On the brink of extinction

Biology and conservation
of Northern Wheatears in the Netherlands

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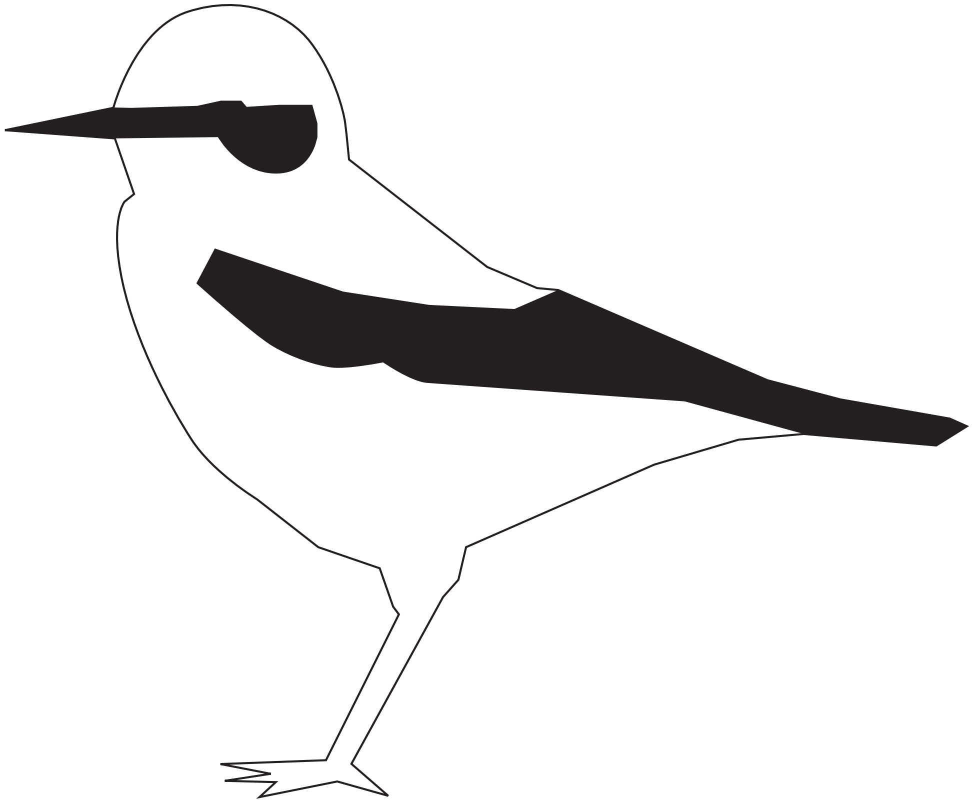
Dit proefschrift is tot stand gekomen in samenwerking met Stichting Bargerveen

Paranimfen:
Chris van Turnhout
Marijn Nijssen

To my family and to birds, for the joy they bring

Contents



Chapter 1

General introduction

H. Herman van Oosten

Factors and approaches in studying population limitation

Degradation of the natural environment due to anthropogenic alterations is a threat to present and future diversity of life. Affected ecosystems often show population increases of a few species but declines of many. Identifying the exact causes of ecosystem deterioration is of greatest importance if we want to preserve species and communities, especially in densely populated regions where (strong) anthropogenic effects will remain present. Sometimes a single factor governs the demise, which may enable finding this particular cause with relative ease. Yet, the decline of populations is generally a result of a mixture of different factors, each contributing to the decline, singly or in relationship with other affecters. The complexity of unraveling this mixture of affecters is far less easy, and may yield several points of action for conservation to become effective.

For example, non-migratory species with a local distribution and life cycle, the unravelling of multiple threats may already be quite a task. For migrants however, finding causality seems even more of a puzzle, as a major part of their life cycle is spent on locations at which completely different stressors may act. Keeping these species in a favourable conservation status is more difficult, as a willing nature manager can only directly influence a limited part of the life cycle.

Understanding population development of a given species boils down to finding environmental drivers of demographic traits; i.e. how reproduction and survival are affected by the environment which the individuals in a population experience during their annual cycle. Clear-cut as this may be, ecological research intended to find proximate causes of population decline is poorly developed and well-meant conservation intentions may, on the whole, be less effective when not based on evidence (Sutherland et al. 2004).

For conservation purposes, it may not be adequate to state that species decline due to habitat changes, as it does not explicitly convey the limiting factors. Without knowledge about the ecological requirements of particular species, (alleged) habitat restoration may not be successful for that species. Population limitations may be related to habitat characteristics such as food availability or quality, predation, and direct and indirect effects of N deposition. They may also concern factors not, or less clearly, inherent to the habitat such as genetic factors. Moreover, interaction of these factors may further increase the complexity of mechanisms limiting populations. Ground-foraging insectivores, for example, are hampered by tall vegetation (due to increased N deposition, land abandonment or decreased grazing pressure) which changes the abundance, diversity and size distribution of arthropod species assemblages through changes in, for instance, microclimate or host plant availability and quality. Therefore, we need information on basic and specific ecological traits of the species we wish to conserve are we to find true causality of decline and to effectively protect the species.

Another approach to find limitations is to study species assemblages according to shared life history characteristics. As a result, these studies provide general factors determining the decline of such ecological groups of species. For instance, migratory terrestrial birds declined most strongly in the Netherlands, compared to other avian ecological groups (Van Turnhout et al. 2010), habitat specialism *per se* makes bird species vulnerable (Julliard et al. 2004) as does single-broodedness compared to species with more broods per year (Jiguet et al. 2007). Yet, interesting and important these findings are in themselves, they often do not provide –nor do they intend to- actual clues to revert declines.

For this thesis, I am interested in the continuing declines among avian species inhabiting Dutch dune grasslands, in relationship with the conservation status of both these bird species and short dune grasslands as an important habitat to the Dutch overall biodiversity. My research focuses on factors limiting population growth of one particular species, the Northern Wheatear (*Oenanthe oenanthe*), as this seems to be the next species in line to become extinct in this habitat and likewise at the national scale of the Netherlands.

Study species

The Northern Wheatear is an insectivorous passerine and the sole member of the genus *Oenanthe* that breeds *circum*-boreal: from eastern Canada across Eurasia to western Alaska, and from sea-level arctic tundra to 4000 m high on Turkish mountain slopes (Glutz von Blotzheim and Bauer 1988). Most individuals winter in Sahelian Africa, which means that birds breeding in Alaska have 14.500 km to fly, there and back again (Bairlein et al. 2012).

Depending on latitude one or two broods per year are produced, each of about 5 sky-blue eggs. Its morphology is adapted to cursorial locomotion (strong legs, large feet, Kaboli et al. 2007) and it forages mainly on the ground on arthropods. Nests are often built in burrows. Given the preferences for short-grown, open fields and conspicuous behavior, the species is much suited for field studies.

The European population declined by 63% since 1990 (PECBMS 2013), yet the global population is not deemed threatened at present (Birdlife 2014). So either European breeding sites are deteriorating more than other sites, wintering conditions differ between western and eastern populations or both. In the Netherlands, the Northern Wheatear has declined from 1900-2500 breeding pairs around 1980 to few relict (sub) populations containing 260-290 breeding pairs in 2012 (Boele et al. 2014). The species now inhabits only nature reserves, so it is not prone anymore to direct loss of habitat due to expansion of cities, highway networks, or other built-up areas, neither directly influenced by changing agricultural management as many other species are (Donald et al. 2001). Unravelling the remaining causes of population decline requires a detailed knowledge of the species' demographic rates, such as reproduction success, survival, and dispersal, and a thorough understanding of ecological factors affecting these demographic parameters. Conservation actions can be formulated once the limiting factors are detected.

Historical decline in relation to ecosystem changes

Breeding numbers of Northern Wheatears have been declining for many decades in the Netherlands and surrounding countries in the lowlands of western Europe (Glutz von Blotzheim and Bauer 1988). The species already declined between 1870 and 1930 in the agricultural landscapes of lowland Europe, from widely spread and common to scarce (Glutz von Blotzheim and Bauer 1988). Afforestation of heathlands and drift-sands, and strong sward-height increase following the myxomatosis epidemic in Rabbits (*Oryctolagus cuniculus*) of the 1950s, rendered large expanses of breeding grounds unsuitable (Glutz von Blotzheim and Bauer 1988). Intensifying of agricultural practises since World War II led to a strong decline of the species in agricultural landscapes, to a final disappearance from such landscapes in the Netherlands during the 1980s (Hustings and Vergeer 2002). Since then, the species is largely confined to nature reserves in the Netherlands, red-listed and therefore exempt from explicit *malheur* as afforestation or otherwise maltreatment of its open habitat.

Strongly increased deposition of fertilizers and acid rain from agriculture and industries have led to an increased availability of nutrients in erstwhile nutrient-poor, sandy soils (Bobbink et al. 2010; Veer and Kooijman 1997), which are characteristic for the breeding habitat of contemporary Northern Wheatears in the Netherlands. Combined with low Rabbit grazing due to a next viral disease (RHD) in the 1990s (Drees et al. 2006; Drees and Van Manen 2005, see Box 1), this has led to rapid encroachment of nitrophilic graminoids at the expense of bare soil and the abundance and diversity of flowering plants and homogenization of vegetation structure (Bobbink et al. 2010; Stevens et al. 2004). The resulting simplification of grassland plant communities has led to impoverishment of arthropod communities (Haddad et al. 2001; Koricheva et al. 2000; Murdoch et al. 1972; Otway et al. 2005; Schaffers et al. 2008; Siemann 1998). In the end, simplification of plant communities potentially cascades up to dietary constraints for insectivores (Britschgi et al. 2006; Schekkerman and Beintema 2007; Vickery et al. 2001), setting the stage for population changes, since first-year survival is often related to body condition of nestlings, which may be in turn affected by decreased prey availability (Brinkhof 1997; Magrath 1991; Naef-Daenzer et al. 2001; Nagy and Holmes 2005; Perrins 1991).

Increased sward heights may severely hamper Northern Wheatears in foraging, since they are adapted to short vegetation (Kaboli et al. 2007). For example, Tye (1992) found that after rapid vegetation increase during spring Northern Wheatears enlarged their territories or refrained from breeding altogether. Similarly, Pärt (2001a) and Low et al. (2010) found that nest predation of Northern Wheatears was higher when nests were located in tall vegetation compared to short vegetation. Lastly, parents taking care of nestlings foraged further from their nests when they were located in tall vegetation than in short vegetation, which probably explained their lower survival (Low et al. 2010). Therefore, encroachment by tall nitrophilic grasses must have had strong consequences for the Northern Wheatears of dune grasslands in the Netherlands.

Demographic changes in relationship to habitat changes, as briefly sketched above, influence each other as well. For example, few nestlings may fledge in years with high nest predation rates yet the number of recruits may not be different the next year compared to years with low nest predation rates. This is because nest predation reduces competition after fledging, due to lower numbers of fledglings, which enables body conditions of surviving chicks to be better before migration. Likewise, the total number of fledglings in years with fewer breeding females (due to, for example, adverse conditions on the wintering sites) may be similar to years with more breeding females because females tend to lay fewer eggs when density is high. However, if food conditions on the breeding sites are insufficient due to habitat alterations, the population will decline since no compensation occurs after heavy winter mortality of adults. Therefore, determining exact rates of fecundity, mortality, immigration and emigration are important, and should be followed by studies to seek (ecological) explanations for the observed demographic rates as to, in the end, try to reverse the decline.

In an effort to try and pinpoint important factors governing contemporary population developments of Northern Wheatears in the Netherlands, we set out to closely study their breeding biology in three populations: (1) coastal population Vogelduin near Castricum, in the Noord-Hollands Dune reserve, (2) coastal population Den Helder, 40 km north of Castricum and (3) inland population Aekingerzand, in National Park Drents-Friese Wold, about 120 km away from both coastal sites. All sites are managed as nature reserves, and access by the public is limited to paths and roads. Below, short descriptions of each site are given.

Site Aekingerzand

This inland population (site A, 268 ha) breeds in heathland with drift sands. Before its restoration from 1990 onwards, this site was largely afforested. Vegetation of drift sands and its edges includes species as the grasses *Corynephorus canescens* and *Arrhenatherum elatius*, the forbs *Jasione montana*, *Filago minima*, *Hieracium pilosella*, *Rumex acetosella* and many lichens. The heathlands consist mainly of the heath species *Calluna vulgaris*, *Empetrum nigrum* and the grass *Deschampsia flexuosa*. After large-scale measures, including removal of trees, scrubs and locally the upper soil layer, breeding numbers (determined as the number of territorial females) increased to 47 in 2008, to decline again to about 17 in 2014.

Site Castricum

The coastal population at Castricum (site C, 74 ha), already mentioned 235 years ago (Nozeman 1789), is within 1 km from the sea. It is located in stabilized, lime-rich dune grasslands (‘grey dunes’) with vegetation dominated by grasses (*Calamagrostis epigejos*, *Ammophila arenaria*), *Carex arenaria*, low scrub (*Salix repens*, *Hippophae rhamnoides*), mosses, lichens, characteristic forbs like *Viola curtisii* and with scattered patches of vegetation-free ground. Numbers in site C decreased from 165 in 1988 to 34 in 2000, and a mere 7 in 2014.

Box 1. Rabbits and Northern Wheatears

Rabbit declines as a consequence of viral diseases (myxomatosis, Rabbit Haemorrhagic Disease (RHD)) are commonly seen as one of the main factors responsible for the Northern Wheatear's demise in the Netherlands. Rabbits are seen as key species as they keep the vegetation short by their grazing and they provide burrows in which Northern Wheatears often breed. After the 1950s outbreak of myxomatosis, a new viral disease was responsible for an estimated 90% decline of the Dutch Rabbit population during the 1990s (Drees et al. 2006; Drees and Van Manen 2005). Therefore, the strong decline of Rabbits may have led to a twofold tragedy: a decline of nesting possibilities and rapidly increasing vegetation height. Indeed, empirical positive correlations across different breeding areas between Rabbit number and number of Northern Wheatear pairs showed a concomitant decline of Rabbit and Northern Wheatear numbers (Van Turnhout et al. 2007). Nevertheless, the positive correlations between Rabbit and Northern Wheatear numbers are sometimes less obvious when compared in more geographic detail (Versluijs et al. 2008, fig. 1). For site Noord-Hollands Dunereserve (NHD) the number of breeding Northern Wheatears neatly follows the numbers of Rabbits. In two other former breeding sites trends seem to deviate: an increasing trend of Rabbit numbers is not followed by recolonisation of Northern Wheatears. Indeed, areas may seem suitable with a high presence of Rabbits and yet, they are not graced by breeding Northern Wheatears (such as area Meijendel in fig. 1).

Attractive through simplicity the above mentioned correlations between nitrogen, Rabbits and Northern Wheatears may seem, they do not always empirically explain the local extinction of populations. Causality cannot be inferred from such correlations.

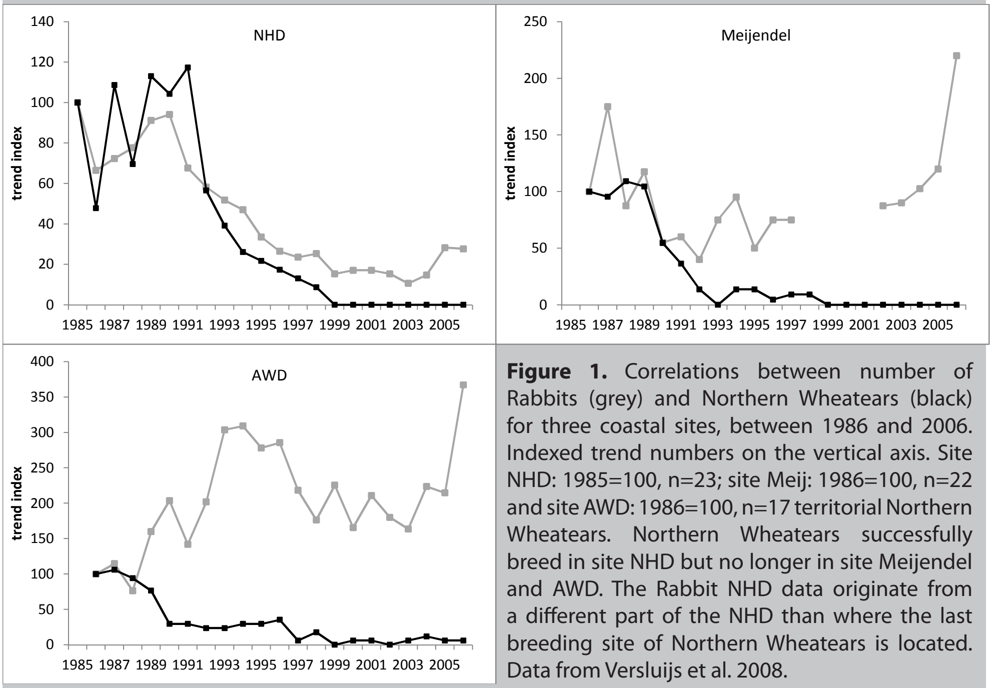


Figure 1. Correlations between number of Rabbits (grey) and Northern Wheatears (black) for three coastal sites, between 1986 and 2006. Indexed trend numbers on the vertical axis. Site NHD: 1985=100, n=23; site Meij: 1986=100, n=22 and site AWD: 1986=100, n=17 territorial Northern Wheatears. Northern Wheatears successfully breed in site NHD but no longer in site Meijendel and AWD. The Rabbit NHD data originate from a different part of the NHD than where the last breeding site of Northern Wheatears is located. Data from Versluijs et al. 2008.

Site Den Helder

Coastal population Den Helder (site D, 160 ha) is separated by 40 km from site C and also within 1 km of the sea. Birds also breed in dune grasslands but, in contrast to site C, the soil is lime poor. Therefore the vegetation is different from site C, with characteristic grasses as *Corynephorus canescens* and *Festuca glauca*, forbs as *Rosa pimpinellifolia*, *Hieracium pilosella* and *Viola canina*, besides many lichens. The site is sandier than site C. Numbers in site D have fluctuated without a clear trend between 1992 and 1998 (min-max 45–69, data Sovon) and between 24–47 pairs during 2007 and 2011.

Overview of the present thesis

This thesis consists of five research chapters, in which major findings are presented and their implications for the Northern Wheatear are discussed.

Chapter two addresses whether population growth in three different Dutch populations is a result of large scale stressors or different demographic factors on a local scale. The outcome can be of considerable importance for practical conservation measures. Species conservation efforts may often be aimed at protecting species on a large scale (e.g. country wide). Yet different populations may in fact be affected by different local stressors or demography. If this is the case, copying a successful restoration strategy from one area into another may lead to failure. As a result we are able to assess how population growth is driven per population and if local conservation measures should be developed or that one measure fits all.

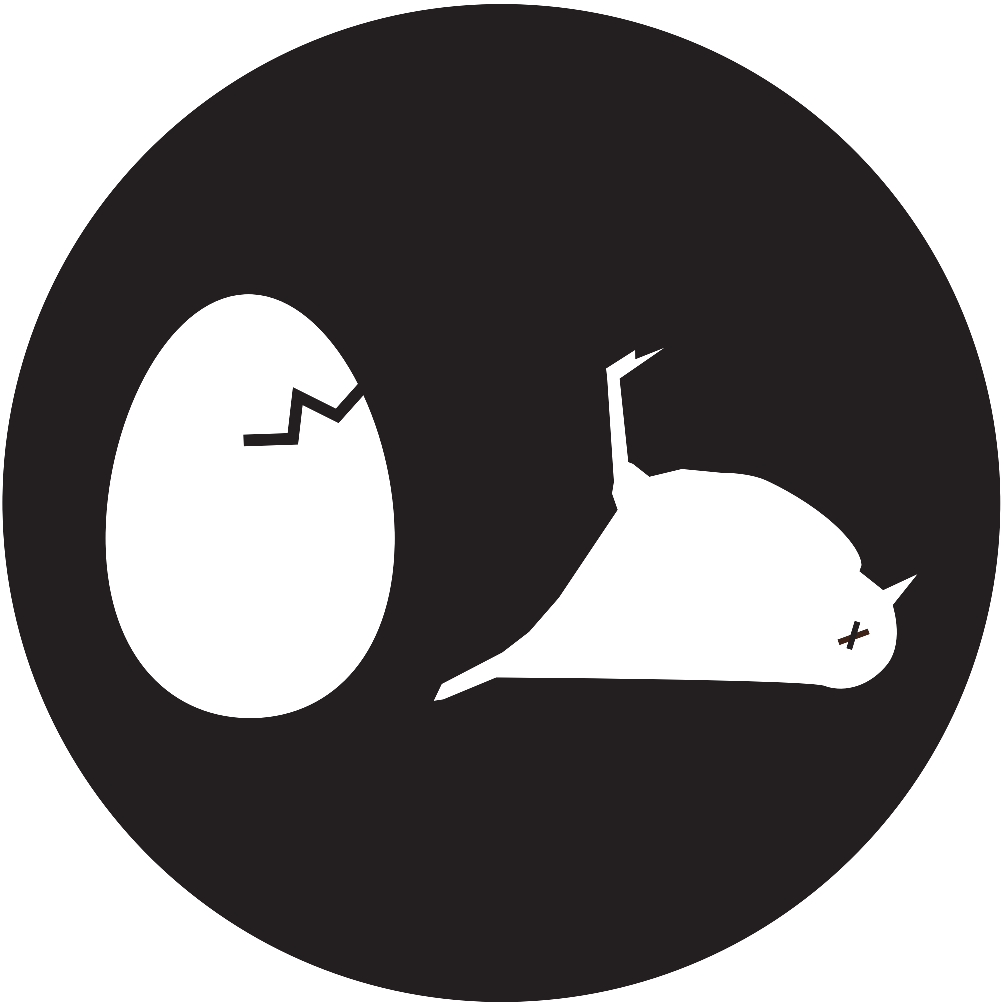
In **chapter three** we study the effects of timing of breeding on survival of juveniles in site Castricum. In many bird species first-year survival declines with fledging date. We disentangle seasonal mortality and extend knowledge on passerine first-year survival a bit further: we study how seasonal mortality differs between early and late fledged Northern Wheatears. Detailed knowledge on first year survival may help conservation efforts since first year survival can exert strong effects on population growth.

Chapter four describes the feeding ecology of Northern Wheatears in one coastal site, with the aim to meaningfully address the importance of different vegetation types for prey and for Northern Wheatears. This may enable tailoring of conservation measures.

Chapter five assesses possible genetic effects of population fragmentation as occurred in northwestern Europe.

In **chapter six**, we investigate presence and possible effects of organic pollutants in the Northern Wheatear foodchain in coastal dunes.

Chapter seven discusses the results of the preceding chapters, provides conservation directions and indicates directions for further research.



Chapter 2

Site-specific dynamics in remnant populations of Northern Wheatears *Oenanthe oenanthe* in the Netherlands

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Abstract

Dynamics of populations may be synchronized at large spatial scales, indicating driving forces acting beyond local scales, but may also vary locally as a result of site-specific conditions. Conservation measures for fragmented and declining populations may need to address such local effects to avoid local extinction before measures at large spatial scales become effective. To assess differences in local population dynamics, we aimed to determine the demographic drivers controlling population trends in three remaining populations of the Northern Wheatear *Oenanthe oenanthe* in the Netherlands, as a basis for conservation actions. An integrated population model (IPM) was fitted to field data collected in each site in 2007–2011 to estimate fecundity, survival and immigration. Sites were 40–120 km apart, yet first-year recruits were observed to move between some of the sites, albeit rarely. All three populations were equally sensitive to changes in fecundity and first-year survival. One population was less sensitive to adult survival but more sensitive to immigration. A life table response experiment suggested that differences in immigration were important determinants of differences in population growth between sites. Given the importance of immigration for local dynamics along with high philopatry, resulting in low exchange between sites, creating a metapopulation structure by improving connectivity and the protection of local populations are important for the conservation of these populations. Site-specific conservation actions will therefore be efficient and, for the short term, we propose different site-specific conservation actions.

Keywords
Elasticity, fecundity, immigration, integrated population model, life table response experiment, survival.

Introduction

Many threatened bird species occur in small populations scattered throughout a fragmented landscape. With decreasing population size, population persistence decreases (Gilpin and Soulé 1986) and, in dispersive animals, small populations sometimes persist only in the presence of a large source population or as part of a metapopulation (Hanski and Ovaskainen 2000). Evaluating population dynamics for conservation management requires high-quality data on the demographic parameters that could be important determinants of population viability: breeding numbers, reproductive success, sex- and age-specific survival and dispersal (Ricketts 2001). Assessing which vital rates drive population dynamics constitutes an important step towards proposing informed conservation measures, and the spatial scale at which they operate (Caughley 1994; Schaub et al. 2012).

Dynamics of populations may be synchronized at large spatial scales, indicating driving forces acting beyond the scale of local sites (Abbott 2011; Blasius et al. 1999; Kendall et al. 2000; Koenig 1999; Lande et al. 1999; Liebhold et al. 2004; Paradis et al. 2000). However, synchrony in population dynamics decreases with decreasing population size, due to increasing demographic stochasticity (Saether et al. 2007; Saether et al. 2011). Therefore, whereas populations of common species follow the waves of synchronized large-scale stressors (Koenig 2002; Saether et al. 2011), rare species, which often occur in small and isolated populations, may require conservation interventions at a more local scale. Vital rates may be affected differentially at local scales due to site-specific conditions.

Knowing the underlying causes for large-scale population fluctuations (e.g. climate change) is important for developing long-term and international conservation strategies, but it might be equally important, and perhaps more effective in the short term, to identify the vital rates that drive local population growth, and how local populations interact (Pulliam 1988). This allows the development of evidence- based and tailored measures to safeguard local populations in the short term until positive effects of long-term, large-scale measures have become effective. Hence, to safeguard rare and localized species at a large geographical scale, it may well be necessary to identify the demographic bottlenecks of remaining local populations. Preferably, studies aimed at understanding drivers of short-term local dynamics and of long-term large-scale dynamics should be undertaken jointly to allow for effective preservation of species. However, finding the appropriate spatial cale for such conservation studies is challenging (Petranka et al. 2004; Schaub et al. 2006). One way is to include several local populations that differ in size and degree of isolation (Schaub et al. 2006).

The Northern Wheatear *Oenanthe oenanthe* occurs in the Netherlands in small and fragmented populations. This migratory passerine is one of the most rapidly declining breeding birds in Europe (Gregory et al. 2009). Since 1990, the European population has declined by over 50% (PECBMS 2013) and numbers in the Netherlands have dropped by at least 80%, from 1900–2500 breeding pairs in the 1970s to 250–290 pairs in 2011 (SOVON 2002, Boele et al. 2013). The species now appears on the Dutch Red

List of Threatened Species. We collected data on population size and demography in three remaining populations of the species in the Netherlands, together holding almost half of the national population. The aim of the present study was to determine which demographic parameters most strongly influenced recent local population growth, as a basis for conservation actions.

We estimated vital rates (fecundity, first-year and adult apparent survival and immigration) for all three local populations by fitting an integrated population model (IPM) to field data. For each population, we performed an elasticity analysis to assess how sensitive the local population growth rate was to changes in vital rates (Jongejans and De Kroon 2005). In this way, we assessed how much the population growth rates would change if each of the vital rates was changed by a given percentage. We complemented these analyse by exploring which demographic processes drive differences in average growth rate between the populations by decomposing these into the contributions of each vital rate in a life table response experiment (LTRE; Caswell 2001) in order to determine how much each of the parameter differences contributed to the difference in population growth rates between the three sites.

Methods

Study species and sites

The Northern Wheatear is an insectivorous long distance migrant breeding from eastern Canada and Greenland across Eurasia to western Alaska (Glutz von Blotzheim and Bauer 1988). In lowland western Europe, numbers have been declining since the 1980s (Burfield and Van Bommel 2004). Once widespread in rural areas, Northern Wheatears have all but disappeared due to agricultural intensification (Glutz von Blotzheim and Bauer 1988). For a variety of reasons, populations in (semi-) natural areas are under pressure as well.

In the Netherlands, Northern Wheatears were widely distributed until the 1980s (Sovon 2002) in sandy, oligotrophic grasslands in coastal dunes and heathlands, where they often bred in burrows of Rabbits *Oryctolagus cuniculus*. The demise of the Dutch population has been attributed to declining Rabbit populations as a result of viral disease. Regional differences in the onset of the Northern Wheatear decline seem to be correlated with differences in the timing of Rabbit declines, with a delay of 5–10 years (Van Turnhout et al. 2007). Being morphologically adapted to foraging on short field ayers (Kaboli et al. 2007), Northern Wheatears aced a deterioration of foraging habitat through grass encroachment in the absence of Rabbits. In addition, large expanses of breeding habitat were lost due to eutrophication and acidification, which stimulated growth of tall grasses, a threat to many oligotrophic systems (Bobbink et al. 2010). As such, the Northern Wheatear is an indicator of the quality of oligotrophic grassland and heathland ecosystems, and representative of several other ground-nesting and ground-foraging bird species (Van Turnhout et al. 2010).

Between 2007 and 2011, we studied three populations of Northern Wheatears in the Netherlands. The inland population at Aekingerzand (site A, 268 ha) is about 140 km from the other two populations. The coastal population at Castricum (site C, 74 ha), present for over 200 years (Nozeman 1789), is separated by 40 km from the coastal population at Den Helder (site D, 160 ha). Populations C and D breed within 1 km of the sea in coastal dunes with vegetation dominated by grasses (*Calamagrostis epigejos*, *Ammophila arenaria*), *Carex arenaria*, low scrub (*Salix repens*, *Hippophae rhamnoides*), mosses, lichens, characteristic forbs such as *Viola curtisii* and with scattered patches of vegetation-free ground. Population A breeds in heathland with drift sands. This site was previously largely forested but was restored from the 1990s by large-scale removal of trees, scrub and, locally, the upper soil layer. All sites are managed as nature reserves, and access by the public is limited to paths and roads (most restricted in D).

Long-term Northern Wheatear population trends differ strongly between sites: after large-scale removal of trees, breeding numbers (determined as the number of territorial females) at site A increased from 2–5 to 30. However, numbers in site C decreased from 165 in 1988 to 34 in 2000 and numbers in site D have fluctuated without a clear trend between 1992 and 1998 (min–max 45–69, data SOVON).

Population census and fecundity

We collected annual data on population sizes, fecundity and sex-based survival at all sites. Breeding success was not quantified for site D in 2010. Data on population size and fecundity were obtained by intensive searching for territory-holding and nesting pairs throughout the breeding season (April–July) in order to establish the number of territories, number of broods and reproductive output of individual nesting attempts. Northern Wheatears regularly produce replacement or true second broods at our study sites (Table 1). Nests were found during construction or at the egg stage by closely observing females. Nests with nestlings were easily found by following feeding parents. Nests were visited several times during a breeding attempt, with a minimum of two visits (census including ringing of nestlings and post-fledging check for dead chicks or unhatched eggs). The number of nests monitored each year was 32–67 at site A, 21–40 at site C and 33–82 at site D.

In nests situated deep inside Rabbit burrows, nest stage (nest building, eggs, young) was determined using an infra-red camera mounted on a stick, connected to a hand-held screen. Nestlings in deep nests were counted and ringed either by carefully shortening the burrow (which never resulted in abandoning the nests) or, rarely, when they appeared outside the burrow. The nest was subsequently excavated to check for any dead chicks or eggs. Families were followed after leaving the burrow to determine the presence of any unringed, and hence missed, juveniles to determine the number of fledglings. These nestlings were captured using spring-traps.

When about 10 days old, nestlings start to walk in the burrow and hence they could be out of reach at the moment of ringing. To avoid missing juveniles, we ringed most nestlings between age 5 and 9 days. As the populations were small and Northern Wheatears are easily detected, we were able to determine the number of breeding females precisely. Even if successful nests were not found, they were found soon after fledging, as family groups are conspicuous and unlikely to be missed. Yearly, up to two nests were found after fledging across populations. To determine possible predation rates in our populations, we counted the numbers of predated nests and number of predated emales, i.e. females not observed following a nest predation event. Unsuccessful females were not easily missed, as visits were frequent and most birds were colour-ringed: the nesting stage was known approximately for each female, and unexpected behaviour (e.g. a female spending time above ground when she was expected to be brooding, or spending time off territory) was followed by a nest check.

Capture-mark-recapture study

In 2007–2010, we individually colour-ringed 404 birds at site A (327 juveniles or nestlings and 77 adults, ≥ 1 year old), 245 at site C (221/24) and 666 birds at site D (538/128), in total 1315 birds. Most adults had already been ringed as nestlings, which explains the low numbers ringed. The sex of ringed nestlings was unknown, but all adults were sexed on the basis of plumage characters (Glutz von Blotzheim & Bauer 1988). Resightings were obtained by dedicated weekly searches in each site during the entire breeding season using telescopes. Resightings in 2007–2011 were used to estimate adult and first-year survival and movements between sites, with inclusion of occasional reports by birdwatchers from the rest of the Netherlands (Elsewhere, 'site' E). Most suitable breeding areas in the Netherlands, apart from our study sites, are surveyed annually as a part of the national breeding bird monitoring programme Boele et al. 2013).

Integrated population model

We developed an IPM for the three populations to estimate demographic variables driving local population dynamics, including immigration, from the joint analysis of population counts, breeding success and capture-mark-recapture data. The ability to estimate immigration rates is a huge advantage of IPMs (Abadi et al. 2010), as immigration may be a very important variable from a conservation perspective (Schaub and Abadi 2011). The model was based on the IPM developed by Schaub et al. (2012). Model parameters were estimated using Monte Carlo Markov chains (MCMCs) in JAGS (Plummer 2003), derived from a script in R (R Development Core Team 2012). Three chains were run for 30 000 iterations each. After a burn-in of 10 000 iterations, every 10th remaining iteration was sampled to estimate the posterior distributions, which we summarized by their mean, standard deviation (sd) and 95% credible intervals. We used uninformative priors for all parameters, with the exception of sampling error of the count data, for which we provided a very narrow variance. The IPM equations are given in Appendix S1.

The IPM described a pre-breeding census for each of the three sites A, C and D. The model did not incorporate direct movements between these populations, as a multistate formulation of the CMR likelihood would make it much more complex. Movements between sites were very rare. However, the IPM estimated the annual immigration rate (immigrants per female present in year t-1) from the joint data. The (absolute) number of immigrants was specified by a Poisson distributed variable, with mean equal to the product of the number of females present in the previous year and the estimated immigration rate. These immigrants probably originated from populations other than those studied, possibly outside the Netherlands. Emigration was not modelled explicitly, but was included in the estimates of apparent survivalrates. The IPM incorporated two age-classes: 1-year-old birds that all started breeding at this age, and older birds, for each population. Immigrants formed a third class, of unknown age (≥1 year). Fecundity and survival were assumed to be identical for both breeding age classes, but first-year (juvenile) survival was estimated separately. A sex ratio of 1:1 was assumed and the female population was modelled. Occasionally, polygynous males were found in our populations, but these were not incorporated in our female-based models.

The population size data entered in the model were the annual numbers of territory-holding and breeding females in the three sites. We modelled these assuming Poisson-distributed errors. In contrast to the IPM of Schaub et al. (2012), we did not use a hierarchical formulation for the demographic rates, as we considered that a 5-year study (resulting in four annual estimates) was too short reliably to separate process and sampling variation.

Fecundity (*f*) was defined for each site and year as the total number of fledged young produced per territorial female. We further decomposed this estimate into contributions of first and second clutches as

$$f = \frac{\mu_1 \times n_1 + \mu_2 \times n_2}{N}$$

where μ_1 and μ_2 are the mean number of fledglings per successful nest of first and second clutches, respectively, n_1 and n_2 the number of successful first and second clutches, and N the number of estimated territorial females. Mean number of fledglings per successful nest was estimated assuming a log-linear relationship to site, year, clutch number (first or repeat clutch) and all pairwise interactions, assuming Poisson errors. For all sites and years, the numbers of successful first and second clutches were assumed to be fixed quantities. For site D in 2010, these numbers were not available and were instead assumed to be stochastic quantities, which we estimated from the relative numbers of first and second clutches in the remaining years in D, as

$$\mathrm{P}(\text{first clutch} \mid n_1, n_2) \sim \text{Binomial}\ (B, p)$$

where *B* represents the number of successful broods for site D in 2010, p represents the probability that a clutch in our dataset is a first clutch, and (1-*p*) the probability that it is a second clutch. This allowed us to estimate n1 and n2 for 2010 at site D and proceed with the above equation.

We used CMR data in combination with a Cormack-Jolly-Seber model using the m-array formulation (Williams et al. 2002) to estimate apparent survival rates. As we were interested in possible sex differences in survival, but the sex of ringed juveniles was unknown and only those that survived could be sexe later when resighted as adults, juvenile and (sex-specific) adult survival rates were estimated from different subsets of the CMR data. First-year survival was estimated from the complete dataset, with a model including age but not sex effects on survival and resighting probability, whereas sex-specific adult survival rates were estimated from the subset of adult birds of known sex, treating the ringing event of birds ringed as adults and the first recapture as adults of birds ringed as juveniles (i.e. the first occasion on which their sex was assessed) as the first encounter. Recaptures of adults thus contribute to both sub-models, but only the adult survival and resighting rates from the second sub-model were included in the projection matrix of the IPM, and thus in the joint likelihood.

We performed prior analyses of the CMR data in program MARK (White and Burnham 1999) to identify the most parsimonious model structure (Burnham and Anderson 2002). For resighting rates, this structure included differences between yearlings and older birds, but no effects of site and year, except for a different value at site D in 2010 (lower due to less intensive fieldwork; Supporting Information Tables S1 and S5 for model selection and parameter estimation). The most parsimonious structure for adult survival included effects (with interactions) of sex and year, but not site, with the exception of a difference in survival for adult females between sites A and C/D. Survival rates of adult males did not appear in the projection matrix of the IPM, but were estimated anyway. The best model for first-year apparent survival included differences between years, but not sexes (as these were unknown) or sites (Tables S2 and S3 for model selection, Table S5 for parameter estimation). However, because we were interested in identifying which site-specific demographic variable was the most influential in driving the dynamics of each local population, we also extended this model structure to one with full site- and year dependency in all vital rates, as well as sex- and age-dependency in apparent survival. This is almost equivalent to three separate local IPMs, with only the information on resighting probabilities being shared among sites. Convergence, as measured by the convergence diagnostic $\hat{r}$, was achieved for all parameters. The diagnostic $\hat{r}$ was 1.008 for fecundity at site A in 2007 and <1.003 for all other parameters including the site-specific survival parameters (Gelman et al. 2002).

Demographic drivers of population change

To assess how annual population growth-rates are affected by proportional changes in the underlying vital rates, we calculated elasticities for vital rates (Caswell 2001; de Kroon et al. 2000). This prospective analysis does not reveal how the populations were affected by actual (realistic) changes in the vital rates, but shows how the populations would change if there was a future change in a demographic rate. To decompose the observed variability in population-specific growth rates as a function of variation in underlying vital rates, retrospectively, we additionally performed an LTRE (Caswell 1989). Performing prospective and retrospective analyses is worthwhile, as factors that govern annual changes and between-population differences are not necessarily the same (Gaillard et al. 2013).

Prospective analysis

For each population, we constructed projection matrices (Caswell 2001) parameterized with mean vital rates obtained from the IPM. The model structure is represented as:

$$\begin{bmatrix} n_j(t+1) \\ n_a(t+1) \end{bmatrix} = \begin{bmatrix} \varphi_j \times \frac{f}{2} + l & \varphi_j \times \frac{f}{2} + l \\ \varphi_a & \varphi_a \end{bmatrix} \begin{bmatrix} n_j(t) \\ n_a(t) \end{bmatrix}$$

where φ_j and φ_a denote the juvenile and adult mean yearly apparent survival, *f* the per-capita reproduction (fecundity) and *I* the per-capita immigration rate. From these models, we calculated and compared elasticity values between populations.

Retrospective analysis

We decomposed differences in population growth rates into contributions from differences in the vital rates between populations. We contrasted the projection matrices of the two populations to that of the best performing population, at site D, in an LTRE. For each of the $m = 4$ vital rates Θ_m, we estimated the mean $\bar{\Theta}_m$ and the difference d_m between each population (*k*) and the reference population D (*ref*).

$$\bar{\theta}_{m,k} = \frac{\theta_{m,k} + \theta_{m,ref}}{2}$$

$$d_{m,k} = \theta_{m,k} - \theta_{m,ref}$$

Next, based on mean $\bar{\Theta}_m$, we estimated the asymptotic growth rate λ_k, and its sensitivity $S_{m,k}$, to each of the vital rates. Essentially, these sensitivities reflect the slope of the population growth rate to changes in the vital rate evaluated at the midpoint between the reference and each particular population. The differences in population growth rates can be approximated by summing over the sensitivities $S_{m,k}$, multiplied by the differences in vital rates $d_{m,k}$:

$$\lambda_k - \lambda_{ref} = \sum (d_{m,k} \times \bar{S}_{m,k})$$

where $C_{m,k}$ denote the contributions of each vital rate to the difference in population growth rate between each population and the reference population.

Results

All results refer to estimations derived from the fully site-specific IPM, as we were primarily interested in site-specific differences in vital rates. The rather small population sizes and conspicuous behaviour of Northern Wheatears resulted in very high annual resighting probabilities for adult birds (posterior mean for all sites and years 0.97 ± 0.01, except for site D in 2010 0.85 ± 0.05) and for first-year birds that returned (posterior mean 0.95

± 0.02, site D in 2010 0.74 ± 0.06). This enhances the precision of our estimates of apparent survival and contributes to the estimation of other demographic parameters, including immigration. Estimated population sizes closely resembled observed population sizes (Fig. 1).

