
Trophic structure of vermetid reef community: High trophic diversity at small spatial scales

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Abstract:

Stable isotopes were used to investigate contributions of autochthonous (i.e. benthic: epilithon and macroalgae) and allochthonous (i.e. pelagic: phytoplankton) organic matter sources to the diet of suspension-feeders, grazers and predators associated to small reef-pools (*cuvettes*) created by the reef-building species *Dendropoma petraeum* in the north-western coast of Sicily (Italy). Contributions of potential food sources were calculated using Bayesian mixing-models and integrated to a multivariate approach to highlight the diversity of C and N pathways within *Dendropoma* *cuvettes*. Both pelagic and benthic organic matter sources were exploited by benthic consumers, although clear differences were revealed in the various species depending on their feeding strategy. Three different trophic pathways were identified: one based mainly on phytoplankton, one based mainly on macroalgae and a third one mainly on epilithon. Suspension-feeders seemed to rely mainly on allochthonous organic matter sources, while grazers showed a wider diet spectrum. Predators revealed a high specialization in each of the three food chains and showed a distinct reliance on organic matter originated from benthic or pelagic sources. Stable isotopes evidenced here a marked differentiation of the trophic niche within the *cuvette*-associated community, which allows minimizing competition in very space-limited conditions.

Highlights

► We investigated the organic matter sources use by vermetid reef *cuvette* community. ► Stable isotope ratios ($\delta^{13}\text{C}$, $\delta^{15}\text{N}$) were used to describe the trophic web. ► Both benthic and pelagic OM sources contributed in different extents. ► Three different trophic pathways were identified within the *cuvette*. ► Predators revealed a high specialization in each of the three trophic pathways.

Keywords: *Dendropoma petraeum* ; Stable Isotope ; Bayesian Mixing Model ; Trophic Niche ; Intertidal

1. Introduction

Nearshore and shallow coastal areas are characterized by food webs with high level of connectance and by a strong benthic-pelagic coupling (Bode et al., 2006). Generally in these systems, the large variety of primary producers, such as seagrasses, macroalgae, microphytobenthos and phytoplankton makes the identification of food sources exploited by benthic organisms and the detection of the main trophic pathways difficult (Bode et al., 2006; Goll ty et al., 2010). In temperate rocky shores communities, macroalgae constitute the most representative vegetation sustaining most primary production (Little et al., 2009). Macroalgae enter the food web mostly via detritic pathways, because of low grazing pressure due to the presence of low digestible components (e.g. polyphenols) (Buchsbaum et al., 1991; Duggins and Eckman, 1997). Moreover, in intertidal rocky shores, herbivores can also largely graze on benthic microalgae (Jenkins and Hartnoll, 2001). Besides this benthic autochthonous production, pelagic allochthonous production (i.e. phytoplankton) actually plays a pivotal trophic role for macrobenthic species (Lefebvre et al., 2009). Thus, both allochthonous and autochthonous organic matter sources support intertidal food webs, even though their respective roles are affected by local environmental conditions, such as substratum features, hydrodynamic forcing and seasonal variation (Lefebvre et al., 2009; Goll ty et al., 2010).

Bioengineer reef building species are known to increase spatial complexity by creating microhabitats, and affecting the local hydro-sedimentary patterns, and ultimately offering numerous spatial niches for a large and often unique diverse associated species (Dubois et al. 2002; Callaway et al., 2010). However, trophic processes within associated reef-building species, such as food partitioning and inter-specific competition, have received little attention so far.

Within rocky intertidal Mediterranean habitats, vermetid reefs are unique and highly diverse biogenic constructions, structurally comparable to coralline reefs in tropical seas (Safriel and Ben-Eliahu, 1991). Indeed, they play a structural role in modifying shape and ecological characteristics of the transitional area between mesolittoral and infralittoral rocky flats, making the habitat more complex and tridimensional. In the Mediterranean Sea, the gastropod *Dendropoma petraeum* represents the main structural vermetid species (Safriel, 1975). In close association with coralline algae (Safriel 1975; Calvo et al., 1998), it builds reefs, which host a highly diverse community. *Dendropoma* reefs can be divided into three different morphological zones, from the inshore towards the open sea: the inner edge (terrestrial boundary), the *cuvette* (pools in the central zone), and the outer edge (sea boundary) (Molinier and Picard, 1953; Chemello and Silenzi, 2011). Each of those zones are fashioned by hydrodynamics features (such as wave exposures) and the reef-building activity of the gastropods. The *cuvette* is the most conspicuous zone, with a high level of habitat heterogeneity and the most diverse associated community (Chemello et al., 1998). Structurally, they are similar to tidal pools in the rocky shore environment (Little et al., 2009). Previous descriptions of benthic assemblages inhabiting these reefs revealed high diversity and species abundance at small scale (i.e. *cuvette* scale) (Chemello et al., 1998; Badalamanti et al., 1998; Mannino, 1992; Goren and Galil, 2001), but the functional ecology of these unique and complex bioconstructions is still poorly understood (Vizzini et al., 2012).

