# A study of the common lizard (*Zootoca vivipara*) population of the Grenspark Kalmthoutse Heide

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## Abstract

#### English

The IPCC reports not only reported higher temperatures in the last decades but also modeled even higher temperatures and droughts for the future. This has a worldwide impact on natural populations, mostly negatively. Ectotherm populations face higher extinction risks considering their vulnerability to those changes. Being already a rare species in Flanders, the need to understand both the situation and the risk of the native populations is raised in priority by local nature conservationists. In this thesis, three different heathlands at the Grenspark Kalmthoutse Heide are studied to determine the population densities of the model species *Zootoca vivipara* and how climate change affects their numbers. This has shown that wet heathlands are the preferred habitat for the model species, as double the number of lizards has been observed compared to dry and dried-up heathlands. Direct impacts of climate change also overshadowed indirect impacts, as prey availability was less optimal in wet heathlands, the lizard's preferred habitats. However, soil moisture, as well as temperature, had a greater impact on prey availability than it had on the population numbers.

#### In Layman's terms

Climate change is one of the main threats to populations, especially reptile populations. As the temperature continues to rise, and droughts become increasingly common, their impact needs to be understood to better protect the affected populations. Research on both droughts as well as hotter environments has become a hot topic in literature. However, studies on both drought and elevated temperatures on local reptilian populations are rare. Local reptilian populations are quite rare in Flanders, making studies on them less evident, but arguably even more needed. In this thesis, the local population of the common lizard *Zootoca vivipara* at the Grenspark Kalmthoutse Heide has been studied. The relative numbers in three different habitat types were studied in hydrological conditions. The found population numbers are then connected to soil moisture conditions and temperature, as well as available food biomass. Wet heathlands habitats contained roughly double the number of specimens, due to their better soil moisture.

## Introduction

#### Climate change and ectotherms

According to the Intergovernmental Panel on Climate Change (IPCC, 2014), the average temperature at the surface of the earth was higher in each of the last three decades than in any preceding decade since 1850. Evidence shows that this rise in surface temperature is caused by anthropogenic activity, mostly due to the emission of greenhouse gases. Global warming is predicted to be between 0.3 and 4.1 °C for 2100 under different scenarios. Of these, the more optimistic scenarios seem no longer realistic, because the necessary actions in that direction have not been undertaken (IPCC 2014, Topic 3).

The observed increase in surface temperature is accompanied by changes in precipitation patterns that are affecting water resources in terms of quantity and quality. On average, the world is predicted to see a net increase in rainfall, but in some places (including western Europe) the amount of water will decrease due to regional drying (Collins et al. 2013).

These changes in temperature and precipitation will most likely be imposed upon the world's biota. Ectothermic animals are considered particularly vulnerable to climate warming (Paaijmans et al. 2013). Ectotherms are highly dependent on temperature and have integrated this dependence into their daily cycle (Folguera et al. 2011). As climate change will not only alter mean temperature, but also daily temperature ranges (Easterling et al. 1997,2000), ectotherms seem especially at risk.

#### The influence of temperature

Sinervo et al (2010) have argued that shifts in temperature due to climate change affect organisms in all biomes and ecosystems, although not equally. Populations (and ultimately species) can mitigate the effects of temperature change in two ways. Given enough time and dispersal capacity, they may shift to more favorable thermal environments, or they may adjust to new climatic conditions by adaptation, behavioral plasticity, or physiological plasticity. If both responses fail, the result will be demographic collapse and extinction.

Lizards, being ectotherms, require solar radiation to attain physiologically favorable body temperatures. However, beyond the optimal temperature, physiological performance will diminish rapidly and if the lizard does not retreat into a cooler place, it will reach a critical thermal maximum temperature above which it will die. Environmental conditions determine the daily time window during which lizards can attain body temperatures that allow activities such as foraging and mating. Under the current climate change, these time windows have narrowed excessively. Lizards that fail to respond to these changes cannot acquire enough energy for maintenance, growth, and reproduction. Such populations and species thus face an increased risk of going extinct (Sinervo et al. 2010). Sinervo et al (2010) further states that, according to their models, the probability of local extinctions in species can account for 6% by 2050, and 20% by 2080. Range shifts would only trivially offset losses, because widespread species with high physiological active body temperatures shift to ranges of endemics, thereby accelerating their demise. Even though the global efforts to reduce  $CO_2$  may avert 2080 scenarios, the projections for 2050 are unlikely to be avoided according to them. They, therefore, conclude that lizards have already crossed a threshold for extinction. Constraints arising from the genetic architecture of thermal physiology together with costly genetic tradeoffs (e.g., growth) and the low heritability for physiological active body temperature adaptation (h<sup>2</sup>=0.17 for their model genus *Sceloporus*) were given as causal drivers.

#### The influence of drought

In contrast to temperature, we know far less about the role of water budgets in limiting terrestrial ectotherms, including lizards (Tracy et al. 2014). Most studies published on the effect of climate change on Lacertilia are focused on the increasing temperature aspect, and less on drought. With a literature review on Web of Science, 635 papers with the keywords "climate change", "lizard" and "temperature" were found. However, when "temperature" was replaced by "drought" or "precipitation", only 100 papers totally were shown, most of which were on the unpredictability of droughts and precipitation.

The IPCC (2014) foresees strong changes in precipitation patterns, with some regions becoming wetter, and others drying out. Prolonged dry conditions are likely to threaten many reptiles in multiple ways. First, they may cause excessive water loss from the body. Evaporative water loss is exceptionally low in "dry-skinned" ectotherms (e.g., reptiles), but they still lose more than 70% of their evaporative water through their skin (Eynan and Dmi'el 1993) and even more in absolute amounts in their feces (Porter et al. 1973). Dehydration due to direct effects of less precipitation leads to changes in behavior to find more water in even unfavorable conditions (Davis and DeNardo 2010) or avoid further water loss, even if the other conditions are favorable (Nagy 1972). According to Kearney et al. (2018), changes in water availability may affect the windows of activity and the distributional patterns of ectotherms even more than the temperature changes. This work will focus on the consequences of less precipitation demographic, and the overall fitness of the model species due to climate change.

Second, climate change is credited to have a progressively increasing impact on environmental degradation (Warner et al. 2009). According to Hill & Caswell (1999), habitat degradation leads to an increasing extinction risk of the species inhabiting it, but this increase was variable with a pattern of habitat loss.

Third, the changes in a habitat not only affect the species studied within but also the food available for that species; in this case, Arthropoda, which are also ectoderm and thus vulnerable in a comparable way as lizards. Chown et al. (2007) argues that climate change could have negative consequences for indigenous species, but negligible effect on invasive ones.

Compared to e.g. birds and flying insects, reptiles are more likely to be threatened by these local changes in precipitation patterns due to their generally reduced dispersal capacity and high dependence on local climatic conditions (Araújo et al. 2011; Araújo et al. 2006). This makes them very vulnerable to local changes and highly hinders their capacity to flee to more favorable climates

#### The influence of unpredictability

Masó et al. (2020) found that the predicted decrease in an environment's predictability, according to the IPCC 2013, could exacerbate the rate of currently observed population decline and extinction. Not only does climate change research predict a decrease in the predictability of precipitation (Collins et al. 2013), but it can also have negative effects on species viability, population growth, and individual performance (Ashander et al. 2016). However, the majority of studies on this subject are theoretical (Ashander et al. 2016) or correlate environmental predictability with life-history traits (Siepielski 2017). The few experimental studies on this topic produced extreme events that severely affected life-history traits (Cherwin and Knapp 2012). Even though it has been predicted that the effect of environmental variance may depend on mean environmental conditions (Lawson et al. 2015).

In this thesis, the impact of drought and temperature will be looked at as direct and indirect factors on the population of the model species *Zootoca vivipara* (Jacquin, 1787).

#### The current situation of Zootoca vivipara

*Zootoca vivipara* is protected under government decree "Natuurdecreet", also known as "Soortenbesluit" under category 1 (Levendbarende hagedis - Ecopedia) and is classified as "Rare". This is however the case for all indigenous amphibians and reptiles in Flanders, as they are protected on a class-, and not species-based level. There is, however, no "Soortenbeschermingsprogramma" (a plan specific for a species on how to protect and restore a species) for *Z. vivipara* (Lijst van alle SPB's - ANB).



*Figure 1: Yearly total Zootoca vivipara observation made in the Grenspark Kalmthoutse Heide from 2011 until 2021. All data was obtained from Waarnemingen.be with permission.* 

Due to the lack of long-term studies monitoring the population densities of lizards in Flanders, there is no scientific evidence on the current local situation. However, individual-based observations, as well as observation collecting sites such as "Waarnemingen.be" indicate a decrease (Fig. 1). Local experts such as André Van Hecke and local nature managers such as those from the Grenspark Kalmthoutse Heide (study site) notice a decrease in observed number in and outside the study area, but no known local cause is yet proven, nor examined.

#### Zootoca vivipara

Of all reptiles, the common lizard (*Zootoca vivipara*) has the widest distribution. The species inhabits much of Europe and Asia, including habitats close to the Arctic Circle (Herczeg et al. 2003) and highlands up to 2000m above sea level (Beebee and Griffiths 2000). (Fig. 2). Despite this, relatively little is known about its current distribution, population numbers, conservation status, and local threat levels (Vacheva and Naumov 2020).

The species was formerly known as *Lacerta vivipara* until the genus *Lacerta* was split into nine genera by Arnold, Arribas & Carranza (2007). Now, it is classified under the genus *Zootoca* (still part of the Lacertidae), where it is the only species. Both its genus and species name mean "live birth," in Greek and Latin respectively (Diaz 2016).



Figure 2: World distribution of Z. vivipara with recognized lineages (in colors) and corresponding subspecies: A) eastern oviparous clade; B) western oviparous clade; C) first central viviparous clade; D) eastern viviparous clade; E) western viviparous clade; F) second central viviparous clade. From Diaz 2016, based on Arribas (2009) and Surget-Groba et al. (2006).

