Spatiotemporal variations in antler development among two neighbouring, yet isolated, red deer populations

Ryan van Mourik & Joost F. de Jong*

Wildlife Conservation & Ecology Group, Wageningen University, Droevendaalsesteeg 3, NL-6708 PB Wageningen, the Netherlands, e-mail: joost.dejong@wur.nl

Abstract: Whereas wildlife occurring in fragmented landscapes is frequently the subject of genetic investigation, there has been relatively little attention for associated spatiotemporal variations in phenotypic traits. For example, many studies have reported the genetic variation and structure of present day red deer (*Cervus elaphus elaphus*) – showing both hybridization with other *Cervus* species and fragmentation – but the consequences for phenotypic traits, even when as conspicuous and elastic as antler size, have not been considered. To examine fine-scale differences in a phenotypic trait in large wildlife species inhabiting fragmented landscapes, we analyzed the antlers of red deer from two neighbouring populations occurring in the Netherlands. To test for area and drought effects, we quantified antler mass, burr circumference and the number of tines from antler series shed in the years 2011 to 2020 by 25 stags of the population at the Hoge Veluwe Estate (n=20) and the relict population at Planken Wambuis (n=5). We found that red deer from the Hoge Veluwe have significantly heavier and larger antlers with more tines (average mass: 3.0 kg; average burr circumference: 23.0 cm, average number of tines: ten) than deer from the neighbouring relict population from Planken Wambuis (2.3 kg, 21.5 cm and eight tines, respectively). Despite the provision of drinking water and pasture management within the Hoge Veluwe, antler development appeared to be significantly diminished by the summer drought of 2018, but only in mature stags (males aged seven years and older). We thus found, on the one hand, a naturally induced variation in a phenotypic trait, despite wildlife management aimed at relaxing natural stressors. On the other hand, the considerable phenotypic variation detected at a small spatial scale illustrated strong anthropogenic influence: it appears that wildlife populations in fragmented landscapes are not only affected genetically, but also phenotypically. We conclude that in fragmented landscapes, deer antlers – and perhaps phenotypic traits of wildlife in general – are strongly shaped by anthropogenic influences, but that naturally induced variation is not completely erased.

Keywords: red deer, antler, phenotype, fragmentation, translocation, drought, wildlife management.

Introduction

Compared to genetic variations, phenotypic variations of wildlife within fragmented landscapes have rarely been investigated. In (north-)western Europe, for example, a multitude of studies has shown that fragmentation, translocation and hybridization have been, and are, altering the genetic variation of large free-ranging mammals (e.g. Hartl et al. 2003, Coster & Kovach 2012, Goedbloed et al. 2012, de Jong et al. 2020). Yet, the question of the effects of these human influences on phenotypic variation has hardly been addressed

* Corresponding author

(but see Otto 2018; and specifically for birds, e.g., Bosse et al. 2017, Strubbe et al. 2020, Benham & Bowie 2021).

Anthropogenic impact on phenotypic variations is likely to be complex, and the diversity of human influences implies it may both enhance and diminish phenotypic variations (Chiyo et al. 2015, Fulgione et al. 2016). In addition, the impact will be conditional on the observed spatial and temporal scales. Translocations may be local and initially increase phenotypic variation; however, in the long run translocations and subsequent gene flow may homogenize phenotypic traits (Gippoliti et al. 2021). In western Europe, translocations and fragmentation have resulted in a genetic mosaic, as is the case with red deer (*Cervus elaphus)* populations in the Netherlands (de Jong et al. 2020). This has possibly led to differences in a conspicuous phenotypic trait, namely the size and form of antlers. Turning to other European wildlife, wild boars' (*Sus scrofa)* hybridization with domesticated pigs has not only altered their coat patterns (aberrant, spotted, hybrids) but, more fundamentally, has possibly increased their litter sizes (Fulgione et al. 2016).

Within the *Cervus* genus, there are considerable variations in body size, coat patterns and antler form – which is mostly present at large spatial scales, but also within Europe and even within countries (Geist 1987, 1998). Landscape openness may be an important driver of this variation. Crown formation at the tip of red deer antlers (a trait developed during the Middle Pleistocene) makes the European form distinct from the Asian (maral) and north-American (wapiti) lineages (Lister 1984). Within European red deer, there are possibly small differences in crown shape, and other antler traits. Hence, given impacts of translocation and fragmentation (both common occurrences for red deer over past centuries), it is likely that phenotypic variations of red deer have been distorted. More specifically, this may have enhanced the spatial diversification of the forms of red deers' antlers , as well as, potentially, new forms, due to hybridization of deer with distinct phenotypes. Meanwhile, the gene flow between connected populations with different ancestries could potentially homogenize phenotypic variations within the species as a whole (de Jong et al. 2020). In the fragmented forest and heathland area of the Veluwe, the Netherlands, some wildlife managers claim that neighbouring red deer populations show clear differences in antler shape and size, and the timing of breeding (J. Leidekker, personal communication) - despite small spatial scales (populations only few kilometres apart). However, to date, these phenotypical variations have not been quantitatively examined.

The natural environment could cause variations in the phenotypic traits of red deer (see Charmantier 2014), as an adaptation to conditions (ecotypes), e.g., camouflage and thermoregulation (Foote et al. 2016), or have a social function (Geist 1987). On the other hand, phenotypic variations may not be an adaptation, but a constraint imposed by the environment. For example, inadequate nutrition, in particular, may limit organismal development. In addition to genetic factors (Peters et al. 2021), the antler size of deer is strongly determined by nutrition (Brown 1990, Torres-Porras et al. 2009, Dryden 2016). Food and water availability varies both spatially (e.g., river floodplains vs. hill lands) and temporally (productive vs. unproductive seasons for plants). As stag antlers grow and are discarded annually they make for an excellent indicator of temporal variations in resource availability (Brown 1990, Dryden 2016).

The increase in the frequency and intensity of droughts in western Europe is particularly influential on the primary production of an ecosystem and overall water availability. Despite the attention given to climate change and the subsequent increase in drought frequency and severity (European Commission 2009), there has been little attention paid to the possible effects of such changes on the phenotypes of large herbivores. One study

found that red deer in the Iberian peninsula produced smaller antlers in years of drought (Azorit et al. 2002, Torres-Porras et al. 2009). In the Veluwe area, wildlife management techniques, such as fertilizing grazing lawns and the provision of drinking pools and salt licks may buffer the effects of extreme environmental variations, such as droughts. Nevertheless, it is not clear if wildlife management can completely mask adverse environmental resources and conditions (a topic worthy of further investigation).

