

OCCURRENCE OF ALLOCHTHONOUS FRESHWATER CRAYFISHES IN LATIUM (CENTRAL ITALY)

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ABSTRACT

The introduction of animal species, transfaunation and habitat destruction are among the greatest threats for biodiversity. In fact, in the water systems of Latium the occurrence of allochthonous crayfishes *Procambarus clarkii*, *Orconectes limosus* and *Astacus leptodactylus* has been observed. The distribution of these species in Latium highlighted some differences between them: *Orconectes limosus* and *Astacus leptodactylus* are present in restricted areas, while *Procambarus clarkii* has a wide distribution throughout the region. The present study confirmed their ecological plasticity, strong capability of invasion and adaptation to new environments of all three species, due to their rapid growth, utilisation of different trophic resources and the frequency and length of their reproduction periods. These aspects can cause irreversible ecologic and economic damage. The eradication of *Procambarus clarkii* would be a difficult task, because of its wide distribution and the many records of the species, especially in the Province of Latina, where complex field irrigation systems are found. All the agencies for environmental protection should increase control activities, with a serious field effort. As regards the other two species, eradication would be easier due to their limited distribution. It is important to avoid the spreading of these species; this can be done through public opinion awareness-raising campaigns and a continuous control on the region, increasing research and supporting it with new laws concerning the introduction and treatment of alien species.

Key-words: allochthonous freshwater crayfishes, *Procambarus clarkii*, *Orconectes limosus*, *Astacus leptodactylus*, central Italy.

PRÉSENCE D'ÉCREVISSES ALLOCHTONES DANS LE LATIUM (ITALIE CENTRALE)

RÉSUMÉ

L'introduction d'espèces animales, la transfaunation et la destruction de l'habitat font partie des menaces les plus graves sur la biodiversité. La présence des écrevisses allochtones *Procambarus clarkii*, *Orconectes limosus* et *Astacus leptodactylus* a été observée dans les eaux douces du Latium. L'étude de la distribution de ces espèces dans le Latium fait ressortir quelques différences : *Orconectes limosus* et *Astacus*

leptodactylus sont limitées à des zones restreintes, tandis que *Procambarus clarkii* a une large distribution sur tout le territoire. Le présent travail confirme la plasticité écologique et la forte capacité d'invasion et d'adaptation à de nouveaux environnements de ces trois espèces, dues à leur croissance rapide, à l'utilisation de différentes ressources trophiques ainsi qu'à la fréquence et à la durée de leurs périodes de reproduction. Ces aspects peuvent engendrer des dommages écologiques et économiques irréversibles. L'éradication de *Procambarus clarkii* serait une tâche difficile, à cause de sa large distribution et des nombreux signalements de cette espèce, surtout dans la province de Latina, où des systèmes complexes d'irrigation sont présents. Toutes les agences de protection de l'environnement devraient augmenter leurs activités de contrôle, avec un effort particulier sur le terrain. Concernant les deux autres espèces, l'éradication serait plus facile du fait de leur distribution restreinte. Il est important de limiter la diffusion de ces espèces ; ceci peut être réalisé grâce à des campagnes de sensibilisation du public et à un contrôle permanent et efficace en favorisant la recherche et par de nouvelles réglementations sur l'introduction et le traitement des espèces allochtones.

Mots-clés : écrevisses allochtones, *Procambarus clarkii*, *Orconectes limosus*, *Astacus leptodactylus*, Italie centrale.

INTRODUCTION

The term "allochthonous" refers to an alien species, which occurs in a given area or ecosystem and that can be found outside its historical distributional area as a result of dispersion caused by man (transfaunation) and not by the intrinsic potential of the species (SCALERA, 2001).

The introduction of animal species (together with habitat destruction) is one of the greatest threats for biodiversity (GENOVESI, 2002). Biological invasions give rise to numerous severe consequences not only at an ecological level, with predation and competition, but also at a genetic, economic (i.e. damages to agriculture, fishing and aquaculture) and sanitary (spreading of pathologies and parasites) level. These problems affect numerous plant and animal species, including freshwater decapods, which have been imported from various countries and are now widespread in Europe (HOLDICH, 2003).

In Italy there are numerous species: *Procambarus clarkii* (Girard, 1852), *Orconectes limosus* (Rafinesque, 1817), *Astacus leptodactylus* (Eschscholtz, 1823), *Pacifastacus leniusculus* (Dana, 1852), *Cherax destructor* (Clark, 1936) and *C. quadricarinatus* (von Martens, 1868). Among these, the last two have never been observed in nature, but are reared in specific aquaculture farms (GHERARDI *et al.*, 1999a).

In the water systems of Latium the occurrence of allochthonous freshwater crayfishes *Procambarus clarkii* and *Orconectes limosus*, Cambaridae coming from North America, and *Astacus leptodactylus*, Astacidae coming from Eastern Europe, has been observed (QUATTROCCHI *et al.*, 1996; SCALICI and GIBERTINI, 2002).

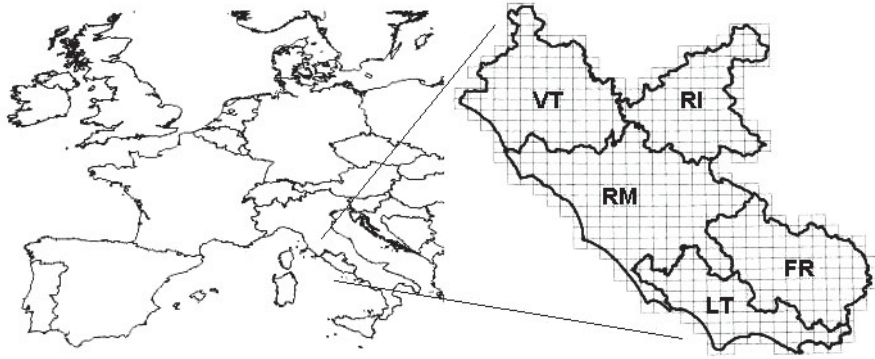
In Italy, *Procambarus clarkii* has been observed for the first time in 1989 in Piedmont (DELMASTRO, 1992a), and later in numerous northern and central provinces (GHERARDI *et al.*, 1999a). In Piedmont and Emilia Romagna this species is already naturalized in many areas of the river Po basin, and one of the major threats concerns its spreading in irrigation canals near Vercelli and Pavia, since the construction of burrows along their banks may damage, causing a collapse, the water system in rice fields, these being the favourite habitat of this species (CORREIA and FERREIRA, 1995; SALVI, 1999; GHERARDI and BARBARESI 2000). In central Italy *Procambarus clarkii* is widespread in Tuscany, especially near the lake Massaciuccoli, in Versilia (province of Lucca, Massa, Carrara), up to the river Magra in the nearby region of Liguria (province of Genoa), near Pisa, in the province

