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Trophic flexibility and prey selection of the wild long-snouted seahorse *Hippocampus guttulatus* Cuvier, 1829 in three coastal habitats

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ABSTRACT

The present study examined the dietary composition of long-snouted seahorse *H. guttulatus* Cuvier, 1829 in Mar Piccolo of Taranto (Apulia, Italy) using a non-destructive flushing method. To assess differences in the selection of prey among different habitats, adult specimens were collected from both highly complex (*Cladophora prolifera* and *Corallina elongata*) and low complex (unvegetated Sandy bottom) habitats. Additionally, samples of benthic fauna were collected from the same habitats and were used to investigate the local availability of potential preys. Results showed significant differences in the diet composition among three studied habitats. Consistently with the results of faunal analysis, gut contents of specimens sampled on *C. elongata* had higher prey abundance and diversity compared to the other two habitats. Dietary composition of seahorses found in the vegetated habitats was similar, and it was mainly based on Amphipoda and Copepoda. However, small prey (< 1 mm) was more consumed on *C. elongata* than on *C. prolifera*. On the sandy bottom, *H. guttulatus* preyed mainly on small crustaceans, but actively selected larger prey (i.e. Galathoidea and Paguroidea). Nematodes were also highly preyed in all habitats, although the high consumption could be possibly attributed to the high abundance and availability of these organisms at the investigated site. *H. guttulatus* showed a great ability to exploit available resources in the best possible manner among different habitats, acting as a specialist predator. With an aim to understand the use of food resources that are available in the environment, the results of this study could help to propose initiatives directed to the seahorse conservation.

Since prey availability does not seem to be a limiting factor in the understanding of variability and consistency of *H. guttulatus* populations, further studies on other environmental and biological aspects could be useful in the assessment of the conservation status of long-snouted seahorses.

2. Introduction

Seahorses (Hippocampinae) are ambush predators that usually rely on their vision to capture prey (Kuiter, 2000). They practice "sit-and-wait" predation strategy, which involves examination of the environment from a hidden place and rapid execution of a surprise attack (Tipton and Bell, 1988; James and Heck, 1994). They rarely swim in search of food and in fact, by using a prehensile tail, they usually grasp a holdfast and remain stationary while waiting for the prey to approach (Felício et al., 2006; Kendrick and Hyndes, 2005). Nevertheless, it is not unusual for seahorses to adopt an active foraging strategy when they search for prey in the water column, sediments, algal beds or phanerogam meadows (Curtis and Vincent, 2005; Felício et al., 2006). Diet

and foraging strategies are well documented in a large number of scientific studies that have shown that most seahorse species principally consume epibenthic invertebrates (e.g. harpacticoid copepods, nematodes, polychaetes), thus reflecting their sedentary behavior (Kendrick and Hyndes, 2005; Castro et al., 2008; Kitsos et al., 2008; Yip et al., 2015). In these studies, crustaceans have been considered as one of the main contributors to the seahorse diet (Foster and Vincent, 2004; Kendrick and Hyndes, 2005; Castro et al., 2008; Yip et al., 2015). Small-sized copepods and amphipods are considered principal target preys (Teixeira and Musick, 2001; Foster and Vincent, 2004; Kendrick and Hyndes, 2005; Castro et al., 2008; Kwak et al., 2008; Yip et al., 2015) probably due to the poor ability of seahorses to capture larger and more vagile prey (Tipton and Bell, 1988). However, occasional consumption

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of larger prey items, such as caridean shrimps and fishes, have also been observed (Woods, 2002; Felício et al., 2006; Castro et al., 2008). It seems that the seahorse's feeding strategies and preferences are highly dependent on the snout length, individual size, abundance and availability of prey (Tipton and Bell, 1988; d'Entremont, 2002; Woods, 2002; Kendrick and Hyndes, 2005; Castro et al., 2008) as well as on the degree of habitat complexity and holdfast availability (Curtis and Vincent, 2005; Correia et al., 2014, 2015).

Although typically associated with structurally complex habitats (Claassens, 2017; Claassens et al., 2018; Manning et al., 2018), several species of seahorses, such as *H. abdominalis*, *H. capensis*, *H. guttulatus*, *H. hippocampus* and *H. kuda*, are able to exploit and hunt on uncovered sandy substrates where there are no potential holdfasts (Bell et al., 2003; Garrick-Maidment and Jones, 2004; Curtis and Vincent, 2005). Moreover, when their preferential habitat is depleted by anthropic impacts, seahorses are able to use artificial structures (swimming nets, pilings) and to occupy less disturbed habitats, which provide refuges from predation and more feeding opportunities (Harasti et al., 2010; Caldwell and Vincent, 2013; Manning et al., 2018). Previous studies have demonstrated that long-snouted seahorses *H. guttulatus* Cuvier, 1829 commonly live and feed in the structurally complex habitats characterized by the high density of seagrasses, vegetation cover and sessile invertebrates that can provide holdfasts and abundant food (Curtis and Vincent, 2005; Caldwell and Vincent, 2013). Some individuals, however, were observed to feed on prey suspending it in the water column by jetting water into the sediment (Foster and Vincent, 2004; Yip et al., 2015).

Like most of its exotic congeners, the Mediterranean *H. guttulatus* primarily feed on Amphipoda, Anomura Decapoda and Mysidacea (Kitsos et al., 2008; Gurkan et al., 2011). However, because of the recent development of consolidated techniques that permitted to study gut contents through stable isotopes analysis (Valladares et al., 2017), the role of smaller prey without calcareous/chitinous exoskeleton (e.g. meiofauna: benthic metazoans, ranging from 30 μm to 1 mm) should be reconsidered. This is probably because soft-bodied taxa, such as nematodes and polychaetes, digest in a short period of time (1–3 h) leaving no visual remains and therefore, they are hardly identifiable by traditional analysis of gut contents, which includes removal of the gut in the laboratory (Alheit and Scheibel, 1982; Scholz et al., 1991). Nevertheless, meiofaunal organisms represent the ubiquitous component of benthic assemblages and important dietary resource for many bottom-feeding fish species (Spieth et al., 2011; Weber and Traunspurger, 2015, 2016).

Using a non-destructive in-situ method, which also enables the identification of soft-bodied species, this study had the twofold purpose: (a) to determine the diet of *H. guttulatus,* and (b) to investigate the differences in the diet composition versus prey availability in different habitats. To achieve these goals, specimens of *H. guttulatus* were collected from three documented preferential habitats in Mar Piccolo of Taranto (for this area; see Gristina et al., 2015, 2017). Additionally, in the same habitats, benthic fauna was sampled to assess the abundance and availability of the potential prey items.

