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## Microhabitat use by the white-clawed crayfish in a Tuscan stream

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(Accepted 8 November 2007)

Habitat modification, pollution, overfishing, poaching, competition from non-indigenous species, and diseases have led to the extinction in Europe of many populations of indigenous crayfish. Under the rationale that any programme of reintroduction should be preceded by a thorough understanding of habitat requirements of the species of concern, we studied the microhabitat use of an *Austropotamobius pallipes* population in Tuscany, central Italy. Microhabitat use was assessed for water depth, current velocity, substrate, percentages of boulders, underwater tree roots, and in-stream vegetation cover. Results show that *A. pallipes*' habitat use is size-partitioned. Smaller individuals mostly occupy stream edges in shallow waters with submerged roots, whereas larger individuals use deeper waters, often associated with boulders. Crayfish spatial distribution is restricted mostly to the microhabitats characterised by extensive cover and slow current velocity. The study highlights the importance of habitat heterogeneity and cover elements for the protection of this indigenous species.

**Keywords:** *Austropotamobius pallipes*; biodiversity conservation; crayfish; freshwater streams; microhabitat use

### Introduction

There is a large consensus today that the most efficient way to protect a species is to conserve its habitat. Habitats can be viewed as a mosaic of small-scale patches of microhabitats (e.g. Robson and Chester 1999), their diversity being often a function of the heterogeneity of these constituent patches. The presence and abundance of many species are strongly affected by several features of microhabitats (e.g. Townsend and Hildrew 1994). This is especially true when the targets of conservation programmes are rare, threatened, or endangered species. It is therefore important not only to assess their spatial distribution at regional and local scales, but also to acquire a thorough understanding of their microhabitat use, so that suitable areas for their reintroduction can be correctly defined (e.g. Sutherland 1998).

In Europe, habitat modification, pollution, overfishing, poaching, competition from non-indigenous species, and the spread of the “crayfish plague” have led to the local extinction of several populations of a number of species of indigenous crayfish (Lindqvist and Huner 1999). Survival of the white-clawed crayfish, *Austropotamobius pallipes* (Lereboullet, 1858), is seriously threatened given that the species is stenoecic with both low resistance (the crayfish is highly vulnerable to disturbances) and low resilience (it is one of a suite of species that hardly recover from major disturbances as a consequence of their slow growth and low fecundity).

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This species is currently distributed 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). The drastic reduction of the geographical range of this species began in the 1860s and continues today (reviewed in Souty-Grosset et al. 2006). For this reason, *A. pallipes* has been given protected status by the European Union Habitats and Species Directive (92/43/EEC, European Council 1992), which designated it as a species whose conservation requires designation of special areas (Annex II) and whose taking in the wild and exploitation may be subject to management measures (Annex V).

Recently, molecular genetic techniques showed a complex situation in the taxonomy of *Austropotamobius*. The taxon is highly structured genetically, and is probably best considered as a species complex (Souty-Grosset et al. 2006). Santucci et al. (1997), Grandjean et al. (2000, 2002) and Fratini et al. (2005) have identified two species in Italy: *A. italicus* from Piedmont to Calabria and *A. pallipes* in north-western Italy (Gherardi et al. 1999). Based on genetic data, the population analysed in this survey is the *A. italicus* species, but since the complex is still under investigation, we prefer to use the original species epithet *pallipes* to be consistent with previous works performed on the same population (e.g. Barbaresi and Gherardi 2001).

This study is part of a more extensive project aimed at identifying sites suitable for *A. pallipes* complex reintroductions. Our main objective was to identify the key features of this species' microhabitat use.

## Methods

### *Study site*

Data were collected during May 2000 in Fosso di Farfereta, a third-order stream in Tuscany (Italy), on the southern side of the Tuscan-Emilian Apennines, 450 m above sea level. This stream was selected because it is known to support stable, abundant, and healthy populations of *A. pallipes*, and is relatively unpolluted and unmodified by anthropogenic intervention (Barbaresi and Gherardi 2001). Data were collected in late spring when individuals are particularly active (Gherardi et al. 1998).

