HIGH DISPERSAL ABILITY OF *AUSTROPOTAMOBIUS PALLIPES* REVEALED BY MICROSATELLITE MARKERS IN A FRENCH BROOK.

N. GOUIN*, C. SOUTY-GROSSET, A. ROPIQUET, F. GRANDJEAN

Laboratoire de Génétique et Biologie des Populations de Crustacés, UMR CNRS 6556, Université de Poitiers, 40 avenue du recteur Pineau, F-86022 POITIERS cedex, France.

Corresponding author : C. SOUTY-GROSSET. E-mail : catherine.souty@univ-poitiers.fr
Tél. : + 33.(0)5.49.45.36.07 ; Fax : + 33.(0)5.49.45.40.15

* Present address : Southwest Foundation for Biomedical Research, Department of Genetics, Laboratory of comparative genetics, P. O. Box 760549, SAN ANTONIO, Texas 78245-0549, USA.

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ABSTRACT

The genetic structure of a large population of the white-clawed crayfish *Austropotamobius pallipes* was assessed with five microsatellite markers by sampling six sites along its extent. The level of genetic polymorphism was relatively high with a mean heterozygosity value of 0.394 for the whole population. No genetic differentiation was observed between the different sampled sites, indicating that this population was panmictic. The possibility of extensive gene flow along this brook, downstream as well as upstream, is discussed.

Key-words : *Austropotamobius pallipes*, microsatellites, gene flow, population structure.

FORTE CAPACITÉ DE DISPERSION D'*AUSTROPOTAMOBIUS PALLIPES* RÉVÉLÉE PAR DES MARQUEURS MICROSATELLITES DANS UN RUISSEAU FRANÇAIS.

RÉSUMÉ

La structure génétique d'une importante population d'écrevisses à pattes blanches *Austropotamobius pallipes* a été appréhendée avec cinq marqueurs microsatellites en échantillonnant six secteurs le long de son aire de distribution. Le niveau de polymorphisme est apparu relativement élevé avec une valeur moyenne d'hétérozygotie de 0,394 pour l'ensemble de la population. Aucune différenciation génétique n'a été révélée entre les différents sites échantillonnés, indiquant que cette population est panmictique. La possibilité d'importants flux géniques étendus le long de ce ruisseau, vers l'aval aussi bien que vers l'amont, est discutée.

Mots-clés : *Austropotamobius pallipes*, microsatellites, flux de gènes, structure des populations.

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INTRODUCTION

It is critically important to understand the dispersal ability of an endangered species, especially when habitat fragmentation becomes more pronounced resulting from human pressures on the environment. Progressively isolating populations, fragmentation tends to favour the erosion of genetic variation, a process that may have catastrophic consequences for the long-term viability of populations (GILPIN and SOULÉ, 1986; LANDE, 1995; SHERWIN and MORITZ, 2000). In this context, a knowledge of the extent to which *A. pallipes* is able to scatter within a brook should help to better anticipate the impact of habitat fragmentation and to plan efficient conservation programmes.

The endangered white-clawed crayfish *A. pallipes* is still widespread in France, but is now confined to headwater river systems (VIGNEUX *et al.*, 1993). Recent genetic analyses, carried out with mitochondrial and RAPD markers, have revealed a high level of genetic structure among French populations sampled within and between different drainages (GRANDJEAN and SOUTY-GROSSET, 2000; GOUIN *et al.*, 2001). These results have been interpreted as the consequence of genetic drift occurring in small isolated populations. If the influence of fragmentation on population differentiation is easy to quantify at this scale, it appears more difficult at the scale of a brook. However, habitat is rarely continuous along rivers. In a recent population study, NEVEU (2000a) revealed differences in crayfish demographic structure between two sites only 2 km apart. Within the same brook, NEVEU (2000b) also pointed out the role of habitat structure in the distribution of white-clawed crayfish. Thus, it seems that populations of *A. pallipes* may be structured within brooks. If this species displays a low dispersal ability, we could expect that such a demographic structure could have an impact on the genetic structure.

