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Role of habitats and sampling techniques on macroinvertebrate descriptors and ecological indicators: An experiment in a protected Mediterranean lagoon

Maurizio Pinna[a,*], Sally Janzen[b], Antonio Franco[a], Valeria Specchia[a], Gabriele Marini[a]

[a] *Department of Biological and Environmental Sciences and Technologies, University of Salento, S.P. Lecce-Monteroni, 73100 Lecce, Italy*
[b] *School of GeoSciences, University of Edinburgh, EH9 3JN Edinburgh, UK*

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ABSTRACT

Macroinvertebrates are commonly applied for ecological investigations and as ecological indicators. However, the role of the sampling technique, effort and habitat on macroinvertebrate descriptors, diversity indices and ecological indicators in transitional water ecosystems is little known yet. This research aims to evaluate the influence of sampling techniques on macroinvertebrate assemblages and ecological indicators comparing box-corer and litterbag techniques, in prairie and unvegetated habitats. The experiment was conducted in a protected Mediterranean shallow lagoon dominated by marine water input. Three types of litterbags were prepared with: i. *Phragmites australis* dry leaves (terrestrial input); ii. *Posidonia oceanica* dry leaves (marine input), and iii. an equal mixture of both leaves. Three replicates of box-corer samples were collected in two sites per habitat, litterbags were submerged and retrieved after 30 days. Macroinvertebrate abundance, species richness, diversity indices and ecological indicators were measured and compared among sampling techniques and between habitats. Macroinvertebrate data was then pooled, analysed and compared to each single technique. Twenty-seven species were sampled overall, 4 species overlapped between box-corer and litterbags, 6 species (26%) were exclusive to the box-corer and 16 species (59%) were caught using only litterbags. Species diversity in litterbags was always higher than in box-corer, but macroinvertebrate assemblages were described better when using data pooled. In prairie, the ecological indicators varied significantly between the data pooled and separate sampling technique. Finally, this research highlights the relevance of using more than one sampling technique to obtain a better description of macroinvertebrate assemblages and the ecological status of Mediterranean lagoons.

1. Introduction

The constant increase of anthropogenic pressures in transitional water ecosystems (TWs) threatens their ecological integrity and leads to the loss of both biodiversity and ecosystem services (Newton et al., 2014). In recent years, much legislation has been developed to provide the assessment of ecological quality of aquatic ecosystems worldwide (e.g., Clean Water Act in the USA, National Water Act in South-Africa, Water Framework Directive and Marine Strategy Framework Directive in Europe). In Europe, several ecological indicators have been proposed under the Water Framework Directive (EC, 2000) for the evaluation of ecological quality of coastal-marine and TWs ecosystems (Pinto et al., 2009; Ponti et al., 2009; Birk et al., 2012). Macroinvertebrate assemblages represent the most common biological quality element used in the development of ecological indicators (Borja et al., 2000; Simboura and Zenetos, 2002; Muxika et al., 2007; Mistri and Munari, 2008; Borja et al., 2009; Basset et al., 2012). They are considered good bioindicators because they are relatively sedentary, have long life spans, belong to various trophic levels and vary in body size (Rosenberg and Resh, 1993; Basset et al., 2004). Moreover they respond to different stressors, both natural and anthropogenic ones. The macroinvertebrates can integrate the response to changes of the physical-chemical features of aquatic ecosystems both in time and space (Pearson and Rosenberg, 1978), may contribute significantly to chemical fluxes at the interface between water and sediment (Cook, 1976), and respond to the anthropogenic pressures according to well-established models (Aller and Aller, 1998). For these reasons, ecological indicators based on macroinvertebrates, such as AMBI, M-AMBI, BENTIX, BITS, BO2A, STAR-ICMI, are largely used in biomonitoring plans of aquatic ecosystems (Borja et al., 2000; Muxika et al., 2007; Miler et al., 2013; Böhmer et al., 2014; Urbanič, 2014).

Although methodologies and indices were already compared for

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[*] Corresponding author.
E-mail address: maurizio.pinna@unisalento.it (M. Pinna).

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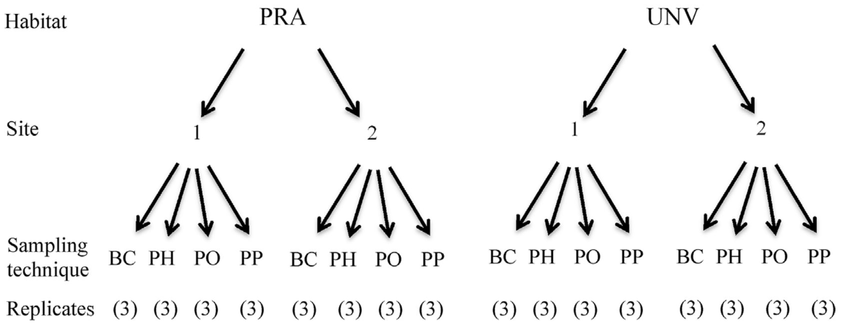

Fig. 1. Schematic representation of the experimental design used in the study. PRA and UNV represent the two sampled habitats: prairie and unvegetated respectively, whereas BC, PH, PO and PP represent the sampling techniques of box-corer, *P. australis, P. oceanica* and the mixed litterbag respectively.