Four consecutive surveys were conducted along a 100 m-long stream-reach (3.3 m width, 0–150 cm water depth) chosen for its high habitat heterogeneity. Water temperature varied from 14 to 14.7°C, pH from 6.5 to 7.3, and conductivity from 454 to 457 µS/cm. Dissolved oxygen content ranged from 7.20 (79.7%) to 9.98 mg/l (98.1%) (Multi-line WTW Instruments). Water hardness was about 70 mg/l as calcium bicarbonate.

Riparian vegetation comprised mainly shrubs of eglantine (*Rosa canina* L.), elder (*Sambucus nigra* L.), fescue (*Festuca heterophylla* Lam.), and trees (*Corylus avellana* L., *Carpinus betulus* L., *Acer campestre* L.) with roots extending into the water. Shadowing canopy produced an abundant leaf detritus that accumulated in pools. Potential predators of crayfish in the stream were fish (trout, *Salmo trutta* L., and chub, *Leuciscus cephalus* L.) and birds (tawny owl, *Strix aluco* L., little egret, *Egretta garzetta* L., grey heron, *Ardea cinerea* L., and corvids of the genus *Corvus*).

### *Crayfish microhabitat assessment*

Quantitative estimates of the crayfish microhabitat use were made during four consecutive days between 21:00 and 24:00 h. The survey was conducted by two

experienced observers wading slowly in the upstream direction. Crayfish were not captured to avoid disturbance but were assessed visually using a face mask. The size of each individual observed (total length, TL) was estimated by comparing the crayfish size and the size, subsequently measured, of a static object in the stream, following Fausch and White (1981). This estimation technique is certainly prone to errors, but, due to the broadness of the size categories under study (see below), we feel confident that the margin of error incurred will not bias the overall size categories. Sexes were not determined.

In each microhabitat where crayfish was observed, water depth (cm) and current velocity (m/s) were measured. Dominant substrate, percentage of boulders >30 cm, in-stream vegetation cover (%), and underwater tree roots were also evaluated within a 250 cm<sup>2</sup> quadrat around the location where the crayfish was found. Substrate categories were based on six classes: mud (1), sand (2), gravel (3), pebble/cobble (4), boulder (5), and bedrock (6) (*sensu* Giller and Malmqvist 1998). Crayfish were deemed to be in proximity to the bank if they were recorded at a distance <50 cm from it; otherwise, crayfish were recorded in the stream bed. Water depth measurement was made using a metre stick and current velocity was obtained using a Valeport 801 electromagnetic flow meter with an accuracy of  $\pm 0.5\%$  of reading. Current velocity was assessed with average measurements at 60% water column depth for depths <75 cm (Bovee 1986). When depth exceeded 75 cm, measurements were taken at 20 and 80% water depth (Bovee 1986). This method is the technique commonly used to assess an organism's habitat preference for current velocity, i.e. comparing data from an individual's location with data on habitat availability. Moreover, the consistency of measurement allows for comparisons between areas on the basis of mesohabitat types. Current velocity at the exact crayfish position was also measured and taken into account in the canonical correspondence analysis performed (see below).

To determine habitat availability, microhabitat variables were measured in multiple cross-stream transects, covering all types of mesohabitats within the study reach. Total depth, average current velocity, substrate type, macrophyte cover, percentage of boulders and underwater tree root measurements were taken at 0.25–1.0 m intervals along each transect. A freehand sketch was performed for each transect in order to help the assessment of the availability of all mesohabitats.

### **Data analysis**

Following Crowe and Underwood (1998) and Olabarria et al. (2002), we adopt here the more neutral term “use”, rather than the often used “preference”, because we have no evidence that an active choice by crayfish can indeed account for the observed pattern of distribution. Microhabitat use was evaluated using Jacobs' index (1974), which is a derivation of Ivlev's electivity index:  $D = (r - p) / (r + p - 2rp)$ .  $D$  was used as an index of the crayfish use towards each class of environmental variables, with values ranging between a maximum of +1 (microhabitat always exploited) and -1 (microhabitat always avoided).  $r$  is the frequency of crayfish using that class of resource and  $p$  is its availability (frequency). In this way, we attempted to minimise any bias derived from differences in the availability of microhabitats. In order to distinguish differences in the microhabitat use between immature and mature crayfish (Lowery 1988), individuals were grouped, according to their total length, into class 1 (total length <60 mm) and class 2 (total length  $\geq$ 60 mm).

