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Knowledge and Management of Aquatic Ecosystems (2011) 401, 24 http://www.kmae-journal.org

DOI: 10.1051/kmae/2011041

The indigenous crayfish *Austropotamobius pallipes* complex in a national park of Central Italy

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Received January 11, 2011
Revised April 27, 2011
Accepted May 6, 2011

ABSTRACT

Key-words: *indigenous crayfish, protected areas, monitoring, management, Central Italy*

The indigenous crayfish *Austropotamobius pallipes* complex has been recently defined by IUCN as an endangered species but our knowledge about its status in Italy is still provisional. An assessment of the most suitable environments for its survival is crucial to preserve the species and to develop appropriate conservation protocols for its management. To this end, during 2008 and 2009, we analyzed eight watercourses in a protected area of Central Italy for *A. pallipes*' presence and for a number of environmental characteristics. Crayfish were found in four out of the eight analyzed watercourses: only one of three old reports was confirmed, while the species has disappeared from the other two. All the streams are characterized by good quality of both water and soil. The differences found for basin and riparian descriptors, canopy cover, shelters and substrate composition were independent of the crayfish presence. Non-indigenous crayfish populations were not recorded in the study area. Among the several causes of crayfish disappearance, overexploitation through illegal fishing, introduction of fish predators and drought seem to be the more likely. These threats should be urgently faced to guarantee the survival of the indigenous crayfish.

RÉSUMÉ

L'écrevisse indigène *Austropotamobius pallipes* complexe dans un parc national de l'Italie centrale

Mots-clés : *écrevisse indigène, zone protégée, évaluation des environnements, surveillance, Italie centrale*

L'écrevisse indigène, complexe *Austropotamobius pallipes,* est menacée, mais nos connaissances sur son statut en Italie sont encore limitées. L'évaluation des environnements les plus appropriés pour sa survie est essentielle pour le maintien de cette écrevisse ainsi que l'élaboration de protocoles de conservation appropriés pour sa gestion. À cette fin, cette étude vise à analyser les caractéristiques abiotiques et biotiques de huit cours d'eaux, la distribution des espèces et la présence d'écrevisses non indigènes dans une zone protégée de l'Italie centrale en 2008–2009. Dans trois d'entre eux, les recherches précédentes avaient montré la présence de l'espèce. Les écrevisses ont été trouvées dans quatre des huit sites analysés, mais des trois anciennes localisations, une seule a été confirmée,

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alors que l'espèce semble avoir disparu des deux autres. Tous les cours d'eau sont caractérisés par une bonne qualité de l'eau et du sol et les différences enregistrées pour les descripteurs de bassin et de rives, le couvert et la composition du substrat étaient indépendants de la présence de l'écrevisse. Des écrevisses non indigènes n'ont jamais été rencontrées dans la zone d'étude. Parmi les causes de la disparition des écrevisses, la sécheresse, la surexploitation par la pêche illégale et l'introduction de poissons prédateurs semblent être les plus probables. Pour protéger la survie de l'écrevisse indigène, ces menaces doivent être prises en compte.

INTRODUCTION

The white-clawed crayfish, *Austropotamobius pallipes* complex, the most widespread indigenous crayfish species in Italy (Aquiloni *et al*., 2010), is at serious risk of extinction due to the increasing number of threats to the integrity of its populations, such as habitat destruction and alteration, pollution, introduction of non-indigenous species, overexploitation, and climate change (Füreder *et al*., 2002; Nardi *et al*., 2005; Gherardi, 2006; Renai *et al*., 2006).

As a result of this alarming situation, *A. pallipes* is included in the Appendix III of the Bern Convention and in the Annexes II and IV of the European Community Directives for the Conservation of Natural Habitats and Wild Flora and Fauna (92/43/EEC and 97/62/EU). In the Red List of Threatened Animals of the International Union for the Conservation of Nature (IUCN) 2010, its status has been moved from vulnerable to endangered (Füreder *et al*., 2010). In Tuscany and Emilia-Romagna regions of Central Italy, this species is protected by the regional laws n° 56/2000 and 15/2006, respectively.

Action plans have been recently undertaken to conserve threatened populations of this crayfish species in most European countries (*e.g.* Holdich and Rogers, 1997; Reynolds, 1997). Updating the current distribution of *A. pallipes* and assessing habitat suitability for its survival are both necessary actions that might help identify the causes of its local extinctions and develop appropriate conservation protocols for its management (Trouilhé *et al*., 2007).

This study aims at (1) investigating the distribution of *A. pallipes* complex in eight watercourses of a protected area in Central Italy (National Park of the "Foreste Casentinesi, Monte Falterona e Campigna"), (2) quantifying the relationships between the crayfish presence/absence and some environmental characteristics and (3) disclosing the possible local threats to the integrity of its populations.

