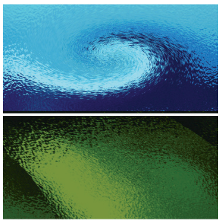
Transitional Waters Bulletin
TWB, Transit. Waters Bull. 4 (2010), n. 2, 105-114
ISSN 1825-229X, DOI 10.1285/i1825229Xv4n2p105
http://siba-ese.unisalento.it



RESEARCH ARTICLE

The Ecological Restoration of the Tarquinia Salterns drives the temporal changes in the benthic community structure

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Abstract

1 - Although wetland ecosystems are able to sustain high degree of biological diversity and provide social and economic value, they are also the most impacted and degraded of all ecological systems.

2 - Natural and human-based alterations are particularly evident in small aquatic ecosystems, where the recovery time can be greatly reduced because of the geological and geomorphological characteristics of the sites.

3 - Although seldom used in lentic environment, benthic communities may represent a suitable tool to measure the recovery effects in aquatic ecosystems, due to several characteristics that make them useful as bioindicator.

4 - Here we study the response of benthic communities to the recovery of the Tarquinia Salterns after their abandon and consequent habitat degradation. The increased deposition of organic and inorganic sediments entailed shallow ponds and laminar circulation, causing eutrophication. The ecological restoration of the site allowed reinstating the water depth and flow and returned environmental parameters (mainly dissolved oxygen) to their typical values.

5 - We measured several characteristics of species assemblage in three different sites of the Tarquinia Salterns, covering a period of ten years spanning from pre-recovery to post-recovery phases.

6 - Our results are twofold: i) they show the feasibility of monitoring wetland restoration activities by investigating the pattern of species assemblage in benthic ecological communities and, ii) show the importance of regional and local processes in the composition and response of benthic communities to perturbations.

Keywords: Solar saltern; Benthos; Ecological restoration

Introduction

Benthic macroinvertebrates are a diverse group of animals highly adapted to a wide range of natural conditions in aquatic environments, where the complex nature of an array of different habitat characteristics, including spatial properties such as habitat area and connectivity, forms heterogeneous, often unevenly distributed populations (Odum, 1983). For instance, small areas with similar benthic habitat are thought to have similar macroinvertebrates composition (Ulfstrand, 1967; Mackay, 1969). Multiple environmental factors influence the structural assemblage of benthic communities, as well as food resources (e.g. detritus and algae). However, benthic habitat is complex and many of the environmental factors that influence the assemblage of invertebrate's species are strongly interrelated (Rabeni and

Minshall, 1977; Williams and Smith, 1996).

Benthic macroinvertebrates are well-suited and used indicators of water quality in many aquatic ecosystems. Several characteristics make them useful as bioindicator: i) ubiquity and, therefore, high probability to be affected by perturbations in aquatic habitats, ii) most species exhibit a range of responses to environmental stress, iii) sedentary relative to other aquatic organisms such as fish and bird species, which permits effective determination of the spatial extent of perturbations, and iv) long life cycles, which allows examination of temporal changes in invertebrates population (Merritt *et al.*, 2008). When subjected to perturbations, benthic communities may respond in different ways, increasing, for instance, the number of opportunistic species (Peterka, 1972), decreasing species diversity (Jonasson, 1969), and increasing species dominance and replacement with increasing eutrophication (Paterson *et al.*, 1970).

Given such characteristics, benthic species and communities as a whole may provide useful information on natural and human-based alterations of natural and artificial ecosystems. Habitat fragmentation and/or alteration can directly or indirectly impact native species through a series of different factors, which result in altered physical conditions (Schoonover *et al.*, 2006), the replacement of native by invasive species (Loewenstein and Loewenstein, 2005), changes in resource availability (Clergeau *et al.*, 1998) and increased sediment, loads, nutrient inputs, and temperatures (Schoonover *et al.*, 2006). The effect of such disturbances is particularly evident in small aquatic environment where the recovery time can be greatly reduced because of the geological and geomorphological characteristics of the sites (Niemi *et al.* 1990).

