

STATUS OF *AUSTROPOTAMOBIOUS PALLIPES* COMPLEX IN THE WATERCOURSES OF THE ALESSANDRIA PROVINCE (N-W ITALY)

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ABSTRACT

Information was gathered on the distribution of white-clawed crayfish *Austropotamobius pallipes* complex in the watercourses of the Alessandria province (NW Italy), on the biological and ecological preferences of the species, and on the features of the biotopes in which it is found. A total of 409 sites on 361 watercourses connected to the main sub-basins of the Po River were analysed, with data gathered during the field research phase, conducted for three consecutive summers from 2002 through 2004. Thirteen percent of the sites investigated were found to currently house crayfish populations. The persistence in time of superficial water and natural morphology of the watercourse were found to be the chief requirements for the presence of crayfish. Data on land use, human density and other factors which potentially limit the survival of crayfish populations were also analysed.

Key-words: *Austropotamobius pallipes* complex, freshwater crayfish, bio-ecological characteristics.

STATUT DU COMPLEXE D'ESPACE D'*AUSTROPOTAMOBIOUS PALLIPES* DANS LES COURS D'EAU DE LA PROVINCE D'ALESSANDRIA (ITALIE NORD-OCCIDENTALE)

RÉSUMÉ

Le projet de recherche a prévu la récolte d'informations sur la répartition du complexe d'espèce *Austropotamobius pallipes* dans les réseaux hydrographiques de la province d'Alessandria (Italie nord-occidentale), sur les caractères bio-écologiques de l'espèce et sur les caractéristiques des milieux où elle a été trouvée. Les prospections de terrain ont été faites durant trois étés consécutifs de la période 2002-2004 et ont permis d'analyser 409 sites sur 361 cours d'eau, appartenant aux principaux sous-bassins du fleuve Pô. Des populations d'écrevisses n'ont été trouvées que dans 13 % des sites prospectés. La persistance temporelle des débits hydriques superficiels et la morphologie naturelle constituent la condition essentielle pour la présence des écrevisses. En outre, les données

ont été analysées par rapport au mode d'utilisation du sol, à la densité démographique et à d'autres aspects qui limitent, potentiellement, la survie des populations astacicoles.

Mots-clés : *Austropotamobius pallipes* complexe, écrevisse, caractéristiques bio-écologiques.

INTRODUCTION

Austropotamobius, the only autochthonous genus of crayfish found in Italian freshwater courses, is present on the Italian mainland but not on the surrounding islands (GHERARDI *et al.*, 1999). Although it is known to be widely distributed throughout the peninsula, information on its range and density is very general and out of date. Habitat Directive 92/43/CEE, received in Italian law (DPR 357/97 and DPR 120/2003), qualifies *A. pallipes* as a species of European community interest for which conservation areas must be set aside (Annex II) and which is subject to periodic sampling for the purpose of population management (Annex V).

Some regional administrations have approved even more stringent legislation. In Piedmont, for example, the capture, transport, trade and keeping in captivity of animals for sale are forbidden except in areas where fishing and sale are permitted and water bodies are repopulated regularly from breeding stock (art. 29, L.R. n. 32 of 2/11/1982). *A. pallipes* is also classified as vulnerable by the IUCN (2000).

The purpose of our research was to obtain a clear picture of the distribution and of the ecological and biological preferences of freshwater crayfish in the Alessandria province (in the south-east of the region of Piedmont) in order to be able to apply communitary conservation law.

Recent research has revealed the presence of two distinct taxonomic entities in Italian fresh waters: *A. pallipes*, found in northwestern Italy and *A. italicus* in northeastern and peninsular Italy (NASCETTI *et al.*, 1997; SANTUCCI *et al.*, 1997; GRANDJEAN *et al.*, 2000; LARGIADÈR *et al.*, 2000; ZACCARA *et al.*, 2004). The results of genetic characterization of all the crayfish populations found in the study area are presently being analysed. Preliminary results of comparison of 16SmtDNA gene sequences confirm that the two species overlap in the province of Alessandria and appear to be sympatric in at least two of the brooks examined (NEGRI *et al.*, 2003). As not enough is yet known about the two species to distinguish them morphologically, the results of this study refer only to the *Austropotamobius pallipes* complex.

