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# AGONISM AND INTERFERENCE COMPETITION IN FRESHWATER DECAPODS

by

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## Summary

We tested the hypothesis that agonistic behavior and interference competition induce species replacements in freshwater decapods. Our model organisms were two crayfish (the indigenous *Austropotamobius italicus* and the non-indigenous and invasive *Procambarus clarkii*) and the indigenous river crab *Potamon fluviatile*. A first experiment was aimed at analyzing the agonistic behavior of similarly-sized males in pairs of the three species combinations. Records were taken for an hour per day during five consecutive days of combats. Results showed that *P. fluviatile* was dominant over the two crayfish species and *P. clarkii* over *A. italicus*, as confirmed by their field distribution. In nature, the non-indigenous species might even reach higher levels of dominance over *A. italicus*. In fact, both the larger body size and the 'stronger' chelae of *P. clarkii* can induce asymmetries in fighting ability. Pairs composed of the non-indigenous crayfish and either *A. italicus* or *P. fluviatile* did not form stable hierarchies, possibly due to the failure of status recognition. In a second set of experiments, the agonistic behavior of the three species combinations was studied in the presence of either food (earthworms) or an artificial shelter (a 10 cm-long piece of a PVC pipe). As expected, resources influenced fighting and dominance translated into a differential capability to compete. In a third experiment, in which we measured shelter use by each species in a non-competitive context, shelters were more extensively occupied by *A. italicus* (which is dependent on natural crevices as hiding places) than by the other two species (which usually dig burrows). We expected that *Potamon* and *Procambarus* would gain less from occupying the offered shelter than *Austropotamobius*, and therefore their defense should be less vigorous. To the contrary, the presence of a rival strengthened the attraction to the shelter of these two species, in particular when river crabs were opposed to *A. italicus*.

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## Introduction

Interference competition occurs between two (or more) sympatric species when they occupy overlapping ecological niches and use similar resources (Wilson, 1975; Huntingford & Turner, 1987). Individuals of the 'stronger' species interfere with individuals of the 'weaker' species by limiting or even impeding the latter's access to food, shelter, sexual partners or to any other limited resource. An obvious consequence is the decreased survival of individuals of the 'weaker' species and/or their lowered opportunities to mate. This form of competition has therefore the immediate effect of reducing fitness of the latter species (Tokeshi, 1999).

Interference is common among many sessile, territorial animals, which need to secure an area of habitat space for their growth and reproduction (*e.g.* Dayton, 1971; Wellington, 1980; Tokeshi & Romero, 1995). However, except for the cichlid fish of Lake Tanganyika (Kuwamura, 1992), cases of motile animals leading to the competitive replacement of the 'weaker' species from a local, if not regional, area have been only rarely described (Tokeshi, 1999).

Indeed, although appealing in its essence, interference has turned out to be an elusive phenomenon, especially in natural settings and in large spatio-temporal contexts. In fact, as claimed by Tokeshi (1999), under such circumstances, researchers have a limited capability to grasp the correct picture of competition. However, time-scale is considerably shortened when non-indigenous species with invasive features act as 'agents of extinction' (Lodge & Hill, 1994) and replace indigenous species with which they share a wide niche overlap (Gherardi, 2000). On the one hand, the literature on biological invasions (*e.g.* Lodge, 1993a, b; Williamson, 1996; Moyle & Light, 1996) has claimed that the principal factor inducing species replacement is predation by invaders on indigenous species. On the other hand, however, competition seems to be underexplored because it is a subtle phenomenon, which is difficult both to identify reliably and to study (Lodge, 1993a).

Since Bovbjerg's (1970) pioneering study, freshwater decapods have been taken as appropriate model organisms to debate the theoretical issues underlying the question of interspecific competition (Gherardi, 2001). The observed disjunct distribution in related taxa (*e.g.* Rhoades, 1944; Aiken, 1965;

Fitzpatrick, 1967; Crocker & Barr, 1968) has been explained as the result of one-sided competition, in which one species suffers from the competitive effect of the other, ‘stronger’ species (Barbaresi & Gherardi, 1997). Contemporaneously, the incidental co-occurrence of two species (*e.g.* Bovbjerg, 1970) led to the analysis of the divergence in resource use producing a co-evolutionary niche separation.

Several crayfish species that have been introduced beyond their natural ranges were found to exert adverse effects on the existing decapod fauna also due to their fighting superiority (Capelli, 1982; Lodge *et al.*, 1986; Holdich, 1988; Hobbs *et al.*, 1989; Hazlett *et al.*, 1992; Holdich & Domaniewski, 1995; Söderbäck, 1995; Guiasu *et al.*, 1996; Momot, 1996; Guiasu & Dunham, 1999; Westman *et al.*, 2002). Since 1974, the red-swamp crayfish, *Procambarus clarkii*, native to north-eastern Mexico and the south-central regions of the USA, has been introduced into several European countries for aquaculture purposes and is now widely distributed in the wild in several water bodies of Europe (Holdich & Gherardi, 1999). In Italy, mixed populations composed of *P. clarkii* and the endemic *Austropotamobius italicus* have been documented in several water bodies (Gherardi *et al.*, 1999a), but some unpublished reports (B. Renai *et al.*, pers. comm.) show that the former species is fast replacing the indigenous crayfish.

In order to assess the impact of the introduced species on *A. italicus*, we conducted a laboratory study, in which, first, we addressed the question whether *P. clarkii* is dominant over the indigenous crayfish in agonistic interactions. Second, we asked whether one species could exclude the other from the access to food and shelter, which are both limited resources in the natural habitats. Our investigation was extended to the dominance/subordinance relationship between the two crayfish and the indigenous river crab, *Potamon fluviatile*. Previously, this latter species was found living segregated from crayfish (Barbaresi & Gherardi, 1997; Gherardi *et al.*, 1999a), but a more recent monitoring campaign (B. Renai, unpubl. data) showed that crabs and either *A. italicus* or *P. clarkii* had come into contact.

Our hypothesis here is that in freshwater decapods agonistic behavior and interference competition have the potential of inducing species replacements. The rationale behind this study was that, if we could identify the major rules governing the invasions, we would be able to predict the future spread and impact of invaders, and eventually devise ways of preserving indigenous species.

## Methods

### *Subjects, collections, and housing conditions*

During June-September 2000 and 2001, a total of 100 individuals per species were collected by hand from three non-overlapping populations in Tuscany (Italy): *A. italicus* (Aus) from the stream Fosso di Farfereta (Florence); *P. fluviatile* (Pot) from the stream Agna (Arezzo); and *P. clarkii* (Pro) from the pond Padule di Fucecchio (Pistoia). For the taxonomic status of *A. italicus*, see Grandjean *et al.* (2002) and Fratini *et al.* (in press).