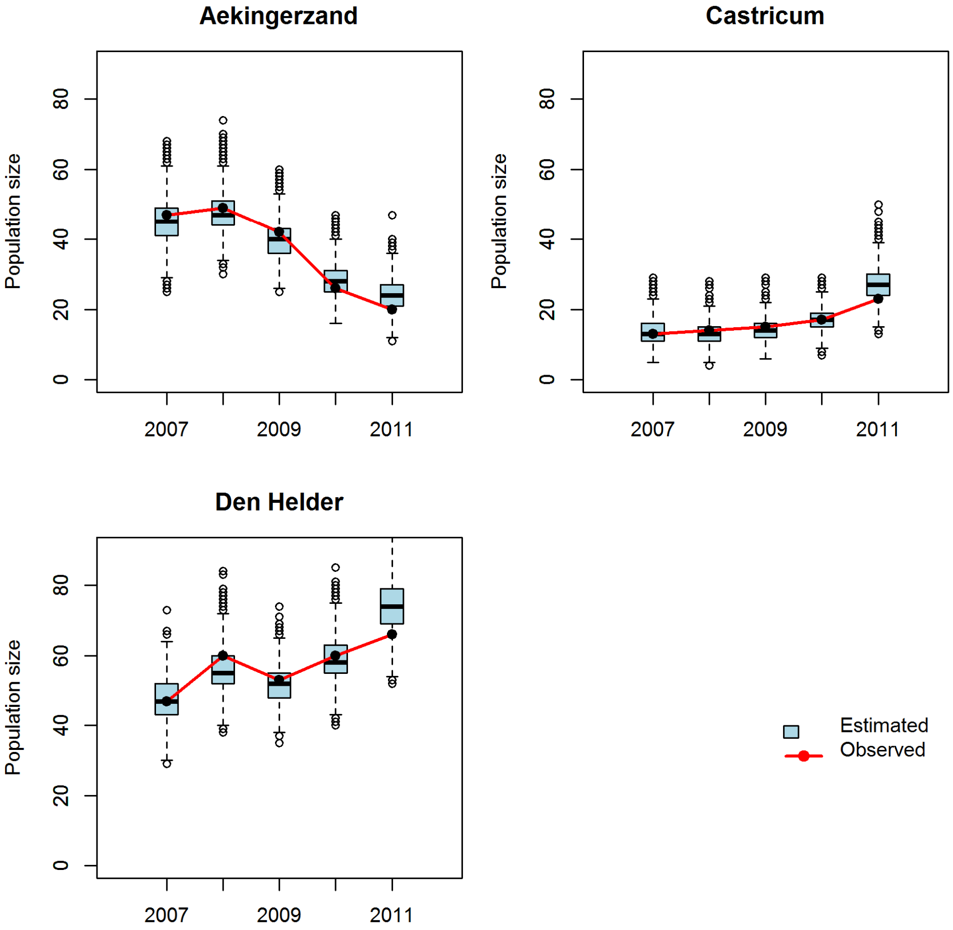

Figure 1. Population sizes (number of territorial females) in three study sites observed and estimated using IPM.

Population sizes and vital rates

Numbers and trends

The three populations contained different numbers of breeding females, site A being intermediate with on average (±1 sd) 37.12 ± 5.12 breeding females annually in 2007–2011, population C the smallest (16.88 ± 3.42) and site D the largest (57.63 ± 6.31). Mean annual growth rate was negative for A, largest for C and also positive for D (Table 2).

Fecundity

Annual fecundity differed between sites and years, being highest at site D and lowest at site A (Table 2). The 95% credible intervals for the difference between sites A and D did not contain 0 (-0.962, -0.089), indicating a significant difference in fecundity. Fecundity per successful nest was highest in site A (4.50), compared with 3.98 for site C and 4.34 for site D.

Survival

First-year survival was particularly variable between years, although averages per site were very similar. Survival of adult females was lowest at site A and highest at D, with C being intermediate. Adult male survival was higher than adult female survival and variable between sites (Table 2). All 95% credible intervals included 0.

Immigration

The smallest population (site C) seemed to receive relatively more immigrants than sites A and D (Table 2). Average absolute numbers (± 1 sd) are 5.6 ± 2.2, 4.3 ± 0.8 and 8.6 ± 3.8 female immigrants annually at A, C and D, respectively. However, these estimates remain quite imprecise as a result of the lack of direct observational data; immigration rates were often close to 0, and nearly all 95% credible intervals for the estimated number of immigrants included 0.

Predation

Predation by Red Foxes *Vulpes vulpes* was frequent at sites A and C (Table 1) but only occasional (one to two events per year) at site D. Females were also regularly predated at site A but not predated at all at site C (Table 1). Of all predation events leading to nest failure, 81% were due to Red Foxes at site A. Other predators included mustelids (probably Stoat *Mustela erminea*), predation of females by Eurasian Sparrowhawks *Accipiter nisus* (rings found near nest) and even mites (Acari). At site C, predation by mice was suspected twice during 2007–2011, and predation by mustelids on three occasions.

Table 1. Mean annual predation rates (sd given in parentheses) by Red Foxes *Vulpes vulpes*, and re-nesting for sites A and C. Predation at site D was rare. On average, nest predation rates by Red Foxes were equally high (22%) but females were often predated as well during a predation event at A but not at C. Predation at site C was not observed in 2008 and 2009, but greatly increased in later years.

	site A	**site C**
% predation of all nests	21.6 (14.5)	21.5 (25.0)
% females predated	12.2 (13.8)	0
% replacement/2nd broods	30.0 (16.6)	61.4 (6.3)

Exchange between sites

Only nine colour-ringed birds (all juveniles) were observed to have moved from one site to another. Three moved from D, and one from C to the island of Texel. Five birds moved from D to C, indicating that there was emigration from D by juveniles. No movement between A and the other sites was observed, and no adults were found to have moved between sites.

Demographic drivers of population change

Prospective analysis

Population growth-rates were equally sensitive to proportional changes in fecundity and first-year survival at all three sites, whereas the growth rate at C was slightly less sensitive to proportional changes in adult survival than at A and D (Fig. 2a). Across populations, population growth rate appeared less sensitive to changes in immigration rate than to changes in fecundity and survival, but population C was almost as sensitive to changes in immigration rate as it was to adult survival (Fig. 2a). The asymptotic population growth rates predicted by projection matrices parameterized with the site-specific mean vital rates were 0.94, 1.39 and 1.17 for A, C and D, respectively. When immigration was removed, asymptotic population growth decreased to 0.78, 0.99 and 1.00, respectively.

Table 2. IPM estimates for demographic parameters by site and year, and averages per site for all years. Values represent site-specific posterior means with standard deviation in parentheses.

A (Aekingerzand)	**2007**	**2008**	**2009**	**2010**	**2011**	**average**
Population size	45.647 (5.968)	47.497 (5.722)	39.739 (5.087)	28.311 (4.312)	24.415 (4.491)	37.122 (5.116)
Fecundity	2.508 (0.266)	2.767 (0.276)	3.129 (0.307)	2.347 (0.318)	5.274 (0.533)	3.205 (0.340)
Female adult survival		0.662 (0.098)	0.372 (0.081)	0.275 (0.067)	0.296 (0.092)	0.401 (0.085)
Male adult survival		0.809 (0.121)	0.611 (0.080)	0.504 (0.073)	0.612 (0.071)	0.634 (0.086)
Juvenile survival		0.182 (0.054)	0.302 (0.041)	0.323 (0.038)	0.446 (0.050)	0.313 (0.046)
Immigration		0.218 (0.182)	0.155 (0.139)	0.113 (0.107)	0.206 (0.187)	0.173 (0.154)
Annual population change		1.055 (0.170)	0.846 (0.136)	0.721 (0.127)	0.877 (0.189)	0.875 (0.156)
C (Castricum)	**2007**	**2008**	**2009**	**2010**	**2011**	**average**
Population size	13.642 (3.317)	13.344 (3.03)	13.898 (2.905)	16.820 (3.160)	26.683 (4.690)	16.877 (3.420)
Fecundity	3.236 (0.514)	3.684 (0.523)	4.683 (0.564)	3.061 (0.433)	1.871 (0.283)	3.307 (0.463)
Female adult survival		0.274 (0.129)	0.469 (0.130)	0.401 (0.116)	0.697 (0.106)	0.460 (0.120)
Male adult survival		0.552 (0.146)	0.457 (0.145)	0.589 (0.136)	0.571 (0.116)	0.542 (0.136)
Juvenile survival		0.288 (0.066)	0.215 (0.051)	0.316 (0.049)	0.441 (0.055)	0.315 (0.055)
Immigration		0.445 (0.397)	0.396 (0.365)	0.357 (0.337)	0.414 (0.384)	0.403 (0.371)
Annual population change		1.032 (0.336)	1.088 (0.316)	1.254 (0.321)	1.628 (0.368)	1.251 (0.335)
D (Den Helder)	**2007**	**2008**	**2009**	**2010**	**2011**	**average**
Population size	47.245 (6.190)	55.911 (6.193)	51.953 (5.507)	58.617 (5.943)	74.399 (7.724)	57.625 (6.311)
Fecundity	3.921 (0.417)	3.546 (0.289)	3.432 (0.278)	3.810 (0.316)	3.971 (0.359)	3.736 (0.332)
Female adult survival		0.527 (0.081)	0.562 (0.067)	0.489 (0.072)	0.441 (0.070)	0.505 (0.073)
Male adult survival		0.576 (0.082)	0.418 (0.072)	0.680 (0.084)	0.626 (0.074)	0.575 (0.078)
Juvenile survival		0.192 (0.035)	0.181 (0.027)	0.360 (0.040)	0.425 (0.044)	0.290 (0.037)
Immigration		0.337 (0.238)	0.135 (0.123)	0.141 (0.126)	0.153 (0.138)	0.192 (0.156)
Annual population change		1.202 (0.2)	0.938 (0.125)	1.138 (0.145)	1.279 (0.159)	1.139 (0.157)

Retrospective analysis

Since population D has been large and stable for many years, we used it as a reference for the other two sites. Survival was in general comparable across the three populations. The largest proportional differences between populations occurred in the per-capita reproduction rate, which was clearly lower at A than in the other populations. Site C was characterized by a relatively high immigration rate (Fig. 2b). Differences between the asymptotic growth rate of A and C compared with D were dominated by different demographic processes (Fig. 2c). The relatively high growth rate at site C appears to be largely due to immigration and, to a lesser extent, first-year survival. Fecundity and especially adult survival were lower compared with site D. The lower population growth rate at site A was mostly due to lower reproduction and lower adult survival. Immigration was comparable to D and contributed only marginally to population growth. First-year survival contributed little, but positively, to population growth. The summed absolute contributions per vital rate ($f = 0.15$, $\Phi_j = 0.11$, $\Phi_a = -0.19$, $I = 0.22$) indicate that, compared with population D, differences in immigration were important in contributing todifferences in population growth between sites. These differences were similar to or greater in importance than differences in female survival or fecundity.

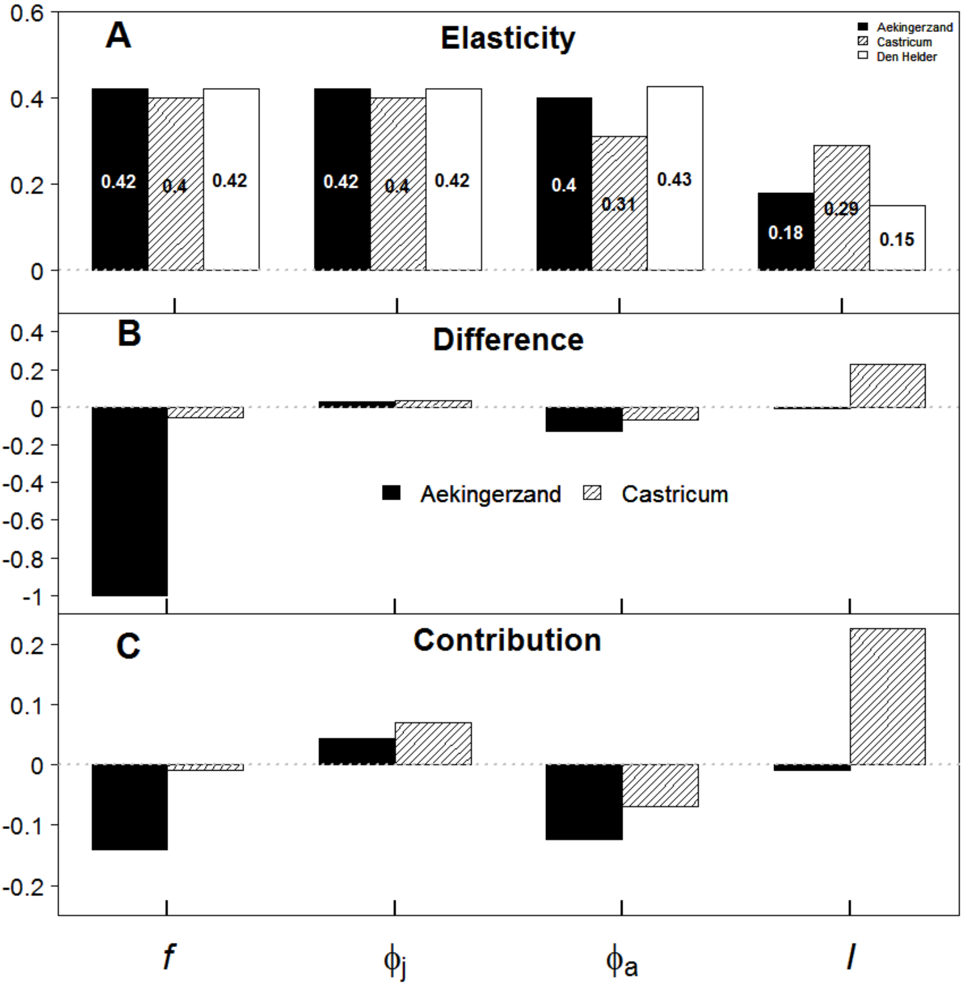

Figure 2. A: Elasticities of asymptotic population growth rate to changes in fecundity (*f*), first-year survival (Φ_j), adult survival (*Φa*), and immigration rate (*I*). Bars include the corresponding value. **B**: Absolute differences in vital rate estimates between populations A, C and reference population D. **C**: Contributions of differences in vital rates to the difference in population specific growth rates, between populations A, C and reference population D.

Discussion

To provide a scientific basis for conservation measures on a national scale, we elucidated demographic bottlenecks for three populations of the threatened Northern Wheatear. By applying an IPM we were able not only to estimate survival and reproduction but also to obtain estimates of immigration rates which, given the often scattered nature of contemporary populations, could well determine their viability (Schaub et al. 2010; Schaub et al. 2013; Ward 2005), and were indeed found to be a factor of importance. The link with conservation management is direct: by combining an IPM with an elasticity and LTRE analysis we show that each population is sensitive to different vital rates, which may be most effectively altered by conservation measures. We show that to safeguard a large-scale (national) population, it is important to safeguard several local populations, each with its own dynamics, with tailored site-specific measures. Furthermore, as immigration contributes strongly to differences in population growth, connectivity between populations should be improved to enhance the likely viability of the populations (Hernańdez-Matías et al. 2013).

Variation between populations

The three populations differed in numbers, trends and vital rates. Moreover, the populations appeared to be controlled by different vital rates and functioned as either a sink or a source. As our fully site-specific model was not favoured over a reduced model with only partial site-effects on survival based on the DIC, estimated site differences should be interpreted with care, but most can be plausibly explained by differences in conditions at the sites.

Only the Aekingerzand (A) population showed a yearly decline during the study period. The LTRE indicated that low fecundity and below average adult female survival contributed most to this poor performance. Nest predation by Red Foxes was frequent, which also led to predation of breeding females at this site. In spite of equally regular nest predation, no females were lost during such events at site C. At site C (and D, where nest predation occurs only occasionally) females bred in vacated burrows of Rabbits and could move deeper into the burrows during an attack by any predator. However, females at site A breed in shallow cavities among the roots of decaying trunks left after tree removal, and are trapped during a predation event. Given the strong population decline observed over the study period, we suggest that the habitat restoration project at this site created an ecological trap for Northern Wheatears: the short and sparse vegetation following tree removal is suitable for foraging and the decaying tree trunks provide plentiful, but dangerous, nesting sites.

Immigration becomes more important in small populations because the same number of immigrants will make a proportionally higher contribution to fluctuations in population size. Additionally, immigration is also important in conditions with lower than average fecundity and adult survival, as is the case for site A. However, the estimated immigration rates and elasticity to immigration were low compared with the other two sites. This might indicate that this inland population is more isolated than the coastal populations, which may prove to be connected to remnant populations on the Dutch Wadden Sea islands. The estimated six immigrant birds may well have originated from adjacent breeding sites in Germany, where small populations still persist (Stiftung Vogelmonitoring Deutschland & DDA, in preparation).

The coastal dune population near Castricum (C) is the smallest of the three, but showed the strongest population growth during the study period. The LTRE analysis suggested that immigration is the main explanation for the higher population growth of C than the other populations. Population C would be very vulnerable to stochastic events without immigration, due to its small size and area of suitable habitat. Although the estimated meanannual number of immigrants was only four, the population is more sensitive to immigration than populations A and D and would not have grown without these immigrants. Despite similar mean nest predation rates at A and C, replacement or second clutches were twice as common at C. Perhaps the high incidence of females predated by Red Foxes precludes production of repeat clutches at A, whereas females were rarely predated at C. The relatively large population at Den Helder (D) escaped the 1990s decline of Rabbits and has been stable for many years (Sovon). D had the highest average fecundity of the three populations. Therefore it could potentially function as a source population for adjacent coastal dune areas. Indeed, population D supplied site C with emigrant birds almost annually, despite there being 40 km between the two sites.

Conservation implications

The fact that all three populations appeared to have their own independent population dynamics has important ramifications for conservation interventions at larger scales. As such, this study may serve as a case study for the many other species that occur in small and often isolated populations. Importantly, we show that dispersal between remaining breeding populations was rare. This may mean that recolonization of sites where the species has become locally extinct will be a slow process. The importance of immigration for small populations was emphasized by the fact that differences in immigration rate contributed the most to differential population growth rates in our study.

Our study further shows that even if measures that cope with large-scale stressors are translated into practical conservation actions, these may be too late or too general for small populations and those with differential demographic bottlenecks. This is particularly true in populations that exhibit a high degree of natal and breeding philopatry. Elucidating demographic bottlenecks for several populations provides opportunities to implement measures that may be effective in the short term. We emphasize the importance of conserving small, relict populations which may, or may not, be connected by mutual migration. Designing conservation plans for several populations requires more extensive funding and time budgets. Indeed, it would be most efficient to plan specific conservation strategies for species at the very onset of decline, when populations are still relatively robust to stochasticity and are more densely spaced, which may allow more frequent migration between sub-populations.

As an illustration of how local demographic studies can result in tailored conservation measures, we briefly present actions to safeguard local populations of Northern Wheatears. For site A we would focus on increasing both fecundity and adult survival. To enhance both vital rates simultaneously, nest protection measures have been implemented since 2010 (wire-mesh covers to prevent excavating of nests by predators). This seems to have been very successful: during the first complete season of applying nest protection, fecundity was 5.27, compared with 2.60 on average for 2007–2010, and no nests or females were predated by Foxes. We also expect that female survival will recover in the coming years. Preservation of the immigration-sensitive population C requires the safeguarding of population D. Because population C may be prone to stochastic effects due to low breeding numbers and being restricted to a small remaining fragment of suitable habitat, it would be beneficial to increase the area of suitable habitat, both adjacent and very close to the existing population. The absence of dominating demographic drivers at site D may indicate a sound balance between fecundity, survival and migration and therefore be indicative of population stability. This is supported by the stability of the population since at least the early 1990s and may be due to the sustained presence of high Rabbit densities. As long as habitat quality remains in its current state, population D seems the most secure of the studied populations. Therefore, we do not recommend high-impact conservation measures in this site yet.

In the longer term, more sustainable conservation actions may encompass rehabilitating natural processes by increasing the effects of aeolian activity (Arens and Geelen 2006). However, we emphasize the joint importance of effective short-term measures and the restoration of such natural processes, as these may become effective only after time periods that exceed the endurance of the remaining populations in the contemporary setting.

Conclusions

In spite of a national and international decline, our results suggest that the remaining local Northern Wheatear populations in the Netherlands are driven by different vital rates. As many other threatened species also occur in scattered populations, we advocate implementing multi-site studies, with populations of different sizes and different degrees of isolation, in order to elucidate conservation actions that can operate locally and in the short term. By safeguarding several local populations one thereby protects the overall population on a larger geographical scale.

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Supporting information

Appendix S1. State-space equations of the IPM.
Tables S1–S5. Model selection and parameter estimations of age-, year-, and site-dependency of survival and resighting probabilities in a Cormack-Jolly-Seber formulation.

Appendix S1 State-space formulation of the integrated population model.

According to our formulation of the sampling process in the likelihood, the number of females (*Y*) counted in each site (*s*) and time (*t*) is given by: $Y_{s,t} \sim Pois[N(Tot)_{s,t}]$, in which $N(Tot)_{s,t}$ denotes the site- and time-specific total number of females estimated from our model:

$$N(Tot)_{s,t} = N(Juv)_{s,t} + N(Ad)_{s,t} + N(Imm)_{s,t}$$

The number of juveniles $N(Juv)_{s,t}$, adults $N(Ad)_{s,t}$ and immigrants $N(Imm)_{s,t}$ are estimated by dynamic expressions of the demographic process as

$$N(Juv)_{s,t} \sim Pois\left[\tfrac{1}{2} f_{s,t-1} \times \varphi(Juv)_{s,t-1} \times \mathrm{N}(Tot)_{s,t-1}\right],$$

$$N(Ad)_{s,t} \sim Bin[\varphi(Fem)_{s,t-1}, \mathrm{N}(Tot)_{s,t-1}],$$

and

$$N(Imm)_{s,t} \sim Pois[c_{s,t-1} \times \mathrm{N}(Tot)_{s,t-1}],$$

respectively. In the above equations, $f_{s,t}$ denotes the per-capita reproduction, $\varphi(Juv)_{s,t}$ the juvenile survival rate, $\varphi(Fem)_{s,t}$ the adults female survival rate, and $c_{s,t-1}$ the per-capita immigration rate, each of which are estimated separately for each site (*s*) and time (*t*) step.

Supporting tables

Model selection and parameter estimations of age-, year-, and site-dependency of survival and resighting probabilities in a Cormack-Jolly-Seber formulation.

Model selection resighting probability *p*

First selection of best model for resighting probability *p*, with most detailed model for survival rate Φ (site*age*time), without individual covariates. In 2010 the ringing and thus resighting effort was much lower in Den Helder, therefore we want to include this in the models for recapture rate. The best model for *p* is a model with different *p*'s for juveniles and adults and a different p for Den Helder in 2010 (Table S1).

Table S1. Model selection for p. (a=age, s=site, t=time, D10=Den Helder 2010)

Model	AICc	Delta AICc	AICc Weights	Model Likelihood	# Par	Deviance
Φ(s*a*t)*p*(a+D10)	2337.53	0.00	0.92	1.00	27	2282.65
Φ (s*a*t)*p*(s+a)	2342.87	5.34	0.06	0.07	28	2285.93
Φ(s*a*t)*p*(s*a)	2346.06	8.53	0.01	0.01	30	2284.98
Φ(s*a*t)*p*(t)	2349.28	11.75	0.00	0.00	27	2294.40
Φ(s*a*t)*p*(a*t)	2351.47	13.95	0.00	0.00	31	2288.32
Φ(s*a*t)*p*(s*t)	2351.95	14.42	0.00	0.00	34	2282.56
Φ(s*a*t)*p*(a)	2352.73	15.20	0.00	0.00	30	2291.65
Φ(s*a*t)*p*(s)	2354.74	17.22	0.00	0.00	27	2299.87
Φ(s*a*t)*p*(s*a*t)	2366.64	29.11	0.00	0.00	46	2272.10

The next step is to select the best model for survival rate Φ, first without the covariates.

Table S2. Model selection for Φ without covariates.

	Model	AICc	Delta AICc	AICc Weights	Model Likelihood	# Par	Deviance
1	Φ(a*t)_p_(a+D10)	2323.92	0.00	0.54	1.00	11	2301.77
2	Φ(s*a+a*t)_p_(a+D10)	2326.69	2.77	0.13	0.25	15	2296.42
3	Φ(a+t)_p_(a+D10)	2326.87	2.95	0.12	0.23	8	2310.78
4	Φ(s+a*t)_p_(a+D10)	2327.52	3.59	0.09	0.17	13	2301.31
5	Φ(s*a+t)_p_(a+D10)	2328.07	4.15	0.07	0.13	12	2303.90
6	Φ(s+a+t)_p_(a+D10)	2330.55	6.63	0.02	0.04	10	2310.42
7	Φ(s*t+s*a+a*t)_p_(a+D10)	2331.94	8.02	0.01	0.02	21	2289.40
8	Φ(s*t+a*t)_p_(a+D10)	2332.53	8.60	0.01	0.01	19	2294.09
9	Φ(s*t+s*a)_p_(a+D10)	2333.53	9.61	0.00	0.01	18	2297.13
10	Φ(s*t+a)_p_(a+D10)	2335.74	11.82	0.00	0.00	16	2303.43
11	Φ(a)_p_(a+D10)	2336.38	12.46	0.00	0.00	5	2326.35
12	Φ(s*a)_p_(a+D10)	2337.50	13.58	0.00	0.00	9	2319.39
13	Φ(s*a*t)_p_(a+D10)	2337.53	13.60	0.00	0.00	27	2282.65
14	Φ(s+a)_p_(a+D10)	2339.78	15.86	0.00	0.00	7	2325.71
15	Φ(t)_p_(a+D10)	2404.64	80.72	0.00	0.00	7	2390.58
16	Φ(s+t)_p_(a+D10)	2407.69	83.77	0.00	0.00	9	2389.59
17	Φ(s*t)_p_(a+D10)	2413.00	89.08	0.00	0.00	15	2382.72
18	Φ(.)_p_(a+D10)	2422.46	98.54	0.00	0.00	4	2414.44
19	Φ(s)_p_(a+D10)	2425.66	101.74	0.00	0.00	6	2413.61

The best model is one without site effects, but with an interaction between age and time. However, both fledging date and condition index differ (significantly) between sites (Roodbergen, unpublished data). Therefore, when testing for the effects of individual covariates, we used a starting model including site effects. The best model including site effects for Φ was model 2 (Table S2). Model 2 consists of an interaction between site and age (s*a) and an interaction between age and time (a*t). Models 3, 4 and 5 performed only slightly worse. However, model 3 does not include site effects and both model 3 and 5 assume a time effect for both age classes, while no age effect was found for adults in the analyses with adults only. When using model 4 as a starting model, the conclusions of the analyses with individual covariates were similar, so we do not show them here.

Including fledging date as a continuous variable improved the starting model significantly (Table S3, compare AICc of models 7 and 10; ΔAICc>2). However, including fledging date as a factor (early/late) improves the starting model even more (compare AICc's of models 2 and 10 and models 2 and 7; ΔAICc>2). Therefore we choose to use the factor instead of continuous variable for fledging date.

Including condition index did not significantly improve the starting model (compare model 9 with model 10; ΔAICc<2). When condition index was included after including fledging date as a factor (as the two are correlated, see Table S4), with or without interaction, this did not improve the model (compare model 4 and 6 with model 2). From this we can conclude that condition does not have an additive effect when correcting for fledging factor. Including an interaction term of site or time with condition index also did not improve the model (compare models 11 and 12 with 9 and 10). The interaction term of site with fledging date improved the slightly, but not significantly (compare model 1 with model 2, ΔAICc<2).

As without individual covariates the best model for Φ was a model with an interaction between age and time only, we tested whether this model was also the best model when including the significant covariate fledging date. This model performed worse than the model including the interaction between site and age (compare model 3 with model 2).

Table S3. Model selection for Φ with covariates (vl=early/late fledging;factor, uc=fledging date; continuous variable and ci=condition index; continuous variable).

Model	AICc	Delta AICc	AICc Weights	Model Likelihood	# Par	Deviance
Φ(s*a+a*t+s*vl)*p*(a+D10)	1647.76	0.00	0.38	1.00	18	1611.37
Φ(s*a+a*t+vl)*p*(a+D10)	1648.89	1.12	0.22	0.57	16	1616.57
Φ(a*t+vl)*p*(a+D10)	1649.50	1.74	0.16	0.42	12	1625.32
Φ(s*a+a*t+vl+ci)*p*(a+D10)	1650.09	2.33	0.12	0.31	17	1615.74
Φ(s*a+a*t+vl*t)*p*(a+D10)	1651.38	3.62	0.06	0.16	19	1612.94
Φ(s*a+a*t+vl*ci)*p*(a+D10)	1652.12	4.36	0.04	0.11	18	1615.73
Φ(s*a+a*t+uc)*p*(a+D10)	1655.10	7.34	0.01	0.03	16	1622.79
Φ(s*a+a*t+s*vl*ci)*p*(a+D10)	1657.07	9.30	0.00	0.01	22	1612.48
Φ(s*a+a*t+ci)*p*(a+D10)	1659.30	11.54	0.00	0.00	16	1626.99
Φ(s*a+a*t)*p*(a+D10)	1659.67	11.90	0.00	0.00	15	1629.39
Φ(s*a+a*t+s*ci)*p*(a+D10)	1661.50	13.73	0.00	0.00	18	1625.10
Φ(s*a+a*t+t*ci)*p*(a+D10)	1664.81	17.05	0.00	0.00	19	1626.38

As including condition did not improve the models for juvenile survival, we again tested for the effects of fledging date, including the juveniles with unknown condition, thereby increasing sample size by 284 individuals (Table S4). Again, including fledging date improved the model significantly (compare models 2 and 5 with model 6; ΔAICc>2), and models with fledging date as a factor performed better than those with fledging date as a continuous covariate (compare model 2 with model 5; ΔAICc>2). Including an interaction term of site or time with fledging date did not improve the model (compare models 3 and 4 with model 2).

The model with an interaction between age and time only, and fledging success performed slightly better than the model including the interaction between site and age. From this, we can conclude that including the interaction between site and age does not significantly improve the model. When comparing the AICc of model 1 from Table S4 with the AICc of model 1 from Table S2, we see that fledging date (factor) again significantly improves the model for Φ.

Table S4. Model selection for Φ with covariates (vl=early/late fledging; factor, uc=fledging date; continuous variable and ci=condition index; continuous variable), including juveniles with unknown condition.

No.	Model	AICc	Delta AICc	AICc Weights	Model Likelihood	# Par	Deviance
1	Φ(a*t+vl)*p*(a+D10)	2315.39	0.00	0.51	1.00	12	2291.21
2	Φ(s*a+a*t+vl)*p*(a+D10)	2316.14	0.76	0.35	0.69	16	2283.83
3	Φ(s*a+a*t+s*vl)*p*(a+D10)	2319.22	3.83	0.08	0.15	18	2282.83
4	Φ(s*a+a*t+vl*t)*p*(a+D10)	2320.56	5.17	0.04	0.08	19	2282.12
5	Φ(s*a+a*t+uc)*p*(a+D10)	2321.87	6.48	0.02	0.04	16	2289.56
6	Φ(s*a+a*t)*p*(a+D10)	2326.69	11.30	0.00	0.00	15	2296.42

Parameter estimates

The estimates from models 1 and 2 of Table S4 are given in Table S5. The estimates for Φ from models 1 and 2 are also shown in figures 1.1 and 1.2. We can see that the pattern in survival rates is very similar for the two models. Survival in early fledging chicks is 0.1−0.16 higher than in late fledging chicks. Moreover, juvenile survival seems to have increased during the study period, whereas adult survival has remained more or less constant between 0.50−0.55. When including site effects in the model, we see that juvenile survival is highest in A, then in C and then in D. In adults, this pattern is reversed, with highest survival in D and lowest in A. Again, we see that resighting rate is lower in juveniles than in adults and in D in 2010, compared to the other years and sites. Resighting rate is similar in the two models.

Table S5. Parameter estimates and 95% confidence limits for models 1 and 2 from Table S4.

Parameter	Estimate	LCL	UCL	Site	Estimate	LCL	UCL
Φ juv early 2008	0.23	0.17	0.29	D	0.21	0.15	0.27
Φ juv early 2009	0.23	0.19	0.28	D	0.20	0.15	0.26
Φ juv early 2010	0.33	0.28	0.39	D	0.29	0.23	0.36
Φ juv early 2011	0.41	0.33	0.49	D	0.36	0.28	0.45
Φ juv late 2008	0.13	0.08	0.20	D	0.10	0.06	0.17
Φ juv late 2009	0.13	0.08	0.19	D	0.10	0.06	0.16
Φ juv late 2010	0.20	0.13	0.28	D	0.16	0.10	0.24
Φ juv late 2011	0.25	0.17	0.36	D	0.20	0.13	0.31
Φ ad 2008	0.55	0.45	0.64	D	0.56	0.46	0.65
Φ ad 2009	0.51	0.44	0.59	D	0.53	0.45	0.61
Φ ad 2010	0.50	0.42	0.57	D	0.52	0.44	0.60
Φ ad 2011	0.57	0.49	0.64	D	0.59	0.51	0.67
Φ juv early 2008				C	0.25	0.17	0.34
Φ juv early 2009				C	0.24	0.17	0.33
Φ juv early 2010				C	0.34	0.26	0.43
Φ juv early 2011				C	0.42	0.32	0.53
Φ juv late 2008				C	0.13	0.08	0.21
Φ juv late 2009				C	0.13	0.08	0.20
Φ juv late 2010				C	0.19	0.12	0.28
Φ juv late 2011				C	0.25	0.16	0.36
Φ ad 2008				C	0.54	0.40	0.67
Φ ad 2009				C	0.51	0.39	0.63
Φ ad 2010				C	0.50	0.38	0.62
Φ ad 2011				C	0.57	0.46	0.68
Φ juv early 2008				A	0.28	0.21	0.38
Φ juv early 2009				A	0.28	0.21	0.35
Φ juv early 2010				A	0.38	0.31	0.46
Φ juv early 2011				A	0.47	0.37	0.56
Φ juv late 2008				A	0.15	0.09	0.24
Φ juv late 2009				A	0.15	0.09	0.22
Φ juv late 2010				A	0.22	0.14	0.32
Φ juv late 2011				A	0.28	0.18	0.40
Φ ad 2008				A	0.51	0.39	0.62
Φ ad 2009				A	0.48	0.39	0.57
Φ ad 2010				A	0.47	0.38	0.56
Φ ad 2011				A	0.54	0.45	0.63
p juv	0.93	0.86	0.96		0.93	0.86	0.96
p ad	0.98	0.94	0.99		0.97	0.93	0.99
p juv D2010	0.67	0.51	0.80		0.70	0.54	0.82
p ad D2010	0.87	0.74	0.94		0.87	0.75	0.94

Chapter 3

Seasonal survival in relation to timing of fledging in a migratory passerine, the Northern Wheatear *(Oenanthe oenanthe)*

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Abstract

Declines of long-distance migrants demand a better understanding of factors affecting population change. As juvenile survival generally is an important determinant of population dynamics, assessing whether juvenile mortality primarily occurs either during post-fledging on the natal site or during migration-winter is important for developing conservation strategies. Here, we assess variation in seasonal survival of juvenile Northern Wheatears (*Oenanthe oenanthe*), a threatened songbird in northwestern Europe, in relation to timing of fledging. By estimating apparent survival based on frequent resightings of fledglings, we show that average two-weekly survival was lower during post-fledging (from fledging to onset of migration) than during migration-winter (from onset of migration to return as breeding bird the next year): both post-fledging survival (0.932 early broods vs 0.880 late broods) and migration-winter survival (0.966 respectively 0.938) were lower for late-fledged juveniles. This resulted in 10-16% lower first-year survival compared to early ones, even though early fledglings need to survive for a longer period during post-fledging. There was no effect of body condition on survival. Apparently, negative effects experienced earlier in life, e.g. during post-fledging, carry-over to affect migration-winter survival. Due to predation of nests, young of repeat clutches fledge late, which results in lower survival. Management practises to increase fledging success may include nest protection measures on the short-term, particularly of first broods in species of high conservation concern, as the Northern Wheatear.

Keywords
post-fledging survival, migration-winter survival, *Oenanthe oenanthe*, seasonal survival, passerine, life-history stages, avian demography, population ecology

Introduction

Survival of juveniles is a major determinant of population dynamics in passerine birds (Saether and Bakke 2000). Yet, survival during different stages of the avian life-cycle remains poorly known, particularly for juveniles (Dybala et al. 2013; Sillett and Holmes 2002). During post fledging, defined as the period between fledging and dispersal and migration from the breeding site (Naef-Daenzer et al. 2001; Vitz and Rodewald 2011), small passerines visually and vocally vanish due to their secretive behaviour. Although important data on immediate post-fledging survival are becoming available by using radio-telemetry (Naef-Daenzer et al. 2001), it remains challenging to gather sufficient sample sizes for a prolonged period after fledging using this method (but see Sim et al. 2013). Mortality appears generally very high during the post-fledging period, which may be partly affected by fledging date (Naef-Daenzer et al. 2001; Smith et al. 1989; Vitz and Rodewald 2011) and poor body condition (Krementz et al. 1989; Nur 1984; Yackel Adams et al. 2006).

It remains, however, largely an enigma how juvenile survival of passerines develops after the post-fledging period (Faaborg et al. 2010). This is unfortunate since juveniles enter a crucial period, particularly for migratory species, in which they prepare for southbound migration by energy consuming post-juvenile moult and by strongly increasing body mass for long-distance flight (Bauchinger and Biebach 2001; Berthold 1996). Furthermore, after first having adapted to forage independently from their parents and to evade predators on their natal sites, juveniles need to adjust to very different prey and predators on migration and wintering sites, after having crossed inhospitable areas such as seas and deserts.

To better understand the evolutionary drivers of passerine life-histories and to identify the most vulnerable life stages for conservation purposes, knowledge on seasonal (here: post-fledging versus migration-winter) and timing (here: fledging date) related first-year survival is important. Although first-year survival can strongly influence population growth of passerines (Robinson et al. 2004), few studies aimed to disentangle mortality at the breeding grounds from winter mortality due to the difficulties described above (Sillett and Holmes 2002; Tarof et al. 2011). Here, we aim to determine if and at what stage of pre-breeding life the frequently reported differences in annual survival between early and late born fledglings arise (e.g. Krementz et al. 1989; Naef-Daenzer et al. 2001; Smith et al. 1989; Verboven and Visser 1998; Vitz and Rodewald 2011). Are these solely explained by a higher post-fledging mortality of late juveniles on the breeding grounds, or is survival of late fledglings (also) lower during the period of migration and wintering?

This question is particularly relevant for conservation of migratory passerines, a group of birds which is strongly declining in the US (Robbins et al. 1989; Sauer et al. 2014) and in Europe (Sanderson et al. 2006; Vickery et al. 2014). If higher post-fledging mortality of late born juveniles would fully explain the differences with early juveniles in annual survival, conservation efforts should focus either on improving nesting conditions or post-fledging conditions on the breeding grounds. Even if, on the other hand, mortality of late born juveniles arises primarily during migration and/or wintering, improving nest success of early nests may be more effective on the short term than actions in the wintering sites, since exact wintering sites and ecology of many species or (sub-) populations are very poorly known and birds from one breeding population may be wintering at different sites.

To investigate seasonal mortality in relation to timing of breeding, we performed frequent resightings of color-marked fledged juveniles at three local populations of a migratory passerine, the Northern Wheatear (*Oenanthe oenanthe*). Due to the species' preferences for short-grown and open vegetation types for foraging (chapter 4), it is very conspicuous throughout the breeding season. In contrast to forest and shrub dwelling passerines which are often difficult to track, it is therefore feasible to gather survival data of a relatively large number of individuals compared to most radio telemetry studies. Specifically, our aims were to (1) determine whether body condition and/or timing of breeding affect overall first-year survival, and reflect to adult yearly survival (2) determine whether post-fledging mortality on the natal site (fledging - August) differs from mortality during migration and winter (September - March), and (3) determine if and how seasonal survival is affected by fledging date.

Methods

Study Species and Sites

The Northern Wheatear is an insectivorous long-distance migrant breeding from eastern Canada and Greenland across Eurasia to western Alaska (Glutz von Blotzheim and Bauer 1988). The species ranks among the top-10 most strongly declining species in Europe (Gregory et al. 2009). Since 1990 the European population has declined by over 50% (PECBMS 2013). In the Netherlands, Northern Wheatears occur in sandy, oligotrophic grasslands in coastal dunes and heathlands where they often breed in burrows of Rabbits (*Oryctolagus cuniculus*).

Between 2007 and 2011 we intensively studied three populations of Northern Wheatears in the Netherlands by color-banding nestlings and adults (chapter two): the inland population at Aekingerzand (site A, 268 ha, 52°55'N, 6°18'E); the coastal population at Castricum (site C, 74 ha, 52°33'N, 4°36'E), and the coastal population at Den Helder (site D, 160 ha, 52°52'N, 4°43'E). Sites are described in more detail in chapter two.

Fieldwork

Northern Wheatears are very conspicuous, strongly philopatric birds which allows for very high annual resighting probabilities (p) between years: $p = 0.97$ for adults and $p = 0.95$ for juvenile birds (chapter two). This provides an excellent setting to study survival in much more detail.

Fieldwork consisted of two parts: (1) to quantify relations between nestling condition, timing of fledging and overall first-year survival in three study populations; (2) to quantify within-year variation in seasonal survival for juveniles, related to timing of breeding, in one intensively studied population (C).

Juvenile and adult yearly survival. During 2007–2010 we marked 1086 nestlings with individual combinations of three colored leg bands and an aluminum band from the Dutch Centre for Avian Migration & Demography (Table 1). Nestlings were marked when 6-12 days old. Fledging success was determined by observation of young after fledging and included a post-fledging nest control to check for remaining dead eggs or nestlings. 229 adult birds were captured using baited spring-traps, banded with a unique combination of colored leg bands and an aluminum band and released. Resightings were obtained by weekly searches in each site during the entire nesting season, using telescopes. Resightings in 2008-2011 were used to estimate overall first-year survival, with inclusion of occasional reports by birdwatchers from the rest of the Netherlands (*n* = 5). Most suitable breeding areas in the Netherlands, apart from our study sites, are surveyed as a part of the national breeding bird monitoring program (Boele et al. 2014). Volunteers performing these counts were specifically asked to watch out for color-banded individuals and report any resightings.

