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Conserving indigenous crayfish: stock assessment and habitat requirements in the threatened Austropotamobius italicus

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ABSTRACT

1. As part of the *Austropotamobius pallipes* species complex, the crayfish *Austropotamobius italicus* is a species of community interest whose preservation requires the designation of Special Areas of Conservation (SACs) (Annex II, EU Habitats Directive). This study aimed at (1) assessing the conservation status of this threatened indigenous species by stock assessment in Central Italy and (2) identifying some aspects of its elective habitat.

2. Surveys were conducted in nine streams harbouring *A. italicus* (streams WI) and in 10 streams where crayfish populations became extinct at least 5 years before the study (streams WO).

3. The results confirmed that *A. italicus* is a K-selected species, with a relatively slow growth rate (males: 0.34; females: 0.37) and a long life expectancy (males: 8.2 years, females: 7.8 years). The extant populations are healthy, showing balanced sex-ratios and well structured age-class compositions. Mortality is mainly due to fishing, which is illegal in Tuscany.

4. Principal Components Analyses showed that the streams WI and WO differ in the abundance of allochthonous plant detritus but not in the taxonomic composition of their macroinvertebrate communities. Age classes were found to be spatially segregated, juveniles mainly using cobbles as substrates and adults seemingly avoiding them.

5. The loss of the pristine riverine landscape seems to have been responsible, together with illegal fishing, for the local extinction of the species. As a consequence, retaining, enhancing, and restoring the habitat and its complexity are required for the preservation of *A. italicus*.

6. The designation of SACs might help in this endeavour if accompanied by programmes aimed at publicizing the need for conservation of this species. Unfortunately, crayfish-focused projects supported by LIFE in Italy since 1992 (4%) and the SACs involved (1.4%) have been relatively few, despite the poor conservation status of this species and its well recognized ecological role.

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KEY WORDS: population size; population structure; habitat requirements; crayfish; *Austropotamobius italicus*; threatened species; SACs

INTRODUCTION

As a consequence of several human activities (e.g. the introduction of non-indigenous species, deforestation, habitat fragmentation, and water quality deterioration; Gherardi *et al*., 2002b), crayfish are among the most imperilled taxa in freshwater systems (Usio, 2007). Of more than 540 crayfish species in the world (Holdich, 2002), only five indigenous species occur in Europe (Souty-Grosset *et al*., 2006). Among them, the genus *Austropotamobius* is widely distributed across western and central European countries, from Spain in the west and the British Isles in the north, to Italy and the Balkans in the south and east (Machino and Holdich, 2006).

In spite of the results of many genetic studies (Grandjean *et al*., 1998, 2002a,b; Fratini *et al*., 2005; Trontelj *et al*., 2005), the taxonomy of this genus, specifically of the *A. pallipes* species complex, is still controversial. Morphological analyses provide ambiguous phylogenetic evidence (Grandjean *et al*., 1998). Avise and Ball (1990) recommend that morphological, biological, and phylogenetic data should be considered together before making a decision about the specific status of a group. Indeed, it is often a confused taxonomy that makes management of threatened species even more problematic than it is already (Frankham *et al*., 2002; Souty-Grosset *et al*., 2006).

The current classification (Grandjean *et al*., 2000, 2002a,b), based on 16S rRNA and supported by allozymatic studies (Santucci *et al*., 1997), defines *A. pallipes* as a species complex composed of two genetically distinct lineages, *A. italicus* and *A. pallipes*. In this paper, the terminology by Fratini *et al*. (2005), although provisional and not officially recognized (Manganelli *et al*., 2006), will be used.

Both *A. italicus* and *A. pallipes* have been found in Italy (Lörtscher *et al*., 1997; Nascetti *et al*., 1997; Santucci *et al*., 1997; Grandjean *et al*., 2000; Largiardèr *et al*., 2000), *A. italicus* being distributed across the entire Italian peninsula and *A. pallipes* being confined to the north-west. The two taxa overlap in the Ligurian Apennine but no hybridization event has ever been recorded (Nascetti *et al*., 1997; Santucci *et al*., 1997).

Austropotamobius pallipes is classified as 'vulnerable' by IUCN (Baillie and Groombridge, 1996) and is listed in Appendix III of the Bern Convention and in Annexes II and V of the EC Habitats Directive 92/43/ECC. It is defined as a species 'of community interest whose conservation requires the designation of Special Areas of Conservation' (Annex II). Unfortunately, legislation in Europe varies among and within countries (Vigneux *et al*., 2002). For instance, in Italy, crayfishing is banned in some regions, e.g. Piedmont, whereas in others, e.g. Veneto, it is allowed with restrictions on size and fishing periods (Mancini, 1986). To make the situation worse, *A. italicus* is not included in any list of species of conservation concern, except in the legislation that applies in the Tuscan Region.

Similarly to *A. pallipes*, *A. italicus* is known to play a role in assuring the services offered by freshwater systems (Gherardi *et al*., 2001). Being among the largest and longest lived freshwater invertebrates (Füreder *et al*., 2003), it exerts direct and indirect beneficial effects on habitats, contributing to energy flow and matter cycling (Souty-Grosset *et al*., 2006). Its occurrence, as shown in other crayfish species, is associated with the availability of boulders, boulder/cobble banks, and riffles (Naura and Robinson, 1998). The *facies* of the substratum accounts for the abundance of this species (Flint and Goldman, 1977), whereas erosion causes loss or reduction of the available habitat (Naura and Robinson, 1998). Among other characteristics of the habitat, fibrous and ramified roots provide shelter to crayfish and act as detritus traps (Bohl, 1987; Smith *et al*., 1996).

