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Use of stable isotope ratios of fish larvae as indicators to assess diets and patterns of anthropogenic nitrogen pollution in estuarine ecosystems

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ABSTRACT

A stable isotope study was carried out to investigate the feeding ecology of the common goby *Pomatoschistus microps* larvae (Krøyer, 1838), and to assess differences in the response of planktonic food web to nutrient enrichment, in two ecosystems from the Southern European coast with different levels of historical pollution (estuaries of the Minho and Lima Rivers). At each estuary and time (July 2012, November 2012, February 2013, and June 2013), the fish larvae of two size classes (class 0: 0–10 mm; class 1: 10–15 mm), particulate organic matter (POM), and pelagic zooplankton were collected. The stable isotope mixing model SIAR revealed that, despite temporal differences in the relative proportion of prey items ingested, in both estuaries *P. microps* larvae feed on both planktonic-hyperbenthic food sources, predating mainly on copepods (from 34% to 60%), Mysidacea (from 16% to 28%), and brachyuran zoea (from 14% to 29%). Fish larvae size classes did not differ significantly for $\delta^{15}N$, and exhibited a very narrow range of the $\delta^{13}C$ signature. Enriched $\delta^{15}N$ values of all biota in the Lima estuary throughout the study period, with a marked nitrogen enrichment in colder months, are indicative of higher anthropogenic inputs of nitrogen (e.g. sewage and industrial discharges, agriculture) into this system. The $\delta^{15}N$ values of fish larvae and other planktonic groups can be a sensitive bioindicator, because they are highly correlated with the nitrogen content of water (ammonium), indicating that this element has transferred through the planktonic food web. Enriched carbon isotope ratios were observed in warmer months, in both estuaries, and the heavier $\delta^{13}C$ values in Lima are best explained by differences in the degree of marine influence. This research emphasises the utility of stable isotopes in trophic interactions studies, highlighting the relevance of the stable nitrogen isotope of zooplanktonic communities as a reliable bioindicator to detect patterns of anthropogenic nitrogen contamination in estuarine ecosystems.

1. Introduction

Stable isotopes of carbon and nitrogen have become a widely used tool for examining food webs, namely dietary patterns, trophic structure and nutrient transfers, across diverse ecosystems (DeNiro and Epstein, 1978, 1981; Eggers and Jones, 2000; West et al., 2006; Layman et al., 2012). In trophic interactions, as one species feeds on another one, the consumer tends to be isotopically heavier than its food source, a process called fractionation factor or trophic enrichment ($\Delta^{13}C$ and $\Delta^{15}N$ for carbon and nitrogen, respectively) (Caut et al., 2009). This effect is more pronounced in nitrogen and leads to increases in $\delta^{15}N$ of approximately 3–4‰ (Wada et al., 1991; Post, 2002) at each trophic level. Furthermore, high levels of $\delta^{15}N$ in basal sources can be used as an alternative method to establish the level of human eutrophication since anthropogenic sources of nitrogen are generally enriched in the heavy isotope when compared to natural sources (Cole et al., 2006; Bannon and Roman, 2008; Baeta et al., 2009a,b). Ratios of carbon isotopes ($\delta^{13}C$) vary substantially among primary producers with different photosynthetic pathways, but change little with trophic transfers, generally from 1 to 2‰ from prey to predator (Peterson and Fry, 1987; Post, 2002). Therefore, $\delta^{13}C$ are typically used to determine which primary producer components are the ultimate carbon source