of Florence (Osmannoro and Sesto Fiorentino), and those of Prato and Pistoia. Their occurrence in Umbria, Abruzzo, Marche and Latium has also been ascertained, especially in the province of Rome and Rieti (GIBERTINI *et al.*, 1998; BARBARESI and GHERARDI, 2000). These last records have been subsequently confirmed by SCALICI and GIBERTINI (2002), who have observed their occurrence in the province of Latina and Viterbo as well. Nonetheless, its occurrence has broadened to many other Italian provinces. New records have been reported even in Sicily (LO VALVO, personal communication). *P. clarkii* is a species with high dispersion and movement power even outside the water. It is able to move periodically covering up to 17 km in 4 days (GHERARDI and BARBARESI, 2000). Moreover, it is a very aggressive species, it can indeed, damage the freshwater community, disturbing it especially on heat. Due to its excavating ability, which implies the construction of tunnels used as burrows or shelters during the dry season and the reproductive period, it can set off even severe landslides and landslips in the banks. The tunnels have a regular form. They are surmounted by a bank of land, similar to a chimney, which could play an important role during the oxygenation (HUNER and BARR, 1991).

Until recently, *Orconectes limosus* was almost unknown in Italy. In fact, its first record in our freshwater courses dates back to 1992 in the Iseo lake (DELMASTRO, 1992b). Subsequently, its occurrence has been signalled in 1993 by GROPPALI in the province of Pavia and later by CONFORTINI and NATALI (1994) in the plains near Verona. In Emilia Romagna its spreading has been highlighted in 1997 (MAZZONI *et al.*). The first record in Latium dates back to 1996 (QUATTROCCHI *et al.*) and its occurrence is limited at the moment to the lake of Piediluco (DÖRR *et al.*, 2001) and to the Salto lake. This last record has been confirmed in 2002 (SCALICI and GIBERTINI, 2002).

In Italy, the occurrence of naturalized populations of *Astacus leptodactylus* has been observed only in the province of Milan, in that of Bologna, in the region of Liguria (river Vara basin, province of La Spezia) and in Latium (GHERARDI *et al.*, 1999a). Its limited expansion in Italian freshwater courses may be due to its high sensibility to the crayfish plague *Aphanomyces astaci* (Shikora, 1902) (GALLUPPI *et al.*, 1996), of which cambarids are healthy vectors for translocation of the pathogen (ALDERMAN and POLGLASE, 1988), and even to other fungal and parasitic infections, like *Thelohania contejeani* Henneguy, 1892, which damages the abdominal muscular fibres (MANCINI, 1986). In particular, aphanomycosis is one of the major causes of rarefactions and disappearance of European crayfish populations, which are extremely sensitive to the disease; meanwhile North-American crayfish shows a strong tolerance (MANCINI, 1986). The transport and trade of exotic species has contributed to its spreading over the entire European territory, endangering the survival of indigenous species. Even though the disease is well known over more than a century and its pathogen has long been identified, there is no efficient method that can fight it and successfully defeat it (MANCINI, 1986). However, a special treatment, using precise $MgCl_2$ concentrations, seems to prevent the development of sporanges and, therefore, the transmission of the pathology through sporulation (RANTAMÄKI *et al.*, 1992).

The main purpose of the present work was to increase the information about these species in order to improve management activities and solve or control the problems involved with their occurrence. This situation requires the knowledge of data, which could allow the institutional authorities to take on accurate managerial decisions. This phase can be achieved through the application of predictive patterns that, nonetheless, require a basic knowledge of the species taken into consideration. More in detail, the need to geographically characterize the species resulted from the awareness that obtaining systematically informational data on the distribution of allochthonous crayfish populations is a prerequisite condition to effectively participate to the management of the allochthonous community and the conservation of the autochthonous one. This type of information enables the intervention on two main factors: the dispersion of allochthonous crayfish and the spreading of pathogen agents. Indeed, the knowledge of the populations' distribution

**Figure 1**

Study area: VT = Viterbo; RI = Rieti; RM = Rome; FR = Frosinone; LT = Latina.

Figure 1

Région d'étude : VT = Viterbo ; RI = Rieti ; RM = Roma ; FR = Frosinone ; LT = Latina.

in a precise area is the first and most important element that helps evaluate the risk of diffusion and plan a logic prevention programme against transmission.

The aim of the present research is to contribute to the increasing knowledge of the distribution and ecology of freshwater allochthonous decapods in water systems of Latium, especially by providing information on the growth, the diet and the reproductive biology (these last aspects are exclusively referred to *P. clarkii* and *O. limosus*).

MATERIAL AND METHODS

Study area

Latium is a region in central Italy, covering an area of 17,203 km² from 41°19' to 42°80'N and from 11°47' to 14°08'E, divided in 5 provinces: Viterbo, Rieti, Rome, Frosinone, Latina (Figure 1). All the investigated environments present anthropic disturbances: lakes are characterized by a moderate eutrophism, while field irrigation canals or pounds show bad levels of water quality.

Sampling procedure

The geographic distribution analysis of allochthonous freshwater crayfishes in Latium was carried out through a bibliographic research, personal communications and field work, the latter conducted during March, May, June, July, August, October, November 2003 and June, July, August 2004. Freshwater crayfishes have been caught making a linear transect along the banks, using hand-nets with different mesh.

The specimens have been measured on the back (along the dorsal mid-line), taking CTL (cephalothorax length, from the tip of the rostrum to the posterior part of the thorax) and TL (total length, from the tip of the rostrum to the end of the telson) using an eyepiece micrometer, then placed in absorbant paper and weighted (W).