Stable isotope analyses (SIA) have been used as a powerful tool to describe the organic matter flow and to resolve trophic relationship in coastal ecosystems (e.g. Vizzini and Mazzola, 2006; Bode et al., 2006; Schaal et al., 2010). Classically, the ¹³C signature patterns are useful to elucidate the origin and pathways of organic matter in food webs, as the primary sources may be isotopically distinct, thus allowing distinctions between pelagic and benthic contributions (France, 1995). The ¹⁵N signals are used to describe food chains lengths and prey-predators relationships (Minagawa and Wada, 1984)

In this study, SIA was used to investigate trophic structure and functioning of vermetid reef intertidal communities and to understand how organic matter sources are exploited by the macrofauna species. In detail, the aims were i) to characterize the food web structure of *D.*

petraeum reef *cuvettes*, ii) to investigate the contribution of autochthonous and allochthonous organic matter sources to the consumers' diet, and iii) to examine how food sources are partitioned within small spatial scales (i.e. within *cuvettes*).

2. Materials and methods

2.1. Study area

The study was carried out along the north-western coast of Sicily (Italy) in the Marine Protected Area (MPA) "Capo Gallo-Isola delle Femmine". This MPA is divided into three different zones of protection: zone A (integral reserve), zone B (general reserve) and zone C (partial reserve). The intertidal rocky shores and the infralittoral seabed host a number of species and communities worthy of protection for their high ecological value and conservation interest, such as *Posidonia oceanica* meadows, sciaphilous communities characterized by the soft red macroalgae *Corallina elongata* and *C. officinalis*, in addition to one of the most extensive vermetid reefs in the Mediterranean (Badalamenti et al., 1992). The sampling area is situated in the zone B of protection located in the eastern part of the reserve (38 12.754 N, 13 17.455 E).

2.2. Sample collection

Macrobenthic organisms and potential food sources, i.e. the autochthonous benthic epilithon (EPI) and macroalgae (MA) and the allochthonous suspended particulate organic matter (SPOM) as a proxy for pelagic phytoplankton, were sampled in May, when primary production reaches maximum values in the Mediterranean. Samples of surface seawater were collected off the reef, so that phytoplankton is not mixed with resuspended material from the reef. EPI, MA and macrobenthos were sampled at low tide in randomly selected reef *cuvettes*, about 150-200 cm in size (equivalent spherical diameters), distant from a few meters. EPI was collected in triplicate by scraping the reef surface with blades. The most abundant and frequent reef MA (i.e. *Ceramium* sp., *Cystoseira* spp. and *Laurencia* sp.) (Mannino, 1992) were collected in triplicate by hand.

To investigate the taxonomic groups representing most of the biomass within the *cuvettes*, sampling focused on the most abundant and representative species typifying the vermetid reef *cuvette* community, from the basis of several previous investigations (Chemello et al., 1998; Badalamenti et al., 1998; Mannino, 1992). Epifaunal organisms were collected using a diverse set of gears, including scrapers and scalpels for molluscs (bivalves and gastropods), nets for small crustaceans and traps for fishes. Endofaunal species associated to microhabitats within the reef-builder *D. petraeum* were collected by carefully extracting small portions of reef; particular care was taken to avoid serious damages to the bioconstruction.

2.3. Laboratory analyses

Once in the laboratory, SPOM samples were obtained by filtering 2 l of pre-filtered (200 μ m) seawater onto pre-combusted (450°C, 4 h) Whatman GF/F filters within 2 hours of collection. Epilithon was observed under a stereo-microscope to identify the main components: benthic diatoms and filamentous algae were found to both dominate samples. For technical reasons, the whole sample was analysed for isotopic ratios as separation techniques did not allow to get pure samples. Epibionts were carefully removed from MA samples. EPI, MA and SPOM samples were analysed separately for $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$. Prior $\delta^{13}\text{C}$ analyses, samples were acidified with drop-by-drop 2N HCl to remove carbonates before drying and grinding (Carabel et al., 2006). For each species of invertebrates and fish, several specimens were pooled, not only to reach the analytical weight in case of small species, but also to limit inter-individual variability. Samples were rinsed with distilled water; muscle tissues were dissected from large individuals (e.g. fishes, large

crustaceans and molluscs) and whole body was used for small individuals. All samples were dried to constant weight at 60°C and ground into a homogeneous powder using mortar and pestle.

