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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 δ^{13} C and δ^{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.

Highlights

► Fish diets were assessed on artificial reefs. ► Stomach content and stable isotope data are similar to those of natural environments. ► Artificial reefs provide diverse food sources for fishes, particularly crustaceans. ► Discrepancy may occur between δ^{15} N and fish diet. ► Trophic level estimation should not be based on δ^{15} N value alone.

Keywords: Artificial reefs ; Fish diet ; NW Mediterranean ; Stable isotopes ; Stomach content ; 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, among 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 (eq. 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 pressure (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 requires thus 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 prev 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 δ^{13} C of the predator is generally slightly higher than the δ^{13} C of its prev (~ + 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 (*ie* δ^{15} N value of the source of organic matter at the base of 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" program, 400 artificial reefs were installed in a 220 ha area between 25 and 35 m depth in the Bay of Marseilles in 2007 and 2008. "RECIFS PRADO" is the largest artificial reef program in the Mediterranean Sea and represents the deployment of a total volume of ~ 27 000 m³ 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 program 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 δ^{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 m³) 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 notake 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 values were higher than this threshold and were removed to prevent this 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 N₂ for δ^{13} C and δ^{15} N,

respectively, according to the equation $\delta X = \left(\frac{R_{\text{sample}}}{R_{\text{standard}}} - 1\right) \times 10^3$, where X is ¹³C or ¹⁵N and R

is the isotope ratio ${}^{13}\text{C}/{}^{12}\text{C}$ or ${}^{15}\text{N}/{}^{14}\text{N}$, respectively. For both $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$, 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+ ($\delta^{15}\text{N}_i - \delta^{15}\text{N}_{\text{TB}}$)/3.4, where i is the fish species, $\delta^{15}\text{N}_i$ is the nitrogen isotopic ratio for species i, 3.4 the theoretical enrichment at each trophic level and $\delta^{15}\text{N}_{\text{TB}}$ the nitrogen isotopic ratio for pelagic or benthic primary production at the base of the trophic network. For pelagic production, the $\delta^{15}\text{N}$ of nanophytoplankton ($\delta^{15}\text{N}$ = 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 ($\delta^{15}\text{N}$ = 3.91 ‰) is the mean annual value measured for macroalgae sampled on the artificial reefs (P. Cresson, unpubl. data).

Fish stomachs were removed and stored in 95 % ethanol. Prey items in stomach contents were sorted under a binocular microscope into their lowest possible taxonomic groups and their wet weight was obtained to the nearest 0.01 mg. The relative importance of prey taxa in a fish species" diet was assessed by the weight percentage of a food type relative to the total

weight of all food ingested. To assess the relative importance of the different prey types for the whole fish assemblage collected on the artificial reefs, relative importance by weight was calculated for each prey species. As pisicivores were heavier than other species and consumed heavy prey, the importance of prey type by weight was corrected by the mean fish weight. Thus, for each fish species, the consumed mass of each food item was divided by the mean weight of the species. The cumulative weight of each food item was then calculated for all fish species consuming this prey. Similarly, the overall occurrence of each prey type was considered as the percentage of species containing this prey (Hyslop, 1980). The trophic level of each fish species was issued from bibliographic data based on stomach content analyses (Barreiros et al., 2002; Darnaude, 2005; Rogdakis et al., 2010; Stergiou and Karpouzi, 2002; Soares et al., 2003).

Hierarchical clustering based on normalized Euclidean distance and Ward's criterion was performed first on mean isotopic ratios to identify groups of species with similar isotopic ratios. The same procedure was applied independently to mean stomach content results to group together species having similar feeding strategies. The results of the two clusterings were compared. All statistical analyses were performed using R software and the "cluster" package (Maechler et al., 2012; R Core Team, 2012)

3. Results

3.1. Stable isotope ratios of fishes

Mean isotope values measured for fishes collected on the artificial reefs displayed a 2 ‰ range for δ^{13} C (-19.73 to -17.66 ‰) and a 7 ‰ (7.83 to 14.87 ‰) for δ^{15} N (Fig. 2), with few spatial or seasonal differences of fish isotopic ratios (Tab. S2). Six groups of species were individualized by hierarchical clustering based on their isotopic ratios (SI1 to SI6). SI1 group comprised three species (Boops boops, Spicara maena and Spicara smaris) and was characterized by the lowest values of both δ^{13} C and δ^{15} N (Table 1). Three groups (SI2 to SI4) with intermediate δ^{13} C and δ^{15} N values were distinguished. One (SI2) was composed of species belonging to the family Labridae (Coris julis, Symphodus mediterraneus and Symphodus tinca), and exhibited relatively low values for δ^{13} C and δ^{15} N. The three *Diplodus* species (Diplodus vulgaris, D. sargus and D. annularis) clustered together in the SI3 group, and exhibited relatively high $\delta^{15}N$ values. The SI4 group, comprising 11 species, was heterogeneous and displayed high δ^{13} C (from - 18.36 to -17.66 ‰) and intermediate δ^{15} N values (from 9.76 to 10.73 ‰). Sphyraena viridensis clustered apart in the SI5 group with low δ^{13} C but rather high δ^{15} N (> 11‰). Finally, two species, *Dicentrarchus labrax* and *Trachurus mediterraneus*, formed the SI6 group, which was characterized by the highest $\delta^{15}N$ ratios (> 13‰).