The CTL measurements have generated polymodal frequency distribution histograms that have been analysed with the FAO-ICLARM Stock Assessment Tools (FiSAT) computer programme (GAYANILO *et al.*, 1996). This programme enables the decomposition of mixed length-frequency distributions into their Gaussian components by means of BHATTACHARYA's (1967) method. The last one is used to estimate the growth rate of fishes,

but it has been also successfully used with many other taxa. For example, reptile (SALVIDIO and DELAGUERRE, 2003), mussels (ARDIZZONE *et al.*, 1996), marine crustaceans (LEONARDI and ARDIZZONE, 1994; MERELLA *et al.*, 1998) and freshwater crayfishes (HOGGER, 1984; ZEKHNINI and CHAISEMARTIN, 1991). With this process, each identified component is subtracted from the remainder of the sample using a Gaussian function. A linear regression analysis for each separate component and a χ^2 goodness-of-fit statistic for the entire samples were then calculated. In addition, a separation index (S.I.) for each pair of adjacent groups was estimated; when $S.I. \leq 2$, separation between components is unreliable (SPARRE and VENEMA, 1996). Moreover, FISAT provides the mean, the standard deviation, and the theoretical number of individuals in each group. The results obtained with the Bhattacharya method have been subsequently used to evaluate the growth. The growth has been calculated with the Von Bertalanffy equation, described as follows:

$$L(t) = L_{inf} \cdot [1 - \exp(-k \cdot (t - t_0))]$$

The mathematical model expresses different growth parameters: L represents the length as a function of the age t , L_{inf} is interpreted as the mean length of old specimens, k is a curvature parameter which determines how fast the crayfish approaches its L_{inf} , t_0 is the initial conditions parameter that determines the point in time when the crayfish have zero length. The latter has no biological significance because on hatching crayfish have well defined dimensions. However, it represents an important parameter to determine the growth curve (SPARRE and VENEMA, 1996). All of the Von Bertalanffy parameters have been calculated by the software STATISTICA Statsoft. In the present research L_{inf} has been calculated by applying the following formula routine (included in FISAT):

$$L_{inf} = L_{max} / 0.95$$

where L_{max} is the maximum size according to SPEDICATO and CANNAS (2000).

Although the physiology of crustaceans is very different from that of fishes, their average body growth appears also to conform to the von Bertalanffy growth model (GARCIA and LE RESTE, 1981). An individual crustacean does not conform to the Von Bertalanffy model, but to some “stepwise curve”, with each step accounting for a moult. However, members of a cohort moult at different times, and therefore the average growth curve of a cohort of crustaceans becomes a smooth curve. For further discussion on the modelling of population dynamics of crustaceans see, for example, JAMIESON and BOURNE (1986) and CADDY (1987). This method has been used with marine crustaceans (ORSI RELINI and RELINI, 1985) and also with freshwater crayfishes (FIDALGO *et al.*, 2001).

Moreover, regression analysis between CTL and other two parameters TL and W (total body weight) have been carried out. The latter was used to obtain the values of the coefficient of condition K_c , calculated according to the following formula;

$$K_c = (W/CTL^b) \cdot 100$$

where b is a species-specific constant and it represents the angular coefficient of the relationship length-weight, which is expressed in terms of natural logarithm:

$$\ln W = b \cdot \ln CTL + \ln K_c$$

From the specimens caught, the stomach has been drawn out as well, dividing contents in order to study the diet. Also the reproductive cycle has been investigated: from the same animals, gonads have been taken away (including vas deferens and androgenic glands for males), first weighted to obtain the gonadic index (G.I.) calculated by applying the following formula:

$$G.I. = W_g / (W - W_g)$$

where W_g is the gonadal weight.

Then, the gonads have been used to prepare histological sections, by fixing them with Bouin, dehydration, inclusion with paraffin, and colouring with Carazzi Hematoxylin and Eosin.

RESULTS

Geographic distribution

The occurrence of freshwater allochthonous crayfish species has been observed in four of the five provinces of Latium, in 21 sites altogether. *Procambarus clarkii* is present in the provinces of Viterbo, Rieti, Rome and Latina (17 sites); *Orconectes limosus* only in the province of Rieti (2 sites), meanwhile *Astacus leptodactylus* in the provinces of Rome and Rieti (3 sites). Table I and Figure 2 show the crayfish's distribution.

Table I

Distribution of allochthonous freshwater crayfishes in Latium. PC = *Procambarus clarkii*, OL = *Orconectes limosus*, AL = *Astacus leptodactylus*.
^{1,2} Dr P.T. COLOMBARI, pers. com.; ³ SCALICI & GIBERTINI, 2002; ⁴ DÖRR et al., 2001; ¹⁰ Dr M. IACONELLI, pers. com.; ¹¹ GIUCCA, 1997; ¹³ Park guard, pers. com.; ¹⁵ Prof. P. MARIOTTINI, pers. com.; ¹⁶ Park guard, pers. com.; ¹⁷ Dr M. LORENZETTI, pers. com.; ¹⁸ Dr G. MOTISI, pers. com.; ¹⁹ Dr D. CELAURO, pers. com.; ²⁰ Dr G. LARICCIA, pers. com.

Tableau I

Distribution des écrevisses allochtones dans le Latium. PC = *Procambarus clarkii*, OL = *Orconectes limosus*, AL = *Astacus leptodactylus*.
^{1,2} Dr P.T. COLOMBARI, com. pers. ; ³ SCALICI & GIBERTINI, 2002 ; ⁴ DÖRR et al., 2001 ; ¹⁰ Dr M. IACONELLI, com. pers. ; ¹¹ GIUCCA, 1997 ; ¹³ Garde-moniteur, com. pers. ; ¹⁵ Prof. P. MARIOTTINI, com. pers. ; ¹⁶ Garde-moniteur, com. pers. ; ¹⁷ Dr M. LORENZETTI, com. pers. ; ¹⁸ Dr G. MOTISI, com. pers. ; ¹⁹ Dr D. CELAURO, com. pers. ; ²⁰ Dr G. LARICCIA, com. pers.