Austropotamobius italicus populations in Italy are subject to the same decrease in number and distribution as observed for *A. pallipes* in its entire range throughout Europe. Threats to these species are many, including habitat fragmentation (Jay and Holdich, 1981), bad management of river basins (Westman, 1985; Lowery and Hogger, 1986; Holdich and Lowery, 1988; Foster and Turner, 1993), overfishing, and the introduction of non-indigenous species (especially *Procambarus clarkii*; Gherardi, 2006) together with their parasites (e.g. *Aphanomyces astaci*; Gherardi and Holdich, 1999). Similarly to *A. pallipes* (Holdich and Reeve, 1991; Reynolds *et al*., 2002; but see Trouilhé *et al*., 2006), *Austropotamobius italicus* is extremely sensitive to slight changes in environmental conditions, so that several authors classify it as a good bioindicator of water quality (Scalici and Gibertini, 2005; Renai *et al*., 2006).

This study assessed the status of some populations of *A. italicus* in Central Italy by providing information about their size and structure. Its main aims were to assess the importance of stock assessment as a reliable indicator of conservation status, and to identify the characteristics of the habitat required for its preservation.

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MATERIALS AND METHODS

Study area

Surveys were conducted between May and October 2003 at night (when crayfish activity is greatest; Barbaresi and Gherardi, 2001) in nine Tuscan streams each harbouring a population of *A. italicus* (streams WI) and in 10 streams where crayfish populations became extinct at least 5 years before the study (streams WO) (Figure 1), as shown by information obtained from previous surveys (F. Gherardi, pers. commun.) and from interviews with local people. The 19 streams belong to four catchments (Magra, Serchio, Sieve, and Arno) and are located in an area of about 300 km^2.

All the study streams run through mountainous or hilly areas at an altitude of 300–800 m, most often surrounded by woods and grazing areas. The riparian vegetation belt (width: 5–30 m) is mainly composed of *Alnus glutinosus*, *Picea abies*, *Populus* sp., and *Salix* sp. The stream bottom is covered by cobbles and boulders that, together with abundant tree roots, are known to provide shelter to crayfish (Naura and Robinson, 1998). Table 1 shows some morphological, chemical, and physical characteristics of the study streams (Renai *et al.*, 2006).

Population abundance, structure, and dynamics

Crayfish were captured by hand by two people walking upstream for 2 h. Surveys were done by turning rocks and searching among roots and detritus. Immediately upon capture, sex was noted and the cephalothorax length (CL), including rostrum, was measured using a vernier caliper. Specimens with CL < 24 mm were defined as juveniles (Grandjean *et al.*, 1998). The occurrence of scars, mutilations, and visible ectoparasites was recorded. After measurement, crayfish were released at the collection site.

For each population, measurements were made of the catch per unit effort (CPUE, the number of crayfish divided by the time spent sampling; Demers and Reynolds, 2002; Scalici and Gibertini, 2005), density (individuals m^{-2}), and biomass (the total weight of the captured crayfish divided by the area of each transect). Crayfish weight (W) was estimated by applying the formulae obtained from preliminary measurements of individuals collected from the same area (B. Renai, unpubl. data): $W = 6 \times 10^{-5}\,\mathrm{CL}^{3.46}$ for males and $W = 310^{-4} \times \mathrm{CL}^{2.96}$ for females.

Histograms of polymodal frequency distributions were generated from data on body sizes and were analysed using Bhattacharya's (1967) method by a routine of the FiSAT (FAO-ICLARM Stock Assessment Tools) computer program (Gayanilo *et al.*, 1996). This method decomposes size–frequency distributions into diverse normal components, every component being identified as an age class. It is based on the assumption that the observed distribution in size classes results from the overlap of diverse normal distributions. The process converts normal distributions into lines that simplify the procedure, linearization being performed by computing the natural logarithms of frequencies. Intercepts and slopes of the regression lines were used to estimate the parameters of each normal distribution. Given a distribution in size classes, the Bhattacharya's (1967) method allows for the iterative computation of regression lines until the total decomposition

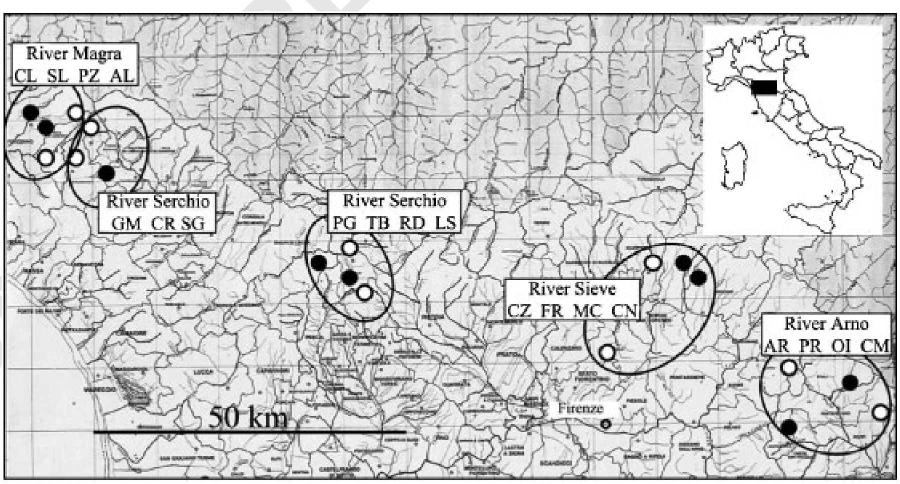

Figure 1. The study area in Tuscany that includes four catchments (Arno, Magra, Serchio, and Sieve). Streams (nine) with extant populations of *A. italicus* and streams where populations became extinct at least 5 years before the study (10) are denoted by black and white dots, respectively. Stream names, abbreviated here, are given in Table 1.

