Supplementary material

Scale-dependent effects of terrestrial habitat on genetic variation in the great crested newt (*Triturus cristatus*)

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Fig. S1 Detailed maps of spatially structured populations (SSP) of the great crested newt. Each map, with the SSP-ID given above, shows the composition of the landscape and the location of the sampled ponds, with the distinction between ponds with less than six samples (N < 6; grey dots) and ponds with at least six samples (N ≥ 6; black dots)

Text S1

Study sites and sampling methods

In 2011 seven spatially structured populations (SSPs) in northern Belgium were visited (Table S1, Fig. 1). Five ponds in each population were sampled using four traps type 'Vermandel' which were placed for 48 hours in April-May. These traps are designed specifically for collecting newts in water while giving them permanent access to air. No crested newts died in the traps. During that time period the traps were emptied twice. The sex of each adult great crested newt was identified on the basis of the sexually dimorphic cloaca, and the pattern on its belly was photographed to identify recaptured newts. A cloacal swab was taken from each individual using nylon flocked swabs. In June and July larvae were caught using dip nets and sampled using skin swabs with cotton buds. Not all sampled ponds contained newts. At the SSP situated in Peer only larvae were caught. In 2012, a limited number of additional samples were taken at some of the seven SSP (Table S1). Another SSP situated in the military base Camp Albert 1er in Marche-en-Famenne (southern Belgium) was sampled in 2010 with special authorisation given the risks to access. Here, toe clippings were taken from adult newts caught in six ponds (with similar procedures as in the other sampling locations). This site is known to hold the largest Belgian SSP of great crested newt (Denoël et al. 2018). Since some of the SSPs delivered only few samples, an additional four SSPs were sampled in March-May of 2018 (Table S1, Fig. 1) where only adults were sampled with cloacal swabs with cotton buds. After sampling, all newts were released back in their pond of origin.

The forensic swabs were air dried for several hours after which they were brought back into their storing tubes. They were stored in the dark at room temperature. DNA-extraction was performed within a month after the samples were taken.

DNA extraction and microsatellite analysis

DNA was extracted from the tissue samples collected in 2010 in Marche-en-Famenne using the DNeasy Blood & Tissue Kit (Qiagen) and eluted in 200 µl AE buffer. The integrity of DNA was assessed on 1% agarose gels and the purity and concentration with the ND-1,000 Nano-Drop spectrophotometer (NanoDrop Technologies). The cotton tips of the swabs were cut away from the sticks and suspended in 100 µl Taq buffer 1x with 5 µl proteinase K, and incubated for three hours at 56 °C. The sample was then heated at 95°C for 20 minutes, for inactivation of the proteinase K. Next, the temperature was brought back down to 15 °C. DNA on the nylon flocked swabs was extracted using the QIAamp DNA Micro Kit as described by the manufacturer (Qiagen).

The set of 31 microsatellites used for genotyping consisted of 12 published loci (Krupa et al. 2002; Drechsler et al. 2013), one locus developed by Krupa et al. (2002) but with adjusted primers and 18 new loci developed in 2014 on the basis of sequences obtained from an Illumina HiSeq2000 flow cell lane by 2 x 100 bp read mode (Genomics Core, KU Leuven, Belgium), using DNA from two tail clips of larvae caught in Duivenbos (Belgium). In total, four multiplex and two simplex sets were created (Table S2). The multiplex PCR amplifications were performed in a solution of 5 µl Multiplex PCR Master Mix (Qiagen), 0.05 to 0.40 µl of each primer set (10 µM), 1 µl DNA. Autoclaved ultrapure water was added to a total volume of 10 µl. Primer concentrations are given in Table S2. The simplex PCR amplifications were performed in 1.5 µl PCR buffer with KCl en $MgCl_2$ (10x), 0.38 µl dNTP (10 mM), 0.60 µl of the primers (10 µM), 0.12 µl Taq-polymerase (5 U/µl), 0.90 µl $MgCl_2$ (25 mM)

(Fermentas), 6.5 µl autoclaved ultrapure water and 5 µl DNA. DNA obtained from the adults was diluted to a concentration of 5 ng/µl. Larval DNA was diluted (1:20) for the simplex reactions but not for the multiplex reactions. PCR conditions for the first multiplex consisted of an initial denaturation step of 15 min at 94 °C, 35 cycles of 30 s at 94 °C, 30 s at 54 °C and 30 s at 72 °C, followed by a final elongation step at 72 °C for 10 min and 15 min at 4 °C, ending at a temperature of 15 °C. The other three multiplex sets had the same PCR program: 15 min at 95 °C, 35 cycles with 30 s at 95 °C, 45 s at 60 °C and 45 s at 72 °C, followed by 10 min at 72 °C, 15 min at 4 °C, ending with a temperature of 15 °C. The simplex PCR reaction had the following conditions: 4 min at 94 °C, 35 cycles as given for the first multiplex but with 50 °C as the annealing temperature, followed by 7 min at 72 °C, 15 min at 4 °C, and ending with a temperature of 15 °C. PCR products were diluted according to the following scheme: 1:20 for multiplex 1 and the adult samples of the simplex sets, 1:100 for the larval samples of the simplex sets and multiplex sets 2 and 3, 1:50 for multiplex 4. We performed the microsatellite genotyping analysis on an ABI 3500 Genetic Analyzer (Applied Biosystems) with the GeneMapper v.4.0 and v.4.3 software packages with fragment sizes based on GeneScan 600 LIZ Size Standard (Applied Biosystems). Negative controls were included in each 96-well PCR to allow for detection of reagent contamination. To test for reproducibility, samples were blindly replicated two to five times within and across well plates, starting from DNA extract. DNA extraction and genotyping were always conducted in the months following the different sampling sessions. Therefore, the number of replicates included varied depending on the number of samples collected at that time, with a minimum of 3 %. Samples with genotypes for at least 50 % of the loci were included for further analysis. A moderate number of samples taken in Viskot showed poor amplification. The overall error rate per locus was 3%.

Recaptures, full siblings and data quality

On the basis of the pattern on the newts' bellies and on their genotypes, recaptured individuals were identified using the Wild-ID program (Bolger et al. 2012) and removed for further analysis. Newts were pit-tagged in Marche-en-Famenne and therefore each individual was sampled only once (Denoël et al. 2018). For the comparison of genotypes the R package allelematch 2.5.1 (Galpern et al. 2012) was used. This approach can handle missing data and takes the genotyping error rate, based on the replicates, into account.

