



UNIVERSITÀ
DEGLI STUDI
FIRENZE

FLORE

Repository istituzionale dell'Università degli Studi di Firenze

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

Questa è la Versione finale referata (Post print/Accepted manuscript) della seguente pubblicazione:

Original Citation:

Conserving indigenous crayfish: stock assessment and habitat requirements in the threatened *Austropotamobius italicus* / S. BRUSCONI; S. BERTOCCHI; B. RENAI; M. SCALICI; C. SOUTY-GROSSET; F. GHERARDI. - In: AQUATIC CONSERVATION-MARINE AND FRESHWATER ECOSYSTEMS. - ISSN 1052-7613. - STAMPA. - 18:(2008), pp. 1227-1239. [10.1002/aqc.935]

Availability:

The webpage <https://hdl.handle.net/2158/252685> of the repository was last updated on

Published version:

DOI: 10.1002/aqc.935

Terms of use:

Open Access

La pubblicazione è resa disponibile sotto le norme e i termini della licenza di deposito, secondo quanto stabilito dalla Policy per l'accesso aperto dell'Università degli Studi di Firenze (<https://www.sba.unifi.it/upload/policy-oa-2016-1.pdf>)

Publisher copyright claim:

La data sopra indicata si riferisce all'ultimo aggiornamento della scheda del Repository FloRe - The above-mentioned date refers to the last update of the record in the Institutional Repository FloRe

(Article begins on next page)

***** PROOF OF YOUR ARTICLE ATTACHED, PLEASE READ CAREFULLY *****

After receipt of your corrections your article will be published initially within the online version of the journal.

PLEASE NOTE THAT THE PROMPT RETURN OF YOUR PROOF CORRECTIONS WILL ENSURE THAT THERE ARE NO UNNECESSARY DELAYS IN THE PUBLICATION OF YOUR ARTICLE

READ PROOFS CAREFULLY

ONCE PUBLISHED ONLINE OR IN PRINT IT IS NOT POSSIBLE TO MAKE ANY FURTHER CORRECTIONS TO YOUR ARTICLE

- § This will be your only chance to correct your proof
- § Please note that the volume and page numbers shown on the proofs are for position only

ANSWER ALL QUERIES ON PROOFS (Queries are attached as the last page of your proof.)

- § List all corrections and send back via e-mail to the production contact as detailed in the covering e-mail, or mark all corrections directly on the proofs and send the scanned copy via e-mail. Please do not send corrections by fax or post

CHECK FIGURES AND TABLES CAREFULLY

- § Check sizes, numbering, and orientation of figures
- § All images in the PDF are downsampled (reduced to lower resolution and file size) to facilitate Internet delivery. These images will appear at higher resolution and sharpness in the printed article
- § Review figure legends to ensure that they are complete
- § Check all tables. Review layout, titles, and footnotes

COMPLETE COPYRIGHT TRANSFER AGREEMENT (CTA) if you have not already signed one

- § Please send a scanned signed copy with your proofs by e-mail. **Your article cannot be published unless we have received the signed CTA**

OFFPRINTS

- § 25 complimentary offprints of your article will be dispatched on publication. Please ensure that the correspondence address on your proofs is correct for dispatch of the offprints. If your delivery address has changed, please inform the production contact for the journal – details in the covering e-mail. Please allow six weeks for delivery.

Additional reprint and journal issue purchases

- § Should you wish to purchase a minimum of 100 copies of your article, please visit http://www3.interscience.wiley.com/aboutus/contact_reprint_sales.html
- § To acquire the PDF file of your article or to purchase reprints in smaller quantities, please visit <http://www3.interscience.wiley.com/aboutus/ppv-articleselect.html>. Restrictions apply to the use of reprints and PDF files – if you have a specific query, please contact permreq@wiley.co.uk. Corresponding authors are invited to inform their co-authors of the reprint options available
- § To purchase a copy of the issue in which your article appears, please contact cs-journals@wiley.co.uk upon publication, quoting the article and volume/issue details
- § Please note that regardless of the form in which they are acquired, reprints should not be resold, nor further disseminated in electronic or print form, nor deployed in part or in whole in any marketing, promotional or educational contexts without authorization from Wiley. Permissions requests should be directed to <mailto:permreq@wiley.co.uk>

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

SARA BRUSCONI^a, SILVIA BERTOCCHI^a, BARBARA RENAI^a, MASSIMILIANO SCALICI^b, CATHERINE SOUTY-GROSSET^c and FRANCESCA GHERARDI^{a,*}

^a *Università degli Studi di Firenze, Dipartimento di Biologia Animale e Genetica, Via Romana 17, 50125 Firenze, Italy*

^b *Università degli Studi Roma Tre, Dipartimento di Biologia, Viale G. Marconi 446, 00146 Roma, Italy*

^c *Université de Poitiers, Laboratoire de Génétique et Biologie des Populations de Crustacés, UMR CNRS 6556, 40 Avenue du Recteur Pineau, F-86022 Poitiers cedex, France*

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.

Copyright © 2008 John Wiley & Sons, Ltd.

*Correspondence to: Francesca Gherardi, Università degli Studi di Firenze, Dipartimento di Biologia Animale e Genetica, Via Romana 17, 50125 Firenze, Italy. E-mail: francesca.gherardi@unifi.it

Received 26 April 2007; Revised 29 August 2007; Accepted 28 October 2007

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; Largiardè *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.

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 km².