#### Appearance and subspecies

Zootoca vivipara is a rather small diurnal lizard (adult snout-vent length approximately 65 mm and a tail up to 110 mm) that mainly feeds on small arthropods. Individuals can be classified into three distinct age classes: juvenile, subadult (yearling), and adult stage (Fig.3). Sexual dimorphism is less pronounced in juveniles. At the juvenile stage, the sex can be determined by ventral scale count (Lecomte et al. 1992), a trait that remains constant throughout the lizard's life (Bauwens and Verheyen 1985). From the subadult stage onwards, at least some secondary sexual traits are recognizable. The females are slightly larger and have a yelloworange, black-spotted ventral side. In contrast, the female's ventral side is paler and lacks black spots. Both viviparous (bringing forth live young) and oviparous (egg-laying) forms can lose their tail to escape a predator (Grzimek 1971). According to Herczeg et al. (2004), the total tail autotomy causes a considerable (19%) decrease in the experimental lizards' bodyweight. However, this has no significant influence on temperature regulation. They suggest that thermoregulation is not significantly affected due to the (small) size of *Z. vivipara*. Their ability to lose their tail would thus not be a factor in this research.



Figure 3: Dorsal and ventral pictures of the juvenile (a, d), subadult (b, e), and adult stages (c, f) of Z. vivipara. The specimens above (a, b, c) are females, those below (d, e, f) males. Note the sexual dimorphism in head size and vent color patterning in adults. Diaz 2016

Despite its Latin name, *Z. vivipara* is one of the few reptiles that exhibit a bimodal reproductive system: some populations are viviparous (live-bearing), while others are oviparous (egg-laying) (Fig. 2). Viviparity presumably evolved under cold environments and allowed the species to colonize new areas of unfavorable climates (Rodriguez-Diaz and Braña 2012), where it hibernates from October until March/April, depending on the weather (Strijbosch 1988). The two main clades with the biggest geographical range are the eastern viviparous clade (*Z. v. sachalinensis*) and the western viviparous clade (*Z. v. vivipara* = *Z. v. pannonica*). The remaining four lineages have smaller geographic ranges and are all situated in mountain ranges: the eastern oviparous clade (*Z. v. carniolica*), the western oviparous clade (*Z. v. louislantzi*), the first central viviparous clade (*Z. v. vivipara* = *Z. v. pannonica*) and the second central viviparous

clade (Z. v. vivipara). (Fig. 2) (Arribas 2009 & Surget-Groba et al. 2006). In 2009, the latter was described as a new subspecies: Z. vivipara louislantzi Arribas. The fieldwork is exclusively done on individuals of the "western viviparous clade" (Zootoca vivipara vivipara), present in the region of the study area. (Diaz 2016)

#### Predators of Zootoca vivipara

Zootoca vivipara has many predators. Despite the many defensive behaviors such as swimming (SWT: Common Lizard) and tail-autotomy (Herczeg et al. 2004), predation is one of the major causes of death (Maslak and Pasko 1999). Newborn Z. vivipara are prey to some invertebrates (Jehle et al. 1996), which will later become the prey of the adult lizards (Strijbosch 1992). Vertebrate predators include larger frogs, other bigger lizards (also from the same species), birds, mammals, and snakes (Maslak and Pasko 1999).

As all the fieldwork was done at the Grenspark Kalmthoutse Heide (see Materials & Methods: Study Site), two of Z. vivipara's known predators are relevant to mention: the common viper (Vipera berus) (Fig. 5) and the smooth snake (Coronella austriaca) (Rugiero et al. 1995) (Fig. 4).





Both are quite uniquely present at the park. (Vipera berus). Picture taken by Their influence was out of scope during

Foregoing research by Thoen et al.

Figure 4: Adult common viper Rollin Verlinde - Vilda.



Figure 5: Adult smooth snake (Coronella austriaca). From Ravon, © Jelger Herder

(1986) not only confirmed that Z. vivipara was prey for both the common viper and the smooth snake, but also that the former chemically detected the presence of the latter and avoided those areas. It was not proven that those cues are interpreted



Figure 6: Adult grass snake (Natrix natrix). From as precise predators but show that at least the distinction Ravon, © Jelger Herder

between predatory species and non-predatory species (grass snake Natrix natrix, Fig. 6) was made.

#### Habitat preferences

Zootoca vivipara is an active thermoregulator, regulating its body temperature like a typical shuttling (back and forth) heliotherm (Avery, 1976). According to Gvozdik (2002), the species is one of the most accurate thermoregulators among reptiles. The cold-blooded characteristics of Z. vivipara are reflected in its habitat preferences and behavior: it is typically found in structurally complex habitats, with a mix of open areas (necessary for basking) and densely vegetated parts (necessary to cool down and hiding).

According van der Werf et al. (2012), the common lizard prefers *Calluna* and *Calluna-Molinia* heathland, where it avoids the grazed sections. It is also considered a characteristic inhabitant of wetter heathlands (Strijbosch, 1988; Glandt, 2001). In those heathlands, purple moor grass (*Molinia caerulea*) often tends to become dominant, if not stopped by management. The lizard's association with purple moor grass (*Molinia caerulea*) was both expected and unexpected according to van der Werf et al. (2012). Purple moor grass degrades heathlands, making it an unexpected variable to increase lizard populations (Corbett & Tamarind 1979). However, by degrading heathlands, it will add more variation to the landscape, as well as provide excellent hiding spots in the dense tussocks, making it also an expected association.

#### Prey preferences and population dynamics

*Zootoca vivipara* is considered in an intermediate position in the food chain (Carretero 2004), as it is both a prey (as discussed previously) and a predator itself (Žagar et al. 2015). Due to their broad range, plenty of research on their diet has been done over the years; in The Netherlands (Strijbosch 1992), Finland (Kopponen & Hietakangas 1972), France (Pilorge 1982), England (Avery 1971), Ukraine (Shcherbak & Shcherban 1980), Russia (Kuranova et al. 2005) and Bulgaria (Vacheva and Naumov 2020). Most of these studies, however, have been carried out in far Northern regions or alpine habitats and will therefore not be discussed, as their prey communities may differ considerably from those in this work's study area.

Strijbosch (1992) analyzed fecal pellets to determine the diet of the Dutch population in lowland heathlands. He concluded that *Z. vivipara* 's diet consists of 34.5% Arachnida and 25.0% of Hemiptera. Only 5.6% was Coleoptera. Vacheva and Naumov (2020) did similar research in Bulgaria, and found sporadic remains of *Z. vivipara*, indicating cannibalistic tendencies can occur. They confirmed that Arachnida was one of the main food sources, as well as Hemiptera. Even though both pieces of research are geographically distant, their outcome was highly similar. Excluding cannibalism, Arthropoda is the main food source, as proven by the multiple studies cited before. The fact that even heavily chitinized prey can be part of the menu (Vacheva and Naumov 2020, Strijbosch 1992) comes as a surprise as they are energetically unprofitable and avoided by most lizards (Vacheva and Naumov 2020). It is, however, observed in other lacertid species in more arid habitats to be a more prominent found source (Adamopoulou & Legacis 2002). The addition of Gastropoda to their diet is however not uncommon (Avery 1966).

García et al. (2009 & 2010) have done well-documented research about heathland Arthropoda and found that the most abundant arthropods present in North Iberian heathland were ants (Hymenoptera, Formicidae), beetles (Coleoptera), spiders (Araneae), and harvestmen (Opiliones), who accounted for 35.0%, 30.9%, 19.6% and 6.8% of the total catch, respectively. They showed that their total arthropod abundance was not significantly affected by the grazer species (Cashmere and Celtiberi goats), herd size, or vegetation type. Species compositions were however significantly affected. Those results are like those of Buchholz et al. (2013), who examined Carabid composition in German heathlands. As the available prey and the consumed prey are quite similar, it confirms that *Z. vivipara* is not a specialist and eats whatever arthropods it can get, with some exceptions like ants.

Low food availability can influence the viability of *Z. vivipara* populations in multiple ways. It may reduce the postnatal growth rate, which will postpone maturation, causing females to start reproducing in their third year instead of their third year. In addition, smaller females typically produce smaller clutches, and offspring of smaller size (Bauwens & Verheyen 1987, Wang et al. 2017). Prey scarcity may also jeopardize the survival of juvenile and (sub)adult stages by impinging on their immune competence (Wang et al. 2017).

As mentioned before, males and females are slightly different in colors and both sexes can have a range of colors, which according to Díaz (2016) had a noticeable effect on mating choice, indicating sexual selection. In his model, the reproductive success of males was best explained when yearling morph frequencies, as well as the adult color morph frequency, were considered, indicating their vital role in mating success. The model also indicated that habitat humidity was an important determinant of male competitiveness and the observed patterns of sexual selection, suggesting that environmental factors can affect the maintenance of genetic variation.

According to Bauwens et al. (1986), the life history varies considerably among populations. Furthermore, Bjørnstad et al. (2004) proved that multiple non-significant survival differences and other minor changes can together have major consequences for populations (Bjørnstad et al. 2004) and that those may not be anticipated by studies of short duration and studies analyzing a few parameters. Therefore, general widespread studies can be inaccurate, and conclusions on population demographics are limited to the studied population.

#### Zootoca vivipara and climate change

When a vertebrate population experiences extreme heatwaves, lower corticosterone secretion leads to behavioral responses and changes in water balance that could represent an adaptive response to avoid overheating, dehydration, and premature death (Jessop et al., 2013). Dupoué et al. (2018) found that wild lizard populations that are more frequently exposed to elevated temperatures express lower plasma corticosterone (stress hormone) during rest. They suggested that this adaptation may help downregulate maintenance costs at rest and energy expenditure during activity, saving energy and water. Less adapted populations are characterized by high metabolism at rest and strong behavioral activity in warmer environments. The inadequate adaptation of the population to those circumstances could induce diverse physiological costs such as higher energy expenditure and a higher risk of dehydration, which eventually has negative impacts on survival and reproduction (Bestion et al., 2015).

More so, a study by Gradient et al. (2001) on the influence of temperature in *Z. vivipara* populations indicated that there was a negligible variation in preferred body temperature, critical thermal minimum, critical thermal maxima, and the tolerance range among four populations of *Z. vivipara* living along an altitudinal and climatic gradient. This supports the view that temperature ranges (preferred, minimal critical, and maximal critical body temperature) are conservative. It would suggest that these lizards cannot respond to long-term temperature fluctuations by changing thermal sensitivity through acclimation or adaptation, due to the lack of initial variation. They reasoned that the species initially does not need genetic variation in this aspect due to being excellent thermal regulators.