Seasonal survival. To investigate first-year survival in more detail we focused on population C. In 2009 and 2010 a total of 119 juveniles were marked in site C (69 in 2009, 50 in 2010). Juveniles were resighted during frequent visits (at least two days per week), specifically aimed at recording color-marked Northern Wheatears and covering the whole study area of 74 ha (Fig. 1). Recordings started when adults arrived on the breeding grounds (from last week of March onwards) and finished when juveniles were no longer present in the breeding site, either because they died or because they left for migration (first week of September).

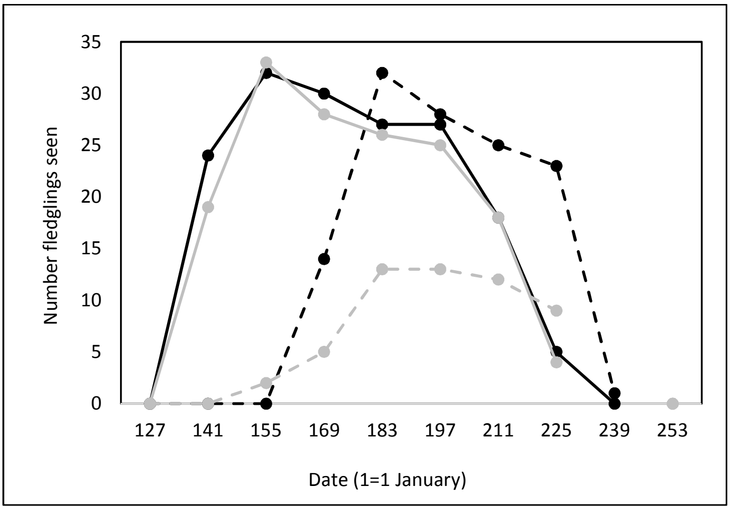

Figure 1. The number of resighted color ringed fledglings of Northern Wheatear in site C per two-week interval. Solid black: early nests 2009 (hatched < 4 June, day 155), solid grey: early nests 2010; dashed black: late nests 2009 (hatched ≥ 4 June), dashed grey: late nests 2010.

Early and late broods. Northern Wheatears regularly produce replacement clutches after failure and also true second broods following a successful first attempt, defined as at least one nestling having fledged. This leads to two distinct peaks of hatchling number during the breeding season (Fig. 2A). To distinguish 'early' from 'late' (replacement/second) broods we decided to use an ecological parameter: the availability of the beetle *Phyllopertha horticola* (Coleoptera: Scarabeidae), which is a bulk prey for nestlings in early broods (chapter 4), but not in late broods due to the beetle's phenology. Birds hatched ≥ 4 June (day 155; day 1= 1 January) were classified as 'late' and birds hatched before that date as 'early', since from early June onwards numbers of *Phyllopertha* decline rapidly (Fig. 2B). Fledging date is determined as 14 days after hatching (Moreno 1987).

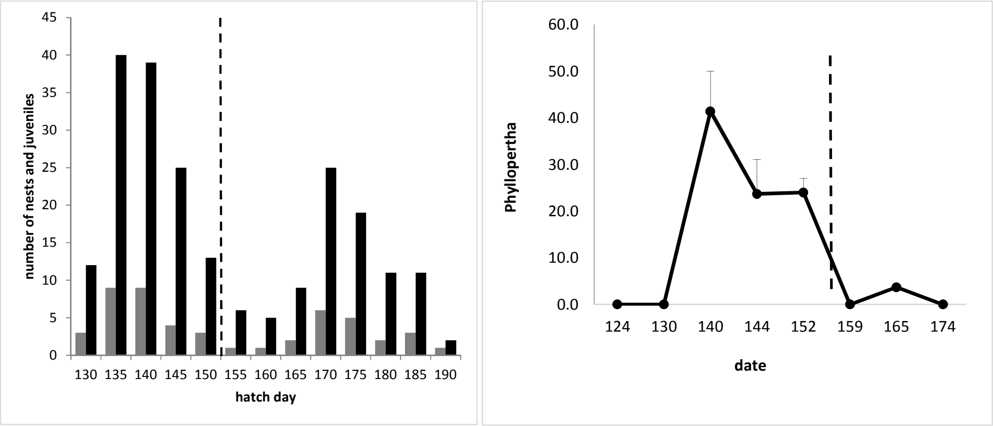

Figure 2. (A) Number of nests (grey bars) and hatchlings (black bars) per five-day interval for site C, where nestling diet has been studied (chapter 4). Data 2007 – 2010, n = 224 hatchlings from 49 broods: 32 early and 17 late. (B) Counts of *Phyllopertha horticola* in 2011 (mean ± SE). The vertical dashed line indicates day 155, which differentiates between early and late nests. Abundance peak of *Phyllopertha* is before this date.

Body Condition. Measurements on the chicks were not always taken at exactly the same ages, and therefore we modelled condition as age-corrected body mass. Based on nestlings with exact known age (nests were visited at the day of hatching) the relation between age and wing length (mm) was determined as age=0.001*(winglength^2)+0.11*winglength+4.2914; (age in whole days, R^2=0.98, *n*=12 broods with 49 nestlings in four years).

Using this formula and wing measurements we determined the age of all nestlings between 5 and 12 days old. Older nestlings start walking and they may be out of reach when taking measurements, perhaps especially when they are in good condition. To avoid the risk of biasing average condition towards young in relative low condition, we decided not to use older nestlings. The relationship between age and weight was then determined based on successfully fledged juveniles, under the assumption that their weight was apparently sufficient to successfully fledge: weight = -26.6 + 8.97*age + 0.46*(age^2) + 0.00404*(age^3); (R^2 = 0.70).

This formula was used to predict weights per age for all nestlings. Subsequently the SD of observed weight per age was calculated. Finally, we divided the residuals of the regression by the SD to obtain an index for condition (Sim et al. 2013).

Statistical Analyses

Analysis 1: relations between juvenile condition, fledging date and survival.

We used Cormack-Jolly-Seber (CJS) models using Program MARK (Lebreton et al. 1992; White and Burnham 1999) to model juvenile survival in relation to fledging date and condition. In all analyses we used Akaike's information criterion corrected for small sample sizes (AIC_c) to evaluate candidate models (Burnham and Anderson 2002). Individual covariates were (1) condition index: 803 nestlings with known condition, (2) fledging date: this variable was included as either a continuous variable (*fc*; day number of fledging, counted from 1 January) or as a factor (*ff*; 'early', 'late'). There were 1077 nestlings with known age, and thus fledging date.

Table 1. Numbers of Northern Wheatears ringed as early and late nestlings and as adults per study site in the Netherlands in the years 2007-2010.

Group\Site	A	C	D	tot
juv early	269	157	500	926
juv late	58	64	38	160
ad	77	24	128	229
total	404	245	666	1315

We first selected the best model for resighting probability (*p*), using the most detailed model for survival rate (Φ), including site (*s*), maturity: juvenile or adult (*m*) and year (y), as Φ(site* maturity*year), without individual covariates. The next step was to select the best model for survival rate Φ, first without the covariates, then including covariates. GOF testing is not possible when including individual covariates (Cooch and White 2013). Therefore, goodness-of-fit was assessed for the most general model without individual covariates, but with an additive effect of the grouping factor fledging date (Φ(site* maturity*year+fledging factor)*p*(site* maturity*year), using parametric bootstrap procedures. The variance inflation factor, $\hat{c}$, was calculated by dividing the observed model deviance by the mean deviance from the simulations. The bootstrap method (1000 simulations) showed that the model fitted the data sufficiently well ($P = 0.18$, with estimated $\hat{c} = 1.12$).

Analysis 2: Juvenile seasonal survival.

During every visit to the study site, all color-marked juveniles seen were recorded. Data were summarized per two week intervals, resulting in seven distinct two week intervals during the breeding season (21 May – 26 August). The next year individuals returning as adults to the breeding grounds were recorded soon after their arrival (median arrival date 2009: day 99, 2010: day 104); the last interval (migration and wintering grounds) therefore consisted of 15 two week intervals. The effective sample size was 496 fledgling interval combinations. In three two-week intervals only few resightings were recorded during 2010, due to very limited field visits (intervals 10, 11 and 18). We therefore decided to exclude these intervals from the analyses.

To be able to separate survival on the breeding site from survival during migration/winter, we used Multi-State models (e.g. Hestbeck et al. 1991) distinguishing two states: breeding season (B) and migration-wintering season (W). In the latter no observations were made. Therefore, state W was an 'unobservable state' (Kendall and Nichols 2002). We assumed that a missing but alive (because it was resighted next year) juvenile at the end of the breeding season could represent either a bird which was still present at the breeding grounds but was not seen, or a bird which had departed for migration. To make sure all juveniles departed for migration, the last encounter occasion during the breeding season (occasion 8) was set at zero for all birds.

We tested for several effects on survival (Φ), resighting probabilities (p) and/or transition rates (ψ) (the probability that an individual moves from one state to the next, for example: from breeding site to migration): (1) time effects (*t*; 7 intervals of 2 weeks); (2) age effects (*a*; two age classes; 0 - 4 weeks after fledging and > 4 weeks after fledging); (3) cohort effects (*c*; 8 cohorts); (4) fledging date (*f*; early: cohorts 1-3 and late: cohorts 4-8); (5) year effects (*y*; 2009 and 2010) and (6) state/seasonal effects (*s*, or *B* and *W*, when referring to the separate survival rates for the breeding (*B*) and non-breeding (*W*) state). To avoid problems with estimability of Φ and p in the last interval, we assumed that p_B was equal to 0.95 in the last interval, as this value was estimated for the whole breeding season from earlier survival analyses (chapter 2).

First we selected the best model for departure probability from the breeding site to the winter site (ψ_{BW}), using the most parsimonious model for survival (Φ) during the breeding season (Φ_B (year*fledging date*age)), a simpler model without age effects for survival outside the breeding season (Φ_W (year*fledging date)) and a model with year and time effects for recapture probability p, (p_B (year*time); $p_W = 0$, *W* being the unobservable state). We assumed that all surviving juveniles would return to the breeding grounds in the next year. (ψ_{WB} from unobservable state to breeding site equals zero for the breeding season intervals 1-7, but equals 1 for interval 8, when birds return from the wintering sites). Indeed, exchange between sites appears very low (chapter 2) We further assume that juveniles do not depart for migration from the breeding site before the 4th (<15 July; $\psi_{BWc,1\text{-}3} = 0$, model 5) or the 5th interval (<29 July, $\psi_{BWc,1\text{-}4} = 0$, all other models) and that all juveniles have departed during the 7th interval (<27 August, $\psi_{BWc,7} = 1$; fig. 1). When selecting the best model for ψ_{BW}, we varied the age-interval combinations at which juveniles had equal probabilities of departure from the breeding grounds (Table S1).

Next, the best model for p_B was selected, using the starting models for survival during the breeding season (Φ_B) and during migration-winter (Φ_W) (with only second order interactions, to improve estimability of parameters) and the best model for ψ_{BW}. The resighting probability at the breeding site will largely depend on the effort made by the researcher to find the colour-marked birds. As the number of visits to the study site differed between intervals, we tested whether p_B differed substantially between intervals and years: p_B(y*t); (2) p_B(y+t); (3) p_B(y); (4) p_B(t) and (5) p_B(.).

Finally, using the best models for ψ_{BW} and p_B, the best fitting model for Φ_B and Φ_W was selected. The Goodness of Fit test was performed on the most parsimonious model with Φ (season*year + year*age + age*fledging date + year*fledging date + year*fledging date*age) and *p*(year*time) and the best model for ψ_{BW} using the bootstrap method.

In the models for survival, we included three co-variables: (1) season, (2) year and (3) fledging date as early/late. As the number of individuals was limited, we decided not to test for interval dependent survival, as this resulted in many inestimable parameters. For the same reason we only included second order interactions between independent variables. As the age class 0-4 weeks was only present during the breeding season, we could only test for age effects in Φ_B, not in Φ_W. Effects of age on Φ_B were tested by including $\Phi_B(a)$, $\Phi_B(f{*}a)$ and $\Phi_B(y{*}a)$.

As we were specifically interested in when the additional mortality of late hatching/fledging chicks (cohorts > 3) occurred, we included two more models, the best model of the above with additional effects of fledging date at state *B*, and the best model of the above with additional effects of fledging date at state *W*. The goodness of fit test showed a near-significant lack of fit of the most parsimonious model ($P = 0.067$). However, the dispersion parameter was small, $\hat{c} = 1.22$. Therefore we corrected the AIC values with this parameter to correct for the possible lack of fit. Because four models had $\Delta\mathrm{AIC_c} < 2$ we used model averaging over these four to obtain the most robust parameter estimates.

Results

Condition and Fledging Date

Body condition index of early fledglings is higher than of late fledglings (GLM in SPSS 21.0, $F = 24.94$, $df = 1$, $P < 0.001$). Body condition also differs between sites ($F = 14.50$, $df = 3$, $P < 0.001$), but not between years ($F = 1.50$, $df = 3$, $P = 0.21$). All interactions between the three variables were not significant ($P > 0.05$).

Fledging date of early juveniles differs between sites (GLM, $F = 15.14$, $df = 3$, $P < 0.001$), with juveniles in site A hatching later than in site D (day 155 respectively 152; *t*-test, $F = 72.45$, $df = 716$, $P < 0.001$), but fledging date did not differ between years ($F = 2.55$, $df = 3$, $P = 0.06$). The interaction site with year is significant ($F = 7.95$, $df = 6$, $P < 0.001$), indicating that variation between sites differs between years.

Fledging date for late juveniles did not differ between sites (Kruskal-Wallis test, $\chi^2 = 1.73$, $df = 2$, $P = 0.42$). Since the number of late broods was sometimes very low, differences between years were not tested. Nevertheless, fledging dates of late nests are shown as averages per site per year in Table 2 (right part), with sample-size.

Table 2. Left of vertical bar: mean body condition index in three study sites Aekingerzand (A), Castricum (C) and Den Helder (D) per year, for early and late juveniles (2007-2011). No late nests were banded in site D, 2010. Differences of mean values per site and fledging groups are significant, per year and all interactions are not. See text. Right of vertical bar: mean fledge day of late broods and sample size per site per year.

		body condition		fledge date late nests	
site	year	early (SE)	late (SE)	mean (SD)	n
A	2007	-0.423 (0.203)	-0.891 (0.371)	177.5 (1.2)	6
	2008	-0.447 (0.096)	-1.366 (0.288)	188.0 (8.1)	10
	2009	-0.320 (0.091)	-0.328 (0.371)	189.5 (3.0)	6
	2010	-0.272 (0.120)	-0.781 (0.344)	189.6 (2.7)	7
C	2007	0.387 (0.235)	-0.436 (0.288)	195.9 (2.0)	10
	2008	0.458 (0.178)	-0.122 (0.227)	186.9 (3.4)	16
	2009	0.499 (0.126)	-0.068 (0.182)	185.2 (4.0)	25
	2010	0.383 (0.115)	0.212 (0.221)	189.5 (6.5)	17
D	2007	0.259 (0.093)	-0.868 (0.455)	196.8 (1.5)	4
	2008	0.231 (0.075)	-0.793 (0.203)	188.5 (7.5)	20
	2009	0.132 (0.071)	-0.372 (0.525)	183.0 (0.0)	3
	2010	0.100 (0.137)	-	-	-

Analysis 1: Juvenile Condition, Fledging Date and Survival

We found that resighting probability was generally high, average 93% (95% CI 86-96%; Table S4) and differed between juveniles and adults, and for study area D in 2010, due to lower observation effort (Table 3). Survival differed between maturity classes and years: in general juvenile birds have a lower survival (24% ± 10% SD) than adults (53% ± 4%), and survival was 10-16% higher in early fledglings compared to late fledglings (Figure 3). No additional effect of condition was found.

Table 3. Selection of the best model (lowest AICc) for resighting probability p in analysis 1 (m = maturity (juvenile/adult), s = site, y = year, D10 = Den Helder 2010). k: number of parameters, w_i: AIC_c-weight of the model.

No.	Model	*k*	AICc	ΔAICc	w_i	Deviance
1	$\Phi(s*m*y)p(m+D10)$	27	2337.53	0.00	0.92	2282.65
2	$\Phi(s*m*y)p(s+m)$	28	2342.87	5.34	0.06	2285.93
3	$\Phi(s*m*y)p(s*m)$	30	2346.06	8.53	0.01	2284.98
4	$\Phi(s*m*y)p(y)$	27	2349.28	11.75	0.00	2294.40
5	$\Phi(s*m*y)p(m*y)$	31	2351.47	13.95	0.00	2288.32
6	$\Phi(s*m*y)p(s*y)$	34	2351.95	14.42	0.00	2282.56
7	$\Phi(s*m*y)p(m)$	30	2352.73	15.20	0.00	2291.65
8	$\Phi(s*m*y)p(s)$	27	2354.74	17.22	0.00	2299.87
9	$\Phi(s*m*y)p(s*m*y)$	46	2366.64	29.11	0.00	2272.10

The best model for survival Φ without covariates was one without site effects, but with an interaction between maturity and year (Table S2 for all models). However, both fledging date and condition index differed significantly between sites (Table 2). Therefore, when testing for the effects of individual covariates, we used a starting model including site effects. The best model including site effects for survival was a model (model 2) with an interaction between site and maturity and an interaction between maturity and time. Models 3, 4 and 5 performed only slightly worse. However, model 3 does not include site effects and both model 3 and 5 assume a time effect for both maturity classes, while no time effect was found for adults in earlier analyses with adults only (chapter 2). When using model 4 as a starting model, the conclusions of the analyses with individual covariates were similar, so we do not show them here.

Including fledging date as a continuous variable improved the starting model significantly (Table 4; compare 7 and 10; $\Delta\text{AIC}_c > 2$). However, including fledging date as a factor (early/late) improved the starting model even more (compare AIC_c's of models 2 and 10 and models 2 and 7; $\Delta\text{AIC}_c > 2$). Therefore we chose to use the factor instead of the continuous variable for fledging date. We did not find an additional effect of condition index on survival (compare model 9 with model 10; $\Delta\text{AIC}_c < 2$ and model 4 and 6 with model 2).

Table 4. Selection of the best model (lowest AICc) for survival rate Φ with covariates in analysis 1 (m = maturity: juvenile/adult, s = site, y = year, D10 = Den Helder 2010, ff = early/late fledging; factor, fc = fledging date; continuous variable and ci=condition index; continuous variable). k: number of parameters, w_i: AIC_c-weight of the model.

No.	Model	*k*	AIC_c	ΔAIC_c	w_i	Deviance
1	Φ(s*m+m*y+s*ff)*p*(m+D10)	18	1647.76	0.00	0.38	1611.37
2	Φ(s*m+m*y+ff)*p*(m+D10)	16	1648.89	1.12	0.22	1616.57
3	Φ(m*y+ff)*p*(m+D10)	12	1649.50	1.74	0.16	1625.32
4	Φ(s*m+m*y+ff+ci)*p*(m+D10)	17	1650.09	2.33	0.12	1615.74
5	Φ(s*m+m*y+ff*y)*p*(m+D10)	19	1651.38	3.62	0.06	1612.94
6	Φ(s*m+m*y+ff*ci)*p*(m+D10)	18	1652.12	4.36	0.04	1615.73
7	Φ(s*m+m*y+fc)*p*(m+D10)	16	1655.10	7.34	0.01	1622.79
8	Φ(s*m+m*y+s*ff*ci)*p*(m+D10)	22	1657.07	9.30	0.00	1612.48
9	Φ(s*m+m*y+ci)*p*(m+D10)	16	1659.30	11.54	0.00	1626.99
10	Φ(s*m+m*y)*p*(m+D10)	15	1659.67	11.90	0.00	1629.39
11	Φ(s*m+m*y+s*ci)*p*(m+D10)	18	1661.50	13.73	0.00	1625.10
12	Φ(s*m+m*y+y*ci)*p*(m+D10)	19	1664.81	17.05	0.00	1626.38

As including condition did not improve the models for juvenile survival, we again tested for the effects of fledging date, including the juveniles with unknown condition but known fledging date, thereby increasing sample size by 274 individuals (34% increase; Supplemental Appendix A). This changed the order of the three best models (model 3 of table 3 becoming the best model now, see table S3), but the conclusions regarding effects of fledging date remain unchanged. For all sites applies that annual survival of early fledged juveniles was higher (10 to 16%) than of late fledged juveniles, and adult survival was higher than for both early and late juveniles (Figure 3).

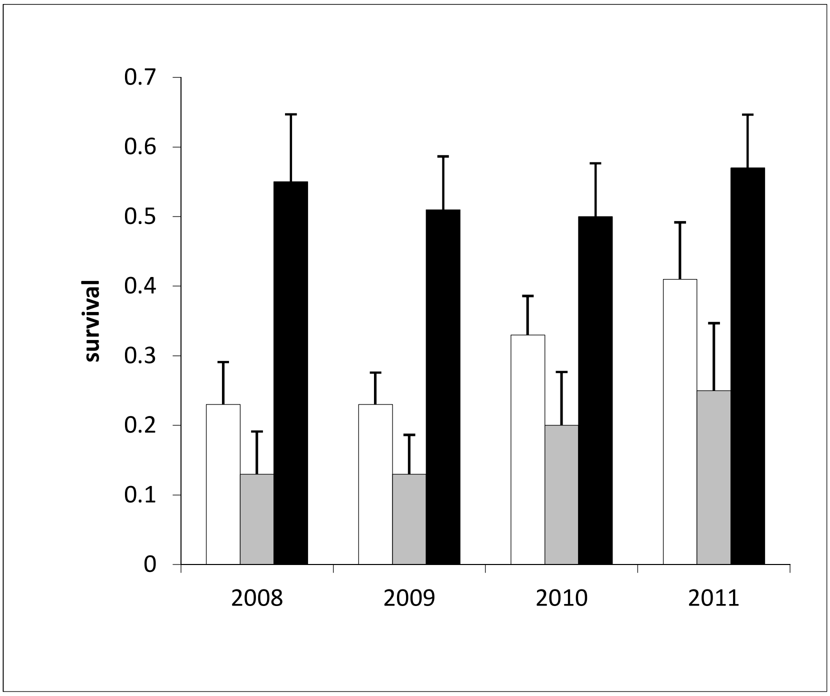

Figure 3. First-year survival for early (white bars) and late (grey bars) broods, and adult survival (black bars), based on model 3 from table 4 (analysis 1), which proved the best model when including an extra 274 chicks with unknown condition. Values (mean and SE) shown per year for all three sites combined.

Analysis 2: Juvenile Seasonal Survival

In analysis 2 we looked in more detail to first-year survival, which we calculated in analysis 1 for early and late fledglings. We divided first-year survival in two periods, (1) post-fledging (fledging to onset of migration) and (2) migration-winter, for both early and late fledglings. We found that average survival per two-week interval is lower during the post-fledging period (early / late fledglings 0.932 / 0.880) than during migration-winter (early / late fledglings 0.966 / 0.938) for both early and late fledglings (Figure 4). However, survival of late fledglings per two-week interval was lower compared to early fledglings for both periods (Figure 4).

The survival model with juvenile survival as a function of season and fledging date, with fixed resighting probabilities [$\Phi(s+f)p(.)$], had most support from the data (smallest $QAIC_c$ value, Table 5), with model 6 for transition probabilities from breeding season to migration-winter season (ψ_{BW}), in which all transitions were fixed at zero (meaning that no juveniles have left the breeding site for migration), except for the 5th and 6th interval of cohort 1 (cohort 1), and the 6th interval of cohort 2 (one parameter) (cohort 2), and the seventh interval, which is fixed at 1 because all juveniles have left the breeding site; Table S1.

The model with an additive effect of state (*B* or *W*) and fledging date was significantly better than the model including the interaction of the two. Including an effect of fledging date in either *B* or *W* only also did not improve the model. Therefore the effect of fledging date did not seem to differ between the two states, suggesting that survival of early and late fledged juveniles differs not only after fledging in the breeding area (fledging - August), but also on migration plus winter (September – March; Figure 4). As the first four models for survival all had $\Delta QAIC_c < 2$ we averaged those models; parameters estimates are given in Appendix 1.

After model averaging, the estimated probability that a juvenile Northern Wheatear leaves the breeding site is 0.589 ± 0.071 for the intervals 5 and 6 (cohort 1) and interval 6 (cohort 2).

Table 5. Model selection for seasonal juvenile survival Φ in analysis 2 (s: season, f: fledging date early/late, W: winter, B: breeding season, k: number of parameters, w_i: AIC_c-weight of the model.

No.	Model	*k*	$QAIC_c$	$\Delta QAIC_c$	w_i	QDeviance
1	Φ(s+f)	5	447.46	0.00	0.25	100
2	Φ(s+W.f)	5	448.94	1.48	0.12	102
3	Φ(s+a+f)	6	449.27	1.80	0.10	100
4	Φ(s+B.f)	5	449.28	1.82	0.099	102
5	Φ(s*f)	6	449.51	2.04	0.088	100

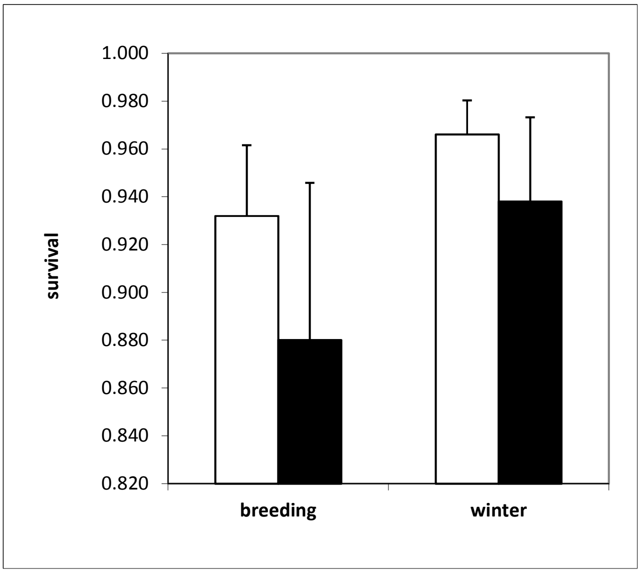

Figure 4. Breeding and winter survival of early fledglings (white bars) and late fledglings (black bars) from the most parsimonious model, given as average (SE) survival per two-week interval.

Discussion

First-year Survival

First-year survival of Northern Wheatears declined with increasing fledging date, in agreement with other studies (reviewed in Maness and Anderson 2013), and was lower compared to adult survival. This date-effect is generally attributed to timing *per se* (the *date hypothesis*; for instance, declining food availability with increasing date) or to earlier breeding of high quality parents (the quality hypothesis; reviewed in Verhulst and Nilsson 2008).

Body condition did not significantly contribute to first-year survival, in spite of significant differences in body condition between early and late nestlings within all three Northern Wheatear populations. Effects of body condition on survival in the literature are equivocal: some studies report positive correlations between condition and survival (Naef-Daenzer et al. 2001; Yackel Adams et al. 2006; Vitz and Rodewald 2011), whereas others do not (Kershner et al. 2004; Sim et al. 2013; Sullivan 1989). As proposed by Krementz et al. (1989) this indicates that condition only affects survival in times of scarcity, such as a lack of food.

Since condition did not influence first-year survival but timing of fledging did, other variables operating after fledging are probably of more importance than nestling condition, at least in the years of research. These variables could be ecological (e.g. more predation and smaller food availability during post-fledging), physiological (e.g. time for molting and before onset of migration shorter for late fledglings), density dependent (independent late fledglings experience more competition due to presence of both adults and early fledglings), or a combination. However, as condition is not only correlated with fledging date, but also with site, these effects cannot be easily identified.

Seasonal Survival in Relation to Timing of Fledging

We further explored first-year survival by dividing between seasons: from fledging to departure for migration and migration-winter. It appeared that (1) daily post-fledging survival at the breeding site is lower than winter survival, irrespective of fledging cohort; and (2) both post-fledging survival and winter survival are lower for late than for early fledglings.

Post-fledging survival

Daily mortality rate of first year wheatears was highest in the period after fledging, before departure for migration, which is in line with a number of other studies (e.g. Naef-Daenzer et al. 2001; Vitz and Rodewald 2011; Sim et al. 2013). Estimated 14-day post-fledging survival was high compared to most other studies on passerine bird species: 0.932 for early fledglings and 0.880 for late fledglings (refer to Appendix 2 for an overview). Only Purple Martin (*Progne subis*; 0.939 for resighted juveniles, Tarof et al. 2011) and Eastern

Meadowlark (*Sturnella magna*; 0.944 including juveniles with unknown fate, Kershner et al. 2004) show higher survival rates. This, and differences within a species between years (Appendix 2), indicate that post-fledging survival may strongly differ between sites, years and timing of nesting. Also, field work methodology may partly influence the outcome: post-fledging survival of Purple Martins as determined by radio-telemetry was 0.868, but based on resightings 0.939 (Tarof et al. 2011).

High post-fledging mortality is generally caused by high predation rates of juveniles with still underdeveloped locomotion, and limited foraging abilities (e.g. Sullivan 1989; Anders et al. 1997; Sim et al. 2013). Unfortunately, we have no information on fledgling predation pressure for our Northern Wheatear populations. Only few avian predators were recorded hunting during the breeding season, mostly Northern Goshawks (*Accipiter gentilis*) albeit Hen Harriers (*Circus cyaneus*) and Merlins (*Falco columbarius*) have been seen chasing Northern Wheatears when migrating through the areas during spring. However, these primarily hunt for larger prey such as Rabbits, which are common in the area. Nest predation by mammals, especially Red Foxes (*Vulpes vulpes*), occurs regularly (chapter 2), yet we do not know if fledged young are also hunted. Therefore, it remains uncertain if predation of juveniles by predators is substantial for Northern Wheatears during post-fledging and if predation pressure differs between early and late fledglings.

Another factor which may particularly affect late fledglings is the often noted seasonal decline of food availability. However, not all ecosystems show strong seasonal peaks in prey abundance. For instance, deciduous forests in temperate regions are prone to a short peak of extremely high arthropod abundance (Both et al. 2010; Feeny 1970; Southwood et al. 2004), but highly productive marshes harbor high densities of arthropods throughout the season (Both et al. 2010; Halupka et al. 2008). Information on changes in food abundance throughout the breeding season is largely unavailable for the Dutch coastal dunes, where our breeding sites are located. Since the post-fledging period of early compared to late fledged Northern Wheatears shifts in time, different prey species or stadia (larva, imago) are available as a result of differences in prey phenology. The scarabid beetle *Phyllopertha horticola*, for example, forms on average 17% of the number of prey fed to nestlings (chapter 4). Yet, due to its phenology this prey is unavailable for late broods, when another scarabid beetle, *Anomala dubia*, takes over as an important prey (H. H. van Oosten, *unpublished data*). Likewise, the number of caterpillars was lower in July than in May/June (H. H. van Oosten, *unpublished data*). During summer grasshoppers (Orthoptera: mainly Acrididae) reach their final size and are often fed to nestlings in late broods (H. H. van Oosten, *unpublished data*), but not in early broods (chapter 4). Late fledglings may depend on grasshoppers due to their large numbers and perhaps due to lower abundance of more easily caught larval prey. In order to meaningfully relate survival to site-specific differences in prey availability during post-fledging, in-depth studies on feeding ecology of Northern Wheatears are necessary.

Migration-winter survival of juveniles

Daily survival rates during migration-winter appear higher than during the post-fledging period for both early and late juveniles. Simultaneously, survival throughout migration and winter remains lower for late compared to early fledged young. This may indicate that effects of stressors earlier in life are carried over to affect migration-winter survival.

The onset of migration is largely endogenous controlled (Berthold 1996), also in Northern Wheatears (Bulte and Bairlein 2013). This allows for limited flexibility with respect to timing of migration, which may be reflected in the remarkable similar pattern of disappearance of juveniles from the breeding sites in both study years. This applies for both early and late fledglings, with the distinction that early fledglings start to disappear before late fledglings do so (Figure 1 and section 'Results: Analysis 2: Juvenile Seasonal Survival'). Our observation that early fledged juveniles may depart before late hatched ones is corroborated by recent studies showing that early fledglings of other migratory songbirds also depart before late fledglings (Savannah Sparrow *Passerculus sandwichensis*, Mitchell et al. 2012; titmice *Paridae*, Meller et al. 2013). Therefore, this apparently more widespread pattern indicates that an early preparation for and onset of migration is advantageous. Early juveniles may choose to leave the natal site as soon as possible to avoid competition for food with adults and late juveniles on the breeding site, to avoid late arrival on stop-overs (which could have repercussions on food availability; Delingat and Dierschke 2000, Moore and Yong 1991), and to avoid late arrival on the wintering sites (best wintering territories (Leisler et al. 1983) already occupied by earlier arriving conspecifics). As a result they may arrive earlier on the wintering site or, alternatively, earlier departure allows a more stretched, less hurried migration period.

Aggressive interactions among fledglings were frequently observed in the field, and fledglings are reported to defend territories against conspecifics already during their post-fledging period (Glutz von Blotzheim and Bauer 1988; Conder 1989). In this scenario, late fledglings probably fare worst both during the post-fledging and the migration-winter period. Not only have late fledglings to compete with adults but also with remaining early fledged juveniles on the natal site which may hamper foraging, as shown for other songbirds (Arcese and Smith 1985; Merilä and Svensson 1997). As a result, net foraging time may be lower than for early fledged juveniles. This may lead to more risky foraging behavior with increased chances of predation (Koivula et al. 1994), lower survival on the natal site and constrained preparation for migration (Berthold 1996; Bauchinger and Biebach 2001). As a result, both post-fledging and migration-winter survival may be lower than for early fledglings. The extent to which yearly and seasonally varying food availability interacts with yearly varying intensities of density-dependent competition remains open for further studies.

Conclusions and Implications for Conservation

First, we have shown that survival of late fledged young of Northern Wheatears is 10−16% lower than for early fledged young. Following the frequent occurrence of nest predation in some sites (chapter 2), replacement broods are regular here and result in a high proportion of late fledged young which generally show a lower survival. We previously showed (chapter 2) that the three populations were equally sensitive to fecundity and first-year survival, but that the contribution of different vital rates (fecundity, first-year survival, female adult survival and immigration) to population growth rate differed between populations. First-year survival contributed positively to population growth (chapter 2). Therefore, if the yearly juvenile output consists mainly of late fledglings with low first-year survival, this may well affect the population growth. Nevertheless, it remains to be seen if indeed, in years with a high incidence of nest predation, a preponderance of late fledglings results in a more negative population growth than expected, based on the absolute number of offspring. For example, the lack of early fledged, thus stronger, conspecifics may ameliorate intra-specific competition (e.g. accessibility to food, foraging time) for late fledglings and hence, increase their first-year survival (Tinbergen et al. 1985).

Second, we have shown that both post-fledging and migration-winter survival in Northern Wheatears are higher in early fledglings than in late fledglings. A next step is to study exact proximate causality, likely related to predation and/or food availability/maternal effects, on survival differences. Which parameters affect migration-winter survival stronger for late fledglings than for early fledglings? The effects of (intra-specific) competition may control both predation chances and foraging time, especially in late fledglings, which in turn affects survival through constrained preparation for migration (body mass increase, molt).

Since survival of (late) fledglings is especially constrained on the breeding site during post-fledging, conservation efforts should focus on the exact nature of these constraints by quantifying food availability and decreasing predation rates. Also, if nest predation or parasitism is frequent to such an extent that only repeat clutches (late nests) provide fledged young, nests should be protected, since survival of late fledglings is much lower than of early fledglings.

Study of seasonal survival of migratory passerines is still in its infancy and many more studies are needed to define how survival develops in the course of seasons and hence, at which season and location conservation measures should be targeted.

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Appendix 1. Averaged estimates of parameters of the best four models (analysis 2: seasonal survival).

Parameter	State	Cohort	Age	Interval	Estimate	SE	Lower	Upper
Φ	B	1-3	≤4 weeks	1-7	0.934	0.017	0.892	0.961
Φ	B	1-3	>4 weeks	1-7	0.936	0.017	0.893	0.963
Φ	B	4-8	≤4 weeks	1-7	0.894	0.034	0.808	0.944
Φ	B	4-8	>4 weeks	1-7	0.897	0.033	0.811	0.947
Φ	W	1-3	all	8	0.955	0.010	0.932	0.970
Φ	W	4-8	all	8	0.917	0.023	0.858	0.952
p	B	all	all	1-6	0.952	0.014	0.917	0.973
p	B	all	all	7	0	NA	NA	NA
p	B	all	all	8	0.95	NA	NA	NA
p	W	all	all	1-8	0	NA	NA	NA
ψ	B→W	1	>4 weeks	5-6	0.589	0.071	0.447	0.718
ψ	B→W	2	>4 weeks	6	0.589	0.071	0.447	0.718
ψ	B→W	all	>4 weeks	7	1	NA	NA	NA
ψ	B→W	all	all	all other	0	NA	NA	NA
ψ	W→B	all	all	1-7	0	NA	NA	NA
ψ	W→B	all	all	8	1	NA	NA	NA

Appendix 2. Overview of post-fledging survival reported in other studies, back-calculated to survival per 14 day intervals.

Species	Particulars	14 days survival	Reference
Hooded Warbler (*Wilsonia citrina*)		0.436	Rush and Stutchbury 2008
Sprague's Pipit (*Anthus spragueii*)		0.513	Fisher and Davis 2011
Dickcissel (*Spiza americana*)		0.574	Berkeley et al. 2007
Rose-breasted Grosbeak (*Pheucticus ludovicianus*)		0.726	Moore et al. 2010
Western Bluebird (*Sialis mexicana*)		0.732	Wightman 2009
Whinchat (*Saxicola rubetra*)		0.744	Tome and Denac 2012
Ovenbird (*Seiurus aurocapilla*)		0.776	King et al. 2006
Wood Thrush (*Hylocichla mustelina*)		0.806	Anders et al. 1997
Worm-eating Warbler (*Helmitheros vermivora*)		0.835	Vitz and Rodewald 2011
Dickcissel (*Spiza americana*)		0.869	Suedkamp Wells et al. 2007
Ovenbird (*Seiurus aurocapilla*)		0.888	Vitz and Rodewald 2011
Eastern Meadowlark (*Sturnella magna*)		0.914	Suedkamp Wells et al. 2007
Larksparrow (*Chondestes grammacus*)	2001/2002	0.522/0.441	Yackel Adams et al. 2006
titmice (Paridae)	May/June	0.775/0.326	Naef-Daenzer et al. 2001
White-throated Robin (*Turdus assimilis*)	pasture/coffee	0.818/0.695	Cohen and Lindell 2004
Ring Ouzel (*Turdus torquatus*)	early/late	0.842/0.536	Sim et al. 2013
Northern Wheatear (*Oenanthe oenanthe*)	early/late	0.932/0.880	this study
Purple Martin (*Progne subis*)	resighting/radio	0.939/0.868	Tarof et al. 2011
Eastern Meadowlark (*Sturnella magna)*	incl unknown fate/known fate	0.944/0.914	Kershner et al. 2004

Supplements

Table S1. Model selection for ψ in analysis 2: seasonal survival.

1. All ψ_{BW}'s equal to zero, except for the 5th and 6th interval, which are estimated separately, and the 7th interval, which is set equal to 1: $\boldsymbol{\psi}_1 = \psi_{BWc,1\text{-}4} = \psi_{BWc,8} = 0$, $\boldsymbol{\psi}_2 = \psi_{BWc,5}$ and $\boldsymbol{\psi}_3 = \psi_{BWc,6}$ and $\boldsymbol{\psi}_4 = \psi_{BWc,7} = 1$
2. All ψ_{BW}'s equal to zero, except for the 5th interval of cohorts 1-4 and 6th interval of cohorts 4 and 5 (one estimate: ψ_2), the 6th interval of cohorts 1-3 (one estimate: ψ_3) and the 7th interval, which is set equal to 1: $\boldsymbol{\Psi}_1 = \Psi_{BWc,1\text{-}4} = \Psi_{BW5,5} = \Psi_{BW6,6} = \Psi_{BWc,8} = 0$, $\boldsymbol{\Psi}_2 = \Psi_{BW1\text{-}4,5} = \Psi_{BW4\text{-}5,6}$, $\boldsymbol{\Psi}_3 = \Psi_{BW1\text{-}3,6}$ and $\boldsymbol{\Psi}_4 = \Psi_{BWc,7} = 1$
3. All Ψ_{BW}'s equal to zero, except for the 5th interval of cohorts 1 and 2 and 6th interval of cohorts 2 and 3 (one estimate: Ψ_2), the 6th interval of cohorts 1-2 (one estimate: Ψ_3) and the 7th interval, which is set equal to 1: $\boldsymbol{\Psi}_1 = \Psi_{BWc,1\text{-}4} = \Psi_{BW3\text{-}5,5} = \Psi_{BW5\text{-}6,6} = \Psi_{BWc,8} = 0$, $\boldsymbol{\Psi}_2 = \Psi_{BW1\text{-}2,5} = \Psi_{BW2\text{-}3,6}$, $\boldsymbol{\Psi}_3 = \Psi_{BW1\text{-}2,6}$ and $\boldsymbol{\Psi}_4 = \Psi_{BWc,7} = 1$
4. All Ψ_{BW}'s equal to zero, except for the 5th interval of cohorts 1 and 2 and 6th interval of cohort 3 (one estimate: Ψ_2), the 6th interval of cohorts 1-2 (one estimate: Ψ_3) and the 7th interval, which is set equal to 1: $\boldsymbol{\Psi}_1 = \Psi_{BWc,1\text{-}4} = \Psi_{BW3\text{-}5,5} = \Psi_{BW4\text{-}6,6} = \Psi_{BWc,8} = 0$, $\boldsymbol{\Psi}_2 = \Psi_{BW1\text{-}2,5} = \Psi_{3,6}$, $\boldsymbol{\Psi}_3 = \Psi_{BW1\text{-}2,6}$ and $\boldsymbol{\Psi}_4 = \Psi_{BWc,7} = 1$
5. All Ψ_{BW}'s equal to zero, except for the 4th interval of cohort 1, the 5th interval of cohorts 2 and the 6th interval of cohort 3 (one estimate: Ψ_2), the 5th and 6th interval of cohort 1 and the 6th interval of cohort 2 (one estimate: Ψ_3) and the 7th interval, which is set equal to 1: $\boldsymbol{\Psi}_1 = \Psi_{BWc,1\text{-}3} = \Psi_{BW2\text{-}4,4} = \Psi_{BW3\text{-}5,5} = \Psi_{BW4\text{-}6,6} = \Psi_{BWc,8} = 0$, $\boldsymbol{\Psi}_2 = \Psi_{BW1,4} = \Psi_{BW2,5} = \Psi_{BW3,6}$, $\boldsymbol{\Psi}_3 = \Psi_{BW1,5\text{-}6} = \Psi_{BW2,6}$ and $\boldsymbol{\Psi}_4 = \Psi_{BWc,7} = 1$
6. All Ψ_{BW}'s equal to zero, except for the 5th and 6th interval of cohort 1 and the 6th interval of cohort 2 (one estimate: Ψ_2) and the 7th interval, which is set equal to 1: $\boldsymbol{\Psi}_1 = \Psi_{BWc,1\text{-}4} = \Psi_{BW2\text{-}5,5} = \Psi_{BW3\text{-}6,6} = \Psi_{BWc,8} = 0$, $\boldsymbol{\Psi}_2 = \Psi_{BW1,5\text{-}6} = \Psi_{BW2,6}$ and $\boldsymbol{\Psi}_4 = \Psi_{BWc,7} = 1$
7. All Ψ_{BW}'s equal to zero, except for the 7th interval, which is set equal to 1: $\boldsymbol{\Psi}_1 = \Psi_{BWc,1\text{-}6} = \Psi_{BWc,8} = 0$ and $\boldsymbol{\Psi}_2 = \Psi_{BWc,7} = 1$

Table S2. Model selection for Φ without covariates in analysis 1, (a = age, s = site, t = time, D10 = Den Helder 2010). *k*: number of parameters, w_i: AIC_c-weight of the model.

