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# Cross-validation of $\delta^{15}N$ and FishBase estimates of fish trophic position in a Mediterranean lagoon: The importance of the isotopic baseline



Giorgio Mancinelli <sup>a,\*</sup>, Salvatrice Vizzini <sup>b</sup>, Antonio Mazzola <sup>b</sup>, Stefano Maci <sup>a</sup>, Alberto Basset <sup>a</sup>

- <sup>a</sup> Department of Biological and Environmental Sciences and Technologies, University of Salento, Ecotekne Centre, Via Monteroni 165, 73100 Lecce, Italy
- b Department of Earth and Marine Sciences, University of Palermo, Via Archirafi 18, 90123 Palermo, Italy

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#### ABSTRACT

FishBase, a relational database freely available on the Internet, is to date widely used as a source of quantitative information on the trophic position of marine fish species. Here, we compared FishBase estimates for an assemblage of 30 fish species sampled in a Mediterranean lagoon (Acquatina lagoon, SE Italy) with their trophic positions calculated using nitrogen stable isotopes.

To assess the influence of the trophic level used to compute the baseline indicator on the robustness of isotopic estimations, we compared the trophic position of fish calculated using the average  $\delta^{15}N$  signature of either basal resource or primary consumer taxa measured at three stations located in the lagoon in July and November 2007.

In general, basal resources showed negligible among-station and inter-season variations in their  $\delta^{15}$ N values; however, they were characterized by a high inter-specific heterogeneity, with signatures varying by approximately 10 per mil units. In contrast, whereas primary consumer signatures showed significant spatial and temporal variations, they were characterized by a lower inter-specific variability.

Fish trophic positions estimated using primary consumers as the isotopic baseline were highly correlated with values provided by FishBase, independently from whether the latter were calculated on either diet data or individual prey items. Conversely, estimations using a basal resources as the baseline indicator were significantly less correlated with FishBase estimates.

The present study emphasized the crucial importance played by inter-specific variability in baseline taxa signatures for a robust assessment of fish trophic position, and confirmed primary consumers as the best candidate for baseline estimation. In addition, our results indicate that, notwithstanding the limitations characterizing the data provided in FishBase, they represent an adequate source of information on the trophic ecology of fish.

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### 1. Introduction

Human-induced removal of key species in marine ecosystems is acknowledged to determine profound effects on the structure and dynamics of food webs, leading to trophic cascades or ecological regime shifts (Pinnegar et al., 2000; Baum and Worm, 2009). In general, large predatory fish species are preferentially overexploited due to their higher economic value (Essington et al., 2006; Estes et al., 2011); the practical result of the process — a shift of the fishing efforts toward species located at lower trophic levels — is actually determined by a reduction in food web length ("fishing down the food web": Pauly et al., 1998; Pauly and Palomares, 2005).

Accordingly, fish trophic position (TP hereafter) is currently recognized as a useful functional indicator of human disturbance, and trends in the mean trophic position of fishery landings are often used as a sustainability and marine biodiversity indicator (Pauly and Watson, 2005; Coll et al., 2008; Branch et al., 2010).

To date one of the most used source of quantitative information on the trophic position of fish species is represented by the webbased relational database FishBase (Froese and Pauly, 2012). The database was developed in 1989 at the International Center for Living Aquatic Resources Management (ICLARM; currently World-Fish Center) in collaboration, among others, with the Food and Agriculture Organization of the United Nations (FAO) and with support from the European Commission (EC). FishBase summarizes key taxonomic, ecological and biological information on 32,500 fish species (as to March 2013), providing for a large proportion of them

<sup>\*</sup> Corresponding author.

E-mail address: giorgio.mancinelli@unisalento.it (G. Mancinelli).

TP values estimated using diet information extracted from publications reporting on stomach contents.

In the last two decades, stable isotope analysis (SIA) has been recognized as a reliable approach to TP estimation alternative to stomach content analysis, as it integrates the food assimilated over a longer time scale, depending on the tissue analyzed (e.g., Gannes et al., 1997; Codron et al., 2012). Trophic positioning relative to a common baseline is generally achieved using  $\delta^{15}N$  signatures (Cabana and Rasmussen, 1996; Vander Zanden and Rasmussen, 1999; Post, 2002). However, this approach is not free from limitations, related, among other factors, to inter- and intra-specific variability in the isotopic signature of baseline species and nitrogen trophic fractionation (reviewed in Post, 2002; Boecklen et al., 2011; see also Woodcock et al., 2012).