Solar saltern in particular, which are constructed for salt extraction and thus subjected to an intensive water management, represent a peculiar aquatic ecosystem where human-based management ensures their ecological maintenance. Moreover, they experience different ecological conditions in a limited spatial context, which create contrasting responses of benthic community to different environmental stress. Benthic macroinvertebrates are largely used in the assessment of streams and rivers, as well as in lenthic waters such as lakes, reservoirs and saltern. By comparing long-term pre-restoration and post-restoration data it can be determined if the macroinvertebrate community has been reset to its original composition and, therefore, evaluate the impact of restoration.

The aim of this work is to determine whether and how benthic community diversity responds to ecological restoration, and to characterize the effects of restoration through the use of benthic community. We used data about macroinvertebrate colonization of three different sites in the area of Tarquinia salterns taken in four different sampling times, covering a period of about 9 years. Our results highlight the positive-ecological-effect of the restoration of benthic communities, which showed an almost complete recovery compared to pre-restoration conditions.

Materials and methods

Study area and field experiment

The study area is the aquatic ecosystem of Tarquinia Salterns, a transitional artificial environment (central Italy, 42°12' N, 11°43' E), composed by a series of pools whose connection is ensured by a channel system, and where the exchange of waters is provided by two connections with the sea, the main of which located north of the area and provided with a water pumping (Fig. 1). Isolation and hydrological connectivity give rise to a wide salinity gradient (Cerfolli *et al.*, 2009; Bellisario *et al.*, 2010), spanning from hypo-saline (mean annual salinity 8.5 gL^{-1}) to

