniles. Similarly, with an average total length of 160 mm (Inns, 2009), *V. berus* were defined as immature if total length was 180 mm or smaller. Any individuals smaller than the cut-off points were removed from the analyses.

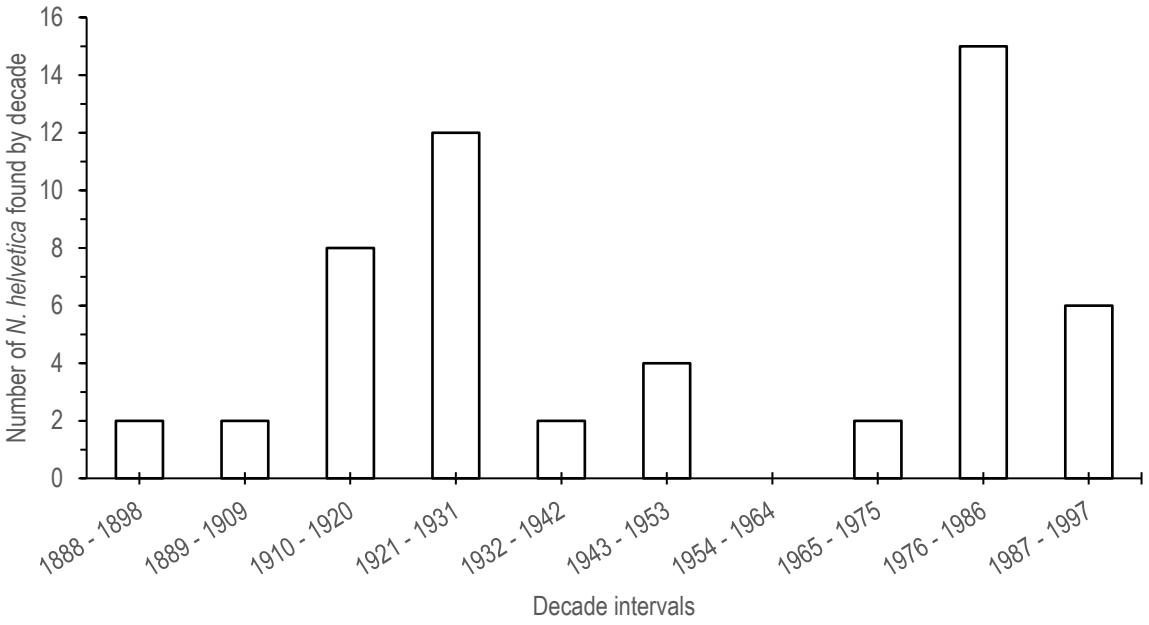


FIG. 1. Male and female *N. helvetica* collected in decade intervals from 1888 to 1997.

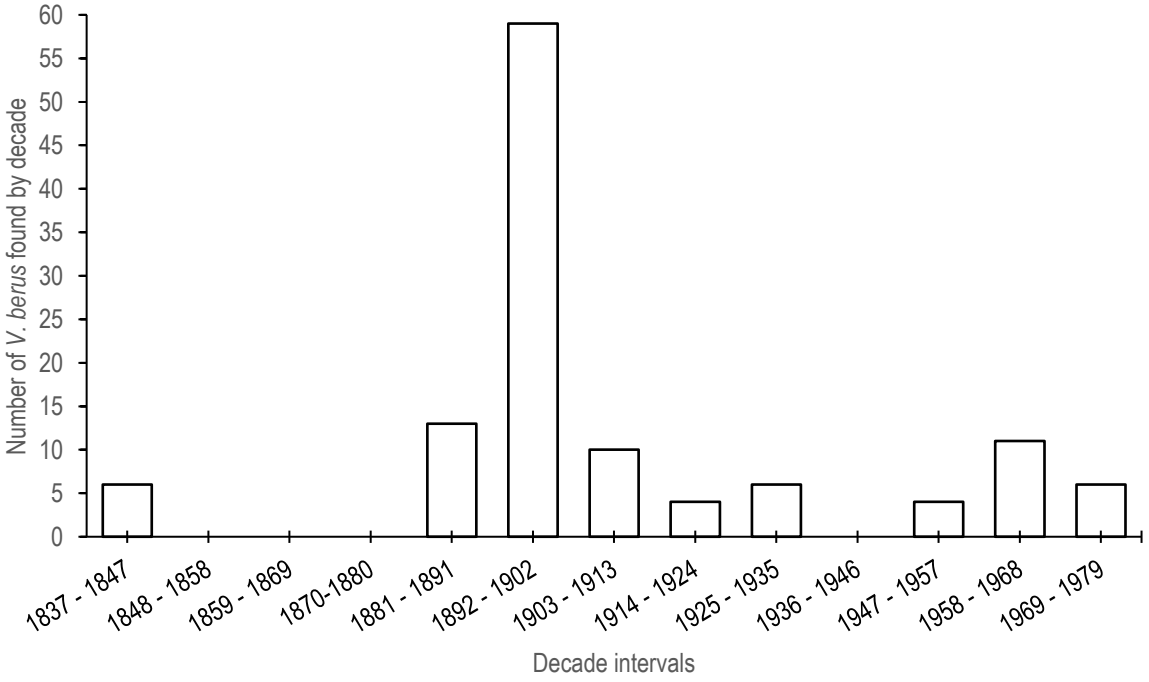


FIG. 2. Male and female *V. berus* collected in decade intervals from 1837 to 1979

PRESERVED SPECIMENS: SHRINKAGE BIAS AND MEASUREMENT

The preservation process is strongly associated with morphological and phenotypic change in reptile specimens (Reed, 2001; Vervust *et al*., 2009; Smith, 1955) with shrinkage in snakes a recognised issue (Natusch & Shine, 2012). It was important to attempt to correct for this bias prior to running analyses.

The NHM's current preservation method for reptile specimens is formalin-fixation for 48 hours followed by long-term storage in either 80% ethanol or industrial methylated spirit (IMS) with most specimens preserved in the latter. Prior to the 1950s, specimens were not formalin exposed and were typically preserved in [an undetermined] spirit (Natural History Museum, London, 2016). It was impossible to establish a corrective factor with any confidence as all historic data analysed were pre-1950. However, *sensu* Reed, (2001), a loss of 6-7% in body length for snakes after six to eight months in alcohol (wherein specimens reach osmotic equilibrium and the shrinkage effect is arrested) may be a tolerable adjustment. For the purpose of the comparative analyses conducted herein, the correction factor of 6.5% based on Reed's mean (6-7%) has been used. This is due to (a) the specific nature of Reed's research as it examined shrinkage in only museum preserved snakes across three times scales (16, 4, <1 years) respectively); (b) the research covered a wide range of specimen lengths (minimum: 241 mm, maximum: 1877 mm); (c) specimens represented four taxa (*Boidae*, *Colubridae*, *Elapidae*, *Viperidae*); and (d) there is little available in the literature referencing snake preservation and effects on specimen length.

As such, analyses for this study were carried out twice: once with original historic measurements, and again with a corrective factor of 6.5% added to length of preserved specimens (derived from Reed, 2001). This correction was then proportionally calculated from the total to adjust SVL and tail measurements accordingly.

Equally, the method used to measure preserved specimens was unspecified in this study, but preserved snakes are often stiff and tightly coiled so it was likely that stretching the specimen along a ruler was impractical (Blouin-Demers, 2003; Natusch & Shine, 2012). Measurements were most likely taken by running a piece of non-elastic string along the ventral midline of individuals and the string subsequently measured with an inflexible ruler (Natusch & Shine, 2012).

CONTEMPORARY DATA

Measurements from contemporary snakes were taken by three experienced researchers independently in two UK counties: Kent and Norfolk (Appendix 2.I.c & d). SVL and tail length were taken manually for all snakes with

total length calculated afterwards. The data had been obtained over a period of nine years (2007 to 2016) from different sites in Kent, but only one site in Norfolk. The total number of *N. helvetica* was 307 (male n=170, females n=137), and 159 for *V. berus* (male n=85, female n=74) after the same criteria used to arrange historic data had been applied (removal of individuals with damaged tails, unknown year of collection, unknown gender). Similarly, the same cut-off points were used for removal of juveniles from the data: (<220 mm for *N. helvetica* and <180 mm for *V. berus*). Plotting frequency distribution charts was unnecessary as all contemporary data had been compiled in the same decade. Measurements were taken using a plastic, transparent squash box and string (Fig. 3) except for five *V. berus* entries which were measured by importing photographs into Image J (Fig. 4) and analysing

FIG. 3. Measuring *V. berus* using the squash box and string method

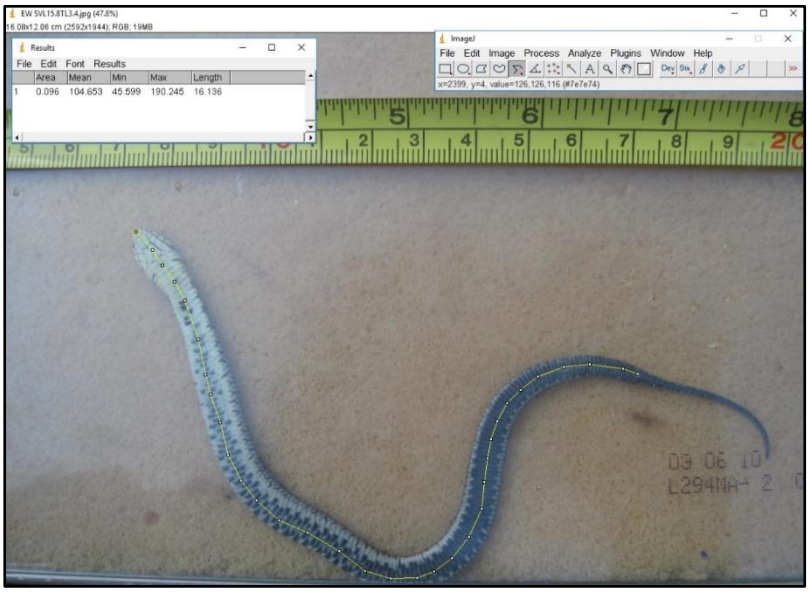


FIG. 4. Measuring *N. helvetica* SVL with Image J

COMPARATIVE ANALYSES

Data were organised by species and then split into gender. These were further divided into three categories for analysis: 'historic uncorrected' (original data from the main historic dataset); 'historic corrected' (historic data corrected by 6.5% to account for specimen shrinkage), and 'contemporary' (data collected between 2007 and 2016). Descriptive statistics (mean and standard error) were calculated in Excel for all measurement categories per species per gender. These data points were cast into line charts which provided a clear picture of how length had changed over time for both male and female *N. helvetica* and *V. berus*. A total of six, univariate two-way ANOVAs (full factorial) were performed in IBM SPSS Statistics v.24 per species. Analyses compared historic uncorrected SVL, tail and total length with contemporary equivalents and historic corrected SVL, tail and total length with contemporary equivalents.

RESULTS

LENGTH TRENDS OVER TIME: *NATRIX HELVETICA*

Uncorrected historic SVL measurements were compared to contemporary measurements and analysed with a two-way ANOVA. The analysis clearly showed SVL was smaller in contemporary snakes for both genders (Fig. 5) $F_{1,333}$=6.35, p=0.012 (corrected: $F_{1,333}$=14.16, p<0.001, Appendix 2.II.a & b). This represented a 4.6% difference in average SVL length for females and an 11.2% difference for males (corrected: 10.3% and 16.5% respectively). As expected, females were larger than males $F_{1,333}$=21.45, p<0.001 (corrected: $F_{1,333}$=22.77, p<0.001). There was no difference in the change over time between gender $F_{1,333}$=0.38, p=0.537 (corrected: $F_{1,333}$=0.61, p=0.433).

Tail length comparisons again showed significant differences over time for both genders (Fig. 6.) $F_{1,333}$=14.39, p<0.001 (corrected: $F_{1,333}$=25.36, p<0.001, Appendix 2.II.c & d). Difference for average female tail length was 14.4% and18.6% in males (corrected: 19.1% and 23.5% respectively). No disparity between gender was found $F_{1,333}$=1.78, P=0.183 (corrected: $F_{1,333}$=1.91, P=0.168), even though male *N. helvetica* are reported to have longer tails than females (Luiselli, 2009). There was no gender and time interaction indicating that the differences over time were the same in both sexes $F_{1,333}$=0.14, p=0.706 (corrected: $F_{1,333}$=0.18, p=0.667).

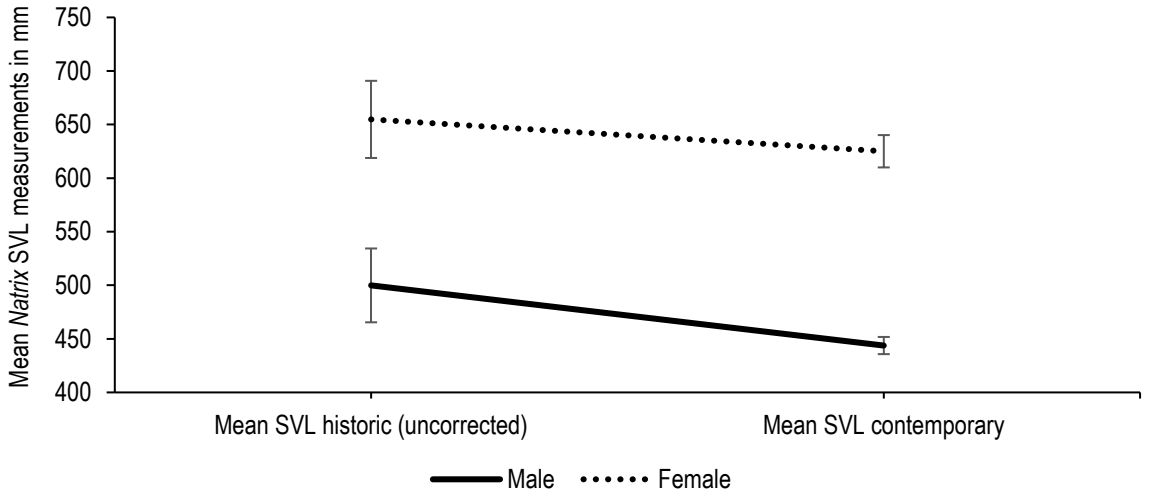


FIG. 5. Mean SVL (±SE) over time for *N. helvetica* with uncorrected historic data

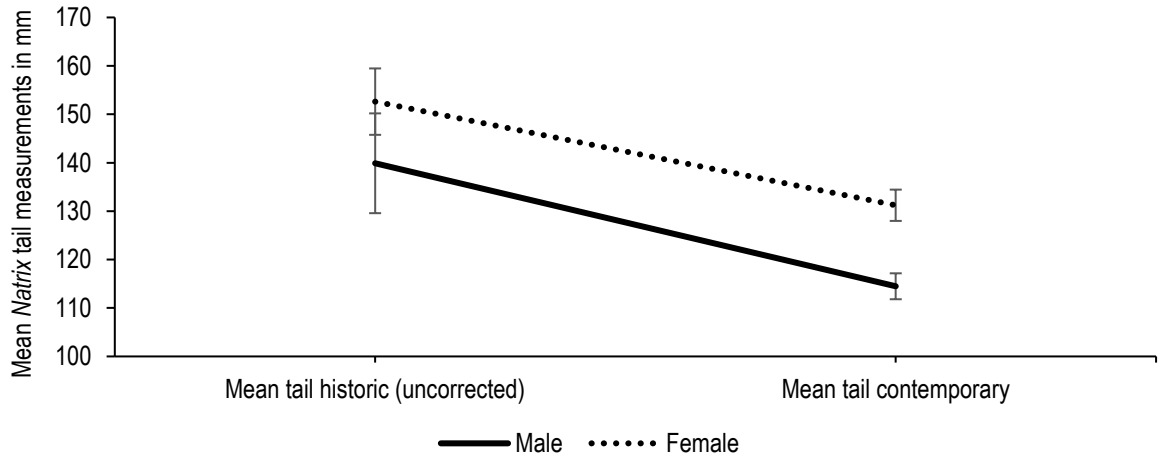


FIG. 6. Mean tail (±SE) over time for *N. helvetica* with uncorrected historic data

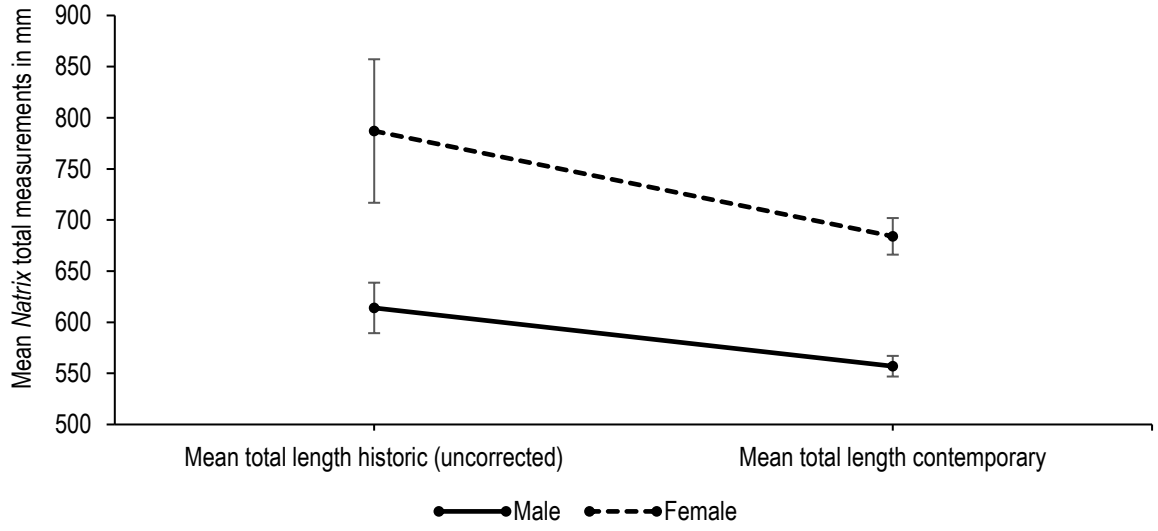


FIG. 7. Mean total (±SE) over time for *N. helvetica* with uncorrected historic data

Total length was calculated for each snake by adding their SVL and tail measurements together. In keeping with the trends already identified, analyses revealed total average length for females was larger than males $F_{1,333}$=17.06, p<0.001 (corrected: $F_{1,333}$=18.11, p<0.001), and there was no significant interaction between genders over time $F_{1,333}$=0.32, p=0.568 (corrected: $F_{1,333}$=0.51, p=0.473). Overall, results strongly supported an average difference in total length between historic and contemporary snakes of both sexes (Fig. 7) $F_{1,333}$=8.39, p=0.004 (corrected: $F_{1,333}$=17.65, p<0.001, Appendix 2.II.e. & f.), with an average difference of 15.2% for females and 13.0% for males (corrected: 20.5% and 18.2% respectively). Corrected data shows that these averages were 20.5% for females and 18.2% for males.

LENGTH TRENDS OVER TIME: *VIPERA BERUS*

There were some similarities between trends observed for *N. helvetica* and those observed for *V. berus*. Female *V. berus* are, on average, larger than males (Forsmann, 1993) and this dimorphism was clearly reflected in the data $F_{1,257}$=5.4, p=0.021 (corrected: $F_{1,257}$=0.72, p=0.394). Moreover, average SVLs for both *V. berus* sexes were smaller than historic snakes (Fig. 8) $F_{1,257}$ =47.41, p<0.001 (corrected: $F_{1,257}$=69.12, p<0.001) with a difference of 12.9% in females and 11.4% for males (corrected 6.3% and 5.9% respectively, Appendix 2.III.a. & b). Interestingly, there is also evidence that males and females appear to be responding differently over time $F_{1,257}$=4.22, p=0.041 (corrected: $F_{1,257}$ =0.33, p<0.561) with a sharper differential in females.

Differences in average tail length for females at 7.3% (corrected: 14%) were again highly significant over time (Fig. 9) $F_{1,257}$=9.49, p=0.002 (corrected: $F_{1,257}$=21.49, p<0.001, Appendix 2.III.c & d), but not for males at 1.2% (corrected: 7%), and a clear difference between genders was identified $F_{1,257}$= 62.36, p<0.001 (corrected: $F_{1,257}$= 88.93, p<0.001). While gender disparity was not unexpected as *V. berus* males have longer tails than females (Forsmann, 1993), there was considerable interaction over time especially evident in females $F_{1,257}$=7.93, p=0.005 (corrected: $F_{1,257}$ =1.51, p<0.219).

Total lengths supported an overall difference between historic and contemporary snakes for both genders (Fig. 10) $F_{1,257}$=42.33, p<0.001 (corrected: $F_{1,257}$=63.57, p<0.001). Females differed by 12.3% in overall length and males by 9.9% (18.6% & 15.7% respectively, Appendix 2.III.e & f). There was no indication that either gender was significantly larger than the other $F_{1,257}$=1.15, p=0.286 (corrected: $F_{1,257}$=0.18, p=0.665, but genders were

responding differently over time with the contrast between females and males more marked in the contemporary data set $F_{1,257}$= 4.76, p=0.030 (corrected: $F_{1,257}$=0.45, p=0.502).

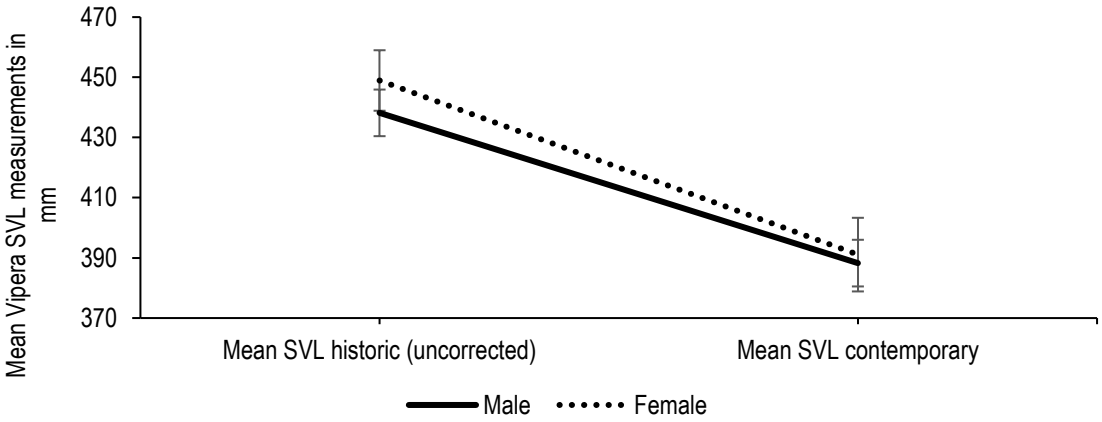


FIG. 8. Mean SVL (±SE) over time for *V. berus* with uncorrected historic data

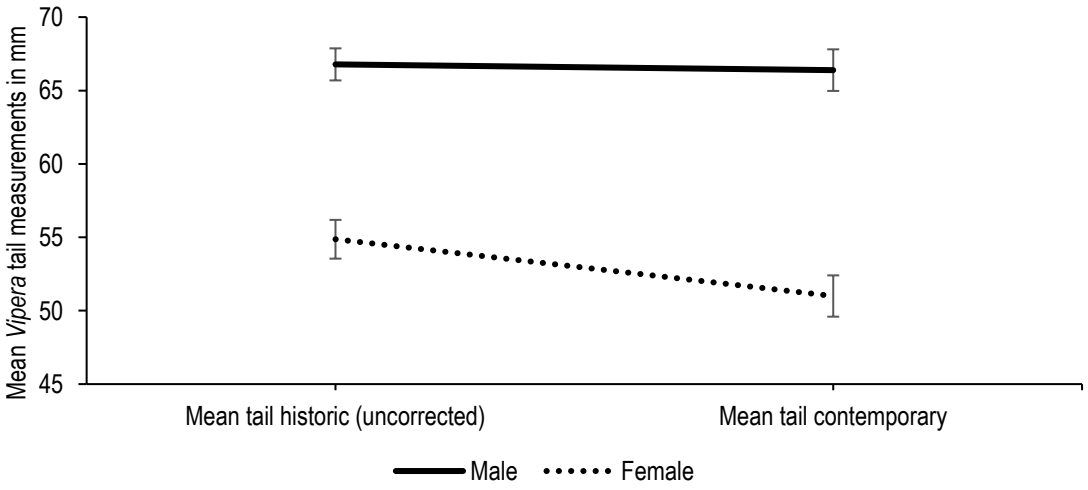


FIG. 9. Mean SVL (±SE) over time for *V. berus* with uncorrected historic data

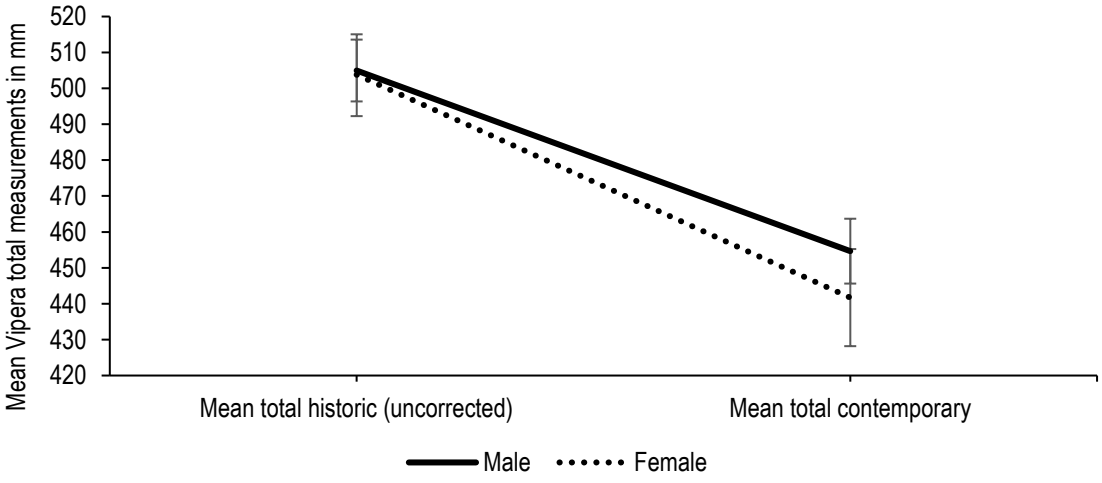


FIG. 10. Mean SVL (±SE) over time for *V. berus* with uncorrected historic data

It can be inferred, therefore, that in both *N. helvetica* and *V. berus,* museum specimens collected 50-100 years ago are larger than live animals measured more recently. The decrease in millimetres is summarised in Table 1.

TABLE 1: Total average length decrease (in mm) over time for *N. helvetica* and *V. berus*, both genders

		Total decrease in mm (uncorrected)	±SD	Total decrease in mm (corrected)	±SD
N. helvetica					
	Male	83	54.7	124	57.6
	Female	123	61.7	176	64.5
V. berus					
	Male	50	18.0	85	18.2
	Female	62	26.9	101	27.8

DISCUSSION

Comparative analyses using the uncorrected data support the hypothesis that contemporary *N. helvetica* and *V. berus* are significantly smaller (i.e. shorter in length) than their historic conspecifics. There is evidence in the data to indicate that this is an ongoing phenomenon for both *N. helvetica* and *V. berus*, and for both genders. Differential effects are apparent between male and female *V. berus*. Accentuated trends were observed on all counts following comparative corrected data analyses because, as per Reed (2001), 6.5% was added to historic measurements to compensate for the shrinkage effects of preservation.

IS CLIMATE CHANGE NEGATIVELY AFFECTING HERPETOFAUNA?

Global studies are increasingly showing that reptile rate of growth is not keeping pace with the effects of climate change, and support for the hypothesis that reptiles are becoming smaller is mounting (Nicholls, 2013). In the UK, temperature rose in the 20th Century by approximately 1°C and the thermal growing season lengthened by close to a month. Predictions for the future indicate that by 2080, temperatures could increase by 2°C to 3.5°C although this could be by as much as 5°C in the southeast (Hulme *et al.,* 2002). There is a considerable body of evidence demonstrating that climate change is impacting on UK species. Hickling *et al*. (2006) showed that range margins for more than ten taxa have shifted significantly northwards. In amphibians and reptiles, distribution shift does not follow this trend and has instead collapsed southwards with species confined in fragmented populations

in a fraction of their former distribution. This response is perplexing as, intuitively, herpetofauna should benefit from warming. They appear, however, to be following a worrying trend observed in other species - most notably butterflies - indicating a lack of dispersal ability which in turn pushes populations into decline (Warren *et al.,* 2001; Hickling *et al.,* 2006).

Indeed, both *N. helvetica* and *V. berus* have declined in recent years and while declines appear to have slowed in the case of *N. helvetica*, they continue to accelerate for *V. berus* (Beebee *et al*., 2009). The reasons for these declines are not immediately clear, but factors such as habitat loss, inbreeding, persecution, pollution, and climate change have been cited as possible causes (Beebee *et al*., 2009). Milder winters are already taking a toll on toads and newts in the UK as they can disrupt hibernation, which in turn leads to poor breeding condition and, in toads, smaller body size (Reading, 2007; Griffiths *et al*., 2010). It is entirely plausible that the same scenario is playing out for UK reptiles. It was recognised almost half a century ago that climate affects spermatogenetic development in *V. berus* (Nilson, 1980).

LARGER SIZE INDICATIVE OF INCREASED REPRODUCTIVE SUCCESS AND FECUNDITY

There is a significant link between body size, reproductive success, and fecundity in snakes. Generally, larger males tend to enjoy greater breeding success. For example, bigger male western diamond back rattlesnakes (*Crotalus atrox*) reach sexual maturity quicker enabling them to breed earlier; and larger male black rat snakes (*Elaphe obsoleta*) sire more offspring per clutch due to greater success during male-male combat and sperm competition (Taylor & Denardo, 2005; Blouin-Demers, 2005). Smaller size can be considered a positive trait in some circumstances - including for *N. helvetica* - as, according to Thorpe (1989), smaller body size promotes vagility which can in turn increase a male's breeding success. However, the reverse was found to be more likely in later studies (Madsen & Shine, 1993). The advantages of having a larger body in *N. helvetica* for both sexes are strongly supported in terms of greater reproductive success. Larger males copulate more frequently than smaller males, and larger females attracted more males (Luiselli, 1996).

New information is emerging pertaining to maternal body size and its effect on the neonatal reptile immune system. There is now evidence that maternal size may play a more important role in this area than previously thought. An examination of blood smears demonstrated that hatchling keelbacks (*Tropidonophis mairii)* born to larger females possessed more azurophils and fewer lymphocytes (Brown & Shine, 2016). The implication of this

is that larger females may produce offspring with a stronger immune system as azurophils have an important pathogen-killing mechanism (Kolaczkowska & Kubes, 2013). Keelbacks are natricine colubrids so it is possible that the above findings may also have implications for *N. helvetica.* Moreover, larger females give birth to larger young. There is a link between hatchling *N. helvetica* size and locomotor performance which, in turn, has implications on young snakes' ability to escape predators as larger hatchlings are faster both terrestrially and aquatically (Hagman *et al*., 2015).

The impact of smaller body size for male *V. berus* is poorly understood, but while larger males are more successful in combat, they may succumb more readily to extended periods of prey unavailability as they require more energy to function than smaller animals (Forsmann, 1993). Fecundity is negatively impacted by smaller body size in female snakes resulting in small egg clutch and litter size. In *V. berus*, this was supported by Andrén & Nilson, (1981) who calculated that there is one extra juvenile for every 2cm of female body length. Survivorship during times of prey scarcity is more likely in larger *V. berus* as they can survive without food for longer than smaller individuals. Moreover, as prey-size limited predators, larger snakes can eat bigger, and consequently more diverse prey items if there is variation in prey size. A study that compared *V. berus* size variation between populations found that snakes were larger where the main prey item (field voles) were bigger (Forsmann, 1993).