2. Materials and methods

2.1. Study area

The study was carried out in Mar Piccolo of Taranto (40°28′N, 17°16′W), which is a semi-enclosed marine system located in the northern part of the Gulf of Taranto (Apulian coast, Ionian Sea) (Fig. 1). Mar Piccolo of Taranto is considered as one of the most heavily polluted water bodies in Italy. Surrounded by urban development, heavy industries and by civil and military shipyards, the water body of Mar Piccolo is affected by heavy metals, hydrocarbons, pesticides and organic wastes (Cardellicchio et al., 2007; Petronio et al., 2012). Despite critical environmental conditions, Mar Piccolo of Taranto hosts a large

and stable population of the European seahorses (both *H. guttulatus* and *H. hippocampus*). Extent and persistence of this population are achieved through the presence of extensive mussel farms, which limit all types of commercial and recreational fishing (Gristina et al., 2015). Furthermore, water eutrophication supports large populations of crustaceans (mostly amphipods), which are potential preys of seahorses (Prato and Biandolino, 2003). Across entire Mar Piccolo, natural rocky substrates are reduced, while the seafloor is dominated by soft sediments that vary from muds to mixed sands. They are sparsely covered by patches of *Cymodocea nodosa,* scattered tufts of brown algae (*Cystoseira* spp.) and large algal beds of *Cladophora prolifera* (Cecere and Petrocelli, 2009). Hard substrates, mainly of anthropogenic origin (wood pools of mussel farms, stone walls along the coastline, rocks, ropes and discarded material), support an algal turf principally constituted of perennial *Cystoseira* C. Agardh, 1820 spp. and other frondose algae (i.e. *Corallina elongata* J. Ellis and Solander, 1786, *Dictyopteris* J. V. Lamouroux, 1809 spp.) (for further description see Gristina et al., 2015, 2017).

In this study, long-snouted seahorses were collected in the area characterized by the presence of a continuous stone wall along the coastline, which shares biocenotic characteristics with the rest of Mar Piccolo (Gristina et al., 2017). Although at least six different habitats were described at Buffoluto site (Gristina et al., 2017), this study focused on three most extensive and continuous habitats: two highly complex (*Cladophora prolifera* and *Corallina elongata*) and one low complex (unvegetated Sandy bottom) habitat, commonly found in Mar Piccolo of Taranto. *Cladophora prolifera* forms large algal beds at 3–3.5 m of depth, *Corallina elongata* forms algal turf on the stone wall along the coastline at approximately 0.5 m of depth, while the unvegetated Sandy bottom is located between the stone wall and algal bed at 1–2 m of depth (for further details see Table 1 in Ape et al., 2019 submitted).

2.2. Sampling and gut analysis

H. guttulatus specimens were collected by SCUBA divers during the first week of June 2018, always between 9 a.m. and 11 a.m. when *H. guttulatus* has more active feeding behavior (Faleiro et al., 2008). A total of 87 individuals (25 on *C. prolifera*, 38 on Sandy bottom and 24 individuals on *C. elongata*) were carefully hand-picked in the investigated habitats. Individuals were selected according to their appearance and on the basis of the signs of pregnancy or unhealthy conditions, such as external lesions. All individuals were sexed and measured for the standard length (SL) to the nearest mm and immediately transferred to the 1 L plastic containers, containing filtered (30 μm mesh size) local seawater mixed with clove-oil (0.05%), a natural anesthetic (Cunha and Rosa, 2006; Castro et al., 2008). Clove-oil has quickly but temporarily effect, and leaves no consequences on the vital status of animals. Flushing method (Kamler and Pope, 2001), adapted from Castro et al. (2008), was used to obtain food items from the guts of seahorses. A catheter of 1 mm in diameter, connected to a needleless plastic syringe and filled with 3 ml of the freshwater, was gently introduced into the snout of each examined specimen. Freshwater was slowly flushed down the snout, inducing regurgitation of food items inside the plastic container. After regurgitation, water inside the plastic bowl was sieved through a 30 μm mesh. The fraction retained on the sieve was preserved in 50 ml tubes and in 4% buffered formalin and Rose Bengal (0.5 g l^{-1}). Immediately after the procedure, seahorses were transferred to a new plastic container with continuously aerated seawater where they stayed until the upright body posture and normal ventilation, considered as the signs of full recovery, have been restored. After the trials, all fish showed full recovery and were released into the wild, approximately at the capture site.

In the laboratory, preserved food items were counted and classified at the major taxa level of taxonomic discrimination using a stereomicroscope. Two size classes of prey organisms were considered: meiofauna (organisms < 1 mm; Giere, 2009) and macrofauna
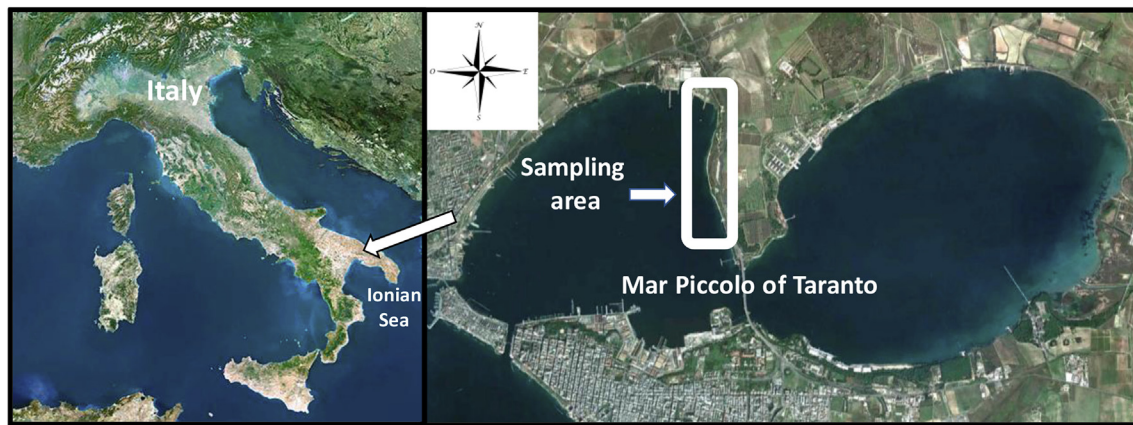
Fig. 1. Study area; white rectangle indicates the sampling area.

(organisms with a size range from 1 mm to 3 cm). In this study, both size classes were considered, while digested matter and unidentifiable fragments were excluded from analyses.

2.3. Ethics statement

This study was carried out in strict accordance with the recommendations of the Aldo Moro University of Bari (AMUB) ethics protocols and it was approved by the Italian Ministry of the Environment, Land and Sea Protection (Permit Number: 6263/2016). Maximum efforts were made to minimize handling and stress of animals.

2.4. Prey sampling and analysis

To examine the availability and abundance of potential prey in the studied area, samples of sediments were collected in the same habitats as the samples of gut contents: *Cladophora prolifera*, *Corallina elongata* and Sandy sediments. The first 2 cm of sediments in case of the Sandy bottom, and 2 cm of both sediments and algal fragments in case of *C. prolifera* and *C. elongata*, were manually sampled in three replicate cores (diameter 3.7 cm, length 20 cm). This sampling method allowed the study of epiphytic, epibenthic and endobenthic organisms, the most

important prey items for *H. guttulatus* (Kitsos et al., 2008). Planktonic prey was not considered because of the difficulties in coupling sampled animals with specific habitats and the accuracy of the sampling procedure whose scale (cores) was not suitable for plankton. Although adopted sampling method does not allow to determine complete faunal spectrum present in Mar Piccolo of Taranto and therefore, *H. guttulatus* potential prey, it however accurately displays the main portion of fauna, and it is sufficient to provide useful information on prey availability within different habitats.