Statistical differences in the patterns of microhabitat use for each variable and between size classes were tested using a Chi-square test. We considered four depth classes (0–20, 20–60, 60–100, and >100 cm), five classes of current velocity (up to 0.05, 0.1, 0.3, 0.5, and >0.5 m/s) and the six categories of substrate as classified above. A canonical correspondence analysis (CCA; ter Braak 1990) was also performed to synthesise the relationship between crayfish size and environmental variables. CCA is an eigenvalue ordination technique usually adopted for direct gradient analysis. It is a constrained form of canonical analysis that uses a weighted-averaging algorithm (between species and between sites) combined with a multiple linear regression using microhabitat measurements as independent variables (Palmer 1993). Species are assumed to show a non-linear and unimodal relationship with environmental variables (following the “uni-modal response model”). Species and sites are displayed in a joint plot ordination diagram (triplot) where symbols (points) and arrows denote the microhabitat variables.

CCA was performed using CANOCO (ter Braak and Šmilauer 1988). To get a finer discrimination of segregation by size for this analysis, crayfish were grouped into three size classes: small (total estimated length <30 mm), medium (total estimated length 30–60 mm), and large (total estimated length ≥60 mm). Prior to analysis, crayfish numbers were transformed using natural logarithms [ $\log(x+1)$ ]. The best microhabitat variables (those retained for analysis) were identified by a forward selection procedure available in version 3.1 of CANOCO (ter Braak and Verdonschot 1995), using a cutoff point of 0.10 (Magnan et al. 1994). A Monte Carlo test (1000 permutations) on both the first axis eigenvalue and the trace (i.e. the sum of all canonical eigenvalues) was used to evaluate the significance of the effects under analysis (ter Braak 1990). CCA model variation was obtained by dividing the canonical eigenvalues by the total inertia, i.e. the sum of all the eigenvalues of a correspondence analysis of the crayfish matrix.

**Results**

A total of 265 crayfish was recorded. Their TL ranged between 15 and 95 mm, the most frequent size classes being between 40 and 70 mm (Figure 1). Crayfish density

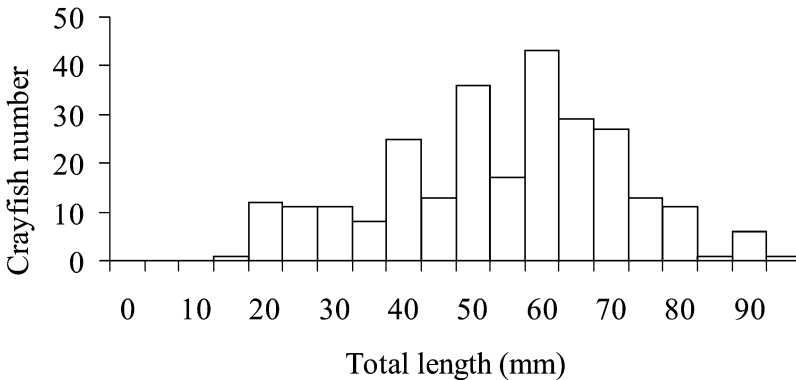


Figure 1. Frequency distribution of the estimated size (total length) of the study crayfish population.

varied between 0.3 and 2.9 individuals/m<sup>2</sup>, with higher densities at sites having a low current velocity (<0.1 m/s).

The study reach showed a high spatial heterogeneity, including all types of mesohabitat (pools, riffles, and runs) and substrate (Table 1). Boulders were more abundant proportionally than tree roots and vegetation cover (Figure 2).

The patterns of microhabitat use for each variable significantly differed between size classes, with the exception of substrate (Table 2). Larger crayfish (TL≥60 mm,

Table 1. Mean values, standard deviation (SD), minimum and maximum values for depth, current velocity, and substrate in the study area.

