

# How consumption and fragmentation of macrophytes by the invasive crayfish *Procambarus clarkii* shape the macrophyte communities of temporary ponds

B. M. Carreira · M. P. Dias · R. Rebelo

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**Abstract** Crayfish are major herbivores in freshwaters, and their activity may change invaded freshwater ecosystems. Macrophyte removal by crayfish results from consumption and fragmentation of plant material and may depend upon the palatability of each species. We studied the impact of *Procambarus clarkii* on the macrophyte community of Mediterranean temporary ponds, testing in laboratory if consumption and fragmentation of five macrophyte species were correlated in palatability tests and in a preference test. We performed an experiment in a natural pond, where we introduced crayfish and estimated its effects on

macrophyte biomass, number of rooted stalks and number of floating cut stalks. *P. clarkii* consumed preferably *Juncus heterophyllus* in both tests and avoided *Carex divisa* and *Ranunculus peltatus* in the preference test. In the presence of preferred species, consumption and fragmentation of the non-preferred species were heavily reduced. In the field trial, the “Crayfish” compartment had 31% less macrophyte biomass, 41% less rooted stalks and 92% more floating cut stalks. Crayfish may remove macrophyte species from the community sequentially, from the most to the least preferred species. Impacts of crayfish in temporary ponds may depend on time of invasion and on the composition of the macrophyte community.

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## Introduction

Biological invasions cause major biodiversity losses worldwide and have strong impacts in particularly vulnerable ecosystems, such as in freshwaters (Lodge et al., 1998; Saunders et al., 2002; Cambray, 2003; Kats & Ferrer, 2003; García-Berthou et al., 2005). Alien freshwater crayfish are opportunist omnivores that may become keystone species and act as ecosystem engineers in invaded areas, due to their central position in the food webs and to the magnitude of the

effects of their benthic activity (Charlebois & Lamberti, 1996; Lodge et al., 2000; Creed & Reed, 2004; Geiger et al., 2005; Flinders & Magoulick, 2007; Hirsch, 2009).

Crayfish reduce macrophyte biomass through both consumption and fragmentation of plant tissue (Nyström & Strand, 1996; Rodríguez et al., 2003; Anastácio et al., 2005; Crehuet et al., 2007; Gherardi & Acquistapace, 2007; Matsuzaki et al., 2009). Macrophytes are often keystone species (Van Nes & Scheffer, 2002) and their removal may have cascading effects in freshwater ecosystems. Sediment resuspension by crayfish increases water turbidity and proliferation of surface microalgae, which decrease macrophyte and periphyton primary productivity, due to reduced light in the water column. This may cause invaded ponds and lakes to shift from a clear to a turbid state, completely changing their communities (Rodríguez et al., 2003). Even more subtle impacts, such as the differential consumption of the most palatable macrophyte species by taxa ranging from pond snails to waterfowl, may affect the macrophyte communities, as well as the entire ecosystem (Elger et al., 2009; Hidding et al., 2010). The crayfish invasion of heterogeneous and species-rich ecosystems, such as ponds, may thus have strong—even contrasting—consequences, depending on the presence of a preferred macrophyte.

In Europe, the red swamp crayfish (*Procambarus clarkii*, Girard, 1852), native to the northeast of Mexico and central-southeast USA, was first introduced in Spain. It is now widespread in central and southern European countries and became the most abundant crayfish species in many of them (Lodge et al., 2000; Correia, 2002; Geiger et al., 2005; Gherardi, 2008). This species feeds on detritus, algae, macrophytes, invertebrates and small vertebrates, playing a major role in freshwater food webs (Gamradt & Kats, 1996; Correia, 2002; Alcorlo et al., 2004; Cruz & Rebelo, 2005; Geiger et al., 2005; Crehuet et al., 2007; Gherardi & Barbaresi, 2008). Its consumption of macrophytes is selective and based mostly on plant traits, such as structure (morphology and hardness), nutritive value and the presence of secondary metabolites, depending also on habitat characteristics (Cronin et al., 2002; Gherardi & Barbaresi, 2008; Matsuzaki et al., 2009).