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Table 1. Chemical (pH, and dissolved oxygen, nitrite, nitrate, and calcium concentration in mg L^{-1}) and physical parameters (temperature, *T*, in °C; conductivity, *C*, in μS s^{-1}), and morphological features (flow velocity in m s^{-1}, mean width in m, and mean depth in cm) of the study streams (abbreviated names in parentheses). Streams WI harbour extant populations of *A. italicus*, while in streams WO the species became extinct at 5 least years before the study

Stream	Basin	pH	O_2	NO_3^-	NO_2^-	Ca^{2+}	T	C	Speed	Width	Depth
With Crayfish (WI)											
Acqua Bianca (SG)	Serchio	8.55	8.19	5.00	0.05	64.75	18.15	345.50	0.67	8.79	31.42
Collegnago (CL)	Magra	8.30	7.61	5.00	0.06	73.50	15.72	362.75	0.25	1.25	23.29
D'Omicio (RD)	Serchio	7.99	8.12	1.75	0.05	47.08	14.97	245.41	0.35	1.47	21.00
Farfereta (FR)	Sieve	7.72	7.48	3.00	0.04	80.25	15.88	405.75	0.01	2.84	24.29
Muccione (MC)	Sieve	8.23	8.04	3.00	0.05	67.00	15.86	462.00	0.44	3.33	25.13
Oia (OI)	Arno	8.06	8.77	4.00	0.04	53.50	15.17	274.83	0.67	4.47	29.33
Prugnano (PR)	Arno	7.68	6.57	2.50	0.04	92.25	14.10	466.00	0.02	2.15	23.21
Selve (SL)	Magra	8.19	7.91	5.00	0.06	66.75	13.34	375.08	0.10	1.11	14.00
Torbecchia (TB)	Serchio	7.99	7.28	3.00	0.03	76.08	15.52	422.50	0.02	1.53	18.79
Without Crayfish (WO)											
Aulella (AL)	Magra	8.27	8.48	5.00	0.05	73.00	15.43	391.50	0.44	2.27	31.70
Arno (AR)	Arno	8.29	8.98	3.50	0.04	61.75	17.44	343.50	0.55	4.09	43.82
Camaldoli (CM)	Arno	8.30	8.85	6.25	0.04	51.50	13.60	273.67	0.53	2.59	27.12
Canaticce (CN)	Sieve	8.08	7.46	5.00	0.09	88.67	15.48	628.25	0.21	1.62	20.41
Carpinelli (CR)	Serchio	8.29	7.91	3.75	0.05	72.25	14.91	339.83	0.16	1.56	16.65
Carza (CZ)	Sieve	8.07	8.49	4.25	0.06	52.00	17.65	544.67	0.03	2.59	35.25
Gambrano (GM)	Serchio	8.15	7.66	5.00	0.05	78.08	15.59	382.08	0.04	1.66	28.58
Liesina (LS)	Serchio	7.57	7.70	1.00	0.04	31.33	17.41	173.75	0.51	3.59	26.91
Pezzola (PZ)	Magra	8.14	7.91	5.00	0.06	42.25	14.20	219.25	0.35	1.82	27.50
Pagano (PG)	Serchio	7.73	7.30	1.50	0.03	48.83	14.08	257.75	0.35	3.39	32.29

of the overall size-frequency distribution. The program provides values for each Gaussian component, i.e. means, standard deviations, numbers of individuals per size class, regression lines (and the respective R^2), and separation index values (SI) for each adjacent group. In particular, SI denotes when two adjacent Gaussians can be separated, i.e. SI $\geqslant 2$ (Sparre and Venema, 1996). In a univoltine population, where SI values decrease below 2, the last class (composed of a few individuals) is included in the previous component. At the end of the separation process, the program provides χ^2-test values. This modal-progression analysis has been used extensively for the assessment of marine and freshwater fish stocks, and less frequently for other taxa, such as reptiles (Salvidio and Delaugerre, 2003), mussels (Ardizzone *et al*., 1996), marine crustaceans (Merella *et al*., 1998), and crayfish (Fidalgo *et al*., 2001; Chiesa *et al*., 2006; Scalici and Gherardi, 2007). To assign an age to each class, April was deemed the date of egg hatching based on information from previous studies conducted in the same area (Gherardi *et al*., 1997).

The results obtained with the Bhattacharya's method were used to evaluate the growth rate of Von Bertalanffy (1938), by the equation (Pauly *et al*., 1992):

$$L(t) = L_\infty\{1 - \exp[-k(t - t_0) - (Ck/2\pi)(\sin 2\pi(t - t_s) - \sin 2\pi(t_0 - t_s))]\}$$

where $L(t)$ is the CL of the individuals at the time t; L_∞ is the mean CL of the oldest individuals, i.e. the 'asymptotic length' (computed as $L_{max}/0.95$, where L_{max} is the maximum recorded length, according to Pauly, 1981); k is the rate at which L_∞ is reached, i.e. the 'curvature parameter'; t_0 is the 'initial condition parameter' and determines when the specimens have a CL equal to 0, C is the amplitude of the curve (i.e. estimation of the influence of season on the growth pattern), and t_s is the summer point (referring to the onset of the first oscillation relative to $t = 0$) (for details see Sparre and Venema, 1996).

