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Contrasting perception of fish trophic level from stomach content and stable isotope analyses: A Mediterranean artificial reef experience

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ABSTRACT

A large complex of artificial reefs was deployed in the Bay of Marseilles, North-Western Mediterranean, for the enhancement of commercial fisheries stocks. Carbon and nitrogen stable isotope and stomach content analyses were performed on 23 fish species collected on the artificial reefs to assess their trophic position and feeding behaviour. Results indicated that fish diets were not modified on the artificial reefs compared to natural environments, nor was the structure of their trophic network. Artificial reefs, with their complex design, provide diverse and abundant food sources for fishes. Ranges of $\delta^{13}C$ and $\delta^{15}N$ of artificial reef fishes were comparable to those recorded in natural Mediterranean environments, with a similar trophic organization. However, some discrepancies appeared when comparing fish trophic level based on isotopic or diet results, which calls for a careful interpretation of stable isotope values as direct indicators of trophic level.

1. Introduction

In a context of multiple human impacts on marine ecosystems, artificial reefs have been widely deployed in marine coastal waters to restore degraded habitats, enhance commercial and recreational fisheries, promote biodiversity and to protect benthic habitats, amongst other management goals (Jensen, 2002; Seaman, 2007). As artificial reefs represent large-scale experiments, they also provide a way to study ecosystem functioning and to elucidate ecological processes (Miller, 2002). Ecological hypotheses can be tested, by comparing biological variables (e.g., colonization kinetics, recruitment, biomasses, species richness, etc.) observed on similar modules modified or untreated to serve as controls (Charbonnel et al., 2002). By the creation of new habitats and increasing food resources, the deployment of artificial reefs can be a useful tool for enhancing fish biomass and sustaining small-scale coastal fisheries (Charbonnel et al., 2002; Scarcella et al., 2011; Steimle and Ogren, 1982). In the Mediterranean Sea, fishing pressures are strong on populations, since 50% of the assessed stocks, like mullets or seabreams, are considered overexploited (FAO, 2012). Artificial reefs are nowadays considered by all stakeholders as an efficient tool to support small-scale fisheries and also to restore coastal zone under strong fishing pressures (Claudet and Pelletier, 2004). As fishing targets generally high trophic level species, overfishing is commonly acknowledged to modify the structure of fish community and decrease fish mean trophic levels (Pauly et al., 1998). A good understanding of human pressures on marine ecosystems thus requires robust indicators of fish trophic level.

Studies on trophic patterns of Mediterranean fish have classically and extensively been performed by stomach content analysis (Bell and Harmelin-Vivien, 1983; Morte et al., 2001; Rosecchi, 1987; Šantić et al., 2011; and references in Stergiou and Karpouzi, 2002). This technique allows for the identification of the prey actually consumed by a fish and gives a "snapshot" of its recent diet. However, some biases linked with accurate prey identification or different rates of prey digestion may be problematic when using this technique. Moreover, the low temporal resolution of this technique requires a large number of samples to obtain a representative view of the dietary patterns of a species (Hyslop, 1980). Some of these biases can be solved using stable isotope analysis. Isotopic ratios of carbon and nitrogen have been used to describe trophic relationships in marine Mediterranean ecosystems (Deudero et al., 2004; Jennings et al., 1997; Pinnegar and Polunin, 2000). When consuming a prey, a predator integrates the C and N isotopic ratios of its prey into its own tissues. A fractionation process occurs at each trophic level, as the $\delta^{13}C$ of the predator is generally slightly higher than the $\delta^{13}C$ of its prey (~+1‰ per trophic level), allowing the use of the carbon isotopic ratio as an indicator of the organic matter origin. The fractionation factor is higher for nitrogen (theoretically +3.4‰ per trophic level) and $\delta^{15}N$ was classically used as a direct indicator of the trophic level of the predator (Post, 2002). Nevertheless, due to biases linked with isotopic ratios of the trophic baseline (i.e. $\delta^{15}N$ value of the source of organic matter at the base of

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the trophic network) and variability of nitrogen fractionation factor, some recently published papers call for a cautious use of $\delta^{15}N$ as a direct indicator of trophic level (Mancinelli et al., 2013; Post, 2002).

Through the "RECIFS PRADO" programme, 400 artificial reefs were installed in a 220 ha area in the Bay of Marseilles in 2007 and 2008. "RECIFS PRADO" is the largest artificial reef programme in the Mediterranean Sea and represents the deployment of a total volume of ~27 000 m^3 of artificial concrete structures. Its aim is to enhance fish biomass in the surroundings of artificial reefs and consequently sustain local small-scale coastal fisheries. This programme represented a valuable opportunity 1) to assess the trophic organization of an artificial reef fish community using stable isotope and stomach content analyses and 2) to compare the use of $\delta^{15}N$ values and diet composition in determining the trophic level of coastal fish.

2. Materials and methods

Fish were collected on two artificial reefs in the "RECIFS PRADO" zone in the Bay of Marseilles, France (Fig. 1). These large reefs (6 m high, 187 m^3) are composed of steel and concrete modules. Their complexity was increased by the addition of bags filled with dead oyster shells (hereafter named oyster bags) creating shelters for small organisms (Charbonnel et al., 2011). Their size and complexity provide habitats of different sizes suitable for most coastal organisms and allow efficient and standardized sampling procedures. The two artificial reefs investigated, one in the north (V3 reef) and the other in the south (V6 reef) of the zone, were chosen according to differences in distance from some organic matter sources (Huveaune River and *Posidonia oceanica* meadows) and management status (Cresson et al., 2012). The whole artificial reef zone is currently a full no-take area but the southern part will be opened to small-scale artisanal fisheries in a few years. V3 and V6 artificial reefs are located at 30 m depth on similar sandy bottom with dead matte of *P. oceanica* (underlying structure of *P. oceanica* meadows constituted of rhizomes and roots intermingled with sediments).

