ORIGINAL PAPER

Complex impact of an invasive crayfish on freshwater food webs

Gentile Francesco Ficetola · Matteo Elio Siesa · Fiorenza De Bernardi ·
Emilio Padoa-Schioppa

Abstract Invasive alien species can have complex effects on native ecosystems, and interact with multiple components of food webs, making it difficult a comprehensive quantification of their direct and indirect effects. We evaluated the relationships between the invasive crayfish, *Procambarus clarkii*, amphibian larvae and predatory insects, to quantify crayfish impacts on multiple levels of food webs, and to evaluate whether crayfish predation of aquatic insects has indirect consequences for their preys. We used pipe sampling to assess the abundance of crayfish, amphibian larvae and their major predators (Ditiscidae, Notonectidae and larvae of Anisoptera) in invaded and uninvaded ponds within a human dominated landscape. We disentangled the multivariate effects of *P. clarkii* on different components of food web through a series of constrained redundancy analyses. The crayfish had a negative, direct impact on both amphibian communities and their predators. Amphibian abundance was negatively related to both predators. However, the negative, direct effects of crayfish on amphibians were much stronger than predation by native insects. Our results suggest that this crayfish impacts multiple levels of food webs, disrupting natural prey-predator relationships.

Keywords Amphibian larvae · Biological invasions · Dragonfly ·
Mesopredator release · Native predators · Redundancy analysis

Introduction

Invasive alien species (IAS) can have both direct and indirect impacts on native species. Direct impacts include processes such as predation, competition, hybridization and spread of diseases (Strayer et al. 2006; Nentwig 2007). Indirect impacts may be more difficult to

G. F. Ficetola (✉) · E. Padoa-Schioppa
Department of Environmental Sciences, Università degli Studi di Milano Bicocca,
Piazza della Scienza 1, 20126 Milan, Italy
e-mail: francesco.ficetola@unimib.it

M. E. Siesa · F. De Bernardi
Department of Life Sciences, Università degli Studi di Milano, Via Celoria 26, 2133 Milan, Italy

detect, but can determine complex and comprehensive consequences (Didham et al. 2007). First, IAS may have indirect impacts because they determine environmental alterations (e.g., habitat modifications, loss of vegetation), which in turn affect native species (Lodge et al. 1994; Strayer et al. 2006). Furthermore, the presence of IAS can modify existing relationships between species and their habitat, for example by magnifying the negative impact of ongoing habitat loss on populations of native species (Didham et al. 2007). IAS can also have complex, interactive impacts on food webs. IAS can become dominant in invaded ecosystems, outnumbering the biomass of native species, and can prey upon/ compete with species at multiple levels of food chains (e.g., on both primary consumers and native predators), thereby modifying the interactive relationships between native species and their natural predators (White et al. 2006; Didham et al. 2007; Nentwig 2007; Miyake and Miyashita 2011). Indirect effects of IAS can have important consequences for management, yet remain a relatively understsudied topic (White et al. 2006; Didham et al. 2007).

The red swamp crayfish, *Procambarus clarkii*, is native to the southern US and northeastern Mexico (Hobbs 1972), but is now invasive at the global scale. This crayfish has multiple impacts on freshwater ecosystems, including the spread of diseases, the alteration of wetland vegetation and trophic state (Gherardi 2006; Gherardi and Panov 2009; Matsuzaki et al. 2009; Dieguez-Uribeondo et al. 2011). It has a wide dietary spectrum, and can feed on both plant and animal items. Nevertheless, animals constitute a substantial proportion of the crayfish' diet, particularly (but not only) in juveniles (Gut-ierrez-Yurrita and Montes 2001; Correia 2003; Alcorlo et al. 2004). *Procambarus clarkii* can prey upon larval amphibians and freshwater insects altering their abundance (Correia 2003; Alcorlo et al. 2004; Cruz et al. 2006; Ficetola et al. 2011b; Miyake and Miyashita 2011; Klose and Cooper 2012). On the other hand, some freshwater insects (e.g., some Hemiptera, larval dragonflies and dytiscid beetles) are major native predators of larval amphibians, and may influence their abundance (Van Buskirk 2005; Van Buskirk and Arioli 2005; Werner et al. 2009).