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): $W = 6 \times 10^{-5} CL^{3.46}$ for males and $W = 310^{-4} \times CL^{2.96}$ 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

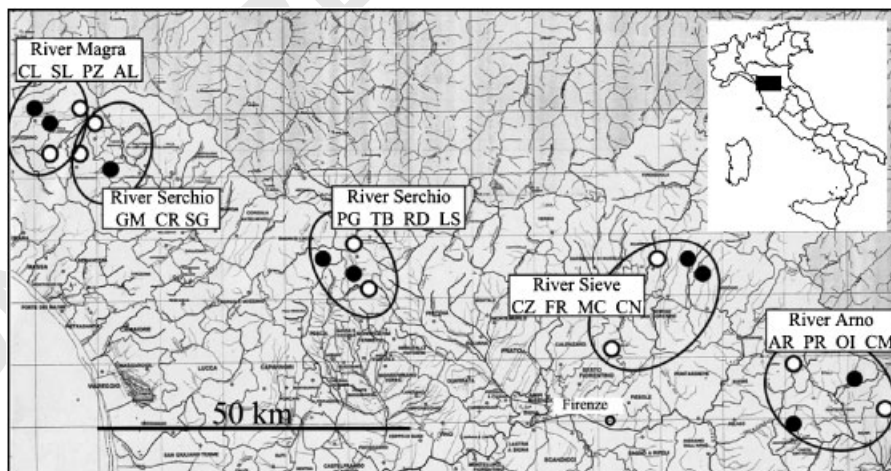


Figure 1. The study area in Tuscany that includes four catchments (Arno, Magra, Serchio, and Sieve). Streams (nine) with extant populations of *A. italicus* and streams where populations became extinct at least 5 years before the study (10) are denoted by black and white dots, respectively. Stream

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

5	Stream	Basin	pH	O ₂	NO ₃ ⁻	NO ₂ ⁻	Ca ²⁺	T	C	Speed	Width	Depth
7	With Crayfish (WI)											
7	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
9	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
11	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
13	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
15	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
17	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
19	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
21	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
23	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

25
 27 of the overall size-frequency distribution. The program
 29 provides values for each Gaussian component, i.e. means,
 31 standard deviations, numbers of individuals per size class,
 33 regression lines (and the respective R^2), and separation index
 35 values (SI) for each adjacent group. In particular, SI denotes
 37 when two adjacent Gaussians can be separated, i.e. $\text{SI} \geq 2$
 39 (Sparre and Venema, 1996). In a univoltine population, where
 41 SI values decrease below 2, the last class (composed of a few
 43 individuals) is included in the previous component. At the end
 45 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

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

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

$$5 \quad t_{max} = (3/k) + t_0$$

7 Characteristics of the habitat

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

14 The width and depth of the wet-bed were measured for each
15 stream every 5-m transect within each reach. The habitat was
16 characterized by recording the percentage of tracts with
17 laminar water flow, and the numbers of ponds and riffles. In
18 two adjacent 15-m transects of each reach, shelter occurrence
19 was assessed by counting the number of crevices in the banks,
20 roots, and boulders, and the substrate composition was
21 analysed in a 1 × 1 m metal frame divided into 16 equal
22 squares launched five times randomly. Inside each square,
23 estimates were made by eye of the percentage area covered by
24 silt, sand (<2 mm diameter), gravel (2–64 mm), cobble (65–
25 256 mm), boulder (>256 mm), and bedrock (fixed rock), and
26 the occurrence of plant detritus (composed of leaves and wood
27 pieces), moss, and periphyton.

28 At the end of each survey, a sample of macroinvertebrates in
29 each reach was collected by kicking and the use of a standard
30 net (mesh size: 290 µm). The taxa occurring in each sample
31 were determined in the laboratory following Campaioli *et al.*
32 (1998) and Ghetti (1997).

33 The degree of environmental integrity was assessed by
34 applying the Fluvial Functionality Index (IFF), a monitoring
35 instrument promoted in 2000 by ANPA (today APAT, the
36 Italian agency for the protection of the environment) and listed
37 in the technical paper of the Water Framework Directive

39 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),
40 number of males (M) and females (F), sex ratio, juvenile/adult ratio (J/A), catch per unit effort (CPUE) (individuals min^{-1}), density (individuals
41 m^{-2}), and biomass (g m^{-2})

43 Stream	N	M	F	Sex ratio	J/A	CPUE	Density	Biomass
44 SG	173	96	77	0.56	0.22*	1.44	0.27	1.61
45 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
47 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
49 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
51 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).

(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–Smirnov 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 thelohanian 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

