EAZA Reptile Taxon Advisory Group

Best Practice Guidelines for Sand Lizard (*Lacerta agilis*)

Version 1

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EAZA Preamble

Right from the very beginning it has been the concern of EAZA and the EEPs to encourage and promote the highest possible standards for husbandry of zoo and aquarium animals. For this reason, quite early on, EAZA developed the “Minimum Standards for the Accommodation and Care of Animals in Zoos and Aquaria”. These standards lay down general principles of animal keeping, to which the members of EAZA feel themselves committed. Above and beyond this, some countries have defined regulatory minimum standards for the keeping of individual species regarding the size and furnishings of enclosures etc., which, according to the opinion of authors, should definitely be fulfilled before allowing such animals to be kept within the area of the jurisdiction of those countries. These minimum standards are intended to determine the borderline of acceptable animal welfare. It is not permitted to fall short of these standards. How difficult it is to determine the standards, however, can be seen in the fact that minimum standards vary from country to country. Above and beyond this, specialists of the EEPs and TAGs have undertaken the considerable task of laying down guidelines for keeping individual animal species. Whilst some aspects of husbandry reported in the guidelines will define minimum standards, in general, these guidelines are not to be understood as minimum requirements; they represent best practice. As such the EAZA Best Practice Guidelines for keeping animals intend rather to describe the desirable design of enclosures and prerequisites for animal keeping that are, according to the present state of knowledge, considered as being optimal for each species. They intend above all to indicate how enclosures should be designed and what conditions should be fulfilled for the optimal care of individual species.

Reference

Gill, I., McGeorge, I., Jameson, T.J.M. Moulton, N., Wilkie, M., Försäter, K., Gardner, R., Bockreiß, L., Simpson, S. and Garcia, G. 2023. EAZA Best Practice Guidelines for Sand Lizard (*Lacerta agilis*) – *First edition*. European Association of Zoos and Aquariums, Amsterdam, The Netherlands.

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Contents

Section 1: Biology and field data

Biology

1.1 Taxonomy:

Order: Squamata - Family: Lacertidae - Genus: *Lacerta* LINNAEUS 1758 - Species: *Lacerta agilis* LINNAEUS 1758

Common Names:

English: Sand lizard

French: Lezard des Souches

Spanish; Castilian: Lagarto ágil

Azerbaijani: Sychrajan Kertenkele

Chinese: 捷蜥蜴

Georgian: Mardi Khvliki

German: Zauneidechse

Italian: Lucertole Agile

Mongolian: Gavshgay Gurvel

Russian: Prytkaya Yashcheritsa

Turkish: Kars Kertenkelesi

Swedish: Sandödla

Subspecies:

A number of subspecies of *Lacerta agilis* are currently recognised, though the validity of many of these are questioned:

The nominotypical subspecies, *L. a. agilis* is distributed in Western Europe and Western Central Europe.

- *L. a. argus* (Laurenti, 1768) inhabits Central Europe, East to the Carpathian mountains and to Eastern Poland.

- *L. a. chersonensis* Andrzejowski, 1832 present in Moldavia, right-bank Ukraine, Belarus, the Baltic States, Leningrad region and south of the neighbouring Karelia. In the east, approximately from the left-bank valley of the river Dnieper a narrow area of intergradation with the neighbouring Eastern subspecies is noted.

- *L. a. bosnica* Schreiber, 1912occurs in the mountains of Croatia, Macedonia, Bulgaria and Greece. There are also records from the Zelegora Mountains, Bosnia and Herzegovina (Buric & Jelic, 2011).

- *L. a. exigua* Eichwald, 1831 occupies the whole Eastern part of the distribution range up to the

Crimean Peninsula, Ciscaucasia in the South and South East Romania (Torok, 1999).
- *L. a. grusinica* (Peters, 1960) inhabits the coast of the Black Sea and sub-montane regions of the Caucasus in the South-West of the Krasnodar Territory, Abkhazia, in the Colchic lowland and Ajaria.
- *L. a. brevicaudata* Peters, 1958 occurs in Northern and Western Armenia, Southern Georgia and on the Southern slopes of the Great Caucasus range within the North Ossetia.
- *L. a. iorensis* Peters and Muskhelishwili, 1968 occurs on the Southern slopes of the Caucasus range: in the valley and ravine of the upper current of the river Lori in Georgia.
- *L. a. boemica* Suchow, 1929 inhabits sub-montane regions of North Ossetia, Ingushetia, Chechnya and Dagestan.
- *L. a. tauridica* Suchow, 1926 inhabits the Southern mountains of Crimea (Agasyan et al, 2010).
- *L. a. garzoni* (Palacios, F. & Castroviejo, J., 1975 & Llorente *et al*, 1997) is known only from a single isolated population in the Pyrenees.
- *L. a. mzymtensis* Tuniyev & Tuniyev, 2008 inhabits the subalpine and alpine meadows of the valley of the upper flow of the Mzymta river, South West Russia.

The most recent and thorough genetic analysis of the *Lacerta agilis* species complex by Andres et al. (2014) found only the subspecies *L. a. chersonensis, L. a. tauridica, L. a. garzoni*, and *L. a. boemica* to be well supported (results supported that *L. a. boemica* deserves species status). The analyses of *L. a. exigua*, *L. a. brevicaudata* and *L. a. grusinica* showed that the three subspecies are genetically similar and could not be separated from each other. Furthermore, they found that the distribution area of the two subspecies *L. a. argus* and *L. a. agilis* needs to be reviewed. The results of Andres et al. (2014)'s study supported the existence of two differentiated lineages, but their distribution pattern is contradictory to past thinking (figure 1). The focus of this best practise guideline is *L. a. agilis* from Western Europe and Western Central Europe.

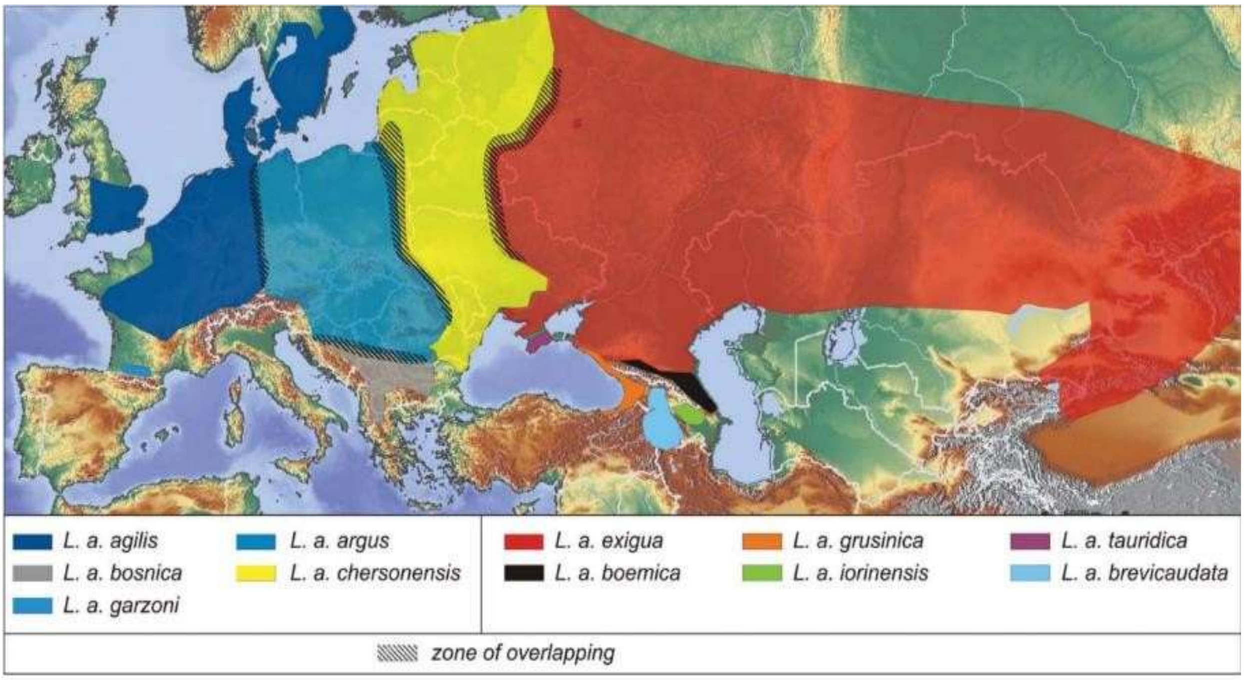


Figure 1. Distribution range of *Lacerta agilis* with division into known subspecies according to Bischoff (1988). From Andres et al, 2014.

1.2 Morphology

L. agilis is a short-legged, rather robust, medium sized lizard and is the smallest member of the "green lizard" group of the family Lacertidae. Sand lizards normally attain a total length of about 18 cm in the north of the range, while some continental specimens can reach 25 cm. The tail is approximately 1.5 times the snout-vent length.

Studies from Dudek (2015) from a Polish population a total of 273 sand lizards were captured (115 adults, 50 subadults, and 108 juveniles; 65.22 mm and 8.95 g, 39.16 mm and 1.79 g, and 32.02 mm and 1.08 g were the respective means; sex ratio 1:0.8). It showed that the body length increases faster than the body mass, meaning that the correlation between these 2 parameters is not linear (figure 2). In another study, (Eplanova & Roitberg, 2015) examined digital images of the ventral body surface in 214 adult or sub-adult individuals (95 males + 119 females), 59 juveniles (29 + 30), and 156 hatchlings of the Eurasian *L. agilis*. Two quantitative traits, the number of transverse rows of ventral scales and the width/length ratio of the anal plate, which are easily recordable from digital images and show no substantial correlation with body size, exhibit pronounced sexual dimorphism. A discriminant function derived from these two characters allows correct identification of the sex in 90% of juvenile individuals when males and females of older stages are used as reference samples (figure 3).

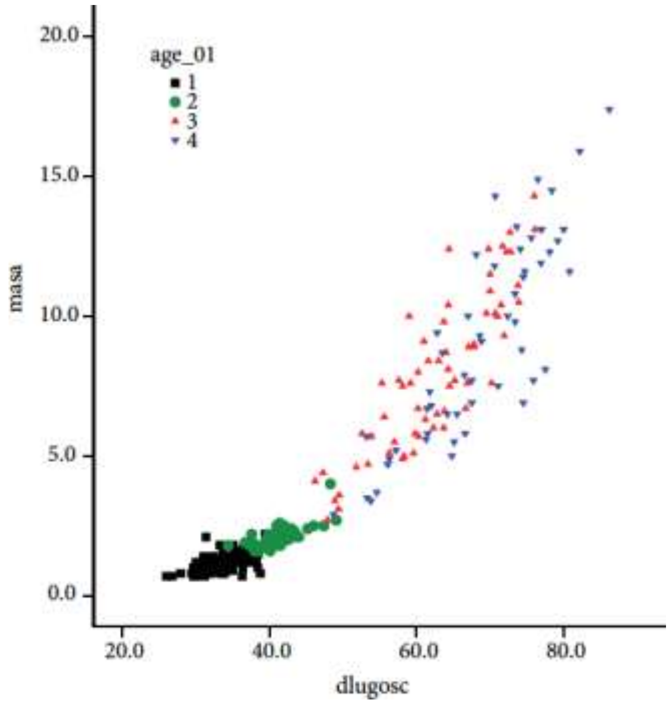


Figure 2. Correlation between lizards' body length and body mass in the 3 age groups (juveniles, subadults and adults; Dudek, 2015).

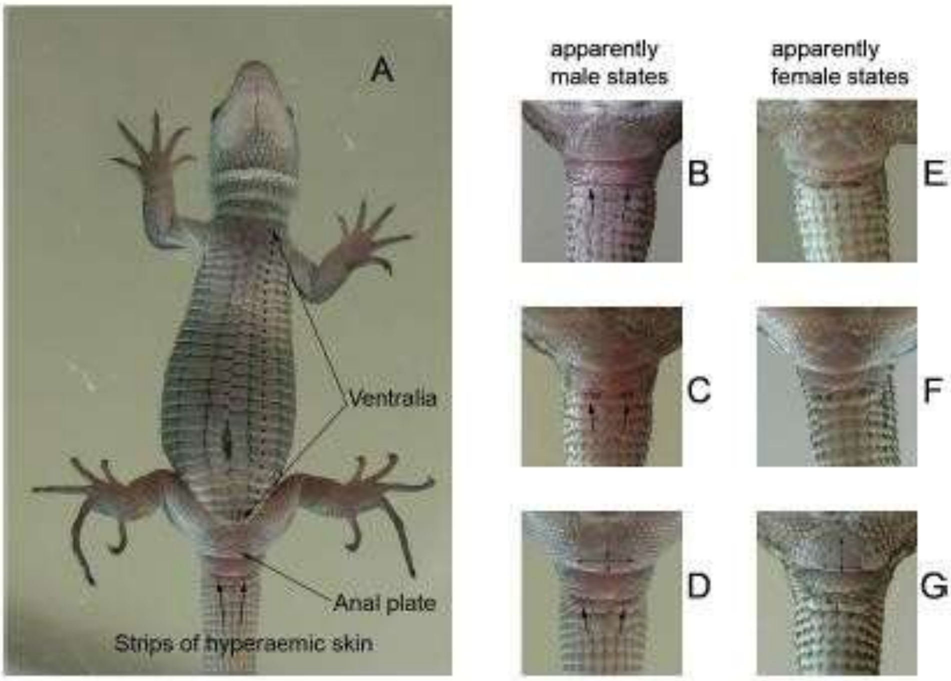


Figure 3. Ventral body surface of hatchling *L. agilis exigua* and study traits (Eplanova & Roitberg, 2015).

Male sand lizards are slightly smaller than females but have noticeably larger heads (Edgar & Bird, 2006; Gvozdik & Boukal, 1998)**.** A study in Bulgaria (Grozdanov & Tzankov, 2014), on the sexual dimorphism showed statistically significant tendencies for larger absolute and relative head size in male sand lizards in comparison with females. The head of both male and female are shovel-shaped to allow them to burrow easily and quickly into the sandy substrate. This is a defence mechanism against potential threats.

Sand lizards exhibit distinct sexual dimorphism in coloration, see figure 4. Males develop bright green flanks in the breeding season (figure 4a), which usually fades prior to hibernation, whereas females retain the light brown or greyish ground colour all year (figure 4b). Males also have some dark reticulated mottling or patches of black scales along the sides, while females generally have a plain background colour.

There is a dark vertebral band composed of numerous brown or black markings, which can be rectangular, circular, semi-circular or irregularly shaped, along with various white spots or lines. A lighter stripe runs down either side of this band and some specimens may also have a broken white vertebral stripe. One or two rows of dark spots with white centres (ocelli) usually occur along the sides of most animals.