No.	Model	*k*	AIC_c	ΔAIC_c	w_i	Deviance
1	Φ(a*t)*p*(a+D10)	11	2323.92	0.00	0.54	2301.77
2	Φ(s*a+a*t)*p*(a+D10)	15	2326.69	2.77	0.13	2296.42
3	Φ(a+t)*p*(a+D10)	8	2326.87	2.95	0.12	2310.78
4	Φ(s+a*t)*p*(a+D10)	13	2327.52	3.59	0.09	2301.31
5	Φ(s*a+t)*p*(a+D10)	12	2328.07	4.15	0.07	2303.90
6	Φ(s+a+t)*p*(a+D10)	10	2330.55	6.63	0.02	2310.42
7	Φ(s*t+s*a+a*t)*p*(a+D10)	21	2331.94	8.02	0.01	2289.40
8	Φ(s*t+a*t)*p*(a+D10)	19	2332.53	8.60	0.01	2294.09
9	Φ(s*t+s*a)*p*(a+D10)	18	2333.53	9.61	0.00	2297.13
10	Φ(s*t+a)*p*(a+D10)	16	2335.74	11.82	0.00	2303.43
11	Φ(a)*p*(a+D10)	5	2336.38	12.46	0.00	2326.35
12	Φ(s*a)*p*(a+D10)	9	2337.50	13.58	0.00	2319.39
13	Φ(s*a*t)*p*(a+D10)	27	2337.53	13.60	0.00	2282.65
14	Φ(s+a)*p*(a+D10)	7	2339.78	15.86	0.00	2325.71
15	Φ(t)*p*(a+D10)	7	2404.64	80.72	0.00	2390.58

16	Φ(s+t)*p*(a+D10)	9	2407.69	83.77	0.00	2389.59
17	Φ(s*t)*p*(a+D10)	15	2413.00	89.08	0.00	2382.72
18	Φ(.)*p*(a+D10)	4	2422.46	98.54	0.00	2414.44
19	Φ(s)*p*(a+D10)	6	2425.66	101.74	0.00	2413.61

Supplemental Appendix A. Model selection for Φ with covariates, including juveniles with unknown condition.

Including fledging date improved the model significantly (Table S3; compare models 2 and 5 with model 6; $\Delta\mathrm{AIC}_c > 2$), and models with fledging date as a factor performed better than those with fledging date as a continuous covariate (compare model 2 with model 5; $\Delta\mathrm{AIC}_c > 2$). Including an interaction term of site or time with fledging date did not improve the model (compare models 3 and 4 with model 2). The model with an interaction between age and time only, and an additive effect of fledging date performed slightly better than the model which in addition included an interaction between site and age. From this we can conclude that including the interaction between site and age does not significantly improve the model.

Table S3. Model selection for Φ with covariates, including juveniles with unknown condition (ff=early/late fledging; factor, fc=fledging date; continuous variable). *k*: number of parameters, w_i: AIC$_c$-weight of the model.

No.	Model	*k*	AIC$_c$	ΔAIC$_c$	w_i	Deviance
1	Φ(a*t+ff)*p*(a+D10)	12	2315.39	0.00	0.51	2291.21
2	Φ(s*a+a*t+ff)*p*(a+D10)	16	2316.14	0.76	0.35	2283.83
3	Φ(s*a+a*t+s*ff)*p*(a+D10)	18	2319.22	3.83	0.08	2282.83
4	Φ(s*a+a*t+ff*t)*p*(a+D10)	19	2320.56	5.17	0.04	2282.12
5	Φ(s*a+a*t+fc)*p*(a+D10)	16	2321.87	6.48	0.02	2289.56
6	Φ(s*a+a*t)*p*(a+D10)	15	2326.69	11.30	0.00	2296.42

All estimates from models 1 and 2 of Table S3 are given in Table S4. The pattern in survival rates is very similar for both models. When including site effects in the model, we see that juvenile survival is highest in A, then in C and then in D.

Table S4. Parameter estimates and 95% confidence limits for models 1 and 2 from Table S3

Parameter	**Estimate**	**LCL**	**FCL**	**Site**	**Estimate**	**LCL**	**FCL**
Φ juv early 2008	0.23	0.17	0.29	D	0.21	0.15	0.27
Φ juv early 2009	0.23	0.19	0.28	D	0.20	0.15	0.26
Φ juv early 2010	0.33	0.28	0.39	D	0.29	0.23	0.36
Φ juv early 2011	0.41	0.33	0.49	D	0.36	0.28	0.45
Φ juv late 2008	0.13	0.08	0.20	D	0.10	0.06	0.17
Φ juv late 2009	0.13	0.08	0.19	D	0.10	0.06	0.16
Φ juv late 2010	0.20	0.13	0.28	D	0.16	0.10	0.24
Φ juv late 2011	0.25	0.17	0.36	D	0.20	0.13	0.31
Φ ad 2008	0.55	0.45	0.64	D	0.56	0.46	0.65
Φ ad 2009	0.51	0.44	0.59	D	0.53	0.45	0.61
Φ ad 2010	0.50	0.42	0.57	D	0.52	0.44	0.60
Φ ad 2011	0.57	0.49	0.64	D	0.59	0.51	0.67
Φ juv early 2008				C	0.25	0.17	0.34
Φ juv early 2009				C	0.24	0.17	0.33
Φ juv early 2010				C	0.34	0.26	0.43
Φ juv early 2011				C	0.42	0.32	0.53
Φ juv late 2008				C	0.13	0.08	0.21
Φ juv late 2009				C	0.13	0.08	0.20
Φ juv late 2010				C	0.19	0.12	0.28
Φ juv late 2011				C	0.25	0.16	0.36
Φ ad 2008				C	0.54	0.40	0.67
Φ ad 2009				C	0.51	0.39	0.63
Φ ad 2010				C	0.50	0.38	0.62
Φ ad 2011				C	0.57	0.46	0.68
Φ juv early 2008				A	0.28	0.21	0.38
Φ juv early 2009				A	0.28	0.21	0.35
Φ juv early 2010				A	0.38	0.31	0.46
Φ juv early 2011				A	0.47	0.37	0.56
Φ juv late 2008				A	0.15	0.09	0.24
Φ juv late 2009				A	0.15	0.09	0.22
Φ juv late 2010				A	0.22	0.14	0.32
Φ juv late 2011				A	0.28	0.18	0.40
Φ ad 2008				A	0.51	0.39	0.62
Φ ad 2009				A	0.48	0.39	0.57
Φ ad 2010				A	0.47	0.38	0.56
Φ ad 2011				A	0.54	0.45	0.63
pjuv	0.93	0.86	0.96		0.93	0.86	0.96
p ad	0.98	0.94	0.99		0.97	0.93	0.99
p juv D2010	0.67	0.51	0.80		0.70	0.54	0.82
p ad D2010	0.87	0.74	0.94		0.87	0.75	0.94

Chapter 4

Habitat selection of brood-rearing Northern Wheatears *Oenanthe oenanthe* and their invertebrate prey

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Abstract

Birds consider both variation in prey abundance and accessibility in their decision where to forage. Acidification and nitrogen deposition affect both prey abundance and accessibility by stimulating growth of nitrophilic grasses at the expense of forbs. Management practises as mowing or grazing primarily affect vegetation structure which also influences the abundance and accessibility of invertebrates. Hence, for effective management and conservation purposes it is paramount to understand the relationships between vegetation structure, densities of preferred prey, and habitat-use of birds. In this study we explore such relationships for the nationally endangered Northern Wheatear *Oenanthe oenanthe* in dune grasslands along the Dutch coast. Our findings support the hypothesis that forager mobility and food accessibility are of greater importance during patch selection than food abundance *per se* in ground foraging birds. The abundance of all potential prey and three of the four most important actual prey groups was highest in tall grass but Northern Wheatears foraged preferentially in short grass. Clearly, grass encroachment by tall grass will diminish habitat suitability for Northern Wheatears due to lowered prey accessibility. We propose that a mixture of short and tall vegetation and landscape management allows for dispersal of arthropods between different (micro) habitats. We provide densities of the important prey in a coastal area where Northern Wheatears still successfully breed. This enables site-managers to efficiently investigate presence and abundance of important prey in seemingly suitable areas but where Northern Wheatears do not breed. Eventually we may be able to discern whether food shortage poses a bottleneck for Northern Wheatears in these uninhabited areas.

Keywords
Northern Wheatear, *Oenanthe oenanthe*, dune, grassland, nitrogen, vegetation structure, diet, arthropods, habitat use

Introduction

Only a quarter of the world's temperate grasslands are left undisturbed (Hannah et al. 1995) and remaining (semi-) natural grasslands in Europe and North America are under high pressure of deposition of atmospheric nitrogen and acidification (Bobbink et al. 2010). The persistence and open character of grasslands is threatened by encroachment of tall, nitrophilic grasses at the expense of abundance and diversity of flowering plants (Bobbink et al. 2010; Stevens et al. 2004). The resulting homogenization of plant communities may well affect arthropod communities (Haddad et al. 2001; Koricheva et al. 2000; Otway et al. 2005; Schaffers et al. 2008; Siemann 1998) which, in turn, can be detrimental for vertebrate insectivores occurring in these grasslands.

Changes in vegetation structure alone could also affect the occurrence of grassland birds. Atkinson et al. (2005) found that vegetation structure rather than the abundance of possible prey determined where birds foraged in agricultural grasslands. Tall grass swards may contain both higher prey species diversity and abundance (Dennis et al. 2008; Morris 2000), but, as tall grass vegetations are far less accessible for small ground-foraging birds (Atkinson et al. 2004), songbirds appeared to preferentially forage on short swards.

So, birds consider both variation in prey abundance and accessibility in their decisions where to forage. As acidification and nitrogen deposition (and other forms of fertilisation) affect both prey abundance and accessibility, the suitability of sites as foraging locations may have altered over time. Management practises counteracting the effects of acidification and nitrogen deposition primarily affect vegetation structure, e.g. by mowing or grazing, which also influence the abundance and accessibility of invertebrates. Hence, for effective management and songbird conservation purposes it is paramount to understand the relationships between vegetation structure, densities of preferred prey, and habitat-use of birds.

In this study we explore such relationships for the nationally endangered Northern Wheatear *Oenanthe oenanthe* in heterogeneous coastal dune grasslands in the Netherlands. These natural grasslands have been affected by encroachment of tall grass resulting from acidification and nitrogen deposition (Kooijman et al. 1998). Northern Wheatears are physically adapted to cursorial locomotion in open habitat (Kaboli et al. 2007) where they 'hop-and-peck' on short vegetation (Conder 1989). Therefore, grass encroachment due to atmospheric deposition of nitrogen (Bobbink et al. 2010) and declined grazing by Rabbits due to viral diseases rendered habitats unsuitable for Northern wheatears to forage. Remaining populations of Northern Wheatears in the Netherlands breed in structurally heterogeneous vegetations where bare sand, low and tall grass swards co-occur on a small spatial scale. We determined the relationships between foraging habitat preference and (1) vegetation type and availability, (2) prey abundance, and (3) prey species composition in interaction with vegetation type, and actual bird diet. In particular, we asked whether Northern Wheatears forage predominantly in habitat patches with the highest densities of their preferred prey types, or in patches where these are less abundant but likely to be more accessible.

In relating foraging behaviour to food abundance, a thorough understanding of diet is paramount, as an insect considered as bird food by a human observer may not be considered so by the insectivorous passerine. Knowing a bird's diet is also crucial to optimise arthropod sampling methods for detecting important prey items, since different arthropods require different sampling methods (Standen 2000). Hence, we will first present results of an extensive Northern Wheatear diet study, performed in the Dutch dune grasslands.

Methods

Study species and study site

Northern Wheatears are small (*c* 25 g) insectivorous passerines which breed from eastern Canada across Eurasia to western Alaska, USA. All birds winter in the African Sahel and eastern Africa (Glutz von Blotzheim and Bauer 1988), as has recently been shown by the use of geolocators for birds from Alaska and eastern Canada (Bairlein et al. 2012), Germany (Schmaljohann et al. 2012b) and the Netherlands (Van Oosten et al. 2014). Field data for this study were collected during breeding seasons of 2007–2010 in coastal dunes in the Noord-Hollands Duinreservaat (NHD; 52°33'N, 4°36'E). Here, Northern Wheatears declined by 90% (from 164 in 1988 to 17 in 2010), as they did elsewhere in the country (Boele et al. 2014; Sovon 2002). The study site of 74 ha is located in stabilised grey dunes, within 1 km from the sea. The highly diverse landscape consists of a mosaic of vegetation succession stages with pioneer vegetation around sandy blow-outs (*Phleo-Tortuletum ruraliformisi*), short semi-natural grasslands (*Taraxaco-Galietum veri*), tall grass vegetation (dominated by *Calamagrostis epigejos* and *Carex arenaria*) and bushes like *Salix repens* and *Hippophae rhamnoides*.

Foraging habitat

We studied foraging behaviour of brood-rearing females in five random territories via burst sampling in 2008 (Dunn and Gipson 1977; Swihart and Slade 1997). Males appeared to often be engaged in territorial disputes instead of feeding nestlings which made effective monitoring of their foraging behaviour less effective. A fix was recorded every 60 seconds, during which period females could transverse their entire territories. A total of 200 fixes were collected per female spread over consecutive days when broods were 4–8 days old, between 17 May and 14 July. Territories were subsequently delineated by a polygon through the outermost fixes (minimum convex polygon, MCP; (Mohr 1947)) in ArcGIS 9.3 software.

To investigate where females find their food within the MCP territory we performed kernel density estimations (KDE) by using Hawth's Analysis Tools (available at http://www.spatialecology.com/htools) in ArcGIS 9.3 software. To select the best fitting smoothing parameter we inspected kernel density estimates resulting from different smoothing parameters (Kie et al. 2010). We applied the same smoothing parameter of value 7 to all five territories. We plotted our chosen utility distribution as isopleths of different values of the likelihood of encountering a foraging female, with 10% increments. As a last step core foraging areas were delineated by the smallest/shortest density isopleth containing 50% of the fixes.

By describing the vegetation characteristics in the field we determined the cover of three different classes of vegetation structure per MCP, KDE and KDE cores: 'pioneer' (open sand with mosses), 'short' (vegetation < 5 cm sward height) and 'tall' (vegetation > 5 cm sward height). Northern Wheatears are reported to mainly forage in vegetation shorter than 5 cm (Conder 1989). We stress that 'tall' mostly was much higher than 5 cm sward height, since vegetation appeared either very short due to grazing by Rabbits, or up to 30 cm when ungrazed.

Nestling diet

To identify prey items, feeding parents were filmed at the nest entrance with automated cameras. During 2008–2010 11 nests were filmed between 18 May and 10 June. Digital video cameras were mounted on tripods 30 cm from the nest entrance under a camouflaged wire-mesh roof. The camera was activated by two infra-red triggers at the entrance when birds entered the nest. Each feeding video captured 6 seconds. Nests were filmed for a maximum of 5 consecutive days, starting 6 days after hatching. Cameras filmed at least 8 hours per day, between 07:00 and 19:00. Filmed arthropods were identified to species level when possible, or grouped to higher taxonomic levels as necessary.

Nestlings were weighed and colorbanded around day 8 and all nestlings from the filmed nests fledged successfully, indicating that food abundance was not limiting in the nestling phase. After seven years of study we are yet to encounter starved nestlings, in spite of dissecting dead nestlings: stomachs are never empty and birds are not emaciated.

Prey habitat

Since Northern Wheatears are primarily ground-foragers we determined occurrence and abundance of soil and litter arthropods by taking sod-cuts. In 10 territories samples were taken in 2007, each consisting of 3 sub-samples. These 10 territories encompassed those in we determined nestling diet during 2008–2010. The samples were divided according to sward height, similar to foraging habitat: 'pioneer' (n = 30 sub-samples), 'short' (n = 30 sub-samples) and 'tall' (n = 30 sub-samples, Table 1), resulting in n = 10 samples per vegetation type. Each sample was 60 x 60 cm and 7 cm deep to encompass the total organic layer where most soil-arthropods occur. Sward height was determined by placing a dowel vertically into the vegetation of each sod cut (Bibby et al. 2000). Sod-cuts were packed individually in plastic containers to prevent arthropods from escaping and were hand-sorted within two days after collecting. Arthropods were stored in 96% EtOH and mostly identified to species-level. We also sampled adult *Phyllopertha horticola* (Coleoptera: Scarabaeidae) which occurred abundantly in late May and early June. In total we established n = 10 plots of 5 x 5 m divided in n = 5 plots per vegetation type ‘short’ and ‘tall’. Each plot was sampled by hand-picking for 10 min in suitable weather during the beetle peak at the end of May.

Statistics

To analyse habitat preferences of Northern Wheatears, we used Jacobs’ preference index (Jacobs 1974) calculated as:

$$D = (r - p) / ((r + p) - 2rp)$$

where r is the proportional use and p the proportional availability of each resource class. D ranges between +1 for maximum preference and -1 for maximum avoidance. In this way we compared vegetation composition of the KDE territory (where the actual foraging occurred) and of the foraging cores to the total available vegetation cover in the MCP territory

To explore covariation between arthropod communities inhabiting different vegetation structures, and nestling diets, we performed a Principal Components Analysis (PCA, gradient < 3) in Canoco 5, after log-transforming the proportional contribution of each arthropod type to reduce effect of extreme values. We included all taxa in the analysis for which we deemed the sampling method (sod-cutting) suitable. This meant we excluded, for instance, grasshoppers (Orthoptera) because sod-cutting is an inappropriate method for sampling grasshoppers. Arthropod types excluded for this reason which were actually fed to nestlings were also excluded from the diet in the PCA. Arthropods present in the samples and sampled in a suitable way but not fed to nestlings were included.

For other analyses, data were tested for normality and homogeneity of variances. The data appeared not to meet the assumption of normality; hence we proceeded with non – parametric tests. To determine whether sampled arthropod densities differed between vegetation types we conducted Kruskall - Wallis tests, followed by Mann - Whitney tests with Bonferroni post-hoc adjustment. To analyse whether abundance of *Phyllopertha horticola* differed between short and tall vegetation we performed a Kolmogorov - Smirnov test, given the higher accuracy at small sample size compared to Mann - Whitney tests (Field 2005).

Table 1. Size (ha) of territory determined by minimum convex polygon (MCP), kernel density estimations (KDE) and size of the KDE cores where 51 ± 2.4 % of all foraging actions occurred. Totals and subdivisions per vegetation type are presented. Values are means ± 1 se.

	MCP	KDE	KDE cores
total	2.06 ± 0.34	1.11 ± 0.09	0.18 ± 0.03
pioneer	0.12 ± 0.12	0.08 ± 0.08	0.02 ± 0.02
short	0.92 ± 0.29	0.72 ± 0.20	0.14 ± 0.14
tall	1.02 ± 0.50	0.32 ± 0.20	0.02 ± 0.02

Results

Foraging habitat

MCP territory size was on average 2.06 ± 0.34 ha (Table 1) of which Northern Wheatears use 55.9 ± 6.5%, for foraging according to the KDE analysis. Foraging appeared to be highly concentrated in certain parts of the territory: 51 ± 2.4% of all foraging actions occurred in 8.8 ± 1.7% of the MCP territory and 16.0 ± 3.0% of the KDE territory (Table 1).

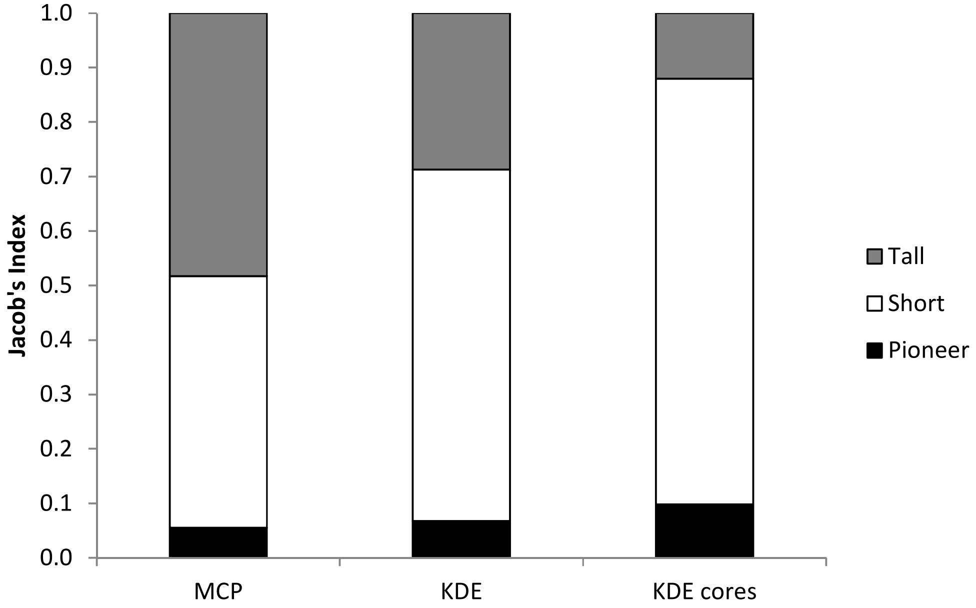

Figure 1. The proportional cover of three different vegetation types (tall, short and pioneer vegetation) differs between total territory size (MCP: Minimum Convex Polygon), the actual part used for foraging (KDE: Kernel Density Estimation) and distinct foraging cores in which 51% of all foraging events occurred (KDE cores).

Vegetation composition differed between MCP, KDE and KDE foraging cores (Fig. 1), with the amount of pioneer and especially short vegetation increasing from MCP to KDE territory and KDE core. Compared to total vegetation availability within the MCP borders, Jacob's (*D*) preference index indicates that females preferred to forage on short (+ 0.36) and pioneer (+ 0.11) vegetation but avoided tall vegetation (- 0.40). Within the foraging cores where 50% of all foraging actions took place preferences become even more pronounced: + 0.61 (short) + 0.30 (pioneer), and - 0.74 (tall).

Nestling diet

In total *n* = 6039 feedings were investigated in 11 nests, 549 ± 311 per nest. These feedings comprised 10 291 detected prey items, 936 ± 553 per nest. Prey types which comprised > 5% of the nestling diet by number were deemed important; these belonged to just four orders (excluding the unidentified arthropods, Table 2), out of 14 orders recorded on film. Important prey were Araneae, larvae of Lepidoptera (especially Noctuidae like *Cerapteryx graminis*, *Mythimna* sp., *Agrotis* sp. but also Nymphalidae, mainly *Issoria lathonia*) and Elaterid beetle larvae (especially *Melanotus punctolineatus*), imagines of the scarabid beetle *Phyllopertha horticola* and Diptera (Asilidae, mainly *Philonicus albiceps*). These four important groups comprised 73.0 ± 2.85% of the total diet fed to nestlings. Variance in diet composition between nests was considerable: caterpillars (Lepidoptera) were fed between 24.5 and 42.3% of a diet, for instance. A complete overview of the diet is provided in Appendix 1.

Table 2. Prey comprising ~ > 5% of the nestling diet, n = 11 broods. Mean and standard error are given in percentages of the total number of prey fed during the diet-study.

Order	Family	Species	mean ± se
Araneae			4.9 ± 1.0
Coleoptera			40.0 ± 5.0
	Elateridae (larvae)		13.8 ± 4.6
	Scarabaeidae	*Phyllopertha horticola*	17.2 ± 3.7
Lepidoptera (larvae)			33.4 ± 1.9
Diptera (imagos)			6.6 ± 1.7
	Asilidae	*Philonicus albiceps*	5.7 ± 1.5
Unidentified arthropods			5.9 ± 1.1

Table 3. Species richness and arthropod abundance differs per vegetation type. Values are means ± SE. Different superscripted letters denote significant differences among groups (see: Results).

	pioneer	short	tall
Number of taxa	3.6 ± 0.5^{a}	12.3 ± 0.6^{b}	21.7 ± 1.2^{c}
Arthropod abundance	6.6 ± 1.0^{a}	25.8 ± 2.1^{b}	84.2 ± 7.4^{c}

Prey habitat

We sampled on average 80.4% of all arthropod taxa fed to the filmed broods. Not sampled were flying insects as Odonata and Diptera, besides Orthoptera for which sod-cutting is not a suitable sampling method. Unidentifed prey fed to nestlings was also counted as not-sampled. The number of arthropod taxa differed significantly between types of vegetation structure (Table 3; H (2) = 67.490, $P < 0.001$) and was highest in tall and lowest in pioneer vegetation. Pioneer harboured fewer taxa than short (U = 49.000, $P < 0.001$) and short fewer than tall (U = 54.000, $P < 0.001$).

The total abundance of arthropods also differed significantly between vegetation types (Table 3; H (2) = 67.933, $P < 0.001$). Pioneer vegetation showed lowest abundance and tall the highest. Abundance in pioneer was lower than in short ($U = 49.000$, $P < 0.001$) and in short lower than in tall vegetation ($U = 54.000$, $P < 0.001$).

Vegetation structure also strongly affected abundance of preferred prey (Table 4; Araneae (H (2) = 56.963, $P < 0.001$) were more common in tall than in short ($U = 26.000$, $P = 0.013$) and occurrence in short did not differ from pioneer ($U = 71.000$, $P = 0.382$). Abundance of Elateridae larvae (H (2) = 49.874, $P < 0.001$) was greater in short than in pioneer ($U = 62.000$, $P < 0.001$) and in tall than in short ($U = 301.500$, $P = 0.011$). *Phyllopertha horticola* was found more abundantly in tall than in short vegetation ($Z = 1.581$, $P = 0.013$). Lepidoptera larvae (H (2) = 14.348, $P = 0.001$) were found more in short than in both pioneer ($U = 218.500$, $P = 0.001$) and tall ($U = 277.500$, $P = 0.006$) vegetations.

Table 4. Abundance of sampled important prey per vegetation type. Values are means ± 1 se. Different superscripted letters denote significant differences among groups (see: Results).

Order	Family	Genus	pioneer	short	tall
Areanea			$0.21^{a} \pm 0.11$	$1.13^{a} \pm 0.19$	$5.20^{b} \pm 0.60$
Coleoptera					
	Elateridae		$1.75^{a} \pm 0.48$	$4.89^{b} \pm 0.58$	$8.37^{c} \pm 1.13$
		Phyllopertha	-	$6.00^{a} \pm 1.21$	$96.00^{b} \pm 22.18$
Lepidoptera			$0.39^{a} \pm 0.17$	$4.97^{b} \pm 1.44$	$0.63^{a} \pm 0.18$

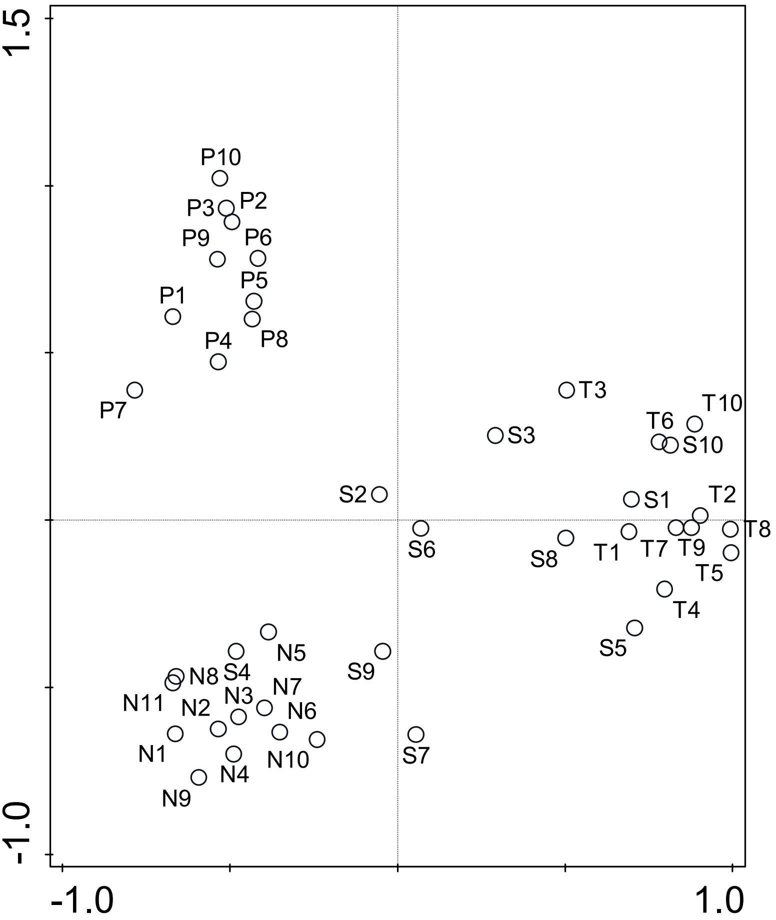

Figure 2. Principal Component Analysis (PCA) indicates separate clustering of arthropod samples from pioneer vegetation (P1-P10), samples of arthropods fed to nestlings (diet data of 11 nests, N1-N11) and arthropod samples from tall vegetation (T1-T10). Samples from short vegetation (S1-10) are widely scattered, bridging the gap between tall vegetation and the diet samples, indicating the existence of different types of short vegetation. Eigenvalue axis 1: 0.37, Eigenvalue axis 2: 0.26.

Relative composition of prey species

PCA analysis (explaining 63% of total variance with two axes: first axis 36.9% and second axis 26.1%) revealed that the species composition differed strongly between samples from the three vegetation types and the actual birds' diet (fig. 2). Samples from pioneer habitat clustered together, as did those from tall habitats. Interestingly, the faunal composition in short grass habitat (the intermediate stage vegetation-wise) did not bridge the gap between pioneer and tall grass communities. Most short grassland samples resembled those from tall grass, but some were much more alike the preferred diet samples from Northern Wheatear nests. The latter (and thus some of the short grassland samples) were relatively rich in caterpillars, weevils and larvae of elaterid beetles. Pioneer vegetation was relatively rich in darkling beetles (and relatives) and tall grasslands in millipedes, centipedes, isopods, and ground beetles.

Discussion

Our findings support the hypothesis that forager mobility and food accessibility are of greater importance during patch selection than food abundance *per se* in ground foraging birds (Atkinson et al. 2005; Fuller et al. 2003; Vandenberghe et al. 2009). The abundance of all potential prey and of three of the four most important actual prey groups was highest in tall grass patches, but Northern Wheatears taking care of nestlings foraged preferentially in short grass vegetations and even preferred pioneer vegetation, with low food abundance, over tall vegetation (cf. Brooke 1979; Conder 1989; Tye 1992). Clearly, grass encroachment by tall grass species resulting from nitrogen deposition and acidification will diminish habitat suitability for Northern Wheatears, due to lowered prey accessibility.

In habitat management, consolidating (and expanding) short grass vegetations will benefit Northern Wheatears most. However, most short grassland sites resemble the fauna community of tall grass vegetations. Based on the dominant species groups in these tall and short grass vegetations, their faunal similarity probably reflects the development of organic layers in the top-soil, and associated detritivore-driven fauna community make-up. Because conditions in the top-soil are similar and many potential prey species can occur in both vegetation types, we hypothesize that the arthropod-rich tall grass patches may serve as a source of potential food items, and thus be beneficial to the birds indirectly (Bonte et al. 2002; Butterfield et al. 1995). As such, the mosaic of tall and short grass vegetations may be a preferable habitat lay-out compared to continuous short grassland (Cole et al. 2010; Morris 2000), but this requires further research.

Interestingly, some fauna communities of short grassland vegetations closely resembled the diet of Northern Wheatear nestlings. These sites were particularly rich in caterpillars, weevil and click-beetle larvae. The diversity in faunal communities in short grasslands was large and it is not known by which factors this variation has arisen. However, we expect the short grasslands to fall into two broad categories: (1) a stage in succession following pioneer grassland and (2) well-developed grassland, which is kept short by grazing activity by Rabbits and ungulates. It remains unclear whether a closer resemblance of the fauna community of a site to the birds' actual diet will relate directly to a higher foraging efficiency and breeding success.

Pioneer vegetations were also preferred by females taking care of nestlings, but, as the density of possible food items proved low here, the significance of pioneer grassland for the foraging birds is likely less than that of short grassland. However, pioneer grasslands are a necessary predecessor to short grasslands without a thick organic top-soil.

We also emphasize that demands of arthropod prey during their complete life-cycle should be subject of attention in grassland management and restoration. Larval stages of *Anomala dubia*, for example, occur in dynamic foredunes (Van Duinen et al. 2005) where we did not observe Northern Wheatears to either forage or breed. Yet, *Anomala dubia* appeared to be a major component of the nestling diet of late broods (Van Oosten, *unpublished data*). It appeared that adult beetles fly inland towards secondary dunes, where they become important prey for Northern Wheatears. This indicates that well-intended conservation measures may be more successful if not solely aimed at restoration of breeding habitat *per se* but at a wider scale.

We show that only few prey groups are important prey for nestlings of the threatened Northern Wheatear. Other dietary studies show that these prey groups are important components of the diet in many locations across Europe (Exnerova et al. 2002; Moreno 1987; Tye 1992). Differences between studies in relative contribution may be partly site-specific or depending on phenology of the prey or sampling method.

We provide densities of those prey in a coastal area where Northern Wheatears still successfully breed. This enables site-managers to efficiently investigate presence of important prey and their abundance in areas which seem very suitable for Northern Wheatears but where they do not occur as breeding birds. Eventually we may be able to discern whether food shortage poses a bottleneck for Northern Wheatears in areas where they do not breed.

Appendix 1. Relative nestling diet of 11 nests, filmed between 18 May and 10 June. L = (mainly) larva, I = (mainly) imago. Values are mean percentage ± 1 se.

Order	Family	Genus / species	L / I	mean ± SE
Haplotaxida			-	0.05 ± 0.03
Araneae			-	4.92 ± 1.03
Diplopoda			-	0.35 ± 0.14
Isopoda			-	0.87 ± 0.31
Odonata		*Enallagma, Orthetrum cancellatum*	I	0.03 ± 0.01
Orthoptera	mainly Acrididae		-	1.61 ± 0.44
Dermaptera			I	0.01 ± 0.01
Hemiptera			-	0.49 ± 0.24
Coleoptera				40.02 ± 5.04
	Alleculidae / Tenebrionidae		L	0.98 ± 0.25
	Carabidae		L	0.2 ± 0.13
	Chrysomelidae		I	0.06 ± 0.06
	Curculionidae	mainly *Philopedon plagiatus*	I	3.32 ± 1.03
	Elateridae			13.76 ± 4.64
		Agrypnus murina	L	4.39 ± 1.59
		Melanotus punctolineatus	L	8.85 ± 3.01
	Lagriidae		I	0.06 ± 0.04
	Scarabaeidae			17.28 ± 3.75
		Phyllopertha horticola	I	17.19 ± 3.73
	Staphylinidae		I	0.85 ± 0.23
Lepidoptera				33.38 ± 1.92
	Arctiidae		I	0.21 ± 0.08
	Geometridae		L	0.7 ± 0.37
	Hesperidae	*Hesperia comma*	L	0.01 ± 0.01
	Lasiocampidae		I	0.2 ± 0.07
	Lycaenidae		I	0.01 ± 0.01
	Noctuidae	mainly *Cerapteryx*, *Agrotis*, *Mythimna*	L	21.4 ± 2.62
	Nymphalidae	mainly *Issoria lathonia*	L	5.68 ± 2.14
	Pyralidae	probably *Synaphe punctalis*	L	0.51 ± 0.28
Diptera				6.59 ± 1.72
	Asilidae	mainly *Philonicus albiceps*	I	5.66 ± 1.53
	Therevidae		I	0.18 ± 0.11
	Tipulidae		I	0.16 ± 0.11
Hymenoptera				1.1 ± 0.39
	Apidae		I	0.05 ± 0.05
	Formicidae		I	0.97 ± 0.39
	Ichneumonidae		I	0.04 ± 0.03
Neuroptera			I	0.01 ± 0.01
Gastropoda			-	2.48 ± 0.36
larva sp			L	5.95 ± 1.06
unidentified sp			-	1.18 ± 0.47

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Chapter 5

Strong genetic isolation in population remnants of a long distance migratory passerine, the Northern Wheatear (*Oenanthe oenanthe*), in the European lowlands

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Abstract

Continuous animal populations often become fragmented due to anthropogenic habitat alterations. These small, fragmented populations are fragile due to demographic and genetic factors, whereby immigration can enhance their long-term viability. Previously, we showed that high philopatry affected local dynamics of three small and remnant subpopulations of Northern Wheatears (*Oenanthe oenanthe*) in the Netherlands. Here, we show that these three populations plus an additional larger population in the European lowlands are highly genetically differentiated based on 28 microsatellite markers. In contrast, we found no evidence for differentiation using two mitochondrial DNA sequences. Our results suggest that populations have recently become isolated from each other. These findings match the demographic results: estimated and observed rates of immigration and dispersal between sub-populations were low. Our results contrast the observed low levels of genetic differentiation over most of a species' range in other long-distance migratory birds. Low dispersal rates in highly mobile birds thus occur when its habitat becomes highly fragmented, and will accentuate stochastic demographic processes and lead to inbreeding, both reducing population viability. As dispersal rates are low, local extinction is unlikely followed by recolonization.

Keywords
Northern Wheatear, *Oenanthe oenanthe,* microsatellite, mtDNA, genetic structuring, passerine, songbird, philopatry, genetic drift, Fst, heterozygosity.

Introduction

Due to a variety of anthropogenic influences, most prominently habitat destruction, populations once distributed continuously may become fragmented, persisting in isolation of each other. These populations are at risk of extinction because of demographic and genetic factors (Gilpin and Soulé 1986). Exchange between populations is important for long-term persistence of small populations (Hanski 1998; Hanski and Gilpin 1997): it buffers against stochastic population fluctuations and against loss of genetic diversity through genetic drift and inbreeding (Brown and Kodric-Brown 1977; Frankham 1996; Slatkin 1987). Connectivity of populations can be determined both demographically and genetically, each with its own merits and shortcomings (Lowe and Allendorf 2010). Demographic connectivity, defined as the degree to which population growth and vital rates are influenced by exchange between populations, is a function of the relative contribution of immigration to population growth. Genetic connectivity, defined as 'the degree to which gene flow influences evolutionary processes in populations' (Lowe and Allendorf 2010) is mainly a function of the absolute number of effective immigrants (Waples and Gaggiotti 2006), defined as breeding individuals that contribute offspring to the next generation. For conservation purposes we need information on both demographic and genetic connectivity to make inferences about the potential persistence of populations. Information on demographic connectivity is needed to assess the contribution of demographic drivers to population growth, such as current immigration or fecundity. Likewise, information on genetic connectivity is required to estimate the overall extent of gene-flow and genetic health of populations.

Previously we showed – using an Integrated Population Model – that the small relict populations of the Northern Wheatear in the Netherlands have high philopatry, and sub-population dynamics were to a varying degree determined by local population processes (chapter 2). These local relict populations comprise an estimated 50% of the breeding Northern Wheatears in the Netherlands, and intensive color ringing allowed estimation of all demographic parameters with high accuracy. The estimated and observed low levels of immigration and small population sizes may quickly lead to genetic differentiation due to drift processes.

In the Netherlands, Northern Wheatears had a wide-spread breeding range 25 years ago, with a large continuous population in the coastal dunes, and a more separate population inland (Sovon 2002; Teixeira 1979; Fig. 1). Dispersal was probably more frequent (as is often the case in more continuous populations, Wiens 1995) and demographic isolation may not have resulted in genetic structuring, although the possibility exists that the coastal and inland populations were separated to some extent. At present, limited immigration may still maintain sufficient gene flow between seemingly isolated populations of this declining species, as has been shown for migratory and philopatric passerines which show little genetic structuring in contemporary fragmented populations (golden-cheeked warbler *Dendroica chrysoparia*, Lindsay et al. 2008; cerulean warbler *Dendroica cerulea*, Veit et al. 2005). Other long-distance migratory species with large ranges (e.g., reed warbler *Acrocephalus scirpaceus*, Prochazka et al. 2011; pied flycatcher *Ficedula hypoleuca*, Lehtonen et al. 2009; barn swallow *Hirundo rustica*, Santure et al. 2010) show little genetic differentiation over most of their breeding ranges, suggesting appreciable dispersal in these highly mobile species.

