



# Seasonal variations in the feeding ecology of *Nephrops norvegicus* in the Adriatic Sea: insights from stomach contents and stable isotope analyses

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**ABSTRACT:** Norway lobster *Nephrops norvegicus* is one of the most important commercial species in the Mediterranean. This study focused on its feeding ecology in an important fishing ground within the Adriatic Sea, using an integrated approach of stomach content (SCA) and stable isotope (SIA) analyses. The simultaneous use of these tools represents a modern and complete approach that provides reliable data over time and reflects the feeding strategy of the analysed species. Monthly sampling was carried out (January–December 2019, July and October 2020; 589 specimens) to investigate seasonal changes in diet and trophic level. Variations in fullness, gonadosomatic and hepatosomatic indices were analysed to explore changes in feeding and variations in energy requirements linked to reproductive needs. Results showed that *N. norvegicus* mostly prey on decapod crustaceans and fish. Seasonal variations in prey consumption were likely linked to the reproductive patterns of the species. SIA results confirmed SCA findings and placed *N. norvegicus* at a high position within the benthic food web. Overall, considering that the Adriatic Sea represents one of the most important and productive fishing areas for Norway lobster, this study provides critical information for an effective ecosystem approach to fisheries management of this resource.

**KEY WORDS:** Norway lobster · Feeding habits · Stomach contents · Isotopic analysis · Trophic position · Benthic food webs · Mediterranean Sea

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## 1. INTRODUCTION

The Norway lobster *Nephrops norvegicus* (Linnaeus, 1758) is a benthic burrowing decapod crustacean inhabiting the continental shelf of the north-eastern Atlantic Ocean and the Mediterranean Sea, at depths of 50–800 m (Bell et al. 2006). The species is of great commercial importance across its entire distribution, with average catches over the period

2010–2019 of 53 493 and 3341 t yr<sup>-1</sup> for the north-east Atlantic Sea and the Mediterranean, respectively (FAO 2021). Within the Mediterranean basin, the Adriatic Sea represents one of the most important and productive fishing areas for Norway lobster (FAO 2021). Here, this species lives at depths of 50–400 m in muddy sediments (Frogliola & Gramitto 1988, Artegiani et al. 1997, Russo et al. 2018). *N. norvegicus* is strongly linked to day–night cycles

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(Aguzzi & Sardà 2008), which affect its behaviour, diet and the possibility of being caught by trawling operations; individuals are captured when they are out of their burrows for feeding and mating (Frogliia 1972, Aguzzi et al. 2021). Females are captured less often during certain periods of the year because they spend more time in their burrows incubating eggs (Newland et al. 1992, Aguzzi & Sardà 2008). Females reach the size at the onset of maturity (SOM) at different sizes, depending on the geographical area (Tuck et al. 2000). In the Mediterranean Sea, the SOM is generally reached at a range of 30–39 mm carapace length (CL) (Mytilineou et al. 1990, Relini et al. 1998). In the Mediterranean, reproduction usually takes place in June–October (with geographical variations; Relini et al. 1998, Mente et al. 2009). Due to its commercial relevance, several authors have investigated the biological and ecological characteristics of *N. norvegicus* (Stachowitsch 1992, Abelló et al. 2002, Aguzzi & Sardà 2007, Aguzzi et al. 2021). However, few studies to date have focussed on its feeding ecology (Baden et al. 1990, Cristo 1998), likely because the trituration process taking place in the foregut by means of the gastric mill (McGaw & Curtis 2013, Cau et al. 2020) makes the identification of prey difficult (Parslow-Williams et al. 2002). Nevertheless, based on the available studies, *N. norvegicus* is classified as a euryphagous and non-selective species, consuming a great variety of crustaceans, fish and mollusks, either as an active predator or a scavenger (Cristo & Cartes 1998, Cristo 1998).

The analysis of stomach contents (SCA) is one of the most widely used techniques to investigate the feeding ecology of a species (Hyslop 1980, Welden et al. 2015). However, this approach presents different shortcomings because of the complexity of taxa identification and, depending on the species' feeding behaviour, the amount of unidentifiable material (Garrison & Link 2000, Parslow-Williams et al. 2002). Moreover, SCA provides a snapshot of the diet of an individual at a certain time and space, thus requiring several samples to obtain a complete picture of the overall feeding ecology of the species (Fanelli et al. 2010). In the last decades, the use of stable isotope analysis (SIA) has proven to be particularly effective in aquatic food web studies (Vander Zanden et al. 2015) with the stable isotopes of nitrogen ( $\delta^{15}\text{N}$ ) and carbon ( $\delta^{13}\text{C}$ ) representing the most used tracers (Divine et al. 2017, McCormack et al. 2019). Carbon isotope composition in living animals usually provides clues to the origin of ingested organic matter through an increase in  $\delta^{13}\text{C}$  of ca. 1‰ per trophic

level, and it is useful for discriminating between pelagic or benthic food (Layman et al. 2012). Nitrogen isotope values are proxies of the trophic level of a species, as  $\delta^{15}\text{N}$  usually increases by 2.5–3.4‰ from prey to consumers (Post 2002, Sweeting et al. 2007, Caut et al. 2009, Layman et al. 2012). Thus, combined measurements of  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  allow trophic relationships within a food web to be revealed (Carlier et al. 2008, Layman et al. 2012). However, recent studies have identified concerns regarding the issue of unquantified error associated with using non-species-specific estimations for modelling and developing dynamic mixing models (Ballutaud et al. 2022). To fully understand temporal changes in the feeding ecology of a species, a combination of the use of SCA and SIA is highly recommended (Genner et al. 2001, Cocheret de la Morinière et al. 2003, Rybczynski et al. 2008).

The combined use of SIA and SCA, therefore, constitutes a robust approach to assess the feeding ecology of a species, its niche width and possible dietary changes in time and space (Bearhop et al. 2004, Fanelli & Cartes 2008, Fanelli et al. 2010, 2011). Together, these techniques can mitigate possible variations in the abundance or type of prey because the calculation of trophic niches through SIA is not influenced in the short term, unlike results obtained exclusively with SCA (Bearhop et al. 2004). These coupled techniques can also determine the trophic status of the species, and limit possible errors in diet determination caused by ontogenetic, environmental and prey composition changes (Genner et al. 2001, Cocheret de la Morinière et al. 2003, Bearhop et al. 2004, Rybczynski et al. 2008). This is especially true for animals such as Norway lobster that do not make large movements on the seafloor, being more territorial and therefore more vulnerable to possible changes in the composition of their prey (Aguzzi & Sardà 2007, 2008). Additionally, such an approach, extended over large temporal scales (i.e. 1 yr of monthly sampling), also allows us to link changes in food availability over time, in relation to natural variations in benthic communities or alterations due to external factors such as fishing pressure, to different food needs of the species during its life cycle and possible changes related to reproductive traits (Fanelli & Cartes 2008, Cartes et al. 2014).