3.2. Stomach content analysis

The analysis of stomach contents revealed the overall importance of crustaceans and fishes as prey for the whole fish assemblage collected on the artificial reefs (Fig. 3). Crustaceans were the most frequent prey item consumed (80 % of occurrence), followed by molluscs (61 %) and polychaetes (52 %). Crustaceans were also the most important prey by weight, followed by primary producers (macroalgae and P. oceanica) and fishes. Five feeding groups were identified by hierarchical clustering on stomach contents (Table 2, Fig. 4): "zooplankton feeders". "muddy/sand bottom mesocarnivores" (hereafter called soft bottom mesocarnivores), "rocky/seagrass bed bottom mesocarnivores" (hereafter called rocky bottom mesocarnivores), "macrocarnivores" and "piscivores". Feeding group designation took into account not only differences in prey consumed, but prey size (larger in macro- than in mesocarnivores) and habitat (water column, soft and hard bottom).

3.3. Comparison of isotopic and dietary results

Full concordance between isotopic and feeding groups occurred only for zooplankton feeders (Fig. 4). The three species of the SI1 group (B. boops, S. maena and S. smaris) fed mainly on zooplanktonic crustaceans (particularly copepods), even if slight differences could be observed between them. The diets of S. maena and S. smaris were almost exclusively composed of zooplanktonic crustaceans (> 75 %), whereas *B. boops* displayed a more diverse diet, including fish eggs, gastropods, macroalgae and P. oceanica in addition to zooplanktonic crustaceans. Rocky bottom mesocarnivores belonged to two different isotopic groups, SI2 for labrids and SI3 for *Diplodus* spp. Labrid species consumed mainly bivalves and polychaetes, but in different proportions (Table 2). S. tinca consumed mostly polychaetes and to a lesser extent bivalves and gastropods, while bivalves dominated the diets of C. julis and S. mediterraneus (86 % and 45 % respectively). The diets of the three Diplodus species (SI3) were more diverse and sessile organisms (macroalgae, ascidians, bryozoans, cnidarians and hydrozoans) were largely consumed. However, each species appeared to rely mainly on one food item, primary producers for *D. annularis*, polychaetes for D. sargus and, to a lesser extent, ascidians for D. vulgaris. Soft bottom mesocarnivores all belonged to the SI4 isotopic group. Stomach contents of *M. variegatus*, *M. surmuletus*, P. acarne, P. erythrinus and T. lastoviza were mainly composed of echinoderms and small benthic crustaceans, along with additional prev like polychaetes or cephalopods (Fig. 5). All macrocarnivores also belong to the SI4 group. The main prey observed in S. notata, S. porcus and S. cabrilla were large benthic crustaceans (brachyurans for S. notata and S. porcus, carids for S. cabrilla, Table 2). The highest discrepancy between isotopic and feeding clustering occurred for piscivores which were part of three different isotopic groups (SI4, SI5 and SI6). Synodus saurus, Scorpaena scrofa and Phycis phycis (SI4), Sphyraena viridensis (SI5), and Dicentrarchus labrax and Trachurus mediterraneus (SI6) displayed largely different isotopic signatures, while all preved mainly on fishes (> 85 %, Table 2, Fig. 5)

3.4. Trophic level estimation

The trophic level calculated from stable isotope values using a pelagic baseline matched the trophic level estimation based on stomach contents for most trophic groups: zooplankton feeders, labrids, soft bottom mesocarnivores, macrocarnivores and pelagic piscivores (Table 3). Calculations based on benthic isotopic baseline always indicated lower trophic levels for these groups. On the contrary, the trophic level of *Diplodus* spp from stomach contents better fitted the isotopic calculation based on benthic baseline, while the pelagic baseline led to a higher estimation. Eventually, the trophic level of benthic piscivores was always lower when calculated from isotopic values than from stomach contents, whatever the trophic baseline used.

4. Discussion

With the exception of gobiids, blennids and pomacentrids which could not be sampled due to their small size, the present study analysed the majority of the fish species observed by underwater visual censuses on the artificial reefs in the Bay of Marseilles (Rouanet et al., 2012). The most abundant species (sparids, scorpaenids, serranids, mullids) included in our

study dominated also in natural rocky habitats (Fasola et al., 1997; Harmelin, 1987; Letourneur et al., 2003).

4.1. Use of artificial reef food resources by fishes

Fish diets on the artificial reefs were rather similar with those observed in natural environments in the area of Marseilles (Bautista-Vega et al., 2008; Bell and Harmelin-Vivien, 1983; Harmelin-Vivien et al., 1989) or in other Mediterranean zones (Fanelli et al., 2011; Kalogirou et al. 2012; Quignard, 1966; Sala and Ballesteros, 1997; Stergiou and Karpouzi, 2002), even if no direct comparison of diets in natural and artificial habitats could be performed in this work.

Stomach contents observed for *Boops boops*, *Spicara smaris* and *S. maena* placed them in the cluster of secondary consumers specialized on zooplanktonic organisms, as already observed (Bell and Harmelin-Vivien, 1983; Stergiou and Karpouzi, 2002). Different proportions of zooplankton and vegetal material were observed in the diet of the more opportunistic *B. boops* (Derbal and Kara, 2008; Fasola et al., 1997).

