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**Biologia marina**

**La dieta dello scampo (*Nephrops norvegicus*) nel Mar Adriatico**

**The diet of Norway lobster (*Nephrops norvegicus*) in the Adriatic Sea**

Tesi di Laurea Magistrale

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# Chapter 1 – Introduction

## 1.1 - Biology and ecology of *Nephrops norvegicus*

The Norway lobster, *Nephrops norvegicus* (Linnaeus, 1758), is a benthic burrowing decapod crustacean that inhabits the Mediterranean Sea and the continental shelves of the North-Eastern Atlantic Ocean, at depths of 50–800 m (Chapman, 1980). The species is distributed in the eastern Atlantic, Morocco, Norway, Iceland, in the Mediterranean and in the Black Sea (figure 1.1).

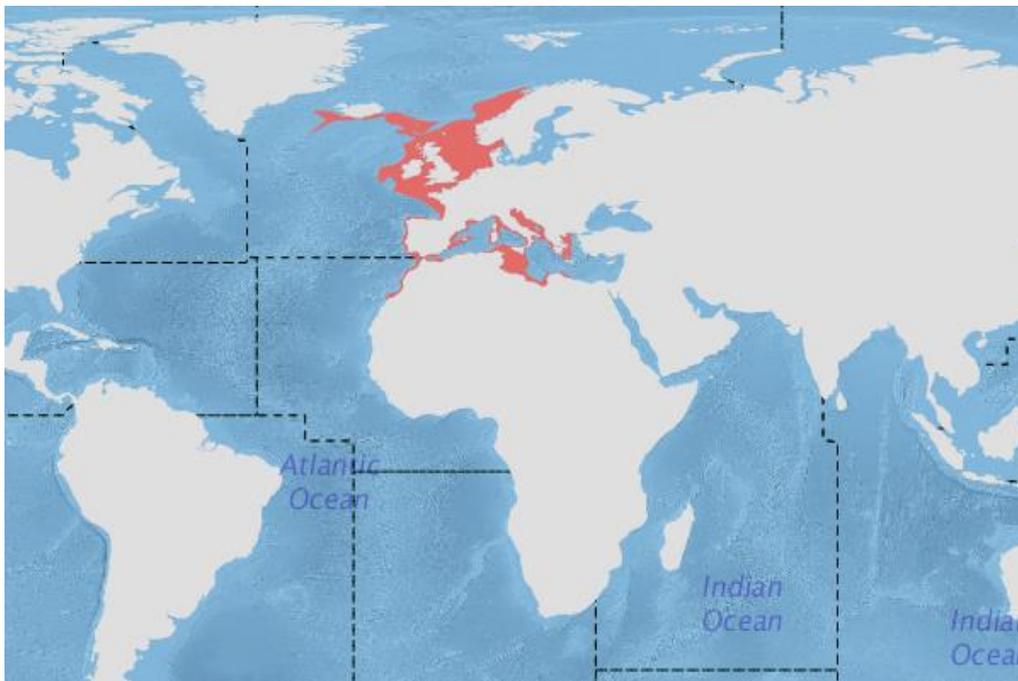


Fig. 1.1 Geographical distribution of *Nephrops norvegicus* (<http://www.fao.org/fishery/species/2647/en>).

The bathymetric distribution of *N. norvegicus* in the Mediterranean extends from the continental shelf to bathyal grounds, at depths from 50 to 400 m. In the Adriatic Sea, the species occurs from ca. 30 m of depth in the Northern part to 400 m in the southern part (Karlovac et al., 1953). The species is of great commercial importance in the North East Atlantic and the Mediterranean, especially in the Adriatic Sea (GFCM Geographical Sub Areas 17 and 18), which is one of the most important and productive fishing areas of the Mediterranean basin. Until 2014, *N. norvegicus* represented here the second crustacean (after the mantis shrimp, *Squilla mantis*) in terms of abundance, in commercial catches, although showing a decreasing trend since 2005. Since 2015 the species has instead been surpassed by a sudden increase of the pink shrimp *Parapenaeus longirostris* (FAO, 2018; Martinelli, 2019).