	Mean	SD	Minimum–maximum
Depth (cm)	41.20	34.60	2–150
Current velocity (m/s)	0.02	0.05	0–0.5
Substrate (1–6)	3.10	1.50	1–6

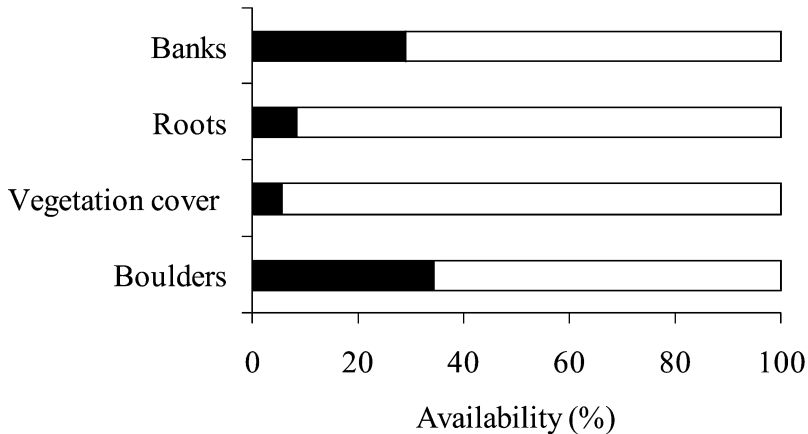


Figure 2. Mean percentages of availability (relative to the total studied area) of bank/bed, roots, macrophytes, and boulders.

Table 2. Statistical differences in microhabitat use ( $\chi^2$  frequencies of co-occurrence) between class 1 (<60 mm estimated total length) and class 2 (≥60 mm estimated total length) for each variable.

	$\chi^2$	df	P
Depth (cm)	46.6	3	<0.001
Current velocity (m/s)	129.1	4	<0.001
Substrate (1–6)	2.0	5	>0.1
Bank/stream bed	3.8	1	<0.05
Roots (%)	6.5	1	<0.05
Macrophyte cover (%)	7.3	1	<0.01
Boulders	9.2	1	<0.01

mean  $\pm$  2SE:  $68.22 \pm 1.48$ ,  $n=126$ ) were most often found in deeper waters and avoided very shallow ones ( $<20$  cm). Smaller crayfish (TL $<60$  mm, mean  $\pm$  2SE:  $41.24 \pm 1.94$ ,  $n=139$ ) exhibited an opposite pattern, avoiding waters deeper than 100 cm and more often using microhabitats with water depth ranging between 20 and 60 cm (Figure 3). Individuals seemed to avoid microhabitats with current velocity  $>0.1$  m/s, particularly riffles (i.e. shallow to moderately deep turbulent habitats). Although no significant differences were observed for substrate use, both size classes used mostly mud and gravel-pebble substrates (Figure 3). Smaller crayfish occurred in areas with abundant submerged roots, near banks (Figure 4), while the larger crayfish mostly used stream-bed microhabitats. Both size classes were found almost exclusively in microhabitats with a high vegetation cover. Boulders were preferentially used by larger crayfish (Figure 4).

The pattern of microhabitat use by the three size classes (small: TL $<30$  mm, mean  $\pm$  2SE:  $22.08 \pm 1.22$ ,  $n=24$  medium: TL=30–60 mm, mean  $\pm$  2SE:  $45.24 \pm 1.48$ ,  $n=115$ , and large: TL $\geq 60$  mm, mean  $\pm$  2SE:  $68.22 \pm 1.48$ ,  $n=126$ ), as shown by the CCA (Figure 5), revealed that depth and vegetation cover (roots and macrophytes) were the most prominent variables influencing crayfish use of microhabitats (Table 3). The first axis was defined by: water depth, percentage of roots, and microhabitat location in the stream (bank/bed) (Figure 5; Table 3). The CCA accounted for about 15% of the total variation in the crayfish size classes abundance ( $P<0.005$ ). The variance inflation of all selected variables was less than 20 (ter Braak 1990). This analysis produced a gradient of crayfish size classes from shallow habitats along the edges, with high cover values, to deeper stream-bed habitats. Small crayfish were associated with shallow edge microhabitats with roots and macrophytes, whereas medium and large crayfish were most often associated with deep waters located in the stream bed. Confirming the results of the previous analysis, the occurrence of smaller crayfish was negatively correlated with water depth and current velocity ( $r=0.41$  and  $0.28$ ,  $P<0.001$ , respectively) but was positively correlated with the cover percentage of roots ( $r=0.41$ ,  $P<0.001$ ), whereas

