

A conceptual model of exotic crayfish (*Procambarus clarkii*) effects on charophyte propagule banks in wetlands

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Un modelo conceptual sobre los efectos del cangrejo rojo americano en los bancos de semillas de carófitos en humedales

Abstract

This paper deals with an evaluation of the environmental impact potential of the American red swamp crayfish (*Procambarus clarkii* Girard) on seed banks in wetlands which it has invaded recently. We put emphasis on stone worts (Charophyta) because of their importance in wetland vegetation in terms of biomass and diversity. We combine laboratory consumption experiments and field surveys to develop a conceptual model on direct and indirect crayfish impacts on vegetation and seed banks. This model can guide us in developing useful management and restoration strategies in the future.

Resumen

Este artículo presenta una evaluación del impacto ambiental potencial del cangrejo rojo americano (*Procambarus clarkii* Girard) sobre los bancos de semillas de las zonas húmedas que recientemente ha invadido. Debido a su importancia en términos de biomasa y biodiversidad de la vegetación de las zonas húmedas, se presta especial atención al grupo de los carófitos (Charophyta). Para ello se realiza una combinación de experimentos de consumo en laboratorio y ensayos de campo que contribuyen a desarrollar un modelo conceptual de los impactos directos e indirectos de los cangrejos sobre la vegetación y el banco de semillas. Este modelo puede servir como guía para desarrollar futuras estrategias de gestión y restauración en los humedales.

Keywords: Seed bank, exotic species, management, sediment, propagules

Introduction

In most parts of the world introductions of exotic species are the first or second (after land use change) most important threat to freshwater biodiversity and ecosystem function (Sala et al., 2000). A case in point is the American red swamp

crayfish (*Procambarus clarkii* Gir.), a species native to the south-eastern USA and north-eastern Mexico, which has nowadays a geographically widespread distribution because of its commercial value. Several studies have dealt with the effective impact of *P. clarkii* on the invaded habitats (reviewed in Geiger et al., 2005) and major

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changes in several freshwater ecosystems have been attributed to its introductions (Dieguez and Söderhall, 1993; Montes et al., 1993; Angeler et al. 2001; Gherardi et al., 2001; Ilhéu et al., 2002). For example, there exists empirical evidence that *P. clarkii* herbivory eradicates submerged vegetation (Feminella and Resh, 1989, Angeler et al., 2003), thereby triggering the shift from the clear-water state to the turbid, phytoplankton-dominated state relatively fast. Nonetheless, considering the enormous feeding plasticity of *P. clarkii* we can expect other, mechanistically distinct, but potentially severe, impacts on vegetation processes in wetlands. For example, Gutiérrez-Yurrita et al. (1998) demonstrated that *P. clarkii* gut contents consist of ca 30% sediments. This suggests a potential direct effect of dense *P. clarkii* populations on seed banks in invaded wetlands, if seeds are accidentally consumed and damaged during gut passage. On the other hand, there shall be no negative impacts on seeds if passage through crayfish guts stimulates germination (Baskin and Baskin, 1998); however, this shall be unrealistic ecologically in the case of *P. clarkii* because of the presence of gastric mills in its stomach that disintegrate ingested particles (Holdich, 2002).

This study was carried out with the aim to evaluate *P. clarkii* effects on a wetland seed bank. We report on the results of two complementary laboratory experiments putting emphasis on charophyte propagules because of their importance, in terms of abundance, in many seed banks (Bonis and Grillas, 2002), and their susceptibility to accidental crayfish consumption because of their small size. These results are combined with our previous field studies (Angeler et al., 2001, 2003) to develop a conceptual model of direct and indirect impacts of this crayfish species on the charophyte component in wetland vegetation, with emphasis on seed banks.

Material and methods

Laboratory experiments

The crayfish specimens used in this study were captured on 26.03.2002 in the Las Tablas de Daimiel National Park (Ciudad Real, central