In this context, the specific objectives of the present study were to (1) investigate the overall feeding ecology of *N. norvegicus* in the Central Adriatic Sea, (2) assess any seasonal variations in its diet and (3) identify potential biological drivers of the observed trends.

## 2. MATERIALS AND METHODS

### 2.1. Study area and sample collection

Specimens of *Nephrops norvegicus* were collected within the 'off Ancona' fishing grounds located in the central Adriatic Sea (Fig. 1). The area is heavily influenced by discharge from the Po River (Artegiani et al. 1997) and is dominated by sandy and muddy sediments (Spagnoli et al. 2010, Santelli et al. 2017). The 'off Ancona' fishing ground is an important area for *N. norvegicus*, where high densities have been observed at ca. 70 m depths (Frogliola et al. 1997). In this area, the reproductive period for this species in terms of ovarian maturation occurs from April–July, with a peak in May (Colella et al. 2018). For females, the SOM is reached at about 30 mm CL (Angelini et al. 2020).

A total of 589 Norway lobster specimens were collected (309 males; 280 females) at depths between 50 and 100 m within the 'off Ancona' fishing ground by the Institute for Marine Biological Resources and Biotechnology of the National Research

Council (CNR-IRBIM) during the biological sampling of commercial catches funded within the European Data Collection Framework (DCF; EU 2017). All sampled individuals were adults; no juveniles were analysed. Monthly sampling was carried out from January–December 2019 and in July and October 2020, on board a commercial bottom trawler (overall length: 25 m; tonnage: 98.5 t; engine power: 480 KW). The net cod-end had a 50 mm diamond mesh according to the Italian legislation in derogation to the European regulations concerning the Mediterranean Sea (EU 2006). Given the difficulty in obtaining samples every month from commercial fishing activity due to marine weather conditions, 2 months were added in 2020 to complete the seasonal sampling. Monthly samples were then merged, following the Northern Hemisphere seasons: 131 individuals collected in winter (January and February), 87 individuals collected in spring (March, April and May), 228 individuals collected in summer (June and July) and 143 individuals collected in autumn (September, October and December).

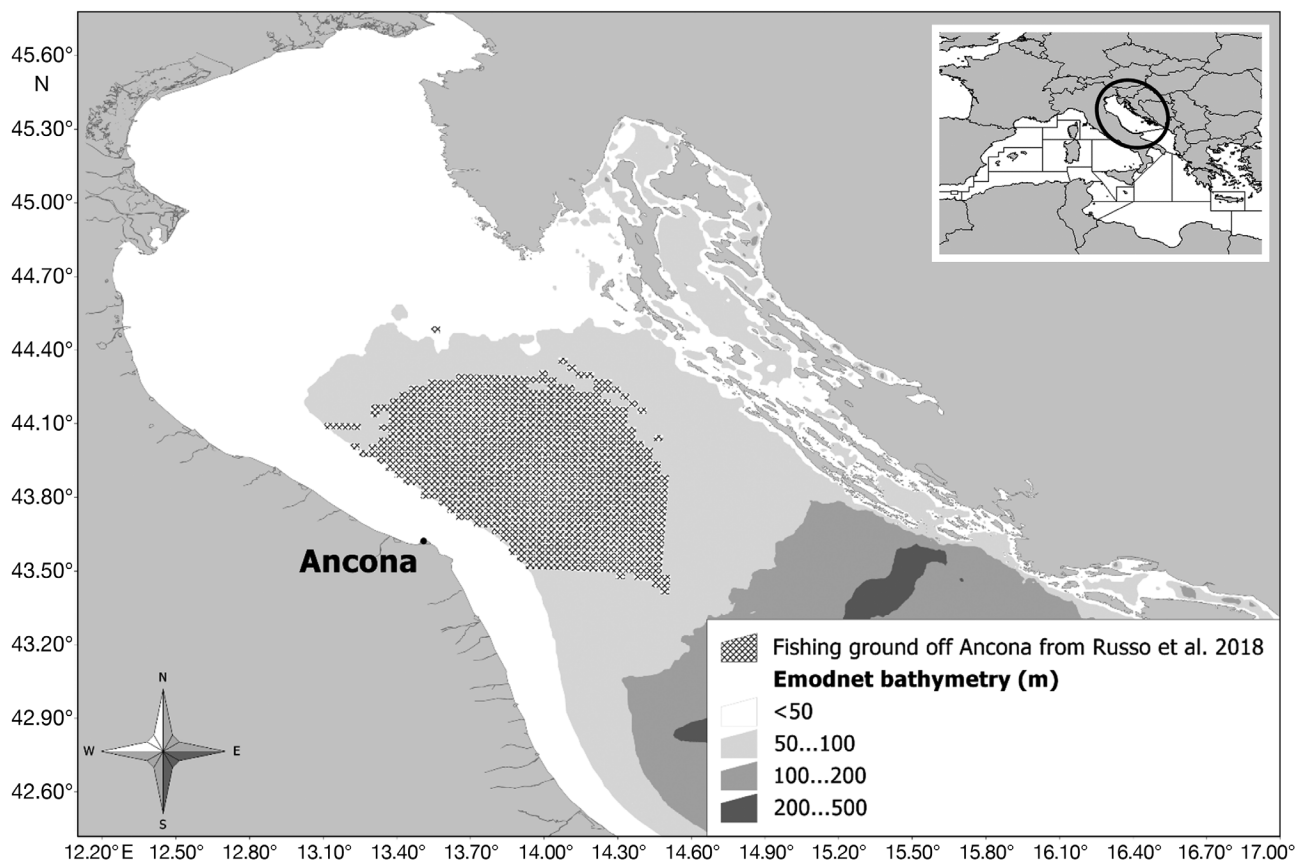


Fig. 1. The 'off Ancona' study area (highlighted by crossed cells), corresponding to the principal *Nephrops norvegicus* fishing ground within the northern-central Adriatic Sea, according to Russo et al. (2018)

## 2.2. Macroscopic and biometric evaluation and SCA

Sex was identified based on the external morphology of the first pair of pleiopods, and macroscopic maturity stage was established only for females, according to ICES criteria (ICES 2010). The sex of each individual was used to calculate the sex ratio as follows: total number of females/total number of females + total number of males. In order to obtain a length frequency distribution (LFD), for each specimen, CL was measured to the lowest mm (using a calliper). The total individual wet weight (WW, in g) was then recorded using a precision scale balance (Radwag WLC 6/ F1/K accuracy: 0.1 g). After dissection with laboratory scissors and tweezers, the gonads, hepatopancreas and full stomach weights (WW, in g) were measured using a Mettler–Toledo XP204 scale (accuracy:  $10^{-4}$  g).