Obtained samples were fixed in 4% buffered formaldehyde in filtered (0.3 μm mesh) seawater solution and then taken to the laboratory for analysis. Except for *C. prolifera*, meiofaunal organisms were extracted from the sediments and algal fragments by a decantation method. This method included adding of the water to each sample, subsequently stirred and decanted in a plastic graduated cylinder (Armenteros et al., 2008). After decantation, the supernatant was firstly sieved through 1 mm mesh to separate larger organisms and then through 30 μm mesh. Procedures of stirring, decanting and filtering were repeated ten times per sample. Because the sample of sediment associated with *C. prolifera* was muddy, it was subjected to the density gradient procedure, recommended for muddy and detrital sediments (Heip et al., 1985). In these samples, the algal fraction was washed in freshwater and then vigorously shaken several times to remove

Table 1
Total abundance (N = mean ± standard deviation), relative abundance (N%), the frequency of occurrence (O%) and preponderance index (IP) of prey taxa found in the gut of *Hippocampus guttulatus* in three different habitats (*Corallina elongata*, Sandy bottom and *Cladophora prolifera*). Higher taxa in bold.

Prey taxa	*Corallina elongata*				Sandy bottom				*Cladophora prolifera*			
	N	N%	O%	IP	N	N%	O%	IP	N	N%	O%	IP
Nematoda	12.4 ± 8.9	22.4	100	23.6	5.0 ± 8.0	36.1	94.1	44.6	4.0 ± 3.5	14.6	96.0	16.1
Copepoda	14.5 ± 10.0	26.1	100	27.5	4.1 ± 3.3	24.8	88.2	28.7	8.7 ± 5.8	32.1	96.0	35.4
Harpacticoida	12.2 ± 8.4	21.9	100		2.4 ± 2.3	14.4	79.4		7.2 ± 5.9	26.5	92.0	
Calanoida	0.0	0.0	0.0		0.2 ± 0.5	1.1	16.7		0.1 ± 0.4	0.3	4.0	
Cyclopoida	0.2 ± 0.5	0.4	16.7		0.1 ± 0.3	0.7	11.8		0.8 ± 2.0	2.8	32.0	
Nauplia	2.1 ± 3.0	3.8	70.8		1.4 ± 1.7	8.6	64.7		0.7 ± 1.3	2.5	28.0	
Polychaeta	0.6 ± 0.8	1.1	50.0	0.6	0.0 ± 0.2	0.2	2.9	0.0	0.2 ± 0.4	0.7	20.0	0.2
Ostracoda	0.5 ± 0.8	0.8	29.2	0.3	0.0 ± 0.2	0.2	2.9	0.0	0.0	0.0	0.0	0.0
Amphipoda	15.7 ± 11.9	28.2	95.8	28.5	0.7 ± 1.2	4.5	38.2	2.3	10.1 ± 9.9	37.4	88.0	37.8
Unidentified	15.3 ± 12.0	27.5	95.8		0.4 ± 1.0	2.5	23.5		9.6 ± 9.9	35.4	84.0	
Caprellidae	0.4 ± 0.7	0.8	29.2		0.3 ± 0.8	2.0	23.5		0.6 ± 0.9	2.1	36.0	
Isopoda	10.0 ± 5.8	18.1	100	19.1	1.6 ± 2.4	9.7	55.9	7.1	2.2 ± 3.1	8.3	72.0	6.9
Unidentified	8.5 ± 5.7	15.4	100		1.6 ± 2.4	9.5	52.9		1.5 ± 2.0	5.6	68.0	
Asellota	1.5 ± 2.0	2.7	58.3		0.0 ± 0.2	0.2	2.9		0.7 ± 2.2	2.7	16.0	
Tanaidacea	0.1 ± 0.3	0.2	12.5	0.0	0.0			0.0	0.0	0.0	0.0	0.0
Galatheoidea	1.0 ± 2.5	1.7	16.7	0.3	3.3 ± 4.7	20.3	61.8	16.5	1.8 ± 3.8	6.5	48.0	3.6
Paguroidea	0.0 ± 0.2	0.1	4.2	0.0	0.3 ± 0.5	1.8	26.5	0.6	0.0	0.0	0.0	0.0
Mysidacea	0.6 ± 2.7	1.1	8.3	0.1	0.1 ± 0.3	0.7	11.8	0.1	0.0 ± 0.2	0.1	4.0	0.0
Bivalvia	0.0	0.0	0.0	0.0	0.0 ± 0.2	0.2	2.9	0.0	0.0	0.0	0.0	0.0
Gastropoda	0.0	0.0	0.0	0.0	0.1 ± 0.6	0.9	8.8	0.0	0.0	0.0	0.0	0.0
Acarina	0.1 ± 0.3	0.2	8.3	0.0	0.1 ± 0.3	0.5	8.8	0.1	0.0	0.0	0.0	0.0
Pycnogonida	0.0	0.0	0.0	0.0	0.0 ± 0.2	0.2	2.6	0.0	0.0 ± 0.2	0.1	4.0	0.0

meiofauna. Meiofauna was sieved through 30 μm mesh after the previous sieving through 1 mm mesh. Sediments were sieved through 1 mm and 30 μm meshes; fraction remaining on the latter mesh was resuspended and centrifuged for three times using Ludox HS40 (density 1.18 g cm^{-3}; Heip et al., 1985). The material obtained by both procedures was preserved in 50-ml tubes, in 4% buffered formalin and stained with Rose Bengal. Prey > 1 mm (i.e. macrofauna) was retained on 1 mm mesh and preserved in 4% formalin. Two size classes of organisms were counted and classified at the major taxa level of taxonomic discrimination using a stereomicroscope. Total abundance and number of taxa were reported to the surface unit (10 cm^{-2}).

2.5. Data analysis

The data obtained from gut contents were analysed using the following indices: percentage frequency of occurrence (%O) (number of guts containing Prey item i ÷ total number of nonempty guts x 100), percentage relative abundance (%N) (number of individuals of Prey item i ÷ total number of all prey items x 100), and index of preponderance (IP), which combines the value of %O and %N and produces a single value for each attribute based on the frequency of occurrence (%O) and relative abundance (%N), using the equation:

$$IP = \frac{\%Ni \; x\%Oi}{\Sigma \; (\%Ni \; x\%Oi)} x100$$

where %N and %O are relative abundance and occurrence of prey i (*sensu* Castro et al., 2008).

Trophic niche breadth was calculated using Levin's standardised index (B) (Hurlbert, 1978; Krebs, 1998) as follows:

$$B_i = 1/n - 1 \left(\frac{1}{\sum_j P_{ij}^2} - 1 \right)$$

where B$_i$ is Levin's standardised index for Predator i, P$_{ij}$ is the proportion of Prey j in the diet of Predator i, and n is the number of prey categories. This index varies from 0 to 1; low values indicate a diet dominated by few prey items (specialised predators) whereas high values indicate a diet dominated by several prey items (generalist predator; Gibson and Ezzi, 1987; Krebs, 1998).

