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RESEARCH ARTICLE

Genetic characterization of Western European noble crayfish populations (*Astacus astacus*) for advanced conservation management strategies

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Abstract One central goal of conservation biology is to conserve the genetic diversity of species in order to protect their adaptive potential. The main objective of this study was to identify management units (MUs) for the threatened noble crayfish (*Astacus astacus*) in Western Europe by utilizing sequence and microsatellite analysis to determine populations in need of focused conservation programs. With the analysis of noble crayfish from 31 sampling sites from Belgium, France, The Netherlands and Germany, and further comparison of this data with a European-wide dataset, we propose four distinct MUs: the French Meuse (MU 1), the French Rhine (MU 2), the Belgian Scheldt and Meuse (MU 3) as well as populations from the French Seine (MU 4). This knowledge enables advanced *A. astacus* conservation management practises in these catchments by distinguishing between outbreeding and inbreeding populations and by preserving the maximum genetic diversity. When required, a high genetic diversity can be conserved by strengthen existing populations via stocking with populations that either bear the most common haplotype or population-specific private haplotypes in order to maintain recent and regional adaptions. Above all, stocking with populations that exhibit haplotypes from outside Western Europe should be avoided in these catchments. This study supports the preservation of the genetic diversity of noble crayfish in Western Europe and provides thus a proposition for advanced conservation management.

Keywords Population genetic diversity · MtDNA sequences · Microsatellite analysis · Species conservation · Artificial stocking · Management units

Introduction

The protection of species diversity is a major goal in species conservation (SCBD 1992). To maintain the genetic diversity within one species, conservation genetics aim to protect genetic variability within and between populations. High genetic diversity is essential for species to adapt to changing environmental conditions (Jump et al. 2009). A reduction in this evolutionary potential can cause local population extinctions (Boulding 2008). Consequently, high genetic diversity is fundamental especially for threatened species. To increase the genetic diversity of populations, artificial cross-stock translocation of individuals is often promoted (Souty-Grosset and Grandjean 2009). However, this may result in outbreeding depression and thus decrease the fitness of a stock (Moritz 1999). In contrast, small isolated populations with lacking gene flow may suffer from inbreeding depression that also reduces fitness. Therefore, one challenge in species' conservation is to find a balance between outbreeding and inbreeding depression (Frankham et al. 2011; Waser and Price 1994).

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To conserve the integrity and the maximum genetic diversity of a species, it is recommended that translocations are only conducted within evolutionary significant units (ESUs) (Ryder 1986). Populations in an ESU are reciprocally monophyletic for mtDNA alleles and show significant divergence of allele frequencies at nuclear loci (Moritz 1994). As Moritz (1994) remarks, this definition may seem overly restrictive, but it is theoretically sound. However, in practice, a Management Unit (MU) is more applicable for population monitoring. A MU can be defined as a population or group of populations with significant divergence of allele frequencies at nuclear or mitochondrial loci compared to other populations or groups of populations, regardless of the phylogenetic distinctiveness of the alleles (Moritz 1994). For species like freshwater crayfish whose distribution has been impacted by anthropogenic influence, we cannot expect strict monophyly for mtDNA alleles. However, because freshwater species often experience restricted gene flow, especially in lotic habitats, an individual management or conservation strategy is required. Consequently, the identification of MUs is an appropriate basis for modern conservation management. Although a MU for freshwater species is expected to be restricted to one river catchment due to limited distribution abilities, this categorization is not valid for all freshwater species (Vonlanthen et al. 2007). Additionally, anthropogenic translocations can alter the genetic distinctiveness of freshwater species as well (Lerceteau-Köhler et al. 2013), an aspect especially severe in species of commercial interest such as freshwater crayfish.

For native crayfish species translocations for restocking purposes should be conducted with special caution because all populations are highly threatened by the spread of the crayfish plague agent (*Aphanomyces astaci*). This highly virulent disease (OIE 2016) has been distributed in most European rivers via invasive crayfish originating from North America (e.g. Pârvulescu et al. 2012; Schrimpf et al. 2013) or even without a host via transport of the *A. astaci* spores (Svoboda et al. 2016). As a result, European native crayfish are currently mostly restricted to isolated ponds, lakes and springs (Holdich 2002).

The European noble crayfish (*Astacus astacus*) shows a decreasing population trend and is listed as vulnerable (IUCN 2013). Its population status is of high concern especially in Western Europe. For instance, in The Netherlands there is only one population remaining (Edsman et al. 2010; Ottburg and Roessink 2012). In Belgium, the species is no longer present in Flanders (Boets et al. 2012) but currently inhabits only Wallonia. Here, however, the number of populations harboured in ponds suffered a 40% reduction between 2001 and 2012 (Cammaerts, unpublished work based on the database of the Département de l'Étude du Milieu naturel et agricole, Gembloux, Belgium). In France the species is close to extinction (Collas et al. 2007; UICN 2012). In Germany most remaining stocks are located in low mountain ranges (Schulz et al. 2008) and many result from anthropogenic artificial translocations for population augmentation.

Previous studies showed that the highest genetic diversities of noble crayfish can be found in south-eastern Europe, the refugial area of this crayfish during the last glacial maximum (Schrimpf et al. 2011, 2014) which lasted from approximately between 26,500 and 19,000 years before present (Clark et al. 2009). Crayfish populations from these refugia recolonized most of the remaining European continent after the retreat of the ice sheet. Due to strong founder effects these populations are usually characterised by low genetic diversity. However, because of the high differentiation of endemic haplotypes from Rhineland-Palatinate in south-western Germany and from Schleswig-Holstein in northern Germany, the existence of additional refugia in Central and Western Europe during the last ice age is still under debate (Schrimpf et al. 2014). As a consequence, an intensive population genetic study of the noble crayfish in its Western European distribution range (France, The Netherlands and Belgium) was required to assess the natural genetic structure and to establish proper management strategies for this threatened species in order to preserve the adaptive potential of the remaining populations.

Therefore, the aim of this study was to answer three questions which are fundamental for future conservation programs of noble crayfish in Western Europe:

1. Can we detect high genetic diversity in Western Europe with distinct haplotypes/alleles that indicate an additional extra-Mediterranean refugium during the last glacial period?
2. Can we reveal genetic similarities or differentiations between populations within the Meuse, Rhine, Scheldt and Seine river catchments in order to identify MUs?
3. Can we detect an anthropogenic influence on noble crayfish population structure to distinguish between autochthonous (indigenous) and allochthonous (artificially translocated) populations?

To answer these questions, we used mitochondrial DNA (cytochrome oxidase subunit I and 16S rRNA) and nuclear DNA (microsatellites) and compared our data from Western Europe to the European wide data set of Schrimpf et al. (2014). Additionally, our study benefits from detailed background information about the studied populations from historic records, which allowed us to compare the genetic results with reconstructions of the populations' origins.

Methods

Study site and molecular analysis

In total, 563 crayfish specimens from 31 sampling sites (Fig. 1; Table 1) from Belgium, France, The Netherlands and Western Germany were collected by hand or trapping devices. Populations originating either from fish farms or hatcheries known to breed crayfish from outside Western Europe were not sampled. The sampling sites were located in the river catchment of the Meuse (N = 18), the Rhine (N = 4), the Scheldt (N = 7) and the Seine (N = 2). We assumed that the crayfish from each site formed a distinct population. Immediately after the lower part of one pereiopod (propodus and dactylus) was removed, specimens were released again at the location where they were caught. Appendages usually regenerate after a few molts. Samples were stored in 96% ethanol until DNA extraction. DNA was extracted from the muscle tissue using a standardized protocol (Sambrook and Russell 2001).

We generated a 350 base pair (bp) fragment of the mitochondrial cytochrome oxidase subunit I (COI) and a 500 bp fragment of the mitochondrial 16S rRNA (16S) for ten individuals per sampling site, using the primer pair ASTCOI (forward primer: 5′-GCGGGGATAGTAGGAACCTC-3′; reverse primer: 5′-ATTTACCGCCCCTAAAATCG-3′) and 16S_1471 and 16S_1472 (Crandall and Fitzpatrick 1996), respectively. Polymerase chain reactions (PCR) were performed according to Schrimpf et al. (2011). PCR products were sequenced on a 3730 DNA Analyzer eight capillary sequencer (Applied Biosystems, MA, USA) by the company SeqIT (Kaiserslautern, Germany). The sequences were edited and aligned with Geneious 5.0.3 (Drummond et al. 2011). The sequences were checked manually for base pair ambiguities, nuclear copies of mitochondrial derived genes, stop codons, and high levels of divergence (Buhay 2009). All haplotypes were submitted to GenBank (Genbank accession numbers will be provided upon acceptance of the manuscript).

To genotype all sampled individuals (N_{max} = 25 per sampling site) we used the six species-specific microsatellite (msat) loci Aas2, Aas6, Aas8, Aas11, Aas766, Aas1198 (Kõiv et al. 2008, 2009). PCR was carried out using a Primus 96 Cycler (Peqlab Biotechnologie GmbH, Erlangen, Germany) under the following conditions: an initial denaturation at 95 °C for 2 min, followed by 35 cycles of 30 s at 95 °C, 30 s at 60 °C, 30 s at 72 °C, and a final extension of 5 min at 72 °C. The 60 °C annealing was replaced by 63 °C for primer Aas11 and by 57 °C for primer Aas8. 2 µl PCR-product were added to 30 µl SLS (Beckman Coulter, Krefeld, Germany). The fragment analysis was performed on a Beckman Coulter CEQ 8000 eight capillary sequencer. Loci were scored using the software GeneMarker version 2.4.0 (State College, PA, USA). Micro-Checker version 2.2.3 was applied to test for scoring error due to stuttering, large allele dropout and null alleles (Van Oosterhout et al. 2004). All loci were tested for linkage disequilibrium with ARLEQUIN version 3.5.1.3 (Excoffier and Lischer 2010). In total, 10% of all samples were randomly chosen for repetition to estimate the genotyping error rate (Bonin et al. 2004). A genotyping error rate of 0.96% was estimated and should not bias our results.

Statistical analysis

Potential refugium in Western Europe

Based on the sequence data, the genetic variation within each sampling site was measured in terms of the number of haplotypes (H_N) and the haplotype diversity (H_D) using DNASP v 5.10.1 (Librado and Rozas 2009). To identify haplotypes and to determine the phylogenetic relationships between haplotypes a median joining (MJ) network (Bandelt et al. 1999) was constructed using the software NETWORK 4.610 (Fluxus Technology, Suffolk, UK). In a second median joining (MJ) network (NETWORK 4.510) the haplotypes of this study were assembled with 46 haplotypes from 540 specimens from the dataset of Schrimpf et al. (2014) in order to compare where the haplotypes of this study have been found before.