MATERIALS AND METHODS

> *STUDY SITES*

The National Park of the "Foreste Casentinesi, Monte Falterona, Campigna" is a *ca*. 36 000 ha protected area in the Tuscan-Emilian Apennines. This area includes one of the most high-quality forested areas in Europe and a variety of animal and plant species of great scientific interest.

The study was carried out in eight watercourses: Arno (A), Acquacheta (AC), B. Campigna (BC), B. Pietrapazza (BP), Corsalone (C), Fiumicello (F), Lama (L) and Oia (O) (Figure 1). Following the protocol of Renai *et al*. (2006), we conducted three samplings, from June to October, for each sites during 2008–2009. We collected data on several parameters along two 10-m longitudinal transects per stream. Transects were chosen so that all the different habitats of the streams (*e.g.* pool and riffles) could be represented in the analysis. Each watercourse was associated with a list of threats to the integrity of crayfish populations (*e.g.* illegal harvesting, introduction of fish, and predators) assessed on a qualitative basis. Information were obtained from grey literature, personal observations and interviews with managers operating in the area (*e.g.* Corpo Forestale dello Stato and Polizia Provinciale).

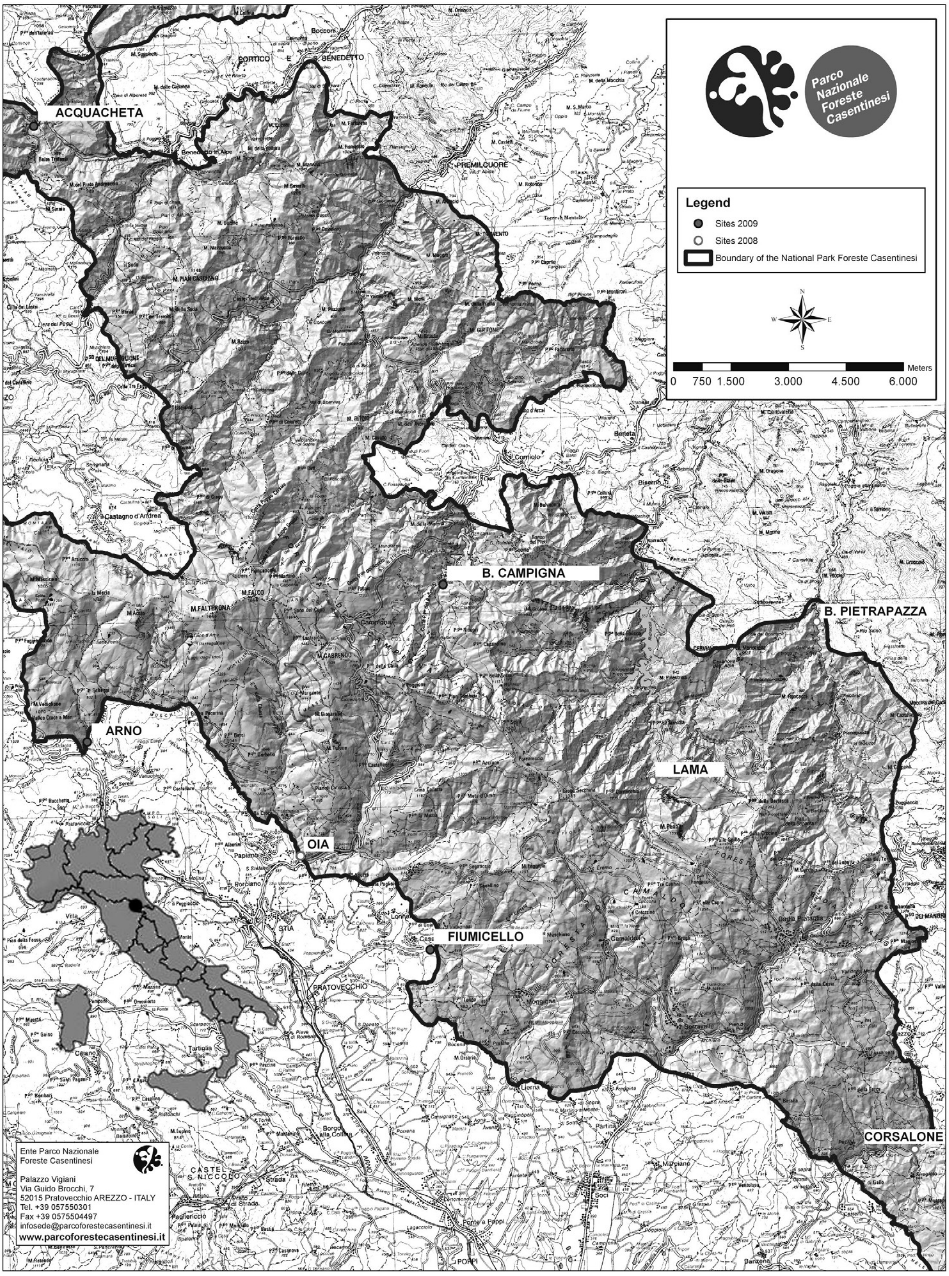

Figure 1
Map of the study sites in the National Park of the "Foreste Casentinesi, Monte Falterona and Campigna".