Given the diverse set of both diversifying and homogenizing factors – of both natural and anthropogenic origin – we were interested in whether spatiotemporal variations in antler development exists in red deer of the Veluwe.

In this study we test for the effects of location and drought on antler development among red deer in the Veluwe. These considerations lead us to ask the following questions:

1. Are there differences in antler development between red deer from the Hoge Veluwe Estate and the relict population of Planken Wambuis?
2. What is the effect of drought on antler development among the red deer population in the Hoge Veluwe Estate?

Using deer antlers as a case study, we aim to increase understanding of phenotypic variations in wildlife that occurs in fragmented, yet spatially proximate, landscapes.

Methods

Approach and study area

We made use of the antler collections of the Hoge Veluwe National Park and the Planken Wambuis Nature Reserve. In Hoge Veluwe, game wardens actively search for shed antlers and, for many stags, almost complete series of antlers are available. At Planken Wambuis antler collection is more *ad hoc*, and there are only a few intact series of antlers from consecutive years available. In both areas, game wardens individually recognize stags and their antlers, which enabled accurate identification, and aging, of shed antlers.

Although adjacent, Hoge Veluwe and Planken Wambuis and their deer populations are separated by a road and fences. Moreover, the two areas differ in many ways and the deer populations have contrasting demographic histories. Whereas the red deer of Hoge Veluwe all stem from founders introduced from other European countries (Pelzers 1991), it is thought that the red deer of Planken Wambuis are, at least partially, autochthonous (Beijer 1897a, 1897b, Schuitemaker 1944, de Jong 2020). Deer densities are higher in Planken Wambuis (5 ind/km^2) than in Hoge Veluwe (3 ind/km^2) (Leidekker & Ouden 2018, Faunabeheereenheid Gelderland 2019).

Data collection

Three parameters were used as indicators for antler development: mass, burr circumference and the number of tines (figure 1). These parameters were chosen because of their use in related studies (Fennessy et al. 1992, Torres-Porras et al. 2009) and the number of parameters was restricted to three for the sake of feasibility.

We measured antler parameters in June 2021 on a total of 240 antlers of red deer stored at local facilities in the Hoge Veluwe National Park and at Planken Wambuis. Antler series (multiple years of one individual) were selectively sampled, focusing on the completeness of the series, the inclusion of antlers from 2019 and 2020, and quality of the antlers (preferring a low level of gnawing). Mass was measured with an analogue measuring scale with an accuracy of 50 grams. Burr circumference was measured with a string around the burr, which was then placed along a ruler, resulting in an accuracy of 5 mm. All externalities above the burr, were regarded as tines. Whenever one

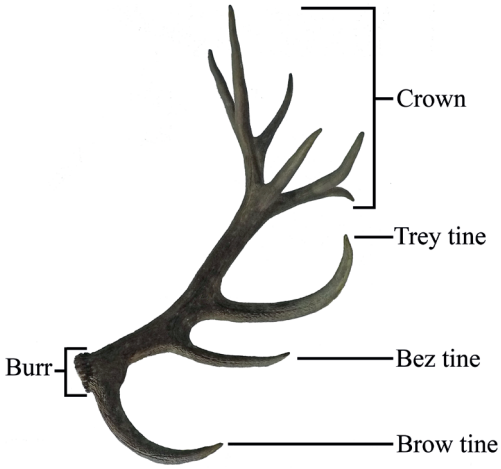

Figure 1. Anatomy of the red deer antler (left side).

or more beams were missing, the tines were not counted for that antler, as it cannot be predicted with certainty whether that beam might have formed a single or multiple tines.

Data processing

The final database contained entries on 198 red deer antlers from Hoge Veluwe and 42 red deer antlers from Planken Wambuis (each side of an antler counting as a separate entry), with the year of formation ranging from 2011 to 2019 (shedding: 2012 to 2020). Entries with an estimated loss of mass of more than 5% due to damage, were excluded from all data processing. Entries for the deer 'Hubertus', were only included in the correlation test. This is because, in contrast to other deer in the study area, this individual was unafraid of humans, and therefore had an advantage over the other deer in foraging. The absolute and relative mass growth were computed for each antler, relative to the preceding antler of the same individual and same side (left/right).

The antlers developed for the rut of 2018 were regarded as been developed under conditions of drought. The year 2018 is widely regarded as a year of exceptional drought with below average rainfall (582 mm rainfall in

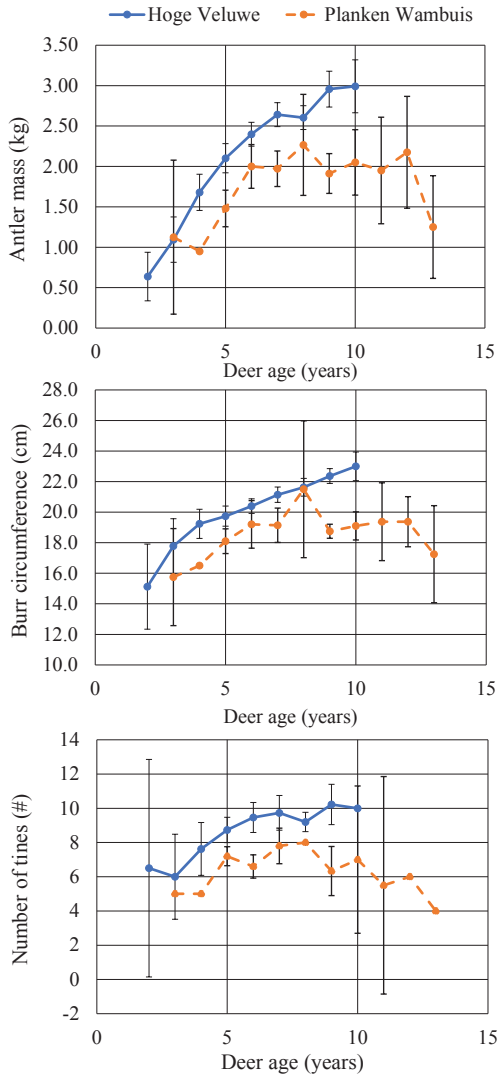

Figure 2. Top: the effect of age on the mass of antlers of red deer from Hoge Veluwe and Planken Wambuis. Error bars display a 95% confidence interval around the estimated mean. Middle: the effect of age on burr circumference of antlers of red deer from Hoge Veluwe and Planken Wambuis. Bottom: the effect of age on the number of tines of antlers of red deer from Hoge Veluwe and Planken Wambuis.