Concerning the msat data, the average number of alleles per locus per site (A) and the expected (H_E) and observed (H_O) heterozygosity were calculated in ARLEQUIN version 3.5.1.3 (Excoffier and Lischer 2010). Each population was tested for deviations of the Hardy–Weinberg equilibrium with GenoDive v 2.0b23 (Meirmans and Van Tienderen 2004). The number of private alleles (A_P, i.e., alleles endemic to populations or regions) per sampling site was calculated with the Genetic Data Analysis (GDA) v 1.1 software (Lewis and Zaykin 2001). In addition, a factorial correspondence analysis (FCA) was conducted with the default settings in GENETIX 4.05 (Belkir et al. 1996–2004) with the msat data of this study and reference data of 289 noble crayfish that were genotyped by Schrimpf et al. (2014) to compare the Western European noble crayfish to a European-wide data set.

MUs in Western Europe

Using sequence and msat data, a hierarchical analysis of molecular variance (AMOVA, Excoffier et al. 1992) with populations from each river catchment partitioned into separate groups was performed and the genetic differentiation in terms Φ_{ST}-values (sequence data) and F_{ST}-values (msat data) among populations and river catchments was estimated with ARLEQUIN v 3.11 (Excoffier and Lischer 2010). Significance was based on 1000 random

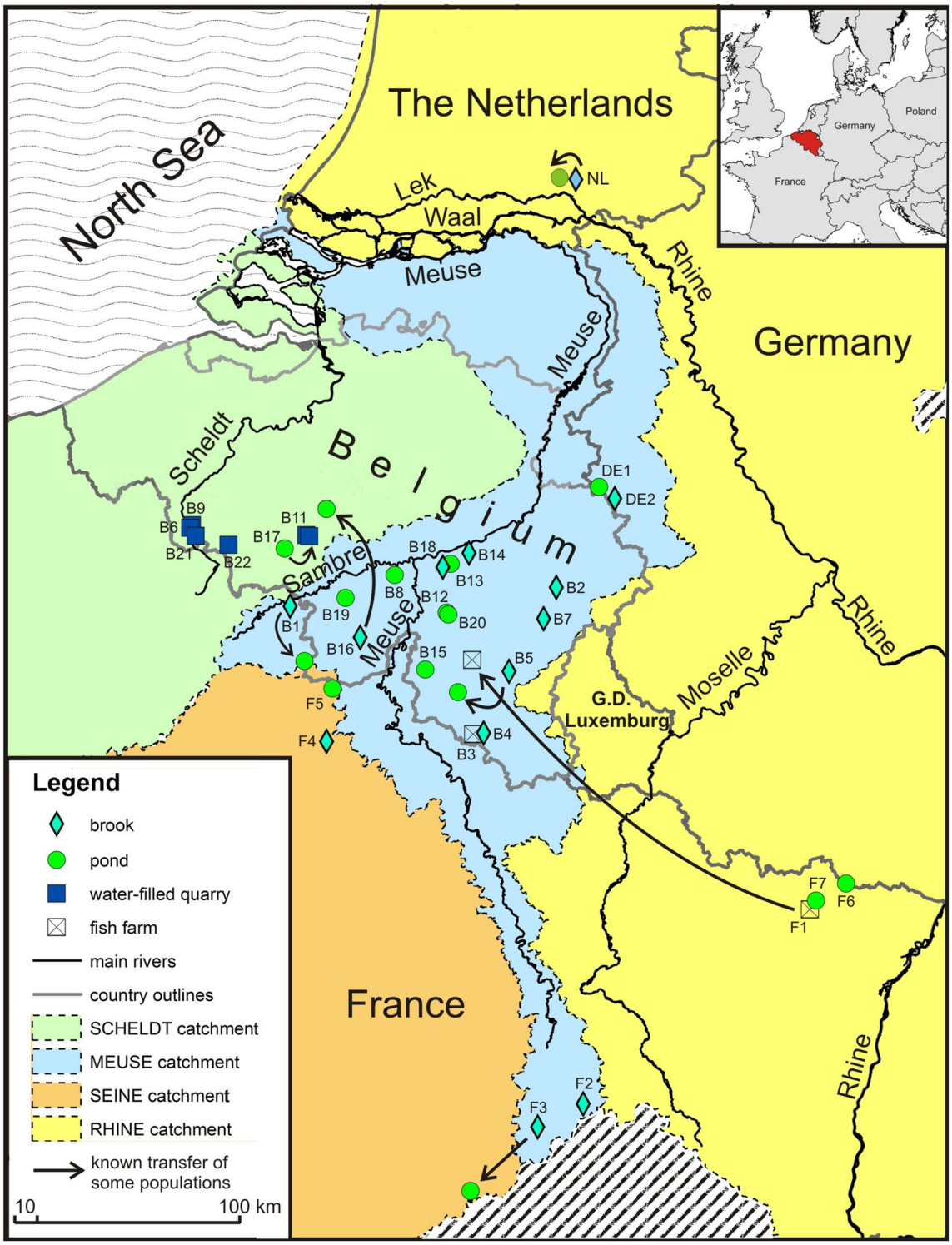

Fig. 1 Map showing the sampling sites of noble crayfish populations in France, Belgium, The Netherlands and Western Germany. Code abbreviations are explained in Table 1. *Arrows* indicate previous known translocations of noble crayfish. (Color figure online)

permutations. We applied the False Discovery Rate (FDR) approach to the p values (Benjamini and Hochberg 1995) of the Φ_{ST}- and F_{ST}-values.

Using msat data, the structure of and the variation among all populations was visualised by a principal component analysis (PCoA) via covariance matrix with data standardization with the software GenAlEx v 6.5 (Peakall and Smouse 2012) where

Table 1 Sampling sites of noble crayfish populations from France (FR), Belgium (B), The Netherlands (NL) and western Germany (DE)

Code	Town	Type	Present cm	Original sub-cm	Origin
Autochthonous population which local origin is indicated by archive					
B2	Lierneux	Pond	Meuse	Meuse, Amblève	Baleur brook, 1880[a]
Populations almost certainly from local origin and known to be present before WWII					
B1	Momignies	Brook	Meuse	Meuse, Sambre	Brooks of Jeumont area (adjacent to Belgium), ca 1930, France[b]
B5	Paliseul	Pond	Meuse	Meuse, Ourthe occidentale	Libramont-Freux area around 1960, but crayfish seems to have been in situ already in 1899[b]
B7	La Roche	Brook	Meuse	Meuse, Ourthe orientale	Said to be local, already known in 1983[a,b]
B12	Ciney	Brook and pond	Meuse	Meuse, Lesse	Said to be local, known since well before WW II[b]
B14	Marchin	Brook	Meuse	Meuse, Houyoux	Said to be local, known since well before WW II[b]
B16	Braine-le-Château	Pond	Scheldt	Meuse, Eau Blanche	From the Roly region, introduced in 1960 in this pond[a]
B18	Gesves	Brook	Meuse	Meuse, Samson	Unknown, present at least as early as 1940[a,b]
Populations possibly from local origin					
B4	Florenville	Brook	Meuse	Meuse, Semois?	Local origin not ascertained, known from 1978[a,c]
B15	Gedinne	Pond	Meuse	Meuse, Houille	Said "to have been never introduced"[b]
B19	Walcourt	Brook	Meuse	Meuse? Sambre?	Unknown, perhaps from local river (le Thyria, where it abounded around 1960)[a,b]
DE2	Aachen	Brook	Meuse	Ruhr	Unknown, maybe local[b]
F2	Contrexéville	Brook	Meuse	Meuse	Possibly transferred from an abbey estate located near the Meuse springs, decennia 1970[b]
F3	Breuvannes-en-Bassigny	Brook	Seine	Meuse	Near the Meuse springs, but has been transferred into a pond in Seine catchment, decennia 1980[b]
F4	Rocquigny	Brook	Seine	Oise	Unknown, possibly local[b]
F5	Signy-le-Petit	Pond	Seine	Oise	Unknown[b]
Populations originating from a possible stocking in past times					
DE1	Aachen	Brook	Meuse	Wurm-Ruhr	Originates from a local brook and pond located in an old estate[b]
B3	Sainte-Cécile	Fish farm	Meuse	Meuse, Semois?	Unknown, but said to be at least local[b] and present before 1980[a]
Population originating from a stocking made before or likely well before 1970					
B6	Tournai	W quarry[g]	Scheldt	?	Unknown, already present in 1996[a]
B9	Tournai	W quarry[g]	Scheldt	?	Unknown, already present in 1985[a]
B11	Ecaussinnes	W quarry[g]	Scheldt	?	Unknown, already present in 1946[a]
B17	Ecaussinnes	W quarry[g]	Scheldt	Scheldt?	Introduced from a fishing pond of a tributary of the River Haine (Scheldt cm), 1966[b]
B21	Antoing	W quarry[g]	Scheldt	?	Unknown, stocked in 1930[a]
B22	Beloeil	W quarry[g]	Scheldt	?	Unknown, already present in 1970[a]
Population originating from a stocking made since 1970 or after this date					
B8	Fosses-la-Ville	Pond	Meuse	?	Said to be from France, 1990, without further precision[b]
B13	Ohey	Pond	Meuse	?	Purchased in a food store of Namur, ca 1970[b]
B20	Ciney	Pond	Meuse	?	Purchased in 2002, said to originate from Poland[b]
Population in The Netherlands					
NL	Arnhem	Pond	Rhine	Rhine	Transferred from a nearby brook, decennia 1980[d]
Population in the Vosges country, perhaps stocked in past times					
F6	Sturzelbronn	Pond	Rhine	Moselle	Local; crayfish already present in Sturzelbronn brook in 1594 and 1789[e]; it may not be ruled out that it originated in a past stocking from Strasbourg by monks of the abbey[f]

Table 1 (continued)

Code	Town	Type	Present cm	Original sub-cm	Origin
Population in the Vosges country, stocked in recent times					
F1	Meisenthal	Hatchery	Rhine	Moselle	Local, present before 1950, stocked in Meisenthal brook[f], sampled at Mirwart fish farm (Belgium)
F7	Lemberg	Pond	Rhine	Moselle	Partly local and from Meisenthal crayfish hatchery[f]

Given is the present catchment (cm), the original sub-catchment (sub-cm) and the origin. The "?" indicates uncertain information. Sampling sites are sorted according to the population history. For species protection, local name and coordinates are not included in this table but can be obtained by enquiry from the authors

[a]DEMNA archives and data base

[b]Owner or manager's personal communication

[c]Balzat and Dussart (1978)

[d]Ottburg and Roessink (2012)

[e]Jehin (2006–2007)

[f]Franckhauser's personal communication

[g]Water-filled quarry

the reference data from Schrimpf et al. (2014) were excluded. The Structure v 2.3.4 software (Pritchard et al. 2000) was used to evaluate the genetic population partitioning. The admixture model with correlated allele frequencies was used without specifying sampling locations. The program was initially run with a number of clusters of K = 1–31 and a burn-in period of 10,000 followed by 50,000 Markov chain Monte Carlo (MCMC) iterations. The analysis was repeated five times. Structure Harvester v 0.6.94 (Earl and von Holdt 2012) was used to determine the most likely number of clusters applying the delta K method (Evanno et al. 2005). The highest delta K was determined at K = 4. A second analysis with K = 4 with a burn-in of 50,000 followed by 500,000 MCMC was repeated ten times.

