

Microcosms as an Ecological Tool to Assess the Environmental Effect Caused by Introduced Species in Temporary Wetlands

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Abstract. Microcosms are small-sized constructed ecosystems that are used as tool for ecological research focused in understanding natural ecosystem functioning and how human activities disturb some ecological processes at different spatial and temporal scales. The many major environmental changes in a system does not allow in fact performing experiments with microcosms, so that studies of trial and error are common and statistical analysis is through the theory of pseudo-experimentation. In this paper we describe different microcosm experiments used to examine the impact of introduced red swamp crayfish (*Procambarus clarkii*) at different densities in biological communities (aquatic macrophytes, algae and macroinvertebrates) in a temporary freshwater wetland. Through accumulation of data from five different the trials we could gain insight into the environmental impact of *P. clarkii*. Crayfish consume all type of macrophytes and algae, but especially the most abundant algae, *Chara connivens*. Final macroinvertebrates density (ind/liter) was reduced about 58% of the initial measure; the specific composition of the macroinvertebrates community also declined considerably as a result of the crayfish. Use microcosm for understanding the ecological functioning of aquatic systems subject to high environmental variations is very important, but it requires patience and dedication to complete them and have reliable results.

Keywords: environmental impact, exotic species, Mediterranean wetlands, *Procambarus clarkii*.

1. Introduction

Many ecologists and conservationists are got tired to report that the introduction and translocation of exotic species is widespread, occurring both deliberately and accidentally all around the world; and that the environmental law is do almost nothing in this concern. Precatory principle of the environmental law it is no applying in countries when solving the poverty problem is a national priority [1]. But, what about in developed countries, such as Spain and Portugal, where priorities are quite different and where non-native species are one of the main threats to freshwater ecosystem functioning and biodiversity? [2]. The impact of non-native species in freshwater is well documented in many permanent aquatic ecosystems by the use of microcosms and other ecological techniques, but very few data are available when the ecosystems affected are temporary with marked hydroperiods and wet-dry seasons [3].

Microcosms, often called enclosures, are small-sized constructed ecosystems that are used as tool for ecological research, i.e. as living machines for understanding natural ecosystems [4]. Notwithstanding recent developments in ecological engineering for improving the design and operation of enclosures, many experiments about crayfish ecology using this tool lack conclusive results because of the great crayfish activity and fast changing environmental conditions in temporary shallow water systems.

Data from many experiments with enclosures are compromised because of failures with enclosure design, sub-estimation of crayfish capabilities, unpredictable weather and to a greater extent, statistical and experimental design. Whereas an experimental design under well-controlled conditions may consist of a

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single treatment, a comparative experiment in the field always involves two or more treatments, and has as its goal the making of one or more comparisons with several repeated samples or treatments [5]. These kinds of experiments are subject to several classes of potential problems, called ‘sources of confusion’: temporal change, procedure effects, experimenter bias, random error, initial or inherent variability among experimental units, and impingement of chance events on an experiment in progress, among others. An experiment is successful to the extent that these factors are prevented from rendering inconclusive or ambiguous results. Many of these potential sources of confusion in an experiment could be minimized by the use of enough replicates and controls. Randomization controls and replications reduce or eliminate potential experimenter bias and variability inherent in the experimental material. The problems arise because these solutions are not feasible in several cases due to the nature of the ecosystems where trials are performed, since regulation of the conditions under the experiments conducted are restricted by physical, biological and chemical environmental heterogeneity. In this sense, Hurlbert [6] severely criticizing how ecologists are designing and analyzing their field experiments, stated that pseudoreplication occurred in many cases, specially in benthological studies with small and middle sized enclosures.