A study in a Pyrenean population, by Perez & Font (2007), measured secondary reflectance peak in the UV in male green colouration. This secondary peak increases sexual dichromatism and male conspicuousness. Moreover, it could have a social signalling function as do similar UV reflecting patches in other lizard species.

There are many variations in the colour and pattern across the range of this species. For example, a red backed "erythronotus" form (figure 4c) occurs in mainland Europe, and is seen more frequently in eastern populations, and males of *L. a. chersonensis* can be completely green (figure 4d). Adult sand lizards have distinctive markings that can be used to identify individuals (Fearnley, 2002; Märtens & Grosse, 1996), a recognition technique that has become popular since the advent of digital photography. Juvenile sand lizards are light brown or tan and have very distinct ocelli (figure 5). Melanistic specimens of *Lacerta agilis* have been recorded but appear to be very rare (Edgar & Bird, 2006). Partial melanism of specimens has also been documented by Smolinsky (2016) and a very rare case of hypomelanism in a female specimen has been recorded by Gvozdik (1999), both cases occurring in Czech Republic.

Figure 4. Showing *L. agilis* polymorphisms: (a) British male *L. a. agilis* (photo credit: Chris Gleed-Owen); (b) British female *L. a.agilis* (photo credit: Chris Gleed-Owen); (c) European erythronotus form (Wikicommons); (d) Male *L. a. chersonensis* (Wikicommons).

Figure 5. *Lacerta agilis* juvenile showing distinct ocelli (photo credit: Chris Gleed-Owen).

1.3 Physiology

Many aspects of the biology and natural history of *L. agilis* have been studied, including a variety of studies into the physiology of the species:

- **Anatomy:** A number of studies have characterised in detail various parts of the anatomy of *L. agilis*: Těsík (1984) characterised the ultrastructure of the tracheal epithelium, (Petko & Ihionvien, 1989) mapped the distribution of substance P (SP), vasoactive intestinal polypeptide (VIP) and serotonin (5-HT) immune-reactive structures in the central nervous system, and (Székely & Matesz, 1988) characterised the topology and organisation of cranial nerve nuclei.

  Skyropa (2020) provided details on the anatomical features of the sand lizard. Features highlighted included eyes with both upper and lower eyelids alongside a blinking membrane. The recesses are located on the lateral surface on the head, a cesspool is formed bordering the tail and trunk area of the body and the male copulatory organs at the tail base have an abundant supply of vessels. Males have better developed femoral pores than females, this trait can be seen in other lizard species.
- **Chromosomes:** A current active area of research on *L. agilis* is the association between life history, sexual dimorphism, and telomeres (repeat sequences of non-coding DNA that cap chromosomes associated with genomic integrity and heavily researched for their role in aging). Olsson et al. (2010) found that tail loss owing to predation causes a shortening of telomeres in males, but not females. This was followed up by a study the next year (Olsson et al, 2011) which found that female sand lizards have longer telomeres than males and better maintain telomere length throughout their lives. Furthermore, telomere length more strongly contributes to life span and lifetime reproductive success in females than in males. More recent work has sought to characterise the karyotype of *L. agilis* and the structure of associated telomeres (Matsubara et al, 2015; Srikulnath et al, 2014), finding a lack of interstitial telomeres and extreme amplification of telomeric repeats on the W sex chromosome.
- **Developmental biology:** *L. agilis* has been used as a model for the study of the development and embryology of Lacertids: Goffinet (1983) characterised the development of the cortical plate in *L. agilis,* Rieppel (1994) characterised the skeleton-genesis of *L. agilis*, and Rupik et al. (2012) characterised reptilian myotomal myogenesis using *L. agilis* as a model.
- **Nuptial colouration:** A study of *L. agilis* by Olsson (1994a) found that the saturation and area of the bright green areas that develop on males during the breeding season, are correlated to male body-mass and body-condition. As such the size and colour of the male “nuptial badge” is an honest signal of male fitness.

1.4 Longevity

The adult phase of life expectancy of British animals was recorded by Corbett as 4 years (Nature Conservancy Council, 1983) giving a total life expectancy estimate of around 6 years. A ten-year study carried out by Berglind (2000) on two populations in Sweden gave an average life expectancy of 5.55 years.

It was found that animals living at higher altitudes (1,900m) have on average a longer life expectancy than those living at lower altitudes (Roitberg & Smirina 1995; Roitberg & Smirina 2006). Long life expectancies in wild sand lizard populations were documented by Strijbosch & Creemers (1988) recording a 12-year-old male and 8-year-old female. Berglind (2005) recorded a 19-year-old female which was also gravid at the time of capture.

A study conducted on Italian populations by Guarino *et al*. (2015) revealed that the adult age, assessed by skeleton-chronology, ranged 2-5 years both in males and females. The study also indicated that there was no significant difference in mean age between the sexes.

Field data

1.5 Conservation status/Zoogeography/Ecology

1.5.1 Distribution - from Edgar & Bird (2006)

Sand lizard distribution has been reviewed in detail by Yablokow, A.W. ed. (1976) and lately Edgar & Bird (2006) and is largely reproduced below:

L. agilis has one of the most extensive distributions of any reptile species in the world. It occurs in 36 countries across Europe, although is largely absent from Iberia and the Mediterranean region (figure 1), and its range extends eastwards to Mongolia and north-west China. *L. a. agilis* has very restricted distribution in Italy, living on two limited areas of the Carnic Alps (Friuli – Venezia Giulia) and the Maritime Alps (Piedmont) (Di Gia & Sindaco, 2004). There are recent new locality records of *L. agilis* in Turkey described by Bulbul *et al* (2019), see figure 6.

A survey conducted by Ceirans (2007), show distribution and habitat for *L. agilis* in Latvia, see figure 7. Records were made more frequently in the coastal lowland, in stretches of valleys of large rivers, and in south-eastern Latvia.

Figure 6. Map showing the locality of *L. a. grusinica* in Turkey. 1. Hepa, Artvin; 2. Yesilkoy, Pazar-Rize (the red diamond represents new locality) (Bulbul *et al*, 2019).

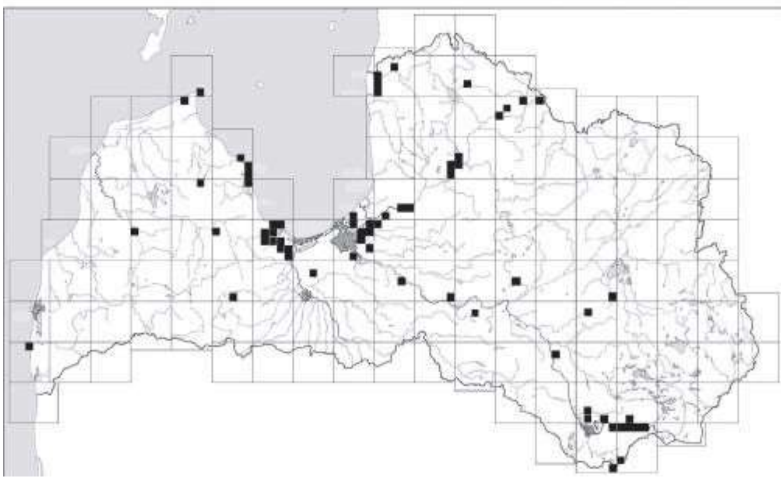


Figure 7. Map showing the distribution of *L. agilis* in Latvia in 1990 – 2006 (Ceirans, 2007).

It is distributed fairly evenly, wherever there is suitable habitat, from sea level to about 2450 m (at Col de France in the Alps).

Sand lizard populations in North-West Europe are largely confined to warm sandy habitats.

The occurrence of this species at these higher latitudes is therefore restricted to biotopes that are already rare themselves. At the very northern edge of its European range, particularly in northern Russia and the Baltic states, sand lizard abundance is mainly limited by climatic factors. Therefore, its populations and distribution here will fluctuate naturally, regardless of the availability of suitable soils and habitats.

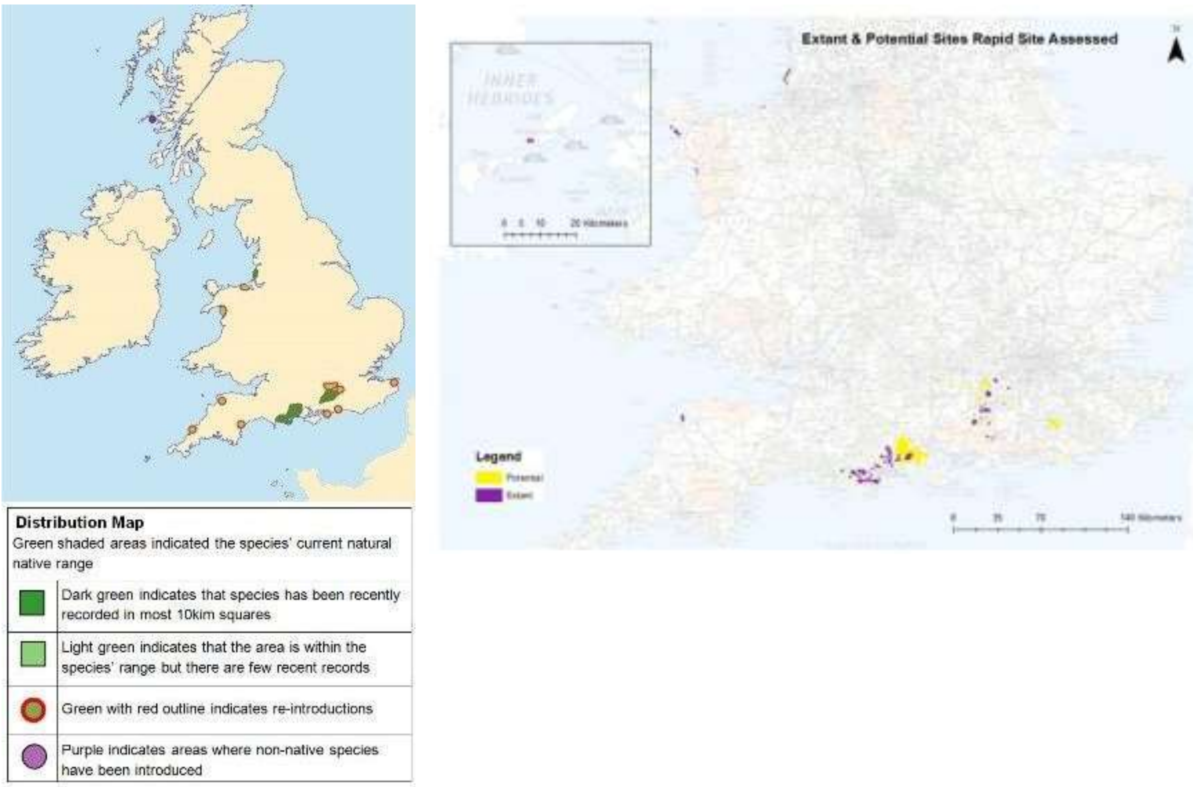
This species is still widespread in northern countries such as Poland and Lithuania, but has suffered its most severe declines as a result of human activities, and has an unfavourable conservation status, in the following countries and regions:

United Kingdom (figure 8)**.** Native populations of *L. agilis* have been lost from the English counties of Berkshire, Cheshire, Cornwall, Devon, East Sussex, Hampshire, Kent, West Sussex and Wiltshire, and this species also became extinct in Wales (Corbett, 1988; Moulton & Corbett, 1999). Sand lizards occur in significant numbers on the lowland heathlands (and one dune system) of Dorset, in southern England, which supports well over half of the UK population. Small numbers of sand lizards also survive on heathlands in Surrey, about 100 km northeast of Dorset, plus the coastal dunes of Merseyside in North-West England (Corbett, 1988). A small introduced population has also survived on the Isle of Coll, off the west coast of Scotland, for over 25 years. There were estimated to be around 6500 adult lizards surviving in the United Kingdom in 1997 (Moulton & Corbett, 1999), with all but a few hundred animals occurring on Dorset heathland, although recent estimates indicate that this number is substantially higher (C. Gleed-Owen, pers. com.). In any event, apart from a number of urban fringe sites, which face severe public pressure, the decline of *L. agilis* in the UK has been effectively halted. Most populations are now stable or increasing and sand lizards have also been reintroduced to several English counties.

Northern France (Normandy) (figure 1)**.** Two subspecies of *L. agilis* occur in France. *Lacerta a. garzoni* is isolated in the eastern Pyrenees, extending slightly into Spain and Andorra, and hence occurs outside the area covered by this Action Plan (although this subspecies is of conservation concern itself). *L. a. agilis* has a largely continental distribution in France – it does not reach the British Channel and is absent from Brittany, the central-west, the south-west and the Mediterranean regions of the country. While it still occurs over extensive areas of eastern and central France, many populations along the northern and western edges of this distribution are isolated and often declining. This trend is especially marked in Limousin and Normandy and is principally due to the loss of heathlands to urbanisation.

A recent field work campaign, in Ariège, aimed to clarify the status and distribution of *L. a. garzoni*, and two localities were found, that appeared to correspond to a small overflow of the population of Upper Cerdagne (département des Pyrénées-Orientales) on to the Atlantic catchment basin via the Puymorens Pass (Pottier, Calvez & Deso, 2007).

Belgium (figure 9). The sand lizard only occurs in the extreme south of Wallonia, south of the Semois river in Luxemburg province (Percsy et al, 1997). This species has suffered an extremely severe decline in Belgium and is apparently now confined to just three localities. One is a heathland complex of approximately 1,500 ha, largely contained within the military training area of Arlon. The other two sites are old stone quarries, of about one ha each, where tiny numbers of lizards inhabit rough grassland with brambles. The Arlon site is regarded as the only viable sand lizard population left in Belgium, albeit with only a few hundred animals surviving, but is threatened by lack of management, especially the encroachment of conifers. This species is considered Critically Endangered in Belgium (Belgium Species List, 2021).



Figures 8. Distribution range of *L. a. agilis* in the United Kingdom (ARC, 2014 & 2019).