Overall, 489 individuals (269 males; 220 females) were used for the SCA and to calculate the fullness index. The contents of each full stomach were extracted and the stomach walls were weighed again; by subtracting the latter measure from the full stomach weight, it was possible to determine the weight of the contents. This procedure was used to calculate stomach fullness (in %), used as an expression of feeding intensity, as follows: content weight / body weight  $\times 100$  (Fanelli et al. 2009). Gonad and hepatopancreas weights were used to determine the gonadosomatic index (GSI; %GSI = gonad weight / body weight  $\times 100$ ) and the hepatosomatic index (HSI; %HSI = hepatopancreas weight / body weight  $\times 100$ ). As some specimens were damaged and it was not possible to obtain gonad and hepatopancreas weights, only 391 individuals (230 males; 161 females) were used to calculate GSI and HSI.

These 2 indices can be good indicators of the nutritional and reproductive condition of a species (Rosa & Nunes 2002a, Zara et al. 2013). The HSI is considered a proxy for energy reserves stored in the liver (Jones & Obst 2000), while the GSI is often used as a proxy for gonad maturity effort and to highlight the different phases of the reproductive cycle (Devlamming et al. 1982). Taking into consideration that females allocate more energy to reproduction than males (Kao et al. 1999, Tsikliras et al. 2010) and that the liver plays an important role in energy storage (Papiol et al. 2014), HSI and GSI were calculated for both sexes as well as in relation to season.

The stomach contents, stored in Petri dishes, were then analysed and each prey item or piece (in the case of hard structures such as crustacean telsons, fish otoliths and cephalopod beaks) was identified to

the lowest possible taxonomic level. The recognized taxa were kept separate in the analyses to best describe the diet. As many prey items were crushed and highly digested, thus making it impossible to weigh them individually, the subjective point method (e.g. Swynnerton & Worthington 1940) was used to determine the contribution of each prey to the overall content of a stomach.

The following indices were then calculated: frequency of occurrence of prey (% $F$  = [number of stomachs containing prey / total number of stomachs]  $\times 100$ ), percentage of numeric abundance of prey (% $N$  = [number of prey / total number of prey]  $\times 100$ ) and percentage of wet weight of prey (% $W$  = [weight of prey / total weight of prey]  $\times 100$ ). These values were then used to calculate the index of relative importance (IRI) according to Pianka (1973) for each taxonomic category, expressed as %IRI =  $(IRI_i / \sum IRI) \times 100$  (Fanelli & Cartes 2008). Trophic diversity was calculated on the prey found in the stomachs of specimens sampled across the sampling period using the Shannon-Wiener index (Shannon & Weaver 1949).

## 2.3. SIA

For SIA, 26 individuals, divided by season (6 in winter, spring and summer; 8 in autumn) and sex (3–4 females and 3–4 males for each season) were selected from the total sample. Specimens were selected according to the average size observed across the sampling period; thus, females used for SIA had a mean ( $\pm$ SD) CL of  $41.5 \pm 10.45$  mm and males had an average CL of  $44.5 \pm 10.15$  mm. The dissected muscle portions were oven-dried at  $60^\circ\text{C}$  for 24 h and then ca. 0.5–1 mg of tissue was put into tin capsules (Fanelli et al. 2010). In order to avoid pseudoreplication, each individual represented a replicate within the factor season (Hurlbert 1984). The samples were then automatically loaded into an elemental analyser (Thermo Flash EA 1112) for the determination of total carbon and nitrogen and then analysed for  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  with a continuous-flow isotope-ratio mass spectrometer (Thermo Delta Plus XP). Stable isotope ratios are expressed in relation to reference international standards (atmospheric  $\text{N}_2$  and PeeDee Belemnite for  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$ , respectively), as:

$$\delta^{13}\text{C} \text{ or } \delta^{15}\text{N} = [(R_{\text{sample}}/R_{\text{standard}}) - 1] \times 10^3 \quad (1)$$

where  $R = {}^{13}\text{C}/{}^{12}\text{C}$  or  ${}^{15}\text{N}/{}^{14}\text{N}$ . Analytical precision, based on standard deviations of internal standards (International Atomic Energy Agency IAEA-CH-6; IAEA-NO-3; IAEA-N-2), ranged from 0.10–0.19‰ for

$\delta^{13}\text{C}$  and 0.02–0.08‰ for  $\delta^{15}\text{N}$ . Additionally, in order to estimate the trophic position (TP) of *N. norvegicus* in the area (see below), samples of primary consumers, i.e. *Anadara kagoshimensis* Tokunaga, 1906 and *Mimachlamys varia* Linnaeus, 1758, collected in the same fishing grounds were prepared for SIA. As lipids were not extracted from the samples, a correction for lipid contents (for samples with C:N ratios >3; Post et al. 2007) was applied, based on the equation proposed by Post et al. (2007), which considers the relationships between C:N ratios and  $\delta^{13}\text{C}$  signatures:

$$\delta^{13}\text{C}_{\text{normalized}} = \delta^{13}\text{C}_{\text{untreated}} - 3.32 + 0.99 \times \text{C:N} \quad (2)$$

where  $\delta^{13}\text{C}_{\text{untreated}}$  is the  $\delta^{13}\text{C}$  of the bulk (not-defatted) samples.

#### 2.4. Statistical analysis on stomach content data

Sex ratios, GSI, HSI and fullness were analysed through univariate analyses. Firstly, data were checked for homogeneity of variance by the Levene test. In case of homogeneity, an ANOVA was run on the data set; alternatively, a non-parametric test was carried out. Differences in mean CL between males and females were tested by Welch's *t*-test, which is used to determine the distribution probability between 2 variables (Hatfield 1998). Univariate analyses were carried out using the R packages 'dplyr', 'ggplot2' and 'car' (Wickham 2016, 2020, Fox & Weisberg 2019). Multivariate techniques were used to analyse changes in diet across seasons. First, non-metric multi-dimensional scaling (nMDS) analysis was carried out on the Bray-Curtis resemblance matrix of 4th-root-transformed prey biomass data. After that, a permutational multivariate analysis of variance (PERMANOVA) was carried out to test for differences among levels of factors (season and sex), using permutation methods and calculating *p*-values through a Monte Carlo test (Anderson et al. 2008). PERMANOVA was run on a 2-factor crossed design, with season (4 levels) and sex (2 levels) as fixed factors. Permutations of residuals were run under a reduced model, and the significance level was set at  $p < 0.05$ . SIMPER was used to evaluate the taxa that contributed most to the similarity/dissimilarity between and within groups. Here, the 'Decapoda' category referred to all unrecognized Decapoda prey beyond this level, apart from swimming crabs of the genus *Liocarcinus* Stimpson, *Goneplax rhomboides* Linnaeus, 1758, and *Brachyura* in general. Finally, canonical analysis of principal coordinates (CAP; Anderson & Willis 2003) was run on the factor found to be significant in PERMANOVA in order to visualise sepa-

ration among samples on the basis of putative factors (along the 2 axes; Gorley 2006). In order to perform these analyses, prey items were assembled by taxonomic groups. Multivariate analyses were carried out with PRIMER v6 PERMANOVA+ software (Gorley 2006, Anderson et al. 2008).