Figure 1
Carte des sites de l'étude dans le parc national de « Foreste Casentinesi, Monte Falterona et Campigna ».

> PHYSICO-CHEMICAL PARAMETERS

We measured air and water temperatures using a thermometer and pH, dissolved oxygen concentration (O_2), ammonium (NH_4^+), calcium (Ca^{2+}), nitrite (NO_2^-), and nitrate (NO_3^-) using colorimetric methods (Aquamerk®, Darmstadt, Germany). The width of each stream and the maximum water depth were measured with a meter stick at the beginning, middle, and end of each transect.

> *BASIN AND RIPARIAN DESCRIPTORS*

Canopy cover. Three photos of the canopy were taken by the ground to the sky at the beginning, middle, and end of each transect, using a digital camera (35 mm objective). Each photo was then transformed in an 8-bit image to evaluate the ratio between light and dark pixels using the software ImageJ 1.32 (Wayne Rasband, National Institute of Health, USA). Thus, we obtained three values of canopy cover for each transect.

Channel substratum and availability of vegetal matter. The analysis was conducted at the beginning of the study. A plastic frame was used to enclose an area of 1×1 m divided into 16 squares. The frame was launched five times for each transect. Inside each square, we visually estimated the percentage of the area covered by: silt ($<$ 0.07 mm diameter), sand (0.07–2 mm), gravel (2–64 mm), cobble (65–256 mm), boulder ($>$ 256 mm), and the availability of organic matter.

Shelter availability. The analysis was conducted at the beginning of the study. We measured the density of natural crevices (*e.g.* number of holes$\cdot$m^{-1}) and the portion of the river bank (in cm) covered by roots, organic debris, mud and rocks along a total of six 1-m long segments randomly chosen on the bank of each transect.

> *ITALIAN EXTENDED BIOTIC INDEX (IBE)*

The analysis was conducted three times for each stream. We used the Italian extended biotic index (IBE) (Ghetti, 1997), a qualitative method based on the occurrence of macroinvertebrates to assess stream quality. Macroinvertebrates were collected using a sampling net (25 cm $\times$ 40 cm with 20 mesh$\cdot$cm^{-2}). Streams were dragged from one bank to the other covering all types of microhabitat. Macroinvertebrates were preserved in vials containing 70% ethanol; in the laboratory, they were identified at the taxonomic level requested by the index (*e.g.* genus for mayflies and stoneflies; family for caddisflies and coleopterans), following the identification keys provided by Campaioli *et al*. (1994), Sansoni (1998) and Campaioli *et al*. (1999). IBE values, ranging between 0 and 14, are obtained from a double entry table. This index reflects water quality on a scale of 1 to 5, where 1 indicates optimal water quality and 5 polluted watercourses.

> *FLUVIAL FUNCTIONALITY INDEX (IFF)*

IFF (Anpa, 2003) integrates the results of the analyses carried out at a micro- (*e.g.*, IBE) and a macroscale (*e.g.* land use). In this way, the fluvial environment may be appraised at a wider scale. The value of IFF ranging from I (the highest river functionality) to V (the lowest river functionality) is obtained by adding the partial scores assigned to each of 14 questions. In the present study, IFF was calculated one time along one transect of 150 m per stream during the period of maximum vegetation (June–July).

> *SOIL BIOLOGY QUALITY INDEX (QBS-AR)*

The analysis was conducted once per stream. This qualitative index (Parisi, 2001) is based on the whole microarthropods community present in a soil sample. In this study, four cubic soil samples (side: 10 cm) were extracted at a distance of 1.5 m from the bank of the streams. Samples were transported to the laboratory and kept in the Berlese–Tullgren funnel extractors under 40 W lamps for seven days. A bottle filled with fixative liquid (75% ethanol and 25% glycerol) was placed under the funnel to collect the microarthropods. The specimens were then observed under a stereomicroscope and identified at the level requested by the index. According to the specimen adaptation to soil environment, a score from 1 to 20 (eco-morphological index, EMI) was assigned to the analyzed individuals. The QBS-ar index results from the sum of these scores within a scale of seven soil quality classes, where higher values correspond to more complex and soil-adapted microarthropods communities.