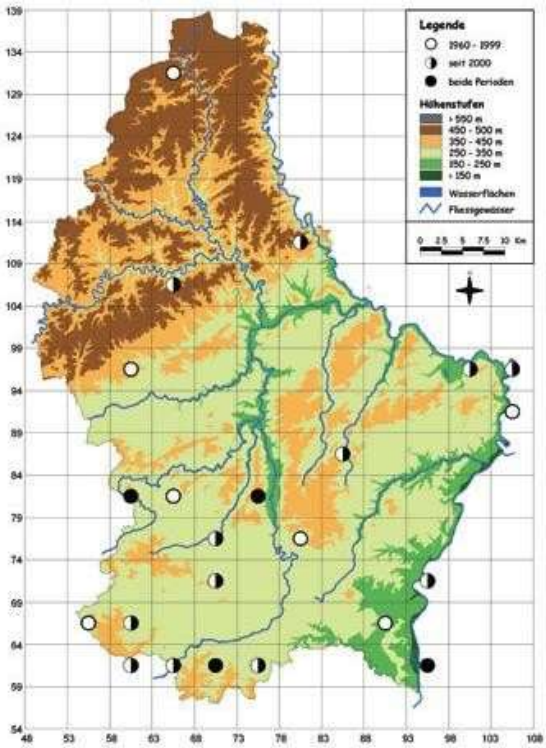


Figure 9. Distribution range of *L. a. agilis* in Luxemburg, Belgium (Proess, 2018).

The Netherlands (figure 10). The main range of the sand lizard in the Netherlands is confined to sandy heathlands, notably the Veluwe and Utrechtse Heuvelrug (Stumpel, 1988), in the centre, east and south-east of the country (Strijbosch, 1985; Zuidwewijk et al, 1998). It also occurs in dune systems along the coast (in the provinces of Zealand and South-Holland), on most of the Wadden islands and on the Frisian islands of Vlieland, Terschelling and Schiermonnikoog. *L. agilis* is considered to be vulnerable in the Red List for the Netherlands (Hom et al, 1996). While the dune populations appear to be stable and are benefiting from positive conservation management (Stumpel, 2004), the island populations are decreasing (RAVON, 1993) and almost all of the heathland populations are under some threat, or have already been lost (Bergmans & Zuiderwijk., 1986). Unfortunately, the prime sand lizard habitat of old, mature heather has been all but eradicated in the Netherlands by inappropriate conservation management, especially overgrazing and mechanised turf cutting (Council of Europe, 2003; Strijbosch, 2002; Stumpel, 2004).

Figure 10. Distribution range of *L. a. agilis* in the Netherlands (Lacerta.de, 2020).

Northern Germany (figure 11). Although *L. agilis* is widespread and often abundant in south and west Germany, its distribution becomes more scattered under the influence of the Atlantic climate in the north of the country (Dierking-Westphal, 1981; Podloucky, 1988; Wollesen & Wrangel., 2002). Here it is mainly confined to heathland and sand dunes in Schleswig-Holstein, Niedersachsen (Lower Saxony) and Nordrhein-Westfalen (North Rhine-Westphalia). In addition, the sand lizard occurs at lower population densities in northern Germany than it does further south. This species experienced a 25-

30% decline in Niedersachsen during the 20th Century. Over 99% of the heathland in northern Germany has been lost and Lüneburg Heath, formerly one of the largest and most important areas for this species in north-west Europe, was devastated by British army training exercises over many decades. Although sand lizards are still extremely rare here, this area is now being re-instated and managed more sympathetically.

Figure 11. Distribution range of *L. a. agilis* in Germany, illustrated by the darker areas (Elbing et al. 1996).

Denmark (figure 12). Sandy habitats are relatively abundant in Denmark so the sand lizard is widely distributed in the country - but is possibly absent from Falster, Lolland and Langeland (Pihl et al, 2001). This species was reported in approximately 50% of the 5 km x 5 km squares investigated in Denmark by Fog (1993). Large populations are known from the Mols Bjerge (Jensen, 1980) and on Røsnæs (Ravn, 1997), although most others are relatively small. Moreover, the sand lizard is decreasing in Denmark and approximately 30% of known populations are thought to have become extinct between 1945 and 1980, with further losses occurring regularly since (Fog, 1993). Pihl et al, (2001) regarded this species as having an “Uncertain” conservation status in Denmark.

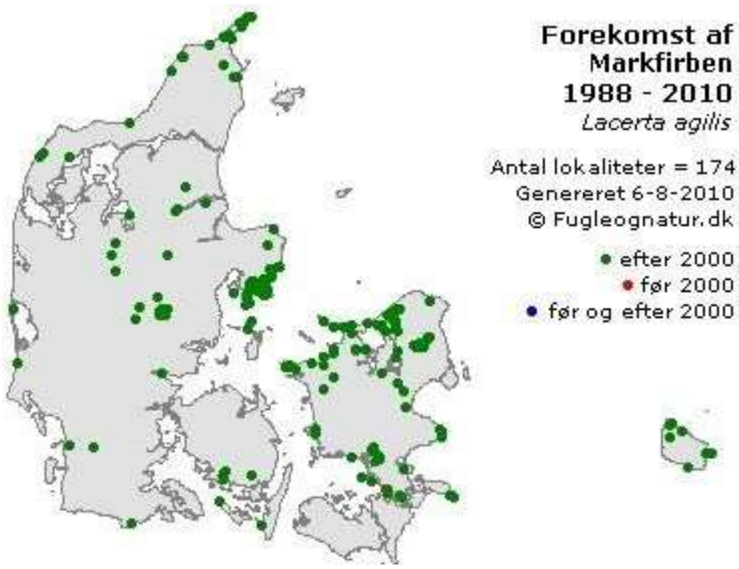


Figure 12. Distribution range of *L. a. agilis* in Denmark (Lacerta.de, 2020).

Sweden (figure 13). The sand lizard occurs at various localities in the southern third of Sweden (Ahlén et al, 1995) and is classified as vulnerable on the Swedish Redlists (Thurfjell et al., 2020). Its main area of distribution is along the South-East and South coastal areas, where it occurs more or less continuously, and quite abundantly in places, in sandy heathland, grassland or rocky habitats. In addition, there are several smaller populations further north, along the northern periphery of the distribution, which occur in pine heath forests on sandy sediments (Andrén et al, 1988; Berglind, 2005). These are isolated from each other, often by more than 100 km, so little genetic interchange seems likely, although there have been several individual sightings of *L. agilis* in areas in between (Andrén et al., 1988). All appear to be small remnants of a past warmer climate (Berglind, 1999, 2005) and it has been estimated that populations occurring in patches of 1 ha or less in extent have >56% chance of extinction within 50 years (Berglind, 2005). In the past, natural forest fires and extensive, small-scale forestry activities created and maintained a dynamic network of open patches, in the early successional stages of forest development, which allowed the species to survive a cooler climate in a number of regions. The introduction of more efficient forestry techniques and, ironically (considering the threat that fires usually pose elsewhere), the better control of forest fires have resulted in a decrease in the number and extent of these warmer, open areas and seem to be the most important factors in the decline of the species in central Sweden (Berglind 1999, 2000 & 2005).

Figure 13. Distribution range of *L. a. agilis* in Sweden (Artfakta, no date).

1.5.2 Habitat - from Edgar & Bird (2006)

Sand lizard habitat requirements have been reviewed in detail by Edgar & Bird (2006) and are reproduced below:

Although sand lizards can be found in a huge variety of habitats in Europe (from urban gardens to alpine pastures), the range-edge populations in the north-west are more or less restricted to sandy habitats, such as lowland heathland and sand dunes, below about 300 m (Märtens & Grosse, 1996; Moulton & Corbett, 1999; Rühmekorf, 1970). Critical elements of sand lizard habitat in the northern range countries include a predominantly south or south-west facing aspect with minimal shading, a diverse vegetation structure providing an intimate mosaic of basking spots and shelter and abundant exposed sand for egg laying purposes (Corbett & Tamarind, 1979; House & Spellerberg, 2006). On lowland heathland sites adult sand lizards are more or less confined to the later successional stages of dry heath, i.e. the mature and degenerate phases of heather growth (Glandt, 1991; Moulton & Corbett, 1999), although dispersing juveniles can be found in other habitats. Areas with a luxuriant ground layer of bryophytes and lichens also seem to be particularly favoured. Dwarf gorses growing amongst heather are common on many sites and may help to protect the lizards from predation. Prime areas for this species may exist within a matrix of less suitable habitat, resulting in localised concentrations of sand lizards. Such areas of optimal habitat create "foci" that are of particular importance to sand lizard conservation. The size and quality of such foci will vary considerably, and an extensive area of good habitat with a varied topography of slopes, gullies, tumuli and banks can support a large number of lizards. Densities of 100-300 lizards per hectare have been recorded

(Moulton & Corbett, 1999; Strijbosch & Creemers, 1988). Since habitats such as lowland heathland and sand dunes are naturally limited in extent in Europe, and the distribution of the sand lizard is further restricted within them by its specific habitat requirements at higher latitudes, populations at the northern edges of the range are far more susceptible to external threats and adverse conditions than they are elsewhere.

1.5.3 Population

L. agilis has one of the most extensive distributions of any reptile species in the world and has presumed very large global population (Agasyan et al, 2010; Edgar & Bird, 2006). Details of the size and status of sand lizard populations in regions on the edge of the species range where significant declines have occurred are given in section 1.5.1.

1.5.4 Threats to Wild Populations – from Edgar & Bird (2006)

Threats to wild sand lizard populations have been reviewed in detail by Edgar & Bird (2006) and are reproduced below:

Habitat Destruction. Reasons for the decline of the sand lizard in Northern Europe have been well established and centre almost exclusively on habitat destruction, plus the associated effects of habitat degradation and fragmentation in the remaining areas (Corbett, 1988; 1989; Corbett & Tamarind, 1979; Council of Europe, 2003; Nature Conservancy Council, 1983; Stumpel, 2004). Lowland heathland has been widely reclaimed for agriculture, mineral extraction, landfill and conifer plantations, and developed for housing, industrial areas, roads and golf courses. Many coastal populations on sand dunes have also been lost to holiday developments, campsites and car parks, as well as to the commercial planting of conifers.

Habitat Degradation. Urban development adjacent to sand lizard habitats has caused many problems due to the associated increase in recreational pressures and deliberate abuse. Due to an unfortunate geographical accident, for example, the last stronghold of the sand lizard in the United Kingdom is situated at the edge of one of the fastest growing urban areas in Europe (Bournemouth/Poole). The huge pressures that have resulted have caused local extinctions of sand lizards and still threaten many populations (Council of Europe, 2003; de Molenaar, 1998; Edgar, 2002; Haskins, 2000; Moulton & Corbett, 1999), especially on the smaller fragments of habitat that still survive. Problems such as arson and post-fire habitat changes, the use of mountain bikes, motorbikes and 4-WD vehicles, uncontrolled horse riding, erosion, vegetation trampling, the dumping of litter and garden waste, pollution, collection by children and dogs and cats are all much more severe on urban fringe sites. The sand lizard is known to be more sensitive to these factors than other reptile species (Strijbosch, 1988; Edgar, 2002). In addition, the costs of wardening urban fringe sites are many times those of rural areas (Haskins, 2000).

Habitat Fragmentation. Habitat destruction has severely fragmented sand lizard habitats in northern Europe, especially in intensively developed areas such as Southern England and the Netherlands. Roads, urban development and agriculture all form effective barriers to natural dispersal and gene flow between sand lizard populations. In the United Kingdom, sand lizards from the small, isolated populations in Surrey and Merseyside now show low genetic diversity (Beebee & Rowe, 2001), as do populations from central Sweden (Gullberg et al, 1998). However, this does not yet appear to a major factor in the decline of sand lizards in Sweden (Madsen et al, 2000) or the Netherlands (Nijman, 1996).

Lack of Habitat Management. Heathlands and dune systems are dynamic, successional habitats. Without external influences periodically reversing succession, or deliberate management by humans, sand lizard habitats may gradually disappear as the woodland climax vegetation develops. However, sand dunes often form the actual climax vegetation in many coastal areas, as do dry heaths on some particularly poor sandy soils. In any event, reptiles are well adapted to succession in natural landscapes and form meta-populations that shift in space and time in response to changing conditions. In modern Europe, however, habitat destruction and fragmentation has often trapped species such as sand lizards on "islands" of semi-natural habitat, surrounded by highly modified landscapes, so natural meta-population dynamics no longer occur. The small size of most sites, plus recreational pressures, self-sown conifers, invasive introduced species, pollution and many other factors have all exacerbated this problem. In order to maintain sand lizard populations in such circumstances, therefore, habitat management is essential (Corbett, 1988; Stumpel, 2004). To make matters worse, several species of invasive plant introduced into Europe are an increasingly severe threat to sand lizard habitats in many areas. The worst offenders include rhododendron, *Rhododendron ponticum*, and pheasant berry, *Gaultheria shallon*, on lowland heathland. Urban fringe heathland sites are also at risk from the introduction of alien plants through the dumping of garden waste. Sea buckthorn, *Hippophae rhamnoides*, although native to parts of Europe, has been widely introduced to sand dune sites elsewhere and is also a serious problem for sand lizards when not controlled.

Inappropriate Habitat Management. The need to manage habitats such as lowland heathland has long been recognised in Europe and significant amounts of funding have been allocated for this in recent years. While the long-term conservation of habitats is of paramount importance, certain forms of management have proved to be detrimental to sand lizards when the particular habitat requirements and ecology of this species have not been adequately considered (Stumpel, 1992; 1999; Moulton & Corbett, 1999). Grazing is an important conservation management tool in Europe and, ideally, an appropriate grazing regime would have positive, or at worst neutral, effects on sand lizards. However, the overgrazing of heathland has destroyed the habitat micro-diversity required by this species in a number of cases (Strijbosch, 2002; Stumpel, 2004) and populations on small sites are particularly vulnerable (Offer et al, 2003). Grazing can also have negative effects for sand lizards on sand dune habitats (Overleg Duinhagedis, 1999), although appears to benefit this species on Dutch dunes (Stumpel, 2004). Similarly, the cutting or forage harvesting of heathland vegetation to maintain this habitat can be extremely damaging to sand lizard habitats, especially when the areas treated are too large, badly sited and too much is cut in any one season. The removal of heathland turfs is often essential to create suitable areas for sand lizard nesting but the large-scale mechanised turf-stripping ("plaagen") carried out in the Netherlands in recent years has been disastrous for many populations of this species (Stumpel, 1992; 2000; 2004).

On many English heaths (Dorset and Hampshire) inappropriate levels of heather management are now having a higher impact on species range, population and habitat. These are legitimate methods of management i.e. cutting, grazing (and burning). However heather management intensity and scale is increasing and remains poorly regulated (ARC, 2014). Controlled burning has also been used to manage some lowland heathland sites and, again, when this technique is used too extensively or is carried out in inappropriate areas, sand lizard habitat has been destroyed. For example, the loss of the sand lizard from the New Forest, in Southern England, is thought to have been the result of over-enthusiastic controlled burning (Tubbs, 1976).