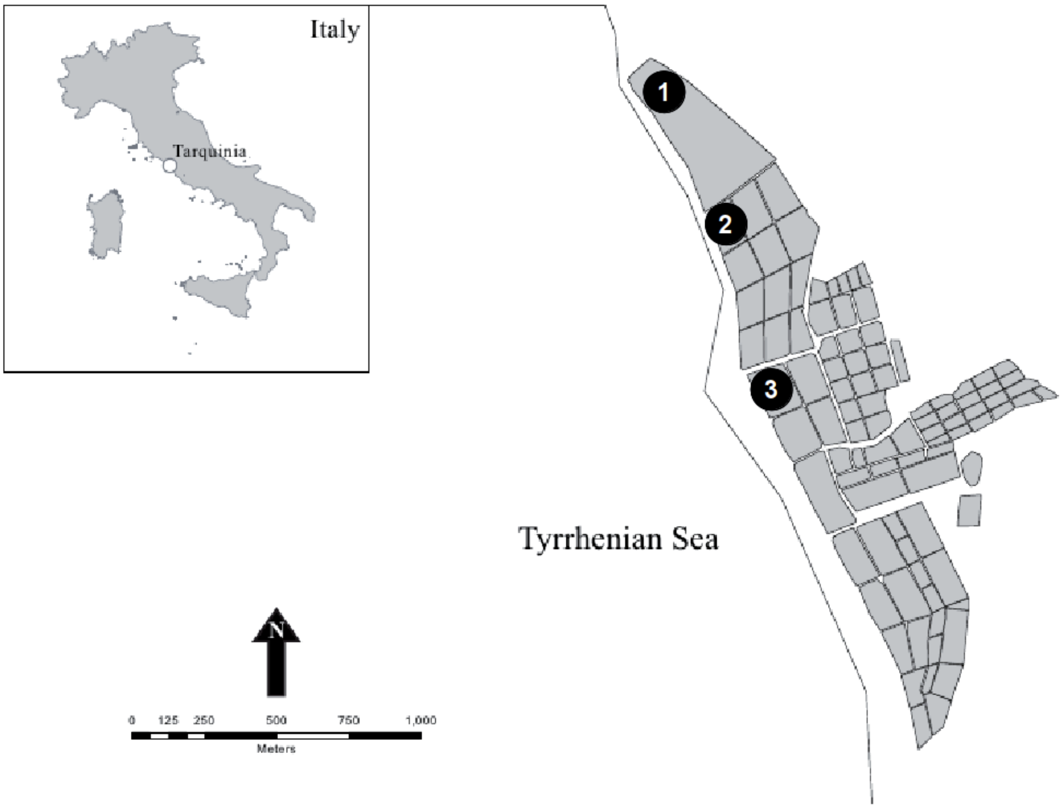

Figure 1. Natural Reserve of Tarquinia Salterns and sampling sites (black circles).

hyper-saline waters (mean annual salinity more than 100 gL^{-1}).

We chose three sampling sites in the area, covering the full spectrum of salinity variation, to analyze the benthic taxonomic distribution from 1997 to 2006.

Quantitative sampling of benthic macroinvertebrates was carried out through a Van Veen Grab (0.06 m^2 and 8 cm depth), from 1997 to 2006 in 3 pools of Tarquinia Saltern. In each pool, 3 replicates were sampled monthly. Samples were sieved using a 0.5 mm mesh size sieve and preserved by adding Formalin solution to yield a concentration of 4% Formalin and 96% sample/seawater (Eleftheriou and Holme, 1984). In the laboratory, the preserved samples were filtered on a 1 mm mesh-size funnel-shaped sieve provided a collector. All sorted benthic macroinvertebrates were identified to the lowest taxonomic level possible using a dissection microscope.

Apparent turnover

Apparent turnover between adjacent years was estimated for each sampling site as:

$$T = 100\,(I + E)/[(S_1 + S_2)\,t)] \qquad (1)$$

Where I is the number of taxa appearing in year 2, E is the number of taxa lost between the censuses, S_1 is the number of taxa in year 1, S_2 is the number of taxa in year 2, and t is the number of years between the censuses (Magnuson *et al.*, 1994). As species turnover rates may strongly depend on sampling interval (Magnuson *et al.*, 1994), we calculated turnover for all possible combinations of sampling intervals for each sampling site.

Richness estimate

We determined long-term species richness in two ways because we wanted to compare the species actually observed with an estimate of the total species pool in each sampling site. We first calculated the cumulative richness, defined as the total number of species actually detected in the entire sampling record. Then, to give an estimate of the total species pool we measured the asymptotic richness, assuming that an infinite number of samples were available. Asymptotic richness is based on the Walford plot (Ricker, 1975) and was obtained by calculating where a plot of the mean cumulative richness at subsample size t versus mean cumulative richness at subsample size $t + 1$ intersects the 1:1 line. The Walford plot provides a simple method for estimating asymptotic richness that is relatively insensitive to sample size (Arnott *et al.*, 1998). Calculations employed the total number of available years of data.

Differences in community assemblage

To search, if any, for differences in community composition during the sampling periods, it has been performed a cluster analysis among the incidence of species within each sampling sites, by using a hierarchical clustering algorithm implemented in the ‘pvclust’ package of R (R Development Core Team, 2010). Hierarchical clustering do not require any specification about the number of clusters to be searched and the starting configuration assessment, but only requires the type of strategy (agglomerative or divisive) and the correct measure of similarity (or dissimilarity).