Spain). 60 individuals were maintained in several 40 L plastic carboys equipped with flower pot refugia at ambient temperature in the laboratory. Water was changed once a week and crayfishes were fed ad libitum with lettuce and sausages (frankfurter) prior to the use in the experiments. The sediments used in this study were collected on 22.03.2002 in the Las Tablas de Daimiel National Park (central Spain), a floodplain wetland infested with several charophytes (*Chara hispida* var. *hispida*, *Ch. hispida* var. *major*, *Ch. canescens*, *Ch. aspera*, and *Tolypella glomerata*), *Ranunculus peltatus*, and *Zannichelia pedunculata*, among submerged species, and *Typha domingensis*, *Cladium mariscus*, *Phragmites australis* and *Scirpus maritimus*, as emergents (Cirujano and Medina, 2002). Only the uppermost 15 cm sediment layer was collected because of the presence of highest viable oospore abundance (Van den Berg et al., 2001; Bonis and Grillas, 2002; authors personal observations). Experiment 1 was an exploratory study to determine the fate of oospores when consumed by *P. clarkii*. We used a total of 8 replicas; 4 replicas with adult males, and 4 replicas with adult females to assess potential sex-dependent differences. Each replica consisted of a 4 L plastic aquarium filled with aged tap water and contained an adult crayfish individual. Feeding of crayfish was suspended for one week to standardise for hunger prior to the beginning of the experiment. Shortly before the start of the experiment, 25 oospores, previously isolated from the sediment, were inserted into a frankfurter sausage cube (ca. 5mm edge length) to facilitate consumption of oospores by *P. clarkii*; each replica received 3 cubes (75 oospores in total). After consumption, fecal pellets were retrieved in regular intervals from the aquaria and observed in the dissecting microscope for the presence and state of oospores. Arcsin transformed data (percentages of damaged and intact oospores) of male and female treatments were statistically compared with one-way ANOVA.

Experiment 2 was designed to assess crayfish impacts on the sediment seed bank, putting emphasis on the abundance of charophyte oospores. 40 l plastic carboys with a surface area of

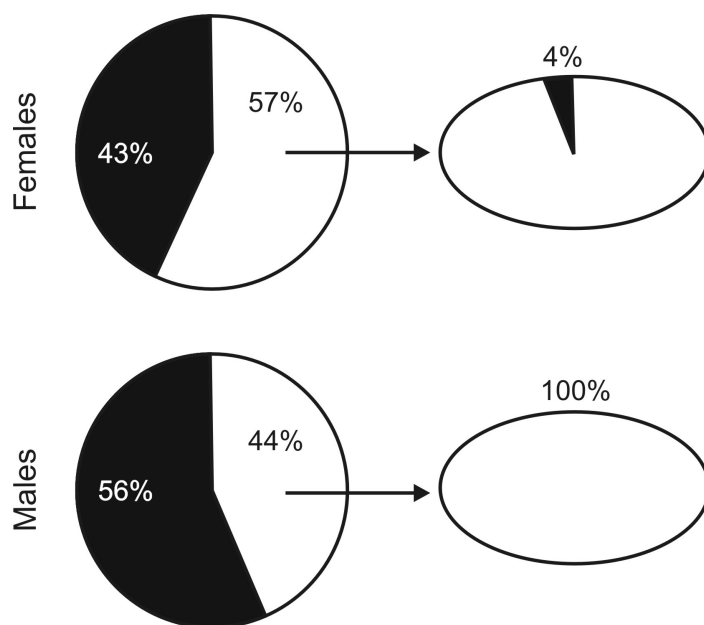


Figure 1. Result of oospore consumption experiment. Round pies showing percentage of ingested (white areas) vs non-ingested (black areas) oospores. Ellipsoid pies show the state of ingested oospores which were recovered from feces; white areas, percentage of damaged/destroyed oospores; black areas, undamaged oospores.

0.25 m², were filled with air-dried wetland sediment to a height of 10 cm and subsequently inundated with aged tap water. Each of three replicates received an adult male and an adult female *P. clarkii* individual, whilst further three replicates remained without crayfish (controls). The experiment started on 05.08.2002 and lasted until 04.11.2002. Replicas were all maintained at 20-25°C, and a 16:8h day-night cycle. Sampling in monthly intervals consisted of recollection of crayfish feces: Crayfish individuals were retrieved from their treatments, washed with distilled water and placed overnight in individual 4l aquaria filled with aged tap water. After 24 h we returned crayfish individuals to their designated replica, and collected feces from the aquarium bottom. Total feces mass was then suspended in 500 ml distilled water and mixed for 2 min. A 50 ml subsample was transferred to sedimentation chambers and the composition of feces evaluated in ca 40 fields using an inverted microscope. Feces particles were

assigned to the following categories: plant detritus, invertebrate and algae remains, and sediment. For the calculation of volumes we assumed simple geometric shapes (spheres, cubes, cuboids). Measurements were taken from at least 50 – 100 particles of each category. Finally the feces composition is expressed as percentage of volume of each category. At the same sampling date, we collected subsamples of sediments (to cover spatial heterogeneity of oospore distribution) of the uppermost sediment layer. Subsamples were gently mixed and oospores counted in 1g (wet weight) of sediment. Crayfish were not fed during the entire duration of this experiment to 1) promote detritivorous feeding of crayfish, and 2) because of development of zooplankton and mosquitolarvae which may have served as a food subsidy. A repeated measures (RM) ANOVA was selected for statistical treatment of the log transformed oospore abundance data.