Anthropogenic translocations in Western Europe

To evaluate the possibility of artificial translocations of noble crayfish we compared the combined COI+16s haplotype distribution of the study area (North Sea catchment) with a European-wide data set (Schrimpf et al. 2014) in the MJ graph with the reference data. An allochthonous haplotype may indicate translocation and stocking from a distant source. Furthermore, the admixture of the populations, based on the Structure results (msats), was evaluated. The admixture model assumes that each individual carries potential ancestry from different clusters.

Results

Potential refugium in Western Europe

In total, out of 309 sequenced individuals, seven combined (COI+16S) haplotypes were detected. The majority of sampled individuals (N = 277) from all four river catchments were invariable (Table 2; Fig. 2) and contained the most common European haplotype, Hap01 (as defined by Schrimpf et al. 2014). 23 sampling sites exhibited only Hap01 ($H_D = 0$). Site B11 was most diverse ($H_D = 0.822$), representing four haplotypes. The median joining network of Western European haplotypes (Fig. 2) showed that most haplotypes were separated by only one bp exchange. Hap41 was most differentiated with ten mutational steps to Hap01.

Regarding the microsatellite data, the microchecker analysis provided evidence for a homozygotes excess and putative null alleles for locus Aas11. The effect of null alleles may reduce the power to correctly assign individuals in the Structure tests to up to 1% and cause a small overestimation of F_{ST} values (Carlsson 2008), however, they will not significantly influence the results. No pair of loci showed significant linkage disequilibrium. Nine populations deviated significantly from the Hardy–Weinberg equilibrium (deficit of heterozygotes, Table 2). The number of alleles and private alleles per population, as well as expected and observed heterozygosity are given in Table 2. The number of alleles and expected heterozygosity was highest at sites F1 (A = 4.5, $H_E = 0.622$) and F7 (A = 4.33, $H_E = 0.631$) and lowest at sites F5 (A = 1.0, $H_E = 0.083$) and B7 (A = 1.5, $H_E = 0.039$).

Figure 3 shows a two-dimensional plot of the FCA, also including the reference msat data. The first two axes account for 3.97 and 3.70% of the total variability of the msat data. Most individuals from Belgium, Germany, The Netherlands and the French Seine and Meuse assemble with the North and Baltic Sea populations from Central Europe. The individuals from the French Rhine group separate from the other populations and the distribution of these

Table 2 Results of the microsatellite and sequence analyses

Code	Microsatellite analysis					Sequence analysis					
	N	A_P	A	H_E	H_O	N	H	H_D	N. div	Hap01	Other Haplotypes
B1	10		1.33	0.076	0.033	10	1	0.00	0.0000	10	
B2	20	1	2.00	0.101	0.096	10	1	0.00	0.0000	10	
B3	20		1.83	0.210	0.223	10	2	0.20	0.0004	9	Hap35 (1)
B4	19		1.50	0.116	0.096	10	1	0.00	0.0000	10	
B5	20	1	2.00	0.122	0.048**	10	1	0.00	0.0000	10	
B6	19	3	2.17	0.118	0.096	10	1	0.00	0.0000	10	
B7	15		1.50	0.039	0.039	10	1	0.00	0.0000	10	
B8	16	1	2.33	0.282	0.243	10	1	0.00	0.0000	10	
B9	19		2.17	0.193	0.213	10	2	0.20	0.0002	9	Hap35 (1)
B11	20	2	2.50	0.293	0.267	10	4	0.82	0.0013	3	Hap35 (3), Hap47 (2), Hap48 (2)
B12	20	2	2.33	0.168	0.164	10	1	0.00	0.0000	10	
B13	20		2.67	0.460	0.302**	10	1	0.00	0.0000	0	Hap40 (10)
B14	19	1	2.33	0.200	0.136**	10	1	0.00	0.0000	10	
B15	20	3	2.17	0.134	0.109	10	1	0.00	0.0000	10	
B16	20		1.83	0.202	0.126	10	1	0.00	0.0000	10	
B17	20		2.83	0.271	0.274	10	2	0.35	0.0004	8	Hap35 (2)
B18	16		1.33	0.050	0.012**	10	1	0.00	0.0000	10	
B19	20	2	1.67	0.188	0.166	9	1	0.00	0.0000	9	
B20	20		2.67	0.218	0.212	10	1	0.00	0.0000	10	
B21	20		2.67	0.306	0.273	10	1	0.00	0.0000	10	
B22	20	1	2.17	0.197	0.176	10	1	0.00	0.0000	10	
DE1	14	3	3.67	0.561	0.365**	10	1	0.00	0.0000	10	
DE2	20		2.00	0.110	0.103	10	1	0.00	0.0000	10	
F1	19	1	4.50	0.622	0.555*	10	2	0.36	0.0043	8	Hap41 (2)
F2	20		1.67	0.192	0.200	10	1	0.00	0.0000	10	
F3	20	1	1.67	0.238	0.255	10	1	0.00	0.0000	10	
F4	10		1.67	0.102	0.112	10	1	0.00	0.0000	10	
F5	12		1.00	0.083	0.014	10	1	0.00	0.0000	10	
F6	16	1	4.50	0.578	0.440**	10	2	0.53	0.0019	6	Hap31 (4)
F7	25		4.33	0.631	0.532**	10	3	0.71	0.0047	4	Hap31 (4), Hap41 (2)
NL	14		1.83	0.144	0.015**	10	1	0.00	0.0000	10	
Total	563	23	2.33	0.507	0.1797	309	7	0.20	0.0007	277	

The number of analysed samples is given for each analysis (N). For the microsatellite analysis the average number of alleles per primer (A), the number of private alleles (A_P), the expected (H_E) and observed (H_O) heterozygosity is shown. Significant deviations from the Hardy–Weinberg equilibrium are indicated (*$p<0.05$, **$p<0.01$). For the sequence analysis, number of haplotypes (H), haplotype diversity (H_D), nucleotide diversity (N. div), the number of individuals that hold the most common haplotype (Hap01) and the other haplotype codes as described in Schrimpf et al. (2014) are indicated. The numbers of individuals corresponding to these haplotypes are indicated in brackets

individuals is also more scattered, indicating a relatively high genetic variation.

MUs in Western Europe

Haplotypes Hap47 and Hap48 were the only haplotypes that occur solely in the study area (Scheldt, population B11; Figs. 2, 6; Table 2). The number of private msat alleles was highest in population B6, B15 and DE1 ($A_P = 3$).

The results of the hierarchical AMOVA are shown in Table 3. The majority of variance was present within populations for sequence as well as msat data (62.94%, $p<0.001$ and 61.97%, $p<0.001$, respectively). In total, 35.82% ($p<0.001$) and 32.27% ($p<0.001$) was attributed to variation among populations within river catchments while variation was minor between river catchments (sequence data: 1.23%, $p=0.262$; msat data: 5.76%, $p=0.044$). Most of the genetic variation was thus represented among crayfish within populations while there was very little variation between river catchments.

While pairwise comparison for mtDNA revealed very low and not significant Φ_{ST}-values between most

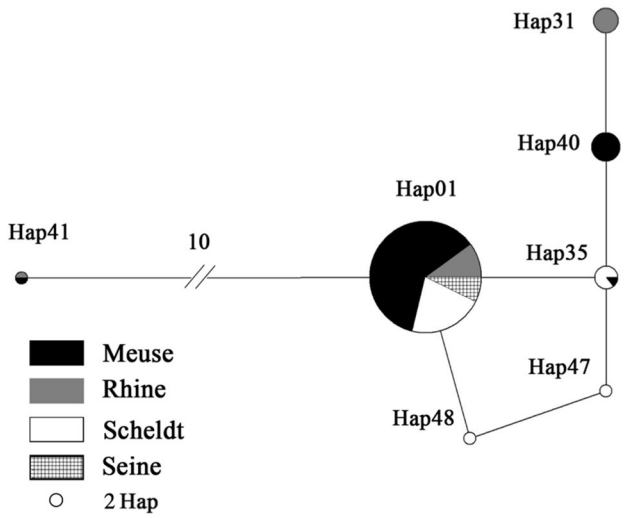

Fig. 2 A median joining network calculated with NETWORK 4.610 showing the genealogical relationships among the concatenated COI and 16S haplotypes from 309 noble crayfish from Western Europe. The size of circles is proportional to the frequency of the represented haplotype. The *circle* in the *lower left corner* represents two haplotypes (2 Hap). Each connecting branch *line* represents one nucleotide substitution. When haplotypes were separated by more than one base pair exchange, the number is given. Haplotype codes on the network correspond to samples listed in Table 2

populations (Table 4 in Appendix 1), Φ_{ST}-values among B11, B13 and F7 and all other populations were high (Φ_{ST}-range: 0.115–1.000; $p < 0.05$) suggesting significant genetic differentiation. According to the msat data, pairwise comparisons between populations revealed significant F_{ST}-values ($p < 0.05$) for most sites (Table 4 in Appendix 1). F3 was the site with the highest differentiation compared to all other sites (mean F_{ST}-value = 0.615). F_{ST}-values were highest between F5 and F2 as well as between F5 and F3, respectively. F_{ST}-values were lower between sampling sites within river catchments (mean F_{ST}-value = 0.194) than amongst river catchments (mean F_{ST}-value = 0.34).