The majority of the fish species sampled on the artificial reefs exhibited a mesocarnivorous diet, based on small invertebrates (crustaceans, molluscs, echinoderms or polychaetes) and benthic primary producers. Taking into consideration the habitat of species allowed clearly distinguishing between rocky and sandy bottom fishes. Based on their diets, labrids and *Diplodus* species were gathered in the same feeding cluster of rocky bottom mesocarnivores. Labrids mainly consumed small benthic invertebrates like molluscs and polychaetes. C. julis is known to prey on gastropods and crustaceans (Quignard, 1966), juvenile echinoderms (Sala, 1997) or bivalves (Bell and Harmelin-Vivien, 1983). The diets of S. tinca and S. mediterraneus are similar and composed of small crustaceans, bivalves, gastropods and polychaetes (Bell and Harmelin-Vivien, 1983). Diplodus spp. can be considered as omnivores as they presented a diversified diet, feeding on a large range of prev. from primary producers to fishes, with a high consumption of diverse sessile invertebrates. Omnivory of Diplodus species is well documented (Derbal et al., 2007; Rosecchi, 1987; Sala and Ballesteros, 1997). The soft bottom mesocarnivores (M. variegatus, M. surmuletus, P. acarne, P. erythinus and T. lastoviza) preyed mainly on echinoderms and small crustaceans, along with other diverse prey like molluscs or polychaetes, consistently with previous results (Bautista-Vega et al., 2008; Fanelli et al., 2011; Fehri-Bedoui et al., 2009). The diet of macrocarnivores (S. notata, S. porcus and S. cabrilla) was also composed of crustaceans, but of larger size (brachyurans and carids), and in larger proportion (> 90 % of the ingested prey weight) than mesocarnivores (Harmelin-Vivien et al., 1989). For all these species, the present study highlighted the importance of crustaceans in artificial reef functioning, which represented the most commonly ingested prey type. These results confirm the major role of crustaceans in fish diets on artificial reefs (Leitão et al., 2007; Relini et al., 2002). Finally, six species (Scorpaena scrofa, Phycis phycis, Trachurus mediterraneus, Sphyraena viridensis, Synodus saurus and Dicentrarchus labrax) mainly preved on fish and could be considered as piscivores. Even if all these predators consume fishes, differences appeared nevertheless amongst them, as they occupied different habitats and consumed different species. Previous works report a high consumption of zooplankton feeding species (B. boops and S. smaris) by S. viridensis, which could be consider as a pelagic piscivore (Kalogirou et al., 2012). P. phycis could be consider as a benthic piscivore, as remains of benthic species (C. julis and S. tinca) were observed in its stomach contents on the artificial reefs. Previous works indicate also the consumption of *B. boops*, *Spicara* spp. or *Chromis chromis* by *S. saurus* and S. scrofa in natural environments (Esposito et al., 2009; Šantić et al., 2011). Finally, T. mediterraneus and D. labrax were high trophic level transient piscivores, as confirmed by the predominance of fish remains in their stomach, consistently to previous works (Pasquaud et al., 2010; Rogdakis et al., 2010).

4.2. Stable isotopes and the trophic structure of fish community

Fishes sampled on the artificial reefs of Marseilles displayed a range of isotopic ratios (~ 2 ‰ for δ^{13} C and 7 ‰ for δ^{15} N) similar to those observed in previous studies of fish assemblages in natural Mediterranean coastal rocky environments (Jennings et al., 1997; Pinnegar and Polunin, 2000; Vizzini and Mazzola, 2009). Using $\delta^{15}N$ as an indicator of trophic level, it could be considered that fishes occupied at least three trophic levels on the artificial reefs studied. The lower level ($\delta^{15}N < 9$ ‰) was occupied by the three zooplankton-feeders (*B. boops*, *S.* smaris and S. maena) which clustered in the SI1 group, the only one to be conserved between the two classifications. The specificity of their diets, both on specific and isotopic points of view, could explain the robustness of this group. The second intermediate trophic level (9 $\% < \delta^{15}N < 13 \%$) gathered most of the species sampled on the artificial reefs. However, four distinct isotopic groups of intermediate trophic level fishes were separated based on both their $\delta^{15}N$ and $\delta^{13}C$ values, and these groups did not match with stomach content clustering. Within the rocky bottom mesocarnivorous group, difference in isotopic values placed labrids (SI2) and *Diplodus* spp. (SI3) in two distinct isotopic groups. The rather low isotopic values observed for labrids were consistent with the consumption of small low trophic level invertebrates, as previously observed in natural environments (Bell and Harmelin-Vivien, 1983; Quignard, 1966; Sala, 1997). Contrarily, the $\delta^{15}N$ observed for *Diplodus* spp. was surprisingly high for species consuming small invertebrates and primary producers, as it was higher than those of carnivorous species like S. porcus. A similar pattern between $\delta^{15}N$ ratios measured in *Diplodus* spp. and *S. porcus* is reported in other Mediterranean rocky environments. In Formentera, Balearic Islands, D. annularis displays a higher δ¹⁵N ratio than S. porcus (9.44 ‰ and 8.93 ‰ respectively, Deudero et al., 2004). In the Bay of Calvi, Corsica, Pinnegar and Polunin (2000) report also higher δ¹⁵N values for D. annularis and D. sargus than for S. porcus (8.39, 9.13 and 7.93 ‰ respectively). These authors explain such high δ^{15} N values by the consumption of fishes by *Diplodus* spp., but have not analysed fish stomach contents. Such an explanation is likely not pertinent here due to the minor importance of fish in the diet of *Diplodus* spp. collected on the artificial reefs. and is confirmed by other studies on their feeding habits (Bell and Harmelin-Vivien, 1983; Derbal et al., 2007; Sala and Ballesteros, 1997). The apparent discrepancy between $\delta^{15}N$ values and diet for Diplodus spp. calls for cautious interpretation of isotopic ratios (see last paragraph). The SI4 group was the most diverse of intermediate trophic level fishes, as it gathered species classified with stomach contents as soft-bottom mesocarnivores, macrocarnivores and benthic piscivores. Finally, the pelagic Sphyraena viridensis clustered apart (SI5) at an intermediate trophic level in spite of a piscivorous diet. The highest trophic level ($\delta^{15}N > 13$ ‰) was represented by two well-known piscivores. Dicentrarchus labrax and Trachurus mediterraneus (Rogdakis et al., 2010; Stergiou and Karpouzi, 2002).