MATERIALS AND METHODS

The study area covered the whole of the Alessandria province in the northwestern Italy, which has a continental climate. The province has a diversified orographic structure with 53% of its 3,560 km² surface area consisting of hills, 12% of mountains and 35% of lowlands. The mountains, which reach a maximum elevation of 1,700 m, are situated in the south-easternmost part of the province. Alessandria is crossed by 2,800 km of watercourses, which make up a dense hydrographic network characterized by different types of water bodies (rivers, streams, brooks, resurgences and artificial canals). It has a human population of about 450,000 living in 190 municipalities of which 116 have fewer than 1,000 inhabitants and of which only 10 have more than 5,000 inhabitants. The average population density is 123 inhabitants/km² (MALACARNE, CUCCO and BOANO, 1999).

Five different land use categories were identified from the territorial map supplied by the administration of the *Provincia di Alessandria*. In terms of total surface area and in

increasing order of human impact, these account for: woodlands, 25%; grasslands, 33%; vineyards/orchards, 21%; sown fields, 17%; urban areas, 4%.

A preliminary picture of the distribution of *Austropotamobius* was put together from bibliographic research, evaluation of collections in some natural science museums (Milan, Pavia, Novara, Bra and Stazzano), and interviews with fishermen and wildlife conservation workers. For three consecutive summers from 2002 through 2004, field investigations were carried out on all the watercourses in Alessandria. Survey sites were identified on maps IGM 1: 25,000 and CTR 1: 10,000 (supplied by the *Provincia di Alessandria*), with no water body being excluded *a priori*. On large watercourses, several 100-500 m stretches were investigated.

Crayfish were searched for mainly by hand in all possible refuges: stones and leaf litter on the bottom of watercourses, holes along the riverbank, and abandoned trash. A fine mesh dip net was used to capture specimens in aquatic vegetation. Their presence was also detected by means of moults. Where watercourses were too turbid or large to capture crayfish by hand, traps were placed for sampling purposes for at least one night (MORIARTY, 1972; BROWN and BREWIS, 1978).

Sites were described by compiling a table of characteristics (see Table I) based on rapid bioassessment protocols (BARBOUR *et al.*, 2002) that includes parameters for the biological and ecological requirements of crayfish (DAGUERRE DE HUREAUX and ROQUEPLO, 1981; SMITH *et al.*, 1996) and items referring to water quality and site resilience (GHETTI, 1997; ANPA, 2000; FONDAZIONE LOMBARDIA PER L'AMBIENTE, 2002). Not all of these parameters will be shown in this paper.

Table I
Variables used in the survey.

Tableau I
Variables utilisées dans l'étude.

General descriptors
<ul style="list-style-type: none"> • date of survey, weather conditions, basin, watercourse, name of site surveyed, altitude (m a.s.l.), air temperature, GPS coordinates, length of surveyed stretch, search effort (time per number of operators), previous reports
Population descriptors
<ul style="list-style-type: none"> • presence/absence of freshwater crayfish or moults; number, sex and size of found freshwater crayfish
Instream habitat descriptors
<ul style="list-style-type: none"> • width of submerged river bed (m), medium and maximum water depth (cm), speed of water flow (6 classes), variety of river course (4 classes), type of main substrate (6 granulometric classes), availability of shelters (5 classes of abundance), presence of aquatic macrophytes (6 classes of abundance), periphyton (6 classes of abundance)
Riparian habitat descriptors (4 quality classes, for each bank)
<ul style="list-style-type: none"> • bank structure and erosion; riparian vegetation type, width and continuity, channel shading (6 classes)
Water quality
<ul style="list-style-type: none"> • total hardness (mg/l CaCO₃), pH, conductivity (µS/cm at 25°C), O₂ dissolved (mg/l) and O₂ %, temperature (°C), community state of aquatic macroinvertebrates (5 EBI classes)

The status of aquatic macroinvertebrate communities was evaluated according to the EBI protocol (GHETTI, 1997) and an index of 1-5 was used to indicate quality, from 1 (unaltered macrobenthic community) to 5 (completely altered macrobenthic community).

Urbanization was expressed as the ratio of inhabitants in the river basin to length of the watercourse from the source to the first sampling point where crayfish were found.

Comparison of environmental parameters in sites where crayfish were found vs. those where crayfish were not found was conducted by means of the Mann-Whitney U test; the Chi-squared test and Spearman's correlation on the SPSS 11.5 package.

RESULTS

Four hundred and nine sites on 361 watercourses were investigated. Only in 235 (57.5%) watercourses was there perennial superficial water, either flowing or in the form of isolated pools. *Austropotamobius pallipes* complex was found in 53 of these (Figure 1). No specimens of allochthonous crayfish species were found. Table II shows the number of sites investigated, water conditions and the number of sites where crayfish were found in each river basin.