To eliminate any factor that could induce an obvious bias to our experiments, only male, hard-shelled, sexually mature specimens with all appendages intact were included in our sampling. In fact, previous studies have shown that hierarchy in decapods is related to gender (*e.g.* Sinclair, 1977), molt stage (*e.g.* Tamm & Cobb, 1978), reproductive condition (*e.g.* Debuse *et al.*, 1999), and mutilations (Gherardi *et al.*, 2000a). We collected only form I individuals of *P. clarkii* (*i.e.* the breeding morphotype of sexually mature males that morphologically differ from the non-breeding morphotype from evident secondary sexual characters, such as prominent copulatory hooks of the 3rd and 4th walking legs, cornified gonopods, and inflated chelae; Huner, 1988), since diverse morphotypes often display differences in their hierarchical status (Guiasu & Dunham, 1997, 1998).

In the laboratory, we measured the carapace length (CL, *i.e.* anterior tip of the rostrum to the posterior edge of the carapace) of each animal. In the population of origin, CL reached a maximum of: 47.7 mm in *A. italicus* (Gherardi *et al.*, 1997), 50.5 mm in *P. fluviatile* (Gherardi *et al.*, 1987), and 70.5 in *P. clarkii* (Gherardi *et al.*, 1999b).

Individuals were marked using a waterproof paint in the center of their carapace and housed individually and visually separated in 36 × 18 × 23 cm plastic aquaria containing two liters of aged (aerated for several days to remove chlorine) tap water. They were kept under a natural 14:10 L:D cycle regime at 23°C and fed daily with 0.1 g of earthworm, which constitutes one common natural food source for these species (Momot *et al.*, 1978; Gherardi, 1987). To remove organic contaminants, water was changed an hour after feeding.

### *Experimental design*

We conducted two sets of experiments on interspecific pairs of the three different species combinations: (1) *A. italicus vs P. fluviatile*, (2) *A. italicus vs P. clarkii*, and (3) *P. fluviatile vs P. clarkii* (hereafter abbreviated as: AusPot, AusPro, PotPro, respectively). This pairwise analysis was designed to simulate the three situations observed in nature. A first experiment ('agonistic dominance') was aimed at examining whether one species had an agonistic advantage over the other by observing interactions between species in the absence of any potential resource. In the second experiment ('competition for a resource'), we explored whether one species could exclude the other from a limited resource (*i.e.* food and shelter). In a third experiment ('shelter occupancy') the use of a shelter in a non-competitive context was analyzed for the three species in individual aquaria.

### *General procedure*

Prior to the experiments, specimens were kept in isolation for 10 days, a period that was considered appropriate to reset any previous agonistic experience (Rubenstein & Hazlett, 1974). Since body size is thought to be the most important indicator of Resource Holding

Potential (Maynard Smith & Parker, 1976) also in crustaceans (Dingle, 1983; Rutherford *et al.*, 1995; Gherardi, 2001), pairs were formed by choosing individuals of the two rival species that differed in their CL by less than 2 mm within a range of 35-45 mm. In no experiment we found a significant difference in the frequency of the paired species that were the larger rivals (Binomial test: $p$ between 0.060 and 0.623). However, these precautions did not account for the different body plan between crayfish and crabs, which remains one bias in our matching of rivals.

Following the procedure of *e.g.* Penn & Fitzpatrick (1963), Capelli & Munjal (1982), Söderbäck (1991), Guiasu & Dunham (1999), and Gherardi & Daniels (2003), all experiments were staged in circular aquaria (diameter: 30 cm) containing 5 l of aged tap water. In Experiment 1, the experimental aquarium was divided transversely into two equal halves by an opaque plexiglass removable divider. Each observation period lasted for an hour and took place at the proximity of dusk (between 18:00-20:00 hours), when more intense activity has been observed in the three species (Gherardi *et al.*, 1988, 2000b; Barbaresi & Gherardi, 2001).

Animals were used only once. After the experiments were completed, indigenous species were maintained in the laboratory for at least a month before being released at the collection sites. This precaution gave us the certainty that *P. clarkii* had not transmitted the crayfish plague to *A. italicus* and *P. fluviatile* (however, plague outbreaks have never been recorded in Italy during the last few decades; Gherardi *et al.*, 1999a). Data from the three experiments were always collected by A.C. and analyzed by F.G.

### *Experiment 1: agonistic dominance*

We observed for an hour the agonistic behavior of ten pairs for each species combination (for a total of 30 pairs analyzed) during five consecutive days. To annul the influence of starvation on agonism (Stocker & Huber, 2001), each individual was regularly fed (and water was changed an hour later) during the isolation phase and at the end of any 1-hr observation. Immediately before their first encounter, the members of each pair were introduced contemporaneously (to avoid the effect of prior residence; Peeke *et al.*, 1995) onto the opposite sides of the divider in the experimental aquarium. After 10 minutes of acclimation, the divider was lifted and individuals were allowed to interact with each other. Before every subsequent 1-hr observation, the two combatants were removed from the experimental aquarium and returned to their individual aquaria for 10 minutes; the divider was inserted and the same procedure was repeated as above.

The events occurring during any 1-hr observation were described on a tape recorder and from these we obtained the following measures:

(1) Total number of fights. A fight began when one opponent approached the other and ended when one of the two individuals ran away, backed off or, if crayfish, tail-flipped away from the other at a distance longer than one body length for at least 10 sec. Tail-flipping away is the backward swimming response of crayfish involving the sudden forward contraction of the abdomen.

(2) Type and intensity of fights. We modified Bovbjerg's (1953) terminology to classify fights into four types of increasing severity: (1) avoidance (AVO, one individual retreats with no overt act by the other); threat (THR, one individual retreats when the other raises its chelae); low intensity interaction (LOW, individuals executed agonistic behavioral patterns of low intensity, *i.e.* touches and pushes); high intensity interaction (HIG, individuals executed

agonistic behavioral patterns of high intensity, *i.e.* grasps and strikes). Behavioral patterns were classified in accordance with: Vannini & Sardini (1971) for crabs, and Bruski & Dunham (1987), Gherardi & Daniels (2003), and Gherardi & Pieraccini (2003) for crayfish. The relative frequency of HIG (in %) was used as an estimate of the intensity of fights.

(3) Duration of fights, distinguishing between fights that lasted <2 min, 2-5 min, and >5 min.

(4) Attacker, *i.e.* the individual that clearly approached the rival. And,

(5) Percentage of dominance, defined as the number of fights won by the dominant individual (*i.e.* the individual that won more than 50% of fights) on the overall number of the fights battled in percent. The winner was the individual that did not retreat or that retreated after the opponent had assumed a body down posture or remained motionless (see Bruski & Dunham, 1987, for the description of these behavioral patterns). In the very few cases when both individuals retreated simultaneously, each was scored as the loser. In no cases did we record a dominance reversal during a 1-hr observation. If the observation ended before an opponent retreated, the fight was not used to determine rank orders.

In 16 instances, without any difference among pair combinations (AusPot: 5, AusPro: 4, PotPro: 6), fights appeared to escalate after an average of three days of cohabitation, yielding to the potential damage of at least one combatant; individuals were thus separated and the observation was considered over.