We studied macrophyte consumption and fragmentation by *P. clarkii* upon macrophyte species from

Mediterranean temporary ponds—high-biodiversity habitats that contain a unique collection of fauna and flora, and included in the European Union list of priority habitats for conservation (Vives, 1996; Oertli et al., 2005; Ruiz, 2008). Our aim was to study the consumption and the fragmentation impacts of *P. clarkii* on different macrophytes and how these contribute to the shaping of the macrophyte community of invaded Mediterranean temporary ponds. We used a mixed approach of laboratory experiments, combining palatability, and preference tests (Jackson & Underwood, 2007; Leberfinger & Bohman, 2010), and a trial in a natural pond. The inclusion of palatability tests in the study of crayfish preference is a novelty compared to previous studies. Palatability tests allow determining the likelihood of consumption of different food items and, therefore, a more accurate interpretation of preference tests results, whether they are carried out in laboratory or in the field. We tested the following hypotheses: (1) consumption and fragmentation preferences should be positively correlated, meaning that macrophyte fragmentation occurs as a result of macrophyte consumption and is proportional to it; and (2) overall impacts in non-preferred macrophytes should be reduced in the presence of preferred macrophytes, as crayfish should consume and destroy mainly the preferred species.

## Materials and methods

### Laboratory experiments

The laboratory experiments design included two distinct sets of tests (following Jackson & Underwood, 2007): palatability tests—in which crayfish were presented with a single macrophyte species at a time; and a preference test—in which crayfish were presented with all macrophyte species simultaneously, providing choice. Crayfish were captured with baited traps and dip-net sweeps in the rice fields of S. Romão and Rio de Moinhos (SW Portugal, 38°14′51.33″N–8°21′10.96″W) between 29 March and 2 May 2010. The crayfish were maintained individually in plastic containers (12.5 × 12.5 × 9.0 cm) under a 12L:12D photoperiod and fed ad libitum every 5 days with commercial fish food Biogranulat Sera Pond (water content 6.4%, protein 32.0%, fat 7.2%, fiber 5.6%, and ash 9.3%). In addition, we fed crayfish the night prior

to the beginning of each test. We used only crayfish without visible injuries and with last known moult occurring more than 2 weeks prior to the test, to give time for crayfish exoskeleton to harden and for crayfish to behave normally. Crayfish post-orbital carapace length (POCL) ranged from 32 to 40 mm (15–25 g wet weight), with no significant differences between sexes ( $F_{1,128} = 2.69$ ;  $P = 0.103$ ) or among the individuals assigned to each test ( $F_{5,128} = 1.56$ ;  $P = 0.173$ ) and no significant interaction between crayfish sex and test ( $F_{5,128} = 1.58$ ;  $P = 0.169$ ). During the tests, crayfish were placed in experimental containers (38.5 × 26.5 × 28.0 cm) that held approximately 4 l of water (collected from a natural spring and changed every day) and a shelter (PVC pipe). We used different individuals in each test to assure independence of the data. Throughout the experiments the mean ( $\pm$ SE) water temperature was measured daily in four randomly selected containers (16.95  $\pm$  0.21°C).

The selection of macrophyte species took into account the following criteria: (i) the presence of a given species in the study pond (see below); (ii) its frequent occurrence in temporary ponds of the study area; (iii) the inclusion of distinct physiognomic types, representing macrophyte diversity in the southwest of Portugal. We used the following five species: *Carex divisa* Huds. (geophyte), *Mentha pulegium* L. (proto-hemicryptophyte), *Juncus heterophyllus* Dufour (hydrophyte), *Baldellia ranunculoides* (L.) Parl. (hydrophyte) and *Ranunculus peltatus* Schrank subsp. *baudotii* (hydrophyte).

Macrophytes were collected immediately prior to each test in temporary ponds located in the vicinity of the study pond. Nine fresh stalks of each species were weighed (after being towel blotted) and dried at 40°C for 5 days to determine macrophyte dry mass per wet weight unit, which differed among species ( $F_{4,40} = 46.943$ ;  $P < 0.001$ ), decreasing from *C. divisa* to *R. peltatus*, according to the sequence presented above.

#### Palatability tests

We tested each of the five macrophyte species in an individual test, accounting for autogenic growth in macrophyte biomass with 15 controls maintained in containers with no crayfish and randomly distributed in the laboratory. For each macrophyte species, we

used a different set of 15 male and 15 female crayfish (also randomly distributed) and each was presented with a single stalk, towel blotted and weighed to the nearest 0.1 mg (fresh weight), and allowed to feed for 24 h. The next day, all stalk fragments were removed and replaced with a new stalk, allowing crayfish to feed for another 24 h, and so on for 5 days. No records were taken in the first 2 days to allow the crayfish to settle and become accustomed to the macrophyte species (Jackson & Underwood, 2007). Each of the following 3 days, we recorded the final fresh weight of the stalk (stalk and its fragments) and the total number of fragments into which the stalk was cut. Stalks were similar in size ( $\approx$  20 cm) and volume, to provide similar encounter rates, and large enough to feed crayfish ad libitum (see Table 1 of supplemental data for weight ranges). After the end of each test, crayfish were maintained in the experimental containers for 2 days and data from crayfish that moulted within this period or during the test were discarded. The palatability tests started on April 7 and ended on May 27 of 2010.