The mortality index (Z) was obtained from the Powell–Wetherall Plot equation (Wetherall, 1986) that computes the asymptotic length and the ratio between the mortality coefficient and the curvature parameter (Z/k) using length-frequency data imported in the FiSAT program. Z is the total mortality, i.e. the sum of natural mortality (M) and the mortality due to fishing (F). M was calculated by the following equation (Pauly, 1980):

$$\log_{10} M = -0.0066 - 0.279 \log_{10} L_\infty + 0.6543 \log_{10} k + 0.463 \log_{10} T$$

where M is the natural mortality, L_∞ is the asymptotic length, k is the curvature parameter, and T is the annual mean habitat

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temperature of the water in which crayfish live. F was obtained subtracting M from Z.

Finally, the expected longevity estimate (t_{max}) was computed from the equation (Gayanilo *et al*., 1996):

$$t_{max} = (3/k) + t_0$$

Characteristics of the habitat

Reaches of 80–150 m in length were designated, one for each study stream. To select them, about 500 m of each stream were investigated to ensure that the environmental characteristics of the surveyed reach (e.g. substrate, water velocity, etc.) averaged those of the entire study stream.

The width and depth of the wet-bed were measured for each stream every 5-m transect within each reach. The habitat was characterized by recording the percentage of tracts with laminar water flow, and the numbers of ponds and riffles. In two adjacent 15-m transects of each reach, shelter occurrence was assessed by counting the number of crevices in the banks, roots, and boulders, and the substrate composition was analysed in a 1×1 m metal frame divided into 16 equal squares launched five times randomly. Inside each square, estimates were made by eye of the percentage area covered by silt, sand (<2 mm diameter), gravel (2–64 mm), cobble (65–256 mm), boulder (>256 mm), and bedrock (fixed rock), and the occurrence of plant detritus (composed of leaves and wood pieces), moss, and periphyton.

At the end of each survey, a sample of macroinvertebrates in each reach was collected by kicking and the use of a standard net (mesh size: 290 μm). The taxa occurring in each sample were determined in the laboratory following Campaioli *et al*. (1998) and Ghetti (1997).

The degree of environmental integrity was assessed by applying the Fluvial Functionality Index (IFF), a monitoring instrument promoted in 2000 by ANPA (today APAT, the Italian agency for the protection of the environment) and listed in the technical paper of the Water Framework Directive (2000/60/EC). IFF is obtained by answering 14 questions, each answer having a numerical weight (ranking from 1 to 30).

Data analyses

Data were analysed using the STATISTICA Statsoft software version 6.0. Frequency data were analysed after using a χ^2-test with Yates correction. For the other analyses, data were first checked for normality and homogeneity of variance using the Kolmogorov–Smirnoff test and, when necessary, were ln $(x+1)$ transformed to remove heteroscedasticity. Von Bertalanffy's parameters were calculated by the use of non-linear regressions. The relationships between crayfish presence/absence and biotic and physical parameters were analysed using *t*-tests, Pearson's correlation tests, two-way ANOVAs followed by Tukey's tests, and Principal Components Analyses (PCA).

RESULTS

In total 1237 crayfish (567 males and 670 females) were recorded. Details of each study population are given in Table 2. Females were more abundant than males in three populations (CL, OI, and TB), while sex ratio (0.35–0.56) did not differ significantly from 1:1 in the remaining five streams. The ratio between juveniles and adults (0.07–0.50) was always biased towards adults with only one exception (RD). CPUE, density, and biomass ranged 0.40–2.12 min^{-2}, 0.18–1.08 m^{-2}, and 1.43 to 10.45 g m^{-2}, respectively. Overall, 14.7% crayfish were found without a cheliped and 18.03% had a regenerated one; 51.17% of them were infected by *Branchiobdella* sp. and 1.45% by *Fusarium* sp. No individual showed apparent symptoms of either thelohania or aphanomycosis.

Size–frequency distributions are shown in Figure 2. Log-transformed CL data differed significantly between sexes ($F = 102.4$, df = 2, 1223, $P = 0.004$), males being larger, and among streams ($F = 0.44$, df = 4, 1213, $P = 0.047$; SL = OI > PR = SG = CL = FR > RD = TB, after Tukey's test), but

Table 2. Details of the *A. italicus* populations analysed in nine study streams (stream names, abbreviated here, are given in Table 1): sample size (N), number of males (M) and females (F), sex ratio, juvenile/adult ratio (J/A), catch per unit effort (CPUE) (individuals min^{-1}), density (individuals m^{-2}), and biomass (g m^{-2})

Stream	N	M	F	Sex ratio	J/A	CPUE	Density	Biomass
SG	173	96	77	0.56	0.22*	1.44	0.27	1.61
CL	200	73	127	0.37*	0.19*	1.67	1.08	10.45
RD	94	48	46	0.51	0.50	0.78	0.29	1.88
FR	156	82	74	0.53	0.29*	1.30	0.64	4.25
MC	113	63	50	0.56	0.12*	0.94	0.18	1.43
OI	254	90	164	0.35*	0.15*	2.12	0.88	8.77
PR	116	65	51	0.56	0.15*	0.97	0.80	7.23
SL	48	20	28	0.41	0.07*	0.40	0.21	2.64
TB	83	30	53	0.36*	0.12*	0.69	0.33	2.32

*Significant differences (p at least <0.05) from the expected 1:1 (after χ^2-tests with Yates correction).