The aim of this paper is to describe the level of genetic differentiation between four discrete remnant populations of Northern Wheatears. We use microsatellite and mtDNA marker data to test three alternative hypotheses about genetic variation. (1) Geographically isolated Northern Wheatear populations show no genetic structure, because there is sufficient gene flow or because the separation is too recent. Genetic variation is similar across the populations. (2) Recent isolation and genetic drift have already resulted in a population genetic structure. In this case, we predict that populations differ with respect to their microsatellites but not (yet) mtDNA. Due to lower mutation rates, mtDNA takes more time to build up genetic variation, and generally shows less variation in populations than microsatellites. Therefore differences in mtDNA between populations indicate deeper time effects. Due to drift processes genetic variation may be lower in the smaller populations compared to a larger German population. (3) Past selection on birds breeding in different habitats (see 'Study species and study sites') has led to more ancient isolation and the formation of genetically widely differing sub-populations. In this case we expect strong population structure, both in microsatellites and in mtDNA, with a likely separation between the coastal and inland populations.

To differentiate between these hypotheses, we analyzed 28 nuclear microsatellite markers found in literature (see Appendix 1 for loci and references) and two mtDNA markers (Aliabadian et al. 2007). The latter were used to distinguish between (sub) species within the genus *Oenanthe* (Aliabadian et al. 2007), and should therefore be suitable for investigating potential differentiation between coastal and inland populations.

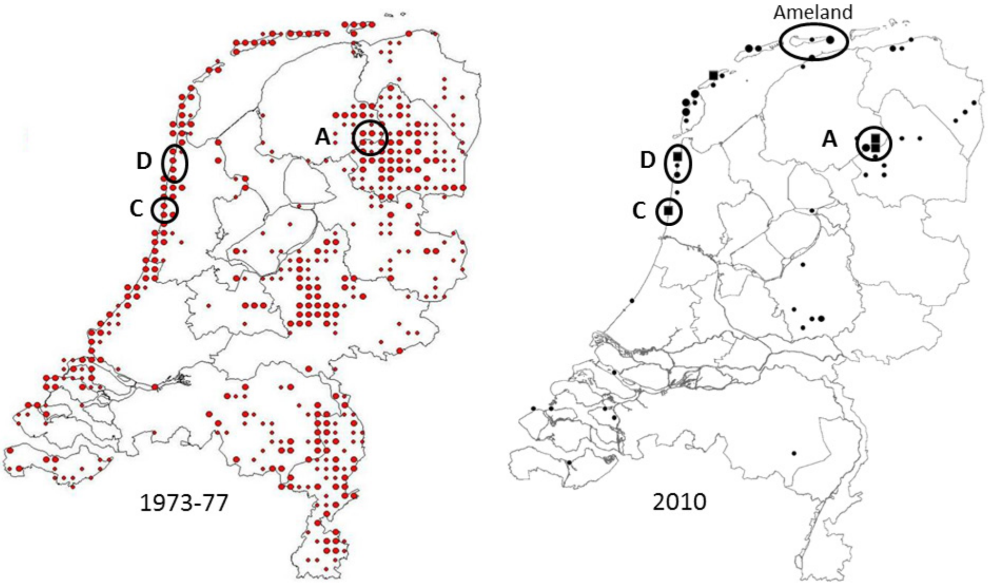

Figure 1. Breeding distribution of Northern Wheatears in the Netherlands, in 1973–1977 (left; Teixeira 1979) and 2010 (right; Boele et al. 2013). The three research sites are denoted with capital letters (see text). Additional DNA was sampled for mtDNA analysis on the island of Ameland.

Methods

Study species and study sites

Northern Wheatears are insectivorous long-distance migrants which breed from eastern Canada across Eurasia to western Alaska (Glutz von Blotzheim and Bauer 1988). The species strongly declines in Europe, where the breeding population decreased by 60% between 1980 – 2011 (PECBMS 2013). In the Netherlands, Northern Wheatears were once widely distributed, but declined by 87 % between 1990-2010 (Boele et al. 2013; Fig. 1). In the European lowlands, they occur in sandy, oligotrophic grasslands in coastal dunes and heathlands where they often breed in burrows of Rabbits (*Oryctolagus cuniculus*) or in holes of remaining trunks of felled trees. Here they forage mostly on the ground, hunting for beetles, beetle larvae and caterpillars (chapter 4). Large expanses of breeding habitat were lost due to eutrophication and acidification, which stimulated growth of tall grasses (Bobbink et al. 2010). This rendered suitable short grown grasslands into inaccessible stands of tall grasses, as *Calamagrostis epigejos*, *Carex arenaria* and *Ammophila arenaria*.

We collected DNA of Northern Wheatear nestlings at four sites: three in the Netherlands (Castricum (C), Den Helder (D), Aekingerzand (A)) and one in Germany (Rhineland-Palatinate, (R)) (Fig. 2) between 2006 and 2013. The coastal population Castricum (site C, 7−24 breeding pairs 2007−2013, also present more than 200 years ago, Nozeman 1789) is separated by 35 km from the coastal population Den Helder (site D, 47−74 breeding pairs 2007−2013). The inland population at Aekingerzand (site A, 11−47 breeding pairs 2007−2013), is 110 respectively 125 km away from both others. Individuals from populations C and D breed in coastal dunes within 1 km from the sea, whereas individuals from population A breed in heathland with drift sands. The German population near Bad Dürkheim, Rhineland-Palatinate (site R, 100 breeding pairs) consists of individuals that breed mostly in vineyards (Buchmann 2001) and is approximately 400 km away from the three Dutch populations. This population is one of eight spatially separated populations being studied intensively since 1995 in a total area of 72 km^2 (Buchmann 2001). Northern Wheatears are threatened with extinction in Germany (Südbeck et al. 2008).

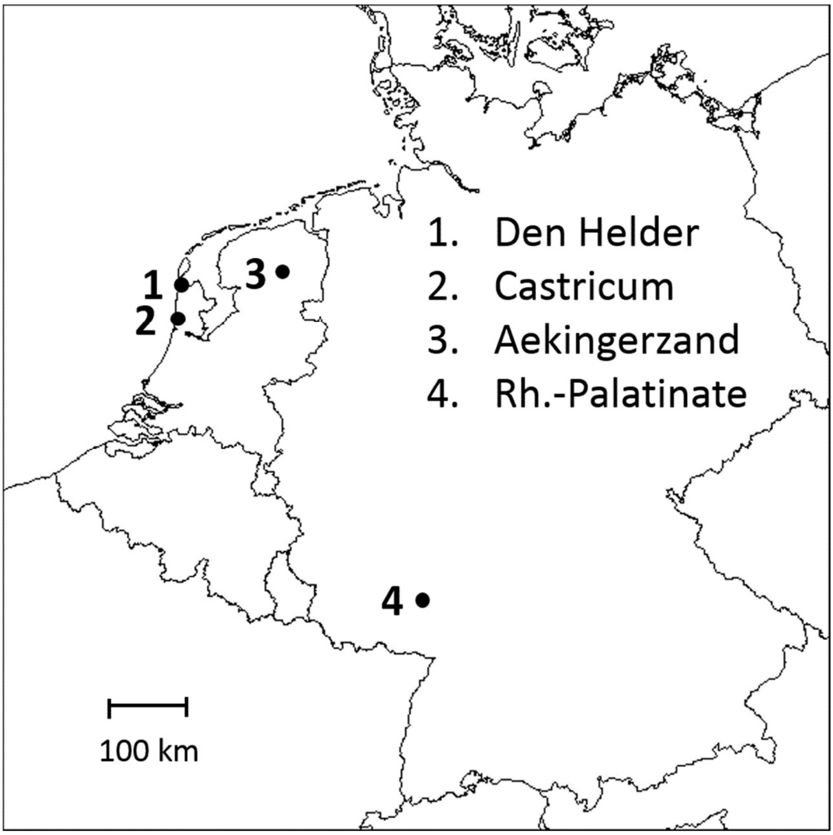

Figure 2. Location of the four sites in northwestern Europe.

DNA extraction, genotyping and sequencing

All Dutch DNA samples stem from growing feathers of nestlings collected when color-banding the birds. The German DNA samples stem from blood obtained by brachial venipuncture of nestlings at the time of banding. DNA from feathers was extracted by using the QuickExtract DNA Extraction Solution (Epicentre), with the following particulars: 90 µl QE solution, 2 cut down growing feathers, 1 hr incubation at 65° C, vortexing every 30 min, heat denaturation 3 min at 95° C and down-spinning twice after denaturation. DNA from blood was extracted using the QIAamp blood extraction kit (Qiagen), following the protocol provided by the manufacturer.

Microsatellites In total we used DNA of 194 putative unrelated birds of four sites (C: 72, A: 58, D: 13, R: 51; one random nestling per nest). Individuals were genotyped with 28 microsatellite markers and sexed with the P2/P8 primer set (Griffiths et al. 1998; Appendix 1). Microsatellite amplifications and sexing were performed in multiplexed PCRs using the Qiagen Type-it Microsatellite PCR Kit (Qiagen, Hilden, Germany) and primer mixes containing four to seven primer pairs (M1 – M6, Appendix 1). Forward primers were labeled at their 5’ end with fluorescent dyes from Life Technologies GmbH

(Darmstadt, Germany). Each 10 µl multiplex PCR contained 1 µl DNA, 5 µl of the 2x Qiagen Type-it PCR Master Mix and 1 µl of one of the six primer mixes (Appendix 1). Cycling conditions were: 5 min initial denaturation at 95˚C, followed by 31 cycles of 30 sec 94°C, 30 sec at the annealing temperature given in Appendix 1, 1 min 72°C; followed by 30 min completing final extension at 60˚C. 1.5 µl of the PCR product was mixed with formamide containing the GeneScan 500 LIZ Size Standard, heat denatured and resolved in POP4 polymer on an ABI 3130xl Genetic Analyzer (all Life Technologies GmbH, Darmstadt, Germany). Raw data were analyzed with GeneMapper 4.0.

Mitochondrial DNA We collected DNA from nine individuals from sites C and D, with additional samples from the island of Ameland (Fig. 1, collectively denoted as 'coastal'), and a further 10 individuals from A and 10 from R, which were all chosen randomly.

We sequenced 695 base pairs (bp) of the mitochondrial cytochrome oxidase subunit 1 (COX1) gene, using the primers BirdF1 TTCTCCAACCACAAAGACATTGGCAC and BirdR1 ACGTGGGAGATAATTCCAAATCCTG (Hebert et al. 2004). Additionally, we sequenced 569 base pairs (bp) of the mitochondrial 16sRNA using the primers 16SA-L CGCCTGTTTATCAAAAACAT and 16SB-H CCGGTCTGAACTCAGATCACGT (Aliabadian et al. 2007). The PCR amplifications were performed in 50 µl reaction volumes consisting of 1x PCR buffer with (NH4)2SO4, 2 mM MgCl2, 200 µM dNTP, 1.25 U Taq DNA Polymerase (Fisher Scientific Germany GmbH, Schwerte, Germany), 1 µl genomic DNA, 0.5 µM of each forward and reverse primer. Cycling conditions were: 5 min 95°C, followed by 35 cycles of 30 sec 95°C, 30 sec 55°C for COX1 or 50°C for 16sRNA, 1 min 72°C; and a final extension at 72°C for 1 min. 10 µl of the PCR product were visualized on an agarose gel. Two times 20 µl were treated 15 min at 37°C with 5 U Exonuclease I and 10 U FastAP Thermosensitive Alkaline Phosphatase (both Fisher Scientific Germany GmbH, Schwerte, Germany), followed by 15 min heat denaturation of the enzymes at 85°C. Subsequently, the PCR products were premixed with 20 pmol primer and send to an external contractor for sequencing (Eurofins MWG GmbH, Ebersberg, Germany).

Data analysis

Within and between population genetic variation was determined by calculating mean number of alleles per locus, observed and expected heterozygosity, F_{ST} and F_{IS} values using microsatellite genotype data for 28 loci in Genepop 3.4 (Raymond and Rousset 1995). Allelic richness, the number of alleles per site corrected for different sample-sizes among sites, was calculated using FSTAT 2.9.3.2 (Goudet 2002). Smallest sample size was 13 individuals (site D). Significance of pairwise F_{ST} values was calculated in GenAlEx 6.5 (Peakall and Smouse 2006). To assess analytical problems due to possible null alleles we investigated whether deviations from the Hardy-Weinberg equilibrium occurred, and we tested for linkage disequilibrium among all marker pairs in Genepop 3.4 (Raymond and Rousset 1995). We conducted an analysis of variance using SPSS 21.0 (IBM Corp. 2012) to investigate whether differences in allelic richness or observed heterozygosity occur among sites.

Using Structure ver. 2.3.4 (Pritchard et al. 2000) we estimated the most likely number of genetic clusters (K) present in our sample and the cluster affiliations of each individual. Structure was run with K ranging from one to seven and five runs per K. Simulations were run with a burn-in period of 500 followed by an additional 500 steps. Individuals can have mixed ancestry in the analyses (admixture model) and allele frequencies are allowed to correlate among populations.

The number of haplotypes of the COX1 and 16SrRNA regions of the mitochondrial DNA was determined per site. Per individual bird the COX1 and 16SrRNA haplotypes were combined to an overall haplotype based on unique COX1 + 16SrRNA allele combinations, to test for population differences by using a chi-square test.

Results

Genetic diversity

Mitochondrial DNA Within the 695 base pair of the mitochondrial COX1 region sequences we found nine polymorphic sites (Table 2A) and four haplotypes. Two of the four haplotypes (H3 and H5) were described by Aliabadian et al. (2007), and occurred in 25 out of 27 birds. One coastal bird and one bird from R each had a different haplotype (Table 2A). The coastal bird differed from haplotype H3 by four transitions and from H5 by two transitions, and was assigned haplotype H6. The Rhineland-Palatinate bird differed in seven transitions from haplotype H3 and in one transition from haplotype H5 and was assigned haplotype H7 (Table 2A). One major haplotype (H3 of 16sRNA) was missing in A. Within the 569 base pairs of the mitochondrial 16SrRNA region sequences two polymorphic sites were found and two haplotypes (Table 2B). Both haplotypes were previously described by Aliabadian et al. (2007).

Table 1. Summary of genetic diversity for the four sites, based on 28 microsatellite loci. Parameters to measure genetic diversity per site are average number of alleles per locus (*A*), allelic richness (A_R), expected heterozygosity (H_E), observed heterozygosity (H_O), and estimations of the inbreeding parameter F_{IS} with *P*-values for heterozygote deficiency in parentheses.

Site	Sample size	*A*	A_R	H_E	H_O	F_{IS} (*P*)
C	72	6.46	4.99	0.65	0.64	0.003 (0.04)
D	13	5.89	5.89	0.67	0.67	0.011 (0.39)
A	58	5.43	4.66	0.63	0.62	0.010 (0.07)
R	51	8.79	6.44	0.71	0.70	0.019 (0.04)

Table 2. Summary of mtDNA genotyping of four Wheatear populations. (A) Haplotypes found within the 695 base pairs of the COX1 mtDNA region, and their frequency per site. Sequence positions are given with respect to Northern Wheatear sequences from Aliabadian et al. (2007). New haplotypes H6 and H7 were not found in that study. (B) Haplotypes found in the 569 base pairs of the mitochondrial 16SrRNA region and their frequency per site. Sequence positions are given with respect to Northern Wheatear sequences from Aliabadian et al. (2007). "Coastal" refers to populations C, D and Ameland.

Haplotype ID	**Position in alignment**									**Site**			
(A) COX1	255	294	336	366	456	483	519	618	672	A	coastal	R	Total
H3	A	T	G	C	C	T	T	C	A	1	2	3	6
H5	G	T	A	G	T	T	C	C	G	7	6	6	19
H6	A	T	G	G	T	T	C	C	G	0	1	0	1
H7	G	C	A	G	T	T	C	C	G	0	0	1	1
Sample size										8	9	10	27

Haplotype ID	**Position in alignment**		**Site**			
(B) 16SrRNA	306	370	A	Coastal	R	Total
H2	A	G	9	7	7	23
H3	G	G	0	3	3	6
Sample size			9	10	10	29

Population differentiation

F_{ST} values were relatively high (mean F_{ST} = 0.08), and all pairwise comparisons were highly significant (Table 3). The number of pairwise significant loci ranged between 21–28 out of 28 loci. The Structure analysis using the 28 non-linked microsatellite loci revealed strong clustering of the populations, in line with the high F_{ST} values: the model with K = 4 genetic clusters had the highest likelihood and the clusters show high congruence with the 4 populations C, A, D and R (Fig. 3). Admixed or immigrant individuals appear to be rare, except in site C in which 15 out of the 72 individuals had more than ~50% likelihood to be assigned to cluster D (site D; figs. 1 and 3).

MtDNA haplotypes based on unique COX1 plus 16SrRNA combinations per individual did not differ between populations 'coastal', A and R (χ^2 = 8.84, df = 8, P = 0.36) and therefore, no deep segregation is apparent between the populations.

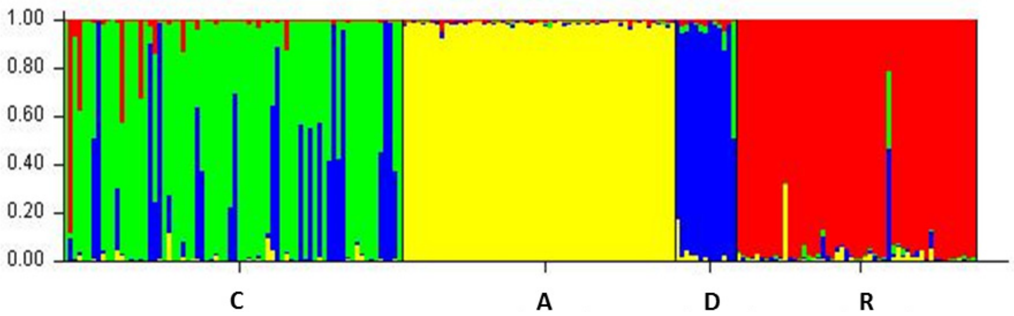

Figure 3. Assignment of individuals to each of K = 4 genetic clusters, without prior population information. Each bar represents the estimated membership coefficient for each individual bird in each cluster. Birds are placed *a posteriori* into their sampling populations by vertical lines.

Discussion

Northern Wheatears declined strongly in European lowlands and now often occur at small and seemingly isolated sites. Demographic analyses showed low dispersal rates between sub-populations (chapter 2), and here we show that this once wide-spread population at present consists of genetically differentiated sub-populations that are likely of recent origin.

Population structure

The presented estimates of genetic structure (F_{ST} values) based on microsatellite markers in our sub-populations of Northern Wheatears are high compared to those reported for other migratory songbirds, especially given the small geographic distances between our populations (mean F_{ST} = 0.08, range 0.05-0.11). For common and widespread species F_{ST} values are typically low and no structuring is reported (e.g. pied flycatchers: mean F_{ST} = 0.01, range 0 – 0.052, *n* = 16 sites covering most of their breeding range, Lehtonen et al. 2009; reed warblers: mean F_{ST} = 0.013, range 0 – 0.064, *n* = 31 sites, Prochazka et al. 2011). Interestingly, this is also true for some strongly declining passerines in rather isolated populations (e.g. black-capped vireo *Vireo atricapilla*: mean F_{ST} = 0.021, Barr et al. 2008; golden-cheeked warbler : mean F_{ST} = 0.008, range 0 – 0.026, *n* = 7 sites, Lindsay et al. 2008 and cerulean warbler: mean F_{ST} = 0.01, range 0 – 0.051, *n* = 14 sites, Veit et al. 2005). The difference between these studies and our study may be due to the very small sub-population sizes in the Northern Wheatears we studied, and to their potentially higher philopatry. In fact, the F_{ST} values we found for the Northern Wheatear are even higher than those typically reported for different subspecies (e.g. seven subspecies of the bluethroat *Lusciania svecica*: mean F_{ST} = 0.042, range 0- 0.17, *n* = 7 subspecies samples at *n* = 11 sites, Johnsen et al. 2006; three subspecies of the reed bunting *Emberiza schoeniclus*: mean F_{ST} = 0.043, range 0 – 0.011, *n* = 3 subspecies at *n* = 13 sites, Kvist et al. 2011).

Consistent with the relatively high and significant F_{ST} values indicating population differentiation, individual Northern Wheatears were genetically assigned to four clusters which closely matched the four populations (Figure 4). Therefore these populations seem to be close to genetic isolation, except for apparent immigration from D birds into the C population (but not vice versa). This pattern of dispersal mirrors the demographic field study well: during 2007-2011 we observed immigration of five birds (four males, one female, with an additional male in 2013) which were color-banded in population D into population C, and birds born in population C were never observed in population D, despite intensive field work and equally high resighting probabilities ($p = 0.97$ for adults and 0.95 for returning first-year birds; chapter 2). Notwithstanding limited dispersal, populations C and D did differ genetically (Table 3); apparently, the number of yearly immigrants was too small to maintain genetic panmixia, or immigrants did not contribute to the genepool because of low reproductive success.

The four studied populations did not differ with respect to their mtDNA sequences. This suggests that population segregation is a recent phenomenon. We postulate that the genetic structuring has been developing due to high philopatry and genetic drift during the last 25 to 35 years, when Dutch breeding numbers declined. Genetic variation was similar among the four sites, but allelic richness was lower in A than in the large German population. Together, these data give strong support for the hypothesis that recent isolation and genetic drift has resulted in a population genetic structure, as shown in the microsatellite data.

The combination of high philopatry and genetic drift in small populations may have resulted in the observed structuring, but why is philopatry so high? Philopatry may be a recent phenomenon, resulting from population fragmentation. Indeed, philopatry typically increases as a response to decreasing population sizes and isolation (Heino and Hanski 2001; Weatherhead and Forbes 1994). Currently, suitable breeding sites for Northern Wheatears are probably rare, and often >10 km from the natal site, so that they may not easily be discovered by individuals prospecting for breeding sites. Alternatively, strong natal and breeding philopatry may be an inherent trait of Northern Wheatears since similar apparent survival rates (observed return rate corrected by resighting probability, White and Burnham 1999) are reported in other populations as well. First-year survival in our three populations was 0.18–0.45 (average 0.31) and 0.27–0.81 (average 0.54) for adults during 2007–2011 (chapter 2), 0.17–0.32 respectively 0.41–0.62 (Uppsala, Sweden during 1996–2006; 80–120 breeding pairs which are part of a larger population, Arlt et al. 2008) and 0.18–0.28 respectively 0.44–0.52 (Fair Isle, Great Britain during 2008–2010; 100 breeding pairs but this is an island population, Seward et al. 2013). Out of more than 2500 resighted Northern Wheatears since 1995 in eight populations of the Rhineland-Palatinate (in total 300 breeding pairs, Buchmann 2001), only 1.6% ($n = 40$ individuals) was observed in a different population than its natal or breeding site (M. Buchmann, *pers. comm.*). This is indicative of strong philopatry in the Rhineland-Palatinate populations as well.

To distinguish between both hypotheses (that strong philopatry is a recent phenomenon or that it is inherent to the species) it would be instructive to conduct a similar study in a large, continuous population, which may perhaps be found in the Scandinavian uplands.

Genetic diversity

Interestingly, we did not observe strong differences in heterozygosity between populations, in spite of varying population sizes in combination with reduced gene flow (as shown by the strong population structuring). Nevertheless, population C and R showed a heterozygote deficit (fewer heterozygotes than expected under Hardy-Weinberg equilibrium). We found that allelic richness was lower in site A than in the large German population, suggesting recent loss of alleles in population A.

Despite small population sizes, overall observed heterozygosity was still in the range of heterozygosities reported in other studies on songbirds (e.g. Brewer's sparrow *Spizella breweri*: mean H = 0.77, Croteau et al. 2007; pied flycatcher: H = 0.76–0.82, Lehtonen et al. 2009; golden-cheeked warbler: mean H = 0.75, Lindsay et al. 2008 and reed warbler: H = 0.55–0.74, Prochazka et al. 2011).

Conservation implications

Inbreeding and genetic drift eventually deteriorate the viability of small populations (Soulé and Mills 1998; Spielman et al. 2004; Westemeier et al. 1998). It is therefore noteworthy that we found heterozygote deficiency in sites C and R, which may be due to inbreeding, and a lower allelic richness in A compared to the largest population R. Contemporary little immigration into the breeding population may deteriorate allelic richness and therefore, the potential for long-term evolutionary adaptation (Lande and Barrowclough 1987).

Another, worrying outcome of our study is that it suggests a rarity of natural recolonization events in otherwise suitable sites or restored ecosystems due to high natal and breeding philopatry. Occasional dispersal was only observed along the coast: we observed two different males in two years, banded as nestlings in C, which established territories in a dune area where Northern Wheatears were extinct (25 km distance from C). Both males remained unpaired. Furthermore, we observed a male, defending a territory on the island of Ameland, 130 km from C where it was born. In spite of these examples of occasional dispersal into abandoned areas (all by males), odds are small that pairs settle and breed in these areas, given (contemporary?) high philopatry.

Low dispersal and immigration may be typical for Northern Wheatears, but also for other declining species in isolated populations (Hansson et al. 2002; Heino and Hanski 2001; Weatherhead and Forbes 1994). Philopatry may increasingly have been selected for since birds not easily find suitable breeding sites due to the scattered occurrence of the breeding habitat. As dispersal has been shown to be heritable in other passerines (Doligez et al.

2009; Duckworth and Kruuk 2009), and can be genetically associated with other flexible behaviors (Dingemanse et al. 2004; Duckworth and Kruuk 2009), selection for genetic philopatry may hamper the possible recovery even if new suitable habitat patches become available. We hypothesize that habitat fragmentation can thus lead to a self-enforcing process, with dispersers becoming increasingly unsuccessful, selecting for philopatric individuals that are less able to adapt flexibly to new situations, leading to isolated populations that cannot easily recolonize new suitable patches and finally reach sizes where stochastic demographic and genetic processes result in local extinction. Therefore, conservation of just a few large populations may not be sufficient.

Practical conservation efforts should be directed to establishing suitable breeding habitat between populations to connect larger populations. For the Dutch coastal dunes suitable habitat characteristics and food densities are available for C which may serve as a baseline for establishing suitable habitat (chapter 4).

Acknowledgements

We are grateful to Remco Versluijs, Stef Waasdorp and Frank Majoor for collecting the Dutch samples, to Martin Buchmann and Wolfgang Fiedler for kindly providing the samples from the Rhineland-Palatinate population, to Sylvia Kuhn for performing the molecular work, and to Arnold van den Burg and Henk Siepel for commenting on the manuscript. We also thank PWN, Landschap Noord-Holland, and Staatsbosbeheer for allowing access to their areas and PWN for allowing access to their field station.

Appendix 1. List of 28 microsatellite loci and a sexing primer used for genotyping *Oenanthe oenanthe*. Primer sequences include information on fluorescence labels used. C, primer concentration in multiplex primer mix; T_a, annealing temperature; N_A, number of alleles in 534 individuals; H_o, observed heterozygosity; H_e, expected heterozygosity. In markers which show an X at Null, offspring-parent mismatches can be explained by the presence of null alleles. † not all alleles of this marker can be separated

Locus	Accession no.	Reference	Primer sequences (O'Connor et al.)	*C* (μM)	Multiplex Mix	*Ta*	Size range (bp)	N_A	*Null*	*Ho*	*He*
ZF_CK303492	CK303492	Jakob Mueller, pers. comm.	F: NED-GCCACYTTCTACCCTSTAGACAAC R: TGTGATGAAGGCRTASGCTG	0.44 0.44	M5	50˚C	141 - 150	4		0.38	0.37
ZF_AC138573	AC148573	Jakob Mueller, pers. comm.	F: PET-ATGYCAACTGAAATGTCAAGGT R: ATGAGGTCACTGAAAGKTWTAATA	0.3 0.3	M6	48˚C	139 - 151	6		0.71	0.67
2F9	/	supplementary information in Heber et al. 2013	F: PET-GCATTTCTGGGCTGTAACAT AAAGGACAATGTAATTGGTG	0.34 0.34	M6	48˚C	72 - 82	4		0.41	0.41
AACC-117	AY703938	Bardeleben et al. 2005	F: VIC-GTAGATGGGGATGGGACAGA R: CAATGACAAATCAACCACCCAAAC	0.11 0.11	M1	56˚C	127 - 155	7	X	0.62	0.68
Calex-05	AM072453	Julia Delingat, pers. comm. Küpper et al. 2007	F: PET-TCCAGCTGAAGTCTTCCGTAAT R: gtttcttTCCACACCTGTTCGCAGTTCAATA	0.54 0.54	M1	56˚C	169 - 184	13		0.73	0.79
Calex-14	AM072462	Küpper et al. 2007	F: PET-GGAGACATTTTCCTACTAAGCA R: AATAGAGTATGTCACAGAGCC	0.46 0.46	M3	52˚C	198 - 208	4		0.41	0.41
CcaTgu14	DV960138.1	Olano-Marin et al. 2010	F: VIC-GTTGTTCYAATTCCAATGC R: CTAAAAATAGCAGTAAAAATACAYAAA	1.06 1.06	M6	48˚C	113 - 131	5		0.41	0.41
CcaTgu19	DV579042.1	Olano-Marin et al. 2010	F: PET-CTGGACCATGACTGCAAGATT R: CAGTGGCAAAKCAGCACCT	0.26 0.26	M2	54˚C	233 - 254	8		0.81	0.75
CcaTgu24	CK316202.1	Olano-Marin et al. 2010	F: VIC-ATCTTGAGTYATGACCTTAAAARTCT R: ATAGCTACAGAAACCTACTTGGGA	0.3 0.3	M5	50˚C	174 - 183	10		0.83	0.81
CcaTgu25	CK305580.1	Olano-Marin et al. 2010	F: PET-CCAGGTYRGCATCAGTACAC R: TAAACCACRCGTGGGCAC	0.8 0.8	M2	54˚C	135 - 151	12		0.83	0.80
CK306783	CK306783.1	supplementary table in Olano-Marin et al. 2010	F: PET-TGGAATCCTTACCAGCAGTT R: TGAAACTCAAGMYTGAACCA	0.36 0.36	M6	48˚C	220 - 232	12		0.88	0.83
ClkpolyQ	GQ847510	Johnsen et al. 2007	F: 6FAM-TTTTCTCAAGGTCAGCAGCTTGT R: CTGTAGGAACTGTTGYGGKTGCTG	0.22 0.22	M2	54˚C	269 - 281	4		0.62	0.62
Gf16	AF081940	Petren 1998	F: 6FAM-CCCTTCAGGGCATGAGTGAGG R: ATGTCATGAACTCAACCAACTCC	0.24 0.24	M1	56˚C	108 - 138	11		0.70	0.71
Mcymu4	U82388	Double et al. 1997	F: PET-ATAAGATGACTAAGGTCTCTGGTG TAGCAATTGTCTATCATGGTTTG	0.5 0.5	M4	52˚C	126 - 158	9		0.68	0.71
oe5	EU573953	Kudernatsch et al. 2009	F: NED-CATTTCTTCTAAAGGTGCTTTGC R: GCTATAGAGCCTTTGAGGTTGAG	0.22 0.22	M2	54˚C	133 - 185	15		0.88	0.87
oe6	EU573955	Kudernatsch et al. 2009	F: NED-TCTTCTCACTTCTTTGTATTTTAGGT R: CCTTCCTAGGATTCCTCCAA	0.42 0.42	M3	52˚C	183 - 207	9	X	0.64	0.72
oe7	EU573954	Kudernatsch et al. 2009	F: VIC-GGAAGGGGCCCATAAGTATC	1.34	M1	56˚C	185 - 197	4	X	0.57	0.59

oe8	EU573956	Kudernatsch et al. 2009	F: PET-TCCAAAGGTTAGTGTTAGTGATTAAGG R: GGCCAGCCTTACTTGACTGG	0.24 0.24	M3	52°C	108 - 120	4		0.60	0.53
oe10	EU573958	Kudernatsch et al. 2009	F: VIC-TCAGCATTCTTGTTGCCTTC R: GCTGAACAAGTGAAAGGATGTG	0.16 0.16	M6	48°C	177 - 203	10		0.68	0.71
PAT MP 2-14†	FM201467	Simeoni et al. 2009	F: 6FAM-GAACAGATAAAGCCAAATTAC R: TAGTGAATGCTTGATTTCTTTG	2.2 2.2	M6	48°C	104 - 136	15	X	0.88	0.85
PAT MP 2-43	AM056063.1	Otter et al. 1998	F: 6FAM-ACAGGTAGTCAGAAATGGAAAG R: GTATCCAGAGTCTTTGCTGATG	0.22 0.22	M2	54°C	121 - 135	8		0.78	0.78
PiJ14-23-CEST	AB204825.1	Olano-Marin et al. 2010	F: VIC-ATCTGGCATKGAAAACTTGG R: CTCCCTGCACCCCAAAC	0.14 0.14	M2	54°C	144 - 162	4		0.56	0.57
PmaC25†	AY260526	Saladin et al. 2003	F: PET-CGTCCTGCTGTTTGTATTTCTG R: CCATGAACCATTTTTAGGGTG	0.3 0.3	M3	52°C	306 - 325	8		0.75	0.67
TG02-088	DV579347	Dawson et al. 2010	F: 6FAM-TGTGTGTTGACAGTATTCTCTTGC R: TTTAAACCTAATAAACGTCACACAGTC	0.26 0.26	M1	56°C	262 - 268	4		0.64	0.67
Tgu05	DV946651.1	Slate et al. 2007	F: NED-CACAGAAAAGTGAGTGCATTCC R: TGGGAAAACATCTTTACCATCA	0.6 0.6	M3	52°C	262-292	13	X	0.69	0.79
Tgu06	CK307697	Slate et al. 2007	F: VIC-CGAGTAGCGTATTTGTAGCGA R: AGGAGCGGTGATTGTTCAGT	0.16 0.16	M4	52°C	172 - 184	7		0.48	0.49
Tgu07	DV948303	Slate et al. 2007	F: 6FAM-CTTCCTGCTATAAGGCACAGG R: AGTGATCACATTTATTTGAATAT	0.24 0.24	M5	50°C	89 - 97	5		0.55	0.58
Titgata68	AY792960.1	Wang et al. 2005	F: PET-ACAGATCAGCATGGTTGCAG R: CATCCACAAGGGCAATCTTT	0.5 0.5	M5	50°C	231 - 325	18	X	0.79	0.80
P2P8*	AF006660	Griffiths et al. 1998	P2: 6FAM-TCTGCATCGCTAAATCCTTT P8: CTCCCAAGGATGAGRAAYTG	0.54 0.54	M5	50°C	345 and 382	/		0.62	0.62

Chapter 6

Dioxin accumulation in terrestrial food chains affects a songbird at low soil pollution

H.H. van Oosten, A.B. van den Burg, D. Arlt, C. Both, N.W. van den Brink, T. Jeppsson, H. de Kroon, W. Traag, H. Siepel

Abstract

Persistent toxics are a threat to global biodiversity, and persistent organic pollutants (POPs) are known to accumulate in the food chain and to have lethal effects on top predators (Gobas et al. 2007; Simonich and Hites 1995), while passerine birds are assumed to be much less affected (Custer et al. 2003; Neigh et al. 2007; Ormerod et al. 2000; Van den Steen et al. 2010). Here we show that in nature reserves with negligible background levels of soil pollution an insectivorous passerine suffers from POPs. We found high concentrations of dioxins in eggs, which were associated with low reproduction due to embryonic malformations. Dioxins reach the birds via a major prey, soil-dwelling insects that bio-accumulate dioxins from the soil. In comparison to birds from a reference population with low dioxin concentrations in eggs, the dioxin effects have the potential to strongly reduce population growth and likely contribute to observed population declines. Our results provide evidence that POPs can bio-accumulate to toxic levels in passerines even when POP concentrations in the soil are well below levels that are considered toxic, levels that are similar to many areas worldwide. We suggest that dioxin accumulation is a widespread, yet generally neglected, factor contributing to the widely occurring declines of insectivorous passerine populations in terrestrial ecosystems.

Keywords
POPs, dioxins, PCB, dead eggs, hatchability, hatching rate, passerine, songbird, malformations,crossed bills, microphthalmia, curled toes.

Introduction

Over fifty years ago, Rachel Carson's *Silent Spring* illuminated the use of persistent toxics like DDT as threat to global biodiversity (Carson 1962). Since then some pesticides have been banned, but ecosystems throughout the world remain being contaminated with other toxic persistent organic pollutants (POPs) like dioxins and PCBs (Simonich and Hites 1995). POPs are by-products of various industrial processes, and are also contained in pesticides. These persistent lipophilic pollutants bio-accumulate resulting in elevated pollutant levels in top predators (Gobas et al. 2007; Simonich and Hites 1995), potentially adversely affecting health, especially in riverine, estuarine and marine environments (Fry 1995; Rhind 2009). Morphological aberrations of avian embryos have been related to elevated POP levels in raptors and other top predators (Fry 1995; Gilbertson et al. 1991). However, only few studies report POP-related pathological findings in insectivores such as passerines (Rhind 2009) and adverse effects of POPs have only been reported in passerines from aquatic ecosystems or industrially contaminated sites (Custer et al. 2005; DeWitt et al. 2006; Markman et al. 2011). The general assumption has become that passerines are less affected by POPs than top predators, either due to lower exposure (Neigh et al. 2007; Ormerod et al. 2000) or to apparent lower sensitivity (Custer et al. 2003; Van den Steen et al. 2010). Here we falsify this conclusion by showing that an insectivorous passerine suffers low reproductive success as a result of bioaccumulation of dioxins at low background soil concentrations of dioxins in nature reserves, with this reduced reproduction having the potential to detrimentally reduce population growth.

We studied the breeding ecology and population dynamics of remnant populations of Northern wheatears (*Oenanthe oenanthe*) in the Netherlands. Population numbers have plummeted from 1900-2500 breeding pairs in 1980 to less than 300 in 2012 (Boele et al. 2014). The remaining small breeding populations occur in sandy grasslands (Hustings and Vergeer 2002). Our two study sites are nature reserves located in the coastal dunes (Vogelduin) and 110 km inland in a heathland-dune area (Aekingerzand). Refer to Supplementary Information for detailed description of study area and species.

Detailed monitoring over 6 years revealed consistently high hatching failure of eggs in the remnant populations. On average 20% (range 11–27% per year) of eggs failed to hatch in Vogelduin, and 14% (range 9–18%) in Aekingerzand during 2008–2013 (n = 230; Table S1). These are significantly higher values compared to a reference population breeding near Uppsala, Sweden, where 6% (range 4–9% per year, 2008–2013, n = 54; Table S1) of eggs failed (generalized linear mixed model (GLMM) with year as random factor: Vogelduin versus Uppsala $Z = 6.35$, $P < 0.001$; Aekingerzand versus Uppsala $Z = 4.23$, $P < 0.001$; n = 355).

Visual inspection of dead embryos from the Dutch populations showed unexpected growth abnormalities, like crossed bills, curled toes and microphthalmia (Table 1). Additionally, an exceptional embryonic disorder was observed, in which the amnion had developed dermal characteristics, most strikingly feather growth on the inside of the amniotic cavity (Fig. S1). We hypothesise that the disordered amnion development could be caused by dioxin-like

Table 1. Overview of the number of embryonic anomalies found in unhatched Dutch Northern Wheatear eggs from two populations (Vogelduin, Aekingerzand) inspected during 2008 – 2013. Of all eggs collected (*n* = 156) 34 % were decayed too much to establish conclusive results. All other eggs were aberrant with defined embryonic anomalies. Eggs often contain multiple anomalies. Fluorescence microscopy showed that the yolk membrane of all inspected eggs with egg yolk infections contained sperm, indicating that all eggs were fertilize

	Amnion feather growth	**Egg yolk infection**	**Curled toes / crossed bill / coelosoma / microphtalmia / no eyes**	**Heart deformities / double heart**	**Other morphological anomalies (see SI)**	**decayed**
Vogelduin (*n* = 118)	9	30	15	4	36	41
Aekingerzand (*n* = 38)	0	7	7	0	15	12

compounds. Dioxins are known to disrupt gene functioning (Ciana et al. 2003; Ohtake et al. 2003) by activating promoters to transcribe oestrogen-responsive genes in tissues where these genes are normally not transcribed (Ohtake et al. 2003). Since development of feathers is controlled by hormones as oestrogen and thyroxin (Spearman 1971), feathering could be indirectly affected by the oestrogen-related actions of dioxins, or additionally by dioxins and PCBs altering the circulating thyroxin-levels in birds (Brouwer et al. 1998). The observed variety of embryo anomalies (Table 1) is consistent with earlier reports for birds suffering dioxin toxicity (Brunstrom and Andersson 1988; Gilbertson 1983; Grasman et al. 1998).

Further support for toxic effects in the Dutch populations was the observed mortality of nestlings without apparent external anomalies and without signs of starvation (18% of all females annually for 2008–2013 had at least one dead nestling, range 7–43% per year). Inspection of the internal organs of five dead nestlings revealed the presence of mottled livers due to necrosis of liver tissue in all nestlings (Fig. S2). Histological examination showed large and pale swollen hepatocytes, and occasional highly vacuolated hepatocytes featuring pycnotic nuclei, a sign of cell death which leads to liver necrosis (Fig. S3). Liver necrosis is known to be caused by PCB's and dioxins (Brooks et al. 1988; Gilbertson et al. 1991).

Overall concentrations of 2, 3, 7, 8 - tetrachlorodibenzo - *p* - dioxin equivalents of dioxins, non-*ortho* and mono-*ortho* PCBs in eggs from the Dutch populations showed elevated levels, more than six times higher than those in Sweden, whereas Dutch populations did not differ from each other (Fig. 1). The concentrations from Dutch eggs are similar to those found elsewhere in avian top-predators such as Northern goshawks (*Accipiter gentilis*, Wiesmuller et al. 2002) and Peregrine Falcons (*Falco peregrinus*, (Malisch and Baum 2007)). Total toxicity values in the range of the average concentration for both Dutch locations (recalculated as per gram wet weight, mean ± s.e.m.: 17 ± 16 pg g^{-1} ww, range 2–89) affected hatching success in wild Wood Duck (White and Seginak 1994) (*Aix sponsa*; lowest-observed-adverse-effect level 20 pg g^{-1} ww).