> CRAYFISH POPULATIONS

The size and sex ratio of populations of *A. pallipes* complex were analyzed using the method of "catch per unit effort" (CPUE). Night-time searching (1 h) was conducted during the period of the species' maximum activity (summer). Crayfish were searched by hand in all possible refuges: stones and leaf litter on the bottom of watercourses, and holes along the river banks. The sex of each captured animal was determined in the field and its total length (TL, from the tip of the rostrum to the tip of the telson) was measured using a digital caliper (accuracy: $\pm$ 0.02 mm). Crayfish were then released at the same place of collection. The presence of non-indigenous crayfish was also investigated in the study sites using the same sampling method.

> DATA ANALYSES

Data were first checked for normality and homogeneity of variance using Kolgomorov–Smirnov and Levene tests, respectively. If these assumptions were not met, appropriate non-parametric tests were applied. Watercourses were compared for their canopy cover, substrate composition and density of shelters by the non-parametric Kruskall–Wallis analyses of variance (statistic: H), followed by multiple comparisons tests. TL of crayfish was compared among streams by the non-parametric Kruskall–Wallis analyses of variance (statistic: H). Frequency data were analyzed by G test with Williams correction. Principal component analysis (PCA) was used to explore differences among the streams for their abiotic characteristics. PERMANOVA (permutation multivariate analysis of variance) was then applied to test differences among streams (statistic: pseudo-F). The similarity matrix was computed using the Euclidean distance. All analyses were based on 999 permutations of residual under a reduced model and Type III sum of squares. Multi-dimensional scaling ordination (MDS) was carried out to explore differences in the community structure of macroinvertebrates among streams and PERMANOVA was used to test differences among streams. The similarity matrix of biotic data was constructed using Jaccard similarity measures. As above, all analyses were based on 999 permutations of residual under a reduced model and Type III sum of squares. The level of significance at which the null hypothesis was rejected is α = 0.05. Multivariate analyses were performed using PRIMER v. 6.1 (Clarke and Gorley, 2006) and PERMANOVA+ for PRIMER routines (Anderson *et al*., 2008).

RESULTS

Crayfish presence was independent of the basin and riparian descriptors (Table I), canopy cover ($H = 35.78$, df = 7, $P < 0.0001$; after multiple comparisons tests: O = L = A = AC = F = BC < BP = C), substrate composition (Table II), and density of potential shelters (density of crevices: $H = 24.23$, df = 7, $P = 0.001$ after multiple comparisons tests: BP < O = L = A = AC = F = C < BC; portion of the river banks covered by roots: $H = 14.17$, df = 7, $P = 0.048$ after multiple comparisons tests: BC < BP = O = A = AC = F = C < L). All the streams are characterized by a good water and soil quality, as indicated by the high values of IBE (1–2), IFF (I–II), and QBS-ar index (3–6) (Table III). Crayfish were present in four out of the eight analyzed watercourses (BC, C, F and L). Crayfish had been previously reported only in L, while in BP and O the old reports were not confirmed.

The differences found among the analyzed watercourses for both abiotic and biotic features (abiotic data: pseudo-F = 2.66, df = 7, P = 0.001; biotic data: pseudo-F = 2.89, df = 7, P = 0.001) were independent of the crayfish presence (Figures 2 and 3).

Table IV reports a list of the potential threats to crayfish for each watercourse. Sites did not differ for the number of potential threats (G = 10.34, df = 7, $P > 0.1$).

CPUE is high in C and BC (84 and 122 individuals captured per hour, respectively), while it is low for L (43 individuals per hour) and F (only 4 individuals per hour). No bias in sex ratio was

Table I
Median value, first (Q1) and third (Q3) quartiles of basin and riparian descriptors measured on each analyzed watercourse. Arno (A), Acquacheta (AC), B. Campigna (BC), B. Pietrapazza (BP), Corsalone (C), Fiumicello (F), Lama (L) and Oia (O).

Tableau I
Valeurs des médianes, premier (Q1) et troisième (Q3) quartiles des descripteurs des bassins et des berges mesurés sur chaque rivière étudiée. Arno (A), Acquacheta (AC), B. Campigna (BC), B. Pietrapazza (BP), Corsalone (C), Fiumicello (F), Lama (L) et Oia (O).