Other Threats. Sand lizard populations in North-West Europe face a number of other, usually localised threats. Eutrophication (nutrient enrichment) of poor soils can dramatically alter heathland vegetation and this can occur adjacent to busy roads, where livestock are given supplementary feed, along paths and tracks heavily used by dog walkers and as a result of agricultural run-off. The atmospheric deposition of nitrates from intensive pig farming has had profound impacts on many heaths in the Netherlands, and not only has this changed heather dominated areas to grassland but has also resulted in the requirement to carry out mechanised turf-stripping (see above). In military training areas, heavy vehicles can devastate vegetation and cause severe soil compaction and night exercises are frequently accompanied by heathland fires started by flares. Introduced pheasants are thought to be causing problems for sand lizards in the United Kingdom and the Netherlands (Bergmans & Zuiderwijk, 1986). Wall lizards (*Podarcis muralis*) have also been widely introduced in the United Kingdom and appear to have replaced sand lizards at one site on sandy coastal cliffs. Wood ants, which often destroy sand lizard nests, have increased in areas of commercial forestry and self-sown conifers on, and adjacent to, many heathland sites in northern Europe. The winter gassing of burrows, which may be used for hibernation by sand lizards, to control rabbits is considered a problem in the United Kingdom.

One threat that is far wider reaching is climate change, although its potential effects on species such as the sand lizard are hard to predict, especially for the many isolated populations that cannot move to adapt. It was once postulated that lower than average May sunshine levels were a factor in the decline of the sand lizard on Merseyside in North-West England (Jackson, 1978, 1979). However, this deteriorating climate theory has since been disproved (Langton, 1988) and human impacts are now seen as wholly responsible.

Sea level rise, erosion and enrichment is a factor for dunes. For heathland there is higher fire risk, which can increase requirement for heather management and increased fire-break mowing can also isolate egg-laying substrates. In England, there is possible 1-2 week earlier egg-laying over the last 6-10 years. It remains very possible that sand lizards could use sand substrate grassland, though most remain intensively grazed and are currently unsuitable (Moulton pers. comm.).

In the future, however, heavier rainfall would destroy more sand lizard nests and reduce juvenile survival rates, whereas drier summers would cause more heathland fires, drought damage to habitats and desiccation of nests. On the other hand, warmer weather could see more double clutches of eggs, as well as improved juvenile survival rates. Moreover, Thomas et al. (1999) theorise that even a small rise in average temperatures could result in a large increase in the area of habitat available in Britain for species like the sand lizard that are living on the edge of their European range. This would therefore mean that sand lizards would be less restricted to heathland and sand dunes in Northern Europe.

1.5.5 Conservation Status

IUCN Red list. *L. agilis* is listed as Least Concern in view of its very wide distribution, tolerance of a degree of habitat modification in some parts of its range and presumed large population. Although it is declining in parts of its range, overall it is unlikely to be declining fast enough (30% or more) to qualify for listing in a more threatened category at the global level. However, numerous subpopulations are threatened across the range of the species (Agasyan et al, 2010).

International Protection. The sand lizard is listed in Annexe II of the Council of Europe's 'Convention on the Conservation of European Wildlife and Natural Habitats' (the Bern Convention) as well as in Annexe IVa of the European Community's 'Directive on the Conservation of Natural and Semi-natural Habitats and of Wild Fauna and Flora, Directive 92/43/EEC' (the Habitats and Species Directive; Edgar & Bird, 2006).

National Protection. European Union member states have drafted laws that transpose the EU Habitats Directive into national legislation so the sand lizard is therefore strictly protected in the United Kingdom, France, Belgium, the Netherlands, Germany, Denmark, Sweden and Estonia at all stages of its life cycle (Edgar & Bird, 2006).

Habitat Protection. While European Union member states are not specifically required to declare Special Areas of Conservation for Annex IV species, the sand lizard is still found within a large number of such sites in Northern Europe due its dependence on Annex I habitats such as heathland and sand dunes. Similarly, this species occurs in many Special Protection Areas, particularly where these have been designated for heathland birds. *L. agilis* is thus well represented in the Natura 2000 site series and the majority of its populations in the United Kingdom, Northern France, Belgium, the Netherlands, Northern Germany, Denmark and Sweden (although less so in Estonia) now occur on habitats protected for other reasons (Edgar & Bird, 2006).

Conservation actions – From Edgar & Bird, 2006

Edgar and Bird (2006) detail conservation actions taken by the international community prior to 2006 in their action plan for the species:

Recommendation 26 of the Standing Committee of the Bern Convention (Council of Europe, 1991) urged the governments of the United Kingdom, Belgium, the Netherlands, Germany, Denmark and Sweden to take appropriate steps to ensure the conservation of sand lizard populations and habitats. Surveys and local conservation activities have since been undertaken in all these countries. In the Belgian region of Wallonia, an agreement has been reached between the National Defence and the Nature and Forest Departments over the future management of the Arlon military training area, and other heaths in the area have been managed with LIFE funding (LIFE96 NAT/B/003034). Comprehensive surveys and management planning have been carried out on sand dune habitats at many sites in the Netherlands (Overleg Duinhagedis, 1999), annual monitoring programmes instigated for reptiles (Zuiderwijk et al, 1998) and detailed ecological and population studies (e.g. van Dijk, 1996) undertaken on sand lizards at Amsterdam, Nijmegen and Wageningen Universities. Conservation programmes have been drawn up for the heaths of Northern Germany (Council of Europe, 1991) and, in particular, restoration and re-creation management is underway at Lüneburg Heath. Detailed surveys have also been recently carried out in Germany (Wollesen & Wrangel, 2002). In Sweden, the universities at Uppsala and Goteburg have been particularly active in conducting wide-ranging research on sand lizards and much conservation management has also been carried out.

Perhaps the most significant conservation efforts for the sand lizard in North-West Europe have involved the intensive work over the past 35 years in the United Kingdom (Corbett, 1969; Webster, 1985; Corbett, 1988; Moulton and Corbett, 1999). *L. agilis* is listed as a priority species for conservation action in the United Kingdom (UK Steering Group on Biodiversity, 1995) and, from 1994 to 1997, was the subject of an English Nature funded Species Recovery Programme (Corbett, 1994; Corbett and Moulton, 1998). A captive breeding and release programme for sand lizards (Langford, 1985; Edgar, 1990; Corbett and Moulton, 1998) has resulted in the re-introduction of this species to six English counties, as well as to north Wales, plus health screening and disease management protocols. Over 80 lowland heathland reserves supporting *L. agilis* are now owned or leased by the Herpetological Conservation Trust, with another three owned by British Herpetological Society. A multi-million pound project, Tomorrow's Heathland Heritage (1999-2005), has also restored and recreated lowland heathland throughout the United Kingdom, and has included work on many sand lizard sites. The extensive management of sand dunes has also been carried out in many coastal areas. English Nature and the Countryside Council for Wales currently fund Biodiversity Action Plan work on sand lizards and management, population monitoring and publicity programmes are undertaken for this species on an annual basis. The former organisation has also commissioned a report to define "Favourable Conservation Status" for *L. agilis* in the UK. Many research projects have been carried out by universities, such as Southampton and Sussex, and include PhD work on this species. As a result, of all these efforts the conservation status of the sand lizard in the United Kingdom has improved enormously in recent years.

In addition, Recommendation 67 of the Standing Committee of the Bern Convention was adopted in 1998 following an appraisal of the continuing threats to the Dorset heathlands (de Molenaar, 1998). Among other actions, the UK Government was recommended to take every effort to prevent further loss and degradation of Dorset heathland, such as ensuring appropriate planning controls are applied, adequately controlling recreational use on the urban and urban fringe sites and taking decisive action to prevent uncontrolled and deliberate fires. This led directly to the establishment of a LIFE funded project in Dorset (LIFE 00 NAT/UK/007079) aimed at tackling urban fringe problems between 2000-2005 (LIFE programme, no date).

Building on pre-2006 conservation actions Edgar & Bird (2006) lay out an action plan for the conservation of *L. agilis* in North-West Europe. The overall goal of this action plan is "to ensure that the decline of range edge populations of sand lizard in North-West Europe is reversed and that these populations are subsequently maintained as a viable and integral part of the habitats and landscapes they occupy." To achieve this goal Edgar & Bird (2006) identify seven objectives:

Objective 1. To plan and carry out field surveys to fill all gaps in current knowledge about the distribution and status of the sand lizard in North-West Europe, specifically the United Kingdom, northern France, Belgium, the Netherlands, northern Germany, Denmark and Sweden, plus Estonia and other Baltic states.

Objective 2. To ensure that any significant, unprotected populations of sand lizard in the countries listed in Objective 1 are safeguarded by suitable national designations.

Objective 3. To define and quantify "Favourable Conservation Status" targets for the sand lizard in the countries listed in Objective 1 in order to plan monitoring programmes and provide an accurate measure of the success of future actions.

Objective 4. To produce management plans (or assist with the amendment of existing plans if necessary) for protected areas that support significant sand lizard populations in the countries listed in Objective 1, taking into account the particular ecological requirements of this species and thereby ensuring that appropriate management regimes are established.

Objective 5. To encourage and support scientific research relevant to sand lizard conservation.

Objective 6. To promote a positive public attitude towards sand lizards in Europe and secure the support of all relevant governments, policy makers, organisations, institutions, landowners and individuals.

Objective 7. To improve international liaison and coordination between all those engaged in surveys, monitoring, habitat management and scientific research (to more effectively achieve Objectives 1-6). Evidence of these goals being achieved has been seen in the UK with the work of the Amphibian and Reptile Conservation Trust (ARC) in partnership with a large number of other organisations. The ARC sand lizard project has involved the acquisition and management of land of significance to the species, monitoring of existing populations to inform management, captive breeding and reintroduction programs, and advocacy work to encourage beneficial policy decisions from the UK government (Amphibian and Reptile Conservation, UK, 2019; Moulton et al, 2011).

1.6 Diet and feeding behaviour

Diet in the wild is almost exclusively composed of arthropods (Nicholson, 1980). Beetles and their larvae (Coleoptera), play an important role in the diet of lizards across all habitats. Other than beetles (Coleoptera), grasshoppers and crickets (Orthoptera), spiders (Arachnida), and butterflies/caterpillars (Lepidoptera) are consumed in quantity. A study carried out in Russia by Shlyakhtin et al. (2019) looked at stomach contents in relation to seasonal variations. The main food source for *L.agilis* being insects, primarily Coleoptera species and the largest daily diet intake was seen in July (2.5-2.7g) and the smallest was in April (1.1-1.2g). Seeds, leaves and flower parts alongside small stones were also found in the samples. In 2020, research into the morphology of the sand lizards was carried out focusing on the digestive features. Skrypka et al. (2020) noted that many researchers state the separation of the oesophagus from the stomach being indicative of animals that eat their food whole. This shows that there is no initial treatment of the food in the mouth prior to swallowing i.e. carnivorous reptiles eating various sized mammals. This feature was not evident in *L. agilis* yet the folding nature of the mucous membrane of the oesophagus allows for a food bolus of multiple insects to be ingested whole. Sand lizards will generally take prey that they can overpower, considering larger and harder invertebrate species.

The dietary habits of a population of *L. agilis* were studied in the Alpine valley Stura di Demonte, northwestern Italy. The faecal contents of 33 adults (16 females and 17 males) and 8 juveniles were analysed. Trophic diversity in prey was negligible between sexes. Juveniles had a lower prey diversity value in comparison with females but not with males. There was a positive relationship between the total number of prey items found in individual faecal contents and lizard body size, and this result may explain the more diverse diet of adults in comparison to juveniles. It was observed that the main taxonomic prey groups were eaten according to their proportion in the environment, with the only exception of ants (Formicidae) that were highly avoided by lizards (Crovetto & Salvidio, 2013).

Nemes (2002) investigated the movement and active foraging behaviour of *L. agilis* early in their yearly period of activity. They often perched under shelter or near bushes being motionless. They sometimes varied the site where they perched in the same area of shelter. When foraging, sand lizards will carry out a pause-travel locomotion alternating between short bursts and pausing. This study found that *L. agilis* flicked their tongues more times per minute than phrynosomatid lizards (ambush species). This benefits sand lizards in active foraging using chemoreception to detect prey and possible trails left behind. This leads to increased prey detection and capture.

1.7 Reproduction

L. agilis are polygamous breeders, both sexes mating with multiple partners. This situation leads to male-male competitive behaviour including displaying, combat, and mate guarding (see section 1.8.5). Combat between males may occur in areas of higher population densities where there are limited basking and mating spaces for all the individuals. In captivity, it has been observed that some males will tolerate the presence of others if they are smaller individuals and don't appear to be a threat or competition for the females (McGeorge, pers. obs.).

Courtship occurs when males and females have emerged from hibernation, and have fed. Males will then start to display their breeding colours. Males will bite and hold on to a female at the base of the tail, this often leaves a small amount of scarring which remains throughout the season. This is a good indicator that the female has been mated. Polyandry of females results in clutches with multiple paternities. *L. agilis* are oviparous, laying eggs in shallow burrows dug in loose sandy soil, leaving them to be incubated by the warmth of the ground.

Sperm competition within the female's reproductive tract can occur. An earlier study by Olsson et al. (1994) established that multiple mating by fully fertile males resulted in multiple paternity of offspring. They concluded that there was no impact on male reproductive success in relation to the time or order in which they copulate with the female. Although, active selection of sperm by females appears to occur in a manner that enhances female fitness (Olsson et al., 1997). Based on this selective process, the sperm of males that are more distantly related to the female are preferentially used for fertilization, rather than the sperm of close relatives (Olsson et al., 1997). This preference may enhance the fitness of progeny by reducing inbreeding depression.

Roitberg et al. (2015), describe a consistently female biased sexual size dimorphism (SSD), in *L. a. agilis*, exhibiting higher clutch size and steeper fecundity compared with an inconsistent variable SSD, in *L. a. exigua. L. a. agilis* shows lower offspring size (egg mass, hatchling mass) and higher clutch mass relative to female mass than *L. a. exigua*, both possible ways to enhance offspring number. SSD difference is due to male size (smaller males in *L. a. agilis*) and, fecundity selection favouring larger females. Together with viability selection for smaller size in both sexes, this explains the female-biased SSD and reproductive characteristics of *L. a. agilis*. The pattern of intraspecific life-history divergence in *L. agilis* is strikingly similar to that between oviparous and viviparous populations of a related species *Zootoca vivipara*.

1.7.1 Developmental Stages to Sexual Maturity

Sand lizard eggs are laid in an advanced stage of development. Captive females have been noted to lay eggs after only ten days of courtship (Rykena, 1987 in: Hartung and Koch, 1988). Clutches that are laid early on in the season are found to have a higher hatch rate, better growth rates and a higher likelihood of juvenile survival (Olsson and Shine, 1997a).