Isotopic analyses were performed with an isotope ratio mass spectrometer (ThermoElectron Delta Plus XP) connected to an elemental analyser (ThermoElectron Flash EA 1112). Isotopic values were expressed in conventional δ unit notation (as parts per mil) in relation to international standards (Peedee Belemnite, for $^{13}\text{C}/^{12}\text{C}$; atmospheric N_2 for $^{15}\text{N}/^{14}\text{N}$), following the formula:

$\delta X = [(R_{\text{sample}} / R_{\text{standard}}) - 1] \times 10^3$, where X is ^{13}C or ^{15}N and R is the corresponding $^{13}\text{C}/^{12}\text{C}$ or $^{15}\text{N}/^{14}\text{N}$ ratio. Analytical precision based on the standard deviation of replicates of internal standards (International Atomic Energy Agency IAEA-NO-3 for $\delta^{15}\text{N}$ and IAEA-CH-6 for $\delta^{13}\text{C}$) was 0.2 ‰ for both $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$.

Trophic levels were calculated for each consumer following Post (2002a); the lowest $\delta^{15}\text{N}$ value in primary consumers was chosen as a baseline.

2.4. Data analysis

Statistical significance of differences in stable isotope ratio among OM sources (SPOM, epilithon, and the macroalgae *Laurencia* sp., *Cystoseira* spp., *Ceramium* sp.) was first assessed with non-parametric Kruskal-Wallis tests on ranks and then with *post hoc* multiple comparison tests (Statistica 8.0 package) when significant. To estimate the proportion of sources contributing to the consumers' diet, we applied a Bayesian mixing model approach, using the software package SIAR (Stable Isotope Analysis in R) (Parnell et al., 2010). When several sources contribute to the diet of an organism, mathematical mixing model can help to estimate the contribution of each end-member to the food web. In this perspective, Bayesian statistics have proven to be of a great help, as they allow models to incorporate variability in organisms and food sources signatures, as well as in trophic fractionation (Parnell et al., 2010). The OM sources considered were EPI and reef MA as autochthonous sources, and SPOM as allochthonous source. Isotopic trophic-step fractionation is no longer considered as a constant value, especially regarding trophic-step enrichment from primary producers to primary consumers, as numerous factors are known to affect discrimination processes in organisms tissues (Martinez Del Rio et al., 2009). Several investigations have provided models as proxy to estimate source-specific trophic enrichment factor (TEF) based on the isotopic signature of the food source (see review in Caut et al., 2009). TEFs were estimated for each of the selected sources based on linear models provided in Caut et al. (2009) for invertebrates, as $\Delta\delta^{13}\text{C} = -0.113 \times \delta^{13}\text{C}_{\text{Source}} - 1.916$ and $\Delta\delta^{15}\text{N} = -0.311 \times \delta^{15}\text{N}_{\text{Source}} + 4.065$. For consumers exhibiting higher trophic levels (secondary consumers and $\text{TL} > 3$), two TEFs were then considered, the first TEF being calculated using Caut et al. (2009) models and the second TEF in accordance with averaged values reported in the literature (1.0‰ for $\delta^{13}\text{C}$ and 3.4‰ for $\delta^{15}\text{N}$) (De Niro and Epstein, 1978; Minagawa and Wada, 1984; Post, 2002a), as in higher trophic chain positions TEFs are reported to be much constant (Vander Zanden and Rasmussen, 2001; McCutchan et al., 2003) and cannot be calculated from models since prey signatures are too diverse. As showed in Table 1, TEF for first trophic step was comprised between -0.6 and +0.5 ‰ for $\Delta\delta^{13}\text{C}$ and +2.0 and +4.0 ‰ for $\Delta\delta^{15}\text{N}$, which fall perfectly within the ranges of reviewed estimates of TEFs (*ibid.*). The standard deviations calculated for TEFs originated from the variability in primary consumers' isotopic signatures.

In order to identify food sources pathways, non-Metric Multidimensional scaling (nMDS) ordination and hierarchical clustering (Euclidean distance, group average mode) were performed on the Bayesian mixing model results. Minimum, maximum (expressed as 95th percentiles) and mean contributions to the consumer diet for each food source were used as factors for each species, to encompass all the variability in the estimation of food sources contributions. Clusters were tested with the similarity profile (Simprof) procedure described in Clarke and Gorley (2006). All the species diets (along with uncertainties) were permuted and compared with what expected under a simple null hypothesis of no differences in primary consumer's contributions to diets. This

procedure hence meaningfully grouped all species that rely on the same basal sources, regardless their feeding mode or trophic level.