Because few data have been published about the dispersion behaviour of the white-clawed crayfish (ROBINSON *et al.*, 2000), we decided to study the structure of a population by using recently isolated microsatellite markers (GOUIN *et al.*, 2000). These genetic markers are highly polymorphic and now commonly used to assess population genetic structure on a fine scale (JARNE and LAGODA, 1996). Their sensitivity to reveal gene flow makes them particularly interesting in conservation biology to estimate the impact of habitat fragmentation on the connectivity between sub-populations (SRIKWAN and WOODRUFF, 2000). In *A. pallipes*, three schemes may be proposed depending on its ability to scatter along the brook. First, if no gene flow occurs between sub-populations, one should observe a high level of genetic structure as described by SLATKIN (1987). In the second case, a low dispersal ability should cause a gradual differentiation of sub-populations due to the reduction of gene flow with the increase in geographic distances (HUTCHINSON and TEMPLETON, 1999). Finally, we may suppose a high level of gene flow among sampled sites. In that case, microsatellites makers should reveal an absence of genetic differentiation (SLATKIN, 1987).

In order to resolve these questions, we propose to analyse the largest population of *A. pallipes* known in the Poitou-Charentes region, that occurring in the brook of Le Magnerolles. Because of its large size, this population should be an interesting model to define the genetic structure of a white-clawed crayfish population and to characterize the dispersal abilities of this species.

MATERIAL AND METHODS

Sampling strategy

As described in Figure 1, the population of *A. pallipes* is spread over 3 000 meters along the Le Magnerolles brook. Six sites were sampled during the summer in 2000, covering the entire extent of that population. A non-destructive sampling strategy was

employed by taking just one pereiopod from the fourth pair. Each leg was separately stored in 95% ethanol until DNA extraction. The number of individuals analysed in this study is reported in Table IV. It is important to note that no evident physical barrier to migration was identified along the brook during this sampling.

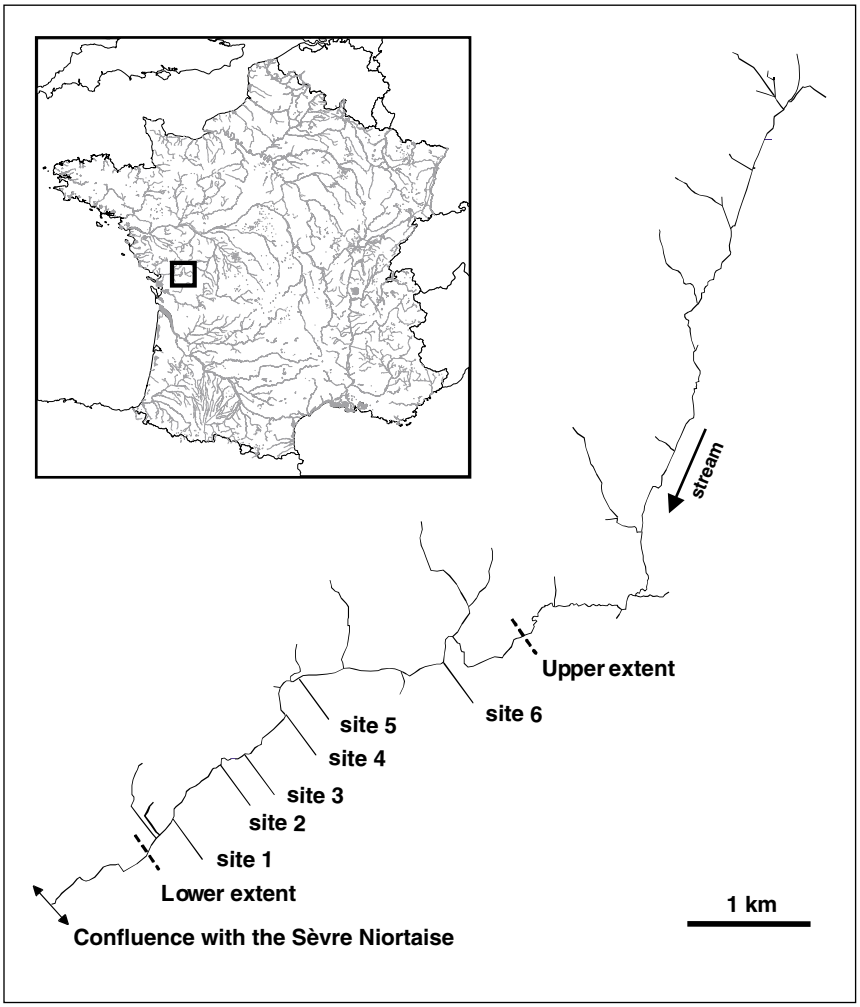

Figure 1
Geographical location and sampling strategy in the brook of Le Magnerolles.