Eighteen reports of crayfish sightings in the Alessandria province were found in the preliminary overview of existing data. Of these only the one for the Piota Stream in the basin of the Orba Stream (SPANÒ and REMAGGI, 1974) was confirmed in the present study. No evidence of crayfish was found either in the main watercourse or in the tributaries to support more recent reports for the basin of the Curone Stream (FERRARI, 1981).

Crayfish were detected at altitude of 100 to 450 m a.s.l. with a majority of sightings (83%) taking place at 150-350 m in the low and medium hills of the Apennine area (Figure 2). In particular, sites with *A. pallipes* complex are more frequent than sites without

Table II

Number of sites surveyed per river basin, showing the number of sites where water was present and the number of sites where *A. pallipes* complex was found. Percentages in round brackets are of the number of sites surveyed per river basin; percentages in square brackets are of the number of sites where water was present per river basin.

Tableau II

Répartition des sites avec présence d'eau et des sites avec *A. pallipes* dans le réseau hydrographique de la province d'Alessandria. Entre parenthèses: pourcentages sur le total des sites étudiés; entre crochets: pourcentages calculés sur le total des sites avec eau, pour chaque bassin.

River basin	Sites surveyed	Water present	<i>A. pallipes</i> present
Belbo	6	2 (33.3%)	0
Bormida - B. Spigno	107	47 (43.9%)	6 [12.8%]
Curone	25	14 (56.0%)	0
Orba	107	80 (74.8%)	29 [36.2%]
Po	64	35 (54.7%)	5 [14.3%]
Scrivia	63	42 (66.7%)	11 [26.2%]
Tanaro	37	15 (40.5%)	2 [13.3%]
	409	235 (57.5%)	53 [22.6%]

