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Influence of sampling effort on ecological descriptors and indicators in perturbed and unperturbed conditions: A study case using benthic macroinvertebrates in Mediterranean transitional waters

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ABSTRACT

The effectiveness and accuracy of biomonitoring programs, based on benthic macroinvertebrates, is strictly related to the sampling design and effort, whereas the feasibility depends on the economic sustainability of sample collection and processing methodologies. In the last decade, how to improve the Rapid Bioassessment Protocols (RBPs) maintaining the accuracy of the results has been a topic recurrently debated among researchers. It is well known that the sample unit size (i.e., surface of the sampled area, SUS) and the sieve mesh size (SMS), selected to collect and to retain benthic macroinvertebrates from soft-bottom samples, may affect the evaluation of the aquatic ecosystem ecological status; however, studies analyzing the combined influence of SUS and SMS on assessment tools are lacking, in particular for transitional water ecosystems. Even if the Water Framework Directive (WFD) suggests rapid and cost-effectiveness sampling effort and procedures, the identification of optimal SUS and SMS is a basic step to improve the RBPs and to meet WFD suggestions. Therefore, this research analyses the effects of four soft-bottom sample unit sizes (0.0225 m^2, 0.0450 m^2, 0.0675 m^2, 0.0900 m^2), and three sieve mesh sizes (4 mm^2, 1 mm^2, 0.25 mm^2) on the selection of benthic macroinvertebrates and, thus, on assessment tools, in a Mediterranean lagoon. A sampling survey was performed in September 2009 at a perturbed and an unperturbed study site in the Lesina lagoon (SE Italian coastline); three replicates were taken for each SUS and SMS using an Ekman–Birge grab (15 cm × 15 cm). The samples were sieved on a column of three sieves, with decreasing mesh size. Benthic macroinvertebrates were sorted, identified, measured, weighted and included in twelve datasets (4 SUS × 3 SMS). Sampling effort (SE) was calculated for each SUS and SMS combination as: $SE = [SUS\, m^2 \times (1/SMS\, mm^2)] \times 100$. Four simple community descriptors (numerical density, taxonomic richness, biomass density, individual body-size) and four ecological indicators (AMBI, BENTIX, BITS, M-AMBI) were compared for each combination of SUS and SMS in both study sites. Simple community descriptors and ecological indicators varied significantly between perturbed and unperturbed study site. The results showed that SMS had significant effects on simple community descriptors and ecological indicators, except for BITS index. Conversely, no significant differences were observed for different SUS analyzing simple community descriptors and ecological indicators, except for taxonomic richness and M-AMBI index. The response of the ecological indicators was only slightly affected by the SMS, whereas SUS choice did not influence the ecological status assessment. Anyway, using the larger SMS (4 mm^2), all ecological indicators showed either the same ecological quality status as the 1 mm^2 and 0.25 mm^2 SMS or, in some cases, one class higher, except for the AMBI index.

1. Introduction

The evaluation of ecological status of aquatic ecosystems, may be carried out through the analysis of ecosystem functions, processes and energetic (Feio et al., 2010; Fonnesu et al., 2004; Gessner and Chauvet, 2002; Sangiorgio et al., 2008; Vignes et al., 2012; Woodward et al., 2012), or measuring one or more taxonomic and not taxonomic descriptors of biological quality elements (BQEs) (Basset et al., 2004; Borja and Dauer, 2008; Orfanidis et al., 2008). Among these, benthic macroinvertebrates have long been used to assess the ecological status, and to detect several kinds of natural and anthropogenic pressures in marine, freshwater and transitional water ecosystems (Evagelopoulos et al., 2008; Pearson and Rosenberg, 1978; Ponti et al., 2008). Benthic macroinvertebrates are considered suitable bioindicators (Ponti et al., 2009; Rosenberg and Resh, 1993), responding to environmental or anthropogenic

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pressures by changing in abundance, taxonomic richness and composition, biomass, body-size and biological traits (Basset et al., 2008; Bremner, 2008; Dauer, 1993; Petchey and Belgrano, 2010; Pinna and Basset, 2004).

The use of biological components, instead of physical–chemical measures, to assess the ecological quality status (EQS) of aquatic ecosystems is also explicitly requested in the Water Framework Directive (WFD, Directive 2000/60/EC), which represents the legislative basis for the management and protection of European water bodies. Efforts made to implement the WFD in transitional water ecosystems have motivated the identification and the development of a large number of ecological indicators, based on e.g., phytoplankton (Alexandrova et al., 2007; Brito et al., 2012), macrophytes (Orfanidis et al., 2007; Sfriso et al., 2009), fish fauna (Delpech et al., 2010; Franco et al., 2009), macroinvertebrates (Basset et al., 2012; Mistri and Munari, 2008; Muxika et al., 2007; Pinto et al., 2009; Ponti et al., 2007), but specific studies on optimal sampling effort (SE) were less considered.

For benthic macroinvertebrates, the effectiveness and usefulness of biomonitoring protocols depend mainly on the time required to obtain the ecological assessment and on the overall costs associated with personnel and sample treatment (Barba et al., 2010; Karakassis et al., 2013; Pinna et al., 2013). Specifically, the estimation of metrics and calculation of ecological indicators requires a considerable effort to collect samples, to sort and to identify the specimens and to measure the individual biomass (Ferraro et al., 1989). In this framework, the simplification of methodologies and effort allocation are topics recurrently debated among researchers aiming at implementing effective Rapid Bioassessment Protocols (RBPs), without compromising the ecological validity and accuracy of the results (Buss and Borges, 2008).