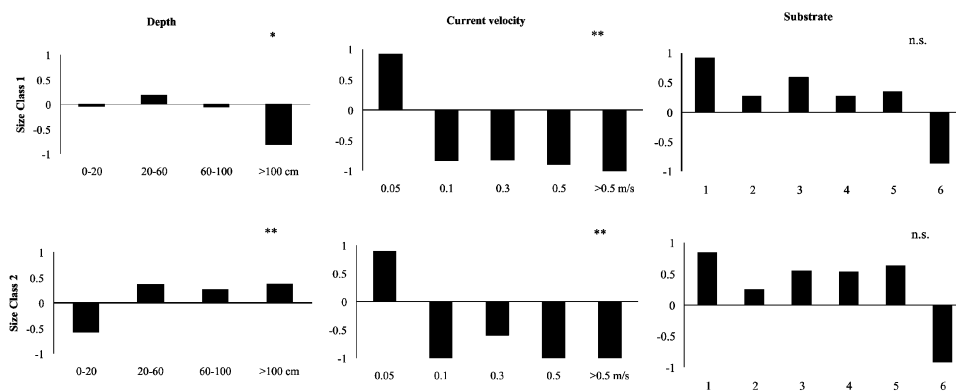


Figure 3. Microhabitat use by crayfish. Calculated electivity scores for water depth (cm), current velocity (m/s), and substrate (1, mud; 2, sand; 3, gravel; 4, pebble/cobble; 5, boulders; 6, bedrock) by class 1 ( $<60$  mm estimated total length) and class 2 ( $\geq 60$  mm estimated total length) individuals. Scores range from 1 (always used) to  $-1$  (always avoided); 0 denotes no use. Asterisk denotes significant deviation from availability (\* $P<0.05$ ; \*\* $P<0.001$ ).



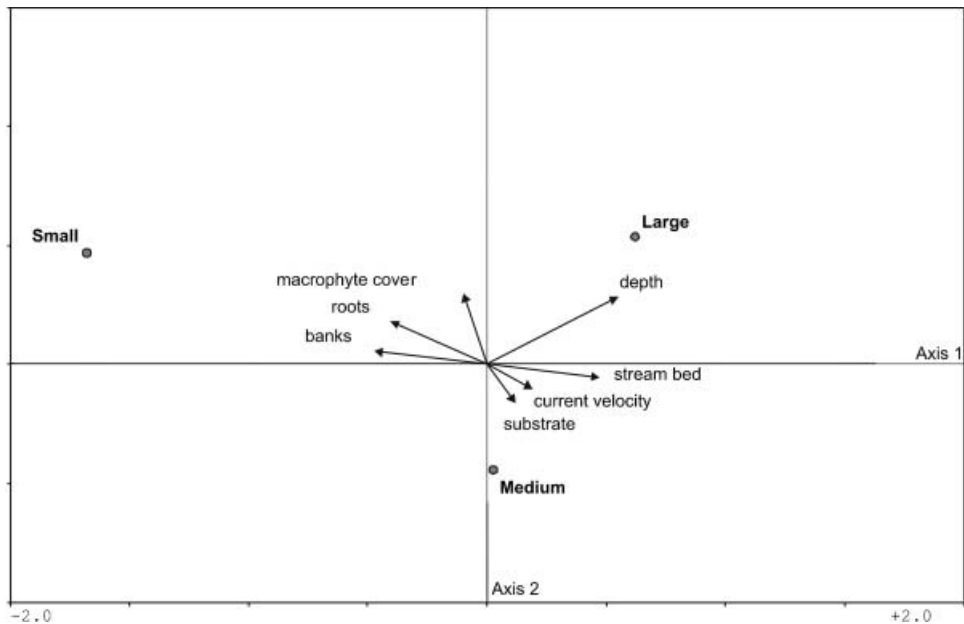
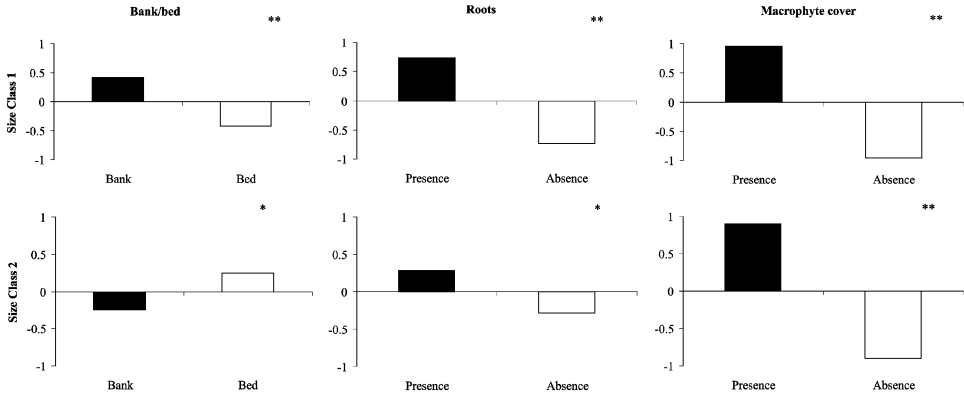


