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1 Radboud University, Institute for Water and Wetland Research, Department of Animal Ecology and Physiology, Nijmegen, The Netherlands
2 EIS Kenniscentrum Insecten, Leiden, The Netherlands
3 German Centre for Integrative Biodiversity Research (iDiv), Leipzig, Germany
4 Stichting Willem Beijerink Biologisch Station, Loon, The Netherlands
5 Natuurmuseum Brabant, Tilburg/De Kaaistoep, The Netherlands
6 Beatrixstraat 1, Goirle/De Kaaistoep, The Netherlands

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Affiliations:	1 Radboud University, Institute for Water and Wetland Research, Department of Animal Ecology and Physiology, Nijmegen, The Netherlands 2 EIS Kenniscentrum Insecten, Leiden, The Netherlands 3 German Centre for Integrative Biodiversity Research (iDiv), Leipzig, Germany 4 Stichting Willem Beijerink Biologisch Station, Loon, The Netherlands 5 Natuurmuseum Brabant, Tilburg/De Kaaistoep, The Netherlands 6 Beatrixstraat 1, Goirle/De Kaaistoep, The Netherlands
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Project management:	Dr. Eelke Jongejans, Department of Animal Ecology and Physiology, Institute for Water and Wetland Research, Radboud University, Heyendaalseweg 135, 6525 AJ Nijmegen, The Netherlands, e-mail: Eelke.Jongejans@ru.nl
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[1] Radboud University, Department of Animal Ecology and Physiology, Nijmegen, The Netherlands

[2] EIS Kenniscentrum Insecten, Leiden, The Netherlands

[3] German Centre for Integrative Biodiversity Research (iDiv), Leipzig, Germany

[4] Stichting Willem Beijerink Biologisch Station, Loon, The Netherlands

[5] Natuurmuseum Brabant, Tilburg/De Kaaistoep, The Netherlands

[6] Beatrixstraat 1, Goirle/De Kaaistoep, The Netherlands

Abstract

Recently, reports of insect declines prompted concerns with respect to the state of insects at a global level. Here we present the results of long-term insect monitoring from two locations, De Kaaistoep, and nature reserves in Drenthe, both in the Netherlands. We report the trends in beetles (Coleoptera), macro-moths (macro-Lepidoptera), caddisflies (Trichoptera), lacewings (Neuroptera), mayflies (Ephemeroptera) and true bugs (Hemiptera), using light traps, and ground beetles (Coleoptera: Carabidae), using pitfall traps. Based on data from light traps, macro-moths, ground beetles, and caddisflies have declined in the mean number of individuals counted per evening, with annual rates of decline of 3.8, 5.0 and 9.2% respectively. Other orders appeared stable (true bugs) or had great uncertainty in the trend estimate (lacewings and mayflies). Based on data from pitfall traps, ground beetles showed a mean annual decline of 4.3% in total annual numbers over the period 1985-2016. However declines appeared stronger after 1995 or when only including traps that were operated for longer periods. Annual trends in total numbers of macro-moths were comparable (but less) to the average of the individual species annual trends. Contrary, annual trends in total numbers of ground beetles were more severe than the average of the individual species trend, suggesting that abundant species may fare worse than rare ones. Our results suggest a reduction in biomass in macro-moths of approximately 61% and in ground beetles of at least 42%, over a period of 27 years. Estimated weights of ground beetles and macro-moths were not significantly related to individual species trends, suggesting that heavy species did not contribute disproportionately to the biomass decline. Our results broadly echo recent reported trends in insect biomass in Germany, even though the comparison is only partly possible.

Contents

Introduction

Insects, despite their huge diversity, and despite their importance to ecosystem functioning, are generally understudied, maybe with the exception of butterflies and wild bees. For those groups that are monitored, most studies reveal declining patterns in recent decades (Van Dyck et al., 2009; Groenendijk and Ellis, 2011). Recently, a large decline in flying insect biomass was reported for German lowland nature reserves (Hallmann et al., 2017), prompting concerns with respect to the state of insects at a global level.

For most insect taxa, no long-term, country-wide monitoring systems exist in the Netherlands. Noticeable exceptions include butterflies, dragonflies and moths (van Swaay et al., 1997; Van Dyck et al., 2009; Groenendijk and Ellis, 2011; Termaat et al., 2015). However, in the Netherlands, two long-term datasets (each from a single location or area) that cover a wider diversity of insects may possibly provide further insights in the state of Dutch insects.

Analysis of insect trends over time poses significant challenges. First, it is often hard to differentiate long-term trends form natural cycles (Fewster et al., 2000; Benton et al., 2002), particularly in absence of prolonged sampling over many years. Secondly, seasonal activity of the insects plays a significant role in the numbers trapped, particularly when species have multiple generations and peaks throughout the year. Thirdly, weather variation, possibly at multiple time spans and with variable time lags, likely influences the population dynamics and activity of the insects (Johnson, 1969; van Wielink, 2017*b*,*a*; Jonason et al., 2014). Hence, sampling characteristics such as timing (both in season as well as during the day) and duration of sampling, can play important roles in the numbers caught, and hence trend estimates. If meaningful trends of insect numbers are to be derived, such aspects need to be corroborated explicitly into the analyses.

Here we report on insect trends, while correcting for sampling and weather aspects, and assess the relative performance of the various insect orders. For the best-studies and most species-rich orders, beetles and macro-moths, we also report trends per family and species. Additionally, based on general weight/length relationships (Sabo et al., 2002; García-

Barros, 2015), we attempt to derive estimates of trends in total biomass, in order to compare these to the recently reported trends in flying insect biomass in Germany (Hallmann et al., 2017).

Methods

Data were collected at two (groups of) sites. For each site we describe the sampling protocols, dataset and statistical analysis. A summary description of available data is given in Table 1. In addition, we obtained data from two KMNI weather stations (for De Kaaistoep data: weather station Gilze-Rijen, for Wijster data: weather station Eelde, at respectively 3.6 and 39.5 km from trapping locations), from which we extracted a number of relevant parameters for effect analysis on insect numbers, as well as for correcting trends.

Table 1: Summary of data used in the present analysis. For each order and family we show the number of samples, number of years and time span, total numbers counted, number of species for which species trends could be determined (with total number of species in the dataset between brackets)

Order	Family	Number of samples	Number of years	Years	Sum counted	Number of locations	Number of Species	Location
Lepidoptera		447	19	1997:2006,2009:2017	49541	1	170(477)	De Kaaistoep
Coleoptera		514	19	1997:2006,2009:2017	239039	1		De Kaaistoep
	Carabidae	511	19	1997:2006,2009:2017	38048	1	59(94)	De Kaaistoep
	Coccinelidae	513	19	1997:2006,2009:2017	9798	1	16(23)	De Kaaistoep
	Silphidae	514	19	1997:2006,2009:2017	382	1	5(6)	De Kaaistoep
Trichoptera		261	10	2006,2009:2017	33540	1		De Kaaistoep
Ephemeroptera		255	10	2006,2009:2017	9713	1		De Kaaistoep
Neuroptera		258	10	2006,2009:2017	936	1		De Kaaistoep
Hemiptera		258	10	2006,2009:2017	49747	1		De Kaaistoep
	Heteroptera	260	10	2006,2009:2017	33523	1		De Kaaistoep
	Cicadomorpha	258	10	2006,2009:2017	9512	1		De Kaaistoep
Coleoptera	Carabidae	239	26	1986:1997,2002:2003,2005:2016	264986	48	98(156)	Drenthe

Figure 1: At De Kaaistoep night-active insects (right) were attracted by light in combination with a white sheet (left). Pictures by Paul van Wielink.

De Kaaistoep

De Kaaistoep is a managed natural area of about 450ha, consisting of heathland, pine forest and grassland. It has been established since 1994 on former arable land. Information about the location, and management history can be found in Felix and van Wielink (2008).

Data in the present analysis have been collected during 628 trapping nights between 1997 and 2017, on average 30 evenings per year (10-77). Data were available for the 1997-2017 period for macro-Lepidoptera and Coleoptera (excluding the years 2007 and 2008), while for Trichoptera, Neuroptera, Hemiptera and Ephemeroptera data were available for analysis only for the years 2006 and 2009-2017. Years 2007 and 2008 lacked information on sampling characteristics (see below) and hence were not included for any order. Among beetles (Coleoptera), light attracted mainly certain ground beetles (Carabidae), some carrion beetles (Silphidae) and labybirds (Coccinelidae). Of the large number of Coleoptera, only ground beetles, ladybirds and some carrion beetles were identified to species up to 2017 (other families up to 2011), accounting for 48000 of 239000 beetle specimens.

Insects were attracted by light in combination with a white cloth (Figure 1) over a period over 1-6 hours per trap night, normally starting around sunset (Figure 2C). Further details of the sampling protocol are given in van Wielink and Spijkers (2013).

It seemed logical that the number of insects caught to be highly dependent on sampling characteristics of each trapping night, and hence, missing information on sampling duration potentially poses a problem during analysis. Information about timing and duration of sampling were available for 82% of the data (n=515), and mostly lacking in the first few years of sampling, as well as in 2007 and 2008. The number of sampling hours varied little among years, but did increase from an average of 3 hours to an average of 4 hours per night after 2009 (Figure 2A&B). Timing of onset of sampling was roughly at sunset throughout the years, with the exception of the first few years in which sampling started on average half an hour after sunset (Figure 2C&D). The starting time of sampling correlated significantly with evaluated sunset moment for specified location (Meeus, 1991; Bivand and Lewin-Koh, 2015) ($R = 96.6\%, df = 514, p < 0.001$). Additionally, the slope of the linear relationship between the starting and sunset moments did not deviate significantly from one ($F = 0.809, p = 0.369$), and the intercept did not deviate from zero ($F = 1.568, p = 0.211$).