	Province	Locality	Site	PC	OL	AL
1	Viterbo	Marta	Marta River	+		
2	Viterbo	Gradoli, Bolsena, Capodimonte	Bolsena Lake	+		
3	Viterbo	Castel Sant'Elia	Fosso del Ponte	+		
4	Rieti	Madonna della Luce	Piediluco Lake		+	
5	Rieti	Rivodutri	Fosso Rivodutri	+		
6	Rieti	Rivodutri	Riserva Nat.Parz. Laghi Lungo e Ripasottile	+		+
7	Rieti	Borgo S.Pietro	Salto Lake		+	
8	Roma	Nazzano	Tevere River			+
9	Roma	Casale Marcigliana	Private area	+		
10	Roma	Dike of Castel Giubileo - Roma	Tevere River	+		
11	Roma	Roma	Low Course of Aniene River	+		
12	Roma	Roma	Fosso di Vaccina	+		
13	Roma	Torre Flavia	Torre Flavia Natural Monument	+		
14	Roma	Ponte Galeria - Roma	Tevere River	+		
15	Roma	Pomezia	Cava Tacconi	+		
16	Roma	Ienne	Private area			+
17	Latina	Foce Verde	Private area	+		
18	Latina	Borgo S.Michele	Private area	+		
19	Latina	Fogliano	Circeo National Park	+		
20	Latina	Caprolace	Circeo National Park	+		
21	Latina	S.Isidoro	Canale Bonifica Pontina	+		

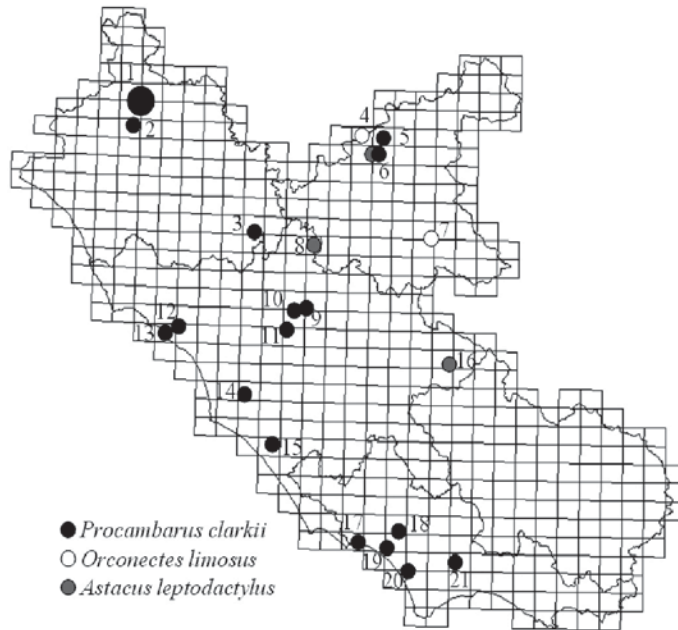


Figure 2
Occurrence of allochthonous freshwater crayfishes in Latium.

Figure 2
Signalements d'écrevisses allochtones dans le Latium.

As for *Astacus leptodactylus*, only the geographical distribution has been investigated, due to the limited number of animals collected during the samplings and to the difficult tracing operations, since the species is present in private areas. Consequently, the following analyses have been carried out exclusively for the cambarids.

Measurements

In total, 153 specimens of *Procambarus clarkii* (92 males and 61 females) coming from the Circeo National Park (province of Latina) caught in July 2003, and 63 individuals of *Orconectes limosus* (33 males and 30 females) coming from the Salto Lake (province of Rieti), caught in July 2004, have been measured, taking CTL, TL and W.

The mean values of CTL are 33.6 mm (± 11.6) for *P. clarkii* females and 36.2 mm (± 11.1) for *P. clarkii* males; 28.1 mm (± 9.7) for *O. limosus* females and 23.9 mm (± 8.7) for *O. limosus* males. The mean values of W are 10.1 g (± 9.7) for *P. clarkii* females and 14.4 g (± 13.8) for *P. clarkii* males; 7.2 g (± 7.6) for *O. limosus* females 11.4 g (± 10.8) for *O. limosus* males. The sex ratio, calculated as n° males/n° females, is 1.5: 1 for *P. clarkii*, and 1.1: 1 for *O. limosus*.

The regression analysis between CTL vs. TL and CTL vs. W for females and for males of both cambarids has been carried out using a low number of specimens, because some of them presented malformations of the rostrum and/or chelae. The results are shown in Table II.

For every function the R^2 correlation value and the statistical significance are respectively shown.

Table II
Regression functions CTL vs. TL and CTL vs. W in *P. clarkii* and *O. limosus*.

Tableau II
Fonctions de régression CTL vs. TL et CTL vs. W de *P. clarkii* et *O. limosus*.

	Sex	Function	N° ind	R ²	p
<i>P. clarkii</i>	F	TL = 1.89·CTL + 2.2	55	0.98	< 0.05
	M	TL = 1.84·CTL + 3.213	76	0.98	< 0.05
	F	W = 0.18·e ^{0.10 CTL}	55	0.94	< 0.05
	M	W = 0.21·e ^{0.10 CTL}	76	0.95	< 0.05
<i>O. limosus</i>	Sex	Function	N° ind	R ²	P
	F	TL = 2.02·CTL + 1.61	30	0.99	< 0.05
	M	TL = 2.31·CTL + 11.12	33	0.92	< 0.05
	F	W = 0.08·e ^{0.14 CTL}	30	0.82	< 0.05
M	W = 0.39·e ^{0.12 CTL}	33	0.90	< 0.05	

The K_c values have been calculated by analysing the length-weight regression. They are $1 \cdot 10^{-2}$ and $0.4 \cdot 10^{-2}$ respectively for *P. clarkii* females and males. As for *O. limosus*, they are $9 \cdot 10^{-3}$ for females and $7.4 \cdot 10^{-1}$ for males. The values of b are 3.16 for *P. clarkii* females and 3.4 for *P. clarkii* males, and 3.28 for *O. limosus* females and 2.2 for *O. limosus* males.

Length-frequency analysis

The length-frequency diagrams were obtained using 1mm size class (Figure 3), using the same samples described in the last paragraph (“measurements”).

The analysis of the diagrams showed a division of the sample into 3 age classes (from 0 + to 2 +) for *P. clarkii*, and into 4 age classes (from 0+ to 3 +) for *O. limosus*. The modal progression analysis on data set has not been processed by sex separately according to ANASTACIO and MARQUES (1995); FIDALGO *et al.*, (2001).

Table III shows the number of specimens for each class, their respective average length, the standard deviation, and the S.I. value. The distribution into age classes obtained with the Bhattacharya method is statistically significant for both *P. clarkii* ($\chi^2 = 19.6$, $gl = 15$, $p < 0.05$) and *O. limosus* ($\chi^2 = 16$, $gl = 5$, $p < 0.05$).