Figure 5. Canonical correspondence analysis ordination of three crayfish size classes (small, <30 mm; medium, 30–60 mm; large, ≥60 mm estimated total length) and environmental variables for the 265 microhabitats.

Table 3. Summary of statistics for the canonical correspondence analysis (CCA) relating crayfish abundance per size class to microhabitat variables (eigenvalues: axis 1=0.25; axis 2=0.052).

	Canonical coefficients		Correlation coefficients	
	Axis 1	Axis 2	Axis 1	Axis 2
Depth (m)	0.49**	0.37**	0.39**	0.13*
Current velocity (m/s)	0.18**	0.025	0.13*	-0.05
Substrate (1-6)	-0.24**	0.19	0.08	-0.08
Roots (%)	-0.27**	0.37*	-0.29**	0.09
Macrophyte cover (%)	-0.34**	0.30*	-0.07	0.14*
Bank	-0.19	0.11	-0.33**	0.027
Stream bed	0.00	0.00	0.33**	-0.027

\* $P < 0.05$ , \*\* $P < 0.001$ .

medium- and large-sized crayfish were positively correlated with current velocity ( $r=0.18$ ,  $P < 0.01$ ) and water depth ( $r=0.36$ ,  $P < 0.001$ ), respectively.

## Discussion

The study population of *A. pallipes* has a clear size-partitioned distribution in the relatively pristine stream it inhabits. Small individuals were found most frequently in stream edges characterised by shallow waters and a high availability of submerged roots, whereas large individuals use deeper waters, associated with boulders.

Several authors have recognised the role that riparian vegetation and its roots have as nursery sites for *A. pallipes* juveniles in the UK (Foster 1995; Smith et al. 1996; Naura and Robinson 1998). The strong affinity of the smaller crayfish for shallow waters has been also found in *Orconectes* spp. (Butler and Stein 1985; Gore and Bryant 1990; Kutka et al. 1996; Englund and Krupa 2000).

Similarly to the other crayfish species studied so far (e.g. *Orconectes propinquus* and *Austropotamobius torrentium*; Kutka et al. 1996; Streissl and Hödl 2004), *A. pallipes* avoids fast currents. On the contrary, this species often uses slow flow microhabitats with an extensive cover, possibly as a result of their feeding habits (Gherardi et al. 2001, 2004). Current velocity affects foraging movements in crayfish (Poff and Ward 1992) and controls their distribution across streams (Hart 1992).

The importance of substrate on crayfish distribution and microhabitat differentiation has been suggested by, for example, Payne (1984) and Streissl and Hödl (2004). However, our study focused for the first time on the relationship between used and available microhabitats and it supported the hypothesis that crayfish occupancy of a given substrate is independent of its availability (crayfish avoid microhabitats without boulders) but is related to crayfish size (larger crayfish are associated with larger substrates). Indeed, an appropriate substrate may provide shelters to protect crayfish from predation (Ilhéu et al. 2003) and to prevent their drift (M. Ilhéu, unpublished data).

It is a reasonable prediction that, within an optimal habitat, predation pressure will be reduced and food in reliable quantity and quality will be available, thereby imposing low energetic costs for the occupying individuals (Sih 1982; Werner et al.

1983). Therefore, differences in the microhabitat use between size classes may result from a balance between diverse (1) predation risks, (2) intraspecific interactions, and (3) feeding requirements.