Eggs typically hatch after a 40-day incubation by the warmth of the soil, but may take up to 60 days in colder conditions (22°C) and as little as 30 days in warmer conditions (31°C) (Jensen, 1982; Li et al, 2013). This is also supported by data compiled by Köhler (2005), see figure 14.

Eggs that are subjected to temperatures consistently below 20°C will significantly reduce hatch rate. In captivity temperatures may be manipulated to give the best outcome. At Chester Zoo the eggs are incubated at between 24 and 25°C and incubation lasts from 40 -45 days.

Hatchlings are typically light brown or tan and have very distinct ocelli (see section 1.2). Snout-vent length (SVL) is between 30 and 60mm in the first year, rising rapidly to 60 to 90mm by the second year. Body size increase typically plateaus at adult size by the fourth year, but growth will continue more slowly throughout an individual's life (Roitberg & Koenig, 2006). Note that there is significant variation in growth rates and body size dependent on subspecies, species from warmer climates typically grow more rapidly and attain larger adult body size than those from cooler climes (Roitberg & Smirina, 2006).

Species	Clutch size	Temperature (° Celsius)	Duration
Lacerta agilis	3-14	21-24	62-63
Lacerta agilis	-	21-29	55-56
Lacerta agilis	-	27-28	41-43
Lacerta agilis	-	28-31	32-36
L. a. argus	-	25-31	35-45
L. a. boemica	4-9	26	45-48
L. a. exigua	3-9	27.5	38
L. a. grusinica	4-14	25-31	38-57

Figure 14. Showing clutch sizes, for *L. agilis* and subspecies, and incubation duration under specific temperature range (Kohler, 2005).

1.7.2 Age of Sexual Maturity

Sexual maturity is typically reached after the second or third hibernation (Saveliev et al, 2006). There is some variation dependent on the subspecies, those from warmer climates reaching maturity earlier than those from cooler climes. Once maturity is reached, males begin to obtain their distinctive colouration (see section 1.2).

1.7.3 Seasonality of Cycling

Seasonal cycles are highly dependent on location. Populations from warmer climates emerge from hibernation earlier in the year and have a longer breeding season than those from cooler climes. *L. agilis* typically emerge from hibernation in April, usually when temperatures are in excess of 12°C, with mating behaviour initiating around a week after emergence (Nuland van & Strijbosch, 1981). Most mating occurs in May, continuing up until mid-June (Amat et al, 2000; Nuland van & Strijbosch, 1981). *L. agilis* typically lay a single clutch per year between June and July, but may lay any time in the breeding season after mating (Nuland van & Strijbosch, 1981; Olsson & Shine, 1997). Field studies by Olsson and Shine (1997) have found that larger females typically lay their clutches earlier in the year and that all females will delay oviposition in response to weather conditions that reduce basking opportunities. Females typically produce one clutch of eggs per season, but may produce a clutch early on in the season and go on to lay another clutch before the end of summer (McGeorge, pers. obs.).

Double-clutching is seen frequently in captive breeding populations, where during favourable spring and summer conditions, the majority of females can lay a second clutch (Gardner, pers. obs.). The end of season pre-hibernation for sand lizards is dependent on the weather conditions and will vary in geographically different zones. Lizards will retire into burrows that they have excavated themselves. This incorporates a tunnel entrance and a circular chamber which is created by the lizard turning around within the substrate to allow space for ease of movement throughout the hibernating period. The entrance to the tunnel will eventually cover over through the effects of wind and rain and thereby keeping the lizard safe and dry for the following winter months. Females need to have regained their body condition after mating and egg-laying and also be prepared for the following breeding season post-hibernation. Males will also need to have regained sufficient weight and body condition to survive the oncoming months. Fat deposits are laid down in the abdominal cavity, liver and more importantly in the tail (Gregory, 1982). Lizards incurring tail loss may require a more extensive period to build up new fat reserves (Blanke, 2006).

It has been noted that lizards in the Chester Zoo and Marwell Wildlife enclosures, disappear rapidly towards the end of August which is likely to be when their fat reserves are at their optimum levels and the lizards stop all activity to preserve them. Occasionally on warm days in the autumn single lizards may be seen out basking for short periods only. Observations made at other sand lizard enclosures have shown that animals that are out in the later months up to November may not survive the winter period. This has been noted in an outdoor vivarium in north-western Germany where an adult female seen out in October was not seen again the following spring (Blanke, 2006).

Spring emergence for sand lizards varies with geography and habitat and unusually long winters may delay this. Lizards in warmer areas are observed emerging earlier (Blanke, 1995). The dependence on temperature is the key factor in the timings of emergence for all animals across Europe.

Hibernacula may be dug into loose sand or soil but where habitats do not have this type of substrate, cavities in rocks or burrows made by other animals such as mice and rabbits may be exploited. Older lizards may use existing hibernating retreats as hiding places during activities in the warmer months (Simms, 1970). Winter retreats should provide good isolation, drainage and sloping ground and as well as sand, soil and gravel, the ground is often covered in dense vegetation with leafy layers and / or cushions of moss (House & Spellerberg, 1983). In the mild English climate, Simms (1970) states a depth of 30cm or more is created for the winter quarters, while Mertens (1947) states depths of 40-60cm for Germany.

Temperatures at hibernacula have been recorded at -15°C to -20°C. Sand lizards are capable of withstanding temperatures below freezing and are even able to survive a temporary freezing of their tissue (Weigmann, 1929). According to Nicholson (1980) most hibernacula are usually occupied by one lizard, but he also found a site that had been used by two lizards and according to Blanke (1995) and Nicholson (1980) animals used the same hibernacula in consecutive years.

1.7.4 Clutch Size

Lacerta agilis typically lay clutches of between 4 and 15 eggs, larger females laying larger clutches (Olsson & Shine, 1997a); a clutch of 17 eggs was recorded at Marwell Zoo in 2020 (Gardner, pers. obs.). After egg laying, the female will move away from the nest site and there is no ensuing parental care of either the nest site or offspring when they later hatch. In fact, hatchling sand lizards are predated by adults if the opportunity arises. In a captive situation, eggs should be removed after laying, incubated artificially and if being reintroduced to the adult group, offspring should be reared to at least sub –adult.

1.8 Behaviour

Daily behaviour is dominated by basking punctuated with short bouts of foraging; aggressive breeding-linked territoriality occurs throughout the spring and early summer. Inclement weather conditions for prolonged periods may affect reproductive activity and can delay or accelerate productivity. Sand lizards, like many other reptiles, utilise burrows to regulate their environmental preferences, retreating to them when conditions are unfavourable (Edgar & Bird, 2006; Alexsson, 2020).

Gollmann & Gollmann (2008) anecdotally recall the following behaviour; ‘when approaching a small, overgrown puddle in a reedy area was noted a conspicuous movement of the water, indicating the dive of a *L. agilis* in Linzer Tiergarten, a large nature reserve at the western outskirts of Vienna, Austria. The lizard was submerged for several minutes. It could be interpreted as a defensive mechanism when potentially predators approach’.

1.8.1 Activity

Annual activity patterns, in this temperature, terrestrial, diurnal species, vary depending on the location of the population. Populations from warmer climates nearer the equator emerge from hibernation earlier and retire to hibernation later in the year. As such, these populations have an extended active season, corresponding behavioural periods (e.g. the breeding season) are therefore also extended.

Animals typically emerge from hibernation in April and retire again between September and November (Nuland van & Strijbosch, 1981; Moulton pers. comm). The breeding season begins almost immediately after emergence running from late April to July (see section 1.7.3), with juveniles typically emerging between July and September (Nuland van & Strijbosch, 1981).

When active, *L. agilis* spend the majority of their time basking or foraging. Individuals often emerge from nightly refugia at 09:00 AM, often retiring and remerging again to avoid peak temperatures at midday. Individuals typically remain active until between 17:00 and 19:00 before retiring for the night (House et al, 1980). *L. agilis* basks for prolonged periods (30-60’) after emergence in the morning and before submergence in the late afternoon (House et al, 1980). When active but not basking *L. agilis* spend time foraging unless involved in breeding behaviour (see section 1.8.5).

1.8.2 Locomotion

Lacerta agilis are mobile using the limbs and associated sinusoidal movement of the body and tail. Locomotion usually proceeds jerkily by short burst of speed. When moving through heavy undergrowth and burrows sinusoidal movement is reduced to produce a slower creeping movement just using the limbs. *L. agilis* is predominantly terrestrial but may climb grasses, bushes, and low tree trunks.

A study conducted by Ekner-Gryb *et al*. (2013) explored how predatory pressure and parasite load influences locomotor performance of wild specimens of the *L. agilis*. Lizards with autotomy ran significantly faster than lizards with an intact tail, but there was no significant difference in running speed between individuals with fresh caudal autotomy and regenerated tails. Parasite presence and load, age and sex had no significant effect on speed. Results indicate that either autotomy alters locomotory behaviour or that individuals with autotomised tails were those that previously survived contact with predators, and therefore represented a subgroup of the fastest individuals. Therefore, in general, predatory pressure but not parasites affected locomotor performance in these lizards.

1.8.3 Predation

Sand lizards are predated by several taxa (Simms, 1970; Olsson, 1993). Wood ants can be a serious threat to incubating eggs and adult sand lizards will also cannibalise their own hatchlings (Corbett & Tamarind, 1979).

L. agilis are predated on by a variety of avian predators including members of the Corvidae and Laridae families, raptors (Dravecky *et al*, 2008), and pheasants (*Phasianus colchicus*) (Edgar & Bird, 2006). In kestrels (*Falco tinnunculus),* 16 % of their diet may be comprised of sand lizards (Kristin 1987).

A variety of mammalian species, both native and introduced may also predate *L. agilis*. Mustelids, foxes and rodents have been observed predating on sand lizards (Märtens, 1996; Woodfine et al. 2017). Domestic and feral cats (*Felis catus*) may be a particularly significant predator where they co-occur with *L. agilis* (Larsen & Henshaw, 2000; Henshaw, 1998).

1.8.4 Sexual Behaviour

During the mating season (see section 1.7.3) *L. agilis* utilise overlapping home ranges over which males will display a level of territoriality (Olsson & Madsen, 2001). During this period females utilise a home range of 160m^2 and males utilise a home range of 1,100m^2 (Olsson & Madsen, 2001). Home range overlap is extensive, and males have both significantly larger and more numerous intra-sexual overlapping than females (approximately five overlapping compared to one). Large males also have significantly more overlap with females than small males do. The smaller females ranges are more widely spaced, female rarely interacting with one another (Olsson, 1988).

Where males encounter females within their overlapping home ranges they will initiate mating. Females are sexually receptive for approximately 10 days and during this period they do not express mate choice but mate with all males capable of courting (Olsson & Madsen, 2001). *L. agilis* have a complex courtship ritual involving an exchange of a series of head nods and limb flicks between the male and female. If an acceptable exchange of signals is achieved the male will progress to biting the flank of the female just above the lower limbs. The female will then begin to walk on for a short time, dragging the male with her. Whilst still grasping the female by the flank the male will bend his body around so that his vent is adjacent to that of the female. Mating will then proceed, the female remaining still whilst the male contracts his whole body. A full and detailed description of mating in *L. agilis* is provided by Kitzler (1941).

After mating males will partake in mate guarding of the female (Olsson & Madsen, 2001). Lateral green area ("badge") size of a male *Lacerta agilis* is strongly negatively correlated with duration of mate guarding (Olsson et al, 2000). This is because males with larger badges tend to be of higher status and are more successful in mate acquisition compared to smaller badged males (Olsson, 1994a; 1994b). As such, larger badged males spend more time acquiring additional partners rather than in a prolonged guarding of an already mated female (Olsson et al, 2000; Olsson & Madsen, 2001).

When mate guarding and encountering each other within their home ranges in the mating season male *L. agilis* perform ritualistic displays that may escalate into physical combat, as described by Olsson (1992): "A displaying male raises itself up on all four limbs and turns its green flank, extended dorso-ventrally, against the opponent. A contest may be settled at this stage but may also escalate into a "ring-dance" and, further, into a "fight" that comprises biting and locking of jaws."

Section 2: Management in Zoos and Aquariums

2.1 Enclosure

The Sand lizard enclosure, at Chester Zoo, was built facing a south to south-westerly direction to maximise natural sunlight opportunities. The surfaces of the dune were created with an average angle of elevation of between 45 and 60 degrees. This not only increases basking times for animals but also affords better viewing opportunities for the zoo guests. Sloping of these areas is only feasible by the planting of native species such as lyme grass (*Leymus arenarius*) and, in particular, marram grass (*Ammophila arenaria*), as the dense root system helps to maintain sand integrity and prevents the potential for landslides to occur. In their coastal habitat these grasses with their extensive rhizome systems allows the plant to thrive under conditions of shifting sands and high winds, to help stabilize and prevent coastal erosion.

The Sand lizard enclosure at Chester Zoo, UK is approximately 19m x 4.5m x 3m. These dimensions do not include the extra height of the overhead netting which is a further 1.5 metres at its highest point. There is a glass door at one end to allow keeper access and a managed footway along the front of the dune to give access to all areas of the enclosure. The enclosure is on show to the general public and has 11 viewing windows wrapped around the full length of the dune (figure 15 & 16), although one of these windows is positioned behind the keeper access barrier.

The base of the dune was originally created by using several tons of compacted hard-core for free draining and limiting the risk of flooding. The dune profile was formed in loose hard-core to provide voids for burrowing / hibernating lizards. This was then covered with sea sand which was collected from the appropriate coastal areas with permissions from the local authorities. The plants were also acquired this way with permission from the local authority. The shape of the dune emulates a natural dune system which may be found in any of the areas occupied by the coastal race of sand lizards. The

retaining wall extends only a nominal distance above the sand level to minimise shaded areas. The North facing wall which belongs to the retaining wall was insulated.
From ground level to the level of the steel girders which form the external structure, the height is 3m and at the highest point of the dune, it measures 1.80m. The width of the dune is approximately 4m and the length of the dune is approximately 19m. No additional supplementary heating was provided, but this could be a consideration if preferred temperature profiles cannot be achieved. The same enclosure design can also be adapted for other European sandy natural habitats panted with heather (*Calluna vulgaris)*.
The overhead nylon netting is supported by 8 steel girders. The overhead protective covering of an outdoor enclosure is vital for the prevention of access by aerial and terrestrial predators, see section 1.8.3. This outside exhibit was originally designed for the management of twenty-five animals in a ratio of approximately even sexes. If only smaller enclosure is possible a lower ratio of males to females can sometimes be a good balance.
This is a detailed description of a dune system created for this species at Chester Zoo, the same may be applied to other zoos across Europe when considering an outside exhibit. Although this is natural way to keep, display and breed the species, they a more difficult to manage due to the size and complexity of the enclosure.
Indoor housing may encompass some of the features of an outside enclosure i.e. the use of sea sand, or suitable alternative, as a substrate, planting and structuring of the dune on a smaller scale. The most important components are the heat and light which needs to emulate as much as possible the same properties of the sun and daylight around the clock, see section 2.1.4.