Field experiments

To construct the conceptual model on crayfish effects on charophyte vegetation in wetlands, we used our previously published data on charophyte aboveground biomass consumption in enclosure experiments (Angeler et al., 2003) [Note that this experiment was not designed to evaluate oospore abundance in the sediment]. Briefly, eight enclosures consisting of transparent polyethylene hose tubing (1 m diameter) were established in a shallow vegetated area of the Las Tablas de Daimiel National Park, a semiarid floodplain wetland in central Spain. Four enclosures contained charophyte vegetation that covered the entire bottom (hereafter referred to as the “charophyte enclosures”), and the other four enclosures were void of vegetation (“sediment enclosures”). The enclosures were driven to ca 40 cm in the sediment and suspended 50 cm above surface, allowing for normal sediment-water and water-air interactions.

The one-week experiment was carried out during a summer draw down. After establishment of the enclosures, adult *P. clarkii* was added in a density corresponding to 4.45 (mean) ± 0.9 (standard deviation) ind m^{-2} in the two charophyte enclosures, and 3.8 ± 0 in the two sediment enclosures. This stocking scheme is well within the natural crayfish density range (e.g., Feminella & Resh, 1989). Experimental individuals received enclosure specific marks with finger nail polish for identification against possible intruders. Upon termination of the experiment, animals were transferred to the laboratory for digestive tract analyses. Throughout the study, we monitored charophyte aboveground biomass in terms of percentage cover as described in Angeler et al. (2003).

Results

Laboratory experiments

Experiment 1: Of the total oospores present in the sausage cubes in experiment 1, 44% and 57% were consumed on average by male and female crayfish respectively. The remaining percentage of propagules were not ingested and lost to the aquarium bottom when crayfish handled the

sausage cubes (Figure 1). When *P. clarkii* consumed oospores, most were destroyed during gut passage (100% destruction male treatment and 96 % in the female treatment; Figure 1). There was no significant sex effect (ANOVA, $p = 0.29$, $F = 2.23$).

Experiment 2: The numbers of oospores per gram sediment (wet weight) before crayfish stocking were 117 ± 17 (average of 3 replicates \pm SD) in the designated crayfish treatment and 99 ± 17 in the control. Oospore abundance decreased consistently in the control and the treatment during the experiment (Figure 2). No significant crayfish treatment effect was observed in comparison with the control (RM-ANOVA, $p=0.13$, $F = 3.69$). Crayfish feces, in terms of volume, were composed on average, and in order of importance, of plant detritus (ca. 80-90%; range observed in 4 samples), sediment (ca. 5-10%), invertebrate and algae remains (ca. 5-10%).

Field experiments

During the one-week enclosure study, crayfish herbivory reduced, as expected, the charophyte cover in the charophyte enclosures. Contrary to expectation, however, crayfish effects on charophyte cover were not very pronounced because crayfish reduced the macrophyte cover by only 35%. This can be explained by a reduction of crayfish density to 1.9 mean ± 0.8 SD in the charophyte enclosures during the experiment. The loss of crayfish was due to mortality and escape from the enclosures. The crayfish individuals which remained in the enclosures showed a diet shift from herbivory to carnivory by scavenging on dead conspecifics. In terms of importance, Chara biomass, meat from dead crayfish, plant detritus, algae and microcrustaceans, and sediment comprised 40, 20, 20, 15, and 5% of gut contents respectively.

In the sediment enclosures crayfish density showed a similar reduction, as was the case in the charophyte enclosures. Here, crayfish density dropped also to 1.9 ± 0.8 ind m^{-2} . Unlike *P. clarkii* individuals in the charophyte enclosures, individuals of the sediment enclosures contained

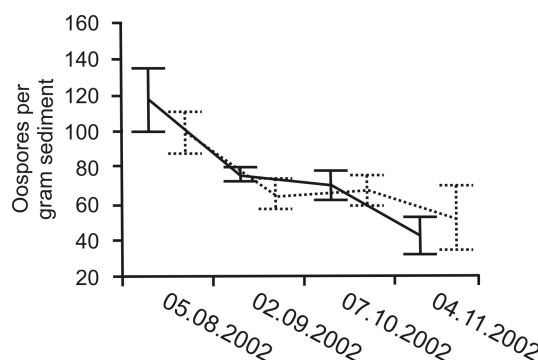


Figure 2. Time course of charophyte oospore abundance in a wetland seed bank being exposed to *P. clarkii* (full line) and in controls (dotted line) in a 4 month laboratory study. Shown are the means \pm SDs of three replicates.