Previous studies on other crayfish species have shown that predation pressure can lead crayfish to change their patterns of activity (Stein and Magnuson 1976) and to vary their habitat use (Stein 1977). It is also well documented that large individuals occur in deep waters whereas small ones are to be found in the shallows (Mather and Stein 1993; Dorn and Mittelbach 1999), this pattern reflecting the occurrence of predatory fish (Englund and Krupa 2000). Indeed, predation risks may vary across a depth gradient as a function of the prey size and predator type (Englund and Krupa 2000; Keller and Moore 2000). Small crayfish experience greater risk from fish than from terrestrial predators and therefore they may be confined to shallower waters (large fish tend to avoid shallow areas; see e.g. Lonzarich and Quinn 1995). Additional protection for small crayfish may be provided by the structural complexity of the stream edges produced by the highly ramified and fibrous roots of the trees from the riparian corridor. Large crayfish are more vulnerable to terrestrial predators, which are less efficient in deep waters (reviewed in Power 1987). Here, boulders can act as refuge against aquatic predators.

The size-partitioned distribution shown in this study may also be the result of intraspecific competition, i.e. aggressive behaviour and cannibalistic predation by larger individuals on smaller size classes, as extensively reported in *Orconectes propinquus* (Capelli 1980; Mather and Stein 1993) and *Procambarus clarkii* (Gutiérrez-Yurrita et al. 1998). Dominance of crayfish individuals is strongly size-dependent; therefore, large crayfish can displace the smaller conspecifics from deeper waters (Vorburger and Ribi 1999; Englund and Krupa 2000) and/or small crayfish can use sheltered microhabitats along the stream edges to avoid the risk of being cannibalised by large conspecifics. In this scenario, habitat complexity is crucial to reduce intraspecific aggression (Tricarico et al. 2005), which is particularly intense at high crayfish densities (Lynda et al. 2004).

Finally, food availability, associated with a diet shift during ontogeny, may also influence *A. pallipes*' microhabitat use. Crayfish switch from a carnivorous diet when they are young (Ilhéu and Bernardo 1993; Gherardi et al. 2004) to a diet composed mostly of vegetal detritus when they reach maturity (Bernardo and Ilhéu 1994; Nyström et al. 1996). Different food resources are often differentially distributed across depth and current gradients (Englund and Krupa 2000). Deep pools contain a large amount of vegetal detritus and can support their occupancy by large crayfish (Reynolds 1979); soaked leaf litter acts as a direct source of food and provides a suitable medium for the growth of microbes, fungi, and several macroinvertebrates. Tree roots can also act as debris-traps by retaining allochthonous leaves (Momot 1984).

Microhabitat use may differ among stream types namely due to variations in morpho-climatic conditions, predation pressure, crayfish density, and population structure. The co-occurrence of different species can also lead to microhabitat partitioning (DiStefano et al. 2003). Although this study presents data from a single population, the sampled location is representative of a well-defined stream type in central Italy, currently or previously inhabited by populations of the *A. pallipes* complex (Renai et al. 2006). Moreover, similar results on microhabitat use have been observed for another white-clawed crayfish population in central Italy (S. Cataudella

and C. Benvenuto, unpublished data), suggesting a consistent pattern. Considering the limited distribution of well-preserved *A. pallipes* complex populations, results from this study may be relevant to infer information on the habitat requirements of the species within similar stream types, mainly for conservation and rehabilitation proposes.

In synthesis, results from this study emphasise the importance of preserving, enhancing or restoring bank-side features, including riparian vegetation, and habitat heterogeneity of streams when the aim is to protect or to reintroduce indigenous crayfish species. This conclusion has obvious relevant implications for conservation and management purposes, including the application of the Water Framework Directive (WFD; European Commission 2000). The WFD requires Member States of the European Union to assess, monitor, and, where necessary, improve the ecological quality status of their waters. This landmark piece of environmental legislation seeks to achieve at least “good ecological status” for all surface water by 2015 and therefore all information on species’ habitat requirements are essential.

Wherever conditions allow for rehabilitation, needs and priority should be selected in order to re-establish habitat complexity and improve land-use management, with the aim of successfully reintroducing white-clawed crayfish populations. Additionally, efforts should be made to minimise global anthropogenic disturbance, and to control for invasion of non-indigenous species.

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