3. Results

3.1. $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ of organic matter sources and consumers

Isotopic values of organic matter sources exhibited wide ranges for both carbon and nitrogen: $\delta^{15}\text{N}$ ranged from $0.3 \pm 0.2\text{‰}$ for epilithon to $6.7 \pm 1.8\text{‰}$ for *Laurencia* sp., while $\delta^{13}\text{C}$ values varied from $-21.8 \pm 0.6\text{‰}$ for SPOM to $-11.9 \pm 0.3\text{‰}$ for epilithon (Table 2). Overall significant differences between organic matter sources were observed for both $\delta^{13}\text{C}$ ($p < 0.001$) and $\delta^{15}\text{N}$ ($p < 0.001$). However post-hoc multiple comparisons showed that no significant differences were found between macroalgal species, hereafter pooled as a unique source called macroalgae (MA).

Consumers' isotopic signatures also exhibited large isotopic carbon and nitrogen ranges (Table 2). The lowest $\delta^{15}\text{N}$ values were for sea sponges Chondrillidae ($6.0 \pm 0.4\text{‰}$), hence used as baseline to calculate trophic levels of other species. Blenniidae finfishes, and especially *Aidablennius sphyinx* ($11.9 \pm 0.6\text{‰}$) exhibited the highest $\delta^{15}\text{N}$ values ($11.9 \pm 0.6\text{‰}$). Estimates of trophic levels allowed the distinction between primary consumers ($2 < \text{TL} < 3$) and secondary consumers ($\text{TL} > 3$), as reported in Fig. 1, which matched the feeding mode retrieved from the literature (Table 2). Carbon signatures of consumers varied markedly, mainly for primary consumers that are well discriminated on the $\delta^{13}\text{C}$ axis, with no overlap. Organisms' signatures spanned from the most depleted values of filter-feeders (from -19.2 to -18.1‰) to more enriched values of grazers (from -17 to -13.7‰). Secondary consumers exhibited a slight narrower carbon range than primary consumers (Fig. 1).

3.2. Mixing model output

Bayesian mixing model outcomes showed a high variability in the relative contributions of the three OM sources (EPI, MA and SPOM) to the consumers' diet (Table 2), as most of the species signatures fell close to the barycentre of the isotopic space defined by the 3 potential food sources; the ranges of possible contributions were expressed as 95th percentiles interval. Overall, EPI percentage contributions exhibited the lowest values for *A. sphyinx*, ranging from 0.0 to 21.4%, while the highest values for *Patella ulyssiponensis*, ranging from 36.7% to 76.3%; MA lowest proportion in diet was for *Cardita calyculata* (from 0.0 to 37.4%), whereas the highest was for *Gammarus* sp. (from 19.0% to 86.6%); SPOM contribution showed the widest range of variation, displaying proportion values from 0.0 to 17.5% for *Patella caerulea*, while from 38.6% to 88.9% for the Nereididae *Perinereis cultrifera* (Table 2).

Overall, three groups of consumers were identified using cluster and nMDS ordination (Fig. 2). As ordination was performed on the Bayesian mixing model results, within each group, species depend on the same basal sources and hence belong to the same trophic pathway. Simprof procedure showed that the three clusters were significantly discriminated ($p < 0.01$). The group A is the larger one and relied largely on SPOM. It included all the filter-feeders, three carnivores/omnivores (i.e. the Eunicidae *Lysidice ninetta*, the Nereididae *P. cultrifera* and *Lipophrys canevae*). The group B had an intermediate size and was characterized by a larger contribution of MA as basal source. It included grazers (*Gammarus* sp., *Hyale* sp., *Patella rustica*, *Parablennius sanguinolentus*) and carnivores/omnivores (*A. sphyinx* and *Coryphoblennius galerita*). The group C was the smallest one and clustered *P. caerulea* and *P. ulyssiponensis*, together with *Phascolosoma granulatum* and *Salaria pavo*. Those species mainly rely on EPI, with the exception of *P. caerulea*, showing a slightly higher mean contribution of MA.