For each species, or order, (k), we model the counts in year t and on day d using Generalized Additive Models (GAM Wood, 2006) and assuming a negative binomial distribution (White and Bennetts, 1996) and a log link to the predictors. GAMs seemed more appropriate than Generalized linear models, as insects counts vary considerably throughout the year, with often multiple peaks (i.e. generations), as well as between years (i.e. non-linear dynamics).

We distinguish six basic models varying in how the year covariate is treated and if weather covariates are included or not (Table 2). We considered linear as well as non-linear trends over time, as well as an annual index (the latter

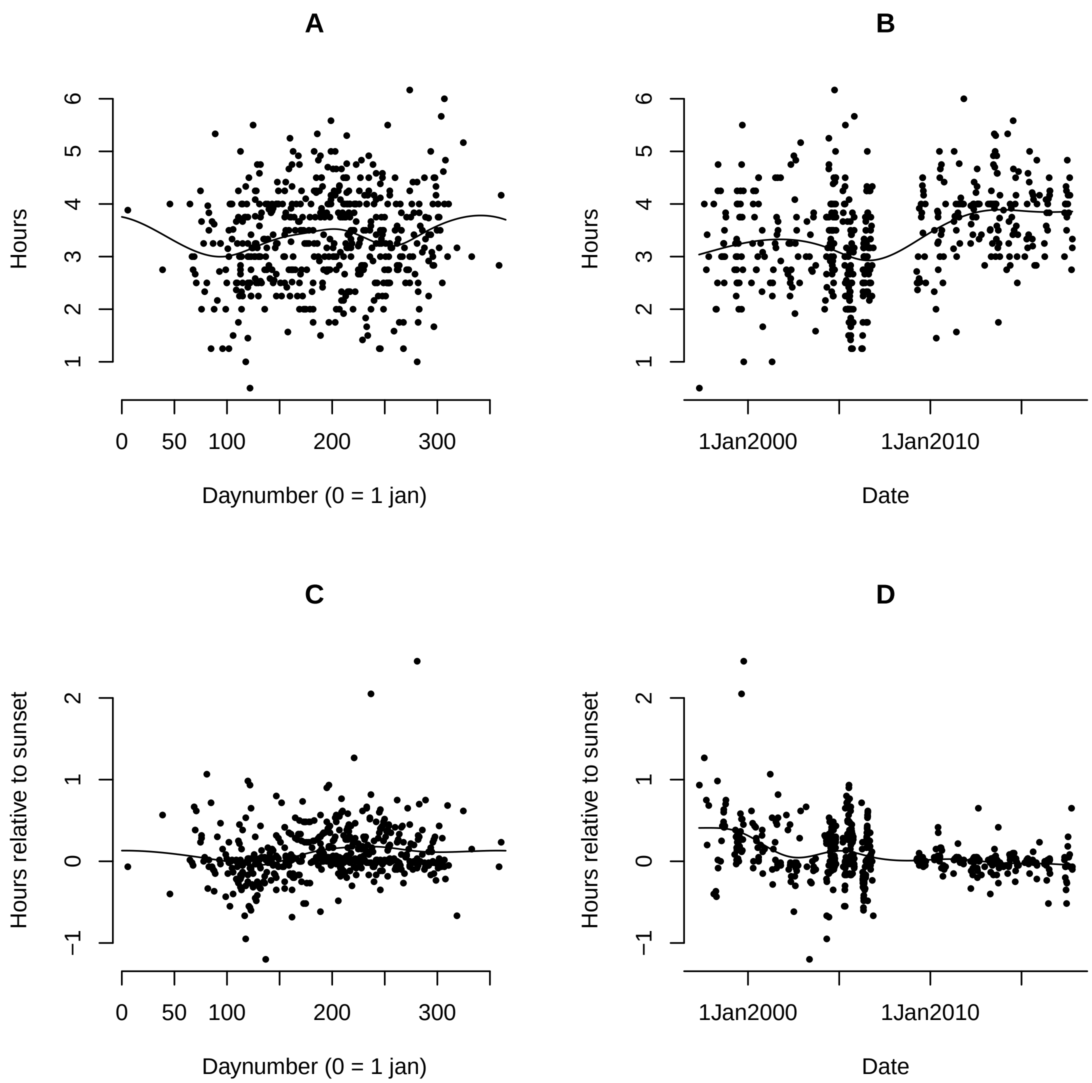

Figure 2: Sampling characteristics for the dataset of De Kaaistoep. **A:** Number of sampling hours per evening against day number. **B:** Number of sampling hours per evening per year. **C:** Starting moment of sampling relative to sunset versus day number. **D:** Starting moment of sampling relative to sunset per year.

for visual assessment). Additionally, in all models we included a smooth seasonal component ($\gamma_s(d)$) and a quadratic component for sampling duration ($h+h^2$), as we expected non-linear responses to sampling duration. Weather covariates include mean temperature, sum of precipitation, mean relative moisture content, and mean wind speed. Additionally, as response variables may have a convex relationship (e.g. optima) to weather variables, we also included quadratic effects. Each weather covariate in $\mathbf{W}$ (including the squared values) was standardized to a zero mean and unit variance.

The different models were compared by the Akaike's Information Criterion (AIC) (Burnham and Anderson, 2003), a measure of parsimony that tries to balance the amount of deviance explained and the number of parameters. Models with the lowest AIC values are preferred, especially when the difference between two models exceeds the value 2 (Burnham and Anderson, 2003).

Table 2: Model expressions considered from each insect response variable for De Kaaistoep data. Covariates t, d and h represent year, day in season, and number of sampling hours respectively, while $\mathbf{W}$ denotes a design matrix with weather covariates and their squared values. γ_s and γ_t represent smooth terms (thin plate splines) while α the intercept, β_t an annual index, β_w weather coefficients, β_h sampling duration coefficient, and ρ the annual log-linear trend coefficient.

Model	Expression	Description
M_0	$\alpha + \gamma_s(d) + \beta_t(t) + \beta_h h + \beta_{h2} h^2$	seasonal trend, discrete annual index, sampling duration
M_1	$\alpha + \gamma_s(d) + \rho \times t + \beta_h h + \beta_{h2} h^2$	seasonal trend, linear annual trend, sampling duration
M_2	$\alpha + \gamma_s(d) + \gamma_t(t) + \beta_h h + \beta_{h2} h^2$	seasonal trend, non-linear annual trend, sampling duration
M_3	$\alpha + \gamma_s(d) + \mathbf{W}\beta_w + \beta_t(t) + \beta_h h + \beta_{h2} h^2$	seasonal trend, discrete annual index, weather effects, sampling duration
M_4	$\alpha + \gamma_s(d) + \mathbf{W}\beta_w + \rho \times t + \beta_h h + \beta_{h2} h^2$	seasonal trend, linear annual trend, weather effects, sampling duration
M_5	$\alpha + \gamma_s(d) + \mathbf{W}\beta_w + \gamma_t(t) + \beta_h h + \beta_{h2} h^2$	seasonal trend, non-linear annual trend, weather effects, sampling duration

Figure 3: One of the pitfall trap locations near Kralo, 2017. Picture by Rikjan Vermeulen.

Wijster

The data used for the present analysis stemmed from the long-term monitoring program using pitfall traps started by Biological Station Wijster (and continued by the Stichting Willem Beijerink Biologisch Station) in two nature reserves in the province of Drenthe, The Netherlands: National Park Dwingelderveld and the fragmented, but increasingly reconnected Hullenzand. The pitfall data have been collected between 1959 and 2016, and concern ground beetles (Coleoptera: Carabidae) collected at in total 48 unique locations. The locations consisted mainly of heathlands, with some forest sites, a forest edge and an abandoned crop field. At each location three square pitfall traps with a circumference of 1 m were installed (Figure 3): one lethal funnel trap with a 3% formaldehyde solution, and 2 live traps. The traps at each location were spaced 10 meters apart. The catch has been identified at weekly intervals. However, for the present analysis we used the annual sums per species and location, as weekly data have not yet been fully digitized and checked. Further details on the sampling protocol and the area are given in den Boer and van Dijk (1994). 158 species of ground beetles were found in the pitfall traps. Because we are only interested in the recent trends in insect abundances, and because sampling protocols were not consistent in the early years, we used only data collected since 1986. For 20 values of yearly catch per species (out of 7778) we suspected erroneous counts, and therefore used multiple imputation (Onkelinx et al., 2017) to derive more reliable estimates for these values based on the correlation structure between years and between other species. Furthermore, years 1998-2001 and 2004 were left out of the analysis as in these years data were incomplete. In total, 7778 records were used in the present analyses, covering over 250000 individual ground beetles (Table 1).