Moreover, to interpret data of gut contents, graphical analysis proposed by Amundsen et al. (1996) was also used. This method, modifying the traditional approach of Costello (1990), is based on a two-dimensional representation of dietary composition where each point represents the frequency of occurrence and the prey-specific abundance (Pi). Prey-specific abundance is defined as the percentage a prey taxon comprises out of all prey items in only those predators in which the given prey occurs. Finally, to compare feeding habits of the long-snouted seahorses with the availability of potential food resources in natural habitats, index of electivity (Ivlev, 1981) has been calculated as follows:

$$E = \frac{r_i - p_i}{r_i + p_i}$$

where E is the measure of electivity, r$_i$ is the relative abundance (%) of prey i in the gut, while p$_i$ is the relative abundance (%) of the same prey item in the sediment samples. This index scales from +1 to −1, where +1 indicates an active selection of a given prey, 0 indicates a random selection or no given prey consumption, while −1 indicates avoidance or inaccessibility of a given prey item (Strauss, 1979). However, the same author (Strauss, 1979) suggested that a value of +1 may indicate the absence of prey item in the habitat sample, while a value – 1 may indicate the absence of prey item in the gut.

To test differences among the gut contents of *H. guttulatus* sampled in three different habitats, univariate and multivariate distance-based permutational nonparametric analyses of variance (PERMANOVA; Anderson 2001; McArdle and Anderson, 2001) were performed on total

abundance, abundance of different size classes (< 1 mm, > 1 mm), taxa richness and community composition of prey items, including habitat (three levels: *Corallina elongata*, Sandy bottom and *Cladophora prolifera*) as fixed factor. PERMANOVAs were based on Bray–Curtis similarity matrices after fourth root transformation of the abundance data, using 9999 random permutations of the appropriate units (Anderson, 2001). When significant differences were found, a pairwise comparison was performed to explore differences among all pairs of factor levels. SIMPER analyses, based on the Bray–Curtis similarity matrix and calculated from the fourth-root-transformed prey abundance data, were performed to assess dissimilarities among gut contents of *H. guttulatus* sampled in three different habitats and to determine prey items which most significantly contribute to the observed dissimilarities. This similarity matrix was also applied to produce a non-metric, multi-dimensional scaling two-dimensional plot (MDS). Moreover, to investigate effects that seahorse size and sex might have on gut contents, a regression analysis between seahorse size and prey abundance (Statistica 6.0, StatSoft) was performed together with the PERMANOVA analysis on prey abundance comparing males-females and using sex as a fixed factor with two levels.

The same statistical design was adopted on Bray–Curtis similarity matrix of the fourth root transformed data of benthic fauna in the sediment to test differences in the availability and abundance of potential prey among three habitats. For this data, due to the restricted number of unique permutations in the pairwise tests, p values were obtained from Monte Carlo samplings (Anderson and Robinson, 2003). Statistical analyses were performed using the PRIMER v6+ software (Plymouth Marine Laboratory; Clarke, 1993; Clarke and Warwick, 1994). In both statistical analysis (gut and sediment contents) and calculation of indices, initially identified taxa were combined to a major taxonomic level. For instance, copepod harpacticoida, cyclopoida, calanoida and nauplia were considered a single taxon (i.e. Copepoda). Therefore, 19 prey items were reduced to 14 items (higher taxa), while 15 taxa found in the sediments were reduced to 12 higher taxa.

3. Results

3.1. Diet composition

Overall standard length (SL) of 59 females and 24 non-brooding males ranged from 90 to 130 mm with a mean value of 101.3 ± 10.0 mm and showed no significant differences among three habitats (PERMANOVA; pseudoF$_{2,82}$ = 1,72 P = 0.19).

A total of 2565 prey items were identified and then classified in 19 prey categories, subsequently reduced to 14 higher taxa (Table 1). Four seahorses with empty guts (4.6% of the sampled individuals) were found on Sandy bottom and were excluded from further analysis. No significant differences (PERMANOVA; pseudoF$_{1,82}$ = 0,90 P = 0.35) in prey abundance comparing males and females and no significant correlation (R = 0.018; P = 0.87) between seahorse size and prey abundance were observed.

Overall analysis of the gut contents showed that the diet of long-snouted seahorses consisted mainly of Nematoda (O% = 96; N% = 23.3%), followed by Copepoda harpacticoida (O% = 94; N% = 27.4), Isopoda (O% = 73; N% = 13.7), Amphipoda (O% = 70; N% = 25.5) and Galatheoidea (O% = 45; N% = 7.0), while other groups provided a percentage contribution lower than 2%. The frequency of occurrence (%) and relative abundance (%) of single taxa among habitats are reported in Table 1.

Significant differences both in the mean prey abundance (PERMANOVA; pseudoF$_{2,82}$ = 40.8, P < 0.001) and the mean number of taxa (PERMANOVA; pseudoF$_{2,82}$ = 7.2, P < 0.001) were found when *H. guttulatus* gut contents were analysed among different habitats (Fig. 2 a, b; see Tables 2 and 3 in Ape et al., 2019 *submitted*). In particular, as revealed by the Pairwise test, mean prey abundance was significantly higher in samples collected on *C. elongata* compared to the other two
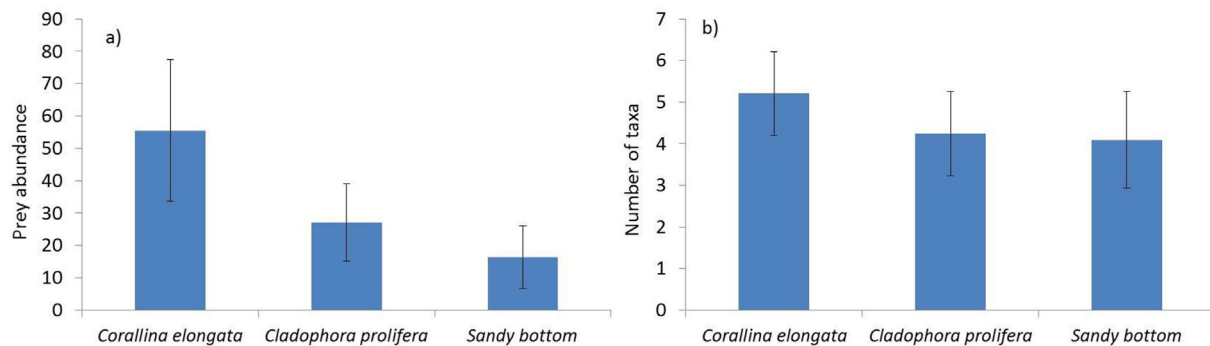
Fig. 2. a) Abundance (mean ± standard deviation) and b) Number of taxa (mean ± standard deviation) of prey items in the guts of *Hippocampus guttulatus* sampled in three different habitats: *Corallina elongata*, Sandy bottom and *Cladophora prolifera*.

habitats ($P < 0.001$). The same test also showed that the mean prey abundance was significantly higher on *C. prolifera* than on Sandy bottom ($P < 0.001$). The mean number of prey taxa, however, was significantly higher in the specimens sampled on *C. elongata* than on *C. prolifera* and Sandy bottom (pairwise, $P < 0.01$) (Fig. 2 b).