In this study we investigated the relationships between *P. clarkii* and freshwater communities of amphibians and predatory insects. We evaluated crayfish impacts on both larval amphibians and their native predators, and tested whether crayfish predation over aquatic insects may result in indirect effects on native amphibians (mesopredator release) (Crooks and Soule 1999). We also quantified the relative importance of native and invasive predators on larval amphibians. Specifically we predicted that: [1] environmental features have a direct impact on the abundance of all components of freshwater communities (the alien crayfish, predatory insects and amphibians); [2] the crayfish has a negative direct impact on the abundance of both native predators and amphibians; [3] native predators have a negative impact on the abundance of amphibian larvae. [4] According to the mesopredator release hypothesis (Crooks and Soule 1999), we predict a positive relationship between crayfish and amphibians, if the impact of insects is not controlled for (i.e., a positive indirect relationship).

Methods

We studied 34 ponds in Lombardy, Northern Italy. The study area (lat: 45.4–45.9°N; long: 8.8–9.4°E) is dominated by agriculture and urban areas, but several natural parks protect forests and important sites for amphibian conservation (Ficetola et al. 2009). Natural vegetation is mostly composed by oak (e.g., *Quercus robur*, *Q. petraea*), hornbeam

(*Carpinus betula*) and maple (*Acer pseudoplatanus, A. campestre*) broadleaved forests (Digiovinazzo et al. 2010). All selected ponds are permanent or semipermanent. Pond substratum was mostly clay, sand and plant detritus.

Procambarus clarkii has been introduced recently in this area (between 2004 and 2006; Fea et al. 2006; Siesa et al. 2011), and several wetlands remain uninvaded. All ponds are used for breeding by amphibians (Ficetola et al. 2011b), and have been selected to have a 50 % ratio of invaded/uninvaded sites. At least one species of brown frog (*Rana latastei* or *R. dalmatina*) laid eggs into each of the study ponds. In late spring 2010, we used pipe sampling (Dodd 2010) to quantitatively assess the abundance of amphibian larvae, aquatic insects and *P. clarkii* in ponds. During this sampling effort, larvae of all amphibian species breeding in the wetlands (Italian crested newt *Triturus carnifex*, smooth newt *Lissotriton vulgaris*, fire salamander *Salamandra salamandra*, Italian tree frog *Hyla intermedia*, common toad *Bufo bufo*, pool frog *Pelophylax* synklepton *esculentus*, agile frog *Rana dalmatina* and Italian agile frog *R. latastei*) were detected. Samples were collected by thrusting a 0.25 m^2 circular pipe sampler through the water column and about 5 cm into the sediment. In all cases, the top of the sampler was above the water line. Small nets (mesh size: 1 mm) were used to remove all animals from the water and the first cm of the sediment (Werner et al. 2009; Dodd 2010). We collected about one sample/100 m^2 (average: four samples per wetland). Detectability analyses showed that this sampling allows a reliable assessment of the distribution of study species (Ficetola et al. 2011b).

Amphibian larvae were identified in the field following standard keys (Lanza et al. 2007) and immediately released. Tadpoles of the frogs *R. dalmatina* and *R. latastei* are morphologically similar, therefore, we pooled them into a single group ("brown frogs"). For each species of amphibian, we calculated abundance as number of larvae/m^2. Individuals of *P. clarkii* were counted and weighed, to measure their wet mass (g/m^2). As potential predators of amphibian larvae, we considered dragonfly larvae (Odonata, Anisoptera), adults and juveniles of Coleoptera Dytiscidae, and Heteroptera of the *Notonecta* genus (the only genus of the Notonectidae family present in the study area) (Ruffo and Stoch 2005; Van Buskirk 2005; Van Buskirk and Arioli 2005). Dytiscids and Notonectids were identified in the field and released; we estimated their abundance as individuals/m^2. Larval dragonflies were stored in ethanol and identified in the laboratory using standard keys (Carchini 1983; Heidemann and Seidenbusch 2002). The impact of dragonflies on native amphibians strongly varies according to their size, as large larvae consume more tadpoles than smaller species or larvae at early development stages (Van Buskirk and Arioli 2005). Furthermore, conservation in ethanol can alter the fresh weight of invertebrates. Larvae were therefore dried in a MMM Incucell V 55 incubator (96 h at 60 °C) and weighed, to estimate dry mass (g/m^2) (Werner et al. 2007). The mass and the summed abundance (individuals/m^2) of dragonflies were used in subsequent analyses, pooling all species of dragonflies. Using a finer taxonomic resolution (e.g., considering the genus or species level) did not improve the performance of analyses, because several species were present in only one or very few wetlands.

