



# Influence of salinity regime on the food-web structure and feeding ecology of fish species from Mediterranean coastal lagoons



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## ABSTRACT

Dual  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  analyses and estimates of biomass were used to characterize the food webs of valuable fish species in three coastal lagoons of the Ebro Delta subjected to contrasting salinity regimes (polyhaline in the Tancada lagoon, mesohaline in the Encanyissada and oligohaline in the Clot lagoon). The  $\delta^{13}\text{C}$  signatures of the entire food-web including primary producers, sediment organic matter and consumers showed the most enriched values in the Tancada lagoon (from approx.  $-4.8\text{‰}$  in sediments to  $-19.7\text{‰}$  in fish) and the most depleted ones in the Clot lagoon (from approx.  $-11.4\text{‰}$  in sediments to  $-25.4\text{‰}$  in fish), consistent with dominant contributions from marine and continental sources, respectively. For  $\delta^{15}\text{N}$ , particularly high values were detected in the submersed vegetation ( $11.3 \pm 0.3\text{‰}$ ) together with more enriched sediment values at lower salinities (by approx.  $2.5\text{‰}$ ), suggesting that historical loadings of agricultural fertilizers are still retained by the systems and transmitted across trophic levels. Negative relationships between  $\delta^{15}\text{N}$  and salinity were also observed for the amphipod *Gammarus aequicauda* and the isopod *Sphaeroma hookeri*, suggesting some consumption of accumulated and resuspended detrital material. In contrast,  $\delta^{15}\text{N}$  signatures of fish showed lower values and inconsistent patterns, possibly because most species have a seasonal use of the lagoons. The biomass of fish species did not show a clear effect of the salinity regime (except for the mosquitofish *Gambusia holbrooki*), but results for mixing models suggest a diet shift from higher contribution of zooplankton size fractions in the Encanyissada (from 57 to 73%) to macrofauna at the other lagoons (from 40 to 67%). We suggest that alterations in salinity might modify the trophic dynamics of the systems from benthic to planktonic pathways, without large-scale differences in  $\delta^{15}\text{N}$  of fish suggestive of similar trophic levels.

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## 1. Introduction

Coastal ecosystems are important sources of valuable goods and services, but human activities such as agricultural and industrial uses modify the hydrology of downstream estuaries and increase the runoff of pollutants and nutrients into coastal waters altering, or destroying natural habitats (Lotze et al., 2006). Measuring and managing the effects of these impacts is important for sustaining coastal fisheries and to preserve and enhance the natural biological diversity. Responses of estuarine food webs to nutrient loading typically encompass the development of phytoplankton blooms and/or fast-growing macroalgae and epiphyte communities (Prado et al., 2012a) that shade submersed plants and cascade up the whole food-web through changes in habitat structure and the qualitative availability of food resources. In addition, hydrological

changes also modify the salinity condition of estuarine areas, which are known to be a major structuring factor for plants (Prado et al., 2012a) and the associated animal communities of fish and invertebrates (Jassby et al., 1995). The interactive effects of freshwater inflows and nutrient loadings on downstream fisheries and habitats are assessed by long-term monitoring of communities (Jassby et al., 1995), but other types of simultaneous studies are also needed to test for differences in the trophic links among ecosystem components, particularly in target species of commercial or conservation interest.

Stable isotope analyses are increasingly used to answer many types of ecological questions, such as identifying trophic position and food web linkages, to elucidate foraging ranges and to trace sources of pollution and organic matter, among other applications (Vizzini and Mazzola, 2004, 2005; Lebreton et al., 2011). For instance, dual examination of  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  compositions has permitted a considerable advance in clarifying the fate of nutrient and organic matter flows and the trophic relations in estuaries and marshes (see review by Fry and Sherr, 1984) adding new insights to