Subsequently, on the aspect of precipitation, Dupoué et al. (2017) did geographically widespread research on *Z. vivipara* populations, investigating the variation in water balance (hydration state and water loss) due to water access and climate. They concluded that the rate of standardized evaporative water loss (SEWL) was similar between the sexes, but males were more dehydrated. They also found that the rate of SEWL was higher in individuals from populations with access to water, but that no population suffered from dehydration. They argued that the differences were caused by permanent and consistent differences among populations related to altitude, slope orientation, and habitat type. This would make the different populations however vulnerable to rapid changes (e.g., climate change). Plasma osmolality and the rate of SEWL were found to not be correlated with environmental humidity but positively correlated to environmental temperature.

Finely, Masó et al. (2019) demonstrated that reduced precipitation predictability (by adding extra precipitation at random or at regular moments) negatively affected the growth of *Z. vivipara* yearlings and the body condition of juvenile females. Furthermore, egg-laying timing was also negatively affected, as eggs were earlier deposited in more predictable conditions. Changes in precipitation predictability significantly affected the adult growth rate and body condition of adult males, but all effects were compensated throughout the experiment, resulting in a full recovery at the end of their growth. In a later study, Masó et al. (2020) also proved that reduced precipitation predictability also decreased the survival rate of both juveniles and adults.

#### The effect of climate change on Zootoca vivipara's prey: Arthropoda

The influence of prolonged periods of drought on arthropod assemblage is of great concern to my study. Based on observations, Remmert (1981) reasoned in some of his hypotheses that arthropods in moist conditions are relatively smaller than those in dry conditions. However, moist conditions also lead to more specimens. In those respects, total biomass can fluctuate independently of precipitation. He did however not test his observations, which thus remain mere hypotheses.

As mentioned before, climate change also affects habitats themselves. Buchholz et al. (2013) examined the current habitat quality of dry sand ecosystems (heathlands) in northwestern Germany using Carabidae as model organisms. They concluded that even though the rarer species are more frequently found in the rare, higher quality habitats, a succession or degradation in habitat does not mean a loss in Carabidae biomass, and thus prey. As Carabidae are known to be useful indicator taxa to analyze shifts in terrestrial ecosystems (Schirmel 2010) their finding gives further incentive for arthropods in general.

A further study on the relationship between plant and Arthropoda biomass and diversity was done by Borer et al. (2012) on five functional groups (forbs, legumes, woody species, and both C3 and C4 grasses). They found no support for a link between plant biomass or diversity to Arthropoda biodiversity. However, Arthropoda biomass was linked to plant diversity, mediated by plant biomass. This mediated effect was of such scale that decreasing the plant diversity artificially, increasing the primary production, lead to a higher Arthropoda biomass. Changes in primary production of the habitat are thus, according to Borer, of higher importance than precipitation or temperature for arthropod biomass.

Lastly, according to Sohlström (2020), climate change reduced arthropod total abundance and diversity but increased arthropod evenness. Detritivores are the most affected in both

abundance and diversity, but as they are not the primary prey group of *Z. vivipara*, this is less concerning for this study. The fact that total abundance is affected, is however problematic considering *Z. vivipara*.

## Thesis objectives

As there is a severe lack of knowledge about the current situation, this thesis aims to estimate habitat-specific population densities in the Grenspark Kalmthoutse Heide, as well as examine the influence drier and hotter climates could have. This will be done by looking at direct factors (drier & hotter) as well as indirect factors (change in available food).

The aim of this work is to examine which could be a driver for the lower population numbers, and how strong their respective influence is:

- Sensors for temperature and humidity will be placed in areas of the Kalmthoutse Heide that were expected to differ in climatic conditions.
- Habitat-linked *Zootoca vivipara* densities will be determined, so that overall population numbers can be better estimated.
- By capturing some specimens, their body mass and length specific to their environments will be known to the population. Based on those numbers, the SEWL can be estimated, examining the direct impact of both temperatures and drought.
- By catching arthropods and determining both their abundance and total dry weight in different habitats, prey availability will be known. This part will be used to determine some of the indirect impacts of temperature and drought.

From these studies, it is theorized that wet habitats have a higher density of *Z. vivipara*, as well as a higher Arthropoda individual and total dry mass. It can also be hypothesized that purely based on the sensors and not the observed habitat types, wetter and cooler environmental conditions lead to higher *Z. vivipara* numbers and more total and individual Arthropoda dry mass. *Zootoca vivipara* numbers would be higher due to more food availability as well as better physical conditions in those environments. Specimen located in habitats that are recently dried out or drying out would also have the most trouble with SEWL and would be both smaller and lighter.

## Materials and Method

#### Study site

The "Grenspark Kalmthoutse Heide" is an extensive cross-border nature reserve. Overall, it is located in between the three large urban agglomerations of Bergen op Zoom, Roosendaal, and Antwerp. It is in both Dutch and Flemish territory, respectively in the provinces of North Brabant (NL) and Antwerp (VL). On the Dutch side, the Grenspark is in the municipality of Woensdrecht. On the Flemish side in the municipalities of Essen, Kalmthout, and Stabroek. Most of the park is protected as Natura 2000 for both birds as a habitat (Fig. 7). (Beleidsplan 2014-2029)



Figure 7: The border of the Grenspark (yellow) with within the area protected under Natura-2000 as part of the Habitat protection (black) and bird protection (blue). Note that on the Flemish side, those borders are highly similar. Beleidsplan 2014-2029

The nature reserve is roughly 60km<sup>2</sup> and consists of heathlands, sand dunes, heathland bogs, and forests. As heathlands and large nature reserves have become guite rare, some of the species found commonly in the area, are considered rare or even endangered. It has iconic species such as the common viper (Vipera berus), smooth snake (Coronella austriaca) (both mentioned before), pine, and stone marten (Martes martes & Martes foina), and the Eurasian eagle-owl (Bubo bubo). It is also home to many rare heathland-dwelling arthropods such as the grayling or rock grayling (Hipparchia semele) and the Alcon blue (Phengaris alcon). (Beleidsplan 2014-2029)

Even though the somewhat resembling species common wall lizard (Podarcis muralis) and the viviparous lizard (Zootoca vivipara) have overlapping distribution, only the latter can be found in the Grenspark Kalmthoutse Heide.

#### Waarnemingen.be

As there are not many previous studies and no monitoring actions done to indicate the abundance or changes of the local population of Zootoca vivipara, the current population situation is unknown. In this thesis, I used two different approaches: observations of the species from the database "Waarnemingen.be" and my own fieldwork. To indicate the changes in population numbers, the observations of the species on "Waarneming.be" in the

last decade will be used (1075 observations). Those will be linked to the wetness of the habitat to unravel possible trends. On the other hand, fieldwork will hopefully indicate links between Z. vivipara population densities, Arthropoda biomass, temperature and soil moisture, and habitat wetness.

Waarnemingen.be collects and verifies the nature observations people send in on a voluntary basis. The last decade of observations from the area was collected *east side of the park is the "Verbindingsweg". Made in Qgis.* 



Figure 8: Open Street map of the area, with the borders of the Grenspark (red line) and all the observations of Zootoca vivipara from Waarnemingen.be. The observations clearly visualise some of the more visited trails within the park. The clear red line on the

(date, coordinates, observer, number of spotted individuals) and plotted in QGIS 3.10.13-A Coruña (Fig. 8) to further analyze. Most of the observations were made by the volunteers of the Grenspark itself, active in various other monitoring programs and qualified to come in the whole area.

In the light of this study, habitat wetness (see further) is of importance. As the observations go back up to a decade, and this information was never noted down, it was impossible to determine the "wetness" of the habitat for each observation. This was done by projecting each location on the Biological Valuation Map (De Saeger et al. 2017) and noting the corresponding general habitat type. These habitats were then translated into wetness as follows:

- 1. wet" = bog, wet heathland, wet grasslands, and channels
- 2. "dry" = dry heathland, degraded heathland, sand dunes, pine forests, oak forests, and calcareous grasslands.

If the observation was made on a road, the "wetness" of the habitats alongside the road was used, provided both sides had the same wetness category. If this was not the case, it was left open. Some habitats were mapped as "degraded heathland" or "heathland" without further indication of whether it was wet or dry heathland. If the patches' wetness was also unknown, the observation was noted down as unknown.

#### Plots



Figure 9: Map of the study area, located north of the province of Antwerp or south of the province of North Brabant. The red line represents the border of the park and the black line the Dutch-Belgian border within the park. Six of the plots are located on the Dutch side (black dots on the left), and nine on the Flemish side (black dots on the right). Each plot it represented whit 2 dots, indicating the exact pitfall locations. Made in Qgis

Within the Grenspark, I selected two areas that each contained dry ("droog", D), wet ("nat", N), and drying ("verdrogend", VER) habitats (one on the Dutch side, one on the Flemish side). Within each of these two areas, I established five series of plots of approximately 15000m2 per plot. Each series consist of a plot located in a dry, a wet, and a dried-up condition, totaling fifteen plots (Fig. 9). All plots are randomly located within their wetness. On every plot, two pitfalls were placed, and sweep samples were taken, to obtain an idea of the available arthropod biomass as food for *Z. vivipara*. On the same plots, lizard counts were held, and one sensor was placed to measure the environmental conditions. Five of the plots were in wet heathland (characterized by *Erica tetralix* and or the appearance of bogs), and 5 in dry heathland (characterized by *Calluna vulgaris*) The last five were in previously wet heathland, but due to changes in water flows, the management or climate, has turned or is in process of being turned into dry heathland (around or in former bogs). All fifteen location-linked variables experienced by multiple plots due to proximity) and are at least 500m apart (Fig.9).

All locations are within the Grenspark Kalmthoutse Heide. The conservation team of Grenspark Kalmthoutse Heide deploys a variety of nature management techniques, but around the plots grazing by sheep (Kempen heath sheep) and beef cattle (Galloway) is the most important one.



Figure 10: Close up of Figure 10, visualizing the plots located on the Dutch side, including the precise locations of the two pitfalls placed in each plot. Made in Qgis.