Environmental features are key determinants shaping freshwater communities. To characterize the abiotic features and the vegetation of each wetland, we recorded in the field six parameters using standard bioassessment protocols: pond surface, maximum depth, water permanence during the study period (permanent/non permanent), canopy cover, riparian vegetation along the shoreline and presence/absence of fish (Table 1). Pond area, depth and hydroperiod were strongly correlated. These variables were therefore synthesized through a Principal Component Analysis, which extracted one component (hereafter: abiotic features) strongly correlated to all the three original variables

Table 1 Environmental variables recorded

Environmental features	Average (range)
Surface[a,b] (m^2)	474 ± 161 (10–5,185)
Maximum depth[a,b] (cm)	72 ± 15 (5–200)
Permanence during the study period (Y/N)[b]	
Fish presence (Y/N, visual estimate)	
Canopy cover (proportion, visual estimate)[c]	0.52 ± 0.05 (0–0.9)
Riparian vegetation (proportion, visual estimate)[c]	0.87 ± 0.04 (0.33–1)

For permanence and fish presence, we do not report average values because these variables can have only values 0–1. Average values are $\pm$ standard errors

[a] Log-transformed prior to analysis

[b] Summarised by PCA prior to perform analyses

[c] Arcsine square root transformed prior to analysis

($P < 0.001$ for all correlations) and explaining 51 % of their variance. Large values of abiotic features indicate large, deep and permanent wetlands.

We used a series of constrained-redundancy analyses (C-RDA) to explore the multivariate relationships between environmental features, crayfish abundance, predatory insects and amphibians. RDA is a canonical analysis that evaluates how much of the variation of the structure of one data set (e.g., community composition) is explained by independent variables (e.g., predators, habitat features) (Borcard et al. 2011). We built six C-RDAs on the basis of a priori hypotheses (Fig. 1) representing expected causal relationships between data sets, while taking into account potential effects of other factors. We hypothesized and tested whether [1] environmental features have a direct impact on the abundance of *P. clarkii*, predatory insects and amphibians, respectively (represented by paths P1, P2 and P3 in Fig. 1); [2] *P. clarkii* has a direct impact on native insects (P4) and amphibians (P5); [3] predatory insects have a direct impact on amphibian larvae (P6). Results of the C-RDAs were then combined, on the basis of a priori hypotheses, in a graphical model that represents relationships among data sets, while controlling for the effects of other parameters (Fig. 1), similarly to how partial regression analyses are combined in path analysis (see Ficetola et al. 2011a for further details). For example, to evaluate variation explained along path P3 in Fig. 1 (i.e., impact of environmental features on amphibians, while taking into account native and alien predators), we used a C-RDA with amphibian larvae as dependent matrix, environmental features as costraining (i.e., independent) matrix, crayfish and predatory insects as conditioning matrix (the effect of which is partialled out). Species abundance data were Hellinger transformed prior to perform C-RDAs (Legendre and Gallagher 2001). To improve statistical power, non-significant conditioning matrices were removed from final analyses; we used a permutation tests (10,000 permutations) to assess significance of C-RDAs (Borcard et al. 2011; Legendre et al. 2011). Subsequently, we used adjusted R^2 values and variance partitioning (Borcard et al. 2011) to assess the amount of variation explained along each path. This procedure was repeated two times. In the first analysis, the abundance of crayfish was expressed as fresh mass, the abundance of dragonflies was expressed as dry mass, while the abundance of the other taxa was expressed as individuals/m^2. This approach was used to scale larger predators in accord with their abilities to consume more preys [e.g., the
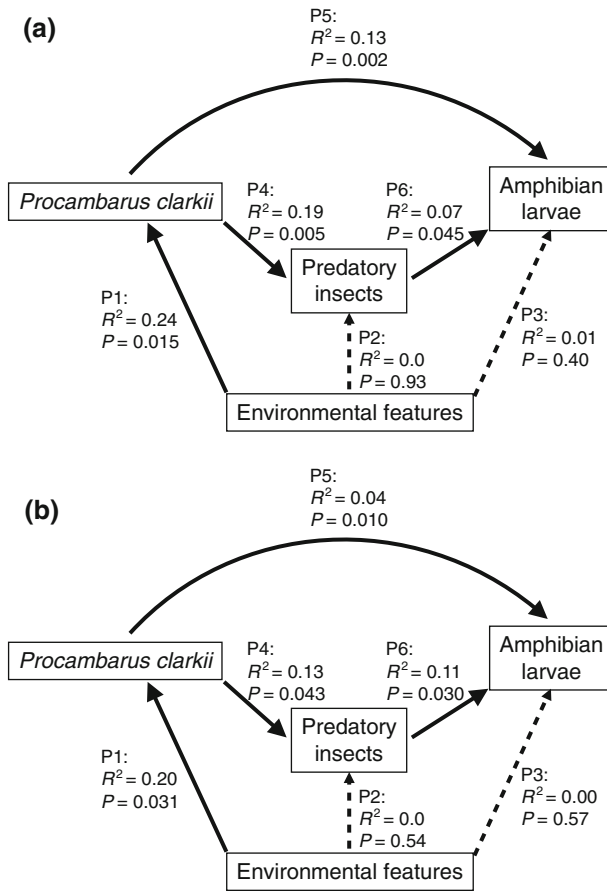
Fig. 1 Path diagrams illustrating the relationships between amphibians, predatory insects, *Procambarus clarkii* and environmental variables, as analyzed using a series of constrained redundancy analyses. *Dashed arrows* represent nonsignificant relationships. *P1* indicates path 1, and so on. *Hypotheses* represented by paths are: P1–P3: environmental features have a direct impact on *P. clarkii*, predatory insects and amphibians, respectively; P4–P5: *P. clarkii* has a direct impact on native insects and amphibians; P6: predatory insects have a direct impact on amphibians. **a** Abundance of *P. clarkii* expressed as fresh mass, abundance of dragonflies expressed as dry mass, abundance of other taxa expressed as individuals/m^2. **b** Abundance of all taxa expressed as individuals/m^2