The dietary composition of *H. guttulatus* among different habitats also displayed significant differences (PERMANOVA; pseudo $F_{2,82} = 14.0$, $P < 0.001$). SIMPER analysis (Table 2) and MDS (Fig. 3 a) showed that there was a higher dissimilarity between Sandy bottom and vegetated habitats than between *C. elongata* and *C. prolifera*. Observed dissimilarity was explained by the presence of abundant Amphipoda, Isopoda, Galatheoidea and Copepoda. In fact, in *C. elongata* and *C. prolifera* habitats, the long-snouted seahorse mainly fed on Amphipoda (IP = 28.5 and 37.8, respectively), Copepoda (IP = 27.5 and 35.4, respectively), Nematoda (IP = 23.6 and 16.0, respectively) and Isopoda (IP = 19.1 and 6.9, respectively) (Fig. 4 a, b and Table 1). Differently, *H. guttulatus* individuals collected on the Sandy bottom mainly fed on Nematoda (IP = 44.6), Copepoda (IP = 28.7), Galatheoidea (IP = 16.5) and Isopoda (IP = 7.1) (Fig. 4 c and Table 1). The lowest value of trophic niche breadth calculated by Levin's index was found on the Sandy bottom (0.26), while the same index on *C. elongata* and *C. prolifera* was 0.33 and 0.38, respectively.

Considering the two prey size classes among all examined habitats, the percentage contribution of prey < 1 mm was higher than that of the prey > 1 mm; prey < 1 was principally represented by Copepoda and Nematoda and its percentage contribution was 54 ± 22% on *C. prolifera*, 63 ± 25% on Sandy bottom and 66 ± 14% on *C. elongata*. Differently, prey > 1 mm was mostly represented by Amphipoda and Isopoda in the vegetated habitats and by Galatheoidea on the Sandy bottom. This prey size class had the lowest values on *C. elongata* (34 ± 15%) and Sandy bottom (35 ± 25%), while the highest percentage contribution was reached on *C. prolifera* (46 ± 22%). Results of PERMANOVA test showed that the number of prey < 1 mm (36.5 ± 17.9 items) was significantly higher (pairwise, $P < 0.001$) on *C. elongata* compared to the other two habitats (10.5 ± 9.2 items on Sandy bottom and 14.6 ± 7.2 on *C. prolifera*). The number of prey > 1 mm (ranging from 5.9 ± 5.8 items on Sandy bottom to 19.0 ± 10.0 items on *C. elongata*) was significantly higher on *C. elongata* only compared to the Sandy bottom (pairwise, $P < 0.001$). The abundance

of two prey size classes and results of the PERMANOVA analysis performed on gut contents are reported in Ape et al., 2019 *submitted* (see Tab. 2, 3 and 4).

3.2. Availability of potential prey

Sampling in three different habitats permitted characterization of benthic assemblages and the identification of organisms/taxa that might be considered as potential prey of seahorses (Table 3).

Mean abundance of benthic organisms ranged from 449.7 ± 74.6 ind.10 cm^{-2} on *C. prolifera* to 1703.5 ± 306.5 ind.10 cm^{-2} on C. *elongata* (Fig. 5 a; Table 3). When compared to the other two habitats, *Corallina elongata* resulted as habitat with the significantly most abundant and diversified (number of higher taxa) faunal community (PERMANOVA, pairwise; $P < 0.01$ for abundance and $P < 0.05$ for number of taxa) (Fig. 5 a, b).

Significant differences in the benthic faunal community composition (PERMANOVA; pseudo $F_{2,82} = 13.5$, $P < 0.001$) among habitats were also observed. SIMPER analysis (Tab. 4) and MDS (Fig. 3b) showed that the highest dissimilarity existed between *C. elongata* and other two habitats. Benthic community of *C. elongata* was dominated by Nematoda, followed by Copepoda harpacticoida, Polychaeta and Amphipoda (Table 3). Sandy bottom and *C. prolifera* showed a very similar benthic community structure (Tab. 4). In these habitats, benthic communities were dominated by Copepoda harpacticoida, followed by Nematoda, Polychaeta and Amphipoda (Table 3).

Among sampled sediments, organisms < 1 mm (meiofauna) represented more than 95% of the total abundance whereas the percentage of organisms > 1 mm was very low; they were mainly represented by Amphipoda and Isopoda, whose values were 0.8 ± 0.3% on *C. elongata*, 1.2 ± 0.5% on Sandy bottom and 4.3% on *C. prolifera*. However, results of PERMANOVA test revealed no significant differences in the abundance of organisms > 1 mm among habitats (from 7 ± 1.7 on Sandy bottom to 15.7 ± 9.5 ind/10 cm^2 on *C. prolifera*). Differently, significant differences (PERMANOVA; pseudo$F_{2,8} = 33.9$, $P < 0.001$) in the abundance of organisms < 1 mm were observed; significantly higher values (pairwise, $P < 0.01$) were found on *C. elongata* (1690.5 ± 308.5 ind.10 cm^{-2}) compared to the other two habitats (430.3 ± 83.4 on *C. prolifera* and 600.0 ± 137.1 ind.10 cm^{-2}

Table 2
The output of SIMPER analyses and PERMANOVA pairwise carried out on the gut contents of *Hippocampus guttulatus*. Prey taxa included in this table were responsible for 60% cumulative dissimilarity among different habitats (*Corallina elongata*, Sandy bottom and *Cladophora prolifera*). (P (perm) = probability level).

| | SIMPER | | PERMANOVA Pairwise | |
	% Dissimilarity		t	P (perm)
Group				
Corallina elongata vs Sandy bottom	46.2	Amphipoda (22%), Isopoda (17%), Galatheoidea (13%), Copepoda (10%)	4.6	0.001
Corallina elongata vs Cladophora prolifera	31.1	Isopoda (20%), Amphipoda (15%), Galatheoidea (14%), Nematoda (13%)	2.9	0.001
Sandy bottom *vs Cladophora prolifera*	41.1	Amphipoda (26%), Galatheoidea (17%), Isopoda (15%), Copepoda (12%)	3.1	0.001

Table 3
Total (N = mean ± standard deviation) and relative abundance (N%) of benthic fauna found in the sediments of three different habitats (*Corallina elongata*, Sandy bottom and *Cladophora prolifera*). In bold the higher taxa.