In the PCoA based on msat data from the present study only (Fig. 4), the data points are distributed in four groups. The first group contains populations from the French Meuse catchment (F2 and F3), the second group comprises of populations from the French Rhine catchment (F6 and F7), from the hatchery in France (F1) and from the German Meuse catchment (DE1). The third group includes noble crayfish from the French Seine (F4, F5) and Belgium (Meuse and Scheldt, B19–22). The fourth group comprises all remaining populations. Sampling site NL, from The Netherlands (Rhine), is situated in-between all groups, while B13 has a position apart from all other groups. In general, the individuals from the French Rhine and Meuse

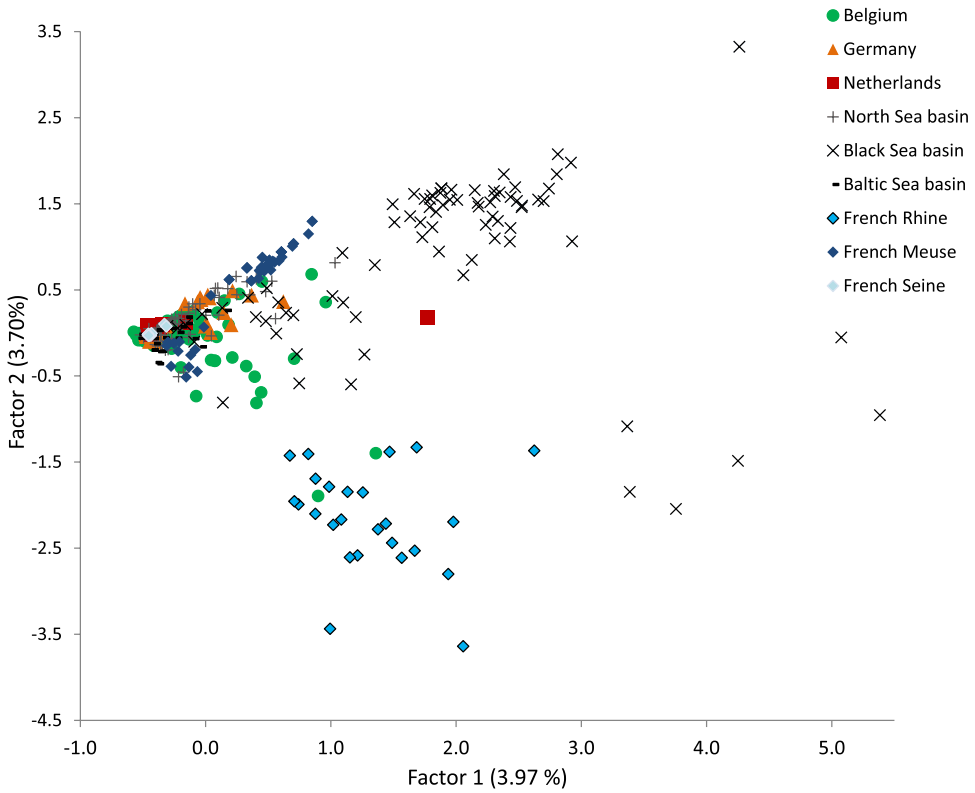

Fig. 3 A factorial correspondence analysis (FCA) based on msat data was calculated with the software GENETIX 4.05 (Belkir et al. 1996–2004) with reference data from 289 noble crayfish from the Black Sea, North Sea and Baltic Sea basins from Schrimpf et al. (2014). The *circles* represent noble crayfish from Belgium, the *triangles* represent individuals from Western Germany, the *diamonds* indicate crayfish from France, the *squares* for crayfish from The Netherlands. The Black Sea basin is indicated by a *cross*, the North Sea basin by a *plus*, and the Baltic Sea by a *minus*. (Color figure online)

Table 3 Results of the analysis of molecular variance (AMOVA) calculated with ARLEQUIN v. 3.11 based on mtDNA and microsatellite allele frequencies (msats) where populations (pop) are grouped according to river catchments

DNA marker	Among groups	Among populations within groups	Within populations
mtDNA			
d.f.	3	27	279
Sum of squares	4469	33,867	52,300
Percentage of variation	1.23	35.82	62.94
Variance components	0.004	0.107	0.187
F-statistic	0.012	0.371***	0.363***
msat			
d.f.	3	27	1091
Sum of squares	114,606	526,289	1063,159
Percentage of variation	5.76	32.27	61.97
Variance components	0.091	0.507	0.974
F-statistic	0.058*	0.342***	0.380***

Significant values of fixation indices (F-statistic) indicated with an asterisk (*$p<0.05$; ***$p<0.001$) base on 1000 random permutations

d.f. degrees of freedom

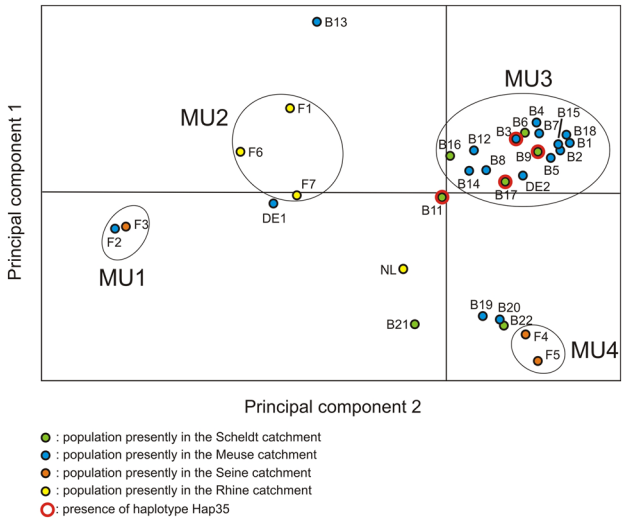

Fig. 4 Plots of the first two axes of a principal component analysis (PCoA) based on msat data implemented using the software GenAlEx v 6.5 (Peakall and Smouse 2012) revealed four groups. Each *dot* represents one population. (Color figure online)

catchments differ in allelic similarity (Fig. 4), while samples presently from the rest of the Meuse, the Scheldt and the Seine show a large allelic similarity.

The Structure analysis based on msat data suggested a clustering of K = 4 as the best model explaining the population structure (Fig. 5). Populations F2 and F3 clearly formed cluster 1 and DE1 had a high affiliation to cluster 1. Populations F1, F6 and F7 comprised cluster 2 with B13 exhibiting a high affiliation to this cluster. Populations B19–B22, F4, F5 and NL group in cluster 3. The remaining populations group in cluster 4. The circles represent the proposed management units (MU) based on the results of both, microsatellite and sequence analyses.

Anthropogenic translocations in Western Europe

Among the seven detected COI+16s haplotypes, four (Hap01, Hap35, Hap40, Hap41) were shared between the study area (North Sea catchment) and the Black Sea, Baltic Sea and Finland areas, respectively (Fig. 6). While Hap01 is the most common European haplotype distributed across all major river catchments and in almost all studied

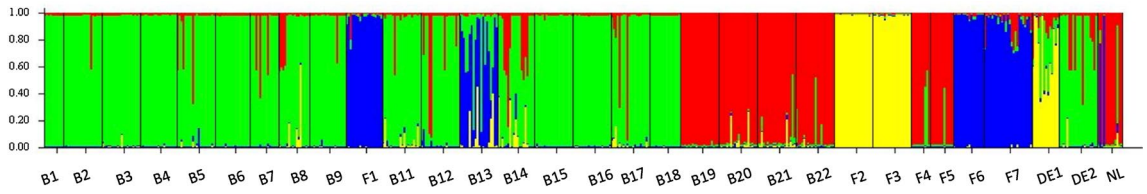

Fig. 5 Genetic population partitioning was evaluated using the Bayesian clustering approach in the program Structure v 2.3.4. The admixture model with correlated allele frequencies was used without specifying sampling locations. Four clusters were considered as most likely by the program. The *colours* correspond to the predicted clusters (1–4) as calculated by Structure. *Yellow* cluster 1, *blue* cluster 2, *red* cluster 3, *green* cluster 4. Each individual is represented by one *vertical column*, which is divided into coloured segments with the length proportional to the individual's estimated affiliation to the specific clusters. (Color figure online)

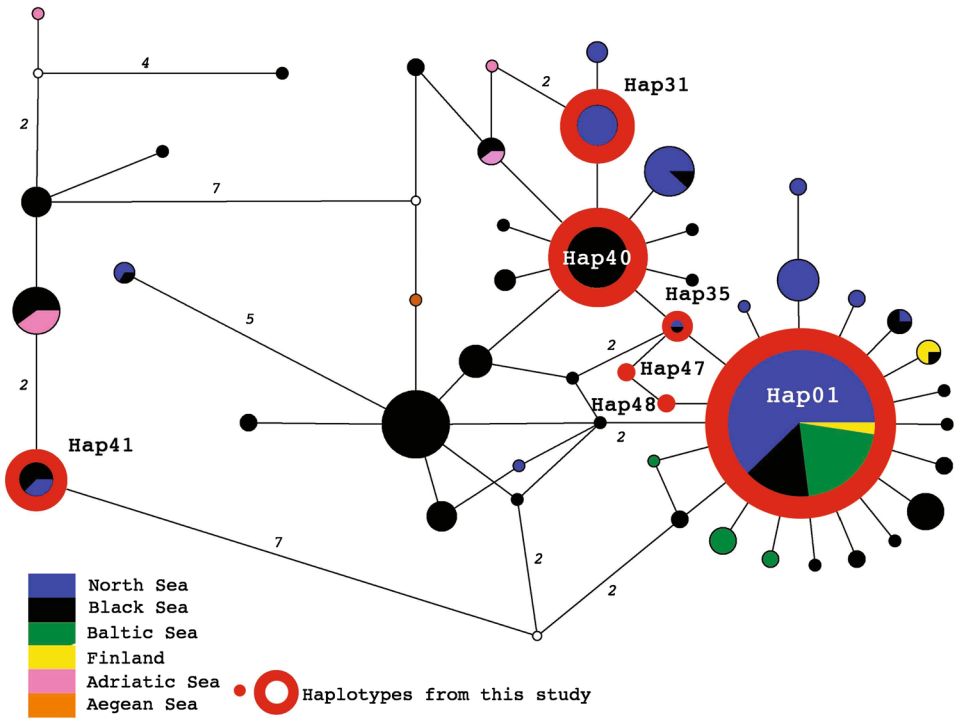

Fig. 6 A median joining network showing the genealogical relationships among concatenated COI and 16S haplotypes from 540 noble crayfish from a European-wide reference dataset (Schrimpf et al. 2014). Haplotypes from this study are highlighted in *red*. Haplotype codes correspond to samples listed in Table 2. See also explanation of Fig. 2. Figure adopted from Schrimpf et al. 2014. (Color figure online)

populations, Hap41 is the most differentiated haplotype, which is shared between the Croatian Danube, the hatchery F1 and the natural population F7.

The structure plot shows that all populations exhibit some admixture of microsatellite alleles from other populations because of mixed genetic heritage, especially in B13, B14, DE1, DE2 and NL (Fig. 5).

Discussion

Potential refugium in Western Europe

The high genetic diversity in Western European noble crayfish supports the hypothesis of an additional extra-Mediterranean refugium during the last glacial period as stated in Schrimpf et al. (2014). Our results indicate that the Belgian and Dutch noble crayfish populations assembled with individuals from the North Sea and Baltic Sea basins, while noble crayfish populations from the French Meuse catchment showed an overlap with samples from the North Sea basin and Black Sea basin (Fig. 3). This denotes for a common origin in south-eastern Europe from where these basins were recolonized (Schrimpf et al. 2014). In contrast, noble crayfish from the French Rhine river catchment (F6 and F7, Table 2) were genetically very diverse and differentiated from all other populations. The sympatric occurrence of both common as well as endemic haplotypes and alleles in the Rhine catchment could be the result of secondary contact of two different recolonization lineages.