4. Discussion

4.1. Contribution of organic matter sources to food web pathways

Vermetid reef *cuvettes* represent an intertidal complex system of pools featured by rich faunal abundance and diversity, as well as rich macroalgae and epilithic community. The spatial complexity of the reef offers large surfaces for macroalgae and epilithon to grow, making those sources of primary importance in the contribution of the diet to local diversity. Allochthonous organic matter input originating from the open sea as phytoplankton (SPOM), enter the *D. petraeum* *cuvettes* in an uneven manner and mainly through wave action. Autochthonous and allochthonous organic matter sources are isotopically distinguishable for both carbon and nitrogen. SPOM showed a lower proportion of the heavy carbon isotope than the autochthonous OM sources, which is consistent with the prevalence of phytoplanktonic components, typically depleted in comparison with benthic organic matter (France, 1995). The SPOM isotopic signature matches literature data (e.g. Harmelin-Vivien et al., 2008; Lefebvre et al., 2009). Macroalgae had a much depleted $\delta^{13}\text{C}$ signal than phytoplankton, while $\delta^{15}\text{N}$ remained quite similar. All investigated macroalgae species had non-significantly different signatures and were considered as a unique pool of organic matter. As a result, if some specialization between macroalgae grazers occurs, SIA did not allow discrimination within the community. Epilithon showed an isotopic signature highly enriched in ^{13}C and depleted in ^{15}N compared to other potential food sources. Such a signature is due to the prevalence of filamentous cyanobacteria (see Vizzini et al., 2012 and references therein). Cyanobacteria, while rarely investigated in isotopic studies – mostly for technical reasons – commonly co-occur with pelagic or benthic microalgae and are suspected to play a significant role as a food resource in sandy sediments (Evrard et al., 2010). However, despite the ubiquitous distribution, their trophic role is still poorly described in rocky shores and their contributions, when evidenced, remain sporadic in time or minor in terms of contribution (Goll  ty et al., 2010; Schaal et al., 2010). However, this investigation clearly evidenced the role of cyanobacteria in the food web *cuvettes* of *D. petraeum* reefs.

The carbon isotopic signatures of the *cuvette* species spanned approximately over the same $\delta^{13}\text{C}$ range as the food sources (ca. 7‰). This indicates that the whole trophic niche of the *cuvette* space (as defined by the diversity of the food sources) was exploited, hence minimizing inter-specific competition for food. Because food sources are isotopically easily discriminated, the results of the mixing models offered here trustworthy estimates of species diets and allowed us to better understand the general food web structure within the *cuvette* habitat. Multivariate analysis revealed the presence of 3 main pathways of organic matter sources, embracing groups of species sharing the same basal sources.

The first trophic pathway had phytoplankton as main basal source, and includes species (identified as group A) with a mean estimated contribution of SPOM of 55% (mean min = 31% ; mean max = 75%). Species identified from the literature as suspension-feeders are represented here with the bivalves *C. calyculata* and *Mytilaster minimus*, as well as the sea-sponges (Chondrillidae). The reef-builder *D. petraeum* also mainly relied on phytoplankton, as previously showed (Vizzini et al., 2012). Even if all the filter-feeders clustered together, they showed differences in the sources contribution, meaning that their trophic niche did not completely overlap, as reported for filter feeders in other studies (Dubois et al., 2007a, 2007b; Schaal et al., 2010). This pathway also includes polychaete species of the families Eunicidae and Nereididae: even if they are often reported as carnivores/omnivores, *L. ninetta* showed trophic level lower than the other predators, whereas *P. cultrifera* exhibited here a predatory behaviour by targeting species from this group A (although the large $\delta^{15}\text{N}$ range suggests that it feeds on various trophic level). It is likely that Nereididae prey upon the most abundant species, namely *D. petraeum*, as predatory polychaetes belonging to this family are often reported in biogenic structures where they prey in tubes from the reefs builder (Dubois et al., 2002). In exhaustive investigations of coastal benthic community, Nereididae also exhibit high nitrogen values (Grall et al., 2006; Dubois et al., 2007a, Schaal et al., 2010). The Blenniidae *L. canevae* has a similar trophic level to *P. cultrifera*, although in the

literature the dominance of algae in gut contents is reported (Velasco et al., 2010). This study showed that they may also prey upon *D. petraeum*. Indeed, while they cannot reach the entire mollusc within its tube, they actually „graze“ the head from *D. petraeum* filtering individuals. The very close species *Lipophrys pholis* was also reported to prey on reef-builder *Sabellaria alveolata* heads (Dubois et al., 2002).

The second pathway was based on macroalgae, being composed of species (group B) that relied mainly on macroalgae as basal sources (on average 48%; mean min = 16%; mean max = 80%). At the bottom of this pathway, the gastropod *P. rustica* and two amphipod species (*Gammarus* sp. and *Hyale* sp.), as well as the finfish *P. sanguinolentus* where all identified as grazers from the literature (Hawkins et al., 1989; Ruffo, 1998, Velasco et al., 2010). However, the mollusc *P. rustica* showed a higher $\delta^{13}\text{C}$ signature than amphipods or *P. sanguinolentus*, suggesting either the grazing on different macroalgae or a larger contribution of epilithon. At the top of this pathway, two carnivorous Blenniidae species (*A. sphyinx* and *C. galerita*) are targeting amphipods as the main component of their diet.