We used generalized additive models to model the annual community abundance and counts per species with a negative-binomial distribution and a log link. We treated trap location as a random effect by making use of the random effects as smooth-terms (Wood, 2006, 2008). We considered six basic models depending on how the year covariate is treated, and if weather covariates are included or not (Table 3). We considered linear as well as non-linear trends over time, as well as an annual index (the latter for visual assessments). Weather covariates include mean temperature, sum of precipitation, mean relative moisture content, and mean wind speed, over the spring months in each year (March-May), and separately over the summer months (June-August). Additionally, we also included quadratic effects of each variable. Each weather covariate in $\mathbf{W}$ (including the squared values) was standardized to a zero mean and unit variance.

The number of years each location was sampled varied between 1 and 22, with 19 of the locations only in one year and 10 locations only in two years. To assess whether out trend estimates are affected by including locations with limited years of sampling, we repeated the analysis by including locations in our models when the number of years sampled exceeded a particular threshold. This threshold was varied between two and ten years, and for each repetition we computed the annual trend coefficient from model M_1, along with the standard error.

Table 3: Model expressions considered for each Ground beetle species of the Wijster dataset. Covariates t represents year, while $\mathbf{W}$ denotes a design matrix with weather covariates and their squared values. γ_t represents a smooth terms (thin plate splines) while α the intercept, β_t an annual index, β_w weather coefficients, b_i random location effect, and ρ the annual log-linear trend coefficient.

Model	Expression	Description
M_0	$\alpha + \beta_t(t) + b_i$	discrete annual index, random location effect
M_1	$\alpha + \rho \times t + b_i$	linear annual trend, random location effect
M_2	$\alpha + \gamma_t(t) + b_i$	non-linear annual trend, random location effect
M_3	$\alpha + \beta_t(t) + \mathbf{W}\beta_w + b_i$	discrete annual index, random location effect, weather effects
M_4	$\alpha + \rho \times t + \mathbf{W}\beta_w + b_i$	linear annual trend, random location effect, weather effects
M_5	$\alpha + \gamma_t(t) + \mathbf{W}\beta_w + b_i$	non-linear annual trend, random location effect, weather effects

Biomass calculation

In order to be able to compare our findings to recent results from Germany (Hallmann et al., 2017), we converted trends in numbers to trends in biomass, using known species length measurements and by the aid of known relationships to weight (Sabo et al., 2002; García-Barros, 2015). Both the Wijster and De Kaaistoep data consist of counts at the species or higher taxonomic level. As weighing insects was not part of the sampling protocols, we estimated mass based on species-specific length ranges. For the Carabidae in the Wijster dataset we looked up the minimum and maximum body length as noted in the ground beetles field guide by Boeken et al. (2002). Per species we averaged the minimum and maximum lengths, and used these averages to estimate mass per species (k), using the mass-length relationship determined by Sabo et al. (2002) for terrestrial insects:

$$mass_k = 0.032 \times length_k^{2.63} \tag{1}$$

where *mass* is in *mg* and *length* in *mm*.

In the case of the macro-Lepidoptera at De Kaaistoep, we looked up species-specific minimum and maximum lengths of the front wings, which is the only size measure provided at the website of De Vlinderstichting (assessed at 11 April 2018). Again we averaged the minimum and maximum lengths (sometimes sex-specific) per species, but now used a Lepidoptera-specific mass-length relationship. García-Barros (2015) measured the mass (mg) and front wing lengths (mm) of 665 specimens. As Garcia-Barros only reported the means and sample sizes per superfamily (his Supplementary Material 5), we analyzed those summary data in a log-log regression analysis with sample size as the weight of the records. Superfamily-specific residuals (ϵ_k) of this regression analysis were stored. The fitted model was then used to estimate the mass of marco-Lepidoptera species based on its average front wing length and the superfamily it belongs to:

$$mass_k = exp(-5.144 + 3.018 \times log(length_k) + \epsilon_k) \tag{2}$$

where for instance the effect sizes (ϵ_k) of Noctuoidea and Geometroidea were 0.218 and -0.126, respectively.

In order to calculate the reduction in biomass over the years, we used the sum of individual species weights ($B_{k,t}$) estimated for a particular year t (for Carabidae in the Wijster dataset) or day d (for macro-Lepidoptera the dataset of De Kaaistoep):

$$B_t = \sum^{K} B_{k,t} \tag{3}$$

and where $B_{k,t} = Y_{k,t} \times mass_k$.

We ran generalized additive models on the resulting responses, using a Gaussian distribution and *log*-link relationship to the covariates. For t he Kaaistoep data, we used the formulation of model M_4 (Table 2) and for the Wijster data model M_1 (Table 3).

Trend classification

We classified order-specific and, where information is available, species-specific trends, based on estimates and significance of the linear trend coefficient ρ (from model M_4 for De Kaaistoep data, and from model M_1 for Wijster data). Classification bins used are given in Table 4.

Table 4: Trend classification. For a given estimated trend coefficient (intrinsic rate of increase: ρ) and significance, the following classification was applied in order to categorize trends of species within orders.

classification trend	p-value	trend category
$\rho < -0.05$	p <0.05	severe decline
$-0.05 < \rho < -0.025$	$p < 0.05$	decline
$\rho < -0.025$	$p > 0.05$	decline (uncertain)
$-0.025 < \rho < 0.025$	-	stable
$\rho > 0.025$	$p > 0.05$	increase (uncertain)
$0.025 > \rho > 0.05$	$p < 0.05$	increase
$\rho > 0.05$	$p < 0.05$	severe increase

Results

De Kaaistoep

Across insect orders, models including weather variables always prevailed over models without (Table 5), and across orders, sampling duration was significantly positively related to the number of insects counted. Given the increase in sampling duration from an average of three hours in the period 1997-2006 to an average of four hours in 2009-2017 (Figure 2B), mean trends over the period were slightly lower when correcting for sampling duration (Supplemetary Figure 2), with the exception for macro-Lepidoptera. Hence, we derived annual trends while accounting for weather variables and sampling duration.

Table 5: AIC table of models per insect order. For model formulations see Table 2. Lowest AIC-value per insect order (i.e. per row) is given in bold.

Insect order	M_0	M_1	M_2	M_3	M_4	M_5
Trichoptera	2399.75	2409.74	2410.98	**2345.17**	2360.18	2360.21
Hemiptera	2731.27	2729.32	2731.19	2592.28	**2590.72**	2591.73
Neuroptera	1028.71	1048.14	1026.04	**1006.95**	1022.93	1009.11
Ephemeroptera	1532.82	1527.96	1527.99	1531.85	**1524.73**	1524.73
Coleoptera	6599.51	6608.07	6608.21	6314.68	6311.04	**6311.02**
Lepidoptera	4788.66	4810.91	4810.99	**4739.43**	4757.39	4757.44

Trends of insects at the order level are depicted in Figure 4. Following correction for sampling duration and weather effects, Hemiptera appeared to be stable, and Neuroptera appeared to decline but not significantly so, and hence the trend was considered to be uncertain. Contrary, caddisflies (Trichoptera), mayflies (Ephemeroptera), beetles (Coleoptera) and moths (macro-Lepidoptera) showed significant negative coefficients. Trends per order are summarized in Table 6. Because apparent declines in Trichoptera and Ephemeroptera might have been dominated by high counts in 2006, we re-analysed these trends while excluding data from 2006. For Ephemeroptera the trend coefficient changed both magnitude and sign ($\rho = 0.010, se = 0.058$, p-value=0.87), hence, we determined that for this insect order the estimated decline is uncertain. For Trichoptera the trend decreased slightly in magnitude but did not change in sign and remained significantly negative (ρ=-0.070, se=0.033, p-value=0.033).

Table 6: Trend evaluation at order level for De Kaaistoep data. For each order, we provide the annual trend coefficient of model M_4, along with standard error, percentage decline and evaluation of the trend (cf. Table 4 and text for Ephemeroptera)

Insect order	Estimate	Standard error	P-value	% decline	Trend evaluation
Trichoptera	-0.096	0.021	<0.001	9.2	severe decline
Hemiptera	-0.006	0.022	0.789	0.6	stable
Neuroptera	-0.047	0.029	0.108	4.6	decline (uncertain)
Ephemeroptera	-0.128	0.037	0.001	12.0	decline (uncertain)
Coleoptera	-0.051	0.010	<0.001	5.0	severe decline
Lepidoptera	-0.039	0.006	<0.001	3.8	decline

Within macro-moth species trends were variable, with on average a decline of 4.1% per year (Figure 5A). The largest group of species (37.5%) showed a declining trend, while only 4.1% showed an increase and the remainder of the species had stable or insignificant trends (Figure 5B). Declines of individual species were positively, but not significantly so, related to mean abundance (mean number of individuals per trapping night; t-value=0.861, p-value=0.392).

Within beetles, total sums of ground beetles (Carabidae) declined severely (ρ=-0.089, se=0.021, p-value<0.001), ladybirds declined (Coccinellidae, excluding the invasive exotic *Harmonia axyridis*, ρ=-0.031, se=0.012, p-value=0.001), while Carrion beetles (Silphidae) were found to increase (ρ=0.035, se=0.016, p-value=0.003). Trends for these families are given in Figure 6. Within ground beetles, species specific trends were highly variable, with on average a decline of 8.3%. A large proportion of species showed declines (44.1%), and only few (6.8%) showed increases (Figure 7).

Within true bugs, for both infraorders for which analyses could be performed, trends appeared stable, or had an uncertain decline, (Heteroptera: ρ=0.009, se=0.024, p-value=0.713, Cicadomorpha: ρ=-0.029, se=0.028, p-value=0.305). Trends for these families are given in Figure 8.