Figure 1
Situation géographique et sites d'échantillonnage le long du ruisseau du Magnerolles.

Genetic analysis

DNA was extracted from 15 mg of muscle tissues, following a Chelex protocol adapted from ESTOUP *et al.* (1996). Tissues were completely dried before the addition of 175 µl of sterile distillated water and 5 µl of proteinase K (20 $mg.ml^{-1}$) and ground with plastic pestles on ice. After adding a further 175 µl of sterile distillated water, tissues were ground a second time. Samples were incubated at 56°C for 4 hours with 20-40% Chelex and placed at 100°C for 8 minutes to inhibit proteinase K. Extractions were stored at -20°C, vortexed and then centrifuged 1 minute at 12 000 rpm before each use.

Genetic diversity was screened at five microsatellite loci using primers specifically developed for *A. pallipes*. Primers for locus Ap2, Ap3 and Ap6 were those published by GOUIN *et al.* (2000). Concerning locus Ap5, a new forward primer (5'_TCTTTATTGTGAGGGGGAAGG_3') was designed to reduce the size of the amplification product. The fifth microsatellite marker used in this study has not been published but was isolated from the same partial genomic library. The sequence of this clone is available in GenBank under accession number AF467810. Two primers were designed for this locus named Ap7: forward 5'_ATGTACGCACTGCTTCACTGG_3' and reverse 5'_TTGTTTGTGGTGTGAATTACCG_3'. Microsatellite polymorphism was analysed using a fluorescent detection method. For this purpose, one of the primers for each locus was 5'-labelled with one of two colours: blue (6FAM) for Ap3, Ap6 and Ap7 loci or green (TET) for Ap2 and Ap5 loci. PCR were carried out in a 12.5 µl reaction volume containing 0.5 unit of AmpliTaq gold DNA polymerase (Perkin Elmer), 1.25 µl of GeneAmp 10X PCR Buffer II (100 mM Tris-HCl, pH 8.3, 500 mM KCl), 1.2 mM $MgCl_2$ (for loci Ap2, Ap3, Ap5, Ap6) or 0.8 mM $MgCl_2$ (for loci Ap7), 60 µM of each dNTP, 400 nM of each primer and 0.5 µl of template DNA (*i.e.* supernatent of the Chelex extraction). Multiplexed PCR was used for Ap2 and Ap3 and for Ap5 and Ap6. All PCRs were performed in a Trio-Thermoblock thermocycler (Biometra, GmBH, Göttingen, Germany), with the following profile: an initial denaturing step of 10 min at 95°C, followed by 30 cycles of 30 sec at 95°C, 30 sec at annealing temperature 64°C (for Ap7) or 60°C (for others), 30 sec (for Ap2/Ap3) or 45 sec (for Ap5/Ap6 and Ap7) at 72°C and a final extension of 10 min at 72°C. These PCR conditions are summarized in Table I. For each sample, 1 µl of the duplex PCR Ap2/Ap3, 3 µl of the duplex PCR Ap5/Ap6, 2.5 µl of the PCR for Ap7, 0.5 µl of GS-500 [TAMRA] internal size standard (red colour, 500 bp) and 13 ml of deionized formamide were mixed. The samples were heated for 5 min at 95°C and chilled on ice before the analysis. Electrophoresis procedures were conducted with an ABI 310 automated sequencer following manufacturer's recommendations. For each sample, 15 sec of injection at 15 kV were used and runs were performed for 24 min at 15 kV at a temperature of 60°C. Analysis was made automatically and allelic size was determined by reference to the internal sizing standard using GENESCAN Analysis version 2.1.

Table I

Polymerase chain reaction (PCR) amplification set composition, $MgCl_2$ concentration, annealing temperature and elongation time for each procedure.

Tableau I

Conditions d'amplification par PCR : multiplexage, concentration en $MgCl_2$, température d'hybridation et durée de la phase d'extension selon les amorces.