Extremely low haplotype diversity was detected in Finland with only one single haplotype in 742 noble crayfish populations (Makkonen et al. 2015) and in Poland with two haplotypes in 50 specimens (Skuza et al. 2016), indicating recently colonised regions. Populations surviving in small extra-Mediterranean refugia that experienced bottlenecks are expected to be characterised by a low diversity, but should have more endemic haplotypes (Maggs et al. 2008). Thus, the occurrence of Hap31 (Fig. 6), a haplotype endemic to the Rhine catchment in south-western Europe where it occurs frequently (Schrimpf et al. 2014), could indicate that these populations have been present in Western Europe for several centuries. This result contradicts earlier assumptions, which argued that the distribution of noble crayfish in France resulted from anthropogenic introductions or a recolonization through the Rhine–Rhone channel (Albrecht 1983). The differentiation, diversity and endemism of haplotypes and alleles from this Rhine catchment could be attributed to an additional refugium of noble crayfish in south-western Europe during the last ice age. This hypothesis originates from Schrimpf et al. (2014) where more endemic haplotypes have been found in the Rhine catchment. The findings of Hap31 in the two populations in France from this study corroborate this hypothesis.

A similar phylogeographic history with an additional western refugium during the last glacial maximum has also been described, e.g., for the spined loach *Cobitis taenia*

(Culling et al. 2006). Moreover, survival in periodically ice-free rivers in central Europe has been assumed for the European grayling *Thymallus thymallus* (Gum et al. 2005) and European bullhead *Cottus gobio* (Vonlanthen et al. 2007). Thus, with the results of this study, an extra-Mediterranean refugium for isolated populations of noble crayfish in Western Europe is very likely. A final proof of this hypothesis would require an increased number of samples or loci. For this the recently developed tetranucleotide microsatellite markers for noble crayfish (Gross et al. 2016) could be used.

MUs in Western Europe

We propose the designation of four management units (MU 1–MU 4) according to genetic differentiation between groups of populations. Based on msat data, we detected a strong differentiation between the French Meuse populations F2 and F3 "MU 1" (Fig. 4) as well as between the French Rhine populations F6 and F7 and Meisenthal hatchery F1 "MU 2" from the remaining sites including the French Seine catchment populations F4 and F5 "MU 4". This differentiation is reflected in particular by the high F_{ST}-values between sites from the French Rhine catchment and from the French Meuse catchment to the remaining sites including "MU 3" (Table 4 in Appendix 1). While the French Rhine harbours at least some distinct mtDNA haplotypes, the French Meuse is characterised only by the common haplotype Hap01, indicating a lower diversity which is typical for recently colonised regions (Hewitt 1999). The genetic differentiation between groups of populations becomes also evident in the Structure analysis (Fig. 5) as well as in the PCoA graph (Fig. 4). While DE1 grouped with "MU 1" in the Structure analysis, it was assigned to "MU 2" in the PCoA. Excluding sampling site DE1 due to the large geographic distance from the MUs 1 and 2 (ca. 180–300 km), we propose three MUs for France that need individual management: the French Meuse river catchment (MU 1), the French Rhine river catchment (MU 2) and the French Seine river catchment (MU 4). The FCA and the PCoA (Figs. 3, 4) showed a genetic similarity within river catchments for noble crayfish, which is common for many freshwater fauna restricted to the water phase. Their dependence on a water body and the limited dispersal potential on land greatly reduces the ability to migrate to other not-connected water bodies and/or catchments as for instance has been shown for the endangered white-clawed crayfish *Austropotamobius pallipes* (Gouin et al. 2006) and the common dace *Leuciscus leuciscus* (Costedoat et al. 2006).

Although genetic similarity of populations within river catchments was large, some genetic differences did occur occasionally. For instance, the French Meuse sites (F2, F3) located near the river springs (Fig. 1) were genetically differentiated from one German Meuse population (DE2) and from the populations presently in the Belgian Meuse catchment (B1–5, B7, B8, B12–15 and B18–20). Since the European landscape has been modified by glaciers and melting water during the last glacial cycles (e.g. Fourneau 2006) this might be a result of changes in the flow direction of the upper river systems after the most recent glaciations. Such a change in the flow direction of river springs was also found for other river systems (Hantke 1993) and may explain why in some cases there is a higher differentiation of populations within a river catchment than between different catchments, as is the case for the European grayling *T. thymallus* (Gum et al. 2005). In such a case, one MU would not correspond to one river catchment. For the Meuse we can therefore propose at least two MUs within one catchment: the French Meuse springs region (MU 1, see above) and the remaining Meuse catchment (MU 3). The proposed MUs harbour 47 private alleles compared to 23 alleles, which are shared between MUs. Five private alleles are distributed in MU 1, 18 in MU 2, 24 in MU 3 and MU 4 harbours no private alleles. The relative high number of private alleles indicates the genetic distinctiveness of the proposed MUs and supports a separate management of the populations within the MUs.

In contrast, we cannot genetically distinguish the populations present in the Belgian Meuse and Scheldt catchments; most of them form one MU ("MU 3") while others (B19–22) are genetically closer to those of the Seine catchment MU4 (Figs. 4, 5). Nevertheless, as a precautionary measure we would recommend that some populations inside the same catchment should be managed separately (e.g. only within catchment translocations) in order to preserve possible recent and regional variability that we could not uncover with the methods used in this study. This recommendation applies especially to population B11 with regionally limited haplotypes Hap47 and Hap48 in the Scheldt catchment, which should be conserved. Since we have only analysed limited samples of noble crayfish populations from Belgium, it is possible that other populations exhibit more private haplotypes.

Anthropogenic translocations in Western Europe

Our msat data exhibited only small signals of genetic admixture among the study populations B13, B14, DE1, DE2 and NL (Fig. 5). This could be an indication that there was less translocations of noble crayfish in Western Europe than previously expected. However, it is known that translocations of noble crayfish have been conducted in Germany, Belgium and France (Albrecht 1983, Franckhauser, personal communication, Collas, unpublished data, Table 1). It is presumed that autochthonous noble crayfish populations from the Scheldt catchment no longer exist in this catchment, except perhaps the

translocated population B17, and that present populations in this catchment most probably originate from restocking with animals from the River Meuse catchment (Cammaerts, personal investigations). The natural genetic structure is probably partly dissolved due to the strong influence of man on the distribution of noble crayfish. The same has been observed in Northern Europe where a similarity between noble crayfish from southern Finland and Sweden can likely be explained by artificial stockings between these countries (Gross et al. 2013). For more details on the anthropogenic influence on the distribution of noble crayfish in Western Europe and stocking recommendations see Appendix 2. Interestingly, the genetic results support the known history of most populations.

Anthropogenic translocations of endangered source populations should be continued in Western Europe in order to minimize extinction risk of the source population. However, the genetic make-up of the source population should be considered. Bláha et al. (2015) found no significant decline in genetic diversity between source and stocked noble crayfish populations one decade (assumed three generations) after stocking and all populations were still viable. However, any noble crayfish transfer has to be preceded by a careful assessment (after Schulz et al. 2002): (1) why is the native crayfish not present (anymore) in the target habitat? (2) The introduction is not causing any negative effects on any other protected species, a problem that can be avoided using newly man-made sites such as water-filled quarries or by establishing priorities between protected species. (3) The stocked waterbody as well as the stocked crayfish are free from the crayfish plague agent.

Additionally, it is important to consider that translocation of wildlife species always bears the risk of transferring diseases (Woodford and Rossiter 1993). For crayfish species the risk is especially high to spread the crayfish plague pathogen *A. astaci*. The finding of latent infected noble crayfish populations makes it possible that an infection is not visible (Jussila et al. 2011; Viljamaa-Dirks et al. 2011). Therefore, careful disease examination of the population should be conducted before any release of the animals into another water body is performed. Infected populations should be excluded from translocation projects. Also movements of fish stocks from infected waters presents a risk of crayfish plague transmission. Predators, fishing gear and any item that has been in contact with contaminated water may transmit the pathogen between waterbodies and should be avoided (OIE 2016).

Conclusions

Within our study area we identified four distinct management units: the French Meuse, the French Rhine and the French Seine catchments as special management units, as well as the remaining catchments Meuse and Scheldt. Although a genetic differentiation could not be detected for the river catchments in Belgium, The Netherlands and Western Germany, as a precautionary measure we recommend separate management practices of some populations to preserve possible recent and regional adaptions and to carefully find a balance between outbreeding and inbreeding depression. To face this challenge in modern *A. astacus* conservation management in Western European catchments, we recommend (1) stocking either with populations that bear population specific private haplotypes or (2) stocking with populations that bear only the most common haplotype H01, and (3) oppose to stocking with populations that exhibit allochthonous haplotypes (populations F1, F7 and B13 and all those from hatcheries working with non-Western European noble crayfish), in order to conserve the maximum genetic diversity in this threatened species.

This study demonstrates that a population genetic analysis can help stakeholders to prioritize conservation actions to specific populations. It also confirms that the designation of ESUs is not applicable for species with anthropogenic influenced population structure. Stocked populations may resemble species with high (artificial) gene flow. In such a case the definition of MUs is the method of choice because it is less strict than ESUs as it does not require the distinctiveness of alleles between groups of populations (Moritz 1994). With the fulfilment of MUs during stockings, the adaptive potential as well as the variation within a species can be preserved.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Appendix 1

See Table 4.

