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Benthic macrofaunal dynamics and environmental stress across a salt wedge Mediterranean estuary

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ABSTRACT

The spatial distribution of benthic macroinvertebrate community in relation to environmental factors was studied along the Ebro Estuary (NE Iberian Peninsula), a salt wedge Mediterranean estuary. Both ordination methods and generalized additive models were performed to identify the different benthic assemblages and their relationship to abiotic factors. Our results showed a strong relationship between macrofaunal assemblages and the predominant environmental gradients (e.g. salinity); thus revealing spatial differences in their structure and composition. Two different stretches were identified, namely the upper (UE) and the lower Ebro Estuary (LE). UE showed riverine characteristics and hence was colonized by a freshwater community; whereas LE was influenced by marine intrusion and sustained a complex marine-origin community. However, within each stretch, water and sediment characteristics played an important role in explaining species composition differences among sampling stations. Moreover, outcomes suggested a total species replacement pattern, instead of the nestedness pattern usually associated with well-mixed temperate estuaries. The sharp species turnover together with the estuarine stratification point out that the Ebro Estuary is working, in terms of ecological boundaries, under an ecotone model. Finally, despite obvious differences with well mixed estuaries (*i.e.* lack of tidal influence, stratification and species turnover), the Ebro Estuary shares important ecological attributes with well-mixed temperate estuaries.

1. Introduction

Estuaries are critical transition zones linking freshwater and marine systems, where a broad range of physicochemical factors co-occur in a small geographical area (Levin et al., 2001; Elliott and Whitfield, 2011). The close connection between riverine and marine habitats assures a rapid and constant exchange of energy, materials and organisms; for this reason, estuaries are commonly described as stressful (*e.g.* Elliott and Whitfield, 2011; Day et al., 2012) and biologically dynamic ecosystems (*e.g.* Ahel et al., 1996; Nebra et al., 2011). Estuarine environmental gradients impose physiological constraints on biota; only a few specialized species are capable of withstanding them, resulting in extremely low-richness communities compared with those from adjacent riverine and marine areas (McLusky and Elliott, 2004; Dauvin, 2007; Elliott and Whitfield, 2011; Day et al., 2012). Among the different abiotic factors of both natural and anthropogenic origin that are known to affect the macrofaunal distribution and diversity within estuaries, the salinity variation has been identified as the main driver in structuring communities. The relationship between tidal action, the salinity gradient and macrofaunal trends has been extensively studied in temperate mixed estuaries (*e.g.* Attrill, 2002; Attrill and Rundle, 2002; Giberto et al., 2007; Whitfield et al., 2012); but the definition of estuary based on the tidal influence and the consequent salinity gradient is still controversial (*e.g.* McLusky, 1999; Elliott and McLusky, 2002; Tagliapietra et al., 2009; Potter et al., 2010; Telesh and Khlebovich, 2010). This controversy becomes relevant when talking about other types of estuaries, such as those where the salinity variation is not the result of marine water dilution (*e.g.* stratified or inverse estuaries).

Since the publication of the Remane diagram (Remane, 1934) and its application to estuaries, several modifications and new models has been developed for explaining the richness pattern of biota along the estuarine salinity gradient (Whitfield et al., 2012). For instance, Attrill and Rundle (2002) proposed a model that

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becomes relevant due to the novelty of introducing ecological boundaries in estuaries, namely ecotone and ecocline, thus reinforcing the fact that boundaries are matter of contemporary ecology as pointed out by Strayer et al. (2003). The riverine-marine interface is the most obvious landscape boundary in aquatic ecosystems (Rundle et al., 1998) and therefore, estuarine boundaries represent challenging environments for carrying out ecological studies. Everything written above is applicable to well-mixed estuaries; but what occurs in non-tidal seas where highly stratified estuaries are formed? When compared to well-mixed estuaries, in highly stratified estuaries: (i) river discharge controls marine intrusion due to the low tidal range (usually tidal amplitude is less than 2 m); (ii) weak mixing drivers enhance water column stratification promoting the formation of a salt wedge; (iii) water density and salinity shows an abrupt change from surface to bottom, friction between fresh and saltwater layers forms a narrow interface called halocline; and (iv) isohalines are arranged horizontally. As a consequence, environmental fluctuations in salt wedge estuaries are not gradual, for instance, there is no salinity gradient along the estuary (Ibáñez, 1993); and in contrast to mixed estuaries, the biota must be adapted to abrupt changes, not only in salinity terms, but also in other environmental features such as water temperature, redox potential (E_h) or pH. Moreover, salt wedge estuaries become rivers when flow is high enough to expel marine intrusion (Muñoz, 1990; Ibáñez et al., 1997); thus, environmental stress for biota is more pronounced (Elliott and Whitfield, 2011). The material and salts exchange is mainly the result of entrainment between layers, together with the turbulence occurring at the salt wedge tip or null point; the halocline only allows scarce transfer of materials between layers, mainly coarse suspension particles and died organisms coming from the upper layer, and salts and nutrients from the lower layer (Dyer, 1997; Lewis, 1997).