### *Experiment 2: competition for a resource*

We analyzed the competition of a total of 120 pairs over two types of limited resources, food and shelter (both with 20 pairs for each species combination). In the first set of experiments (food competition, FO), experimental animals were starved during the last five days of isolation and then presented in pair with abundant food (10 earthworms, the same food item they were familiar to) contained in a 1.5 × 0.5 × 0.5-cm cage made of 0.3-mm gauge wire mesh; previous observations had not revealed any attraction of the study species to the empty box. In the second set of experiments (shelter competition, SH), the shelter was a 10 cm-long piece of a rigid, gray and straight PVC pipe (4 cm internal diameter). This artificial shelter had the length and the internal diameter suitable for the size range of the individuals under study (Nakata & Goshima, 2003); preliminary tests had shown that different colored PVC pipes were occupied to the same extent by the three study species (to the contrary, black shelters were preferred by *Procambarus zonangulus* and *P. clarkii* studied by Blank & Figler, 1996). During isolation, individuals were accustomed to the shelter for the first five days of isolation. Both types of resources were glued onto the center of the experimental aquarium. Because of their dimension, FO and SH could only be defended and occupied by one animal at a time.

At the beginning of the experiments, the two individuals were placed simultaneously into the opposite halves of the aquarium and their behavior was observed for an hour. In addition to the measures described for Experiment 1, we also recorded the time spent by an individual defending FO or occupying SH. An animal was designated as being in the shelter if at least three-fourth of its carapace was inside it.

### *Experiments 3: shelter occupancy*

We measured the time that each of 20 individuals per species spent inside the artificial shelter in a non-competitive context. Animals belonged to the same range of body size as in the other two experiments; they were subjected to the same procedure of isolation as in the shelter competition experiment and were tested in the same experimental aquaria as above. We analyzed shelter occupancy of the first and the last day of a five-day long experiment.

### Statistical analysis

We followed the procedures found in Sokal & Rohlf (1969) and Siegel & Castellan (1988). Since the assumptions of normality of data and homogeneity of variance were not met also on log-transformed data and some measures represented ordinal data, we applied nonparametric tests. Mann-Whitney tests (statistic: $U$), Kruskal-Wallis analyses of variance (statistic: $H$), and Schreirer-Ray-Hare tests (statistic: *SRH*) were used to examine differences between independent samples. Related samples were analyzed by the Wilcoxon matched-pairs signed-ranks tests (statistic: $T$) and Friedman two-way analyses of variance (statistic: $Fr$). When the null hypothesis was rejected by Kruskal-Wallis and Friedman tests, Multiple Comparisons tests allowed us to determine which pairs of samples differed significantly, $\alpha$ levels being adjusted by Bonferroni correction to reduce type I error. Nonparametric measures of correlation were done with Spearman rank correlation tests (statistic: $r_s$, Student's $t$ for samples larger than 10). The same test was used in Experiment 1 to analyze the eventual changes with the time of combat in the different measures taken; the analysis was done on median values computed for every day of combat (from day 1 to day 5, AusPot: $N = 10, 7, 6, 6, 5$; AusPro: $N = 10, 6, 6, 6, 6$; PotPro: $N = 10, 5, 4, 4, 3$). Text and figures provide medians and interquartile ranges (first-third quartiles), which are the statistics most appropriate for describing the central tendency of scores in the ordinal scales analyzed by nonparametric tests. $p$ values of less than 0.05 were considered statistically significant.

The results from Experiment 2 for the two conditions of 'food competition' (FO) and 'shelter competition' (SH) were compared with the results from the first hour of combat of Experiment 1 (condition 'without resources', WR). The time of shelter occupancy in SH was compared with the same time obtained in the non-competitive context of Experiment 3.

## Results

### Experiment 1: agonistic dominance

#### Number, duration, and intensity of fights

Fights significantly decreased in their number from day 1 to day 5 in AusPot ($r_s = -1$, $N = 5$, $p < 0.01$), but remained constant in AusPro ($r_s = -0.08$, $N = 5$, $p > 0.1$) and PotPro ($r_s = 0.5$, $N = 5$, $p > 0.1$) (Fig. 1A). There was no significant difference among the three species combinations, analyzed separately per day ($H$ between 3.276 and 4.583, df $= 2$, $p > 0.1$), except for day 3 in which AusPot pairs engaged in a significantly lower number of fights than the other two interspecific pairs ($H = 6.779$, df $= 2$, $p < 0.05$). The majority of fights (61%) lasted less than 2 min, while

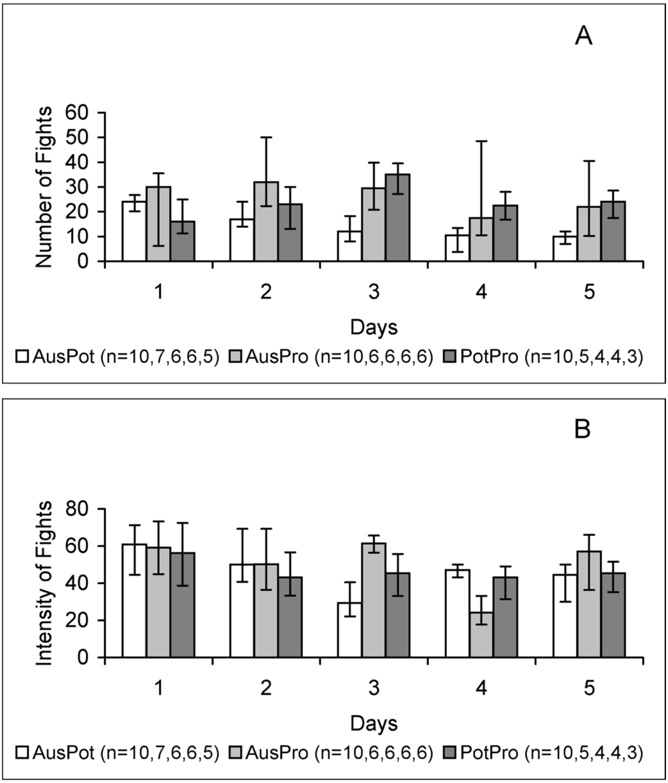

Fig. 1. Median values (and interquartile ranges) of number (A) and intensity (B) of fights throughout the five days of combat per species combination. Species combinations were: *Austropotamobius italicus vs Potamon fluviatile* (AusPot), *A. italicus vs Procambarus clarkii* (AusPro), and *P. fluviatile vs P. clarkii* (PotPro). The intensity of fights is estimated from the relative frequency (in %) of high intensity interactions. Fight number significantly decreased from day 1 to day 5 in AusPot ($p < 0.01$), but not in AusPro and PotPro ($p > 0.1$). Fight intensity remained constant with time in every species combination ($p > 0.1$).

the remaining 35% and 4% lasted 2-5 min and >5 min, respectively. Fight intensity remained constant with the time of combat in the three species combinations (AusPot: $r_s = -0.7$, $N = 5$, $p > 0.1$; AusPro: $r_s = -0.3$, $N = 5$, $p > 0.1$; PotPro: $r_s = -0.3$, $N = 5$, $p > 0.1$; Fig. 1B).