	A	AC	BC	BP	C	F	L	O
Canopy cover								
Median	49.5	60.6	73.4	104	109.5	62.7	48	44
Q1	45.7	55.50	64.06	103.25	97.75	52.46	45.5	42
Q3	53.6	62.55	76.79	107.75	152.75	72.39	49.75	46
Substrate composition								
Boulder								
Median	54.69	11.25	27.19	31.25	60.63	46.3	0.63	65.63
Q1	48.93	8.36	25.31	18.99	53.13	36.3	0	47.81
Q3	70.16	17.97	33.98	64.06	74.85	79.7	7.81	86.25
Cobble								
Median	24.69	79.06	68.12	68.59	30.00	36.4	43.91	23.12
Q1	21.47	62.73	58.99	35.94	16.72	2.58	35.78	8.52
Q3	29.455	84.92	74.22	71.10	44.69	49.9	49.37	36.72
Gravel								
Median	6.69	4.69	2.65	0	0	8.29	43.91	0
Q1	1.33	2.81	0.16	0	0	0.31	38.12	0
Q3	16.22	5.31	10.31	0	0	18.7	49.37	3.75
Silt								
Median	0	0	0	0	0	0	0	0
Q1	0	0	0	0	0	0	0	0
Q3	0	0	0	0	0	0	0	0
Organic matter								
Median	9.37	0	0	31.25	0	6.25	100	0
Q1	0	0	0	25	0	1.56	95.31	0
Q3	18.75	0	0	64.06	18.75	12.5	100	100
Sand								
Median	0.78	0.94	0	0	0	0	0	3.44
Q1	0	0	0	0	0	0	0	0.16
Q3	13.84	7.89	0	0	0	0	22.5	14.77
Shelters availability								
Holes								
Median	3	4.5	6	0.5	5	4.5	0	2.5
Q1	2	3.75	5	0	3	3	0	1
Q3	4.25	6.25	8	3	6.25	5.25	6.75	5
Roots								
Median	2.5	0	0	0	0	0	2.5	5
Q1	0	0	0	0	0	0	0	0
Q3	10	8.75	0	32.5	0	0	77.5	22.5

Table II
Substrate composition compared among the analyzed watercourses after Kruskall–Wallis tests (statistic: H), followed by multiple comparisons tests. Significant differences are denoted in bold. See Table I for the correspondence between abbreviations and streams.

Tableau II
Composition du substrat comparée entre les rivières analysées par test de Kruskall-Wallis (statistique : *H*), suivi d'un test de comparaisons multiples. Les différences significatives sont indiquées en gras. Voir le tableau I pour la signification des abréviations.

	H	df	*P*		Hierarchy
Boulder	47.25	7	**< 0.0001**		**L = AC < BC < BP = F = A < C = O**
Cobble	37.69	7	**< 0.0001**		**O < A = F = C = L < BP < BC < AC**
Silt	12.47	7	0.09		not significant
Organic matter	41.26	7	**< 0.0001**		**BC = AC < F < C = A < O < BP < L**
Sand	27.86	7	**< 0.0001**		**BC = F = C = BP < AC = A < L = O**
Gravel	43.38	7	**< 0.0001**		**C = BP < O = BC = AC = A < F < L**

Table III
Value (mean ± SE) of physico-chemical parameters measured on each analyzed watercourse. The values of IBE, IFF and QBS-ar are also reported. See Table I for the correspondence between abbreviations and streams.

Tableau III
Valeurs (moyennes ± SE) des paramètres physico-chimiques de chaque rivière étudiée. Les valeurs des paramètres IBE, IFF et QBS sont aussi indiquées. Voir le tableau I pour la signification des abréviations.

	A	AC	BC	BP	C	F	L	O
Air temperature	16.67 ± 1.67	19.33 ± 3.84	21.67 ± 2.67	17.33 ± 2.33	22.33 ± 3.53	22.50 ± 1.32	17 ± 3	17.67 ± 1.33
Water temperature	12 ± 0.58	17 ± 4.04	15.67 ± 1.20	14.33 ± 2.73	14 ± 1	15.33 ± 1.45	12.67 ± 1.73	13 ± 1
pH	8	8.17 ± 0.17	8.33 ± 0.17	8.33 ± 0.17	8.17 ± 0.17	8.08 ± 0.08	7.83 ± 0.17	8.33 ± 0.17
O_2	8.90 ± 1.55	6.70 ± 1.51	6.27 ± 1.79	6.03 ± 1.03	6.60 ± 0.60	7.17 ± 0.69	5.77 ± 1.86	6.17 ± 1.69
NH_4^+	0.05 ± 0.03	0.02 ± 0.02	0.07 ± 0.02	0.03 ± 0.02	0.03 ± 0.02	0.05	0.05 ± 0.05	0.07 ± 0.02
Ca^{2+}	46.67 ± 6.67	84.33 ± 6.17	74 ± 4.16	70.67 ± 3.71	94.67 ± 4.37	102 ± 9.45	62.67 ± 5.46	60 ± 5.77
NO_2^-	0	0	0	0	0.02 ± 0.02	0	0.02 ± 0.02	0
NO_3^-	5.83 ± 5.83	3.33 ± 3.33	0	13.33 ± 4.17	15 ± 2.50	5.83 ± 5.83	12.50 ± 2.50	17.50 ± 4.33
Width of each stream	5.13 ± 0.55	5.78 ± 1.01	4.09 ± 0.34	4.50 ± 0.29	7.77 ± 0.09	2 ± 0.32	6.30 ± 0.70	7.27 ± 0.15
Maximum water depth	19.24 ± 4.67	8 ± 3.12	14.39 ± 2.14	11 ± 1.73	13.50 ± 1.32	10.72 ± 3.67	13.50 ± 1.44	16.67 ± 2.33
IBE	1-1-1	1-1-2	1-1-1	1-1-1	1-1-1	1-1-1	1-1-1	1-1-1
IFF	I	I	I	II	I	I	I	I
QBS	4	5	6	5	3	5	3	5

