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A rapid bioassessment tool for the evaluation of the water quality of transitional waters

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A B S T R A C T

The term transitional waters encompasses a wide variety of ecosystems with highly variable environmental conditions and naturally impoverished biological communities. These characteristics have led to a considerable delay in the design and implementation of ecological assessment tools within the European Water Framework Directive. Chironomids are a very important faunal group of transitional waters in terms of relative abundance and species richness. In the present investigation a water quality index based on the chironomid pupal exuviae assemblages was developed. Thirty-seven sites were surveyed along the Catalonian coast (North-East Spain) for their Chironomid pupal exuviae. The index registered a significant response to the causes of eutrophication and its symptoms. Different taxonomic resolution levels were tested and a final tool is proposed based on a genus level identification of chironomid taxa and a classification system based on sensitive/tolerant taxa abundances. The tool has the advantage of being cost-effective (cheap, involving low time-consumption and easy to use) and of integrating all the habitats within the ecosystem.

1. Introduction

Since the publication of the Water Framework Directive (WFD) in 2000 (European Commission, 2000), many biotic indices have been developed, validated and intercalibrated, in order to assess the ecological status of the European water bodies (Hering et al., 2010; http://www.wiser.eu). The ecological status assessment tools are already available for most of the established water mass typologies (e.g. rivers and lakes). Nonetheless there is still some controversy regarding the evaluation tools to be used in transitional waters, in which the implementation of the WFD has been considerably delayed (Zaldívar et al., 2008; Hering et al., 2010). Transitional waters are defined in the WFD as "bodies of surface water in the vicinity of river mouths which are partly saline in character as a result of their vicinity to coastal waters, but which are substantially influenced by freshwater flows". In being located in coastal areas, which are the most populated areas in the world, these ecosystems are subjected to strong anthropogenic pressures that often have unpredictable effects over their physico-chemical properties. Transitional waters are naturally rich in nutrients (Levin et al., 2001), being difficult to distinguish between human-induced and natural organic enrichment (Elliott and Quintino, 2007). These features, together with the difficulty of finding and defining reference sites (Basset and Abbiati, 2004), have delayed the design and validation of biological indices for the assessment of the ecological status of transitional waters. There is a need to find organisms that can be present over wide environmental gradients from which a reliable water quality index can be built. In this context chironomids (non-biting midges) are a good candidate. They have been often neglected in transitional waters due to their difficult taxonomy, but they are present in these ecosystems at high densities and relatively high diversities (Bayo et al., 2001; Sahuquillo et al., 2006; Dimitriadis and Cranston, 2007; Sahuquillo et al., 2007; Cañedo-Argüelles and Rieradevall, 2009). Furthermore they have proved to be good indicators in rivers (Warwick, 1990; Wilson and Ruse, 2005; Raunio and Paasivirta, 2008) and lakes (Ruse, 2002; Raunio et al., 2010; Ruse, 2010), and they can signal eutrophication symptoms such as oxygen depletion (Brodersen and Quinlan, 2006).

The chironomidae (Diptera) are holometabolous insects with four life stages (egg, larva, pupa and adult). Larvae grow in the water

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associated to the different available habitats (some species burrow in the sediment, while others are attached to macrophytes or swimming free while looking for prey). Eventually the late fourth instar larvae develop wing pads, moult to pupae and then swim to the water surface where adults cast their pupal skin (exuviae) and emerge to mate (Ferrington et al., 1991). Since all the chironomid larvae inhabiting a given water mass will eventually undergo this metamorphosis, pupal exuviae collection has a great potential for characterizing the whole chironomid community. Chironomidae pupal exuviae collection was first used by Thienemann (1910), but it did not become a widespread technique until the 1970s (Coffman, 1973; Wilson and Bright, 1973). From then on it has been mainly used for the characterization and bio-assessment of rivers (McGill et al., 1979; Rieradevall and Prat, 1986; Raunio and Paasivirta, 2008) and lakes (Ruse, 2002; Raunio et al., 2010; Ruse, 2010), but it has never been applied to transitional waters. Among the advantages of using chironomid exuviae we can highlight that: 1) they are easy to collect, 2) they integrate information from all the habitats present in a water body, and 3) the preparation and identification are easier and faster than that of larvae. More recently Wilson and Ruse (2005) compiled most of the available information and developed a Chironomid Pupal Exuviae Technique (CPET), a tool for harmonizing field and laboratory methods for the monitoring and quality assessment of both lentic and lotic freshwaters. Since then the CPET methodology has been adopted as a European Standard (EN 15196:2006), and recognized in different European states, such as in Spain in the norm AENOR UNE-EN 15196:2007.