Further potential bias in estimates of genetic variation can come from sampling close relatives due to the sampling design, such as sampling newt larvae in ponds (Goldberg and Waits 2010). Although the removal of siblings is under discussion and should be performed with caution (Waples and Anderson 2017), empirical studies on pond-breeding amphibians found that the presence of full siblings could affect the tests to find problematic markers and bias population structure (Sánchez-Montes et al. 2017; O'Connell et al. 2019). However, small numbers of full siblings do not appear to significantly affect genetic estimates such as allelic richness and heterozygosity (Peterman et al. 2016; Sánchez-Montes et al. 2017). We identified all pairs of full siblings among larvae within ponds using the software COLONY 2.0.6.5 following a maximum likelihood approach (Wang 2004; Jones and Wang 2010). The method also takes error rate and allelic drop out in consideration. The latter was estimated based on replicated genotypes with the software Pedant 1.0 (Johnson and Haydon 2007). The following settings were used: full likelihood method, random mating, polygamous males and females and three runs of medium length. The adults sampled within a SSP were included as potential parents. Pairs of individuals were perceived as full sibs with a minimum probability of 0.95.

In Temse and Tommelen full-sib families were found in two ponds each (Table S1). Only one genotype per full-sib family was kept in the dataset for further analysis.

To investigate possible deviations from Hardy–Weinberg equilibrium (HWE), we used the test available in GENEPOP 4.7 (Rousset 2008). To assure no Wahlund effect could influence the test results, we performed tests at the pond-level. We implemented a correction for multiple testing with the Bonferroni correction with a nominal level of 5%. Significant deviances of HWE can be caused by sampling families or multiple cohorts (Hansen et al. 1997; Jankovic et al. 2010). We therefore also performed tests after excluding genotypes of larvae and juveniles and/or reducing the number of adult genotypes to a maximum of 30 (when possible). The presence of null alleles was assessed with the program GENEPOP using the maximum likelihood method, following the expectation maximisation algorithm of Dempster et al. (1977) and with software program ML-NULLFREQ (Kalinowski and Taper 2006). Finally, GENEPOP was applied to test for linkage disequilibrium (LD) between pairs of loci, again with Bonferroni correction.

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Table S1 Spatially structured populations (SSP) of great crested newts sampled in 2010-2012 and 2018. Number of sampled individuals (N) is given per development stage. Numbers between parentheses next to the number of adults/juveniles are the remaining number of samples after excluding low quality genotypes and after excluding recaptures (no recaptures in MF), respectively. The numbers between parentheses next to the number larvae are the remaining number of samples after excluding low quality genotypes and after excluding full siblings, respectively. See Fig. 1 for SSP locations

SSP	SSP-ID	Pond	Year	N juvenile	N adults	N larvae
De Brand	DB	DB2	2011		4	1
		DB2	2012		12 (11; 9)	
		DB3	2011	1	26	2
		DB3	2012		37 (31; 25)	
		DB5	2011		25 (24; 16)	2
		DB5	2012		10 (6; 5)	
		DB15	2012	1	10 (9; 9)	
Tommelen	TO	TO0	2011	1	8	
		TO1	2011	1	4	9
		TO2	2011	1	5	5
		TO3	2011	2	6	9
		TO4	2011	1	11 (10; 10)	25 (25; 23)
		TO5	2011		1	42 (42; 37)
Zemst	ZE	ZE1	2011		1	
		ZE2	2011		1 (0; 0)	
		ZE5	2012		5 (4; 4)	
		ZE6	2012		50 (43; 38)	
Temse	TE	TE1	2011	9	3	28 (28; 17)
		TE2	2011	12 (11; 11)	16 (15; 15)	25 (25; 21)
		TE2	2012		15 (14; 14)	
		TE4	2011	3	30 (29; 28)	52 (47; 47)
		TE4	2012		28	
		TE5	2011	1	6 (1; 1)	12
Wetteren	WT	WT1	2011	2	10 (10; 9)	
		WT1	2012	1	32 (31; 29)	
		WT2	2011	10 (9; 9)	5	
		WT2	2012		5	
		WT4	2011	5	15 (15; 14)	21
		WT4	2012		45 (45; 41)	
		WT5	2011	1		
		WT6	2011		2	6
		WT6	2012		24 (22; 22)	
Wervik	WR	WR2	2011		13	
		WR3	2011		23 (23; 22)	27 (26; 26)
De Panne	DP	DP1	2011	1	6 (6; 5)	
		DP2	2011	1	4	
		DP3	2011	3 (2;2)	10	26 (24; 24)
		DP4	2011		5	
		DP5	2011	1	2	

SSP	SSP-ID	Pond	Year	N juvenile	N adults	N larvae
Peer	PE	PE0	2011			7 (6; 6)
Marche-en-Famenne	MF	MFH4	2010		30 (29)	
		MFH7	2010		30	
		MFH9	2010		30 (20)	
		MFK2	2010		30	
		MFJ22	2010		29	
		MFJ33	2010		30	
Antitankgracht	ATG	P13	2018		5 (3; 3)	
		P15	2018		36 (31; 31)	
		unknown	2018		20 (18; 17)	
Meertsheuvel	MH	Bos2	2018		5	
		L-perceel	2018		2	
		Stad1	2018		51 (39; 38)	
		Stad2	2018		15 (11; 11)	
Bos van Aa	BvA	4A	2018		76 (68; 66)	
		12s	2018		86 (84; 84)	
		12l	2018		37	
		2007-Thuis	2018		3	
Viskot	VK	PA	2018		34 (28; 28)	
		PB	2018		31 (26; 26)	
		PC	2018		1	
		PD	2018		7 (4; 4)	
		PE	2018		45 (18; 18)	
		PF	2018		31 (19; 19)	

Table S2 The set of 31 microsatellite loci for *Triturus cristatus*, including published and new markers. The different multiplex (MP) and simplex (SP) sets for PCR amplifications are given, the fluorescent labels, as well as the volume of primers (10 μM) added to the PCR mix described in Text S1