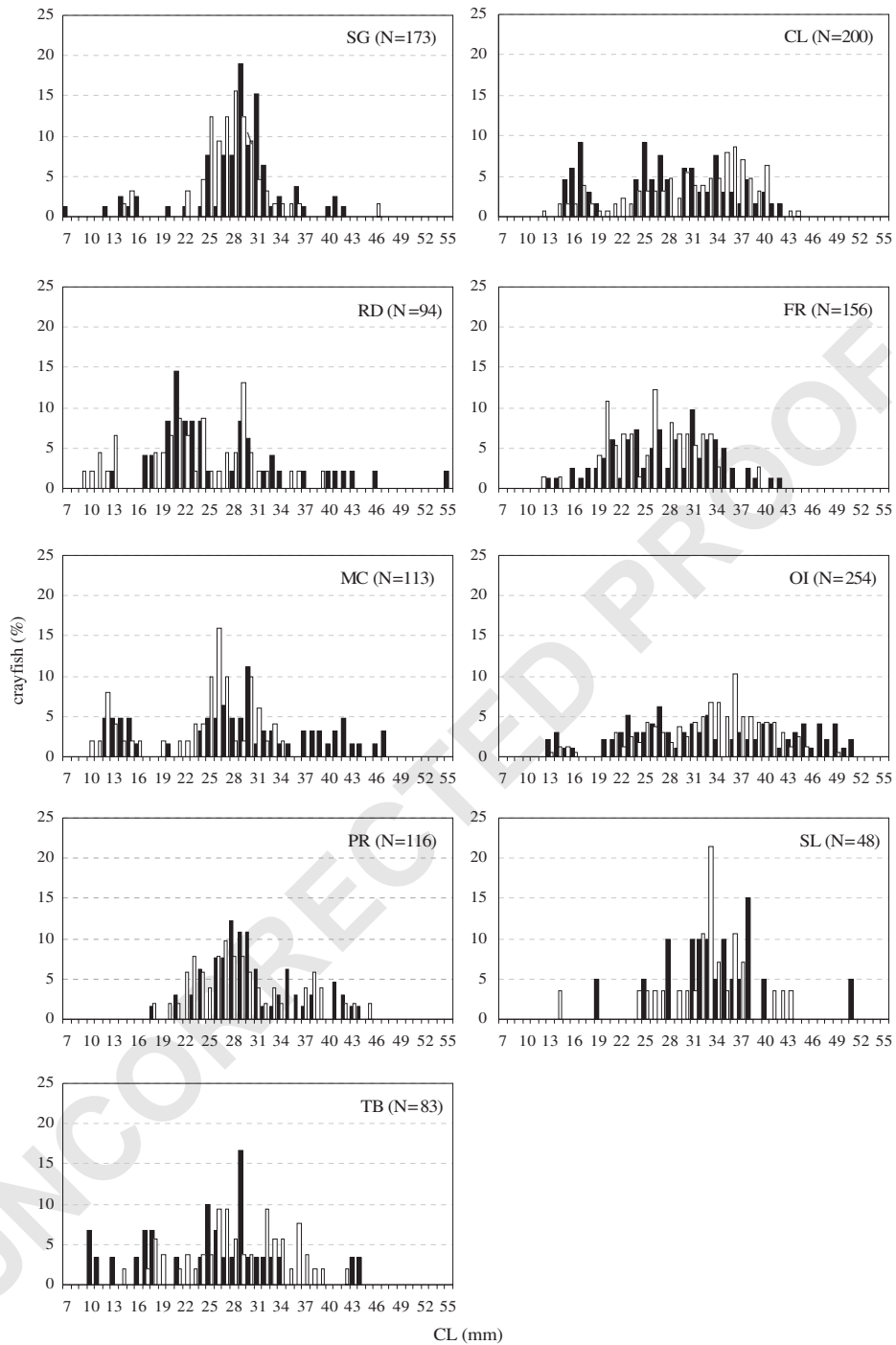


Figure 2. Size frequencies of males (black) and females (white) for each of the nine study streams (stream names, here abbreviated, are given in Table 1).

1 not for the interaction sex \times stream ($F = 1.73$, $df = 2, 1227$,
 3 $p = 0.14$). This analysis showed that two streams (SL and OI)
 5 contained populations with larger individuals, while the
 7 populations of RD and TB were composed of significantly
 9 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

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

Stream	Age	Males				Females			
		<i>N</i>	Mean CL (SD)	R^2	SI	<i>N</i>	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
21	50					5	36.37 (2.20)	0.7	2.11
23 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
25						13.89	28.54 (1.57)	0.46	3.85
						0.82	32.5 (0.7)	1	3.49
27 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
29 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
35 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
37	50	17.850	46.44 (2.31)		3.57	42.17	38.85 (2.404)	0.70	2.58
39 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
41						7	38 (1.1)	1	5.36
						3	44.17	0.75	0.56
43 SL ⁺	28	16	35.5 (1.2)		20	13	33 (1.18)	0.76	
						5.19	36.36 (0.623)	1	3.72
45 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
49						7.97	31.5	1	3.09
						2.67	34.45	1	3.57

51 * 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.

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

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

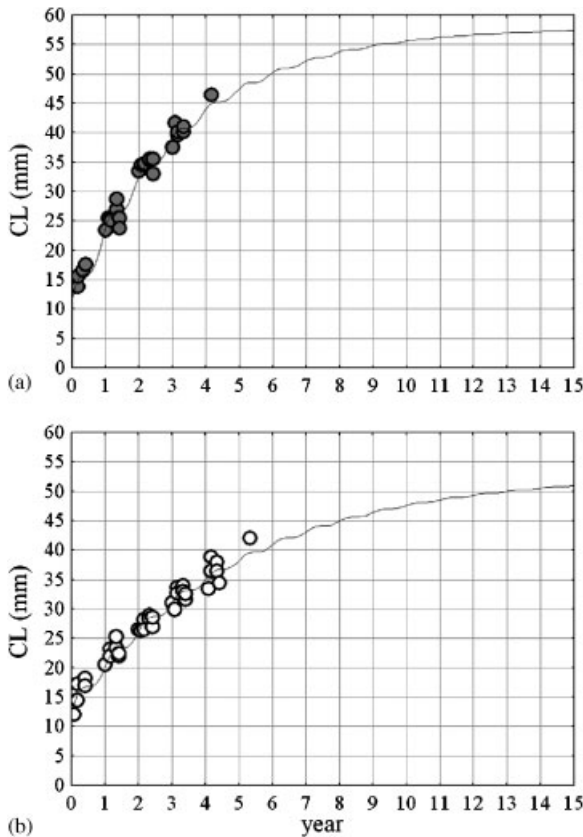


Figure 3. Growth curves for males (a) and females (b). Data from different populations have been pooled for the analysis.