predatory impact of a large dragonfly larvae is higher than the impact of small larvae, and the overall food intake of very small crayfishes may be smaller than the intake of large crayfishes (Van Buskirk and Arioli 2005; Werner et al. 2007)]. In a second analysis, the abundance of all taxa was expressed as individuals/m^2, which improved homogeneity among taxa. Furthermore, *P. clarkii* juveniles are proportionally more carnivorous than adults (Gutierrez-Yurrita and Montes 2001; Correia 2003). As the overall number of juvenile crayfishes was much higher than the number of adults (about 80 % of captured individuals), this analysis gives more weight to the impact of juvenile crayfishes. We performed analyses using the package VEGAN in R 2.12 (R Development Core Team 2010; Oksanen et al. 2011).

Results

Procambarus clarkii was detected in 50 % of ponds, with abundance ranging from 1 to 23 individuals/m^2 (2 to 118 g/m^2; average $\pm$ SE: 33.5 $\pm$ 7.6 g/m^2). In nearly all the invaded ponds, we detected both juvenile and adult forms of *P. clarkii*. We detected larvae of seven dragonflies and eight amphibians (Table 1); *Salamandra salamandra* and *Bufo bufo* were detected in less than 10 % of ponds and excluded from analyses. *Notonecta* or Dytiscidae were detected in 24 % of ponds (Table 2).

   Crayfish abundance was significantly related to pond features (permutation test, $P = 0.015$; Fig. 1a: path P1). Crayfish were more abundant in large, permanent wetlands with limited emerging vegetation. After taking into account the effect of crayfish, relationships between environmental features and predatory insects or larval amphibians were not significant ($P \geq 0.4$ for both taxa; paths P2–P3). The abundance of all predators was negatively related to *P. clarkii* ($P = 0.005$; path P4; Table 3). Similarly, after accounting for potential effects of predatory insects, there was a negative relationship between most amphibians and *P. clarkii* ($P = 0.002$; path P5; Table 4). Finally, when taking into account the effect of crayfish, there was a significant relationship between amphibian larvae and aquatic insects ($P = 0.045$; path P6), newts being negatively associated with Dytiscidae and *Notonecta*, and anurans being negatively associated with dragonflies (Fig. 2). The direct effects of crayfish abundance explained 19 % of variation of predator communities and 13 % of variation of amphibian communities, while the relationship between predators

Table 2 Average abundance ($\pm$SE) of amphibian and dragonfly larvae detected through pipe sampling in the study wetlands