Multiplexed amplification sets	$MgCl_2$	Annealing temperature	Elongation time
Ap2-Ap3	1.2 mM	60˚C	30 sec
Ap5-Ap6	1.2 mM	60˚C	45 sec
Ap7	0.8 mM	64˚C	45 sec

Data analysis

Genetic polymorphism was estimated for each sampled site as the number of alleles per locus (A), observed heterozygosity (H_o) and expected heterozygosity (H_e), computed with GENETIX 4.01 (BELKHIR *et al.*, 1998). Conformity with Hardy-Weinberg equilibrium at each locus and in each sample was tested using the probability test of GUO and THOMPSON (1992) available in GENEPOP 3.2a (RAYMOND and ROUSSET, 1995). In order to define the population structure, the null hypothesis of homogeneity in allelic distribution among each sampled site was tested with a Fisher's exact test. This test was

performed through 1 000 iterations using the Markov chain method with GENEPOP 3.2a. Analysis of molecular variance was used to assess a global differentiation index between sites with Arlequin 2.0 (SCHNEIDER *et al.*, 2000). Since a low ability of *A. pallipes* to spread upstream could lead to a gradual loss of genetic diversity along this axis, we tested the organization of the heterozygosity at each and overall loci with the Mann-Withney test (MANN and WHITNEY, 1947). This test was performed by calculating the magnitudes of the differences between the rank of the sampled site positions and the rank from the lower to the higher level of polymorphism.

RESULTS

Allele frequencies by site are reported in Table II. The number of alleles at each locus was relatively low, ranging from 2 at Ap2 and Ap6 to 5 at Ap5. All loci were polymorphic within each of the six samples analysed. The expected heterozygosity varied from 0.135 to 0.670 depending on locus and sample (Table III). The per-sample expected heterozygosity averaged over loci ranged from 0.372 in site 2 to 0.410 in site 3 (Table IV). The mean value reached 0.394 for the whole population. Because no test was significant, the probability test revealed that the frequencies of the genotypes observed within each sampled sites were at Hardy-Weinberg equilibrium (Table III; Table IV).

Table II

Allelic frequencies at each locus and each sample and statistical analysis of the distribution of allele frequencies between sites for each locus (the name of each allele correspond to its size in base pairs).

Tableau II

Fréquences alléliques pour chaque locus et chaque site échantillonné ; analyse statistique de la distribution des fréquences alléliques entre les sites et pour chaque locus (le nom de chaque allèle correspond à sa taille en paires de base).

Alleles	Site 1	Site 2	Site 3	Site 4	Site 5	Site 6	Homogeneity test
Ap2							$p = 0.928$
184	0.206	0.087	0.278	0.167	0.237	0.158	
190	0.794	0.913	0.722	0.833	0.763	0.842	
Ap3							$p = 0.654$
148	-	-	-	0.048	-	0.026	
150	0.912	0.913	0.861	0.927	0.816	0.842	
154	0.088	0.087	0.111	0.024	0.184	0.132	
158	-	-	0.028	-	-	-	
Ap5							$p = 0.383$
331	0.059	0.109	-	0.071	0.118	0.083	
333	0.029	0.022	0.028	-	-	-	
335	0.529	0.435	0.472	0.405	0.412	0.556	
337	0.323	0.348	0.444	0.357	0.412	0.361	
341	0.059	0.087	0.056	0.167	0.059	-	
Ap6							$p = 0.846$
344	0.676	0.783	0.778	0.786	0.868	0.737	
348	0.324	0.217	0.222	0.214	0.132	0.263	
Ap7							$p = 0.995$
264	0.589	0.544	0.639	0.548	0.583	0.632	
266	0.412	0.413	0.333	0.429	0.417	0.368	
268	-	0.043	0.028	0.023	-	-	

No significant differences in allele frequency distributions were observed among the different sites (Table II), indicating the absence of genetic structure within this population of *A. pallipes*. The analysis of molecular variance confirmed this result by revealing a global index of differentiation F_{ST} equal to -0.008, which was not significant (p = 0.94). Moreover, none of the tests performed on the organization of the heterozygosity were significant (data not showed), revealing no reduction of the genetic diversity from the lower to the upper extent of the population of Le Magnerolles.

Table III

Total number of alleles per locus (A), F_{IS}, observed (H_o) and expected (H_e) heterozygosity for each locus and sample.

Tableau III

Nombre total d'allèles par locus (A), F_{IS}, hétérozygoties respectivement observée (H_o) et attendue (H_e) pour chaque locus et chaque échantillon.