*Nephrops norvegicus* belongs to the Phylum Arthropoda, Subphylum Crustacea, Class Malacostraca, Order Decapoda, Suborder Pleocyemata, Infraorder Astacidea, Superfamily Nephropoidea, family Nephropidae (Stachowitsch, 1992).

## *Morphology*

*Nephrops norvegicus* is a crustacean decapod with 13 segments covered by an unsegmented carapace, a rigid structure that protects the branchial chambers (cephalothorax, separated into cephalon and thorax), a six-segment abdomen (pleon), with 10 walking legs, that is the posterior part of the body and it is composed by a series of movable somites and ending with the telson, a false somite that enables the lobster to swim (figures 1.2 and 1.3).

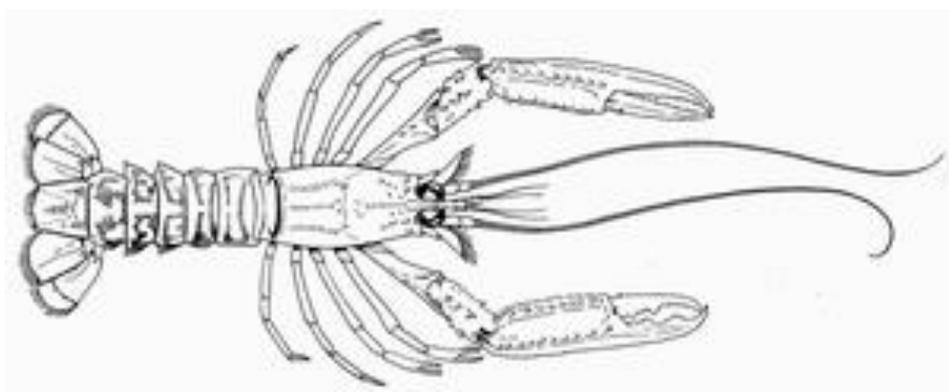


Fig 1.2 *Nephrops norvegicus* (Linnaeus, 1758) (<http://www.fao.org/fishery/species/2647/en>)

However, as all astacids, this species preferably walk rather than swim. The cephalon of *N. norvegicus* is equipped with compound pedunculated eyes, two pairs of antennae, the first one being called “antennules”, and the second are the “second antennae”; these structures compose the sensory system. The antennae are followed by a pair of mandibles and two pair of jaws (Stachowitsch, 1992).