The parameters of the Von Bertalanffy growth curve, calculated by applying the mean values of the single age classes, are: $L_{inf} = 62.1$ mm; $k = 0.79$, $t_0 = -0.1$ for *P. clarkii*, and $L_{inf} = 54.7$ mm; $k = 0.71$; $t_0 = -0.2$ for *O. limosus*. Figure 4 shows the curves that have been obtained.

Diet analysis

To carry out evaluations on the feeding habits of *P. clarkii* and *O. limosus*, 151 (90 from males and 61 from females) and 61 (33 from males and 28 from females) coming from the two main sites (Circeo National Park in 2003 and Salto Lake 2004) stomachs have been respectively analysed. As for the quantitative analysis, in total 28 empty stomachs has been found in *P. clarkii*, corresponding to 18.54%; and 12 in *O. limosus*, corresponding to 19.67%.

As for the qualitative analysis, in the full stomachs of the red swamp crayfish the contents observed have been divided into 4 categories: animal components (crayfishes

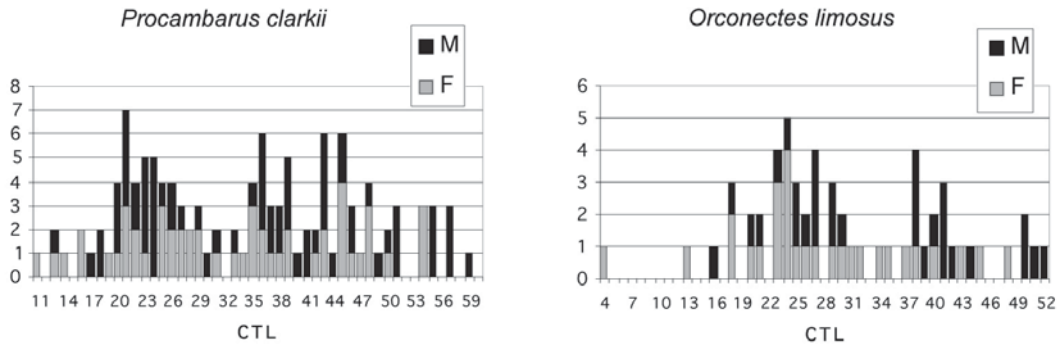


Figure 3
Length-frequency diagrams of *P. clarkii* and *O. limosus* (M = males, F = females).

Figure 3
Diagrammes de longueur-fréquence de *P. clarkii* et *O. limosus* (M = mâles, F = femelles).

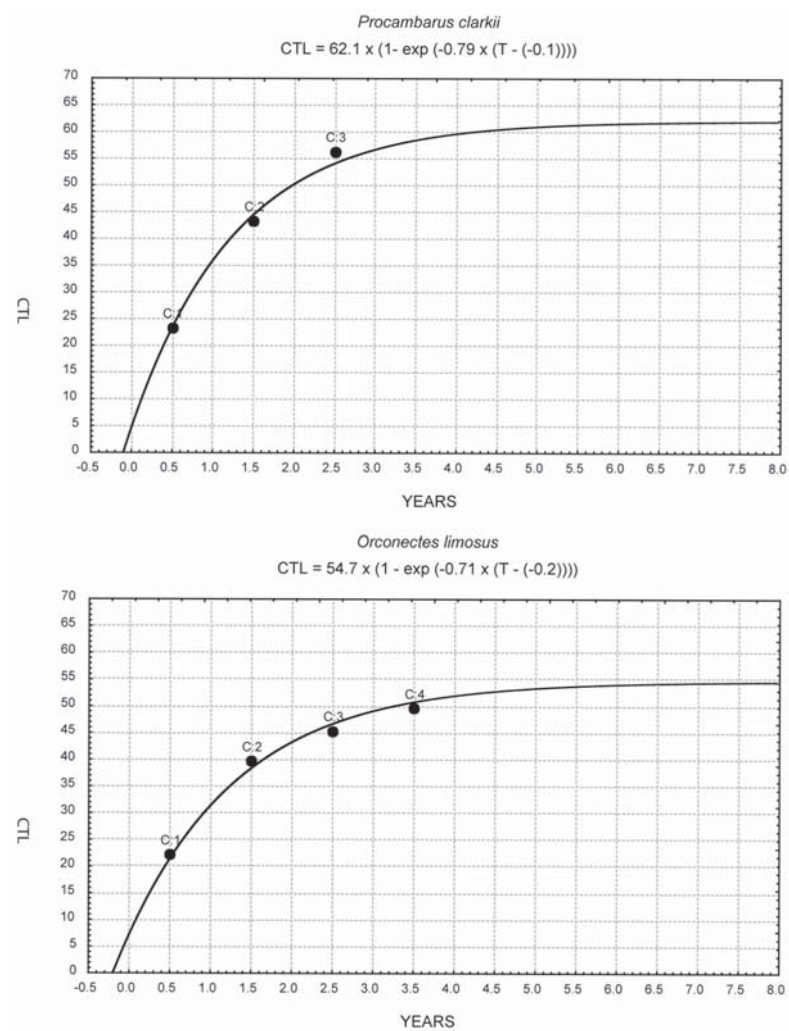


Figure 4
Growth curves of *P. clarkii* and *O. limosus*.

Figure 4
Courbes de croissance de *P. clarkii* et *O. limosus*.

Table III

Age classes of *P. clarkii* and *O. limosus* (N = number of individuals, M = mean length, DS = standard deviation, S.I. = separation index).

Tableau III

Classes d'âge de *P. clarkii* et *O. limosus* (N = nombre d'individus, M = longueur moyenne, DS = écart type, S.I. = index de séparation).