Locus	A		Site 1	Site 2	Site 3	Site 4	Site 5	Site 6
Ap2	2	A	2	2	2	2	2	2
		H_o	0.294	0.174	0.444	0.238	0.263	0.316
		H_e	0.327	0.159	0.401	0.278	0.361	0.266
		F_{is}	+0.130	-0.073	-0.079	+0.167	+0.297	-0.161
Ap3	4	A	2	2	3	3	2	3
		H_o	0.176	0.174	0.222	0.143	0.263	0.263
		H_e	0.161	0.159	0.254	0.135	0.301	0.273
		F_{is}	-0.067	-0.073	+0.123	-0.034	+0.151	+0.062
Ap5	5	A	5	5	4	4	4	3
		H_o	0.589	0.565	0.500	0.571	0.556	0.500
		H_e	0.607	0.670	0.576	0.627	0.617	0.551
		F_{is}	+0.062	+0.178	+0.159	+0.113	+0.128	+0.121
Ap6	2	A	2	2	2	2	2	2
		H_o	0.412	0.348	0.333	0.238	0.158	0.316
		H_e	0.438	0.340	0.346	0.337	0.228	0.388
		F_{is}	+0.089	-0.000	+0.064	+0.315	+0.333	+0.212
Ap7	3	A	2	3	3	3	2	2
		H_o	0.471	0.522	0.556	0.571	0.500	0.526
		H_e	0.484	0.532	0.480	0.516	0.486	0.465
		F_{is}	+0.059	+0.042	-0.130	-0.084	0.000	-0.104

Table IV

Sample size (N), observed (H_o) and expected (H_e) heterozygosity averaged over loci and exact probability of conformity to Hardy-Weinberg equilibrium (p).

Tableau IV

Taille de l'échantillon (N), valeur moyenne des hétérozygoties respectivement observée (H_o) et attendue (H_e) pour tous les loci et probabilité exacte d'équilibre de Hardy-Weinberg (p).

Sample	N	H_e	H_o	*p*
Site 1	17	0.403	0.389	*0.980*
Site 2	23	0.372	0.357	*0.987*
Site 3	18	0.410	0.422	*0.378*
Site 4	21	0.389	0.362	*0.622*
Site 5	19	0.404	0.355	*0.664*
Site 6	19	0.389	0.395	*0.809*

DISCUSSION

Microsatellite polymorphism

Although the allelic diversity was relatively low, the degree of polymorphism displayed by microsatellite markers in this study appeared very high. While using allozymes SANTUCCI *et al.* (1997) and LARGIADÈR *et al.* (2000) reported a mean heterozygosity value in *A. pallipes* of 0.001 and 0.015 respectively, but microsatellites revealed a value of 0.394 for the population occurring in Le Magnerolles brook. Even if comparison of the level of polymorphism might be inappropriate by the use of only one population, these markers seem able to reveal more genetic variability in the white-clawed crayfish than do allozymes. Such a difference between nuclear markers is not surprising and has been reported in numerous species such as the brown trout *Salmo trutta* (ESTOUP *et al.*, 1998; CAGIGAS *et al.*, 1999). The crayfish population of Le Magnerolles has been already studied using RAPD markers (GOUIN *et al.*, 2001), and displayed a genetic diversity of 0.446 estimated by the Shannon diversity index. Although this index and heterozygosity estimates are not directly comparable, values are similar for this population indicating that we may expect to observe the same level of polymorphism in *A. pallipes* with microsatellites. Nevertheless microsatellite markers have a great advantage compared to RAPDs since they are co-dominant and therefore more informative (JARNE and LAGODA, 1996). Thus the ability of microsatellites to reveal polymorphism in *A. pallipes* and their co-dominance confer on these markers a high potential to analyse population genetic structure in this species.

Population structure

The five microsatellites used in this study revealed the absence of genetic differentiation among the six sites sampled along the brook of Le Magnerolles. Since sampling encompassed the whole extent of the population, we can consider it to be panmictic. This result suggests that white-clawed crayfish are able to spread along a distance of at least 3 000 meters, maintaining the genetic homogeneity within the population as suggested by SLATKIN (1987). It also implies that the discontinuity of freshwater habitats, while responsible for the distribution of *A. pallipes* (NEVEU, 2000a, b), would have no effect on its genetic structure in the absence of barriers to migration.