SAMPLING AND MEASURER BIAS

Sampling bias is a known problem when conducting herpetological surveys and it can easily lead to misrepresentation of size frequency distributions in study populations (Rodda *et al.,* 2015a). Observer bias is particularly troublesome when methods such as visual searches – the success of which can vary greatly according to skill and experience of the observer(s) – are employed (Willson, 2016). Other sources of bias can be assigned to species-specific life traits such as habitat association, variation in colour or behaviour, and response to capture, or the sampling method employed; how traps are designed, and how the area and habitats are sampled (Willson, 2016). For example, evidence strongly supports that smaller snake species as well as the juveniles of larger snake species are much more likely to use refugia, introducing a sampling bias to collection efforts (Herczeg *et al*., 2007; Gregory &Tuttle, 2016; Halliday & Blouin-Demers, 2015). It is unknown whether historic specimens were collected opportunistically (i.e. out in the open), but as the use of artificial refugia is a relatively recent development it is likely

that this was the case. Contemporary snakes were caught both opportunistically and targeted under refugia. Given that smaller snakes from both species in this study favour cover objects, this sampling bias cannot be discounted.

Measurer bias can, according to Setser (2007), be allocated to three categories which are: variation between measurements made by a single measurer using a single technique; variation between measurements made by different measurers using the same technique; and variation between measurements made using different techniques. Whether snakes are shrinking or whether evidence of shrinkage could in fact be due to measurer bias or sampling error is a controversial topic (Madsen & Shine, 2001; Luiselli, 2005). The best way to measure a snake is debatable. What is certain is the conventional measurement to take is the SVL as this eliminates the issue of measuring snakes with part of their tail missing and biasing results. Various methods have been developed to measure snakes including pinning and stretching the animal alongside a ruler, immobilisation in a squash box and running a string down the ventral midline, and anaesthetising the animal and stretching it along a ruler although the latter is only suitable in a laboratory environment. All these methods have associated biases and, according to some studies, reflect varying degrees of inaccuracy (Bertram & Larsen, 2004; Penning *et al*., 2013) with Cundall *et al*. (2016) stating that snakes do not in fact have a single length, rather a range of normal operating lengths. The implication is that it is virtually impossible to obtain a precise, actual size.

More recently, image analysis software as a tool for snake measuring is gaining popularity (Cundall *et al.,* 2016; Astley *et al.,* 2017). Software enables key measurements to be obtained from a photograph as long as a measurement scale is included alongside the snake when the image is taken. There is still a risk of measurements bias as it can be difficult to ascertain the location of the anal plate - required as a measurement landmark - especially if the photograph is of low quality or the recorder lacks experience. It is also possible that there may be inter and intra-measurer variation when using software as well as differences between the software packages themselves.

VALIDITY OF COMPARING PRESERVED SPECIMENS AND LIVE ANIMALS

The multitude of preserved specimens held in institutions globally provide scientists with valuable biological libraries. These collections facilitate research on species that may otherwise be hard to conduct in the wild and provide pathways into new areas of study. Moreover, the data that can be extracted from historic collections offer insight to the morphological and evolutionary changes biodiversity and populations experience over time.

There are many preservation methods but a common, contemporary technique fixes specimens in buffered 10% formalin. The specimen is then washed in water and immersed in 70% ethanol, (80% ethanol or IMS at the NHM). Other common, but less reliable methods include preservation in pure formaldehyde or in solutions with additives such as Bouin's or Gilson's solution (Vervust *et al.,* 2009). Earlier methods of preserving, as in the case of the pre-1950s snakes in this study, simply involved immersing specimens in alcohol or spirit. Storing specimens in this way is associated with significant dehydration and, in snakes, shrinkage in length (Klauber, 1943, Reed, 2001).

LIMITATIONS

Historic data in this study originates from NHM specimens that were preserved using different methods. Those pre-dating 1950 may not be formalin-fixed and are stored in 'spirit' while post 1950s specimens may be fixed in 10% formalin and stored in either 80% ethanol or IMS. Consequently, there may be some variation in preservation effects on body size, but it is impossible to determine whether this is the case in this study. Simmons (2014) states that information is frequently unavailable from collections on how specimens were handled, whether they were fixed (and how they were fixed), and the method used for preservation.

The study referred to Reed (2001) to obtain the 6.5% correction value to address shrinkage. However, Reed's research used a different preservation method and, unlike this study, included a fixing stage. It is possible that the correction factor of 6.5% does not compensate accurately for shrinkage of historic specimens in this case. It is recommended that researchers conduct a pilot study, where possible, to help establish corrective factors for the specific taxa of interest (Reed, 2001), although other studies indicate this may be required at species level (Lee, 1982; Vervust *et al.,* 2009).

The historic sample size for male *N. helvetica* was very small (n=9). Additionally, measurements from snakes captured in Jersey were included in both historic and contemporary datasets as once snakes had been filtered according to the criteria required to conduct analyses, there were inadequate historic entries to compare male *N. helvetica* (Appendix 2.I.a). This introduces two limitations to the study. The first is these snakes are not representative of UK *N. helvetica* populations. Secondly, Jersey *N. helvetica* are an island population and there may be morphological differences, notably in size, between the Jersey population and those found in the UK. Individuals can vary in size between populations although this could be down to prey availability rather than

genetics (Madsen & Shine, 1993). The study could be improved by removing male *N. helvetica* from the study completely due to the low sample size and potential misrepresentation due to inclusion of Jersey measurements in the analyses.

Inter-observer bias is a common issue in studies involving measurements and it cannot be disregarded here. While historic measurements were taken by one researcher, contemporary measurements were taken by four. The introduction of inter-observer bias in morphometrics is more significant than previously thought, and researchers are increasingly highlighting its confounding effects (Lee, 1990; Hayek *et al.,* 2001; Roitberg *et al.,* 2011; Cundall *et al*., 2016). A sampling bias is also present as snakes in the contemporary set were both opportunistically collected as well as sampled for under refugia. Smaller *N. helvetica* and *V. berus* both favour cover objects Additionally, the methods used to collect snakes in the museum collection are unknown.

CONCLUSIONS

Survival for reptiles is inexorably linked to climate where even a small variation in temperature can detrimentally impact on their ecology and physiology (López-Alcaide & Macip-Ríos, 2011). The synergistic effects of global warming on reptile phenology, distribution traits and behaviour patterns are well documented (Whitfield-Gibbons *et al.,* 2000) and are key factors increasing the threat of extinction for this taxon worldwide.

Current research suggests climate change is impacting UK herpetofauna (Hickling *et al*., 2006), and that both *N. helvetica* and *V. berus* are in decline (Beebee *et al*., 2009). Distribution shifts indicate that reptiles may not be able to keep pace with the rate of climate change, and action will most likely be needed in the future to facilitate their movement (Sutherland *et al*., 2008). The full implications of decreasing body size in UK snakes are unclear, but the evidence so far suggests it may be far-reaching.

CHAPTER 3: OBSERVER ABILITY TO DETECT *NATRIX* IN THE FIELD USING PLASTICINE SNAKE MODELS

SUMMARY

The ability to detect snakes in the field could be influenced by phenotypic and morphological variables attributable to the target species. These variables include body size, colouration, and body positioning. To test what effect - if any - these variables had on detectability, plasticine model *N. helvetica* were distributed along a predetermined transect in likely reptile habitat. The transect was walked by two inexperienced groups (Group A and Group B), and an Experienced Observer independently, and model observations recorded. All groups detected more larger models than small models. Overall, the Experienced Observer found 27% more models than Group A and 20% more models than Group B. Moreover, the Experienced Observer found more small models than Group A and Group B (42% and 33% respectively). This study was unique as it was the first of its kind to use phenotypically accurate representations of a target species to assess detectability during a visual survey.

INTRODUCTION

A major issue associated with cryptic species surveying is the recording of false negatives whereby the species is present but goes undetected at the site (Fitzpatrick *et al*., 2009). Failure to take detectability into account can detrimentally impact analytical accuracy in key areas such as population structure, abundance, and species richness. With the engagement of volunteers increasing in biodiversity surveying and monitoring programmes – Schmeller *et al*., (2009) found that 86% of participants in European biological monitoring schemes were volunteers – the resulting data are often viewed critically (Lewandowski & Specht, 2014). Indeed, Fitzpatrick *et al*., (2009) caution against mixing participants with differing experience levels in the same survey as this can introduce sampling variation and increase the likelihood of both false negatives and positives.

Cryptic reptile species can be difficult to observe in the field especially in the case of smaller individuals and without the use of artificial refugia (Gregory & Tuttle, 2016; Halliday & Blouin-Demers, 2015). When out in the open, body size, body positioning, and the presence / absence of the yellow collar distinctive to *N. helvetica* could influence detectability. Using plasticine snake models, this study examines the impact of both size bias and detectability on survey efforts. Other considerations linked to detectability includes the target species' behaviour,

phenological traits, morphology, cryptic nature, size and life stage as well as the sampling method and capture technique employed to obtain data (Wilson, 2016; O'Donnell *et al*., 2015; Mazerolle *et al*., 2007).

While the use of replica model organisms in ecological studies is not novel (Bateman *et al*., 2016; Bittner, 2003; Madsen, 1987; Mitrovich & Cotroneo, 2006; Posa *et al.,* 2007, Saporito *et al*., 2007), the use of species-specific models to investigate detectability has been little explored. In Honduras, Albergoni *et al.* (2016) examined the effectiveness of volunteers visually surveying for model herpetofauna, including snakes. However, the models were unnaturally brightly coloured representing generic body form over any species-specific characteristics. Some of the models were bright pink or purple, which could have made spotting them more likely. The plasticine models for this study reflected the natural colouration of *N. Helvetica* and blended into the habitat very effectively making observing them more challenging and more realistic. This ensured that surveying effort would be more reflective of a real-world scenario and consequently strengthen analyses when considering experience level.

This aim of this study was twofold. Firstly, to assess what influence – if any – different model body positioning and phenotypic traits would have on detectability for both inexperienced and experienced participants. Secondly to find out whether it would be easier for groups to observe large models or small models in the field.

METHODOLOGY

PREPARING SNAKE MODELS

Models were made from non-toxic, pre-coloured modelling plasticine (Newplast®) using the colour 'ginger' for the heads and bodies, and 'yellow' for the distinctive collar and eyes. Eight different model types were identified, coded, and then created (Table 1).

TABLE 1. Coded *Natrix helvetica* model types. Each model type n=13

Large models (n=52, <1000mm >955mm)		Small Models (n=52, <500mm >455mm)	
LCY	large, coiled, collared	SCY	small, coiled, collared
LCN	large, coiled, no collar	SCN	small, coiled, no collar
LUY	large, uncoiled, collared	SUY	small, uncoiled, collared
LUN	large, uncoiled, no collar	SUN	small, uncoiled, no collar

The dimensions of a Newplast® block are 220mm (length); 50mm (width); and 30mm (depth). Each block is made up of 32 individual cords which can be easily peeled away from the block as needed. The cords are arranged in strips of four. Making all 104 models required 45.5 blocks (23.3kg) of ginger Newplast® and one block of yellow

(500g). Large *N. helvetica* models were made using 20 cords (312.5g of Newplast®) per model while small models were made using 8 cords (125g of Newplast®) per model. Heating blocks of Newplast® in a preheated kitchen oven at 50°C for approximately 2 minutes made the material more pliable and easier to mould into shape.

Models were made by hand. A large, clean surface was used to roll the Newplast® into a snakelike shape gradually working along the body to form a tapered tail. No differentiation was made between gender. A thick section was left unrolled at the other end for the head, which was then formed by manually pressing the section into shape. Yellow collars and eyes were added after the main model structure had been made, but there was no specific standardization in fabrication apart from ensuring size and positioning were morphologically correct. Each model was left to set by cooling on greaseproof paper at room temperature.

Once the models had cooled and become rigid, morphological details were added. Flank patterning and neck stripes around the yellow collars were replicated using a small paintbrush (Master Art "Premier" size 3) and black exterior masonry paint (B&Q Black Smooth Masonry Paint 50ml Tester Pot). The dark colouring around the yellow iris of the eye and the circular pupil were drawn on using black, indelible pen (Sharpie Ultra Fine Tip Permanent Marker). The dorsal and ventral surfaces were left unmarked (Figs. 1 & 2). When the paint had dried, the models were then packed in layers on greaseproof paper and put into boxes for transporting.

FIG. 1. Four types of uncoiled model
A Large, uncoiled, no collar (LUN)
B Small, uncoiled, no collar (SUN)
C Large, uncoiled, collared (LUY)
D Small, uncoiled, collared (SUY)

FIG. 2. Four types of coiled model
E Large, coiled, collared (LCY)
B Large, coiled, no collar (LCN)
C Small, coiled, collared (SCY)
D Small, coiled, no collar (SUN)

EXPERIMENT SITE

The study took place at Soakham Downs, an established reptile surveying site in Kent. It has four reptile species present including occasional *N. helvetica* and is monitored by the Durrell Institute of Conservation and Ecology (DICE), University of Kent. The site is visited by students who survey for reptiles as part of an undergraduate fieldwork module. Thirty tin refugia are already in place (Appendix 3.I.) and students walk a predetermined but unmarked transect of roughly 350m over an area approximately 3000m^2 (Fig. 3) checking tins and recording the reptiles that they find under them. Students also look for any reptiles openly basking along the transect. Soakham Downs was selected as the best fit for the detectability study as it is a relatively small site, easily accessible and represents excellent reptile habitat.

To ensure adequate data were collected and that enough participants were available, the study was timed to coincide with two inexperienced student groups (Group A (n=9) and Group B(n=10)) undertaking fieldwork over two days. Group A took part in the study on day one and Group B took part on day two. An experienced observer also participated on day two, walking the transect alone and recording model observations independently from the inexperienced student group. Students were not present when the Experienced Observer was looking for models.

MODEL PLACEMENT

The type order by which models would be placed was randomised by inputting each model code thirteen times into Excel and using the [=RAND()] function (Appendix 3.II.). On the day before the first group of students were due to survey, models were placed in likely reptile habitat within 5m of the transect walk but >1.5m from tins. Likely habitat was defined as an area with thick undergrowth and natural cover (Figs. 4 & 5) and precluded placements that would be too obvious or unusual such as the middle of a path or on a tree branch. A unique number from 1 to 104 was allocated to each model and written in indelible pen on the ventral surface to prevent repeat observations by the same group. Model locations were logged by GPS (eTrex30) (Appendix 3.II.) to facilitate retrieval when the experiment had concluded.

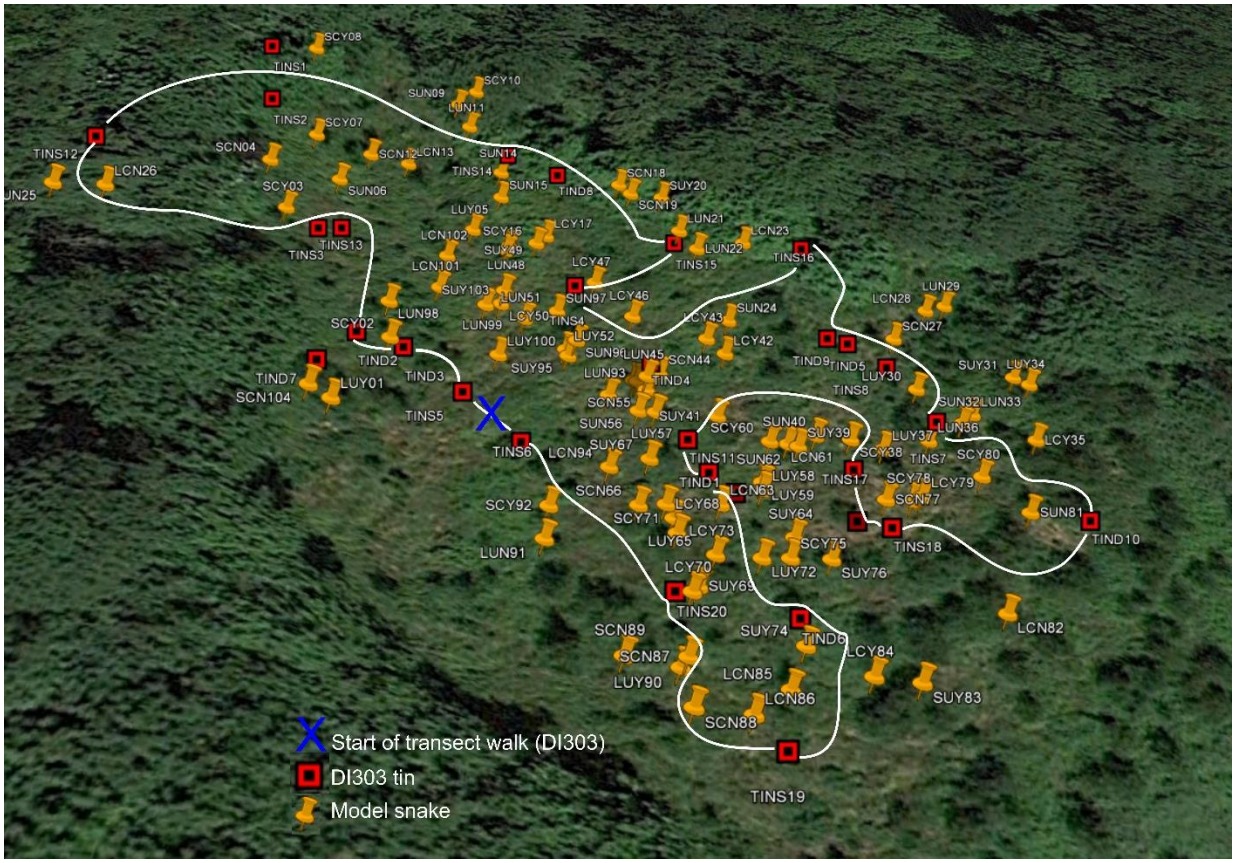


FIG. 3. Transect and tin / model placement at Soakham Downs mapped using Google Earth

FIG. 4. Example of model type large, uncoiled, collared (LUY) placed in likely reptile habitat

FIG. 5. Example of model type large, uncoiled no collar (LUN) placed in likely reptile habitat

TRANSECT WALK

Both inexperienced groups were accompanied by experienced reptile surveyors (Group A by three surveyors and Group B by two). The surveyors did not participate in the study but were present to help guide the students around the transect and to record observations made.

Prior to walking the transect, the groups were shown an example of a model snake and informed that they should try and detect as many as they could whilst on the walk. They were not told how many models were present at the site. They were asked to not touch the models and to call out if they found any. This was to ensure that model placement did not change as well as eliminate any bite risk from *V. berus* commonly found in the area. When an observation was made, an experienced reptile surveyor identified the model using its unique number and it was recorded in a notebook.

A specific time limit to walk the transect was not allocated but Group A and Group B took roughly an hour and a half to complete the transect while the Experienced Observer took two hours. Groups walked the same predetermined transect late morning / early afternoon in similar weather conditions in good light and worked

independently from each other. Group A participating on day one (8th April 2016) and Group B on day two (11th April 2016). The Experienced Observer also participated on day two but independently from Group B.

RESULTS

COMPARISON OF SURVEY GROUPS

Observations were collated per group (Group A, Group B, and Experienced Observer) and per model type (Appendix 3.III.a & b.). Overall, the Experienced Observer found the most models (27% more than Group A and 20% more than Group B, Table 2,), but all three groups found more large models than small. Ten models were not found by any group, 90% (n=9) of which were small (Fig. 6).

TABLE 2. Overall number / percentage of models found by each group

	Group A	Group B	Experienced observer
Represents % of total models	53	58	72
Overall model total found (of n=104)	55	60	75
Represents % of total small models	44	37	64
Overall small model total found (of n=52)	23	19	33
Represents % of total large models	62	79	81
Overall large model total found (of n=52)	32	41	42

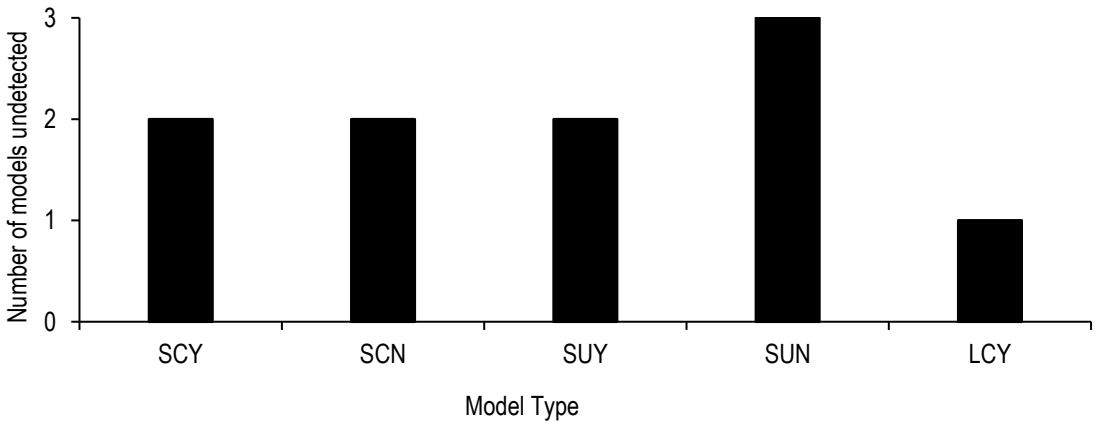


FIG. 6. Models undetected by any group
SCY=small, coiled, collar
SCN=small, coiled, no collar
SUY=small, uncoiled, collar
SUN=small, coiled, no collar
LCY=large, coiled, collar

The sum of each model type detected vs undetected by each group was then cast into 2x2 contingency tables and analysed using chi-square (df1, no Yates correction, two-tailed P value). All three groups found more large models than small models (Appendix 3.IV.a – d.), and significantly so for Group B and the Experienced Observer (Fig. 7). Only Group B was influenced by a trait other than size, with observers finding more snakes with collars (n=35) representing 67% of the total compared to 48% of total (n=25) for those without (Fig. 8). There was a tendency for all groups to observe more uncoiled snakes than coiled snakes, but this was only significant for Group B (Fig. 9).

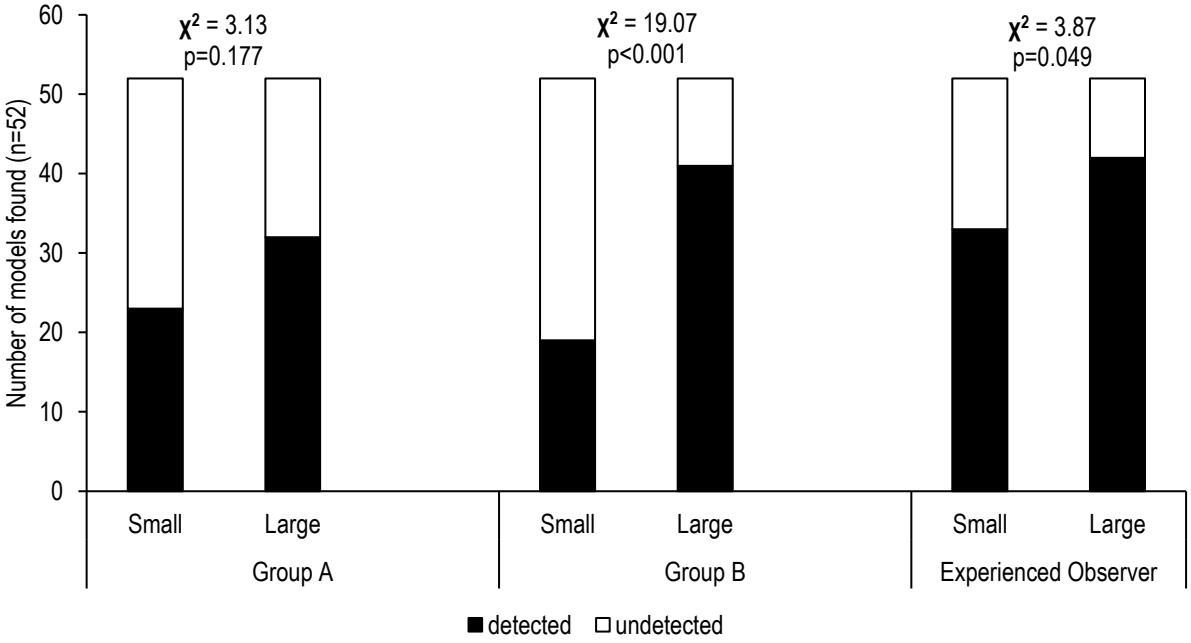


FIG. 7. Intra-group comparison for small vs large model detection

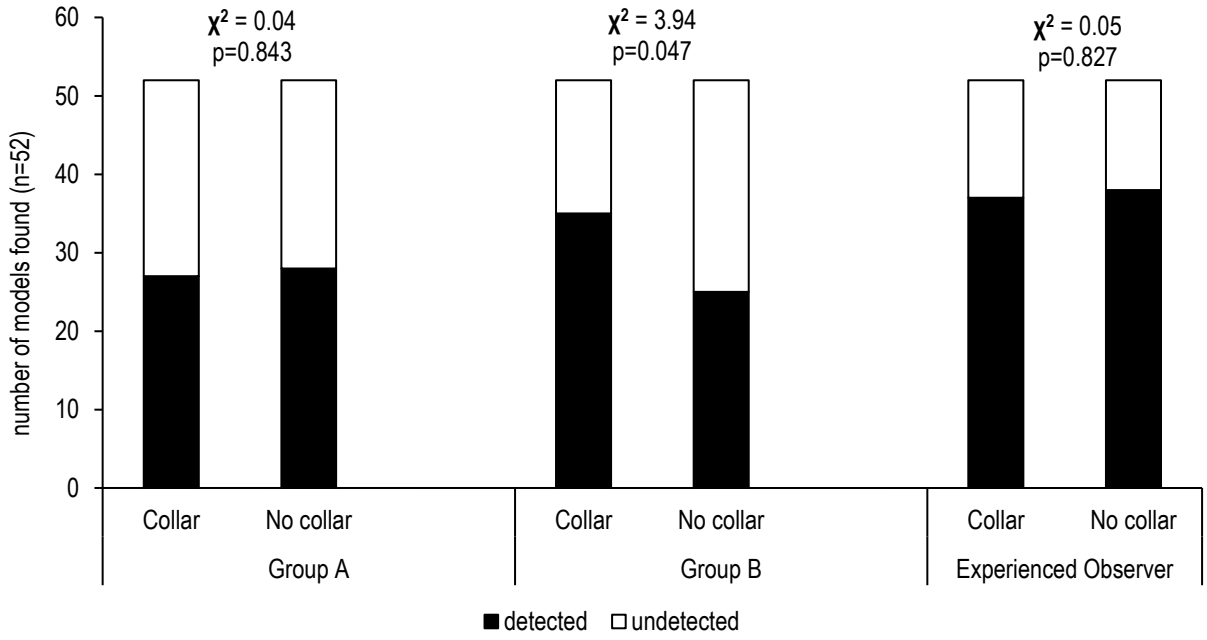


FIG. 8. Intra-group comparison for collared vs no collar model detection

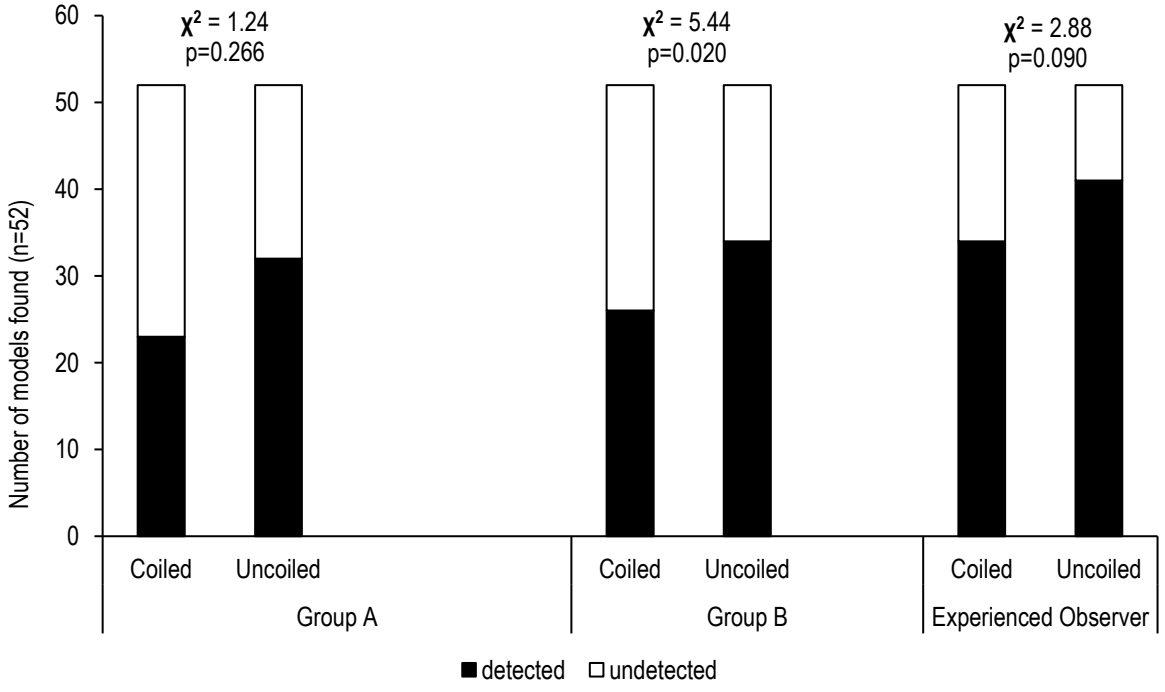


FIG. 9. Intra-group comparison for coiled vs uncoiled model detection

Once the 'large' variable had been removed from the analyses, only Group B observed more uncoiled snakes than small, coiled snakes (Appendix 3.V.a.). There were no distinctions between other groups or other traits (Appendix 3.V.b.).

INDIVIDUAL GROUPS VS EXPERIENCED OBSERVER

Some differences in detectability were noted when the groups were compared separately to the Experienced Observer. Keeping with the trend, model size was again statistically significant (Fig. 10) with the Experienced Observer finding more small models than either Group A or Group B but only more large models than Group A. Positioning and phenotypic variables were statistically significant in some cases (Fig. 11) but never across both groups for the same variable (Appendix 3.VI.a-d.). Variables that were not significant can be found in Appendix 3.VII.a. &. b.