#### 2.5. Statistical analysis of isotope data

Standard ellipse areas (SEA) for the  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values were calculated using Bayesian statistics in the R package 'SIBER' (Jackson et al. 2011), to evaluate the area of the isotopic niche of the species in the various seasons. After calculating the SEA, a Bayesian SEA analysis was used to calculate the posterior distribution of the covariance matrix for each group ( $\text{SEA}_c$ ); the  $\text{SEA}_c$  used the summary statistics calculated earlier to add the maximum likelihood estimates to the Bayesian estimates (Jackson et al. 2011). Trophic positions based on stable isotope data ( $\text{TP}_{\text{SIA}}$ ) were estimated according to the following equation (Post 2002):

$$\text{TP}_{\text{SIA}} = [(\delta^{15}\text{N} - \delta^{15}\text{N}_b) / \Delta_n] + \lambda \quad (3)$$

where  $\delta^{15}\text{N}$  and  $\delta^{15}\text{N}_b$  are, respectively, the nitrogen isotopic signature of each Norway lobster specimen and that of the benthic and pelagic baselines taken from Fanelli et al. (2022) and E. Fanelli (unpubl. data; carnivorous and omnivorous zooplankton),  $\Delta_n$  is the trophic enrichment expected at each trophic level (3.4‰), according to Post (2002), and  $\lambda$  is the TP of the baseline, set at 2 as a primary consumer (Carlier et al. 2007, Rigolet et al. 2014). Additionally, the R package 'TROPHICPOSITION' was used to calculate the TP, which allows the incorporation of a Bayesian model to calculate a consumer's TP using stable isotopes with 1 or 2 baselines (Quezada-Romegialli et al. 2018a,b). For TP estimation, we used data from Fanelli et al. (2022) for the pelagic baseline and from E. Fanelli (unpubl. data) for the benthic baseline. Before running mixing models to determine the main contributors to the diet of *N. norvegicus*, a mixing polygon simulation was run using the R package 'splancs' (Rowlingson & Diggle 2021), thus taking into account the isotopic signatures of consumers and the mean and standard deviation of dietary source isotopic signatures and trophic enrichment factors (Parnell et al. 2013). Particulate organic matter (POM) was also tested in the model as a probable food source. It was not possible for us to directly sample POM in the study area; thus, for the analysis we used values from another site within the Adriatic Sea



reported in Faganelli et al. (2009). The mixing polygon was calculated for each iteration of source data, and each point in the polygon was used to determine whether consumers are within or on the edge of the mixing polygon. Iterations continued until the variance of the mixing polygon's area stabilised (Smith et al. 2013). This analysis allowed us to select the most suitable food sources to be used in the mixing model, built using the R package 'SIMMR' (Parnell et al. 2013). The results were used to investigate proportions of different prey in the *N. norvegicus* diet previously identified on the basis of SCA results and refined through the mixing polygon routine. This analysis can discriminate various food sources from the isotope values taken from the organisms' tissue samples (Parnell et al. 2013). Prey used for mixing models were the brachyuran crabs *G. rhomboides* and *Liocarcinus depurator* Linnaeus, 1758, the bivalve *M. varia*, and the European anchovy *Engraulis encrasicolus* Linnaeus, 1758. All isotope data used in the analyses are presented in Table 1.

### 3. RESULTS

#### 3.1. Biological features

The LFD obtained from all sampled *Nephrops norvegicus* specimens showed a unimodal distribution (Fig. S1 in the Supplement at [www.int-res.com/articles/suppl/m695p109\\_supp.pdf](http://www.int-res.com/articles/suppl/m695p109_supp.pdf)). Males ranged from 15–75 mm CL (mean  $\pm$  SD: 47.3  $\pm$  10.15 mm), while females ranged from 20–75 mm CL (42.6  $\pm$  10.45 mm). The LFD differed significantly between

Table 1. Isotopic values of all species used for stable isotope analysis. All prey isotopic values are from E. Fanelli (unpubl. data) except *Sardina pilchardus* and *Trachurus* sp., which are from Albo-Puigserver et al. (2016)

Species	$\delta^{15}\text{N}$	$\delta^{13}\text{C}$	$\delta^{15}\text{N}_{\text{SD}}$	$\delta^{13}\text{C}_{\text{SD}}$
<i>Anadara demiri</i>	7.8	-19.5	0.7	0.2
<i>Anadara kagoshimensis</i>	8.7	-18.5	1	0.6
<i>Mimachlamys varia</i>	7.3	-19.8	0.2	0.1
<i>Tritia mutabilis</i>	11	-19.2	0.1	0.1
<i>Astropecten irregularis</i>	8.9	-18.8	0.8	1
<i>Medorippe lanata</i>	10.6	-17.6	0.5	0.8
<i>Goneplax rhomboides</i>	8.4	-18.7	1.2	0.7
<i>Liocarcinus depurator</i>	11.1	-18.1	0.7	0.7
Phytoplankton	3.3	-20.5	0.6	0.5
Plankton omnivorous	4.4	-20.6	0.5	0.3
Plankton carnivorous	5.1	-19.8	0.4	0.3
<i>Engraulis encrasicolus</i>	8.6	-18.8	0.7	0.3
<i>Sardina pilchardus</i>	8.1	-20.0	0.3	0.4
<i>Trachurus</i> sp.	9.0	-19.0	0.4	0.3

the 2 sexes (Welch's *t*-test,  $t = 4.4$ ,  $df = 266.5$ ,  $p < 0.001$ ). In general, males were slightly more abundant during winter (0.35), spring (0.37) and autumn (0.44), while in summer (0.60) the number of females was higher. The highest GSI value for females was recorded in summer (11.33) and the lowest in autumn (0.04), while males' GSI remained constant throughout the year, ranging from 0.09–1.85 (Fig. 2). The HSI values for both females and males were relatively constant throughout the year, except for females in autumn where the maximum values occurred (for females, HSI ranged from 0.05–6.15; for males, from 0.42–5.61; Fig. 3). Overall, there were significant differences between males and females for both GSI and HSI ( $p < 0.05$ ). Differences in the GSI index were significant for all factors tested (sex and season; Table 2). According to pairwise comparisons, there were significant differences only for female specimens across seasons, with spring differing from summer and summer differing from autumn and winter (both pairwise comparisons:  $p < 0.05$ ). The results for HSI were significant ( $p < 0.05$ ) for all factors tested (sex and season; Table 3a). For females, pairwise comparisons showed that HSI changed significantly across seasons, with autumn differing from all other seasons (both pairwise comparisons:  $p < 0.05$ ). GSI and HSI values for males remained constant across seasons. The fullness index, calculated separately for females and males by season, showed a relatively constant trend (Fig. 4), with a slight increase observed for males in autumn. However, there were no significant differences for any of the factors tested (Table 3b).