I chose to study plots in both the Dutch (series 1 & 2) and Flemish (series 3, 4 & 5) part of the area (see Fig. 10, 11 & 12). However, the Dutch part has a limited amount of heathland, causing the plots to be closer to each other. In those cases, the plots were separated by landscape elements such as walking trails with a row of trees and/or ditches. To distinguish between the plots, the wet ones were named N for "nat", meaning "wet" in Dutch. D was used for "droog" or "dry" and VE for "verdrogend/verdroogd" or "drying up/dried up". The plots were named in Dutch but will carry English names or abbreviations in this thesis for easy reference. The numbers indicate the series but have also further meaning for distinguishable purposes. The two pitfalls on every plot received names consisting of the plot number, followed by either 1 or 2 to distinguish the pitfalls. For instance, pitfalls 3D1 and 3D2 are the two pitfalls placed in the "dry" plot of series 3.



Figure 11: Locations of the plots situated on the Flemish side of the park, with precise location of each pitfall placed during this thesis. Made in Qgis.

Wet (N) plots were plots consisting of vegetation indicating wet heathland conditions: bogs, Juncus effusus, and overall wet heathland vegetation. This is, in contrast, to dry (D) plots, which had only alive vegetation that indicated dry heathland, mostly dry heathland vegetation. Dry plots could have landscape elements indicating a wetter time (e.g., ditches), but could not have wet vegetation, healthy or dying at the moment of the study. Dried up or drying up plots (VE) are plots that are all located around the remains of what was a bog. Due to this, the original vegetation was wet, but due to the drop in water quantity over the years, is now losing or have lost its typical wet vegetation and is transitioning to dry heathland. To be classified as a drying up/dried up plot, the plot still had to have the remains of wet vegetation while also having dry vegetation, indicating the transition. In contrast to wet plots, the drying up/dried up plots also have dry vegetation, and the wet vegetation could even be visibly suffering with the new, drier conditions. The plots in the drying up/dried up category are either going from open, dried-up bogs to dry heathland or have lost the battle against grasses and other pioneer vegetation due to drier conditions and are now in the middle of management to aid the transition into dry heathland. The two Dutch VE plots are located at the side where once the bog "Klein meer" was, the Flemish counterparts are located around the dried-up spatula-shaped bog "Langven", now only a dried-up bog bottom. For a complete description of every plot, see Appendix – Locations.

#### Sensors

For the measurements of moisture and temperature, 15 TOMST TMS-4 (Michelská, Czech Republic) sensors were used with the help of Prof. Dr. Meysman. One of those was placed at each plot, next to a pitfall, at the locations shown in Fig. 10 & 11. The TMS-4 dataloggers measure air and soil temperature as well as soil moisture thanks to three temperature sensor units and one soil moisture sensor unit (Fig 12). Their high-capacity lithium battery has a lifespan of approximately 10 years before a recharged battery is needed. Combined with the large memory, capable of 524,288 data recordings, each TMS logger is autonomous and requires minimal maintenance. The data was stored in the sensors themselves to be downloaded later as the wireless transfer was not available for the duration of the TOMST



Figure 12: Photograph of the sensor model used, with the location of the 4 sensory units and other important parts. TOMST

research. The models I got lacked the middle shield present in Fig. 12. The whole design resembles an herb, and the strategic sensory sectors are of relevance for most organisms as they represent the shades of the leaf's top structure of the plant, the soil surface region, and the region of the in-ground roots. The temperature sensor unit is a MAXIM/DALLAS Semiconductor DS7505U+, with a resolution of 0.0625 °C and with an accuracy of ±0.5 °C. The soil moisture sensor unit uses the time-domain transmission method. Briefly, high-frequency-shaped electromagnetic pulses (ca 2.5 GHz) are sent through the device. When reaching the counting unit (the sensor unit itself), another pulse is sent. This occurs within a 640-microsecond time window. Pulses are counted as raw moisture signals (50–200 MHz). The number of pulses counted is directly related to the soil moisture content, with higher soil moisture reducing the count of pulses received. The counts are then inverted and scaled to the numerical range of 1–4095 (raw TDT data), referred to as (raw) soil moisture counts (Wild et al. 2019).

With the aid of the included digging tool (a steel pin), the green part of the sensor was put into the ground so that temperature sensory unit two was just located at soil surface level. Due to technical difficulties, the sensors were only placed together with the first lizard counts and pitfalls in July (two months after the beginning of the other measurements).

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The preset interval of 15 minutes was retained, as advised by the producer. TOMST suggested to use protective cages with the sensors, as they are vulnerable to physical damage. The biggest disadvantage of this approach is that they made the sensors easier to notice by passers-by (and to be stolen). To limit this noticeability, dead branches were placed over the sensors as a protective barrier, which also caused them to be protected from large mammals.

From 19/07/2021 to 6/11/2021, data was collected by the sensors. Only the data collected from July to September was used in overall models, as this marks the end of the field period. Data outside this period could not be used to compare with my other data and was thus not of importance in this aspect. The whole dataset was however still used for conclusions on the abiotic variables. Data was collected after the field period and transferred by TMD adapter by a free software published by the TOMST (Lolly Manager (1.29)).

#### Prey densities

Pitfall trapping and sweep sampling were the two methods used to sample the density of the arthropod fauna in all the plots: pitfall trapping is the traditional method used to examine the substrate-dwelling fauna of heathlands (Garcia et al. 2010, Buchholz et al. 2013, Usher 1992), but sweep sampling may capture airborne arthropods more readily. Two pitfalls were placed on every plot, as indicated in Fig. 11 & 12. As the fieldwork was long going and the falls would continuously be placed at the same spot, a



Figure 13: Pitfall similar to those placed during the fieldwork. The rand of the pitfall is slightly lower than the ground level to make sure insects fall in instead of going around. ©WordPress

short PVC tube was placed in the ground to keep the hole open. To prevent unnecessary casualties, this was covered up or filled with sticks during the non-active trapping periods. In doing so, all possible measures were carried out to prevent the entrapment of organisms in the PVC tubes during non-measurement periods. The pitfall itself was made of a plastic cup (11cm diameter top, 9.5cm diameter bottom, 7.5cm high) and filled with soap suds by roughly two centimeters. To prevent the unlikely trapping of amphibians, reptiles, and small mammals, a plastic bottlecap was also placed in the cup, allowing such animals to climb out of the cup. Pitfalls were placed once every month and left open for seven consecutive days. I tried to restrict the trapping of arthropods to weeks with favorable weather conditions, i.e., periods that would allow lizard foraging activity. Therefore, I avoided weeks with cold and wet weather, if possible.

The locations of the traps were chosen randomly in the plots and placed around 50 meters apart to have an as complete sampling of the area as possible. The only exception was plot 3N, due to this area being sensitive to flooding, potential pitfall locations were limited and caused the distance between the two pitfalls to be only 20 m.

In the context of this research, the arthropod community catch is the food source of the species in which I am interested. As of yet, there is no preference known in *Zootoca vivipara*, thus all arthropods were of equal importance. The only exception to this are airborne ants. These ants are known to not be consumed by *Zootoca vivipara* (Pilorge 1982) and were subsequently excluded in the sorting process of dirt, vegetation, and water. This allowed us to account for arthropods by total dry mass and numbers.

As flying insects are also a fit food source for *Zootoca vivipara* but are far less likely to be caught in pitfalls, a dragnet was swept twenty times at every plot per month. This was only done when the weather was favorable, and the vegetation was dry. Due to those requirements, and the aim to do it in the same week as the pitfalls were placed to avoid time bias, some plots could not be sampled every month.

The obtained samples were put in the freezer (Öko Arctis Super, Nürnberg, Germany) for a minimum of 12 hours at -18°C to kill every insect that was still alive. After that, the arthropods and worms were separated from the dirt and other non-interested materials and organisms (including non-flying ants), counted, and conserved in 70% Ethanol. Once every two months all caught insects were transported to the University of Antwerp Campus Drie Eiken (Universiteitsplein 1, 2610 Antwerp). There, the insects were manually separated from the conservation fluid, dried for 24h at 70°C (Binder B 28, Tuttlingen, Germany), and then weighed per pitfall/sweep sample (Mettler Toledo XS205 Dual Range Balance, ±0.01mg, Columbus, USA).

During the collection of Arthropoda (hundred-fifty pitfalls and seventy-five sweep samples), multiple samples were lost or not even taken due to wet weather making the use of a dragnet impossible, the emptying and/or destruction of pitfalls by birds, sheep, and Galloways. Those were not left out, but rather noted down as N/A, for statistical purposes. Pitfall data was collected from 5/5/2021 until 14/9/2021. There was a dataset consisting of the measured total dry mass per sample, and one of the individual average dry masses, obtained by dividing the total dry mass by the number of arthropods dried in that sample.

#### Lizard counts

At least once every month, every plot was visited to determine the number of present lizards. This was done on days on which weather conditions allowed lizards to be active, i.e., with sufficiently high temperatures and limited amounts of clouding. Due to this, in combination with very numerous prolonged periods of wet and cold weather ("Belgian summer"), not every plot was counted every month. At every count, the cloud coverage was noted down (on a scale of 8, 0 being no clouds and 8 being a full sky coverage), the wind force in Beaufort (based on observation), and the local temperature according to The Weather Channel (https://weather.com/), the date, and if it was in the morning or afternoon, as well as who was participating in the count. Loïc van Doorn helped with the first three lizards count in May 2021, subsequent counts were done alone. As the counts were thus mostly done by only one person, there is no observer bias to test, making this variable non-significant

Due to the lack of previous studies considering the local population and the open nature of the dominant habitat type (heathland), plot counts were done (Herpetofauna Inventory and Monitoring – Department of Conservation ). At every plot picked and monitored by the pitfalls and the field sensors (<u>Prey densities</u> and <u>Sensors</u>), a 30-minute search for lizards was done, looking maximal 2,5 meters from the observer.

I attempted to catch every observed lizard to determine the sex, and measure and weight it (Mitutoyo Absolute Digimatic Coolant Proof Calipers IP66,  $\pm 0.01$ mm, Kanagawa, Japan, and Pesola PPS200,  $\pm 0.02$ g, Schindellegi, Switzerland) to obtain information on their body condition. Caught specimens were handled with care and released as soon as possible, to minimize stress and the risk of tail autotomy. The time spent attempting to catch lizards was not included in the searching time, so every sampling location was searched for exactly 30 min regardless of the number of caught specimens or attempts to. In total, forty-seven lizard counts were done on the plots, and every plot was at least counted twice resulting in sixty-nine observed (visual and/or auditory) specimens. However, of the sixty-nine observed specimens only two were caught, forcing me to abandon this part of the research plan.