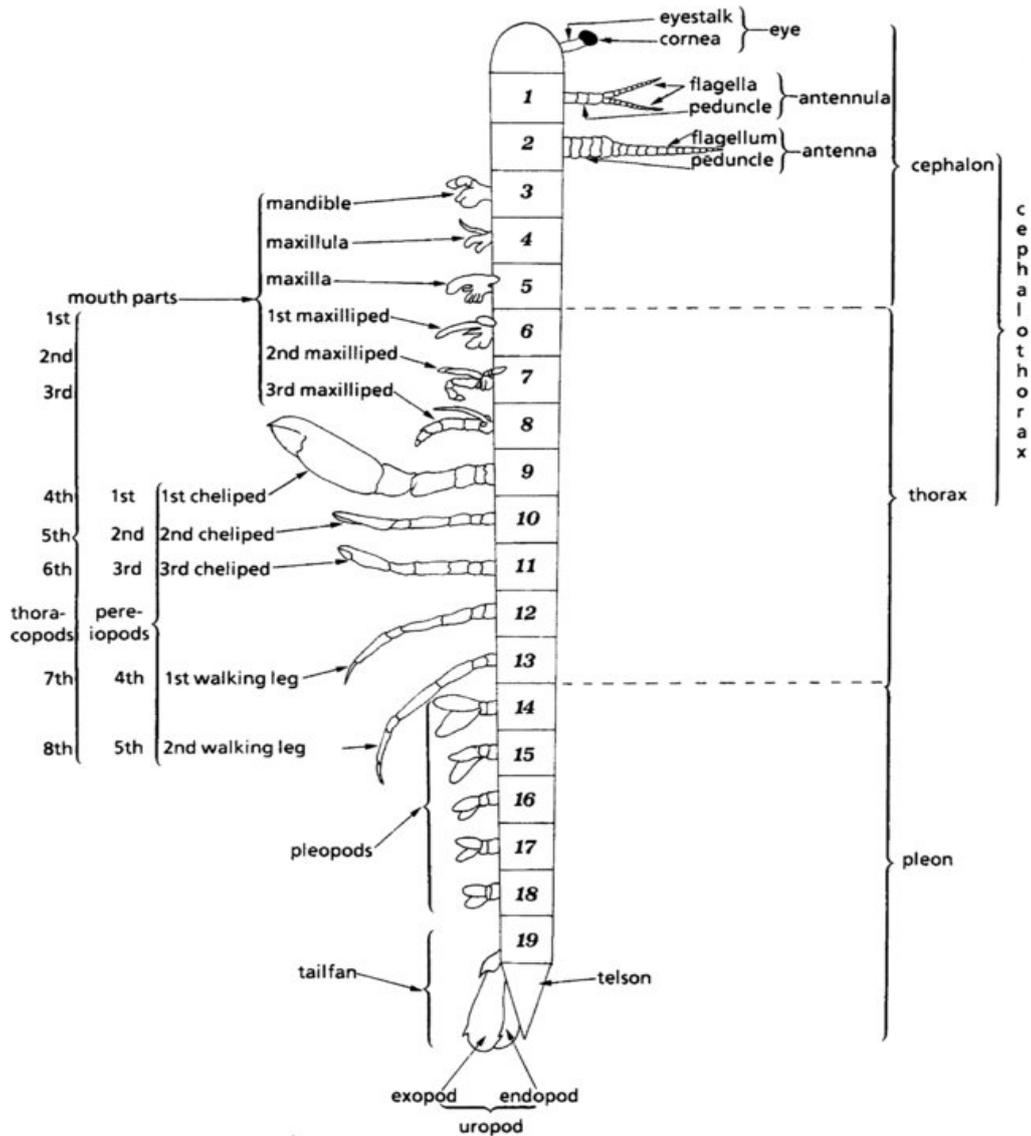


Fig. 1.3 Schematic illustration of lobster's segmented body plan and the appendages that arise from these body segments (from Holthuis, 1991).

### *Reproduction*

The species has separated sexes with internal fertilization and visible external organs for reproduction (figure 1.4); it spawns once per year (Farmer, 1974).

The spermatogenesis in males occurs throughout the year, whereas the ovaries

of the females mature in spring, when the moulting also occurs after the egg-hatching stage. The post-moult period in late spring seems to be the only period available for copulation (Farmer, 1974). Rotllant et al. (2012) described the gross morphology of the male reproductive system and demonstrated that it is similar to that of other decapods species, as reviewed by Krol (1992). Appendix masculina is an accessory that distinguishes males from females in decapods and it is located on the endopod (inner side) of the second pleopods of the species (Wenner et al., 1985).



Fig. 1.4 Ventral vision of a female on the left and male on the right of *Nephrops norvegicus*.

Appendix masculina is a visible rounded body structure, completely absent from females, which along with the petasma, is associated with the sperm transfer and copulation (Lampri et al., 2015). In particular, during the copulation period the first pair of pleopods creates an open-ended tube that its tip is inserted inside the sperm receptacle of the female. Then, the appendix masculina, with the appendix interna, functioning similarly to a piston facilitates the sperm transfer inside the tube created by the first pleopods (Cobb et al., 2012). According to Sainte-Marie, 2007, males with partially ablated petasmata or with ablated appendices masculinae did not copulate with females.