Thus, in spite of apparently similar diets based on fish, piscivorous species displayed different isotopic ratios and were classified in three isotopic groups (SI4, SI5 and SI6). The comparable δ^{13} C values of *Sphyraena viridensis* (SI5) and *Boops boops, Spicara maena* and *S. smaris,* along with the ~ 3 ‰ higher δ^{15} N of *S. viridensis*, testified of the consumption of zooplanktivorous fishes by this piscivore, as observed by Kalogirou et al. (2012). The three benthic piscivorous species (*S. saurus, S. scrofa* and *P. phycis*) displayed δ^{15} N values close to those of crustacean-eating mesocarnivores (SI4). This might appear surprising for fish consumers, generally considered to be at the highest trophic level in the Mediterranean (Stergiou and Karpouzi, 2002), but could be explained by the consumption of low trophic level benthic fish species. Finally, the high δ^{15} N value of *T. mediterraneus* and *D. labrax* (SI6) confirmed the high trophic level of these two piscivores (Pasquaud et al., 2010; Rogdakis et al., 2010).

4.3. δ^{15} N: a direct indicator of trophic level?

Carbon and nitrogen stable isotope ratios have been widely used to assess trophic patterns in fish communities, sometimes replacing time-consuming stomach content analysis (Deudero et al., 2004; Jennings et al., 1997; Pinnegar and Polunin, 2000). In this approach δ^{15} N is considered as a direct indicator of the trophic position of consumers. However, recently published results demonstrated that different factors, like trophic baseline, isotopic fractionation and consumer metabolism among others, may bias direct trophic level interpretation (Mancinelli et al., 2013; Mill et al., 2007; Post 2002; Schmidt et al., 2004). Results of the current study illustrated some of these biases. Discrepancies were observed between the classifications based on stable isotope or stomach content analyses for all trophic groups except zooplankton feeders. The case of Diplodus spp. illustrated the influence of both trophic baseline isotopic ratios and trophic enrichment factor on the trophic level estimation of herbivorous (or omnivorous) versus carnivorous species (Mancinelli et al., 2013; Mill et al., 2007). A simplistic interpretation of the high *Diplodus* δ^{15} N could place these species at a high trophic level, just below the piscivorous D. labrax and T. mediterraneus, as suggested by the calculation of their trophic level from isotopic values. Such a position was incongruous with their omnivorous feeding patterns, as they consumed large amounts of algal matter and sessile invertebrates, along with other low trophic level prey. *Diplodus* $\delta^{15}N$ $(\sim 11 \text{ }\%)$ was surprisingly $\sim 2 \text{ }\%$ higher than those of labrids, which belonged to the same trophic group of rocky bottom mesocarnivores (Fig. 4). Their $\delta^{15}N$ value could reflect the importance of algae or seagrass in their diet as benthic primary producers classically present higher δ^{15} N than phytoplankton (Nadon and Himmelman, 2006). The calculation of *Diplodus* spp. trophic level using a benthic baseline, rather than a pelagic one, resulted actually in a more consistent trophic level value, similar to the labrids one, and lower than those of piscivores. Recent works demonstrated also that fractionation factor associated with herbivory is higher than the usually-accepted 3.4 ‰ value, due to differences in the enzymatic and digestive systems of herbivorous species (Mill et al., 2007; Wyatt et al., 2010). The combined effects of prevalent benthic vegetal material in the diet of *Diplodus* spp., along with a higher fractionation factor, explained thus likely their high $\delta^{15}N$ values. This demonstrated how direct simplistic interpretation of isotopic ratios can lead to wrong inferences of consumers" diet and trophic level.

In the Mediterranean Sea, piscivorous species are considered to represent the highest trophic level for fishes, with a mean value close to or higher than 4 (Stergiou and Karpouzi, 2002). In the present study, the large range of δ^{15} N values (10.1 - 14.9 ‰) recorded in the six piscivorous species analysed would place them at two distinct trophic levels. The lower trophic level of S. scrofa and P. phycis, when calculated from stable isotopes, was probably due to their consumption of crustaceans, in addition to fish. But some strictly piscivorous species, like S. viridensis and S. saurus, displayed an isotopic-calculated trophic level lower than 4, as they consume zooplankton-eating or small benthic fishes, which presented themselves low $\delta^{15}N$ values. Inferring their diet and trophic level only from their $\delta^{15}N$ values that were similar to those of meso- and macrocarnivores (Fig.5), would have led to misinterpretations. On the contrary, the high $\delta^{15}N$ of *D. labrax* and *T. mediterraneus* were consistent with a trophic level higher than 4, as previously calculated by Rogdakis et al. (2010). Using the specific 3.8 % fractionation factor proposed for fish muscle $\delta^{15}N$ by Sweeting et al. (2007), *D. labrax* prey should exhibit $\delta^{15}N$ values close to 10 ‰, similar to those recorded for macrocarnivorous and other piscivorous fish species, which confirmed the consumption of high trophic level fishes by this predator. The current results confirmed the separation amongst fish-eating species proposed by Stergiou and Karpouzi (2002). A trophic level lower than 4 appear consistent for species eating fish and crustaceans. But a trophic level close to or higher than 4 for all fish-eating species can be questioned, since some species exhibit low $\delta^{15}N$ while preying only on fishes. Thus, "piscivore" is not always synonymous with "top-predator".