A total of 339 fishes belonging to 32 species were sampled on the artificial reefs by spear fishing and trammel nets in summer and winter 2010. Species for which only one or two individuals were collected were discarded and the resulting 325 fishes belonging to 23 species (Table 1) were used for isotopic and stomach content analyses. Details on the number of fish actually sampled at each season on each reef are presented in Table S1.

In the laboratory, each fish was measured (standard length, to the nearest cm) and weighed (total mass, to the nearest g) before dissection. White dorsal muscle was taken for isotopic analyses before freeze-drying and grinding. In temperate fishes, lipid concentration in white muscle is generally low and this tissue was demonstrated to be the most suitable for stable isotope analysis (Pinnegar and Polunin, 1999). Lipid content was assessed by the C/N ratio. A C/N value lower than 3.5 generally indicates a lipid content too low to bias the isotopic ratios (Sweeting et al., 2006). In the whole dataset, less than 10 were higher than this threshold, and were removed to prevent this lipid bias.

Stable isotope measurements were performed with a continuous-flow isotope-ratio mass spectrometer (Delta V Advantage, Thermo Scientific, Bremmen, Germany). Results are expressed in δ notation relative to PeeDee Belemnite and atmospheric N_2 for $\delta^{13}C$ and $\delta^{15}N$, respectively, according to the equation $\delta X = \left(\frac{R_{sample}}{R_{standard}} - 1\right) \times 10^3$, where X is ^{13}C or ^{15}N and R is the isotope ratio $^{13}C/^{12}C$ or $^{15}N/^{14}N$, respectively. For both $\delta^{13}C$ and $\delta^{15}N$, measurement precision is <0.1‰ (replicate measurements of internal laboratory standards, acetanilide). The trophic level of fish species based on isotopic analysis was assessed using the formula adapted from Badalamenti et al. (2002): $TL_i = 1 + (\delta^{15}N_i - \delta^{15}N_{TB}) / 3.4$, where i is the fish species, $\delta^{15}N_i$ is the nitrogen isotopic ratio for species i, 3.4 the theoretical enrichment at each trophic level and $\delta^{15}N_{TB}$ the nitrogen isotopic ratio for pelagic or benthic primary production at the base of the trophic network. For pelagic production, the $\delta^{15}N$ of nanophytoplankton ($\delta^{15}N = 1.77$‰, Rau et al., 1990) was used as previous results confirmed its dominance in the Bay of Marseilles (Gregori et al., 2001). Value used for benthic production ($\delta^{15}N = 3.91$‰) is the

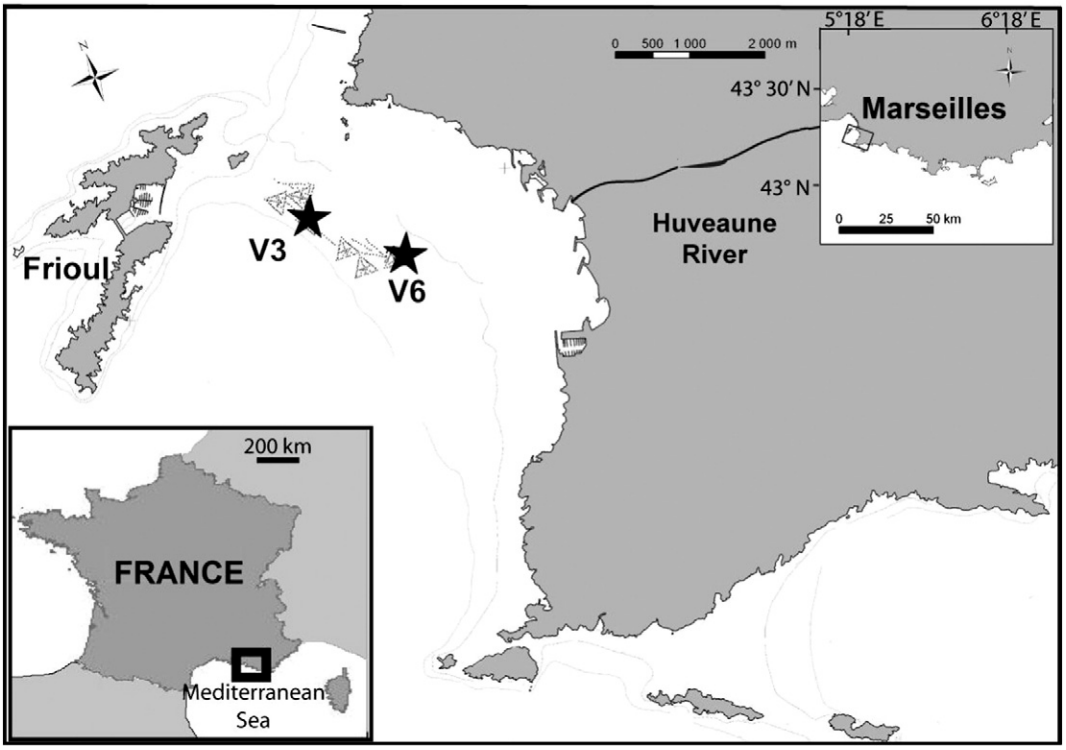

Fig. 1. Location of artificial reefs (V3 and V6) in the Bay of Marseilles.