#### Timetable

The fieldwork started in April 2021, when both the male and female lizards are out of hibernation (Bauwens 1981), but the first batch of arthropod samples could not be analyzed due to difficulties with preserving the samples. The lizard activity season in 2021 ended in late September, so arthropod data for May 2021-September 2021 were considered. Due to extremely good weather in April 2022, the field counts were done for the last time for

additional comparable data on lizards, to supplement the data gathered in the bad summer. The sensors were placed on the 19 and 20 of July 2021 (later than the other field parts due to technicalities) and removed at the end of the field period (October).

#### Data analysis

All statistical analysis was done in R version 4.0.3 and for all calculations, all variation is expressed in standard deviation, unless it was a direct measurement, in which case the measurement error provided by the manufacturer was used.

As the 1075 observations were over 10 years (see <u>Waarnemingen.be</u>), I counted the number of lizards observed in wet and dry habitats and each of the broad habitat categories used in the Biological Valuation Map. A ratio of the observed specimen in the wet habitats that year over total observed yearly numbers was then calculated. Spearman correlation test ('pspearman package, Savicky 2022) was then used to determine if there was a relation between the two, to see if observed numbers were linked to habitat wetness. A Probit regression (aod package, Lesnoff and Lancelot 2012 and ggplot2 package, Wickham and Chang 2014) was used to determine the trend in those numbers and the relation between the wetdry ratio and observed numbers. A multi-factor ANOVA with a Fitted Linear Model (total yearly observations in the function of observations in BWK categories) (car package, Fox et al; 2022) was used to compare the yearly observation numbers with the habitat types where fifteen or more observations were made.

Nested (time in quarters of an hour since start was nested in the variable month, which was important for the other datasets) two-way analysis of covariance (ANCOVA) (packages tidyverse by Wickham 2022, ggpubr by Kassambara 2020, rstatix by Kassambara 2021 and broom by Robinson, Hayes and Couch 2022) was used to determine if the sensors differentiate significantly in all four measured variables over time and if this was only between wetness types or also between the plots. The student's t-Test was in addition done to test if the temperature differed depending on the height of the sensory units on the same sensor. To be able to do this, the downloaded data was cut to obtain only the data from the fieldwork, as the sensors work from assemblage until they are broken, or the battery runs out. An extra column was made for the average temperature and moisture count for every wetness type, for the three temperature measurements, and the one soil moisture count to be able to compare the obtained data to the wetness type

One outlier was removed due to the collection of abnormal amounts of carrion beetles, which were lured by the smell of rotting insects as the pitfall was unexpectedly completely dried out.

This sample was for said reason obtained differently than the others and thus was not comparable to the rest. Multi-factor ANOVA was done to test the significance of wetness, plot, month, and sampling method for both datasets on the respectively total and average dry mass. The student's t-test was done to determine the significance of the three wetness types. All tests were done on both the total dry mass and the individual dry mass. One-way ANOVA was used to determine total and average dry mass significance in the datasets for each plot and the plots grouped per wetness.

On part of the lizard counts, as Shapiro-Wilk tests (package dplyr, MIT 2022) indicated no abnormalities, multiple Fitting Linear Models were made for the nested multiple-way ANOVA to determine the importance of time (day, month, and year), month, day nested in month, plot, wetness, plot nested in wetness, cloud cover, temperature, wind, and observers. Based on the results and literature, unimportant variables were left out in further models. Also, additional Student t-Tests were done to determine the significance of the three wetness types on counted lizards.

For the incorporation of the various aspects, multiple Fitted Linear Models for multiple-way ANOVA were made, to test the influence of temperature, soil moisture, and total and individual arthropod dry weight in lizard numbers. Another model was made to test all the previous variables on Arthropod dry mass. The last model was made for a one-way ANOVA, to test if individual and total Arthropoda dray mass were linked significantly. Comparing the three fieldwork projects (sensors, pitfalls, and lizard counts), only the data that is comparable in the same timeframes are used. Due to the late placement of sensors, only data from July 2021 until September 2021 is used. For every month and every wetness type, the lizard counts, the average temperatures, soil moisture counts of the sensors, and total dry mass is used.

## Results

#### Waarnemingen.be

The waarnemingen.be database holds 1075 records for Z. vivipara within the Grenspark. These observations, all between 2011 and 2021, came from seventy different observers, and most of them concerned observations of single individuals. The total number of lizards observed has declined over the years (Fig. 14 Top: GLM with Poisson log-linear distribution, F(21) =236.6, P<0.001). The proportion of lizards that were seen in wet versus dry habitats did not change in the time period considered (Probitregression, slope=0.012, se=0.008, z=1.52, P=0.13). The total number of both observations of the species in Belgium (Fig. 14 Bottom) as the number of reptiles spotted in the Grenspark increased in the same time frame.



Figure 14: Top: Yearly observations of Zootoca vivipara from Waarneming.be within the park (blue) and the percentage of those observations made in wet habitats according to the BWK (orange). Both graphs seem to follow the same trend since 2014, but statistical analysis denies this. **Bottom**: Observations of Zootoca vivipara in Waarnemingen.be, in Belgium with observations (blue), total specimens (orange), and observers (green). As 2022 is still ongoing, those numbers are not yet representative. Overall, the numbers are going up, in contrast to those in the study area. @Waarnemingen.be

2007 2008 2009 2010 2011 2012 2013 2014 2015 2016 2017 2018 2019 2020 2021 2022

Lizards were seen more often in heathland bogs (as they can swim) (F(1) = 143.76, Pr = 0.0069), and dry Calluna (F(1) = 36.86, Pr = 0.026). Both habitat types raised the predicted number of observations according to the same Fitting Linear Model used for the ANOVA.

#### Sensors

Graphics were made for the three temperature sensor units in every placed sensor and the moisture count (Fig. 15 and Appendix Fig. A1).



Figure 15: Soil moisture counts from every sensor placed during this thesis. The wet plots (blue) take up the higher lines and the dry (yellow and orange) and dried-up ones (green) the lower levels. The soil in the dry plots reaches the wet plots levels in case of rainfall but loses those levels when the precipitation stops, unlike in the wet plots. After rainfall, the dried-up plots almost immediately drop back to lower levels, indicating their limited capacity of holding water in their soil

Statistical analysis indicated (Fig. 15 and Appendix Fig. A1 Moisture), that the tree wetness types differ significantly not only for moisture count but also for every other sensory unit (except the surface temperature (T2) between dry and wet plots.) (Table 1 left side). Time (quarter-hours after the start of measurements nested in a month) proved also to be a significant factor for all of them (Table 1 right side) as confirmed by the p-values from a nested multiple-way ANCOVA.

Table 1: On the left are the P-values of a two-sample t.test for the four sensory units, per wetness type. For every sensory unit, the difference between the sensors placed in the wet, dry, and dried-out plots was significant (green), except for the temperature measured just above the ground (T2) between the sensors placed in dry plots (D) and those placed in wet plots (N) (red). On the right, the results of multiple two-way ANOVAs to determine if time was a significant factor. In the first row, the average temperature is given for every sensory unit (T1, T2, T3, and Moisture) per wetness type. For all wetness types and sensory units, this factor was significant. Also given: the degrees of freedom (Df) and the F value (F). The high p-values are normal as it is colder at night than in the daytime. However, the tests gave useful indicatory values.

Two-sample t.test	T1	T2	Т3	Moisture		Wet			
Wet - Dry	9.65E-11	0.1087	<2.2E-16	<2.2E-16		T1	T2	Т3	Moisture
Wet - Dried up	<2.2E-16	<2.2E-16	<2.2E-16	<2.2E-16	Av. Value (°C, °C, °C and RMC)	14.49	14.13	14.00	2265.69
Dry - Dried up	<2.2E-16	<2.2E-16	<2.2E-16	<2.2E-16	sd	2.88	6.36	6.80	433.60
					F	122.46	14.69	13.03	310.40
					Df	73	73	73	73
					P(r )	< 2.2e-16	< 2.2e-16	< 2.2e-16	< 2.2e-16
						Dry			
					Av. Value (°C, °C, °C and RMC)	14.34	14.07	13.80	1752.98
					sd	3.50	6.48	6.74	416.39
					F	86.82	16.31	14.47	1202.20
					Df	73	73	73	73
					P(r )	< 2.2e-16	< 2.2e-16	< 2.2e-16	< 2.2e-16
						Dried up			
					Av. Value (°C, °C, °C and RMC)	15.30	14.57	14.35	1236.03
					sd	4.31	7.01	6.92	312.37
					F	52.11	13.56	13.34	462.80
					Df	73	73	73	73
					P(r )	< 2.2e-16	< 2.2e-16	< 2.2e-16	< 2.2e-16

#### Prey densities

For the total dry mass in both sampling methods, the model indicates variation between months (F(1) = 2.35, Pr = 0.127). Total dry mass by dragnet sampling in between the wetness types does not differ significantly (F(1) = 0.05 Pr = 0.826) (Wet= 0.029g  $\pm$  0.031g (N=25), Dry= 0.016g  $\pm$  0.018g (N=25), Dried-up= 0.027 g  $\pm$  0.021g (N=25)), but does differ significantly in the total pitfall dry mass between the three wetness types (F(1) = 6.13 Pr = 0.015) f (Wet= 0.34g  $\pm$  0.42g (N=50), Dry=  $\frac{1}{1000}$ 

Average arthropod drymass



Figure 16: Average total pitfall sample dry mass, per wetness type. Regardless the few outlines for every type (mostly due to Silphidae), there is a visual difference between the three types. Wet habitats have lower sampling dry mass than dried-up and dry plots. The latter two have similar quartile values, but dried-up plots note higher average.

0.58g (N=50)) (Fig. 16). The significance is mainly caused by the significant difference between wet and dried-up pitfall samplings (T = -2.63, p = 0.010). Surprisingly, the Dried-up pitfalls yield a higher total dry mass than both those of the Wet and Dry plots. Interestingly, even though the monthly variation is not significant, it is the case of dragnet sampling in wet plots (F(1) = 4.65 Pr = 0.042) (May and August having higher values).