found for any population (C: m = 53, f = 73, G = 3.17, df = 1, $P > 0.05$; L: m = 34, f = 31, G = 0.14, df = 1, $P > 0.1$; BC: m = 92, f = 92, G = 0, df = 1, $P > 0.1$; F: m = 2, f = 2, G not computed). Differences among watercourses were found for TL (Table V) (females: H = 46.87, df = 2, P = 0.0001; males: H = 68.48, df = 2, P = 0.0001), without any difference between sites subject or not to poaching. Non-indigenous crayfish were not found in any site.

DISCUSSION

Our study shows that populations of *A. pallipes* complex are present in four out of the eight analyzed sites, but only in one of the three old reports (Lama) its presence has been confirmed. Compared to other crayfish populations in Tuscany (Brusconi *et al*., 2008), CPUE is high in Corsalone and B. Campigna, but it is low in Lama and Fiumicello.

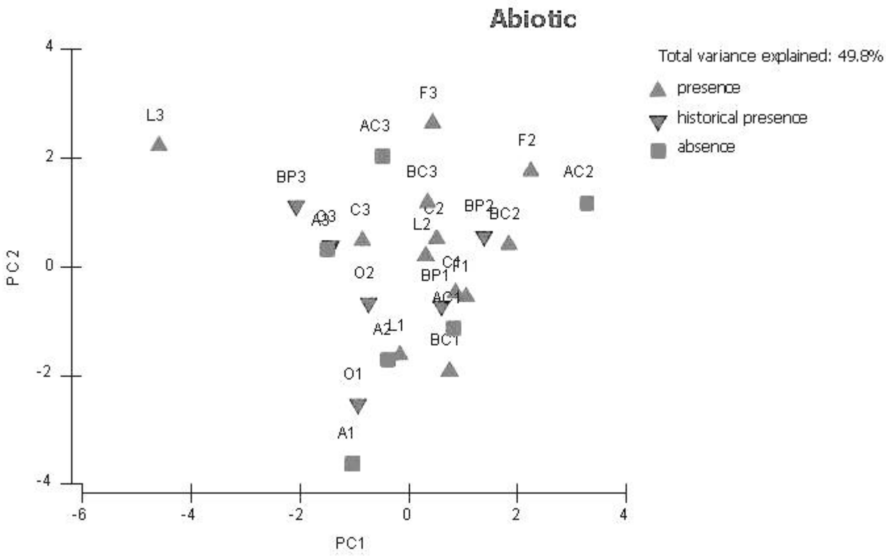

Figure 2
Two-dimensional scatter plot of the first and second principal components of abiotic characteristics of the study sites. Data labels indicate: location, samplings, and crayfish distribution.

Figure 2
Graphe des première et seconde composantes principales des caractéristiques abiotiques des sites étudiés.