assessing the ecological quality status in estuarine and marine coastal environments (Borja and Dauer, 2008), the efficiency of sampling techniques in detecting changes and spatial variations of macroinvertebrate assemblages and ecological indicators in Mediterranean lagoons is poorly understood. Similarly, there is a lack of studies linking sampling techniques to ecological status classification (Pinna et al., 2013; Pinna et al., 2014; Sangiorgio et al., 2014). In TWs, ecological studies and biomonitoring plans with macroinvertebrates are usually carried out using only box-corers, grabs or other devices (e.g., plastic tubes or boxes). These methods are quantitative and allow collecting samples anytime; however they underestimate the vagile fauna, they are time consuming and their use is limited to favourable substrate conditions such as soft and unvegetated bottoms (Tolonen and Hämäläinen, 2010; Pinna et al., 2013). Moreover TWs are characterized with high habitat patchiness, the use of a single technique can lead to a misclassification of the ecological quality status (Pinna et al., 2013, 2014). The choice of sampling technique in TWs therefore remains a point of discussion for some researchers (Quintino et al., 2011). Recent studies have indicated that a combination of techniques, suitable for multiple habitat sampling, would be required for a more realistic characterization of macroinvertebrate assemblages present in the aquatic ecosystems although it is time consuming and expensive (Davies, 2001; Clapcott et al., 2012; Collier et al., 2013; Marini et al., 2013; Di Sabatino et al., 2014, 2015). Some researchers have also suggested that the assessment of aquatic ecosystem health and integrity should be reached with both community structure descriptors and ecosystem functional properties (Sandin and Solimini, 2009; Sangiorgio et al., 2014). Indeed ecosystem functions have already been proposed for assessment of aquatic ecosystem quality (Gessner and Chauvet, 2002; Bergfur, 2007; Castela et al., 2008).

The aim of this research was to compare two main sampling techniques, box-corer and litterbag, for studying macroinvertebrate assemblages and ecological indicators in Mediterranean coastal lagoons. Macroinvertebrates were collected in two habitats of a protected Mediterranean lagoon dominated by marine water input using a box-corer and litterbag technique. Three different litterbag series were performed with *Phragmites australis* (Cav.) Trin. ex Steud. 1841 leaves detritus, *Posidonia oceanica* (L.) Delile 1813 leaves detritus and an equal mixture of both leaves. The specific objectives of the research were: i. to test the null hypothesis of no significant differences between the macroinvertebrate assemblages collected with the box-corer and litterbags; ii. to analyse the resulting ecological indicator based on the box-corer and litterbag samples; iii. to investigate whether a single technique and data pooled from various techniques result in similar outcomes in terms of descriptors and ecological indicators; iv. to examine the response of sampling techniques with respect to prairie and unvegetated habitats.

2. Materials and methods

2.1. Site description

The sampled shallow Mediterranean transitional water ecosystem lies within the uninhabited Sant'Andrea Island, Gallipoli, Italy (40.049599N, 17.949530 E). The flat limestone plate extends over about 50 ha and is the largest island of the Apulian Ionian coast. Sant'Andrea Island is included in the Natural Regional Park "Isola di Sant'Andrea e litorale di Punta Pizzo" since 2006. The island hosts endemic species (e.g., *Limonium japygicum* Groves, 1887) and protected species living in the lagoon (e.g., *Pinna nobilis* Linnaeus, 1758); so that the island has been identified as Site of Community Interest (SCI) and Special Protection Area (SPA) by European Union. The existing protection and conservation measures on top of the fact that the island has been uninhabited since 1970, have limited the anthropogenic pressures on the island, resulting in pristine environmental conditions. The sea surrounding the island is dominated by sensitive habitats such as *P. oceanica* meadows and coralligenous reefs.

The lagoon is located in the Northeast of the island, extending over about 4 ha with an average depth of 0.5 m. *Nanozostera noltii* Hornem., 1832 and *Cymodocea nodosa* Ucria, Asch., 1870 are distributed patchily in the soft bottom and vegetated areas. The water in the lagoon experiences very saline conditions and is indeed dominated by marine water input. Freshwater inputs are limited to precipitations and to the underground seapage.

2.2. Experimental design

The preliminary monitoring of the lagoon highlighted the presence of two bottom habitats: a prairie habitat characterized by seagrasses (PRA) and an unvegetated habitat (UNV). Two sampling sites per habitat were chosen inside the lagoon. Macroinvertebrate samples were collected by box-corer (BC), *P. australis* litterbags (PH), *P. oceanica* litterbags (PO) and by litterbags with equal mixtures of both leaf detritus (PP). Overall, we considered four sampling techniques. Three replicates were collected in each sampling site and per technique. The Fig. 1 schematically illustrates the experimental design of our research. The box-corer was used at the start of the experiment (12 June, 2015) whereas litterbags were collected 30 days after their submersion at the beginning of the experiment. Abundance, community structure, species richness, diversity indices and two ecological indicators (AMBI and M-AMBI) were measured and compared between habitats and sampling techniques. Ecological status classes of AMBI and M-AMBI ecological indicators were also computed and compared in order to analyse possible differences among sampling techniques and habitats. The ecological quality status classes of AMBI are easily calculated using the AZTI – Tecnalia software (Borja et al., 2004; Muxika et al., 2007; http://www.azti.es/). The calculation of ecological status classes of M-AMBI requires the definition of reference values/conditions for the three variables applied in the index and combined in the factorial analysis. This is recognised as a critical steps, because an incorrect definition of the reference conditions can greatly modify results and ecological quality status. The reference conditions are chosen inside of a specific category of aquatic ecosystems and classify based on ecoregion, typology, etc. Since it is often difficult to find 'true' no-impacted sites (Muxika et al., 2007) extend datasets and previous information