2018, opposed to an average of 880 mm over the other years in the decade - meteorological measuring station in De Bilt, Netherlands) (KNMI 2021), combined with above average

temperatures and number of sun hours, causing higher evapotranspiration (den Ouden 2018, Sluijter 2018).

Statistical analyses were run in IBM SPSS Statistics v28.0.0.0 (190). To test the differences in antler development between Hoge Veluwe and Planken Wambuis, we conducted an univariate General Linear Model (GLM) with the three measured parameters as dependent factors, area as a fixed factor and age as a random factor.

Strong mutual correlations between the three measured parameters made it possible to exclude burr circumference and number of tines in the further tests on the effects of drought and yearly developmental growth (see appendix 1-3). Hence, we were confident in only using mass as an indicator. This indicator was preferable over the other two variables as it had the largest range in data and the lowest relative measuring error. We modelled antler growth in both areas by conducting a linear regression analysis. Mass was entered as the dependent variable, and age and (age)^2 as independent variables, yielding a 2nd degree function describing antler growth with age. To test for the effect of drought on the annual change in antler mass (both absolute and relative) of red deer from Hoge Veluwe, we conducted another univariate GLM, using the drought of 2018 as the fixed (binary) factor. In addition, we conducted Mann-Whitney-U tests on the absolute and relative mass change, comparing antlers from the same age class in dry and normal circumstances.

Results

Spatial variation in antler mass

Red deer antler mass could be explained by area and age (GLM; area: df=1, F=26.095, P<0.001; age: df=11, F=9.600, P=0.002; no interaction effect: F=2.008, P=0.056). Stags from Planken Wambuis typically reached their peak antler mass at the age of eight, with a value of approx. 2.3 kg. In the Hoge Veluwe, stags reached their peak mass at the age of ten (or possibly older), with a value of approx. 3 kg. In Planken Wambuis, antler mass began to decrease among stags older than eight years (Figure 2a). Antler mass differed between the two areas at all ages, with higher masses among the Hoge Veluwe population. By age ten, when Hoge Veluwe stags reached their peak antler mass, the difference in mass amounts to 50%: in Planken Wambuis antler mass of ten year old stags was typically 2 kg, compared to 3 kg for stags from Hoge Veluwe (figure 2a).

Similarly, burr circumference could be explained by area and age (GLM; area: df=1, F=31.810, P<0.001; age: df=11, F=7.340, P=0.003; no interaction effect: F=1.408, P=0.204). At all ages, Hoge Veluwe deer had a greater burr circumference than the deer from Planken Wambuis. Peak circumferences were reached at the ages ten or higher at Hoge Veluwe and at eight years at Planken Wambuis,, with approximate values of 23.0 cm and 21.5 cm, respectively. At Planken Wambuis, a decrease in circumference was seen after eight years and, more notably so, after twelve (figure 2b).

Lastly, the number of tines could also be explained by area and age (GLM; area: df=1, F=44.035, P<0.001; age: df=11, F=4.328, P=0.014; no interaction effect: F=0.997, P=0.438). Deer from Hoge Veluwe typically formed antlers with more tines than similarly aged deer from Planken Wambuis. Deer from Hoge Veluwe had the most tines (10) at the age of 9-10 years and the most tines found on a stag from Planken Wambuis was eight, on an eight year old individual. (figure 2c).

The effect of age on antler mass in both areas could be explained by linear regression analysis with a quadratic function (Hoge Veluwe: P<0.001 and F=146.501; Planken Wambuis: P<0.001 and F=16.686). For Hoge Veluwe, the relationship was numerically expressed as *Mass = -0.667 + 0.734*Age + -0.038*Age^2* (coefficients yielded resp. t=-2.448, P=0.015; t=8.016, P<0.001; t=-5.180, P<0.001). The

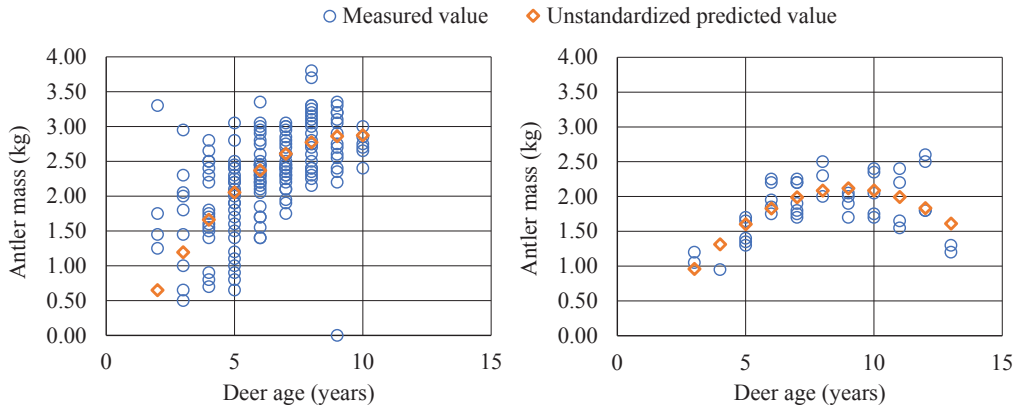

Figure 3. The effect of age on the antler mass of red deer in Hoge Veluwe (left) and Planken Wambuis (right). Non-standardized predicted values were obtained from linear regression models predicting the following relations: for Hoge Veluwe: Mass = -.667 + 0.734*Age + -0.038*Age^2; and for Planken Wambuis: Mass = -0.482 + 0.577*Age+ -0.032*Age^2.

model predicted maximum antler mass at age ten, with value of 2.871 kg (figure 3). For Planken Wambuis, the relationship can be numerically expressed as *Mass = -0.482 + 0.577*Age + -0.032*Age^2* (coefficients yielded resp. t=-1.158, P=0.254; t=5.354, P<0.001; t=-4.924, P<0.001). The model predicted maximum antler mass at age nine, with a value of 2.121 kg (figure 3). From this it follows that red deer from Hoge Veluwe typically achieve a higher maximum antler mass than those from Planken Wambuis.