Here it has been used an agglomerative algorithm, starting at the bottom and at each level recursively merge a selected pair of clusters into a single cluster, producing a grouping at the next higher level with one less cluster. Each level of the hierarchy represents a particular grouping of the data into disjoint clusters of observations, and the entire hierarchy represents an ordered sequence of such groupings, consisting of two groups with the smallest intergroup dissimilarity. Uncertainty in hierarchical cluster analysis has been calculated via multiscale bootstrap resampling for each cluster in hierarchical clustering, where the p-value of significance indicates how strong the cluster is supported by data. The algorithm provides two types of p-values, called AU (Approximately Unbiased) and BP (Bootstrap Probability). AU is computed by multiscale bootstrap resampling and represents a better approximation to unbiased p-value than BP, which is computed by normal bootstrap resampling. Clusters with AU values larger than 95% provides a minimum level of significance of 0.05. To evaluate the distances between clusters, it has been used a agglomerative method with 1,000 replicates based on the analysis of variance (Ward's linkage), attempting to minimize the sum of squares of any two (hypothetical) clusters that can be formed at each step.

Results

A total of 27 species were detected in the sampling sites. The mean annual richness of each sampling site ranged from 7 to 17 species, with an average of 13 (Table 1), whilst the total number of species observed in each sampling site ranged from 13 to 26, with an average of 20. For pool 1 and 2 an average of only 65% of the cumulative species pool was detected each year, whilst for pool 3 this percentage decreases to 35%. The asymptotic estimate predicted a species pool lower than the cumulative species richness over the entire sampling period for all sampling sites (Table 1). If we assume that communities are not stable but in transition to a new state, then this might indicate that long sampling periods are needed to detect all species that

Table 1 - Species richness. Mean annual richness was calculated over 10 years subdivided in 4 sampling periods (1997, 2003-2004, 2005 and 2006). Cumulative richness is the total number of species found during entire sampling period. The asymptotic richness was calculated based on a Walford plot of cumulative richness at year *t* versus cumulative richness at year *t* + 1.

Sampling site	Long-term richness		
	Mean	Cumulative	Asymptotic
Pool 1	17	26	16
Pool 2	13	21	11
Pool 3	7	13	7
Mean	12.667	20.000	11.333
SD	4.964	6.557	4.509

occur in a given sampling site. Even after the sampling period, we had recorded an average of 67% of the total species pool, based on an asymptotic estimate of species richness. Only in pool 3 the percentage of recorded species vs. the asymptotic estimate is 87%, which might indicate the extreme low richness and species turnover of this sampling site.

Among-year differences in apparent turnover rates differed among sampling sites (One-way ANOVA, Tukey a posteriori multiple comparison test, F = 6.227, p = 0.05), especially between pool 1 and 2 with respect to pool 3 that have showed higher turnover rates between all adjacent years. A great number of acquired species (paired t-test, p = 0.05) have been measured after the recovery period (Fig. 2), whilst an opposite trend has been found during the periods before the restoration activity (Fig. 2), where in all sampling sites have been lost a significant number of species. Among all sampled species, about 22% is characterized by a general equilibrium between *E* and *I* (see Eq. 1), which is indicative of their continued presence in the system during the sampling period (Table 2). Moreover, in all sampling sites we observed a general increase (Table 2) in the ratio of *I* to *E* during the second half of the sampling period (i.e. following the restoration activities), which was indicative of the tendency of the system to acquire species, i.e., the entry of new species or the restoration of those lost behind the recovery phase.

Species turnover rates usually alter species composition over time, as observed by the hierarchical clustering showing similarities in community composition over years. After 1,000 bootstrap of the original incidence matrices, all sampling period formed independent clusters (Fig. 3). Years straddles the recovery period (2003-2004 and 2005) always clustered together at distance far from other sampling periods. Sampling periods before (1997) and after (2006) the recovery period formed two distinct clusters, although their small distance clearly indicates similar community composition, which was indicative of the return to initial conditions in terms of species richness as a result of restoration activities.