Taxa	*Corallina elongata* N (ind.10 cm^{-2})	N%	Sandy bottom N (ind.10 cm^{-2})	N%	*Cladophora prolifera* N (ind.10 cm^{-2})	N%
Nematoda	790.0 ± 388.0	46.4	122.0 ± 13.5	20.1	92.3 ± 11.0	20.5
Copepoda	398.5 ± 78.5	23.4	403.0 ± 136.8	66.4	262.0 ± 73.0	58.3
Harpacticoida	322.0 ± 52.0	18.9	244.0 ± 72.3	36.9	226.0 ± 58.9	50.3
Cyclopoida	8.0 ± 4.0	0.5	2.3 ± 1.5	0.4	4.3 ± 1.5	1.0
Nauplia	68.5 ± 29.5	4.0	176.7 ± 65.6	29.1	31.7 ± 14.5	7.0
Polychaeta	379.5 ± 13.5	22.3	66.3 ± 21.0	10.9	49.0 ± 15.1	10.9
Ostracoda	22.0 ± 1.0	1.3	0.0	0.0	7.0 ± 4.0	1.6
Amphipoda	73.0 ± 2.0	4.3	13.7 ± 3.5	2.3	33.7 ± 13.7	7.5
Isopoda	30.5 ± 13.5	1.8	0.7 ± 1.2	0.1	3.7 ± 1.2	0.8
Unidentified	5.5 ± 4.5	0.3	0.7 ± 1.2	0.1	2.0 ± 0.0	0.4
Asellota	25.0 ± 9.0	1.5	0.0	0.0	1.7 ± 1.2	0.4
Tanaidacea	4.5 ± 3.5	0.3	0.0	0.0	0.0	0.0
Bivalvia	1.0 ± 0.0	0.1	0.3 ± 0.6	0.1	1.0 ± 1.7	0.2
Gastropoda	3.5 ± 1.5	0.2	0.3 ± 0.6	0.1	0.0	0.0
Acarina	0.5 ± 0.5	0.0	0.0	0.0	0.0	0.0
Turbellaria	0.7 ± 1.2	0.0	0.7 ± 1.2	0.1	0.7 ± 1.2	0.1
Ophiuroidea	0.0	0.0	0.0	0.0	0.3 ± 0.6	0.1
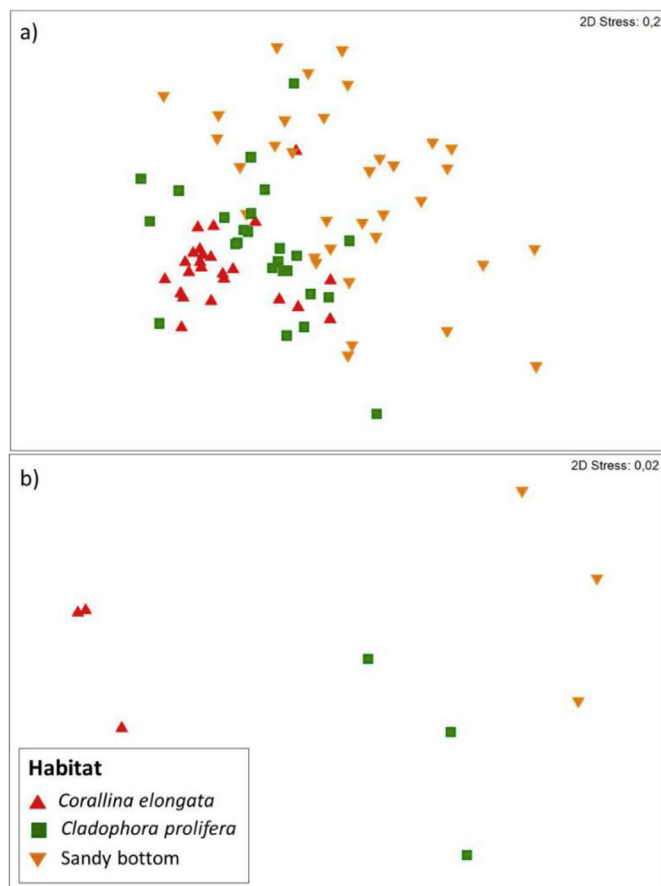
Fig. 3. Multi-dimensional scaling (MDS) analysis performed using a) taxonomic composition of gut contents of *Hippocampus guttulatus* and b) taxonomic composition of fauna in three different habitats: *Corallina elongata*, Sandy bottom and *Cladophora prolifera*.

on Sandy bottom). The abundance of two prey size classes and results of the PERMANOVA analysis performed on sediment fauna are reported in Ape et al., 2019 submitted (see Tab. 2, 5 and 6).

3.3. Selectivity of prey

Ivlev's electivity index, applied to the fourteen prey items from both gut contents and habitats showed a high selection of Galatheoidea, Mysidacea and Isopoda in all habitats (E > 0.82) (Fig. 6 a, b, c). Amphipoda showed a mixed trend since they were actively selected in vegetated habitats (E = 0.74 on *C. elongata* and E = 0.67 on *C. prolifera*) but only randomly preyed at Sandy bottom (E = 0.33) (Fig. 6 a, b, c). Copepoda and Nematoda were randomly eaten in all habitats (ranging from E = - 0.46 on Sandy bottom to E = 0.06 on *C. elongata* and from E = - 0.35 on *C. prolifera* to E = 0.28 on Sandy bottom, respectively). Polychaeta was avoided or was inaccessible in all habitats (E < - 0.87). Acarina resulted highly selected on *C. elongata* and Sandy bottom. Ostracoda was actively selected and preyed on Sandy bottom, randomly eaten on *C. elongata*, and completely avoided on *C. prolifera* (E = - 1.00). Bivalvia and Gastropoda were highly selected on Sandy bottom (E = 0.53 and 0.88 respectively), while completely avoided or inaccessible in vegetated habitats. Pycnogonida, a negligible prey item in the gut contents of *H. guttulatus*, resulted selected on Sandy bottom and *C. prolifera*.

4. Discussion

The present study demonstrated that the diet of *H. guttulatus* is mainly based on small crustaceans, in particular Amphipoda and Copepoda, and it also highlighted the consistency of the observed feeding pattern with findings from previous studies (Kitsos et al., 2008; Gurkan et al., 2011; Valladares et al., 2017). Moreover, this study demonstrated that soft-bodied organisms and large-sized crustaceans, such as Galatheoidea and Paguroidea, are also important resources in the seahorse's diet. In agreement with Castro et al. (2008), flushing method adopted by this study and short time-lapse between the capture of individuals and collection of gut contents allowed to appreciate the importance of prey without calcareous/chitinous exoskeleton, such as nematodes. However, high abundance of nematodes in the gut contents might be the result of prey availability rather than the seahorse's preference since peculiar environmental features of Mar Piccolo of Taranto (high trophic level, organically enriched sediments) favor the settlement of rich Nematoda community (Mirto et al., 2000; Vezzulli et al., 2003) in all studied habitats.