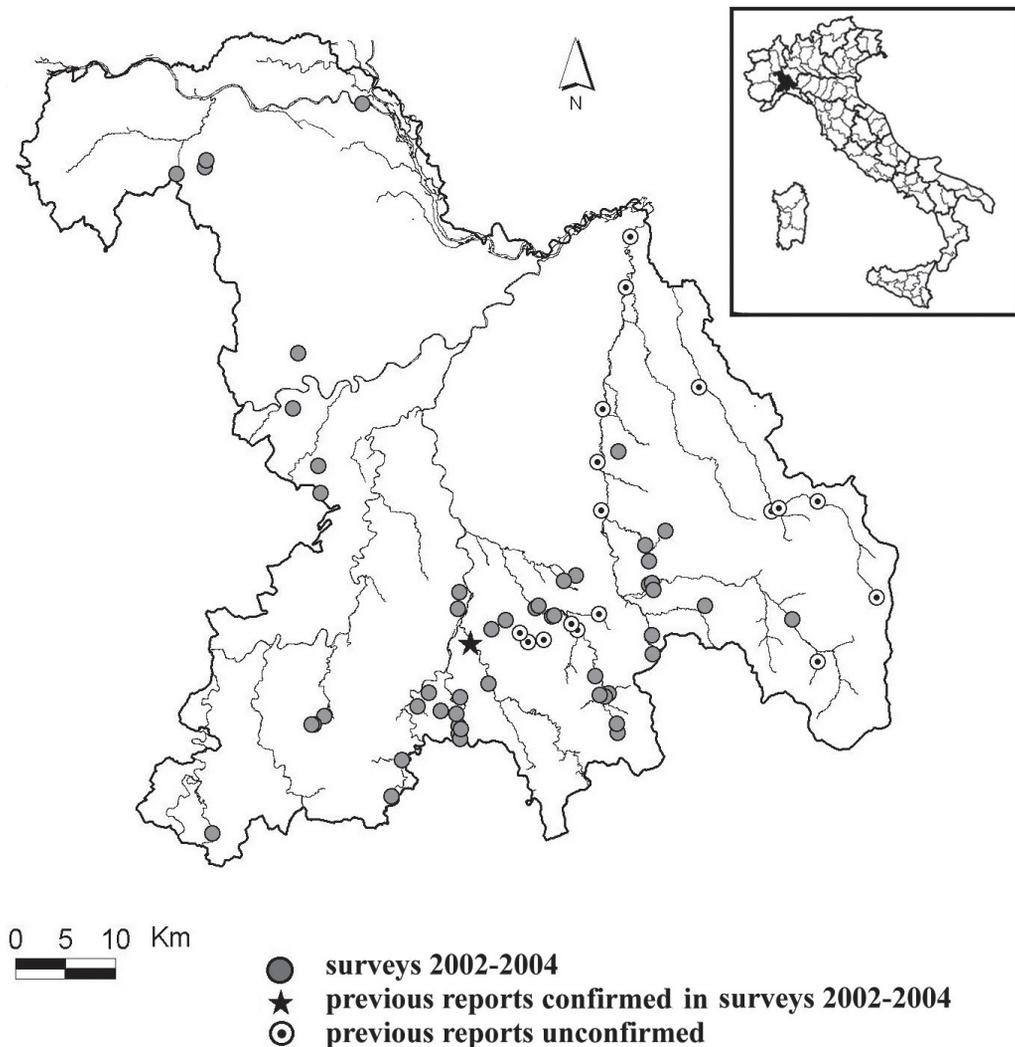


Figure 1
Distribution of *A. pallipes* complex populations in the province of Alessandria.

Figure 1
Distribution des populations d'*A. pallipes* complexe dans la province d'Alessandria.

crayfish at 250-350 m, and less frequent at the lowest and highest elevations (Pearson $\chi^2 = 15.565$; $df = 4$; $p = 0,004$).

Water temperatures of 10.6-33°C were found at sites where water was present, with the highest values being measured in the basin of the Bormida – B. Spigno Stream. At sites where crayfish were found, water temperatures were mostly below 21°C (84.3% of sites) with the highest temperature of 26°C being measured in the Piota Stream (basin of the Orba Stream). Ranges of physical and chemical water parameters for the sites where crayfish were found are shown in Table III and in Figure 3.

Most crayfish populations (42/53 = 79%) were found in sites where urbanization is below 100 inhabitants/km, with 36 sites lying in river basins where urbanization was

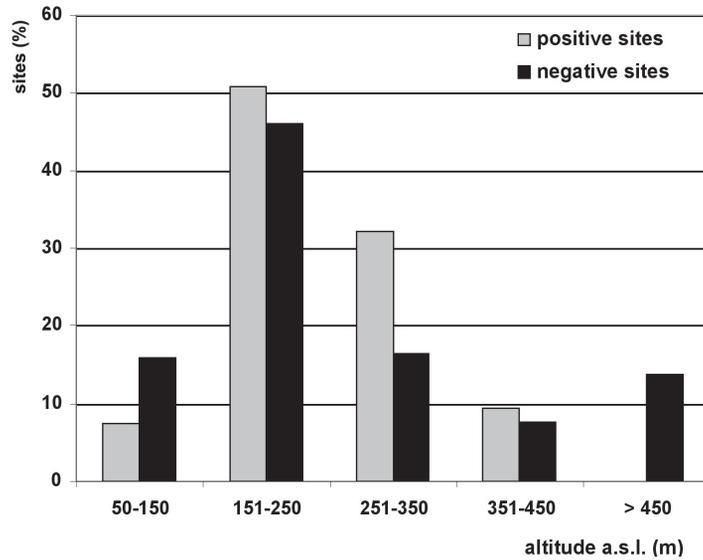


Figure 2
Presence and absence of *A. pallipes* complex according to altitude interval.

Figure 2
Répartition des sites étudiés dans les différentes classes altitudinales.

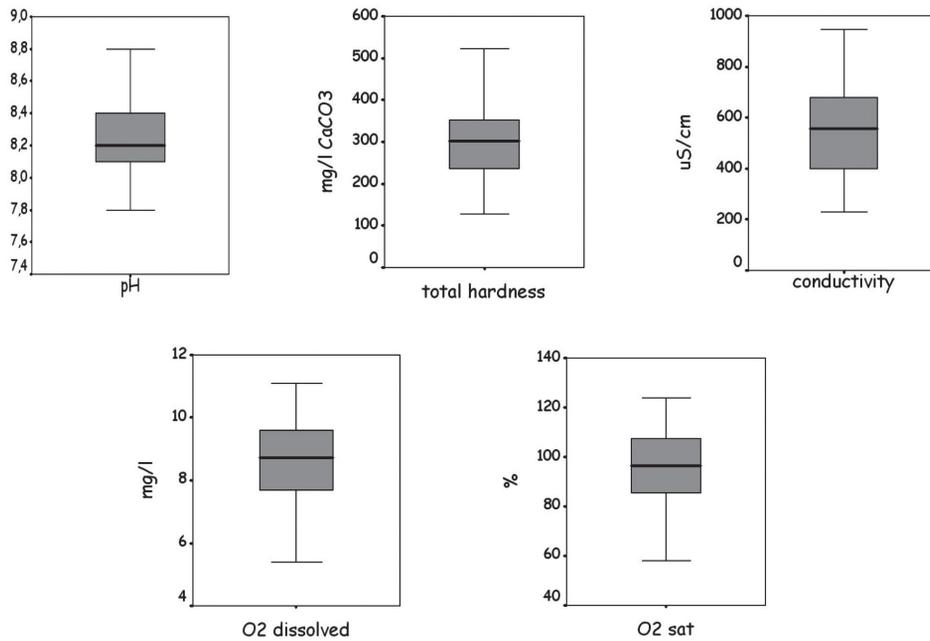


Figure 3
Boxplots of values of chemico-physical parameters measured on the sites with crayfish.