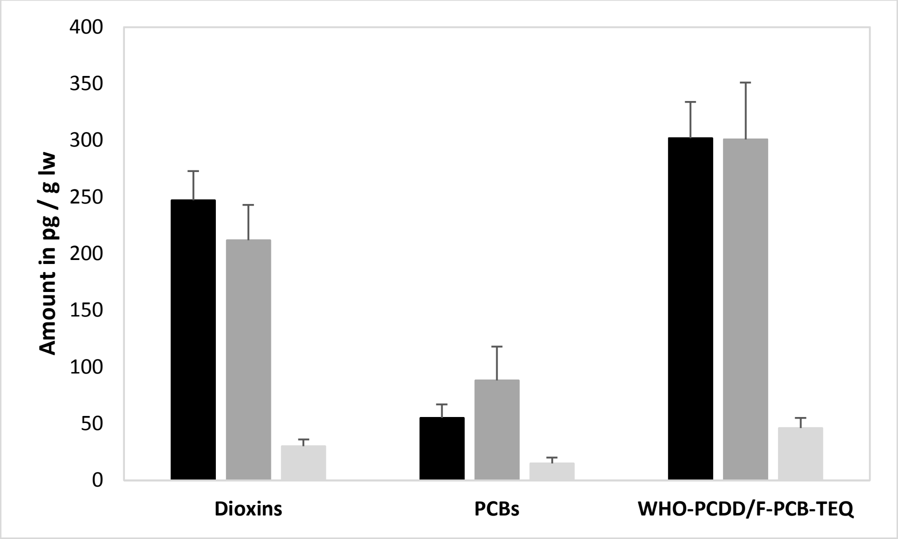

Figure 1. Concentrations of dioxins (WHO-PCDD/F-TEQ), PCBs (WHO-PCB-TEQ) and overall toxicity (WHO-PCDD/F-PCB-TEQ) in eggs of Northern Wheatears collected in three locations. Number analyzed: Vogelduin (black) *n*=21 of *n*=15 females, Aekingerzand (dark-grey) *n*=14 of *n*=14 females, Uppsala (Sweden; grey) *n*=11 of *n*=10 females. Concentrations of dioxins and PCBs calculated as WHO-TEFs and given as pg g-1 fat (mean ± s.e.m.). Kruskal-Wallis test, x^2=22.70, *P*<0.001 for all studied populations (WHO-PCDD/F-PCB-TEQ), followed by Mann – Whitney U tests, U=8.00, *P*<0.001 for pooled Dutch versus Swedish eggs, whereas Dutch populations did not differ from each other (U=135.00, *P*=0.686).

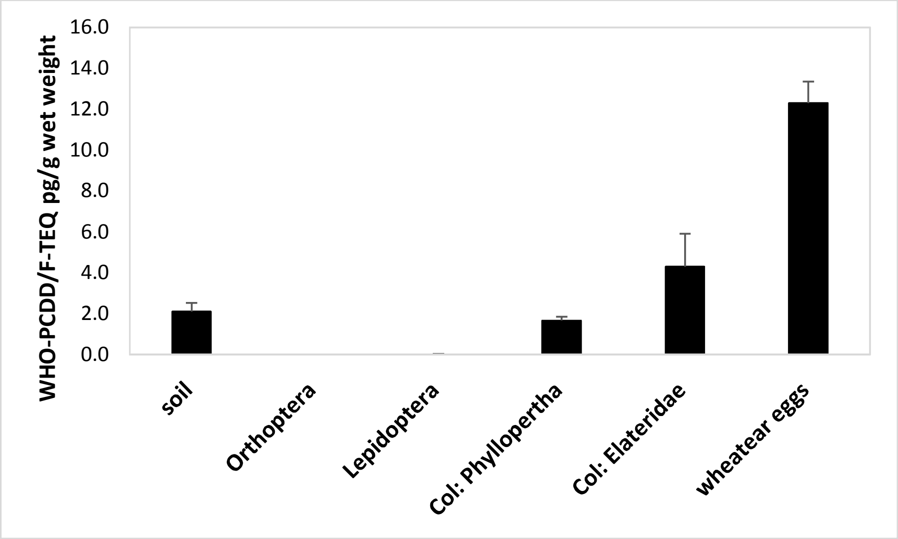

Figure 2. Concentrations of dioxins (WHO-PCDD/F-TEQ in pg g-1 wet weight, mean ± s.e.m) in the food chain of Northern wheatears in the coastal Vogelduin population. Sample sizes are 3 for soil (pooled samples consisting of 4 subsamples each) and insects (pooled samples consisting each of at least 10 individual insects) and 21 for wheatear eggs. Dioxins bio-accumulate in particular in soil dwelling larvae, especially in species with a multi-year development as Elateridae (Coleoptera). Aboveground herbivorous taxa (Orthoptera, Lepidoptera) bio-accumulate insignificant amounts of dioxins.

Analysis of dioxin concentrations in the local food-chain showed low concentration in the soil, clear bio-accumulation in some major prey species, and even higher concentrations in Northern wheatear eggs (Fig. 2). Especially soil-dwelling invertebrates accumulated more dioxins than aboveground herbivorous prey (Mann – Whitney $U < 0.001$, $P = 0.002$). Passerine females need to deposit nutrients for their eggs on a daily basis (Perrins 1996). Given the approximated energy needs for a laying female wheatear, we calculated that around 200 larval Elateridae would cover her daily energy needs, whereas 100 larvae contain the amount of POPs found in a single egg. These calculations strongly suggest that the observed concentrations of dioxins in Northern wheatear eggs from the Dutch population have a local origin, and are not brought from their wintering grounds or migratory stop-overs. This is further supported by the low dioxin concentrations in eggs of the Swedish reference population (Fig. 1), which generally winters in the same African wintering grounds. Tests for residues of organochlorinated pesticides (OCPs, such as DDT; Table S2) in eggs were at low concentrations and similar for the Dutch and Swedish populations. The observed low OCP concentrations lead us to conclude that the effects are not caused by pesticides.

A simulation study further suggests that the reduced hatching observed in the field is sufficient to contribute to the observed population declines. A comparison of potential population growth scenarios with dioxin-reduced hatching rate (80% hatching success) and reference hatching rate (94% hatching success) showed that dioxin-poisoning translated to a reduction of population growth rate (λ) from 1.07 to 0.98. Based on a starting population of 17 females (current Vogelduin population) this change in λ corresponds to a projected difference in population size of 20 breeding females after 10 years (34 vs. 14 females; Fig. S4). We conclude that bio-accumulated dioxins acting through reduced hatching rate can have a substantial negative effect on population growth.

The soil dioxin concentrations in the Dutch nature reserves that we studied are similar to those in rural sites in many other industrialized countries (e.g. Germany, UK, Austria, Spain, Italy, Denmark, USA; Tables S3 and S4). As dioxins bind to organic matter, adverse effects for insectivores may be most profound in habitats with a shallow organic soil layer (see Methods), such as the vast semi-desert areas of the globe. Our results show that at these widely occurring background soil concentrations, dioxins bio-accumulate in soil-dwelling arthropods which inhabit the organic layer of these soils. We have shown evidence that bio-accumulation of dioxins leads to adverse effects in a passerine species that depends on soil-dwelling prey. As this applies for many other species in this guild of birds, dioxins may have contributed to the worldwide decline of passerines. Similar effects are to be expected in other vertebrates that rely on similar prey. Bio-accumulation of initially low dioxin concentrations up into the food chain is likely to be a widespread phenomenon potentially threatening biodiversity in many parts of the world.

Acknowledgements

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Supporting Information

Study areas and species

Since 2007 we have been studying the breeding ecology and population dynamics of two remaining Dutch populations of Northern wheatear (*Oenanthe oenanthe*), a long-distance migratory insectivorous songbird which winters in the African Sahel. Population numbers have plummeted since 1980 in the Netherlands, with population declines suggested to be mainly due to decline in Rabbit (*Oryctolagus cuniculus*) populations and atmospheric nitrogen deposition (both changing species-rich short-grown and open semi-natural grasslands into mono-specific stands of tall grasses (Bobbink et al. 2010)) reducing the amount of suitable habitat and nest site availability. Small and isolated populations of a few dozen breeding pairs are holding on in isolated, sparsely vegetated dune grasslands, where they prey mostly on arthropods and breed in abandoned burrows of Rabbits or in decaying stumps of felled trees.

One studied population inhabits a coastal dune area (Vogelduin, Noord-Hollands Dune Reserve, NHD, 52° 55´N 06° 55´ E). The second population is situated 120 km inland (Aekingerzand, 52° 33´N 04° 36´ E). Both areas have been designated Natura2000 sites, i.e. are part of the pan-European network of protected nature reserves. The NHD Reserve, 5300 ha, is partly used for the production of drinking water for nearly the whole province of North-Holland. The quality of the water is constantly monitored. Aekingerzand is part of the National Park Drents-Friese Wold (6000 ha) which was established in 2000. For selected comparisons we use reference data from a population of Northern wheatears in Sweden near Uppsala where birds inhabit pastures, crop fields, farmyards and unmanaged grasslands (Arlt et al. 2008).

Northern wheatear diet

While we have no direct information on females' diet during egg-laying we have detailed data on nestling diet for the Vogelduin population (chapter 2). By filming feeding parents at n = 11 nests between 2008 and 2010 we found that nestling diet was comprised mainly by the beetle *Phyllopertha horticola* (Scarabeidae; imagos), click beetle larvae (Elateridae), caterpillars, spiders, adult Asilidae (Diptera) and grasshoppers (Orthoptera: Acrididae) (chapter 4). Due to their phenology, prey like *Phyllopertha horticola*, adult Asilidae and grasshoppers present in the nestling diet are not yet available during egg laying. However, click beetle larvae are present year-round due to their multi-year larval development, and we expect click beetle larvae to comprise a significant proportion of females' diet during egg-laying. Food is an important source of nutrients for egg formation in passerines, which cannot produce eggs from internal nutrient stores ("daily surplus users" (Nager 2006)).

Table S1. Summary of relevant breeding parameters of Northern Wheatears from both Dutch sites (Vogelduin and Aekingerzand) and the Swedish site (Uppsala). The number of breeding females (# females), number of nests inspected (# nests), percentage of nests with at least one failed egg (% nests ≥ 1 failed egg) and the percentage of failed eggs in all nests with at least 1 egg hatched (% egg failure) per study site per study year.

	Vogelduin				**Aekingerzand**				**Uppsala**			
	# females	**# nests**	**% nests ≥ 1 failed egg**	**% egg failure**	**# females**	**# nests**	**% nests ≥ 1 failed egg**	**% egg failure**	**# females**	**# nests**	**% nests ≥ 1 failed egg**	**% egg failure**
2008	14	19	58	22	48	44	50	15	24	24	21	4
2009	15	20	50	27	41	26	46	18	17	17	18	8
2010	17	19	53	19	26	11	55	11	11	11	45	9
2011	24	27	41	13	22	21	43	13	25	25	16	6
2012	14	15	47	11	16	12	33	9	29	29	24	6
2013	7	5	60	27	16	15	60	12	19	19	26	4

Hatching and embryonic anomalies

Due to intensive fieldwork we collected detailed demographic data and each year from 2008 to 2013 we monitored 95– 00% of all nests at both sites. Eggs that did not hatch in an otherwise successful nest were counted as failed. This procedure excludes nests where females were predated upon, or nests that were disturbed by other external causes (e.g. burrow collapse). During our study 11–27% of eggs failed to hatch in the Vogelduin population and 9–18% in Aekingerzand (Table S1). Hatching failure appeared to be high compared to published rates from other studies. In Germany, between 3–5% of eggs failed to hatch in several adjacent populations (Buchmann 2001) and hatching failure in two Swedish populations was 5% and 8% (Moreno 1987). Those rates are matched by the 4–9% failure rates for the Swedish reference population (Table S1). Remarkably, 20% of eggs failed to hatch in one population in the UK of which 89% contained dead early embryos, which were arrested in their development (Currie et al. 1998). Unfortunately no information on the occurrence of embryonic malformations, or on possible causes for the hatching failures, is provided in that study. In other studies it remained unclear how hatching failure was determined, which makes it impossible to compare the data (Conder 1989).

When the nestlings were ringed around day 8, eggs which failed to hatch were collected and stored at 4°C. Eggs were later opened and visually inspected. In all failed eggs which were not decayed too much to establish conclusive results, embryonic anomalies were observed (Table 1). Exposure to dioxin-like POPs has been shown to cause growth deformities like crossed bills, curled toes and microphthalmia in a variety of bird species

(Brunstrom and Andersson 1988; Gilbertson 1983; Grasman et al. 1998). However, such growth deformities have also been reported as an effect of nutrient deficiencies, as shown in poultry studies (Romanoff 1972). As such, these anomalies are expected in the presence of elevated dioxin levels, but they do not totally exclude other causative factors. Besides the above morphological anomalies we found others (Table 1). These include underdeveloped extremities, shortened lower mandibles, mottled livers, cerebral hemorrhage, dilated veins and intestine loops outside the body. Egg yolk infections (Fig. S5) appear at a first glance as normal, fresh eggs with transparent and fluid egg white and a normal, globular egg yolk.

Figure S5. Infection of the egg yolk. Egg white is normal (transparent and fluid) whereas the egg yolk appears normal but is very thick upon touching ('pudding'), as shown in this figure.

However, whereas normal egg yolk flows out upon breaking the yolk membrane, in yolk infections the egg yolk is very thick ('pudding'). Apparently bacteria inside the female enter the egg yolk before it is enveloped in protective egg white. It indicates a lowered immune system activity of the female, which could be due to disease or dioxins and PCBs (Jones and de Voogt 1999). In $n = 9$ eggs of $n = 3$ females the development of the amniotic membrane had progressed beyond its normal limits: the amnion had thickened, lost its transparency and grew feathers (Fig. S1). Progressed development typifies aberrant DNA transcription. Such anomalies can arise either due to genetic disorders or the presence of factors that erroneously stimulate DNA transcription. Dioxins have hormone mimicking characteristics and are known to interfere with hormone receptors and DNA transcription (Ciana et al. 2003; Ohtake et al. 2007; Ohtake et al. 2003). The anomaly of the amniotic membrane led us to investigate possible eco-toxicological explanations for the observed high egg failure rates.

Another cause reducing breeding success was occasional mortality of nestlings without any externally visible trauma. Due to the time-lag between time of death and time of actual collection, the rapid onset of decay of organs prevented conclusive *post-mortem* autopsy. After three years of repeated observations we were able to discern *a priori* which nestlings were dying, since they were transferred outside the nest by the parents. We collected $n = 4$ dying nestlings from $n = 3$ females in 2011 and $n = 1$ dying nestling in 2013 for subsequent fresh autopsy and histological study of their livers. To compare the histology of the necrotic Northern wheatear livers to healthy livers, we opted to use an apparently healthy juvenile Eurasian blackbird (*Turdus merula*), a common breeding bird in the Netherlands (Hustings and Vergeer 2002). This individual was freshly collected as prey of Northern Sparrowhawk (*Accipiter nisus*), which one of us follows closely for years. The nestling Eurasian blackbird was of similar age (8 days) as the dying Northern wheatears (7 days). Since liver function, anatomy and structure are similar among bird species (Hickey and Elias 1954; Romer and Parsons 1986), comparison of livers of two songbirds within the same family is valid, and allowed us not to sacrifice a healthy nestling of a Dutch red-list bird species.

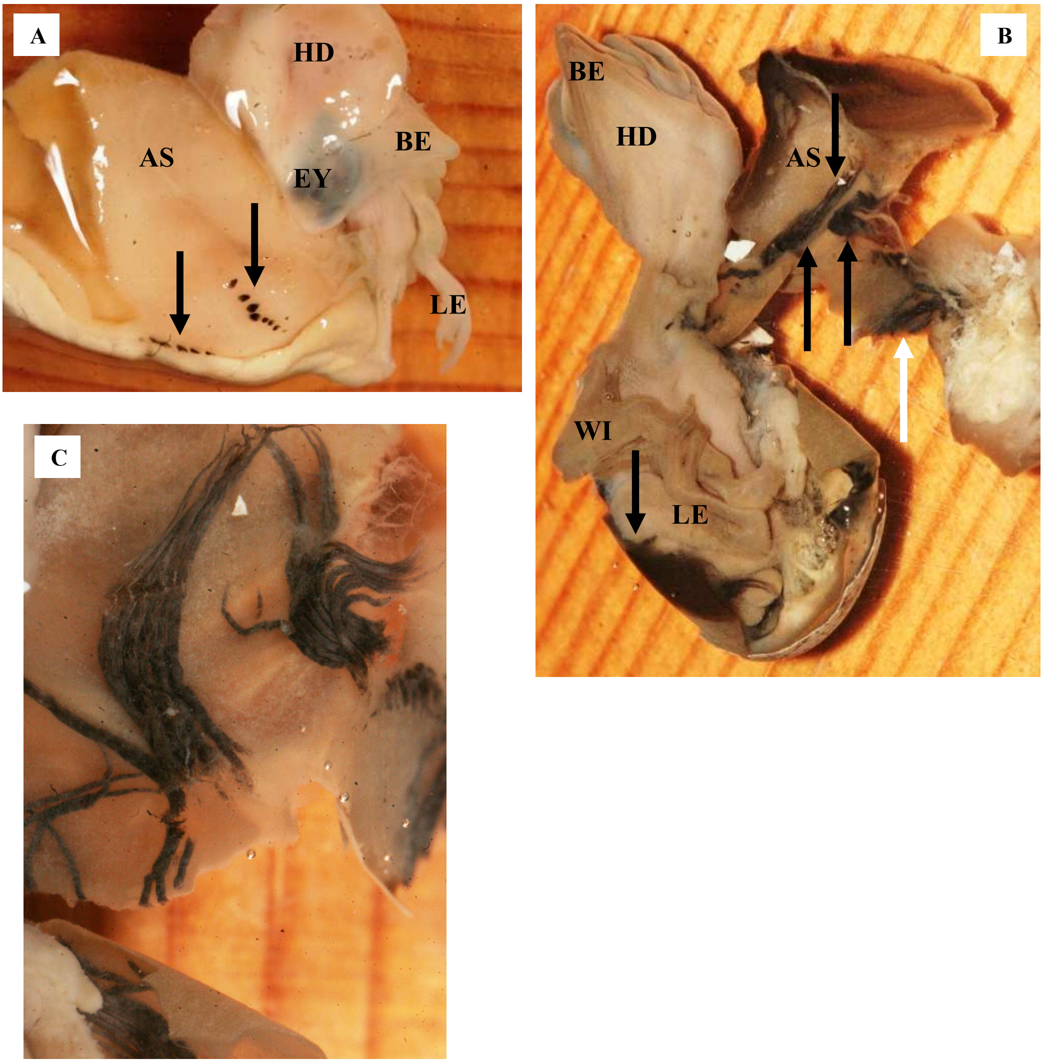

Figure S1. Northern wheatear embryos in which the amniotic sac developed dermal characteristics and grew attached to embryos. Embryo A is younger than embryo B. Both embryos show feather growth on the inside of the amniotic cavity (black arrows). The feathers indicated by the white arrow in figure B are shown in more detail in figure C. As = amniotic sac, Be = beak, Ey = eye, Hd = head, Le = leg, Wi = wing. This anomaly was found in *n* = 9 eggs of *n* = 3 females.

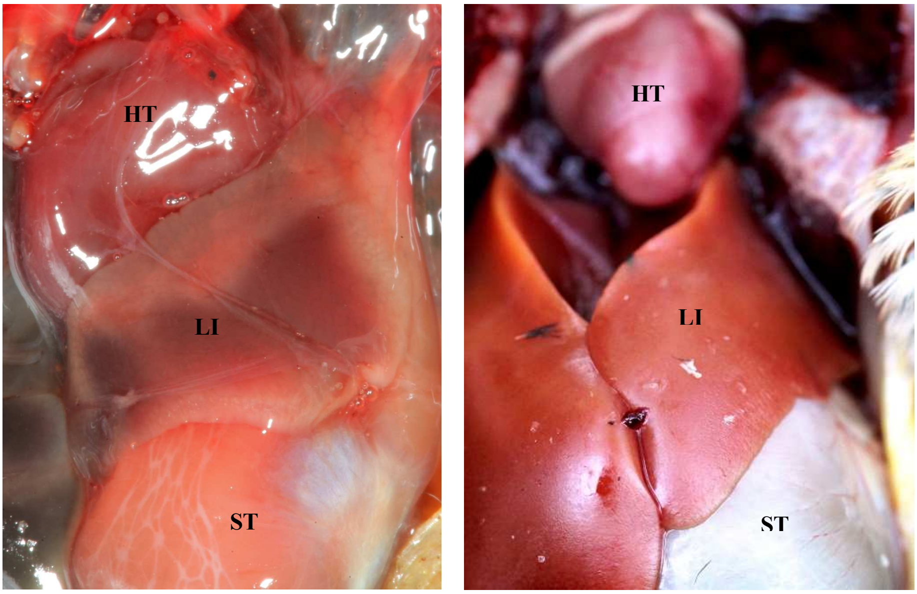

Figure S2. Necrotic and control liver. Left: Necrotic liver from of Northern wheatear nestling 7 days old. Liver had a mottled phenotype due to liver necrosis. Right: Healthy liver of 8 days old Eurasian blackbird (Turdidae: *Turdus merula*) nestling shows normal, regular coloration. Ht = heart, Li = liver, St = stomach

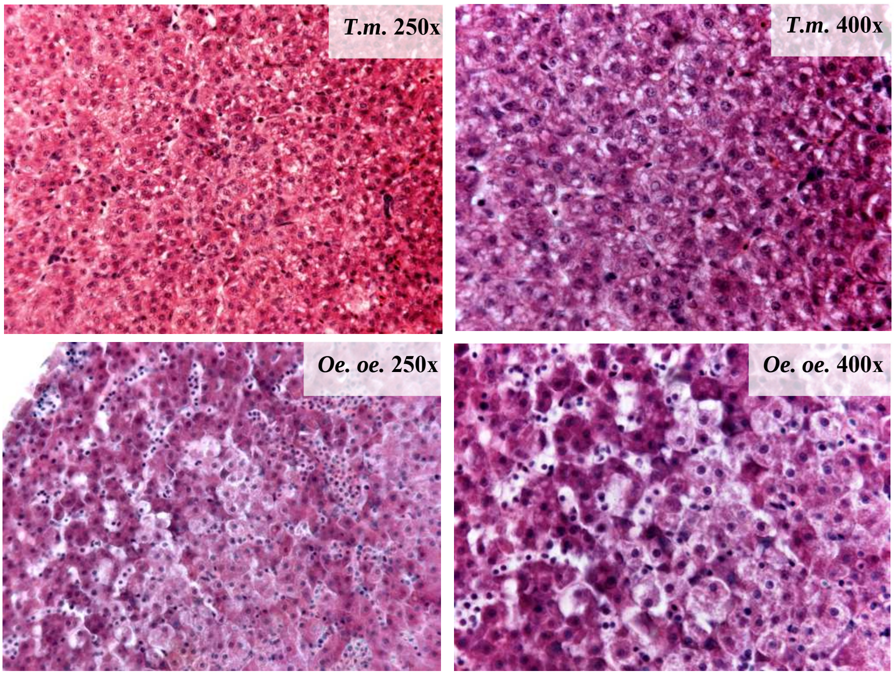

Figure S3. Histology of necrotic and control liver (hematoxylin and eosin stained). Control liver of Eurasian Blackbird (*Turdus merula*, *T. m.*, upper two images) show regular cells. The mottled liver of Northern Wheatear (*Oe. oe.*, lower two images) shows distinctly large and pale, swollen hepatocytes. Occasional highly vacuolated hepatocytes show pycnotic nuclei, a sign of cell death and, subsequently, liver necrosis.

Contaminant analyses: dioxins and PCBs

To test whether dioxins and dioxin-like compounds (here: PCBs) could be involved in causing the hatching failures, embryo and liver anomalies, we collected egg samples from different individuals. We collected $n = 35$ eggs from $n = 29$ females (Fig. 1). Of these eggs, $n = 8$ from $n = 6$ females were collected as freshly laid eggs and $n = 27$ from $n = 23$ females were collected as failed eggs. Freshly collected eggs (only collected in 2011) did not differ in dioxin and PCB levels from unhatched eggs from 2011 ($n = 6$) (Mann-Whitney test, $U = 23.00$, $P = 0.897$ for dioxins; $U = 21.00$, $P = 0.699$ for PCBs, measured as pg/g fat). We collected a further 11 failed eggs and nestlings of $n = 10$ females from the Swedish reference population. We anticipated lower POP pollution in Sweden, as Sweden is less industrial, and POP concentrations in locally produced foods for human consumption (butter) are lower in Sweden than in the Netherlands (Weiss et al. 2005).

To investigate how dioxins bio-accumulate in the food chain on the Dutch breeding grounds we analyzed soil and arthropod (Northern Wheatear prey) samples. In both Vogelduin and Aekingerzand we collected $n = 3$ soil samples, each of which consisted of 4 subsamples taken in a 2x2 m square and containing all soil between 0–10 cm depth. Standing crop, mosses and other debris were removed, and the samples were dried in a stove at 70°C for 48 hours. Prey samples were collected in Vogelduin only since we have detailed knowledge on the local nestling diet only for this population (chapter 2). Of the main prey Orthoptera: Acrididae (imagos), Lepidoptera (larvae), Coleoptera: *Phyllopertha horticola* (imagos) and Elateridae (larvae) we collected $n = 3$ samples each consisting of at least 10 individuals collected in territories of Northern Wheatears. Animals were stored at -17° C. Concentrations of dioxins and PCBs were determined by applying WHO-TEFs (World Health Organisation Toxic Equivalent Factors, Van den Berg et al. 1998).

Contaminant analyses: pesticides

Pesticides such as DDT are also known to be poisonous for wildlife as birds and their mode of action may be similar to dioxins and PCBs (Fry 1995). Therefore, the reported detrimental effects may be caused by pesticides as DDT and derivatives, which are still used in the African wintering areas. We also determined the concentration of pesticides belonging to the ‘dirty dozen’ (Stockholm Convention, (2008)) in eggs and nestlings from Vogelduin, Aekingerzand ($n = 5$ eggs each) and the Swedish reference site Uppsala ($n = 10$ nestlings; Table S2).

Most of the pesticides occurred in low concentrations (2014) in eggs of Northern Wheatears. Only *pp*’-DDE showed slightly elevated concentration (Table S2) and concentration did not differ between the three sites (Kruskal-Wallis test, $\chi^2 = 4.552$, $P = 0.208$). *pp*’-DDE levels were similar to maximum allowed residue levels in consumer eggs in the European Union (Commisson 2008), which makes it unlikely that the adverse effects as we describe for Northern Wheatears are caused by pesticides.

Upon receipt, the samples were all stored in the freezer at -20° C. After thawing, the samples were thoroughly homogenized and a homogeneous (sub) sample was taken for analysis of dioxins, PCB's and organochlorinated pesticides (OCP's). Mass labeled internal standards were spiked to the sub-sample. The lipids and contaminants were extracted from the samples by the Smedes method (Smedes 1999). This method allows extraction of the non-bound lipids (triglycerides) and the 'bound' lipids (e.g. phospholipids). The latter are particularly relevant in case of non-fatty biota and soil sample matrices, as was the case in current study. After extraction of the samples, the lipid yield was determined.

After extraction, the lipids were dissolved in a 1/1 (v/v) mixture of ethylacetate/cyclohexane (15 ml) of which 12.5 ml was injected on a gel permeation chromatography (GPC) system for removal of the lipids. The fraction containing the dioxins, PCB's and OCP's was collected and the solvent was reduced to 1 ml Some extracts were cleaned through lipid removal using Al2O3 column chromatography and afterwards the volume was reduced to 1 ml 50 microliter out of 1 ml extract was taken for analysis of OCP's by comprehensive multidimensional gas chromatography coupled to mass spectrometry (GCxGC-MS).

Table S2. Concentrations of pesticides in eggs and nestlings of Northern Wheatears collected in Vogelduin (*n* = 5 eggs), Aekingerzand (*n* = 5 eggs) and Uppsala (*n* = 10 nestlings). All concentrations are low, given in ng g^{-1} ww. Concentrations lower than 1 were not determined. Pp'-DDE given in mean ± s.d.

	Vogelduin	Aekingerzand	Uppsala	Maximum allowed residue levels chicken egg EU (ng g^{-1};45)
Biphenyl	<1 - 1.01	<1 – 3.95	<1	10
pp'-DDD	<1 – 1.86	<1	<1 – 9.11	50
pp'-DDE	57.98 ± 94.19	76.73 ± 70.79	67.47 ± 85.57	50
pp'-DDT	<1 – 7.28	<1	<1 – 12.80	50
Dieldrin	<1	<1 – 26.99	<1 – 11.91	20
Diphenylamine	<1	<1 – 2.21	<1	50
HCB	<1 – 2.5	<1 – 1.96	<1 – 3.75	20
HCH beta-	<1	<1	<1 – 1.24	10
HCH gamma- (Lindane)	<1	<1	<1 – 2.89	10
Heptachlor epoxide (iso B)	<1 – 1.96	<1 – 8.35	<1	20
Oxychlordane	<1 – 1.06	<1 – 3.12	<1	5
Permethrin I	<1	<1	<1 – 1.76	50
Permethrin II	<1	<1	<1 – 1.65	50
Phenylphenol o-	<1 – 1.23	<1 – 1.88	<1	50

The details of the applied method can be found elsewhere (Van der Lee et al. 2008). The dioxins, dioxin-like (dl-) PCBs and the non-dioxin-like (ndl-)PCB's were determined by GC coupled to high resolution mass spectrometry (HRMS). The details of this method are published elsewhere (Uçar et al. 2011; Van Leeuwen et al. 2009). The quality of the applied methods is controlled by analysis of blank samples, the use of (mass labeled) internal standards, recovery experiments and the participation in proficiency tests. RIKILT is ISO 17025 accredited for the analysis of dioxins and PCB's in biota and soil samples. Table S5 provides information on which dioxins and PCBs have been analyzed.

Food chain: from soil to click beetle larva to egg

We found that click beetle larvae (Coleoptera: Elateridae) bio-accumulated the highest levels of dioxins (Fig. 2) and click beetle larvae are likely to comprise a significant proportion of females' diet during egg-laying (see "Northern Wheatear diet", above). Therefore, we use this prey to explore the ecological relevance of the dioxin concentration found in soil and arthropods on the Dutch breeding grounds. We estimated how many click beetle larvae a female would theoretically need to consume to be able to deposit the amount of dioxins in one egg.

Fresh weight of one click beetle larva is 0.0692 ± 0.0316 g (average ± s.d., *n* = 22). The concentration of dioxins (WHO-PCDD/F-TEQ) in click beetle larvae is 4.28 pg/g wet weight. It follows that an individual larva contains 0.0692 * 4.28 is 0.30 pg WHO-PCDD/F-TEQ. On average WHO-PCDD/F-TEQ concentration in one egg of Northern Wheatears from the Vogelduin population is 12.3 ± 4.8 pg g^{-1} wet weight (*n* = 14). Since one egg weighs 2.565 g, excluding shell (Schönwetter and Meise 1967), the average amount of WHO-PCDD/F-TEQ sums up to 2.565 * 12.3 is 31.5 pg per egg. Hence, to be able to deposit 31.5 pg WHO-PCDD/F-TEQ in one egg, a female has to consume 31.5 / 0.30 larvae, which results in 105 click beetle larvae per egg.

Northern Wheatears feeding nestlings spend 87 kJ per day (Moreno 1989). The energy content of mealworms (Coleoptera: Tenebrionidae) is 9.4 kJ / g (Finke 2002). Assuming 65% assimilation efficiency of beetle larvae (Kacelnik 1984), a female needs to consume 87 / (0.65 * 9.4) is 14.2 g of beetle larvae per day. Since one click beetle larva weighs 0.069 g, a female would therefore need 14.2 / 0.069 is 206 larvae per day. This is double the amount required for dioxin-deposition as observed in one egg. We found previously 4.9 larvae per 0.36 m^2 (*n* = 30 samples) and that a Northern Wheatear territory contains 0.92 ha of preferred short vegetation (chapter 4). This translates to 125,222 larvae per 0.92 ha.

A Northern Wheatear weighs approximately 25 g during the breeding season (Glutz von Blotzheim and Bauer 1988) and one egg corresponds to ca. 10% of the body mass. This large percentage can only be achieved by additional feeding during laying. One egg is laid every 24 hours which implies that during one day enough food must be obtained to produce one egg. Click beetle larvae likely comprise a significant proportion of females' diet during egg-laying. Given the amount of larvae needed for self-maintenance during nestling feeding we calculated that a female should be able to consume the required amount of click beetle larvae a day. Thus, the concentrations of dioxins that we find in these larvae could well result in the high concentration found in eggs, even with low soil contamination on the breeding grounds, and irrespective of possible additional contamination in the wintering areas.

The high variation in contaminant content between eggs of different individuals within the areas may be due to the strongly differing dioxin and PCB contents between the arthropod prey types. Individuals likely differ in diet either due to differences in small-scale local availability and individuals responding to differences in relative prey densities, and/or differences in prey choice (Bolnick et al. 2003).

Effects of egg hatchability on population growth

To evaluate the potential effects of the difference in hatching success between the Dutch Vogelduin population and the Swedish Uppsala reference population on population growth rate (λ) we used a simple deterministic unstructured, single-sex population model, based on the female population. The model was defined as:

$$N_{t+1} = N_t(F+S) = N_t \lambda$$

$$F = p_{breeding} \times \frac{clutch\ size}{2} \times hatching\ rate \times survival_{1y}$$

with S = $\text{survival}_{\text{female}}$. We calculated λ for two scenarios, (1) 'dioxin-affected' using the 80% hatching rate from Vogelduin and (2) 'reference' using the reference 94% hatching rate from Uppsala. All the other parameters characterised the Dutch Vogelduin population (estimated using an integrated population model following (Schaub and Abadi 2011)) and were identical between the two scenarios, namely:

Probability of breeding ($p_{breeding}$) = 1
Clutch size = 4.13
First-year survival = 0.315
Adult female survival = 0.46

Population growth rate in the 'dioxin' scenario was negative with $\lambda_{\text{dioxin}} = 0.98$ indicating a declining population. In contrast, the 'reference' scenario showed a clearly increasing population with $\lambda_{\text{normal}} = 1.07$. If these two growth rates are used to deterministically project the current population of 17 breeding females (the average number of breeding females 2007-2011 in the Vogelduin population) over 10 years, the 'dioxin' scenario results in 14 breeding females while the 'normal' scenario results in 34 females, a strong increase (Fig. S4). This simple simulation indicates that reduction of hatching success is strong enough to change a growing population into a declining population, given the current values of demographic parameters of the wheatear population. Note that the purpose of this population model is only to evaluate the effects of hatching success on potential population growth, based on the current demography of the Vogelduin population. This model is not meant to provide accurate projections of the Vogelduin population, since it ignores e.g. density dependence as the population size increases, variability in vital rates between years and immigration into the population.

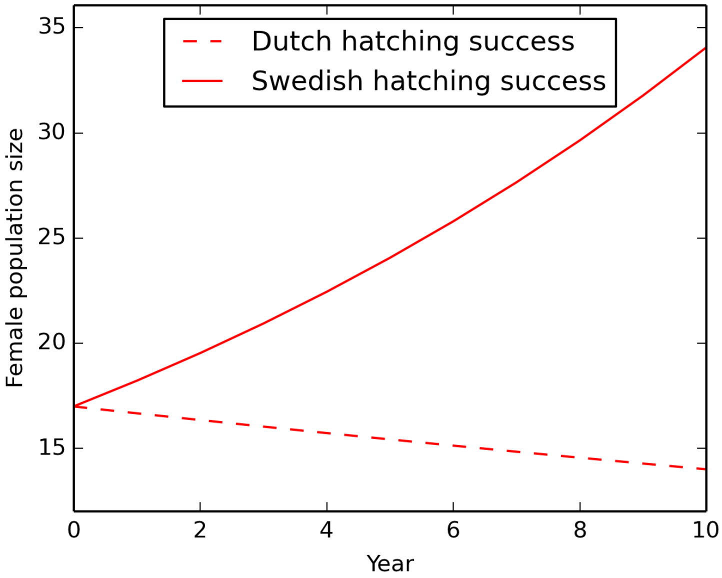

Figure S4. Projected population development under low hatching rate (80%) and 'reference' hatching rate (Uppsala, 94%). Starting population $n = 17$ females, which is the average Vogelduin population for 2007 – 2013.

Dutch and international soil POP contamination

Because degradation of dioxin-like compounds in the soil is in the order of decades to a century, the toxic legacy of pollution from decades ago can still be largely present in the soil (Sinkkonen and Paasivirta 2000). Soil concentrations may continue to rise as long as atmospheric input exceeds the rates of loss (Kjeller et al. 1991), even when output to the atmosphere decreases through legislation.

The background values for dioxins and PCBs in the Netherlands as determined in this study are in the range of values reported previously for the Netherlands (range 1.5–16.4 ng I-TEQ kg^{-1}, $n = 32$ sites on a national 40 x 40 km^2 grid, (Van den Berg et al. 1994)). In fact, a 1994 analysis provided a value of 2.6 ng I-TEQ kg^{-1} (Van den Berg et al. 1994) near Vogelduin, which closely matches our 2.56 WHO-PCDD/F-PCB-TEQ in Vogelduin. The Dutch values are similar to those found in rural parts of other European industrialized countries, as for instance the United Kingdom (Creaser et al. 1989; Creaser et al. 2007), Germany (Fiedler et al. 2002), Austria (Boos et al. 1992), Italy (Didomenico et al. 1993), Spain (Eljarrat et al. 2001), urban areas of Denmark (Vikelsøe 2004), New Zealand (Buckland et al. 1998) as well as the USA (Reed et al. 1990; USEPA 2007; Yake et al. 2000; Tables S3 and S4). This could mean that insectivorous birds and other insectivorous animals in other industrialized countries suffer from POP related aberrations in a similar way as shown in this study.

Most dioxins bind to the upper soil layer (< 15 cm) (Brzuzy and Hites 1995; Kitunen and Salkinojasalonen 1990; Reed et al. 1990; Vikelsøe 2004) and most soil-dwelling arthropods occur in the organic layer of the soil (e.g. detritivores). In soils with a shallow organic layer we hypothesize that most arthropods occur in a dioxin-polluted environment their entire lives, in contrast to deep organic soils where arthropods can migrate between more and less contaminated organic soil layers. We therefore expect especially birds that find their prey in sandy soils with only a shallow organic layer to be prone to dioxin accumulation.

Table S3. Soil concentrations of dioxins (WHO-PCDD/F-TEQ), PCBs (WHO-PCB-TEQ) and overall toxicity (WHO-PCDD/F-PCB-TEQ) in pg g^{-1} wet weight (mean ± s.d.). Number of soil samples analysed: Vogelduin *n* = 3, Aekingerzand *n* = 3.

	Dioxins	**PCBs**	**WHO-PCDD/F-PCB-TEQ**
Vogelduin	2.06 ± 0.75	0.50 ± 0.18	2.56 ± 0.91
Aekingerzand	2.05 ± 0.35	0.46 ± 0.11	2.50 ± 0.45

Table S4. Soil dioxin concentrations, measured in TEQ, for several countries and locations. Values given as presented in sources; single values concern averages, others min-max ranges. Dutch values are not distinctively different from those in other countries.

country	**location**	**TEQ (pg / g soil)**	**source**
United Kingdom	rural	3.3	60
Germany	urban centre	3	62
	urban fringe	2	62
	rural	1	62
Austria	rural	1.3 - 3.8	63
	urban	2.0 - 8.6	63
Italy	rural	0.1 - 4.3	60
Spain	rural	0.3 - 3.1	64
Denmark	rural	0.7	66
	urban	6.2	66
New Zealand	grassland	0.5 - 0.9	67
USA	field	1.8	68
USA	urban	0.1 - 19	69
	open land	0.04 - 4.6	69
USA	rural	5.2	70
Netherlands	dune / heathland	2.5	this study

Table S5. Analyzed congeners of dioxins and PCBs.

congener
2,3,7,8-TCDF
1,2,3,7,8-PeCDF
2,3,4,7,8-PeCDF
1,2,3,4,7,8-HxCDF
1,2,3,6,7,8-HxCDF
2,3,4,6,7,8-HxCDF
1,2,3,7,8,9-HxCDF
1,2,3,4,6,7,8-HpCDF
1,2,3,4,7,8,9-HpCDF
OCDF
2,3,7,8-TCDD
1,2,3,7,8-PeCDD
1,2,3,4,7,8-HxCDD
1,2,3,6,7,8-HxCDD
1,2,3,7,8,9-HxCDD
1,2,3,4,6,7,8-HpCDD
OCDD
WHO-PCDD/F-TEQ [lb]
PCB 81
PCB 77
PCB 126
PCB 169
WHO-NO-PCB-TEQ [lb]
PCB 123
PCB 118
PCB 114
PCB 105
PCB 167
PCB 156
PCB 157
PCB 189
WHO-MO-PCB-TEQ [lb]
WHO-PCB-TEQ [lb]
WHO-PCDD/F-PCB-TEQ [lb]

Chapter 7

Synthesis

H. Herman van Oosten

Synthesis

In this thesis I aimed to explore contemporary limiting factors for Northern Wheatears in the Netherlands. The large-scale demise of the Northern Wheatear over most of western Europe has generally been attributed to afforestation of heathlands, intensification of agriculture, decreased grazing by sheep and Rabbits, and N deposition stimulating sward heights and density (Collar 2005; Glutz von Blotzheim and Bauer 1988). In the preceding chapters I have shown that Northern Wheatear populations are constrained by multiple factors during different life-stages, which act together on the remaining populations. This combination of factors includes novel aspects of population limitation in birds.