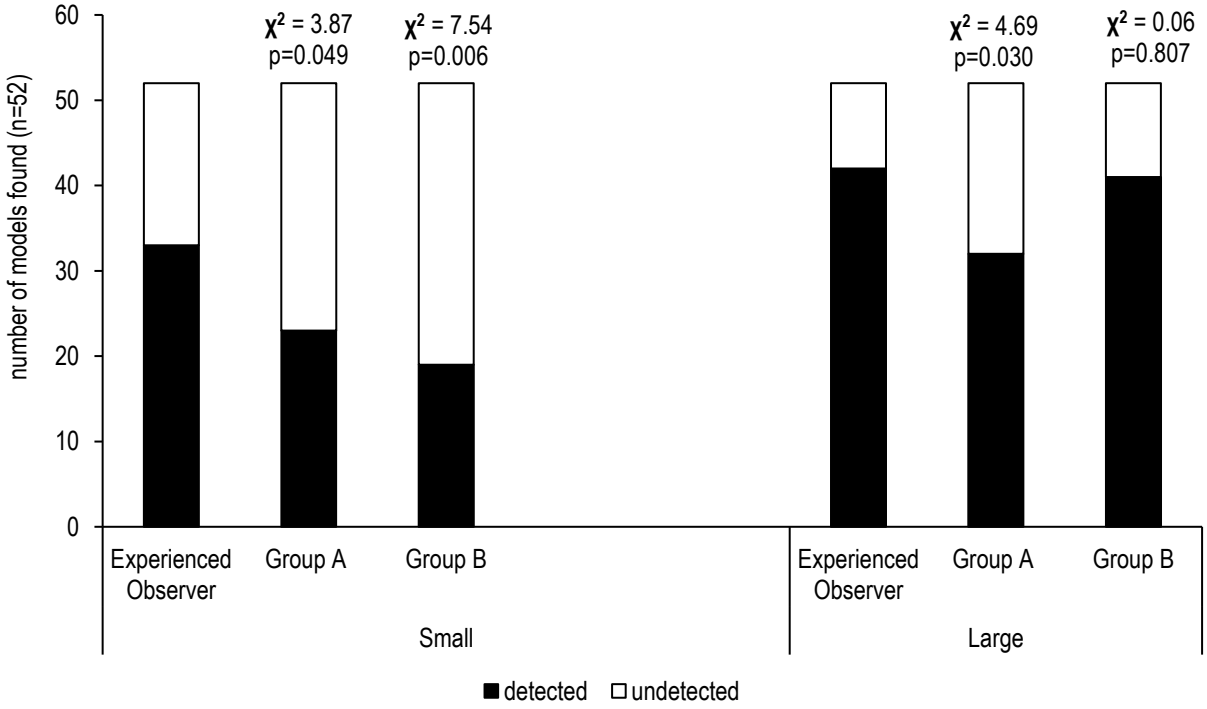


FIG. 10. Detection of small and large models: Group A vs Experienced Observer and Group B vs Experienced Observer

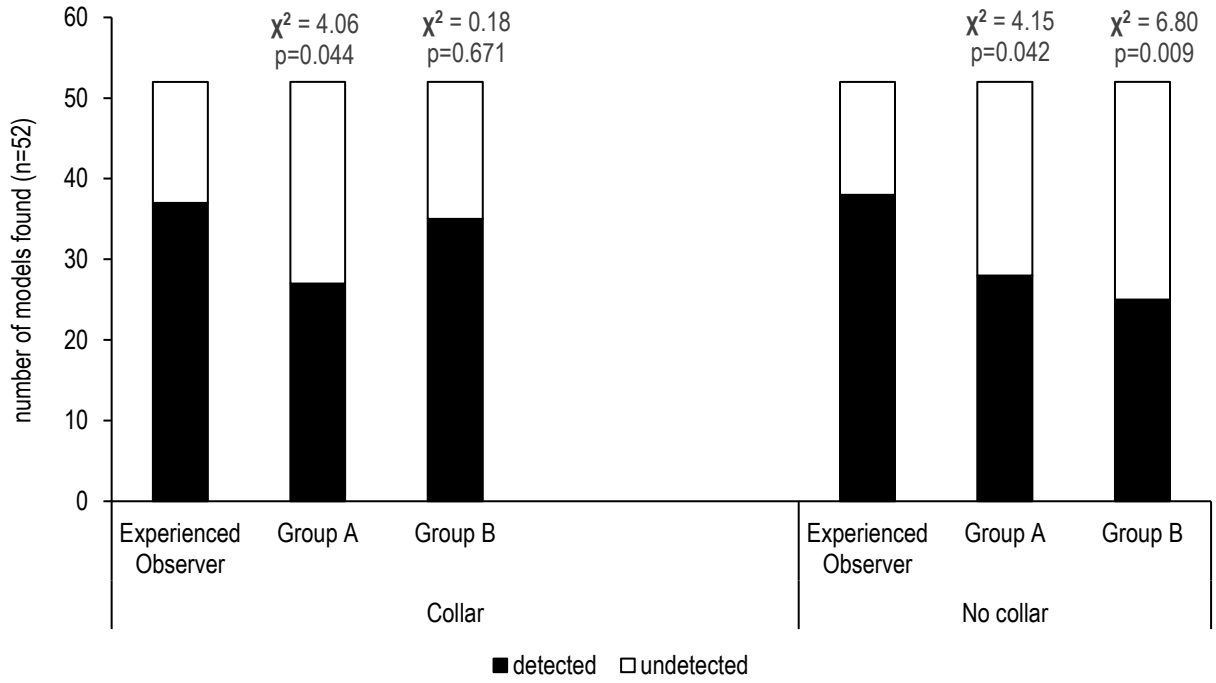


FIG. 11. Detection of collar and uncollared models: Group A vs Experienced Observer and Group B vs Experienced Observer

In sum, this study showed that large models were easier to find than small models for inexperienced and experienced observers alike. Ninety percent of models unlocated by any group were small. The presence / absence of collar and coiled / uncoiled variables were predominantly not significant. However, Group B did find more uncoiled small models than coiled small models. Group B also found more collared snakes overall when combining large and small models. When analyses were carried out independently for Group B on large models collar vs no collar, and small models collar vs no collar the results were not significant on both counts Appendix 3.V.a).

DISCUSSION

SAMPLING TECHNIQUE AND SIZE BIAS

Size bias related to sampling technique is a well-documented issue when it comes to population monitoring and sampling surveys for snakes in the field. This is especially true for smaller species, cryptic species and for snakes in earlier life stages (Gregory & Tuttle, 2016; Wilson, 2016; Halliday & Blouin-Demers, 2015). Interestingly, size bias is also apparent when using models. Albergoni *et al.* (2016) documented that volunteers conducting a visual survey for replica herpetofauna in Honduras observed more large models than small. A finding further supported by this study.

Determining an appropriate sampling method for target species requires knowledge of ecology and behaviour. For example, a programme in Guam that used traps baited with mice to capture invasive brown treesnakes (*Boiga irregularis*) was effective for adult snakes but failed to trap immature snakes due to ontogenetic shift (Rodda *et al*., 2007). The cryptic nature of many immature reptiles also confounds detectability. Rodda *et al.* (2015b) recorded a considerable capture disparity between juvenile and adult lizards with '…*a remarkably consistent undersampling of juveniles and a modest oversampling of prime adults in each of the five species [of lizards] we studied*'. In fact, mid-sized adults were 50% oversampled and juveniles were 24-79% undersampled.

The same trend applies to *N. helvetica* whereby adults are more likely to be found in the open and immature snakes under refugia (Gregory and Tuttle, 2016; Reading, 1997). This underpins the importance of selecting a sampling method most suited to (a) the study species and (b) uses techniques that minimizes size bias as much as possible. Mitigating for size bias is attainable, but it requires careful consideration. Conducting a simple visual encounter survey (VES) for a species such as *N. helvetica,* for example, would be an unsuitable sampling method as it would introduce a size bias. Eliminating size bias totally is most likely impossible – it could be the case that larger snakes are simply easier to spot.

DETECTION AND VOLUNTEER CONSIDERATIONS

Volunteer ability to adhere to sampling method protocol, complete different tasks, and collect and record high quality data can determine the success or the failure of a conservation project (Albergoni *et al.,* 2016). As the recruitment of volunteers into biodiversity monitoring schemes continues to increase so do the questions surrounding the reliability of volunteer-derived data (Lewandowski & Specht, 2014). For example, occupancy modelling seeks to account for imperfect detection while estimating the occupancy probability ψ that a target species is present (or absent) from randomly selected study areas (O'Donnell & Semlitsch, 2015). However, this type of modelling requires a minimum of two independent surveys each recording presence / absence data at each study site. It has been documented numerous times that different observers have different identification skills and differing approaches to search effort (Albergoni *et al.,* 2016; Freilich & LaRue Jr. 1998; Lewandowski & Specht, 2014) but inter-observer variation, and variation between experienced and inexperienced observers remains relatively understudied (Fitzpatrick *et al.,* 2009). In some cases, volunteer bias can be beneficial. Snall *et al*. (2011) suggest that volunteer-led opportunistic survey schemes focused on rare species yield comparatively more data

than systematic schemes with strict protocols. Clearly there is a need for more research in this area. Moreover, developing methods that enable researchers to better engage with volunteers will produce better quality data.

Volunteer characteristics can influence accurate data collection remarkably. Physical fitness, education background, visual acuity and hearing, previous biological surveying experience, and commitment and willingness to undertake tasks are all elements that can bias data collection (Newman *et al*., 2003; Mazerolle *et al*., 2007). Moreover, volunteer group size should be tailored to the survey work required as detectability decreases the larger the group size. This is most likely due to participants becoming distracted (Albergoni *et al*., 2016).

An easy aspect to overlook is the timing of the survey. Dim light or very bright light could affect visual acuity, and inclement weather may not only adversely affect visibility but also participant motivation to complete the study (Albergoni *et al*., 2016; Mazerolle *et al*., 2007). Moreover, the height at which observers are focusing on during biological surveys can have an influence on detectability. The study conducted by Albergoni *et al*. (2016) showed that volunteers recorded more model sightings at middle-level (43%) with little difference between ground level models (29%) and top-level models (28%).

Finally, the duration of the survey should be taken into account. An anuran call survey found that a 15-minute survey yielded more results than surveys conducted over five or ten minutes. Longer survey times showed a pattern of decreasing detection efficiency. The authors stated that, in the case of volunteers, excessive survey duration could decrease volunteer willingness to visit other sites during the same survey period. It may also detrimentally impact volunteer retention and, consequently, annual observations may vary (Pierce & Gutzwiller, 2004).

REPLICA MODEL ORGANISMS IN ECOLOGICAL RESEARCH

Using plasticine models can be hugely beneficial in ecological research. The pliable nature of plasticine makes it possible to record data that would otherwise be exceptionally difficult to obtain. Unlike materials used in other studies such as wood, plastic and rubber (Andrén & Nilson, 1981; King, 1987; Smith, 1977), plasticine is easily adapted to specific morphological plans and it comes in many different hues so species colouration is relatively easy to replicate. Additional phenotypic traits such as spots or stripes can simply be painted or drawn on. For particularly lifelike models, a technique has been developed that uses a master mold and silicon yielding excellent results (Yeager *et al.,* 2011).

Plasticine has been used in predator-prey interaction studies as it retains beak, claw, and teeth imprints (Madsen, 1987; Bittner, 2003). This property enabled Mitrovich and Cotroneo (2006) to establish that ground squirrels attack smaller rattlesnakes more aggressively than larger ones, and that smaller snakes are attacked in the head area while larger snakes are attacked around the tail. Likewise, researchers in Costa Rica used 800 plasticine frogs to determine that bright colouration did function as an aposematic signal (Saporito *et al.,* 2007), and in the Philippines plasticine caterpillars and nests were used to establish if there was a link between habitat degradation and predation (Posa *et al*., 2007). Researchers now had a new, highly effective tool to investigate antipredator behaviour of prey.

Plasticine models are not just limited to predator prey studies. As this study has clearly shown, plasticine is also an excellent tool to explore two key unknowns in surveying – size bias and detectability for target species.

LIMITATIONS

There are several factors to consider should this study be repeated. One such factor was lack of observers' concentration to the task at hand. In both inexperienced groups, there was clear variability between participants in their engagement with searching. This was more of an issue with Group B than Group A. This is relevant as the impact of volunteers' willingness and motivation to participate in a survey can affect the quality of data collected (Lewandowski & Specht, 2014). One individual in Group B was responsible for a considerable number of model finds but each group had individuals that were more adept at finding models than the rest. Students were asked if they had any field experience prior to walking the transect – none of them did – but some were clearly more motivated to search and had longer attention spans than others. It would be good practice to ascertain participant experience level as well as willingness to take part in a study such as this.

It is possible that Group B had a slight advantage over Group A when looking for models. The grass and vegetation had been flattened by Group A in some areas the previous day, so this may have enhanced detectability within Group B. To eliminate this bias, it would have been advisable to allow some time to elapse before running the second transect. This would have allowed the grass and vegetation to recover so formerly trampled patches would not have been discernible to Group B.

Results could have been strengthened if the same timeframe had been accorded across the groups and the Experienced Observer. The Experienced Observer took half an hour more than the groups to complete the

transect. It is feasible, therefore, that the total end count would have been less than it was had the Experienced Observer been restricted an hour and a half window – the time it took for both inexperienced groups to complete the transect.

CONCLUSIONS

Phenotypically accurate models such as the plasticine snakes in this study are a useful tool for researchers to gain a better understanding of detectability biases, volunteer ability, and the accuracy of data observers record. This is important for two reasons. Firstly, the dependency on volunteer data drawn from biological surveys has increased dramatically in recent times. This could be due to online engagement through 'citizen science' monitoring programs and easy data upload to monitoring schemes (Schmeller *et al*., 2009). Secondly, volunteer data are often excluded from final analyses due to the concern that it is fundamentally flawed (Lewandowski & Specht, 2014). Dependent on the sampling methodology employed, researchers can use models to test for detectability bias in advance. This can help determine whether skilled observers, inexperienced volunteers or a combination of both best suit a study as well as tweak existing methodologies to ensure detectability biases are considered. By targeting sampling methods to the skill level of participants, researchers can obtain excellent results without significant variation between skill levels (Freilich & LaRue Jr. 1998; Oldekop *et al.,* 2011; Newman *et al*., 2003).

CHAPTER 4. MEASURING TECHNIQUES AND MEASURER BIAS

SUMMARY

Measuring a snake can be remarkably difficult. Different methods and different measurers may yield different values for the same measurement. Accounting for such variation and bias is therefore important when carrying out comparative analyses of morphology. Three experiments were conducted to test for biases associated with snake biometrics as they relate to accuracy, precision, and repeatability. *N. helvetica* and *V. berus* measurements recorded manually using the squash box and string technique by one researcher and measured again by another researcher using the image analysis software, Image J, were compared. Some variation between measurements was found, but only for *V. berus* tails. The second experiment tested variation in *N. helvetica* SVL and tail lengths taken by three independent measurers each using the image analysis programs Image J, Snake Measure Tool, and Serpwidget. While no inter-program differential was noted, inter-measurer variation was high with one participant consistently recording longer lengths. Finally, the same *N. helvetica* was repositioned five times in a squash box and a photograph taken for each position. SVL and tail measurements were recorded ten times for each photo in Image J, Snake Measure Tool, and Serpwidget by one researcher. Measurements from the same snake recorded in different positions yielded different lengths. While inter-measurer, intra-measurer, and inter-method biases are addressed in the literature in some form, this is the first experiment of its kind to test variation in measurements obtained digitally for the same snake in different positions.

INTRODUCTION

Morphometrics are an integral part of systematics, ecological and evolutionary research. Body size can provide valuable details about biological, physiological, and ecological traits. Growth rate and patterns can show how an organism interacts with its environment, and are indicators of variation among populations, species, and individuals (Madsen & Shine, 2000.a). Since the landmark study suggesting marine iguanas (*Amblyrhynchus cristatus)* 'shrink' in response to environmental stressors (Wikelski & Thom, 2000), considerable efforts have been made to investigate the phenomenon and determine what the implications of body size are for ectotherms (Koons *et al.,* 2009; Bendik & Gluesenkamp, 2012; Caruso *et al*., 2015; López-Calderón *et al*., 2016). Analysing body sizes requires measurements, yet measurement error is often surprisingly high in morphometric studies (Measey *et al.,*

2003), and with an elongate and elastic body that can contort, contract, and stretch, measuring a snake is remarkably difficult.

There are many considerations that influence which method will be most suitable for the task as different species require different techniques. Moreover, the physical state of the snake will influence the measurements recorded. For example, a live snake has muscle tone enabling it to coil and twist. Measurements may be shorter in this case than measurements taken from the same snake if it was anaesthetised (Setser, 2007; Rivas *et al*., 2008; Natusch & Shine, 2012). Immobilisation with a squash box and then tracing the ventral midline either in pen or with string was found to be particularly useful when measuring small, venomous snakes (Bertram & Larsen, 2004), but the technique is not without its own imprecisions as if the snake is improperly restrained, it can move in the box (Blouin-Demers, 2003).

Increasingly, the use of image analysis software to measure morphological characteristics is becoming popular among researchers (Measey *et al*., 2003; Penning *et al.,* 2013). The process is straightforward and simply requires researchers to import a photograph of the organism they wish to measure into the program. Appropriate calibrations and scale are set and then measurements can be taken using the in-program tools. Depending on which software is used, researchers can record measurements using a 'segmented line tool' or 'curved line tool'. A segmented line inserts a straight vector line from each point (node) set on the image whereas curved line use cardinal splines to curve vector lines between nodes. Several studies that examine variation between measurers using the digital method state that biases are still difficult to control for and may be introduced to the data (Cundall *et al*., 2016; Astley *et al*., 2017).

Reflecting the studies of Cundall *et al*. (2016), Experiment 1 compares measurements obtained using a squash box and string with those made using digital image analysis software. While recording biometrics for snakes using image analysis software is not novel, testing different software between different users (Experiment 2), and analysing the effect of different positions of the same snake has not been previously examined (Experiment 3). This chapter's aim is to explore the variation that may be incurred when: different measurers measure the same snakes; variation between different methods of measuring snakes; and variation between measurements of different images of the same snake.

METHODOLOGY

OVERVIEW

All participants were researchers who had practical experience measuring snakes and with the techniques used in the experiments. For experiments that examined variation involving multiple measurers (Experiments 1 and 2), participants were unaware of data values previously recorded. Measurement parameters were identical across experiments with SVL and tail lengths all measured according to Figure 1, either manually or digitally (dependent on experiment). Total lengths were not physically measured but obtained by adding SVL and tail length together for each snake. There was no restriction on snake size, and juveniles were included. Likewise, no distinction was made between snake gender, and individuals with damaged tails were not removed. *N. helvetica* and *V. berus* were measured in Experiment 1, but only *N. helvetica* data were analysed in Experiment 2 and 3.

N. helvetica and *V. berus* were captured in Kent except for the *N. helvetica* in Experiment 3, which was caught in Berkshire. Snakes were restrained using a squash box (see Chapter 2, Fig. 3, p.24), and photographs were taken from roughly 0.5m directly overhead using mobile phone or digital cameras. Any blurred or indistinct pictures or any images where part of the snake was hidden, were discarded. Searches for snakes were conducted visually from March to September either in the morning from 0900 – 1100 hrs and the afternoon from 1400 – 1730 hrs by four researchers, and mainly at known reptile sites. These were Kings Wood, Kent, and Buckleberry Common, Reading (Berkshire). Snakes out in the open, usually basking, were caught whenever possible. Refugia were located at some sites. These were turned and any snakes hiding underneath quickly captured. Care was taken when catching *V. berus* and thick gardening gloves worn at all times when processing them. Snakes were measured, photographed, and released where they had been found.

MEASURING SNAKES

The measurement conventionally collected to determine a snake's length is the snout-to-vent-length (SVL) (Setser, 2007; Penning *et al*., 2013). Two measurements were taken for all three of the experiments in this study. These were SVL and tail length. A third measurement, total length, was not physically measured, but obtained by adding SVL and tail lengths together. As per convention, SVL measurements started from the uppermost tip of the rostral scale then followed the ventral midline as closely as possible down to the base of the anal plate. The tail length was taken from the base of the anal plate to the tip of the final sub-caudal scale (Fig. 1).

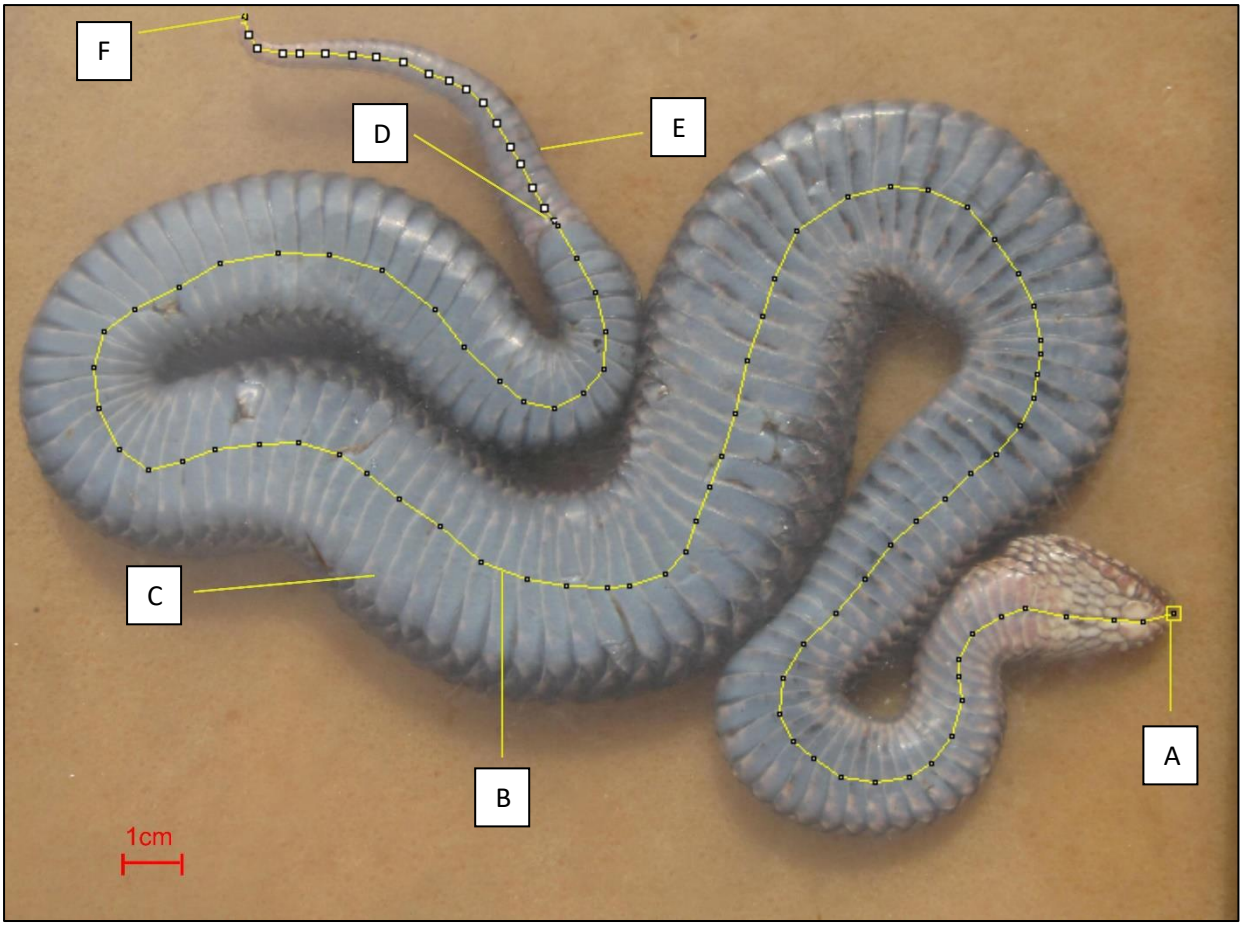


FIG. 1. Measurement points to determine a snake's length:
A Rostral scale
B Ventral midline
C Ventral shields (single)
D Anal plate
E Sub-caudal scales (divided)
F Tail tip

Locating the anal plate for both *N. helvetica* and *V. berus* is relatively straightforward as the sub-caudal scales are divided whereas ventral shields are singular (Fig. 2). However, for inexperienced observers locating the anal plate can be difficult especially for *N. helvetica* as the ventral colouration tends to be darker than *V. berus.* This makes the plate less visible (Fig. 2). It is important to ensure, therefore, that all participants can identify the key measurement points successfully, possibly practising their measuring technique in advance (Cundall *et al*., 2016). All participants in this study were experienced and, apart from providing instructions on how to successfully conduct Experiment 2, it was unnecessary to demonstrate to participants how and where to take measurements.

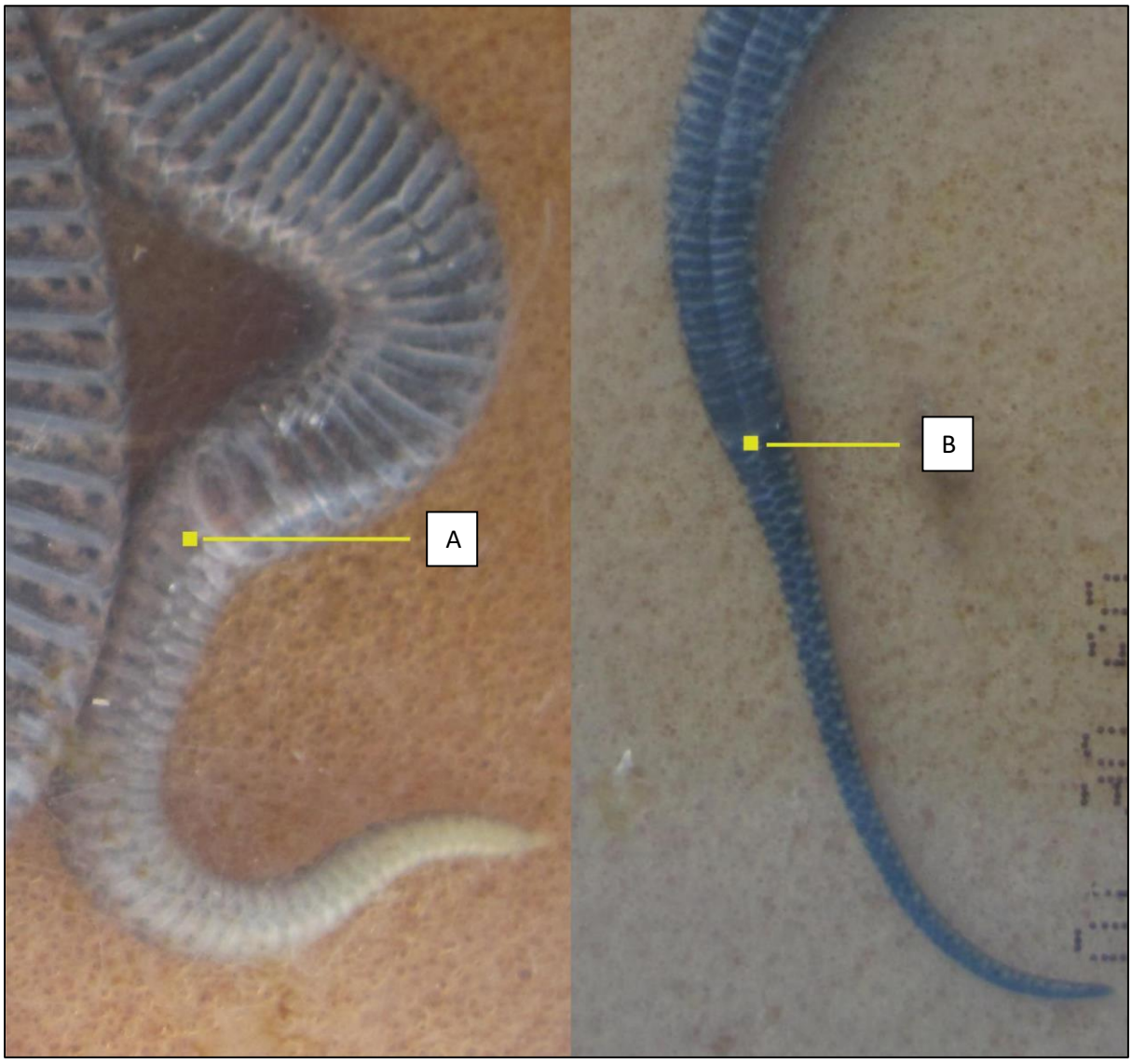


FIG. 2. Anal plate location: A = *V. berus* / B = *N. helvetica*

USING A SQUASH BOX

The squash boxes used in this study were large Ferrero Rocher chocolate boxes (216mm by 216mm, depth 44mm): transparent, plastic containers with tight-fitting, removeable lids (see Chapter 2, Fig. 3, p.24). A section of upholstery foam was cut to size that would fit snugly inside each box. Ensuring the foam was a tight fit was essential as, once the lid of the box was secured, the foam had to exert enough pressure on the snake to immobilise it. If the foam was not a good fit, snakes were able to right themselves or coil over so when the box was inverted to measure along the snake's ventral midline, the researcher was presented with the dorsal surface instead. This was problematic as it not only increased time spent in the field but also put additional stress on the snake as the animal had to be taken out of the box, repositioned, and secured again. Foam which did not fit properly was

discarded and a new piece cut. A reference guide of 1cm was drawn on the bottom of the box in indelible pen (Sharpie Ultra Fine Tip Permanent Marker, Fig. 3). On boxes where the 1cm guide was not present, a ruler or tape measure was placed on the box instead (Fig. 4). A photograph was then taken with a section of the ruler / tape measure within the frame. This was to ensure a scale was available to calibrate programs when the photos were digitally processed

To use the squash box, the snake was placed inside the box with the foam removed. The foam was then quickly placed back on top of the snake and gently pressed down, restraining it. The lid was then put back on which effectively secured the snake *in situ*. The box was then inverted so the snake's ventral surface was uppermost for measuring with string and to take a photograph for digital analysis. The string used to take the measurements was non-elastic and the tips had been dipped in wax to prevent them fraying. Different string varies in elasticity so to prevent bias, the same string was used to take all squash box measurements. Extra care was taken when larger snakes were in the squash box as they were stronger, and more able to curl their tail over their body, or push their tail and head from out under the foam. Finally, care was taken to ensure there was no glare from the sun on the box when taking photographs. This prevented taking pictures that were overexposed and guaranteed that all parts of the snake were visible when images were imported into image analysis programs.

Fig. 3. Pen measure reference. Image © Mikaella Lock

Fig. 4. Tape measure reference. Image © Richard Griffiths

IMAGE ANALYSIS SOFTWARE

The fundamental process of importing images, setting a scale, and taking measurements was very similar across all three software packages with just a few inter-program variations. The programs used were the core version of Image J (i.e. no additional plug-ins or customisations); Snake Measure Tool, and Serpwidget's Snake Measurer (referred to herein as Serpwidget).

All three programs are freely available online. Image J and Snake Measure Tool are downloadable packages, and Serpwidget is an online platform allowing the upload of images via a server.

- Image J: https://imagej.net/Welcome
- Snake Measure Tool: https://sourceforge.net/projects/snakemt/
- Serpwidget http://serpwidgets.com/main/measure

An image was imported into the program and a scale set which enabled the program to convert pixels into measurements (in this case, centimeters). To set the scale, a line segment was drawn from one point of the reference of known size in the photograph to the other. The programs' measurement tool(s) were then calibrated to reflect the scale. There are specific menus in Image J and Snake Measure Tool for this function, while value and measurement unit boxes are displayed in the browser for Serpwidget. Once calibrations were complete, SVL and tail measurements were taken by clicking points down the length of the snake following the ventral midline as closely as possible (Fig. 1). The number of 'clicks' was unspecified and varied between measurer and between programs. Segmented line tools in their default state were used to take measurements in Image J and Snake Measure Tool (Fig. 5). The curve line measurement, which automatically applied splines (Fig. 6) was taken in Serpwidget. Programs were re-calibrated for each image.

Photographs were easily importable into Image J and Snake Measure Tool in their raw state. Serpwidget, however, has a 4MB limit for image uploads. The file size for some images was reduced using graphic design software (Xara Xtreme Pro 5) as they exceeded Serpwidget's upload limit at their original size. All images used in all three experiments were 300 dpi JPEGs with no filters applied at the time photographs were taken or during the analysis process.

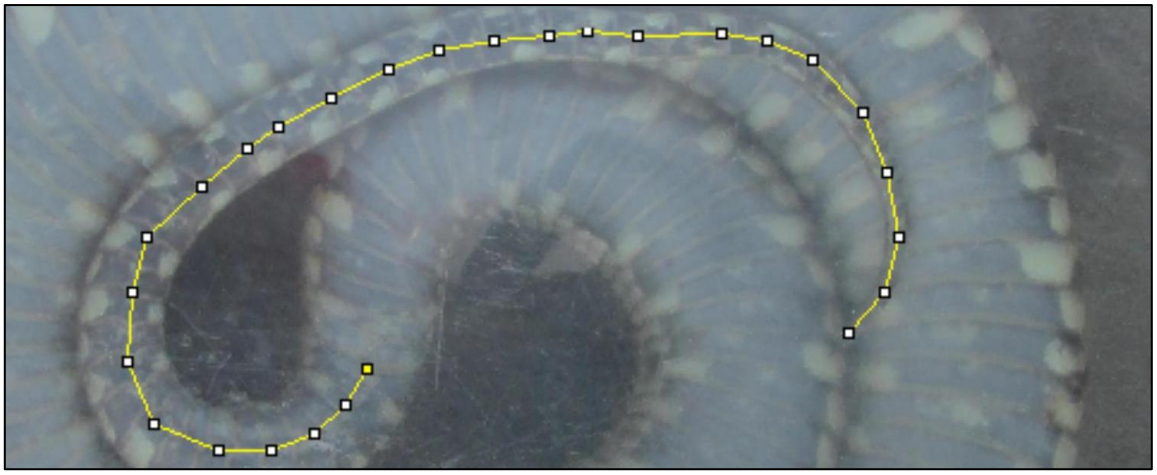


Fig. 5. *N. helvetica* tail measurement in Image J using segmented tool.