In this study we explore how environmental factors influence macrofaunal community distribution in a distinctive estuarine boundary, by focusing on several different aspects. Therefore, the aims of this paper are: first, to relate the distribution and abundance of the different macroinvertebrate species to environmental variables and river disturbances by means of ordination methods, and second, to analyze the response of the macrofaunal community at the population and species level to the variation in limnological features along the whole estuarine stretch.

2. Materials and methods

2.1. Study site

The study was conducted in the Ebro Estuary (Fig. 1) located in the NE of the Iberian Peninsula (40°43′10″ N, 0°40′30″ E). The Ebro River flows into the Mediterranean Sea and forms a Type 4 (salt wedge or highly stratified) estuary (Hansen and Rattray, 1966; Muñoz and Prat, 1989; Muñoz, 1990; Ibáñez, 1993) of about 32 km long, with a mean width of 240 m and a mean water depth of 7 m. The tidal range is low, *ca.* 0.20 m (Cacchione et al., 1990), and its low influence promotes the formation of a salt wedge controlled by the river discharge (advance, retreat and time of permanence). Briefly, when river flow exceeds 350–400 $m^3\ s^{-1}$ the salt wedge is pushed seawards and the estuary works as a river or ‘fluvial estuarine stretch’, conversely the salt wedge reaches its maximum landwards (*ca.* 30–32 km from the river mouth) at flows lower than 100 $m^3\ s^{-1}$ (Ibáñez, 1993; Ibáñez et al., 1997).

The basin is strongly regulated by approximately 190 dams, managing water for hydropower production, irrigation, and human consumption. Large reservoirs have altered the annual flow not only by modifying the natural seasonal flow pattern (Muñoz and Prat, 1989) but also by preventing flood frequency and intensity (Ibáñez et al., 2012; Rovira et al., 2012a). The annual mean flow has decreased since the beginning of the century to the present (Muñoz and Prat, 1989; Ibáñez et al., 1996). In particular, Mequinenza and Ribarroja reservoirs (located on the main river about 100 km upstream from the river mouth) have a significant regulatory effect over river flow in the lower Ebro River (Ibáñez et al., 2012; Rovira et al., 2012a), and therefore are considered to be the final factor responsible for the salt wedge dynamics and macrofaunal trends along the Ebro Estuary. Water regulation virtually assures the presence of the salt wedge in the same position for long periods (Ibáñez et al., 1995; Sierra et al., 2004; Falcó et al., 2010; Nebra et al., 2011).

2.2. Sampling design and laboratory procedures

In order to cover the whole study area, both the estuarine reach and the uppermost stretch potentially accessible by the salt wedge during low flow periods, nine sampling stations were established from the river mouth to 37 km upstream (Fig. 1). Each station was sampled seasonally (from summer 2007 to spring 2008); on each sampling occasion, three sediment samples were collected using a Ponar grab (0.046 m^2) for benthic macroinvertebrates, and sediment traits, including grain size characterization and total organic matter content (TOM). Macrofauna samples were washed in situ (using pumped bottom water) through a 0.5 mm mesh sieve to separate macroinvertebrates from finest sediment particles, and the organisms retained were immediately fixed with buffered 10% formalin. Later in the laboratory, all the macroinvertebrates were sorted, counted and identified under a stereomicroscope to the lowest possible taxonomic level, usually to species level. Bottom water samples were analyzed for total chlorophyll, pheophytin and nutrient concentrations, including phosphate (PO_4), total phosphorous (P_T), ammonium (NH_4), nitrate (NO_2), nitrite (NO_3), total nitrogen (N_T) and orthosilicate (SiO_4). An YSI 556 multi-parameter probe was used to measure water temperature (°C), dissolved oxygen (mg l^{-1}), oxygen saturation (%), pH, salinity and conductivity (mS cm^{-1}). Additionally, hydromorphological characteristics were also measured (*i.e.* depth, flow velocity and water transparency) (see Appendix A). Data available at the Ebro Basin Authority web site (http://www.chebro.es; station 9027: Tortosa), were used to calculate the permanence time of the salt wedge for each sampling station and season as a function of the daily average river flows according to Ibáñez (1993).

2.3. Data analysis

The macroinvertebrate abundance and environmental variables data sets were analyzed by means of multivariate ordination techniques, including both indirect (Principal Component Analysis and Detrended Correspondence Analysis; PCA and DCA, respectively) and direct (Canonical Correspondence Analysis; CCA) gradient techniques. A PCA was carried out to explore the relationships and association patterns among environmental variables in the sample sets. Kaiser-Meyer-Olkin's (KMO) measure of sampling adequacy was used to assess the usefulness of a PCA; KMO ranges from 0 to 1 and should be >0.5 if variables are sufficiently interdependent for PCA to be useful (Tabachnick and Fidell, 2001). On the other hand, the structure of the macroinvertebrate community was investigated by means of DCA (*i.e.* indirect gradient technique) and CCA (*i.e.* direct analysis method). Indirect gradient analysis only uses the species × sample matrix in the ordination, whereas in direct techniques the ordination results are constrained to optimize their linear relationship to the environmental variables. Indirect and direct gradient analyses are complementary because although direct gradient analysis provide