## Types of fights

In every species combination, the relative frequency of each type of fights did not significantly vary between day 1 and day 5 ($r_s$ between 0.075 and 0.7, $N = 5$, $p > 0.05$), except for AVO in PotPro ($r_s = 0.9$, $N = 5$,

$p < 0.05$), which increased with the time of combat. Overall, in every species combination the execution of behavioral patterns of contact between opponents (LOW, 20-34.9%, and HIG, 55.8-58.3%) was more frequent than AVO (2.1-5%) and THR (0-3.3%). This was confirmed by comparing median values of the five days of combat (AusPot: $Fr = 25.144$, df = 3, $p < 0.001$; AusPro: $Fr = 20.097$, df = 3, $p < 0.001$; PotPro: $Fr = 23.452$, df = 3, $p < 0.001$; after a Multiple Comparisons test and Bonferroni correction: AVO = THR < LOW = HIG, $p < 0.01$).

## Tail-flipping away

Tailflips were exclusively executed by Aus. Their occurrence was independent of both the rival species (for each day: $U$ between 7.5 and 31, $N$ between10&10 and 5&6, $p > 0.05$) and the day of combat (with Pot: $r_s = -0.775$, $N = 5$, $p > 0.1$; with Pro: $r_s = -0.5$, $N = 5$, $p > 0.1$).

## Attacks

Pot was less often the attacker than the other two species (median: 0; $H = 12.860$, df = 2, $p < 0.01$; after Multiple Comparisons test and Bonferroni correction: Pot < Aus = Pro, $p < 0.02$), whatever the species of the rival was ($H = 0.406$, df = 1, $p > 0.1$). A similar independence of attacks from the rival species was shown in both Aus (median: 2.5; $H = 1.235$, df = 1, $p > 0.1$) and Pro (median: 3.5; $H = 0.33$, df = 1, $p > 0.1$). The number of attacks increased with the percentage of dominance in both Aus ($r_s = 0.45$, $t = 2.138$, df = 18, $p < 0.05$) and Pot ($r_s = 0.476$, $t = 2.294$, df = 18, $p < 0.05$), but not in Pro ($r_s = -0.062$, $t = -0.264$, df = 18, $p > 0.1$).

## Dominance

Dominance, which was not correlated with the size difference between rivals ($r_s = -0.293$, $t = 1.62$, df = 28, $p > 0.05$), remained constant with time in every species combination (AusPot: $r_s = 0.3$, $N = 5$, $p > 0.1$; AusPro: $r_s = 0.7$, $N = 5$, $p > 0.1$; PotPro: $r_s = 0.3$, $N = 5$, $p > 0.1$). As shown after a comparison among median values of the five days of combat, dominance by Pro ($U = 3$, $N = 10, 10$, $p < 0.002$; Fig. 2C) was significantly lower when this species was opposed to Pot than when it had to fight with Aus. On the contrary, dominance by Aus ($U = 27$, $N = 10, 10$, $p > 0.1$; Fig. 2A) and by Pot ($U = 44$, $N = 10, 10$, $p > 0.1$; Fig. 2B) was independent

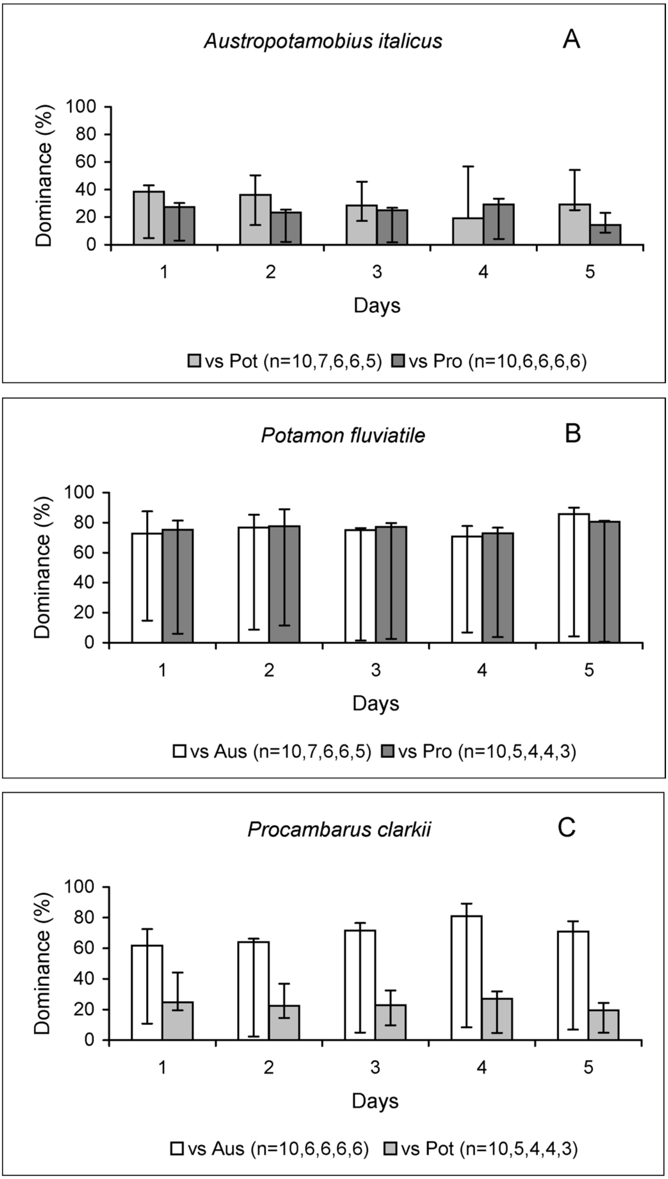

Fig. 2. Median values (and interquartile ranges) of dominance (%) by each species over the two rival species throughout the five days of combat. Aus, Pot, and Pro mean *Austropotamobius italicus*, *Potamon fluviatile*, and *Procambarus clarkii*, respectively. Dominance remained constant with time in every species combination ($p > 0.1$). Dominance by Pro was significantly lower when opposed to Pot than to Aus ($p < 0.002$). Dominance by Aus ($p > 0.1$) and by Pot ($p > 0.1$) was independent of the rival species.

of the rival species. Overall, Pot was dominant over Pro, and Pro over Aus ($H = 31.913$, df $= 2$, $p < 0.001$; $p < 0.01$ after Multiple Comparisons test and Bonferroni correction).

### *Experiments 2 and 3: competition for a resource and shelter occupancy*

#### Number, duration, and intensity of fights

Pairs of the three species combinations engaged in a similar number of fights ($SRH = 0.524$, df $= 2$, $p > 0.1$), but a significant difference was found among conditions ($SRH = 9.57$, $p < 0.01$), with WR > FO > SH ($p < 0.01$ after Multiple Comparisons test and Bonferroni correction) (Fig. 3A). Fight duration, estimated from the percentage of fights lasting more than 5 min, was significantly longer in SH than in WR and FO ($H = 9.001$, df $= 2$, $p < 0.02$; $p < 0.01$ after Multiple Comparisons test and Bonferroni correction) (Fig. 3B). While in AusPot and AusPro, the fight intensity did not change among conditions (AusPot: $H = 0.038$, df $= 2$, $p > 0.1$; AusPro: $H = 2.83$, df $= 2$, $p > 0.05$), in PotPro it was significantly higher in WR and FO than in SH ($H = 8.987$, df $= 2$, $p < 0.02$; $p < 0.01$ after Multiple Comparisons test and Bonferroni correction) (Fig. 3C).