This paper reviews a series of comparative experiments conducted to evaluate the environmental impact of different densities (biomass/m²) of the crayfish *P. clarkii* under natural conditions in temporary freshwater marsh in Doñana National Park (Spain). The experiments we performed using different enclosure designs with the purpose of showing that despite of difficulties presented by experiments with enclosures under high natural environmental variations, they are useful tools for assessing the effect of this invasive species on freshwater communities (macrophytes, algae and other macroinvertebrates). This exotic crayfish species was introduced into the Iberian Peninsula in 1973, and its importance is due to its trophic and reproductive behavior, that may alter the structure and function of the ecosystems invaded. This has turned the red swamp crayfish into a key ecological species of these ecosystems, modifying the routes of energy transfer and the availability of food resources and refuge for other species [7]. We expected that the crayfish should affect the ecosystem at least in three ways: 1) by reducing biomass of submersed macrophytes and the number of species, and thereby reducing habitat complexity. In turn, these reductions should indirectly affect invertebrate biomass and species richness; 2) if crayfish can reduce the abundance of periphyton-grazing snails then, increased crayfish density should have a positive, indirect effect on periphyton triggering what is called cascading effect or trophic cascade; 3) selective predation, on invertebrates may cause a decrease in the number of invertebrates taxa with increasing crayfish density.

2. Methodology

2.1. Fieldwork

Over four years, three prototype designs and five short and long-term experiments were conducted to get the final product. During this time, principles that govern freshwater marsh ecosystems in Doñana National Park (DNP) were formulated and tested. The five microcosm devices used in the first field experiment consisted of 4m² of 5mm mesh size enclosures, submersed 30cm, open top and bottom, which may be operated as a system closed to the exchange of crayfish, but open to the exchange of macroinvertebrates, other matter and to running water. This microcosm was located in the Hinojos’s fresh-water marsh (DNP). The second experiment, in the sandy area that joined with the natural temporary freshwater marsh, close to the Martinazo (DNP), was developed using eight 0.3mm mesh size enclosures of 2m² each, submersed 20cm, open bottom and close top by the same mesh, each enclosure was 1m apart from the others. These enclosures were placed in two columns in the middle of the ecosystem. The third experiment was conducted in the fresh-water marsh zone that joined with Lucio el Palacio (DNP), using 10 cage enclosures of 1m² each with 0.5mm mesh size, close top and bottom, and each enclosure was 1m separate from the others. These enclosures were placed in two columns at 50m from the littoral zone. The 10 cage enclosures of 1m² were also used in fourth, fifth and sixth experiments and were placed in Lucio del Bolín (DNP) and in Lucio el Palacio, respectively. In both experiments each enclosure was separated approximately 1m from each other but in hazard distribution in the middle of the pond, submersed 15cm.

Low crayfish biomass density ranged from 20 to 170gm⁻². And high density ranged from 200 to 400gm⁻². Crayfish used for low-density enclosures were juveniles, whereas crayfish for high density were adults. Only

healthy, intermolt crayfish were used. Each experiment had at least two enclosures without crayfish, acting as controls. Macrophyte and macroinvertebrates biomass and water quality (dissolved oxygen, temperature, alkalinity, hardness, pH) were recorded at the beginning, in the middle and at the end of each experiment.

2.2. Data analyses

Statistical *t*-test analyses were conducted separately on the results of experiments 1 and 2. Values and means of subsamples were analyzed within an ANOVA framework in trials 3 and 4 (three replicates, blocked on crayfish density) and experiment 5 (2 replicates per treatment combination). Shannon-Wiener diversity index, H' , were computed to measure macroinvertebrates taxa diversity in enclosures at the beginning and at the end of the trial 5. To compare both indices we used the next variation of a *t*-test: $t = (H'_1 - H'_2) / \sqrt{S_{H'_1 - H'_2}^2}$, where $S_{H'_1 - H'_2}^2 = \sqrt{S_{H'_1}^2 + S_{H'_2}^2}$, and $S^2 H'$ is the variance of H' for each observation [8]. One tailed as well two tailed hypotheses may be tested by this procedure. Also the population diversity indices may be hypothesized to differ by some value, other than zero, in which case the other computer of *t* would be $|H'_1 - H'_2|$ [9]. Shannon-Wiener index was chosen because it is appropriate for a random sample of an entire community; such a sample will probably not contain representatives of each species in the entire community. However, the lack of data on rare species has little effect on the value of H' . Finally, this index is suitable to compare diversity indices from two collections of data. Macrophyte biomass losses were compared across density and sex -ratio assays using Mann-Whitney U Test, while crayfish impacts on different macrophytes species were compared using the Wilcoxon paired-sample Test and Friedman repeated measures ANOVA as well as by the Shorygin forage ratio (an index of food selectivity). In many cases dependent variables were log-transformed to produce homogeneous variance when indicated by residual analysis. Levels of type I error (a conclusion that there is an effect when none actually exists) were set at 0.05 for main effects and 0.1 for interactive effects so that we may be more conservative with respect to type II error [10].