Name	MP/SP	Label	Repeat	Primer sequences (5'-3')	Primer volume (μl)	Ref.
Tcri13	MP1	Pet	(GT)36 interrupted	F: GTGATGGTTGCCAAGC R: GATCCAAGACACAGAATATTTAG	0.10	Krupa et al. 2002
Tcri29	MP1	Ned	(TTTC)22 (CA)11	F: CGAGTTGCCCAGACAAG R: GATCACATGCCCATGGA	0.10	Krupa et al. 2002
Tcri35	MP1	Ned	(GAAA)32 interrupted	F: CCAACTGGTATGGCATTG R: GATCACAGAAACTCTGAATATAAGC	0.10	Krupa et al. 2002
Tcri46	MP1	Vic	(TTTC)23	F: CAAGTTTCCTCTGAAGCCAG R: GTTTCTTGCCTGACAAAGTAATGCTTC	0.10	Krupa et al. 2002
Tcri27	SP1	Fam	(GAAA)27 interrupted	F: GATCCACTATAGTGAAAATAAATAATAAG R: CAAGTTAGTATATGATATGCCTTTG	0.60	Krupa et al. 2002
Tcri36	SP2	Ned	(GAAA)36 interrupted	F: GATCATCTGAATCCCTCTG R: ATACATTCATGACGTTTGG	0.60	Krupa et al. 2002
TRCR501	MP2	Fam	(AACAT)11	F: GGTATGAAGTAGGATTGTGTTCAGA R: AAATAACCATTTCTGTGAACGACT	0.20	
Tcri43c	MP2	Vic	(AAAG)18	F: CATCTGTTTCTGAAGTAACTGAAAGAT R: AGGTCGACCACCCTAACTGT	0.20	Adjusted from Krupa et al. 2002
TRCR427	MP2	Fam	(AGAT)10	F: TTGGCTAGAGGGATAAATGGA R: CATGTAGAAGCAATGGGACATC	0.20	
Tc50	MP2	Vic	(ACTC)18	F: GCGGATACATGGTCTTCGTT R: TTCAGTTAAAAGTGTCCTCTGTGG	0.20	Drechsler et al. 2013
TRCR408	MP2	Vic	(ATCC)15	F: CAACTTGTAACTGTGCCTGAAA R: AACCGGAAATCACATTTCTAACA	0.20	
TRCR414	MP2	Ned	(AATC)13	F: GCAAGGGAGGGATACTTGAA R: CCCTCAGTAACTTCCTGGCA	0.10	
TRCR424	MP2	Ned	(AGAT)11	F: AGGGCTGACTGACTTGTTCC R: TCTTGGAGTTTCACGCCTTC	0.10	
Tc52	MP2	Ned	(ATTG)17	F: GGCTCTTCGACTGAATGGAG R: CGGTCAATTGGTTGTAGCAG	0.10	Drechsler et al. 2013
Tc74	MP2	Pet	(AATC)13	F: TCTGTGACATGTCCTGATAGTGAA R: TAGCACCATGAGACCCTCAC	0.20	Drechsler et al. 2013
TRCR403	MP3	Fam	(AGAT)13	F: GCACTGAGTCAAAGTAATTTATTGAT R: TGTTGAACTGGCTCTGCACT	0.20	
Tc70	MP3	Fam	(ACAT)14	F: GGGTTGCAAAGCACCTTAAT R: TACCTGGGTCCTCCTCCAAG	0.20	Drechsler et al. 2013
TRCR417	MP3	Fam	(AATC)12	F: AGTCGTGCCATACAAGAAACC R: TATGGGATGCCAGCACAGT	0.20	
Tc66	MP3	Vic	(ATCC)18	F: CCTTTGTACACCACTGGCAAA R: TGGTCCTATAAAGCCATCTTGG	0.20	Drechsler et al. 2013
TRCR502	MP3	Vic	(AACAT)10	F: GCCATCCAAGTTATTCAAGCA R: ACTCATCGGAGTGACGGTTT	0.05	

Name	MP/SP	Label	Repeat	Primer sequences (5'-3')	Primer volume (μl)	Ref.
TRCR416	MP3	Ned	(AAAG)12	F: CAATTGTTCTGCCAAAGGCT R: TCCAGGAAGAAACCTGACCTT	0.20	
TRCR406	MP3	Ned	(AGAT)15	F: TGCCAGAAACCTACCCATGT R: GCAACTCACTCACCCAAACA	0.20	
TRCR302	MP3	Pet	(AGC)16	F: GTGGCTGAGATCCATCACCT R: TCAAACCTGTCCCACACTGA	0.10	
TRCR401	MP3	Pet	(AGAT)20	F: GCAGGAAATGGGAAATAACAT R: AAATCCCAGTGCCATCTCAT	0.40	
TRCR425	MP4	Fam	(AGAT)10	F: GCATGCCCACCCAATTATAC R: CGTACGGTTAATGAGGAAGC	0.20	
TRCR423	MP4	Fam	(AGAT)11	F: TCTTCAACTGCCTGGTCTGT R: GAGTGCCTCGCACACTTT	0.40	
TRCR402	MP4	Vic	(AGAT)18	F: TGTGTCCTGGAGAAGTGTGC R: CAGTGCTGCGTCCTTTGTTT	0.40	
TRCR421	MP4	Vic	(AATC)11	F: GACCCTGAAGGGTGATTAGC R: TCAGATTTGGGTGCATGAG	0.10	
Tc68b	MP4	Ned	(ATCC)24	F: AAAGTGCACTCTTTCTCTGAAGC R: TGCAAAGTGCATGTGTGACT	0.20	Drechsler et al. 2013
TRCR422	MP4	Ned	(AATC)11	F: CCGGGACACTTCTGTTGAA R: GCCCAGGTCGACACATCTT	0.05	
TRCR407	MP4	Pet	(AGAT)15	F: CCTTGTCCCTTATAGGTTGATGC R: GAATTACATGGTAAGACCTGACTAACT	0.20	

Table S3 Mean, median, minimum and maximum distances among the ponds included in the models within each spatially structured population (SSP)

SSP	Number of ponds	Mean distance (m)	Median distance (m)	Minimum distance (m)	Maximum distance (m)
DB	4	956	843	438	1676
TO	6	177	183	19	299
ZE	1	-	-	-	-
TE	4	564	567	56	1029
WT	4	176	162	104	306
WR	2	1702	1702	1702	1702
DP	2	811	811	811	811
PE	1	-	-	-	-
MF	6	120	106	67	220
ATG	1	-	-	-	-
MH	2	194	194	194	194
BvA	3	307	339	135	448
VK	4	227	217	73	408

Table S4 Land cover types and landscape features included as explanatory variables in the mixed models, with mean values and ranges (between parentheses; when number of ponds > 1) per spatially structured population (SSP) and buffer radius. Each landscape variable is followed by a short description