87% of plant detritus in their gut contents, with the remainder falling in the categories of sediment and algae and invertebrates.

Discussion

From a mechanistic point of view, results of laboratory experiment 1 indicate that *P. clarkii* has the potential to damage charophyte propagules, which can be attributed to gastric mills in crayfish guts that efficiently grind ingested food particles (Holdich, 2002). Under environmentally more realistic scenarios (laboratory experiment 2), however, the abundance of charophyte oospores in seed banks with and without exposure to *P. clarkii* was not significantly different, notwithstanding our stocking scheme reflects a slightly higher *P. clarkii* density than is found in nature (e.g., Feminella and Resh, 1989). Here, the feeding ecology of *P. clarkii* (Momot, 1995) could help in interpreting these results.

Plant detritus was the most important food source for crayfish in our study. Sediment consumption was low, contrasting with the observations made by Gutiérrez-Yurrita et al. (1998). However, we acknowledge the distinct environmental settings and/or experimental frames used in both studies. While Gutiérrez-Yurrita et al. (1998) did not evaluate charophyte oospore destruction in the sediments, our study does not

suggest that *P. clarkii* significantly reduces the abundance of oospores in the wetland seed bank through consumption, even though it has the potential to do so (see results from laboratory experiment 1). This suggests that *P. clarkii* does not directly reduce seed bank potential through detritivory and accidental seed ingestion, and provides some optimistic perspectives on the restoration of site-specific vegetation of a wetland if *P. clarkii* abundances can be effectively managed.

The results of our field study and those of other authors (Feminella and Resh, 1989) suggest that the standing crop of submerged macrophytes can be significantly reduced by *P. clarkii*, thereby contributing in the shift from a macrophyte dominated, clear-water state to a phytoplankton-dominated, turbid water state (Angeler et al., 2003). Based on the preliminary line of evidence provided here on the impacts in the wetland propagule bank, and the current state of investigation related to the impacts of crayfish herbivory in wetlands, we provide a conceptual model on direct and indirect crayfish impacts on the charophyte component of wetland vegetation (Figure 3).

Because we have not found a significant reduction of charophyte oospores in the sediment, the model suggests that the direct impact of *P. clarkii* on the charophyte propagule bank through detritivory and accidental seed consumption should be relatively low. By contrast, an indirect impact in

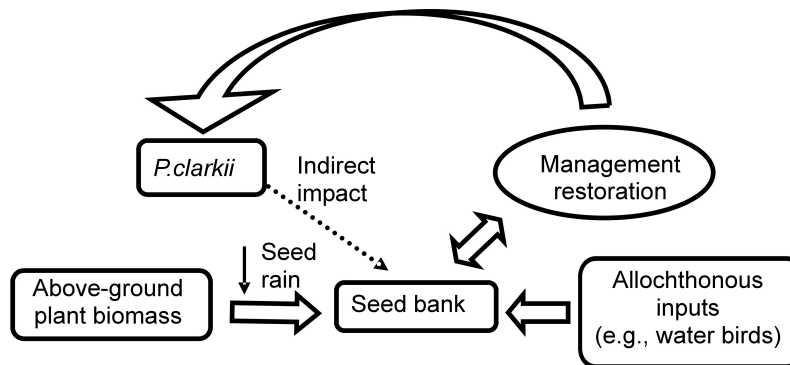


Figure 3. A conceptual model of direct and indirect effects of the exotic crayfish, *Procambarus clarkii*, on wetland seed banks, with emphasis on charophyte propagules. The model incorporates mechanisms related to effects of herbivory on above-ground plant biomass, and detritivory associated with benthic foraging in environments free from vegetation. Thick arrows suggest a strong influence, while faint dotted arrows suggest a low impact. For further details see text.

the propagule banks shall be given because of the elimination of above-ground plant biomass including the associated reproductive units, thereby truncating the replenishment of the propagule bank through “seed rain”. However, dormant plant propagules and animal resting eggs can be viable throughout decades (Baskin and Baskin, 1998, Thorp and Covich, 2001), suggesting that the available stock of propagules in the wetland sediments could give rise to structurally and functionally equivalent vegetation types to those existing prior to crayfish invasions, if *P. clarkii* populations can be effectively managed. Furthermore, allochthonous inputs of propagules through dispersal by waterfowl could somehow mitigate the reduced in situ seed production. Because *P. clarkii*'s preferential feeding mode is carnivory, management strategies could incorporate *P. clarkii* autecological requirements to relieve wetland vegetation from herbivory pressure. This, in turn, could help reinstate the seed rain to the sediments.