#### Preference test

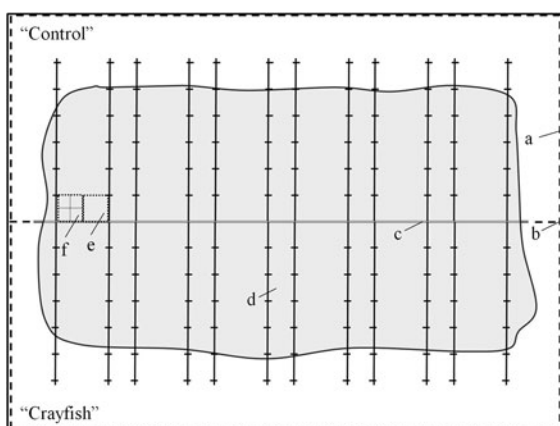
To allow for choice, we presented a different set of crayfish (15 males and 15 females) with five stalks (one of each macrophyte species), again using 15 controls to account for autogenic change in macrophyte biomass. Using the same approach as in the palatability tests, we allowed crayfish to feed for 24 h, replacing all stalks for fresh ones every 24 h for 5 days. The stalks and fragments of each macrophyte species were separated and in the final 3 days we recorded stalk final fresh weight (including the stalk and its fragments), and the total number of fragments into which the stalks were cut. Crayfish were followed for 2 days after the test and data were discarded if moult occurred within this period or during the test. The preference test started on May 12 and ended on May 17 of 2010.

#### Field trial

The trial took place in a pond situated in the field station of Herdade da Ribeira Abaixo (Serra de Grândola; 38°6′25.59″N–8°34′12.53″W), that fills during the autumn (October–November) and dries by the end of spring (May–June). The pond, with

ca. 40-m<sup>2</sup> surface, has been monitored since 2002 and, although the presence of isolated individuals was recorded (Rui Rebelo, pers. obs.), does not sustain a *P. clarkii* population. On November 2009, the pond was surrounded with a cattle barbed fence 90-cm high (Fig. 1a) and a plastic net 20-cm high (1-mm mesh size) was set along the sides and attached to the ground, to prevent colonization by crayfish (Fig. 1b). The pond was divided into two similar compartments (“Control” and “Crayfish”) with a quadruple plastic sleeve attached to wooden poles and buried 30 cm into the ground (Fig. 1c).

To estimate the initial macrophyte cover in each compartment, access corridors (0.5-m wide, separated by 1 m) were delimited with floating rope marked every 0.5 m (Fig. 1d), establishing a 0.5 × 0.5 m grid over the pond surface (Fig. 1e). Each square (0.25 m<sup>2</sup>) was photographed using a tripod, and further divided in four 0.0625 m<sup>2</sup> squares through image processing (Fig. 1f), delimiting 207 squares in the “Control” compartment and 237 squares in the “Crayfish” compartment (differences in *n* result from irregularities in the pond margins). We estimated cover of all macrophyte species by assessing their occurrence in these 0.0625-m<sup>2</sup> squares. Although the comparison of macrophyte cover between compartments at the start of the experiment was only qualitative and macrophyte biomass may have differed, we find it reasonable to assume it was similar (see Table 2 of supplemental data for cover values species by species).



**Fig. 1** Representation of the experimental pond setup and of macrophyte sampling design at day 1: cattle barbed fence (a); plastic net (1-mm mesh size) (b); quadruple plastic sleeve (c); pond access corridors (d); virtual 0.5 × 0.5 m grid over the pond surface (e); and units used to sample macrophyte cover (f)

On 6 May 2010, 100 male crayfish with a POCL of 32–40 mm (capture location and method were the same as for the laboratory tests) were introduced in the “Crayfish” compartment (ca. 5 ind m<sup>-2</sup>, similar to the densities observed in nature; Matsuzaki et al., 2009). Crayfish were marked on the uropods following the methodology proposed by Guan (1997). Traps baited with canned sardine were set every 4 days in the “Control” compartment to check for crayfish colonization. The trial ended 21 days later and 70 crayfish were recaptured from the “Crayfish” compartment (none from the “Control”) with baited traps and frozen at –18°C. Their stomach contents were examined later and the different food items weighed. The remaining crayfish may have died as a result of agonistic interactions and at least seven individuals were predated. After the removal of crayfish, nine 25 × 25 cm squares were placed in randomly selected squares of the grid of each compartment and all rooted macrophytes inside were removed, identified, dried, and weighed to the nearest 0.1 g. To count the floating macrophyte stalks, in each compartment a 1 × 0.5 × 0.5 m bottomless plastic box was set vertically in the water column in five random squares and all floating stalk pieces were collected, identified, and counted.