Looking at the average individual weight of every caught arthropod, the sampling method indicates a trend towards higher mass for pitfalls (F(1) = 2.12, Pr = 0.147, pitfalls = 0.02g/prey  $\pm$  0.02g/prey (N=150) & sweep = 0.002g/prey  $\pm$  0.002g/prey (N=75)). Monthly variation was also significant (F(1) = 2.34, Pr = 0.128), increasing in time. In contrast to what was the case for total dry mass, the effect of an individual plot is highly significant (F(1) = 23.00, Pr<0.001). There is also a significant difference between the dry plots for pitfall sampling (F(1) = 9.26 Pr = 0.004), indicating that at least one of them is an outlier. Further testing indicated that 5D (0.031g/prey  $\pm$  0.024g/prey , N = 10) is the outlier, having on average a heavier prey items than 1D (0.010g/prey  $\pm$  0.009g/prey , N = 10) and 2D (0.01g/prey  $\pm$  0.02g/prey ,N = 10) (df = 11.56, p = 0.028 & df = 15.78, p = 0.046). Wetness is a significant factor (F(1) = 6.50 Pr = 0.012) (Table 2). Additionally, a significant monthly variation in the dry plots' pitfalls (F(1) = 3.73 Pr = 0.059) and the dried-up plots pitfalls (F(1) = 9.27 Pr = 0.004) was found, were mass increased in time.

Table 2: Summary of the prey sampling, organized per sampling method and wetness type. Not only are there clear differences between the two methods, in which pitfalls catch more and heavier prey, but also in wetness type. The dried-up plots have in both methods high total and individual dry mass, especially in average individual dry mass.

	Wetness type			
	Wet	Dry	Dried up	
	Pitfalls			
Total sampel dry mass (g)	0.358	0.490	0.904	
sd (g)	0.412	0.464	1.778	
Average number of specimen per sample	31	30	42	
Average individual mass (g)	0.011	0.016	0.022	
	Sweep samples			
Total sampel dry mass (g)	0.029	0.016	0.027	
sd (g)	0.031	0.017	0.020	
Average number of specimen per sample	20	15	17	
Average individual mass (g)	0.001	0.001	0.002	

#### Lizard Counts

Due to the cold and wet summer weather, counts were lower than expected (typical "Belgian summer"). For the wet plots, on average 2.13 ± 1.23 (15 counts) lizards were observed per 30 minutes. In contrast, only 1.2 ± 1.42 (15 counts) and 1.12 ± 0.93 (17 counts) were observed for respectively dry and driedup plots per count (Fig. 17). A nested multipleway ANOVA (Shapiro-Wilk tests indicated no abnormalities in wetness types) showed no significant influence of temperature (F(1) = 2.45), Pr = 0.132, wind (F(1) = 3.80, Pr = 0.073), observer (F(1) = 0.66, Pr = 0.423), date (F(7) =1.13, Pr = 0.386) nor month (F(1) = 0.77, Pr = preferred habitat of Zootoca vivipara and that dry habitats 0.397) or morning/afternoon (F(1) = 0.01, Pr =

0.946). Also, none of the nested variables were significant; date in month (F(6) = 1.01, Pr = 0.453), morning/afternoon in day (F(6) = 0.61, Pr =0.720). However, "wetness" (F(2) = 3.99; Pr = 0.026)(see further), plot (F(14) = 4.24, Pr < 0.001) (Table 2 and Appendix Fig. A2) and cloud cover (F(1) = 6.51,Pr = 0.016) (more clouds yielded less specimen) (Appendix Fig. A3) where significant according to the model.

In wet habitats, the number of Z. vivipara spotted was significantly higher (Two Sample t-test: t = 2.76, p = 0.010) than in dried-up plots, and wet habitats tend to have more observations than dry ones (Two Sample t-test: t = 1.99, p = 0.057) (Fig. 16). There was no indication that dry and dried-up habitats would differ (Two Sample t-test: t = 0.19, p = 0.85). Of the plots, some have significant (Summary Fitting Linear Model) higher or lower observed specimen; 1N (t = 3.85, Pr < 0.001), 2N (t = 2.61, Pr =

0.013), 2VE (t = 2.69, Pr = 0.011), 5N (t = 2.138, Pr = 0.040) and the "Wilgenduinen" (4D) (t = 3.563, Pr = 0.001). (Table 3) Cloud cover was the last significant factor in the total number of



Figure 17: Boxplot of the number of observed lizards per 30 minutes, sorted per wetness type. Dry plots had clearly the lowest numbers, except two outliers, both done at 4D "Wilgenduinen". Wet plots had, except three outliers, consistently the highest numbers. In the dried-up plots, the most variation in sited lizards was noted down, but no outliers. In this figure, we see that wet plots are the contain far less specimen.

Table 3: The average number of observed lizards per 30 minutes, for every plot. For every plot, also the number of counting repetitions is given. Some plots note far higher numbers (1N, 2N, 2VE, 4D, and 5N). Due to lower numbers and the limited number of counts done per plot, the standard deviation is very high, sometimes even higher than the average.

Plot	Average	sd	Counts
1D	0.50	0.58	4
1N	3.00	1.41	4
1VE	1.25	0.50	4
2D	1.66	1.15	3
2N	2.33	0.58	3
2VE	2.25	0.50	4
3D	0.33	0.58	3
3N	1.66	0.58	3
3VE	0.00	0.00	3
4D	3.00	2.00	3
4N	1.00	1.41	2
4VE	0.33	0.58	3
5D	0.50	0.71	2
5N	2.00	1.00	3
5VE	1.33	0.25	3

observations according to the Fitted Linear Model, resulting in an estimated 1.81 lizards per count with no clouds (t = 7.73, Pr < 0.001). Per increasing scale of cloud cover (on eight), the number of predicted lizards observed will lower by 0.16 (t = -2.18, Pr = 0.034) (Appendix Fig. A3).

#### The influence of environmental conditions and prey availability on lizard densities

To determine the influence of environmental conditions and prey availability on lizard densities, a model containing the analyzed datasets was used. Arthropod dry mass is a significant factor according to the model (F(1) = 9.61, P(r) = 0.005), with a significant positive correlation (estimated = 0.900 extra observed lizards/30minutes per gram caught dried arthropods, P(r) = 0.005). Temperature however has no significant linear impact (-0.068 lizards per count/°C, P(r) = 0.722) but is a significant factor (F = 22.39, P(r) < 0.001). The last factor, soil moisture, was again significant (F(1) = 29.95, P(r) < 0.001) and positive correlated (+0.0004 lizards per count/moisture count, P(r) = 0.518).

The effects of soil moisture, temperature, and lizard numbers on total arthropod dry mass per pitfall were tested with a similar model. Soil moisture counts were significant (F(1) = 119.1819, P(r) < 0.001) for arthropod dry mass, but lowered the total dry mass by increasing count (-0.0008g/count, P(r) = 0.022). Temperature was also found significant according to the model (F = 57.90, P(r) < 0.001), and caused a significant decline (-0.346g/count, P(r) < 0.001). The last factor in my model was the number of lizards observed per plot, and this was not only significant (F(1) = 9.61, P(r) = 0.005) but also positively correlated (+0.328g/observed lizard, P(r) = 0.005).

## Discussion

#### Waarneming.be

My analysis based on the data from Waarnemingen.be confirmed a decrease in observations, as was previously noted by a Dutch plots monitoring (RAVON, Schubben en Slijm, nr 21, 2014), and further supported the claims of local experienced specialists such as André Van Hecke. I cannot exclude that this is due to a decrease in time looking for *Z. vivipara*, as effort or field time is not noted down in the databank. However, both the number of observations of *Zootoca vivipara* in Belgium (Fig.14 Bottom) as well as the number of snake observations in the study area grow in the same period, indicating that observing reptiles in and outside the study area is still popular. The most likely explanation is thus a decreasing number of *Z. vivipara* in the study area. The presence of two healthy snake predator populations, which are not present in other *Z. vivipara* populations, could be a factor. However, the seemingly decline in other *Z. vivipara* populations according to Van Hecke indicates that other factors are also in play.

The unconfirmed relation between the wet percentage and the yearly observations can be caused by numerous reasons, of which the most plausible are the outlier in the year 2011 and observation bias due to habitat structure. However, removing the values of 2011 drops the p-value to a third, but did not result in any significance, concluding that this is not the only explanation. If only the last seven years are taken in to account, the relations are however significant. The cause of the mismatch in the first years is unknown to me. I suspect however that the link in the later years is due to that the lower numbers result in a lower likelihood to spot fleeing lizards. In these aspects, the more open, drier habitats have the advantage as the movement is more visible and audible, increasing the observations.

Looking at habitats in specific during the decade of data from Waarnemingen.be, I aim to find indications of dependence on specific habitats causing increases or decreases in yearly numbers. Wet habitats are established as preferred habitats, leading to the suspicion that those could be of importance in total yearly numbers. The analysis indicates that the habitats in which observations made are indicatory for high yearly numbers are on the opposite sites of the wetness spectrum: heathland bogs and dry heathland-sand dunes. As swimming is one of the abilities of *Z. vivipara* to escape predators (Bauwens & Thoen 1981)., observations made in heathland bogs are possible but rather rare as it is an unusual place to look. It is however a likely habitat a specimen could run to, as they prefer wet heathlands (Glandt, 2001) which can often be found near bogs. The chances of spotting this behavior are

therefore slim, and thus more probable if the yearly observations are high. On the other hand, open habitats such as sand dunes are less preferable habitats, as they are low in prey (Borer 2012) and hiding places. Presumably, observations made in such habitats are due to high population numbers, causing forced movement of some individuals to less preferable habitats.

Information contained in public databases depending on observations provided by volunteers should however always be looked at skeptically, as only presence is noted, thus not excluding the absence of a species, merely noting the lack of observations. It also has both species as observer bias, as some species are more popular and thus harder locked for and the observation more likely to be shared, and the skill of observing and identifying species differs per observer. Therefore, the results from Waarnemingen.be are more indicatory than conclusive.

#### Abiotic variables

As the plots were chosen based on their perceived soil wetness, the fact that the five plots labeled as wet turned out to have the highest soil moisture counts came as no surprise. However, the plots with the dry vegetation had higher moisture counts than the dried-up plots, and those values dropped slower than those of the dried-up plots (Fig. 15) after wet periods. As found by Sowerby et al. (2008), soil exposed to droughts has a reduction in its water-holding capacity, which was according to them most likely linked to an increase in hydrophobicity in the soil. This would explain why the dried-up soils could not, in contrast to the dry plots, hold a high moisture count after rainfall as they have changed from wet conditions to dry conditions without the needed soil adaptation.