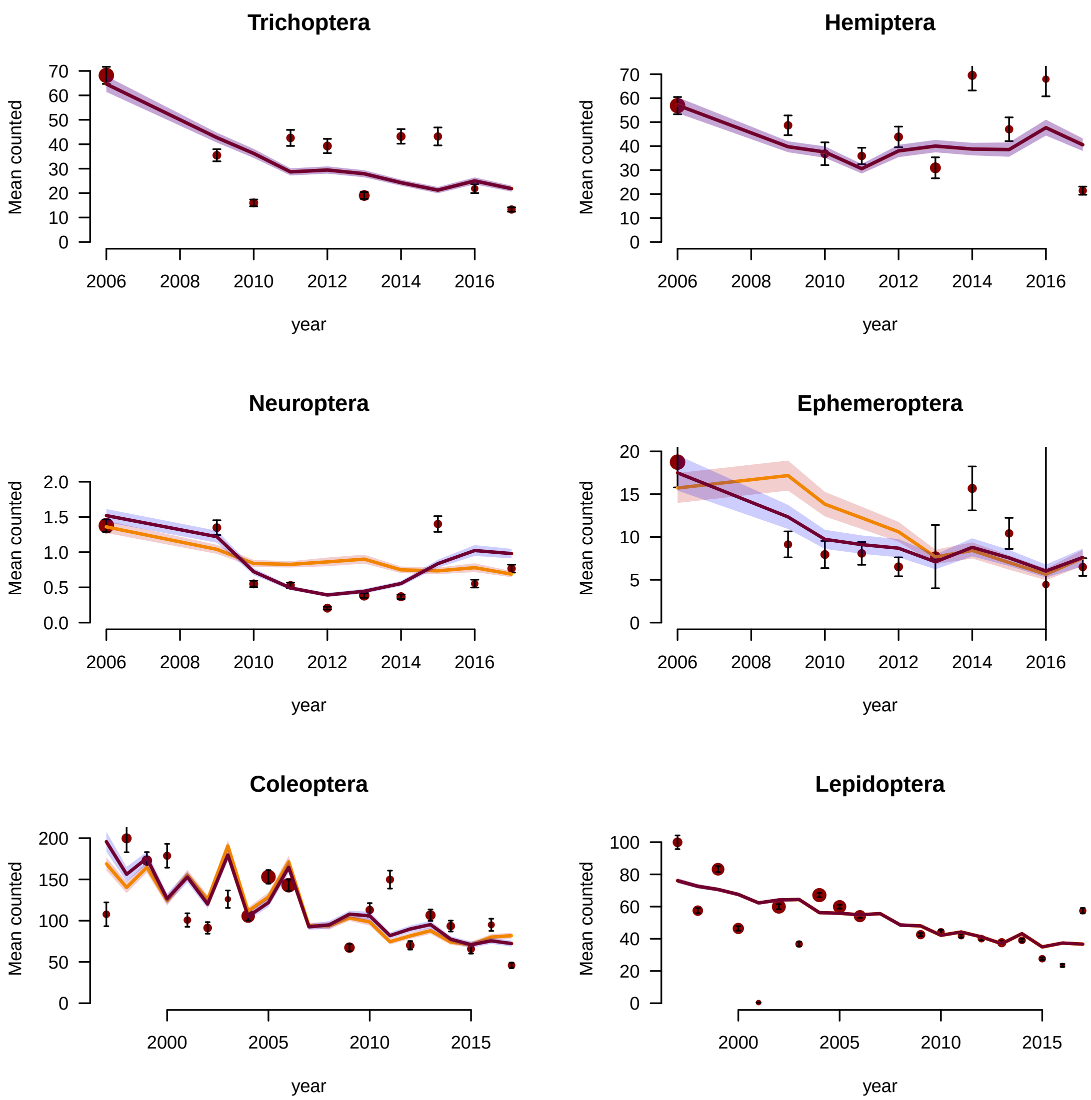

Figure 4: Trends in numbers counted per evening of six orders of insects at De Kaaistoep. For each order, the annual indices (points, model M_3), and trend estimates of the linear (orange, model M_4) and non-linear (blue, model M_5) trends are given. Evidence for non-linearity is only apparent in Neuroptera, Ephemeroptera and Coleoptera, while for the remainder of the orders results of models, model M_4 and, model M_5 are indistinguishable.

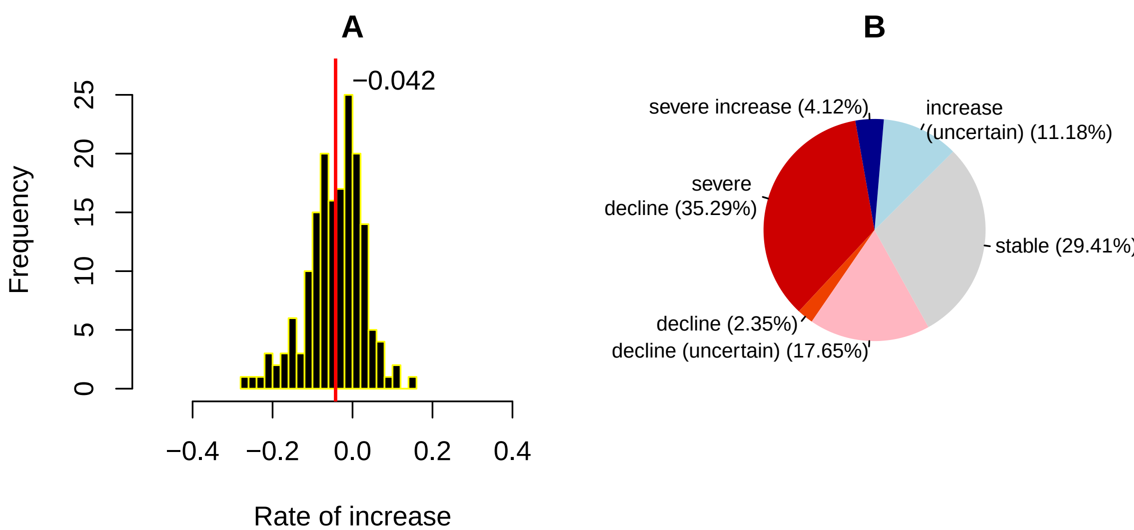

Figure 5: Trends in numbers counted per evening for individual macro-moth species (Lepidoptera, n=170 species) at De Kaaistoep. **A**: Distribution of trend coefficients, as estimated form model M_4. **B**: Pie diagram showing proportion of species in each of the six trend categories.

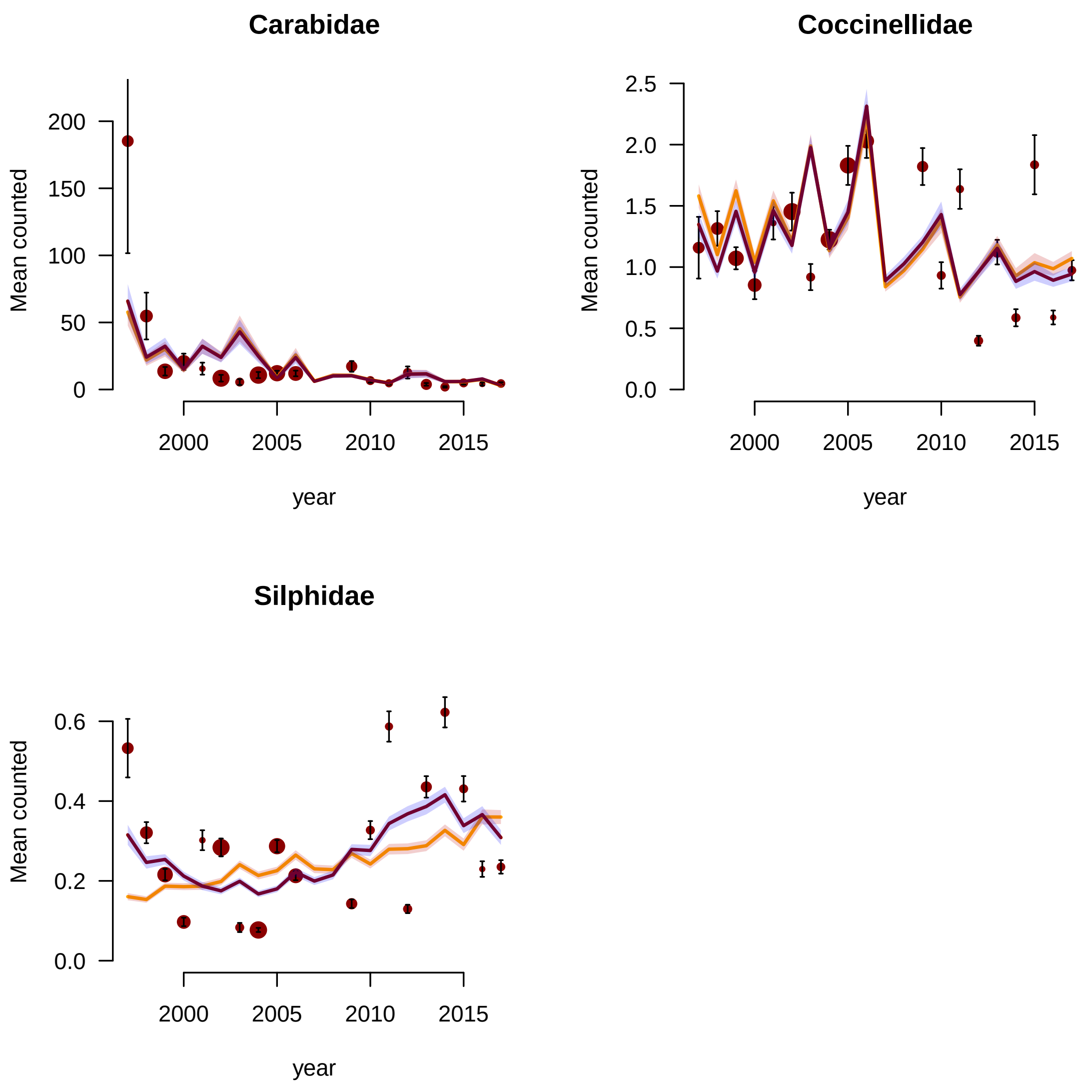

Figure 6: Trends in numbers counted per evening of three families of beetles (Coleoptera) at De Kaaistoep. For each family, the annual indices (points, model M_3), and trend estimates of the linear (orange, model M_4) and non-linear (blue, model M_5) trends are given. In ground beetles and ladybirds the linear and non-linear trends are largely indistinguishable, while for carrion beetles evidence suggest non-linear trends over the study period.