3. Results

P. clarkii effects on macrophytes were not determined in experiments 1 and 2 because of the low water retention period in Doñana's fresh-water marsh. Experiments 3 and 4 were partially successful because they came to determine the effects of crayfish density on macrophytes, but the results were inconclusive in the first case because a strong wind flipped the cages before completing the experiment and in the second case an unexpected flood suddenly changed the environmental conditions of the freshwater temporary marsh.

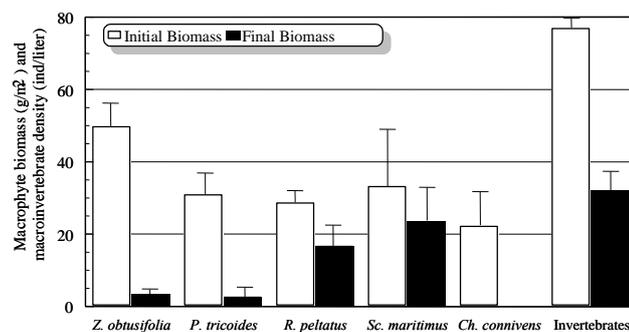


Fig. 1: Aquatic macrophyte biomass (g wet weight/m²) and macroinvertebrates density (ind/liter) inside of the enclosures at the beginning and at the end of the fifth experiment. Enclosures were placed at Lucio del Palacio. Lines show standard error.

Data from trial 5 were almost finished because of a high-speed water flow that entered into the system at the end of the experiment. This sudden flow moved the substrate of the enclosures and the enclosures themselves, allowing some crayfish to escape. Figure 1 shows algae and hydrophyte mean biomass (g wet weight/m²) and macroinvertebrates density (ind/liter) inside of the enclosures at the beginning and at the end of the trial.

The macroalga *Chara connivens* was totally consumed, whereas the macrophytes *Zannichellia obtusifolia* (from 49.85 to 3.4g m⁻²) and *Potamogeton tricoides* (from 31 to 2.7g m⁻²) were the secondary food items consumed by crayfish. On the other hand, *Scirpus maritimus* and *Ranunculus peltatus* were the less

consumed macrophytes, although these both species were severely damaged not only due to direct consumption but also due to fragmentation and cutting by crayfish.

The final macroinvertebrates density (ind/liter) was reduced about 58% of the initial measure. Figure 2 presents the list of macroinvertebrates taxa recorded inside of the enclosures at different sampling times. In the first sampling period, at the beginning of the experiment, the macroinvertebrates community was structured on 16 families with Physidae, Chironomidae and Corixidae the most abundant in number of individuals (196, 179 and 70 ind/enclosure, respectively). At the end of the trial, just 9 families were recorded in enclosures, Physidae and Chironomidae were again the most abundant, followed by Haplontaxidae (127, 56 and 12 ind/liter respectively), the latter taxon moving from position six to position three.