### Statistical analysis

Daily biomass consumption was calculated as follows:

$$\text{Consumed biomass} = (W_i \times A - W_f) \times W_s,$$

where  $W_i$  is the initial fresh weight of a stalk;  $A$  is the mean autogenic change in controls (final/initial stalk fresh weight);  $W_f$  is the stalk final fresh weight; and  $W_s$  is macrophyte mean proportion of dry mass (dry/fresh mass). When the estimates of macrophyte consumption were negative we considered them to be null. We assume that these negative values could only result from very small or non-existent macrophyte consumption combined with the weighing error (0.1 mg), since we also detected negative values in controls.

We used Spearman correlation tests to check for correlation between the consumed biomass and the number of fragments of each macrophyte species in each test. We used linear mixed models to analyse consumed biomass and the number of fragments produced by crayfish in the palatability tests. In both models macrophyte species and crayfish sex were set as fixed factors and POCL as a covariate. As consumed

biomass and number of fragments were recorded in 3 different days for each individual we included day as a repeated measure and set crayfish individual as a random factor. The contrast matrix between all fixed factors was built using Bonferroni corrections.

In the preference test, the consumption of a specific macrophyte was dependent on the consumption of the other species. To analyze these data, we performed randomization tests, comparing, for each macrophyte, biomass consumed by each sex with the distribution of 1,000 random permutations of the results matrix (the same procedure was followed regarding the number of fragments). As a test statistic, we used the 99-percentile of the observed value in the generated value distribution (Manly, 1997).

To compare total macrophyte biomass consumed and the total number of fragments produced by male and female crayfish in the preference test, we used linear mixed models with sex as a fixed factor and set POCL as a covariate. Again, we included day as a repeated measure and set crayfish individual as a random factor.

The field trial suffers from pseudoreplication issues and we followed Hulbert's (1984) approach comparing treatment mean values and their variability without applying statistical tests. At the beginning of the trial, we compared macrophyte cover; and at the end of the trial, we compared specific biomass, number of rooted stalks and number of floating stalks from "Control" and "Crayfish" compartments. Randomization analyses were carried out using R software (R Development Core Team, 2010) and all the other analyses were performed using the software IBM SPSS Statistics 20.

## Results

### Laboratory experiments

#### Palatability tests

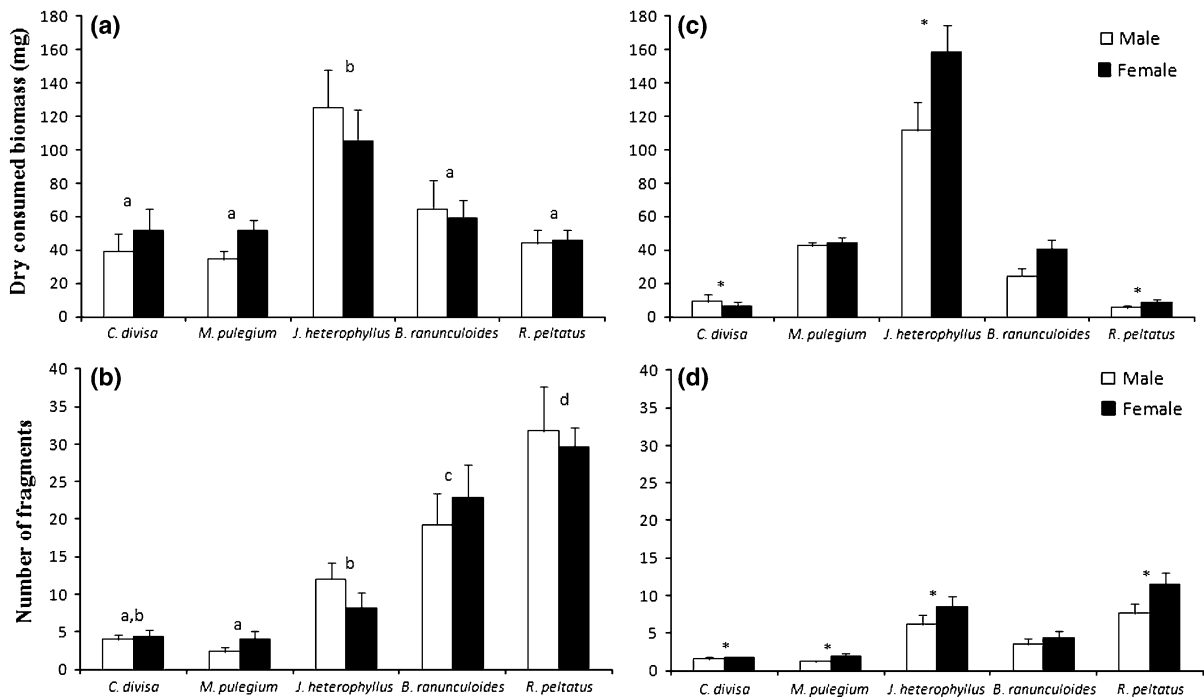
Consumed biomass differed among macrophyte species ( $F_{4,139,118} = 11.582$ ;  $P < 0.001$ ), but not between crayfish sexes ( $F_{1,139,067} = 0.023$ ;  $P = 0.880$ ) and was positively affected by crayfish size ( $Z = 5.624$ ;  $P < 0.001$ ). We found no significant interaction between consumed biomass and crayfish sex ( $F_{4,139,118} = 0.666$ ;  $P = 0.617$ ). *J. heterophyllus* was the most consumed species with  $114.9 \pm 14.7$  mg day<sup>-1</sup> (mean  $\pm$  SE), up