The third food pathway included species (group C) mainly based on epilithon, which yielded an average contribution of 42% (mean min = 30%; mean max = 62%). This group is composed of the two gastropods, *Patella caerulea* and *P. ulyssiponensis*, known to scrape the reef surface with their radula (Hawkins et al., 1989). The sipunculid *P. granulatum* belongs to this pathway, but exhibited a much higher $\delta^{15}\text{N}$ value than the Patellidae species. Little is known on sipunculids' diet, but they are mainly considered as deposit-feeders (Murina, 1984). This species is found in the crevices and in empty *D. petraeum* tubes, where it scrapes off food from the substratum for epilithon and macroalgae detritus: it is likely that it also behaves as a scavenger during its borrowing activity, feeding on died organisms, hence explaining its high $\delta^{15}\text{N}$ value. *S. pavo* is a Blenniidae species living in crevices and this study shows that it is likely to prey on Patellidae species.

The clustering of species into three different trophic pathways is based on the result of similarities in food sources contributions in species diets estimated with Bayesian statistics. While this approach led here to three obvious – statistically distinct – clusters, one should also consider that species are trophically plastic. This MDS approach produces here hard clustering, because a clear-cut decision for each object (species) is made. Further applications of this approach need to pay special attention to species at the edges of the clusters, hence considering they may belong to several clusters. In this perspective, fuzzy clustering method allows for some ambiguity in the data, which often occurs in practice, especially for trophic behaviour.

4.2. Structure and functional diversity within *cuvette* food web

Isotopic data on the main species representing most of the diversity and the biomass of the benthic assemblage associated to the *cuvette* of *D. petraeum* reefs revealed that both allochthonous and autochthonous food sources contribute to the food web. *Cuvettes* are morphological depressions created by vermetid biogenic reefs and are in many ways similar to tidal pools on rocky shores. Intertidal tides pools are stressful environments for benthic organisms, with immersion and emersion periods bringing allochthonous food (phytoplankton) (Little et al., 2009). This study showed that within the *cuvette* food web, the whole trophic niche is exploited by species inhabiting the *cuvettes*: indeed, we showed here that the number of species is evenly distributed between allochthonous (45% of species) and autochthonous (55% of species) food sources. This could contribute to regulate primary production within the *cuvettes* and to minimize intra- and inter-specific food competition in a stressful environment subject to short-term changes. By closely looking at the grazers trophic group, for example, it is worth noticing an even split between species focusing mainly on macroalgae and species focusing on epilithic microalgae. Even within the Patellidae, one of the most abundant and diverse group in rocky shores (Little et al., 2009), mixing models revealed inter-specific differences in their diets. Calculation of the trophic levels also revealed an equal distribution of predators between each food pathway. While fish species (i.e. *L. canevae*) and polychaetes (i.e. *P. cultrifera*) feed on suspension-feeders, other fishes

(i.e. *A. sphinx* and *C. galerita*) prey on macroalgae grazers and *S. pavo* prey on epilithon grazers. These results are supported by previous investigations of gut contents of Blenniidae from rocky pools, revealing high degree of trophic specialization (Velasco et al., 2010). Interestingly, the Eunicidae species did not exhibit a predatory behaviour, as suggested from the literature (Fauchald and Jumars, 1979) and hence limits the trophic competition with other polychaete predators (e.g. Nereididae species), as previously showed in soft sediment between Eunicidae and Glyceridae species (Dubois et al., 2007a). The diversity in predator trophic niches can reduce the top-down effect in the community organization (e.g. Estes et al., 2001) and we hypothesized here that the diversity in top-down pressures could contribute to the stability and the resilience of *cuvettes* food web.

Variations in $\delta^{15}\text{N}$ ranges give an estimate of the length of the food chain, and provide a proxy of the complexity in trophic interactions (Post, 2002b). Autochthonous and allochthonous food pathways exhibited very similar food chain lengths and all the predators exhibited very similar $\delta^{15}\text{N}$ signatures. This pattern leads to a wide range in $\delta^{13}\text{C}$ at the top of the food chain. This isotopic space pattern is uncommon (the isotopic space is actually not pyramidal-shaped), because rocky shore communities classically revealed that top predators all prey upon all species, coupling the diverse pathways, hence showing a narrower $\delta^{13}\text{C}$ range (Gollety et al., 2010). Conversely predators investigated here revealed a high level of specialization in each of the three food chains, showing that even at small spatial scale (meter-scale), high specialisation of predators leads to the exploitation of the whole trophic niche, which allows minimizing competition.