	Abundance (N/m^2)		Dry weight (g/m^2)	
	With crayfish	Without crayfish	With crayfish	Without crayfish
a: Amphibians				
Triturus carnifex	0.53 $\pm$ 0.53	7.00 $\pm$ 2.29		
Lissotriton vulgaris	0.59 $\pm$ 0.48	54.80 $\pm$ 28.34		
Salamandra salamandra	0.24 $\pm$ 0.17	0.06 $\pm$ 0.06		
Hyla intermedia	0.00 $\pm$ 0.00	18.94 $\pm$ 13.45		
Bufo bufo	0.00 $\pm$ 0.00	0.08 $\pm$ 0.08		
Pelophylax synklepton *esculentus*	0.00 $\pm$ 0.00	21.37 $\pm$ 11.56		
Rana dalmatina + *R. latastei*	19.22 $\pm$ 7.84	152.20 $\pm$ 87.71		
b: Odonata, Anisoptera				
Aeshna isosceles	0.00 $\pm$ 0.00	0.18 $\pm$ 0.18	0.000 $\pm$ 0.000	0.002 $\pm$ 0.001
Aeshna cyanea	0.65 $\pm$ 0.48	0.65 $\pm$ 0.36	0.082 $\pm$ 0.059	0.063 $\pm$ 0.046
Aeshna mixta	0.00 $\pm$ 0.00	0.08 $\pm$ 0.08	0.000 $\pm$ 0.000	0.002 $\pm$ 0.002
Libellula quadrimaculata	0.00 $\pm$ 0.00	0.12 $\pm$ 0.12	0.000 $\pm$ 0.000	0.003 $\pm$ 0.003
Orthetrum albistylum	0.06 $\pm$ 0.06	0.00 $\pm$ 0.00	0.003 $\pm$ 0.003	0.000 $\pm$ 0.000
Orthetrum cancellatum	0.06 $\pm$ 0.06	0.00 $\pm$ 0.00	0.004 $\pm$ 0.004	0.000 $\pm$ 0.000
Sympetrum sanguineum + S. *striolatum*	3.52 $\pm$ 2.37	21.24 $\pm$ 7.11	0.022 $\pm$ 0.014	0.216 $\pm$ 0.086
c: Other predators				
Ditiscidae	0.31 $\pm$ 0.15	1.18 $\pm$ 0.94		
Notonecta	0.00 $\pm$ 0.00	0.59 $\pm$ 0.59		

Table 3 Pearson's correlation between the abundance of *Procambarus clarkii* and each taxon of amphibian larvae and predatory insects

Taxon	Correlation with *Procambarus clarkii*	
	(a) Using abundance r	(b) Using mass r
Lissotriton vulgaris	**−0.56**	**−0.65**
Triturus carnifex	**−0.51**	**−0.58**
Hyla intermedia	**−0.34**	**−0.39**
Rana latastei + *R. dalmatina*	**−0.41**	**−0.58**
Pelophylax s. *esculentus*	**−0.36**	**−0.41**
Dragonflies	**−0.45**	**−0.47**
Dytiscidae	−0.004	−0.08
Notonecta	−0.14	−0.16

In (a) the abundance of all taxa is measured as individuals/m^2. In (b), the abundance of *P. clarkii* is measured as fresh mass, the abundance of dragonflies is measured as dry mass, while for other taxa it is measured as individuals/m^2. Significant correlations ($P < 0.05$) are in bold. $N = 34$ in all correlations

and amphibians explained much less variation (Fig. 1a). Results of analyses remained nearly identical if the abundance of crayfish and dragonflies is expressed as individuals/m^2 instead than mass (Fig. 1b).

The mesopredator release hypothesis predicts a positive relationship between crayfish and amphibians, if the impact of insects is not controlled for (Crooks and Soule 1999). A simple (unconstrained) RDA between crayfish and amphibians explained a significant amount of variation (analysis measuring crayfish abundance as fresh mass: $P < 0.0001$; analysis measuring crayfish abundance as individuals/m^2: $P = 0.002$) but, contrary to the expectation of the mesopredator release hypothesis, the relationship was negative for all amphibians (Table 3).

Discussion

The invasive crayfish, *P. clarkii*, had strong impacts on multiple levels of a pond food web, and reduced the abundance of both predators and primary consumers, confirming our prediction [2] (Table 1; Table3). Most taxa of amphibians and predatory insects were also found in invaded wetlands, but in these wetlands they attained very low abundance, compared to the uninvaded ones. The crayfish impacts on native communities is likely caused by direct predation, but might also involve indirect relationships (Lodge et al. 1994; Klose and Cooper 2012). For instance, crayfish may reduce the abundance of insects through consumption (path P4 in Fig. 1; Correia 2003; Alcorlo et al. 2004; Miyake and Miyashita 2011). Furthermore, indirect relationships between the invasive crayfish and the native predatory insects may be mediated by crayfish predation over amphibian larvae, which may determine a limited food availability for insects (White et al. 2006).