Figure 3
Boîtes à moustaches des valeurs des paramètres chimico-physiques mesurés dans les sites avec écrevisses.

Table III
Values of chemical and physical parameters in sites where *A. pallipes* complex was found.

Tableau III
Valeurs des paramètres chimico-physiques relevés dans les sites avec écrevisses.

Parameter	N	Mean	Range
Water temperature (°C)	50		11-26
pH	48	8.22	7.6-8.8
Total hardness (mg/l CaCO ₃)	50	301.6	127-545
Conductivity (µS/cm at 25°C)	48	540.8	197-945
O ₂ dissolved (mg/l)	48	8.56	4.5-15.7
O ₂ sat (%)	48	96.0	47-170

0 inhabitants/km. Only one site, at 1,366 inhabitants/km, had an urbanization ratio above 600 inhabitants/km (see Figure 4).

Results of the Mann-Whitney U test of environmental parameters for sites where *A. pallipes* complex were found vs. sites where they were not found are reported in Table IV. Only factors for which the difference was statistically significant or nearly significant are shown. Crayfish were found to be significantly more frequent in unchannelled stretches of watercourses which offer abundant shelters, and are characterized by a coarse granulometry bottom substrate and wide diversification of microhabitats (Figure 5).

It was not possible to apply the EBI protocol at 79 sites where the superficial water flow was fragmented into isolated pools; or where flow velocity was almost 0.

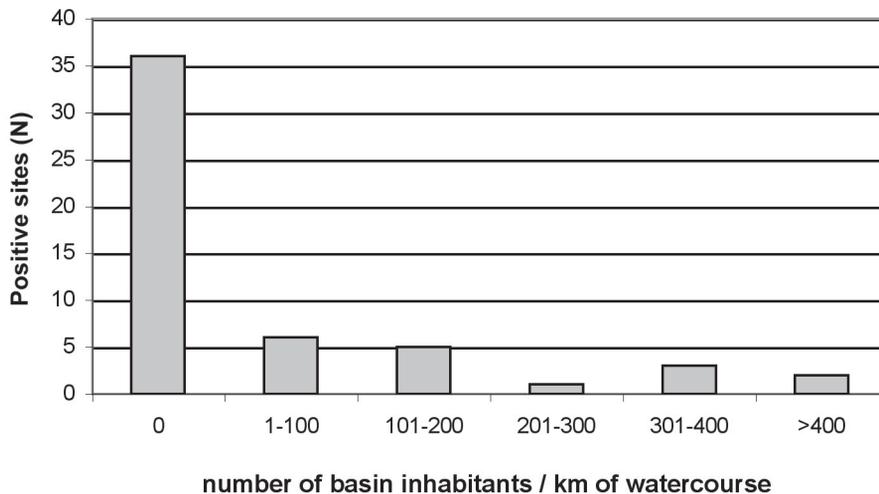


Figure 4
Level of urbanization in sites where *A. pallipes* complex was found.

Figure 4
Niveau d'urbanisation des sites avec écrevisses.

Table IV

Comparison of environmental characteristics in sites with *A. pallipes* complex vs. sites without *A. pallipes* complex (Mann-Whitney U test).

Tableau IV

Comparaison de quelques caractéristiques environnementales des sites avec ou sans écrevisses (test U de Mann-Whitney).

Variable	Z	n tot	p
Predominant granulometric class	- 3.260	170	0.001**
Abundance of shelters	- 3.188	170	0.001**
E.B.I. class	- 3.159	127	0.002**
Variety of river course	- 2.462	170	0.014*
Land use category	- 1.876	409	0.061

Nevertheless, crayfish were more frequent where the biological quality of the water was the best (Figure 6).

Comparing condition of water courses vs. land use (Figure 7) for all the sites (409), persistence of superficial water flow and the presence of isolated pools of water were found to be significantly more frequent in the most natural areas (woodlands and grasslands); whereas drying up of the water course was more frequent in agricultural and urban areas (Mann-Whitney U Test: $Z = -3.41$; $p = 0.001$).