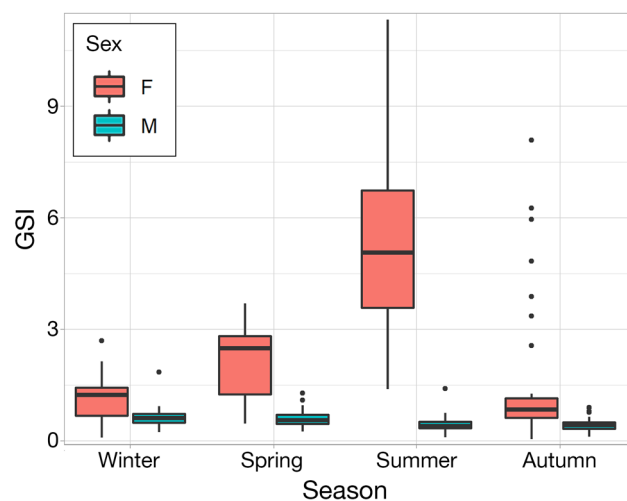


Fig. 2. Seasonal variation of the gonadosomatic index (GSI) with median values (horizontal black lines), value beyond interquartile range (vertical black lines) and outliers (black dots), obtained for the sampled male and female *Nephrops norvegicus* individuals

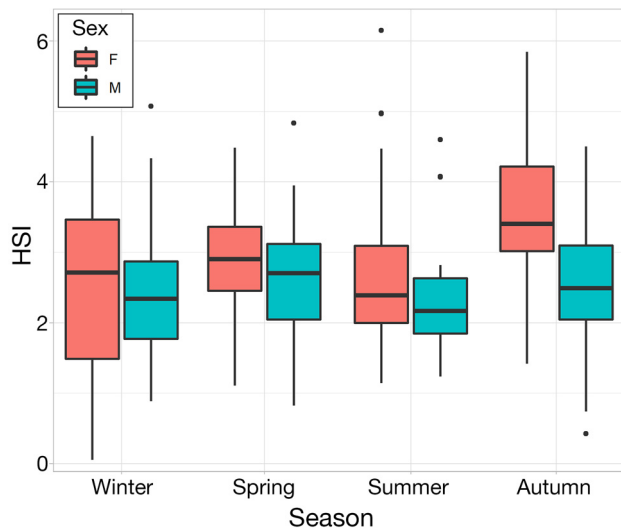


Fig. 3. Seasonal variation of the hepatosomatic index (HSI) obtained for the sampled male and female *Nephrops norvegicus* individuals. Details as in Fig. 2

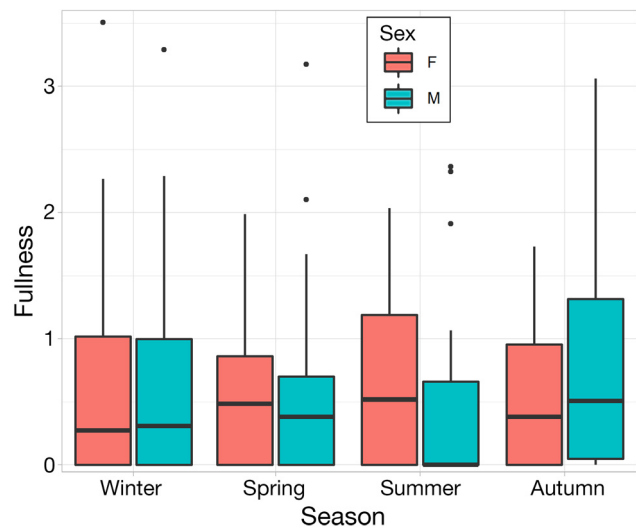


Fig. 4. Seasonal variation of the fullness index of obtained for the sampled male and female *Nephrops norvegicus* individuals. Details as in Fig. 2

Table 2. Nonparametric ANOVA carried out for the gonadosomatic index of *Nephrops norvegicus* by sex and season. The residual value (RD) represents the difference between the single GSI value and the mean of all obtained values

	df	RD	Mean RD	F	p
Sex	1	129.361	129.361	772.016	<0.001
Season	3	79.431	26.477	158.013	<0.001
Sex × season	3	88.402	29.467	175.859	<0.001

Table 3. Results of ANOVA carried out for the (a) hepatosomatic index and (b) fullness index of *Nephrops norvegicus* by sex and season

	df	SS	MS	F	p
(a)					
Sex	1	14.09	14.092	15.888	<0.001
Season	3	13.90	4.634	5.224	0.00156
Sex × season	3	11.57	3.856	4.347	0.00509
(b)					
Sex	1	0.64	0.638	1.334	0.249
Season	3	2.04	0.678	1.418	0.237
Sex × season	3	2.85	0.950	1.986	0.115

### 3.2. Diet composition

Out of 489 analysed stomachs, 329 were full. The analysis of diet composition in terms of %W showed that *N. norvegicus* mostly fed on decapods, fish and polychaetes throughout the year, with a total of 35 different taxa found (Box 1). Individuals of different sizes were consumed, mainly of small and medium

sizes. Only one plastic item was found in the stomach contents. The crabs *Goneplax rhomboides* and *Liocarcinus* spp. represented the most abundant items in all seasons (22 and 29% of all decapods, respectively). Fish showed the greatest abundance in summer, while polychaetes were more abundant in autumn (Fig. 5). There were significant differences in the diet, in terms of consumed prey, only for the factor season, with summer being significantly different from autumn ( $p < 0.05$ ; Table 4). There were no significant differences in diet between the 2 sexes ( $p > 0.05$ ), which showed similar results for prey consumed. The CAP analysis showed a separation among seasons, with the first axis (CAP1) separating mostly spring and summer samples and the second (CAP2) separating winter and spring (Fig. S2). SIMPER analysis showed a strong contribution of decapod crustaceans and fish in every season (Table S1). In winter, spring and autumn, the most abundant prey were decapod crustaceans followed by fish. In summer, the prey with the greatest contribution was fish, followed by *Liocarcinus* spp. and *G. rhomboides*.