In accordance with our prediction [3], amphibian abundance was negatively related to their native predators (Fig. 1, path P6), which were scarce in invaded wetlands (path P4; Table 1). Based on Crooks and Soule (1999), an indirect positive effect could be a plausible outcome of crayfish on amphibians (mesopredator release). However, the direct, negative impact of *P. clarkii* on amphibians (path P5) was much stronger than its indirect

Table 4 RDA scores representing the relationships between the abundance of *Procambarus clarkii* and the abundance of amphibians

	Relationship with *P. clarkii.*	
	(a) Using abundance	(b) Using mass
Lissotriton vulgaris	−0.38	−0.53
Triturus carnifex	−0.25	−0.30
Hyla intermedia	−0.26	−0.22
Rana latastei + *R. dalmatina*	0.18	0.06
Pelophylax s. *esculentus*	−0.18	−0.18
% Constrained variation	18 %	14 %
% Conditional variation	10 %	19 %
Total variation	0.35	0.35

Scores are estimated by a C-RDA taking also into account predatory insects. Negative scores indicate a negative relationship between crayfish and amphibian taxa, after taking into account the effect of insects. In (a) the abundance of all taxa is measured as individuals/m^2. In (b), the abundance of *P. clarkii* is measured as fresh mass, the abundance of dragonflies is measured as dry mass, while for other taxa it is measured as individuals/m^2. We also report the amount of variation explained by the independent effect of crayfish (constrained variation), the amount of variation explained by the conditioning matrix, and the total variation of models
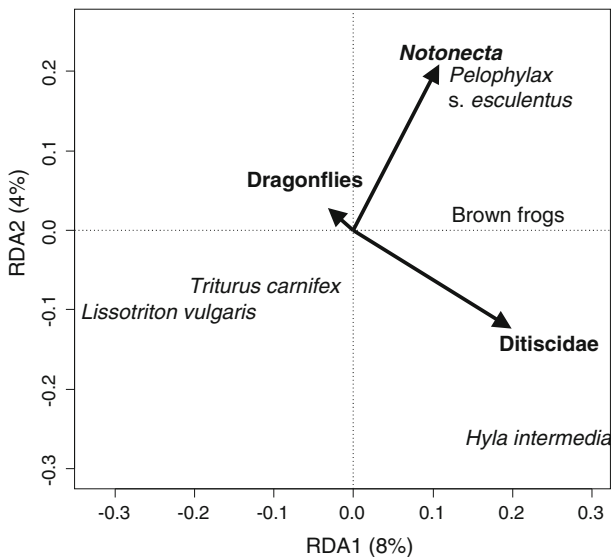
Fig. 2 Constrained redundancy analysis relating the abundance of amphibian larvae to predatory insects, while taking into account the abundance of *P. clarkii*. *Arrows* represent the correlations between taxa abundances; only the first two axes are shown. In parenthesis, the amount of variation explained by RDA axes

effects, mediated through the reduction of native predators. The direct impact of *P. clarkii* explained twice the variation explained by native insects (path P6), and *P. clarkii* explained only partially the variation of predator communities (path P4). Therefore, prediction [4], formulated on the basis of the mesopredator release hypothesis, was not supported by data.

Omnivorous crayfish may become dominant species in freshwater food webs, controlling the abundance of both macrophytes and animals at multiple trophic levels (Lodge et al. 1994; Covich et al. 1999; Usio and Townsend 2002; Klose and Cooper 2012). In our study case, the crayfish impact on amphibian larvae (i.e., the basal trophic level) was so strong that they did not really benefit from the reduction of their predators: the reduction in native predators was overwhelmed by the direct, negative impact by crayfish (Table 3). Our results confirm those obtained in other freshwater systems. For instance, in New Zealand streams, the crayfish *Paranephrops zealandicus* reduces the abundance of predatory insects, but the majority of primary consumers are not facilitated by the scarcity of predators because of the negative direct impact of crayfish (Usio and Townsend 2002).

In natural conditions, both amphibians and freshwater insects strongly depend on pond features. Presence of fish, hydroperiod and canopy cover are major environmental gradients determining the composition of amphibian communities. For instance, newts usually prefer semi-permanent ponds without fish, brown frogs are often associated with shaded ponds, while pool frogs and tree frogs are associated with sunny ponds (Van Buskirk 2003; Ficetola and De Bernardi 2004; Van Buskirk 2005). Similarly, hydroperiod, sun exposure and pond size influence the richness and the composition of dragonfly communities (McCauley et al. 2008; Hamasaki et al. 2009). Therefore we expected a significant relationship between pond features and the composition of amphibian and insect communities (prediction [1]). However, our data did not confirm this prediction, because crayfish impacts was extremely strong, overwhelming relationships between native communities and wetland features (Usio and Townsend 2002). In practice, native communities were poorly represented in all invaded wetlands, independent of environmental features: several wetlands, having environmental features potentially suitable for amphibians and insects, actually host very poor native communities. This is a further indication that this IAS overshadows the processes structuring freshwater communities at multiple levels.