Finally, we acknowledge that exotic species are only one facet, besides contamination and alteration of the hydrological cycle that contribute to the degradation of wetlands. The survival of

wetland environments in the future will likely depend on remedial actions taking synergistic approaches.

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References

- Angeler, D.G., Sánchez-Carrillo, S., Garcia, G. and Alvarez-Cobelas, M., 2001. The influence of *Procambarus clarkii* (Cambaridae, Decapoda) on water quality and sediment characteristics in a Spanish floodplain wetland. *Hydrobiologia*, 464:89-98.
- Angeler, D.G., Sánchez-Carrillo, S., Alvarez-Cobelas, M., Cirujano, S. and Medina, L., 2003. Exotic crayfish activity and its effects on water quality: preliminary implications for the alternative stable equilibria in Mediterranean wetlands. *Journal of Mediterranean Ecology*, 4: 13-21.

- Baskin, C. C. and Baskin, J. M., 1998. Seeds: ecology, biogeography, and evolution of dormancy and germination. Academic Press, New York.
- Bonis, A. and Grillas, P., 2002. Deposition, germination and spatio-temporal patterns of charophyte propagule banks: a review. *Aquatic Botany*, 72: 235-248.
- Cirujano, S. and Medina, L., 2002. Plantas acuáticas de las lagunas y humedales de Castilla – La Mancha. Real Jardín Botánico (CSIC) and Junta de Comunidades de Castilla – La Mancha., pp. 1-340.
- Dieguez, J. and Söderhall, K., 1993. *Procambarus clarkii* Girard as a vector for the crayfish plague fungus, *Aphanomyces astaci Schikora*. *Aquaculture and Fisheries Management*, 24: 761-765.
- Feminella, J.W. and Resh, V.H., 1989. Submerged macrophytes and grazing crayfish: an experimental study of herbivory in a California freshwater marsh. *Holarctic Ecology*, 12:1-8.
- Geiger, W., Alcorlo, P., Baltanas, A. and Montes, C., 2005. Impact of an introduced Crustacean on the trophic webs of Mediterranean wetlands. *Biological Invasions*, 7: 49–73.
- Gherardi, F., Renai, B. and Corti, C., 2001. Crayfish predation on tadpoles: A comparison between a native (*Autropotamobius pallipes*) and an alien species (*Procambarus clarkii*). *Bulletin Francais de la Peche et de la Pisciculture*, 361: 659-668.
- Gutierrez-Yurrita, P.J., Sancho, G., Bravo, M.A., Baltanas, A. and Montes, C., 1998. Diet of the red swamp crayfish *Procambarus clarkii* in natural ecosystems of the Doñana National Park temporary freshwater marsh (Spain). *Journal of Crustacean Biology*, 18:120-127.
- Holdich, D.M. (Ed.), 2002. *Biology of Freshwater Crayfish*. Blackwell Publishing, Oxford.
- Ilhéu, M., Guilherme, P. and Bernardo, J.M., 2002. Impact of red swamp crayfish (*Procambarus clarkii*) on aquatic invertebrate and macrophyte assemblages: a case study in the south of Portugal. *Verhandlungen der Internationalen Vereinigung für Theoretische und Angewandte Limnologie*, 28: 144-147.
- Momot, W.T., 1995. Redefining the role of crayfish in aquatic ecosystems. *Reviews in Fisheries Science*, 3: 33–63.
- Montes, C., Bravo, M.A., Baltanas, A., Gutierrez, P.J. and Duarte, C.M., 1993. Ecological basis for an integral management of the American red swamp crayfish (*Procambarus clarkii*) in the Doñana National Park. ICONA, Madrid.
- Sala, O.E., Chapin, F.S III., Armesto, J.J., Berlow, E. J., Bloomfield, R. and Dirzo, E., Huber-Samwald, E., Huenneke, L.F., Jackson, R.B., Kinzig, A., Leemans, R., Lodge, D.M., Mooney, H.A., Oesterheld, M., LeRoy Poff, N., Sykes, M.T., Walker, B.H., Walker, M. and Wall, D.H., 2000. Biodiversity scenarios for the year 2100. *Science*, 287: 1770-1774.
- Thorp, J. H. and Covich, A. P., 2001. *Ecology and Classification of North American Freshwater Invertebrates*, second edition. Academic Press, San Diego.
- Van den Berg, M.S., Coops, H. and Simons, J., 2001. Propagule bank buildup of *Chara aspera* and its significance for colonization of a shallow lake. *Hydrobiologia*, 462: 9-17.