to more than twice the consumption of any other species (42.6–61.4 mg day<sup>-1</sup>) (Fig. 2a). Daily consumed dry biomass was similar for the non-preferred macrophytes:  $48.5 \pm 6.7$  mg day<sup>-1</sup>, which represented about 0.6–2.4% of the mean crayfish wet body weight. The number of fragments also differed among macrophyte species ( $F_{4,131,514} = 38.070$ ;  $P < 0.001$ ), but not between crayfish sexes ( $F_{1,131,487} = 0.047$ ;  $P = 0.828$ ) and was positively affected by crayfish size ( $Z = 6.145$ ;  $P < 0.001$ ). We found no significant interaction between number of fragments and crayfish sex ( $F_{4,131,514} = 0.627$ ;  $P = 0.644$ ). *R. peltatus* was the most fragmented species ( $30.7 \pm 3.1$  fragments day<sup>-1</sup>), followed by *B. ranunculoides* which also had high number of fragments ( $21.3 \pm 3.0$  fragments day<sup>-1</sup>), whereas *J. heterophyllus* ( $10.0 \pm 1.4$  fragments day<sup>-1</sup>), *C. divisa* ( $4.2 \pm 0.5$  fragments day<sup>-1</sup>), and *M. pulegium* ( $3.2 \pm 0.6$  fragments day<sup>-1</sup>) were less fragmented (Fig. 2b).

For female crayfish correlations between consumed biomass and the number of fragments were significant for each of the species tested, except for *R. peltatus*. For male crayfish these correlations were significant only for *C. divisa*, *J. heterophyllus* and *B. ranunculoides* (Table 1).

#### Preference test

Crayfish of both sexes selected *J. heterophyllus* ( $P < 0.001$ ) and avoided *C. divisa* ( $P < 0.001$ ) and *R. peltatus* ( $P < 0.001$ ). Crayfish did not select or avoid *M. pulegium* ( $P = 0.632$  for males and  $P = 0.352$  for females) and *B. ranunculoides* ( $P = 0.108$  for males and  $P = 0.283$  for females), which consumption was intermediate to that of the other species (Fig. 2c). Male crayfish consumed  $194.7 \pm 21.5$  mg day<sup>-1</sup> of macrophyte dry biomass (2.8–474.8 mg day<sup>-1</sup>), while female crayfish consumed  $258.6 \pm 19.5$  mg day<sup>-1</sup> (7.8–644.2 mg day<sup>-1</sup>), which represented  $4.6 \pm 0.5\%$  and  $6.3 \pm 0.5\%$  of male and female crayfish wet body weight, respectively. Regarding fragmentation, crayfish selected *J. heterophyllus* ( $P < 0.05$ ) and *R. peltatus* ( $P < 0.001$ ) with  $7.3 \pm 1.0$  and  $9.5 \pm 1.1$  fragments day<sup>-1</sup> each. Crayfish did not fragment *C. divisa* ( $P < 0.001$ ) and *M. pulegium* ( $P < 0.001$ ), with  $1.6 \pm 0.1$  and  $1.6 \pm 0.2$  fragments day<sup>-1</sup> each (Fig. 2d). *B. ranunculoides* was neither selected nor avoided with  $3.9 \pm 0.6$  fragments day<sup>-1</sup> ( $P = 0.27$  for males and  $P = 0.16$  for females).