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Tables

Table 1. Estimates of Trophic Enrichment Factors using food source signatures, after models for invertebrates developed by Caut et al. (2009) for $\Delta\delta^{13}\text{C} = -0.113 \times \delta^{13}\text{C}_{\text{Source}} - 1.916$ and $\Delta\delta^{15}\text{N} = -0.311 \times \delta^{15}\text{N}_{\text{Source}} + 4.065$.

Source	Mean $\delta^{13}\text{C}$ TEF	sd	Mean $\delta^{15}\text{N}$ TEF	sd
EPI	-0.6	0.0	4.0	0.1
MA	-0.2	0.2	2.2	0.4
SPOM	0.5	0.1	2.0	0.3

Table 2. $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ mean values (‰), trophic level (TL) and percentage contribution of organic matter (OM) sources to the diet (mixing model output) of consumers. The feeding mode of consumers based on literature data is provided. N: number of samples; sd: standard deviation; low: lower 95th percentile proportion; high: higher 95th percentile proportion.

OM sources and consumers	Feeding mode	Code	N	$\delta^{13}\text{C}$		$\delta^{15}\text{N}$		TL		% EPI			% MA			% SPOM		
				mean	sd	mean	sd	mean	sd	low	high	mean	low	high	mean	low	high	mean
Autochthonous OM sources																		
Epilithon			9	-11.9	0.3	0.3	0.2											
<i>Ceramium</i> sp.			9	-15.8	1.3	5.5	0.4											
<i>Cystoseira</i> spp.			9	-15.5	1.1	5.8	0.3											
<i>Laurencia</i> sp.			9	-14.3	1.2	6.7	1.8											
Allochthonous OM sources																		
SPOM			6	-21.8	0.6	6.5	0.9											
Consumers																		
Porifera																		
Chondrillidae	Filter feeder ^a	1	3	-18.4	0.3	6.0	0.4	2.0	0.1	2.1	59.1	29.8	0.0	45.9	19.5	17.5	73.1	50.7
Mollusca																		
<i>Cardita calyculata</i>	Filter feeder ^b	2	5	-18.7	0.5	6.2	0.6	2.1	0.2	1.3	46.0	24.3	0.0	37.4	13.9	40.8	76.0	61.8
<i>Dendropoma petraeum</i>	Filter feeder ^c	3	9	-18.1	0.2	7.9	0.7	2.5	0.2	7.6	29.6	18.7	8.8	42.7	25.7	47.5	63.2	55.6
<i>Mytilaster minimus</i>	Filter feeder ^d	4	3	-18.4	0.4	7.9	0.4	2.6	0.1	0.2	29.1	14.8	2.2	52.9	29.4	38.0	72.9	55.8
<i>Patella caerulea</i>	Grazer ^e	5	5	-13.7	0.8	6.7	0.6	2.2	0.2	30.4	61.3	45.0	30.9	66.6	49.1	0.0	17.5	5.8
<i>Patella rustica</i>	Grazer ^e	6	3	-14.5	1.2	7.6	0.1	2.5	0.0	5.3	51.7	29.3	13.3	81.2	46.6	0.0	51.5	24.1
<i>Patella ulyssiponensis</i>	Grazer ^e	7	3	-14.3	0.1	6.1	1.0	2.0	0.2	36.7	76.3	55.5	3.4	55.5	32.9	0.0	22.5	11.6
Crustacea, Amphipoda																		
<i>Gammarus</i> sp.	Grazer ^f	8	4	-16.3	1.0	8.3	0.8	2.7	0.2	0.0	38.7	17.3	19.0	86.6	53.1	7.8	51.2	29.6
<i>Hyale</i> sp.	Grazer ^f	9	4	-17.0	0.7	8.0	0.7	2.6	0.2	0.8	34.1	18.0	16.6	70.6	43.9	22.1	53.5	38.1
Anellida, Polychaeta																		
<i>Lysidice ninetta</i>	Carnivorous/omnivorous ^g	10	4	-16.9	1.4	9.6	0.9	3.1	0.3	10.2	58.8	36.8	0.0	48.0	20.9	13.8	66.4	42.3
<i>Perinereis cultrifera</i>	Carnivorous/omnivorous ^g	11	3	-18.6	0.5	11.4	2.1	3.6	0.6	0.0	28.1	11.8	0.0	44.3	19.7	38.6	88.9	68.6
<i>Sabella</i> sp.	Filter feeder ^g	12	3	-19.2	0.8	7.0	0.0	2.3	0.0	4.3	46.3	27.0	0.0	47.6	22.1	21.4	79.5	50.9
Sipuncula																		
<i>Phascolosoma granulatum</i>	Omnivorous ^h	13	6	-14.7	0.1	10.3	0.4	3.3	0.1	29.7	52.7	40.8	14.4	49.3	32.3	18.3	35.0	26.9
Fish																		
<i>Aidablennius sphyinx</i>	Carnivorous/omnivorous ⁱ	14	5	-16.5	0.9	11.9	0.6	3.7	0.2	0.0	21.4	8.8	26.2	70.1	48.6	27.4	57.8	42.5
<i>Coryphoblennius galerita</i>	Carnivorous/omnivorous ⁱ	15	3	-15.2	0.6	11.6	0.3	3.6	0.1	0.6	42.8	21.6	14.8	81.3	47.3	8.6	51.7	31.1
<i>Lipophrys canevae</i>	Omnivorous ^{i, l}	16	3	-17.1	0.6	11.6	0.7	3.6	0.2	0.0	29.4	13.1	6.2	61.5	36.5	32.1	68.5	50.5
<i>Parablennius sanguinolentus</i>	Grazer ^l	17	3	-16.2	0.5	8.5	0.5	2.7	0.1	0.0	46.8	23.5	10.2	82.6	46.1	7.0	50.0	30.5
<i>Salaria pavo</i>	Carnivorous/omnivorous ^{i, l}	18	3	-14.6	0.6	10.2	0.6	3.2	0.2	20.9	56.4	38.9	7.1	63.8	36.4	6.0	42.9	24.7