Land cover type / feature	Buffer radius (m)	DB	TO	ZE	TE	WT	WR	DP	PE	MF	ATG	MH	BvA	VK
Grassland and shrubs: proportion of the buffer area covered with grassland and shrubbery not used for agricultural purposes (map layer BBK)	50	0.04 (0.01-0.06)	0.54 (0.19-0.92)	0.02	0.15 (0.06-0.21)	0.48 (0.28-0.75)	0.15 (0.06-0.25)	0.69 (0.39-0.98)	0	0.28 (0-0.55)	0.13	0.1 (0.1-0.11)	0.04 (0-0.07)	0.12 (0.07-0.17)
	100	0.01 (0.01-0.02)	0.46 (0.21-0.74)	0.03	0.1 (0.06-0.17)	0.41 (0.24-0.53)	0.15 (0.05-0.25)	0.57 (0.28-0.87)	0.02	0.28 (0.11-0.45)	0.06	0.04 (0.03-0.04)	0.05 (0.01-0.12)	0.1 (0.04-0.16)
	250	0.05 (0.02-0.09)	0.42 (0.33-0.46)	0.05	0.12 (0.08-0.14)	0.27 (0.27-0.29)	0.12 (0.03-0.2)	0.37 (0.31-0.44)	0	0.4 (0.35-0.46)	0.12	0.02 (0.02-0.03)	0.08 (0.05-0.12)	0.09 (0.04-0.18)
	500	0.07 (0.04-0.1)	0.3 (0.26-0.34)	0.16	0.12 (0.12-0.14)	0.23 (0.22-0.24)	0.09 (0.05-0.13)	0.35 (0.3-0.39)	0.03	0.43 (0.4-0.48)	0.18	0.06 (0.06-0.06)	0.1 (0.09-0.11)	0.14 (0.06-0.17)
Pasture: proportion of the buffer area covered with grassland and shrubbery used for agricultural purposes (map layer BBK)	50	0.6 (0.55-0.67)	0 (0-0)	0.49	0.22 (0-0.34)	0 (0-0)	0.68 (0.44-0.92)	0 (0-0)	0.05	0 (0-0)	0.38	0.44 (0.43-0.45)	0.61 (0.51-0.77)	0.49 (0.32-0.66)
	100	0.43 (0.33-0.55)	0.02 (0-0.07)	0.34	0.23 (0.1-0.33)	0.03 (0-0.11)	0.58 (0.38-0.79)	0 (0-0)	0.18	0 (0-0)	0.14	0.4 (0.34-0.47)	0.38 (0.3-0.49)	0.39 (0.16-0.59)
	250	0.39 (0.26-0.56)	0.03 (0-0.03)	0.33	0.25 (0.17-0.34)	0.07 (0.05-0.11)	0.34 (0.17-0.5)	0 (0-0)	0.37	0 (0-0)	0.15	0.4 (0.29-0.5)	0.31 (0.26-0.33)	0.3 (0.24-0.37)
	500	0.37 (0.28-0.43)	0.07 (0.02-0.09)	0.25	0.27 (0.1-0.34)	0.15 (0.13-0.18)	0.32 (0.23-0.41)	0 (0-0)	0.35	0 (0-0)	0.08	0.32 (0.3-0.34)	0.3 (0.26-0.34)	0.31 (0.28-0.33)
Arable land: proportion of the buffer area covered with agricultural fields used for growing crops (map layer BBK)	50	0.03 (0-0.09)	0 (0-0)	0.07	0.43 (0.17-0.69)	0.1 (0-0.3)	0.02 (0-0.03)	0 (0-0)	0.92	0 (0-0)	0.24	0.37 (0.36-0.39)	0.01 (0-0.03)	0.01 (0-0.04)
	100	0.16 (0-0.39)	0 (0-0)	0.14	0.52 (0.32-0.7)	0.12 (0-0.3)	0.14 (0.14-0.15)	0.01 (0-0.02)	0.78	0 (0-0)	0.51	0.5 (0.46-0.55)	0.09 (0-0.22)	0.14 (0.02-0.31)
	250	0.22 (0.1-0.37)	0 (0-0)	0.16	0.46 (0.43-0.51)	0.24 (0.18-0.27)	0.41 (0.38-0.44)	0 (0-0)	0.6	0 (0-0)	0.48	0.5 (0.35-0.65)	0.18 (0.08-0.25)	0.26 (0.23-0.33)
	500	0.2	0.01 (0-0.03)	0.15	0.37 (0.33-0.44)	0.26 (0.24-0.28)	0.45 (0.43-0.47)	0 (0-0)	0.58	0 (0-0)	0.38	0.46 (0.43-0.49)	0.23 (0.16-0.27)	0.22 (0.12-0.35)
Trees: proportion of the buffer area covered with trees (map layer BBK)	50	0.3 (0.27-0.36)	0.42 (0.06-0.79)	0.37	0.15 (0.02-0.31)	0.36 (0-0.7)	0.12 (0-0.25)	0.3 (0-0.6)	0	0.31 (0.13-0.54)	0.16	0.04 (0.04-0.04)	0.33 (0.21-0.4)	0.34 (0.22-0.45)
	100	0.37 (0.25-0.48)	0.39 (0.09-0.63)	0.31	0.1 (0.02-0.2)	0.4 (0.21-0.51)	0.08 (0-0.17)	0.21 (0-0.43)	0.01	0.35 (0.22-0.47)	0.25	0.03 (0.02-0.04)	0.46 (0.39-0.51)	0.34 (0.26-0.42)
	250	0.31 (0.25-0.36)	0.23 (0.15-0.26)	0.16	0.09 (0.06-0.14)	0.35 (0.28-0.41)	0.04 (0-0.07)	0.12 (0.02-0.23)	0	0.37 (0.29-0.43)	0.21	0.06 (0.02-0.1)	0.41 (0.35-0.45)	0.32 (0.28-0.36)
	500	0.34 (0.29-0.36)	0.14 (0.13-0.14)	0.15	0.1 (0.07-0.18)	0.25 (0.23-0.26)	0.03 (0.02-0.04)	0.13 (0.06-0.21)	0.02	0.46 (0.42-0.49)	0.24	0.13 (0.12-0.14)	0.33 (0.28-0.42)	0.26 (0.23-0.3)