#### Tail-flipping away

The frequency of tailflips by Aus in the presence of Pot did not vary with conditions ($H = 2.241$, df $= 2$, $p > 0.1$), while in the presence of Pro this behavior was more often executed in FO ($H = 6.221$, df $= 2$, $p < 0.05$; $p < 0.01$ after Multiple Comparisons test and Bonferroni correction).

#### Attacks

The frequency of attacks was independent of the rival species ($SRH$ between 0.001 and 1.149, df $= 2$, always $p > 0.1$), but, except for Pot ($H = 4.613$, df $= 2$, $p > 0.05$; Fig. 4B), it was affected by the condition (Aus: $H = 14.752$, df $= 2$, $p < 0.001$, Fig. 4A; Pro: $H = 12.167$, df $= 2$, $p < 0.01$, Fig. 4C). In fact, both Aus and Pro attacked the rival more often in WR than in FO and SH (Aus: $H = 14.87$, df $= 2$, $p < 0.001$; $p < 0.01$ after Multiple Comparisons test and Bonferroni correction; Pro: $H = 12.22$, df $= 2$, $p < 0.01$; $p < 0.01$ after Multiple Comparisons test and Bonferroni correction).

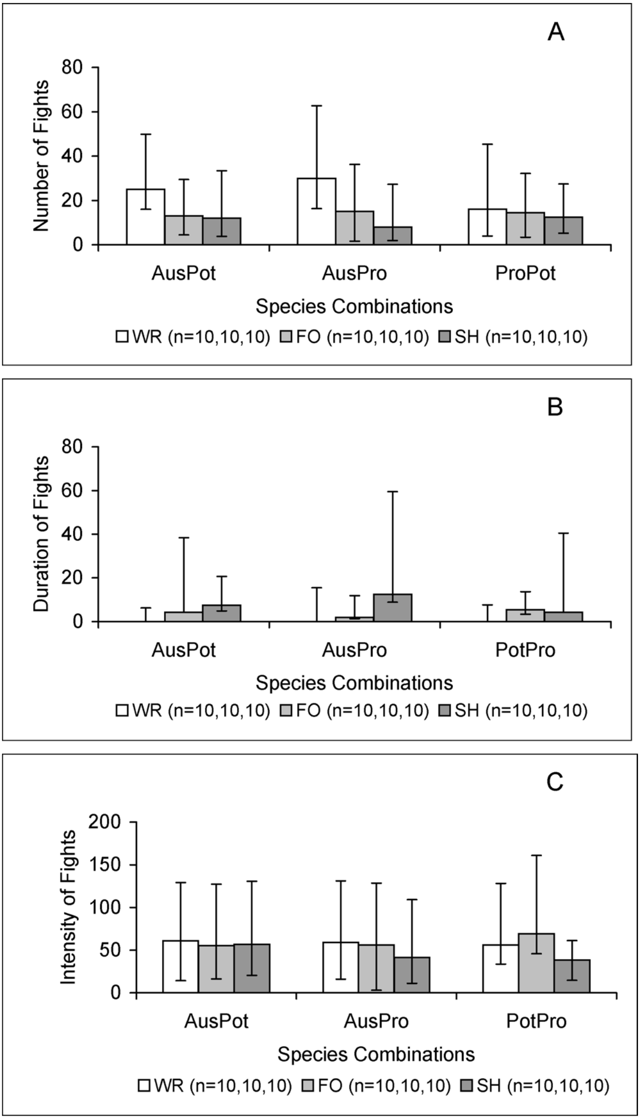

Fig. 3. Median values (and interquartile ranges) of number (A), duration (B), and intensity (C) of fights in the three species combinations for every condition. WR = without resources, FO = in the presence of food, SH = in the presence of an artificial shelter. Duration of fights is estimated from the relative frequency (in %) of fights lasting more than 5 min. See Fig. 1 for the meaning of AusPot, AusPro, and PotPro. The number of fights differed among conditions (WR > FO > SH, $p < 0.01$) and their duration was longer in SH than in WR and FO ($p < 0.01$). Only in PotPro, fight intensity was significantly higher in WR and FO than in SH ($p < 0.01$).

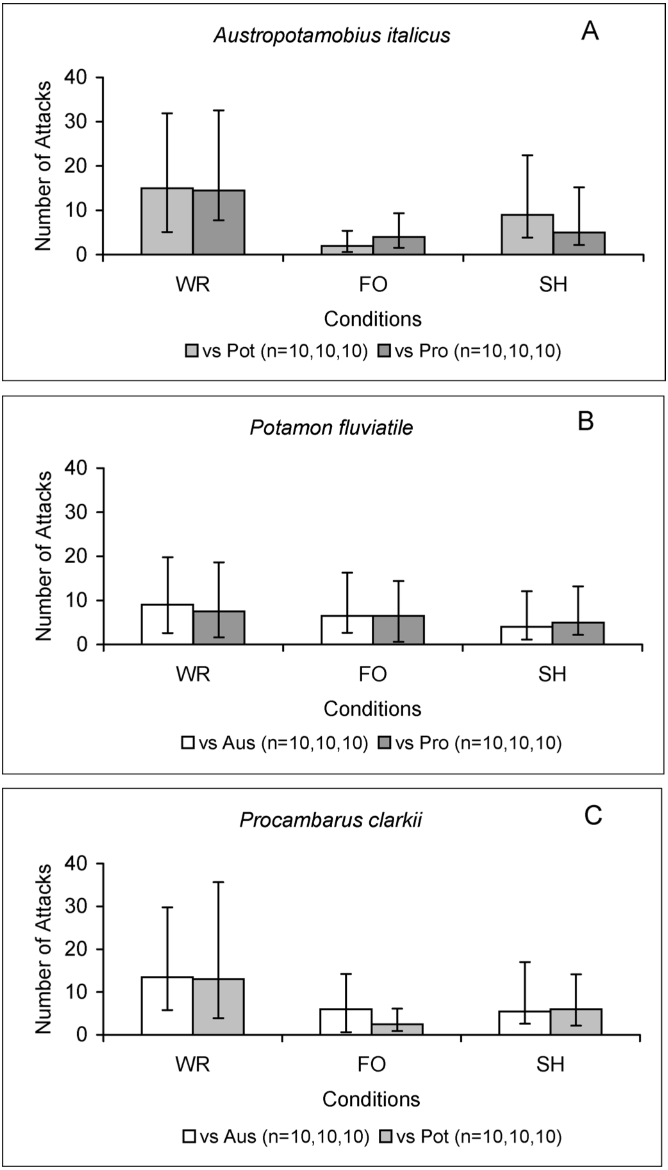

Fig. 4. Median values (and interquartile ranges) of the number of attacks performed by *Austropotamobius italicus* (A), *Potamon fluviatile* (B), and *Procambarus clarkii* (C) towards the two rival species in the three conditions. See Fig. 2 for the meaning of Aus, Pot, and Pro and Fig. 3 for the meaning of WR, FO, and SH. The number of attacks was affected by the condition in Aus ($p < 0.001$) and Pro ($p < 0.01$), but not in Pot ($p > 0.05$).