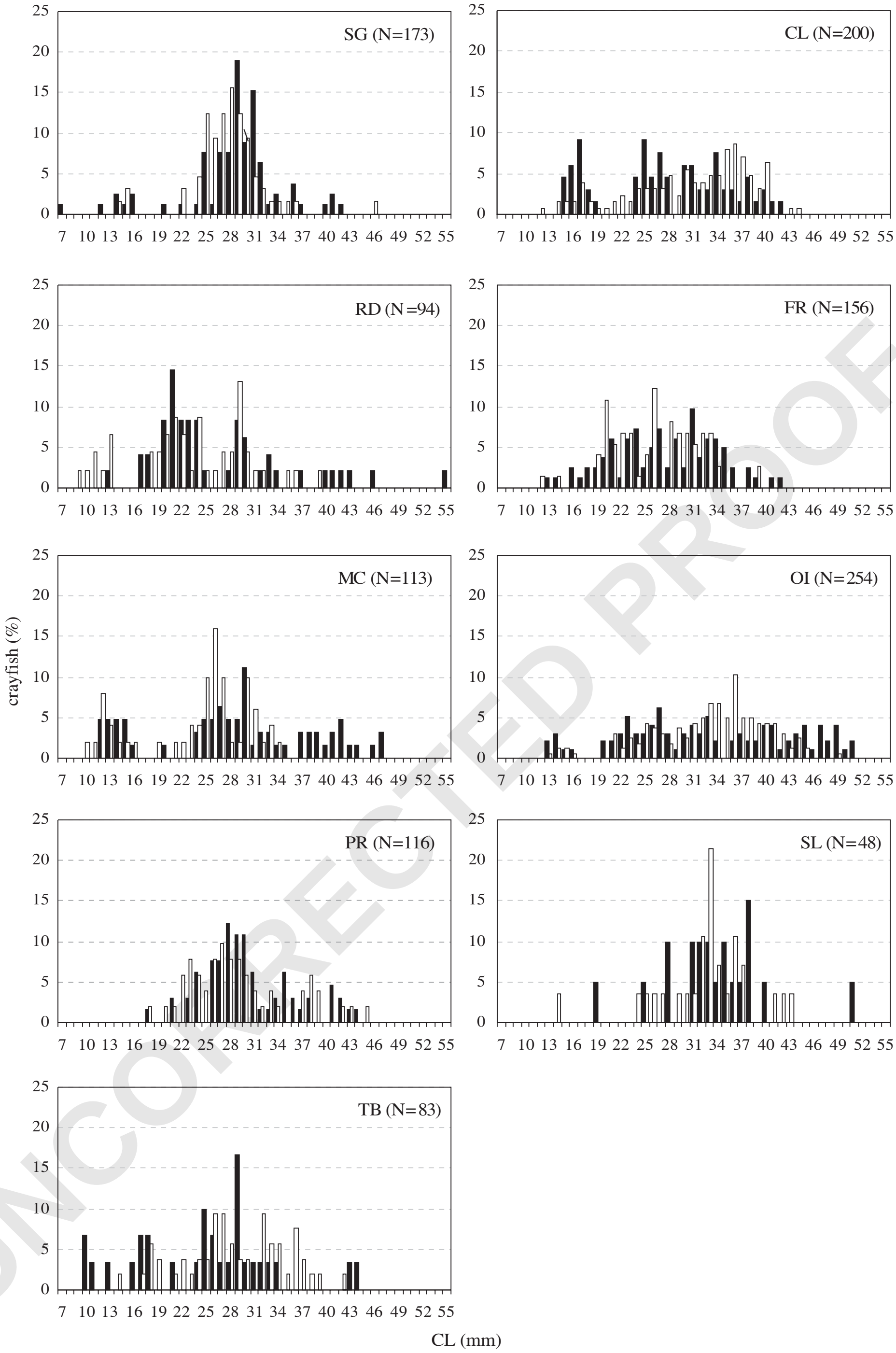

Figure 2. Size frequencies of males (black) and females (white) for each of the nine study streams (stream names, here abbreviated, are given in Table 1).

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not for the interaction sex *x* stream ($F = 1.73$, df $= 2$, 1227, $p = 0.14$). This analysis showed that two streams (SL and OI) contained populations with larger individuals, while the populations of RD and TB were composed of significantly smaller individuals.

Table 3 gives the age (in months) for each size class, obtained by applying Bhattacharya's (1967) method, the number of individuals collected, their respective mean CL (and SD), R^2, and the SI values. Age classes ranged from 1 in SL to 5 in OI in males, and from 2 in SL to 5 in all the study

Table 3. Analyses of the cephalothorax length (CL)-frequencies after the application of the Bhatthacharya's method in the nine study streams (stream names, here abbreviated, are given in Table 1)