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

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 ($\leq 5 \text{ g m}^{-2}$) and abundant ($> 5 \text{ g m}^{-2}$) 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

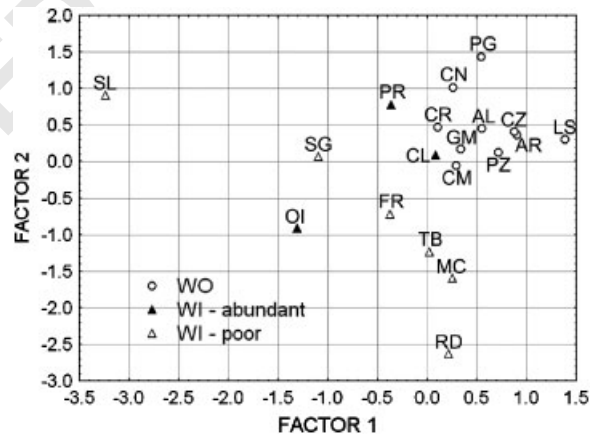


Figure 4. Scatterplot obtained from the abundance of plant detritus, moss, and periphyton compared for streams WO and streams WI with abundant (biomass $> 5 \text{ g m}^{-2}$) and poor (biomass $\leq 5 \text{ g m}^{-2}$) crayfish populations. See Table 1 for the meaning of WO and WI and of stream abbreviations.

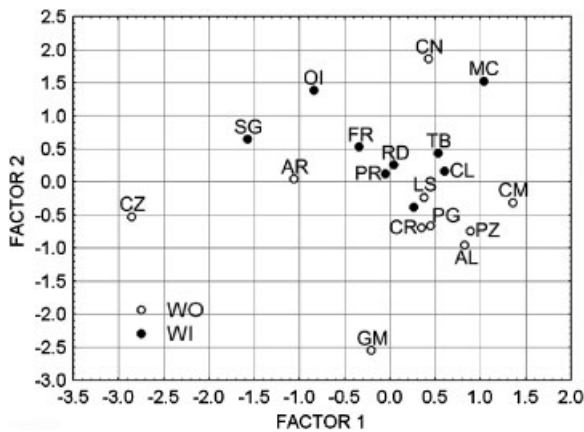


Figure 5. Scatterplot obtained from the composition of macroinvertebrate communities compared for streams WO and WI. See Table 1 for the meaning of WO and WI and of stream abbreviations.

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 *Thelohalium* 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 Bertalanffy'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 the macroinvertebrate communities, taken as a proxy of the past

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

7 Acting in concert with overexploitation, the loss of pristine
 8 riverine landscape seems to have been responsible for the local
 9 extinction of crayfish populations, at least in central Italy.
 10 Riparian vegetation is a source of allochthonous plant detritus,
 11 which is known to provide food to benthic consumers,
 12 including crayfish (Momot, 1984; Richardson, 1991; Nakano
 13 *et al.*, 1999; Usio, 2000). In fact, although adult *A. italicus*
 14 seem to prefer moss in laboratory choice experiments
 15 (Gherardi *et al.*, 2004), when their foraging behaviour was
 16 recorded in the field, they were most often observed visiting
 17 patches of coarse detritus and woody debris (Gherardi *et al.*,
 18 2001), vegetal material being the main item found in their gut
 19 (Gherardi *et al.*, 2004). These results clearly show the influence
 20 that the inputs from streamside vegetation exert on crayfish
 21 populations, as suggested for other members of stream
 22 community (Allan *et al.*, 2003). A significantly larger
 23 abundance of plant detritus was found in the streams with
 24 extant populations, when compared with the streams where
 25 crayfish have become extinct. Riparian vegetation also
 26 provides shade that maintains cool water temperatures and,
 27 together with fallen branches and large woody debris (C.
 28 Benvenuto *et al.*, unpublished data), offers shelter against
 29 predators.

30 Finally, the evidence that, in contrast to the adults, *A.*
 31 *italicus* juveniles most often use cobbles, seems to suggest that
 32 age classes are segregated in the habitat (Arrignon and Roche,
 33 1981; Foster, 1995; Smith *et al.*, 1996; Neveu, 2000; Reyjol and
 34 Roqueplo, 2002). Such segregation might have the effect of
 35 decreasing competition for shelters between age classes (Stein,
 36 1977; Momot, 1993; Lodge and Hill, 1994; Gherardi, 2002).
 37 Indeed, cobbles guarantee to juveniles the regular availability
 38 of periphyton and macroinvertebrates (Foster, 1995), both of
 39 which are the items most often found in their gut (Goddard,
 40 1988; Gherardi *et al.*, 2001).

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

50 As a consequence of these results, retaining, enhancing, and
 51 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'Environnement'). 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).