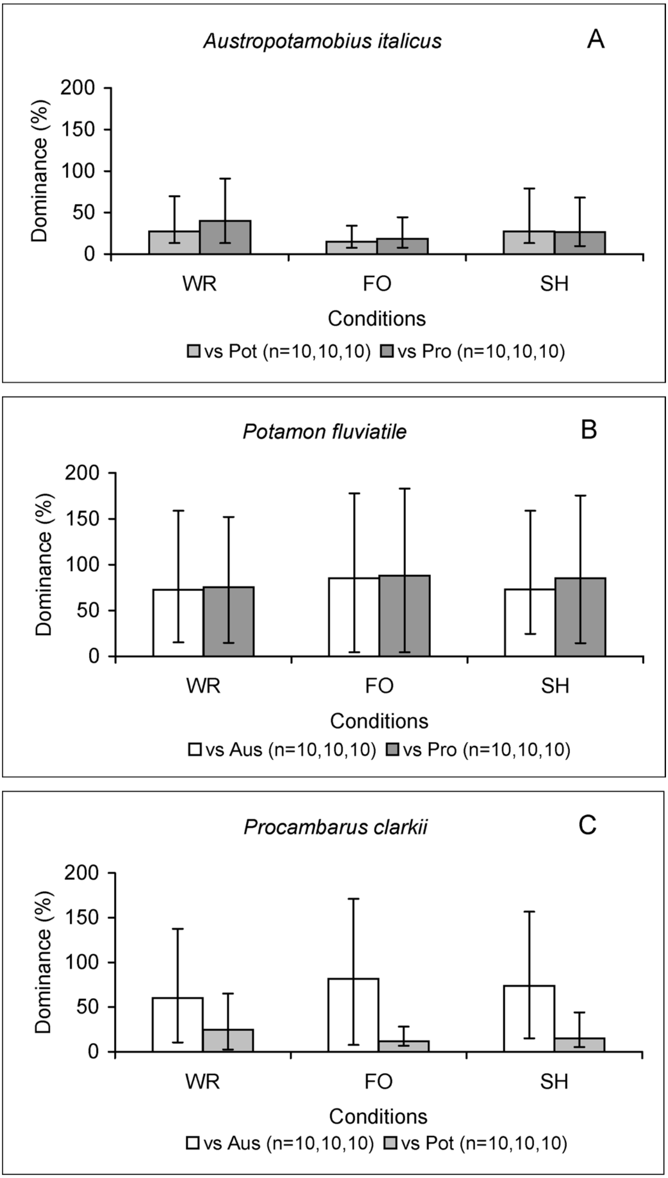

Fig. 5. Median values (and interquartile ranges) of dominance (%) by each species over the two rival species compared among conditions. See Fig. 2 for the meaning of Aus, Pot, and Pro. See Fig. 3 for the meaning of WR, FO, and SH. Dominance by Aus and Pot was independent of the rival species ($p > 0.1$), but changed with the condition ($p < 0.01$). Pro reached higher dominance levels with Aus ($p < 0.01$), without any significant difference with conditions ($p > 0.1$).

## Dominance

In Aus and Pot, dominance did not depend on the rival species (Aus: $SRH = 1.452$, df = 2, $p > 0.1$; Pot: $SRH = 0.268$, df = 2, $p > 0.1$), but changed with the condition (Aus: $SRH = 10.29$, df = 2, $p < 0.01$; Pot: $SRH = 10.85$, df = 2, $p < 0.01$; species × condition, Aus: $SRH = 0.709$, df = 2, $p > 0.1$; Pot: $SRH = 1.3$, df = 2, $p > 0.1$) (Figs 5A and 5B). Aus ($H = 10.344$, df = 2, $p < 0.01$) and Pot ($H = 10.915$, df = 2, $p < 0.01$) won relatively less and more fights, respectively, in FO than in WR and SH. In contrast, Pro reached higher dominance levels when its rival was Aus ($SRH = 39.11$, df = 2, $p < 0.01$), without any significant difference with conditions ($SRH = 0.202$, df = 2, $p > 0.1$; interaction: $SRH = 4.459$, df = 2, $p > 0.1$) (Fig. 5C).

## Defense of food

Often, individuals maintained their contact with food and defended it from the rival without necessarily consuming it. Pot was able to defend food for a longer time than the other two species (*vs* Aus, $H = 4.165$, df = 1, $p < 0.05$; *vs* Pro, $H = 1.463$, df = 1, $p > 0.1$), followed by Pro (*vs* Aus, $H = 4.1$, df = 1, $p < 0.05$).

## Shelter occupancy

Experiment 3 showed that, in the absence of a rival, Pro was the only species that significantly increased the time of shelter occupancy from day 1 to day 5 ($T = 41.5$, $N = 20$, $p < 0.01$; in contrast: Aus: $T = 73$, $N = 20$, $p > 0.05$; Pot: $T = 117$, $N = 20$, $p > 0.05$). In the non-competitive context, Aus occupied the shelter for a longer time than Pro and Pot in decreasing order ($H = 28.526$, df = 2, $p < 0.001$; $p < 0.02$ after Multiple Comparisons test and Bonferroni correction). Surprisingly, in the presence of a rival, Aus drastically reduced ($H = 19.596$, df = 2, $p < 0.001$; Fig. 6A) and Pot drastically increased ($H = 19.167$, df = 2, $p < 0.001$; Fig. 6B) the time of shelter occupancy without any effect of the rival species, while no significant changes were found in Pro ($H = 3.697$, df = 2, $p > 0.1$; Fig. 6C).