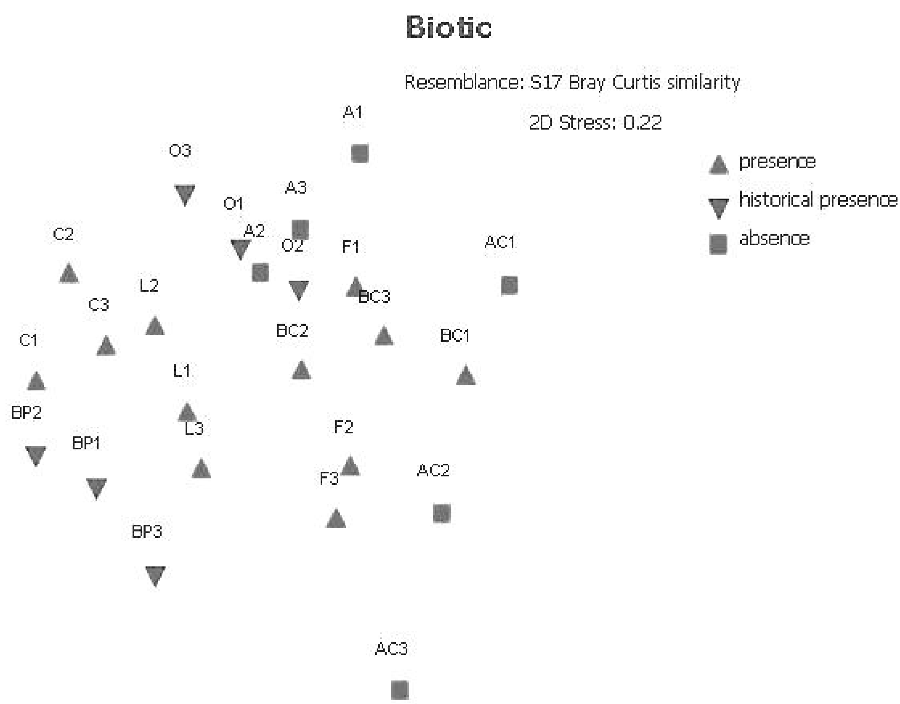

Figure 3
Non-metric multidimensional scaling ordination (MDS) of the community structure of macroinvertebrates of the study sites. Data labels indicate: location, samplings, and crayfish distribution.

Figure 3
Ordination par cadrage non-métrique multidimensionnel (MDS) de la structure des communautés de macroinvertébrés dans les sites d'étude.

Table IV
Comparison among the analyzed watercourses for the threats to which indigenous crayfish populations are or may be subject. Threats are assessed on the basis of their presence (denoted with 1) and absence (denoted with 0). UN = unknown. See Table I for the correspondence between abbreviations and streams.

Tableau IV
Liste pour les rivières étudiées des menaces auxquelles les populations d'écrevisses indigènes sont ou non soumises. La présence d'une menace est notée 1, son absence 0 et UN pour non connue. Voir le tableau I pour la signification des abréviations.

	A	AC	BP	O	BC	C	F	L
Indigenous crayfish population (present, absent, past)	absent	absent	past	past	present	present	present	present
Non-indigenous crayfish population	0	0	0	0	0	0	0	0
River crab population	0	0	0	0	0	1	0	0
Sporadic pollution from domestic discharges and farms	1	0	1	1	1	1	1	0
Events of total drought in the last 20 years	0	1	0	0	0	0	1	0
Abstraction for domestic supply, irrigation or stock-watering	1	0	UN	1	UN	1	1	0
Introduction of fish predators or restocking	1	0	1	1	1	1	1	0
Fish predators already present (*e.g. Salmo* (*trutta*) *trutta* Linnaeus, 1758)	1	1	1	1	1	1	1	1
Fragmentation of watercourses by artificial barriers (*e.g.* dikes)	1	0	1	1	1	1	1	1
Presence of settlements within a range of 3 km as the crow flies	1	0	1	1	1	1	1	0
Easily practicable tracks or roads	1	0	1	1	1	1	1	0
Illegal harvesting	0	0	1	1	UN	1	1	0
Number of threats	7	2	7	8	6	9	9	2

Table V
Median value, first (Q1) and third (Q3) quartiles of the total length of crayfish, distinguished per sex, of the three analyzed watercourses hosting an A. pallipes *complex population (L = Lama, C = Corsalone and BC = B. Campigna).*

Tableau V
Valeurs des médianes, premier (Q1) et troisième (Q3) quartiles de la longueur totale des écrevisses pour chaque sexe des trois rivières étudiées hébergeant le complexe *A. pallipes* (L = Lama, C = Corsalone et BC = B. Campigna).

	L	C	BC
Females			
Median	38.13	54.86	77.20
Q1	35.67	42.52	70.00
Q3	74.79	61.13	82.03
Males			
Median	48.58	54.13	83.08
Q1	38.20	44.00	75.91
Q3	71.25	59.84	88.73

Interestingly, the number of crayfish captured in Lama (43) is similar to the number (48) captured in an hour during a sampling conducted in 1999 in the same period of the year (Cenni, 2001) using the same method. The persistence of *A. pallipes* complex in Lama is probably due to the few threats to crayfish population integrity (see Table IV), including the low accessibility of this site to people: this watercourse is in fact located in the centre of the park and is reached by a few uneven roads. On the contrary, Oia and B. Pietrapazza are very close to human settlements, being thus more subject to overexploitation, habitat destruction, and pollution. However, there is no difference between watercourses with or without crayfish for all the recorded parameters as already reported in Renai *et al*. (2006). Besides, the sites without crayfish have similar physico-chemical values as those required by the species (Holdich, 2003), along with high values of IBE, IFF and QBS. PCAs and MDSs did not discriminate among watercourses with, without and with historical presence of *A. pallipes* complex. As previously shown by Renai *et al*. (2006), the characteristics of all the analyzed streams were favorable to the survival of the species.