The results obtained on the feeding habits and trophic position of artificial reef fishes in the Bay of Marseilles demonstrates that combining stable isotope and stomach content analyses remains a necessary approach to clarify fish trophic relationships, especially given that different prey types may present similar isotopic signatures (Layman et al., 2012). Even within one ecosystem, the crude comparison of $\delta^{15}N$ values of organisms does not necessarily reflect their trophic level and diet composition. The isotopic values measured in a consumer result not only from the isotopic ratios of its prey, but also from varying fractionation factors and food component routine (Perga and Grey, 2010; Schmidt et al., 2004; Sweeting et al., 2007). Thus, the correct interpretation of trophic relationships of organisms based on stable isotopic signatures is a highly complex task, which does not preclude the knowledge of their biology and feeding behaviour.

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Figures



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

Fig. 2: Mean isotopic ratios (δ^{13} C and δ^{15} N) of fishes sampled on artificial reefs. Species grouped together by the hierarchical clustering analysis are represented with similar symbols (white square: SI1; circle: SI2; triangles: SI3, diamonds: SI4, black square: SI5; crosses: SI6). Colours of the diamonds in the SI3 group represent the diet of the species (black: piscivores; grey: macrocarnivores; white: muddy/ sand bottom mesocarnivores). For graphical convenience, standard deviations are not plotted and the names of some species are abbreviated (*S. medit. Symphodus mediterraneus, S. not. Scorpaena notata, M. sur: Mullus surmuletus, P. ery: Pagellus erythrinus*).



Fig. 3: Percentage of occurrence (black bars) and cumulative corrected mass (grey bars) of the main taxonomic groups of prey consumed by all the fish species analysed. Cnid. : Cnidarians



Fig.4: Hierarchical clustering tree based on stomach content analysis. The superimposed SI1 to SI6 indications represent the isotopic groups formed by an independent hierarchical clustering based on stable isotope ratios. They were added to evidence differences in clustering run with the two data sets.



Fig. 5: Food spectra of nine species belonging to the SI4 group according to similar SI ratios but to three trophic groups according to different diets. Numbers after species names represent the number of stomachs analysed.



Tables

Table 1: Family, species, mean standard length (SL) and range (minimal and maximal length), mean mass and range (minimal and maximal mass), stable isotope ratios ($\delta^{13}C \pm sd$, $\delta^{15}N \pm sd$); numbers of fishes (n indiv), of stable isotope analyses (n SIA) and of stomach content analyses (SCA) realized.

Family	Species	SL (mm)	Mass (g)	δ ¹³ C (‰)	sd	δ ¹⁵ N (‰)	sd	n indiv	n SIA	n SCA
Carangidae	<i>Trachurus mediterraneus</i> (Steindachner, 1868)	259 (236-277)	231 (215-245)	-18.74	0.77	14.87	2.98	5	10	4
Centracanthidae	<i>Spicara maena</i> (Linnaeus, 1758)	131 (104-170)	62 (35-101)	-19.51	0.33	7.83	0.25	10	26	6
	<i>Spicara smaris</i> (Linnaeus, 1758)	141 (116-154)	63 (45-104)	-19.73	0.41	8.61	0.77	9	13	5
Labridae	<i>Coris julis</i> (Linnaeus, 1758)	109 (68-144)	19 (5-45)	-18.62	0.55	9.74	0.31	13	18	13
	<i>Symphodus mediterraneus</i> (Linnaeus, 1758)	109 (95-131)	41 (25-75)	-19.25	0.26	9.17	0.52	5	18	16
	<i>Symphodus tinca</i> (Linnaeus, 1758)	158 (115-213)	110 (35-250)	-18.78	0.84	9.55	0.52	6	15	12
Moronidae	<i>Dicentrarchus labrax</i> (Linnaeus, 1758)	325 (267-430)	811 (370- 1870)	-18.20	2.53	13.92	0.6	4	12	3
Mullidae	<i>Mullus surmuletus</i> Linnaeus, 1758	142 (91-216)	75 (20-225)	-17.88	0.72	9.94	0.67	35	44	19
Phycidae	<i>Phycis phycis</i> (Linnaeus, 1766)	334 (333-335)	668 (650-685)	-17.95	0.3	10.73	0.33	3	9	3
Scorpaenidae	<i>Scorpaena notata</i> Rafinesque, 1810	112 (68-148)	65 (10-195)	-17.66	0.39	10.15	0.5	33	41	27