Figure 8 shows that the latter two land uses corresponded with a significant decline in the biological quality of the water, which was the best in areas where human impact is the lowest i.e. woodlands and grasslands (Spearman correlation: $\rho = 0.56$; $n = 127$; $p = 0.001$). Most sites (77%) with crayfish populations are found in wooded areas (18 findings) and grassland (23 findings).

DISCUSSION

This comprehensive survey of the *A. pallipes* complex throughout the hydrographic system of the Alessandria province has substantially increased our knowledge of the distribution of this notoriously elusive species, which has been of little interest to man in recent years.

Although crayfish populations have been found at elevations of 600-1,000 m a.s.l. (FÜREDER *et al.*, 2003), those investigated in the present study in the province of Alessandria were found at lower elevations in the low-medium hills of the Apennines where temperatures are more consistent with the known ecological and biological needs of the species. TACHET (2002) describes *A. pallipes* as a stenothermic psychrophile with a preference for temperatures below 15°C and according to MANCINI (1986), can tolerate 25°C only for brief lapses of time. However, thirty-two percent of the crayfish populations examined in this study inhabit sites where the summer water temperature is between 21 and 25°C, with 26°C being recorded at one site. ALONSO (2001) reported average annual temperatures at sites inhabited by crayfish of 8.8 to 11.4°C, with extremes of 0.6 and 24°C. These findings suggest less strict stenothermy than was thought and a capacity to tolerate summer temperatures that stray widely from optimal values.

The data gathered for other parameters also yield values outside of some ranges found in the literature: the average water conductivity of 600-945 $\mu\text{S}/\text{cm}$ found in 34% of

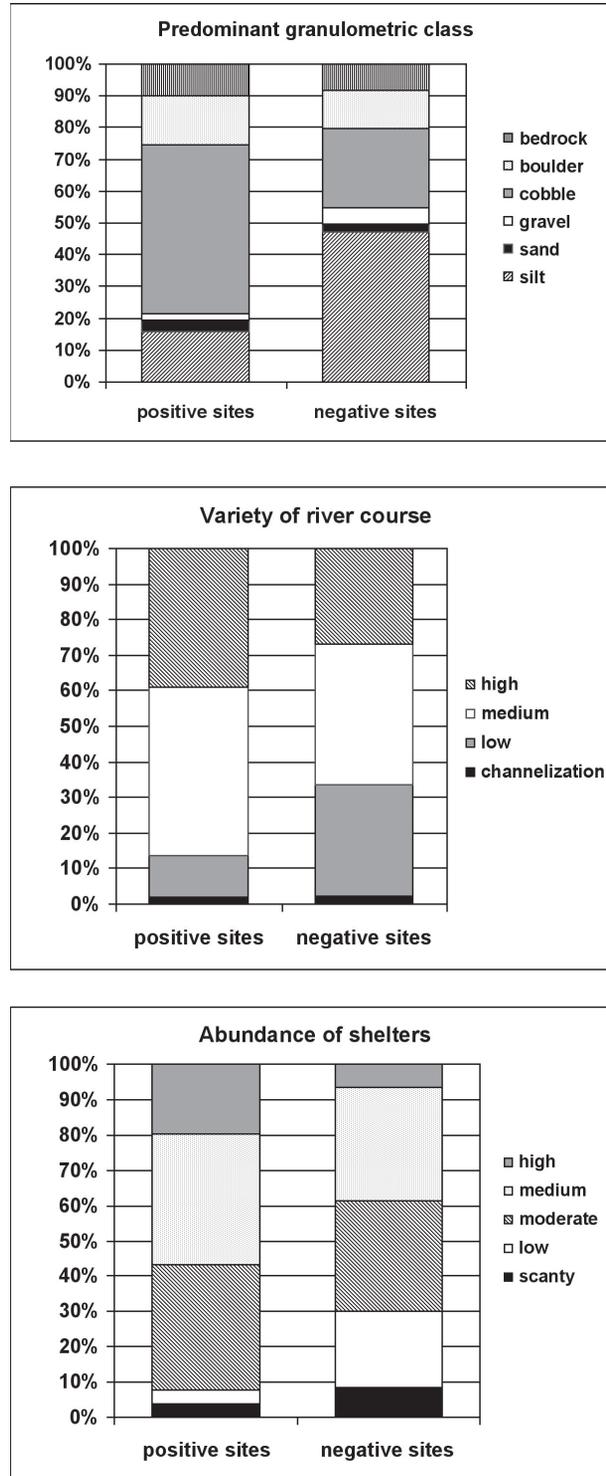


Figure 5
A. pallipes complex presence and absence according to instream habitat descriptors.