Long-snouted seahorses are usually associated with structurally complex habitats, such as seagrasses, brown algae and anthropic debris (Curtis and Vincent, 2005; Lazic et al., 2018). However, as stated by James and Heck (1994) and Felício et al. (2006), seahorses are showing trophic flexibility and can modify their foraging strategy depending on the habitat complexity. In complex vegetated habitats, seahorses can use an "ambush" foraging strategy when they wait for prey from a hidden position and then launch a rapid surprise attack (Sih et al.,
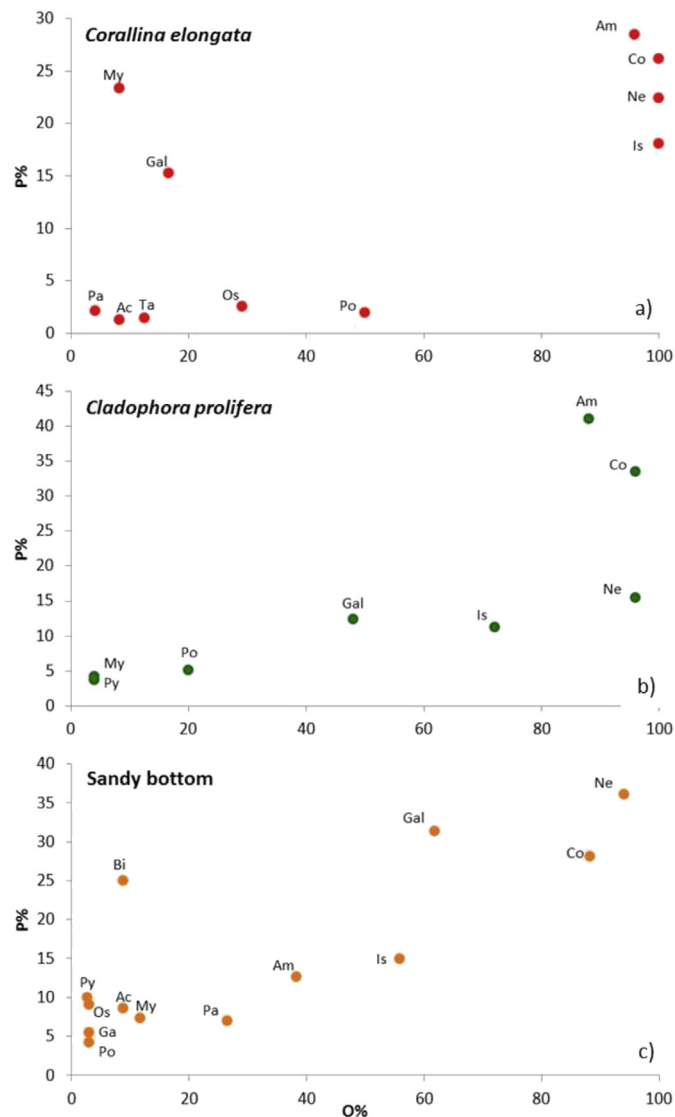
Fig. 4. Feeding strategy diagram. Prey-specific abundance (P%) plotted against frequency of occurrence (O%) of prey items in the diet of *Hippocampus guttulatus* sampled in three different habitats: a) *Corallina elongata*, b) *Cladophora prolifera* and c) Sandy bottom. Prey items: Amphipoda (Am), Copepoda (Co), Nematoda (Ne), Isopoda (Is), Galatheoidea (Gal), Mysidacea (My), Polychaeta (Po), Ostracoda (Os), Tanaidacea (Ta), Acarina (Ac), Paguroidea (Pa), Pycnogonida (Py), Bivalvia (Bi) and Gastropoda (Ga).

1998). In uncovered areas (e.g. sand), however, they are able to pursue prey actively and feed while swimming (James and Heck, 1994; Felício et al., 2006). Accordingly, as shown in this study, the long-snouted seahorse *H. guttulatus* is capable of modulating feeding strategy by catching the prey that is more accessible in a specific habitat.

Unlike what reported by Gurkan et al. (2011) and Curtis and Vincent (2005) in structurally complex habitats such as *P. oceanica* meadows, in this study, the diet of *H. guttulatus* seems to be only rarely and occasionally based on the planktonic prey. Instead, it seems largely focused on benthic or epibenthic organisms both when referred to the bare sandy bottom, where seahorses can 'crawl' or 'walk' on sediments (Hicks and Coull, 1983; Ryer, 1988) while actively searching for prey, and vegetated habitats, where seahorses by adopting a "sit and wait" foraging strategy (James and Heck, 1994; Felício et al., 2006) can exploit the prey in algal and adjacent bottoms habitats. However, considering the importance of planktonic prey in the diet of juvenile seahorses (Kanou and Kohno, 2001; Castro et al., 2008), further investigations at different life-stage levels (juveniles and adults) should also include planktonic components.

H. guttulatus individuals collected on *C. elongata* fed primarily on crustaceans (Amphipoda, Copepoda and Isopoda) and Nematoda. Moreover, observation of the benthic community and analysis of the Ivlev's electivity index demonstrated that Isopoda, Galeotheoidea and Mysidacea are actively selected, while Nematoda and Copepoda harpacticoida are only randomly selected, probably due to their high abundance within the benthic assemblage. Previous studies have described high density and diversity of benthic fauna associated with *Corallina* spp. Habitat (Hicks, 1977; Gibbons, 1988). This could also explain the high number of prey ingested by *H. guttulatus* that in this complex habitat could efficiently use "sit and wait" foraging strategy (James and Heck, 1994; Felício et al., 2006), in accordance with what has already been observed in other small predator fish, such as blennies *Bellapiscis medius* (Coull and Wells, 1983) and *Clinus superciliosus* (Gibbons, 1988) on *Corallina* spp. Furthermore, complexity of *C. elongata* turf and the consequent high availability of small-sized prey (< 1 mm), generally preferred by juvenile seahorses (Woods, 2002; Foster and Vincent, 2004; Castro et al., 2008), can explain the role of this habitat as a recruitment area of *H. guttulatus* juveniles (Gristina et al., 2017).

Diet of *H. guttulatus* on *Cladophora prolifera* was mainly based on Amphipoda (selectively predated), Copepoda and Nematoda (only randomly eaten), while Polychaeta, Ostracoda and Bivalvia appeared to be completely avoided or inaccessible in this habitat. *C. prolifera* beds, due to their rapid turnover, give rise to a rich detritus food chain suitable for detritivorous crustaceans (Levinton and McCartney, 1991), thus supporting the adult fraction of *H. guttulatus* population (Gristina et al., 2017). Results of the present study seem to encourage this statement since the consumption of adult-preferable larger prey (> 1 mm) in this habitat was higher respect to the other two habitats and was principally based on amphipods.
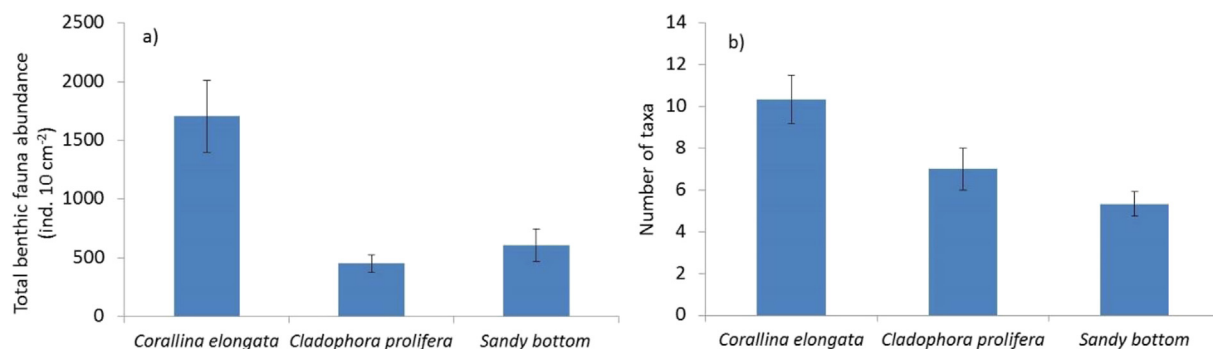

Fig. 5. a) Abundance (mean ± standard deviation) and b) Number of taxa (mean ± standard deviation) of benthic fauna organisms in the sediments sampled in three different habitats: *Corallina elongata*, Sandy bottom and *Cladophora prolifera*.

Table 4
The output of SIMPER analyses and PERMANOVA pairwise carried out on the benthic community composition. Taxa included in this table were responsible for 60% cumulative dissimilarity among different habitats (*Corallina elongata*, Sandy bottom and *Cladophora prolifera*). (P (perm) = probability level).