**Fig. 2** Mean macrophyte biomass daily consumed (+SE) and mean number of fragments originated daily (+SE) by male and female crayfish while feeding in the palatability tests (**a, b**) and during the preference test (**c, d**). Biomass values refer to macrophyte dry biomass consumed per day (mg) and species are arranged according to an increase in both branching degree and

water content. Letters over bars indicate significant differences ( $P < 0.05$ ) between macrophyte species in the palatability tests (contrast matrix was built using Bonferroni's correction). Asterisks were added over bars on (**c**) and (**d**) (preference test) whenever the observed values stood below the 0.5 percentile or above the 99.5 percentile of the generated distributions

**Table 1** Correlations between consumed biomass and number of fragments produced by male and female crayfish in the palatability tests and in the preference test

	Palatability		Preference	
	Male	Female	Male	Female
<i>C. divisa</i>	0.690*	0.769*	0.400	0.273
<i>M. pulegium</i>	0.427	0.852*	0.420	0.825*
<i>J. heterophyllum</i>	0.902*	0.853*	0.671	0.599
<i>B. ranunculoides</i>	0.846*	0.825*	0.673	0.137
<i>R. peltatus</i>	0.495	0.555	0.426	0.433

Significant correlations after Bonferroni's correction ( $P < 0.05$ ) are marked with asterisk

Females consumed 32.8% more biomass than males ( $F_{2,30.027} = 128.786$ ;  $P < 0.001$ ) and produced 53.4% more fragments ( $F_{2,20.493} = 62.141$ ;  $P < 0.001$ ). While consumed biomass was not affected by crayfish size ( $Z = 0.983$ ;  $P = 0.327$ ) the number of fragments was positively affected ( $Z = 2.421$ ;  $P < 0.05$ ). In general, with the exception of *J. heterophyllum*, all

species were less fragmented in the preference test than in the palatability tests. This reduction in fragmentation was particularly high in *R. peltatus* and *B. ranunculoides*. These two species are among the most fragmented species in both the palatability tests and in the preference test, but were much less fragmented in the preference test (Fig. 2b, d).

In this test, correlations between consumed biomass and the number of fragments were significant for fewer macrophyte species. For females, we only found a significant correlation between consumed biomass and the number of fragments for *M. pulegium* (Table 1). Whereas for males, we did not find any significant correlations for any of the macrophyte species tested (Table 1).

#### Field trial

After 21 days, macrophyte total biomass was 31% lower in the "Crayfish" than in the "Control" compartment (82% for *J. heterophyllum* and 72% for

*Callitriche cf. brutia*) (Table 2). We also found that the total number of rooted stalks was 41% lower in the “Crayfish” than in the “Control” compartment (84% for *C. cf. brutia* and 60% for *J. heterophyllus*) (Table 2). Furthermore, we found that total number of floating cut stalks was 92% higher in the “Crayfish” than in the “Control” compartment (94% for *C. cf. brutia*, 89% for *J. heterophyllus* and 85% for *R. peltatus*) (Table 2). The results of these three parameters suggest that crayfish changed the relative proportions of the different macrophyte species in the pond. The analysis of crayfish stomachs revealed that 85% of its volume was plant material ( $\approx 33\%$  larger plant fragments and  $\approx 52\%$  shredded plant tissue) and that 15% were animal remains (macroinvertebrates and amphibians).

## Discussion

We found that crayfish were selective, that consumption preference was not always tied to the amount of fragmentation and that the predominance of these impacts varied among macrophyte species. We also found that, in the presence of preferred species, consumption and fragmentation of the non-preferred species were greatly reduced, suggesting that crayfish may remove macrophyte species from the community sequentially, from the most to the least preferred species. Several authors observed macrophyte fragmentation behaviour by crayfish (Nyström & Strand, 1996; Rodríguez et al., 2003; Anastácio et al., 2005; Crehuet et al., 2007; Gherardi & Acquistapace, 2007; Matsuzaki et al., 2009), but this study is the first to

associate consumption and fragmentation with crayfish food preference and to explain how both impacts contribute for the reduction of macrophyte biomass. We showed that although consumption and fragmentation are generally positively associated, both types of impact can independently have strong effects on macrophytes. Finally, this work illustrates the magnitude of crayfish impacts through the extent of the damage caused in the macrophyte community of the study pond in only 3 weeks.