Anyway, the RBPs are useful only if they ensure the accuracy of the obtained results, using the fastest and less expensive techniques and methodologies (Cañedo-Argüelles et al., 2012; Metzeling and Miller, 2001; Oliveira et al., 2011). Until now, a reduction in the time spent to process the samples and in the biological monitoring program costs have been achieved by decreasing the sampling effort, e.g., by limiting the number of samples/replicates in the macroinvertebrates collection or by using laboratory sub-sampling (Mavrič et al., 2013; Metzeling and Miller, 2001; Vlek et al., 2006), by using “surrogates” of species identification (e.g., identification of genus or family; Dauvin et al., 2003), and by retaining the larger body-size fraction of macroinvertebrates (Pinna et al., 2013). The development of RBPs for macroinvertebrates has been performed mainly in freshwater ecosystems (Hughes et al., 2012; Lorenz et al., 2004; Somers et al., 1998), while studies on optimal sample unit size (SUS) and/or sieve mesh size (SMS) to collect representative and accurate benthic macroinvertebrate samples, are mostly lacking for transitional water ecosystems.

Hence, the aim of this research was to compare the efficiency and accuracy of twelve sampling effort conditions based on the combination of four SUS (0.0225 m^2, 0.0450 m^2, 0.0675 m^2, 0.0900 m^2), and three SMS (4 mm^2, 1 mm^2, 0.25 mm^2) on the most common assessment tools (simple community descriptors and ecological indicators), to a perturbed and to an unperturbed study site in the Lesina lagoon (SE Italy). The specific objectives were to analyze: (1) the difference in numerical density, taxonomic richness, biomass density and individual body-size (IBS) of benthic macroinvertebrate assemblages, between study sites, among SUS and SMS; (2) the influence of sampling effort on ecological indicators (AMBI, BENTIX, BITS, M-AMBI) both in perturbed and unperturbed study site; (3) the patterns of variation of simple community descriptors in relation to changes in sampling effort. In addition, we suggested an optimal combination of SUS and SMS to obtain accurate results limiting the sampling effort, and to test whether the optimal combination detects the variation in the type and magnitude of perturbation pressures between study sites.

2. Materials and methods

2.1. Description of sampling site

Lesina lagoon (41.88° N; 15.43° E) is a non-tidal, shallow and mesohaline transitional water ecosystem, located in the South-eastern Italian coastline. It is one of the largest lagoons in Italy with the maximum axis about 24.4 km, a total area of 51.4 km^2, and an average depth of 0.8 m; the catchment area is about 600 km^2 (Vignes et al., 2009 and references therein; Fig. 1). The lagoon is separated from the Adriatic sea by a sand-bar about 18 km long, characterized by typical Mediterranean coastal vegetation, like *Gracilaria gracilis* [(Stackhouse) Steentoft, Irvine & Farnham, 1995] and *Cladophora* sp. (Kützing, 1843). The hydrological regime is strongly influenced by continental freshwater inputs and by local meteorological conditions, especially winds and rains. The residence time of the waters is estimated to be about 70–100 days. Temperature and salinity follow a seasonal trend, with minimum values in winter and maximum values in summer. Water temperature generally ranges from 3 °C to 32 °C and salinity from 5 PSU to 51 PSU. Potentially, the lagoon has a low vulnerability to human activities; however, urban and agricultural wastewater discharges enter the lagoon particularly in the western part of the lagoon, leading to well know pulse eutrophication events (Basset et al., 2013; Specchiulli et al., 2009; Vignes et al., 2009). In the early summer 2008, a strong dystrophic crisis occurred in the western part of the lagoon, resulting in hypoxic conditions for a few weeks over an area up to 2 km^2, significantly affecting all ecosystem compartments (Specchiulli et al., 2009). Nutrient load from wastewaters, reduced hydro-dynamism and extreme climate events have been advocated as major causes of the dystrophic events (Basset et al., 2013; Vignes et al., 2009).

2.2. Sampling design

Sampling campaign was carried out in September 2009 at a perturbed site (WSL01 – located in the western lagoon) and an unperturbed site (WSL05 – located in the eastern lagoon) of the Lesina lagoon (Fig. 1). Soft-bottom samples were collected in the framework of the WISER project, funded by the EU FP7 Programme in which Lesina lagoon was one of five transitional waters included in the project. This specific research was performed only in the Lesina lagoon with the aim to analyze the effect of sampling effort reduction on assessment tools both in perturbed and unperturbed conditions. To reach the aim, Lesina lagoon offers two optimal and well known areas characterized by above conditions (Basset et al., 2013; Borja et al., 2011). In particular, Basset et al. (2013) shows that the two areas (here named as LA1 and LA2) are different (perturbed and unperturbed) for almost the whole year.

The benthic macrofauna of the Lesina lagoon is a typical brackish-water assemblage for south Italian transitional waters (Alemanno et al., 2007; Mancinelli, 2010, 2012; Mancinelli et al., 2007, 2009, 2013a,b; Menéndez et al., 2003; Ponti et al., 2008; Potenza and Mancinelli, 2010; Vignes et al., 2012).

The sampling design was implemented to analyze the influence of four sample unit sizes and three sieve mesh sizes on established macroinvertebrate assessment tools (simple community descriptors: numerical density, taxonomic richness, biomass density, individual body-size; ecological indicators: AMBI, BENTIX, BITS, M-AMBI) (Table 1). Four sample unit sizes (SUS) 0.0225 m^2, 0.0450 m^2, 0.0675 m^2, 0.0900 m^2, three sieve mesh sizes (SMS) 4 mm^2, 1 mm^2, 0.25 mm^2, and three replicates for each SUS and