Females carry the eggs with the pleopods until the nauplius hatch (Stachowitsch, 1992). Female thorax is characterized by the thelycum: this is a seminal receptacle, a blind pouch that is not connected to the female reproductive tract but opens externally between the last pereopods to receive spermatophores during copulation. Spermatophores are stored along the wall opposite the opening and are later extruded by the contraction of muscles associated with the pouch; the rest of the pouch is filled with a “sperm plug” of hard material (Waddy et al., 1995, Talbot et al., 1991). The other female pleopods promote the attachment of the eggs after spawning.

## *Ecology*

Norway lobster is a sedentary species that inhabits burrows constructed in muddy sediments, spending a large part of the day inside the burrow (Abelló et al., 2002). The underground lifestyle of Norway lobster influences its behaviour and physiology. From the burrows they exercise a strong territorial control of the surrounding areas. The animals come out of the burrows mainly to procure their supplying, but the strong territorialism of the nearby individuals can influence the collection of food, therefore the diet will be based on what is found in the burrows. Moreover, other social behaviours are influenced by the social hierarchy, such as mating and moult, which in fact occur out of the burrows (Aguzzi et al., 2008).

*Nephrops norvegicus* is a euryphagous and non-selective species, consuming a great variety of crustaceans, fish, and molluscs, either as an active predator or scavenger, but there are still few studies on its diet and food consumption (Cristo et al., 1998).

### **1.2 – Scope of the work**

In this study the analysis of the stomach contents of *Nephrops norvegicus* collected in two different study areas, off Ancona and in the Pomo/Jabuka pit, was carried out. Off Ancona specimens were collected monthly across one year

of sampling (from January 2019 to December 2019), while at the Pomo/Jabuka pit samples come from two surveys carried out in May and October 2019.

In this context, the objective of this study was to determine:

- 1) the overall feeding ecology of the species in the Northern-Central Adriatic;
- 2) seasonal variations in the feeding behaviour of specimens collected off Ancona;
- 3) the feeding ecology of the species in the Fishery Restricted Area (FRA) of the Pomo/Jabuka Pit, where fishing activities with bottom-contact gears have been banned since 2017 (Italian Decree, DM 466 1/6/2017);
- 4) possible differences in the diet of specimens from the two sites, off Ancona, where the species was collected in highly-fished grounds, and on Pomo/Jabuka pits, where trawl-fishing is banned, and
- 5) possible relationships of such differences with the biological condition of the species.

Few studies have been done on the diet of *Nephrops norvegicus* for Adriatic populations and it is important to better understand the ecology of the species in this area and possible implications for fishery management measures.



Fig. 1.5 Stomach of *Nephrops norvegicus* open during content analysis.

## Chapter 2 - Material and methods

### 2.1 – The study area: the Adriatic Sea

#### *Geomorphology*

The Adriatic Sea is an epi-continental basin, the depth increases from north to south and it is divided in three sub-basins, Northern, Central and Southern Adriatic (figure 2.1).