| Land cover type / feature	Buffer radius (m)	SSP												
		DB	TO	ZE	TE	WT	WR	DP	PE	MF	ATG	MH	BvA	VK
Surface water: proportion of the buffer area covered with surface water (map layer GRB)	50	0.06 (0.03-0.09)	0.18 (0.09-0.29)	0.02	0.15 (0.07-0.23)	0.02 (0-0.04)	0.03 (0.02-0.03)	0 (0-0)	0	0.11 (0.04-0.21)	0.17	0.03 (0.03-0.04)	0.02 (0-0.03)	0.09 (0-0.22)
	100	0.02 (0.01-0.03)	0.12 (0.09-0.26)	0	0.07 (0.03-0.12)	0.01 (0-0.02)	0.01 (0.01-0.01)	0 (0-0)	0.02	0.11 (0.05-0.14)	0.11	0.01 (0.01-0.01)	0.01 (0-0.01)	0.08 (0.01-0.13)
	250	0.02 (0.01-0.03)	0.22 (0.2-0.24)	0.02	0.06 (0.01-0.08)	0.07 (0.03-0.08)	0.01 (0.01-0.01)	0 (0-0)	0	0.05 (0.05-0.06)	0.04	0.01 (0.01-0.01)	0.01 (0.01-0.01)	0.07 (0.03-0.09)
	500	0.03 (0.01-0.04)	0.09 (0.09-0.09)	0.04	0.05 (0.02-0.06)	0.04 (0.04-0.05)	0.01 (0-0.01)	0 (0-0)	0	0.02 (0.02-0.02)	0.03	0.01 (0.01-0.01)	0.03 (0.01-0.04)	0.09 (0.09-0.09)
Distance to the nearest pond (m)	all	141 (79-216)	11 (8-15)	29	216 (99-354)	89 (70-98)	11 (9-13)	484 (189-779)	20	11 (5-19)	20	193 (188-198)	193 (125-328)	58 (19-125)
Roads (m): total length of paved roads within the buffer (map layer GRB)	50	0 (0-0)	23 (0-92)	0	38 (0-99)	0 (0-0)	0 (0-0)	0 (0-0)	0	70 (48-90)	0	29 (0-59)	0 (0-0)	136 (0-266)
	100	0 (0-0)	289 (0-511)	0	199 (116-237)	0 (0-0)	121 (0-243)	0 (0-0)	0	187 (181-195)	0	92 (0-183)	41 (0-123)	386 (0-677)
	250	36 (0-100)	3220 (473-4765)	618	1377 (621-2018)	273 (0-545)	838 (432-1243)	207 (0-414)	0	574 (492-835)	439	817 (759-875)	862 (745-1026)	3571 (2937-3806)
	500	1436 (0-2245)	32668 (25274-	4134	4960 (2882-5987)	8687 (8203-9170)	3488 (2303-	813 (603-1023)	2454	1728 (1562-1900)	3037	2579 (2579-2579)	4431 (3858-5564)	11640 (9071-
Number of lentic systems: total number of lentic systems within the buffer (map layer Watervlakken v. 1.0)	50	1 (1-1)	16 (6-31)	5	2 (1-2)	1 (1-1)	1 (1-1)	3 (1-4)	1	9 (7-11)	2	1 (1-1)	1 (1-1)	2 (1-3)
	100	1 (1-2)	35 (23-72)	6	2 (1-3)	3 (2-3)	2 (1-2)	5 (3-6)	1	31 (25-34)	4	1 (1-1)	1 (1-1)	3 (1-3)
	250	5 (2-8)	116 (93-142)	12	4 (2-6)	10 (7-13)	4 (3-4)	8 (7-8)	2	113 (103-124)	6	3 (2-3)	2 (1-4)	7 (5-9)
	500	15 (7-20)	147 (146-148)	17	10 (9-13)	19 (17-22)	10 (9-11)	11 (10-12)	4	145 (145-145)	7	5 (4-6)	7 (5-10)	11 (10-13)

Table S5 Mean (and standard deviation) of several indexes per locus averaged over all sampled ponds with a minimal sample size of six, including total number of alleles per locus

Locus	Total A	A	A_R	H_O	H_E	F_{IS}
Tcri13	13	4.31 (1.44)	3.01 (1.84)	0.47 (0.20)	0.48 (0.18)	0.02 (0.16)
Tcri29	16	4.33 (1.20)	3.17 (0.66)	0.54 (0.14)	0.59 (0.14)	0.06 (0.17)
Tcri35	15	5.33 (1.58)	4.20 (1.97)	0.66 (0.17)	0.70 (0.14)	0.06 (0.16)
Tcri46	9	4.21 (1.34)	3.25 (0.79)	0.58 (0.17)	0.61 (0.16)	0.05 (0.18)
Tcri27	13	6.18 (2.13)	4.32 (1.01)	0.72 (0.18)	0.75 (0.09)	0.05 (0.22)
Tcri36	16	4.00 (1.88)	3.02 (1.06)	0.55 (0.24)	0.56 (0.20)	0.03 (0.20)
TRCR501	4	2.23 (0.93)	1.75 (0.66)	0.24 (0.23)	0.25 (0.22)	0.03 (0.22)
Tcri43c	11	5.44 (2.12)	3.85 (0.90)	0.65 (0.13)	0.69 (0.10)	0.05 (0.13)
TRCR427	19	7.54 (2.54)	4.87 (1.18)	0.72 (0.21)	0.77 (0.14)	0.08 (0.22)
Tc50	23	8.82 (3.18)	5.12 (1.20)	0.78 (0.13)	0.79 (0.10)	0.00 (0.10)
TRCR408	6	2.54 (0.82)	2.07 (0.53)	0.35 (0.18)	0.34 (0.16)	-0.01 (0.18)
TRCR414	4	2.41 (0.72)	1.99 (0.50)	0.38 (0.21)	0.39 (0.20)	0.04 (0.20)
TRCR424	7	3.87 (1.20)	3.08 (0.76)	0.59 (0.20)	0.58 (0.18)	-0.02 (0.17)
Tc52	6	3.21 (0.89)	2.51 (0.50)	0.46 (0.15)	0.45 (0.12)	0.00 (0.17)
Tc74	7	2.59 (1.07)	1.96 (0.70)	0.26 (0.20)	0.28 (0.20)	0.06 (0.18)
TRCR403	10	4.44 (1.67)	3.23 (1.03)	0.57 (0.23)	0.56 (0.22)	-0.01 (0.11)
Tc70	5	2.59 (1.02)	1.76 (0.57)	0.21 (0.19)	0.21 (0.18)	0.02 (0.22)
TRCR417	8	2.69 (1.00)	2.10 (0.72)	0.37 (0.26)	0.37 (0.24)	-0.02 (0.19)
Tc66	9	3.33 (0.96)	2.58 (0.55)	0.46 (0.16)	0.49 (0.14)	0.06 (0.21)
TRCR502	5	2.69 (0.98)	2.08 (0.70)	0.35 (0.24)	0.35 (0.23)	0.01 (0.19)
TRCR416	8	3.77 (1.13)	2.66 (0.60)	0.45 (0.14)	0.48 (0.14)	0.05 (0.16)
TRCR406	26	8.13 (2.49)	4.94 (1.06)	0.77 (0.13)	0.78 (0.10)	0.02 (0.12)
TRCR302	5	2.38 (0.85)	1.90 (0.68)	0.27 (0.23)	0.29 (0.23)	0.01 (0.18)
TRCR401	17	6.77 (2.21)	4.23 (0.97)	0.69 (0.16)	0.71 (0.14)	0.03 (0.13)
TRCR425	28	10.85 (3.78)	5.84 (1.33)	0.84 (0.10)	0.84 (0.08)	0.00 (0.10)
TRCR423	9	4.23 (1.11)	3.04 (0.77)	0.58 (0.21)	0.55 (0.17)	-0.03 (0.17)
TRCR402	19	8.33 (2.63)	5.24 (1.11)	0.76 (0.11)	0.82 (0.08)	0.07 (0.11)
TRCR421	6	1.87 (0.86)	1.46 (0.52)	0.12 (0.15)	0.13 (0.16)	0.07 (0.24)
Tc68b	14	6.49 (1.92)	4.16 (0.84)	0.72 (0.13)	0.71 (0.12)	-0.02 (0.12)
TRCR422	3	1.72 (0.60)	1.47 (0.41)	0.14 (0.14)	0.15 (0.15)	0.01 (0.17)
TRCR407	22	9.23 (2.78)	5.38 (1.29)	0.80 (0.16)	0.81 (0.11)	0.03 (0.12)