Discussion

Benthic macroinvertebrates are a diverse group of animals that are highly adapted to a wide range of natural conditions in aquatic environments, especially in shallow, fragmented water bodies, where they form heterogeneous and unevenly distributed populations. The high variability of species richness and diversity found in the three sampling sites reinforce the role of multiple variables such as local habitat conditions in

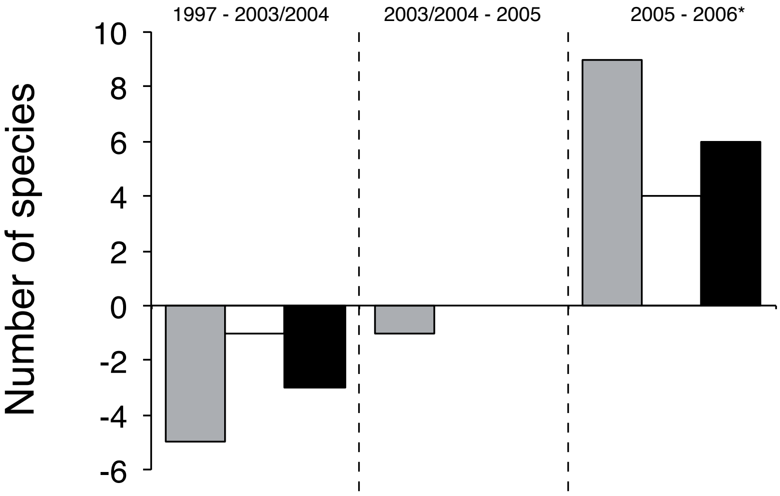

Figure 2. Number of species loss/acquired within censuses. Grey bars are for pool 1, white for pool 2 and black for pool 3.

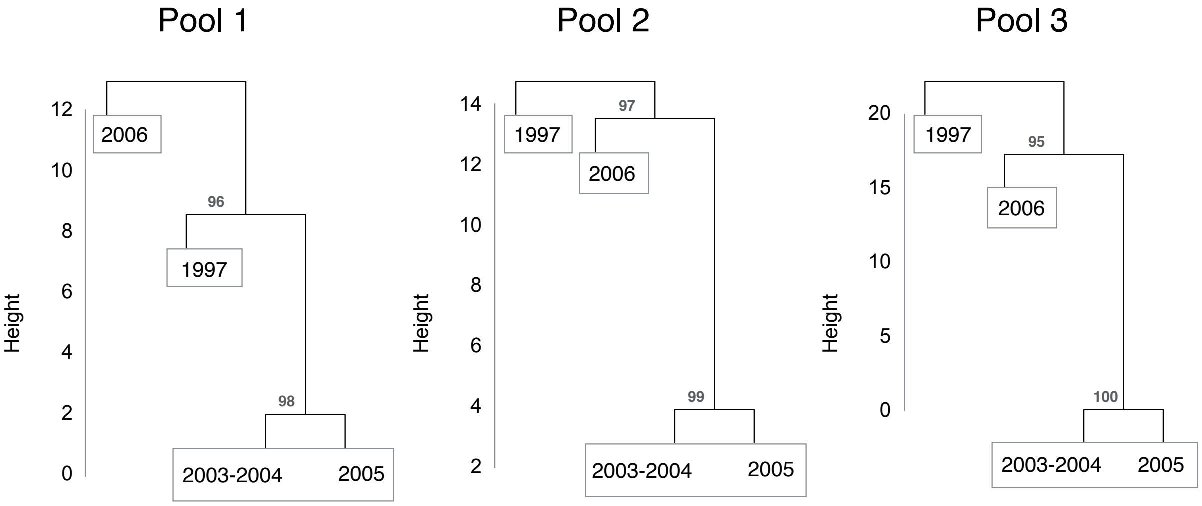

Figure 3. Hierarchical clustering of macroinvertebrates assemblage in each sampling site. Numbers on the branches represent the p-value of significance that indicates how strong the cluster is supported by data. A value of 95 represents the minimum significance level of 0.05.

structuring macroinvertebrate assemblages at small spatial scales (Rabeni and Minshall, 1977; Reice, 1980; Palmer *et al.*, 2000). Substrate characteristics that influence macroinvertebrate microdistribution include mineral versus plant material, living versus decomposing plants, particle size of mineral substrate, organic detritus availability, food

Table 2 - Incidence table of benthic species in the three sampling pools. For each sampling site it has been reported the ratio of *E* to *I* for each species before (R_b) and after (R_a) the restoration activities, and the total number of species at equilibrium (=), gained (+) or loss (-) during the censuses. R_b and R_a indicate if a species has been lost (-), acquired (+) or remained in equilibrium (=) between consecutive censuses.