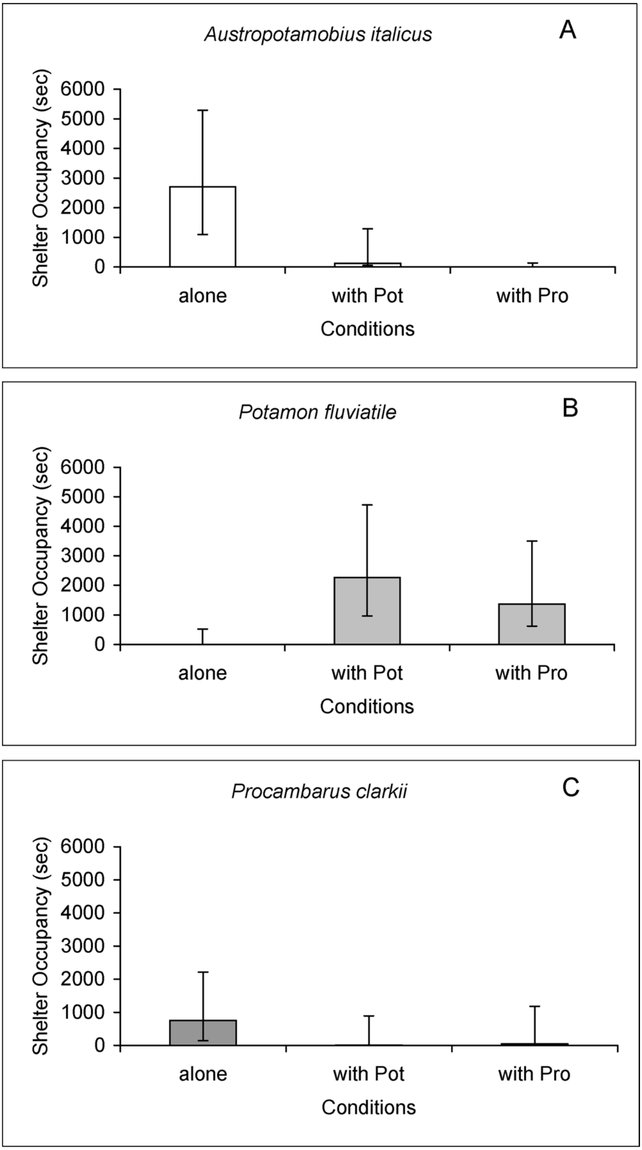

Fig. 6. Median values (and interquartile ranges) of the time of shelter occupancy in *Austropotamobius italicus* (A), *Potamon fluviatile* (B), and *Procambarus clarkii* (C) in the absence (alone) and in the presence (with Aus, with Pot or with Pro) of an opponent. See Fig. 2 for the meaning of Aus, Pot, and Pro. Sample sizes were 10 for the condition 'alone' and for every species combination. In the presence of a rival, Aus drastically reduced ($p < 0.001$) and Pot drastically increased ($p < 0.001$) the time of shelter occupancy, while no significant changes were found in Pro ($p > 0.1$).

## Discussion

### *Combating without resources*

Our study clearly showed that, even in the absence of any resource (Söderbäck, 1991), the non-indigenous *P. clarkii* is dominant over the indigenous *A. italicus*. This result is consistent with the replacement patterns observed in the field (Holdich *et al.*, 1999) and with other laboratory studies, in which agonism between non-indigenous and indigenous crayfish has been investigated in other species combinations (Capelli & Munjal, 1982; Butler & Stein, 1985; Söderbäck, 1991; Hazlett *et al.*, 1992). However, fighting superiority seemed not to be related to the potential of preying on the subordinates, as suggested for *Pacifastacus leniusculus* in England confined with *Astacus leptodactylus* and *A. pallipes* (Holdich *et al.*, 1995). That *A. italicus* is also subordinate to *P. fluviatile* confirms previous data (Barbaresi & Gherardi, 1997) and constitutes a further hint in favor of the hypothesis that the competitively superior river crab has displaced the indigenous crayfish since the Pleistocene, when the two species converged into Italy after migrating from eastern European regions (Pretzmann, 1987).

The river crab *P. fluviatile* appeared capable of outcompeting *P. clarkii*, notwithstanding that in natural populations the latter species usually reaches larger body dimensions than the crab. To our knowledge, this is the second described case of indigenous species of freshwater decapods that is dominant over non-indigenous species. The first is the crayfish *Astacopsis franklinii*, endemic to Tasmania: in the laboratory, this species dominated interactions, gained first possession of a shelter, and maintained it when opposed with equally-sized specimens of the non-indigenous *Cherax destructor* (Elvey *et al.*, 1996).

It can be questioned how well this study conducted under artificial conditions could reflect the situation in nature. Recently, Bergman & Moore (2003) showed that, at least for *Orconectes rusticus* and *O. virilis*, intraspecific fights analyzed in the laboratory scored frequencies, duration, and intensity levels significantly higher than field encounters, possibly because the confinement in small aquaria increases the ‘critical reaction’ effect (Hediger, 1950) by providing too narrow a space for escapes. However, laboratory experiments are still viewed as invaluable in elucidating the behavioral mechanisms and the environmental components that affect aggression (Bergman &

Moore, 2003). Besides, there are no good reasons to assume that dominance relationships recorded in the laboratory would be reversed in nature.

Here, we showed the existence of asymmetries in fighting ability that act as reliable predictors of *P. clarkii*'s dominance over *A. italicus*. First, although we used similarly-sized individuals in our experiments, natural populations were usually skewed towards the largest sizes in *P. clarkii*, on one hand, and towards the smallest sizes in *A. italicus*, on the other (B. Renai, pers. observ.). And a difference in body size has been repeatedly assumed to be a major determinant of dominance order in crayfish (Bovbjerg, 1953, 1956; Rabeni, 1985; Pavey & Fielder, 1996). For instance, Vorburger & Ribi (1999) demonstrated that the negative impact exercised by *Pacifastacus leniusculus* on the indigenous *Austropotamobius torrentium* increased with the relative size of the former, the larger and faster growing species being favored in interference competition. Therefore, being on average smaller, the indigenous *A. italicus* may be particularly vulnerable to the non-indigenous *P. clarkii* in natural habitats even more than in our laboratory experiments. Second, chelae of similarly-sized *P. clarkii* were usually longer (by about 3 mm) compared to that of *A. italicus* (F. Gherardi, unpubl. results); this suggests that the non-indigenous crayfish has further advantages in agonistic encounters with the indigenous species since its weapons are considerably bigger. Preliminary data (A. Cioni, unpubl. results) showed that chelae are also significantly more efficient (*i.e.* the 'strength' exercised is higher) in *P. clarkii* and *P. fluviatile* than in *A. italicus* due to their different architecture.

A third asymmetry between *A. italicus* and *P. clarkii* is related to their behavior: only the former species escaped at the end of interspecific contests using tailflips. The execution of this behavior induces the subordinate individual to occupy a more dangerous habitat; in fact, by swimming above the substrate and higher in the water column, the crayfish makes itself more vulnerable to fish predation (Guiasu & Dunham, 1999).

In accordance to the interpretation of hierarchy as a means to mitigate agonism, once a dominance relationship has been established, the phenomenon of 'social inertia' occurs (Wilson, 1975). This implies a significant reduction in the strength of agonism (see *e.g.* Issa *et al.*, 1999) and the shaping in the loser of a typical subordinate behavior (Copp, 1986; Guiasu & Dunham, 1999; Gherardi & Daniels, 2003). In the species combinations under study, we found several anomalies to this view. In fact, (1) the frequency of fights did not decrease over successive encounters (except for the pair AusPot); (2) they lasted for a relatively short time; (3) fights were always characterized by the execution of strong agonistic behaviors, while threats were seldom performed; (4) tailflips, signaling the subordinate status of *A. italicus*, were performed independently of the rival species and their frequency was almost the same from the first encounter; (5) at about the third day of cohabitation, more than 50% of combats might result in an injury to one of the two rivals; and (6) independently of the rival species, the dominant crab was seldom the attacker. Also, dominance remained constant during the five days of combat.