		N	M	DS	S.I.
<i>P. clarkii</i>	0 +	45	23.21	3.36	–
	1 +	66.95	42.41	4.32	4.76
	2 +	13.34	56.81	2.53	4.05
<i>O. limosus</i>	0 +	28	22.06	5.12	–
	1 +	11.77	39.57	1.73	5.11
	2 +	2.23	45.30	1.68	3.37
	3 +	2.98	49.50	1.21	2.92

parts – limbs, stomach, carapace fragments –, ostracods, insects – fragments and larvae of Diptera, elytra of Coleoptera, heads of Hymenoptera, Hemiptera –, mollusc eggs, and fish remains – otoliths, scales, branchial bones); vegetal components (algae, fragments of stalks and seeds of *Ceratophyllum*, buds and vegetal fibers); detritic component (mucilage, sand and fine particulate sediment); nylon fragments and threads have been classified as other. The same analysis for the American crayfish has pointed out 4 categories as well: animal components (crayfishes parts – limbs, carapace fragments – complete insects or their fragments – Diptera, Hemiptera –, and not identified bone fragments); vegetal components (algae, fragments of seeds, stalks and buds); detritic component (mucilage, sand and fine particulate sediment); nylon fragments and threads, plastic and polystyrene have been classified as other. Finally, occurrence frequencies of the 4 food items (animal,

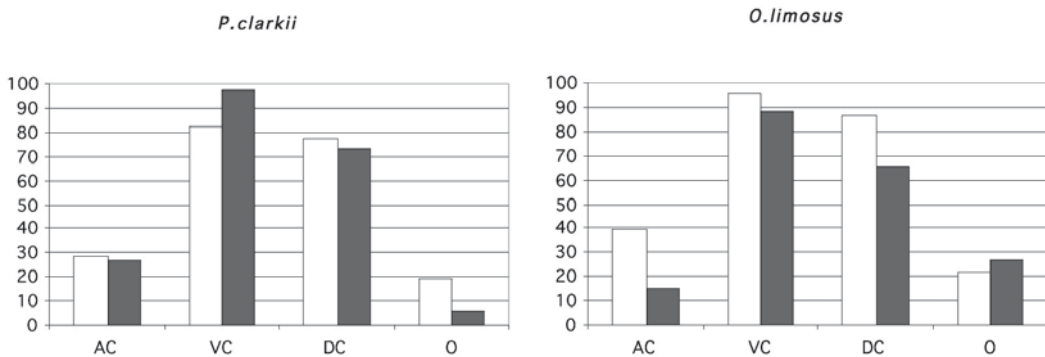


Figure 5

Frequency of occurrence of the stomach content (Y-axis expressed as%) in *P. clarkii* and *O. limosus*: females in white and males in grey (AC = Animal component, VC = Vegetal component, DC = Detritic component, O = Other).

Figure 5

Fréquences dans l'estomac (exprimé en % sur les ordonnées) de *P. clarkii* et *O. limosus*: femelles en blanc et mâles en gris (AC = composant animal, VC = composant végétal, DC = composant détritique, O = autre).

vegetal, detritic, and other) have been calculated for both species (Figure 5). For both *P. clarkii* and *O. limosus*, the differences found in the occurrence frequencies between males and females are not statistically significant, except for the animal component of the latter ($\chi^2 = 9.58$, $gl = 1$, $p < 0.01$).

Histology and gonadic index

The analysis of the histological slides has allowed to identify the different maturity phases of the gonads in the cambarids and to determine the period and the minimum size at which the mature gametes are present. In the female *P. clarkii* the mature oocytes have been observed to be more frequent in July (57% of specimens caught), in individuals with a minimum size of 27.75 mm CTL. Gonads proved to be empty in the 14% of individuals caught in July and in the 50% of that of October. Moreover, an ovigerous female was caught in October 2003. As for the males, mature spermatozoa have been observed in all individuals, the highest values of which has been observed in March (60% of specimens). In this month also mature spermatophores (characterized by a layered wall) have occurred, they were present in the 20% of the individuals, even if they have been observed with higher frequency in the samples of July (60% of individuals) and October (50% of individuals). Furthermore, from March until July, and especially in March (20% of specimens caught), empty gonads have been studied. The minimum size at which mature spermatozoa have been observed was 20.7 mm CTL.

The mean values of the gonadic index in *P. clarkii* are $0.32 (\pm 0.3)$ for females and $0.15 (\pm 0.2)$ for males.

In *O. limosus*, among the females caught mature gonads have not been observed. This has prevented the identification of the minimum maturity size. On the contrary, as for the males, mature spermatozoa have been found in the specimens caught in June (50% of the sample) and August (60%), with mature spermatophores in individuals that were caught in July (100% of specimens) and August (40%). Moreover, in June a juvenile with 4.5 mm CTL was caught. The minimum size at which mature spermatozoa are present is 24.85 mm CTL.

The mean values of the gonadic index in *O. limosus* are $0.31 (\pm 0.2)$ for females and $0.36 (\pm 0.3)$ for males.

In both species and sexes, the values of the gonadic index have not shown a linear relationship with CTL (Figure 6).

DISCUSSION

The distribution of the allochthonous freshwater crayfishes in Latium highlighted some differences between them: *Orconectes limosus* and *Astacus leptodactylus* are present in restricted areas, while *Procambarus clarkii* has a wide distribution throughout the region. These differences may be traced back to the great number of records obtained, in relation to the occurrence of the red swamp crayfish, which is by now well known even among people who are not experts in this field. The great interest for this species from a commercial and fishing point of view, could represent the basis of the rapid dispersion that has been observed in the past years, which, together with the great vagility of the species (GHERARDI and BARBARESI, 2000), has contributed to the creation of the present situation. As for *O. limosus*, the fishing interest towards this species seems to be limited to the Salto lake populations. It may be possible that its introduction, as for *Astacus leptodactylus*, was accidental, during restocking activities of *Austropotamobius italicus* in the 80s (MANCINI, 1986).

The last observations carried out on cambarids show and confirm their plasticity (especially in *P. clarkii*), the way in which they are able to exploit the numerous resources of environments in which they have been introduced.

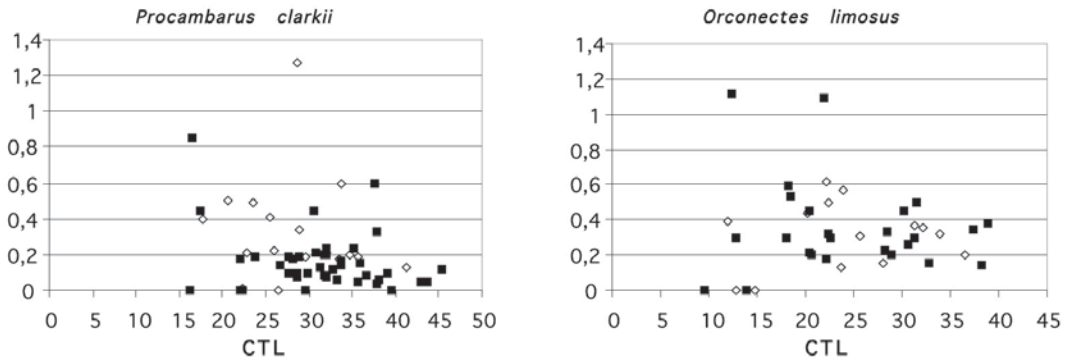


Figure 6

Relationship between G.I. and CTL in *P. clarkii* and *O. limosus* (females in white and males in black).