	<i>Scorpaena porcus</i> Linnaeus, 1758	142 (84-251)	128 (25-400)	-17.67	0.49	9.74	0.41	28	37	6
	<i>Scorpaena scrofa</i> Linnaeus, 1758	177 (124-217)	275(80-535)	-18.07	0.22	10.06	0.23	5	18	3
Serranidae	<i>Serranus cabrilla</i> (Linnaeus, 1758)	139(114-169	61(25-105)	-18.36	0.22	9.79	0.23	20	43	14
Soleidae	<i>Microchirus variegatus</i> (Donovan, 1808)	87 (72-100)	19 (10-30)	-18.14	0.34	10.2	0.14	7	21	6
Sparidae	Boops boops (Linnaeus, 1758)	158 (101-222)	69 (30-195)	-19.90	0.29	8.46	0.32	33	33	30
	<i>Diplodus annularis</i> (Linnaeus, 1758)	123 (94-188)	66 (25-185)	-18.85	1.1	11.72	1.65	48	34	28
	<i>Diplodus sargus</i> (Linnaeus, 1758)	158 (142-195)	158 (100-285)	-18.77	0.69	11.54	0.73	5	13	4
	<i>Diplodus vulgaris</i> (Geoffroy Saint- Hilaire, 1817)	115 (65-173)	76 (10-195)	-18.14	0.78	11.59	0.83	20	58	12
	Pagellus acarne (Risso, 1827)	118 (97-190)	47 (20-145)	-17.76	0.6	10.64	0.56	20	36	11
	Pagellus erythrinus (Linnaeus, 1758)	147 (115-162)	72 (40-105)	-17.77	0.26	10.81	0.6	5	32	3
Sphyraenidae	<i>Sphyraena viridensis</i> Cuvier, 1829	394 (373-414)	355 (295-395)	-19.33	0.85	11.07	0.65	5	10	3
Synodontidae	<i>Synodus saurus</i> (Linnaeus, 1758)	207 (161-235)	115 (40-170)	-18.30	0.46	10.5	0.79	3	9	2
Triglidae	<i>Trigloporus lastoviza</i> (Bonnaterre, 1788)	148 (102-195)	76 (20-155)	-17.9	0.56	9.76	1.07	3	9	2

Table 2: Weight percentage for prey taxa in stomach content, prey representing less than 1% are presented with + symbol. Trophic group were determined by hierarchical clustering on stomach contents. Zoo: zooplankton feeders, MesoRB: rocky/ seagrass bottom mesocarnivores, MesoSB: muddy/ sandy bottom mesocarnivores, Macroc: macrocarnivores, Pisc: piscivores. SI group: groups resulting from the clustering analysis on SI ratios. Prim prod: primary producers, Cepha: cephalopods, Gastr: gastropods, Bival: bivalves, Polyc: polychaetes, Zoopk: zooplanktonic crustaceans, Crust: benthic crustaceans, Echin: echinoderms, Ascid: ascidians. Groups with minor contribution were pooled in "Others" and represented by superscript letters (b: bryozoans, c: cnidarians, h: hydrozoans, f: foraminifera, u: unidentified matter).

Species	Trophic group	SI group	Prim prod	Cepha	Gastr	Bival	Polyc	Zoopk	Crust	Echin	Ascid	Fish	Others
Boops boops	Zoo	1	4	-	10	1	-	69	-	-	-	14	2 ^{c+u}
Spicara smaris	Zoo	1	2	-	20	-	-	78	-	-	-	-	-
Spicara maena	Zoo	1	-	-	-	-	9	91	-	-	-	-	-
Coris julis	MesoRB	2	-	-	+	86	3	-	11	-	-	-	-
Symphodus tinca	MesoRB	2	-	-	10	21	41	-	8	-	-	-	29 ^{b+f+u}
S. mediterraneus	MesoRB	2	3	-	6	47	+	-	6				37 ^u
Diplodus annularis	MesoRB	3	87	-	+	+	+	-	+	-	2	6	2 b+h+f+u
Diplodus sargus	MesoRB	3	6	-	+	1	86	-	-	-	7	-	-
Diplodus vulgaris	MesoRB	3	-	-	6	-	10	-	-	8	47	-	29 ^{b+h}
Microchirus variegatus	MesoSB	4	-	-	11	-	21	-	19	49	-	-	-
Mullus surmuletus	MesoSB	4	2	-	+	13	13	-	47	24	-	-	+ ^u
Pagellus acarne	MesoSB	4	3	-	6	6	7	-	20	56	2	-	-
Pagellus erythrinus	MesoSB	4	-	-	32	-	-	-	45	24	-	-	-
Trigloporus lastoviza	MesoSB	4	-	62	-	-	-	-	38	-	-	-	-
Scorpaena notata	Macroc	4	-	-	-	3	-	-	97	-	-	-	-
Scorpaena porcus	Macroc	4	+	-	-	-	+	-	99	-	-	-	-

Table 2 (continued)

Serranus cabrilla	Macroc	4	-	-	-	-	+	-	91	-	-	9	-
Synodus saurus	Pisc	4	-	-	-	-	-	-	-	-	-	100	-
Scorpaena scrofa	Pisc	4	-	-	-	-	-	-	15	-	-	85	-
Phycis phycis	Pisc	4	-	-	-	-	-	-	4	-	-	96	-
Sphyraena viridensis	Pisc	5	-	-	-	-	-	-	+	-	-	100	-
Dicentrarchus labrax	Pisc	6	-	-	-	-	-	-	-	-	-	100	-
Trachurus mediterraneus	Pisc	6	-	-	-	-	-	-	1	-	-	99	-

Table 3: Comparison of trophic levels of artificial reef fishes. Trophic levels (TL) were issued from bibliographical data when based on stomach contents, with superscript letters standing for the reference used, or were calculated from the stable isotopic values recorded in this study, using the formula of Badalamenti et al. (2002): NT_i = 1 + ($\delta^{15}N_i - \delta^{15}N_B$) / 3.4, with $\delta^{15}N_i$ the $\delta^{15}N$ value of fish species i, 3.4 the theoretical trophic enrichment factor, and $\delta^{15}N_B$ the isotopic ratio of the trophic baseline. Nanophytoplankton ($\delta^{15}N = 1.77 \%$) or macroalgae ($\delta^{15}N = 3.91 \%$) were used as proxies of pelagic or benthic trophic baselines.