Stream	Age	Males				Females			
		N	Mean CL (SD)	R^2	SI	*N*	Mean CL (SD)	R^2	SI
SG	4	3	16.5 (1.2)	0.12		17	25.27 (0.88)	1	
	16	35	28.721 (1.21)	0.5	10.1	33.66	28.39 (1.73)	0.92	2.37
	28	4	36 (0.95)	1	6.24	3.96	33.97 (1.67)	0.38	3.27
	40	4	41 (0.84)	0.8	6.09				
CL	2	16	15.56 (1.77)	0.16		8	17.21 (1.11)	1	
	14	11.90	25 (0.84)	1	7.1	23.89	21.97 (1.41)	0.27	3.76
	26	20	34.77 (2.71)	0.14	5.48	42.96	26.47 (2.46)	0.36	2.22
	38	2.84	40.2 (0.66)	1	3.21	21.12	32.67 (1.49)	1	3
	50					5	36.37 (2.20)	0.7	2.11
RD^{*+}	17	8	23.672 (0.77)	1		8	16.83 (2.91)	0.37	
	29	4	33 (0.85)	1	11.5	14.55	22.43 (1.6)	0.27	2.47
						13.89	28.54 (1.57)	0.46	3.85
						0.82	32.5 (0.7)	1	3.49
FR^{*+}	12	40	23.356 (3.56)	0.12		6	20.5 (1.2)	1	
	24	26.75	33.371 (1.7)	0.34	3.79	7	26.5 (1.04)	1	5.34
	36	2.84	37.5 (1.24)	1	2.79	13.66	30.998 (2.16)	0.14	2.8
MC*	1	10	13.83 (1.34)	0.75		8	12 (2.9)	1	
	13	8	25.5 (1.57)	1	7.992	6	26.23 (2.31)	0.9	5.45
	25	10.99	34.52 (4.46)	0.5	2.991	9.85	29.91 (1.18)	1	2.10
	37	5.70	41.64 (0.91)	0.94	2.64	4.52	33.46 (0.248)	0.6	4.95
OI^{*+}	2	6	13.77 (0.81)	1		6	14.5 (1.2)	1	
	14	28	25.42 (2.41)	0.36	7.204	9	23.21 (1.10)	1	7.87
	26	23.890	34.38 (3.38)		3.086	21	28.25 (1.48)	0.95	3.53
	38	11.770	39.57 (1.52)		2.11	36.88	33.6 (1.66)	0.98	3.04
	50	17.850	46.44 (2.31)		3.57	42.17	38.85 (2.404)	0.70	2.58
PR^{*+}	16	43	26.897 (2.43)	0.59		13	23.34 (1.4)	0.91	
	28	9.46	35.288 (0.97)	0.87	4.92	22.66	28.91 (1.62)	0.66	3.68
	40	8.38	40.187 (1.69)	0.74	3.66	3.21	33.21 (0.675)	1	3.74
						7	38 (1.1)	1	5.36
						3	44.17	0.75	0.56
SL^{+}	28	16	35.5 (1.2)		20	13	33 (1.18)	0.76	
						5.19	36.36 (0.623)	1	3.72
TB^{*+}	5	5	17.5 (1.2)	1		6	18.23	1	
	17	7	25.5 (1.05)	0.87	7.087	3.99	22	1	4.54
	29	11.040	35.54 (3.95)	0.3	4	12.87	26.91	0.66	4.08
						7.97	31.5	1	3.09
						2.67	34.45	1	3.57

* and $^{+}$ Significant differences for males and females, respectively, after χ^2-tests. Ages are in months; *N* is the theoretical number of individuals; R^2 is the output of correlation tests; and SI denotes the Separation Index.

streams in females. A relatively low abundance of juveniles were recorded in PR, SL, and RD.

The parameters of the Von Bertalanffy's growth function were computed from the mean values of the age classes. It was assumed that all the analysed populations were subject to the same growth rate because the study streams, located in the same geographic area, have similar climatic characteristics (Renai *et al.*, 2006). For this reason, the same asymptotic length (L_∞) was assigned to all the populations. Data were pooled and a single growth curve for males and females was plotted (Figure 3), showing that life expectancy (i.e. t_{max}) is 8.2 and 7.8 years for males and females, respectively. Von Bertalanffy's parameters, distinguished between sexes, are given in Table 4.

The effects of vegetal material (specifically plant detritus) on the occurrence and abundance of *A. italicus* populations were investigated by the use of a PCA. Streams WI, without any distinction between streams with poor ($\leqslant 5\,g\,m^{-2}$) and abundant ($>5\,g\,m^{-2}$) populations, were discriminated from streams WO from the percentage of detritus (Figure 4). The first two principal components reached 63.64% of the total variance. The sum of the first two principal component eigenvalues amounted to 45.50% of the inertia.

Streams WI and WO did not differ in the taxonomic composition of their macroinvertebrate communities, as shown by the application of a second PCA (Figure 5). The first two principal components reached 26.02% of the total variance. The mean abundance of Plecoptera, Ephemeroptera, and Trichoptera (i.e. the taxa most sensitive to chemical pollution) did not differ significantly between types of streams ($G = 0.125$, df $= 1$, $P > 0.05$).

Most of the characteristics relating to stream morphology (i.e. width and depth of the wet-bed, the percentage of tracts

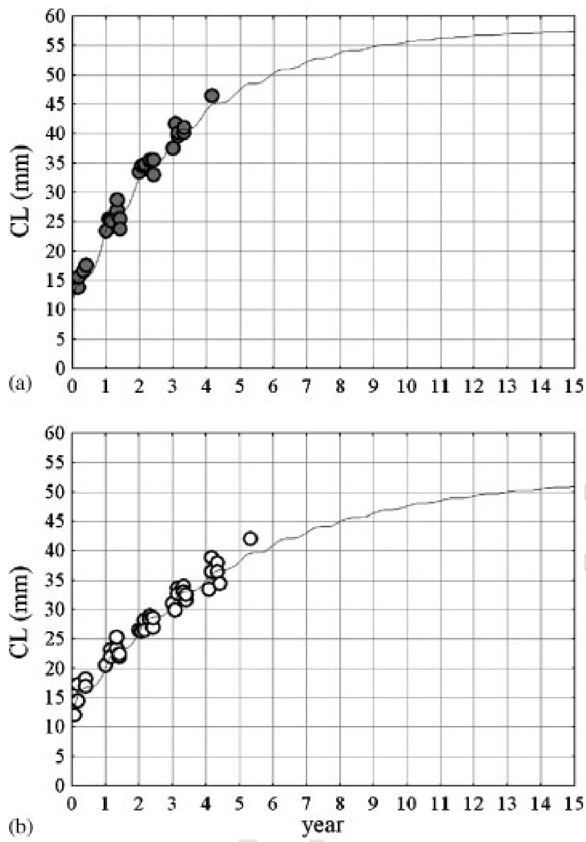

Figure 3. Growth curves for males (a) and females (b). Data from different populations have been pooled for the analysis.