Here, we calculated the trophic position of 30 fish species collected in a Mediterranean lagoon at three sampling stations and in two seasons using stable nitrogen isotope ratios ( $\delta^{15}$ N). The main scope of the study was to test their relationship with FishBase estimates. Only a few studies have attempted to compare TP values calculated experimentally using SIA and FishBase data, with nonunivocal results. Good concordance was found in some cases (Kline and Pauly, 1998), while in other cases weak, or seasonallydependent relations were observed (Faye et al., 2011; Carscallen et al., 2012). In addition, we verified whether the strength of the SIA-FishBase relationship was influenced by the trophic level chosen to calculate the  $\delta^{15}N$  baseline. For freshwater systems, there is general agreement that primary consumers more than basal resources may provide the most appropriate baseline indicator (Cabana and Rasmussen, 1996: Vander Zanden and Rasmussen, 1999, 2001; Post, 2002); noticeably, no information are to date available for lagoons and other coastal environments. Here, we calculated baseline indicators using either basal resources or primary consumers, and tested whether the different baselines affected the relationship between  $\delta^{15}N$ - and FishBase estimates of fish trophic positions. Inter-specific variability in the isotopic signatures of baseline resources was taken into consideration by implementing a bootstrap procedure for TP estimation.

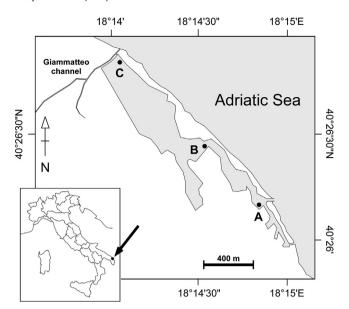
# 2. Materials and methods

## 2.1. Study site

The study was carried out in the Acquatina Lagoon  $(40^{\circ}27'22''N-18^{\circ}12'24''E)$ , a non-tidal brackish-water basin located on the Adriatic Sea in SE Italy (Fig. 1). Complete information on the system morphology and oceanography is provided in Belmonte (2009) and in Maci and Basset (2009). In brief, the lagoon is a shallow (1.2 m average depth) coastal water body about 2 km long with a surface area of 0.45 km². The basin is connected with the Adriatic Sea by a mouth located in the southward area and receives freshwater inputs from the Giammatteo channel in the northward area (Fig. 1).

Dense stands of the seagrass *Cymodocea nodosa* (Ucria) Ascherson cover more than 50% of the bottoms, while other seagrasses of the genera *Ruppia* and *Zostera* together with macroalgae of the genera *Ulva*, *Dyctiota*, and *Cystoseira* are locally abundant (Petrocelli and Cecere, 2009). *Cymodocea nodosa* represents the main source of autochthonous plant detritus to the benthic system; secondary allochthonous inputs are represented by *Phragmites australis* (Cav.) Trin. ex Steudel and *Posidonia oceanica* (L.) Delile leaf litter, the latter entering the lagoon during storm events (Maci and Basset, 2009; Mancinelli, 2012a).

The benthic macrofauna of the basin is mainly characterized by a brackish-water assemblage of invertebrate species typical of other lagoons and coastal habitats of the area (e.g., Mancinelli and



**Fig. 1.** The Acquatina lagoon. The three sampling stations (A–C) used during the study period are shown.

Rossi, 2001; Mancinelli et al., 2005, 2007, 2009; Alemanno et al., 2007a, 2007b; Mancinelli, 2010; Potenza and Mancinelli, 2010; Vignes et al., 2012). Among primary consumers, small-sized crustaceans such as gammaridean amphipods and sphaeromatid and idoteid isopods characterize the epifaunal assemblage associated to seagrasses and macroalgal beds (Mancinelli, 2012a). This group of taxa also represents the most important contributor to the diet of the diverse ichthyofauna of the lagoon (Prato et al., 2009), dominated by representatives of the families Sparidae, Atherinidae, Moronidae, Engraulidae, and Gobiidae (De Mitri, 2004; Maci and Basset, 2009).

### 2.2. Sampling procedures

Three sampling stations were located in the Acquatina lagoon (Fig. 1). Station A and C were characterized by muddy bottoms and thick layers of decomposing *Posidonia oceanica* leaf litter. In contrast, station B was located in the central part of the basin and was characterized by dense stands of *Cymodocea nodosa*.

The fish assemblage was sampled twice, in June and November 2007, using fyke nets. They were constituted by three hoop-nets equipped with wings, set up radially at the end of a 20 m-long blocking net tethered to the shore. Each hoop net had two funnels, and a mesh size ranging from 12 mm for the wings to 6 mm for the end. Nets were recovered after 24 h from deployment. Captured fish were stored on ice and transferred to the laboratory.

At each sampling station, representative submerged vegetation was sampled in the area surrounding the fyke net by hand. For each species, several leaves/fronds were collected, with a minimum of 3 replicates sampled at random positions. Leaf litter was sampled from natural bottom accumulations. For the analysis of sediment organic matter, triplicate samples of the uppermost (0–1 cm depth) sediment layer were collected using a PVC core (i.d. 4 cm) and stored in sterile containers. Simultaneously, representatives of primary consumers species were collected using a hand-towed scoop net (20  $\times$  10 cm, 25  $\mu m$  mesh size) swept through the submerged vegetation and accumulated leaf litter. Upon collection, floral and invertebrate samples were placed in separate sterile falcon tubes or plastic bags, labeled and stored on ice. Litterbags were used in combination with scoop net samplings to increase the

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