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previous studies based on foraging observations, assimilation rates, and/or gut and fecal contents.  $\delta^{15}\text{N}$  measurements offer an indicator of the organism trophic level ( $\delta^{15}\text{N}$  enrichment usually ranges between 2‰ and 5‰ and the overall theoretical value between trophic levels is considered to be around 3.4‰; DeNiro and Epstein, 1981), whereas  $\delta^{13}\text{C}$  measurements indicate the sources of nutrition for consumers ( $\delta^{13}\text{C}$  is only enriched by approx. +0.4‰ among trophic levels; Vander Zanden and Rasmussen, 2001). Dual isotope studies of  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  have also been helpful in resolving contributions of marine vs. terrestrial sources of organic matter (Fry and Sherr, 1984) which were traditionally quantified using optical techniques of low resolution or data-intensive and difficult to parameterize. For nitrogen, the mean of published  $\delta^{15}\text{N}$  values in terrestrial plants is approx. 4‰ less positive than that of marine algae and phytoplankton and can influence the isotopic composition of organisms feeding in either source, although ranges overlap significantly (see review in Schoeninger and DeNiro, 1984; France, 1995). Despite this, nitrogen-rich organic wastewater from land-based human activities with highly enriched  $\delta^{15}\text{N}$  values (approx. +3 to +5‰) may disperse widely in estuarine and marine habitats, increasing  $\delta^{15}\text{N}$  values of consumers (Vizzini and Mazzola, 2004; Vizzini et al., 2005). For carbon, the concentrations of dissolved organic carbon (DOC) in riverine freshwater usually exceed those of marine origin (Fry, 2002) and result in different contributions to the organic matter budgets of estuarine systems. In addition, the  $\delta^{13}\text{C}$  composition of terrestrial organic matter in freshwater systems tends to be lighter than that in marine and estuarine food-webs (Schoeninger and DeNiro, 1984; Deegan and Garritt, 1997), and differences in dissolved inorganic carbon (DIC)  $\delta^{13}\text{C}$  values may vary from -10‰ in riverine freshwater to +2‰ in surface seawater (Fry, 2002) resulting from the presence of  $^{13}\text{C}$ -depleted  $\text{CO}_2$  derived from the decomposition of terrestrial organic matter (Boutton, 1991). The incorporation of  $^{13}\text{C}$ -depleted  $\text{CO}_2$  by aquatic macrophytes and phytoplankton during photosynthesis may be effectively transmitted to consumers and extend through the food-web. Therefore, species in habitats subjected to contrasting contributions of freshwater and marine water (i.e., distinctive salinity gradients) may have different  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  signatures in much the same way that organisms moving between isotopically distinct food-webs can carry with them information on the location of previous feeding (Schoeninger and DeNiro, 1984). The extent of these differences in  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  may be variable across types of organisms, depending on species type and variable factors such as diet type (Prado et al., 2012b). In particular, fish isotopic signatures appear to be highly responsive to salinity variations (approx. 6 and 7‰ higher values of  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$ , respectively in marine than in freshwater habitats; Schoeninger and DeNiro, 1984) and additional effects in species abundance are also common. Both complementary approaches may help to elucidate overall impacts of the salinity regime in community structure, as well as functional changes in the whole system.

Coastal lagoons in the Ebro Delta (Southern Catalonia, NW Mediterranean) are subjected to persistent human influence, including rice agriculture (approx. 70% of the total Delta surface), waterfowl hunting and traditional fishing. From the end of the 19thC. to the late 1980s, rice farming strongly determined the hydrological cycle of the coastal lagoons, due to periodic disposal (May to November) of drainage water rich in nutrients and agricultural chemicals (e.g., PCBs, DDTs and HCB), which enhanced phytoplankton and greatly reduced submerged vegetation and associated populations of fish and waterfowl (Comín et al., 1990). In 1990, after the area was declared Natural Park, local authorities transferred most of the agricultural drainage to the bays and initiated freshwater inputs from the Ebro River with lower nutrients and organic matter, in order to improve the habitat for fish and

game birds. However, seasonal freshwater inflows (from late spring to fall) still reverse the natural hydrological cycle of the lagoons, which attain lower salinities in summer and higher in winter. In addition, inputs of freshwater favored the successful establishment of non-native species of fish and invertebrates that threaten the persistence of local species such as the Iberian toothcarp *Aphanius iberus* (Vargas and De Sostoa, 1997; Caiola and Sostoa, 2005) and cause important economic losses.