Species	P1						P2						P3					
	1997	2003/2004	R_b	2005	2006	R_a	1997	2003/2004	R_b	2005	2006	R_a	1997	2003/2004	R_b	2005	2006	R_a
Hydrobia acuta	1	1	=	1	1	=	1	1	=	1	1	=	1	1	=	1	1	=
Cerithium vulgatum	1	1	=	1	1	=	0	1	+	1	0	-	0	1	+	0	0	
Nassarius corniculus	1	1	=	1	1	=				0	1	+						
Ovatella myosotis							0	1	+	1	0	-						
Nassarius costulatus				0	1	+				0	1	+						
Haminoea sp.				0	1	+												
Abra ovata	1	1	=	1	1	=	1	1	=	1	1	=	0	1	+	1	1	=
Modiolus adriaticus	1	1	=	1	0	-												
Mytilaster sp.	1	0	-				1	0	-									
Cerastoderma glaucum	1	0	-	0	1	+	0	1	+	1	1	=	1	1	=	1	1	=
Nainereis laevigata	1	1	=	1	1	=	0	1	+	1	1	=						
Spio decoratus	1	1	=	1	1	=	1	1	=	1	1	=	0	0		0	1	+
Capitella capitata	1	1	=	0	1	+	1	1	=	0	1	+	1	0	-	0	1	+
Neanthes irrorata	1	0	-				1	0	-	0	1	+	1	0	-	0	0	
Armandia cirrhosa				0	1	+	0	0		0	1	+						
Perinereis cultrifera	1	1	=	1	1	=	1	1	=	1	0	-				0	1	+
Ophiodromus pallidus				0	1	+	0	0		0	1	+						
Amphiglena mediterranea	1	0	-															
Idotea baltica	1	1	=	1	1	=	1	1	=	1	1	=						
Sphaeroma serratum	1	1	=	1	1	=												
Corophium insidiosum	1	1	=	1	1	=	1	0	-	1	1	=	1	0	-	1	1	=
Erichtonius pallidus				0	1	+												
Microdeutopus spp				0	1	+												
Gammarus aequicauda	1	0	-	0	1	+	1	1	=	1	1	=	1	0	-	0	1	+
Oligochaeta	1	1	=	1	1	=	1	1	=	1	1	=				0	1	+
Chironomus spp. (larvae)	1	1	=	1	1	=	1	1	=	1	1	=	1	1	=	1	1	=
Other Diptera (larvae)				0	1	+	0	0		0	1	+	1	0	-	0	1	+
E = *I*			14			12			9			10			3			5
E < *I*			0			10			4			7			2			6
E > *I*			5			1			3			3			5			0

retention ability, heterogeneity, and stability (Allan, 1975; Cobb *et al.*, 1992; Boyero, 2003).

Theoretical studies suggest that species composition of 'island-like' habitats is dynamic (MacArthur and Wilson, 1967). Nonetheless, there have been few assessments of the rates of influx or disappearance of species in patchy systems such as solar saltern. We have reported high apparent turnover rates in each of three sampling sites, and this tendency was particularly evident between distant pools (i.e. pool 1 and 2 with respect to pool 3), which are indicative of the spatial effect on community composition.

Moreover, this may be the result of a core group of species being present every year, whilst the remaining species being intermittently present and absent. Indeed, we have found that 22% of sampled species are characterized by a general equilibrium between 'extinction' and 'immigration', which is indicative of their continued presence in the system during the sampling period.