Such anomalies are of interest, since they might signify that the interspecific pairs under study, and specifically the pair *A. italicus* and *P. clarkii*, are unable to maintain a stable dominance hierarchy (see also Guiasu & Dunham, 1999). In the decapod literature, three mechanisms (that are however not mutually exclusive alternatives) have been claimed to induce significant changes in dominant and subordinate behavior. The first refers to the 'winner and loser' effects (Dugatkin, 1997; Bergman *et al.*, 2003); the second assumes the existence of a form of individual recognition (see *e.g.* Karavanich & Atema, 1998); the third possibility for hierarchy maintenance is the recognition of the opponent's 'aggressive state' (Winston & Jacobson, 1978), which occurs through the identification of some cues (for example a pheromone) exclusively controlled by an individual's internal state. The latter was found to rule the dominance structure of several crayfish species (see *e.g.* Zulandt Schneider *et al.*, 1999, 2001; Gherardi & Daniels, 2003). As a consequence, research on dominance relationships in decapods would benefit from an understanding of why the subordinates, in particular in crayfish contests, fail in recognizing the status of an opponent in interspecific, but not in intraspecific (Vannini & Gherardi, 1981; Copp, 1986; Gherardi *et al.*, 2001), pairs.

### *Combating with resources*

An energetically costly and time-consuming behavior like fighting is only adaptive when rewarded in terms of the prior access to resources (Case & Gilpin, 1974) and, as a consequence, an elevated social status only gives advantages when it assures the acquisition and maintenance of such resources (Wilson, 1975). In particular, shelters are valuable resources for crayfish

(Bovbjerg, 1970), serving to minimize predation risks from mammals, fish or other crayfish (Garvey *et al.*, 1994; Lodge & Hill, 1994; Englund, 1999; Hill & Lodge, 1999; Englund & Krupa, 2000) and in some species to attract mates (Bergman & Moore, 2003). Hobbs (1991) even suggested that shelter availability is the 'principle resource bottleneck' in crayfish populations. There is evidence that shelters can be limited in crayfish populations (Bovbjerg, 1970; Capelli & Magnuson, 1983; Lodge & Hill, 1994) and that an inferior ability in competing for them may lead to a higher susceptibility to fish predation (*e.g.* Olsen *et al.*, 1991; DiDonato & Lodge, 1993; Garvey *et al.*, 1994).

We therefore expected that the presence of food and the availability of a shelter could influence fighting (see Capelli & Hamilton, 1984, for intraspecific contests). In fact, we found that fights, although being less numerous, had a longer duration when individuals competed for a shelter than when they battled in its absence. In the presence of food, *P. clarkii* attacked its rival more often than in its absence and *P. fluviatile* won a relatively higher number of fights; in this situation, conflicts reached higher levels of agonism (at least when the crab competed with *P. clarkii*) and ended more often with tailflips by *A. italicus*.

Dominance hierarchies usually translate into a differential capability to compete for either the acquisition of food or the occupancy of a shelter. In this study, we found that *P. fluviatile* spent the most time (and *A. italicus* the least time) in contact with the offered food and inside the shelter. This validates the hypothesis that, at least among freshwater decapods, fighting superiority can to a certain extent explain species replacements, since it allows for the exclusive use of resources by the 'stronger' species.

Our results are consistent with the findings of *e.g.* Deng *et al.* (1993), who suggested that *P. clarkii* may be superior to the subordinate *P. zonangulus* when competing for food. At the same time, the establishment of a dominance order has been shown to be a key factor involved in the acquisition and defense of shelters in several marine (*e.g.* in fiddler crabs: Hyatt & Salmon, 1978; in lobsters: O'Neill & Cobb, 1979; Cowan & Atema, 1990), and freshwater decapods (*e.g.* in crayfish: Penn & Fitzpatrick, 1963; Bovbjerg, 1970; Capelli & Munjal, 1982; Garvey *et al.*, 1994; Blank & Figler, 1996). Similar results were obtained in three dyads studied so far and composed of an invasive and an indigenous crayfish species, *i.e. Pacifastacus* *leniusculus* and *Astacus astacus* in Sweden (Söderbäck, 1991), *P. leniusculus* and *Austropotamobius torrentium* in Austria (Vorburger & Ribi, 1999), and *P. leniusculus* and *Cambaroides japonicus* in Japan (Usio *et al.*, 2001; Nakata & Goshima, 2003).

When we tested each species separately, shelters were more extensively used by *A. italicus* than by *P. clarkii* and *P. fluviatile*. The types of shelter we offered are potentially less attractive for *P. clarkii* and *P. fluviatile*, which do not depend on available crevices as hiding places, as *A. italicus* does, but usually dig burrows into soft substrates (Huner & Barr, 1991; Gherardi *et al.*, 1987). Given that differences in pay-off influence the outcome of animal conflicts (Parker, 1974), we expected that, due to their lower preference for the offered type of shelter, *P. fluviatile* and *P. clarkii* would gain less from occupying the shelter than *A. italicus* and therefore would defend it less vigorously. This was observed by Vorburger & Ribi (1999) in *P. leniusculus* which, although being dominant in agonistic encounters, competed less efficiently for the offered shelter than the subordinate *A. torrentium*. Surprisingly, in our case the presence of a rival strengthened the attraction to the shelter for *P. fluviatile* and *P. clarkii*. In particular, the river crabs were able to monopolize the resource when opposed to *A. italicus*. To our knowledge, this is the first report that shows an increase of resource use under the risks of competition (the fact that crayfish increase resource use under the risks of predation was shown by Söderbäck, 1992; Hill & Lodge, 1994; Garvey *et al.*, 1994).

## Conclusions

Obviously, caution is needed when the issue of species replacement is analyzed, especially when studies are conducted in the simplified laboratory environment. In freshwater decapods, competitive interference is not the only mechanism into action which may explain species distribution: the observed distribution patterns might be explained by other plausible mechanisms, as discussed by Söderbäck (1995). These include certain life history traits, such as higher individual growth rates, earlier sexual maturity and higher fecundity (Huner & Lindqvist, 1995; Gherardi *et al.*, 1999b), recruitment failure (Söderbäck, 1995), differential susceptibility to predation (Butler & Stein, 1985; Lodge *et al.*, 1986; DiDonato & Lodge, 1993; Mather

& Stein, 1993; Garvey *et al.*, 1994), and reproductive interference (Capelli & Capelli, 1980; Berrill, 1985; Butler & Stein, 1985; Söderbäck, 1994; Holdich *et al.*, 1995). In addition, *Procambarus* is known to carry the crayfish fungus *Aphanomyces astaci* (the agent of the crayfish plague), which has caused the local extinction of some European indigenous crayfish populations (Alderman & Polglase, 1988).

And, at least in some instances (Lodge *et al.*, 1986), competition models seem to be inadequate to explain the dynamics of crayfish assemblages. In nature, populations certainly interact with more than one other population and in most cases species compete intraspecifically and interspecifically not just for one resource but for an array of resources. As a consequence, the outcomes of interspecific interactions may be variable and are probably affected by other community structuring forces such as predation, parasitism, and disturbance.

Several reviews have underlined the relevance of behavioral issues in conservation biology (Sutherland, 1998) and the role of behavior in animal invasions (Holway & Suarez, 1999). Consistent with these views, our main result that agonistic behavior and interference competition have the potential to explain species replacements might help our understanding of, and ability to manage biological invasions.

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