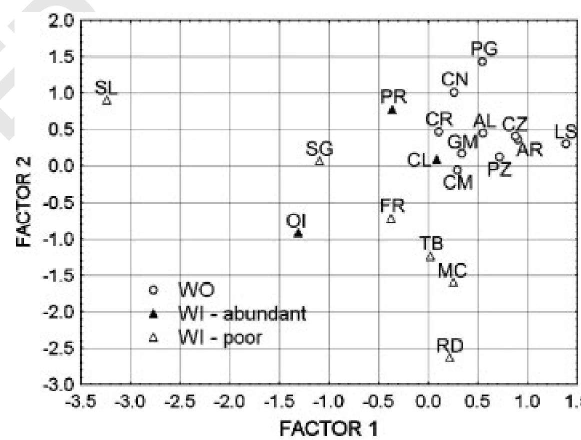

Figure 4. Scatterplot obtained from the abundance of plant detritus, moss, and periphyton compared for streams WO and streams WI with abundant (biomass $>5\,g\,m^{-2}$) and poor (biomass $\leqslant 5\,g\,m^{-2}$) crayfish populations. See Table 1 for the meaning of WO and WI and of stream abbreviations.

Table 4. Von Bertalanffy's parameters for *A. italicus* males and females, i.e. curvature parameters (k), mean lengths of old individuals (L_∞), initial condition parameters (t_0), the expected longevity estimate (t_{max}), amplitudes (C), summer points (t_s), total mortalities (Z), natural mortalities (M), and mortalities due to fishing (F)

	k	L_∞	t_0	t_{max}	C	t_s	Z	M	F
Males	0.34	57.89	−0.64	8.2	0.92	0.104	10.04	1.3×10^{-5}	10.04
Females	0.37	52.11	−0.29	7.8	0.96	0.085	11.25	3.9×10^{-6}	11.25

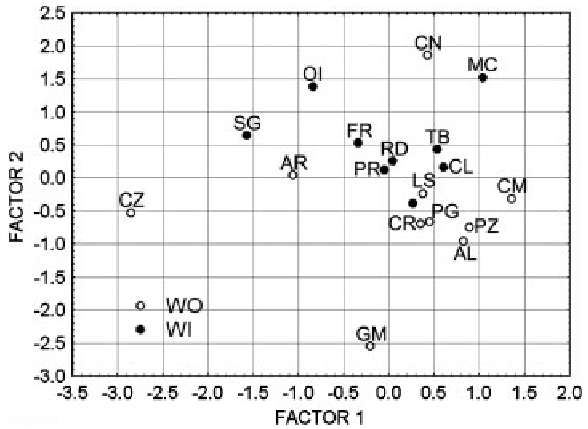

Figure 5. Scatterplot obtained from the composition of macroinvertebrate communities compared for streams WO and WI. See Table 1 for the meaning of WO and WI and of stream abbreviations.

with laminar water flow, the numbers of pools and of riffles, shelter occurrence, and IFF) did not show any significant correlation with the population structure (r between 0.01 and 0.85, df $= 9$, P always > 0.05). On the contrary, significant results were found for the *facies* of the substratum: crayfish numbers increased with percentage of cobbles when they belonged to the age classes 0+ ($r = 0.48$, df $= 9$, $P = 0.057$) and 1+ ($r = 0.73$, df $= 9$, $P = 0.026$), but decreased when they belonged to older age classes (2+: $r = -0.33$, df $= 9$, $P = 0.0587$; 3+: $r = -0.78$, df $= 9$, $P = 0.014$; 4+: $r = -0.77$, df $= 9$, $P = 0.015$; and 5+: $r = -0.1$, df $= 9$, $P = 0.0579$).

DISCUSSION

This study shows that *A. italicus* has been subject to a rapid, drastic contraction in its distribution range in Tuscany; in fact, the species is found today in nine of the 19 streams where it occurred previously only 5 years before this survey. An alarming decrease in the abundance of *A. italicus* populations has also been noted in the last 30 years if compared with previous surveys (Mancini, 1986). Indeed, the biomass and the CPUE values of the study populations are comparable with those recorded in *A. italicus* and *A. pallipes* populations of other Italian regions and European countries (Füreder *et al.*, 2003; Lyons and Kelly-Quinn, 2003; Scalici and Gibertini, 2005). This might suggest that the trend documented here is a reflection of a general phenomenon occurring across the entire distribution range of the *A. pallipes* species.

Overall the study populations seem to be healthy, showing a balanced sex-ratio and a relatively low number of injured individuals; even fewer crayfish were found to be parasitized by *Fusarium* sp. (*Branchiobdella* sp. is apparently harmless to this species; Gherardi *et al.*, 2002a) and none is affected by *Thelolaia* sp. or *Aphanomyces astaci*. Application of Bhattacharya's (1967) method confirms that the study populations (except two) are well structured in their age-class composition, with four classes of males and five classes of females. On the other hand, the low frequency of juveniles found in nearly all the study streams may be because of their elusive behaviour, which makes them difficult to find.

The analysis of Von Bertallanfy's parameters supports the results of preliminary studies on a population of *A. pallipes* in England (Brewis and Bowler, 1982) and confirms the information obtained on the growth rate of captive individuals (Pratten, 1980). *Austropotamobius italicus* is a K-selected species, with a relatively slower growth rate (0.34 and 0.37 for males and females, respectively) and a longer life expectancy (8.2 and 7.8 years for males and females, respectively) when compared with other crayfish species analysed with the same method, such as *Orconectes limosus* (Chiesa *et al.*, 2006), *Pacifastacus leniusculus* (Smietana and Krzywosz, 2006), and *P. clarkii* (Correia, 1993; Gutiérrez-Yurrita *et al.*, 1996; Chiesa *et al.*, 2006; Scalici and Gherardi, 2007).