Figure 5
 Caractéristiques environnementales des sites avec ou sans écrevisses.

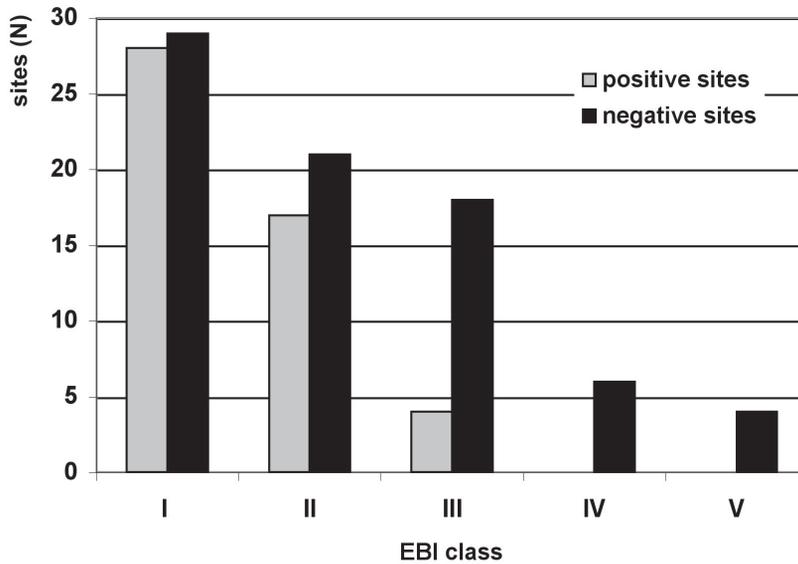


Figure 6
A. pallipes complex presence and absence according to site biological quality.

Figure 6
 Niveaux de qualité biologique (classes EBI) dans les sites avec ou sans écrevisses.

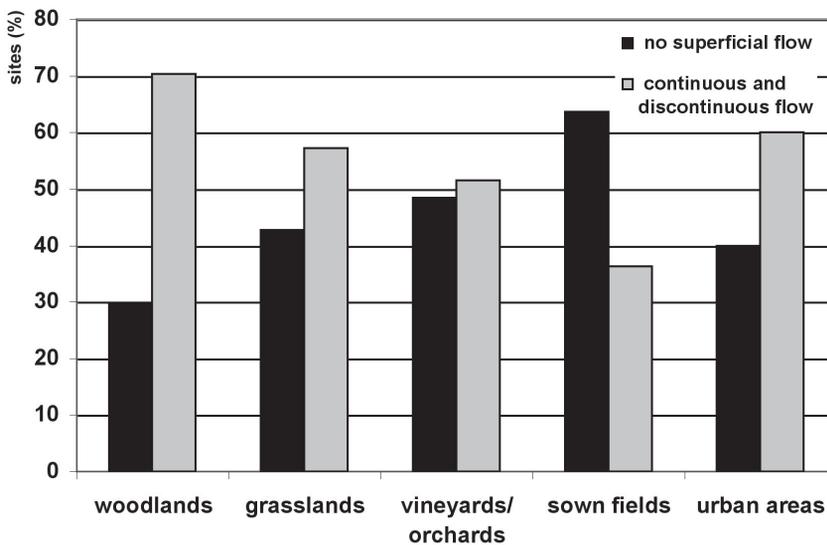


Figure 7
 Presence and absence of superficial water according to land use category.

Figure 7
 État hydrique des sites étudiés regroupés par catégories de mode d'utilisation du sol.

sites occupied by crayfish is much higher than the value of 607 $\mu\text{S}/\text{cm}$ reported by RALLO and GARCIA-ARBERAS (2002), lying within the range of 457-1,638 $\mu\text{S}/\text{cm}$ reported by ALONSO (2001). Again, RALLO and GARCIA-ARBERAS (2002) report an average water