The effects of drought on antler mass change

For deer from the Hoge Veluwe, absolute antler mass change was not related to age (figure 4) (GLM; df=7, F=3.317, P=0.123) and relative antler mass change decreased with age (figure 4) (GLM; df=7, F=15.449, P=0.005). Antler growth rates were higher among younger than older individuals: in juvenile males, antlers typically increased by 175% compared to the previous year; in stags, this change was typically 120%. Notably, at the age of eight both absolute and relative antler mass change showed relatively low increases under normal circumstances (figure 4).

Outcomes of statistical tests on the effect of the 2018 drought on antler development were equivocal. Relative antler development was not affected by the drought (GLM; drought: df=1, F=3.500, P=0.123; drought*age: df=4, F=1.330, P=0.264). In contrast, absolute antler mass was affected by the interaction between drought*age, with the main effect of drought being close to significance (GLM; drought: df=1, F=5.646, P=0.072; drought*age: df=4, F=3.449, P=0.011). Under normal rainfall conditions red deer stags aged seven and older typically increased their antler mass by 300 to 500 grams, in the dry year the antler mass of most stags did not grow or even decreased (estimated average growth of 0 gram or lower). Once again, notably, at age eight relatively low values for mass change were observed under normal circumstances (figure 4).

Mann-Whitney-U tests corroborated the observed patterns for the effect of drought on mass change (both absolute and relative). In five and six year old deer no effect of drought was observed on absolute nor relative mass change (5-abs: z=-0.892, P=0.205; 5-rel: z=-0.183, P=0.462; 6-abs: z=-0.300, P=0.401; 6-rel: z=-0.634, P=0.279) Similarly, deer of eight years old, which had relatively low mass

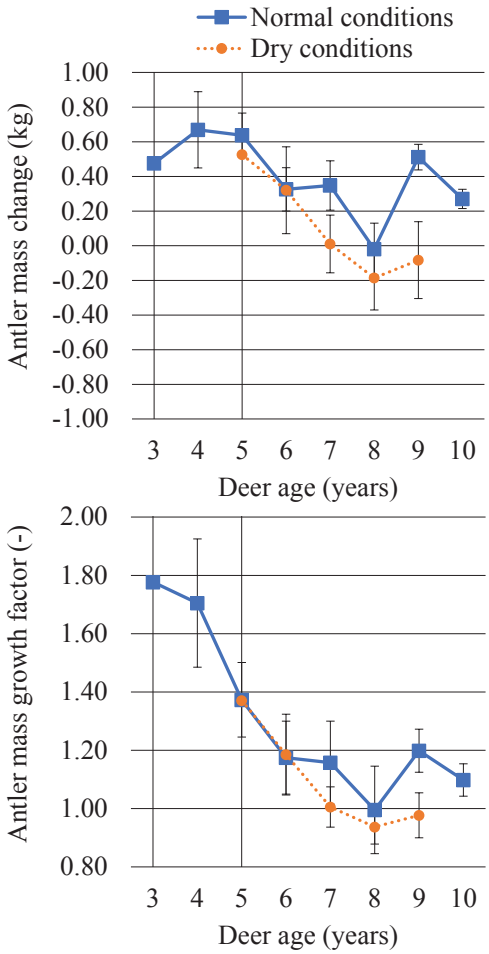

Figure 4. Absolute (top) and relative (bottom) mass change of red deer antlers from Hoge Veluwe, plotted against deer age. Mass change is calculated relative to the preceding year. Antlers developed during the drought of 2018 are regarded as having developed in 'dry conditions' (582 mm annual rainfall), opposed to antlers formed in the other years between 2011 and 2020, which are regarded to have developed under 'normal conditions' (880 mm average annual rainfall). Error bars display 95% confidence intervals around the estimated means. Raw data plots are included in appendix 3a and 3b.

change values under normal precipitation conditions, experienced no discernible effects from the drought on antler mass change (abs: z=0.956, P=0.175; rel: z=-1.198, P=0.126). For deer of seven and nine years old, there was a significant effect of drought on both relative and absolute mass change, indicating lower mass change values in the year of the drought (7-abs: z=-2.354, P=0.008; 7-rel: z=-2.382, P=0.008; 9-abs: z=-3.214, P<0.001; 9-rel: z=-3.188, P<0.001).

Discussion

To investigate fine-scale spatiotemporal variations in the development of antlers of red deer occurring in a fragmented landscape, we measured mass, burr circumference and the number of tines of antler series of red deer originating from 2012 to 2020, from two neighbouring populations in the Netherlands that have contrasting demographic and management history. Diverse anthropogenic influences may have caused diversification (due to e.g., translocations and isolation due to fragmentation) but also homogenization (due to e.g., connectivity measures and wildlife management interventions) of antler development. We found strong differences in antler development between the relict population of Planken Wambuis and the neighbouring population from the enclosed Hoge Veluwe Estate: antlers in the estate population were up to 50% larger. Furthermore, the results showed an inhibiting effect of the 2018 summer drought on antler development, though only in mature stags (i.e., stags of seven years and older). We thus found spatiotemporal fine-scale variations in a phenotypic trait, which – as we argue below – is caused by a combination of natural and anthropogenic influences.

Explanations for the observed fine-scale variations in antler development

The observed phenotypic differences between the two adjacent red deer populations are most likely explained by anthropogenic activities. We suspect the differences in antler phe-

Figure 5. Series of seven subsequent antlers from a red deer stag from Hoge Veluwe.

notypes are best explained by the contrasting genetic ancestry of the two populations (autochthonous relicts vs. an imported estate population) (Schuitemaker 1944, Beijer 1987a, 1987b). Furthermore, landscape and habitat fragmentation have led to long-term isolation of large mammals occurring in the Netherlands. This was already known to prevent genetic heterogenization among red deer (de Jong et al. 2020), and with our findings can now also be regarded to have led to 'phenotypic fragmentation'.

It is unlikely that the physical environments of the two samples was significantly different, given that the areas are directly adjacent, contain similar soil and vegetation types and are both open for recreation during daytime. There are two possible anthropogenic explanations for the observed differences. First, differences exist in wildlife management. Whilst the approaches at the two sites are largely similar (water ponds, salt licks, fertilised grazing lawns, etc.), Hoge Veluwe provides more intense management. Moreover, in Hoge Veluwe deer are kept at lower densities (approx. 3 ind/km^2) than in Planken Wambuis (approx. 5 ind/km^2) (Leidekker & Ouden 2018, Faunabeheereenheid Gelderland 2019). Alternatively, observed differences in antler weight may have a genetic cause; the two populations have different genetic ancestry, with Hoge Veluwe having a higher proportion of allochthonous genetic variations due to importing red deer from other European populations (de Jong et al. 2020).