At Nordens Ark, Sweden, sand lizards are housed in an open air indoor enclosure. The enclosure measures approx. 3 meters long and between 2 to 1 meter wide. The enclosure is illuminated by two 150W metal halide lamps. For the basking sites 70W HiD lamps (Econlux, Solar Raptor) are used as the main source for light, heat and UVB radiation.

Figure 15. Showing the front of the *L. agilis* artificial dune at Chester Zoo. (Chester Zoo).

Figure 16. Showing side view of the *L. agilis* artificial dune at Chester Zoo. (Chester Zoo).

Figure 17. Showing staff entrance to the *L. agilis* artificial dune at Chester Zoo. Note the board placed inside the door, to prevent escapes once the door is opened. (Chester Zoo).

Figure 18. Showing view from the entrance door of the *L. agilis* artificial dune at Chester Zoo. Note the 35cm walk way around the windows allowing full keeper access to the dune. (Chester Zoo).

The sand lizard enclosure at Marwell Zoo, UK, consists of a south-facing outer fruit-cage measuring approximately 20 x 9 m and 2.5 m in height. The external enclosure was first established in 1989 (as described in Woodfine et al, 2017). It was upgraded over winter 2018-19 to consist of low breeze block walls (90 cm high and extending ~40 cm below ground), topped by an outward-facing galvanised steel overhang extending 20 cm, and double electric fence directly below this to deter rodents. A renewed timber frame on top of the walls supports high tensile nylon netting over the entire enclosure (figure 19). Within the outer fruit-cage are several vivaria surrounded by gravel walkways: a captive breeding adult vivaria (~12 x 5 m), a smaller research area (~5.5 x 4 m), a quarantine area (~5.5 x 1 m) and two juvenile rearing vivaria (~6 x 2 m). Each vivarium is delimited by transparent acrylic sheets measuring 30-40 cm above ground level and buried at a depth of 30 cm, topped with inverted plastic piping. Habitat within the vivaria is dry heathland-grassland mosaic, with heathland soil and plants rescued from Canford Heath and transplanted during the initial construction of the vivarium (Woodfine et al, 2017).

Figure 19. Showing side view of the *L. agilis* outdoors enclosures with dry heathland-grassland mosaic at Marwell Wildlife. (Marwell Wildlife).

In Münchner Tierpark Hellabrunn, Germany, the sand lizard enclosure is outdoors having a surface of 6m^2 keeping three lizard breeding pairs with common toads (*Bufo bufo*). Three of the walls are glass with a back stone wall. The overhead nylon netting is supported by eight steel girders. The enclosure is protected from aerial predators with a nylon netting (5 cm diameter) which is retractable to provide access to keepers. The interior is totally naturalised with rocks, logs, and natural vegetation (figure 20 & 23). The lizards overwinter in the outdoor enclosure. To avoid flooding, a hole of approx. 1 m diameter and 1m depth has already been dug and filled with stones, soil and leaves to keep the soil as loose as possible so that the animals can crawl into it. In addition, a water basin above the hole protects this area from water entry as much as possible so that the soil does not freeze over (figures 21 & 22).

Figures 20, 21 & 22. Showing view from the glass enclosure of the *L. agilis* at Münchner Tierpark Hellabrunn (top). Note the artificial pool (bottom left), natural vegetation (bottom right) and back concrete wall. (Münchner Tierpark Hellabrunn).

Figure 23. Showing view from above the glass enclosure of the *L. agilis* at Münchner Tierpark Hellabrunn. Note the black netting retracted on one side. (Münchner Tierpark Hellabrunn).

2.1.1 Boundary

At Chester Zoo, the outdoor enclosure is on show to the general public. The door allowing keeper access is behind a gate preventing public access. There is a board measuring 50cm in height immediately behind the door so that when the door is opened, any lizards by the door may not escape, see figure 17. There is no stand-off barrier to the public and therefore no impediments to viewing the animals close up.

The height of the windows enclosing the dunes would need to be adjusted according to the height of the dune and the vegetation growing from it itself. The window height is 1m, which stands clear of the dune by 0.5m in a vertical straight line from the 35cm gap left between the window and dune (figure 18). This takes into account any potential of escape by lizards that may climb up the vegetation and launch themselves across to the outer barrier and also provide full keeper access around the dune. At Nordens Ark, the enclosure is on show for the public but being an open air enclosure and allowing visitors to come close to some of the species there is only a 30-centimetre-high glass pane in the front. The sand lizard enclosure at Marwell Zoo is off-show to the public.

2.1.2 Substrate

The substrate for the coastal form of sand lizard should be natural sea sand. The appropriate permissions would be required to excavate any quantity of this from local beaches. If this is not available to collections too far away from a coastal region, a commercial product is available called wash sand and extra calcium could be added to this in the form of ground oyster shell. For other races occupying heathland habitats, a mix of soil, peat and fine gravel would be appropriate.

For nesting and rearing chambers, the same type of substrate may be utilised. Hibernacula would be constructed from the same substrate within the enclosure and any cavities created by the rockwork would add structure and integrity to chosen hibernating sites.

2.1.3 Furnishings and Maintenance

The Sand lizard is a temperate species found across Europe and ideally would be kept outside in a naturalistic environment. This should also be reflected in the type of substrate, furnishings and planting used so that the enclosure emulates the natural environment as much as possible.

The substrate for the coastal form of Sand Lizard should be comprised of sea sand, locally sourced and acquired with permissive licences from the local authority. To provide the shape of a naturally landscaped dune, compacted hard-core with the addition of loose hard-core may be used. The sand dropped over the top of the shaped rock work will allow the lizards to comfortably burrow without the sand losing its integrity. This should also be backed up by the provision of plants that are locally sourced and acquired with the requisite licences provided by the local authority. Plants such as lyme grass (*Leymus arenarius*) and marram grass (*Ammophila arenaria)* will establish their roots in the sand dune which will also provide the sand with the necessary integrity to maintain stability. The sand lizards will create their own burrows and hibernating sites. Other plant species' which may be used for ground cover are stonecrop from the large genus of *Sedum* plants in the family Crassulaceae and dewberry, a group of species in the genus *Rubus*. As the dune system has sufficient hard core beneath the substrate, this should allow drainage of any amount of natural rainfall.

Other furnishings may include cork bark pieces, rocks, wood slabs or slate/clay tiles. It's not likely that these items would be seen in their natural habitat but they can be useful as a creation of vantage points for males establishing territories, visual break-ups and basking sites for all.

A vitally important feature of a dune or heath enclosure is basking sites and these should be created at regular intervals, as they will be selected by males and guarded as a territory. The basking sites needs to be kept clear of planting and should have a gentle slope, angled at around 45 to 60 degrees, to allow maximum benefit from solar radiation.

2.1.4 Environment

Modern husbandry practices encourage an evidence-based approach, utilising data from the field to influence captive management (Arbuckle, 2013). Appropriately placed naturalistic outdoor enclosures in countries where *L. agilis* occur are subjected to natural parameters of heat, light, humidity and seasonality. Intervention may be required if the summer is exceptionally hot and dry and extra watering of plants and sand is needed. Temperatures as high as 48°C have been recorded at the top of the dune, at Chester Zoo, in the summer and even at the lowest level, temperatures regularly exceeded 28°C. At these elevated temperature levels animals would retreat into the dune for shade. See figure 24 below climate chart for the habitat of Sand lizards in the UK.

Environmental parameters for juveniles in indoor housing, the following is recommended: ambient day temperature 22-24°C, ambient night temperature 15-17°C, basking spot 26-28°C, UV-Index range 0.7-3.0 (Baines et al, 2016).

It is important to select the most appropriate equipment when providing artificial heat to animals. Short-wavelength heaters, such as quartz-halogen heaters, emit the same type of infrared radiation as the sun (infrared-A) and are currently recommended as day time basking heat sources for larger areas if you need to heat a large area. For small enclosures, e.g. to rear juveniles however, these are not practical and incandescent/halogen/MH spot lamps an equally good source of IR-A and light. IR-A radiation penetrates through skin to the subcutaneous tissues, whereas most of IR-B and all of IR-C (i.e. long-wave IR) from other heating units other than incandescent lamps, do not (Porter 1967; Schroeder et al, 2007; Baines, pers. obs.). Short-wave infrared heaters are also much more effective for providing basking warmth and less likely to cause skin damage from localised overheating of the skin surface. Moreover, these shorter wavelengths have a biological effect upon living cells unrelated to warmth. They activate genes responsible for a wide range of effects, which include acceleration of healing and protection against UV damage (Schieke et al, 2003; Schroeder et al, 2007; Baines, pers. comm.). IR-A units come in a variety of wattages and sizes, so careful selection, control and mounting is required depending on the enclosure size (figure 25).

Exposure to appropriate levels of UVB, as found in natural sunlight, is critical for the health and wellbeing of reptiles and other taxa. UVB has a range of effects on the skin, including disinfection and immune modulation, but its most important effect is the enabling of vitamin D3 synthesis, vital for maintaining calcium homeostasis. Adequate vitamin D3 is essential for life. Low calcium levels affect muscle function, growth and reproduction; severe vitamin D3 deficiency results in metabolic bone disease (Baines, pers. comm.). T5-HO UVB Lighting come in a variety of sizes and provide a versatile lighting option when the UVB fluorescent tubes are used in reflective fixtures (figure 26), and combined with infrared heat lamps and either metal halides or LEDs for additional visible light, to ensure sunlight stimulation. (Baines, pers. comm.). Baines et al. (2016) provide further details of providing and selecting appropriate UV radiation levels for all species of reptiles and amphibians. Infrared (heat), visible light and UV must cover the same area, and be distributed as evenly as possible across the entire basking zone, avoiding small spots of concentrated heat. Basking zones should be large enough to encompass the entire body of the animal, or several animals if they are housed together (Gill, pers. comm.). In all cases, the key thing is to provide animals with appropriate thermal and photo gradients with opportunity to regulate themselves.

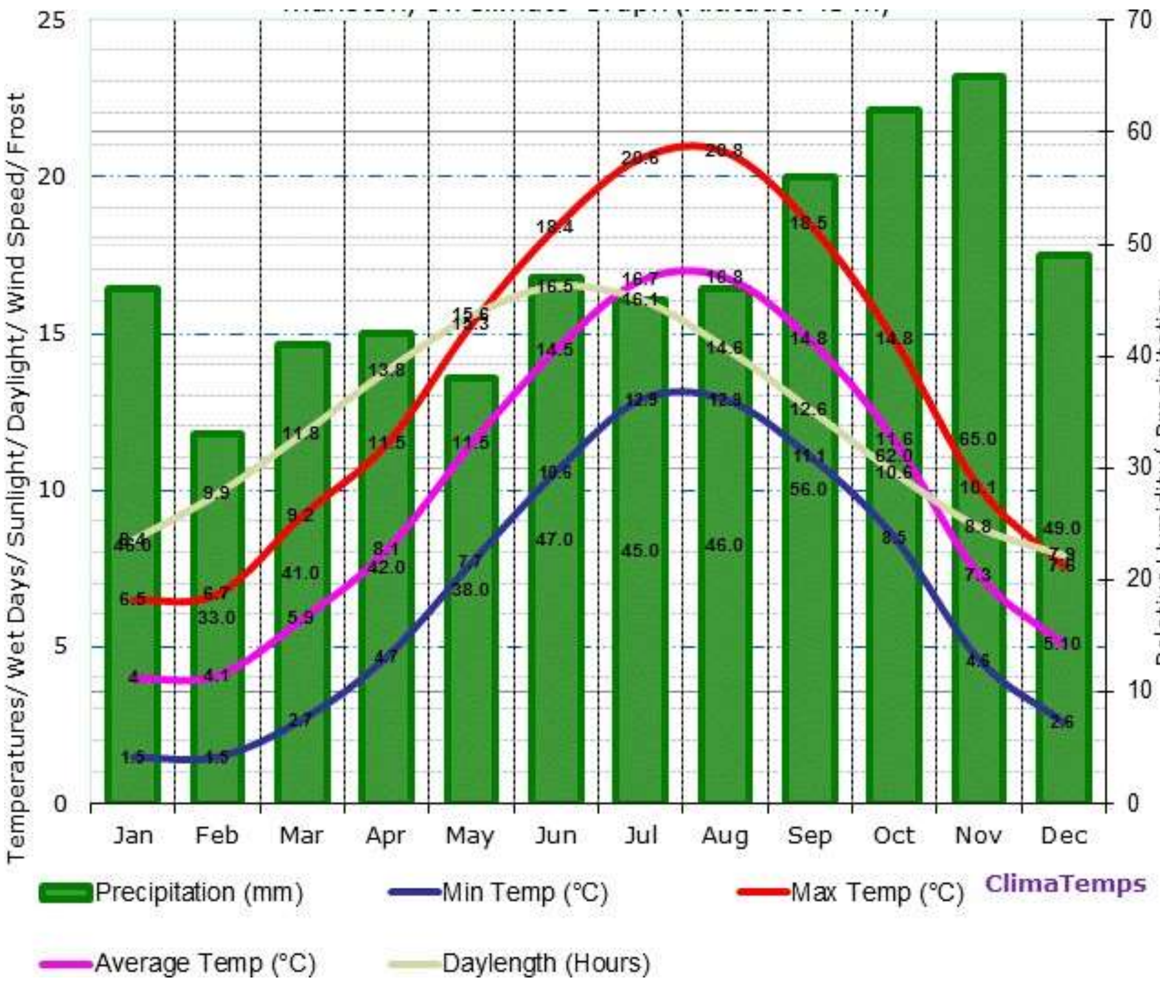


Figure 24. Showing environmental data for *L. agilis* habitat in Manston, UK (2014).

Figure 25. Showing some units that emit short wave infrared (IR-A) radiation:
(a) 35W GU10 halogen spot lamp (https://www.electricalworld.com); (b) 80W halogen (PAR38) lamp (http://www.cp-lighting.co.uk); (c) 1.5kW quartz-halogen bask zone heater (http://www.equinesunswitch.co.uk/Goodwood.html).