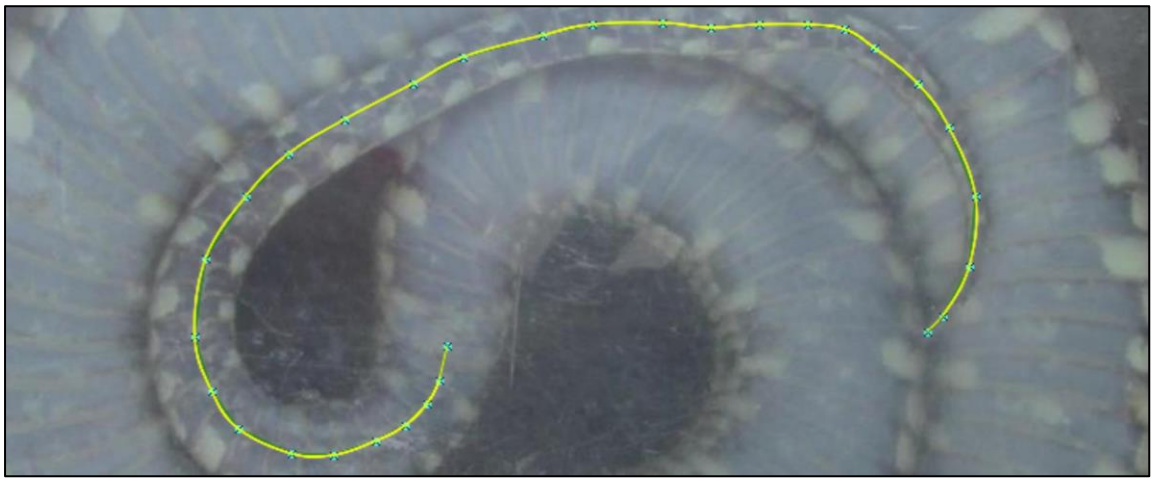


Fig. 6. *N. helvetica* tail measurement in Serpwidget using curve line tool demonstrating splines (same snake as Fig. 5)

EXPERIMENTS

1: SQUASH BOX AND STRING VS DIGITAL TECHNIQUE

Two independent researchers measured SVL and tail measurements for *N. helvetica* (n=17) and *V. berus* (n=52) caught in Kent. No distinction was made for gender, life stage, or damaged tails. One researcher used the squash box and string technique (Figs. 3 & 4) to obtain measurements in the field. Once snakes were measured using the string technique, a photograph of the ventral surface was taken for each animal. These photographs were then given to a second researcher to measure SVL and tail lengths in Image J. The second researcher was unaware of the measurements recorded by researcher one. Images were processed in Image J using the segmented line tool. String measurements were compared with digital measurements using paired sample *t*-tests performed in SPSS v24. Total measurements were obtained by adding SVL and tail lengths together and were

included in the analyses (Figs. 7 & 8). The aim of the experiment was to determine if measurements recorded using the string method would differ significantly from the same measurements recorded in Image J.

2: COMPARATIVE ANALYSES OF SOFTWARE AND MEASUREMENT VARIATION BETWEEN MEASURERS

Ten photographs of *N. helvetica* restrained in a squash box (Appendix 4.I). were selected 'at random' from a set of 21 images Randomisation was achieved by renaming all photographs with a simple code (e.g. Asnake, Bsnake, Csnake), inputting the codes to Excel and using the =RAND() function. The first ten images in the list generated by Excel were then recoded again (format: E_nn_a, E_nn_b, etc) for distribution to participants in the experiment. Photographs had been taken in Kent in 2015 and displayed the snakes' ventral surface along with a measurement reference (ruler or tape measure dependent on image). No distinction was made for gender, life stage, or damaged tails. The string was not included in the images.

Three researchers took part in Experiment 2. All were proficient computer users and experienced field workers who had measured snakes using image analysis software previously and were familiar with the key morphological landmarks. The ten images, along with a 'practice image', were given to the researchers with an explanation of how to complete the experiment. Researchers were asked to download Image J and Snake Measure Tool using HTML links provided as well as access Serpwidget online. One researcher had experience with all three software packages while the other researchers only had experience with Image J. The practise image was provided so researchers who had not used Snake Measure Tool and Serpwidget were able to familiarise themselves with the programs' functions prior to measuring images for the experiment. With the issue of the 4MB upload limit previously recognised for Serpwidget, image size was reduced for all photographs. These size-reduced images were used in all three programs.

Researchers were instructed to measure snake SVL and tail length once for each of the ten photographs using all three software packages. Each photograph was therefore measured three times, but only once in each program, with researchers each recording a total of sixty measurements (SVLs n=30, tails n=30) (Appendix 4.II). They were instructed to use the segmented line tool in Image J and Snake Measure Tool, but to record the curved line measurement in Serpwidget. They were emailed an Excel spreadsheet to input their results and, upon

completion of the experiment, researchers were asked to email the spreadsheet back. Total measurements were obtained by adding SVL and tail lengths together, and were included in the analyses

Once all results had been received, the measurements were sorted and analysed with a three-way ANOVA (univariate, full factorial) and *post hoc* Tukey test. The aim of the experiment was twofold as it sought to determine variation between programs and variation between measurers while controlling for differences in sizes between snakes.

3: SNAKE POSITIONING AND ITS EFFECT ON REPEATED MEASUREMENTS

Experiment 3 was conducted by one researcher. An adult, male *N. helvetica* was captured in Berkshire, immobilised in a squash box and a photograph taken of the ventral surface. The box was then opened, the foam removed, and by lightly lifting the body with fingers, the snake was gently encouraged, to reposition itself. The foam was placed back on top of the snake, the lid secured, and the box inverted so a second photograph could be taken. This repositioning was prompted a further three times, resulting in a total of five photographs, each one capturing the same snake in a different position in the squash box. Care was taken to ensure the full ventral surface of the snake was visible prior to taking the photographs. All images taken on the same day using an iPhone 6 camera.

SVL and tail length were measured for each 'positioning' photograph (n=5) ten times in each image analysis program: Image J, Snake Measure Tool, and Serpwidget. In total, 300 measurements were taken (SVL n=150, tail n=150) with 100 measurements taken per program (SVL n=50, tail n=50). As in Experiment 2, measurements for Experiment 3 were recorded using segmented line tool in Image J and Snake Measure Tool, and curved line tool in Serpwidget. Image size was reduced so they could be uploaded to Serpwidget, but they were analysed at their original size in Image J and Snake Measure Tool. Total measurements were obtained by adding SVL and tail lengths together and were included in the analyses. Measurements were analysed with three-way ANOVAs. The aim of the experiment was to test whether varying position for the same snake resulted in significantly different measurements as well as examine any interaction between the programs and the varying positions of the snake.

TABLE 1. Summary of experimental designs testing measuring techniques and measurer bias

Experiment One	**Aim 1:** Determine difference (if any) between measurements taken using Squash vs Image J				
	Researcher (s)	*N helvetica*	*V. berus*	Measurements	Recording Method
	R[1]	n=17	n=52	SVL & TL	Squash & string
	R[2]	n=17	n=52	SVL & TL	Image J
** One researcher measured snakes in the field using squash, second researcher measured photos of the same field-caught snakes with Image J.*					
Experiment Two	**Aim 1**: Determine variation (if any) between three measuring software programs **Aim 2**: Determine variation (if any) between the measurements recorded by three measurers				
	R[1]	n=10	0	SVL & TL	Image J, Snake Measure Tool & Serpwidget
	R[2]	n=10	0	SVL & TL	Image J, Snake Measure Tool & Serpwidget
	R[3]	n=10	0	SVL & TL	Image J, Snake Measure Tool & Serpwidget
** Researchers measured the same images using all three software packages.*					
Experiment Three	**Aim 1**: Determine whether snake positioning in squash would influence measurements **Aim 2**: Determine whether snake positioning would influence measurements taken between three software programs				
	R[1]	1	0	SVL & TL	Image J, Snake Measure Tool & Serpwidget *
** Images were taken of the same snake repositioned in a squash box five times.*					

RESULTS

EXPERIMENT 1: SQUASH BOX AND STRING VS DIGITAL TECHNIQUE

N. *helvetica (n=17)* SVL, tail, and total measurements were recorded by two independent researchers, each using a different measuring technique: squash box and string method and digital measurement in Image J. Data for the two methods was compared using paired sample *t*-tests performed in SPSS v 24. There was no significant difference between measurements recorded using the squash box and string and Image J program on all counts. (Fig. 7).

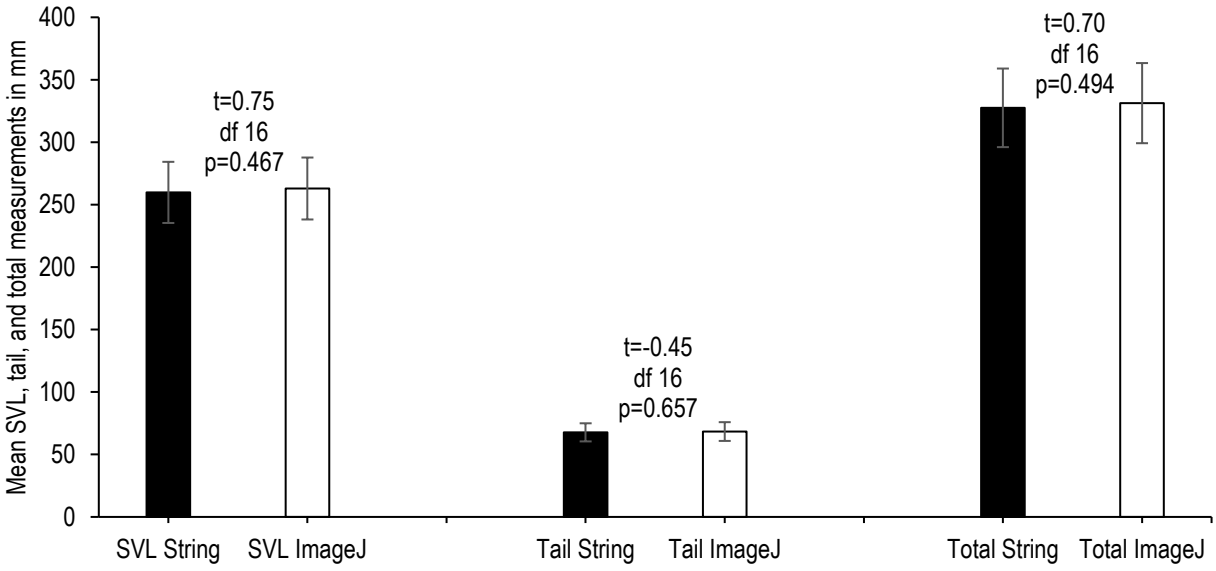


Fig. 7. Comparative *N. helvetica* measurements (mean lengths ±SE) taken by string method and ImageJ and compared using paired *t*-tests.

The analyses were repeated for *Vipera berus* (n=52) SVL, tail and total measurements. In this case, difference in tail length were highly significant between squash box and string and the Image J measuring methods (Fig. 8). A slight significance was observed for total lengths, but this was likely due to the tail length disparity increasing variance for totals. Tail measurements recorded in ImageJ were longer than those obtained by string method with a percentage increase of 4.24% for tails. SVL and total percentage increase between Image J and the squash box and string method were greater by 1.39% and 1.75% respectively.

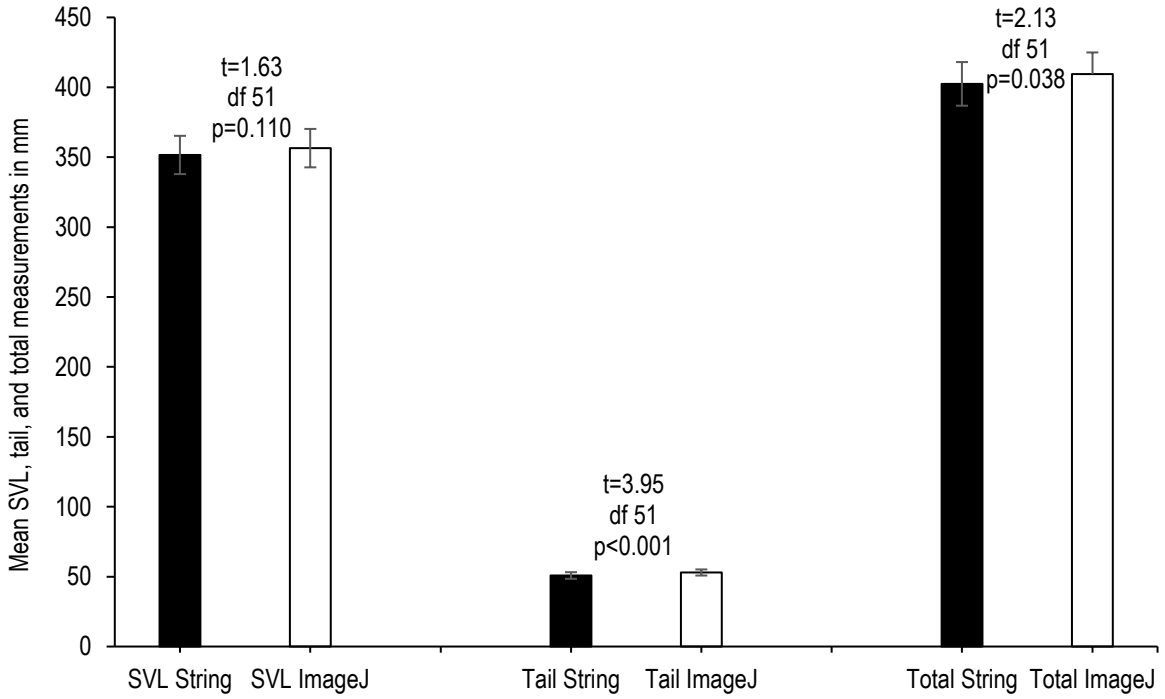


Fig. 8. Comparative *V. berus* measurements (mean lengths ±SE) taken by string method and ImageJ and compared using paired *t*-tests.

EXPERIMENT 2: COMPARATIVE ANALYSES OF SOFTWARE AND MEASUREMENT VARIATION BETWEEN MEASURERS

Differences in *N. helvetica* SVL and tail measurements were compared between three experienced researchers using three software packages: ImageJ, Snake Measure Tool, and Serpwidget. The SVL and tail lengths were taken from ten photographs, each of a different *N. helvetica*, and measured once in each software package. Totals were not physically measured but added up from SVL and tail measurements. Each participant measured SVL and tail lengths from the same ten photos independently. Data were then analysed with three-way ANOVA to test for variance between programs and *post hoc* Tukey test to compare variance between the three measurers.

SVL MEASUREMENTS

Results for SVL measurements (Fig. 9.) showed significant variation between participants $F_{2,76}$=21.05, p<0.001, but no significant difference in measurements between software $F_{2,76}$=1.30, p=0.280. There was a significant difference between the size of snakes used in the study $F_{9,76}$= 980.22, p<0.001, but this was expected as no parameters had been set for a specific size class.

Tukey HSD confirmed that measurement variation was high among all three participants but most notably for participant one who systematically took longer SVL measurements than participants two and three respectively

(p=0.007 and p<0.001). There was some variation between participants two and three (p=0.028) with participant three measuring systematically lower than both participants one and two.

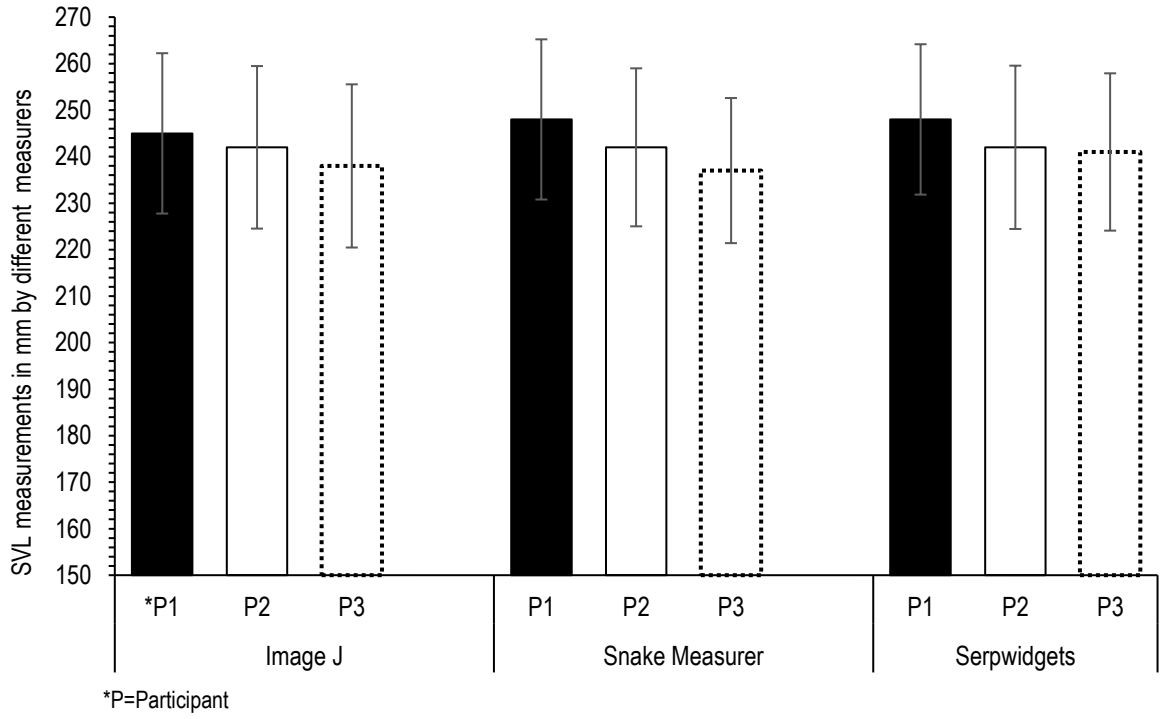


Fig. 9. Comparative software and measurement variation between participants: SVLs (mean lengths ±SE).

TAIL MEASUREMENTS

Tail measurement variation (Fig. 10) between participants was found to be significant $F_{2,76}$= 5.59, p=0.005. However, Tukey HSD showed that while there was no significant variation between participants two and three p=0.995, there was between participant one and two p=0.016, and participant one and three p=0.019. Again, variation between software was not significant $F_{2,76}$= 0.18, p=0.836.

TOTAL MEASUREMENTS

There was again significance in measurement variation between participants $F_{2,76}$= 697.68, p<0.001 but not between software $F_{2,76}$=0.77, p=0.464, (Fig. 11). Following *post hoc* tests, participant one was found to be systematically recording significantly longer measurements across all three software packages than participant two and three (p<0.001).

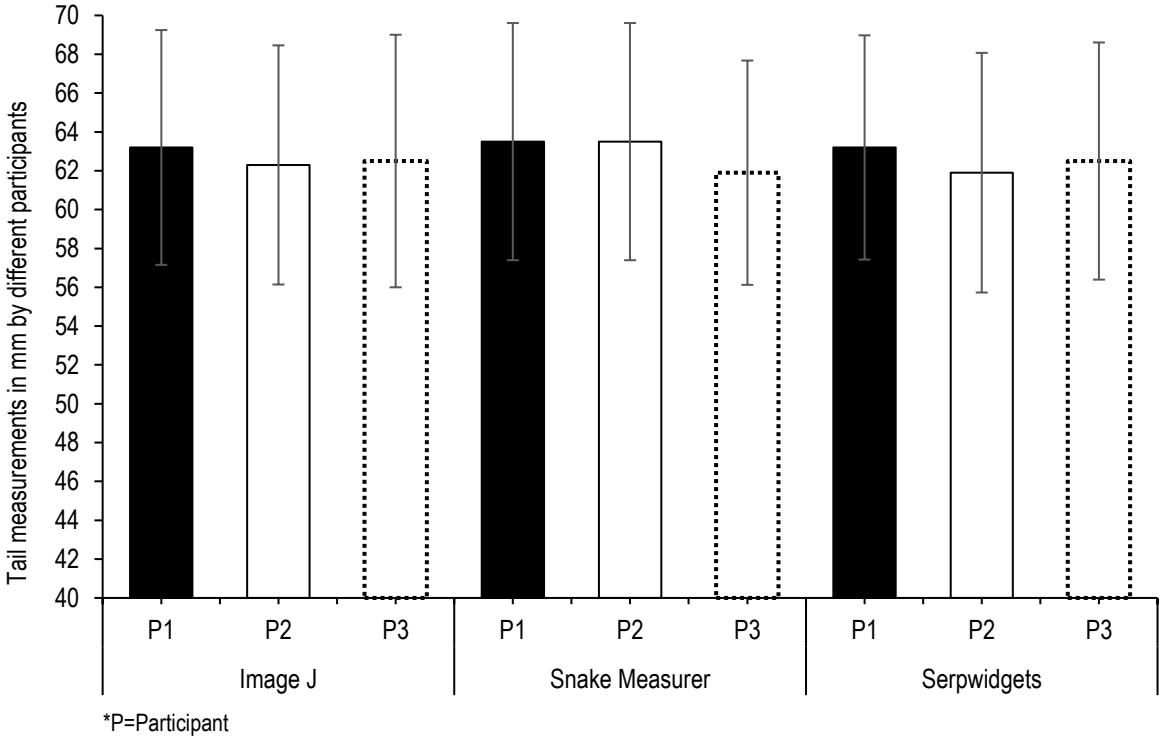


Fig. 10. Comparative software and measurement variation between participants: Tails (mean lengths ±SE).

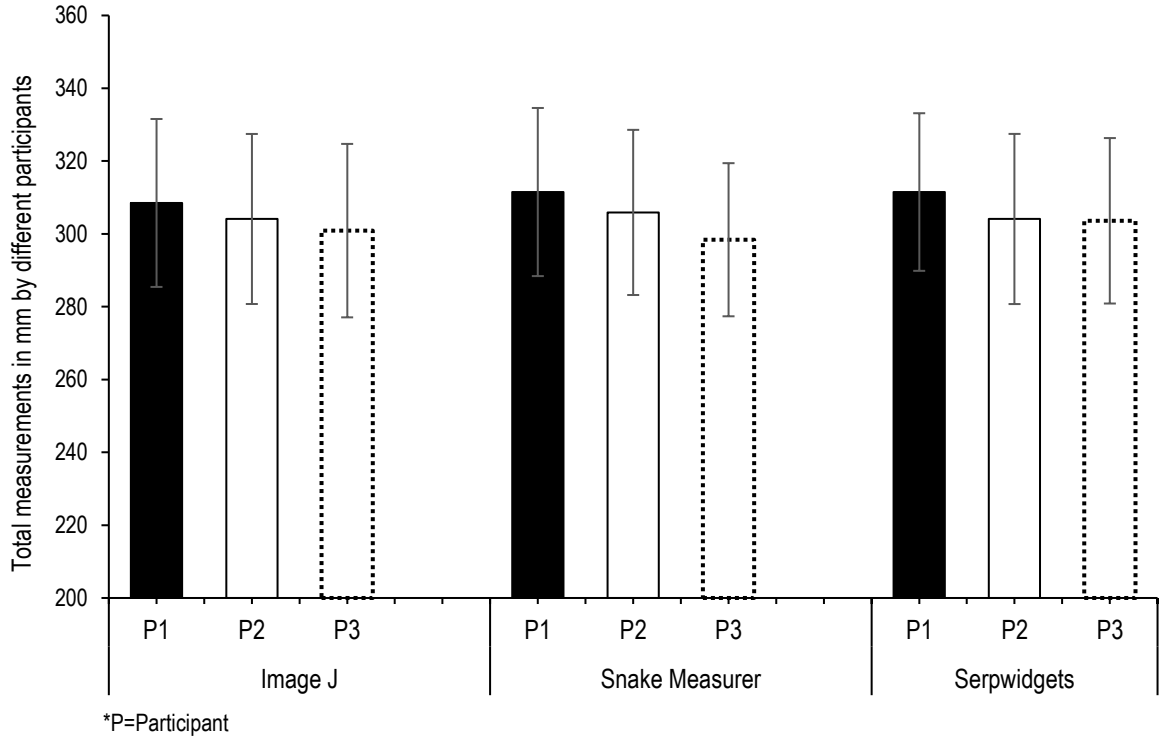


Fig. 11. Comparative software and measurement variation between participants: Totals (mean lengths ±SE).

EXPERIMENT 3: SNAKE POSITIONING AND ITS EFFECT ON REPEATED MEASUREMENTS

The same *N. helvetica* was randomly re-positioned in a squash box five times. One photograph was taken per position. SVL and tail were measured ten times per image in ImageJ, Snake Measure Tool and Serpwidget while totals were obtained by adding SVL and tail measurements together. All measurements were taken by the same experienced researcher and were analysed with three-way ANOVA to determine variation between programs, difference between measurements, and to examine interaction between position and software.

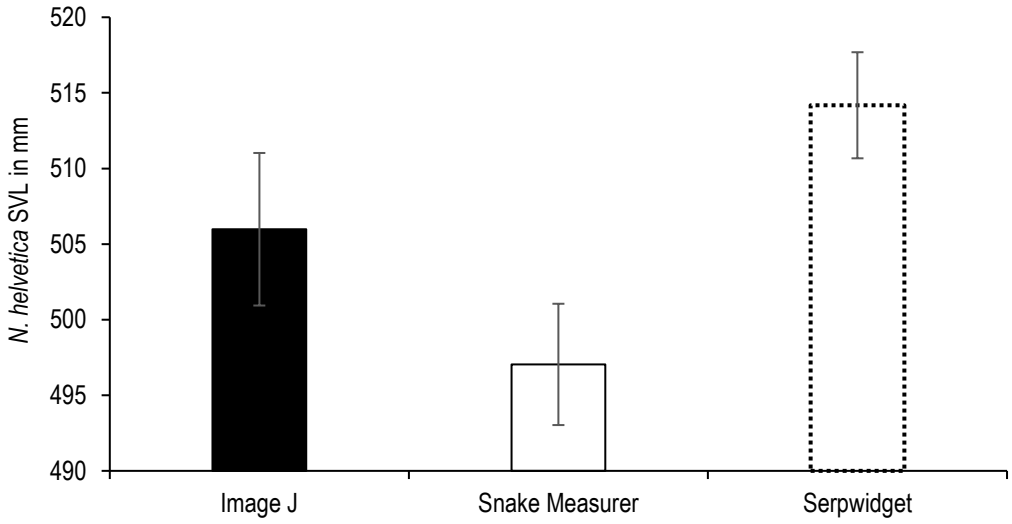


Fig. 12. SVL measurements (mean lengths ±SE) from three programs for the same *N. helvetica* in five different positions

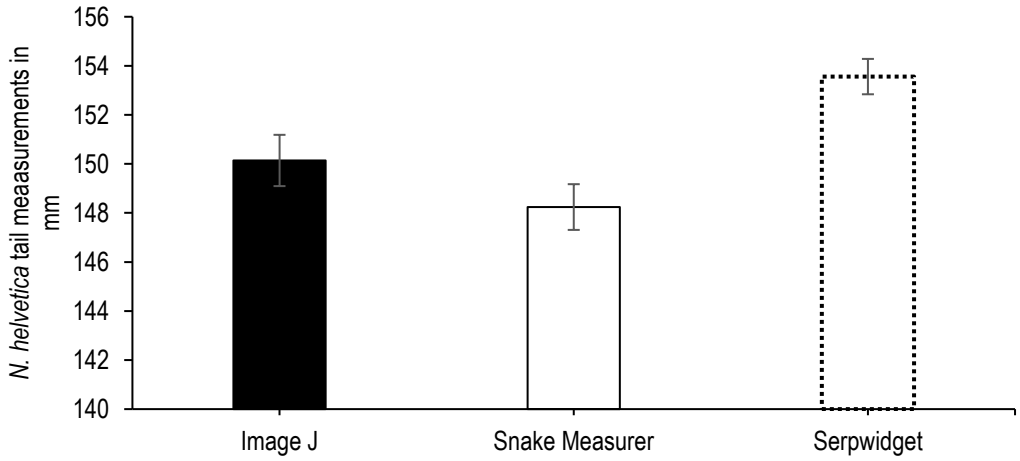


Fig. 13. Tail measurements (mean lengths ±SE) from three programs for the same *N. helvetica* in five different positions

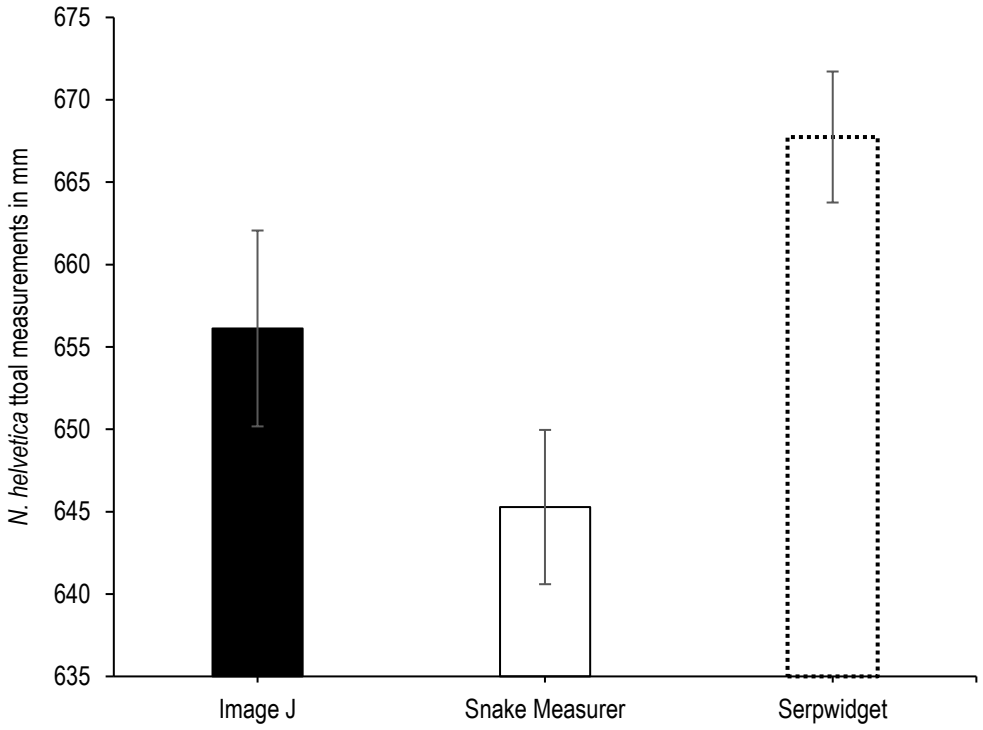


Fig. 14. Total measurements (mean lengths ±SE) from three programs for the same *N. helvetica* in five different positions

For all three measurements (Figs. 12-14), it was found that position influenced measurement highly significantly: SVL: $F_{4,135}$=78.03, p<0.001; tail: $F_{4,135}$=9.35, p<0.001; and total: $F_{4,135}$=53.41, p<0.001. Totals were included predominantly for illustrative purposes as these measurements had not been physically recorded in the software packages.

A comparison between software indicated significance, again on all counts (Appendix 4.III), however a *post hoc* Tukey test showed that this significance was between Snake Measure Tool and Serpwidget (p<0.001). Differences between Image J and Snake Measure Tool, and Image J and Serpwidget were not significant.

Finally, the interaction between positions and programs was analysed and found to be highly significant, again on all counts: SVL: $F_{8,135}$=6.90, p<0.001; tail: $F_{8,135}$=8.15, p<0.001; and total: $F_{8,135}$=7.36, p<0.001. The value obtained when taking a measurement therefore depends on the position of the snake in relation to the software used.

DISCUSSION

Experiment 1 showed that measurements taken by two independent researchers using different methods (squash box and string, and Image J) were comparable, with measurement differences statistically not significant in all cases for *N. helvetica*. When the paired sample *t*-tests were conducted for *V. berus*, it was surprising that difference in tail length was highly significant yet SVL was not. *V. berus* have shorter tails than *N. helvetica*, and measuring these shorter lengths appears to have been more problematic. The reason for this was unidentified, but tail measurements were, on average, 2 mm (±SE 2.4) longer in Image J than the squash box. The total number of *V. berus* (n=52) measurements available for analysis were higher than *N. helvetica* (n=17), so it is possible analysing greater number of individuals exposed this bias.

Multiple measurers recording morphometrics, particularly when precision and accuracy are key elements of the study, has long been associated with the introduction of significant bias (Palmeirin, 1998; Measey *et al., 2003*; Rivas *et al.,* 2008; Goodenough *et al.,* 2010). Experiment 2 found this to be the case also. Even though the researchers who took part were all experienced, measurement variation for both SVL and tail lengths with two of three pairwise comparisons were significantly different. *Post hoc* analysis further evidenced this bias. In line with other studies (Cundall *et al*., 2016), one measurer consistently recorded longer lengths than the others. The systematic nature of the longer lengths recorded by one measurer indicates that intra- measurement error was not an issue here. It is possible that this measurer was recording measurements with a greater number of clicks and / or spline fitting. There was no significant difference between the three image analysis programs used in this experiment.