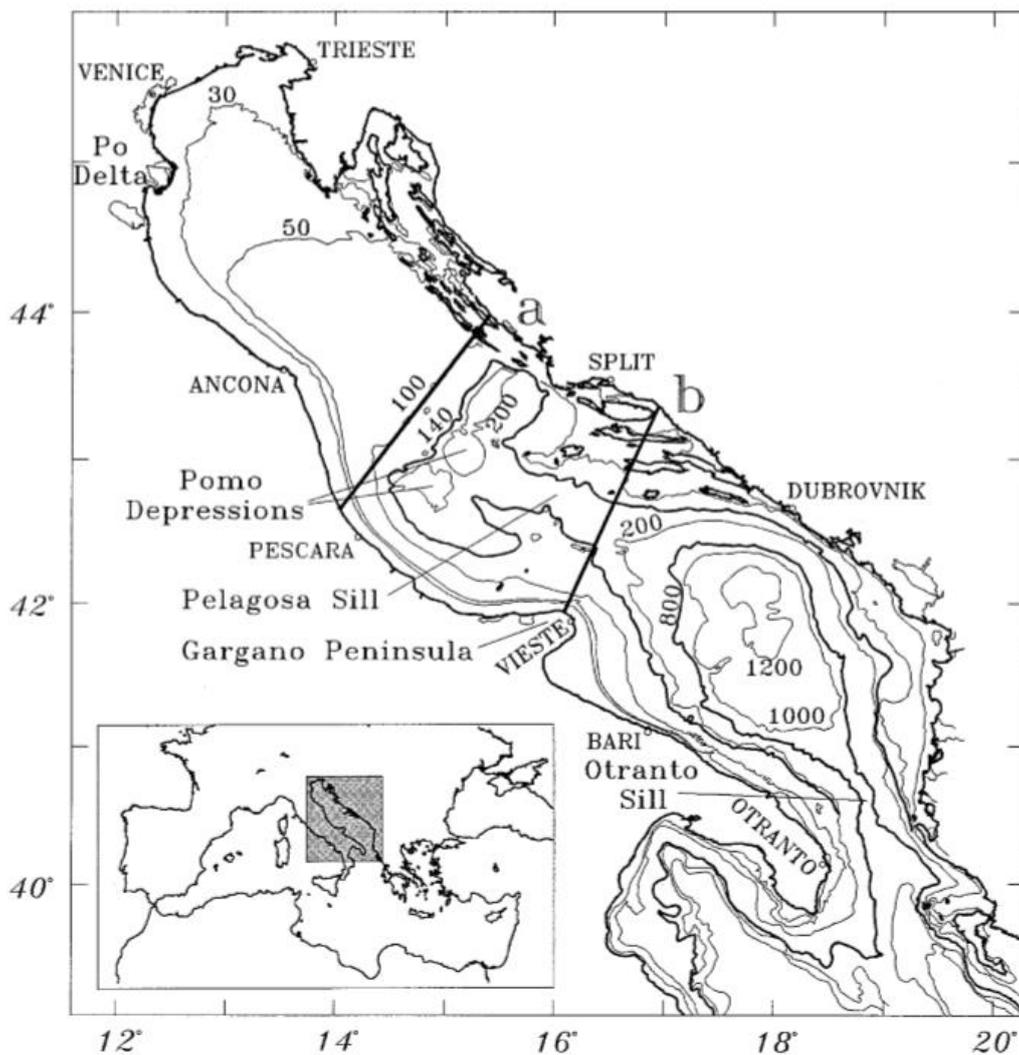


Fig. 2.1 The Adriatic Sea and the division in sub-basins (from Artegiani et al., 1996).

The northern sub-basin has a maximum depth of 100 m and is characterised by an extremely shallow mean depth (about 30 m), it is the area most influenced by the Po river discharge. The middle sub-basin is a transition zone between the northern part and the southern sub-basin, which shows somewhat open sea conditions. The central sub-basin has a depth comprises from 100 m to a maximum of 270 m of depth, in the Pomo/Jabuka pits (Artegiani et al., 1997). The southern sub-basin extends up to Otranto with a maximum depth of 1200 m (Russo et al., 1996).

#### *Oceanographic characteristics*

In winter the temperature is considerably low, about 11 °C, in the northern region and along the western coast, while in the rest of the basin it is less rigid, about 14°C. In summer the surface temperature is rather uniform over the entire Adriatic Sea. The temperature gradient in front of the Po River mouth reaches a maximum in autumn. In this season a large-scale frontal area divides the western and eastern parts of the basin. In summer, there is a sharp thermocline at 30 m depth in the Northern and at 50 m in the Central Adriatic (Artegiani et al., 1997a), the surface temperature is uniform over the entire basin, being 23-24 °C in the open sea (Cataudella et al., 2011).