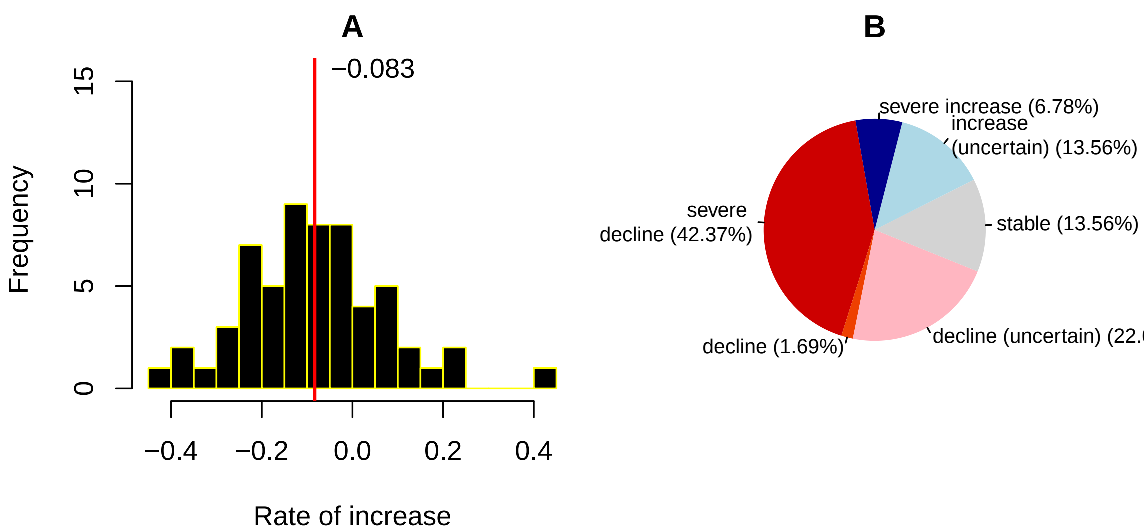

Figure 7: Trends in numbers counted per evening of ground beetle species (Coleoptera: Carabidae, n=56) at De Kaaistoep. **A**: Distribution of trend coefficients, as estimated form model M_4. **B**: Pie diagram showing proportion of species in each of the six trend categories.

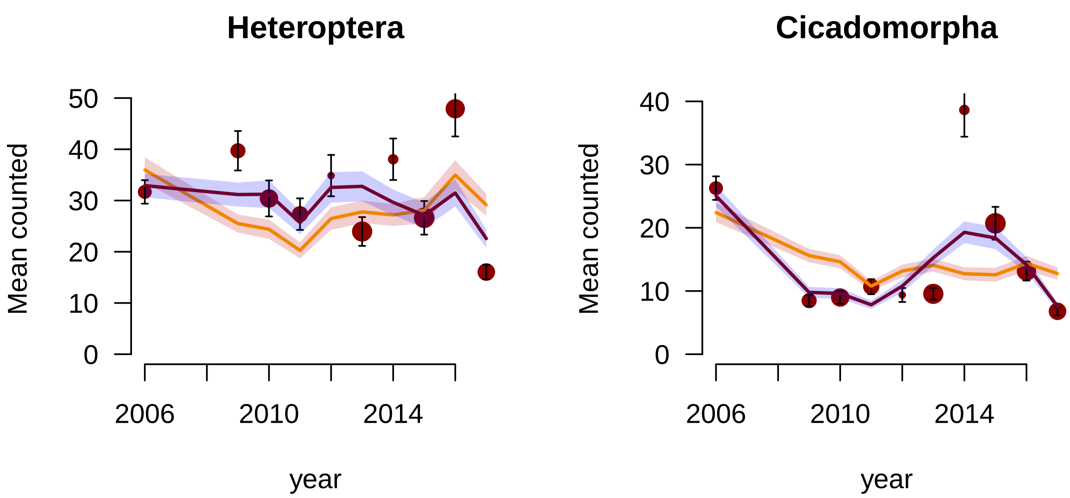

Figure 8: Trends in numbers counted per evening for two infraorders of true bugs (Hemiptera) at De Kaaistoep. For each infraorder, the annual indices (points), and trend estimates of the linear (orange) and non-linear (blue) trends are given. For both infraorders non-linear models better explained the annual trends in mean numbers counted per evening.

Wijster

Year totals over all species of ground beetles showed a declining pattern regardless of the considered model. However, non-linear trends significantly better explained year totals as compared to linear (AIC_l=3773.63, d.f=33.48, versus AIC_{nl}=3768.26, df=35.54). Models considering weather variables did not improve model fit, regardless if they were measured over spring (March-May) or summer (June-August). Hence, we present trends based on models that ignore weather effects. The linear trend coefficient was significantly negative (ρ=-0.044, se=0.006, $p-$value<0.001, 4.34% decline per year, Figure 9A). results of the non-linear trend model however showed that the trend initially increased, followed by a decline starting after 1995 (Figure 9A). The linear annual trend since 1995 showed even steeper declines (ρ=-0.060, se=0.009, $p-$value<0.001), implying a 5.6% annual decline since 1995.

Furthermore, the number of years that a given location was sampled was found to be of influence on the trend estimates. Including only locations with more than two years of sampling for example resulted in a trend coefficient of ρ=-0.051 (se=0.005), i.e. 4.97% annual decline rate. Restricting the inclusion criteria for locations according to the number of sampling years even further resulted in even more negative trends (up to 5.45% annual rate of decline, Supplementary Figure 3).

Between species, average decline amounted to 2.96% per year (Figure 9B), which is less steep than the trend of the year totals. As such, most species (56.6%) showed stable trends, or had insignificant trend slopes, while 37.8% of the species showed clear declines (most of which severe declines) and 5.6% of species showed positive trends (Figure 9C). Interestingly, individual species trends were significantly negatively related to the mean abundance of each species over the period 1986-2016 ($t-$value =-2.674, p-value=0.009, Figure 9D).