Total A: total number of different alleles; A: mean number of alleles; A_R: allelic richness; H_O: observed heterozygosity; H_E: expected heterozygosity; F_{IS}: inbreeding coefficient
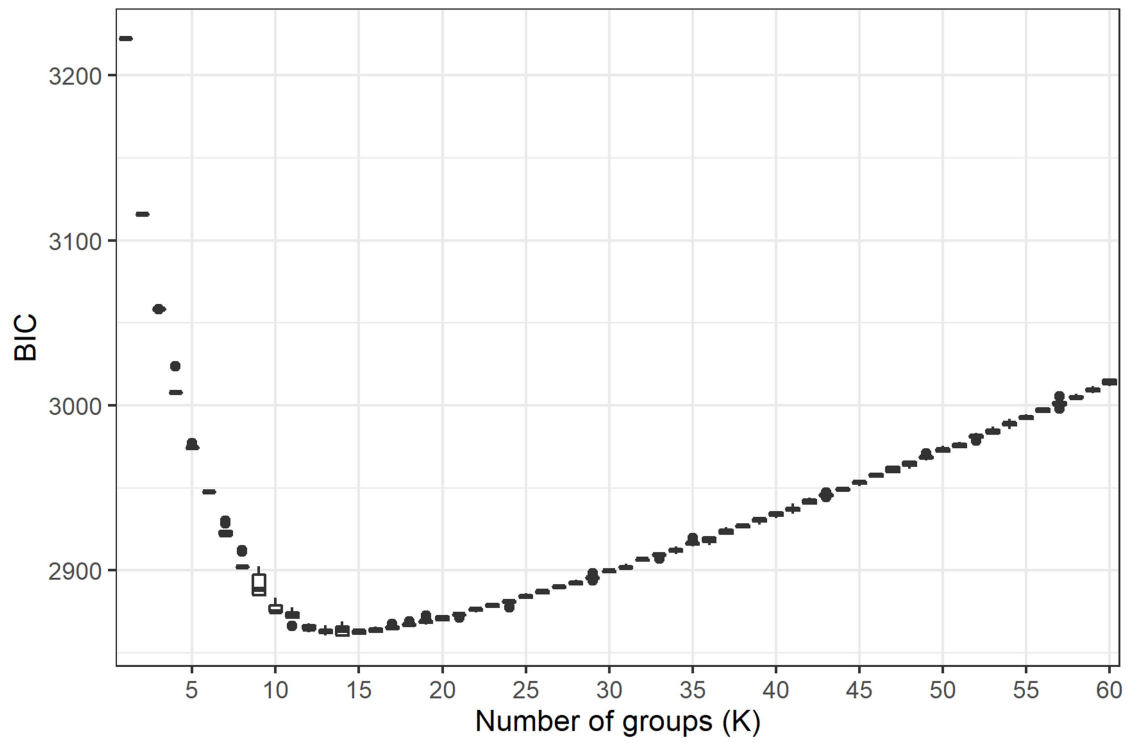
Fig. S2 Bayesian Information Criterion (BIC) values for all 60 groups evaluated with the K-means procedure. Boxplots of BIC for each K were constructed based on ten replicates
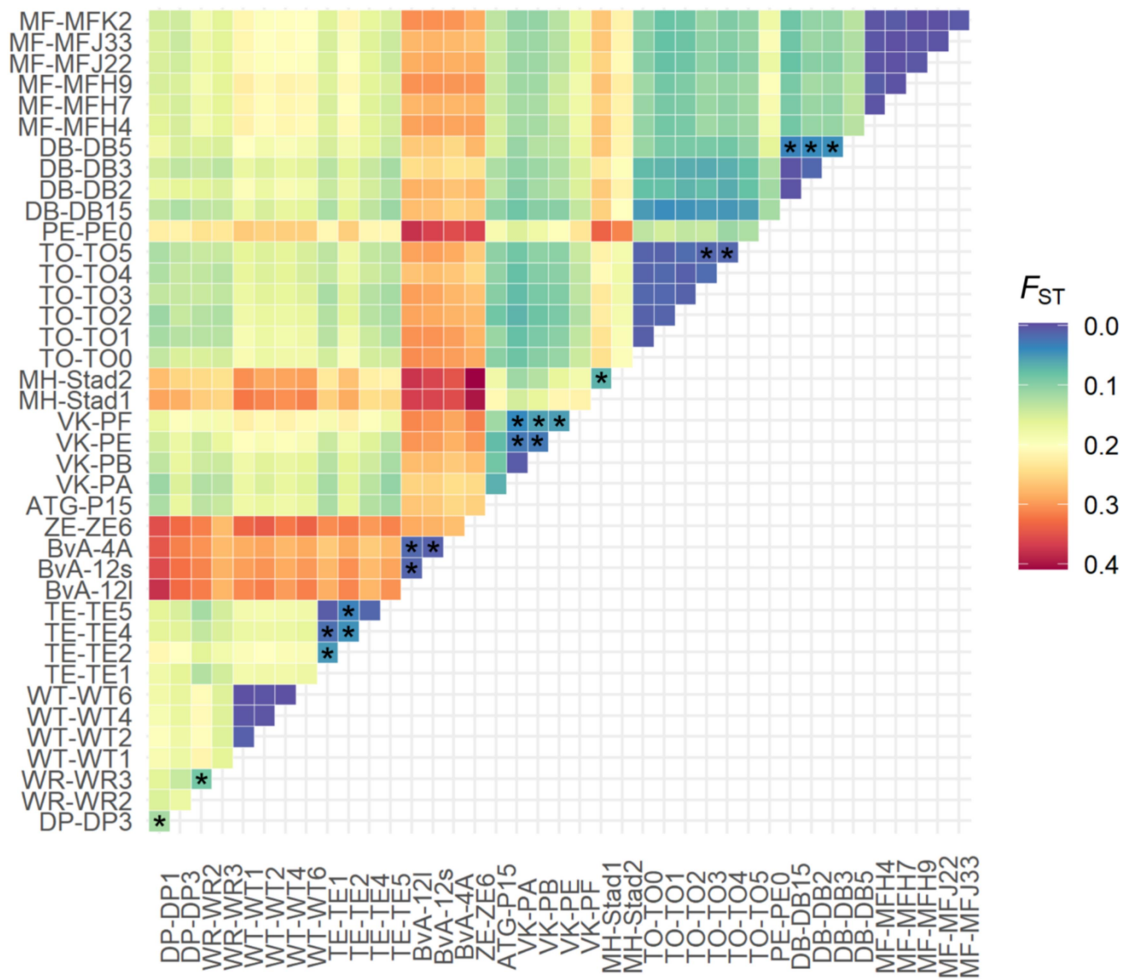
Fig. S3 Heatmap of pairwise F_{ST}. Every Pond-ID is preceded with the ID of the spatially structured population (SSP). F_{ST} values among ponds from different SSPs were all significant at the 95% level; significant F_{ST} among ponds within SSPs are indicated with *.