Experiment 3 explored an aspect of snake morphometrics that is novel in the literature. Re-positioning the same *N. helvetica* introduced a remarkable bias to SVL and tail lengths. When the dynamic morphology and movement of a snake's body is considered, this is perhaps not surprising. Muscle tension may influence intervertebral distance essentially making a snake's body longer or shorter (Astley *et al*., 2017). If a snake is more compressed, (a fundamental aspect of using a squash box), this reduces stretching which can vary lengths (Cundall *et al*., 2016). Greater compression also effectively flattens the body more. Astley *et al*. (2017) demonstrated that stouter and wider snakes yielded measurements with greater variation although this observation was made using measurements taken from the dorsal surface digitally, and vertebral column was used as a guide

rather than ventral midline. Nevertheless, it is clear that different positions will yield different results not only when measuring snakes, but probably other taxa as well. Moreover, the experiment demonstrated the imprecision of repeatability when position is changed. The variation between repeated measurements was about ± 5 mm even though they were recorded by the same measurer. The results indicate that bias could be better addressed if snake positioning was standardised.

Undoubtedly restraint and position standardisation would need to account for the requirements of different genera. A standard restraint and positioning method that works for a grass snake, is very unlikely to be suitable for a green anaconda. A plea to standardise body size measurements was published by Siegel, (1988). Digital image analysis is rapidly proving to be an exceptionally useful tool not only for biometrics but in many other aspects of ecology, systematics, and evolutionary research too (Davis *et al*., 2004; Davis & Grayson, 2007; Siegenthaler, 2017). There is now a need for standardisation in how images are produced and how they are processed in the various image analysis applications available.

LIMITATIONS

In Experiment 1, the mixed method of one researcher using the squash box and string method to obtain measurements, and the other researcher using Image J is limited in terms of interpretation as the method of measurement is confounded by using two different measurers. A fundamental issue for both Experiment 2 and Experiment 3 was the comparison of measurements obtained using the segmented tool option in ImageJ and Snake Measure Tool with measurements obtained using the curved line tool in Serpwidget. Spline fit is a tool used in image analysis software to curve straight, segmented lines. This process potentially elongates segmented line measurements as they are converted to splines. There is a possibility that measurements recorded in Image J and Snake Measure Tool underestimate lengths. This potential bias was not tested for. It would be useful to repeat all the experiments with the spline fit option applied across all three image analysis programs.

The number of clicks a measurer uses to record measurements in image analysis software can have an impact on accuracy with underestimates increasing the more clicks made (Astley *et al*., 2017). It would be useful to attempt to create a method that standardises the number of ‘clicks’ and/or relative distance between ‘clicks’ when taking measurements. Further research is needed in this area.

CONCLUSIONS

From the literature and from the results obtained from these experiments, clearly there is a need to not only standardise measurement methods, but to consider redefining the convention of absolute precision when reporting measurements, certainly in the case of live snakes (Cundall *et al.,* 2016). It appears that there is no single length for a snake and, *sensu* Cundall *et al*. (2016), snakes operate within a range of different sizes as part of their normal behaviour. Accepting this range of sizes gained from repeated measurements and determining the mean average and standard deviation of that range could a better approach. The findings of this study support that statement. Moreover, it has been repeatedly demonstrated that multiple measurers significantly confound studies that rely on determining the lengths and sizes of snake morphology regardless of the measurement method (Penning *et al*., 2013; Cundall *et al*., 2016; Astley *et al*., 2017). Digital image analysis can minimise many of the biases and confounding variables associated with snake biometrics. It does not, however, resolve issues such as variation due to position and how the software is used. There remains considerable research effort in this area to fully understand how the technology can be best applied to researcher needs and study aims.

CHAPTER 5: GENERAL DISCUSSION

THE VALUE OF MORPHOMETRICS IN RESEARCH

Evolving around 300 million years ago during the Upper Carboniferous, reptiles are among the most successful creatures on Earth (Piñeiro *et al*., 2012). Indeed, evolution over such a lengthy timescale has ensured that the herpetofauna represent some of the most ecologically diverse and morphologically fascinating creatures on the planet (Inns, 2009). Data from preserved specimens - both standalone or in conjunction with contemporary data - can provide unique and valuable insights over time, and the line between ecology and evolution are increasingly blurring (Dietl, 2013). As the comparative study of historic vs contemporary snake lengths has shown, body measurements may reveal patterns and trends that would otherwise be difficult to identify.

Morphometrics across taxa have evidenced that some species of North American songbirds have adapted wing shapes over the last century in response to habitat loss and fragmentation (Desrochers, 2010). Following dam installations, blacktail shiner (*Cyprinella venusta*) are becoming deeper bodied with smaller heads. These adaptions increase locomotor performance in this species of fish (Hass *et al.,* 2010). The invasion of cane toads (*Bufo marinus*) has had a deleterious impact on indigenous species in Australia. Morphometrics have shown that the body size and relative head size in two snake species (*Hemiaspis signata* and *Tropidonophis mairii*) are decreasing in response (Phillips & Shine, 2004). Snakes are gape-limited predators, and a smaller head size subsequently decreases the risk of ingesting the large, and highly toxic cane toad (Phillips & Shine, 2004).

The role of taking morphometrics in herpetology is invaluable as measurements can reveal crucial information about species delineation, phylogenetic analyses and deepen understanding of evolutionary change based on an organism's physical traits (Watters *et al.,* 2016). Moreover, as collections age, the year of collection is becoming increasingly important as species become rarer or extirpated (Winker, 2004). Morphometrics studies can greatly contribute to biodiversity conservation – as recognition of their value grows, perhaps so too will their use in future studies.

BIASES AND MEASUREMENT VARIABILITY

Using preserved specimens as proxies to living organisms presents some limitations as different preservation techniques (e.g. ethanol, freezing, formalin, IMS) can distort (Lee, 1982); alter colouration (Shetter, 1936; Smith,

1955); bloat (Bernal & Clavijo, 2009), and shrink (Reed, 2001; Vervust *et al.,* 2009) ectotherm specimens to varying degrees. The consensus among researchers is that smaller intra-species specimens are most heavily influenced by preservation changes, and this is apparent across many taxa including snakes (Klauber, 1943), frogs (Lee, 1982), fish (Martinez, *et al.,* 2013); lizards (Vervust *et al.,* 2009) and crabs (Rufino *et al.,* 2004). Perhaps many of these biases may be overcome by conducting a pilot study to determine precise correction factors prior to undertaking full analyses (Reed, 2001). The type of instrument that is being used to take the measurements must also be considered as this too may be a source of bias (Roitberg *et al.,*2011) as well as measurer experience (Bernal & Clavijo, 2009). In some studies, measurer inexperience accounted for 10-30% of total sample variance (Yezerinac *et al*., 1992) with smaller morphometrics resulting in higher measurer error (Bernal & Clavijo, 2009).

Increasingly, researchers are depending on image analysis software as a tool to conduct measurement studies. Software has been shown to be more precise and more accurate than all other manual methods of measuring snakes (Astley *et al*., 2017). However, as evidenced in this study, researchers must consider the elasticity and considerable flexibility of a snake's morphology. Moreover, as image analysis software usage becomes more frequent in scientific research, so too does the evidence of limitations and bias linked to image analysis. Issues are not limited solely to the organism, its position, or its morphometric characteristics. The technology behind not only image analysis programs, but also the equipment used to take photographs, warrants further study to establish how they may be best applied to study requirements. Different cameras will produce different images; taking photographs from varying heights may distort images; and the angle at which a photograph is taken will influence measurements taken in image analysis software. The remit of this study did not embrace investigation of potential differentials and variations associated with photography equipment and software. However, it is important that the researcher is aware of the potential biases that can be introduced when using digital methods. A particularly in-depth evaluation of these issues is discussed in Astley *et al*. (2017).

CLIMATE CHANGE AND FUTURE PROJECTIONS

The synergistic effects of global warming on reptile phenology, distribution traits and behaviour patterns are well documented (Whitfield-Gibbons *et al.,* 2000), and are key factors increasing the threat of extinction for this taxon worldwide. Globally, the mean temperature warmed by 0.74°C during the 20th Century with a 0.4°C temperature increase occurring from 1970 (Walmsley *et al.,* 2007). Reptile survival is inexorably linked to climate

where even a small variation in temperature can detrimentally impact on their ecology and physiology (López-Alcaide & Macip-Ríos, 2011).

Reptile ecologies and distributions are frequently synchronised with rainfall pattern, cloud cover and humidity levels, and are a delicate balance easily tipped by climate change (Bickford *et al.,* 2010). Phenological timing for many ectothermic taxa is changing because of climate change (Walther *et al.,* 2002). For example, the common frog (*Rana temporaria*) is spawning earlier at the national level in the UK (Carroll, *et al.,* 2009). Some multivoltine insects such as the European species, the wall brown butterfly (*Lasiommata megera*), depend upon environmental cues as larva to either develop fully or winter diapause. Fluctuations of temperature and photoperiod may be contributing to the loss of entire generations in this butterfly species (Van Dyck *et al.,* (2015). Climate-induced local extinctions and range shifts of prey species will also have a significant impact on predatory reptiles (Bickford *et al.,* 2010). This is particularly the case for snakes where fitness, reproduction and survivorship are very strongly linked to prey base density (Bickford *et al.,* 2010; Madsen & Shine, 2000.b).

Sex determination in many egg-laying reptiles such as crocodilians, turtles and some lizards is temperature-dependent (Bull, 1980; Crews *et al.,* 1994). This places many egg-laying reptiles at risk of becoming single sex populations within the next century (Bickford *et al.,* 2010). Females may select cooler nesting sites to mitigate the effects of climate change, but this may not be adequate to offset it entirely (Bickford *et al.,* 2010; Telemeco *et al*., 2009). Conventionally it has been thought that adaption was an incredibly slow process, but researchers now know that this is not the case (Yom-Tov & Geffen, 2010). What is apparent is that ecology and evolution are inextricably linked; some species are rapidly evolving in response to environmental change while some are not (Urban *et al.,* 2013). It has been predicted, based on climatic envelope models, that between 11-49% of endemic reptiles will become extinct by 2080 (Thomas et *al.,* 2004). However, for some reptile species, strong plasticity or high genetic variance that brings about phenotypic changes to fit a new climate may offset the damage caused by increasing temperatures (Urban *et al.,* 2013).

Regardless of a paucity of research on climate change and the body size relationship in reptiles (Böhm *et al*., 2016), evidence is mounting that this phenomenon is indeed occurring (Whitfield Gibbons *et al*., 2000; Walther *et al.,* 2002; Ashton & Feldman, 2003). Basal metabolic rate (BMR) in reptiles is directly linked to external temperature, and as temperature increases so does BMR (Gillooly *et al.,* 2001). Increased temperature is therefore indicative of a higher metabolic rate, which in turn means a need to consume more food in to maintain average

body size. Prey densities are decreasing in line with climate change so affected reptile populations will either reduce or species will adapt and become smaller through natural selection (Bickford *et al.,* 2010). Smaller body size impacts on fitness in reptiles as smaller females are less fecund (Du *et al.,* 2005) and neonates born to smaller mothers may have a greater susceptibility to disease (Brown & Shine, 2016). It is important to note that temperature increases do not affect all reptiles in the same way. Western diamondback rattlesnake (*Crotalus atrox*) size increased in colder, wetter areas (Amarello *et al.,* 2010), while Palestine viper (*Daboia [Vipera] palaestinae*) size increased in more arid areas (Volynchik, 2012).

There is considerable conjecture around the shrinking phenomenon (Pincheira-Donoso & Meiri, 2013; Vilela *et al.,* 2014; Yom-Tov & Geffen, 2011). However, the link between body size and making ecological predictions should, *sensu* Vilela *et al.* 2014, be a fundamental goal, and using biometrics to determine trends may be considerably easier than assessing ecological aspects to determine the same (Vilela *et al.,* 2014; Pincheira-Donoso, 2013).

PROJECTED CLIMATE SCENARIOS AND THE FUTURE OF SNAKES IN THE UK

In the UK, mean temperature increased by 1°C during the 20th Century resulting in the thermal growing season lengthening by a month (Walmsley *et al.,* 2007). Projections indicate that many species will be affected by climatically destabilised habitat that is no longer suited to their ecological and biological requirements (Berry *et al.,* 2002; Berry, ;2003). Moreover, species with limited dispersal ability or where habitat is too fragmented to colonise, may not be able to keep pace with the inevitable changes warmer temperatures will bring (Berry *et al.,* 2003; Hickling *et al.,* 2006).

Based on climate envelope models by Dunford & Berry (2012), potential outcomes for snakes in the UK have been predicted under a low global temperature scenario with an increase of 2°C from the 2020s to the 2080s, and a high scenario with an increase of 3.9°C. Only four out of thirteen species of UK native reptiles and amphibians are predicted to have stable ranges in both scenarios. For *N. helvetica* the forecast is quite promising as the species should be relatively robust thanks to its wide distribution and a future increase of available climate space (Dunford & Berry, 2012). This is not the case for *V. berus* (or the UK's only other species of snake, the smooth snake, *Coronella austriaca*). Patchy distribution is a considerable impediment to *V. berus* shifting range to appropriate climate space (Dunford & Berry, 2012). Moreover, its natural habitats - grasslands, heathland, maritime

cliffs and sand dunes - are considered some of the most at risk from climate change (Dunford & Berry, 2012; Berry, *et al.,* 2002; 2003). Population declines in *V. berus* have been accelerating since the early 2000s (Beebee *et al.,* 2009), but perhaps even more alarmingly, populations are not shifting northwards as might be expected (Walmsley *et al.,* 2007; Hickling *et al.,* 2006). In fact, *V. berus* populations are collapsing southwards resulting in pockets of snakes surviving in a fraction of their former distribution (Hickling *et al.,* 2006). This is of considerable concern as under both climate change scenarios, *V. berus* is projected to lose all climatic space in the south, southeast, central and northern England by 2080 (Dunford & Berry, 2012).

CONCLUSIONS

The size of a snake can help determine many ecological trends and physiological traits (Böhm *et al*., 2016; Walther *et al.,* 2002; Whitfield Gibbons *et al*., 2000). This study supported the hypothesis that both *N. helvetica* and *V. berus* may have decreased in average length over time in the UK. Corrective factors were applied where possible, and analyses were conducted to test for any biases that may have influenced this finding. Detectability was investigated in the field using phenotypically accurate plasticine models. This demonstrated that larger model snakes are easier to detect along a transect. This is a bias that may have influenced the data within both historic and contemporary datasets as collection of snakes in the field may be skewed towards larger individuals given they are easier to detect. Furthermore, it was found that an experienced observer is likely to find more model snakes than a group of inexperienced observers. This may have implications for surveys which are highly dependent on volunteers and those with less training in the field. A comparison between two different measurement methods (squash box and string, Image J) showed very little variation in general. However, the study was limited as the comparison was made between two researchers each using a different measurement method (squash box and Image J). This could be improved if only one researcher had recorded measurements for both methods. While this study resulted in little variation, it may be advisable that researchers do not mix methods when recording measurement data as this may affect the validity of results if not corrected for during analyses (Roitberg *et al.,* 2011; Cundall *et al*., 2016). It was determined that while there was little significance between image analysis programs, measurer variation was highly significant. In line with other studies conducted in this area (Bernal & Clavijo, 2009; Cundall *et al.,* 2016; Lee, 1982), the findings also support that only one measurer should record measurements in studies that require a high level of precision.

Positioning of a snake can vary measurements considerably. Following these findings, perhaps a new convention for reporting snake biometrics should focus less on determining precise values and instead report measurements within an acceptable error range. Some morphometric studies suggest that measuring snake biometrics to the nearest 5 mm may be suitable (Campbell & Murphy, 1984; Cundall *et al.,* 2016; Forsmann, 1993). However, whether 5 mm would prove to be a suitable error range for all snakes is debatable given the enormous inter-species and intra-species differences in lengths. Moreover, given the significant bias introduced by body positioning, clearly there is a need to standardise snake positioning when measuring biometrics digitally and, most

likely, manually as well. However, the elastic nature of a snake's body could make 'a standard position' very difficult to define let alone attain.

The thesis supports the hypothesis that multiple measurers taking multiple measurements can be an unreliable practice. Caution is urged in these circumstances, and it is recommended that an error baseline is determined so a corrective factor can be applied.

The decrease in body length determined by this thesis for *N. helvetica* and *V. berus* in the UK warrants further exploration to firstly understand what the implications of this may be for future populations so mitigation plans may be formulated; and secondly to ascertain whether 'shrinking' is occurring in other native herpetofauna. There is already evidence that common toads (*Bufo bufo*) are responding to warmer winters with reductions in female body size (Reading, 2006) and female palmate newts (*Lissotriton [Triturus] helvetica*) lay fewer eggs at higher temperature (Galloy & Denoël, 2010).

Finally, anthropogenic climate warming is likely to become the most significant threat to species across many regions, including the UK, and will be further exacerbated by loss of habitat as well as fragmentation. Moreover, species that are able to persist may well face the threat of invasives, putting vulnerable endemic species even more at risk (Bickford *et al.,* 2010; Thomas *et al.,* 2004; Walther *et al.,* 2002). Climatic modelling results are stark and climate change impact is now inevitable, however realising a minimum climate change scenario will reduce the number of projected extinctions significantly (Berry *et al.,* 2002; Bickford *et al*., 2010, Thomas *et al.,* 2004). There is still time.

REFERENCES

Albergoni, A., Bride, I., Scialfa, Jocque, M. & Green, S. (2016). How useful are volunteers for visual biodiversity surveys? An evaluation of skill level and group size during a conservation expedition. *Biodiversity Conservation* **25**, 133-149.

Allen, J. A. (1877). The influence of physical conditions in the genesis of species. *Radical Review* **1**, 108-140.

Amarello, M., Nowak, E. M., Taylor, E. N., Schuett, G. W., Repp, R. A., Rosen, P.C., & Hardy Sr, D. L. (2010). Potential environmental influences on variation of body size and sexual size dimorphism among Arizona populations of the western diamond-backed rattlesnake (*Crotalus atrox*). *Journal of Arid Environments* **74**, 1443-1449.

Anderson, P. K., Cunningham, A. A., Patel, N. G., Morales, F. J., Epstein, P.R. & Daszak, P. (2004). Emerging infectious diseases of plants: pathogen pollution, climate change and agrotechnology drivers. *Trends in Ecology and Evolution* **19**, 535-544.

Andrén, C. & Nilson, G. (1981). Reproductive success and risk of predation in normal and melanistic colour morphs of the adder, *Vipera berus*. *Biological Journal of the Linnean Society* **15**, 235-246.

Ashton, K. G. & Feldman, C. R. (2003). Bergmann's rule in nonavian reptiles: turtles follow it, lizards and snakes reverse it. *Evolution* **57**, 1151-1163.

Astley, H. C., Astley, V. E., Brothers, D., Mendelson III, J. R. (2017). Digital analysis of photographs for snake length measurement. *Herpetological Review* **48**, 39-43.

Bateman, P. W., Fleming, P. A. & Wolfe, A. K. (2016). A different kind of ecological modelling: the use of clay model organisms to explore predator-prey interactions in vertebrates. *Journal of Zoology* **301**, 251-262.

Beebee, T. J. C., Wilkinson, J. W. & Buckley, J. (2009). Amphibian declines are not uniquely high amongst the vertebrates: trend determination and the British perspective. *Diversity* **1**, 67-88.

Bendik, N. F. & Gluesenkamp, A. G. (2012). Body length shrinkage in an endangered amphibian is associated with drought. *Journal of Zoology* **290**, 35-41.

Bennie, M. M. G. 'Shrinking snakes: are grass snakes (*Natrix natrix*) in the United Kingdom Becoming Smaller?' (*Unpublished Bachelor of Science dissertation*, University of Kent, 2013).

Bergmann, K. (1847). Über die verhältnisse der Wärmeökonomie der Thiere zu ihrer Größe. *Gottinger Studien* **3**, 595-708.

Bernal, M. H. & Clavijo, J. A. (2009). An essay on precision in morphometric measurements in anurans: inter-individual, intra-individual and temporal comparisons. *Zootaxa* **2246**, 32-44.

Berry, P. M., Dawson, T. P., Harrison, P.A. & Pearson, R. G. (2002). Modelling potential impacts of climate change on the bioclimatic envelope of species in Britain and Ireland. *Global Ecology and Biogeography **11***, 453-462.

Berry, P. M., Dawson, T. P., Harrison, P.A., Pearson, R. G. &Butt, N. (2003). The sensitivity and vulnerability of terrestrial habitats and species in Britain and Ireland to climate change. *Journal for Nature Conservation* **11**, 15-23.

Bertram, N. & Larsen, K.W. (2004). Putting the squeeze on venomous snakes: accuracy and precision of length measurements taken with the 'squeeze box'. *Herpetological Review* **35**, 235-237.

Bickford, D., Howard, S., Ng, D. J. J. & Sheridan, J. A. (2010). Impacts of climate change on the amphibians and reptiles of Southeast Asia. *Biodiversity Conservation* **19**, 1043-1062.

Bittner, T. D. (2003). Polymorphic clay models of *Thamnophis sirtalis* suggest patterns of avian predation. *Ohio Journal of Science* **103**, 62-66.

Blouin-Demers, G., Weatherhead, P. J., Shilton, C. M., Parent, C. E. & Gregory, P. B. (2000). Use of inhalant anesthetics in three snake species. *Contemporary Herpetology* **4**, 1-11.

Blouin-Demers. G. (2003). Precision and accuracy of body-size measurements in a constricting, large-bodied snake (*Elaphe obsoleta*). *Herpetological Review* **34**, 320-323.

Blouin-Demers, G., Lisle Gibbs, H. & Weatherhead, P. J. (2005). Genetic evidence for sexual selection in black ratsnakes, *Elaphe obsoleta*. *Animal Behaviour* **69**, 225-234.

Böhm, M., Williams, R., Bramhall, H. R., McMillan, K. M., Davidson, A. D., Garcia, A., Bland, L. M., Bielby, J. & Collen, B. (2016). Correlates of extinction risk in squamate reptiles: the relative importance of biology, geography, threat and range size. *Global Ecology and Biogeography* **25**, 391-405.

Brown, G. P. & Shine, R. (2016). Maternal body size influences offspring immune configuration in an oviparous snake. *Royal Society Open Science* **3**: doi 160041 [Accessed online 15/01/2017].

Browne, R. K. (2014). Measuring amphibian morphometrics with ImageJ. *Amphibian and Reptile Conservation. Protocols 2*. [Accessed online 02/12/2017 http://www.redlist-arc.org/Protocols.html].

Bull, J. J. (1980). Sex determination in reptiles. *The Quarterly Review of Biology* **55**, 3-21.

Burrell, A. S., Disotell, T. R. & Bergey, C. M. (2015). The use of museum specimens with high-throughput DNA sequencers. *Journal of Human Evolution* **79**, 35-44.

Campbell, J. A. & Murphy, J. E. (1984). Reproduction in five species of Paraguayan colubrids. *Transactions of the Kansas Academy of Science* **87**, 63-65.

Carroll, E. A., Sparks, T. H., Collinson, N. & Beebee, T. J. C. (2009). Influence of temperature on the spatial distribution of first spawning dates of the common frog (*Rana temporaria*) in the UK. *Global Change Biology* **15**, 467-473.

Caruso, N. M., Sears, M. W., Adams, D. C. & Lips, K. R. (2015). Widespread rapid reductions in body size of adult salamanders in response to climate change. *Global Change Biology* **20**, 1751-1759.

Cope, E. D. (1896). The primary factors of organic evolution. *Open Court Publishing Company*, New York, USA, 492.

Crews, D., Bergeron, J. M., Bull, J. J., Flores, D., Tousignant, A., Skipper, J. K. & Wibbels, T. (1994). Temperature-dependent sex determination in reptiles: proximate mechanisms, ultimate concerns, and practical applications. *Development Genetics* **15**, 297-312.

Cundall, D., Deufel, A., MacGregor, G., Pattishall, A. & Richter, M. (2016). Effects of size, condition, measurer, and time on measurements of snakes. *Herpetologica* **72**, 227-234.

Davis, A. K., Farrey, B. & Altizer, S. (2004). Quanitifying monarch butterfly larval pigmentation using digital image analysis. *Entomologia Experimentalis et Applicata* **113**, 145-147.

Davis, A. K. & Grayson, K. L. (2007). Improving natural history research with image analysis: the relationship between skin color, sex, size and stage in adult red-spotted newts (*Notophthalmus viridescens viridescens*). *Herpetological Conservation and Biology* **2**, 65-70.

Desrochers, A. (2010). Morphological response of songbirds to 100 years of landscape change in North America. *Ecology* **91**, 1577-1582.

Dunford, R. W. & Berry, P. M. (2012). Climate change modelling of English amphibians and reptiles: *Report to the Amphibian and Reptile Conservation Trust (ARC-Trust*). Environmental Change Institute, University of Oxford, Oxford. [Accessed online 19/11/2017. http://www.researchgate.net/publication/261680126]

Dietl, G. P. (2013). The great opportunity to view stasis with an ecological lens. *Palaeontology* **56**, 1239-1245.

Du, W., Ji, X. & Shine, R. (2005). Does body volume constrain reproductive output in lizards? *Biology Letters* **1**, 98-100.

Ewins, P. J. (1985). Variation of black guillemot wing lengths post-mortem and between measurers. *Ringing & Migration* **6**, 115-117.

Feldman, A. & Meiri, S. (2013). Length-mass allometry in snakes. *Biological Journal of the Linnean Society* **108**, 161-172.

Fisher, M. C., Henk, D. A., Briggs, C. J., Brownstein, J. S., Madoff, L. C., McCraw, S. L. & Gurr, S. J. (2012). Emerging fungal threats to animal, plant and ecosystem health. *Nature* **484**, 186-194.

Fisher, R. N. & Shaffer, H. B. (1996). The decline of amphibians in California's Great Central Valley. *Conservation Biology* **10**, 1387-1397.

Fitch, H. S. (1940). A biogeographical study of the *Ordinoides artenkreis* of garter snakes *(Thamnophis*). *University of California Publications in Zoology* **44**, 1-150.

Fitzpatrick, M. C., Preisser, E. L., Ellison, A. M. & Elkinton, J. S. (2009). Observer bias and the detection of low-density populations. *Ecological Applications* **19**, 1673-1679.

Forsman, A. (1991). Variation in sexual size dimorphism and maximum body size among adder populations: effects of prey size. *Journal of Animal Ecology* **60**, 253-267.

Forsman, A. (1993). Growth rate in different colour morphs of the adder (*Vipera berus*) in relation to yearly weather variation. *OIKOS* **66**, 279-285.

Forsman, A. (1994). Growth rate and survival in relation to relative head size in *Vipera berus*. *Journal of Herpetology* **28**, 231-238.

Fox, W. (1948). Effects of temperature on development of scutellation in the garter snake *Thamnophis elegans atratus*. *Copeia* **1948**, 252-262.

Franklinos, L. H. V., Lorch, J. M., Bohuski, E., Rodriguez-Ramos Fernandez, J., Wright, O. N., Fitzpatrick, L., Petrovan, S., Durrant, C., Linton, C, Baláž, Cunningham, A. A. & Lawson, B. (2017). Emerging fungal pathogen *Ophidiomyces ophiodiicola* in wild European snakes. *Scientific Reports* **7**, 3844. doi:10.1038/s41598-017-03352-1 [Accessed online: 17/12/2017].

Freilich, J. E. & LaRue Jr, E. L. (1998). Importance of observer experience in finding desert tortoises. *Journal of Wildlife Management* **62**, 590-596.

Galloy, V. & Denoël, M. (2010). Detrimental effect of temperature increase on the fitness of an amphibian (*Lissotriton helveticus*). *Acta Oecologica* **36**, 179-183.

Gardner, J. L., Peters, A., Kearney, M. R., Joseph, L. & Heinsohn, R. (2011). Declining body size: a third universal response to warming? *Trends in Ecology and Evolution* **26**, 285-291.

Gillooly, J. F., Brown, J. H., West, G. B., Savage, V. M. & Charnov, E. L. (2001). Effects of size and temperature on metabolic rate. *Science* **293**, 2248-2251.

Garner, T. W. J., Walker, S., Bosch, J., Hyatt, A. D., Cunningham, A. A. & Fisher, M. C. (2005). Chytrid fungus in Europe. *Emerging Infectious Diseases* **11**, 1639-1641.

Gloger, C. L. (1833). Das Abändern der Vögel durch Einfluβ der Klimas. Breslau, Germany.

Goodenough, A. E., Stafford, R., Catlin-Groves, C. L., Smith, A. L. & Hart, A. G. (2010). Within- and among-observer variation in measurements of animal biometrics and their influence on accurate quantification of common biometric-based condition indices. *Annales Zoologici Fennici* **47**, 323-334.

Gregory, P. T. (2004). Sexual dimorphism and allometric size variation in a population of grass snakes (*Natrix natrix*) in southern England. *Journal of Herpetology* **38**, 231-240.

Gregory, P. T. & Tuttle, K. N. (2016). Effects of body size and reproductive state on cover use of five species of temperate-zone natricine snakes. *Herpetologica* **72**, 64-72.

Griffiths, R. A., Sewell, D. & McCrea, R.S. (2009). Dynamics of a declining amphibian metapopulation: survival, dispersal, and the impact of climate. *Biological Conservation* **143**, 485-491.

Hagman, M., Löwenborg, K. & Shine, R. (2015). Determinants of anti-predator tactics in hatchling grass snakes. *Behavioural Processes* **113**, 60-65.

Halliday, T. R. & Verrell, P. A. (1988). Body size and age in amphibians and reptiles. *Journal of Herpetology* **22**, 253-265.

Halliday, W. & Blouin-Demers, G. (2015). Efficacy of coverboards for sampling small northern snakes. *Herpetology Notes* **8**, 309-314.

Harris, M. P. (1980). Post-mortem shrinkage of wing and bill of puffins. *Ringing & Migration* **3**, 60-61.

Hartley, C., Newcomb, R., Russell, R. & Stevens, J. R. (2006). Amplification of DNA from preserved specimens shows blowflies were preadapted for the rapid evolution of insecticide resistance. *PNAS* **103**, 8757-8762.

Hass, T. C., Blum, M. J. & Heins, D. C. (2010). Morphological responses of a stream fish to water impoundment. *Biology Letters* **6**, 249-263.

Hayek, L. C., Heyer, R. W. & Gascon, C. (2001). Frog morphometrics: a cautionary tale. *Alytes* **18**, 153-177.

Head, J. J. & Polly, D. P. (2007). Disassociation of somatic growth from segmentation drives gigantism in snakes. *Biology Letters* **22**, 296-298.

Herczeg, G., Gonda, A., Perälä., Saarikivi, J., Tuomola, A. & Merilä, J. (2007). Ontogenetic differences in the preferred body temperature of the European adder *Vipera berus*. *Herpetological Journal* **17**, 58-61.

Hickling, R., Roy, D. B., Hill, J. K., Fox, R. & Thomas, C. D. (2006). The distributions of a wide range of taxonomic groups are expanding polewards. *Global Change Biology* **12**, 450-455.

Holmes, F. & Amphibian and Reptile Conservation. (2016). *Reptile identification*. [Accessed online 07/11/2017 https://www.arguk.org/info-advice/id-guides/241-reptile-id-guide-2016-compressed/file].

Hopkins, J. J., Allison, H. M., Walmsley, C. A., Gaywood, M. & Thurgate, G. (2007). Conserving biodiversity in a changing climate: guidance on building capacity to adapt. *DEFRA*. [Accessed online 18/09/2017. www.biodiversitysouthwest.org.uk/docs/Conserving%20biodiversity%20in%20a%20changing%20climate.pdf].

Houston, D. & Shine, R. (1994). Low growth rates and delayed maturation in arafura filesnakes (Serpentes: *Acrochordidae*) in tropical Australia. *Copeia* **1994**, 726-731.