ACKNOWLEDGEMENTS

This study was part of the Galileo project (2004–2005):
 'Environmental quality of freshwaters for the conservation
 and management of the threatened crayfish (genus
Austropotamobius'. Grants were provided by Un.D.A.Felix
 and the Italian Ministry of Agriculture (MIPAF, 'Sesto Piano
 Triennale per la Pesca e l'Acquacoltura').

REFERENCES

- Allan JD, Wipfli MS, Caouette JP, Prussian A, Rodgers J.
 2003. Influence of streamside vegetation on inputs of
 terrestrial invertebrates to salmonid food webs. *Canadian
 Journal of Fisheries and Aquatic Sciences* **60**: 309–320.
- Arrignon J, Roche B. 1981. Population of the crayfish
Austropotamobius pallipes pallipes Lereb. In a brook of
 Corsica, France. *Freshwater Crayfish* **5**: 229–238.
- Ardizzone GD, Belluscio A, Gravina MF, Somaschini A.
 1996. Colonization and disappearance of *Mytilus
 galloprovincialis* Lam. On an artificial habitat in the
 Mediterranean Sea. *Estuarine, Coastal and Shelf Science*
43: 665–676.

- 1 Avise JC, Ball RM. 1990. Principles of genealogical
concordance in species concepts and biological taxonomy.
3 In *Evolutionary Biology*, Futuyama D, Antonovics J (eds).
Oxford University Press: Oxford, UK; 45–67.
- 5 Baillie J, Groombridge B. 1996. *IUCN Red List of Threatened
Animals*. IULN: Gland, Switzerland.
- 7 Barbaresi S, Gherardi F. 2001. Daily activity in the white-
clawed crayfish, *Austropotamobius pallipes*: a comparison
9 between field and laboratory studies. *Journal of Natural
History* **35**: 1861–1871.
- 11 Bhattacharya CG. 1967. A simple method of resolution of a
distribution into Gaussian components. *Biometrics* **23**: 115–
135.
- 13 Bohl E. 1987. Comparative studies on crayfish brooks in
Bavaria (*Astacus astacus* L., *Austropotamobius torrentium*
15 Schr.). *Freshwater Crayfish* **7**: 287–294.
- 17 Brewis JM, Bowler K. 1982. The growth of the freshwater
crayfish *Austropotamobius pallipes* in Northumbria.
Freshwater Biology **12**: 187–200.
- 19 Campaioli S, Ghetti PF, Minelli A, Ruffo S. 1998. Manuale
per il riconoscimento dei macroinvertebrati delle acque dolci
italiane. Museo di Storia Naturale di Trento, Trento, Italy.
- 21 Chiesa S, Scalici M, Gibertini G. 2006. Occurrence of
allocthonous freshwater crayfishes in Latium (Central
23 Italy). *Bulletin Français de la Pêche et de la Pisciculture*
380–381: 883–902.
- 25 Cornell HV, Lawton JH. 1992. Species interactions, local and
regional processes and limits to the richness of ecological
27 communities: a theoretical perspective. *Journal of Animal
Ecology* **61**: 1–12.
- 29 Correia AM. 1993. Length-weight relationship for two
populations of red swamp crayfish, *Procambarus clarkii*
(Decapoda Cambaridae) from Portugal. *Freshwater Crayfish*
31 **9**: 442–450.
- 33 De Luise G. 1991. Diffusione, allevamento e ripopolamento in
Friuli del gambero d'acqua dolce. Chiandetti, Reana del
35 Rojale, Italy.
- 37 Demers A, Reynolds JD. 2002. A survey of the white-clawed
crayfish, *Austropotamobius pallipes* (Lereboullet), and of
water quality in two catchments of Eastern Ireland. *Bulletin
Français de la Pêche et de la Pisciculture* **367**: 729–740.
- 39 Douglas M, Lake PS. 1994. Species richness of stream stones:
an investigation of the mechanisms generating the species-
area relationship. *Oikos* **69**: 387–396.
- 41 Fidalgo MR, Carvalho AP, Santos P. 2001. Population
dynamics of the red swamp crayfish, *Procambarus clarkii*