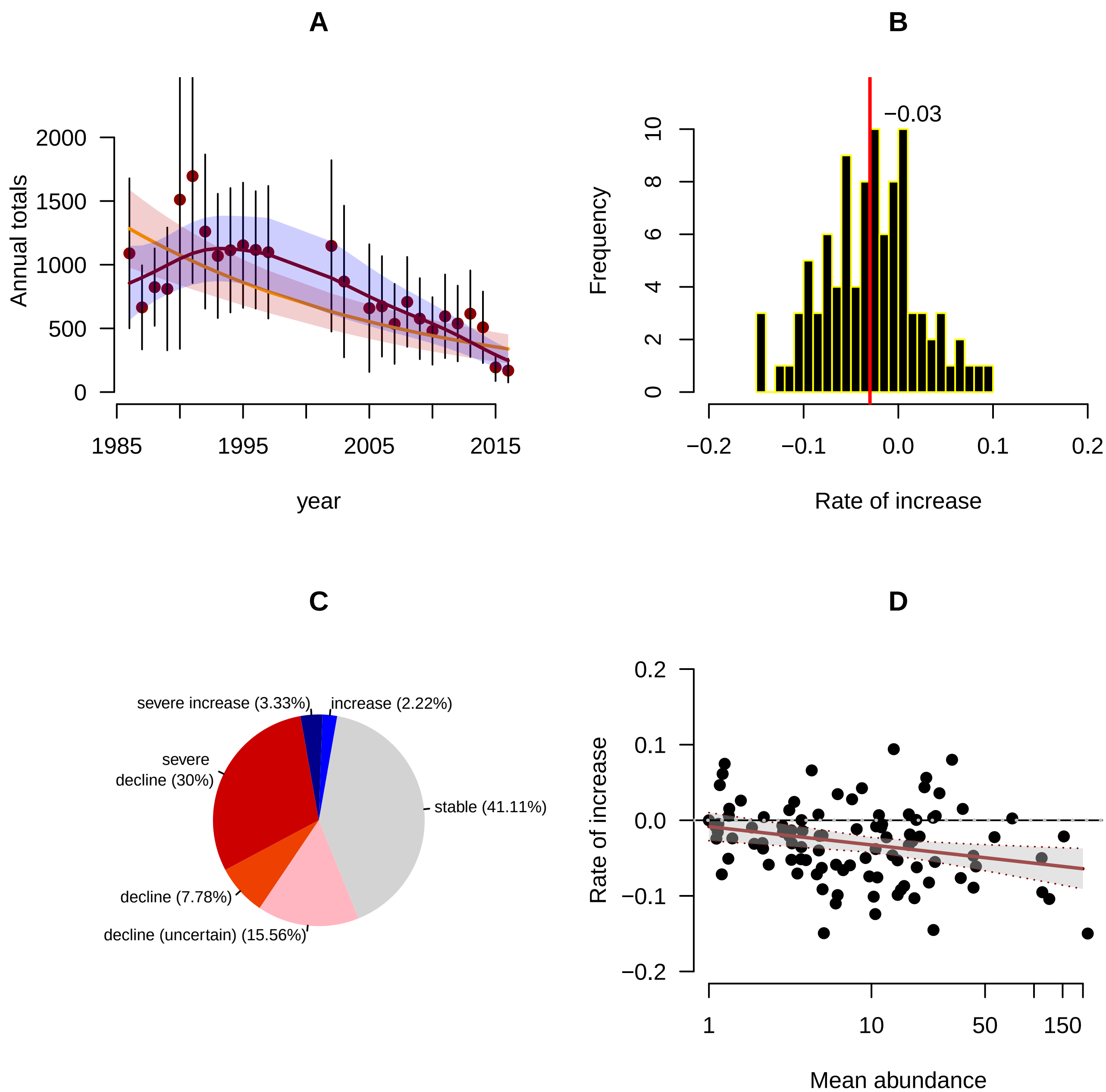

Figure 9: Trends in total numbers counted per year for carabid beetle species (Coleoptera: Carabidae) at Drenthe. **A**: Points represent mean number of individuals (totals over all species, as estimates of M_0), while orange and blue lines depict predictions of linear M_1 and non-linear M_2 models respectively. **B**: Distribution of trend coefficients, as estimated from model M_1, for 98 species. **C**: Pie diagram showing proportion of species in each of the six trend categories. **D**: Trend - abundance relationship for ground beetles at the Wijster locations.

Biomass

For the macro-Lepidoptera at De Kaaistoep, we found an severe decline in total biomass (ρ=-0.036, se=0.006, $p-$value<0.001, i.e. 3.3%(se=0.52) mg/year (Figure 10). The species trends were negatively, but not significantly so, related to estimated weight of the species (t-value=-1.248, p-value=0.214). For the ground beetles of Wijster, we found an average decline in biomass of 1.99% (se=0.48) per year, which is considerably less than that of numbers per species or total sums of individuals (Figure 11). However, considering only the period after 1995, the rate of decline in biomass appeared a lot more severe (ρ=-0.0414, se=0.006, $p-$value<0.001), implying an average 4.1% (se=0.53) decline per year.

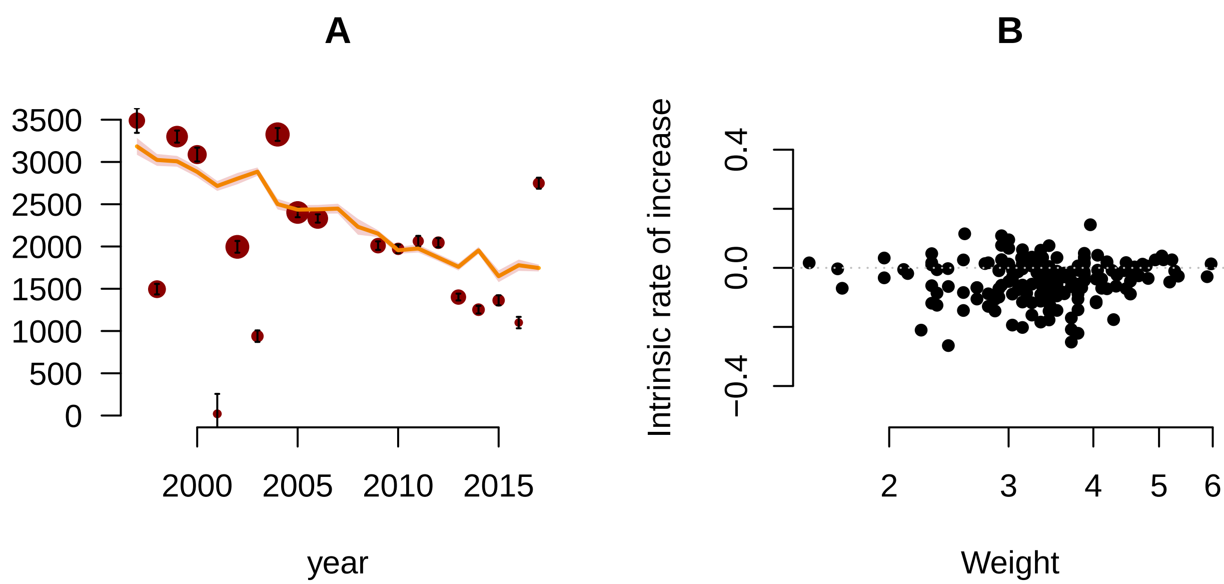

Figure 10: Biomass trend of macro-moths (Lepidoptera) at De Kaaistoep. **A**: Average annual biomass per trapping night against year. **B**: Species trend against weight of species for 170 species of macro-Lepidoptera. The relationship is not significant (t-value=0.841, p-value=0.403)

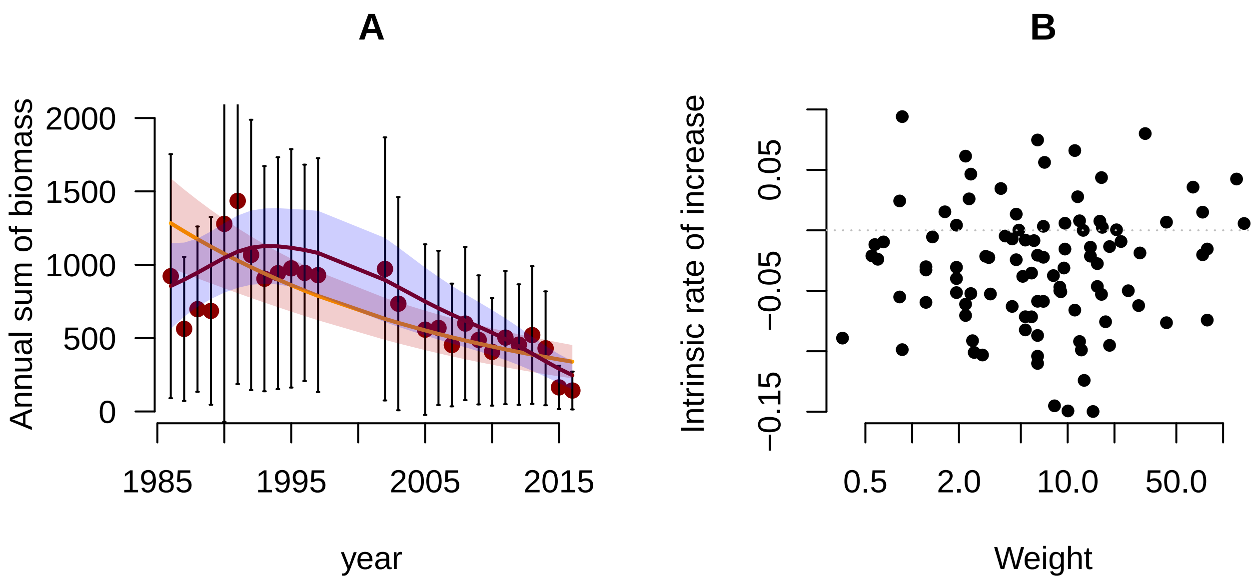

Figure 11: Biomass trend of ground beetles (Coleoptera: Carabidae) at Drenthe. **A**: Total annual biomass over time. **B**: Species trend against weight of species for 98 species of ground beetles. The relationship is not significant (t-value=1.571, p-value=0.120)

Discussion

We reported trends of six orders of insects at De Kaaistoep, and one order at Wijster. At order level, macro-Lepidoptera, Coleoptera and Trichoptera (caddisflies) at De Kaaistoep, and Coleoptera (only Carabidae) at Wijster, showed severe declines. Only Hemiptera appeared to be stable, while the negative trend for Neuroptera was statistically not significant and for Ephemeroptera not consistent enough over the study period. The average species trends for macro-moths (based on light trap data) were negative, and comparable (but slightly less steep) than the trend of the total numbers. Contrary, annual trends in total numbers of ground beetles (based on pitfall data) were more sever that the average of the individual species trend, and together with a significant negative relationship between species trends and their mean abundance, suggest that common species may fair worse than less common ones.

For macro-moths, the biomass reductions amounted to 3.3% per year. Over an extrapolated period of 27 years this amounted to a reduction of 61%, which is close to (but less than) the reported declines in Germany for total flying insect biomass (Hallmann et al., 2017, -76%) over 27 years. Ground beetles of the Wijster dataset also showed a negative biomass trend, although at a less strong rate (mean = 1.99% per year). Over a period of 27 years, this would amount to 42% reduction in total biomass. However, after 1995 the average rate of decline in biomass was more severe (4.1%), which, over a period of 27 years, would amount to 67.3%. Even higher rates of decline can be found depending on which locations are included (i.e. including only long series of locations results in even more negative annual trends, Supplementary figure 3). Given the latter, our results for the Drenthe heathlands and forests for ground beetles are likely to be conservative. Furthermore, biomass decline in ground beetles appeared less severe than the decline in numbers. In part, this can be explained by the fact that medium weight range common species are most in decline, while heavy species have variable trends.