Hulme, M., Jenkins, G. J., Lu, X., Turnpenny, J. R., Mitchell, T. D., Jones, R. G., Lowe, J., Murphy, J. M., Hassell, D., Boorman, P., McDonald, R. & Hill, s. (2002). Climate Change Scenarios in the United Kingdom: *The UKCIP02 Scientific Report*. Tyndall Centre for Climate Change Research, University of East Anglia, Norwich.

Inns, H. (2009). The species accounts. In: *Britain's Reptiles and Amphibians*. Hampshire, *UK: WILDguides*. P70-74.

Jayne, B. C. (1982). Comparative morphology of the semispinalis-spinalis muscle of snakes and correlations of locomotion and constriction. *Journal of Morphology* **172**, 83-96.

Jockush, E. L. (1997). Geographic variation and phenotypic plasticity of number of trunk vertebrae in slender salamanders, *Batrachoseps* (Caudata: *Plethodontidae*). *Evolution* **51**, 1966-1982.

King, R. B. (1987). Color pattern polymorphism in the Lake Eerie water snake, *Nerodia sipedon insularum*. *Evolution* **41**, 241-255.

Klauber, L. M., (1943). Tail-length differences in snakes with notes on sexual dimorphism and the coefficient of divergence. *Bulletin of the Zoological Society of San Diego* **18**, 1-60.

Klauber, L. (1956). Rattlesnakes. Their habitats, life histories, and influence on mankind. *University of California Press*, Berkeley and Los Angeles, USA.

Kolaczkowska, E., & Kubes, P. (2013). What is a neutrophil? From 'Neutrophil recruitment and function in health and inflammation'. *Nature Reviews Immunology* **13**, 159-175.

Koons, D. N., Birkhead, R D., Boback, S. M., Williams, M. I. & Greene, M. P. (2009). The effect of body size on cottonmouth (*Agkistrodon piscivorus*) survival, recapture probability, and behavior in an Alabama swamp. *Herpetological Conservation and Biology* **4**, 221-235.

Laughlin, D. C. (2003). Geographic distribution and dispersal mechanisms of *Bouteloua curtipendula* in the Appalachian Mountains. *American Midland Naturalist* **149**, 268-281.

Lee, J. C. (1982). Accuracy and precision in anuran morphometrics: artifacts of preservation. *Systematic Zoology* **31**, 266-281.

Lee, J. C. (1990). Sources of extraneous variation in the study of meristic characters: the effect of size and inter-observer variability. *Systematic Zoology* **39**, 31-39.

Lee, J. L., Thompson, A. & Mulcahy, D. G. (2016). Relationships between numbers of vertebrae, scale counts, and body size, with implications for taxonomy in nightsnakes (Genus: *Hypsiglena*). *Journal of Herpetology* **50**, 616-620.

Lewandowski, E. & Specht, H. (2014). Influence of volunteer and project characteristics on data quality of biological surveys. *Conservation Biology* **29**, 713-723.

Lindell, L.E., Forsman, A. & Merilä. J. (1992). Variation in number of ventral scales in snakes: effects on body size, growth rate and survival in the adder, *Vipera berus*. *Journal of Zoology* **230**, 101-115.

Lindell, L. E. (1994). The evolution of vertebral number and body size in snakes. *Functional Ecology* **1994**, 708-719.

Lindsey, C. C. (1975). Pleomerism, the widespread tendency among related fish species for vertebral number to be correlated with maximum body length. *Journal of the Fisheries Board of Canada* 1975, 2453-2469 [Accessed online 12/09/2017 https://doi.org/10.1139/f75-283].

López-Alcaide, S. & Macip-Ríos, R. (2011). Effects of climate change in amphibians and reptiles. In Grillo, O. & Venora, G. (eds.). *Biodiversity Loss in a Changing World*, InTech, 163-184.

López-Caldéron, C., Feriche, M., Alaminos, E., & Pleguezuelos, J. M. (2016). Loss of largest and oldest individuals of the Montpellier snake correlates with recent warming in the southeastern Iberian Peninsula. *Current Zoology* **63**, 607-613.

Lorch, J. M., Knowles, S., Lankton, J. S., Michell, K., Edwards, J. L., Kapfer, J. M., Staffen, R. A., Wild, E. R., Schmidt, K. Z., Ballmann, A. E., Blodgett, D., Farrell, T. M., Glorioso, B. M., Last, L. A., Price, S. J., Schuler, K. L., Smith, C. E., Wellehan, J. F. X. & Blehert, D. S. (2016), Snake fungal disease: an emerging threat to wild snakes. *Philosophical Transactions of the Royal Society* **371** [Accessed online 13/11/2017 http://dx.doi.org/10.1098/rstb.2015.0457].

Lourdais, O., Shine, R., Bonnet, X., Guillon, M. & Naulleau, G. (2004). Climate affects embryonic development in a viviparous snake, *Vipera aspis*. *OIKOS* **104**, 551-560.

Luiselli, L. (1996). Individual success in mating balls of the grass snake, *Natrix*: size is important. *Journal of Zoology* **239**, 731-740.

Luiselli, L. (2005). Snakes don't shrink, but 'shrinkage' is an almost inevitable outcome of measurement error by the experimenters. *OIKOS* **110**, 199-202.

Madsen, T. (1987). Are juvenile grass snakes, *Natrix*, aposematically coloured? *OIKOS* **48**, 265-267.

Madsen, T. & Shine, R. (1993). Phenotypic plasticity in body sizes and sexual size dimorphism in European grass snakes. *Evolution* **47**, 321-327.

Madsen, T. & Shine, R. (2000.a). Silver spoons and snake body sizes: prey availability early in life influences long-term growth rates of free-ranging pythons. *Journal of Animal Ecology* **69**, 952-958.

Madsen, T. & Shine, R. (2000.b). Rain, fish and snakes: climatically driven population dynamics of Arafyura filesnakes in tropical Australia. *Oecologia* **124**, 208-215.

Madsen, T. & Shine, R. (2001). Do snakes shrink? *OIKOS* **92**, 187-188.

Martinez, P. A., Berbel-Filho, W. M. & Jacobina, U. P. (2013). Is formalin fixation and ethanol preservation able to influence in geometric morphometric analysis? Fishes as a case study. *Zoomorphology* **132**, 87-93.

Mayer, M., Shine, R. & Brown, G. P. (2016). Bigger babies are bolder: effects of body size on personality of hatchling snakes. *Behaviour* **153**, 313-323.

Mazerolle, M. J., Bailey, L. L., Kendall, W. L., Royle, A. J., Converse, S. J. & Nichols, J. D. (2007). Making great leaps forward: accounting for detectability in herpetological field studies. *Journal of Herpetology* **41**, 672-689.

Measey, G. J., Bernardo Silva, J. & Di-Bernarndo, M. (2003). Testing for repeatability in measurements of length and mass in *Chthonerpeton indistinctum* (Amphibia: Gymnophiona), including a novel method of calculating total length of live caecilians. *Herpetological Review* **34**, 35-39.

Mills, E. L., Pittman, K. & Munroe, B. (1982). Effect of preservation on the weight of marine benthic invertebrates. *Canadian Journal of Fisheries and Aquatic Sciences* **39**, 221-224.

Mitrovich, M. J. & Cotroneo, R. A. (2006). Use of plasticine replica snakes to elicit antipredator behavior in the California ground squirrel (*Spermophilus beecheyi*). *The Southwestern Naturalist* **51**, 263-267.

Natusch, D. J. D. & Shine, R. (2012). Measuring body lengths of preserved snakes. *Herpetological Review* **43**, 34-35.

Nelson, C. E. (1971). Effects of differential preservation on dermal pustularity of the microhylid frogs Gastrophryne and Hypopachus. *Herpetological Review* **3**, 93.

Newman, C., Buesching, C. D. & Macdonald, D. W. (2003). Validating mammal monitoring methods and assessing the performance of volunteers in wildlife conservation – "*Sed quis custodiet ipsos custodies*?" *Biological Conservation* **113**, 189-197.

Nicholls, H. (2013). In a hotter world, it's survival of the smallest. *New Scientist* **2903**, 02/09/2013, 40-43.

Nilson, G. (1980). Male reproductive cycle of the European adder, *Vipera berus*, and its relation to annual activity periods. *Copeia* **4**, 729-737.

O'Donnell, K. M. & Semlitsch, R. D. (2015). Advancing terrestrial salamander population ecology: the central role of imperfect detection. *Journal of Herpetology* **49**, 533-540.

Ohlberger, J. (2013). Climate warming and ectotherm body size – from individual physiology to community ecology. *Functional Ecology* **27**, 991-1001.

Oldekop, J. A., Bebbington, A. J., Berdel, F., Truelove, N. K., Wiersberg, T. & Preziosi, R. F. (2011). Testing the accuracy of non-experts in biodiversity monitoring exercises using fern species richness in the Ecuadorian Amazon. *Biodiversity Conservation* **20**, 2615-2626.

Olsson, M., Gullberg, A. & Tegelstrom, H. (1996). Mate guarding in male sand lizards (*Lacerta agilis*). *Behaviour* **133**, 367-386

Palmeirim, J. M. (1998). Analysis of skull measurements and measurers: can we use data obtained by various observers? *Journal of Mammalogy* **79**, 1021-1028.

Penning, D., Gann, E., Thomas, W., Carlson, T., Mittelhauser, J., Bilbrey, L. & Cairns, S. (2013). An evaluation of techniques for measurements of snake length. *Collinsorum* **2**, 20-24.

Phillips, B. L. & Shine, R. (2004). Adapting to an invasive species: toxic cane toads induce morphological change in Australian snakes. *PNAS* **101**, 17150-17155.

Pierce, B. A. & Gutzwiller, K. J. (2004). Auditory sampling of frogs: detection efficiency in relation to survey duration. *Journal of Herpetology* **38**, 495-500.

Pincheira-Donoso, D. & Meiri, S. (2013). An intercontinental analysis of climate-driven body size clines in reptiles: no support for patterns, no signals of processes. *Evolutionary Biology* **40**, 562-578.

Piñeiro, G., Ferigolo, J, Ramos, A. & Laurin, M. (2012). Cranial morphology of the Early Permian mesosaurid *Mesosaurus tenuidens* and the evolution of the lower temporal fenestration reassessed. *Comptes Rendus Palevol* **11**, 379-391.

Posa, M. R. C., Sodhi, N. S. & Koh, L. P. (2007). Predation on artificial nests and caterpillar models across a disturbance gradient in Subie Bay, Philippines. *Journal of Tropical Ecology* **23**, 27-33.

Pounds, A. J., Bustamente, M. R., Coloma, L. A., Consuegra, J. A., Fogden, M. P. L., Foster, P. N., La Marca, E., Masters, K. L., Merino-Viteri, A., Puschendorf, R., Ron, S. R., Sánchez-azofeifa, G. A., Still, C. J. & Young, B. E. (2006). Widespread amphibian extinctions from epidemic disease driven by global warming. *Nature* **439**, 161-167.

Pyke, G. H. & Ehrlich, P. R. (2010). Biological collections and ecological / environmental research: a review, some observations and a look to the future. *Biological Reviews* **85**, 247-266.

Queral-Regil, A. & King, R. B. (1998). Evidence for phenotypic plasticity in snake body size and relative head dimensions in response to amount and size of prey. *Copeia* **2**, 423-429.

Quinn, H. & Jones, J. P. (1974). Squeeze box technique for measuring snakes. *Herpetological Review* **5**, 35.

Reading, C.J. (2007). Linking global warming to amphibian declines through its effects on female body condition and survivorship. *Oecologia* **151**, 125-131.

Reading, C. J. (1997). A proposed standard method for surveying reptiles in dry lowland heath. *Journal of Applied Ecology* **34**, 1057-1069.

Reed, R. N. (2001). Effects of museum preservation techniques on length and mass of snakes. *Amphibia-Reptilia* **22**, 488-491.

Reichling, S. B. & Gutzke, W. H. N. (1996). Phenotypic consequences of incubation environment in the African elapid genus *Aspidelaps. Zoo Biology* **15**, 301-308.

Ricklefs, R. E. (1980). Old specimens and new directions: the museum tradition in contemporary ornithology. *Auk* **97**, 206-208.

Rivas, J. A., Ascanio, R. E. & Muñoz, M. D. C. (2008). What is the length of a snake? *Contemporary Herpetology* **2008**, 1-3.

Rodda, G. H., Dean-Bradley, K., Campbell, E. W., Fritts, T. H., Lardner, B., Yackel Adams, A. A. & Reed, R. N. (2015a). Stability of detectability over 17 years at a single site and other lizard detection comparisons from Guam. *Journal of Herpetology* **49**, 513-521.

Rodda, G. H., Yackel Adams, A. A., Campbell, E. & Fritts, T. H. (2015b). General herpetological collecting is size-biased for five Pacific lizards. *Journal of Herpetology* **49**, 507-512.

Rodda, G. H., Savidge, J. A., Tyrrell, C. L., Christy, M. T. and Ellingson, A. R. (2007). Size bias in visual searches and trapping of brown treesnakes on Guam. *Journal of Wildlife Management* **71**, 656-661.

Rohr, M. C., Henk, D. A., Briggs, C. J., Brownstein, J. S., Madoff, L. C., McCraw, S. L. & Gurr, S. J. (2012). Emerging fungal threats to animal, plant and ecosystem health. *Nature* **484**, 186-194.

Roitberg, E. S., Orlova, V. F., Kuranova, V. N., Bulakhova, N. A., Zinenko, O. I., Ljubisavljevic, K., Shamgunova, R. R., Carretero, M. A., Clasen, A., Fokt, M. & Böhme, W. (2011). Inter-observer and intra-observer differences in measuring body length: a test in the common lizard, *Zootoca vivpara*. *Amphibia-Reptilia* **32**, 477-484.

Rufino, M., Abelló, P. & Yule, A. B. (2004). The effect of alcohol and freezing preservation on carapace size and shape in Liocarcinus depurator (Crustacea, Bachyua). In: *Morphometrics – Applications in Biology and Paleontology, Elewa, A. M. T., Ed., Springer* 45-54.

Saint Girons, H. (1978). Morphologie externe compare et systémique des Viperes d'Europe (Reptilia, Viperidae). *Revue Suisse Zoologie* **85**, 565-595.

Saporito, R. A., Zuercher, R., Roberts, M., Gerow, K. G. & Donelly, M. A. (2007). Experimental evidence for aposematism in the dendrobatid poison frog *Oophaga pumilio*. *Copeia* **4**, 1006-1011.

Schmeller, D. S., Henry, P., Julliard, R., Gruber, B., Clobert, J., Dziock, F., Lengyel., Nowicki, P., Deri, E., Budrys, E., Kull, T., Tali, K., Bauch, B., Settele, J., Van Swaay, C., Kobler, A., Babji, V., Papastergiadou, E. & Henle, K. (2009). Advantages of volunteer-based biodiversity monitoring in Europe. *Conservation Biology* **23**, 307-316.

Seigel, R. A. & Ford, N. B. (1988). A plea for standardization of body size measurements in studies of snake ecology. *Herpetological Review* **19**, 9-10.

Selander R. K. & Johnston, R. F. (1967). Evolution in the house sparrow. I. Intrapopulation variation in North America. *Condor* **69**, 217-258.

Setser, K. (2007). Use of anaesthesia increases precision of snake length measurements. *Herpetological Review* **38**, 409-411.

Sheridan, J. A. & Bickford, D. (2011). Shrinking body size as an ecological response to climate change. *Nature Climate Change* **1**, 401-406.

Shetter, D. S. (1936). Shrinkage of trout at death and preservation. *Copeia* **1936**, 60-61.

Siegenthaler, A., Mondal, D. & Benvenuto, C. (2017). Quantifying pigment cover to assess variation in animal colouration. *Biology Methods and Protocols* **2017**, 1-8.

Simmons, J. E. (2014). Effects of fixatives and preservatives on specimens. In: *Fluid Preservation: A Comprehensive Reference.* Maryland, United States of America: Rowman & Littlefield. 73-80.

Smith, H. M. (1955). Effect of preservatives upon pattern in a Mexican garter snake. *Herpetologica* **11**, 165-168.

Smith, S.M. (1977). Coral-snake pattern recognition and stimulus generalization by naïve great kiskadees (Aves: Tyrannidae). *Nature* **265**, 535-536.

Snall, T., Kindvall, O., Nilsson, J. & Part, T. (2011). Evaluating citizen-based presence data for bird monitoring. *Biological Conservation* **144**, 804-810.

Suarez, A. V. & Tsutsui, N. D. (2004). The value of museum collections for research and society. *Bioscience* **54**, 66-74.

Sutherland, W. J., Bailey, M. J., Bainbridge, I. P., Brereton, T., Dick, J. T. A., Drewitt, J., Dulvy, N. K., Dusic, N. R., Freckleton, R. P., Gaston, K. J., Gilder, P. M., Green, R. E., Heathwaite, A. L., Johnson, S. M., Macdonald, D. W., Mitchell, R., Osborn, D., Owen, R. P., Pretty, J., Prior, S. V., Prosser, H., Pullin, A. S., Rose, P., Stott, A., Tew, T., Thomas, C. D., Thompson, D. B. A., Vickery, J. A., Walker, M., Walmsley, C., Warrington, S., Watkinson, A. R., Williams, R. J., Woodroffe, R. & Woodroof, H. J. (2008). Future novel threats and opportunities facing UK biodiversity identified by horizon scanning. *Journal of Applied Ecology* **45**: 821–833.

Taylor, E.N. & Denardo, D.F. (2005). Sexual size dimorphism and growth plasticity in snakes: an experiment on the western diamond-backed rattlesnake (*Crotalus atrox*). *Journal of Experimental Zoology* **303**, 598-607.

Telemeco, R. S., Elphick, M. J. & Shine, R. (2009). Nesting lizards (*Bassiana duperreyi*) compensate partly, but not completely, for climate change. *Ecology* **90**, 17-22.

Thomas, C. D., Cameron, A., Green, R. E., Bakkenes, M., Beaumont, Y. C., Collingham, B. F. N., Erasmus, Ferreira de Siqueira, M., Grainger, A., Hannah, L., Hughes, L., Huntley, B., Van Jaarsveld, A. S., Midgley, G. F., Miles, L., Ortega-Huerta, M. A., Townsend Peterson, A., Phillips, O. L. & Williams, S. E. (2004). Extinction risk from climate change. *Nature* **427**, 145-148.

Thorpe, R.S. (1989). Pattern and function of sexual dimorphism: a biometric study of character variation in the grass snake (*Natrix*, Colubridae) due to sex and its interaction with geography. *Copeia* **1989**, 53-63

.

Tinkle, J. L., Gartner, G. E. A., Jayne, B. C. & Garland, J. R. (2017). Ecological and pylogenetic variability in the spinalis muscle of snakes. *Journal of Evolutionary Biology* **30**, 2031-2043.

Urban, M., Richardson, J. L. & Freidenfelds, N. A. (2013). Plasticity and genetic adaption mediate amphibian and reptile responses to climate change, *Evolutionary Applications*. [Accessed online 21/10/2017. http://doi.org/10.1111/eva.12114].

Van Dyck, H., Bonte, D., Puls, R., Gotthard, K. & Maes, D. (2015). The lost generation hypothesis: could climate change drive ectotherms into a developmental trap? *OIKOS* **124**, 54-61.

Vervust, B., Van Dongen, S. & Van Damme, R. (2009). The effect of preservation on lizard morphometrics – an experimental study. *Amphibia-Reptilia* **30**, 321-329.

Vilela, B., Villalobos, F., Rodríguez, M. A. & Terribile, L. C. (2014). Body size, extinction risk and knowledge bias in new world snakes. *PLoSOne* **9**, e113429 [Accessed online 23/11/2016].

Vogelsang, T. J. & Hans Franses, P. (2005). Are winters getting warmer? *Environmental Modelling and Software* **20**, 1449-1455.

Volychik, S. (2012). Morphological variability in *Vipera palaestinae* along an environmental gradient. *Asian Herpetological Research* **3**, 227-239.

Walmsley, C.A., Smithers, R.J., Berry, P.M., Harley, M., Stevenson, M.J., Catchpole, R. (2007). *MONARCH – Modelling Natural Resource Responses to Climate Change – a synthesis for biodiversity conservation*. UKCIP, Oxford, United Kingdom.

Walther, G. R., Post, E., Convey, P., Menzel, A., Parmesan, C., Beebee, T. J. C., Fromentin, J., Hoegh-Guldberg, O. & Bairlein, F. (2002). Ecological responses to recent climate change. *Nature* **416**, 389-395.

Wandeler, P., Paquita, E. A. H. & Keller, L. F. (2007). Back to the future: museum specimens in population genetics. *Trends in Ecology and Evolution* **22**, 634-642.

Watters, J. L., Cummings, S. T., Flanagan, R. L. & Siler, C. D. (2016). Review of morphometric measurements used in anuran species descriptions and recommendations for a standardized approach. *Zootaxa* **4072**, 477-495.

Whitfield Gibbons, J., Scott, D. E., Travis, R. J., Buhlmann, K. A., Tuberville, T. D., Metts, B. S., Greene, J. L., Mills, T., Leiden, Y., Poppy, S. & Winne, C. T. (2000). The global decline of reptiles, déja vu amphibians. *BioScience* **50**, 653-666.

Willson, J. D. (2016). In *Reptile Ecology and Conservation, A Handbook of Techniques*. 1st Ed. Edited by C. Kenneth Dodd, Jr. Oxford University Press: Oxford.

Wikelski, M. & Thom, C. (2000). Marine iguanas shrink to survive El Niño. *Nature* **403**, 37-38.

Winker, K. (2004). Natural History Museums in a Postbiodiversity Era. *BioScience* **54**, 455-459.

Yates, T. L., Mills, J. N., Parmenter, C. A., Ksiazek, T. G., Parmenter, R. R., Vande Castle, J. R., Calisher, C. H., Nichol, S. T., Abbott, K. D., Young, J. C., Morrison, M. L., Beaty, B. J., Dunnum, J. L., Baker, R. J., Salazar-Bravo, J. & Peters, C. J. (2002). The ecology and evolutionary history of an emergent disease: Hantavirus pulmonary syndrome: evidence for two El Niño episodes in the American Southwest suggests that El Niño-driven precipitation, the initial catalyst of a trophic cascade that results in a delayed density-dependent rodent response, is sufficient to predict heightened risk for human contraction of hantavirus pulmonary syndrome. *Bioscience* **52**, 989-998.

Yeager, J., Wooten, C. & Summers, K. (2011). A new technique for the production of large numbers of clay models for field studies of predation. *Herpetological Review* **42**, 357-359.

Yezerinac, S. M., Lougheed, S. C. & Handford, P. (1992). Measurement error and morphometric studies: statistical power and observer experience. *Systematic Biology* **41**, 471-482.

Zhang, L., Kouba, A., Wang, Q., Zhao, H., Jiang, W., Scott, W. & Zhang, H. (2014). The effect of water temperature on the growth of captive giant Chinese salamanders (*Andrias davidianus*) reared for reintroduction: a comparison with wild salamander body condition. *Herpetologica* **70**, 369-377.

APPENDICES

APPENDIX 2.I.a & b.

2.I.a: *N. helvetica* historic measurements: (uncorrected)

Male				Female			
SVL	Tail	Total	Location	SVL	Tail	Total	Location
643	171	815	East Sussex	876	201	1077	Wiltshire
542	159	701	West Sussex	720	158	878	Hampshire
635	181	816	Surrey	675	169	844	Hampshire
423	113	536	Worcestershire	575	136	711	Hampshire
606	168	774	Jersey	743	170	913	Surrey
561	156	718	Jersey	628	151	779	Norfolk
494	133	627	Jersey	539	135	674	Cambridgeshire
582	176	758	Jersey	907	195	1102	Wales
304	83	388	Jersey	606	148	754	Jersey
				397	94	491	Jersey
				514	132	646	Jersey
				469	116	585	Jersey
				842	194	1036	Jersey
				866	191	1057	Jersey
				498	126	624	Cambridgeshire
				323	83	406	Isle of Wight
				663	158	821	Wales
				774	163	937	Wales
				822	175	997	Wales
				504	135	639	Hampshire
				810	175	985	East Sussex
Entries not included as post 1950s				**Entries not included in subset as post 1950s**			
520	131	651	England (unknown)	657	138	795	Hampshire
484	128	612	England (unknown)	596	140	736	Essex
618	172	790	Essex	536	126	662	Essex
341	97	438	Essex	552	141	693	Essex
507	138	645	Surrey	707	170	877	Devon
519	133	652	London	530	130	660	Devon
519	132	651	Kent	321	76	397	Warwickshire
473	126	599	Devon	644	139	783	Devon
471	144	615	Surrey	632	146	778	Staffordshire
602	147	749	Essex	798	168	966	Kent
430	112	542	Devon	717	154	871	Hertfordshire

2.I.b: *V. berus* historic measurements (uncorrected)

Male				Female			
SVL	Tail	Total	Location	SVL	Tail	Total	Location
390	58	448	Dorset	453	50	503	Kent
488	76	564	Sussex	453	60	512	Hampshire
503	77	580	Sussex	545	65	610	Suffolk
400	65	466	Surrey	500	63	563	Suffolk
483	70	554	Essex	528	66	595	Suffolk
453	74	526	Lancashire	521	70	591	Suffolk
473	79	552	Lancashire	394	49	442	Suffolk
509	75	583	Hampshire	433	55	488	Hampshire
467	68	535	Wales	511	70	582	Norfolk
465	70	535	Scotland	458	59	517	Norfolk
461	69	530	Scotland	409	50	458	Suffolk
273	43	316	Scotland	448	64	511	Surrey
506	65	571	Scotland	462	61	523	Surrey
430	70	500	Scotland	440	55	496	Surrey
455	68	524	Scotland	495	59	554	Surrey
412	58	470	Scotland	484	52	537	Herefordshire
504	72	576	Scotland	509	52	561	Hampshire
453	70	523	Scotland	426	49	475	Hampshire
501	78	579	Scotland	509	60	568	Wales
490	70	560	Scotland	490	55	545	Wales
438	72	510	Scotland	504	57	561	Wales
381	65	445	Scotland	450	56	506	Wales
394	60	453	Scotland	468	59	526	Wales
428	65	494	Hampshire	540	69	610	Scotland
437	67	504	Hampshire	557	72	629	Scotland
458	73	531	Hampshire	265	32	296	Scotland
383	67	451	Hampshire	456	47	503	Scotland
468	65	533	Surrey	496	62	557	Scotland
393	65	458	Surrey	462	57	519	Scotland
420	61	481	England (unknown)	176	22	197	Wales
373	60	433	Hampshire	469	61	530	Scotland
492	79	571	Essex	491	58	549	Scotland
488	66	554	Northumberland	417	56	473	Scotland
385	60	445	East Sussex	429	52	482	Scotland
347	57	404	Sussex	477	64	540	Scotland
426	60	486	Surrey	486	51	538	England (unknown)
343	52	396	Surrey	505	73	578	Scotland
423	65	488	Surrey	485	57	542	Scotland
410	65	475	Kent	488	62	550	Scotland
531	63	594	Kent	468	70	539	Scotland

463	78	540	Kent
404	65	468	Essex
432	60	492	Hampshire
438	68	506	Kent
449	72	521	Wales

Entries not included as post 1950s

371	59	430	Hampshire
444	85	529	Buckinghamshire
524	75	599	Essex
417	64	481	Essex
380	64	444	Surrey
507	72	579	West Sussex
471	71	542	Essex

499	57	556	Hampshire
482	63	545	Surrey
452	54	506	Surrey
426	37	464	England (unknown)
537	61	597	Kent
422	50	472	Hampshire
424	50	474	Hampshire
473	51	525	Hampshire
384	51	436	Kent
464	60	524	Surrey
510	65	575	Essex
424	53	478	Surrey
249	32	281	Dorset
438	57	495	Surrey
398	45	443	Wales
296	35	331	Wales
336	40	376	Wales

Entries not included as post 1950s

370	47	417	Scotland
537	68	605	Scotland
582	67	649	Scotland
521	62	583	Buckinghamshire
272	34	306	Essex
439	53	492	Somerset
460	55	515	Essex
545	60	605	Buckinghamshire
476	60	536	Buckinghamshire
646	81	727	Sussex
168	27	195	Essex

2.I.c: *N. helvetica* contemporary measurements

Male				Female			
SVL	Tail	Total	Location	SVL	Tail	Total	Location
522	139	661	Kent	645	146	791	Kent
520	145	665	Kent	660	153	813	Kent
530	150	680	Kent	670	150	820	Kent
408	130	538	Kent	620	120	740	Kent
522	130	652	Kent	583	110	693	Kent
540	140	680	Kent	690	120	810	Kent
548	135	683	Kent	305	70	375	Kent
503	130	633	Kent	610	142	752	Kent
483	140	623	Kent	640	135	775	Kent
395	89	484	Kent	612	143	755	Kent
515	135	650	Kent	610	132	742	Kent
562	140	702	Kent	713	150	863	Kent
505	137	642	Kent	670	150	820	Kent
328	90	418	Kent	680	140	820	Kent
518	140	658	Kent	720	160	880	Kent
490	130	620	Kent	573	130	703	Kent
505	140	645	Kent	660	162	822	Kent
510	125	635	Kent	693	152	845	Kent
515	140	655	Kent	755	125	880	Kent
483	135	618	Kent	680	150	830	Kent
480	130	610	Kent	630	150	780	Kent
555	145	700	Kent	736	166	902	Kent
508	141	649	Kent	715	150	865	Kent
448	112	560	Kent	725	145	870	Kent
501	130	631	Kent	700	142	842	Kent
466	107	573	Kent	697	148	845	Kent
437	139	576	Kent	684	141	825	Kent
454	151	605	Kent	700	141	841	Kent
359	112	471	Kent	663	145	808	Kent
317	90	407	Kent	680	157	837	Kent
255	81	336	Kent	375	63	438	Kent
470	145	615	Kent	557	120	677	Kent
266	65	331	Kent	486	85	571	Kent
283	89	372	Kent	645	79	724	Kent
530	130	660	Norfolk	691	117	808	Kent
350	100	450	Norfolk	357	92	449	Kent
360	110	470	Norfolk	298	74	372	Kent
510	130	640	Norfolk	268	69	337	Kent
460	110	570	Norfolk	309	80	389	Kent
600	150	750	Norfolk	282	72	354	Kent
455	125	580	Norfolk	220	47	267	Kent
630	140	770	Norfolk	226	50	276	Kent
510	110	620	Norfolk	216	48	264	Kent
510	140	650	Norfolk	259	61	320	Kent
385	125	510	Norfolk	205	51	256	Kent
590	185	775	Norfolk	238	63	301	Kent
640	38	678	Norfolk	610	140	750	Norfolk

554	156	710	Norfolk	670	120	790	Norfolk
406	108	514	Norfolk	840	150	990	Norfolk
500	110	610	Norfolk	600	120	720	Norfolk
460	120	580	Norfolk	830	110	940	Norfolk
520	136	656	Norfolk	570	120	690	Norfolk
478	112	590	Norfolk	640	134	774	Norfolk
423	130	553	Norfolk	656	154	810	Norfolk
620	172	792	Norfolk	670	180	850	Norfolk
560	238	798	Norfolk	410	80	490	Norfolk
430	88	518	Norfolk	625	123	748	Norfolk
533	129	662	Norfolk	554	120	674	Norfolk
292	72	364	Norfolk	660	144	804	Norfolk
570	150	720	Norfolk	630	135	765	Norfolk
201	49	250	Norfolk	472	113	585	Norfolk
190	55	245	Norfolk	470	110	580	Norfolk
340	102	442	Norfolk	440	98	538	Norfolk
517	197	714	Norfolk	600	131	731	Norfolk
318	132	450	Norfolk	730	210	940	Norfolk
440	115	555	Norfolk	530	113	643	Norfolk
503	131	634	Norfolk	560	131	691	Norfolk
522	143	665	Norfolk	291	55	346	Norfolk
390	111	501	Norfolk	192	52	244	Norfolk
546	32	578	Norfolk	634	147	781	Norfolk
557	132	689	Norfolk	484	116	600	Norfolk
420	124	544	Norfolk	625	143	768	Norfolk
510	144	654	Norfolk	255	60	315	Norfolk
470	100	570	Norfolk	730	144	874	Norfolk
460	130	590	Norfolk	750	201	951	Norfolk
515	135	650	Norfolk	332	80	412	Norfolk
490	120	610	Norfolk	610	130	740	Norfolk
560	142	702	Norfolk	490	80	570	Norfolk
350	75	425	Norfolk	731	141	872	Norfolk
490	150	640	Norfolk	636	177	813	Norfolk
345	61	406	Norfolk	713	141	854	Norfolk
363	95	458	Norfolk	488	129	617	Norfolk
544	138	682	Norfolk	770	180	950	Norfolk
610	75	685	Norfolk	700	160	860	Norfolk
324	87	411	Norfolk	966	128	1094	Norfolk
650	152	802	Norfolk	740	140	880	Norfolk
510	250	760	Norfolk	305	50	355	Norfolk
521	129	650	Norfolk	315	90	405	Norfolk
430	120	550	Norfolk	440	106	546	Norfolk
300	68	368	Norfolk	268	72	340	Norfolk
325	87	412	Norfolk	770	157	927	Norfolk
434	118	552	Norfolk	485	116	601	Norfolk
530	110	640	Norfolk	640	145	785	Norfolk
471	191	662	Norfolk	844	202	1046	Norfolk
513	149	662	Norfolk	775	165	940	Norfolk
330	91	421	Norfolk	525	118	643	Norfolk
550	135	685	Norfolk	742	136	878	Norfolk
290	82	372	Norfolk	670	163	833	Norfolk
565	157	722	Norfolk	280	62	342	Norfolk
463	137	600	Norfolk	182	40	222	Norfolk
481	129	610	Norfolk	450	120	570	Norfolk