Here I will review the results from two perspectives: (1) causes of population limitation of Dutch Northern Wheatears, based on this thesis and literature; (2) what the Northern Wheatear tells us about the 'quality' of the dune ecosystem. I finish with some, in my opinion, interesting and yet unresolved research questions.

Main results from this thesis

Using detailed demographic studies of three remnant small populations in the Netherlands, I showed that populations are limited by different demographic parameters (fecundity, mortality, immigration, emigration) and that dispersal between populations is very rare (chapter 2). By studying pre-breeding survival per season for early and late fledglings, I showed that overall first-year survival is higher for early fledglings, as a result of both higher post-fledging and migration-winter survival compared to late fledglings (chapter 3). I document in detail the importance of just a few arthropods as prey for nestlings, most of which reach the highest densities in tall grass vegetation, where, however, Northern Wheatears do not forage. By analysing microsatellites for four populations, I show that population structure is very strong for Northern Wheatears, implying that exchange between populations is very low, and that this condition has been established well before the study period. Lastly, I found indications of persistent organic pollutants such as dioxins to strongly affect hatchability of eggs.

Individually and combined, these five studies have ramifications for conservation of the species in the Netherlands. Figure 1 visually captures factors (putatively) influencing the number of breeding females from one year to the next. I only considered females, because their number determines the effective population size. The relationships indicated in this figure will be further discussed in the following paragraphs.

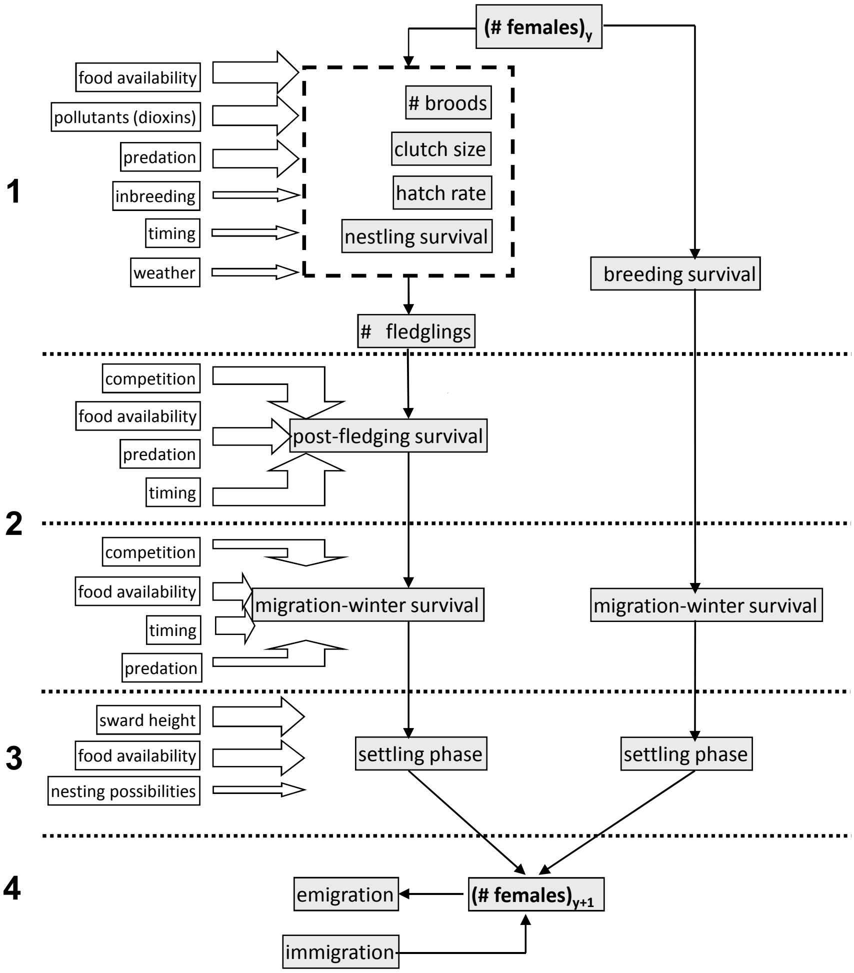

Figure 1. Factors influencing contemporary population growth during different stages in the yearly cycle of a population Northern Wheatears in the coastal dunes of the Netherlands, from starting number of females (y) to the number of females the following year (y+1). Thickness of the arrows roughly indicates the estimated effect-size of a factor: thick arrows indicate a strong effect and thin arrows a less strong effect. Part (1) encompasses factors influencing productivity of a female between laying and fledgling (*the nesting phase*); part (2) encompasses factors influencing pre-breeding survival, divided in survival of fledglings until leaving the natal site (post-fledgling survival) and survival of fledglings and adults during the migration and winter period (together: *pre-breeding survival*); part (3) shows factors influencing decision making in the settling phase upon returning to the breeding sites (*settling phase*); part (4) concerns *dispersal*, denoted as emigration out of the populations and immigration into the population.

Limitations during the nesting phase

Food availability

The overall success of the nesting phase, from nest building until fledging of young, is determined by female decisions upon which extraneous factors operate. This is a highly energy demanding phase, as the adults try to maximise current reproduction without incurring the loss of potential future reproduction. This makes the nestlings prone to food limitation as parents will shun food shortages that might arise to their offspring (Martin 1987; Moreno and Hillström 1992). Food availability and nest predation risk are considered the main ecological factors shaping life-history strategies in birds (Lack 1947; Nagy and Holmes 2004; Nagy and Holmes 2005; Ricklefs 2000; Skutch 1949; Zanette et al. 2006). Hence, a female has to choose her breeding site in such a way to maximize food availability and to minimize predation risk.

I found that most prey for Northern Wheatears occur abundantly in tall vegetation, but here they are unavailable, since Northern Wheatears do not forage in tall grass (chapter 4), most likely because they are morphologically adapted to short vegetation (Kaboli et al. 2007). As tall grass patches may function as source from which arthropods enter the short vegetation, the importance of tall grass patches is a hitherto overlooked element of the ‘Northern Wheatear landscape’ of Dutch coastal dunes. Parental Northern Wheatears in Uppsala, Sweden, breeding in tall vegetation, foraged further away from the nest than parents breeding in short vegetation, thereby increasing the daily workload (Low et al. 2010). So it appears that a certain extend of tall vegetation may enhance food abundance whereas too much coverage diminishes this effect.

One can argue that arthropod abundance is higher in tall vegetation as a consequence of very low predation pressure by Northern Wheatears and other insectivores which also prefer to forage on short vegetation (Atkinson et al. 2004; Atkinson et al. 2005). Nevertheless, arthropod diversity is generally higher in tall vegetation as a result of increasing structural complexity of the vegetation and build-up of organic soils (Dennis et al. 2008; Morris 2000).

Although I have no data showing the explicit importance of food abundance on reproductive output of Northern Wheatears, food is a main factor concerning reproductive output in birds in general (Martin 1987). Increased food abundance often relates to earlier laying, increased clutch size and increased reproductive output (reviewed in Martin 1987). Starvation of nestling Northern Wheatears is (almost) never reported for the Dutch breeding sites, indicating that food abundance in the occupied territories is not limiting the nestling phase. Yet, the varying frequency with which females start a repeat or second brood (54-71% of all females per year during 2007-2011, site C) may indicate food limitations to occur later in the breeding season, as Nagy and Holmes (2005) experimentally showed that double-broodedness increased after providing extra food in Black-throated Blue Warblers *Dendroica caerulescens*.

Predation risk

Hungry nestlings beg more for food, which triggers the parents to intensify foraging, but which also attracts hungry predators. Nest predation is frequent in sites C and A (chapter 2), with on average 21% of all first nests being predated with variation between years and early and late broods (fig. 2). Predation is a common factor influencing nest success in other mainland populations as well: 28% in England (data 1976-1977) (Tye 1980) and 33.5% of nests of 1st calendar year and 25.7% of adult nests in Sweden (data 1993-1999) (Pärt 2001a).

Predation strongly affects the number of fledged juveniles in C and A, though in slightly varying ways (chapter 2). Population C breeds in Rabbit burrows, which is advantageous since females survive by simply walking deeper in the burrow during a predation event by Red Foxes, and still can produce a second or third nest. Population A, by contrast, breeds in decaying tree trunks, in shallow hollows, where females are trapped during a predation event by Red Foxes and often predated as well, preventing a second nesting possibility. Up to 33% of all breeding females were depredated upon in one year (2009), of which 79% by Red Foxes.

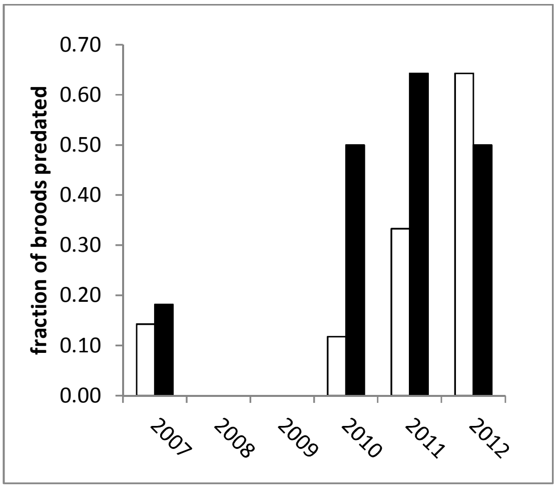

Figure 2. The fraction of all broods being depredated by Red Foxes per year, early (white bars) and late (black bars) broods in site C. Number of broods per year (early / late): 2007 25(14/11); 2008 20(13/7); 2009 23(14/9); 2010 29(17/12); 2011 38(24/14); 2012 22(14/8), only broods certain to be, or not to be, predated by foxes.

In Sweden, predation was found to be higher in tall vegetation compared to low vegetation for Northern Wheatears (Low et al. 2010; Pärt 2001a), with the main predators Weasels (*Mustela nivalis*), Stoats (*M. erminea*) and Adders (*Vipera berus*). One of the reasons of higher nest predation in tall vegetation is that voles, the main prey of the above predators, most abundantly occur under cover (Low et al. 2010; Pärt 2001a). Yet, Tye (1992) did not find a relationship between vegetation height and predation in England; predation rates were similar for both vegetation types. The main nest predator in Dutch populations is the Red Fox, with only occasional suspicion of predation by stoats or weasels. It remains to be seen whether foxes also prefer cover during their night time hunt, since they are not being predated upon themselves, whereas much smaller (mammalian) predators may fall victim to larger predators (such as foxes).

Predation is next to food availability the other main factor shaping life-history characteristics in birds (Martin et al. 2000; Skutch 1949; Slagsvold 1982) and strongly affecting annual reproductive output. The loss of females and nestlings by depredation affects population growth (chapter 2). Across all years (2007-2014), repeat clutches (excluding true second broods, which follow a successful first brood) (clutch size 5.03 ± 1.02 SD, $n = 33$ broods) are smaller than first clutches (5.62 ± 0.81, $n = 79$, Mann-Whitney $U = 872.50$, $p = 0.003$) in site C. Therefore, predation of first broods not only leads to lower first-year survival of the, by definition, late fledglings resulting from repeat broods, but the reproductive output is also smaller in repeat broods.

Egg failures, inbreeding and POP-contamination

Up to 27 % hatching failure in otherwise successful nests reduces the total number of fledglings. Because of its limiting capabilities, it is important to unravel causality of low hatching success (Hemmings et al. 2012). Around 10 % of eggs fail to hatch in wild birds (Koenig 1982), which can be due to a huge number of possible causes, totalling to this figure. Among the causes of egg failure are male and/or female infertility (e.g. Wetton and Parkin 1991), toxic agents (Fry 1995; Gilbertson et al. 1991), nutritional factors, addling, and inbreeding or other genetic factors (Bensch et al. 1994; Keller and Waller 2002; Kempenaers et al. 1996).

Low hatchability of eggs, especially in site C, is not present in the reference population near Uppsala. In site C, an average 20% of all eggs do not hatch while in Uppsala this is 6%. Every other parameter being equal between sites, low egg hatchability seems to be the most significant difference between the Uppsala and Castricum populations. Indeed, increased hatching success by 14% would make the Castricum population a growing one (chapter 6). So, it is important to understand the causes of hatching failure.

Of all inspected not decayed eggs that failed to hatch ($n = 103$, sites C and A combined), 36 % failed before the onset of embryo formation. These all showed bacterial contamination of the egg yolk, most likely originating from oviducal contamination. All eggs of which perivitelline membranes were inspected contained sperm. As such, it is most likely that the eggs were fertilised, but embryo development ceased before reaching a detectable size. Still, eggs may not have been fertilized, because of low sperm quality or due to genetic incompatibility between males and females, for example due to inbreeding (Hemmings et al. 2012), the likelihood of which will be discussed below.

After fertilization, 64 % of eggs failed in the embryo stage. A first question is whether the observed embryo mortality may be due to inbreeding. For now, I can in this respect only reflect upon the genetic diversity and heterozygosity of the observed populations.

Breeding among close relatives would be expected given the population sizes of 7 females in C (2013 and 2014) and 12 respectively 18 in A (2013 and 2014), in combination with very few immigrations per year (Jensen et al. 2007; Keller 1998; Marr et al. 2006; Van Noordwijk and Scharloo 1981). Indeed, two pairs consisting of (genetic) brothers and sisters nested in site C in 2014, but it remains uncertain whether the male was also the true genetic father (extra-pair offspring are common: 21% of all nestlings in 27 complete nests during 2009-2012, H. H. van Oosten, *unpublished results*). As populations have declined further since the start of my study, an increasing importance of inbreeding effects can be expected. Heterozygosity (H_O) is not particularly low compared to other Northern Wheatear populations and other songbird species. However, comparison between studies using different sets of markers must be made cautiously since H is a relative measure, depending on the type and allele frequencies of the markers used (Jensen et al. 2007).

The extent and possible effects of inbreeding remain to be studied further, preferably by a pedigree analysis (e.g. Jensen et al. 2007; Keller and Arcese 1998), which may be feasible in the future since blood samples are being collected in sites C and A since 2009. It remains to be seen if the observed low hatchability is caused by inbreeding, especially since some embryonic aberrations indicate hormone disrupting effects. These could be caused by organic pollutants such as dioxins (Fry 1995; Gilbertson et al. 1991) which are present in the eggs.

Dioxins present in only background concentration in the soil accumulate in soil dwelling larvae but not in aboveground arthropods as grasshoppers and caterpillars (chapter 6). Therefore, the degree of pollution in an insectivore, for example the Northern Wheatear, depends on the ecology of the prey and the relative availability of below- and aboveground prey for the insectivore. Hatchability was much higher in Uppsala, Sweden (average 94 %) than in Castricum (average 80 % during 2008-2013), and dioxin concentrations were six times lower; concentrations in Castricum are within the range of possible toxic effects and in Uppsala dioxin levels are far below such values. This means that either soil pollution is less than in Dutch areas, or that the diet consists of relatively more aboveground prey in Uppsala. Both could result in the low concentration in Swedish eggs. For now, we have indications for dioxins to affect hatching rate yet we need experiments and/or data from other populations to verify or falsify alleged dioxin effects on Northern Wheatears. Too, we need to explore hatching rates among other species with similar diets but which occur in large populations and are therefore exempt from possible deleterious effects of inbreeding.

Other embryo anomalies indicate poor food quality in terms of amino acids. Similar finds are still under study in heathland and forest ecosystems in the Netherlands, where amino acid synthesis in plants is disrupted due to nitrogen deposition and poor mineral nutrition of plants (Van den Burg et al. 2014; Vogels et al. 2011).

To conclude, average hatching failure of 20% strongly affects reproductive output. As modelled in chapter 5, if hatching failure would be similar to the population near Uppsala, Sweden (average 6%) population growth could be positive for site C, although the model does not incorporate immigration.

Weather and timing of breeding

Two additional extraneous factors which influence the total number of yearly fledglings per female are weather and timing of breeding. As a result of heavy rain in site C, sand occasionally flows into breeding burrows, covering the nests. Females escape but the nest contents are buried. Sand avalanches are unknown as a limiting factor in site A due to very different nestling sites (decaying tree trunks). In comparison with the effects of food and predation, this effect is not so large (five out of 187 nests 2007–2014) but has significance when populations are very small (e.g. two out of seven first broods were buried this way in 2014).

Lastly, timing (arrival and of starting the first nest) may constrain the number of fledglings per female Northern Wheatear. Timing has been shown to have strong effects on reproduction: reproductive success is reported to decline with season (Hochachka 1990; Perrins 1970; Verhulst et al. 1995), also for Northern Wheatears (Brooke 1979; Oberg et al. 2014), which points towards a more general decline in breeding conditions over the breeding season (Perrins 1965; Svensson 1997; Verboven and Visser 1998; Verhulst et al. 1995), yet the exact nature of which parameters decrease remains scarcely known.

Little is known about seasonal effects on food availability for Northern Wheatears: Tye (1992) reports a decrease in prey numbers (the bulk of which were ants) but an increase in total sampled arthropod biomass from July onwards (because of grasshoppers reaching their final instar?), based on data from one year. I found taxonomic differences in diet compositions of early and late broods (H. H. van Oosten, *unpublished data*) and that body condition of late nestlings (repeat or true second broods) is on average lower compared to early nestlings (chapter 2). Causality remains unstudied and probably multifarious, but it may be indicative of lower food availability and/or quality in the course of the breeding season. In corroboration with this idea, females with second broods commence breeding after the first or second egg is laid, leading to asynchronous hatching of young in those broods (H. H. van Oosten, *unpublished data*), which may indicate parental adjustment to food shortage (Lack 1954; Stenning 1996). Still, about 60 % of the Castricum females start a repeat or true second brood. Perhaps asynchronous hatching results in at least few offspring in good body condition to be on par with competing older early fledglings and adults once they leave the nest, as has been shown for Great Tits (*Parus major*) (Garnett 1981; Sandell and Smith 1991).

In spite of still limited knowledge of effects of timing (in relation to food availability and predation) on annual reproductive output, we do know that timing of fledging has strong effects on survival of fledgling Northern Wheatears (chapter 3, see also the next section). Because nestling condition did not significantly relate to first-year survival, the timing-related survival of fledglings is probably not a carryover effect from the nestling phase in spite of early nestlings having a higher body condition index than late nestlings.

Limitation outside the breeding phase

After fledging juveniles have to learn to survive on their own and mortality is generally high during this period (Krementz et al. 1989; Naef-Daenzer et al. 2001; Nur 1984; Smith et al. 1989; Vitz and Rodewald 2011; Yackel Adams et al. 2006), although post-fledging mortality does not always show a peak directly after fledging (Kershner et al. 2004; Tarof et al. 2011, chapter 3).

I show in chapter 3 that first-year survival of Northern Wheatears is higher for early fledged than for late fledged juveniles, as is shown for other birds (Krementz et al. 1989; Naef-Daenzer et al. 2001; Smith et al. 1989; Verboven and Visser 1998; Vitz and Rodewald 2011). Mean first-year survival for early / late fledglings is for *2008* 0.23/0.13; *2009* 0.23/0.13; *2010* 0.33/0.20 and *2011* 0.41/0.25. In spite of a lower survival of late offspring, on average 60% of females produce a repeat or true second brood per season in C. This is explained by the fact that ~50% of females succumb per year (chapter 2) and females should therefore try to maximize the number of fledglings per year rather than across years (Nagy and Holmes 2005).

Predation not only decreases the number of fledglings but may also indirectly affect survival of fledged young by causing fledglings of repeat clutches to be in the late cohort. If predation on fledged Northern Wheatears changes during the course of the breeding season remains unknown but for songbirds in different ecosystems (titmice inhabiting forests), predation pressure by Eurasian Sparrowhawks (*Accipter nisus*) on fledglings increases in time, thereby affecting late fledglings stronger than early ones (Götmark 2002; Naef-Daenzer et al. 2001).

Albeit speculative, intra-specific competition among fledglings may hamper late fledglings in foraging, since aggressive interactions are frequently observed among Northern Wheatears. Therefore, survival of late fledglings may be enhanced in years of high predation rates due to ameliorating competition with stronger, early fledged conspecifics. Tinbergen et al. (1985) show for Great Tits that first-year survival of fledglings from first broods does not depend on density but survival of fledglings from second broods strongly depends on density of first brood fledglings, perhaps related to large age-differences. In corroboration with this idea, Both et al. (1999) show for Great Tits that being heavy is especially important for first-year survival in years with high densities. Therefore, one would expect either fewer second broods or smaller second broods in years of high density of first broods and/or high nestsucces of first broods (Both et al. 1999; Tinbergen et al. 1985).

Furthermore, I show that lower first-year survival of late fledglings was not only caused by lower post-fledging survival on the natal site, but survival was also lower during the migration-winter period per two-week interval, compared to early fledglings (Chapter 3). Note that even though post-fledging mortality per two-week interval is higher than for migration-winter intervals, the total mortality during the complete migration-winter period is higher than the total post-fledging period, since the duration is much longer (15 intervals) than the post-fledging period (maximal 6 intervals).

Conditions affecting post-fledging survival leave their traces to also affect survival in the remainder of the year (carryover effects, (O'Connor et al. 2014)), perhaps in combination with factors affecting survival that have different intensities in the course of time, thereby affecting late fledglings stronger than early ones. Carryover effects may concern migration in sub-optimal body condition (Bauchinger and Biebach 2001; Berthold 1996) due to hampered foraging by aggressive older fledglings on the natal site (Arcese and Smith 1985; Merilä and Svensson 1997), resulting in lower first year survival. For example, first-year survival of Northern Wheatears significantly increased after supplying extra food during the post-fledging period, compared to an unfed control group (Seward et al. 2013).

Early fledglings of Northern Wheatear appear to leave the natal site earlier than late fledglings (fig. 3.1), as do other migratory songbirds (Savannah Sparrow *Passerculus sandwichensis* (Mitchell et al. 2012) and Great, Blue *Cyanistes caeruleus* and Coal Tit *Periparus ater* (Meller et al. 2013). Thus, leaving early seems to be advantageous with reasons to be found either on the breeding site (food, competition and predation), the migration plus winter period, or both. Late departure may result in later arrival on stop-overs, which could have repercussions on food availability (Delingat and Dierschke 2000; Moore and Yong 1991), and in later arrival on the wintering sites when the best wintering territories (Leisler et al. 1983) may have been occupied by earlier arriving conspecifics.

Populations of Northern Wheatears are not only constrained by processes on the breeding grounds as described in this thesis, but also on migration or on the wintering grounds. Only five months are spent in the Netherlands, and seven elsewhere. Yet, we know next to nothing about their migration and wintering ecology, only that they winter in Sahelian Africa (Collar 2005; Moreau 1972; Schmaljohann et al. 2012a; Van Oosten et al. 2014) and that the more westerly winter quarters are occupied by western breeders and eastern quarters by breeders from the east of their vast area of distribution (Collar 2005; Förschler and Bairlein 2011; Glutz von Blotzheim and Bauer 1988).

Poorly developed knowledge on wintering ecology of many Afro-Palearctic migrant bird species hampers possible conservation efforts directed to the wintering grounds, in spite of sometimes pronounced effects of wintering conditions on population growth in the northern breeding sites. Populations of many Afro-Palearctic migrant bird species have declined in Europe, especially species wintering in dry, open habitats (Sanderson et al. 2006; Vickery et al. 2014; Zwarts et al. 2009). Human population increase is much more pronounced in the western Sahel, as is the increase of livestock, which leads to changes in the natural habitat by felling trees and over-grazing by livestock. We show elsewhere that two Dutch Northern Wheatears winter in Mauritania and Mali/Ivory Coast (Van Oosten et al. 2014), implying that they are prone to strong alterations of their winter habitat. Northern Wheatears intuitively suffer less from forest clearance than do arboreal species. Nevertheless, Wilson and Creswell (2010) show that highest densities of wintering Northern Wheatears in Nigeria occur in areas with some trees left and not in areas devoid of trees. The species may, therefore, be prone to increasing winter limitations as well, if the human population continues to grow with concomitant large-scale adverse alterations of the landscape where Northern Wheatears winter.

Limitations during settling

Upon approaching last year's breeding (adults) or natal site (first year birds), Northern Wheatears must decide in which territory site to settle. Preferably this is a site with high (expected) food availability and low predation risk. Since territory quality strongly determines reproductive success in Northern Wheatears (Arlt and Pärt 2007; Currie et al. 2000; Pärt 2001b), this is an important choice.

Northern Wheatears must estimate the territory suitability for the remainder of the breeding season, based on clues available upon arrival. I show that Northern Wheatears preferentially forage in short vegetation (chapter 4), therefore vegetation height may well be an important clue for territory quality, as suggested by Tye (1992). Indeed, a higher number of fledglings were produced and nest predation chances are lower in territories with short vegetation, as opposed to tall (Pärt 2001a), which lead to differential population growth rates between habitats with short and tall vegetation (Arlt et al. 2008). Yet vegetation height increases in nutrient rich grasslands during the growing season except for when grazing effectively counters growth. Therefore, a positively assessed territory may turn out bad once nestling time approaches due to strongly increased vegetation height. Near Uppsala, Sweden, around 95% of all territory sites had very low sward heights early spring, yet half of them had tall vegetation later in the breeding season (Arlt and Pärt 2007). Likewise, Tye (1992) observed that cessation of grazing by sheep increased vegetation height which was very short at the time of arrival. Lastly, I excluded grazing by Rabbits in the field by erecting vegetation exclosures in Northern Wheatear territories in site C. As a result, vegetation rapidly increased from a few centimeters in April 2008 to 15 cm in July 2008 (H.H. van Oosten, *unpublished results*), which would hamper foraging greatly (Kaboli et al. 2007). As a direct effect of vegetation growth in the breeding season, Tye (1992) reports already settled Northern Wheatears to expand their territories, to desert the area and to have delayed breeding as a result of territory disputes. In line with these results, Exnerova et al. (2002) found that foraging territories of Northern Wheatear were larger in densely vegetated grasslands than in soil-banks, indicating hampered accessibility to prey in tall grasslands.

Indeed, being able to predict territory quality throughout the season is very important yet difficult to realize. Pärt et al. (2011) show that, although vegetation height during settling early spring poorly predicts vegetation height later that season, vegetation height at the moment when nestlings are fed is correlated between years. This cue may be of use for prospectors, i.e. birds already looking where to breed the next year. Tye (1992) found prey abundance in spring to positively correlate to prey abundance during breeding for Northern Wheatears breeding on the Breckland, England, yet males appeared to use vegetation clues to pick a territory, even though vegetation is a worse predictor of breeding time food availability.

Clearly, the predictability of territory quality, by whichever cues, remains open to much further work and the quality of predictions and the nature of cues used may be largely influenced by stability of the ecosystem the birds require, and specific site characters.

Limitation by dispersal

I show that the observed and estimated immigration rates into breeding populations are very low (chapter 2), and have led to strong genetic structuring (chapter 5). The low frequency of immigration could have repercussions for population viability in the course of time, as discussed below.

Philopatry typically increases as a response to decreasing population size and isolation (Heino and Hanski 2001; Weatherhead and Forbes 1994) and dispersal has been shown to be a heritable trait in passerines (Doligez et al. 2009; Duckworth and Kruuk 2009). Therefore, philopatry may be increasingly selected for when populations and the amount of suitable habitat decline. Alternatively, if philopatry is not selected for when the amount of suitable habitat declines, progressively fewer immigrants are expected to enter increasingly isolated breeding populations as shown for Yellow-headed Blackbirds (*Xanthocephalus xanthocephalus*) (Ward 2005), leading to population decline if recruitment of local origin and progressively lower number of immigrants no longer offset local mortality. Either way, selection or no selection for philopatry are two sides of the same 'small-and-scattered-populations-are-fragile' coin: if mortality is higher than the number of recruits in highly philopatric populations, they are ultimately doomed. On the other side: if mortality is higher than the number of recruits in populations with low philopatry, these populations are doomed if the number of surrounding source populations declines to such extend that immigration becomes increasingly rare (Dale 2001; Hanski 1998; Ward 2005).

Philopatry may decrease soon after the onset of population fragmentation and isolation, since dispersing individuals can still find inhabited populations. When fragmentation and isolation become more intense, individuals no longer successfully find other populations and philopatry should increase (Brown and Kodric-Brown 1977; Dale 2001). Increased philopatry during population decline may then slow or reverse the local decline. If increased philopatry plus fecundity offsets mortality, the population is in equilibrium and probably largely regulated by density dependent effects. If other close-by suitable breeding sites are available for colonization, philopatry may decrease again (Heino and Hanski 2001), since selection for philopatry is not advantageous anymore. Yet, if other suitable sites are not readily available, low numbers of individuals, stochastic events, and deteriorating allelic diversity (due to genetic drift and inbreeding) may ultimately threaten population viability (Frankham 1995; Frankham 1996; Gilpin and Soulé 1986; Lande 1993; Lande et al. 1999).

Apparent survival rates in populations C and A, which basically are a measure of the degree of philopatry as well, are in the same range as calculated for other populations (Table 1), in spite of different latitude-related environmental conditions. The sites listed in Table 1 differ regarding degrees of isolation: the English site concerns a real island, 40 km away from the nearest other island. Only once has a male ringed as nestling on Fair Isle been observed as a breeding bird on the nearest island. Given the presence of a bird-observatory and visiting birders, the lack of observed ringed birds elsewhere probably reflects truly very low emigration rates (Seward et al. 2013). Survival estimates from Sweden are based on a core area of 8 km^2, centrally within the total study area of 60 km^2 in which all breeding pairs were identified. Therefore, all individuals recruiting within 6 km from the core area were detected (Arlt et al. 2008). Since the median dispersal distance was 1300 m, permanent emigration outside the core area is rare at best. Lastly, out of more than 2500 resighted Northern Wheatears in eight populations of the Rhineland-Palatinate, Germany, only 1.6% (40 individuals) was observed in a different population than the natal or the breeding site (M. Buchmann, *pers. comm.*).

Thus, this all suggests that the high degree of philopatry is inherent to Northern Wheatears rather than acquired by strong selection during population declines. Nevertheless, one can argue that all populations concern in fact relicts of a putative much wider occurrence in the past, which makes a separation of inherent versus acquired philopatry difficult. A solution may be to study philopatry in areas where the species still has a much larger, continuous population as perhaps the Scandinavian uplands.

Table 1. First year and adult survival of the three Dutch sites compared to a Swedish and English site. The years of study for the Netherlands (2008-2011) and England (2008-2010) were much shorter than for Sweden (1993-2006), which probably affects the mean survival estimates. Estimations are given as: mean (95% CI) for the Netherlands, min-max average survival for six vegetation types combined for Sweden (Arlt et al. 2008); first year survival mean (95% CI), adult: min-max average survival per year of males plus females for England (Seward et al. 2013).

Site	first year survival	adult survival
Netherlands (site A)	0.31 (0.23-0.41)	0.52 (0.38-0.72)
Netherlands (site C)	0.32 (0.21-0.43)	0.50 (0.28-0.78)
Netherlands (site D)	0.29 (0.22-0.37)	0.54 (0.41-0.70)
Sweden (Uppsala)	0.17-0.32	0.34-0.62
England (Fair Isle)	0.28 (0.17-0.42)	0.30-0.69

Practically, this implies that chances for populations to disappear are currently larger than for populations to reappear in former breeding sites (in the Netherlands), even when such sites seem suitable regarding food and nesting sites, although data on both are still scarce (H. H. van Oosten, *unpublished data*). Nevertheless, occasional emigrants (three first-year males) were observed outside the natal site in abandoned areas. These males defended a territory but two of them eventually showed up in the natal site (site C) in the same season, having been unsuccessful in attracting a female in the abandoned site. The third bird did not show up in his natal site but it remains unknown if he bred in the previously abandoned site. It is interesting to note that these three males (and two other males from site C which established territories within existing populations, chapter 2) did not first show up in their natal site. One could expect them to first assess conditions in their natal site and, if not pleasing because of, for instance, a high density of occupied territories, only then to move on. Yet we did not first observe them in their natal sites, despite intensive fieldwork. Perhaps they prospected other sites during post-fledging dispersal and flew straight there to attempt to breed the following spring. Some further support for this idea is available: twice and in two different years, I observed a juvenile Northern Wheatear in site C for a few weeks during late summer which was born in site D a few months earlier (40 km distance). Remarkably, both these birds returned as breeding birds to site C, which they had prospected, and not to their natal site, D. This observation is in concordance with findings by Pärt et al. (2011), who found that 79% of returning adults established territories at sites they had been prospecting the preceding year as non-breeding adults. Thus, however little we know on requirements during late summer, it may be worthwhile to study those requirements and check their availability in abandoned areas, to persuade young birds to positively assess those areas while prospecting.

Feed-back mechanisms and prospects of remaining populations

Thusfar I showed, by using my results and results of others as reported in literature, which factors are limiting during which phase of the year, with an emphasis on the breeding season. Here I want to discuss briefly on feed-back mechanisms between the different demographic factors (fecundity, mortality, immigration and emigration) for a small population: site C. Besides, I want to reflect on possible future population developments if no conservation measures are being taken for all three sites (C, A and D).

Fecundity in C is affected by two main factors, predation and low hatchability of eggs. In years with high nest predation rates, such as 2012 when 64 % of all first broods in site C were predated upon, lowered hatchability of eggs (chapter 6) has little effect on the number of fledglings. Yet in years with very limited predation, up to 27 % hatching failure in otherwise successful nests reduces the total number of fledglings. Clutch size does not seem to be affected by density of breeding pairs, since clutch size of first broods in 2011 (year with most breeding pairs; 5.45 ± 1.0 SD, n = 20) did not differ from 2007 (year with few breeding pairs; 5.58 ± 0.67, n = 11; Mann-Whitney $U = 112.50$, $p = 0.75$) nor did second and repeat clutches differ between all years (Kruskall-Wallace $\chi^2 = 11.85$, $p = 0.11$). Perhaps the number of breeding pairs during the study period has never been high enough as to be influenced by density effects, or sample sizes are too small to detect effects (although effect-size must be limited if not detected with present sample sizes).

Small population sizes may lead to increased post-fledging survival of juveniles, due to ameliorated competition with older conspecifics (Tinbergen et al. 1985). Theoretically, small population sizes with fewer fledglings may then still provide equally many recruits the next year compared to years with many fledglings which suffer from more intense intraspecific competition after fledging, resulting in lower (post-fledging and migration-winter) survival.

The rates of immigration are very low, as shown in chapters 2 and 4. Yet, immigration is important for population growth, especially in site C, because this population is very small. As a consequence, every immigrant counts considerably to the population. Given the small numbers of immigrants, all three populations depend largely on their own fecundity and survival. As long as these two factors offset mortality and emigration, the population may be stable for a long time –unless stochastic events (e.g. disease, fire, deviant sex-ratio, genetic drift), inbreeding or sex-specific emigration affect population growth (Dale 2001). I have no strong indications yet for some of these events to affect population growth, but, admittedly, I did not pay enough attention to some of these events.

Under current population size and immigration rates, effects on e.g. allelic richness are to be expected, as is inbreeding (as reported above, two genetic brother-sister pairs were noted in site C during 2014). There are also indications of sex-specific dispersal: during 2007–2014, 11 males (temporarily) emigrated > 25 km from their natal sites (C and A) but only one female, which is interesting in the light of the generally held view that mostly females emigrate from the population (Clarke et al. 1997; Greenwood 1980; Weatherhead and Forbes 1994). Perhaps females are only under recorded in other sites because they are less visible than males?

To conclude, demographic factors may influence each other although indications for e.g. density dependent effects are limited. As long as fecundity and survival offset mortality and the paucity of immigrants, remaining populations are safe in short term. Yet, nestling and female mortality was very high in sites C and A, which showed a decline. Since realizing increasing immigration is much less feasible in the contemporary setting, both populations will probably go extinct if no measures are being taken. No adequate number of immigrants will probably safe those populations.

Additionally, we do not know if wintering conditions deteriorated with increasing human populations. And even if Northern Wheatear mortality during winter is higher than previously or even if wintering-effects carryover (sensu O'Connor et al. 2014) to affect breeding condition of females, measures taken on the breeding ground may still strongly increase local population sizes in spite of different large-scale limiters acting in the wintering site (Zwarts et al. 2009) and vice versa.

The Northern Wheatear in the dune ecosystem

With the results of this thesis in mind, I will here reflect on possible interactions between the population development of Northern Wheatears on the one hand and vegetation changes, arthropod diversity and population trends among different birds of dune grasslands on the other hand. Next, I present some ideas on conservation and management, partly directly based on my findings and partly based on expert-judgement, a term indicating the absence of proof but the presence of logically derived decisions, inferred from present-day knowledge. Thereafter, I do suggestions for interesting and fun future work and I finish with a general conclusion.

Changes in insectivores' assemblages and habitat quality of coastal dune grasslands

Population developments across species

Like Northern Wheatears, a number of other bird species have strongly declined or disappeared altogether from the Dutch coastal dunes in the recent past, such as (with year of probable disappearance as regular breeding bird): Little Owl *Athene noctua* (1977, Van Ommering and Verstrael 1987), Red-backed Shrike *Lanius collurio* (1982, Scharringa et al. 2010), Skylark *Alauda arvensis* (1993, Reisen 2011, few left northern mainland dunes (Scharringa et al. 2010)) and Whinchat *Saxicola rubetra* (2007, Scharringa et al. 2010), which have been preceded in the 1950s by Stone Curlew *Burrhinus oedicnemus* (Van den Berg and Bosman 1999), and a little later by Montagu's Harrier *Circus pygargus* (Bijlsma et al. 2001). Yet not all insectivores in dune grasslands declined, such as Meadow Pipit *Anthus pratensis*, Stonechat *Saxicola torquata* and Woodlark *Lullula arborea*, the latter also requiring sandy, open vegetation (Arlettaz et al. 2012).

Habitat quality as measured by vegetation height

Since ground-foraging songbirds prefer to forage in accessible short vegetation (Atkinson et al. 2004; Atkinson et al. 2005, Chapter 4), increases in sward height and density should eventually lead to a decrease in the amount of suitable habitat and changes in arthropod communities, as discussed in chapter 1. Changing arthropod communities may be important contributors to differential population developments of insectivorous birds in semi-natural grasslands.

Since average body size of arthropods declines with increasing vegetation succession (Siemann et al. 1999; Steffan-Dewenter and Tscharntke 1997), smaller birds can persist since size of insectivorous predators correlates positively with size of arthropod prey (Brandl et al. 1994; Fisher and Dickman 1993), provided accessibility to prey in the vegetation. One such persisting species is the Meadow Pipit, which is 20 % smaller in weight than Northern Wheatears occurring in the same dune grasslands. Meadow Pipits prefer to forage on short vegetation patches in between taller ones (Douglas et al. 2008; but see van Klink et al. 2014a; Vandenberghe et al. 2009) and they are still common breeding birds in the Netherlands (Boele et al. 2014), also in coastal dunes (Reisen 2011; Scharringa et al. 2010). Perhaps most passerines are adapted to feed their young with specific prey (as do Northern Wheatears, chapter 4), as reflected by the many subtle but distinct differences in beak shapes.

The exact prey-species will differ between sites, but sizes and loading for Northern Wheatears could be similar (within the same sub-species). For instance, the number of prey per feeding in site C and eastern Sweden (Moreno 1987) was very similar: 1.7 ± 0.9, respectively 1.7 ± 0.4 for males and 1.5 ± 0.4 for females (mean ± SD; Netherlands: sexes combined, n = 2765 filmed feedings out of 18 early and late broods 2008-2010). By contrast, I found the number of prey per feeding for Meadow Pipits in site C to be higher than for Northern Wheatears: 3.9 ± 0.7 (n = 302 feedings out of 3 nests 2009).

Preliminary data indicate that adult Tipulidae are an important prey of Meadow Pipits in site C, which is in concordance with other studies (Douglas et al. 2008; Evans et al. 2005), and this prey is of no importance to Northern Wheatear, in spite of often foraging alongside Meadow Pipits and hence, having the opportunity to catch Tipulids. A detailed food-ecological study of abundant (Meadow Pipit) and declined (Northern Wheatear) species would shed light on the relations between increased vegetation succession, changes in arthropod abundance and diversity, and differing trends in avian insectivore populations.

Habitat quality as measured by organic pollutants

Dietary differences between insectivorous species also influence the uptake of dioxins and other persistent organic pollutants from the soil. Exposure to organic pollutants from the soil is highly dependent on the diet: above-ground larvae do accumulate dioxins in insignificant amounts compared to their soil-dwelling counterparts. As I show in chapter 6, dioxins accumulate in soil-dwelling larvae, even in only slightly contaminated soils, to reach high concentrations in Northern Wheatear eggs. Therefore, accumulation in songbirds with a diet primarily consisting of above-ground larvae may be much less than in Northern Wheatears, which for a large part feed their young with prey originating from the top soil. Sensitivity of birds to dioxins and similar substances differs among species, depending on the exact nature of the aryl hydrocarbon receptor to which dioxins bind (Farmahin et al. 2013a; Farmahin et al. 2013b; Head et al. 2008; Karchner et al. 2006; Manning et al. 2012). So, adverse effects may not be apparent if the species is less sensitive. The extent to which our Northern Wheatears suffered from dioxins remains to be further studied, primarily by elucidating the nature of the aryl hydrocarbon receptor.

Habitat quality as measured by area suitable foraging habitat and food abundance

A strongly declined availability of short and open grassland offers smaller foraging sites for specialized birds, in increasing isolation from each other (chapter 2 and 5). It appears that foraging conditions in the remaining (study) populations are not affecting population growth more than expected given predation pressure: starvation of nestlings is very rare, first-year and adult survival rates are similar to those reported for other populations and we have no indications that first-year and adult mortality outside the natal or breeding site is very high (chapter 3). In short, there are no direct indications for structural food shortage in our three remaining relict populations.