The temperature measurements for the sensors on the other hand did not visually seem to differ in the three different wetness types (Appendix Fig. A1). but the ANCOVA indicates significant differences. The dried-up plots noted the higher average temperatures and the highest temperatures making them in combination with water problems, less suitable, as well as the less preferred dry habitats. Due to the long timespan and the fluctuation caused by day and night, the differences between the wetness types were unclear in the figures. As the temperature is strongly related to vegetation structure and water, the differences in wetness types were to be expected.

#### Prey biomass

Dried-up plots had by far the most prey biomass, and plots were even more significantly different than the three wetness categories, it indicates that besides wetness other factors

also play a significant role in my arthropod biomass model. Literature suggests that those factors include, among other things, primary production according to Borer (2012), and grazing according to Garcia (2009). The latter is however rather similar in most plots (Appendix - Locations). That primary production shows as an important factor, and explains why dried-up/drying up plots yield a higher arthropod dry mass. The transition from wet to dry vegetation causes the opportunity for new plants and thus the pioneers' stage in succession, with the typical fast-growing plants occupying the free space first. Dry and wet plots are in their end phase of succession, and thus both their primary production is far lower, supporting fewer consumers, resulting in less arthropod dry mass.

In my study, both monthly, as well as wetness variation in total and individual average arthropod dry mass, is mostly caused by changes in abiotic conditions. Comparable results were found by Palacios-Vargas et al (2007) and Remmert (1981).

The difference in sampling method (pitfall - dragnet) is only tested for individual average dry mass, as the methods are non-comparable in yield and thus total dry mass. Individual average dry mass was however significantly higher in pitfalls, as lower body mass is an adaptation to flight. More active flyers would have a relatively lower body mass to lower the energetic cost of flight, explaining the significant difference. Furthermore, caught specimens were of totally different groups: the pitfalls mostly caught Araneidae and Carabidae, were as the sweep net mostly caught flies and mosquitoes, far smaller build species.

All the results from dragnet sampling are less robust, as there was no repetition per month, as well as generally far lower sample size and active duration. This makes the results more coincidental, as well as outliers.

#### Lizard densities

As the preference of *Z. vivipara* for wetter Calluna and Calluna-Molinia heathland was known (van der Werf et al. 2012, Strijbosch 1988 & Glandt 2001), making the significance in lizard densities depending on wetness type with wet habitat as preferred habitat as predicted. The high variation in counted lizards in dried-up plots (Fig. 17) is presumably due to the variation in the five dried-up plots themselves, ranging from very open patches up to highly heterogenetic areas. This is again reflected in the outlier 4D ("Wilgenduinen") with its range of vegetation.

The fact that I did not see an effect of both the time factors (month and day) came as a surprise, as the behavior of *Z. vivipara* changes during the field period, making it more likely to be

spotted during mating season (more bold behavior to find and mate with a female) and the timeframe in which the females are pregnant and thus slower (Bij12 2017). The fact that environmental temperature did not influence the number of lizards seen is probably an effect of my choice to perform counts during favorable weather conditions only.

The wind was relatively neglectable as a variable, as this was windless or a slight breeze, arguably undetectable on ground level. The diurnal behavior of the species explains the fact that the model could not prove a significant influence of morning against afternoon counts. If the sampling would have been done in smaller time frames (e.g. 8 am-10 am), some significance may surface.

Cloud cover was of significance to the number of observed lizards, but the temperature was not. In contrast to temperature, cloud cover was more variable during the day and thus also during the fieldwork than temperature, making it more likely to affect the data. As described by Gvozdik (2002), *Z. vivipara* is an ectotherm, making it receptible to temperature changes and in need to sunbathe. Blockage of the sun by clouds takes away the ability of the lizards to sunbathe and thus affects its detectable presence.

That plots are a more significant factor in my model than wetness highlights that, as with the arthropods sampling, the measured temperatures and soil moisture variables used to divide the fifteen plots into three groups are not the sole significant factors. Other possible factors could be grazing (Strijbosch 2002), presence of predators (Thoen et al. 1986), food availability (will be discussed further), and maybe even other inter-species interactions.

#### The influence of environmental conditions and prey availability on lizard densities

To quote Prof. Dr. Van Diggelen (Excursion Habitat Restoration 2022): "Habitat restoration is 90 à 95% abiotic conditions", indicating the importance of abiotic factors in any ecosystem. As abiotic conditions are thus the major factor for any species' presence and wellbeing, this thesis will heavily rely on the found abiotic conditions to confirm or confute the hypotheses. Based on the integrated data (<u>Results integrated data</u>) I constructed a figure indicating the correlations of the different field aspects of this study. The resulting figure (Fig. 18) is in line with accepted findings; that *Z. vivipara* prefers wetter conditions (Glandt 2001)., thus soil moisture had a positive (green) correlation. I also expected a negative influence of temperature (Jessop et al. 2013), which supports my first hypothesis. However, even though



(black arrow) was found in my model. Food availability was also of significance (green arrow between prey availability and lizard densities), which was in line with the finding of Wang et al. (2017). However, based on the individual and total arthropod dry mass, both moisture soil and temperature have а

influence

temperature was significant,

no clear linear influence

of

the

Figure 18: Visualisation of the resulting integration of the count's, prey, and the soil moisture and temperature measurements. P values from the ANOVA's were used to determine significant interactions, and are visualised with arrows, where the thickness reflects the significance. Positive interactions are coloured green, negative ones red. All values are put on the power of five or ten, to ease the comparison. Both abiotic values have a strong negative impact on prey availability, but their indirect impact on the lizard population density is less than their direct impact. Flowchart made in ClickCharts.

negative effect (red arrows), contrary to my hypothesis. The effect of soil moisture was already predicted based on arthropod data only, in the assumption that the wetness of the plots was rightly indicated by its vegetation. The relations found by my study, and the comparable size of their influence, is however only applicable in the Grenspark, as no comparable data was gathered in other areas. My results thus also corroborate the hypotheses stated by Remmert (1981): in this study, the greater numbers of arthropods do not compensate for the reduction of average individual body mass, which both are caused by wetter conditions. Wetter conditions lead to higher primary production, supporting more individuals, and due to smaller individuals needing less food but evaporating relatively more by having a higher surface areavolume ratio. This causes smaller individuals to have lower mortality rates in those conditions than their bigger counterparts, making them more abundant.

Interestingly, both models stated a positive correlation between arthropod dry mass and observed lizards. This is presumably linked to better food available for both (Borer et al. 2012 and Wang et al. 2017).

Due to climate change, a drier and hotter climate is predicted and already measurable (IPCC 2014), which is bad news for *Z. vivipara* (Masó et al. 2020, Diaz et al. 2007). My results indicate that a drier climate would negatively affect the common lizard, and as higher temperatures increase water loss, so would the higher temperature. The more unpredictable nature of both heatwaves as droughts due to climate change would arguably further worsen the impact (Masó et al. 2020). However, only increasing temperature would presumably negatively affect the arthropod dry mass available for *Z. vivipara*, as a drier climate would, according to my data, be beneficial.

The abiotic factors have a greater impact on prey availability than on *Z. vivipara* numbers themselves, according to the p-values. However, prey availability has a smaller impact on the *Z. vivipara* population than the abiotic factors according to their respective p-values. The main cause of decreasing *Z. vivipara* observations would thus be the abiotic factors temperature and soil moisture. Past years had dry and hot summers, leading to droughts (Mos 2020). The drying out of some habitats and bogs would have been a factor in declining *Z. vivipara* numbers, due to its negative impact on the individual (Glandt 2001, Dupoué et al. 2018, Gradient et al. 2001) and demographic health (Díaz 2016).

All proved or confute hypotheses are based on data gathered in a relatively limited period, and only from one study area, reducing the reliability of any found relations. Furthermore, as Bauwens et al. (1986) and Bjørnstad et al. (2004) discovered, the life history of populations varies considerably among populations, making my finding highly specific for this population and may not be accurate for other populations.

#### Limitations of the study

Due to corrections in the field method, the first month of fieldwork (April) was lost. Technical issues resulted in the late placement of the sensors for soil moisture counts and temperature resulting in only three months of comparable data, undermining the significance of any found results. Further research in this area would thus be preferred, over a longer period.

Furthermore, due to wet weather, the number of lizard counts done was lower than planned as well as the lack of dragnet sampling for all the Dutch plots in July. The destruction or unwanted collection of the pitfalls by local wildlife also resulted in the following pitfalls to yield no data: 3N2 in May, 3VE2, 4VE1&2, and 5VE1 in June, and 1D2 in August. *Zootoca vivipara* were not only shyer than observed in the decade before the study, but also in fewer numbers (see <u>Results: Waaremingen.be</u>). The lack of experience in catching small lizards further hindered catch success.

## Conclusion

In this thesis, the aim was to have a concrete idea of habitat-specific density numbers of the local population and their extinction risks due to climate change. This research showed that a hotter climate, with more dry heathland results in less available Arthropoda dry mass as prey for *Z. vivipara*, as well as reducing its population numbers and overall fitness in those habitats. Relatively wetter and colder heathland conditions are more favorable for higher *Z. vivipara* population numbers. Drier soil was, however, a positive factor for Arthropoda, in contrast to its effect on lizard populations. A hotter and drier climate will further endanger the local population, and a further decline without interference is expected.

Wet heathland habitats remain a preferred habitat for *Z. vivipara*, doubling the numbers for dry and dried-up heathlands. The conservation of the former habitats would thus be recommended for protection of the *Z. vivipara* populations.

The influence of plant species and vegetation cover is yet to be determined, as well as the physiological adaptations of *Z. vivipara* to the drier and hotter climates. The latter was initially part of this thesis but had to be cut out due to a lack of initial data for the model.

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## Appendix

#### Locations

N1 was located at N51.14355° E4.360807° and consisted around small, sometimes drying-up bogs. Around the bogs, the following indicatory plant species were present: *Drosera intermedia*, which starts to disappear when *Erica tetralix*, appears, together with *Juncus effusus*. At the borders, *Molinia caerulea* and *Salix sp.* start to grow, together with some *Rubus sp.* and *Pinus sylvestris*. The trees are however shopped down and sometimes even completely removed regularly as part of the "Programma Aanpak Stikstof" (PAS) project (2020-present) to preserve the heathland restored by the Life Helvex project (2014-2020). No grazers were used in this plot. (Mos 2020) The border of the plots was indicated by the start of *Calluna vulgaris*. The site has water supply issues, complemented by dry summers, but still maintains it wet vegetation.