43 (Girard, 1852) from the Aveiro Region, Portugal
(Decapoda, Cambaridae). *Crustaceana* **74**: 369–375.
- 45 Flint RW, Goldman CR. 1977. Crayfish growth in Lake
Tahoe: Effects of habitat variation. *Journal of the Fisheries
Research Board of Canada* **34**: 155–159.
- 47 Foster J. 1995. Factors influencing the distribution and
abundance of the crayfish *Austropotamobius pallipes*
49 (Lereboullet) in Wales and the Marches, UK. *Freshwater
Crayfish* **7**: 78–98.
- 51 Foster J, Turner C. 1993. Toxicity and field simulated farm
waste episodes to the crayfish *Austropotamobius pallipes*
(Lereboullet): elevated ammonia and reduced dissolved
oxygen concentrations. *Freshwater Crayfish* **9**: 249–258.
- Frankham R, Ballou JD, Briscoe DA. 2002. *Introduction to
Conservation Genetics*. Cambridge University Press:
Cambridge, UK.
- Fratini S, Zaccara S, Barbaresi S, Grandjean F, Souty-Grosset
C, Crosa G, Gherardi F. 2005. Phylogeography of the
threatened crayfish (genus *Austropotamobius*) in Italy:
implications for its taxonomy and conservation. *Heredity*
94: 108–118.
- Freeman BJ, Freeman MC. 1994. Habitat use by an
endangered riverine fish and implications for species
protection. *Ecology of Freshwater Fish* **3**: 49–58.
- Füreder L, Oberkofler B, Hanel R, Leiter J, Thaler B. 2003.
The freshwater crayfish in South Tyrol: heritage species and
bioindicator. *Bulletin Français de la Pêche et de la
Pisciculture* **370–371**: 79–95.
- Gayanilo FC, Sparre P, Pauly D. 1996. The FAO ICLARM
stock assessment tools (FiSAT) user's guide. *Computerized
Information Series (Fisheries)* **8**: 1–126.
- Gherardi F. 2002. Chpt. 7. Behaviour. In *Biology of
Freshwater Crayfish*, Holdich DM (ed). Blackwell: Oxford,
UK; 258–290.
- Gherardi F. 2006. Crayfish invading Europe: the case study of
Procambarus clarkii. *Marine and Freshwater Behaviour and
Physiology* **39**: 175–191.
- Gherardi F, Acquistapace P, Santini G. 2001. Foraging in the
white-clawed crayfish, *Austropotamobius pallipes*—a
threatened species. *Archiv für Hydrobiologie* **152**: 339–351.
- Gherardi F, Acquistapace P, Santini G. 2004. Food selection
in freshwater omnivores: a case study of crayfish
Austropotamobius pallipes. *Archiv für Hydrobiologie* **159**:
357–376.
- Gherardi F, Cenni F, Crudele G, Mori M. 2002a. Infestation
rate of branchiobdellids in a population of *Austropotamobius
pallipes* from a stream in central Italy: preliminary results.
Bulletin Français de la Pêche et de la Pisciculture **367**:
785–792.
- Gherardi F, Holdich DM. 1999. *Crayfish as Europe as Alien
Species. How to Make the Best of a Bad Situation?* A.A.
Balkema: Rotterdam, The Netherlands.
- Gherardi F, Smietana P, Laurent P. 2002b. Interactions
between non-indigenous and indigenous crayfish
species. *Bulletin Français de la Pêche et de la Pisciculture*
367: 899–907.
- Gherardi F, Villanelli F, Dardi P. 1997. Behavioral ecology of
the white-clawed crayfish *Austropotamobius pallipes* in a
Tuscan stream: preliminary results. *Freshwater Crayfish* **11**:
182–193.
- Ghetti PF. 1997. Indice Biotico Estesio (I.B.E.). I
macroinvertebrati nel controllo della qualità degli ambienti
di acque correnti. Manuale di applicazione. Provincia
Autonoma di Trento: Trento, Italy.
- Goddard JS. 1988. Food and feeding. In *Freshwater
Crayfish: Biology Management and Exploitation*,
Holdich DM, Lowery RS (eds). Croom Helm: London,
UK; 145–166.