The majority of macro-Moths are attracted to light, as are Mayflies (Ephemeroptera) and Caddisflies (Trichoptera). We expect that our results based on data from De Kaaistoep represent a large proportion of these species in the Netherlands. Contrary however, for beetles, particularly ground beetles, only a minority of species is attracted to light, and hence our results from De Kaaistoep are probably less representative of all species occurring in the area. The Wijster dataset is likely to be more representative for Carabidae species. However, both sampling methods (light traps in De Kaaistoep and pitfall traps in Drenthe) broadly suggest declines in ground beetles in the Netherlands. For carrion beetles (Silphidae, as counted at the light traps), the trends are considered unreliable as their counts are highly dependent on the presence of carrion in the area.

Mayflies are aquatic insects, while Caddisflies have an aquatic larval stadium. The declines observed presently are surprising because at De Kaaistoep, water quality is thought to have improved over recent years, with sensitive aquatic species (for example larvae of Odonata) showing positive population trends (van Wielink and Spijkers, 2012).

Further analysis of De Kaaistoep data may need to deal with autocorrelation in the residuals (See Supplementary figure 4). Although it is unlikely that the trends calculated while accounting for autocorrelation will change in sign or magnitude, the standard errors may increase and hence also the uncertainty around the trends.

Comparison to the German results (Hallmann et al., 2017) remains difficult because we do not possess data on most day-active species. Additionally, both light traps and pitfall traps in this study likely sampled different species and numbers to the malaise traps, as have been deployed by the Krefeld Entomological Society in Germany.

Conclusions

Insects in Dutch nature reserves, particularly macro-moths, ground beetles and Caddisflies, appear to be in severe decline according to the studied datasets, as are lacewings and mayflies, albeit with less certainty. Together with recent reports on butterflies (van Swaay et al., 2018) at the national level, the limited information that is available suggests that insects in the Netherlands are in decline too, similar (but slightly less negative) to the trends reported for the German nature areas (Hallmann et al., 2017). As such, we conclude that the declines in insects may be a widespread phenomenon, not limited to nature areas in Germany only.

Standardized networks to monitor the state of insects in the Netherlands is largely absent, or limited to few species groups only. Structural funding and facilitation for developing such monitoring networks, possibly using citizen science, is highly required at the moment, as this would provide the information necessary to assess the state of entomofauna in the Netherlands, investigate drivers, and to develop conservation guidelines. Further work should concentrate on formulating and testing plausible causes for the declines observed presently.

Acknowledgements

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References

Benton, T. G., D. M. Bryant, L. Cole, and H. Q. Crick. 2002. Linking agricultural practice to insect and bird populations: a historical study over three decades. Journal of Applied Ecology **39**:673–687.

Bivand, R., and N. Lewin-Koh, 2015. maptools: Tools for Reading and Handling Spatial Objects. URL `http://CRAN.R-project.org/package=maptools`.

Boeken, M., K. Desender, B. Drost, T. van Gijzen, B. Koese, J. Muilwijk, H. Turin, and R. Vermeulen. 2002. De loopkevers van Nederland en Vlaanderen (Coleoptera, Carabidae). Jeugdbondsuitgeverij, Nederland .

Burnham, K. P., and D. R. Anderson. 2003. Model selection and multimodel inference: a practical information-theoretic approach. Springer Science & Business Media.

den Boer, P. J., and T. S. van Dijk. 1994. Carabid beetles in a changing environment. 94-6, Wageningen Agricultural University.

Felix, R., and P. van Wielink. 2008. On the biology of *Calodromius bifasciatus* and related species in De Kaaistoep (Coleoptera: Carabidae). Entomologische Berichten **68**:198–209.

Fewster, R. M., S. T. Buckland, G. M. Siriwardena, S. R. Baillie, and J. D. Wilson. 2000. Analysis of population trends for farmland birds using generalized additive models. Ecology **81**:1970–1984.

García-Barros, E. 2015. Multivariate indices as estimates of dry body weight for comparative study of body size in Lepidoptera. Nota Lepidopterologica **38**:59–74.

Groenendijk, D., and W. Ellis. 2011. The state of the Dutch larger moth fauna. Journal of Insect Conservation **15**:95–101.

Hallmann, C. A., M. Sorg, E. Jongejans, H. Siepel, N. Hofland, H. Schwan, W. Stenmans, A. Müller, H. Sumser, T. Hörren, et al. 2017. More than 75 percent decline over 27 years in total flying insect biomass in protected areas. PLoS ONE **12**:e0185809.

Johnson, C. G. 1969. Migration and Dispersal of Insects by Flight. Methuen and Co. Ltd.; London.

Jonason, D., M. Franzen, and T. Ranius. 2014. Surveying moths using light traps: effects of weather and time of year. PLoS ONE **9**:e92453.

Meeus, J. H. 1991. Astronomical Algorithms. Willmann-Bell, Incorporated.

Onkelinx, T., K. Devos, and P. Quataert. 2017. Working with population totals in the presence of missing data comparing imputation methods in terms of bias and precision. Journal of Ornithology **158**:603–615.

Sabo, J. L., J. L. Bastow, and M. E. Power. 2002. Length–mass relationships for adult aquatic and terrestrial invertebrates in a California watershed. Journal of the North American Benthological Society **21**:336–343.

Termaat, T., R. H. van Grunsven, C. L. Plate, and A. J. van Strien. 2015. Strong recovery of dragonflies in recent decades in The Netherlands. Freshwater Science **34**:1094–1104.

Van Dyck, H., A. J. van Strien, D. Maes, and C. A. van Swaay. 2009. Declines in common, widespread butterflies in a landscape under intense human use. Conservation Biology **23**:957–965.

van Swaay, C., G. Bos, R. van Grunsven, J. Kok, K. Huskens, J. van Deijk, and M. Poot. 2018. Vlinders en libellen geteld. Jaarverslag 2017. Rapport VS2018.006, De Vlinderstichting, Wageningen.

van Swaay, C. A., D. Maes, and C. Plate. 1997. Monitoring butterflies in the Netherlands and Flanders: the first results. Journal of Insect Conservation **1**:81–87.

van Wielink, P., and H. Spijkers. 2013. Insects nightly attracted to light at a single site in De Kaaistoep, The Netherlands. Orders, families and species identified in 1995–2011. Entomologische Berichten **73**:200–214.

van Wielink, P. S. 2017*a*. *Harmonia axyridis* (Coleoptera: Coccinellidae): 13 jaar gevolgd met lichtvangsten in De Kaaistoep, Noord-Brabant. Entomologische Berichten **77**:97–105.

van Wielink, P. S. 2017*b*. Negentien jaar lichtvangsten van lieveheersbeestjes in De Kaaistoep (Coleoptera: Coccinellidae). Entomologische Berichten **77**:127–139.

van Wielink, P. S., and H. Spijkers, 2012. Fauna in de Oude en Poppelsche Leij. Pages 41–54 *in* T. Peeters, A. van Eck, and T. Cramer, editors. Natuurstudie in De Kaaistoep Verslag 2012, 18e onderzoeksjaar. TWM Gronden BV, Natuurmuseum Brabant and KNNV-afdeling Tilburg, Tilburg.