Table 4 Pairwise FST- and ΦST-values between sampling sites calculated with ARLEQUIN, v. 3.11

	B1	B2	B3	B4	B5	B6	B7	B8	B9	B11	B12	B13	B14	B15	B16	B17
B1		*0.000*	*0.000*	*0.000*	*0.000*	*0.000*	*0.000*	*0.000*	*0.000*	**0.395**	*0.000*	**1.000**	*0.000*	*0.000*	*0.000*	*0.111*
B2	*0.015*		*0.000*	*0.000*	*0.000*	*0.000*	*0.000*	*0.000*	*0.000*	**0.395**	0.000	**1.000**	0.000	0.000	0.000	0.111
B3	***0.061***	***0.061***		*0.000*	*0.000*	*0.000*	*0.000*	*0.000*	*0.111*	**0.284**	0.000	**0.947**	0.000	0.000	0.000	0.000
B4	***0.115***	**0.128**	***0.070***		*0.000*	*0.000*	*0.000*	*0.000*	*0.000*	**0.395**	0.000	**1.000**	0.000	0.000	0.000	0.111
B5	*0.031*	*0.038*	**0.098**	**0.113**		*0.000*	*0.000*	*0.000*	*0.000*	**0.395**	0.000	**1.000**	0.000	0.000	0.000	0.111
B6	*0.014*	*0.016*	*0.061*	*0.086*	*0.058*		*0.000*	*0.000*	*0.000*	**0.395**	0.000	**1.000**	0.000	0.000	0.000	0.111
B7	*0.033*	*0.035*	***0.075***	**0.081**	*0.034*	*0.008*		*0.000*	*0.000*	**0.395**	0.000	**1.000**	0.000	0.000	0.000	0.111
B8	***0.102***	**0.089**	***0.062***	**0.181**	**0.110**	**0.093**	**0.099**		*0.000*	**0.395**	0.000	**1.000**	0.000	0.000	0.000	0.111
B9	**0.087**	***0.064***	**0.071**	**0.201**	**0.139**	**0.100**	**0.136**	*0.029*		**0.284**	0.000	**0.947**	0.000	0.000	0.000	0.000
B11	**0.269**	**0.260**	**0.183**	**0.307**	**0.282**	**0.246**	**0.275**	**0.115**	**0.200**		**0.395**	**0.713**	**0.395**	**0.395**	**0.395**	*0.198*
B12	**0.132**	**0.133**	**0.082**	**0.111**	**0.134**	**0.119**	**0.111**	**0.089**	**0.140**	**0.143**		**1.000**	*0.000*	*0.000*	*0.000*	*0.111*
B13	***0.420***	**0.446**	**0.357**	**0.385**	**0.442**	**0.389**	**0.370**	**0.331**	**0.416**	**0.347**	0.265		**1.000**	**1.000**	**1.000**	**0.901**
B14	**0.304**	**0.273**	**0.212**	**0.308**	**0.290**	**0.256**	**0.236**	**0.146**	**0.242**	**0.186**	**0.163**	**0.323**		*0.000*	*0.000*	*0.111*
B15	*0.011*	*0.025*	**0.073**	**0.144**	***0.053***	***0.062***	**0.071**	**0.115**	**0.098**	**0.267**	**0.126**	**0.432**	**0.300**		*0.000*	*0.111*
B16	**0.373**	**0.349**	**0.259**	**0.343**	**0.387**	**0.316**	**0.332**	**0.228**	**0.300**	**0.134**	**0.158**	**0.309**	**0.100**	**0.365**		*0.111*
B17	**0.131**	**0.108**	**0.099**	**0.202**	**0.151**	**0.128**	**0.159**	***0.048***	**0.078**	**0.063**	***0.108***	**0.379**	**0.177**	**0.136**	**0.195**	
B18	*0.023*	*0.000*	***0.062***	***0.114***	*0.052*	*0.005*	*0.021*	**0.122**	**0.099**	**0.288**	**0.141**	**0.433**	**0.288**	*0.030*	**0.361**	**0.147**
B19	**0.351**	**0.347**	**0.316**	**0.346**	**0.300**	**0.327**	**0.306**	**0.254**	**0.361**	**0.290**	**0.228**	**0.464**	**0.352**	**0.337**	**0.395**	**0.273**
B20	**0.315**	**0.297**	**0.268**	**0.364**	**0.287**	**0.295**	**0.288**	**0.196**	**0.294**	**0.260**	**0.252**	**0.476**	**0.302**	**0.296**	**0.380**	**0.219**
B21	**0.363**	**0.360**	**0.282**	**0.366**	**0.371**	**0.350**	**0.343**	**0.205**	**0.303**	**0.206**	**0.206**	**0.393**	**0.280**	**0.361**	**0.295**	**0.234**
B22	**0.339**	**0.317**	**0.283**	**0.348**	**0.297**	**0.321**	**0.312**	**0.229**	**0.316**	**0.270**	**0.233**	**0.488**	**0.305**	**0.313**	**0.363**	**0.224**
DE1	**0.449**	**0.476**	**0.355**	**0.453**	**0.469**	**0.437**	**0.432**	**0.274**	**0.420**	**0.195**	**0.291**	**0.296**	**0.345**	**0.467**	**0.327**	**0.315**
DE2	***0.109***	**0.101**	**0.095**	**0.104**	**0.062**	**0.119**	**0.084**	***0.100***	**0.151**	**0.243**	*0.049*	**0.397**	**0.215**	**0.111**	**0.294**	**0.141**
F1	**0.365**	**0.398**	**0.340**	**0.386**	**0.394**	**0.355**	**0.341**	**0.266**	**0.363**	**0.280**	**0.282**	**0.196**	**0.318**	**0.389**	**0.334**	**0.318**
F2	**0.704**	**0.711**	**0.624**	**0.673**	**0.691**	**0.675**	**0.663**	**0.564**	**0.677**	**0.536**	**0.555**	**0.448**	**0.600**	**0.702**	**0.605**	**0.613**
F3	**0.702**	**0.707**	**0.621**	**0.671**	**0.691**	**0.670**	**0.657**	**0.560**	**0.675**	**0.537**	**0.541**	**0.459**	**0.581**	**0.701**	**0.590**	**0.610**
F4	**0.433**	**0.368**	**0.310**	**0.423**	**0.354**	**0.360**	**0.355**	**0.221**	**0.337**	**0.262**	**0.258**	**0.479**	**0.287**	**0.358**	**0.367**	**0.231**
F5	**0.618**	**0.513**	**0.425**	**0.529**	**0.483**	**0.487**	**0.489**	**0.352**	**0.484**	**0.375**	**0.354**	**0.547**	**0.441**	**0.485**	**0.498**	**0.362**
F6	**0.475**	**0.511**	**0.446**	**0.492**	**0.498**	**0.471**	**0.451**	**0.366**	**0.476**	**0.369**	**0.372**	**0.292**	**0.409**	**0.497**	**0.430**	**0.420**
F7	**0.345**	**0.369**	**0.317**	**0.348**	**0.359**	**0.333**	**0.318**	**0.251**	**0.347**	**0.253**	**0.242**	**0.246**	**0.291**	**0.371**	**0.299**	**0.290**
NL	**0.450**	**0.438**	**0.374**	**0.451**	**0.430**	**0.408**	**0.385**	**0.247**	**0.385**	**0.263**	**0.283**	**0.370**	**0.181**	**0.451**	**0.272**	**0.275**

	B18	B19	B20	B21	B22	DE1	DE2	F1	F2	F3	F4	F5	F6	F7	NL
B1	*0.000*	*0.000*	*0.000*	*0.000*	*0.000*	*0.000*	*0.000*	*0.111*	*0.000*	*0.000*	*0.000*	*0.000*	*0.333*	**0.230**	*0.000*
B2	0.000	0.000	0.000	0.000	0.000	*0.000*	*0.000*	*0.111*	*0.000*	*0.000*	*0.000*	*0.000*	*0.333*	**0.230**	*0.000*
B3	0.000	0.000	0.000	0.000	0.000	*0.000*	*0.000*	*0.106*	*0.010*	*0.000*	*0.000*	*0.000*	*0.262*	**0.202**	*0.000*
B4	0.000	0.000	0.000	0.000	0.000	*0.000*	*0.000*	*0.111*	*0.000*	*0.000*	*0.000*	*0.000*	*0.333*	**0.230**	*0.000*
B5	0.000	0.000	0.000	0.000	0.000	*0.000*	*0.000*	*0.111*	*0.000*	*0.000*	*0.000*	*0.000*	*0.333*	**0.230**	*0.000*
B6	0.000	0.000	0.000	0.000	0.000	*0.000*	*0.000*	*0.111*	*0.000*	*0.000*	*0.000*	*0.000*	*0.333*	**0.230**	*0.000*
B7	0.000	0.000	0.000	0.000	0.000	*0.000*	*0.000*	*0.111*	*0.000*	*0.000*	*0.000*	*0.000*	*0.333*	**0.230**	*0.000*
B8	0.000	0.000	0.000	0.000	0.000	*0.000*	*0.000*	*0.111*	*0.000*	*0.000*	*0.000*	*0.000*	*0.333*	**0.230**	*0.000*
B9	0.000	0.000	0.000	0.000	0.000	*0.000*	*0.000*	*0.106*	*0.010*	*0.000*	*0.000*	*0.000*	*0.262*	***0.202***	*0.000*
B11	**0.395**	**0.377**	**0.395**	**0.395**	**0.395**	**0.395**	**0.395**	**0.199**	**0.412**	**0.395**	**0.395**	**0.395**	**0.209**	**0.184**	**0.395**
B12	*0.000*	*0.000*	*0.000*	*0.000*	*0.000*	*0.000*	*0.000*	*0.111*	*0.000*	*0.000*	*0.000*	*0.000*	*0.333*	**0.230**	*0.000*
B13	**1.000**	**1.000**	**1.000**	**1.000**	**1.000**	**1.000**	**1.000**	**0.532**	**1.000**	**1.000**	**1.000**	**1.000**	**0.500**	**0.278**	**1.000**
B14	*0.000*	*0.000*	*0.000*	*0.000*	*0.000*	*0.000*	*0.000*	*0.111*	*0.000*	*0.000*	*0.000*	*0.000*	***0.333***	**0.230**	*0.000*
B15	*0.000*	*0.000*	*0.000*	*0.000*	*0.000*	*0.000*	*0.000*	*0.111*	*0.000*	*0.000*	*0.000*	*0.000*	*0.333*	**0.230**	*0.000*
B16	*0.000*	*0.000*	*0.000*	*0.000*	*0.000*	*0.000*	*0.000*	*0.111*	*0.000*	*0.000*	*0.000*	*0.000*	*0.333*	**0.230**	*0.000*
B17	*0.111*	*0.097*	*0.111*	*0.111*	*0.111*	*0.111*	*0.111*	*0.111*	*0.124*	*0.111*	*0.111*	*0.111*	***0.211***	**0.181**	*0.111*
B18		*0.000*	*0.000*	*0.000*	*0.000*	*0.000*	*0.000*	*0.111*	*0.000*	*0.000*	*0.000*	*0.000*	*0.333*	**0.230**	*0.000*

Table 4 (continued)