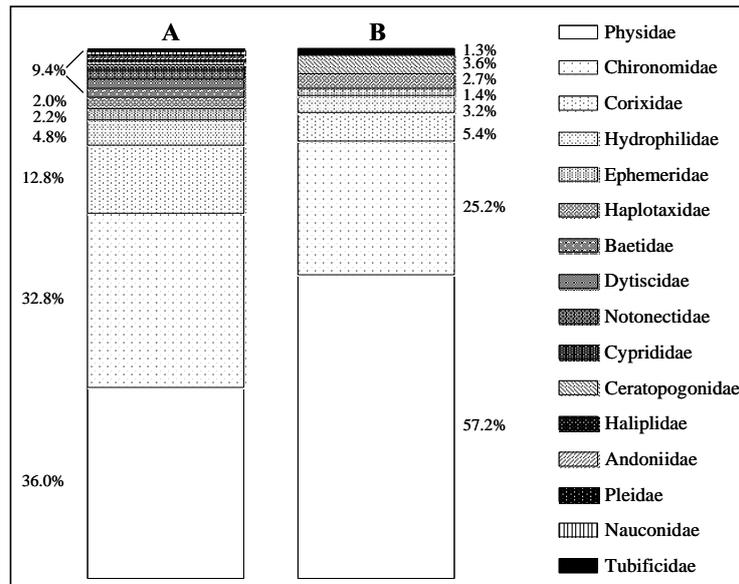


Fig. 2: List of macroinvertebrates taxa (families) recorded inside of the enclosures placed at Lucio del Palacio. Percentage of occurrence of individuals of the most abundant families (A) at the beginning and (B) at the end of the fifth experiment.

Table 1 reveals that the diversity of macroinvertebrates taxa in enclosures at the beginning of the trial is not the same as in the enclosures at the end of the experiment ($p < 0.001$). The macroinvertebrates community was clearly more diverse at the beginning than at the end of the experiment ($H' = 0.7454$; $H' = 0.554$, respectively).

Table 1: Comparison between two Shannon-Wiener diversity indices of macroinvertebrates taxa in enclosures at the beginning and at the end of trial 5. Enclosures were placed at Lucio del Palacio.

Sampling time	n	H'	S _{H'}	S _{H'1} -S _{H'2}	v	t estimated	t _{0.001(2), 242}
Beginning the trial	545	0.7454	0.00045				
				0.039	242	4.91	3.330***
Ending the trial	222	0.5540	0.00102				

n= sample size (occurrence of macroinvertebrates); H'=Shannon-Wiener index; S_{H'}=variance of each H'; S_{H'1}-S_{H'2}= t-test variance; v= degrees of freedom. ***P < 0.001.

4. Discussion

Because most of the studies was begun and completed in one of the lowest precipitation cycles which the region has experienced in many decades (rain deficit was >240mm per year), many of our experiments failed due to the low water retention period in the enclosures. On the other hand, the high locomotive activity of

crayfish was the other factor, which exerted negative influence in the experiments performed. Thus, the development of the final enclosure design was a long learning process. By this view, the most significant point we see is the trial and error process of finding what works best, and what works best can be found from scientific principles [4]. We can state that trial and error efforts to find the maximum performance is aided by variations and choices provided by the dynamic oscillation of the weather and crayfish behavior. Another way to reduce error is using other statistical analysis such as Monte Carlo simulations or fuzzy logic models, these techniques proved to be important when were applied to plot less sample data sets, and in all cases the error of the estimate when were measured as relative root mean square error, was considerably reduced with increasing sample size. Certainly we cannot control weather, but we can increase microcosm numbers, and thus, increase sample size.

Each experiment, taught us how ecosystems respond to a specific stress factor: for instance, the effect of large herbivore mammals on macrophyte biomass and growth in fresh-water marsh was observed and assessed in experiments 1 and 2, supporting the hypothesis that the reduction of the macrophyte biomass must not be ascribed to crayfish activity alone, but also to the pressure of herbivore mammals that graze in and tread the fresh-water marsh. In addition, new hypotheses concerning relationships between herbivores and macrophytes derived from experiments 3 to 5 are being tested in other experiments conducted in our labs.

5. Conclusions

These enclosure studies both qualitatively and quantitatively demonstrated that *P. clarkii* acts on ecosystems by different manners and at several levels modifying the routes of energy transfer and the availability of food resources and refuge for other species in the ecosystems.

Ecological models derived from experimental data using microcosm permit analysis future potential ecological scenarios, including ecological models concerning the impact and consequences of future climate change on different levels of the ecosystem function and structure.

In order to avoid the statistical problems associated with data analysis, in the design of experiments with microcosms it is very important avoid pseudoreplication, since differences in analysis may lead to differences in conclusions regarding the significance of experimental treatment effect.

Well-designed enclosures offer increased complexity; sustainable and experimental condition control and thus, very good statistical analyses.

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7. References

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