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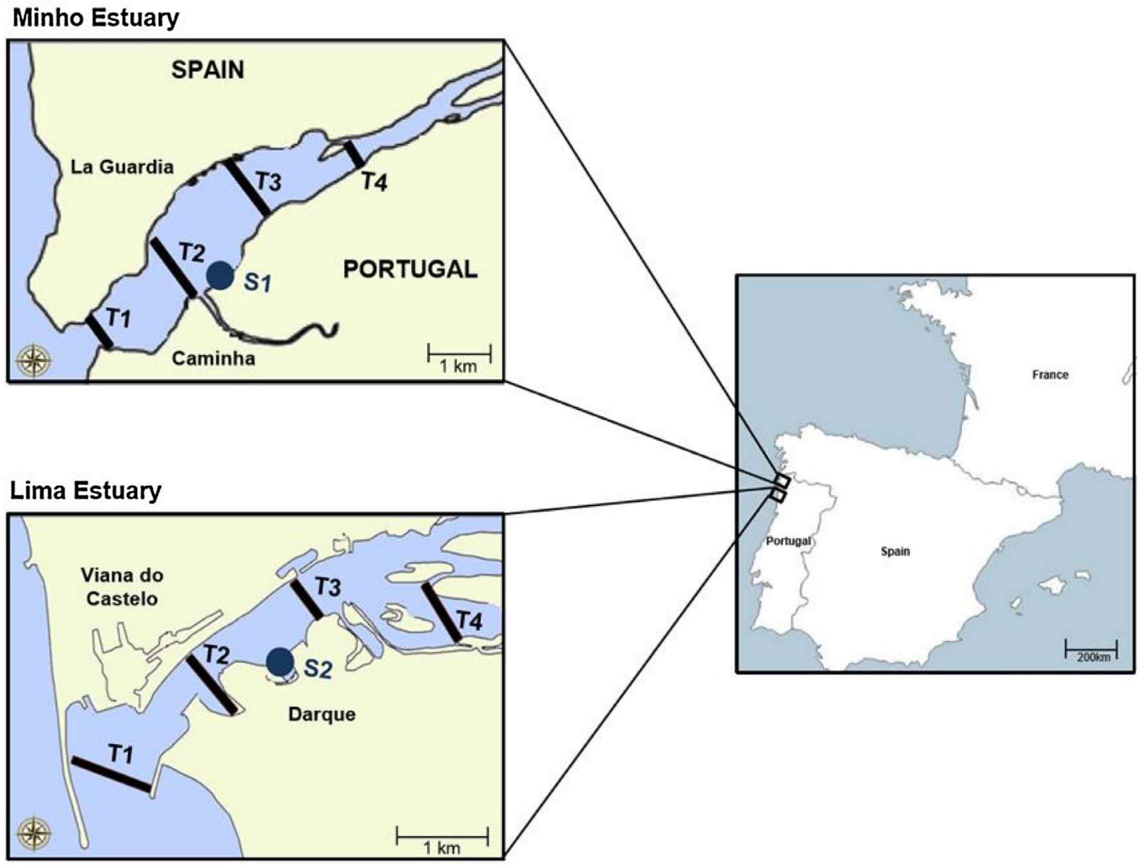

		Transect			
		T1	T2	T3	T4
Minho	Coordinates	41º52'12.76''N	41º52'52.96''N	41º53'39.14''N	41º54'17.27''N
		8º51'32.08W	8º50'46.14''W	8º49'58.61''W	8º49'1.66''
	Transect depth (m)	5.16±0.73	3.99±1.02	2.98±1.5	3.35±0.97
Lima	Coordinates	41º40'52.25''N	41º41'15.05''N	41º41'30.88''N	41º41'29.14''N
		8º50'26.21''W	8º49'36.42''W	8º48'53.63''W	8º47'40.91''W
	Transect depth (m)	8.55±2.37	7.65±2.39	5.65±1.14	3.74±0.99

Fig. 1. Location of selected sampling transects (T1-T4) in both estuaries (Minho and Lima Rivers Estuaries) from the Southern European coast. The selected sampling sites for the capture of zooplanktivorous fish are also indicated in the figure (S1-S2). The transect coordinates and average depths are presented in the table.

assimilated by higher trophic level consumers (Fry and Sherr, 1984; Peterson and Fry, 1987).

Estuarine ecosystems constitute transition zones where freshwater from land drainage and river basins mixes with seawater, creating some of the most biologically productive areas on Earth (Levin et al., 2001). Such environments are effective nurseries for several marine fish species, and provide shelter and food for several other resident ones that complete their entire life cycle within the estuarine system (Dolbeth et al., 2007, 2008). Resident species are quite relevant in estuarine food webs as intermediate predators, being consumers of plankton and benthos, and prey of several larger fishes, decapods, and birds, playing a crucial role in the overall dynamics and functioning of the estuarine ecosystem. The feeding ecology of fish larvae has been studied extensively in marine environments, whereas there are only a few studies on estuarine fish larvae worldwide, and most are completely concentrated in low-middle latitudes (15 – 30°N/S) (Llopiz, 2013), and this is somehow surprising given the levels of productivity in estuaries and the trophic role of fish larvae in the estuarine food web. Predatory success in estuarine nursery areas by fish larvae is crucial for good body condition, growth rate and ultimately for larval survival (Strydom et al., 2014). Fish larvae are typically zooplanktivorous, feeding on a variety of micro- and meso-zooplankton, often dominated by copepods, with a shift from smaller to larger prey through larval ontogeny (Llopiz, 2013). The common goby *Pomatoschistus microps* (Krøyer, 1838) is among the most abundant species in estuaries, lagoons and along the shores of Europe, and despite its high abundance and importance in estuarine trophic webs (Maes et al., 2003; Parmanne and Lindstrom, 2003; Salgado et al., 2004; Leitão et al., 2006; Dolbeth et al., 2008), to the best of our knowledge, only one study, Thiel et al. (1996), based on gut content analysis, has examined the diet of *P. microps* larvae.

The planktonic larval phase of fishes is characterised by high recruitment variability and mortality, and its maintenance is greatly