- 1 Grandjean F, Cornuault B, Archambault S, Bramard M,
Otrebsky G. 2000. Life history and population biology of the
3 white-clawed crayfish *Austropotamobius pallipes*, in a brook
from the Poitou-Charentes Region (France). *Bulletin
5 Français de la Pêche et de la Pisciculture* **356**: 55–77.
- 6 Grandjean F, Frelon-Raimond M, Souty-Grosset C. 2002a.
7 Compilation of molecular data for the phylogeny of the
genus *Austropotamobius*: one species or several? *Bulletin
9 Français de la Pêche et de la Pisciculture* **367**: 671–680.
- 10 Grandjean F, Gouin N, Frelon M, Souty-Grosset C. 1998.
11 Genetic and morphological systematic studies on the
crayfish *Austropotamobius pallipes* (Decapoda: Astacidae).
Journal of Crustacean Biology **18**: 549–555.
- 12 Grandjean F, Harris DJ, Souty-Grosset C, Crandall KA.
13 2002b. Systematics of the European endangered crayfish
species *Austropotamobius pallipes* (Decapoda, Astacidae),
15 with a re-examination of the status of *Austropotamobius
berndhauseri*. *Journal of Crustacean Biology* **22**: 667–681.
- 16 Gutiérrez-Yurrita PJ, Ilhéu M, Montes C, Bernardo J. 1996.
17 Morphometrics of red swamp crayfish from a temporary
Marsh (Doñana National Park, Sw. Spain) and a temporary
19 stream (Pardiela stream, S. Portugal). *Freshwater Crayfish*
21 **11**: 384–393.
- 22 Holdich DM. 2002. Present distribution of crayfish in Europe
and some adjoining countries. *Bulletin Français de la Pêche
23 et de la Pisciculture* **367**: 611–650.
- 24 Holdich DM, Lowery RS. 1988. *Freshwater Crayfish: Biology,
25 Management and Exploitation*. Croom Helm: London, UK.
- 26 Holdich DM, Reeve ID. 1991. The distribution of freshwater
27 crayfish in the British Isles with particular reference to
crayfish plague, alien introductions and water quality.
29 *Aquatic Conservation: Marine and Freshwater Ecosystems*
1: 139–158.
- 30 Jay D, Holdich DM. 1981. The distribution of the crayfish,
31 *Austropotamobius pallipes*, in British waters. *Freshwater
32 Biology* **11**: 121–129.
- 33 Largiadèr CR, Herger F, Lortscher M, Scholl A. 2000.
34 Assessment of natural and artificial propagation of the
white-clawed crayfish (*Austropotamobius pallipes* species
35 complex) in the alpine region with nuclear and
37 mitochondrial markers. *Molecular Ecology* **9**: 25–37.
- 38 Lodge DM, Hill A. 1994. Factors governing species
39 composition, population size, and productivity of cool-
water crayfishes. *Nordic Journal of Freshwater Resources* **69**:
41 111–136.
- 42 Lörtscher M, Stucki TP, Clalüna M, Scholl A. 1997.
43 Phylogeographic structure of *Austropotamobius pallipes*
populations in Switzerland. *Bulletin Français de la Pêche et
44 de la Pisciculture* **347**: 649–661.
- 45 Lowery RS, Hogger JB. 1986. The effects of river engineering
and disease on a population of *Austropotamobius pallipes* in
47 the river Lea, UK. *Freshwater Crayfish* **6**: 94–99.
- 48 Lyons R, Kelly-Quinn M. 2003. An investigation into
49 disappearance of *Austropotamobius pallipes* (Lereboullet)
population in the headwaters of the Nore River, Ireland and
51 the correlation to water quality. *Bulletin Français de la Pêche
et de la Pisciculture* **370–371**: 139–150.
- Machino Y, Holdich DM. 2006. Distribution of crayfish in
Europe and adjacent countries: updates and comments.
Freshwater Crayfish **15**: 292–323.
- Mancini A. 1986. Astacicoltura. allevamento e pesca dei
gamberi d'acqua dolce. Edagricole, Bologna, Italy.
- Manganelli G, Favilli L, Fiorentino V. 2006. Taxonomy and
nomenclature of Italian white-clawed crayfish. *Crustaceana*
79: 633–640.
- Merella P, Alemany F, Carbonell A, Quetglas A. 1998. Fishery
and biology of Norway lobster *Nephrops norvegicus*
(Decapoda: Nephropidae) in Mallorca (western
Mediterranean). *Journal of Natural History* **32**: 1631–1640.
- Momot WT. 1984. Crayfish production: a reflection of
community energetic. *Journal of Crustacean Biology* **4**:
35–53.
- Momot WT. 1993. The role of exploitation in altering the
processes regulating crayfish populations. *Freshwater
Crayfish* **9**: 101–117.
- Nakano S, Miyasaka H, Kuhara N. 1999. Terrestrial-aquatic
linkages: riparian arthropod inputs alter trophic cascades in
a stream food web. *Ecology* **80**: 2435–2441.
- Nardi PA, Bernini F, Bo T, Bonardi A, Fea G, Ferrari S, Ghia
D, Negri A, Razzetti E, Rossi S. 2004. Il gambero di fiume
nella Provincia di Alessandria. Provincia di Alessandria,
Italy.
- Nascetti G, Andreani P, Santucci F, Iaconelli M, Bullini L.
1997. Struttura genetica di popolazioni italiane di gambero
di fiume (*Austropotamobius pallipes*) e strategie per la sua
conservazione. *Atti della Società Italiana di Ecologia* **18**:
205–208.
- Naura M, Robinson M. 1998. Principles of using River
Habitat Survey to predict the distribution of aquatic species:
an example applied to the native white-clawed crayfish
Austropotamobius pallipes. *Aquatic Conservation: Marine and
Freshwater Ecosystems* **8**: 515–527.
- Neveu A. 2000. Étude des populations d'*Austropotamobius
pallipes* (Crustacea, Astacidae) dans un ruisseau forestier de
Normandie. II. Répartition en fonction de la structure des
habitats: stabilité et variabilité au cours de cinq années.
Bulletin Français de la Pêche et de la Pisciculture **356**: 99–
122.
- Nyström P. 2002. Ecology. In *Biology of Freshwater Crayfish*,
Holdich, DM (ed). Blackwell: Oxford, UK; 192–235.
- Pauly D. 1980. On the interrelationships between natural
mortality, growth parameters and mean environmental
temperature in 175 fish stocks. *Journal du Conseil CIEM*
39: 175–192.
- Pauly D. 1981. The relationships between gill surface area
and growth performance in fish: a generalization of
Von Bertalanffy's theory of growth. *Meeresforschung* **28**:
251–282.
- Pauly D, Soriano-Bartz M, Moreau J, Jarre-Teichmann A.
1992. A new model accounting for seasonal cessation of
growth in fishes. *Australian Journal of Marine and
Freshwater Research* **43**: 1151–1156.
- Picchi S, Scalera R, Zaghi D. 2006. Il bilancio di LIFE Natura
in Italia — Indicazioni e prospettive per il futuro. Ministero