Appearances and/or disappearances of species from censuses can be caused by several mechanisms, including some that are artifactual in nature. Artifactual mechanisms include chance detection of rare species, and variation in population densities above and below detection limits because of changes in abundance or spatial distribution. The actual mechanisms include: i) areal dispersal (Jenkins, 1995), ii) downstream dispersal, iii) human introductions of exotic and native taxa (Yan and Pawson, 1997), iv) emergence from sediment egg banks (Hairston, 1996), v) extinctions resulting from environmental disturbances, vi) chance extinctions related to random variation in birth and death rates, and vii) unusual weather or climatic conditions (Stemberger *et al.*, 1996).

Some of these mechanisms are probably not important drivers of turnover in our study site. Different microhabitat conditions may exert a strong influence on species turnover, as we can see from the similar community composition of pool 1 and 2, which experiences almost similar environmental conditions than that of pool 3. However, the apparent turnover in our study site is likely to be a consequence of extinction/colonization dynamics related to the habitat changes occurred along the study period. In 1997 the salt production in the study site ceased, and the consequent reduction of the maintenance activities resulted into a reduction of water flow and an increasing of organic and inorganic matter sedimentation. This alteration of the hydrological and trophic status of the salterns resulted in hypoxic sediment layers and in several episodes of eutrophication (Cimmaruta *et al.*, 2010). The ecological restoration of the salterns started in 2003 and ended at the beginning of 2006, when both the abiotic and biotic habitat components showed a benefit from the rescue. For example, oxygen concentrations raised from hypoxic (2.2 ppm) to normal (6.3 ppm) and the abundance of waterbirds showed its maximum values in 2006 (Cimmaruta *et al.*, 2010).

Our results show that benthic communities are naturally structured by different microhabitat conditions, which have the ability to influence the pattern of species occurrence, as a consequence of ecological processes acting at local (i.e. site) scale. Therefore, the spatial configuration of the pools affect the composition of benthic communities, which respond differently to environmental fluctuations in terms of species richness and diversity, where pools in spatial proximity had similar asymptotic richness and apparent turnover. On the other hand, on a regional scale (i.e. whole system level), all sampling sites have had similar responses following the recovery, resettling around initial conditions.

Although a general and positive trend as a result of restoration activities, communities did not return to their original composition. The distribution and composition of aquatic macroinvertebrate communities are influenced by a variety of environmental factors such as habitat characteristics, water quality, sediment quality, food quality and quantity along with biological factors such as competition and predation (Peeters *et al.*, 2004). The relatively small size of the study site and the close spacing of the pools should minimize habitat fragmentation and allow invertebrates to disperse throughout the area, especially as this whole wetland is inundated during heavy rainfall (Cimmaruta *et al.*, 2010). Under such conditions, invertebrates could move freely between the drains. Whether such quick colonization would occur in more fragmented wetlands that are surrounded by more modified catchments is unknown, but may be unlikely if there are barriers that impede colonization processes. Habitat restoration in wetland ecosystems is not always successful (Malakoff, 1998; Zelder, 2000), and may even unintentionally decrease habitat diversity, although benefiting only few taxa. However, our results show how restoration met its conservation objective of returning the ecosystem to a more natural condition.

Wetland ecosystems frequently support high biological diversity, in addition to providing hydrological, water quality, social and economic values (Mitsch and Gosselink, 2000). On the other hand, they are also among the most impacted and degraded of all ecological systems (Davis and Froend, 1999). Given such characteristics, a fundamental part of restoration programs is the need to monitor their effectiveness. In this way, the success or otherwise of the restoration can be determined, as well as the need for additional management intervention if progress toward the restoration target is not achieved. Here we have shown the feasibility of monitoring wetland restoration activities by investigating the pattern of species assemblage in ecological communities, providing useful information about the ecological status of benthic communities in a fragmented system.

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