With single antler weights of 2 to 3 kg, the antler sizes of red deer from the Veluwe are small in comparison to other European populations. Although in 'harsh' environments, such as the Isle of Rhum, Scotland, antler weights barely reach 1 kg (Kruuk et al. 2002), in many parts of Europe single antler weights of 5 kg and higher are common (Geist 1987, p. 251; Geist 1998, table A7). High antler weights are related to supplementary feeding or management, but also to fertile soils. A few decades ago, when supplementary feeding was still widespread practice in the Veluwe area antler weights there reached 5 kg (van den Hoorn 1996, p. 179).

Despite the effect of location on the level of antler development (more developed antlers at Hoge Veluwe), the relation between age and development remained largely unaffected. Maximum antler mass was reached by stags at similar ages (age ten at Hoge Veluwe and age

eight at Planken Wambuis; figure 3). Our findings on the age-mass relation were in line with expectations based on other studies (e.g. Clements et al. 2010). Thus, although sizes differ, the general antler development at the individual level are similar in the two areas (figure 3, with an example series of antlers in figure 5).

Explanations for the observed variation in antler development induced by drought

Antler development of red deer stags in the Netherlands generally concludes in mid-July, indicated by the stags shedding the velvet from their antlers, although scientific sources on this are lacking (Vodnansky 2009). The most severe circumstances of the drought of 2018 developed over the course of July, once antler development had almost finished. Nonetheless, the months of May and June saw below average precipitation and above average evapotranspiration, and after these months a potential precipitation deficit of approx. 175 mm had been recorded (national average). Throughout July this deficit rapidly increased to a value of approx. 300 mm, which then decreased slightly, ending up at approx. 280 mm by the end of September (Sluijter et al. 2018). The local potential precipitation deficit for Hoge Veluwe followed a similar pattern to the national average, with a deficit of approximately 180 mm, 300 mm and 260 mm at the 1st of July, August and September, respectively (appendix 4) (KNMI 2018). So while the precipitation deficit worsened the most dramatically over the course of July, about 60% of the deficit had been established by the end of June. This implies that, though not at maximum severity, the deer did in fact experience conditions of drought during antler development stages in 2018.

The findings on the effects of the 2018 drought showed the impact that natural circumstances can have on animals, despite active management. Our results are in line with conclusions from similar studies on Iberian red deer, for which less developed antlers in males exposed to conditions of drought have also been reported (Torres-Porras et al. 2009). The physiological explanation for the observed relation could be an overall (near) catabolic status – in energy or protein – leaving little opportunity for production including antler development. Alternatively, deer could have experienced a shortage of specific minerals required for antler development, in particular calcium and phosphorus. From an ecological perspective, there are several potential explanations: (i) during droughts, deer have more energy expenditure due to higher thermoregulatory costs; (ii) during droughts, deer have lower nutrient intake, because weather conditions and thermoregulation (including more passive behaviour) limit their foraging activity (Bugalho & Milne 2003); (iii) during droughts, deer have lower nutrient intake, because droughts cause a deterioration of forage quality and abundance.

Regarding explanation (i), within the study area, close forest stands provide sheltered microclimates, which reduce the need for active thermoregulation, e.g., through sweating and panting (Parker & Robbins 1984, Thompson et al. 2021). In addition, in the study area, red deer are already tuned to daytime avoidance, adopting a crepuscular activity pattern (Ensing et al. 2014). Regarding explanation (ii), irrespective of droughts, red deer in the study area are always limited in their foraging activity, because of their aforementioned pattern of avoiding daylight activity. At first, explanation (iii) appears unlikely, given the availability of fertilized grazing lawns and lick stones. However, the grazing lawns only provide part of the deer's diet, and most of the provided lick stones only contain sodium and chlorine (thus, lacking, *inter alia*, the calcium and phosphorus used in antler construction). During the drought, game wardens observed that, in comparison to other years, the deer were in relatively poor

overall condition (Ruseler, personal communication). Also, in the year of the drought, wardens observed that deer started rutting later than usual, which may be a consequence of an overall lower body condition. Thus, we think that lower nutrient availability is the most likely underlying cause of the reduced antler development during the drought year.

The absence of an inhibiting effect of drought in deer younger than seven years old differed from other studies. A 2001 study by Torchhammer et al. showed that drought affected the body mass of young deer more severely than the body mass of older animals. In fact, inhibited growth in a deer's first year (due to e.g., drought) can reduce antler development in subsequent years (Forchhammer et al. 2001, Torres-Porras 2009). Although our study did not take body mass into account, these observations seem to conflict with our findings, as antler mass positively generally correlates with body mass.

We consider it intriguing that the antler development of juvenile deer was unaffected by the drought, as this contradicts our expectations. Since juvenile individuals experience the largest yearly increases in antler growth, this would suggest they require relatively more nutrients than older deer. However, adult deer with maximum antler development do require the absolute maximum amount of resources for antler growth, which could explain why they appeared to be more affected by the drought. On the other hand, this is contradicted by the timing of antler development in stags of different age. With increasing age, antler development starts earlier in the year, while the end date remains constant (Clements et al. 2018). Given that the drought occurred in late spring and early summer (i.e. the final stages of antler development), individuals of all ages experienced the same negative impacts due to the drought. From this it follows that the older stags had a relatively shorter time of negative impact on their antler development due to the drought. This suggests that antler development in younger stags would be more susceptible to the effects of drought, due to a relatively longer period of dry circumstances.

To clarify these differences in the susceptibility of deer of different ages to drought, future studies could include relative antler mass in comparison to body mass and evaluate how this ratio develops with age and responds to drought. Furthermore, the mobilization of calcium and phosporus from the skeleton for antler growth at different ages could be included, as this may determine the underlying mechanisms for the different responses to drought (and possibly other environmental factors).

Furthermore, the absence of a significant effect of drought on eight year old deer is difficult to explain (since deer of the ages of seven and nine *were* affected). Considering the small sample sizes, there is a possibility for a type II error (i.e., a false negative: the effect was also present in eight year old stags, but not found). Future research should aim to obtain larger sample sizes (especially in drought years), and preferably test for multiple times of drought at multiple locations.