Fresh biomass consumed in palatability and preference tests was similar to the  $440 \text{ mg day}^{-1}$  recorded for *P. clarkii* by Cronin et al. (2002). In the palatability tests, crayfish consumed 0.6–2.4% of their mean weight in macrophyte biomass, a range of values close to the amount that Avault et al. (1981) used to feed crayfish (3%). In the preference test, crayfish consumed 4.6–6.3% of their body weight in macrophyte biomass, a range of values close to what Ilhéu & Bernardo (1993) claim sufficient to feed crayfish ad libitum (7%).

The palatability tests indicate that *P. clarkii* preferred *J. heterophyllus* and this was even more obvious in the preference test. *R. peltatus* and *B. ranunculoides* were the most fragmented macrophytes in the palatability tests, but in the preference test the number of fragments of these species decreased drastically. This suggests that, when given a choice, *P. clarkii* consumes mainly the preferred species, which greatly reduces its impact on other species. This is further supported by the significant correlation between consumed biomass and number of fragments of *J. heterophyllus* for both sexes in the preference test. However, in the case of *R. peltatus* the lack of

**Table 2** Field trial results of “Control” and “Crayfish” compartments: macrophyte biomass (mg), number of rooted stalks and number of floating stalks on day 21 (mean  $\pm$  SE)

Species	Biomass (mg)		Rooted stalks		Floating stalks	
	“Control”	“Crayfish”	“Control”	“Crayfish”	“Control”	“Crayfish”
<i>M. pulegium</i>	50.9 $\pm$ 19.5	52.1 $\pm$ 25.5	3.4 $\pm$ 1.4	2.6 $\pm$ 0.5	0.0 $\pm$ 0.0	1.4 $\pm$ 0.6
<i>P. paludosa</i>	36.7 $\pm$ 28.9	40.2 $\pm$ 22.9	1.0 $\pm$ 0.6	0.9 $\pm$ 0.6	0.4 $\pm$ 0.2	12.4 $\pm$ 8.3
<i>J. heterophyllus</i>	1,450.6 $\pm$ 343.1	265.8 $\pm$ 70.5	41.7 $\pm$ 9.0	16.7 $\pm$ 8.4	4.4 $\pm$ 1.8	40.8 $\pm$ 9.8
<i>B. ranunculoides</i>	231.4 $\pm$ 88.8	195.5 $\pm$ 93.1	6.8 $\pm$ 1.8	2.6 $\pm$ 1.5	0.8 $\pm$ 0.5	1.2 $\pm$ 0.5
<i>R. peltatus</i>	2,988.6 $\pm$ 623.5	2,964.1 $\pm$ 557.9	96.7 $\pm$ 20.7	104.6 $\pm$ 12.0	1.6 $\pm$ 0.7	10.6 $\pm$ 2.8
<i>Callitriche</i> sp.	22.0 $\pm$ 7.9	22.5 $\pm$ 7.2	6.9 $\pm$ 2.3	7.9 $\pm$ 1.9	0.0 $\pm$ 0.0	15.0 $\pm$ 6.7
<i>Callitriche cf. brutia</i>	217.5 $\pm$ 94.8	60.7 $\pm$ 18.8	85.6 $\pm$ 44.1	13.9 $\pm$ 3.8	0.6 $\pm$ 0.2	10.6 $\pm$ 5.6
Total	5,301.4 $\pm$ 533.0	3,662.0 $\pm$ 498.1	264.9 $\pm$ 40.8	156.8 $\pm$ 7.2	8.8 $\pm$ 2.3	114.4 $\pm$ 12.3

correlation between consumed biomass and the number of fragments suggests that crayfish may fragment macrophytes without consuming them, as *R. peltatus* was the most fragmented, but one of the least consumed species.

Crayfish preference for *J. heterophyllus* was probably due to a combination of preferable macrophyte traits, since among the macrophytes tested this was the only species with filamentous morphology, low water content and no secondary metabolites. Cronin et al. (2002) showed that low water content and high concentrations of proteins and nitrogen are positively associated with a high nutritive value in plants. *C. divisa* had the lowest water content, but was the least branched; whereas *R. peltatus* was very branched and tender, but had a high water content and possibly secondary metabolites which are frequent in *Ranunculaceae* (Núñez & Castro, 1991). Our laboratory tests support similar food preferences for male and female crayfish. However, in the preference test, females consumed 32.8% more biomass than males and produced 53.4% more fragments. This could be an effort to replenish energy channelled to reproduction, since crayfish were captured during April, shortly after the spring reproductive period (Cruz et al., 2004).