The fact that *A. pallipes* could spread over such a distance might appear surprising, particularly upstream. However, recent population studies using radio-tracking have been carried out to understand dispersal ability in crayfish. ROBINSON *et al.* (2000) have revealed that males of *A. pallipes* were able to cover about 4.2 meters per day and females about 1.7 meters. Comparable results were obtained by ARMITAGE (2000), who recorded a male that covered 101 meters in a month. In *Astacus astacus*, SCHÜTZE *et al.* (1999) pointed out that dispersal activity fluctuated with season. While activity was low during the winter, crayfish were able to spread up to 830 meters downstream and 546 meters upstream in 15 days during the summer. These authors also recorded individuals having covered 2 439 meters between June and August. All these studies tend therefore to argue that crayfish are able to scatter over relatively large distances along streams, downstream as well as upstream. In this context, even if dispersion occurs more frequently downstream (SCHÜTZE *et al.*, 1999), we may consider that white-clawed crayfishes could have spread upstream in the brook of Le Magnerolles. The genetic homogeneity revealed in this brook could then be explained by extensive gene flow in both directions, downstream as well as upstream.

According to these results, there appears to be no barrier preventing upstream migration of white-clawed crayfishes in the brook of Le Magnerolles. If the downstream dispersal is obviously the easiest way to spread, especially for juveniles, we may assume that adults are able to cover long distances upstream. As described in *A. astacus* by SCHÜTZE *et al.* (1999), the upstream dispersal is more likely to occur during the summer.

Because of the lower level of water and the lower speed of the stream, crayfishes could expend less energy to cover long distances during this period. This ability to spread upstream could be viewed as an adaptation to find refuge pools with better water quality during the dry season, and could increase survival in the case of a drastic drought. It could also confer a selective advantage to the crayfish able to go upstream by increasing the chance to maintain their genes in the population. But the adaptive nature of dispersal, if any, needs to be clarified, as do all the factors that could stimulate dispersal such as local high densities and/or competition between males. Further population studies using radio-tracking methods that are better adapted to crayfish would help to answer these questions. Moreover, as we don't really know the ability of *A. pallipes* to overcome obstacles to dispersal, it could be interesting to compare the genetic structure of populations occurring in brooks having different topologies and known barriers such as artificial dams.

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REFERENCES

ARMITAGE V., 2000. Observations of radio tracked crayfish (*Austropotamobius pallipes*) in a northern British river. *Proceedings of the Crayfish Conference*, Leeds, 63-69.

BELKHIR K., BORSA P., GOUDET J., CHIKHI L., BONHOMME F., 1998. GENETIX, logiciel sous Windows TM pour la génétique des populations, Laboratoire Génome et Populations, CNRS UPR 9060, Université de Montpellier II, Montpellier (France).

CAGIGAS M.E., VAZQUEZ E., BLANCO G., SANCHEZ J.A., 1999. Combined assessment of genetic variability in populations of brown trout (*Salmo trutta* L.) based on allozymes, microsatellites, and RAPD markers. *Marine Biotechnology*, *1*, 286-296.

ESTOUP A., LARGIADÈR C.R., PERROT E., CHOURROT D., 1996. Rapid one-tube DNA extraction for reliable PCR detection of fish polymorphic markers and transgenes. *Molecular Marine Biology and Biotechnology*, *5 (3)*, 295-298.

ESTOUP A., ROUSSET F., MICHALAKIS Y., CORNUET J.M., ADRIAMANGA M., GUYOMARD R., 1998. Comparative analysis of microsatellite and allozyme markers: a case study investigating microgeographic differentiation in brown trout (*Salmo trutta*). *Molecular Ecology*, *7*, 339-353.

GILPIN M., SOULÉ M.E., 1986. Minimum viable populations: processes of species extinction. *In* : *Conservation biology: the science of scarcity and diversity* (SOULÉV M.E., ed.), 19-34. Sinauer Associates, Sunderland, MA.

GOUIN N., GRANDJEAN F., SOUTY-GROSSET C., 2000. Characterization of microsatellite loci in the endangered freshwater crayfish *Austropotamobius pallipes* (Astacidae) and their potential use in other decapods. *Molecular Ecology*, *9*, 636-637.

GOUIN N., GRANDJEAN F., BOUCHON D., REYNOLDS J.R., SOUTY-GROSSET C., 2001. Genetic structure of the endangered freshwater crayfish *Austropotamobius pallipes*, assessed using RAPD markers. *Heredity*, *87*, 80-87.