General implications of the phenotypic variations

Whereas the impacts of anthropogenic activities on genetics have received much attention (Hartl et al. 2003, Coster & Kovach 2012, Goedbloed et al. 2012, de Jong et al. 2020), there has been less research on their influence on phenotypes (but see Crispo et al. 2010, Fulgione et al. 2016, Thompson et al. 2019). Our study shows that humans not only induce genetic variations among large mammalian wildlife, but also phenotypic variations. Antlers are a clear 'phenotypical showcase'; almost literally showing that the morphology of wildlife can be shaped by humans. Across the species range, vertebrates may have striking intra-specific phenotypic variations (Kingdon 1990) and there are certainly many sub-

tle or invisible phenotypic traits as well. All these traits are potentially subject to anthropogenic alterations. Studies have addressed concerns about the mixing of subspecies and races due to anthropogenic wildlife translocations, which could have consequences on the adaptive capacity of species (Gippoliti et al. 2018, Thompson et al. 2019, Gippoliti et al. 2021). Additionally, selective hunting focused on individuals with impressive phenotypical features, causes populations in general to have less impressive features - e.g., current day elephants typically have smaller tusks than half a century ago, due to hunting focused on animals with large tusks (Chiyo 2015). In the Hoge Veluwe, the contrary occurs: to assert negative selection on poor body condition, hunting is directed towards animals with a low body condition (J. Leidekker, personal communication).

Furthermore, if the drought of 2018 was a manifestation of climate change, then climate change affects antler development, and thus animal phenotypes. In any case, our findings show that natural disturbances are not easily mitigated by wildlife management interventions. In the study area, red deer are helped to overcome scarcity by the installation of water pools, the provision of lick blocks (although mostly salt), and mowing and the fertilization of meadows. While anthropogenic effects can be strong, as addressed earlier, it becomes apparent that natural fluctuations in phenotypic traits are still present.

Conclusions

In this study, we have investigated the effects of spatial and temporal variations in environmental circumstances on the antler development of red deer in an area of mostly heathland in the Netherlands. After doing measurements on 240 antlers from two sites in the Dutch Veluwe region and conducting statistical tests, we conclude firstly, that red deer from the relict population in Planken Wambuis grow less developed antlers than those from the population from the Hoge Veluwe Estate; and secondly, that antler development by red deer of more than seven years old from Hoge Veluwe was inhibited by drought. The former shows that in human-dominated landscapes antler development is steered by a combination of natural and anthropogenic forces.

Whilst it was known the red deer populations in Hoge Veluwe and Planken Wambuis were genetically differentiated because of their long-term isolation, our study found how the latter has also led to 'phenotypic fragmentation'. Thus, anthropogenic actions can shape what an animal looks like. The results emphasise the impact that fragmentation and population isolation can have on wildlife.

The drought of 2018 was found to have an inhibiting effect on antler development in the Hoge Veluwe Estate, and thus influenced the phenotypes of this large wild mammal. This indicates that droughts can impact red deer antler development, despite mitigating wildlife management interventions. Furthermore, if the summer drought of 2018 was a manifestation of climate change, then our study showed that climate change affects the exterior of mammals (i.e., their appearance). Furthermore, our results indicate that natural disturbances are not easily or fully compensated for by management interventions. With the presence of water pools, salt licks, and meadow management, the deer at Hoge Veluwe still experienced an inhibiting effect on antler development as a result of the summer drought. Despite the earlier mentioned strength of anthropogenic impacts, a complete 'eradication' of natural influence on animal phenotype therefore appears difficult. With droughts expected to become more frequent and severe due to climate change, wildlife managers should re-evaluate their management actions and anticipate the possible consequences of climate change on animal phenotypes (and, possibly, related animal performance).

Future research should ideally consider multiple years of drought rather than one, and test for drought at multiple locations rather than one. A possible interaction effect of location and drought could then also be included. Furthermore, the relation between deer age and susceptibility to droughts could be explored in more detail, as the unexpected differences between adult and juvenile antler development under conditions of drought would make for an interesting study.

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Samenvatting

Effect van herkomst en droogte op geweivorming door edelherten van Planken Wambuis en Hoge Veluwe

Hoewel de invloeden van mens en omgeving op de genetica van zoogdieren in West-Europa uitgebreid bestudeerd zijn, is er weinig aandacht geschonken in onderzoek aan mogelijke effecten op de fenotypes. Het doel van deze studie is om meer kennis te vergaren over de relaties tussen fenotypische variatie, en variatie (op kleine schaal in tijd en plaats) van omgevingsvariabelen. De focus van deze studie ligt op de geweivorming van edelherten (*Cervus elaphus elaphus*) in West-Europa, als duidelijk fenotypisch kenmerk. Onze resultaten zijn gebaseerd op gemeten variabelen (massa, omtrek boven de roos, en aantal enden) aan in totaal 240 edelhertgeweien, gevormd tussen 2011 en 2020, uit twee natuurgebieden in de Veluwe. Verder presenteren wij hier de gevonden effecten van de zomerdroogte van 2018 op de geweivorming van edelherten in Nationaal Park Hoge Veluwe. Allereerst is er kwantitatief bewezen dat de geweien van edelherten op de Hoge Veluwe (gem. massa: 3.0 kg; gem. omtrek: 23.0 cm, gem. aantal enden: 10) verder ontwikkeld zijn dan van de herten op Planken Wambuis (2.3 kg, 21.5 cm, 8 enden, respectievelijk). De genotypische verschillen tussen de populaties van de twee gebieden waren reeds bekend, als resultaat van decennialange isolatie door de landschapsversnippering in Nederland. Maar uit onze resultaten blijkt nu ook, dat het gefragmenteerde, antropogene landschap direct beïnvloedt hoe geweien van edelherten eruit zien, en daarmee tot een zekere "fenotypische fragmentatie" leidt. Dit zal zich hoogstwaarschijnlijk niet beperken tot de zichtbare fenotypische kenmerken, maar zal ook invloed hebben op de onzichtbare kenmerken. Ten tweede tonen onze uitkomsten aan dat de zomerdroogte van 2018, over het algemeen, de geweiontwikkeling van edelherten boven de 6 jaar oud heeft geremd. De onderzochte geweien, die zich had-

den ontwikkeld tijdens deze tijd van droogte, vertoonden een relatief lagere groei t.o.v. het voorgaande jaar, dan geweien die ontwikkeld waren in jaren met gemiddelde neerslagcijfers. De droogte heeft deze invloed kunnen uitoefenen, ondanks de aanwezigheid van meerdere proactieve (natuur)beheermaatregelen. Hoewel de invloed van de mens op de natuur significant is, blijkt hieruit dat de invloed van de omgeving op fenotypische ontwikkeling niet gemakkelijk gecompenseerd wordt door antropogene interventies. Indien de zomerdroogte van 2018 verklaard kan worden als een gevolg van klimaatverandering, betekent dit dat klimaatverandering directe impact heeft (of kan hebben) op het uiterlijk van wilde dieren in West-Europa.