430	120	550	Norfolk	485	109	594	Norfolk
475	117	592	Norfolk	516	122	638	Norfolk
560	142	702	Norfolk	310	74	384	Norfolk
425	121	546	Norfolk	570	166	736	Norfolk
530	140	670	Norfolk	650	144	794	Norfolk
560	146	706	Norfolk	560	150	710	Norfolk
326	88	414	Norfolk	710	160	870	Norfolk
450	135	585	Norfolk	770	150	920	Norfolk
530	143	673	Norfolk	278	58	336	Norfolk
430	94	524	Norfolk	360	110	470	Norfolk
458	118	576	Norfolk	345	75	420	Norfolk
370	101	471	Norfolk	764	188	952	Norfolk
493	91	584	Norfolk	683	149	832	Norfolk
480	88	568	Norfolk	534	107	641	Norfolk
540	123	663	Norfolk	328	72	400	Norfolk
280	51	331	Norfolk	312	82	394	Norfolk
424	112	536	Norfolk	495	115	610	Norfolk
561	116	445	Norfolk	816	184	1000	Norfolk
300	75	375	Norfolk	358	86	444	Norfolk
220	52	272	Norfolk	285	70	355	Norfolk
310	80	390	Norfolk	720	150	870	Norfolk
285	55	340	Norfolk	650	145	795	Norfolk
553	137	690	Norfolk	770	75	845	Norfolk
330	89	419	Norfolk	664	156	820	Norfolk
550	140	690	Norfolk	630	153	783	Norfolk
280	66	346	Norfolk	770	80	850	Norfolk
510	117	627	Norfolk	690	140	830	Norfolk
420	94	514	Norfolk	650	150	800	Norfolk
448	122	570	Norfolk	560	120	680	Norfolk
580	165	745	Norfolk	538	102	640	Norfolk
576	138	714	Norfolk	670	158	828	Norfolk
506	136	642	Norfolk	630	120	750	Norfolk
460	119	579	Norfolk	604	144	748	Norfolk
335	55	390	Norfolk	688	152	840	Norfolk
320	82	402	Norfolk	325	80	405	Norfolk
430	110	540	Norfolk	385	85	470	Norfolk
560	122	682	Norfolk				
480	130	610	Norfolk				
340	90	430	Norfolk				
395	101	496	Norfolk				
272	63	335	Norfolk				
440	103	543	Norfolk				
326	58	384	Norfolk				
440	115	555	Norfolk				
685	171	856	Norfolk				
500	130	630	Norfolk				
372	88	460	Norfolk				
348	74	422	Norfolk				
350	80	430	Norfolk				
180	48	228	Norfolk				
334	84	418	Norfolk				
385	101	486	Norfolk				
378	112	490	Norfolk				
310	70	380	Norfolk				

376	117	493	Norfolk
296	72	368	Norfolk
328	83	411	Norfolk
450	96	546	Norfolk
318	78	396	Norfolk
330	83	413	Norfolk
310	80	390	Norfolk
255	65	320	Norfolk
294	68	362	Norfolk
308	72	380	Norfolk
510	140	650	Norfolk
588	164	752	Norfolk
580	68	648	Norfolk
288	62	350	Norfolk
460	126	586	Norfolk

2.I.d: *V. berus* contemporary measurements

Male				Female			
SVL	Tail	Total	Location	SVL	Tail	Total	Location
470	67	537	Kent	202	43	245	Kent
428	75	503	Kent	201	32	233	Kent
497	81	578	Kent	415	62	477	Kent
425	69	494	Kent	544	69	613	Kent
419	63	482	Kent	181	29	210	Kent
381	71	452	Kent	178	26	204	Kent
444	75	519	Kent	526	75	601	Kent
371	69	440	Kent	505	66	571	Kent
415	85	500	Kent	377	40	417	Kent
401	64	465	Kent	433	58	491	Kent
365	65	430	Kent	457	50	507	Kent
391	55	446	Kent	211	26	237	Kent
414	78	492	Kent	458	57	515	Kent
416	71	487	Kent	431	51	482	Kent
202	31	233	Kent	496	54	550	Kent
339	56	395	Kent	158	25	183	Kent
334	49	383	Kent	197	27	224	Kent
373	61	434	Kent	359	45	404	Kent
398	79	477	Kent	389	51	440	Kent
382	65	447	Kent	392	43	435	Kent
343	61	404	Kent	413	57	470	Kent
373	62	435	Kent	441	49	490	Kent
375	70	445	Kent	445	49	494	Kent
378	64	442	Kent	478	62	540	Kent
385	67	452	Kent	511	60	571	Kent
391	59	450	Kent	168	35	203	Kent
376	58	434	Kent	176	25	201	Kent
162	30	192	Kent	220	28	248	Kent
405	74	479	Kent	390	58	448	Kent
442	88	530	Kent	404	53	457	Kent
434	75	509	Kent	405	62	467	Kent
400	75	475	Kent	432	59	491	Kent
312	60	372	Kent	451	64	515	Kent
413	75	488	Kent	463	61	524	Kent
497	82	579	Kent	459	62	521	Kent
380	62	442	Kent	339	43	382	Kent
445	74	519	Kent	475	60	535	Kent
454	79	533	Kent	435	66	501	Kent
374	52	426	Kent	310	43	353	Kent
415	71	486	Kent	321	44	365	Kent
420	73	493	Kent	426	54	480	Kent

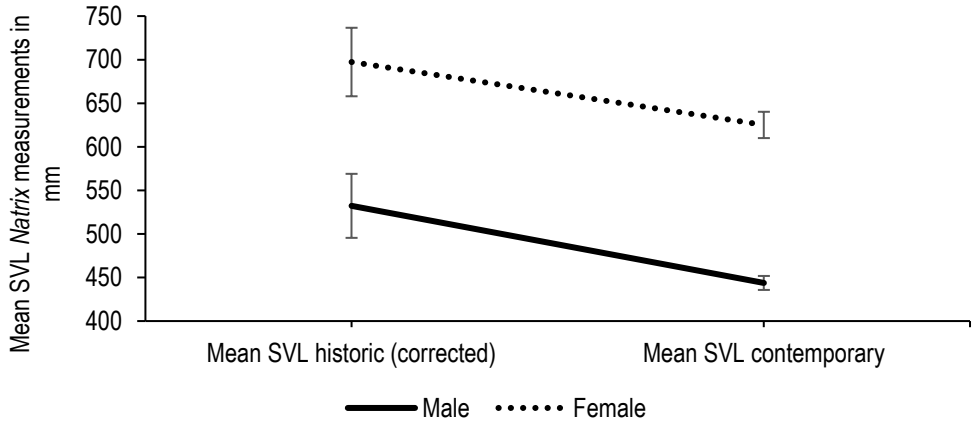
362	66	428	Kent	404	59	463	Kent
480	84	564	Kent	413	54	467	Kent
420	64	484	Kent	418	49	467	Kent
495	75	570	Kent	495	53	548	Kent
444	80	524	Kent	440	51	491	Kent
389	69	458	Kent	530	67	597	Kent
260	45	305	Kent	436	58	494	Kent
504	83	587	Kent	456	56	512	Kent
425	70	495	Kent	480	64	544	Kent
425	76	501	Kent	394	46	440	Kent
409	78	487	Kent	238	35	273	Kent
512	85	597	Kent	431	57	488	Kent
438	74	512	Kent	475	54	529	Kent
418	70	488	Kent	201	35	236	Kent
388	67	455	Kent	176	26	202	Kent
340	60	400	Kent	455	59	514	Kent
400	63	463	Kent	470	58	528	Kent
420	69	489	Kent	431	57	488	Kent
440	75	515	Kent	475	54	529	Kent
272	42	314	Kent	466	54	520	Kent
410	70	480	Kent	445	52	497	Kent
383	79	462	Kent	290	35	325	Kent
354	57	411	Kent	338	39	377	Kent
396	71	467	Kent	439	54	493	Kent
455	79	534	Kent	443	57	500	Kent
368	75	443	Kent	437	56	493	Kent
296	54	350	Kent	434	53	487	Kent
354	57	411	Kent	533	63	596	Kent
396	71	467	Kent	466	52	518	Kent
480	72	552	Kent	303	41	344	Kent
466	76	542	Kent	446	60	506	Kent
384	77	461	Kent	379	52	431	Kent
368	62	430	Kent	431	63	494	Kent
378	62	440	Kent				
415	58	473	Kent				
351	66	417	Kent				
351	51	402	Kent				
302	56	358	Kent				
445	68	513	Kent				
378	64	442	Kent				
442	88	530	Kent				
162	33	195	Kent				
225	38	263	Kent				
163	24	187	Kent				

APPENDIX 2.II.a & b

2.II.a. Mean SVL over time for *N. helvetica* with historic data corrected by 6.5% for shrinkage

Source	Type III Sum of Squares	df	Mean Square	F	P
Gender	467536.14	1	467536.14	22.77	<0.001
Time	290788.27	1	290788.27	14.16	<0.001
Gender * time	7740.84	1	7740.84	00.38	0.537
Error	6748229.18	333	20264.95		

2.II.b: Mean SVL (±SE) over time for *N. helvetica* with historic data corrected by 6.5% for shrinkage

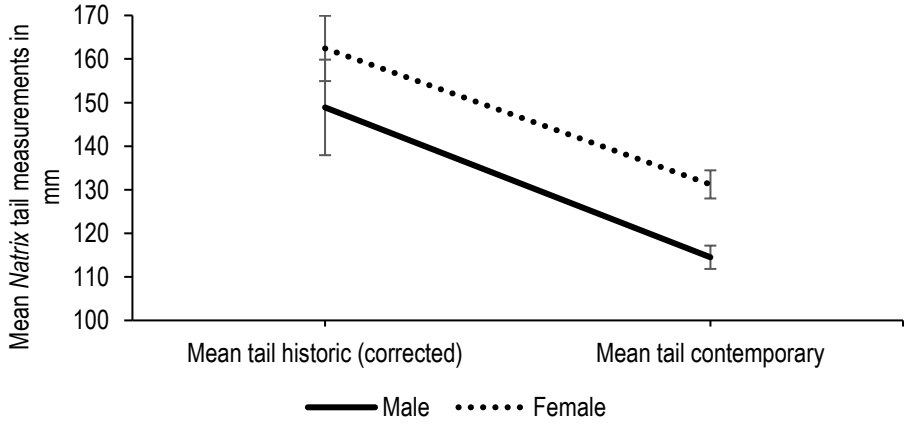


APPENDIX 2.II.c & d.

2.II.c. Mean tail over time for *N. helvetica* with historic data corrected by 6.5% for shrinkage

Source	Type III Sum of Squares	df	Mean Square	F	P
Gender	2480.47	1	2480.47	01.91	0.168
Time	32921.86	1	32921.86	25.36	<0.001
Gender * time	240.45	1	240.45	00.19	0.667
Error	428379.24	333	1286.42		

2.II.d: Mean tail (±SE) over time for *N. helvetica* with historic data corrected by 6.5% for shrinkage

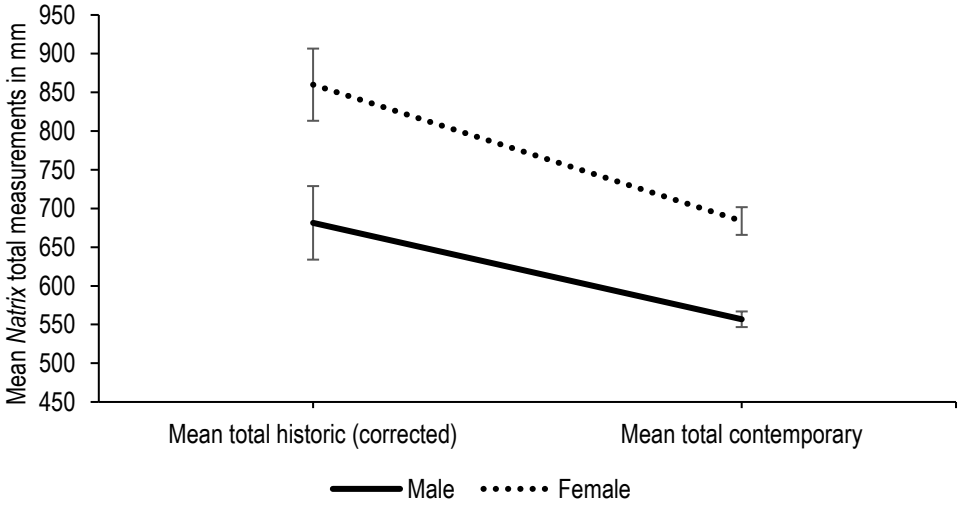


APPENDIX 2.II.e & f.

2.II.e. Mean total over time for *N. helvetica* with historic data corrected by 6.5% for shrinkage

Source	Type III Sum of Squares	df	Mean Square	F	P
Gender	542627.00	1	542627.00	18.11	<0.001
Time	526149.06	1	526149.06	17.56	<0.001
Gender * time	15482.91	1	15482.91	00.52	0.473
Error	9849568.02	333	29578.28		

2.II.f. Mean total (±SE) over time for *N. helvetica* with historic data corrected by 6.5% for shrinkage

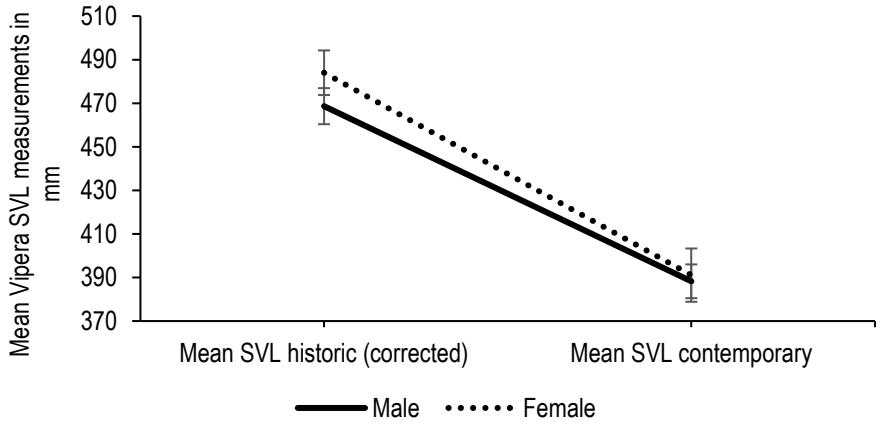


APPENDIX 2.III.a & b.

2.III.a. Mean SVL over time for *V. berus* with historic data corrected by 6.5% for shrinkage

Source	Type III Sum of Squares	df	Mean Square	F	P
Gender	5652.97	1	5652.97	00.81	0.369
Time	470581.06	1	470581.06	67.37	0.000
Gender * time	2683.96	1	2683.96	00.38	0.536
Error	445923.11	279	1256.12		

2.III.b. Mean SVL (±SE) over time for *V. berus* with historic data corrected by 6.5% for shrinkage



APPENDIX 2.III.c & d.

2.III.c. Mean tail over time for *V. berus* with historic data corrected by 6.5% for shrinkage

Source	Type III Sum of Squares	df	Mean Square	F	P
Gender	13783.32	1	13783.32	101.99	0.000
Time	2868.43	1	2868.43	21.23	0.000
Gender * time	174.412	1	174.412	01.29	0.257
Error	37568.95	279	135.14		

2.III.d. Mean tail (±SE) over time for *V. berus* with historic data corrected by 6.5% for shrinkage

APPENDIX 2.III.e & f.

2.III.e. Mean total over time for *V. berus* with historic data corrected by 6.5% for shrinkage

Source	Type III Sum of Squares	df	Mean Square	F	P
Gender	1782.21	1	1782.21	00.20	0.653
Time	546929.55	1	546929.55	62.09	0.000
Gender * time	4226.77	1	4226.77	00.48	0.489
Error	2448610.49	279	8807.95		

2.III.f. Mean total (±SE) over time for *V. berus* with historic data corrected by 6.5% for shrinkage

APPENDIX 3.I

Code	GPS point
S1	TR0355349273
S2	TR0355549255
S3	TR0358549263
S4	TR0357149242
S5	TR0357049237
S6	TR0360749241
S7	TR0360549249
S8	TR0358549236
S9	TR0360849231
S10	TR0359549234
D1	TR0356349245
D2	TR0358249250
D3	TR0357849244
D4	TR0359949251
D5	TR0359549236
S11	TR0354049258
S12	TR0355749254
S13	TR0357249268
S14	TR0358249258
S15	TR0359749259
S16	TR0359949238
S17	TR0360249234
S18	TR0359549213
S19	TR0357349229
S20	TR0359749224
D6	TR0356049238
D7	TR0357149269
D8	TR0359749252
D9	TR0362049234
D10	TR0359049239

Fieldwork Soakham Downs (Kent) tin locations

APPENDIX 3.II.

Unique Number	Code	GPS point	Unique Number	Code	GPS point	Unique Number	Code	GPS point
1	LUY	TR0356249237	46	LCY	TR0358249254	91	LUN	TR0357649230
2	SCY	TR0356549245	47	LCY	TR0357149254	92	SCY	TR0358249244
3	SCY	TR0355649256	48	LUN	TR0357349258	93	LUN	TR0357849237
4	SCN	TR0355449261	49	SUY	TR0357149252	94	LCN	TR0358349235
5	LUY	TR0355449263	50	LCY	TR0357449250	95	SUY	TR0357849246
6	SUN	TR0355949262	51	LUN	TR0357849247	96	SUN	TR0358049251
7	SCY	TR0355349273	52	LUY	TR0357749245	97	SUN	TR0356349248
8	SCY	TR0355649274	53	LCN	TR0358349244	98	LUN	TR0357149252
9	SUN	TR0356849272	54	LCY	TR0358449244	99	LUN	TR0357449244
10	SCY	TR0357249275	55	SCN	TR0358349241	100	LUY	TR0356849251
11	LUN	TR0356949270	56	SUN	TR0358449242	101	LCN	TR0356749256
12	SCN	TR0356149266	57	LUY	TR0358649239	102	LCN	TR0357549255
13	LCN	TR0356849266	58	LUY	TR0359249235	103	SUY	TR0356049236
14	SUN	TR0357249265	59	LUY	TR0359249239	104	SCN	TR0356449246
15	SUN	TR0357249263	60	SCY	TR0359749241			
16	SCY	TR0357549258	61	LCN	TR0359349236			
17	LCY	TR0357649259	62	SUN	TR0358749234			
18	SCN	TR0358249264	63	LCN	TR0359549231			
19	SCN	TR0358549263	64	SUY	TR0358649232			
20	SUY	TR0358749261	65	LUY	TR0358349232			
21	LUN	TR0358749257	66	SCN	TR0358449237			
22	LUN	TR0359249259	67	SUY	TR0358449236			
23	LCN	TR0359249251	68	LCY	TR0358749227			
24	SUN	TR0358549249	69	SUY	TR0358749225			
25	SUN	TR0350349253	70	LCY	TR0358549231			
26	LCN	TR0360349250	71	SCY	TR0359249229			
27	SCN	TR0360649253	72	LUY	TR0358749231			
28	LCN	TR0360949256	73	LCY	TR0359449222			
29	LUN	TR0360549246	74	SUY	TR0359349229			
30	LUY	TR0360449245	75	SCY	TR0359649231			
31	SUY	TR0361349248	76	SUY	TR0359849223			
32	SUN	TR0360849243	77	SCN	TR0360149235			
33	LUN	TR0361449249	78	SCY	TR0360349235			
34	LUY	TR0361549242	79	LCY	TR0360849238			
35	LCY	TR0360649241	80	SCY	TR0361249236			
36	LUN	TR0360249240	81	SUN	TR0360949227			
37	LUY	TR0359949238	82	LCN	TR0360249220			
38	SCY	TR0359949240	83	SUY	TR0359949221			
39	SUY	TR0359549239	84	LCY	TR0359349220			
40	SUN	TR0358949240	85	LCN	TR0359149216			
41	SUY	TR0359049247	86	LCN	TR0358649222			
42	LCY	TR0359049250	87	SCN	TR0358849216			
43	LCY	TR0358549245	88	SCN	TR0358149221			
44	SCN	TR0358449244	89	SCN	TR0358749219			
45	LUN	TR0358349250	90	LUY	TR0357749229			

Model code randomisation using Excel function =RAND() with GPS points

APPENDIX 3.III.a.

Model	Number	GrpA	GrpB	EO
SCY	2			Y
SCY	3	Y		
SCY	7			
SCY	8			
SCY	10	Y		
SCY	16			
SCY	38			
SCY	60	Y	Y	Y
SCY	71	Y	Y	Y
SCY	75			Y
SCY	78	Y		Y
SCY	80			Y
SCY	92		Y	
		5	3	6
GrpA+GrpB pooled:			6	

Model	Number	GrpA	GrpB	EO
SCN	4	Y		
SCN	12			
SCN	18	Y		
SCN	19	Y	Y	Y
SCN	27			Y
SCN	44			Y
SCN	55	Y		Y
SCN	66			Y
SCN	77		Y	Y
SCN	87			Y
SCN	88	Y	Y	Y
SCN	89	Y		Y
SCN	104			
		6	3	9
GrpA+GrpB pooled:			7	

Model	Number	GrpA	GrpB	EO
SUY	20	Y	Y	Y
SUY	31			Y
SUY	39	Y	Y	Y
SUY	41	Y	Y	Y
SUY	49	Y	Y	Y
SUY	64		Y	Y
SUY	67			Y
SUY	69		Y	Y
SUY	74			
SUY	76	Y	Y	Y
SUY	83	Y	Y	Y
SUY	95		y	Y
SUY	103			
		6	9	11
GrpA+GrpB pooled:			9	

Model	Number	GrpA	GrpB	EO
SUN	6		Y	
SUN	9			
SUN	14	Y	Y	Y
SUN	15	Y		
SUN	24			
SUN	25			Y
SUN	32	Y		Y
SUN	40			Y
SUN	56	Y	Y	Y
SUN	62			Y
SUN	81	Y		
SUN	96			
SUN	97	Y	Y	Y
		6	4	7
GrpA+GrpB pooled:			7	

Total model observations per group per small model type

APPENDIX 3.III.b.

Model	Number	GrpA	GrpB	EO
LCY	17	Y	Y	Y
LCY	35		Y	Y
LCY	42		Y	
LCY	43			
LCY	46		Y	Y
LCY	47	Y	Y	Y
LCY	50	Y	Y	Y
LCY	54		Y	Y
LCY	68	Y	Y	Y
LCY	70	Y	Y	
LCY	73	Y	Y	Y
LCY	79	Y		Y
LCY	84	Y	Y	Y
		8	11	10
GrpA+GrpB pooled:			11	

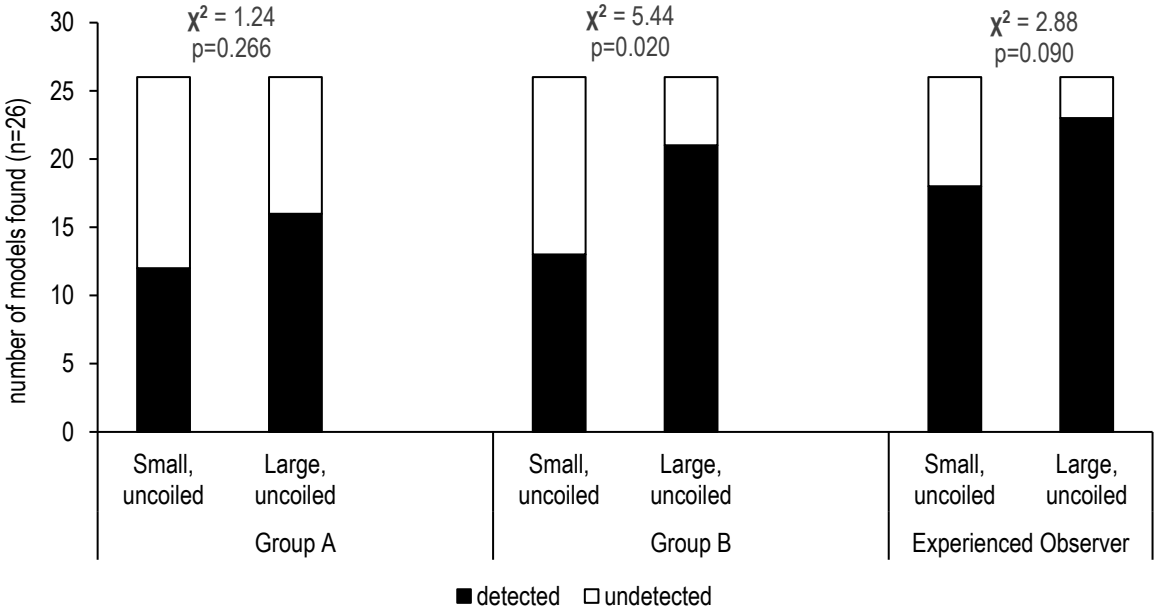
Model	Number	GrpA	GrpB	EO
LCN	13	Y	Y	
LCN	23	Y	Y	
LCN	26	Y	Y	Y
LCN	28			Y
LCN	53		Y	
LCN	61			Y
LCN	63			Y
LCN	82	Y	Y	Y
LCN	85	Y	Y	Y
LCN	86	Y	Y	Y
LCN	94	Y	Y	Y
LCN	101	Y		
LCN	102		Y	Y
		8	9	9
GrpA+GrpB pooled:			10	

Model	Number	GrpA	GrpB	EO
LUY	1	Y	Y	
LUY	5	Y		
LUY	30		Y	Y
LUY	34		Y	Y
LUY	37		Y	Y
LUY	52		Y	Y
LUY	57	Y	Y	Y
LUY	58	Y	Y	Y
LUY	59	Y	Y	Y
LUY	65	Y	Y	
LUY	72	Y	Y	Y
LUY	90		Y	Y
LUY	100	Y	Y	Y
		8	12	10
GrpA+GrpB pooled:			13	

Model	Number	GrpA	GrpB	EO
LUN	11	Y	Y	Y
LUN	21	Y	Y	Y
LUN	22	Y		Y
LUN	29	Y	Y	Y
LUN	33	Y	Y	Y
LUN	36	Y	Y	Y
LUN	45			Y
LUN	48	Y	Y	Y
LUN	51	Y	Y	Y
LUN	91		Y	Y
LUN	93		Y	Y
LUN	98			Y
LUN	99			Y
		8	9	13
GrpA+GrpB pooled:			10	

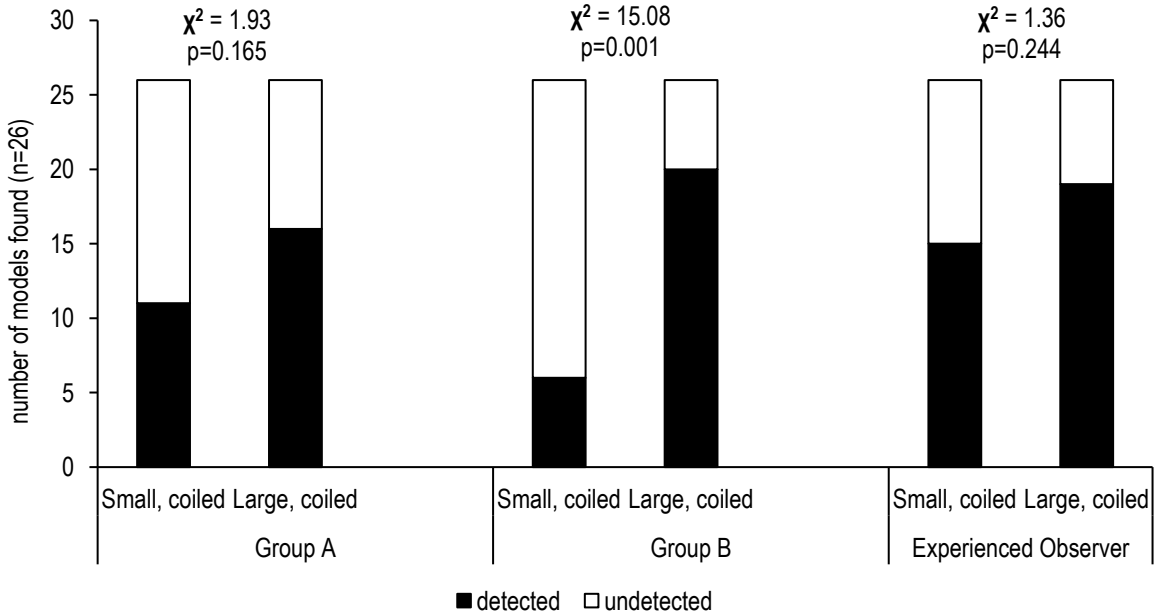
Total model observations per group per large model type

APPENDIX 3.IV.a.



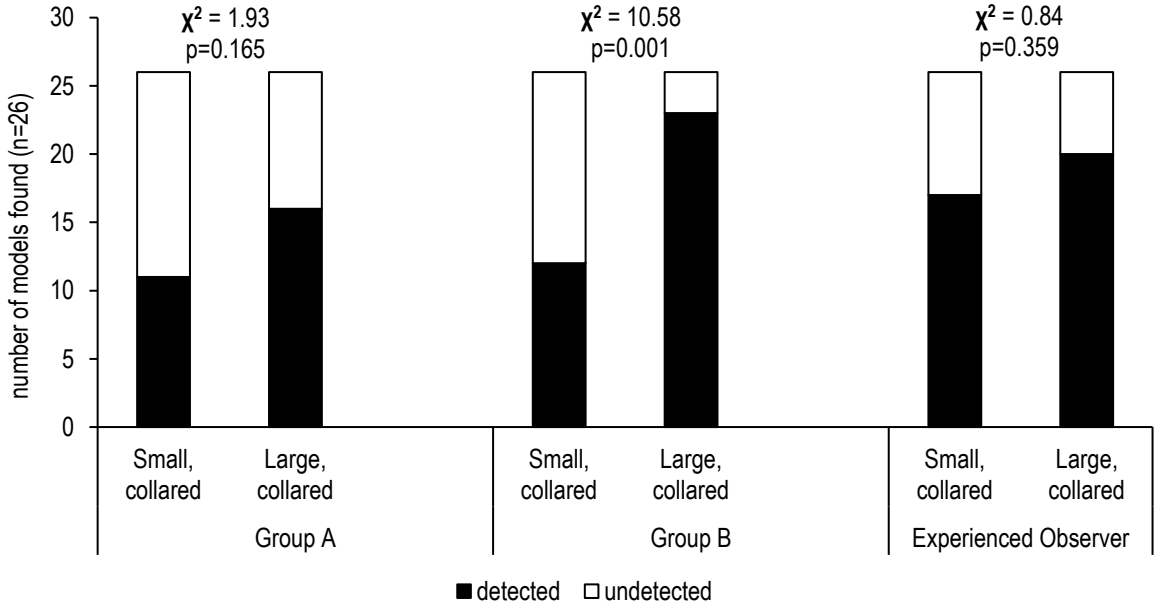
Intra-group comparison for small, uncoiled vs large, uncoiled model detection

APPENDIX 3.IV.b.