Trankia areuna	Creation	Stomach	Stable isotope TL			
Trophic groups	Species	content TL	Pelagic	Benthic		
Zooplankton	Boops boops	2.5 ^a	3.0	2.3		
feeders	Spicara smaris	3.0 ^ª	3.0	2.4		
	Spicara maena	3.2 ^a	2.8	2.2		
	Mean	2.8 ± 0.3	2.9 ± 0.2	2.3 ± 0.2		
Labrids	Coris julis	3.3 ^ª	3.3	2.7		
	S. tinca	3.3 ^a	3.3	2.7		
	S. mediterraneus	3.2 ^a	3.2	2.5		
	Mean	3.3 ± 0.1	3.3 ± 0.1	2.6 ± 0.1		
Diplodus spp.	Diplodus annularis	3.4 ^a	3.9	3.3		
	Diplodus sargus	3.4 ^a	3.9	3.2		
	Diplodus vulgaris	3.1 ^a	3.9	3.3		
	Mean	3.3 ± 0.2	3.9 ± 0.4	3.3 ± 0.4		
Muddy or sand	M. variegatus	3.4 ^b	3.5	2.8		
bottom	Mullus surmuletus	3.3 ^a	3.4	2.8		
mesocarnivores	Pagellus acarne	3.7 ^a	3.6	3.0		
	Pagellus erythrinus	3.3 ^a	3.7	3.0		
	Trigloporus lastoviza	3.5 ^a	3.3	2.7		
	Mean	3.4 ± 0.2	3.5 ± 0.2	2.9 ± 0.2		
Macrocarnivores	Scorpaena notata	3.5 ^a	3.5	2.8		
	Scorpaena porcus	4.0 ^a	3.3	2.7		
	Serranus cabrilla	3.4 ^a	3.4	2.7		
	Mean	3.6 ± 0.3	3.5 ± 0.1	2.8 ± 0.1		
Benthic	Scorpaena scrofa	4.1 ^a	3.4	2.8		
piscivores	Synodus saurus	4.5 ^c	3.6	2.9		
	Phycis phycis	4.1 ^a	3.6	3.0		
	Mean	4.2 ± 0.2	3.5 ± 0.2	2.9 ± 0.2		
Pelagic	Dicentrarchus labrax	4.3 ^d	4.6	3.9		
piscivores	T. mediterraneus	3.5 ^a	4.9	4.2		
	S. viridensis	4.3 ^e	3.7	3.1		
	Mean	4.0 ± 0.5	4.4 ± 0.7	3.8 ± 0.7		
a: Stergiou and Kar	rpouzi (2002) ; b: Darna	ude (2005) ; c	: Soares et a	al. (2003) ; d:		

Rogdakis et al. (2010) ; e: Barreiros et al. (2002)

Supporting information

Supporting information may be found in the online version of the paper

Fomily	Family Species	Wi	nter	Summer		
ганну	Species	V3	V6	V3	V6	
Carangidae	Trachurus mediterraneus		5			
Centracantidae	Spicara maena	1	2	3	4	
	Spicara smaris	7	1		1	
Labridae	Coris julis	8	5			
	Symphodus mediterraneus		2	1	2	
	Symphodus tinca		1	2	3	
Moronidae	Dicentrarchus labrax	1	3			
Mullidae	Mullus surmuletus	4	12	13	6	
Phycidae	Phycis phycis	1	2			
Scorpaenidae	Scorpaena notata	12	6	8	7	
	Scorpaena porcus	5	4	10	9	
	Scorpaena scrofa	2		2	1	
Serranidae	Serranus cabrilla	6	5	3	6	
Soleidae	Microchirus variegatus		1	6		
Sparidae	Boops boops	17	3	9	4	
	Diplodus annularis	7	33	3	5	
	Diplodus sargus		2	2	1	
	Diplodus vulgaris	4	5	5	6	
	Pagellus acarne	11	6	2	1	
	Pagellus erythrinus			3	2	
Sphyraenidae	Sphyraena viridensis		5			
Synodontidae	Synodus saurus		2	1		
Triglidae	Trigloporus lastoviza		1	2		

Table S.1: Actual sampling at each season and each reef

Table S.2: Significant spatial or seasonal variations of mean isotopic ratios (δ^{13} C and δ^{15} N) of fishes on Marseilles" artificial reef (n \geq 9). S: summer, W: winter. P test significance (*** for p-value \leq 0.001, ** for p-value \leq 0.01, * for p-value \leq 0.05). Post-Hoc: results of post-hoc comparison of means

n indiv	Spacias	Factor	Values (‰)	test	Statistics	Р	Post-Hoc
TI ITUIV	Species			$\delta^{13}C$			
10	Spicara maena	Season	S: -19.68 ± 0.27 W: -19.18 ± 0.12	ANCOVA	7.69	*	S < W
				δ^{15} N			
28	Scorpaena porcus	Season	S: 9.57 ± 0.35 W: 9.94 ± 0.40	ANOVA	9.03	**	S < W
9	Spicara smaris	Season	S: 9.26± 0.02 W: 8.42 ± 0.78	ANOVA	9.29	*	S > W
35	Mullus surmuletus	Reef	V3: 9.83 ± 0.84 V6: 10.03 ± 0.46	ANCOVA	4.29	*	V3 < V6

Table S.3: Parameters of the linear regression between stable isotopes values and standard length of fishes. Significant relationships are written in bold. n: number of individuals. Linear relationships were considered to be significant when $n \ge 10$, $r \ge 0.5$ and $p \le 0.05$. Due to the low number (3) of individuals, no regression was performed for *Phycis phycis, Synodus saurus* and *Trigloporus lastoviza*.