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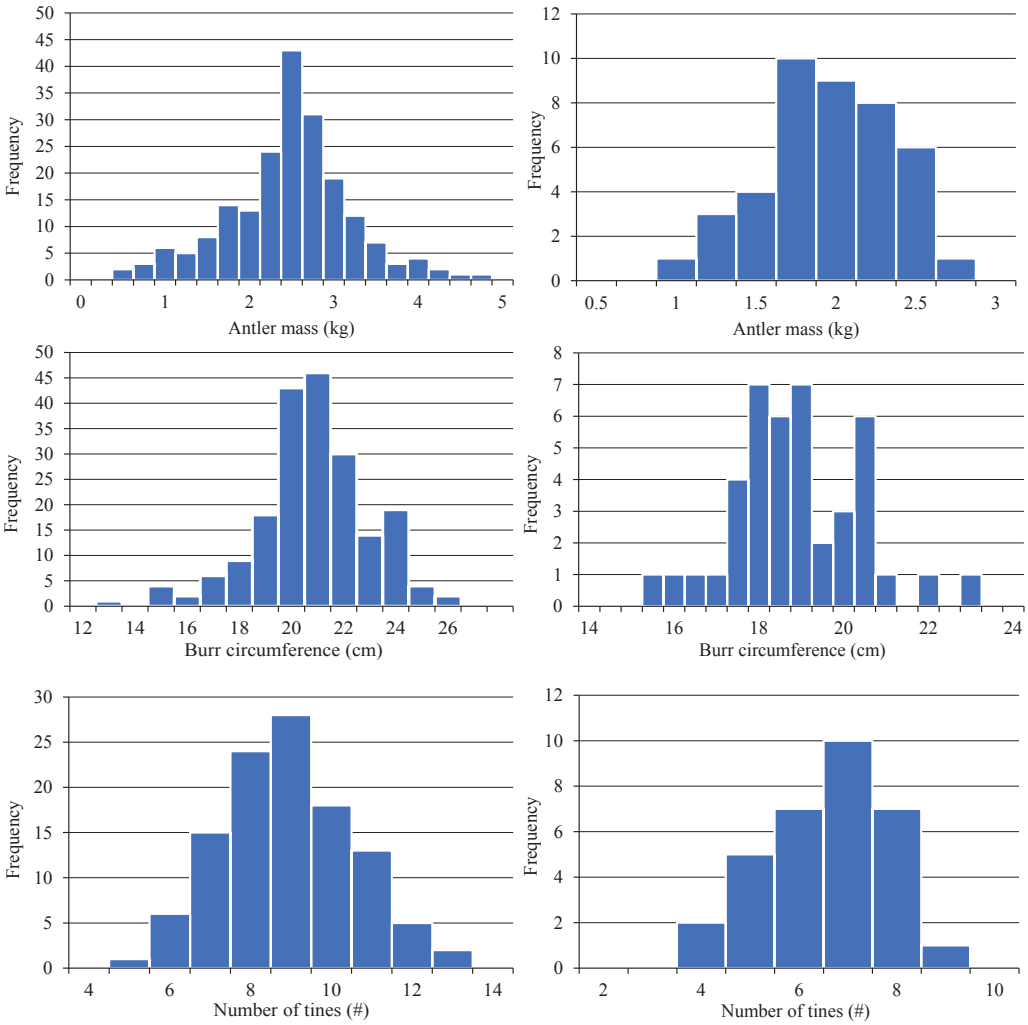
Appendices:



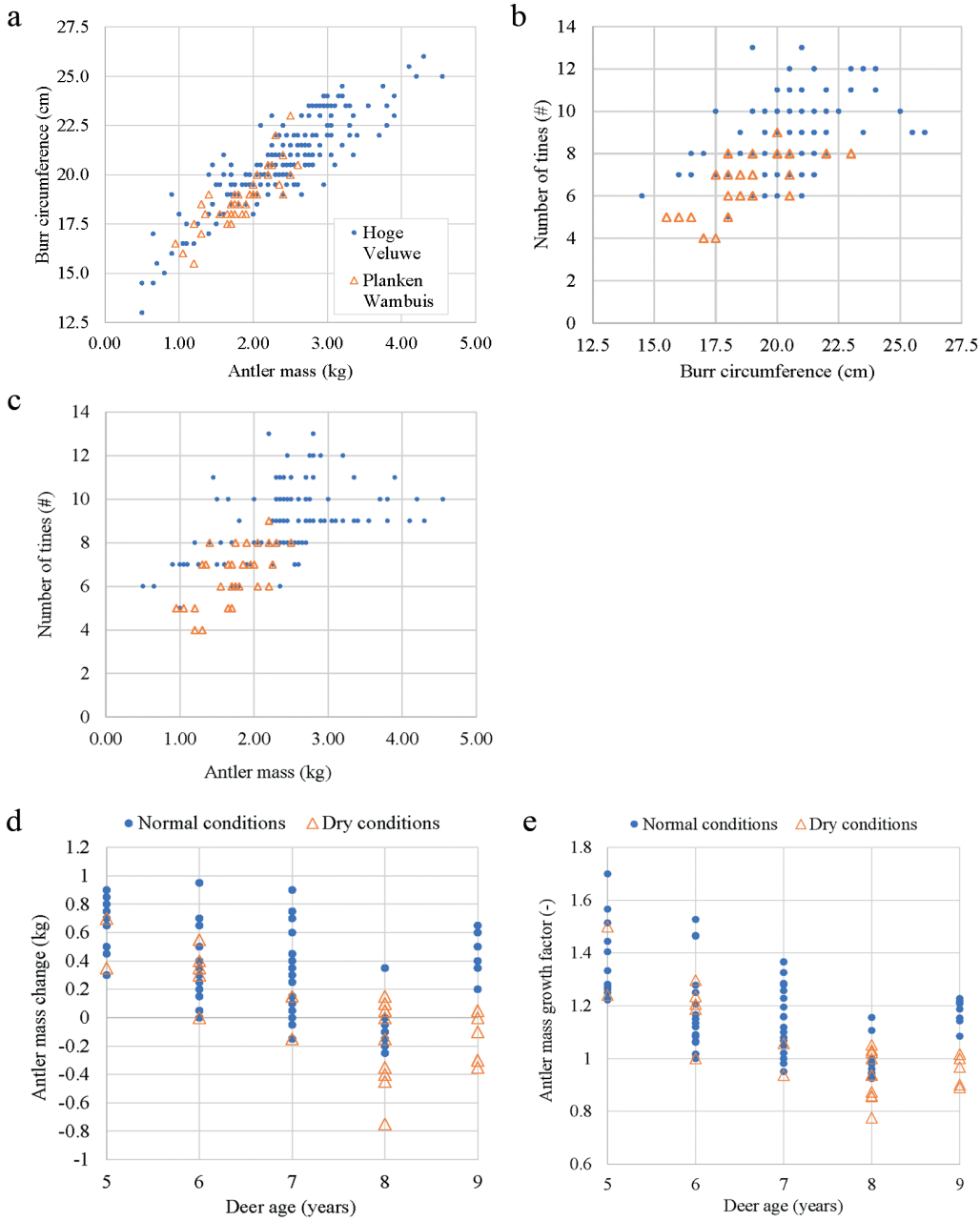
Appendix 1. Histograms of measured red deer antler parameters, from top to bottom: mass, burr circumference and number of tines. Left column: measurements of antlers from Hoge Veluwe: right column; measurements of antlers from Planken Wambuis.