a Bell, 2008; b Avila, 2003; c Barash and Zenziper, 1985; d Safriel and Sasson-Frostig, 1988; e Hawkins et al., 1989; f Ruffo, 1998; g Fauchald and Jumars, 1979; h Murina, 1984; i Goldschmid et al., 1984; l Velasco et al., 2010.

Figures

Figure. 1. $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ mean values (‰) and standard deviations for organic matter sources (■), invertebrates (●) and fish (△) in the study area. EPI: epilithon; MA: macroalgae; SPOM: suspended particulate organic matter; consumers are coded in Table 1. Shaded grey vertical bars represent the $\delta^{15}\text{N}$ encompassed by primary consumers (PC) and secondary consumers (SC) according to calculated TL.

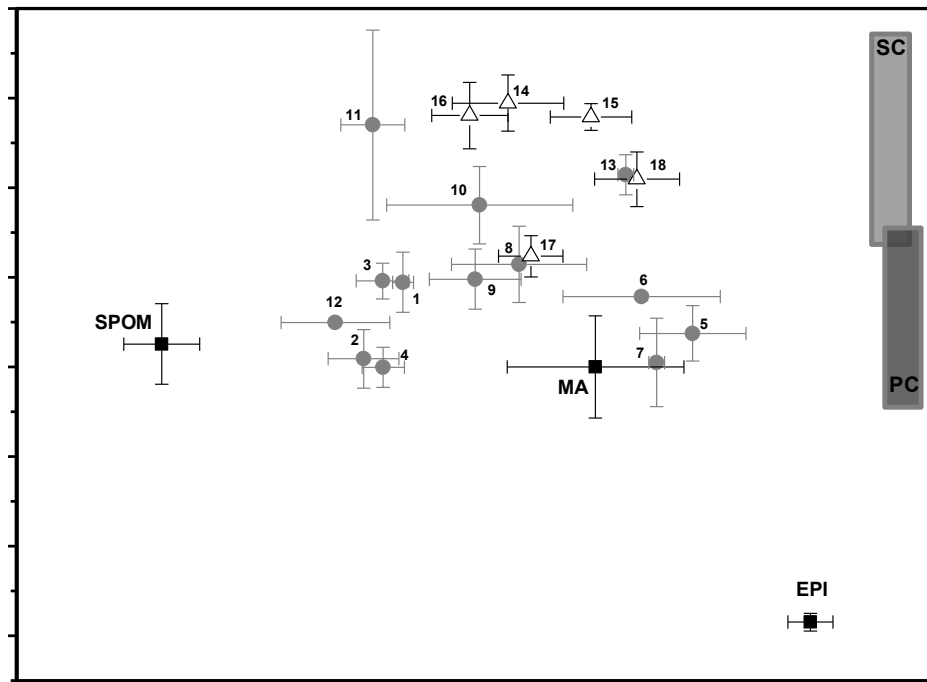


Figure. 2. Dendrogram of hierarchical cluster analysis (a) and non metric multidimensional scaling (nMDS) ordination (b) of similarity matrix (Euclidian distances) on percentage contributions of organic matter sources to the consumers diets (lower 95th percentile, higher 95th percentile and mean value). Significant clusters (A, B and C) corresponding to trophic pathways were tested using the Simprof procedure. Ff: filter feeders; Gr: grazers; C/O: carnivores/omnivores. Consumers are coded in Table 1.