The data from our field trial suffer from pseudoreplication issues, since they represent a single pond and, for that reason, data interpretation and the conclusions should be taken with caution. Nevertheless, we think that the field trial supports our findings from the laboratory tests, since *P. clarkii* acted as a specialist, consuming preferentially *J. heterophyllus* and *C. cf. brutia*. Macrophyte fragmentation was less selective and seemed more important in the pond than in the laboratory, which can be due to differences in water depth. In the laboratory, water depth was low and crayfish could easily access the floating stalks, cutting them multiple times. In the pond, water depth was higher and the mishandling of stalks would make it more likely for crayfish to cut a new stalk, instead of fragmenting the same floating stalk several times, having a higher impact on the macrophyte community. Overall, the striking 31% difference in the macrophyte biomass of “Control” and “Crayfish” compartments after 21 days suggests that a spring occupation of the pond (3 month) would lead to a severe impact on the macrophyte community. This change in the relative proportions of the pond macrophytes is likely to have direct implications for the community dynamics.

### Crayfish invasion of temporary ponds

The absence of fish (crayfish predators) in temporary ponds may render these ecosystems more favourable to crayfish than more permanent waters (Rodríguez et al., 2005). However, their temporary character poses a high challenge for crayfish to establish populations, as the summer drought reduces their numbers. Impact on macrophytes probably occurs in two phases—first on the preferred species and later on the remaining, previously avoided species. Therefore, consumptive impacts on the macrophyte community might be more important in the early stages of crayfish invasion, while the importance of fragmentation will increase over time, especially after the removal of all the preferred species. After this event, the macrophyte community may suffer a faster and more generalised deterioration.

In temporary habitats, such as Mediterranean ponds, the extent of the impacts of an invasion by *P. clarkii* may depend greatly on the macrophyte composition of the invaded community. In ponds where preferred species are abundant, consumption and fragmentation impacts should be high, but limited to these species, with little or no impacts on the remaining macrophytes. Even so, a high impact in these species alone could change the macrophyte community composition through changes in the relative abundance of the preferred and non-preferred species. On the contrary, in ponds without preferred species there may be an unexpectedly high and generalized impact on all macrophytes in the community, since crayfish may fragment these macrophytes while searching for the most palatable species. This shows that consumption and fragmentation impacts are not necessarily proportional and may vary with the composition of the macrophyte community.

To fully understand the consequences of crayfish herbivory on the macrophyte community of temporary ponds, it is also important to consider how crayfish invasion may affect the seed bank dynamics. We did not assess this issue, but differential reduction in macrophyte species biomass may prevent seed formation or even give a competitive advantage to the less consumed species, which could increase the proportion their seeds in the seed bank.

Biological invasions are a major driver of biodiversity change in Mediterranean freshwater ecosystems and the Iberian Peninsula is a particularly threatened region (Sala et al., 2000; Cuttelod et al., 2008). The loss of



macrophytes from the community, or even the reduction of their biomass, diminishes substrate available for periphyton, an important resource for some grazers, such as tadpoles and aquatic snails, which have a high number of rare species in these habitats. In addition, the removal of macrophytes from the southwest Iberian temporary ponds also contributes to the exclusion of amphibians, such as the near threatened *Triturus pygmaeus* and the endemic *Pelodytes ibericus* (Cruz et al., 2006a, b), as well as of the endangered dragonfly *Coenagrion scitulum* (Torralba-Burrial et al., 2011), the latter probably through a decrease in quality and quantity of suitable oviposition sites. There were also recorded shifts from a clear to a turbid state, where primary productivity is dominated by planktonic algae (Rodríguez et al., 2003), with a simplification of the food web structure, potentially followed by a loss of biodiversity.

This work shows how an alien species, described as an opportunist may impact macrophyte species differently, often in a very selective way. Macrophyte consumption and macrophyte fragmentation are not always associated and both impacts may affect macrophytes strongly and independently. Interestingly, our work shows that crayfish may remove macrophyte species sequentially from the community. Furthermore we show that crayfish impact on the macrophyte community of a temporary pond can be extensive, even in a short period. The distinct species composition and hydroperiod of Mediterranean habitats may allow for this alien species to fit in the food web in different, even unexpected, ways.

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