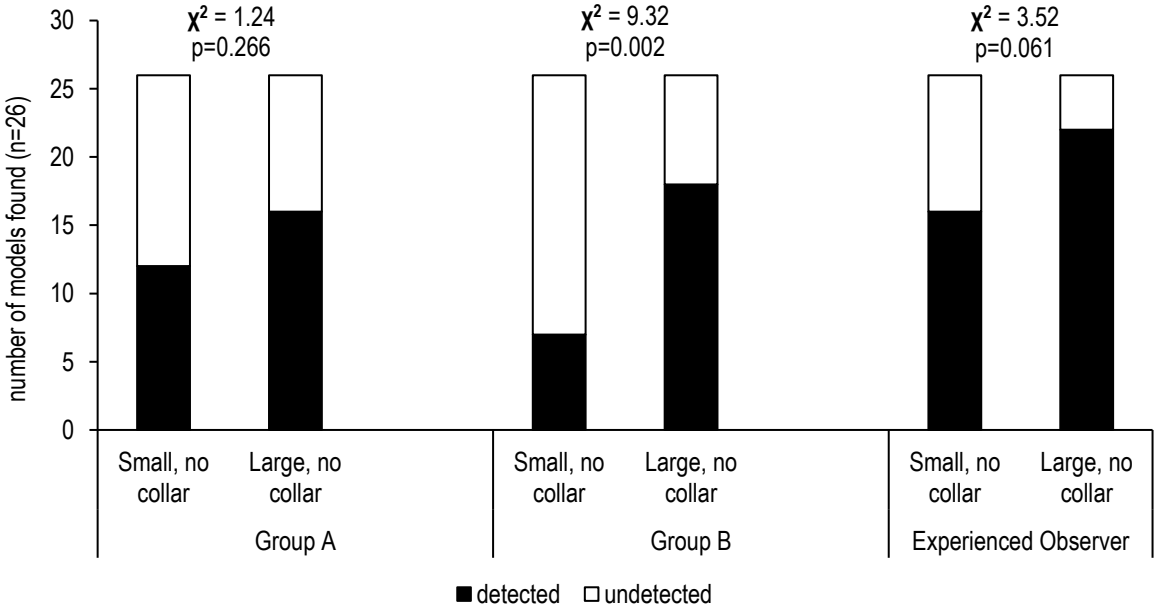
Intra-group comparison for small, coiled vs large, coiled model detection

APPENDIX 3.IV.c.



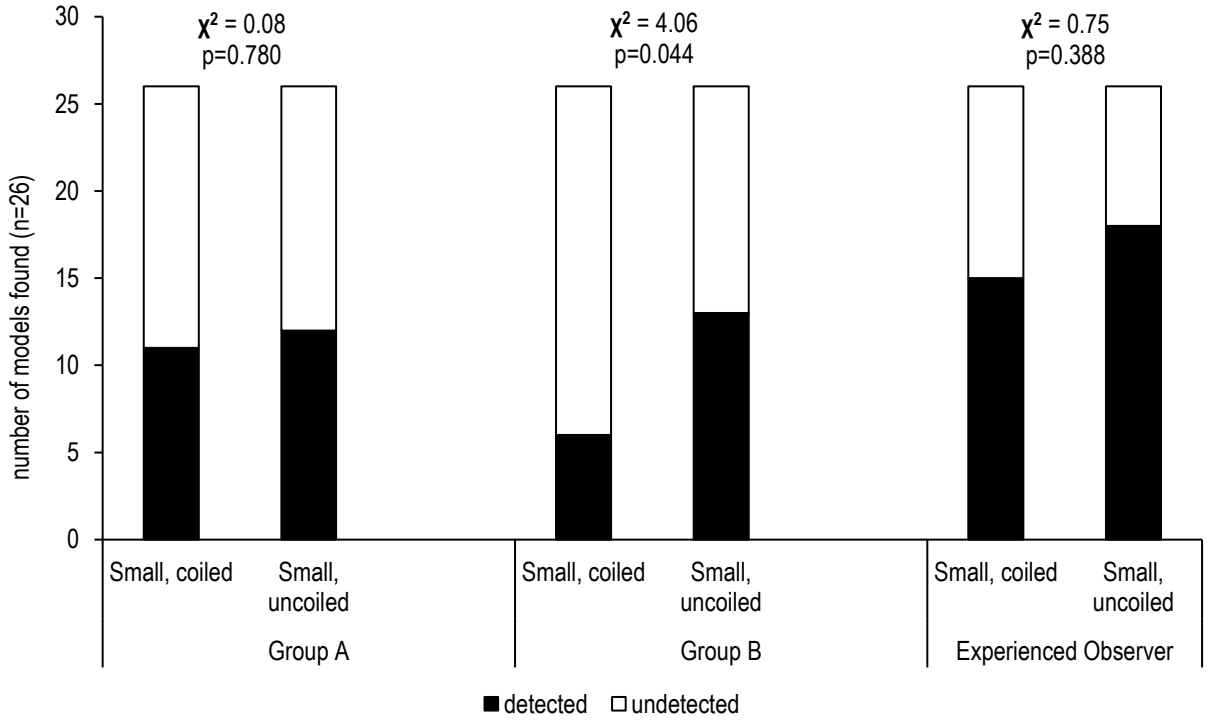
Intra-group comparison for small, collared vs large, collared model detection

APPENDIX 3.IV.d.



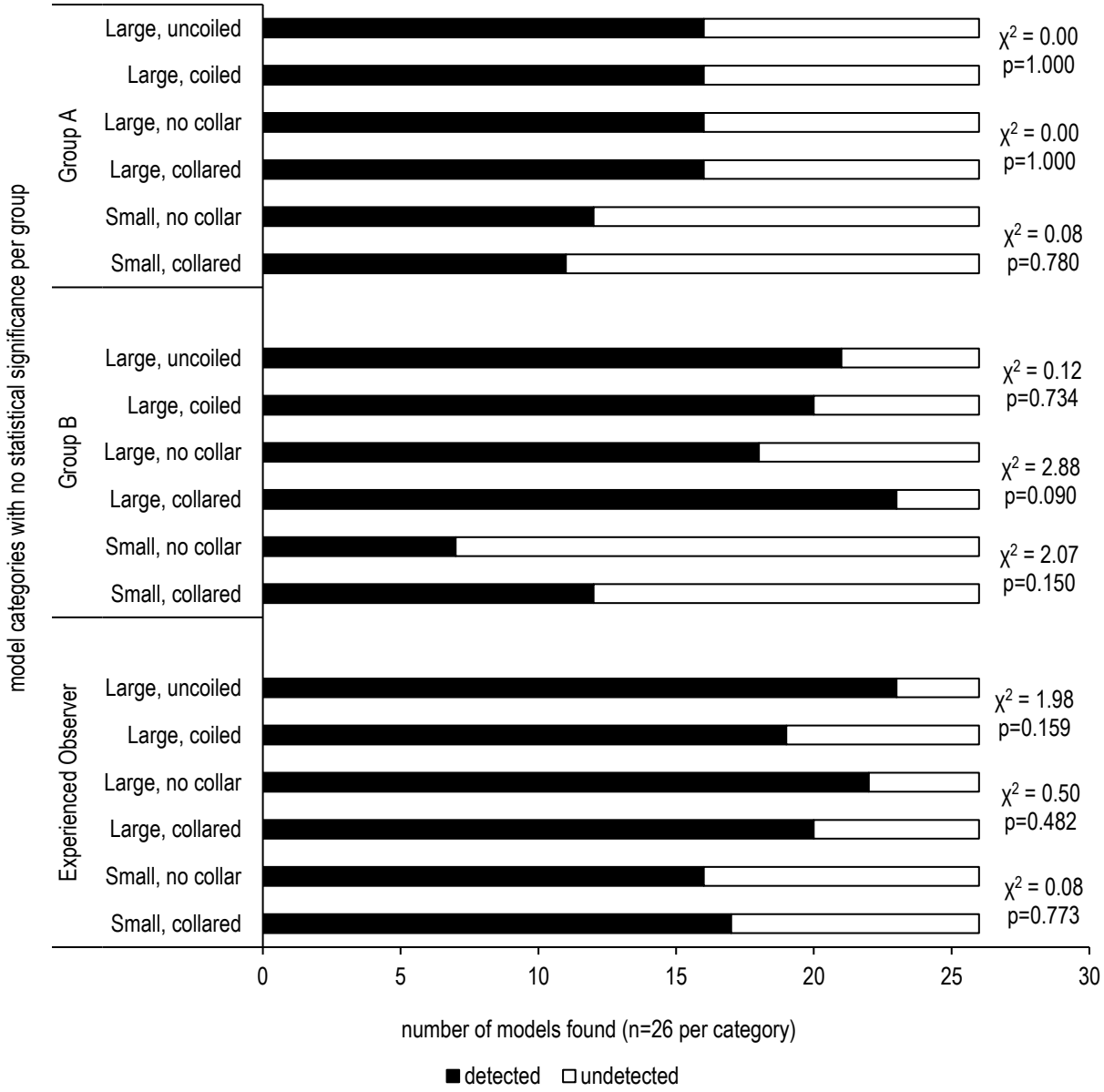
Intra-group comparison for small, no collar vs large, no collar model detection

APPENDIX 3.V.a.



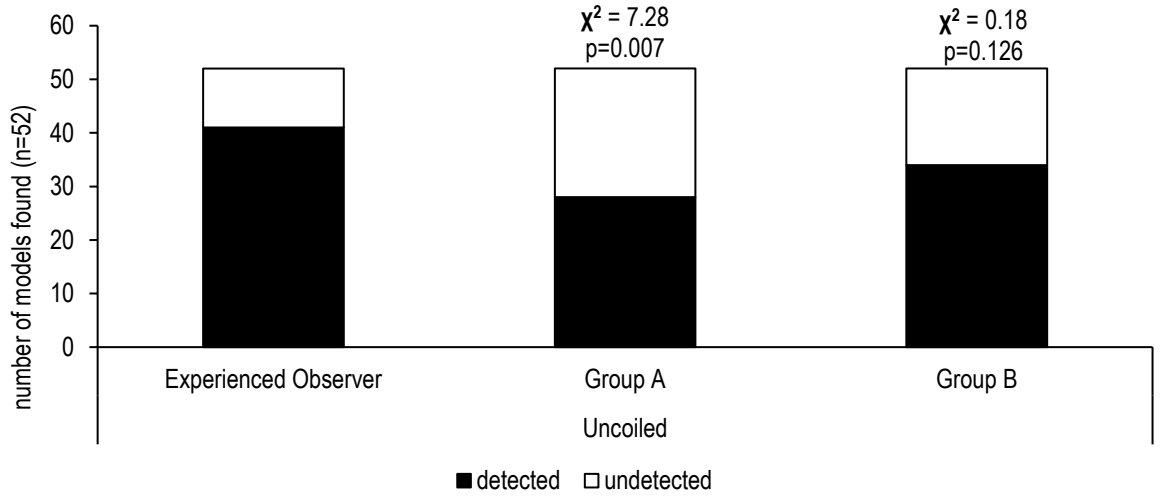
Intra-group comparison for small, coiled vs small, uncoiled model detection

APPENDIX 3.V.b.



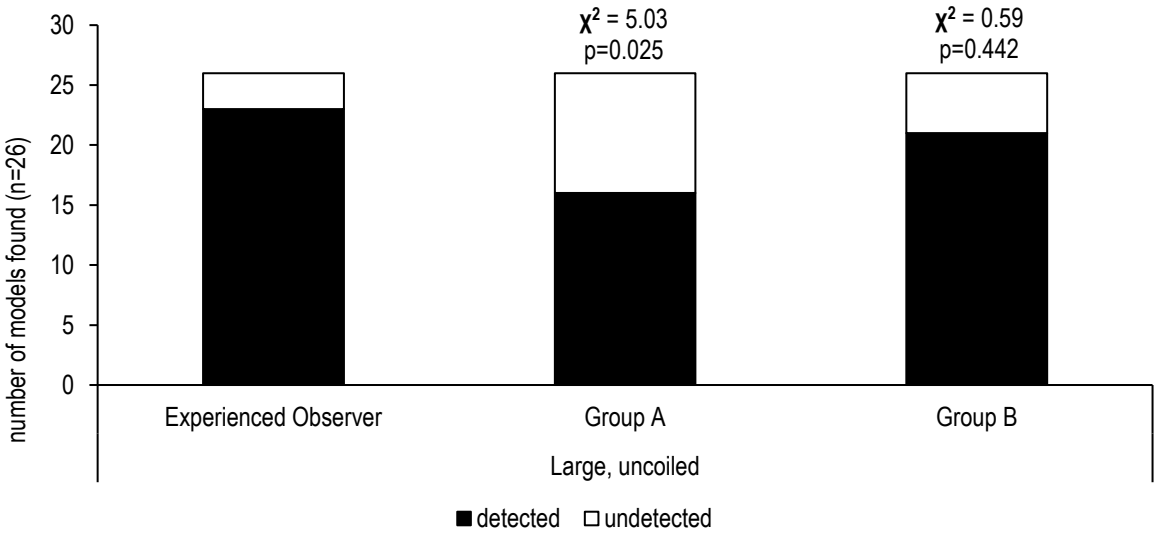
Intra-group analysis for detection of small and large categories n=26 models

APPENDIX 3.VI.a.



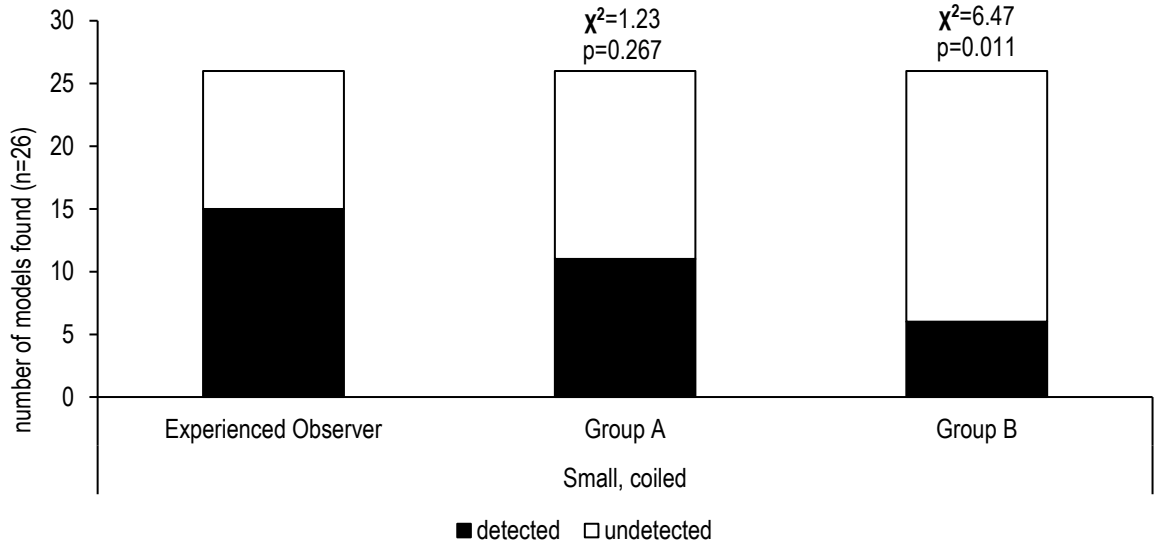
Detection of uncoiled models: Group A vs Experienced Observer and Group B vs Experienced Observer

APPENDIX 3.VI.b.



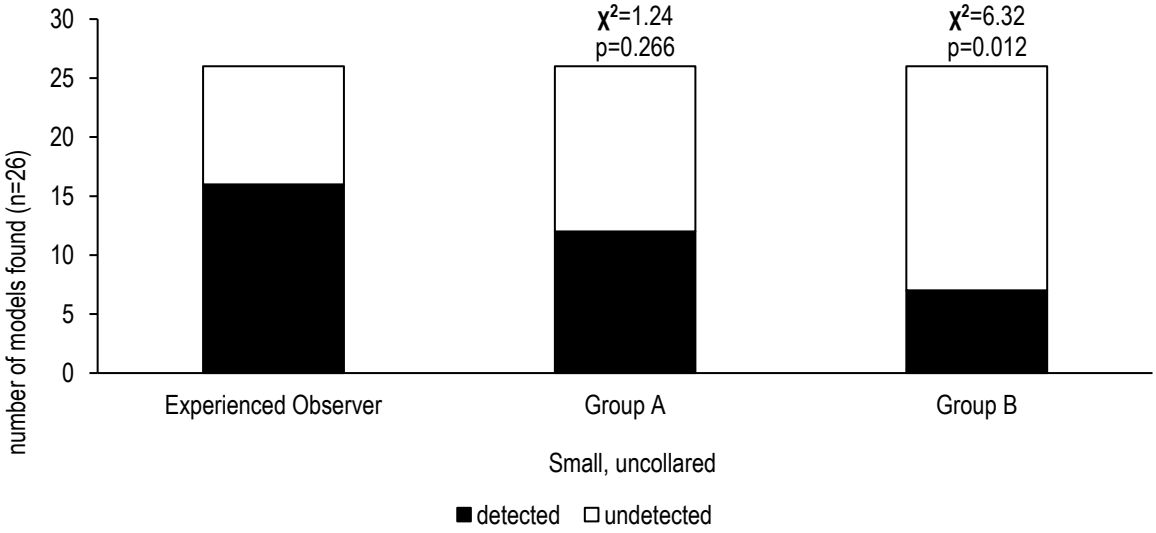
Detection of large, uncoiled models: Group A vs Experienced Observer and Group B vs Experienced Observer

APPENDIX 3.VI.c.



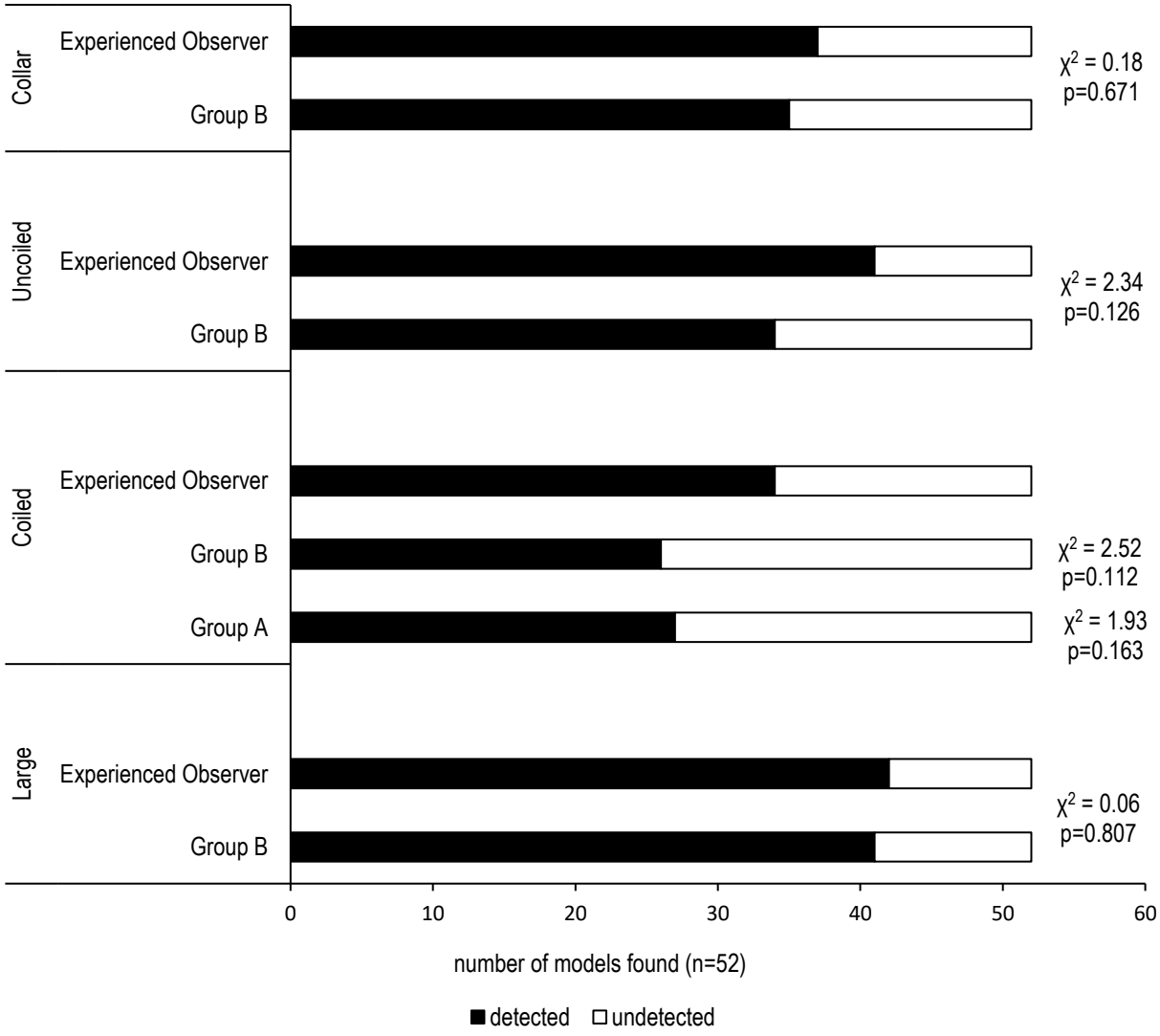
Detection of small, coiled models: Group A vs Experienced Observer and Group B vs Experienced Observer

APPENDIX 3.VI.d.



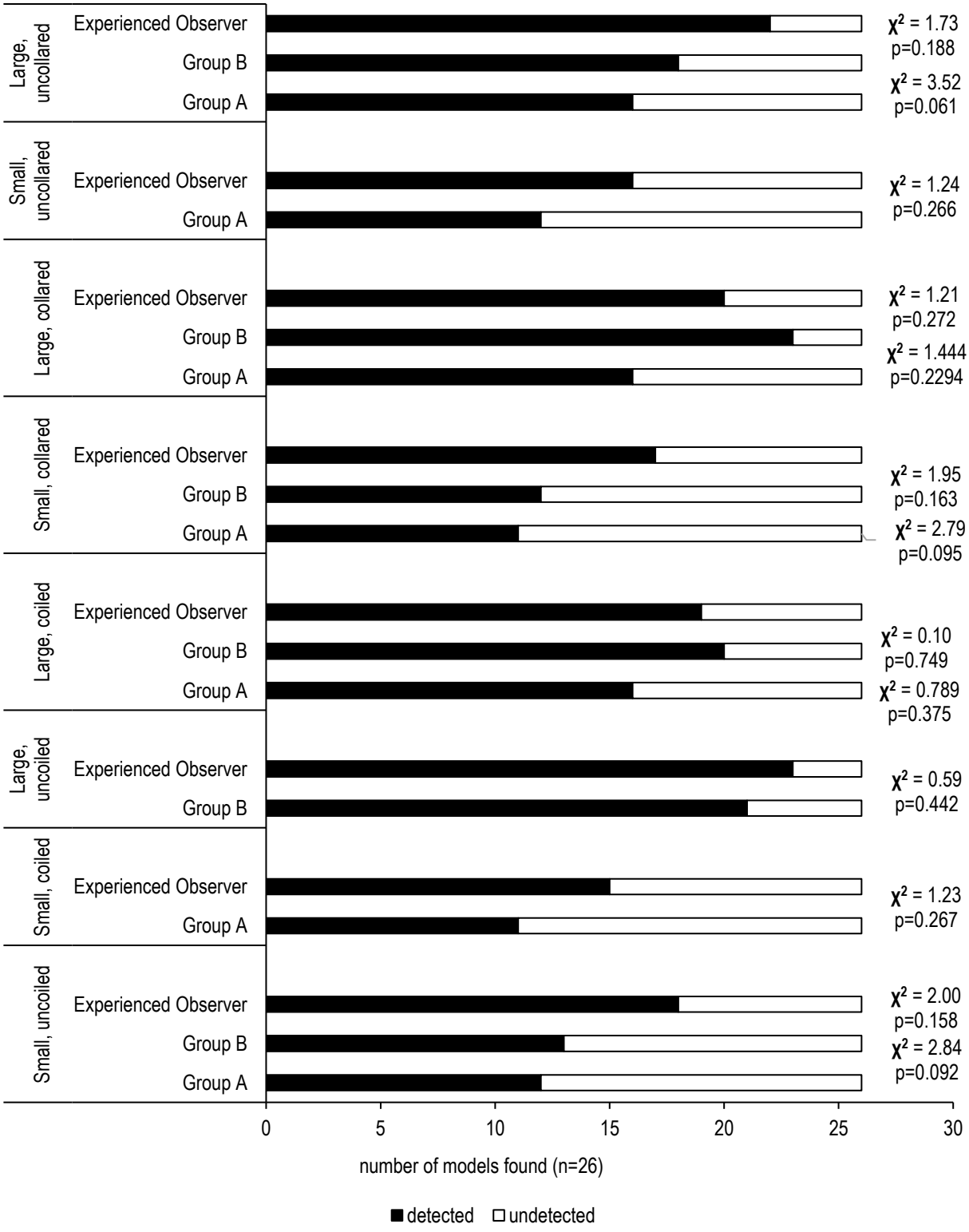
Detection of small, uncollared models: Group A vs Experienced Observer and Group B vs Experienced Observer

APPENDIX 3.VII.a.



Model variables (n=52) detected by Group A vs Experienced Observer and Group B vs Experienced Observer:

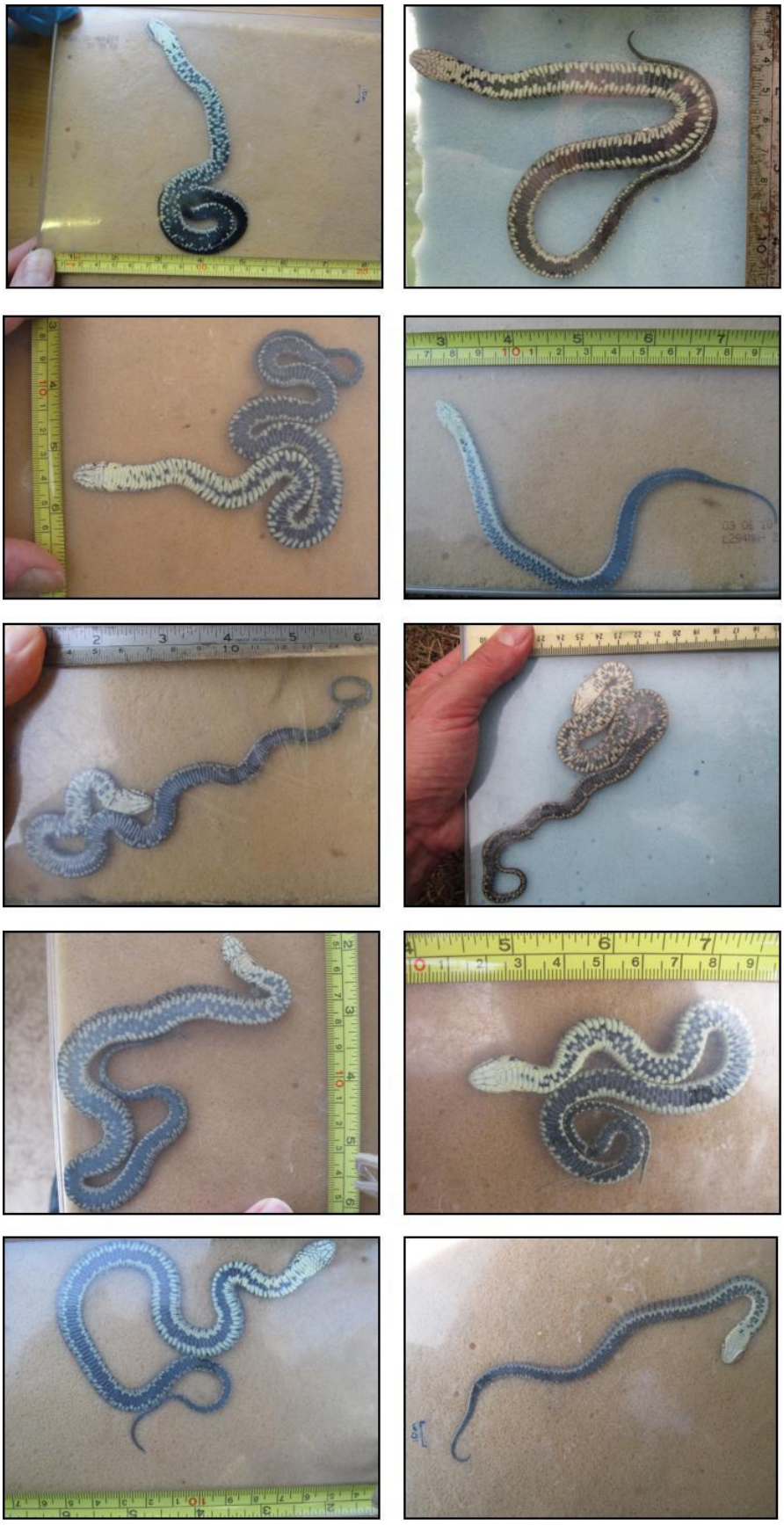
APPENDIX 3.VII.b.



Model variables (n=26) detected by Group A vs Experienced Observer and Group B vs Experienced Observer

APPENDIX 4.I.

N. helvetica restrained in squash boxes used in Experiment 2



APPENDIX 4.II.

N. helvetica SVL, tail and total measurements taken by three researchers using three different software packages

	ImageJ					
Image code	SVL(R1*)	SVL(R2**)	SVL(R3***)	Tail(R1)	Tail(R2)	Tail(R3)
E_nn_a	161	164	168	33	34	34
E_nn_b	223	214	218	60	58	58
E_nn_c	301	293	283	73	70	69
E_nn_d	257	257	250	64	64	64
E_nn_e	330	328	332	98	98	101
E_nn_f	252	241	234	69	69	69
E_nn_g	170	162	153	42	40	38
E_nn_h	211	206	203	50	47	47
E_nn_i	264	264	253	60	59	59
E_nn_j	284	289	290	83	84	86

	Snake Measurer Tool					
Image code	SVL(R1)	SVL(R2)	SVL(R3)	Tail(R1)	Tail(R2)	Tail(R3)
E_nn_a	177	164	170	36	33	35
E_nn_b	223	214	214	60	59	60
E_nn_c	290	296	290	69	70	71
E_nn_d	263	266	251	66	67	64
E_nn_e	343	317	302	100	96	94
E_nn_f	244	245	233	68	70	68
E_nn_g	169	164	162	42	41	40
E_nn_h	212	206	200	49	48	47
E_nn_i	264	264	260	59	59	58
E_nn_j	295	288	283	86	85	82

	Serpwidget					
Image code	SVL(R1)	SVL(R2)	SVL(R3)	Tail(R1)	Tail(R2)	Tail(R3)
E_nn_a	174	162	169	36	33	33
E_nn_b	227	214	218	61	58	58
E_nn_c	300	295	289	71	70	70
E_nn_d	262	262	262	65	66	68
E_nn_e	320	327	320	96	97	97
E_nn_f	250	239	240	68	68	70
E_nn_g	173	162	154	42	40	40
E_nn_h	212	208	210	49	47	47
E_nn_i	272	267	263	60	56	60
E_nn_j	293	286	286	84	84	82

APPENDIX 4.III.

ANOVA tables for SVL, tail and total measurements: comparisons between software, Experiment 3

	Source	Type III Sum of Squares	df	Mean Square	F	P
SVL	Position	81963.20	4	81963.20	78.03	<0.001
	Software	7349.05	2	7349.05	13.99	<0.001
	Position * software	14494.68	8	14494.68	06.90	<0.001
	Error	35450.40	135	262.60		
TAIL	Position	957.37	4	957.37	09.35	<0.001
	Software	726.81	2	726.81	14.19	<0.001
	Position * software	1669.59	8	1669.59	08.15	<0.001
	Error	3456.50	135	25.60		
TOTAL	Position	93855.71	4	93855.71	53.40	<0.001
	Software	12616.36	2	12616.36	14.36	<0.001
	Position * software	25861.17	8	25861.17	07.36	<0.001
	Error	59320.10	135	439.41		