Table S6 Mean relatedness (and variance) within and among ponds and the mean difference between both in each spatially structured population (SSP) with at least two ponds sampled

SSP	r within ponds	r between ponds	Δr
BvA	0.102 (0.017)	0.091 (0.149)	0.011**
MH	0.111 (0.022)	0.0614 (0.013)	0.050**
VK	0.076 (0.015)	0.048 (0.007)	0.028**
DB	0.062 (0.009)	0.029 (0.003)	0.033**
DP	0.069 (0.012)	0.037 (0.004)	0.032**
MF	0.052 (0.005)	0.048 (0.005)	0.004*
TE	0.069 (0.008)	0.032 (0.003)	0.036**
TO	0.054 (0.009)	0.034 (0.003)	0.021**
WR	0.063 (0.008)	0.015 (0.001)	0.048**
WT	0.063 (0.009)	0.061 (0.008)	0.002

r: the mean DyadML coefficient; Δr: mean difference in relatedness within and among ponds; **: significant at the 98% confidence level; *: significant at the 95% confidence level

Table S7 Model selection table for each response and buffer size with models displaying ΔAICc ≤ 2

Response	Buffer radius (m)	Intercept	Within-SSP-number of lentic systems	Within-SSP-distance to nearest pond	Within-SSP-surface water	Within-SSP-grassland and shrubs	Within-SSP-pasture	Between-SSP-pasture	Within-SSP-arable land	Within-SSP-trees	Between-SSP-trees	Within-SSP-roads	df	AICc	ΔAICc	weight
A_R	50	3.236	-0.01720	-0.00105	1.732				0.4093				7	7.6	0	0.365
		3.236	-0.01748	-0.00093	1.805								6	8.0	0.4	0.300
	100	3.235	-0.01354	-0.00116	3.182		0.5486		0.8171				8	-0.2	0	0.416
		3.235	-0.01497	-0.00102	3.217		0.4772		0.6439	-0.2360			9	0.7	0.87	0.269
	250	3.238		-0.00091									4	15.3	0	0.364
		3.237		-0.00087			0.3928						5	16.8	1.49	0.173
	500	3.237		-0.00103					-1.521				5	12	0	0.409
		3.237		-0.00101	-2.492				-1.724				6	14	1.99	0.151
F_{IS}	50	-0.04105		-0.00017			-0.2252			-0.06211			6	-121.3	0	0.393
		-0.04124		-0.00021			-0.2116						5	-119.5	1.82	0.158
		-0.05901		-0.00017			-0.2252	0.05936		-0.06211			7	-119.4	1.90	0.152
	100	-0.04128								-0.1725			4	-121.1	0	0.339
		-0.04122		-0.00008						-0.1576			5	-119.7	1.31	0.176
		-0.06903								-0.1725	0.1054		5	-119.6	1.50	0.160
	250	-0.04153								-0.3472			4	-116.6	0	0.178
		-0.04139								-0.3395		-0.00001	5	-116.3	0.24	0.158
		-0.0414	0.001209							-0.3538			5	-116.1	0.50	0.139
		-0.04145							-0.1325	-0.3930			5	-115.2	1.35	0.091
		-0.0413							-0.1433	-0.3888		-0.00001	6	-115.2	1.35	0.090
		-0.06449								-0.3472	0.1062		5	-115.0	1.62	0.079
		-0.04147		-0.00008						-0.2940			5	-114.8	1.74	0.075
	500	-0.04172		-0.00014									4	-113.7	0	0.188
		-0.04193											3	-113.4	0.31	0.161
		-0.04157		-0.00017					-0.2720				5	-113.2	0.45	0.150
		-0.06473		-0.00014							0.1098		5	-112.1	1.62	0.084
		-0.04184							-0.2071				4	-112.0	1.67	0.082
		-0.04148		-0.00018		-0.1722			-0.3407				6	-111.9	1.79	0.077
		-0.06392									0.1041		4	-111.9	1.83	0.075

Response	Buffer radius (m)	Intercept	Within-SSP-number of lentic systems	Within-SSP-distance to nearest pond	Within-SSP-surface water	Within-SSP-grassland and shrubs	Within-SSP-pasture	Between-SSP-pasture	Within-SSP-arable land	Within-SSP-trees	Between-SSP-trees	Within-SSP-roads	df	AICc	ΔAICc	weight
LD-N_e	50	2.919	0.8957								3.751		5	53.6	0	0.389
		2.962									3.65		4	54.8	1.17	0.217
		2.886	0.9163						2.287		3.841		6	55.0	1.38	0.195
	100	3.122			16.13						2.823		5	56.0	0	0.333
		3.18									2.705		4	56.5	0.45	0.266
		3.887			16.13								4	58.0	1.99	0.123
	250	4.063								4.803			4	80.6	0	0.322
		4.076											3	81.6	1.05	0.190
		4.067		0.00225									4	82.0	1.41	0.159
		4.060					1.556			5.023			5	82.6	1.97	0.120
	500	3.877			-42.94								4	57.4	0	0.283
		3.199			-42.94						3.057		5	57.5	0.12	0.267
		3.937											3	58.3	0.87	0.183
		3.304									2.780		4	58.7	1.32	0.147
SA-N_e	50	3.694		-0.00243									4	27.2	0	0.277
		3.687	-0.06326	-0.0026	1.887								6	27.9	0.72	0.193
		3.691	-0.03734	-0.00243									5	28.1	0.86	0.180
		3.700											3	28.5	1.28	0.146
		3.692		-0.00296					0.6597				5	28.9	1.70	0.119
	100	3.687		-0.00289		1.174			-1.104				5	25.4	0	0.356
		3.479		-0.00289					-1.104	0.7302			6	27.0	1.63	0.158
		3.686		-0.00305	1.88				-1.084				6	27.1	1.77	0.147
		3.694		-0.00243									4	27.2	1.86	0.141
	250	3.686		-0.00228								-0.00019	5	24.3	0	0.35
		3.692										-0.00020	4	26.0	1.69	0.15
	500	3.434		-0.00243							1.157		5	27.1	0	0.264
		3.694		-0.00243									4	27.2	0.10	0.251
		3.437									1.165		4	28.1	0.99	0.161
		3.700											3	28.5	1.38	0.133
		3.692		-0.00263	1.951								5	29.1	1.96	0.099

SSP: spatially structured population, LD-N_e: LD-based estimates of N_e, SA-N_e: SA-based estimates of N_e, df: degrees of freedom

Table S8 Model averaged results of the top models with ΔAICc ≤ 2 for each of the response values and buffer sizes. Coefficients are given for each covariate still included in the model