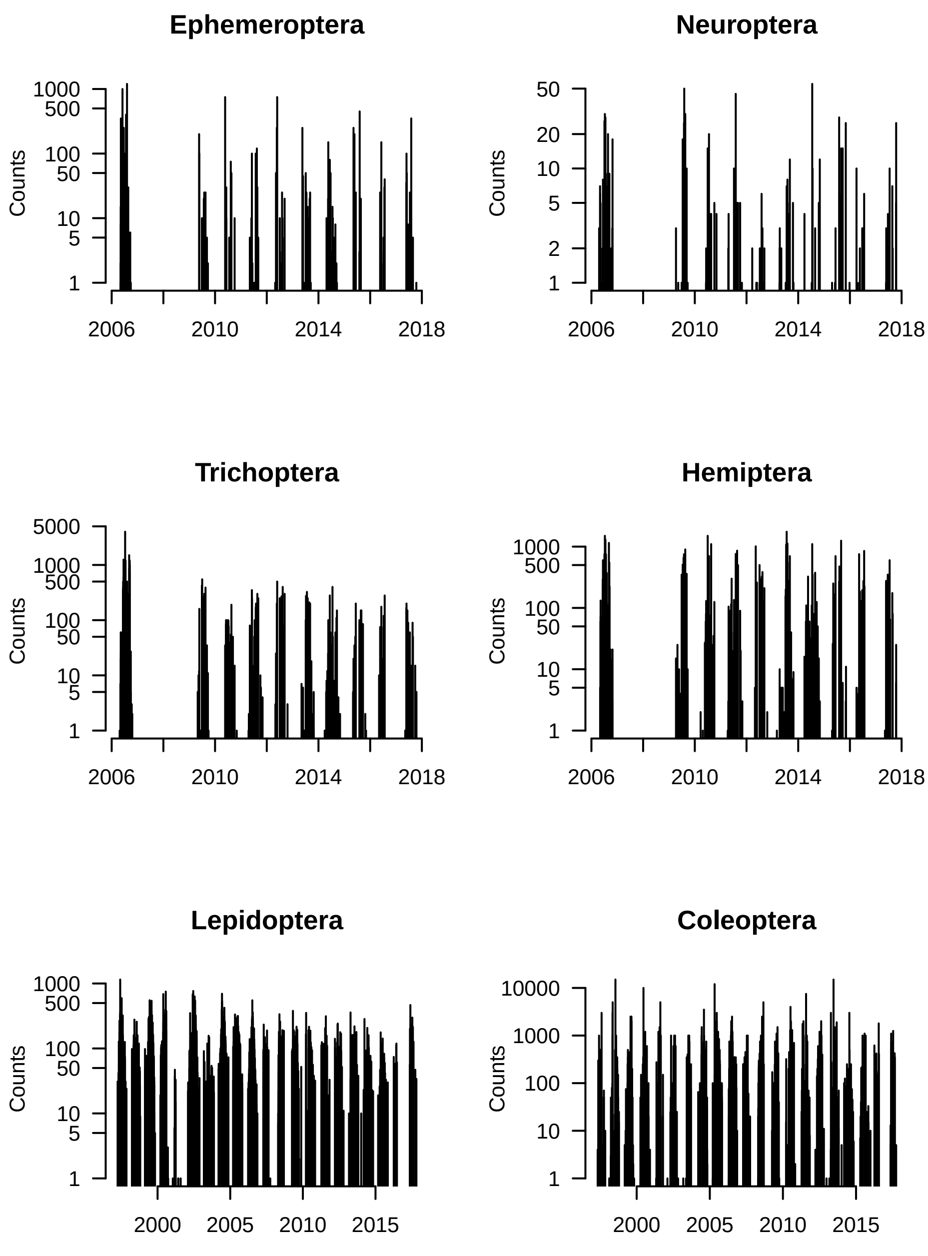
White, G. C., and R. E. Bennetts. 1996. Analysis of frequency count data using the negative binomial distribution. Ecology **77**:2549–2557.

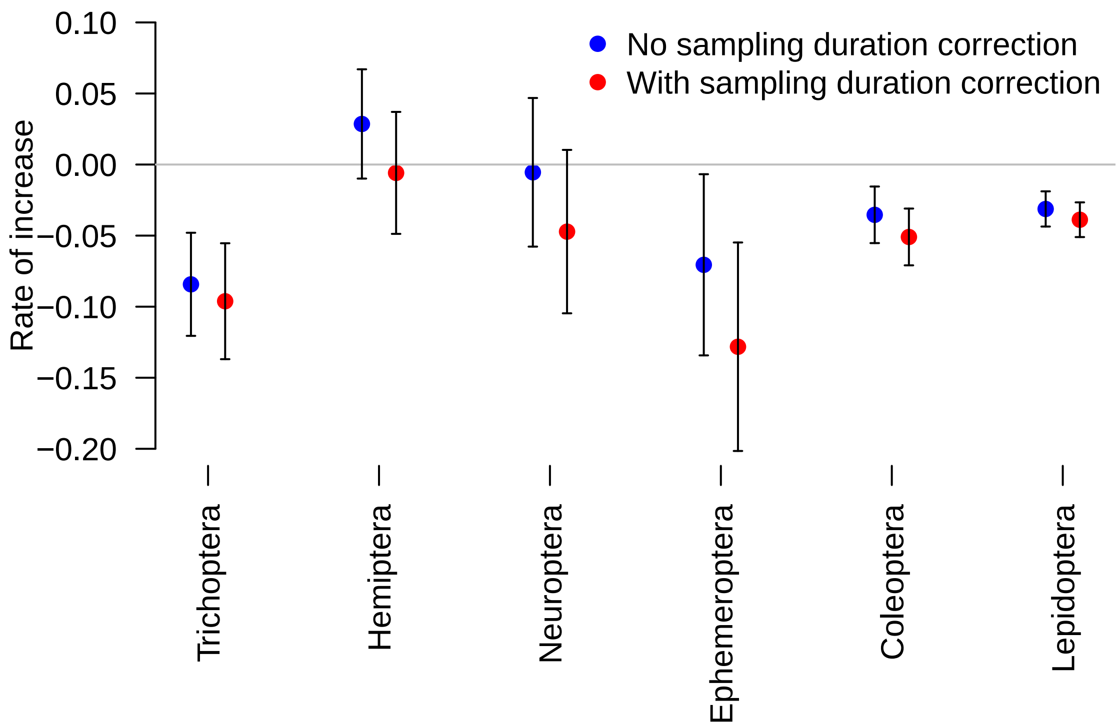
Wood, S. N. 2006. Generalized Additive Models: an Introduction with R. Chapman and Hall/CRC.

Wood, S. N. 2008. Fast stable direct fitting and smoothness selection for generalized additive models. Journal of the Royal Statistical Society: Series B **70**:495–518.

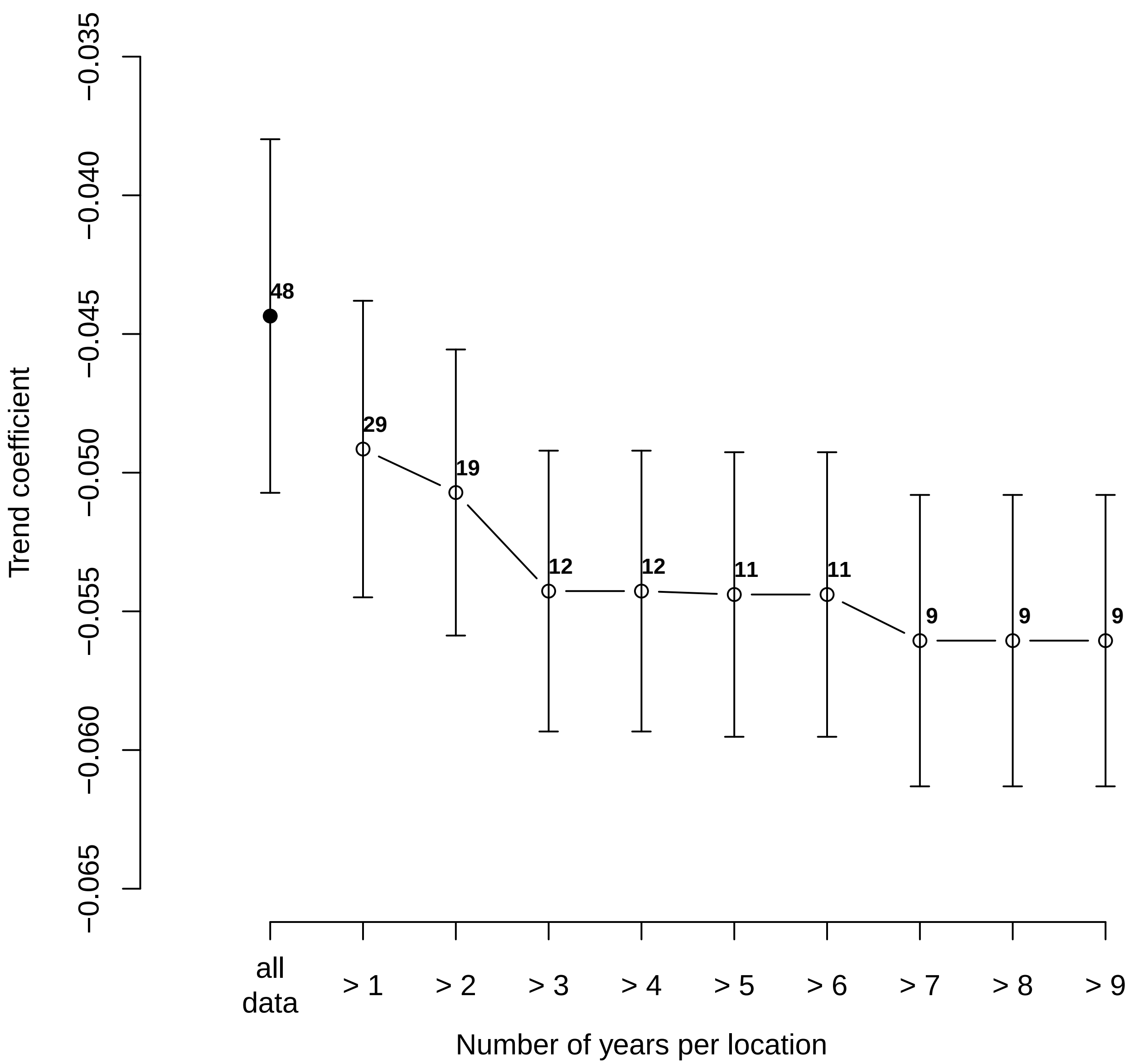
Supplements

Supplementary Figure 1: Raw counts at order level for data of De Kaaistoep





Supplementary Figure 2: Mean annual intrinsic rate of increase for each insect order based on data from De Kaaistoep, with and without correction for sampling time effort. Estimates are based on subset data for which information on sampling was available



Supplementary Figure 3: Annual trend coefficient (± 1se) for subsets of the Wijster data. Each point depict the trend as calculated for all locations that were sampled in more than x years. Numbers are the number of locations included in the data.

Supplementary Figure 4: Residual autocorrelation functions for data in De Kaaistoep per order. Some autocorrelation is detected up to several months.