GRANDJEAN F., SOUTY-GROSSET C., 2000. Mitochondrial DNA variation and population genetic structure of the white-clawed crayfish *Austropotamobius pallipes pallipes*. *Conservation Genetics*, *1*, 309-319.

GUO S.W., THOMPSON E.A., 1992. Performing the exact test of Hardy-Weinberg proportion for multiple alleles. *Biometrics*, *48*, 361-372.

HUTCHINSON D.W., TEMPLETON A.R., 1999. Correlation of pairwise genetic and geographic distance measures: inferring the relative influences of gene flow and drift on the distribution of genetic variability. *Evolution*, *53 (6)*, 1898-1914.

JARNE P., LAGODA P.J.L., 1996. Microsatellites, from molecules to populations and back. *TREE*, *11*, 424-428.

LANDE R., 1995. Mutation and conservation. *Conservation Biology*, *9*, 782-791.

LARGIADÈR C.R., HERGER F., LÖRTSCHER M., SCHÖLL A., 2000. Assessment of natural and artificial propagation of the white-clawed crayfish (*Austropotamobius pallipes* species complex) in the Alpine region with nuclear and mitochondrial markers. *Molecular Ecology*, *9*, 25-37.

MANN H.B., WHITNEY D.R., 1947. On a test of whether one of two random variables is stochastically larger than the other. *Ann. Math. Stat.*, *18*, 50-60.

NEVEU A., 2000a. Etude des populations d'*Austropotamobius pallipes* (Crustacea, Astacidae) dans un ruisseau forestier de Normandie. I. Structures démographiques et croissance : stabilité et variabilité au cours de six années. *Bull. Fr. Pêche Piscic.*, *356*, 71-98.

NEVEU A., 2000b. Etude des populations d'*Austropotamobius pallipes* (Crustacea, Astacidae) dans un ruisseau forestier de Normandie. II. Répartition en fonction des habitats : stabilité et variabilité au cours de cinq années. *Bull. Fr. Pêche Piscic.*, *356*, 99-122.

RAYMOND M., ROUSSET F., 1995. GENEPOP (Version 1.2): Population Genetics software for exact tests and ecumenicism. *Journal of Heredity*, *86*, 248-249.

ROBINSON C.A., THOM T.J., LUCAS M.C., 2000. Ranging behaviour of a large freshwater invertebrate, the white-clawed crayfish *Austropotamobius pallipes*. *Freshwater Biology*, *43*, 1-13.

SANTUCCI F., IACONNELLI M., ANDREANI P., CIANCHI R., NASCETTI G., BULLINI L., 1997. Allozyme diversity of European freshwater crayfish of the genus *Austropotamobius*. *Bull. Fr. Pêche Piscic.*, *347*, 663-676.

SCHNEIDER S., ROESSLI D., EXCOFFIER L., 2000. A software for population genetics data analysis. Genetics and Biometry Laboratory, University of Genova, Switzerland. http://anthropologie.unige.ch/arlequin

SCHÜTZE S., STEIN H., BORN O., 1999. Radio telemetry observations on migration and activity patterns of restocked noble crayfish *Astacus astacus* (L.) in the small River Sempt, North-East of Munich, Germany. *Freshwater Crayfish*, *12*, 688-695.

SHERWIN W.B., MORITZ C., 2000. Managing and monitoring of genetic erosion. *In* : *Genetics, demography and viability of fragmented populations* (YOUNG A.G. and CLARKE G.M., Eds.), Cambridge University Press, 9-34.

SLATKIN M., 1987. Gene flow and the geographic structure of natural populations. *Science*, *236*, 787-792.

SRIKWAN S., WOODRUFF D.S., 2000. Genetic erosion in isolated small-mammal populations following rainforest fragmentation. *In* : *Genetics, demography and viability of fragmented populations* (YOUNG A.G. and CLARKE G.M., Eds.), Cambridge University Press, 149-172.

VIGNEUX E., KEITH P., NOEL P., 1993. *Atlas préliminaire des Crustacés Décapodes d'eau douce de France.* Coll. Patrimoines Naturels, Vol. 14, S.F.F., B.I.M.M.-M.N.H.N., C.S.P., Ministère de l'Environnement, Paris, 55 p.