Group	SIMPER % Dissimilarity		PERMANOVA Pairwise t	P (perm)
Corallina elongata vs Sandy bottom	32.6	Ostracoda (16%), Isopoda (14%), Nematoda (14%), Polychaeta (12%), Tanaidacea (10%)	4.7	0.003
Corallina elongata vs Cladophora prolifera	26.9	Nematoda (19%), Polychaeta (15%), Tanaidacea (12%), Gastropoda (12%), Isopoda (8%)	2.3	0.003
Sandy bottom *vs Cladophora prolifera*	19.1	Ostracoda (27%), Isopoda (17%), Bivalvia (9%), Copepoda (9%)	2.1	0.043
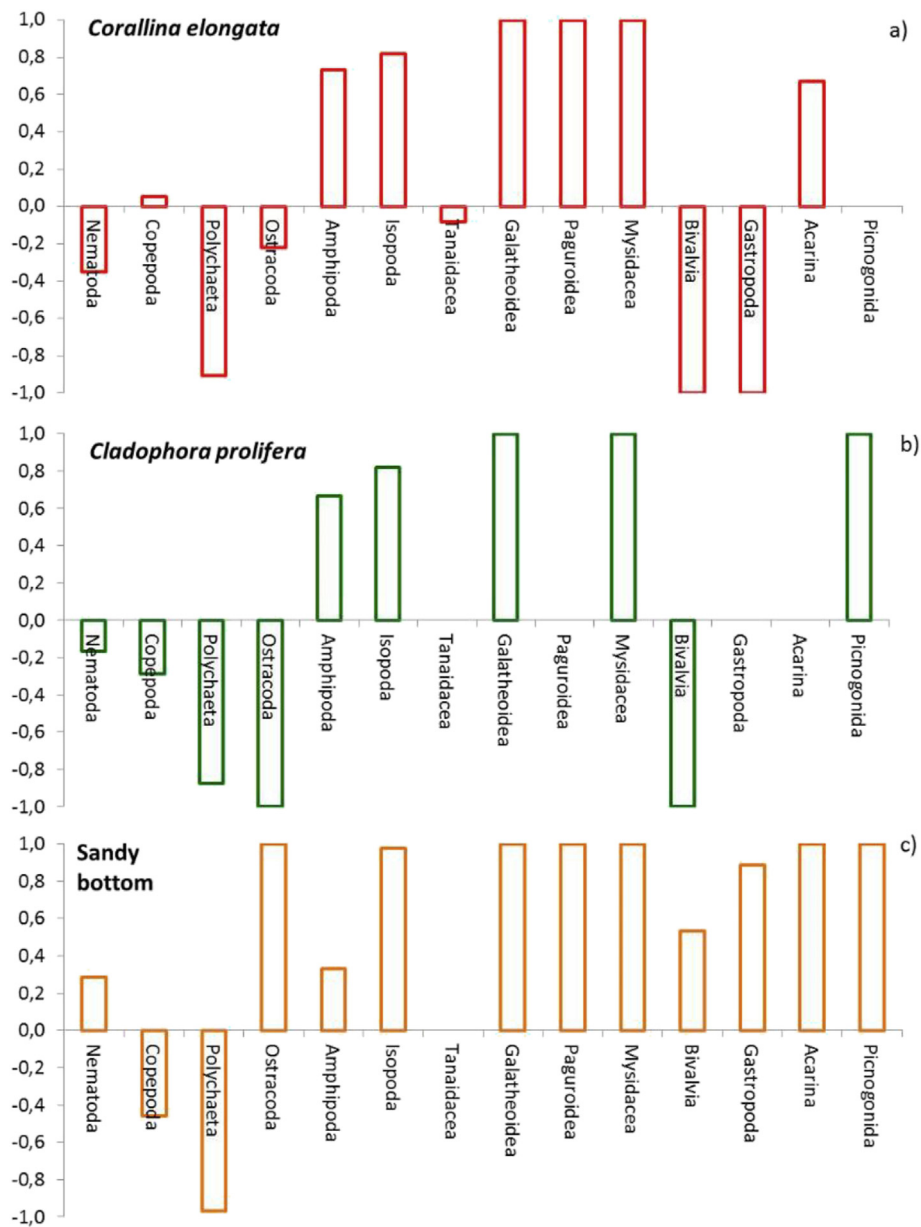
Fig. 6. Ivlev's electivity index applied to the fourteen prey items in the guts of *Hippocampus guttulatus* in the environment of three different habitats: a) *Corallina elongata*, b) *Cladophora prolifera* and c) Sandy bottom.

On the Sandy bottom, *H. guttulatus* displayed different feeding habits in comparison with vegetated habitats. In this habitat, long-snouted seahorses preferred Nematoda, Copepoda and Galatheoidea. Ivlev's electivity index underlined a random predation of Nematoda, Copepoda and Amphipoda, a complete avoidance or inaccessibility of Polychaeta and an active selection of other prey taxa, among which Ostracoda, Gastropoda and Bivalvia were selected only in this habitat, probably because more accessible. In this habitat, a particularly interesting result

regarded capture and ingestion of Paguroidea. This prey item (ranging from 5 mm to approximately 2 cm), as well as Galatheoidea, was actively selected by *H. guttulatus*. However, these organisms were not present in the sampled benthic community probably due to the adopted sampling technique (cores of 3.7 cm in diameter) that was not suitable for the sampling of large vagile fauna. As suggested by Woods (2002) and Felício et al. (2006), *H. guttulatus* can consume prey that is larger (1–2 cm) than its maximum mouth gape by breaking it into small

pieces. On the contrary, in this study, large Paguroidea were found completely intact in the guts, suggesting that long-snouted seahorses are able to extract Paguroidea from the shell by sucking and then ingest it while integer (see the video presented in Ape et al., 2019 *submitted*).

5. Conclusions

Results discussed in the present paper describe the capacity of long-snouted seahorses to feed on both complex and barren substrates. Although these results have shown that the higher number of prey is consumed in vegetated habitats that on the sandy bottom, differences in dietary composition evidenced that *H. guttulatus* is able to actively feed on specific prey items and modulate prey exploitation by adapting to different habitats and consequent prey abundance and availability. In accordance with this, it was observed that seahorses are able to relocate in a new habitat and modulate the prey choice when their elective habitat become depleted by the anthropic impact (Caldwell and Vincent, 2013).

However, decline and fragmentation of coastal habitats (Calizza et al., 2013) are considered as the main threats to European seahorse populations (both *H. guttulatus* and *H. hippocampus*) (Foster and Vincent, 2004).

Due to the ability of *H. guttulatus* to relocate and modulate its feeding habits by adapting to the habitat, the impacts that coastal habitat loss and fragmentation could have on seahorse populations do not seem determined by food availability.

In light of this, and as suggested by Manning et al. (2018) for *H. whitei*, holistic approach that takes into account both environmental and biological aspects has to be considered in order to understand variability, consistency and conservation status of *H. guttulatus* populations subjected to human pressures.

Authors' contributions

AF, SM and MG conceived the ideas and designed methodology; AF, GC, SM, CP, TL, and MG collected the samples; FA and MG analysed the data; AF, SM and MG led the writing of the manuscript. All authors contributed critically to the drafts and gave final approval for publication.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ecss.2019.04.034.

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