Figure 26. Growth Technology "Lightwave" hydroponic unit T5 LW24-HO, 60cm, 4 tube (https://www.growthtechnology.com/product/lightwave-t5-lw24-ho/). Available in other sizes depending on coverage required.

2.1.5 Dimensions

For up to six pairs, a dune/heath system measuring approx. 20m x 4m x 3m would allow sufficient basking sites and territories. The height of dunes will vary as it is dependent on the depth of substrate used to form the dune. A single pair would live satisfactorily in a much more scaled down version, i.e. an enclosure measuring 2 x 1m. However, if another male were to be introduced, the site would need to be increased in size to allow both males to establish their own territories without the potential for conflict. Housing fewer males and females will help reduce conflict between males.

Hatchling and juvenile specimens may be housed indoors in groups dependent on sizes of individuals. An enclosure measuring 90cm x 45cm x 45cm will comfortably house a group of six hatchlings for rearing through to the juvenile stage (Figure 27; Exo Terra Natural Terrarium Large PT2613; http://www.exo-terra.com/download/sales_sheets/Sales_Sheet_Natural_Terrarium_Large.pdf).

Where individuals can be seen to grow at a lesser or greater rate than their siblings, they will need to be separated out into further rearing units.

Figure 27. Showing enclosure used for rearing juvenile *L. agilis*. Product from Exo-terra. (Chester Zoo). Enclosure; Exoterra Natural Terrarium Large PT2613; http://www.exo-terra.com/download/sales_sheets/Sales_Sheet_Natural_Terrarium_Large.pdf

2.1.6 Hibernation

Animals kept in indoor enclosures are not exposed to environmental conditions that prepare them for winter as those kept in outdoor enclosures. To prepare animals kept indoors bot temperature and photoperiod should be dropped to mimic natural conditions as closely as possible. Sand lizards kept at Nordens Ark are acclimatized to lower temperatures over a 4-week period were temperature and daylight hours are reduced. As the temperature drops the animals will stop feeding and start to hide within the enclosure. As a way to allow the animals to fully digest any prey items a last feeding is done 3 weeks prior to hibernation. Animals are then moved to plastic boxes (SmartStore™ Classic 31, 50 x 39 x 26 cm) furnished with a substrate of gravel or wood shaving with multiple hides for individual lizards to utilize for this Nordens Ark has used dry sphagnum moss and PVC pipes, also present is a shallow water dish (figure 28). Boxes are then moved to a fridge or as in the case of Nordens Ark a repurposed cellar equipped with a cooling system to allow for a stable temperature. Initially temperature is set to 15°C and over a period of 7 days temperature is reduced to around 4,5-5°C, at this temperature animals are kept for 1 month before reversing the temperature back to 15°C again.

Figure 28. Plastic box adecuated for hibernation of *L. agilis* at Nordens Ark. (Nordens Ark).

2.2 Feeding

All feeding is temperature dependent. As soon as the adults emerge from hibernation, provided the temperatures are above 12°C, they will be ready to feed. If the animals are being managed in an outside naturalistic setting, there will be wild food readily available. To ensure that all individuals are receiving a healthy, balanced diet and a sufficient amount of food, target feeding may be employed. It's important to know the animals well either by visual recognition, by understanding where each animal has established its territory (and this may change through the season) or by a counting system. The latter method may be challenging if the number of individuals in the enclosure exceeds ten and the enclosure is of a considerable size.

On emergence from hibernation, animals should be checked daily on fine, dry days and offered food. As the spring days become warmer and dryer, food may be offered more frequently and at the onset of summer, animals should be visited two to three times per day. Feeding is weather-dependent so on days when it is raining heavily or the ambient temperature drops to below 12°C, it is unlikely that specimens will be seen out. However, if there is any solar heat even on cool days and the substrate has warmed up, there is a strong possibility that specimens will be out basking.

Regardless of age, unless there are health issues, specimens will feed with similar regularity. Familiarity with feeding also engages the attention of the specimens so that they lose any nervousness and come close enough to take food from the feeder’s fingers. This is particularly advantageous for the keeper as animals may be scrutinised more clearly and females may be monitored for potential eggs. This period of familiarity is limited to the warmer months and when food is no longer the catalyst, specimens resume their state of alertness.

Outside enclosures will harbour natural populations of suitable live food such as caterpillars, spiders, ants, moths, beetles, flies, grasshoppers and other invertebrate larvae. Offering a captive–bred diet allows for the opportunity to gut-load or coat the food with a mineral/vitamin supplement, which is particularly beneficial to reproductive females.

In captivity, the diversity of prey items for insectivorous species is limited and often nutritionally inadequate, leading to nutrient deficiencies in animals. Zoological institutions utilise gut-loading, an insect supplementation technique, to compensate for these nutrient short fallings (Attard, 2013). It is crucial that live food species are given the best possible care, including the provision of a balanced and appropriate diet at all times. This will ensure that they will in turn provide a nutritionally valuable meal to a predator species (Spencer & Spencer, 2006).

2.2.1 Basic diet

In the wild any invertebrates that are easily over-powered, including beetles (Coleoptera), beetle larvae, woodlice (Oniscidea), crickets and grasshoppers (Orthoptera), small arachnids, moths (Lepidoptera) etc. may be consumed.

In captivity there are a number of invertebrate species that are cultured commercially and can be fed to sand lizards, once gut loaded. These include, but not limited to, African black crickets (*Gryllus campestris*), brown house crickets (*Acheta domesticus)*, locusts (*Schistocerca gregaria)* and wax worm larvae (*Galleria mellonella)*. Spencer & Spencer (2006) provide further options of prey species that could also be fed to sand lizards, and in addition detail of how to care and culture these prey species in a captive setting. Hatchlings at Nordens Ark have fed on Four-spotted bean weevils (*Bruchus quadrimaculatus*).

2.2.2 Special dietary requirements

If the substrate is sourced naturally, there will be a plentiful supply of sea / snail shells crushed and mixed in to it. If the substrate has been artificially sourced an extra source of calcium may be added using crushed cuttlefish bone (https://en.wikipedia.org/wiki/Cuttlebone), powdered oyster shell or pure calcium carbonate (https://en.wikipedia.org/wiki/Calcium_carbonate). When feeding directly an additional supplement of a commercially available reptile multi-vitamin may be used to dust the food appropriately, under the specific product guidelines.

2.2.3 Method of feeding

Feeding may begin after the sand lizards have emerged from hibernation and temperatures remain fairly stable at around 12 – 15°C or above. It is important to correlate feeding with days when it is dry and warm i.e. with solar radiation. A good indicator would be to feel the sand to check how it is warming up. Very often solar radiation will heat up the sand and even when sunshine is sporadic, the sand will remain warm for some time. Also, the lizards will make it obvious when they are hungry as they become familiar with the movements of the keeper and may follow them around the enclosure. On cool, rainy days it would be pointless attempting to feed as the lizards are unlikely to emerge. Therefore, it would be prudent to always consider the weather conditions before offering any feed items. In perfect conditions feeding of appropriate size prey should occur two to three times per day for both adults and juveniles. See section 2.5 on food presentation forming part of behavioural enrichment.

2.2.4 Water

Appropriately sized water sources should be placed within the enclosure to give the animals the opportunity to access a fresh water supply, although it is unlikely that they would be used by this species. Hydration regularly occurs through morning dews and in instances of extended periods of extreme heat, for a large enclosure a generous hosing/misting down of the whole enclosure multiple times a day.

2.3 Social Structure

Sand lizards are solitary in the wild and generally only meet up for the purposes of mating. Males encroaching on other male territories will be quickly chased off.

Numbers of individuals in enclosures will largely depend on the amount of space available, but as a guideline a pair or a trio per square metre should be comfortable. Males are territorial and will require prime basking spots and areas to court females without having to fend off other males. If there are other males in a spacious enclosure, provided there are sufficient basking and mating areas for them, this won’t be an issue. Males need to be able to challenge each other in a safe environment i.e. one that has the amount of space to allow escape or retreat. Nordens Ark keep sand lizards in same sex groups and males show no excessively aggressive behaviour towards each other as long as they have sufficient basking sites and no females are present within the enclosure.

2.3.1 Sharing Enclosure with Other Species

It is preferable to maintain this species without adding other species to the enclosure. This will reduce the potential for competition for basking sites and feeding opportunities. In the wild, the common lizard (*Zootoca vivipara*) and sand lizards do overlap in habitats, and if the needs of both species could be catered for sufficiently then there would be no reason not to mix. Do consider here that if animals are being released, then origin and disease status of both species should be the same.

If animals are to be released, a licence, the owner’ consent and a disease risk assessment must be followed, and long-term post-release monitoring is an essential part of a responsible conservation translocation (IUCN, 2013). At Nordens Ark, Sweden, sand lizards have been kept together with eastern Hermann's tortoise (*Testudo hermanni boettgeri*) without any signs of discomfort or stress from either species. Elevated basking sites have been provided for the lizards to utilize without competing with the tortoises (figure 29).

Figure 29. Sand lizards kept together with eastern Hermann's tortoise (*Testudo hermanni boettgeri*) at Nordens Ark. (Nordens Ark).

2.3 Breeding

In an outside naturalistic environment, there is no need for climatic intervention by adding additional heat or light sources, see section 2.1.4. Breeding will occur in the spring as soon as the temperatures begin to rise and animals emerge from winter hibernation. Males and females may live alongside each other throughout the year and stimulation to breed will come from natural sources i.e. sunlight, increased temperature and a plentiful supply of food. In an indoor setting mating behaviour will begin after a winter dormancy.

2.4.1 Mating

Male lizards will establish territories which will include a prime basking area and rivals will be chased off at every opportunity. The male will attempt to claim and keep a female in his territory for the purpose of courtship and mating. Mating occurs in the spring as soon as the temperatures have risen and continue into the summer months. Females have been known to be gravid from August and have laid eggs as late as the end of August although this would be unusual (McGeorge, pers. obs.).

Olsson & Madsen (2001) describe promiscuity in populations in Sweden, where females mate multiply and exhibit positive relationship between the number of partners and offspring viability. This relationship is most likely the result of variable genetic compatibility between mates arising from post copulatory phenomena, predominantly assortative fertilization with respect to parental genotypes.

2.4.2 Egg Laying

Egg laying may occur 4 -6 weeks after copulation. The female will gradually start to increase in girth until the shape of the eggs may be seen through the skin, taking on a “pebbled” appearance. At this point, the female may be removed and placed in a nesting chamber for safe delivery of the eggs. The period leading up to egg laying is especially important as the female needs to be fed frequently on a heavily supplemented diet i.e. a mineral/vitamin supplement should be generously sprinkled on all food offered. At Chester Zoo, Nutrobal (https://www.vetark.co.uk/pages/Nutrobal-for-reptiles.aspx) is the vitamin supplement that is used when supplementing prey items for sand lizards. Prior to egg laying the female will refuse food which is a good indicator to check for any nesting test sites.

The nesting chamber, within an outdoor enclosure, must be positioned carefully to ensure it is exposed to natural light, sun and shade, in order to provide options for a gravid female. A sand substrate of around 18cm depth and suitable furniture such as cork bark pieces and slates placed in strategic positions in the chamber will optimise the chances of a successful oviposition. The female will be very careful to hide where the eggs have been laid but her aim will be to lay against a hard surface or wall after digging down approximately 10cm and then tunnelling across to the final position.

At Marwell Zoo, at least two sand patches measuring approx. 2 m^2 are maintained during the main breeding enclosure for oviposition. These are monitored and photographed at least daily during the egg laying season to assess presence of test burrows. Once burrows are backfilled (indicating oviposition has occurred), the location of these is marked on the surface. If multiple females are test-burrowing at similar times, which is common, excavation is delayed until this behaviour has stopped and all clutches are then excavated concurrently to limit disturbance. Weather conditions are closely monitored, and should unfavourable conditions prevail (e.g. extreme heat, periods of heavy rain), excavation is undertaken during the least active times of day to both minimise risk of egg failure and limit disturbance to any gravid females.

2.4.3 Incubation

The eggs should be carefully uncovered, extracted and transferred to an incubator (figure 30).

In a study done on the island of Wangerooge, North Sea, two clutches of eggs were found deposited at 8 and 7.5 cm depth and hatched after 56 and 55 days respectively (Elbing, 1993).

Female sand lizards' fat reserves are at their lowest after egg production (Llorente & Carretero, 2000), and therefore should be allowed to remain in the nesting chamber for a few days and fed well before releasing her back out into the enclosure.

It is important to handle eggs carefully after removing them from the nest site and place them into a container filled with the same media that they were laid in to prevent the eggs from turning or getting jostled on the way to the incubator. Eggs should not be rotated from the position in which they were laid. Sometimes, two or more eggs will be stuck together in situ; attempts should not be made to separate these, but to excavate and incubate them as they are grouped. For incubation media, vermiculite (https://www.pro-rep.co.uk/portfolio/vermiculite/) is a tried and tested product and has been used for over 30 years, at Chester Zoo, for incubating *L. agilis* eggs (McGeorge, pers. obs). Mixed in a 1:1 ratio by weight with water and added to a small plastic container with ventilation holes in the lid. The media should half fill the container. Small indentations should be made in the substrate and each egg placed in the centre of the indentation which should create an air space around each egg before placing the entire box inside an incubator. See figures 31 to 32 for incubation setups for the species at Chester Zoo.

At Chester Zoo, incubation temperatures set at 24°C have effectively given hatch times of around 45 days. Consistent temperatures below 20°C will result in a poor hatch with poorly developed young, unlikely to survive after hatching. Hatching Sand lizard eggs at the above temperature of 24°C has realised an even number of sexes. However, the last clutch of eggs that were hatched at the same temperature, in July 2019, were comprised of two males and seven females (McGeorge, pers. obs). Idrisova (2018) observed that eggs that were incubated greater than 29°C had the highest frequency of deviations and malformations.

At Marwell Zoo, eggs are incubated at a temperature of 25°C and humidity of 90% in Rcom Reptile Max 90 incubators. Eggs are kept within their clutches in vermiculite in separate tubs (each measuring ~20 x 10 cm), which are positioned within the main incubator tray. Every day, eggs are checked and aerated by removing and 'wafting' the tub lids. Any signs of infertility (e.g. developing mould) are monitored and eggs removed and discarded as needed. Post-excavation, fertile eggs will have a pink glow when held to the light; as the eggs swell, develop and near hatching this becomes less obvious. Some clutches (often second clutches) have been observed to be particularly pink, with the shell appearing very translucent; this has not affected the viability of eggs, which have successfully hatched (Gardner, pers. comm.).