			δ ¹³ C	δ ¹⁵ N			
		n		r p		r	р
Carangidae	Trachurus mediterraneus	5	δ ¹³ C= -22.4 + 1 x 10 ⁻² SL	0.32 0.37	δ ¹⁵ N= 41.8 - 1 x 10 ⁻¹ SL	-0.59	0.07
Centracanthidae	Spicara maena	10	δ ¹³ C= -21.0 + 1 x 10 ⁻² SL	0.75 0.00	δ ¹⁵ N= 8.6 – 6 x 10 ⁻³ SL	-0.51	0.01
	Spicara smaris	9	δ ¹³ C= -22.3 + 2 x 10 ⁻² SL	0.61 0.03	δ ¹⁵ N= 9.6 – 7 x 10 ⁻³ SL	-0.13	0.67
Labridae	Coris julis	13	δ ¹³ C= - 18.8 +1 x 10 ⁻³ SL	0.06 0.82	δ ¹⁵ N= 9.4 + 3x 10 ⁻³ SL	0.24	0.33
	Symphodus mediterraneus	5	δ ¹³ C= -18.8 -4 x 10 ⁻³ SL	-0.22 0.43	δ ¹⁵ N= 6.0 + 3 x 10 ⁻² SL	0.73	0.00
	Symphodus tinca	6	δ ¹³ C= -19.7 + 6 x 10 ⁻³ SL	0.32 0.24	δ ¹⁵ N= 10.3 - 4 x 10 ⁻³ SL	-0.45	0.09
Moronidae	Dicentrarchus labrax	4	δ ¹³ C= -20.9 + 8x 10 ⁻³ SL	0.21 0.50	δ ¹⁵ N= 9.5 + 1 x 10 ⁻³ SL	-0.19	0.54
Mullidae	Mullus surmuletus	35	δ ¹³ C= -18.6 + 5 x 10 ⁻³ SL	0.21 0.18	δ ¹⁵ N= 8.2 + 1 x 10 ⁻² SL	0.51	0.00
Scorpaenidae	Scorpaena notata	33	δ ¹³ C= -18.9 + 1 x 10 ⁻² SL	0.51 0.00	δ ¹⁵ N= 9.2 + 8 x 10 ⁻³ SL	0.28	0.07
	Scorpaena porcus	28	δ ¹³ C= -17.1 - 3 x 10 ⁻³ SL	-0.31 0.07	δ ¹⁵ N= 10.1 – 3 x 10 ⁻³ SL	-0.25	0.13
	Scorpaena scrofa	5	δ ¹³ C= -17.1 + 5 x 10 ⁻³ SL	-0.93 0.00	δ ¹⁵ N= 9.7 + 2 x 10 ⁻² SL	0.34	0.16
Serranidae	Serranus cabrilla	20	δ ¹³ C= -18.3 - 9 x 10 ⁻⁵ SL	-0.01 0.97	δ ¹⁵ N= 9.0 + 5 x 10 ⁻³ SL	0.34	0.03
Soleidae	Microchirus variegatus	7	δ ¹³ C= -18.3 + 2 x 10 ⁻³ SL	0.06 0.80	δ ¹⁵ N= 10.1 + 1 x 10 ⁻³ SL	0.08	0.74
Sparidae	Boops boops	33	δ ¹³ C=-20.1 + 1 x 10 ⁻³ SL	0.11 0.50	δ ¹⁵ N=8.3 + 7 x 10 ⁻⁴ SL	0.06	0.72
	Diplodus annularis	48	δ ¹³ C= -19.0 + 8 x 10 ⁻⁴ SL	0.02 0.89	δ ¹⁵ N= 5.7 + 5 x 10 ⁻² SL	0.62	0.00
	Diplodus sargus	5	δ ¹³ C= -23.6 + 3 x 10 ⁻² SL	0.47 0.09	δ ¹⁵ N= 11.7 – 1 x 10 ⁻³ SL	-0.03	0.92
	Diplodus vulgaris	20	δ ¹³ C= -17.9 - 1 x 10 ⁻³ SL	-0.05 0.76	δ ¹⁵ N= 13.1 – 1 x 10 ⁻³ SL	-0.37	0.03
	Pagellus acarne	20	δ ¹³ C= -18.6 + 7 x 10 ⁻³ SL	0.30 0.10	δ^{15} N= 11.2 – 4 x 10 ⁻³ SL	-0.19	0.31
Sphyraenidae	Pagellus erythrinus Sphyraena viridensis	5 5	δ^{13} C= -19.0 + 9 x 10 ⁻³ SL δ^{13} C= -7.5 - 3 x 10 ⁻² SL	0.58 0.02 -0.65 0.04	δ^{15} N= 6.7 + 3 x 10 ⁻² SL δ^{15} N= 7.3 – 1 x 10 ⁻² SL	0.81 0.28	0.00 0.44