### 3.3. Stable isotope and Bayesian statistics

The  $\delta^{13}\text{C}$  values ranged from  $-18.2$  to  $-20.6\text{‰}$ , with the maximum observed in winter and the lowest in summer.  $\delta^{15}\text{N}$  values varied from  $8.5$ – $11\text{‰}$ , with the maximum observed in spring and the lowest in winter (Fig. 6a). The estimation of SEAc showed that *N.*

Box 1. List of prey items found in the sampled *Nephrops norvegicus* stomachs. \*unidentified material

<b>FORAMINIFERA</b>	Caridea
Foraminifera*	<i>Processa</i> sp.
Uvigerina sp.	Crangonidae
<b>PORIFERA</b>	<i>Pontophilus spinosus</i>
Porifera*	Alpheoidea
<b>GASTROPODA</b>	<i>Alpheus glaber</i>
Gastropoda*	Paguridae
Rissoidae	Penaeoidea
Turritellidae	<i>Parapaeneus longirostris</i>
<b>BIVALVIA</b>	<i>Solenocera membranacea</i>
Bivalvia*	Pandaloidea
<i>Anadara kagoshimensis</i>	<i>Plesionika heterocarpus</i>
<i>Kurtiella bidentata</i>	<b>ECHINODERMATA</b>
<i>Mytilus galloprovincialis</i>	Echinodermata*
Pectinidae	Echinoidea
<b>CEPHALOPODA</b>	<b>OSTEICTHYES</b>
Cephalopoda*	Osteichthyes*
Sepiolidae	Pleuronectiformes
<i>Sepiola affinis</i>	Clupeiformes
<b>POLYCHAETAE</b>	<i>Sardina pilchardus</i>
<i>Sternopsis scutata</i>	<i>Engraulis encrasicolus</i>
<b>CRUSTACEA</b>	Carangidae
Crustacea*	<i>Trachurus trachurus</i>
Amphipoda	Mullidae
Hyperidae	<i>Mullus barbatus</i>
Isopoda	<b>PLANT DEBRIS</b>
Decapoda	Debris of land plant
Brachyura	Algae
<i>Goneplax rhomboides</i>	<b>PLASTIC REMAINS</b>
<i>Liocarcinus depurator</i>	

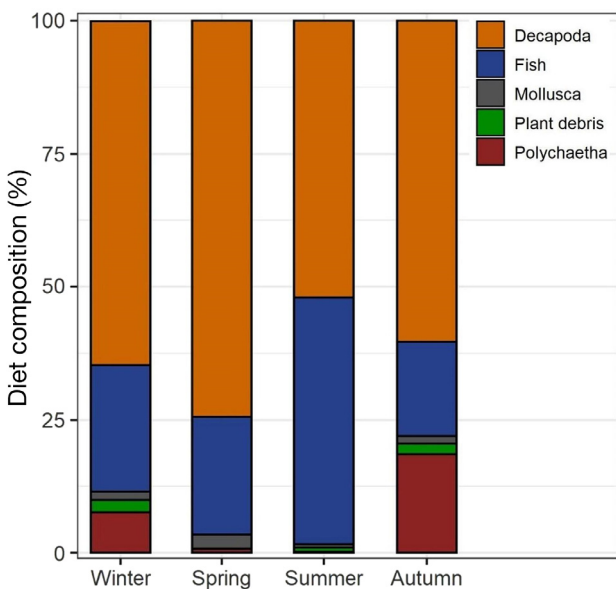


Fig. 5. Seasonal composition of the diet of *Nephrops norvegicus* in terms of the percentage of wet weight of prey. To better highlight the various categories of prey, taxa have been merged (Decapoda contains Brachyura, *Liocarcinus* spp. and *Goneplax rhomboides*)

Table 4. Results of PERMANOVA (a) main test and (b) pairwise comparisons for the diet composition of *Nephrops norvegicus* by sex and season

(a) Source	df	MS	Pseudo-F	p (perm)
Season	3	7737.2	2.06	0.01
Sex	1	1622.6	0.43	0.82
Season × sex	3	2395.3	0.64	0.83

(b) Groups	t	p (perm)
Winter, spring	0.43	0.96
Spring, summer	1.28	0.15
Summer, autumn	2.13	0.01
Autumn, winter	0.96	0.48

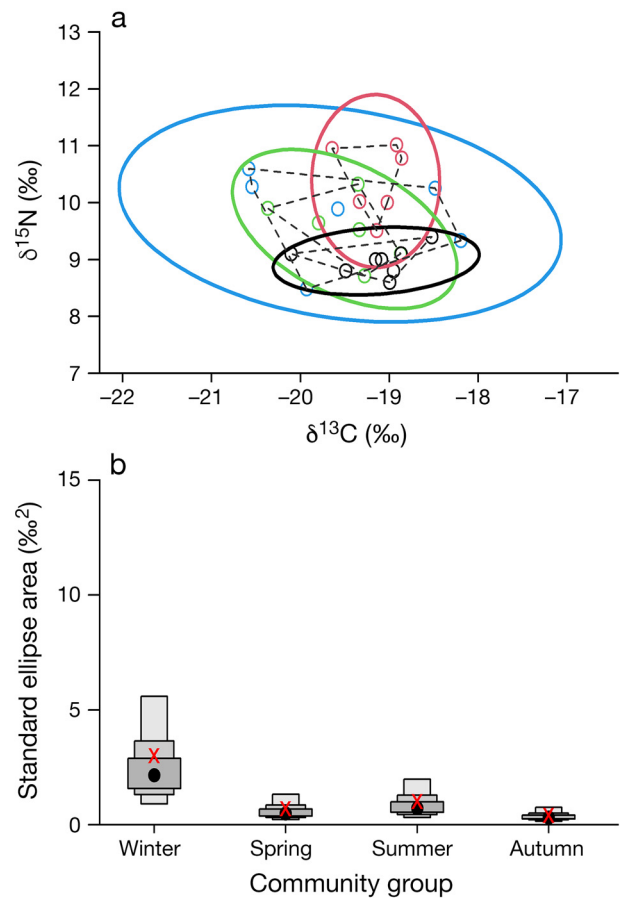


Fig. 6. (a) Standard ellipses encompassing ca. 95% of the data drawn for each group independently on  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$ , the dotted lines representing each individual showing the area occupied by each group (season). Ellipses are drawn per season (light blue: winter; red: spring; green: summer; black: autumn). (b) Standard ellipse area illustrating posterior estimates of the Bayesian SEA for each group, each box represents the communities with credible intervals at 25–50 and 75%, the red cross represents the median and the black point the mean value, used to compare individual groups (seasons)



*norvegicus* exhibited the widest niche width in winter and summer (Fig. 6b).

The 'TROPHICPOSITION' analysis indicated that the contribution of the benthic baseline was higher compared to that of the pelagic baseline (Fig. 7). The Norway lobster was generally located at the fourth trophic level, with winter TP = 4.17, spring TP = 4.37, summer TP = 4.08 and autumn TP = 3.90.