In the present investigation chironomid pupal exuviae were collected following CPET along the Catalonian coast (Northwestern Spain) to provide a cost-effective tool for the water quality assessment of transitional waters. The index was named EQAT, which is the Catalan acronym for "Chironomid Exuviae in Transitional Waters". The specific objectives were to: 1) characterize the relation of the chironomid community with the environment; 2) design a water quality index based on the pollution tolerance of the recorded taxa at different taxonomic resolution levels, and 3) test the reliability of the index.

2. Methods

2.1. Sampling

For the index design samples were collected in 37 permanent shallow water bodies (including coastal lagoons, ponds and shallow lakes) associated to several wetland areas along the Catalonian coast (Table 1). Thirteen water bodies were sampled in 2004–2005, and 24 in 2007. The sampling surveys were conducted on three different occasions (May, June and October) which comprised the maximum emergence periods of chironomids in the study area (Sànchez-Millaruelo et al., 2009). Some of the sampling sites, together with additional sites, were used in order to test the correlation of the index with different trophic variables (Table 1). The data-set used for correlation analyses comprised 66 independent samples collected in shallow water bodies of the Llobregat Delta during June and September of 2004 and March and May of 2005 (Table 1).

Chironomid exuviae were collected along the leeward shore of the water masses using a 250 μm mesh size hand-net. In the case of large and complex lagoons up to three sites per visit were explored in each water body. The net was gently passed through the water surface for about 10 min and the collected material was emptied into a plastic tray containing water in order to count the collected exuviae. If approximately 200 exuviae were counted, no further sampling was conducted, if not, the procedure was repeated for a maximum time of 20 additional minutes. The samples were preserved in ethanol 70°. Once in the laboratory the samples were sieved through a 250 μm mesh and placed in a Petri dish. Chironomid pupal exuviae were removed from debris and identified to family level using binocular magnifying lens. Then they were dehydrated using ethanol 96°, mounted permanently in Euparal® on a microscope slide and identified to species (Langton and Visser, 2003) using a high magnification (400×) microscope.

In order to characterise the water physico-chemical conditions, surface water conductivity, pH, temperature and dissolved oxygen were measured *in situ* using a field multi-parametric sensor. Two litres of surface water were collected and kept cold until laboratory analysis. One litre was used for the analyses of dissolved inorganic nutrients (NH_4^+, NO_3^-, NO_2^-, total nitrogen, PO_4^{3-}, and total phosphorus) and total organic carbon (TOC). The other litre was filtered to analyze phytoplanktonic chlorophyll-a (except for the water bodies used for testing the index, where chlorophyll-a data was not analyzed). All analyses were performed following standard methods (Greenberg et al., 1999).

2.2. Data analysis

2.2.1. Raw data treatment

For each site the three biological samples (May, June and October) were merged into one single sample, from which the relative abundance of each species was calculated according to the total species abundance (%). Given the multiplicative relationship between the species and the environmental variables, the species data were square root transformed prior to statistical analysis as recommended by Lepš and Šmilauer (2003). All the environmental variables were standardized in order to work within the same measurement units. Those variables that registered a standard deviation higher than the mean were log-transformed as recommended by Legendre and Legendre (1998). A Pearson correlation test was performed between all the environmental variables, and when two variables registered a correlation coefficient higher than 0.95 one of them was excluded from the multivariate analysis.

2.2.2. Exploratory analysis

A detrended correspondence analysis (DCA) was performed in order to explore the type of relationship between the species and the environmental data. The first axis of the DCA analysis had a gradient length (λ) of 5.06 standard deviation (SD) units, and thus the data were suitable for unimodal analysis (Lepš and Šmilauer, 2003). The direct relation between the species and the environmental data was explored by unimodal canonical correpondence analysis (CCA) using biplot-scaling centred on interspecies distances. DCA, and CCA were performed using CANOCO 4.5 software (ter Braak and Šmilauer, 2002). The CCA analysis included all the environmental variables as active predictor variables and the TRIX index (Vollenweider et al., 1998) as a supplementary variable. The TRIX index is based on chlorophyll-*a*, total nitrogen and total phosphorus concentrations and on oxygen saturation values, and it was designed to characterise the trophic state of coastal marine waters. It is calculated using the following formula proposed by Vollenweider et al. (1998):

$$\mathrm{TRIX} = (\mathrm{Log}_{10}(\mathrm{chl}\ a \times |\mathrm{DOdev}| \times \mathrm{DIN} \times \mathrm{TP}) + 1.5)/1.2$$

where chl-a, DIN and TP refer to the chlorophyll-a, dissolved inorganic nitrogen and total phosphorus concentrations (mg l^{-1}) respectively, and IDOdevI is the oxygen absolute deviation from saturation (%). Forward selection was used as a stepwise procedure in order to test which variables significantly explained residual variation in species composition.

The degree of nestedness of the species data-set was analyzed in order to test whether the chironomid species distribution among