All the study watercourses (with and without crayfish) are subject to several threats to crayfish population integrity. Obviously, most of them are difficult to quantify due to the scarcity of data about, for example, rainfall, illegal fishing, fish restocking. Our assessment has been thus simply qualitative.

Excluding non-indigenous crayfish species (absent from the study sites), and water overabstraction and sporadic events of chemical pollution from domestic discharges (both difficult to monitor), overexploitation through illegal fishing, introduction of fish predators and habitat alteration constitute the most serious threats to crayfish populations in the study area.

In part, local extinction of *A. pallipes* complex can be ascribed to illegal fishing. Indeed, in Tuscany poaching is one of the main causes of the disappearance of *A. pallipes* complex (Renai *et al*., 2006). Fishing, in fact, induces a drastic decrease in the population size; if a population is already at low density due to other human-induced threats (Scalici and Gibertini, 2005), its overexploitation might further reduce genetic diversity (Bertocchi *et al*., 2008), thus increasing the vulnerability to both environmental stress and random events. There are today strict regulations that limit crayfishing, but in some areas poaching is part of cultural traditions, as reported in small villages inside the park or at its boundaries also for other species of conservation concern (*e.g.* the river crab *Potamon fluviatile*). Events of illegal fishing have been recorded in some surveyed sites, also in the watercourses where crayfish are still present. Size distribution in the populations subject to poaching seems not to be skewed toward the smaller size classes, as expected since poachers prefer large individuals. The frequency of smaller size classes in Corsalone and Lama with respect to B. Campigna may be due to an intrinsic populations difference or to a different intensity of poaching through the years.

The introduction of fish predators (Englund, 1999) is another threat to crayfish: a large part of these stream, as other streams in the park (Baratti *et al*., 2006), have been subject to fish restocking in the last decade, thus predator pressure should have been increased.

The river crab *P. fluviatile* is able to out-compete *A. pallipes* (Barbaresi and Gherardi, 1997). However, in the study area it is only present in the stream where crayfish are abundant, although in different areas, with crayfish occupying the upper part and crabs the lower part of the same stream. Further studies are necessary to understand whether this is the result of competition between species.

Finally, drought may have lead some *A. pallipes* complex populations to local extinctions, as previously recorded in other watercourses of Tuscany (Renai *et al*., 2006). Indeed, during last summers (*e.g.* 2003) many watercourses were dried out for months with the consequent reduction in suitable habitats. The reduced water availability is not only associated with climate change, but it is also due to water abstraction for irrigation and domestic purposes (as reported in Fiumicello; G. Mazza, pers. com.). Fragmentation of watercourses due to dikes, as in the stream Oia, is an additional cause of reproductive isolation of crayfish in sub-populations, with the consequent reduced genetic diversity.

The local extinction of *A. pallipes* complex is certainly due to the combination of the above mentioned threats. To be effective, any attempt to manage crayfish should quantify each threat and remove all of them or at least mitigate their impact. Management must be carried out following guidelines based on the exhaustive knowledge of crayfish biology and ecology that have been acquired by the several research groups in the latest years. We are confident that only through a constant and constructive exchange between management and scientific research will we be able to identify the problems and to find appropriate solutions. Restoring aquatic habitats, reintroducing indigenous crayfish, preventing the introduction of non-indigenous species and implementing legislation are all actions required with urgency in order to guarantee the survival of indigenous crayfish (Holdich *et al*., 2009). Finally, particular attention should be paid to inform and educate non-specialists and raise awareness in them, particularly for what concerns illegal fishing, since the success of any action may be ensured only by the continuous support and participation of the public at large (Gherardi, 2010).

ACKNOWLEDGEMENTS

We thank G. Cusseau for the French translation and L. Sacchini, A. Bottaci, A. Zoccola, N. Siemoni, M. Lucchesi, F. Cianferoni, F. Zinetti, A. Fani, M. Magherini, A. Gennai, G. Crudele, V. Gonnelli, A. Cini and R. Cervo for their precious help during this research. Thanks are also due to S. Cannicci and G. Santini for statistical suggestions. This study is part of a scholarship funded by the National Park of the "Foreste Casentinesi, Monte Falterona e Campigna", entitled "Conservation of species and habitats in the wetlands of the National Park".

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