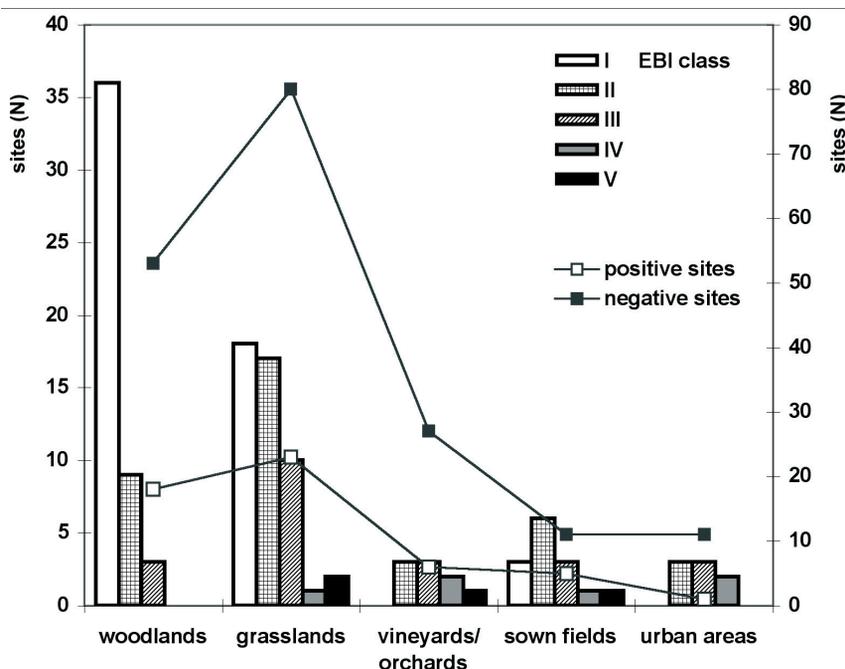


Figure 8

Bar graph: number of sites per EBI category by land use categories. Line graph: frequency of presence and absence (number of sites) of *A. pallipes* complex per land use category is shown on the secondary y-axis.

Figure 8

Niveau de qualité biologique de l'eau et présence/absence des écrevisses dans les différentes typologies d'utilisation du sol. L'axe secondaire des ordonnées (à droite) indique la fréquence (N) des sites avec ou sans écrevisses.

hardness in sites occupied by crayfish of 12.5°F with a maximum value of 20.4°F, not far off the value of 22.9°F reported by SMITH *et al.* (1996). However, 90% of crayfish-inhabited sites in the province of Alessandria had water hardness values of over 20.0°F, with a maximum value of 54.5°F being registered. TROSCHER (1997) reports an average dissolved O₂ saturation of 85% with a minimum of 56%, in this study, 24% of crayfish-inhabited sites had lower dissolved oxygen saturations, with 47% being recorded for one site.

A. pallipes complex was also found to be more versatile than is usually thought in terms of the biological quality of the water. Although most populations live in sites where water quality was high (EBI classes I and II), four populations were found in sites with an EBI classification of III. These observations agree with those of the AERC (1998) and of LYONS and KELLY-QUINN (2003). The *A. pallipes* complex thus appears to be able to tolerate some degree of deterioration in the biological quality of the water it lives in, although populations living in such sub-optimal conditions are more vulnerable to extinction.

The statistical test results, shown in Table IV, suggest that the *A. pallipes* complex prefers watercourses whose natural morphology has remained intact. Human interventions like channelling and straightening of river banks reduce the habitability of the water course for *A. pallipes* complex by simplifying it and reducing the availability of microhabitats and refuges; and result in a reduction in granulometry of the bottom substrate. The degree of

naturalness of watercourses was found to be an important requisite for crayfish, confirming findings made in France (DAGUERRE DE HUREAUX and ROQUEPLO, 1981) and in Spain (MARTÍNEZ, RICO and ALONSO, 2003).

The human impact on crayfish populations emerged clearly from the distribution pattern of crayfish-inhabited sites and human population density. Sixty-eight percent of crayfish populations were found in areas where there are no human inhabitations; and the frequency of crayfish-inhabited sites precipitated even at the lowest human population densities. Two of the effects responsible for this decline are probably a lowering of the biological quality of the water in human-inhabited areas and the availability of surface water, which is affected by land use. The high demands for water in intensive and specialized agriculture result in the drying up of watercourses in summer, when there is no rain. Forty-two and a half percent of watercourses investigated were completely dry and surface water in another 7.1% was fragmented into pools whose persistence was uncertain.

CONCLUSIONS

A substantial contraction of the distribution range of *A. pallipes* complex in the Alessandria province of Italy is indicated by the fact that only one of 18 previous reports was confirmed in this study. The present distribution pattern may be the outcome of the recently adopted practises of recalibrating watercourse sections and of dredging, and of an increase in the catchment of water sources to supply demands from new types of crop cultivation and increasing tourist urbanization in hill and mountain areas.

The results of this study on the one hand suggest that further investigation of the habitat requirements of *A. pallipes* complex is necessary, since the crayfish was absent from a large number of sites where environmental indices reflected what are thought to be favourable conditions. On the other, the *A. pallipes* complex appears to have a wider ecological niche than is generally thought, putting in question the meaningfulness of the bioindicators for this species.

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