Response	W/B	Covariate	Buffer radius of 50 m					Buffer radius of 100 m				
			Estimate	SE	CI	Z	p	Estimate	SE	CI	Z	p
A_R		intercept	3.2358	0.1701	2.9024; 3.5691	20.473	<0.0001	3.2348	0.1754	2.8910; 3.5786	18.442	<0.0001
	W	distance to the nearest pond	-0.0010	0.0003	-0.0015; -0.0005	3.771	0.0002	-0.001	0.0002	-0.0016; -0.0006	4.648	<0.0001
	W	number of lentic systems	-0.0173	0.0076	-0.0322; -0.0024	2.276	0.0228	-0.014	0.0035	-0.0210; -0.0072	3.982	<0.0001
	W	surface water	1.7649	0.4877	0.8091; 2.7207	3.619	0.0009	3.1957	0.6774	1.8681; 4.5233	4.718	<0.0001
	W	arable land	0.2248	0.2726	-0.3091; 0.7591	0.825	0.4096	0.7490	0.2445	0.2697; 1.2283	3.063	0.0022
	W	pasture						0.5205	0.2367	0.0565; 0.9845	2.199	0.0279
	W	trees						-0.0928	0.1574	-0.4012; 0.2157	0.589	0.5555
F_{IS}		intercept	-0.0450	0.0204	-0.0849; -0.0050	2.206	0.0274	-0.0478	0.0242	-0.0952; -0.0005	1.980	0.0477
	W	distance to the nearest pond	-0.0002	0.0001	-0.0003; -0.00003	2.290	0.0220	-0.00002	0.00005	-0.0001; 0.0001	0.400	0.6890
	W	pasture	-0.2221	0.0688	-0.3569; -0.0873	3.230	0.0012					
	B	pasture	0.0128	0.0400	-0.0655; 0.0911	0.322	0.7478					
	W	trees	-0.0481	0.0375	-0.1216; -0.0254	1.284	0.1993	-0.1686	0.0526	-0.2718; -0.0655	3.204	0.0014
	B	trees						0.0250	0.0717	-0.1155; 0.1655	0.349	0.7274
LD-N_e		intercept	2.9230	0.4559	2.0294; 3.8166	6.411	<0.0001	3.2739	0.5014	2.2911; 4.2567	6.529	<0.0001
	W	number of lentic systems	0.6582	0.5597	-0.4388; 1.7551	1.176	0.2396					
	W	surface water						10.1918	10.7626	-10.9026; 31.2862	0.947	0.3440
	W	arable land	0.5570	1.2977	-1.9865; 3.1006	0.429	0.6677					
	B	trees	3.7457	1.5388	0.7298; 6.7616	2.434	0.0149	2.2974	1.6346	-0.9064; 5.5011	1.405	0.1600
SA-Ne		intercept	3.6924	0.0990	3.4983; 3.8864	37.3	<0.0001	3.6471	0.1546	3.3441; 3.9500	23.598	<0.0001
	W	distance to the nearest pond	-0.0021	0.0015	-0.0051; 0.0008	1.427	0.1540	-0.0028	0.0012	-0.0052; -0.0004	2.313	0.0208
	W	number of lentic systems	-0.0207	0.0326	-0.0847; 0.0433	0.634	0.5260					
	W	surface water	0.3981	0.9358	-1.4361; 2.2323	0.425	0.6710	0.3443	1.0676	-1.7481; 2.4367	0.323	0.7471
	W	arable land	0.0856	0.3281	-0.5575; 0.7287	0.261	0.7940					
	W	trees						-0.9067	0.6452	-2.1714; 0.3579	1.405	0.1599
	B	trees						0.1438	0.4291	-0.6972; 0.9848	0.335	0.7376

Response	W/B	Covariate	Buffer radius of 250 m					Buffer radius of 500 m				
			Estimate	SE	CI	Z	p	Estimate	SE	CI	Z	p
A_R		intercept	3.2375	0.1643	2.9154; 3.5596	19.701	<0.0001	3.2367	0.1667	2.9100; 3.5634	19.418	<0.0001
	W	distance to the nearest	-0.0009	0.0003	-0.0015; -0.0004	2.866	0.0042	-0.0010	0.0003	-0.0016; -0.0005	3.539	0.0004
	W	surface water						-0.6723	1.9482	-4.4911; 3.1459	0.345	0.7299
	W	arable land						-1.5756	0.6611	-2.87125; -0.2799	2.383	0.0172
	W	pasture	0.1264	0.2915	-0.4450; 0.6978	0.434	0.6646					
F_{IS}		intercept	-0.0437	0.0189	-0.0807; -0.0067	2.316	0.0206	-0.0461	0.0205	-0.0863; -0.0059	2.248	0.0245
	W	distance to the nearest	-0.00001	0.00004	-0.00008; 0.00006	0.203	0.8389	-0.0001	0.0001	-0.0003; 0.0001	0.919	0.3581
	W	number of lentic systems	0.0002	0.0006	-0.0009; 0.0014	0.354	0.7235					
	W	arable land	-0.0308	0.0821	-0.1918; 0.1301	0.376	0.7071	-0.1029	0.1833	-0.4621; 0.2564	0.561	0.5746
	W	pasture						-0.0162	0.0696	-0.1527; 0.1202	0.233	0.8155
	W	trees	-0.3517	0.1489	-0.6435; -0.0599	2.362	0.0182					
	B	trees	0.0104	0.0497	-0.0869; 0.1077	0.209	0.8342	0.020	0.0695	-0.1153; 0.1570	0.300	0.7642
	W	roads	-0.000004	0.000008	-0.000021;	0.518	0.6048					
LD-N_e		intercept	4.0664	0.2026	3.6694; 4.4634	20.074	<0.0001	3.5879	0.5090	2.5903; 4.5855	7.049	<0.0001
	W	distance to the nearest	0.0005	0.0012	-0.0018; 0.0027	0.695	0.6950					
	W	surface water						-26.8538	27.4232	-80.6023; 26.8946	0.979	0.3270
	W	pasture	0.2366	0.9103	-1.5476; 2.0208	0.260	0.7950					
	W	trees	2.7185	3.1187	-3.3941; 8.8311	0.872	0.3830					
	B	trees						1.3916	2.0004	-2.5292; 5.3124	0.696	0.4870
SA-N_e		intercept	3.6880	0.0987	3.4943; 3.8812	37.366	<0.0001	3.5733	0.1929	3.1952; 3.9515	18.520	<0.0001
	W	distance to the nearest	-0.0016	0.0014	-0.0044; 0.0012	1.129	0.2588	-0.0017	0.0016	-0.0047; 0.0014	1.058	0.2900
	B	trees						0.5431	0.7652	-0.0047; 0.0014	0.710	0.4780
	W	grassland and shrubs						0.2135	0.9326	-1.6143; 2.0414	0.229	0.8190
	W	roads	-0.0002	0.0001	-0.0004; -0.00002	2.253	0.0242					

W: within spatially structured populations, B: between spatially structured populations, LD-N_e: LD-based estimates of N_e, SA-N_e: SA-based estimates of N_e, SE: standard error, CI: 95% confidence interval