The estimate of *A. italicus* mortality rate (Z) also provides useful information about the possible anthropogenic and environmental factors threatening this species. Natural mortality seems to be low, possibly because of the scarcity of predators, the absence of parasites and of diseases in general, and no recent pollution events. Conversely, the mortality as a result of fishing is high, confirming the previous hypothesis (Renai *et al.*, 2006) that this activity, illegal in Tuscany, has been the main cause of local extinction of *A. italicus* in several basins. In fact, fishing has been found to cause a drastic reduction in the carrying capacity of some populations (Scalici and Gibertini, 2005) and seems to have contributed to decreased genetic diversity (Santucci *et al.*, 1997; S. Bertocchi *et al.*, unpublished data) that makes the affected populations highly vulnerable to both environmental stressors and stochastic events. In Tuscany, although pollution incidents and/or drought events may have had a considerable impact in the past, illegal fishing seems to have reduced the integrity of crayfish populations more than habitat degradation. A companion study (Renai *et al.*, 2006) has shown that some physicochemical parameters of the habitat associated with recent pollution events, such as pH, temperature, and water chemistry, have had only a limited effect on crayfish occurrence in the study area. Neither does *A. italicus* seem to be affected by the taxonomic composition of macroinvertebrate communities, taken as a proxy of the past

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history of pollution (see, in contrast, Trouilhé *et al*., 2003 and Scalici and Gibertini, 2005). Indeed, although susceptible to organic pollution (Trouilhé *et al*., 2006), the closely related *A. pallipes* is able to survive in poor quality waters, being found in acid, peaty areas in moorlands and in eutrophic angling lakes (Demers and Reynolds, 2002).

Acting in concert with overexploitation, the loss of pristine riverine landscape seems to have been responsible for the local extinction of crayfish populations, at least in central Italy. Riparian vegetation is a source of allochthonous plant detritus, which is known to provide food to benthic consumers, including crayfish (Momot, 1984; Richardson, 1991; Nakano *et al*., 1999; Usio, 2000). In fact, although adult *A. italicus* seem to prefer moss in laboratory choice experiments (Gherardi *et al*., 2004), when their foraging behaviour was recorded in the field, they were most often observed visiting patches of coarse detritus and woody debris (Gherardi *et al*., 2001), vegetal material being the main item found in their gut (Gherardi *et al*., 2004). These results clearly show the influence that the inputs from streamside vegetation exert on crayfish populations, as suggested for other members of stream community (Allan *et al*., 2003). A significantly larger abundance of plant detritus was found in the streams with extant populations, when compared with the streams where crayfish have become extinct. Riparian vegetation also provides shade that maintains cool water temperatures and, together with fallen branches and large woody debris (C. Benvenuto *et al*., unpublished data), offers shelter against predators.

Finally, the evidence that, in contrast to the adults, *A. italicus* juveniles most often use cobbles, seems to suggest that age classes are segregated in the habitat (Arrignon and Roche, 1981; Foster, 1995; Smith *et al*., 1996; Neveu, 2000; Reyjol and Roqueplo, 2002). Such segregation might have the effect of decreasing competition for shelters between age classes (Stein, 1977; Momot, 1993; Lodge and Hill, 1994; Gherardi, 2002). Indeed, cobbles guarantee to juveniles the regular availability of periphyton and macroinvertebrates (Foster, 1995), both of which are the items most often found in their gut (Goddard, 1988; Gherardi *et al*., 2001).

In summary, the complexity of the riverine landscape that comprises riparian vegetation and the diverse substrates in the river bed ensures both food and protection to *A. italicus* and allows for the maintenance of healthy populations of this threatened species. This conclusion confirms the results of a recent survey of *Cambaroides japonicus* populations in Japan (Usio, 2007), which demonstrated the existence of a significant association between this species and early successional tree species in the riparian vegetation.

As a consequence of these results, retaining, enhancing, and restoring the diversity of the habitat (Simberloff, 1988; Freeman and Freeman, 1994; Rabeni and Sowa, 1996; Sutherland, 1998), with its mosaic of microhabitat patches (Cornell and Lawton, 1992; Robson and Chester, 1999), seem to be the only options available for conserving *A. italicus* and other indigenous species in stream communities (Douglas and Lake, 1994; Townsend and Hildrew, 1994).

Special Areas of Conservation (SACs) within the Natura 2000 network (designated under the EC Habitats Directive) might help, if associated with programmes aimed at both publicizing the need for the conservation of this species and increasing public awareness of the threats to its existence. Unfortunately, in Italy, invertebrates in general and crayfish in particular have attracted little attention from managers and policy-makers. Since 1992, only six Italian projects focused on crayfish (compared with 145 other projects) have received financial support from the EC through LIFE ('L' Instrument Financier pour l'Environement'). Protective action, including re-introduction programmes, for the *A. pallipes* species complex has been conducted in only 35 SACs (none exclusively devoted to its conservation) of the 2503 Natura 2000 sites designated in Italy between 1992 and 2005 (about 1.4%) (data from Picchi *et al*., 2006). These figures are decidedly low, when compared, on the one hand, with the poor conservation status of this species in Italy as this and other previous studies have shown (De Luise, 1991; Salvidio *et al*., 2002; Nardi *et al*., 2004; Renai *et al*., 2006), and on the other, its well recognized ecological role, (Nyström, 2002).

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