N2 was located south-west warts of N1, at N51.409160° E4.370388°, and was more homogenous than N1. It consisted of a lower patch of heathland, surrounded by higher-located walking trails. Multiple small bogs indicate the wetness of the area. The vegetation was dominated by full-grown *Erica tetralix* but has multiple, parallel disturbance lines due to previous topsoil removal with heavy machinery (Life Helvex 2014-2020), where *Molinia caerulea* takes over. Sheep were used to keep the grasses in check by a yearly high-density grazing, from the ongoing PAS project which started in 2014. (Mos 2020)

N3 is located between the Stappersven and the Verbindingsweg at N51.40346° E4.440022°. The plot is on the banks of the bog, dominated by tussock-forming *Molinia caerulea* with traces of *Erica tetralix*, and has many ditches running through it. It is a known place for *Zootoca vivipara* under the local volunteers and is hard to walk through due to its wetness in combination with high purple moor grass tussocks. Under the BWK classification, this area was noted down as wet heathland (4010) and degraded heathland (cm). No management was in place on this site, as far as my records go (2011).

N4 is due east of N3, at N51.400295° E4.422472°. It is located far from any roads or walking trails and situated in a lower part surrounded by old sand dunes now covered by dry heathland. Due to this being lower than its surrounding parts, the groundwater reaches closer to the surface than in its surrounding dry heathlands, making it sometimes even up to ground level after a wet period. The vegetation alternates between heavily *Molina caerulea*-dominated patches and ones that are dominated by *Erica tetralix*. Some young pine trees are trying to break through, however, none of them are higher than 50cm. The southern border has some

young *Betula pendula* (max 3m high). According to the BWK, the plots cross two patches, both wet heathland (4010), but different in their BWK classification. The northern part is classified as heathland (c) and is still recovering from the 2011 fire. The Southern part is wet heathland with dry heathland parts (ce + cg). No dry heathland parts were however crossed during any insect trappings nor lizard counts. Galloways can roam here, since at least 2019, and in both 2019 and 2020, there is a record of extensive sheep grazing. Some parts of the site are yearly mowed since at least 2016, as fire prevention measurements.

N5 is located south of N3 and was located near Het Moerken (invisible on the map, uncharted on most maps). It is surrounded by dry heathland but consists entirely of degraded wet heathland around the pond called "Het Moerken". Unlike the previous degraded heathland patches, *Erica tetralix* and *Molina caerulea* are equally dominant. According to the BWK, the area consists of multiple patches described as wet heathland (ce & 4010), wet degraded heathland (4010 but cm), and *Aira*-alliance and open grassland on sand dunes (hac & 2330). This is however outdated, as the situation described by the BWK is no longer present at N5 but pushed back by the wet heathland. I have no record of any management in this plot (going back until 2011), but Galloways can roam here (were never sited near or in the plot).

D1 was the most northern plot of them all, located at N51.415165° E4.36145°. It was also the only plot not entirely situated in heathland, due to the limited amount of walking distance of the other plots. The local habitat can be described as an open pine (*Pinus sylvestris*) forest with degraded dry heathland patches heavily dominated by *Molina caerulea*. Some young *Betula pendula* are also present. No special management was in place (records going back until 2008; Mos 2020).

D2 was located (N51.407763° E4.3691630° at the southern part of the small heathland patch on the Dutch side. It used to be a lot wetter at least two decades ago (by the age of the trees), but every ditch has long lost any trace of water. Those ditches were stream-upwards dammed to keep the polluted waters from agriculture out of the park. The area itself is higher located than the surrounding areas and is completely covered in mostly dried-out *Molina caerulea*. Young *Calluna vulgaris* started to grow but was far from established. Noticeably, multiple tree trunk remnants and branches were dried out on the ground. Sheep were sometimes present during the field period to preserve the present vegetation by "drukbegrazing" (extensive grazing), as part of the PAS project since 2014. Since 2018, an additional three hundred sheep are used for second grazing (one in spring, one in summer). (Mos 2020) D3 was the most northern plot on the Flemish side and was the only one located in an area closed to the public during bird breeding season. After consultation with the local conservation authorities (Joey Braat, Boswachter Ecologie Staatsbosbeheer West-Brabant (Dutch Part); Jef De Winter, Agency of Nature and Forests (ANB) (Flemish part) and Martin Mos of Natuurmonumenten (Dutch part)), a plot was laid out that was used outside the protected period. The BWK has the area noted as cmb+cg and 4030, meaning degraded heathland with *Molina caerulea* with spots of dry heathland and wet heathland, and ce + 4010, both meaning wet heathland. The area was indeed a variation of dry heathland with strokes of *Molina caerulea*. It was located on a somewhat flat sandhill and surrounded by wetter heathland. The fieldwork on this plot was always done in the dry parts. Since at least 2019, Galloways roam this area, and in 2020, some horses were also used.

D4 was at the start of the famous "Wilgenduinen" (N51.3988889° E4.437222222°, which was an old sand dune, rooted by a few *Betula pendula* trees and had a rather open dry heathland vegetation dominated by Calluna vulgaris. It was another known *Zootoca vivipara* location and was known as the source of lizard specimens for previous research (Bauwens 1981, Bauwens & Thoen 1981, Bauwens et al. 1983, Bauwens & Verheyen 1987, Van Damme et al. 1990 and Gvoždík and Van Damme 2003). According to the BWK, this site was wet degraded heathland (cm and 4010), even though the complete lack of indicating species present. In 2019, some trees were removed, and at least since 2019, sheep are herded here.

D5 was found south of N4 (at N51.397437° E4.42338°) and was a *Molinia caerulea*-dominated grassland, with few developed patches of *Calluna vulgaris* and a handful of full-grown *Pinus sylvestris*. Of all plots, D5 varied most in elevation. Labeled as dry heathland on young sand dunes (cg and 2310) and open grassland on sand dunes (hac and 2330), the BWK confirmed the present vegetation. Galloways also roam the area as management, and sheep also graze extensively here. Both records going back until 2019

VE1 was an open area at N51.412597° E4.363723°, where little grows. However, the borders of this parch were quite diverse with *Salix sp., Betula pendula, Molinia caerulea, Juncus effusus,* and multiple species of dry-soil moss, lichen, and various herbs. On the northern side of the plot, some higher-growing grasses are growing, on what seems to be less dried-out soil. *Calluna vulgaris* was not yet present. This site had its topsoil removed as part of the Life Helvex project (2014-2020) and is machine-cut as a form of management since 2021. (Mos 2020)

VE2 was very much like VE1, but more enclosed due to higher located trails. The open patch was here by far smaller, and so was the overall patch. The "green border" was however also

thicker. The wetter parts were more prominent, but still, no surface water, nor any typical wet heathland species were present. *Calluna vulgaris* has not yet appeared. VE2 was located at the bottom of what was the bog (N51.407623° E4.367498°). Sheep were stationed here for a brief period during the fieldwork, as local management. This was part of the high-density grazing, active as part of the PAS project since 2014. Since 2018, an additional three hundred sheep are used for a second grazing (one in spring, one in summer). (Mos 2020)

VE3 was located on the northeast side of what was formally the "Langven" (N51.400433° E4.42982°). The parts close to the used-to-be banks are covered with now dying *Erica tetralix*. Higher up, the sand dunes are covered with starting dry heathland vegetation and some *Pinus sylvestris*. The remains of more pine trees are still lying around. Grasses cover most parts of the area and are kept short by Galloways. The BWK was a little bit outdated here, as it states the previous pre-management state; *Thero-Airion* with pine trees and birch on sand dunes (hacb + pins + bet, 2330). Galloways can also roam here but are cited more frequently in plot VE5. In 2019, extensive sheep grazing took place.

VE4 was located on the west side of the dried-up "Langven", at N51.399298° E4.43201°, just before the bog used to get narrower (right at the heft-plate transition of the spatula shape). The vegetation was almost identical to VE3, except for more *Erica tetralix* and *Calluna vulgaris*. This was reflected in the original, pre-dried out state in the BWK, in which it was noted down as degraded wet heathland (cm + 4010) and higher up as dry heathland with some trees (*Pinus sylvestris* in this case) (cgb + 2310). The Galloways were also present in this area but were observed more frequently at plot VE5. Extensive sheep grazing also took place here in 2019.

VE5 was located at the southeast side of the dried-up bog (N51.397222° E4.43138°). The vegetation was again similar but had more pine trees. This also makes it the preferred shadow place of the Galloways. The bog-bordering side was wet heathland (cm, 4010), but was now converting to dry heathland. The other side is as stated, richer in pine trees, and thus noted down as such (ppmh). Those trees are partially shopped down in 2020. Also, in 2019, extensive sheep grazing took place.



Figure A1: Boxplot visualization of the sensor data, per sensory unit and per wetness type. The variation increases with dept of the sensory unit for temperature, as T1 (the highest placed) has the lowest quantile distance, and T (in the ground) h-the highest. All three the temperatures are visually highly similar between the wetness types but are significantly different. This is caused by a far higher variation throughout the day than between wetness types. The Moisture counts on the other hand visualize clearly the difference between the three wetness types. Wet plots confirm they are wetter, whereas dry plots do not note down the lowest boxplot. Dried up plots measured the lowest Moisture counts, as well as the most variable outliers.



Figure A2: A plot-based distribution of the observed lizards per 30 minutes, colored per wetness type. All dry plots have, as always, the yellow color, the wet plots are colored blue and dried-up green. No plot has outliers, but some plots have a far greater variety and higher values. Wet plots have consistently higher values, confirming those plots as preferred habitats. Both dry and dried-up plots have a high variation between the plots and have both low as well high values for the plots, with limited variation in almost every plot.

#### **Observed lizards**



Figure A3: Visualization of the numbers of lizards observed per 30 minutes, sorted based on cloud cover (on 8). The blue and yellow coloration is solely for better linking each boxplot with its cloud cover value. As most counts were done under a bright sky, the first boxplots have the highest variation. However, with some exceptions, a downwards trend is visible, highlighting the negative impact cloud cover has on lizard observations.