Figure 6

Rapport entre I.G. et CTL de *P. clarkii* et *O. limosus* (femelles en blanc et mâles en noir).

Indeed, *P. clarkii* is able to adapt itself to new environments and shows a high tolerance to the most variable environmental conditions (HOBBS *et al.*, 1989), which, associated to a high growth rate (HUNER, 1978) and a successful reproductive strategy, allows for the development of highly dense populations.

This can be reinforced by the results that have been obtained on the b constant, which is always higher than 3 and represents an allometric growth (which is due to a higher weight increase, more than to the length). These values may be compared to those obtained by other authors in Spain (GUTIÉRRIEZ-YURRITA *et al.*, 1994; GUTIÉRRIEZ-YURRITA *et al.*, 1996; GUTIÉRRIEZ-YURRITA and MONTES, 1999) and Portugal (CORREIA, 1993). The constant K_c as well shows similar values between the two sexes, as previously observed by other authors (CORREIA, 1993; GUTIÉRRIEZ-YURRITA *et al.*, 1994; GUTIÉRRIEZ YURRITA *et al.*, 1996). The *sex ratio* shows higher values for males, unlike shown in other studies (i.e. ANASTÁCIO and MARQUES, 1995; FIDALGO *et al.*, 2001), but it complies with what has been observed by OLUOCH (1990) in Kenya, COSTA *et al.* (1996) in the Azores, GUTIÉRRIEZ-YURRITA *et al.* (1996) and PÉREZ-BOTE *et al.* (2000) in Spain, FRUTIGER *et al.* (1999) in Switzerland and DELMASTRO (1999) in Italy. Here, GHERARDI *et al.* (1999b) have observed a balanced *sex ratio*, except for June (when males prevail) and October (when females prevail). Thus, this parameter seems to vary according to the geographic area and the period in which the caught is carried out.

The analysis carried out with FISAT, has allowed for the identification of 3 age classes for *P. clarkii*, according to what has already been observed by LOZANO-GUERRA and ESCAMILLA-NIÑO (1995). The authors have described three size groups: subadults, adults and adults that survive through a second reproductive season. Moreover, HUNER (2002) sustains that their life has a maximum duration of 4 years, while the mean life in nature does not exceed the 12-18 months, conversely FRUTIGER *et al.* (1999) have described how the majority of the individuals live through the third year and how the bigger ones can live up to five years of age. As for the values of the von Bertalanffy growth parameters, for *P. clarkii* L_{inf} corresponds to what has already been observed by FIDALGO *et al.* in 2001, ($L_{inf} = 62.01$ mm), but the k and t_0 values have been proved different. However, they are very similar to those observed by ANASTÁCIO and MARQUES in 1995 ($k = 0,7$).

Orconectes limosus as well shows a high invasive ability due to its resistance to pollutants, to the crayfish plague and to high fecundity. This has frequently led to the substitution of the autochthonous species in many Eastern European Countries (PIELOW, 1938; SCHWENG, 1973; STYPINSKA, 1979; ORZECOWSKI, 1984). The values of the von Bertalanffy growth parameters of *O. limosus* have shown how this species has a slower growth rate compared to the red swamp crayfish. The growth constant b of males is < 3 , while that of the females is > 3 . This matches what has been observed by VAN DEN BRINK *et al.* (1988), for non-ovigerous females ($b = 3,58$). However, it shows different results for the males ($b = 3,82$ see VAN DEN BRINK *et al.*, 1988). In our case, the results show that in the latter the length increases more with regard to the weight, while as for females the weight increases more than the length, this is probably due to the bigger energy need, which is necessary for the development of the eggs. This corresponds to what we have observed even for k_c . However, it differs from the results of the authors who have been precedently quoted and who have not highlighted significant differences in the growth between males and females. As for the *sex ratio* the results show an almost equal relationship between the sexes, confirming what has been observed by BOTT (1950) and by VAN DEN BRINK *et al.* (1988), who have observed seasonal variations of the *sex ratio*, with almost equal values for males and females during the summer. Our results have underlined the existence of 4 age classes, while SHULTZ and SMIETANA (2001) have described up to 6 age classes for the females. HAMR (2002) claims that the mean life has duration of 2 years, and the maximum duration is 4 years (ANDREWS, 1907; KOSSAKOWSKI and ORZECOWSKI, 1975; SMITH 1981; VAN DEN BRINK *et al.*, 1988, MOMOT, 1988).

As for the diet, both species have shown a great ability to utilize the various trophic resources, confirming the fact that they are omnivorous species. However, without distinction between the sexes, it emerges that the vegetal component clearly prevails, followed by the detritic and animal components, as already observed for *P. clarkii* (GUTIÉRRIEZ YURRITA *et al.*, 1998). The occurrence of cannibalism (NYSTRÖM, 2002) has to be confirmed, because the crayfish fragments found may be derived from exuviae and not from living animals. The preference for the vegetal component may result extremely noxious for the water plants (GARCÍA-MURILLO *et al.*, 1993; GUTIÉRREZ-YURRITA and MONTES, 1999; ACQUISTAPACE *et al.*, 2004). In *P. clarkii*, the absence of feeding differences between males and females, already observed by GUTIÉRRIEZ YURRITA *et al.* (1998), may be due to the fast growth of the species. Indeed, metabolic differences between the sexes have not been previously observed (GUTIÉRRIEZ YURRITA *et al.*, 1994). Conversely, in *O. limosus*, the differences in the animal component between males and females may be due to the metabolism of the latter, whose oogenesis would require a higher quantity of energy.