The mixing polygon simulation showed that consumer values were inside the 95% mixing region (the outermost contour in Fig. S3), allowing us to select the prey for the following mixing models. POM was eventually discarded from the Norway lobster's diet, as it was not a probable food source; indeed, given its great variability, it did not fit well in the polygon. The prey that showed the highest contribution to the *N. norvegicus* diet, according to the SIMMR output, was *Liocarcinus depurator*, while that showing the lowest was *G. rhomboides* (Fig. 8a). Seasonal differences in prey contribution were also evidenced by the model, with *L. depurator* mostly contributing to Norway lobster's diet in winter, spring and summer, followed by *Engraulis encrasicolus*. A clear shift toward greater consumption of fishes occurred in autumn, when the crustacean component decreased (Fig. 8b).

#### 4. DISCUSSION

This is the first study integrating SCA and SIA to analyse the feeding ecology of *Nephrops norvegicus* in the Adriatic basin at a seasonal level. Results confirmed that the Norway lobster behaves as a scavenger and, to a lesser extent, also as an active benthic predator, mostly feeding on decapod crustaceans (especially the brachyuran crabs *Liocarcinus* spp. and *Goneplax rhomboides*), fish, polychaetes and bivalves. This finding is in agreement with what has been observed in other studies (Cristo 1998, Cristo & Cartes 1998, Santana et al. 2020). Overall, the trophic diversity of prey was low, and as a generalist predator, its diet tends to reflect local variations in food availability (Parslow-Williams et al. 2002). Indeed, the 2 decapod species found to be prevalent in *N. norvegicus* diet (*G. rhomboides* and *Liocarcinus* spp.) are particularly frequent across the whole study area

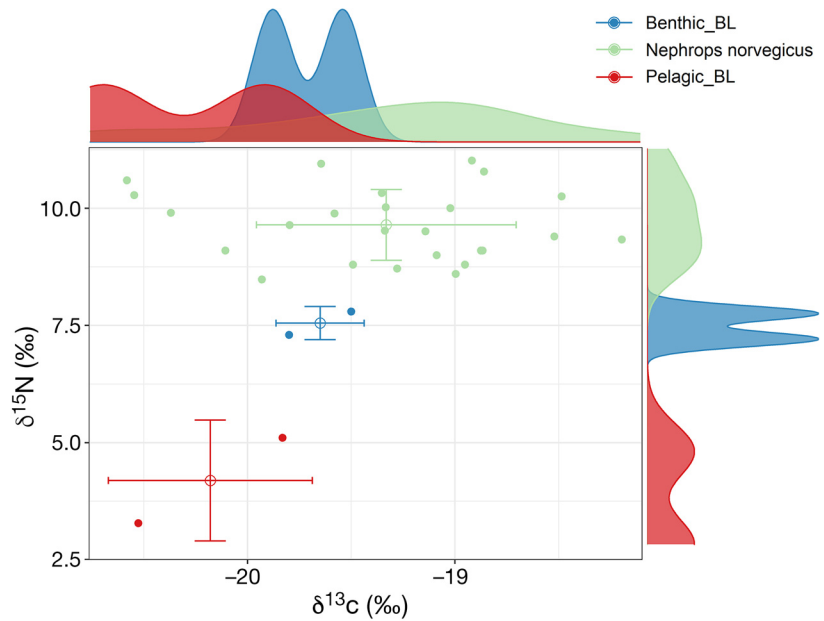


Fig. 7. Trophic position plot for the  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  isotopic signals of *Nephrops norvegicus* and of the considered benthic (Benthic\_BL) and pelagic (Pelagic\_BL) baselines

(Santelli et al. 2017). Furthermore, these 2 species have little to no commercial value and are often discarded by bottom trawl fisheries (Sánchez et al. 2007), which may affect food availability for benthic scavengers (Ramsay et al. 1997, Depestele et al. 2018). Therefore, as a consequence of the high trawling effort occurring in the Adriatic Sea (Russo et al. 2020), the availability of these decapod species as discard on the seabed may influence the feeding behaviour and fullness of *N. norvegicus*. Fish also represent important food items, according to the scavenging behaviour of the species (Nickell & Atkinson 1995, Cristo & Cartes 1998, Depestele et al. 2018). Moreover, some otoliths were found in stomach contents, especially of *E. encrasicolus* and *Sardina pilchardus* Walbaum, 1972, suggesting that the fishes were not consumed whole. Fish fragments or dead pelagic fish on the bottom could be present due to discard from fishery activities (Tsagarakis et al. 2014).

##### 4.1. Seasonal variations in the biological condition of *N. norvegicus*

The GSI index increased in females during summer, in agreement with the reproductive period of the species as reported for the Adriatic Sea and other areas (Rosa & Nunes 2002b, Mente et al. 2009, Colella et al. 2018). For males, no fluctuations in the index across seasons were observed, consistent with