- dell'Ambiente e della Tutela del Territorio, Direzione Generale Protezione della Natura, Rome.
- 3 Pratten DJ. 1980. Growth in the crayfish *Austropotamobius pallipes* (Crustacea: Astacidae). *Freshwater Biology* **10**: 401–412.
- 5 Rabeni CF, Sowa SP. 1996. Integrating biological realism into habitat restoration and conservation strategies for small streams. *Canadian Journal of Fisheries and Aquatic Sciences* **53**: 252–259.
- 7 Renai B, Bertocchi S, Brusconi S, Grandjean F, Lebboroni M, Parinet B, Souty Grosset C, Trouilhé MC, Gherardi F. 2006. Ecological characterisation of streams in Tuscany (Italy) for the management of the threatened crayfish *Austropotamobius pallipes* complex. *Bulletin Français de la Pêche et de la Pisciculture* **380–381**: 1095–1114.
- 9 Reynolds JD, Gouin N, Pain S, Grandjean F, Demers A, Souty-Grosset C. 2002. Irish crayfish populations: ecological survey and preliminary genetic findings. *Freshwater Crayfish* **13**: 584–594.
- 11 Reyjol Y, Roqueplo C. 2002. Preferential habitat analysis of white-clawed crayfish, notably juveniles, in three brooks of Corrèze, France. *Bulletin Français de la Pêche et de la Pisciculture* **367**: 741–762.
- 13 Richardson JS. 1991. Seasonal food limitation of detritivores in a montane stream—an experimental test. *Ecology* **72**: 873–887.
- 15 Robson BJ, Chester ET. 1999. Spatial patterns of invertebrate species richness in a river: the relationship between riffles and microhabitats. *Australian Journal of Ecology* **24**: 599–607.
- 17 Salvidio S, Mori M, Lattes A, Galli L, Arillo A. 2002. The freshwater crayfish *Austropotamobius pallipes* (Lereboullet, 1858) in Liguria, NW Italy: implications for management at the regional level. *Bulletin Français de la Pêche et de la Pisciculture* **367**: 663–670.
- 19 Salvidio S, Delaugerre M. 2003. Population dynamics of the European leaf-toed gecko (*Eleuptyes europaea*) in NW Italy: implications for conservation. *Herpetological Journal* **13**: 81–88.
- 21 Santucci F, Iaconelli M, Andreani P. 1997. Allozyme diversity of European freshwater crayfish of the genus *Austropotamobius*. *Bulletin Français de la Pêche et de la Pisciculture* **347**: 663–676.
- 23 Scalici M, Gibertini G. 2005. Can *Austropotamobius italicus meridionalis* to be used as a monitoring instrument in Central Italy? Preliminary observations. *Bulletin Français de la Pêche et de la Pisciculture* **376–377**: 613–625.
- 25 Scalici M, Gherardi F. 2007. Structure and dynamics of an invasive population of the red swamp crayfish (*Procambarus clarkii*) in a Mediterranean wetland. *Hydrobiologia* **583**: 309–319.
- 27 Simberloff D. 1988. The contribution of population and community biology to conservation science. *Annual Review of Ecology and Systematics* **19**: 473–511.
- 29 Smietana P, Krzywosz T. 2006. Determination of the rate growth of *Pacifastacus leniusculus* in lake Pobledzie, using polymodal length-frequency distribution analysis. *Bulletin Français de la Pêche et de la Pisciculture* **380–381**: 1229–1243.
- 31 Smith GRT, Learner MA, Slater FM, Foster J. 1996. Habitats features important for the conservation of the native crayfish *Austropotamobius pallipes* in Britain. *Biological Conservation* **75**: 239–246.
- 33 Souty-Grosset C, Holdich DM, Noël PY, Reynolds JD, Haffner P. 2006. *Atlas of Crayfish in Europe*. Muséum national d'Histoire naturelle Paris (Patrimoines naturels, 64): France.
- 35 Sparre P, Venema SC. 1996. Introduction à l'évaluation des stocks de poissons tropicaux. Document technique sur les pêches, 306/1, Rev.1, FAO, Rome, Italy.
- 37 Stein RA. 1977. Selective predation, optimal foraging, and the predator-prey interaction between fish and crayfish. *Ecology* **58**: 1237–1253.
- 39 Sutherland WJ. 1998. The importance of behavioural studies in conservation biology. *Animal Behaviour* **56**: 801–809.
- 41 Townsend CR, Hildrew AG. 1994. Species traits in relation to a habitat template for river systems. *Freshwater Biology* **31**: 265–275.
- 43 Trontelj P, Machino Y, Sket B. 2005. Phylogenetic and phylogeographic relationship in the crayfish genus *Austropotamobius* inferred from mitochondrial COI gene sequences. *Molecular Phylogenetics and Evolution* **34**: 212–226.
- 45 Trouilhé MC, Ricard F, Parinet B, Grandjean F, Souty-Grosset C. 2003. Management of the white-clawed crayfish (*Austropotamobius pallipes*) in Western France: abiotic and biotic factors study. *Bulletin Français de la Pêche et de la Pisciculture* **370–371**: 97–114.
- 47 Trouilhé MC, Souty-Grosset C, Grandjean F, Parinet B. 2006. Physical and chemical water requirements of the white-clawed crayfish (*Austropotamobius pallipes*) in western France. *Aquatic Conservation: Marine and Freshwater Ecosystems*. DOI: 10.1002/aqc793.
- 49 Usio N. 2000. Effects of crayfish on leaf processing and invertebrate colonisation of leaves in a headwater stream: decoupling of a trophic cascade. *Oecologia* **124**: 608–614.
- 51 Usio N. 2007. Endangered crayfish in northern Japan: distribution, abundance and microhabitat specificity in relation to stream and riparian environment. *Biological Conservation* **134**: 517–526.
- Vigneux E, Thibault M, Marnell F, Souty-Grosset C. 2002. National legislation, EU directives and conservation. *Bulletin Français de la Pêche et de la Pisciculture* **367**: 887–898.
- Von Bertalanffy L. 1938. A quantitative theory of organic growth. *Human Biology* **10**: 181–213.
- Westman K. 1985. Effects of habitat modification on freshwater crayfish. In *Habitat Modifications and Freshwater Fisheries*, Alabaster JS (ed). Butterworths: London, UK; 245–255.
- Wetherall JA. 1986. A new method for estimating growth and mortality parameters from length frequency data. *ICLARM Fishbyte* **4**: 12–14.