It should be remarked that the effect of crayfish on amphibians was stronger than the overall impact of all native predators, even tough only 50 % of wetlands were invaded, and in some of them the abundance of *P. clarkii* was low because these ponds are at the early stages of the invasion. We might expect that a polytrophic generalist such as *P. clarkii*, which may feed on animals, plant items, carrion and detritus, would prey with lower efficiency and less impact than insects, for which live tadpoles are a major food item. However, several features may allow *P. clarkii* to have a stronger impact than native predators. First, *P. clarkii* is larger than any other invertebrate native of European stagnant water, and lack of co-evolution hampers the responses of prey (White et al. 2006; Gomez-Mestre and Diaz-Paniagua 2011). Second, different crayfish life history stages have different trophic specialization: young crayfish are more carnivorous than adults (Gutierrez-Yurrita and Montes 2001; Correia 2003; Alcorlo et al. 2004). Furthermore, the ability to exploit multiple resources may allow *P. clarkii* maintaining high densities also when preys are scarce, as in these areas crayfish may shift to a detritus or plant-based diet. Highly generalist alien predators may thus have stronger impacts on native communities than expected, because they can deplete their preys while maintaining high densities through the exploitation of other resources (e.g., plants).

References

Alcorlo P, Geiger W, Otero M (2004) Feeding preferences and food selection of the red swamp crayfish, *Procambarus clarkii*, in habitats differing in food item diversity. Crustaceana 77:435–453

Borcard D, Gillet F, Legendre P (2011) Numerical ecology withy R. Springer, New York

Carchini G (1983) Odonata. CNR, Verona

Correia AM (2003) Food choice by the introduced crayfish *Procambarus clarkii*. Ann Zool Fenn 40:517–528

Covich AP, Palmer MA, Crowl TA (1999) The role of benthic invertebrate species in freshwater ecosystems—Zoobenthic species influence energy flows and nutrient cycling. Bioscience 49:119–127

Crooks KR, Soule ME (1999) Mesopredator release and avifaunal extinctions in a fragmented system. Nature 400:563–566

Cruz MJ, Pascoal S, Tejedo M, Rebelo R (2006) Predation by an exotic crayfish, *Procambarus clarkii*, on Natterjack Toad, *Bufo calamita*, embryos: its role on the exclusion of this amphibian from its breeding ponds. Copeia 2006:274–280

Didham RK, Tylianakis JM, Gemmell NJ, Rand TA, Ewers RM (2007) Interactive effects of habitat modification and species invasion on native species decline. Trends Ecol Evol 22:489–496

Dieguez-Uribeondo J, Aquiloni L, Martin MP, Gherardi F (2011) The North American crayfish *Procambarus clarkii* is the carrier of the oomycete *Aphanomyces astaci* in Italy. Biol Invasions 13:359–367

Digiovinazzo P, Ficetola GF, Bottoni L, Andreis C, Padoa-Schioppa E (2010) Ecological thresholds in herb communities for the management of suburban fragmented forests. For Ecol Manage 259:343–349

Dodd CKJ (ed) (2010) Amphibian ecology and conservation. A handbook of techniques. Oxford University Press, Oxford

Fea G, Nardi PA, Ghia D, Spairani M, Manenti R, Rossi S, Moroni M, Bernini F (2006) Dati preliminari sulla distribuzione in Lombardia dei gamberi d'acqua dolce autoctoni e alloctoni. Atti Soc It Sci Nat Mus Civ St Nat Milano 147:201–210

Ficetola GF, De Bernardi F (2004) Amphibians in an human-dominated landscape: the community structure is related to habitat features and isolation. Biol Conserv 119:219–230

Ficetola GF, Padoa-Schioppa E, De Bernardi F (2009) Influence of landscape elements in riparian buffers on the conservation of semiaquatic amphibians. Conserv Biol 23:114–123

Ficetola GF, Marziali L, Rossaro B, De Bernardi F, Padoa-Schioppa E (2011a) Landscape - stream interactions and habitat conservation for amphibians. Ecol Appl 21:1272–1282

Ficetola GF, Siesa ME, Manenti R, Bottoni L, De Bernardi F, Padoa-Schioppa E (2011b) Early assessment of the impact of alien species: differential consequences of an invasive crayfish on adult and larval amphibians. Divers Distrib 17:1141–1151

Gherardi F (2006) Crayfish invading Europe: the case study of *Procambarus clarkii*. Mar FreshwBehav Phy 39:175–191

Gherardi F, Panov VE (2009). *Procambarus clarkii* (Girard), red swamp crayfish/crawfish (Cambaridae, Crustacea). In: Pages 316 *in* DAISIE. Handbook of alien species in Europe. Springer, Dordrecht