This is important for two reasons. Firstly, it implies that the description of foraging habitat, food preferences and food abundances (chapter 4) can validly be seen as a baseline against which site managers can check habitat suitability of restored grasslands in calcareous dunes. Secondly, expanding the area size of populations is important, not at first to increase the number of breeding pairs but to decrease the impact of possible stochastic events, such as predation which may be depending on just a few individual predators which specialize in digging up Northern Wheatear nests. Everything else being equal, spreading chances of stochastic effects stabilizes the population as a whole (Hanski 1998).

Habitat quality as measured by predator abundance

Red Foxes are the main nest predator in sites C and A though occasionally other species cause nest mortality as well (e.g. mice, mustelids, lice, humans; chapter 2). Red Foxes were extinct in the coastal dunes until the late 1960s (Mulder 2005), after which they increased and stabilized around the mid-1990s (data: www.zoogdiervereniging.nl). At least in the southern coastal dunes of the Netherlands, the density of Red Foxes is among the highest reported outside towns (Mulder 2000). It has been generally assumed that mustelids declined strongly after population increases of Red Foxes.

Generally, Rabbits are by far the most important prey for Red Foxes, yet strong declines of birds, e.g. various gulls Laridae, are attributed to predation by Red Foxes (Mulder 2005). It remains uncertain if also songbirds declined after the advent of Red Foxes. Nevertheless, I show that their predation strongly affects reproductive output of Northern Wheatears, in some years, in sites C and A. Especially in small populations one or few foxes specialized in digging up nests may cause extinction, in the end. Therefore, nest protection measures should be taken into practice when depredation of nests by Red Foxes is strong.

Conservation and restoration

Species-specific studies seem not to be *en vogue* in “the landscape era” for a while (Simberloff 1998), when it comes to conservation of ecosystems. Yet, I would strongly plea not to forget the species which are, in the end, the stars in the landscape and easily observed indicators of quality. It is remarkable that, although many species are protected by law (even including aims for establishing a minimum population size), autecological studies about those species are only seldomly performed due to lack of funding. It goes without saying that ‘everything we need to know about a certain species is already published and therefore, we use those data’, quickly loses meaning in a rapidly changing world. We still lack knowledge about what particular species precisely need at the level of detail required for population and habitat restoration for those species. Study a few more species, with opposing trends but roughly similar diets and ecology, and I am quite sure it will be very revealing, fundamentally and practically.

If we would not have studied the Northern Wheatear with an open mind, we would not have found accumulation up to toxic levels of dioxins, which may affect many other insectivores in the dune ecosystem as well. Nor would we know the importance of tall vegetation patches in dune grasslands, which should not only be short and sandy. Lastly, now we know we really have to expand and connect the existing population of Northern Wheatears, since they seem very little inclined to disperse and successfully settle elsewhere.

Short-term conservation actions

Few breeding populations remain in the Netherlands and surrounding countries do not fare much better. The species recently became (almost) extinct in Belgium (D. Verbelen, *in litt.*) and is threatened with extinction as a breeding bird in Germany (Südbeck et al. 2008). This bleak situation, combined with strong philopatry in remaining Dutch populations, does not offer much hope for immigration from further away to rescue our by now small populations. What we still have has to survive, are we not to lose the species for a long time. Fortunately, things can be done immediately.

Nest protection

The primary task is to safeguard populations on the short term, by enhancing nest success (number of fledged young) in populations suffering from nest predation. I am aware that nest predation is a very normal and widely occurring phenomenon to which birds are generally well adapted. Yet, remaining populations of Northern Wheatears are very small (especially sites C and A) and in my opinion this warrants nest protection, if we are not to lose the species from those sites. Nest protection is easily done, by covering nests with mesh-wire, except from the entrance. This proves to be 100 % effective against predation by Red Foxes. It prevents foxes from excavating burrows front-first and from digging down straight to the nest. This method is cheap and takes very little time and effort.

Increase suitable habitat around populations

Single, small populations are very fragile as they are sensitive to sporadic stochastic events. Therefore, the spatial distribution of remaining populations must be increased over either a larger number of (adjacent) breeding cores (meta-population setting) or, preferable if possible, the extant population must be spatially extended (the single large reserve has gained preference over several small ones, since small populations still are vulnerable). To accomplish this, one needs funds and acres. Fortunately, remaining breeding populations are situated within nature reserves, already providing the acres.

To compensate for the lack of grazing by Rabbits and increased vegetation growth due to N deposition, managers deploy large herbivores as cattle and horses. Especially horses graze the vegetation very short, which results in a (at least structure-wise) suitable sward height for Northern Wheatears. In due course, arthropod abundance and diversity should be compared to values reported in chapter four. Overall, increasing grazing intensity to a level optimally controlling grass height negatively affects arthropod diversity due to disturbance, trampling, or due to competition for food (e.g. flowering plants). The expectations are that grazing by large herbivores contributes positively to faunal diversity when increasing vegetation heterogeneity outweighs mentioned negative results. Therefore, a generally low grazing pressure is recommendable, coupled with regular arthropod sampling to check for changes, be they positive or negative, of the faunal assemblages (Van Klink et al. 2014b).

In this respect, it is of practical interest that the highest abundance of preferred prey occurs in tall vegetation, where the birds do not forage. The notion that Northern Wheatears strictly need short vegetation needs some adjustment, which may be vital concerning the abundance of food and prey-specific accumulation of pollutants.

Nestboxes

One can consider offering nest-boxes (see for design recommended by the British Trust for Ornithology (BTO) on http://www.bto.org/sites/default/files/u15/downloads/publications/guides/nestbox.pdf) in sites, proven to be suitable in terms of available foraging area and food abundance. Provide many nest boxes, since Northern Wheatears appear to be remarkable picky in choosing their nesting burrow. We have observed, more than once, different breeding pairs to choose the very same burrow for nesting, in spite of dozens of other nesting opportunities. There may be ways to attract new pairs to suitable areas nearby existing remnant populations, by a combination of song play back during the arrival time, combined with the presence of dummy birds. It could be argued that the place to do this is south of existing populations, as individuals pass through these areas when migrating to their breeding sites.

Long-term measures

For more durable and less labour intensive actions, large scale process-approach measures (such as restoring abiotic dynamics to coastal dunes by allowing Aeolian activity to shape and form the landscape) may turn out to be successful eventually. Unfortunately, the results of the current projects are not yet positive for Northern Wheatears. Yet, we stress that both local, species-aimed, short-term measures should be applied, next to long-term landscape approaches. Landscape restoration by natural processes may take too long for remaining populations to survive, and therefore to grace the recreated landscape with their presence.

Suggested future work

As this thesis spans quite diverse topics, they may be seen as mere starting points for much further research on Northern Wheatears and the guild of ground-foraging insectivores.

1. It would be very instructive, both from an applied and more fundamental view, to study dietary differences (or similarities) of a variety of songbirds belonging to this guild, including species that increase and species that decrease in numbers. In similar habitat as the Northern Wheatears, Meadow Pipits occur in probably stable or increasing populations, as do Woodlarks and Stonechats. Given the increased vegetation mass, insect assemblages changed. Perhaps bird species smaller than Northern Wheatears, such as Meadow Pipit and Stonechat, depend on smaller prey which may in turn have habitat requirements nowadays more commonly found than those of insects upon which

Northern Wheatears prey. We have some indications that meadow pipits indeed feed their young with different, smaller prey. Too, the above bird species may be prone to different levels of predation to which they adjust for example feeding rates and clutch size. There remains much to learn from such comparative studies, with probably a huge relevance for management.

2. Arthropod abundance, availability and diversity in abandoned areas should be aim of intensified studies, in relation to management practises. In spite of high philopatry, it seems unlikely that seemingly suitable sites remain unsettled because of a mere few dozen kilometre distance.

3. Genetic differences exist between four studied populations. It would be instructive to know if more populations have their own genetic signature. If so, one may in great detail be able to study migratory connectivity between breeding population and wintering location in Sahelian Africa. By collecting DNA of Northern Wheatears on the wintering grounds one may assign birds to the breeding populations. Simultaneously, one can apply novel miniature GPS loggers to pinpoint exactly the wintering locations of individuals, thereby enabling field studies on the exact wintering sites of the populations of choice!

General Conclusion

This thesis is centred upon current limitations of Northern Wheatear populations on the breeding grounds in the Netherlands. The most important findings are that (1) dispersal between remnant populations is very limited, which has been the situation for some time; (2) fledgling mortality rate is higher on the breeding grounds than during migration-winter, especially for chicks from late broods; (3) food abundance is not limiting nestling survival but increases when patches of tall grasses are present within short dune grasslands; (4) hatching of eggs is limited, especially in site C, which may be due to dioxin toxicity; (5) predation is the most important threat to the short-term survival of the studied populations. These factors are not necessarily the ones causing the rapid decline of past few decades: it has been suggested that the Dutch demise is mainly due to habitat loss and deterioration as a result of increased N deposition and a crashed Rabbit population. In the end, determining which factors caused the decline are of less importance, conservation-wise, than factors governing contemporary populations growth, since conservation and site management lays in the hand of present-day site managers, researchers and policy makers.

As a result of very little dispersal between sites, conservation of individual populations is of the highest priority are we to conserve the species for the Netherlands at all in the short term. The single most determining factor is nest predation (sites C and A), which is, fortunately, easily to prevent against by nest protection measures. Habitat quality should be secured and the surface area of suitable habitat needs to be expanded, counteracting rapid vegetation succession, mostly due to nitrogen deposition. If Rabbit populations will again collapse, grazing by sheep should be commenced immediately since otherwise sward height will increase very rapidly.

My study, in which I closely worked together with fellow wheatearologists, proves the value of studying limitations of a single species, which is characteristic of a certain ecosystem, by a top-down analysis. In a bottom-up approach I would, for example, never have found the possible effects of dioxins of egg hatchability, which may be far more widely applicable to other species and ecosystems beyond Northern Wheatears of short dune grasslands. This study unveils that we still know next to nothing about how different population trends among insectivorous dune grassland birds can be explained. If one is to optimize site management to preserve the insect and avian communities, such explanations should be established by field study.

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Summary

Past decades witnessed a strong decline of many songbirds that forage on or near the ground in the Netherlands. Little is known about its direct causation but foraging conditions have notably deteriorated. Ground-foraging songbirds prefer to forage on short vegetation as it allows better accessibility to the ground. Nitrophilic grasses have expanded during the last decades and now cover large expanses of former nutrient poor habitats. This has been a result of acidification, N deposition and, until recently, strongly declined grazing by Rabbit (*Oryctolagus caniculus*). Therefore, the amount of suitable short-grown habitat has declined. And yet, localities exist where some of these songbirds still occur or, interestingly, do not occur in spite of apparent suitable conditions for ground-foraging birds. One of these species is the Northern Wheatear (*Oenanthe oenanthe*), the main character of this thesis. It still occurs in the northern coastal dunes, the Wadden Islands and very localized inland, with only a single relatively large remaining population. Breeding numbers have started their most recent decline in the 1980s. The central aims of this thesis are to determine how the persisting populations are limited on the Dutch breeding grounds and if conservation of this species is feasible (**chapter one)**.

To find answers, three populations were studied from 2007 onwards and, with a varying intensity, have been so ever since. These sites are: a coastal population near Castricum (C), harboring about 7 – 24 pairs between 2007 and 2013; site Den Helder (D), another coastal population 40 km north of C with quite stable breeding numbers of about 50 females; and site Aekingerzand, the largest inland population with about 17 to 50 pairs and about 120 km away from both coastal sites. All sites are managed as nature reserves and access by the public is limited to paths and roads.

It appeared that population size in the three sites is driven by different demographic parameters (**chapter two**). Population size in site C, being the smallest of all, was sensitive to immigration, which on average appeared to be rare, but this factor contributed most strongly to yearly variation in population growth here. Population growth in A was most strongly affected by low female survival. Site D did not appear to be influenced by any particular factor, indicating that population growth is stable. Variation in limiting factors between populations is an important find, as it implies that conservation measures should be fine-tuned to a local scale; measures effective for one population may thus not be so in the next. Site C would probably benefit from creating additional suitable habitat towards site D, to enhance immigration and therefore, decrease sensitivity to stochastic events. Site A would benefit from direct nest protection, as females are often depredated during a nest predation event. Site D would benefit from ‘doing nothing’ as long as conditions stay as they are. The fact that dispersal is very rare between populations has consequences for conservation because safeguarding one large putative source population may not result in emigrants to settle in abandoned areas.

In **chapter three** I explore first-year survival for early and late fledged Northern Wheatears. Furthermore, survival estimations were divided in two periods: post-fledging (fledging to migration) and migration-winter. By using very frequent repeat observations we show first-year survival of early fledglings is higher than of late fledglings (10-16% higher). Survival per two-week interval during post-fledging and migration-winter is higher for early fledglings than for late fledglings. Compared to most other songbirds, post-fledging survival is very high. Overall migration-winter mortality is higher than post-fledging due to the much longer duration of the winter period. I conclude that the factors affecting post-breeding survival of late fledglings continue to exert their effects during migration-winter. However, lower survival of late-fledglings may also be due to different factors operating on migratory stop-over sites related to ongoing season, such as food availability. Conservationwise, the results emphasize the importance of increasing the number of early fledglings, for instance by nest protection, since young from repeat clutches are late fledglings by definition, which survive worse.

In **chapter four**, the relationships between arthropod abundance in vegetation of different height (pioneer, short < 5cm and tall > 5cm), vegetation preferences of foraging parental Northern Wheatears, and the diet of nestlings were determined. It appeared that Northern Wheatears strongly preferred to forage on short vegetation, most pronounced in feeding cores (foraging sites were 50% of all foraging actions took place). Nevertheless, highest overall arthropod abundance and species richness occurred in tall grass vegetation, where most of the preferred prey (> 5% in diet numerically) also occurred. Only caterpillars occurred more in short compared to tall vegetation. This implies that patches of tall vegetation may be important elements in the Northern Wheatear habitat, as they may function as sources from where arthropods disperse into short vegetation. Therefore, I conclude that restoration of dune grasslands should not only aim at short and open vegetation, but also that patches with tall grasses should occur, as to increase prey abundance for insectivores as the Northern Wheatear.

In **chapter five** I explore whether the observed high degree of philopatry has led to genetic differences between populations, being the three Dutch sites and a site located in the Rhineland-Palatinate, southwest Germany. It appeared that strong genetic (microsatellite) differences exist between all four sites. The fact that even both coastal populations (sites C and D) differ from each other, indicates that populations have been very little prone to dispersal between sites. It remains uncertain whether strong philopatry is an inherent trait in Northern Wheatears or an acquired taste, as a result of declining populations in a matrix of increasingly unsuitable habitat. I conclude that limited dispersal between populations is not enough to cause panmixia among remaining populations, and chances of recolonizing restored sites are small, especially when located more than a few kilometres away from existing breeding sites.

In **Chapter six** I report of low hatching rates of eggs (average 80% of all eggs) and embryonic malformations in those eggs. Some of the malformations (feather growth on the amniotic sac) indicate the effects of hormone-mimicking substances, such as persistent organic pollutants. Indeed, concentrations of especially dioxins were high, presenting a possible cause. Further, the accumulation of dioxins from soil into the Northern Wheatear food chain was established: it appeared that soil concentrations were low and similar to those in other industrialized countries. Furthermore, above-ground herbivores such as caterpillars and grasshoppers contained hardly any dioxins but soil-dwelling prey, especially long-lived larvae of click beetles, accumulated dioxins. In the end, Northern Wheatears bio-accumulate high concentrations by consuming such ground-dwelling prey. Interestingly, hatching rates in a Swedish reference population were much higher (94%) and dioxin concentrations six fold lower. I conclude that we have indications for dioxins to affect hatching of eggs, and to subsequently affect population growth.

Chapter seven contains the overall synthesis, conservation measures and suggested future work. When compared to other breeding populations first-year survival rates are all similar, indicating that population growth is not limited more than is to be expected based on literature. Adult survival is also similar, except for site A where many breeding females were depredated during breeding by Red Foxes. Simple nest protection measures proved 100% effective against foxes. The number of fledglings produced per female differed slightly between sites with site D highest and site A lowest. Productivity was perhaps somewhat lower than in, for instance, the Uppsala population (Sweden). This is noteworthy, especially since double breeding is very rare in Uppsala. Nest predation rates are not distinctly different between the Dutch populations and others, so predation rates do not explain the lower number of fledglings per Dutch female. Low hatchability of eggs in the studied Dutch populations, especially in site C, is not present in Uppsala. In site C, an average 20% of all eggs do not hatch while in Uppsala this is 6%. Every other parameter being equal between sites, low egg hatchability limits the number of fledglings and limits population growth as an additional factor to fecundity and survival.

Conservation of all remaining populations is important if one is to safeguard the species as a breeding bird for the Netherlands. Recolonisation of abandoned or restored sites away from the birds' present distribution is not to be expected in the short term, as a result of strong philopatry. The biggest dangers for small populations, sites C and A, is being small in itself. Every depredated nest or female decreases the population considerably. Depredation of 64% of all nests, as in site C during 2012, poses a large threat by strongly limiting the number of offspring. For sites C and D applies that if Rabbits decline as they did in the 1950s and again in the 1990s, the vegetation becomes unsuitable for Northern Wheatears within one breeding season. In site A, sheep are the main grazers. To decrease vulnerability to stochastic events, site managers should aim to increase the coverage of suitable habitat adjacent or very close to existing occupied sites. Too, nest protection measures should be taken to increase the number of fledglings, especially of first broods. Restoring ecosystems with the idea that (animal) species will return by themselves may not apply for Northern Wheatears, at least not in the short term.

Samenvatting

In de afgelopen decennia zijn veel zangvogels die op of nabij de bodem hun voedsel vinden afgenomen in Nederland. Hoewel weinig bekend is over directe oorzaken, zijn de de foerageeromstandigheden over het algemeen sterk verslechterd. Deze groep zangvogels foerageert vooral in korte vegetatie omdat bodemprooien daar beter toegankelijk zijn dan in hoge vegetatie. Stikstofminnende grassen zijn sterk toegenomen in bedekkingsgraad, ook in voormalige voedselarme milieus. De oorzaken voor deze uitbreiding zijn verzuring, vermesting en, tot zeer recent, de sterk afgenomen konijnenbegrazing. Dit alles heeft geleid tot een afname van geschikt, kort-begroeid en open habitat. En toch zijn er plekken waar deze zangvogels nog voorkomen of, wat erg interessant is, plekken waar ze juist niet voorkomen hoewel de omstandigheden geschikt lijken. Een van deze soorten is de Tapuit (*Oenanthe oenanthe*), het onderwerp van deze studie. De aantallen broedvogels zijn sterk afgenomen sinds eind jaren '80, begin jaren '90. Deze vogel komt nog steeds voor in de noordelijke vastelandsduinen, de Waddeneilanden en lokaal in het binnenland, waar nog maar een relatief grote populatie gedijt. Het hoofddoel van mijn onderzoek is te achterhalen hoe de resterende populaties gelimiteerd zijn in hun groei, en hoe de soort beschermd kan worden.

Om antwoorden te vinden zijn drie populaties bestudeerd vanaf 2007 en, met een variërende intensiteit, dat zijn ze tot de dag van vandaag. Deze populaties zijn: een duinpopulatie nabij Castricum (C), waar 7 – 24 vrouwtjes broedden tussen 2007 en 2014; een duinpopulatie tussen Groote Keeten en Den Helder (D), ongeveer 40 km noord van C met een stabiele populatie van ongeveer 50 vrouwtjes; en een populatie op het Aekingerzand, in Nationaal Park Drents-Friese Wold. Dit is de grootste binnenlandse populatie met tussen de 17 tot 50 vrouwtjes, ongeveer 120 km verwijderd van beide kustpopulaties. Alle drie de locaties zijn natuurgebieden en toegang is beperkt tot wandelpaden en wegen (**hoofdstuk 1**).

Het blijkt dat de populatiegroei per locatie door verschillende demografische factoren wordt gestuurd. Demografische factoren zijn geboorte, sterfte en migratie, die variëren met leeftijd (**hoofdstuk 2)**. Populatiegroei in C, de kleinste populatie, is meer dan in beide andere locaties gevoelig voor immigratie. Hoewel immigratie over het algemeen een zeldzaam fenomeen blijkt te zijn, verklaart deze factor toch de variatie in populatiegroei tussen locaties. Populatie A wordt het sterkst beïnvloed door lage overleving van vrouwtjes. Populatie D blijkt door niet een factor specifiek te worden beïnvloed; dit indiceert dat deze populatie stabiel is (zoals ook uit tellingen blijkt). Dat populaties door verschillende factoren worden geremd is een belangrijke vondst, omdat het indiceert dat beschermingsmaatregelen moeten worden aangepast aan lokale omstandigheden; maatregelen die in een populatie effectief zijn, zijn dat dus niet meteen in een andere populatie. Populatie C zal waarschijnlijk profiteren van uitbreiding van geschikt habitat in de richting van populatie D, om zo immigratie te stimuleren en ook de gevoeligheid voor toevallige (stochastische) effecten (zoals brand, predatie, ziekte) te verkleinen. In locatie A is nestbescherming een goede optie omdat vrouwtjes vaak op het nest gepredeerd worden. In populatie D moet niets worden gedaan zolang de omstandigheden niet veranderen. Het feit dat dispersie tussen populaties een zeldzaam fenomeen is heeft consequenties voor bescherming: het beschermen van een grote bronpopulatie resulteert waarschijnlijk niet snel in emigranten die in verlaten gebieden gaan broeden.

In **hoofdstuk drie** onderzoek ik de overleving in het eerste levensjaar van vroeg en laat uitgevlogen jonge Tapuiten. Vervolgens is deze overleving in meer detail bekeken: voor zowel de periode na-uitvliegen-tot-wegtrek als de periode trek-winter. Door analyse van zeer frequente waarnemingen van gekleurringde uitgevlogen jongen laten we zien dat eerste-jaars overleving van vroege jongen 10-16% hoger is dan van late jongen. Daarnaast blijkt de overleving per tweewekelijks interval gedurende na-uitvliegen en gedurende trek-winter hoger te zijn voor vroege jongen dan voor late jongen. Toch is de totale trek-winter overleving lager dan na-uitvliegen omdat de periode trek-winter veel langer duurt. Ik concludeer dat factoren die na-uitvliegen de overleving beïnvloeden, wellicht ook nog effect hebben gedurende trek-winter. Daarnaast kunnen andere factoren specifiek voor trek-winter een rol spelen bij de lagere overleving van late jongen, zoals voedselbeschikbaarheid op de rustplaatsen tijdens trek. Deze studie laat zien dat het voor beschermingsdoeleinden van belang is met name de aantallen vroege jongen te laten groeien, bijvoorbeeld door nestbescherming, omdat jongen uit herlegsels (na predatie van het eerste nest) automatisch late jongen zijn, die slechter overleven.

In **hoofdstuk vier** onderzoek ik de relaties tussen arthropoden abundantie in vegetatie van variërende hoogte (pioniersvegetatie, lage vegetatie < 5cm en hoge vegetatie > 5cm), de vegetatievoorkeur van voedselzoekende Tapuiten, en het dieet van nestjongen. Het blijkt dat Tapuiten een sterke voorkeur hebben voor lage vegetatie, met name in zogenaamde foerageerkernen (plekken binnen het territorium waar 50% van alle foerageeracties plaatsvindt). Toch komen zowel de grootste aantallen als de meeste soorten arthropoden voor in hoge vegetatie, waar ook de meeste geprefereerde prooien voorkomen (prooien die > 5% van het numerieke dieet uitmaken). Alleen rupsen komen vooral voor in korte vegetatie. Dit alles betekent dat plekjes met hoge vegetatie belangrijke elementen kunnen zijn in het Tapuitenhabitat, omdat ze als bronnen kunnen functioneren van waaruit arthropoden zich kunnen verspreiden in lage vegetatie. Daarom concludeer ik dat herstel van duingraslanden niet alleen gericht moet worden op korte en open vegetatie, maar dat ook plekjes met hoge vegetatie moeten voorkomen, om zo bij te dragen aan een groot aantal prooien voor insectivoren als de Tapuit.

In **hoofdstuk vijf** onderzoek ik of de hoge mate van filopatrie (terugkeer naar dezelfde geboorte- danwel broedlocatie) heeft geleid tot genetische verschillen tussen de drie Nederlandse populaties en een populatie in de Rheinland-Pfalz, in Zuidwest-Duitsland. Het blijkt dat sterke genetische (microatelliet) verschillen bestaan tussen alle vier de populaties. Het feit dat zelfs beide duinpopulaties (C en D) van elkaar verschillen, indiceert dat populaties inderdaad erg weinig individuen uitwisselen. Het is nog onzeker of deze zeer sterke filopatrie een inherente eigenschap is van Tapuiten, of verkregen als aanpassing aan een afnemende hoeveelheid geschikt habitat in een groter wordende zee van ongeschikt habitat. Ik concludeer dat de bescheiden mate van dispersie tussen populaties onvoldoende is om genetische vermenging in stand te houden, en dat de kansen op herkolonisatie van herstelde gebieden klein zijn, vooral wanneer deze gebieden op wat grotere afstand van bestaande broedpopulaties liggen.

In **hoofdstuk zes** laat ik zien dat gemiddeld slechts 80% van de eieren uitkomen (in C) en dat embryo's afwijkingen vertonen. Enkele van deze afwijkingen, zoals veergroei op het amnionvlies, indiceren de aanwezigheid va hormoon-imiterende stoffen, zoals persistente organische gifstoffen. De concentraties van met name dioxines zijn inderdaad verhoogd, waardoor deze stoffen als mogelijke veroorzaker kunnen worden gezien. Daarnaast heb ik de accumulatie van dioxine in de voedselketen van de Tapuit onderzocht: van de bodem via prooien tot in de Tapuit. De hoeveelheden dioxine in de bodem zijn laag en goed vergelijkbaar met die in andere geïndustrialiseerde landen. De concentraties in bovengrondse prooien als rupsen en sprinkhanen zijn verwaarloosbaar. Bodembewonende prooien accumuleren wel dioxines, met name lang-levende larven van kniptorren. Uiteindelijk accumuleren dioxines in Tapuiten door het verslinden van zulke prooien. Het is interessant dat het uitkomstpercentage van eieren in een Zweedse referentiepopulatie veel hoger is (94%) en dat dioxineconcentraties zes keer lager zijn. Ik concludeer dat er indicaties zijn voor een negatief effect van dioxines op het uitkomen van Tapuiteneieren, en dat de popuatiegroei zo geremd wordt.

Hoofdstuk zeven bevat de synthese, aanwijzingen voor beschermingsmaatregelen en suggesties voor verder werk. Vergeleken met buitenlandse Tapuitenpopulaties is de eerste-jaarsoverleving even hoog, wat indiceert dat populatiegroei is niet meer gelimiteerd wordt door eerste-jaarsoverleving dan verwacht op grond van de literatuur. Ook adulte overleving is hetzelfde, behalve voor A waar veel vrouwtjes zijn gepredeerd door Vossen. Eenvoudige nestbeschermingsmaatregelen blijken 100% effectief te zijn tegen Vossen. De aantallen uitgevlogen jongen per vrouwtje verschillen wat tussen de populaties, in D het hoogst en in A het laagst. Bovendien was deze jongenproductie wat lager dan in, bijvoorbeeld, de Zweedse populatie. Dit is van belang, vooral omdat de Zweedse vogels veel minder vaak een tweede legsel produceren. De mate van nestpredatie verschilt niet sterk tussen de Nederlandse populaties en die in het buitenland, dus predatie verklaart niet het lagere aantal uitgevlogen jongen per Nederlands vrouwtje. Het lage uitkomstpercentage van eieren, met name in C (80%), is afwezig in Uppsala, Zweden (94%). Indien alle andere parameters hetzelfde zijn tussen de populaties, beperkt het lage uitkomstpercentage het aantal uitgevlogen jongen, en de populatiegroei, als additionele factor naast overleving.

Bescherming van alle resterende populaties is van belang wanneer we de Tapuit als broedvogel voor Nederland willen behouden. Herkolonisatie van verlaten of herstelde gebieden verder weg van resterende populaties is niet te verwachten op korte termijn, als gevolg van sterke filopatrie. Het grootste gevaar voor kleine populaties, C en A, is dat ze klein zijn. Hierdoor verkleint elk gepredeerd nest of elk gepredeerd vrouwtje de populatiegroei relatief sterk. Predatie van 64% van alle nesten, zoals in C gedurende 2012, is een grote bedreiging voor deze kleine populatie omdat het aantal uitgevlogen jonge sterk gereduceerd is. Voor C en D geldt dat als Konijnen weer sterk gaan afnemen, zoals in de jaren ’50 en in de jaren ’90, de vegetatie snel ongeschikt wordt voor Tapuiten, binnen een broedseizoen. In A zijn schapen de belangrijkste grazers. Om de kwetsbaarheid ten aanzien van stochastische effecten te verkleinen, kunnen beheerders de oppervlakte geschikt habitat aangrenzend of zeer nabij nog bestaande populaties vergroten. Tevens moeten nestbeschermingsmaatregelen worden genomen om het aantal uitgevlogen jongen te vergoten, vooral van eerste broedsels. Herstel van ecosystemen met het idee dat (dier)soorten vanzelf zullen terugkeren lijkt niet te gelden voor Tapuiten, althans niet op de korte termijn.

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Herman,
3 november 2014, Wageningen.

Curriculum Vitae

List of publications

Hendrik Herman van Oosten zag op 10 juni 1978 zijn eerste levenslicht in Zutphen, waar het 18 jaar lang goed toeven was. Vanaf 1990 zat hij op het Baudartius College waar hij in 1996 zijn gymnasiumdiploma in de wacht sleepte. Nog leuker was het gevogel, aanvankelijk met de 7x50 en rode Peterson van pa, daarna met de 8x42 quick focus ('Het Kneus'), de Tirion (met handtekening van Nico de Haan) en vanaf 11 juni 1994 met De Johnson, met JNM, met Jeroen Voerknecht of alleen. Gouden tijden!

In september 1996 begon hij aan zijn studie Biologie en rondde die af, als populatiebioloog, in augustus 2002 aan de Landbouwuniversiteit Wageningen / Wageningen University / Wageningen UR. Prachtig drie maanden vogels inventariseren in Noord-Israel met Bas van de Meulengraaf (dat is tussen de bedrijven door inderdaad gebeurd), daarna fijn 12 maanden lang *Trichogramma* wespen bestuderen op Entomologie onder leiding van Richard Stouthamer & Ties Huigens in 2001. Tenslotte, in 2002, een maand of vijf op dampend Borneo naar gedraaide en fraaie *Opisthostoma* slakjes gekeken –overigens niet alleen naar slakjes- met Merijn Salverda en onder aangename leiding van Menno Schilthuizen. Toen was de koek op en begon het post-studie bestaan –beginnend met een half jaar accu's en remschijven sjouwen, magazijnen leegruimen en schoonmaken, vooral veel schoonmaken, alles om een reis van drie maanden naar Peru te bekostigen, voor de vogels.

In 2004 heeft Herman een jaar overvliegende trekvogels geteld in De Blauwe Kamer en in 2005 een jaar als onderzoeker gewerkt als entomoloog, samen met Tom van Noort, op de University of California te Riverside, bij Richard Stouthamer. Vervolgens met het daar verdiende geld weer een maand naar Peru gegaan en, sinds april 2007, in dienst bij Stichting Bargerveen. Daar begon hij met het tapuitenwerk, begeleiden van studenten en anderszins. Een mooie en leerzame tijd, met een promotietraject erin verweven aan de Radboud Universiteit te Nijmegen. Op-en-af heeft Herman bij Stichting Bargerveen gewerkt tot september 2013, afgewisseld door trouwen met Agata, gevogel op *the bird continent* en de geboortes van zijn twee kinderen (Kamiel en Julia in 2012 en 2014).

Tussen december 2013 en mei 2014 werd hij voor vijf maanden aangesteld bij Dierecologie, IWWR, van de Radboud Universiteit om het tapuitenproefschrift af te schrijven. Nu, hier is het resultaat!

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Some photos

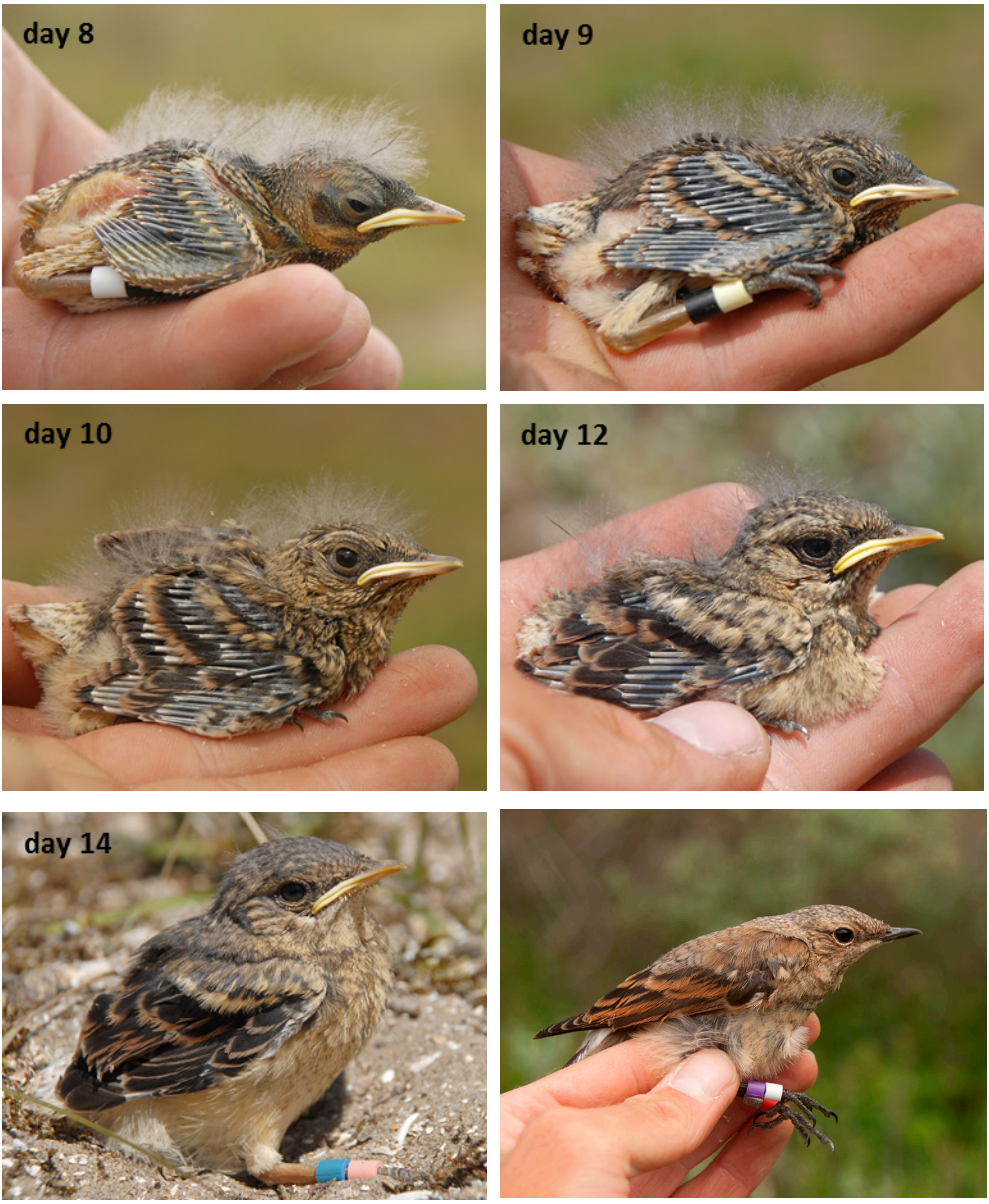
Photo 1. Adult male: a Northern wheatear at its best, April 16th 2011. Born June 17th 2009, last observation August 13th 2011 at Vogelduin (photo R. Versluijs)

Photo 2. Female at Vogelduin, July 13th 2009. Born June 19th 2008, last observation July 14th 2009 (photo R. Versluijs)

Photo 3. Rarely observed nest built under dense Crowberry (*Empetrum nigrum*) at Aekingerzand, June 3rd 2007 (photo S. Waasdorp)

Photos 4 and 5. Nest predation by red foxes is frequent and a heap of sand at the nesting burrow is a bad omen. Indeed, after some excavation the nest is torn apart outside the burrow. Vogelduin May 23rd 2013, BLNN.1 (photos H.H. van Oosten)

Photo 6-11. Nestling appearance, weight and wing length. The same nestling (brood ZVW.2 2009) portrayed between days 2-7 of age. Day 2: wing 8mm and weight 4 gr; day 3: 9.5mm and 6 gr; day 4: 11 mm and 9 gr; day 5: 15.5 mm and 11.75 gr; day 6: 20 mm and 14.2 gr; day 7: 26 mm and 16.1 gr (photos R. Versluijs)



Photos 12-17. Day 8: 32.5 mm and 19.8 gr; day 9: 38 mm and 21.9 gr; day 10: 43.5 mm and 23.3 gr. Day 11: no photo. Day 12 and 14: different bird than preceding one (brood ZZ.1 2009). Fledged nestling not yet molting into first-winter plumage, June 24th 2013 (brood KB.1 2013)

Photo 18. Unfortunate but still alive nestling: broken beak, muscle along wing partly devoured. Predator mice or shrews? Vogelduin, July 9th 2010 (907) (photo H.H. van Oosten).

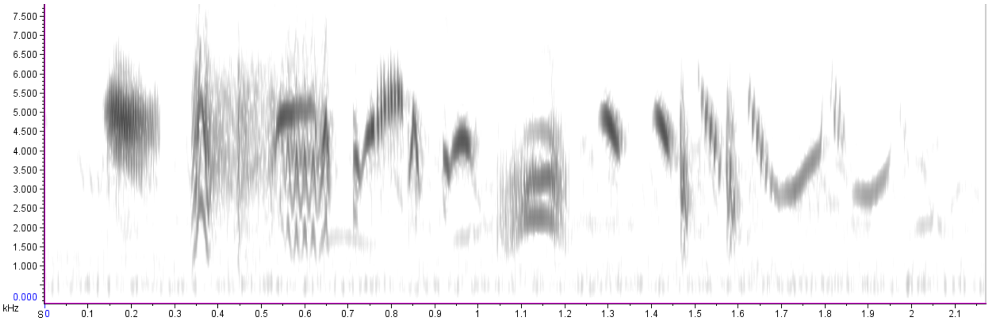


Photo 19. A song worth seeing. Little is known about the song of the Northern Wheatear. Songbirds have two voiceboxes, we mankind but one, which they can use simultaneously –imagine the neural control of all the muscles involved! At 0.6 sec (horizontal axis) you can see both voiceboxes in action: one sings the triangular phrase and the other the broad, dark horizontal tone. 2nd cy male at Aekingerzand, April 2012 (recording and sonogram H.H. van Oosten).

Verschenen in de serie ‘Mechanisms and constraints in biodiversity conservation and restoration’:

1. Verberk, W.C.E.P. 2008. Matching species to a changing landscape – aquatic invertebrates in a heterogeneous landscape.

2. Remke, E. 2009. Impact of atmospheric deposition on lichen-rich, coastal dune grasslands.

3. Van Kleef, H.H. 2010. Identifying and crossing thresholds in managing moorland pool macroinvertebrates.

4. Vermonden, K. 2010. Key factors for biodiversity of urban water systems.

5. Van Turnhout, C.A.M. 2011. Birding for science and conservation. Explaining temporal changes in breeding bird diversity in the Netherlands.

6. Schipper, A.M. 2011. Multiple stressors in floodplain ecosystems. Influences of flooding, land use and metal contamination on biota.

7. Van Duinen, G.A. 2013. Rehabilitation of aquatic invertebrate communities in raised bog landscapes.

8. Van Noordwijk, C.G.E. 2014. Through arthropod eyes. Gaining mechanistic understanding of calcareous grassland diversity

9. Verbrugge, L.N.H. 2014. Going global: perceiving, assessing and managing biological invasions

10. Van Oosten, H.H. 2015. On the brink of extinction. Biology and conservation of Northern Wheatears in the Netherlands.

Het samenwerkingsverband tussen Natuurplaza en de Radboud Universiteit Nijmegen (IWWR) ontwikkelt, bundelt en verspreidt kennis op het gebied van herstel en behoud van biodiversiteit en ecosystemen.

In Natuurplaza participeren:
- Stichting Bargerveen
- Vereniging SOVON vogelonderzoek Nederland
- Stichting reptielen, amfibieën en vissenonderzoek Nederland (RAVON)
- Stichting floristisch onderzoek Nederland (FLORON)
- Zoogdiervereniging
- Vereniging onderzoek flora en fauna (VOFF)

Deze combinatie van organisaties verbindt het verspreidingsonderzoek met het wetenschappelijk onderzoek. De koppeling van veldwaarnemingen in ruimte en tijd met ecologische lab-, veld- en beheerexperimenten resulteert in innovatieve kennis. De Natuurplaza partners hebben een breed en actief netwerk van vrijwilligers. Hierdoor is er een continue vernieuwing, ontwikkeling en doorstroom van kennis, waardoor maatschappelijke vraagstukken snel en adequaat aangepakt kunnen worden.

Stichting Bargerveen heeft als doel het ontwikkelen van kennis voor systeemgericht natuurherstel met focus op fauna en het (inter)nationaal verspreiden van kennis. Daarnaast is het bijdragen aan de opleiding van studenten en junior onderzoekers een belangrijke doelstelling. De onderzoeksvragen die de stichting beantwoordt komen voort uit de problemen waar beheerders of beleidsmakers tegenaan lopen. Veel thema's zijn gerelateerd aan de effectiviteit van de beheersmaatregelen, alsmede aan de VER-thema's, zoals verzuring, vermesting en verdroging. Het onderzoek wordt uitgevoerd in binnen- en buitenland en in een scala van landschappen, waaronder de kustduinen, stuifzanden, kalkgraslanden, laagveenwateren, hoogvenen, heide en vennen.

Stichting Bargerveen is met de andere Natuurplaza partners gehuisvest binnen de Radboud Universiteit Nijmegen en werkt nauw samen met de afdelingen dierecologie en -ecofysiologie, milieukunde, aquatische ecologie en milieubiologie.

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