	B18	B19	B20	B21	B22	DE1	DE2	F1	F2	F3	F4	F5	F6	F7	NL
B19	**0.379**		*0.000*	*0.000*	*0.000*	*0.000*	*0.000*	*0.097*	*0.000*	*0.000*	*0.000*	*0.000*	**0.316**	**0.213**	*0.000*
B20	**0.335**	**0.135**		*0.000*	*0.000*	*0.000*	*0.000*	*0.111*	*0.000*	*0.000*	*0.000*	*0.000*	*0.333*	**0.230**	*0.000*
B21	**0.388**	**0.172**	**0.139**		*0.000*	*0.000*	*0.000*	*0.111*	*0.000*	*0.000*	*0.000*	*0.000*	***0.333***	**0.230**	*0.000*
B22	**0.358**	***0.118***	***0.049***	**0.123**		*0.000*	*0.000*	*0.111*	*0.000*	*0.000*	*0.000*	*0.000*	*0.333*	**0.230**	*0.000*
DE1	**0.485**	**0.391**	**0.391**	**0.264**	**0.416**		*0.000*	*0.111*	*0.000*	*0.000*	*0.000*	*0.000*	*0.333*	**0.230**	*0.000*
DE2	**0.121**	**0.238**	**0.264**	**0.268**	**0.232**	**0.404**		*0.111*	*0.000*	*0.000*	*0.000*	*0.000*	*0.333*	**0.230**	*0.000*
F1	**0.396**	**0.369**	**0.370**	**0.312**	**0.395**	**0.225**	**0.370**		*0.124*	*0.111*	*0.111*	*0.111*	**0.174**	*0.026*	*0.111*
F2	**0.719**	**0.615**	**0.626**	**0.492**	**0.635**	**0.303**	**0.647**	**0.399**		*0.000*	*0.000*	*0.000*	***0.350***	**0.245**	*0.000*
F3	**0.716**	**0.611**	**0.631**	**0.497**	**0.637**	**0.329**	**0.637**	**0.402**	**0.204**		*0.000*	*0.000*	*0.333*	**0.230**	*0.000*
F4	**0.432**	**0.145**	*0.024*	**0.141**	*0.035*	**0.413**	**0.288**	**0.372**	**0.670**	**0.668**		*0.000*	*0.333*	**0.230**	*0.000*
F5	**0.590**	**0.178**	***0.108***	**0.219**	**0.149**	**0.492**	**0.408**	**0.437**	**0.727**	**0.726**	**0.063**		*0.333*	**0.230**	*0.000*
F6	**0.511**	**0.429**	**0.443**	**0.366**	**0.463**	**0.271**	**0.460**	**0.099**	**0.429**	**0.426**	**0.444**	**0.509**		*0.015*	*0.333*
F7	**0.373**	**0.279**	**0.297**	**0.230**	**0.309**	**0.206**	**0.317**	**0.114**	**0.368**	**0.357**	**0.303**	**0.359**	**0.090**		**0.230**
NL	**0.465**	**0.340**	**0.307**	**0.240**	**0.304**	**0.318**	**0.354**	**0.290**	**0.543**	**0.514**	**0.286**	**0.423**	**0.352**	**0.246**	

Results for mtDNA data are shown above the diagonal and microsatellite data are shown below the diagonal. Significant values are indicated in bold ($p < 0.05$), non significant values are in italics. Bolditalic values were not significant under the FDR (Benjamini and Hochberg 1995)

Appendix 2

Detailed discussion about the stocking history of the analyzed populations

Our study benefits from detailed information about the history of the studied populations. The differentiated Hap41 found in the Meisenthal hatchery (F1) and in a natural population in the Rhine catchment in France (F7, Lemberg) indicates a stocking event because this haplotype and related ones are frequently distributed in the Croatian Danube catchment (Hap 41 in Fig. 6 and in Schrimpf et al. 2014). A parallel evolution of Hap41 is highly unlikely because it differs from the most common haplotype Hap01 by 10 mutations. The hatchery (F1) was founded with crayfish from the Meisenthal brook in the Vosges, where ponds were dug out in the past to supply power to the local glass industry (Franckhauser, personal communication). We thus assumed that the population from the hatchery was stocked with Danubian crayfish. In its geographic vicinity, sampling site F6 (Sturzelbronn in the Vosges, Fig. 1) is located, which belongs to the same MU (Fig. 4). Noble crayfish were present in the former Sturzelbronn abbey estate already in 1594 and 1789 (Jehin 2006–2007). Therefore, it is most probable that population F6, characterized by the Western-European haplotype Hap31, existed here already historically. It should be preserved by priority. The presence of F6 should explain the presence of both Hap31 and Hap41 in the F7 population. Because noble crayfish stockings likely occurred many times in this region more natural populations from the French Rhine should be analysed to estimate the influence of stockings from the past.

Although some of the sampled populations, especially those harboured in water-filled quarries such as B6 and B9 on the one hand and B11 and B17 on the other hand (all Scheldt catchment) live only a few hundred meters apart from each other in a same locality, they were stocked independently (Table 1), from a distinct origin and in distinct quarries without any exchange between them. While the origin of B9 and B11 is unknown, the origin of B17 is a pond in the Haine-Scheldt catchment. However, as this population may have been stocked in past times, its very first origin is unknown. Interestingly, these stocked populations and the fish farm B3 (Meuse catchment) all carried the haplotype Hap35, which was previously found in the Romanian Danube catchment (Schrimpf et al. 2014). However, since haplotype Hap35 differed by only one base pair exchange from Hap01 it is possible that Hap35 has evolved independently twice. In this case the occurrence in both regions would not necessarily be a sign for a translocation of noble crayfish from Romania to Belgium, but could be an indication of a relationship between the Belgian populations. According to the msat data, the respective Belgian populations (B3, B9, B11, B17) were distinct from noble crayfish from the Black Sea catchment, but they did show some similarity to the remaining noble crayfish from central and Western Europe (Fig. 3). Especially interesting is population B11, which additionally holds two private haplotypes (Hap47, Hap48), that have not been found elsewhere in Europe before. The private haplotypes explain the high Φ_{ST}-values between B11 and all other populations (Table 4 in Appendix 1). As long as we cannot provide evidence for an alien origin of these private haplotypes we

recommend special protection of population B11 in the Scheldt catchment.

Population B13 was highly differentiated from all other populations (high Φ-values and F_{ST}-values, Table 4; Figs. 4, 5), even from the geographically very close and presumably autochthonous population B18. All 10 sequenced individuals of population B13 carried a haplotype (Hap40, Table 2) that was previously found in the Romanian Danube catchment (Schrimpf et al. 2014). Interestingly, this population was stocked with crayfish purchased in a Belgian food store in the 1970s (S. Kanjester, personal communication; Table 1). Since both the historic record and the genetic results indicate an allochthonous origin, population B13, along with other populations with allochthonous haplotypes (populations F1, F7), should not be used for further stockings in Belgium. Also other crayfish with Danubian (see Schrimpf et al. 2014) or other foreign origin should not be used.

Small isolated populations like the last known Dutch noble crayfish population (NL) are at high risk from inbreeding depression (Frankham et al. 2012). Individuals in small populations have a lower fitness and the extinction probability is increased especially in changing environments (Willi et al. 2006). Haplotype and allele diversities are indeed already low in the Dutch population (Table 2). Additionally, as for the Belgian and the French Oise catchment populations, the extinction risk for noble crayfish in The Netherlands is especially high due to the wide distribution of American crayfish species (Koese and Soes 2011) and the crayfish plague (Tilmans et al. 2014). Consequently, this population should be a focus of special management.

References

Albrecht H (1983) Besiedlungsgeschichte und ursprüngliche holozäne Verbreitung der europäischen Flußkrebse (Decapoda: Astacidae). Spixiana 6:61–77

Balzat N-H, Dussart A (1978) Guide de la Pêche en Ardenne. Duculot, Paris-Gembloux, pp 384

Bandelt H-J, Forster P, Röhl A (1999) Median-joining networks for inferring intraspecific phylogenies. Mol Biol Evol 16:37–48

Belkhir K, Borsa P, Chikhi L, Raufaste N, Bonhomme F (1996–2004) GENETIX 4.05, logiciel sous Windows TM pour la génétique des populations. Laboratoire Génome, Populations, Interactions, CNRS UMR 5171, Université de Montpellier II, Montpellier

Benjamini Y, Hochberg Y (1995) Controlling the false discovery rate: a practical and powerful approach to multiple testing. J R Stat Soc B 57(1):289–300

Bláha M, Žurovcová M, Kouba A, Policar T, Kozák P (2015) Founder event and its effect on genetic variation in translocated populations of noble crayfish (*Astacus astacus*). J Appl Genet 57:99–106

Boets P, Lock K, Adriaens T, Mouton A, Goethals PLM (2012) Distribution of crayfish (Decapoda, Astacoidea) in Flanders (Belgium): an update. Belg J Zool 142(1):86–92

Bonin A, Bellemain E, Bronken Eidesen P, Pompanon F, Brochmann C, Taberlet P (2004) How to track and assess genotyping errors in population genetic studies. Mol Ecol 13:3261–3273

Boulding EG (2008) Genetic diversity, adaptive potential, and population viability in changing environments. In: Carroll SC (ed) Fox conservation biology: evolution in action. Oxford University Press, New York, pp 199–219

Buhay JE (2009) "COI-like" sequences are becoming problematic in molecular systematic and DNA barcoding studies. J Crustac Biol 29:96–110

Carlsson J (2008) Effects of microsatellite null alleles on assignment testing. J Hered 99:616–623

Clark PU, Dyke AS, Shakun JD, Carlson AE, Clark J, Wohlfarth B, Mitrovica JX, Hostetler SW, McCabe AM (2009) The last glacial maximum. Science 325:710–714

Collas M, Julien C, Monnier D (2007) Note technique: La situation des écrevisses en France. Résultats des enquêtes nationales réalisées entre 1977 et 2006 par le Conseil Supérieur de la Pêche. Bull Fr de la Pêche Piscic 386:1–39

Costedoat C, Chappaz R, Barascud B, Guillard O, Gilles A (2006) Heterogeneous colonization pattern of European Cyprinids, as highlighted by the dace complex (Teleostei: Cyprinidae). Mol Phylogen Evol 41(1):127–148

Crandall KA, Fitzpatrick JF Jr (1996) Crayfish molecular systematics: using a combination of procedures to estimate phylogeny. Syst Biol 45(1):1–26

Culling MA, Janko K, Boron A, Vasil'ev P, Côté IM, Hewitt GM (2006) European colonization by the spined loach (*Cobitis taenia*) from Ponto-Caspian refugia based on mitochondrial DNA variation. Mol Ecol 15:173–190

Drummond A, Ashton B, Buxton S, Cheung M, Cooper A, Duran C, Field M, Heled J, Kearse M, Markowitz S, Moir R, Stones-Havas S, Sturrock S, Thierer T, Wilson A (2011) GENEIOUS Pro v 5.0.3. (computer program). http://www.geneious.com

Earl DA, von Holdt BM (2012) STRUCTURE HARVESTER: a website and program for visualizing STRUCTURE output and implementing the Evanno method. Conserv Genet Res 4(2):359–361

Edsman L, Füreder L, Gherardi F, Souty-Grosset C (2010) *Astacus astacus*. IUCN 2013. IUCN Red List of Threatened Species. Version 2013.2. http://www.iucnredlist.org. Accessed 10 Feb 2015

Evanno G, Regnaut S, Goudet J (2005) Detecting the number of clusters of individuals using the software STRUCTURE: a simulation study. Mol Ecol 14:2611–2620