As for the reproduction, these preliminary observations on gonadal maturation, may suggest that the *P. clarkii* breeding occurs in Latium between the summer and the autumn. Besides, the occurrence of an ovigerous female in October confirms what has been reported by other Italian authors, GHERARDI *et al.* (1999b) for the province of Florence, and DELMASTRO (1999) for Piedmont, where ovigerous females have been found during the summer. Most probably, the species is able to breed twice a year, even during the spring, as already observed by some authors (CANO and OCETE, 1997; GUTIÉRREZ-YURRITA and MONTES, 1999), outside its original distributional area. However, to confirm this hypothesis further researches are necessary, to clearly identify the breeding period, with a special attention to the occurrence of ovigerous females, and females with larvae. As suggested by the same GUTIÉRREZ-YURRITA and MONTES (1999), this species is very likely able to breed during the whole year: the entire process may vary in relation to the environmental conditions, allowing the species to easily adapt itself to the most varied ecosystems and naturalise even in geographic areas that are different to its primary distributional area. HUNER (1981) suggests that in favourable conditions there may be up to three generations each year. Studying reproduction has highlighted how the sexual

maturity may be reached in females at 27.75 mm CTL, corresponding to 54.7 mm TL, and in males at 20.7 mm, equal to 41.3 mm TL. HUNER (2002) describes this value as very variable, ranged from 45 mm to 125 mm TL or more. Moreover, the duration of the reproductive period, the rapid growth, the early sexual maturity and the short mean life, may even explain the result observed confronting CTL with the gonadic index, which was difficult to interpret, and supplied by the histological method. As for *O. limosus*, the occurrence in July and August of sexually mature males matches the data reported by HAMR (2002) for Quebec (primary distributional area), where the breeding takes place in spring (March-April) and again in September-October. Besides, the occurrence in June of a newly independent juvenile confirms what reported by the same author even in Europe, where the breeding takes place in spring and eggs are carried by the female until June-July, period in which they hatch. Also VAN DEN BRINK *et al.* (1988) in the Netherlands have observed the occurrence of ovigerous females from March until June, with the highest value in April, and in May females with juveniles attached to the abdomen. This may suggest the existence of a wide reproductive interval that allows the species to breed more times during the year. However, even in this case, further researches are necessary to clearly identify the breeding period in the population examined. In males observed by us, the sexual maturity is reached at 24.85 mm CTL. HAMR (2002) quotes a unitary value of 25-35 mm CTL for European populations, and 45 mm CTL for those of Quebec. Even in this case, the frequency of the breeding periods may explain the result of the gonadic index with respect to CTL, which is difficult to interpret.

CONCLUSION

Identifying managerial activities that may eradicate or at least reduce the great problems caused by the allochthonous crayfish, represents a difficult operation, due to the current situation in Latium, region which shows, especially in the province of Latina and partially in that of Rome, a morphology of the territory and of the rain-collecting basins water system extremely heterogeneous and complex. In these conditions the eradication of the allochthonous species, especially the red swamp crayfish, would require a considerable economic and field effort.

It is necessary to aid the manual removal of exotic crayfishes with other more efficient methods. Even though the feasibility of eradication and monitoring activities of populations of non-native crayfishes has been discussed in several studies (i.e. HOLDICH *et al.*, 1999; HOWARD, 2000; KEMP, 2000; SIBLEY and NÖEL, 2002), insecticides, herbicides, or fungicides seem to potentially represent one of the most efficient instruments (LAURENT, 1995). However, these may bring forth a great number of negative effects on other animal and plant species.

While bringing along an eradication programme it is, nonetheless, necessary to consider the economic importance that these species have acquired in the past years. To bring into force a managerial activity that concerns the monitoring or the eradication, it is necessary that the methods used 1) are safe for the environment, 2) represent a high probability of success, 3) are inexpensive, 4) do not harm human health, 5) are explicable to the public (HOLDICH *et al.*, 1999). Due to the difficulties in identifying methods that comprehend all these characteristics, the best compromise, which is acceptable from an ecologic, economic and ethic point of view, must be achieved according to a process defined as "situationality" (GHERARDI and ANGIOLINI, 2002). In this case, as for the eradication of *O. limosus* only from the Salto lake, the eradication is difficult to carry out, since it may influence the economy of a small but consistent social reality, which uses this species as an important resource and which is long called by locals "ours" (autochthonous). In this context, the planning of environmental managerial interventions, compatible with the socio-economic and cultural needs of the local populations, is fundamental, in order

to get their approval and involve them in their achievement, increasing the probability of success. Moreover, the impossibility of approaching, in some cases, populations of allochthonous crayfishes, as for the Turkish crayfish, should make one reflect. In this case private property has represented a great obstacle.

Nonetheless, it is necessary to act as soon as possible to avoid the worsening of the current conditions, using simultaneously, if possible, different strategies. Control is the conservation activity that seems to have the greatest possibility of success. In this context, the use of pheromones is a method proposed by other authors (HOLDICH *et al.*, 1999; KEMP, 2000; SIBLEY and NÖEL, 2002) as a potential means of control of invasive allochthonous species. STEBBING *et al.* (2004) have studied the use of these substances showing how, with the attraction of reproductive *Pacifastacus leniusculus* males, it is possible to control the populations of undesired crayfishes. This has to be integrated with a constant and practical monitoring of the territory, which may certainly reduce the possibility of dispersion of these species. In this sense even an instrument like the GIS (Geographic Information System) may contribute to the identification of potentially apt areas to the dispersion and the colonization from undesired species (SCALERA, 2001). Furthermore, territorial control means prevention of transfaunation. Internationally speaking, as for this sector, more than 140 countries, among which Italy, have adhered to a regulation of the Convention on International Trade in Endangered Species of Wild Flora and Fauna (CITES). However, inside national boundaries there are no specific laws that regulate fish transport, especially of decapods, and not even specific organisms dealing with this problem (MAIO, 2002). This allows free transport in every area of the region and of the national territory, with clearly obvious consequences.

Thus, it is important to highlight the need to regulate legal instruments suitable to control the fishing activities and prohibit a further introduction of the species in other sites. Even the establishment of areas similar to the no-go English areas (HOLDICH and REEVE, 1991), anyhow criticised by some experts of the field, may represent a useful solution, provided that it is reinforced by valuable laws and tangible controls.

Moreover, it is important to be aware of the scarce interest from citizens to these problems and simultaneously start a programme of environmental education on more levels (that means for a wide public starting from students up to adults and those who have limited knowledge of this field). This may greatly help to create awareness, not only in the public opinion, but also in every person dealing with sportfishing and professional fishing.

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