Here we combine the use of fishing captures and stable isotopes to investigate the influence of the salinity regime on the abundance and food-web structure of five fish species in coastal lagoons of the Ebro Delta and to elucidate possible differences in the trophic status of the systems. We selected three lagoons with contrasting seasonal variations in salinity (Tancada: approx. 17–33‰; Encanyissada: approx. 11–30‰; and Clot: approx. 6–14‰, in summer and winter, respectively) resulting from different degrees of freshwater inflow and seawater flux, and we evaluated: (1) the abundance of commercial fish species (the European eel, *Anguilla anguilla*, the mullet, *Liza* spp., and the sand smelt *Atherina boyeri*), and species of conservation concern (the common goby *Potamochistus microps*, the Iberian toothcarp *Aphanius iberus*, and the eastern mosquitofish *Gambusia holbrooki*), and (2) the  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  composition of fish species and food sources and their relationship with salinity. Finally, (3) isotope mixing models were used to assess possible differences in food source contributions to fish diets at each lagoon.

## 2. Materials and methods

### 2.1. Study sites

The study was conducted in three coastal lagoons of the Ebro Delta receiving freshwater inflows from several irrigation canals from April–May till December as well as marine water through canals permanently connected to the sea. The Encanyissada lagoon is the largest in size (418 ha) and is connected to the Alfacs Bay through a natural outlet and to the Clot lagoon (56 ha) through a flood gate (Fig. 1), whereas the Tancada lagoon has an intermediate size (185 ha) and is connected to the Alfacs bay through three narrow canals. Seasonal monitoring since 2007 (Prado et al., 2012a) shows winter polyhaline waters in the Encanyissada and Tancada lagoons (salinity  $28.09 \pm 0.09$  and  $27.10 \pm 0.16$  respectively) and mesohaline in the Clot lagoon ( $11.6 \pm 0.16$ ), whereas summer salinities are lower but still polyhaline in the Tancada ( $18.71 \pm 0.69$ ), mesohaline in the Encanyissada ( $12.56 \pm 0.83$ ) and oligohaline in the Clot lagoon ( $3.63 \pm 0.09$ ). The angiosperm *Ruppia cirrhosa* dominates the submersed vegetation in the Encanyissada lagoon (with some mixed areas with *Potamogeton pectinatus*) and is the only plant species in the Tancada lagoon. In contrast, the oligomesohaline regime of the Clot lagoon favors the development of *P. pectinatus* with intersperse *R. cirrhosa* and mixed stands of *P. pectinatus* and *Najas marina* in some reduced areas. Water nutrients and chlorophyll are similar among the lagoons ( $\text{NO}_2 + \text{NO}_3$ :  $9.5\text{--}65 \mu\text{g l}^{-1}$ ,  $\text{PO}_4$ :  $13.5\text{--}25 \mu\text{g l}^{-1}$ , Chl A:  $0.15\text{--}3.1 \mu\text{g l}^{-1}$ ) except for higher levels of ammonia in summer in the Tancada lagoon ( $\text{NH}_4$ :  $11\text{--}59$  and  $233\text{--}284 \mu\text{g l}^{-1}$  in the Encanyissada and Clot lagoons and in the Tancada lagoon, respectively). The three lagoons are similar in depth (80–90 cm in all monitoring sites), and have a temperature range from 25 to 28 °C in summer to 9–12 °C in winter.

### 2.2. Collection and preparation of samples

#### 2.2.1. Fish samples

Twelve multimesh nylon gillnets 30 m long by 1.5 m high were set in the lagoons during March–April 2010 in order to capture

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