Appendix 2. Output of Pearson correlation tests conducted on the following measured antler parameters: antler mass, burr circumference and number of tines. Top: Hoge Veluwe. Bottom: Planken Wambuis.

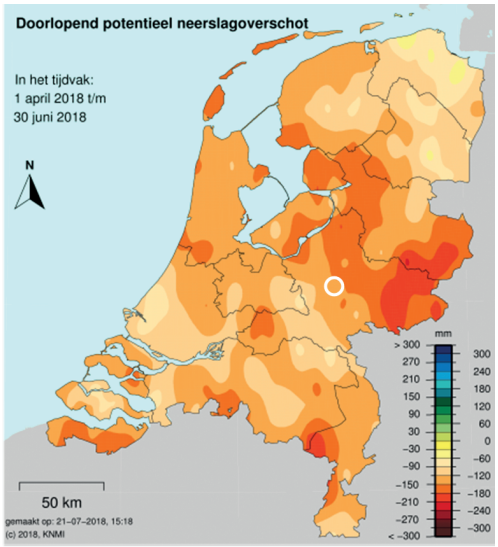
		Antler mass	Burr circumference	Number of tines
Mass	Pearson correlation	--		
	n	198		
Burr circumference	Pearson correlation	0.872**	--	
	Sign. (2-tailed)	<0.001		
	n	198	198	
Number of tines	Pearson correlation	0.504**	0.467**	--
	Sign. (2-tailed)	<0.001	<0.001	
	n	112	112	112

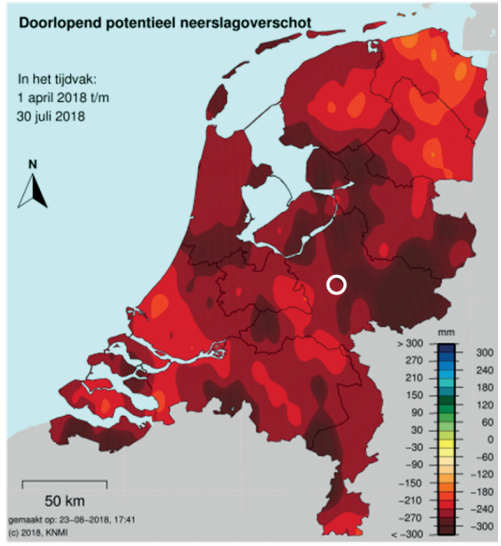
		Abstract:	Burr circumference	Number of tines
Mass	Pearson correlation	--		
	n	42		
Burr circumference	Pearson correlation	0.848**	--	
	Sign. (2-tailed)	<0.001		
	n	42	42	
Number of tines	Pearson correlation	0.624**	0.633**	--
	Sign. (2-tailed)	<0.001	<0.001	
	n	32	32	32

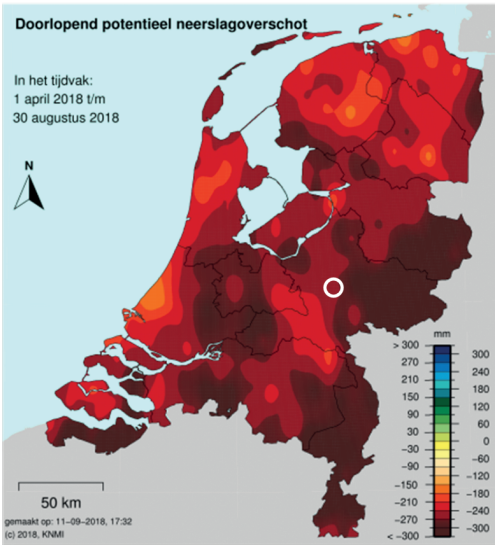
** Correlation is significant at the 0.01 level (2-tailed).



Appendix 3. a-c: Plots of burr circumference v. antler mass (a), of the number of tines v. burr circumference (b), and of burr circumference v. antler mass (c) of the sampled red deer antlers from Hoge Veluwe and Planken Wambuis. d and e: Plots of absolute antler mass change v. deer age (d) and of antler mass growth factor v. deer age (e) for sampled antlers from Hoge Veluwe. Mass change is calculated relative to the preceding year. Antlers developed during the drought of 2018 are regarded as formed under 'dry conditions' (582 mm annual rainfall), opposed to antlers formed in the other years between 2011 and 2020, which are plotted as having developed in 'normal conditions' (880 mm average annual rainfall).







Appendix 4. Continuous potential rainfall deficit in the Netherlands on 30 June (upper left), 30 July (upper right) and 30 August (bottom). The white circle indicates the location of the Hoge Veluwe National Park. Taken from KNMI (2018), https://www.knmi.nl/nederland-nu/klimatologie/geografische-overzichten/archief neerslagoverschot, altered by R. van Mourik.