Gomez-Mestre I, Diaz-Paniagua C (2011) Invasive predatory crayfish do not trigger inducible defences in tadpoles. Proc R Soc B 278:3364–3370

Gutierrez-Yurrita PJ, Montes C (2001) Bioenergetics of juveniles of red swamp crayfish (*Procambarus clarkii*). Compar Biochem Physiol Mol Integr Physiol 130:29–38

Hamasaki K, Yamanaka T, Tanaka K, Nakatani Y, Iwasaki N, Sprague DS (2009) Relative importance of within-habitat environment, land use and spatial autocorrelations for determining odonate assemblages in rural reservoir ponds in Japan. Ecol Res 24:597–605

Heidemann H, Seidenbusch R (2002) Larves et exuvies des libellules de France et d'Allemagne (sauf de Corse). Société Française d'Odonatologie

Hobbs HH (1972) Biota of freshwater ecosystems, identification manual 9: Crayfishes (Astacidae) of North and Middle America. Water Pollution Control Research Series. US Environmental Protection Agency, Washington, DC

Klose K, Cooper SD (2012) Contrasting effects of an invasive crayfish (*Procambarus clarkii*) on two temperate stream communities. Freshw Biol 57:526–540

Lanza B, Andreone F, Bologna MA, Corti C, Razzetti E (eds) (2007) Fauna d'Italia, vol XLII. Amphibia, Calderini, Bologna

Legendre P, Gallagher ED (2001) Ecologically meaningful transformations for ordination of species data. Oecologia 129:271–280

Legendre P, Oskanen J, ter Braak CJF (2011) Testing the significance of canonical axes in redundancy analysis. Methods Ecol Evol 2:269–277

Lodge DM, Kershner MW, Aloi JE, Covich AP (1994) Effects of an omnivorous crayfish (*Orconectes Rusticus*) on a fresh-water littoral food-web. Ecology 75:1265–1281

Matsuzaki SS, Usio N, Takamura N, Washitani I (2009) Contrasting impacts of invasive engineers on freshwater ecosystems: an experiment and meta-analysis. Oecologia 158:673–686

McCauley SJ, Davis CJ, Relyea RA, Yurewicz KL, Skelly DK, Werner EE (2008) Metacommunity patterns in larval odonates. Oecologia 158:329–342

Miyake M, Miyashita T (2011) Identification of alien predators that should not be removed for controlling invasive crayfish threatening endangered odonates. Aquatic Conserv Mar FreshwEcosyst 21:292–298

Nentwig W (ed) (2007) Biological invasions. Springer, Berlin

Oksanen J, Blanchet FG, Kindt R, Legendre P, O'Hara RB, Simpson GL, Solymos P, Stevens HH, Wagner H (2011) Vegan: community ecology package version 1.17–6. www.r-project.org

R Development Core Team (2010) R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna

Ruffo S, Stoch F (eds) (2005) Checklist e distribuzione della fauna italiana. Memorie del Museo Civico di Storia Naturale di Verona, 2a serie, sezione Scienze della Vita, Verona

Siesa ME, Manenti R, Padoa-Schioppa E, De Bernardi F, Ficetola GF (2011) Spatial autocorrelation and the analysis of invasion processes from distribution data: a study with the crayfish *Procambarus clarkii*. Biol Invasions 13:2147–2160

Strayer DL, Evinver VT, Jeschke JM, Pace ML (2006) Understanding the long-term effects of species invasions. Trends Ecol Evol 21:645–651

Usio N, Townsend CR (2002) Functional significance of crayfish in stream food webs: roles of omnivory, substrate heterogeneity and sex. Oikos 98:512–522

Van Buskirk J (2003) Habitat partitioning in European and North American pond-breeding frogs and toads. Divers Distrib 9:399–410

Van Buskirk JV (2005) Local and landscape influence on amphibian occurrence and abundance. Ecology 86:1936–1947

Van Buskirk JV, Arioli M (2005) Habitat specialization and adaptive phenotypic divergence of anuran populations. J Evol Biol 18:596–608

Werner EE, Skelly DK, Relyea RA, Yurewicz KL (2007) Amphibian species richness across environmental gradients. Oikos 116:1697–1712

Werner EE, Relyea RA, Yurewicz KL, Skelly DK, Davis CJ (2009) Comparative landscape dynamics of two anuran species: climate-driven interaction of local and regional processes. Ecol Monogr 79:503–521

White EM, Wilson JC, Clarke AR (2006) Biotic indirect effects: a neglected concept in invasion biology. Divers Distrib 12:443–455