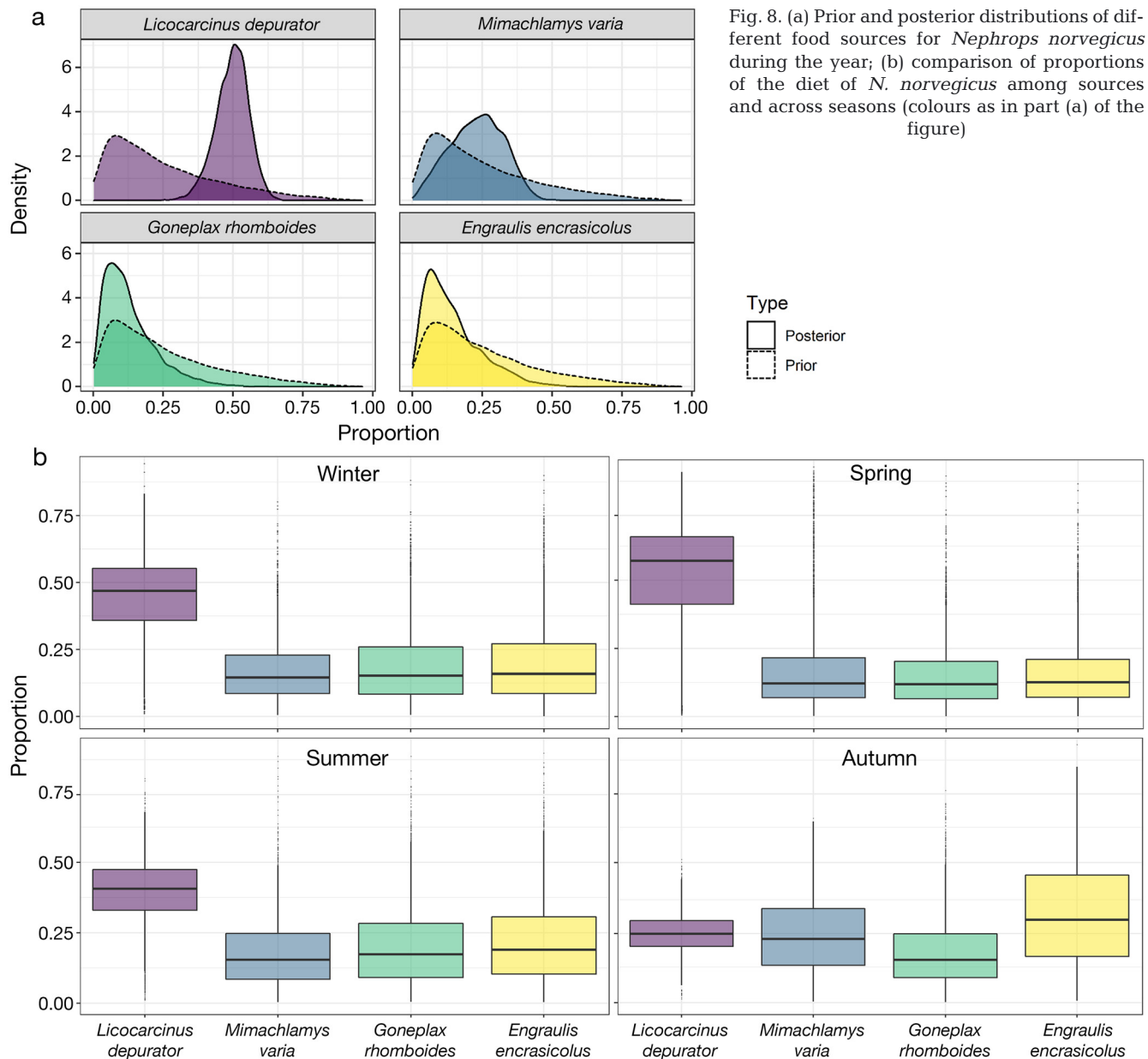


Fig. 8. (a) Prior and posterior distributions of different food sources for *Nephrops norvegicus* during the year; (b) comparison of proportions of the diet of *N. norvegicus* among sources and across seasons (colours as in part (a) of the figure)

a pattern of continuous spermatogenesis (Farmer 1974, Sardà 1998). In females, the HSI index showed an increase in autumn after the reproductive period, with a trend contrasting that described for the Atlantic Ocean (Rosa & Nunes 2002b). These results could be attributed to the increase in fullness values which occurred in autumn and recovery after reproductive activity. Indeed, females need to invest in lipids to produce eggs, especially for yolk formation (Gibson & Barker 1979, Dall 1981, Relini et al. 1998). Stomach fullness was relatively constant throughout the year (with slightly higher values in autumn), suggesting continuous predatory behaviour during all

seasons, as observed in the north-eastern Atlantic (i.e. Irish Sea; Parslow-Williams et al. 2002), with no differences between sexes.

#### 4.2. Seasonal variations in the feeding ecology

Changes in feeding habits of the Norway lobster are linked to both the availability of prey on the bottom and changes in energy demand throughout the year (i.e. for reproduction; Bell et al. 2006). When emerging from their burrows, Norway lobsters do not usually travel long distances (Vigo et al. 2021). The strong ter-

territoriality shown by this species could influence seasonal variations in its diet when mobile prey availability varies over time and space according to seasonal patterns; for example, *Liocarcinus depurator*, one of the most important prey species, undergoes offshore migrations during the summer (Onay & Bilgin 2021) in the Black Sea. Seasonal migrations have also been reported for other relevant prey in Norway lobsters' diet (see Fouzai et al. 2012). The possible fluctuations in the abundance of prey could influence the biological conditions of the species. In the study area, small pelagic species found in the lobsters' stomach contents, especially *S. pilchardus*, may have been discarded by bottom trawl fisheries (confirmed by DCF data stored at CNR-IRBIM) and, to a lesser extent when fish are small or damaged, also by pelagic trawlers (Santojanni et al. 2005). While decapod crustaceans were abundant prey items in the *N. norvegicus* diet throughout the year, higher consumption of fishes occurred in spring and summer. The higher contribution of the fish prey signature in the predator's tissue in autumn (as obtained by mixing models based on  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  values) was explained by a delayed time of incorporation following consumption. Prey contribution as determined by SIA generally confirms SCA results (Polunin & Pinnegar 2002, Post 2002). Isotopic values change across seasons, with variations related to food availability also reflected in the analysis of the breadth of trophic niches (Post 2002, Decottignies et al. 2007). Although differences between sexes across seasons due to reproductive activities were expected (Rosa & Nunes 2002a, Colpo & López-Greco 2018), males and females exhibited similar feeding strategies. Similar results of inter-sex feeding behaviour for *N. norvegicus* were described by Santana et al. (2020) in Clew Bay (Ireland).

Although other authors (Murray & Cowie 2011, Carreras-Colom et al. 2022) have reported plastic items in the stomach contents, in our study this factor was not relevant. However, recognition of these items could have been masked by other prey and/or their small size, as the presence of microplastic fragments and fibres in the gut (stomach and intestine together) of Norway lobsters from the same study area have already been reported by Martinelli et al. (2021).

#### 4.3. Position of *N. norvegicus* in the benthic food web

Analysis of the TP of *N. norvegicus* suggests that the species occupies a high trophic level, as already observed for other decapods (Fanelli et al. 2013),

with minimal differences across seasons. The scavenging behaviour of the species, whereby it consumes other high trophic level species (i.e. presenting high isotopic values) such as fish or large crustaceans (sometimes similar in size to *N. norvegicus*), supports such high TP value attribution (Cristo 1998, Santana et al. 2020). The consumption of prey at different trophic levels can vary according to the area, and consequently influence the TP of *N. norvegicus* (Hinz et al. 2017). The TP model confirmed that *N. norvegicus* mostly relies on benthic food items (albeit including fish). In this study the variability in TP can indicate diversified consumption based on prey availability, including discard; this is indeed common in species that can be active predators as well as scavengers, like *Callinectes sapidus* Rathbun, 1896, a crustacean with a similar feeding ecology (Carrozzo et al. 2014). However, changes in this pattern can be expected in the future; for example, a decrease in fisheries discards due to application of the Landing Obligation (EU 2013) could affect the TP of *N. norvegicus*.

## 5. CONCLUSIONS

This work contributes to our knowledge of the feeding ecology of *Nephrops norvegicus*. The results confirm the scavenging behaviour of the species, and the strong relationship of its diet with the local benthic community, with *Liocarcinus* spp. and *Goneplax rhomboides*, being common species in the study area. Furthermore, this study highlights possible links between the feeding ecology of the species and the availability of fisheries discard. The results of the combined use of SIA and SCA allowed us to obtain a complete description of the feeding habits of *N. norvegicus* throughout the year and link this behaviour to changes in GSI, HSI and fullness indices. *N. norvegicus* is of great commercial importance in the study area which represents an important and heavily exploited fishing ground. The TPs recorded here can be used in future food-web models within the study area. Therefore, the results of the present study are of paramount importance within the context of an ecosystem approach to fisheries.

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