Excoffier L, Lischer HEL (2010) Arlequin suite ver 3.5: a new series of programs to perform population genetics analyses under Linux and Windows. Mol Ecol Resour 10:564–567

Excoffier L, Smouse PE, Quattro JM (1992) Analysis of molecular variance inferred from metric distances among DNA haplotypes: application to human mitochondrial-DNA restriction data. Genetics 131:479–491

Fourneau RO (2006) Initiation à la géomorphologie de la Wallonie, région d'Europe. Cercle des Naturalistes de Belgique asbl, pp 172

Frankham R, Ballou JD, Eldridge MDB, Lacy RC, Ralls K, Dudash MR, Fenster CB (2011) Predicting the probability of outbreeding depression. Conserv Biol 25:465–475

Frankham R, Ballou JD, Dudash MR, Eldridge MDB, Fenster CB, Lacy RC, Mendelson JR, Porton IJ, Ralls K, Ryder OA (2012) Implications of different species concepts for conserving biodiversity. Biol Conserv 153:25–31

Gouin N, Grandjean F, Souty-Grosset C (2006) Population genetic structure of the endangered crayfish *Austropotamobius pallipes* in France based on microsatellite variation: biogeographical inferences and conservation implications. Freshwater Biol 51:1369–1387

Gross R, Palm S, Kõiv K, Prestegaard T, Jussila J, Paaver T, Geist J, Kokko H, Karjalainen A, Edsman L (2013) Microsatellite markers reveal clear geographic structuring among threatened noble crayfish (*Astacus astacus*) populations in Northern and Central Europe. Conserv Genet 15:809–821

Gross R, Kõiv K, Pukk L, Kaldre K (2016) Development and characterization of novel tetranucleotide microsatellite markers in the noble crayfish (*Astacus astacus*) suitable for highly multiplexing and for detecting hybrids between the noble crayfish and narrow-clawed crayfish (*A. leptodactylus*). Aquaculture. doi:10.1016/j.aquaculture.2016.04.015

Gum B, Gross R, Kuehn R (2005) Mitochondrial and nuclear DNA phylogeography of European grayling (*Thymallus thymallus*): evidence for secondary contact zones in central Europe. Mol Ecol 14:1707–1725

Hantke R (1993) Flussgeschichte Mitteleuropas. Ferdinand Enke-Verlag, Stuttgart

Hewitt GM (1999) Post-glacial re-colonization of European biota. Biol J Linn Soc 68:87–112

Holdich D (2002) Distribution of crayfish in Europe and some adjoining countries. Bull Fr Pêche Piscic 367:611–650

IUCN (2013) IUCN Red List of Threatened Species. Version 2013.2. http://www.iucnredlist.org. Accessed 2 Jan 2016

Jehin P (2006–2007) Rivières, étangs et pisciculture dans les Vosges du Nord avant la Révolution. Ann Sci Rés Bios Trans Vosges du Nord-Pfälzerwald 12:91–112

Jump AS, Marchant R, Peñuelas J (2009) Environmental change and the option value of genetic diversity. Trends Plant Sci 14(1):51–58

Jussila J, Makkonen J, Vainikka A, Kortet R, Kokko H (2011) Latent crayfish plague (*Aphanomyces astaci*) infection in a robust wild noble crayfish (*Astacus astacus*) population. Aquaculture 321:17–20

Koese B, Soes M (2011) De Nederlandse rivierkreeften (Astacoidea, Parasacoidea). Entomologische Tabellen 6:107

Kõiv K, Gross R, Paaver T, Kuehn R (2008) Isolation and characterization of first microsatellite markers for the noble crayfish, *Astacus astacus*. Conserv Genet 9:1703–1706

Kõiv K, Gross R, Paaver T, Hurt M, Kuehn R (2009) Isolation and characterization of 11 novel microsatellite DNA markers in the noble crayfish, *Astacus astacus*. Anim Genet 40:124–126

Lerceteau-Köhler E, Schliewen U, Kopun T, Weiss S (2013) Genetic variation in brown trout *Salmo trutta* across the Danube, Rhine, and Elbe headwaters: a failure of the phylogeographic paradigm? BMC Evol Biol 13:176

Lewis PO, Zaykin D (2001) Genetic data analysis: computer program for the analysis of allelic data. Version 1.0 (d16c). (computer program). http://www.lewis.eeb.uconn.edu/lewishome/software.html

Librado P, Rozas J (2009) DnaSP v5: a software for comprehensive analysis of DNA polymorphism data. Bioinformatics 25:1451–1452

Maggs CA, Castilho R, Foltz D, Henzler C, Jolly MT, Kelly J et al (2008) Evaluating signatures of glacial refugia for North Atlantic benthic marine taxa. Ecology 89(11):108–122

Makkonen J, Kokko H, Jussila J (2015) Mitochondrial cytochrome oxidase I gene analysis indicates a restricted genetic background in Finnish noble crayfish (*Astacus astacus*) stocks. Knowl Manag Aquat Ecosyst 416:21

Meirmans PG, Van Tienderen PH (2004) GENOTYPE and GENODIVE: two programs for the analysis of genetic diversity of asexual organisms. Mol Ecol Notes 4:792–794

Moritz C (1994) Defining 'evolutionary significant units' for conservation. Trends Ecol Evol 9:373–375

Moritz C (1999) Conservation units and translocations: strategies for conserving evolutionary processes. Hereditas 130:217–228

OIE (Office international des épizooties) (2016) Crayfish plague (*Aphanomyces astaci*). Chapter 2.2.1. In: World Organization for Animal Health (ed) Manual of diagnostic tests for aquatic animals 2016. Office International des épizooties, Paris, pp 101–118. http://www.oie.int/fileadmin/Home/eng/Health_standards/aahm/current/chapitre_aphanomyces_astaci.pdf. Accessed 20 June 2016

Ottburg F, Roessink I (2012) Europese rivierkreften in Nederland; vaststellen, veiligstellen, versterken en veilige leefgebieden. Wageningen, Alterra. Alterra-rapport 2341, pp 42

Pârvulescu L, Schrimpf A, Kozubíková E, Resino SC, Vrålstad T, Petrusek A, Schulz R (2012) Invasive crayfish and crayfish plague on the move: first detection of the plague agent *Aphanomyces astaci* in the Romanian Danube. Dis Aquat Org 98:85–94

Peakall R, Smouse PE (2012) GenAlEx 6.5: genetic analysis in Excel. Population genetic software for teaching and research-an update. Bioinformatics 28:2537–2539

Pritchard JK, Stephens M, Donnelly P (2000) Inference of population structure using multilocus genotype data. Genetics 155:945–959

Ryder OA (1986) Species conservation and systematics: the dilemma of subspecies. Trends Ecol Evol 1:9–10

Sambrook J, Russell DW (2001) Rapid isolation of Mammalian DNA. In: Molecular cloning: a laboratory manual. Cold Spring Harbor Laboratory Press, New York

SCBD (Secretariat of the Convention on Biological Diversity) (1992) Convention on biological diversity. http://www.cbd.int. Accessed 10 Feb 2014

Schrimpf A, Schulz H, Theissinger K, Pârvulescu L, Schulz R (2011) First large-scale genetic analysis of the vulnerable noble crayfish *Astacus astacus* reveals low haplotype diversity of Central European populations. Knowl Manag Aquat Ecosyst 401:35

Schrimpf A, Chucholl C, Schmidt T, Schulz R (2013) Crayfish plague agent detected in populations of the invasive North American crayfish *Orconectes immunis* (Hagen, 1870) in the Rhine River, Germany. Aquat Invasion 8(1):103–109

Schrimpf A, Theissinger K, Dahlem J, Maguire I, Pârvulescu L, Schulz H, Schulz R (2014) Phylogeography of noble crayfish (*Astacus astacus*) reveals multiple refugia. Freshwater Biol 59:761–776

Schulz R, Stucki T, Souty-Grosset C (2002) Roundtable session 4A. Management: reintroductions restocking. Bull Fr Pêche Piscic 367:917–922

Schulz H, Gross H, Dümpelmann C, Schulz R (2008) Flusskrebse Deutschlands. In: Füreder L (ed) Flusskrebse: Biologie–Ökologie–Gefährdung. Folio, Wien, pp 71–81

Skuza L, Keszka S, Panicz R, Šmietana P (2016) Molecular characterization of the noble crayfish (*Astacus astacus* L.) population from Pomeranian lakes (north-western Poland) based on mitochondrial DNA. Knowl Manag Aquat Ecosyst 417:3

Souty-Grosset C, Grandjean F (2009) Populations translocation events and impact on natural habitats. In: Gherardi F, Corti C, Gualtieri M (eds) Biodiversity conservation and habitat management, encyclopaedia of life support systems. EOLSS Publisherc Co. Ltd., Oxford

Svoboda J, Mrugała A, Kozubíková-Balcarová E, Petrusek A (2016) Hosts and transmission of the crayfish plague pathogen *Aphanomyces astaci*: a review. J Fish Dis. doi:10.1111/jfd.12472

Tilmans M, Mrugała A, Svoboda J, Engelsma MY, Petie M, Soes DM, Nutbeam-Tuffs S, Oidtmann B, Roessink I, Petrusek A (2014) Survey of the crayfish plague pathogen presence in The Netherlands reveals a new *Aphanomyces astaci* carrier. J Invertebr Pathol 120:74–79

UICN (2012) La liste rouge des espèces menacées en France: Crustacés d'eau douce de France métropolitaine. Dossier de presse du 7 juin 2012, pp 25. http://www.uicn.fr/Liste-rouge-crustaces-d-eau-douce.html. Accessed 26 Aug 2013

Van Oosterhout C, Hutchinson WF, Wills DPM, Shipley P (2004) Micro-checker: software for identifying and correcting genotyping errors in microsatellite data. Mol Ecol Notes 4:535–538

Viljamaa-Dirks S, Heinikainen S, Nieminen M, Vennerstrom P, Pelkonen S (2011) Persistent infection by crayfish plague *Aphanomyces astaci* in a noble crayfish population—a case report. Bull Eur Assoc Fish Pathol 31:182–188

Vonlanthen P, Excoffier I, Bittner D, Persat H, Neuenschwander S, Largiadèr CR (2007) Genetic analysis of potential postglacial watershed crossings in Central Europe by the bullhead (*Cottus gobio* L.). Mol Ecol 16(21):4572–4584

Waser NM, Price MV (1994) Crossing distance effects in *Delphinium nelsonii*: outbreeding and inbreeding depression in progeny fitness. Evol Int J org Evol 48:842–852

Willi Y, Van Buskirk J, Hoffmann AA (2006) Limits to the adaptive potential of small populations. Annu Rev Ecol Evol Syst 37:433–458

Woodford MH, Rossiter PB (1993) Disease risks associated with wildlife translocation projects. Rev Sci Tech Int Off Epizoot 12(1):115–135