Figure 30. Showing a clutch of eggs laid in the enclosure at Chester Zoo. Eggs have been uncovered by a keeper to be removed for incubation. (Chester Zoo).

Figure 31. Showing a clutch of eggs placed and arranged in incubation box and media. (Chester Zoo).

Figure 32. Showing incubation boxes arranged within the pro hatch incubator (http://pro-racks.com/prohatch.html) at Chester Zoo. (Chester Zoo).

2.4.4 Hatching

Incubation periods are temperature dependent. Typically, cooler incubation temperature will result in longer hatching periods and higher temperatures will result in shorter incubation periods (Kohler, 2005) See section 1.7.1 and figure 14. Elbing (1993) records average incubation temperature between 21.5°C and 21.6°C result in incubation periods of 56 and 55 days retrospectively. The incubators used at Chester Zoo are pro hatch incubators (http://pro-racks.com/prohatch.html), see figure 32.

In the 3-24 hours prior to hatching, eggs will begin to 'sweat', forming small droplets on their surface. Hatchling lizards will make a small opening in the shell with their egg tooth before their head, followed by the rest of their body, emerges; they can sit semi-emerged for several hours. Eggs within clutches will typically hatch within a maximum of 24-48 hours of each other. At Marwell Zoo, hatchlings are released into outdoor rearing areas within their clutch 24 hours after the final egg from that clutch has hatched.

2.4.5 Development and Care of Young

Hatchlings may be reared in specially prepared outdoors enclosures or similarly prepared indoors enclosures (figures 15-18 & 27). Care needs to be taken when setting up outside to allow for extreme temperature changes and the potential of flooding from heavy rainfall. Any nursery chambers should have sufficient drainage installed to allow water to escape and plenty of hides/ cover for escape from extreme temperatures. The substrate would need to be sufficiently deep enough to allow the young lizards to burrow away from any potential dangers i.e. predation.

2.4.6 Rearing

Hatchlings may be reared in a protected outdoor enclosure or in an indoor vivarium set up with supplementary artificial heating and lighting, see section 2.1.4. For outdoor enclosure, it's important to provide both shaded and open areas to allow for basking and feeding opportunities and protection from excessive heat or cold. For a clutch of up to a maximum of twelve hatchlings a floor space measuring not less than 30cm x 30cm would be appropriate. As the hatchlings develop any that grow faster than their siblings need to be separated out into separate enclosures. Young sand lizards are food oriented and tail or body bites are common. If animals are not separated due to differences in size, deaths may occur.

Rearing enclosures should be furnished with cork bark, slates or flat stones, chopped grasses or scrubby low-lying plants, have a soft sandy or soil substrate and access to a fresh, shallow water supply, see figure 22. For an indoor enclosure, an overhead spot lamp of between 20 and 35W should be sufficient to create a heated basking space. Artificial lighting is necessary to provide UVB and visible light, see section 2.1.4.

Newly hatched and first instar brown and black crickets (*Gryllus* sp.) and flightless fruit-flies (*Drosophila* sp.) may be offered initially, once daily, until feeding has been observed by all of the hatchlings. Several feeds in there will be a noticeable change in the demeanour of the hatchlings. They will become increasingly avaricious and ready to bite anything that moves. When this happens feeding should be increased to twice daily but taking care not to flood the enclosure with food items and only feeding what may be consumed within the next 2-3 hours. Experience from raising juveniles communally at Nordens Ark has proven un-successful with individuals competing over same food items resulting in injuries and in some cases death. Fresh water should always be available in a shallow dish but a daily misting over the entire enclosure is important to replicate the dew that would naturally form overnight in an outside situation and which would be licked up by the hatchlings.
Hatchlings at Marwell Zoo are reared in naturalistic outdoor vivaria measuring 6 x 2 m. A maximum of fifty-sixty individuals are held in each vivaria. Hatchlings have been observed to compete over large wild-sourced prey, ultimately enabling its consumption by the individuals involved. No direct signs of aggression between hatchlings of similar age have been observed and communal basking is common (Gardner, 2021)

2.4.7 Population Management

Breeding recommendations are often linked to 'in-situ' work that operate either locally or regionally through environmental agencies. See sections 1.5.3 to 1.5.5.2. For this species an EAZA Studbook programme, supporting the reintroduction programmes in UK was previously discussed on the core of the captive breeding and reintroduction programme for UK. However, the captive breeding for reintroduction programme for Chester Zoo didn't continue due to lack of new release sites and as a result the Studbook did not materialise. Marwell Wildlife continues with the captive breeding programme in collaboration with Amphibian and Reptile Conservation Trust and have recently supported a PhD examining protocols around reintroduction of the sand lizard (examining pre, during and post-release elements) (Gardner, 2021) .

2.5 Behavioural Enrichment

Food presentation: Varying food items and food delivery; scatter feeds, targeted feeds and utilising slow-release feeders would all provide a certain level of enrichment to these lizards. Also varying these feeding methods would ensure that individuals, within a group are more likely to have the opportunity to feed.
Furnishings: As this is a naturalistic exhibit dependent on locally sourced vegetation and substrate, extra furnishings for the benefit of the lizards must be carefully considered. Pieces of slate and natural cork bark may be introduced as visual barriers and basking opportunities. Water containers may be

strategically placed around the exhibit although it's not likely that they will be utilised by the lizards if it is an outside area.

Angles of dune system: In a naturalistic setting the dune system should have angles of elevation to allow maximum exposure to sunlight. This may be replicated in an indoor vivarium by sloping substrate over built up furnishings and placing spot / heat lamps directly over the highest points of the slopes. By creating these elements for the lizards in their environment will maximise options of environmental stimuli and promote natural behaviours.

2.6 Handling

2.6.1 Individual Identification and Sexing

Adult males and females are sexually dimorphic by colour, see figure 33. Males are various shades of green and females are brown. Hatchlings are more difficult to assess as they all appear brown after emerging from the egg. From the summer post-first winter males eventually start to show the greenish tinges on their flanks, around two months of age, which sets them apart from the females. The colouring for the males may vary a lot which makes individual recognition easier but for ease of identification, photographs and implanted passive integrated transponder (PIT-tags) may be appropriate to distinguish animals. Recommendations, taken from Divers et al. (2005) for PIT tagging lizards with a snout-vent length (SVL) less than 12.5cm, are to implant intracoelomic (WSAVA, 2020) or subcutaneously on the caudal half of the left flank (BVZS, no date).

Figure 33. Showing sexual dimorphism in *Lacerta agilis* (First-nature, 2020).

2.6.2 General Handling

Animals should be handled with care and only when essential. For example, for general health checks, collecting morphometric data or transport to another facility. As a rule, animals subjected to these forms of handling do not become over-stressed if they have been long-term captives.

2.6.3 Catching/Restraining

If animals are kept in a naturalistic environment with a lot of vegetation, it would be prudent to select a suitable moment when the animal is in an open space. Capture using herpetology nets or by hand (Di Gia & Sindaco, 2004; Ekner-Grzyb et al., 2013; Dudek et al., 2015 & 2016) should be concise so that the animal is restrained in a way so as not to apply too much pressure to any parts of the animal's body. The tail should be avoided during capture as there is a high likelihood that it may be dropped. Juveniles may be gently picked up by applying pressure to the mid-section of the body, just behind the forelegs, and wrapping the thumb and first two fingers together in a pinched position. For adult specimens, capture similarly but carefully hold the tail just behind the hind legs for extra support. Adjust your hand position accordingly so that both handler and lizard are comfortable and stress free (see figure 34).

Figure 34. Showing safe restraint of *L. agilis* (Chester Zoo).

2.6.4 Transportation

Ideally sand lizards should be transported in a well-ventilated plastic container with natural substrate and appropriate refugia like cork (carefully placed to avoid shifting that could squash animals) and grasses, to make the animals at ease during transportation (see figure 35 & 36). If animals are being transported during the non-hibernating months, temperature ranges from 18^{o}C - 22^{o}C should be maintained. Several animals may be transported together in one container and for a maximum of 6 adults the minimum measurement for this should be 30cm x 30cm x 30cm. For the same sized container, a maximum of 12 juveniles or 20 hatchlings may be transported. Other type of transport is using soft breathable cotton bags (e.g. similar used for bats, birds and small mammals) keeping the animals in the darks and reducing stress.

Figure 35. Showing transport container used by Chester Zoo, UK.

Figure 36. Showing an aerial view of transport container used by Chester Zoo, UK.

2.7 Veterinary: Considerations for Health and Welfare

Animals should be examined visually daily. Any unusual behaviours should be noted and monitored. The eyes should be clear and bright. The scales should be in alignment with the body i.e. no lifted or protruding scales. The mouth should close neatly so that both jaws are in alignment.

Regular observations will indicate any health issues. Adult females are likely to incur mating injuries which will leave scarring but this is natural and unlikely to require veterinary attention. The loss of tails or tips of tails may occur in extreme mating rituals between male and female, or, combat between two males, but again unlikely to require veterinary attention.

Routine health screening should be undertaken including of captive breeding adults, and hatchlings prior to reintroduction (for example using Disease Risk Analysis and Health Surveillance documentation such as Shadbolt and Sainsbury, 2021).

Skin sloughing needs to be monitored when managing the species in indoor vivaria as the humidity levels need to be well balanced.

New specimens coming into an institution should be isolated from the rest of the collection during quarantine period. In addition, new animals should be subjected to suitable screening based on recommendations from the institution's veterinarians.

There are a number of studies documenting infestations of the ectoparasite *Ixodes ricinus* on *L. agilis* (Bauwers *et al*, 1983; Gryczyñska-Siemiatkowska *et al*, 2007; Foldvari *et al*, 2009). *I. ricinus* is a three-host tick species, distributed primarily in Europe. It is involved in the transmission of a large variety of pathogens of medical and veterinary importance including e.g. tick-borne encephalitis virus and *Borrelia burgdorferi* causing Lyme borreliosis (Ecdc, 2014). Majláthová et al. (2008) took samples from sand lizards captured in different localities including Romania, Poland and Slovakia. From these samples, PCR amplification techniques identified borrelial infections with varied results. *B. burgdorferi* was absent in specimens from Poland but samples tested from both Slovakia (45%) and Romania (57%) were positive for this species. *B. lusitani* was confirmed as present in all biopsies tested.

L. agilis is well studied on the topic of parasite community ecology, sand lizards being an obvious host choice. In a study relating parasites to habitat range, 30 localities of the Ukraine and Bulgaria were sampled. Sharpilo et al. (2015) found 30 helminth species of which only 3 were lizard specialists comprising of trematodes (10), nematodes (13), cestodes (4) and acanthocephalans (3). Similarly, a study carried out by Lewin (1992) found 17 parasitic species including trematode, nematodes and acarines. Of these, again 3 lizard specific species including *Metaplagiorchis molini, Ophionyssus saurarum* and *Plagiorchis mentulatus*. Other parasites were considered more generalist species. Although, *I. ricinus* has been a reason for transmission of pathogens, it is not proven to be a vector of blood parasites such as Haemogregarinid (Apicomplexa and Adeleorina). Research by Majláthová et al. (2010) investigated the presence of blood parasites present in both common lizards *(Zootoca vivipara)* and sand lizards in western Poland. There was a significant difference between the two regarding frequency resulting in *l. agilis* having less than the *Z. vivipara*.

Salmonella are also a consideration regarding handling and keeping of any reptiles. Studies cover this topic more regarding captive pets such as *Trachemys scripta* spp. in the interest of human health. One study carried out to sample wild sand lizards in Poland to screen for Salmonellosis. Out of 38 lizards swabbed and faeces analysed, 24% proved positive for the presence of *Salmonella enterica houtenae*. Due to this subspecies being rarely encountered as a threat to human health, the paper concluded that this was not of great concern, but further research was needed (Dudek et al., 2016).

On the death of any specimen, a detailed post-mortem should be performed as soon as possible. All dead specimens should undergo a gross post-mortem, histopathology and faecal parasitology, and all samples should be preserved for further investigation or future studies (Lopez, pers. comm.). This information could provide vital information that could be used to safe guard other specimens.
See appendix 1 for sample post-mortem form.

Section 3: References

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Section 4: Appendix 1

Sand Lizard (*Lacerta agilis*) post-mortem examination form

Path No.: ……………. ZIMS/ ID No.:……………. Species:………………………………………

Tag/chip No.:………………………… Age: Hatchling / juvenile / young adult / adult / old adult

Sex:………….. Weight:…………g SV length:........... mm

Hatched:…../…../…. Found dead:…../…../…. Post mortem:…../…../….

Samples saved: (Formalin, Frozen, Alcohol)

	Fm	FR	OH
GIT contents			
Stomach			
Pancreas			
SI			
LI			
Liver			
Heart			
Spleen			
Lung			
Trachea			
U. Bladder			
Kidney			
Adrenal			
Testes/ovaries			
Oviduct			
Head			
Sp. Cord			
Muscle			
Skin			
Carcase			
32 ectoparasites			

Samples sent for laboratory tests:

Parasitology:

Bacteriology:

Histology:

Other (virology, etc):

Provisional Gross Diagnosis:

Prosector:……………………………………………

Reported to Vet? Phone call / staff present at PM / written note Date:…../…../…..

Note: collect samples from all organs labelled in grey in the table. Frozen samples must be always maintained frozen. Thawing and refreezing samples will damage them.

For fixing tissues in formalin or Alcohol use a ratio of 10:1 fixative to tissue by volume and ensure all pieces are a maximum of 10mm cube. Prior to sending samples in formalin or alcohol, remove as much fixative as possible while maintaining tissue wet, close lid well. Wrap up pot in sufficient tissue paper to absorb any spillage and double bag.

Take pictures at all stages of examination including any lesions observed. Add a ruler for assessment of dimensions.

Condition score: Obese / fat / good / thin / emaciated

State of preservation: Good / fair / poor / marked autolysis

Storage since death: Fresh / Refrigerated / ambient temperature / frozen / fixed with:

History and clinical signs

External observations including ectoparasites, skin condition, external lesions:

Skin:

Eyes:

Other:

Specific Internal Macroscopic Pathological Findings:

Alimentary system

Buccal cavity and tongue:

Oesophagus:

Stomach (and contents):

Small intestine (and contents):

Large intestine (and contents):

Rectum, cloaca:

Liver:

Pancreas:

Muscular and skeletal apparatus

Cardiovascular System

Heart: Other:

Aorta:

Respiratory System

Nares/rostrum:

Trachea:

Lungs:

Lymphatic System

Thymus:

Spleen:

Urinary System

Kidneys and adrenal:

Ureters:

Bladder:

Reproductive System

Oviducts / Ovaries:

Testes:

Nervous System