In all the seasons, there are some strong salinity frontal areas, particularly along the western coast, related to the river runoff. Frontal structures are determined

by the strong gradients between the low salinity waters, which are always present along the western side of the Adriatic Sea, and the interior basin salinity field (Artegiani et al., 1997). Salinity shows large-scale irregularity during the spring–summer seasons, an indication of higher baroclinic eddy activity. The maximum values of salinity are found in winter, when the 38.3 psu isohaline includes all the offshore area of the entire basin, while the minimum values of salinity occur in summer. There is an area of relatively high salinity, greater than 38.5 psu, in the middle Adriatic, which persists in all seasons. In spring the noticeable influence of the Albanian rivers' runoff is shown by a wide area with salinity lower than 38.0 psu in front of the southeaster coastline (figure 2.2) (Cataudella et al., 2011).

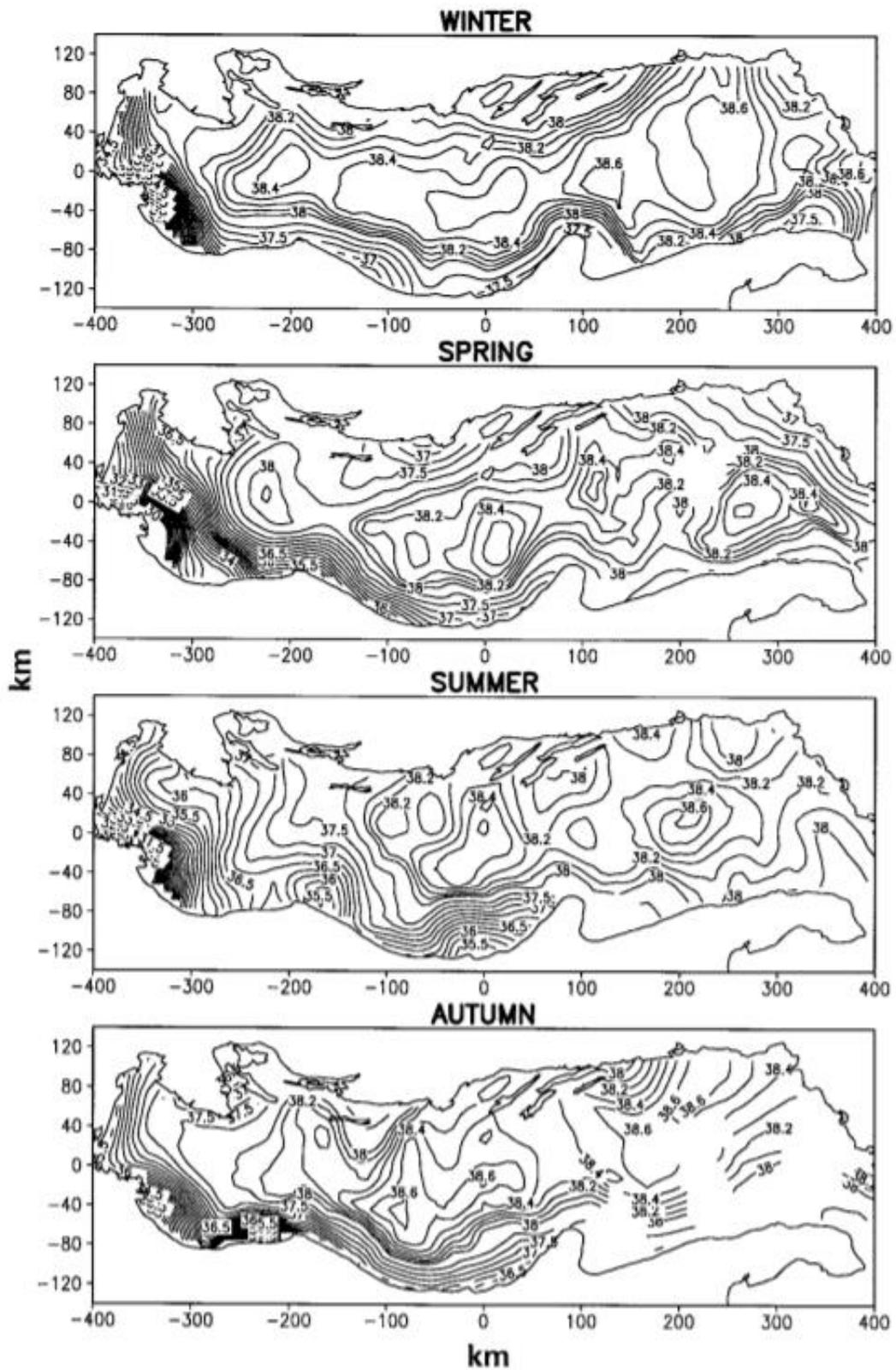


Fig. 2.2 Salinity average value expressed in psu during the seasons in the Adriatic Sea (from Artegiani et al., 1997).

The general circulation is of cyclonic type; from the eastern Mediterranean, water masses enter through the Otranto channel. The return flow is given by the cold waters of the Northern Adriatic (NAdDW), which, formed in winter, flow towards the south along the Italian coast and in the middle Adriatic, then a branch will form deep waters (MAdDW) present in the area of Pomo (figure 2.3). The general circulation is composed of currents and vortices that changes in all the different seasons (Cataudella et al., 2011).

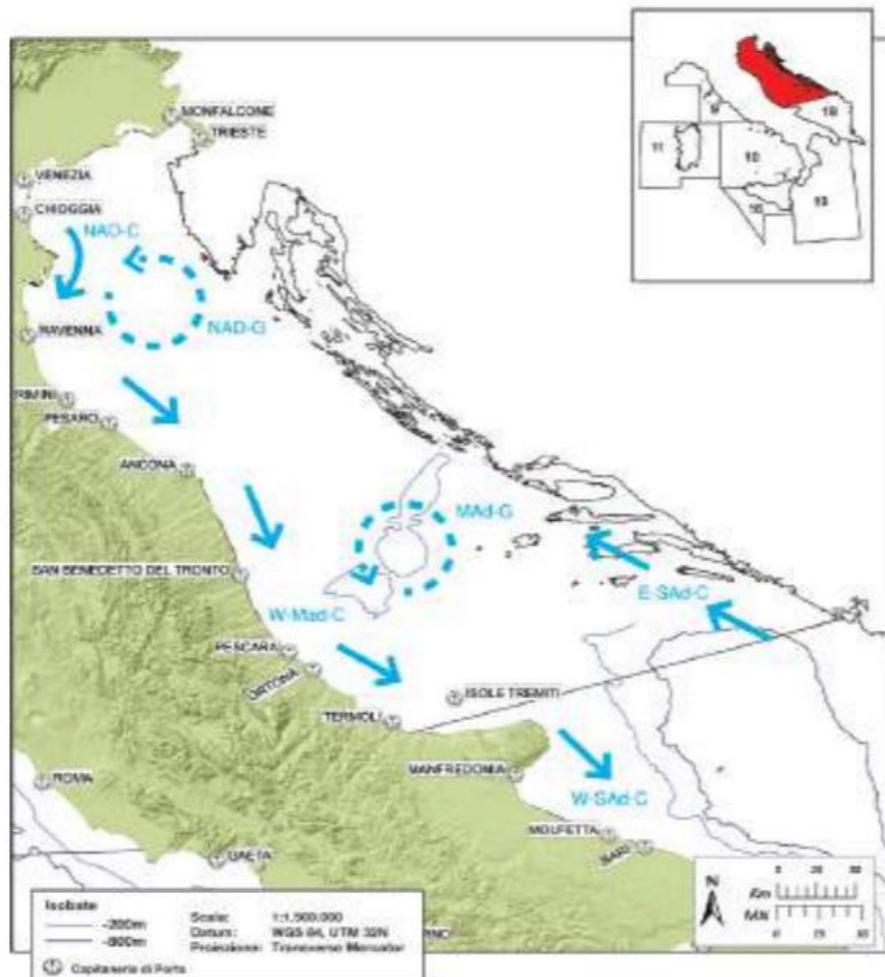
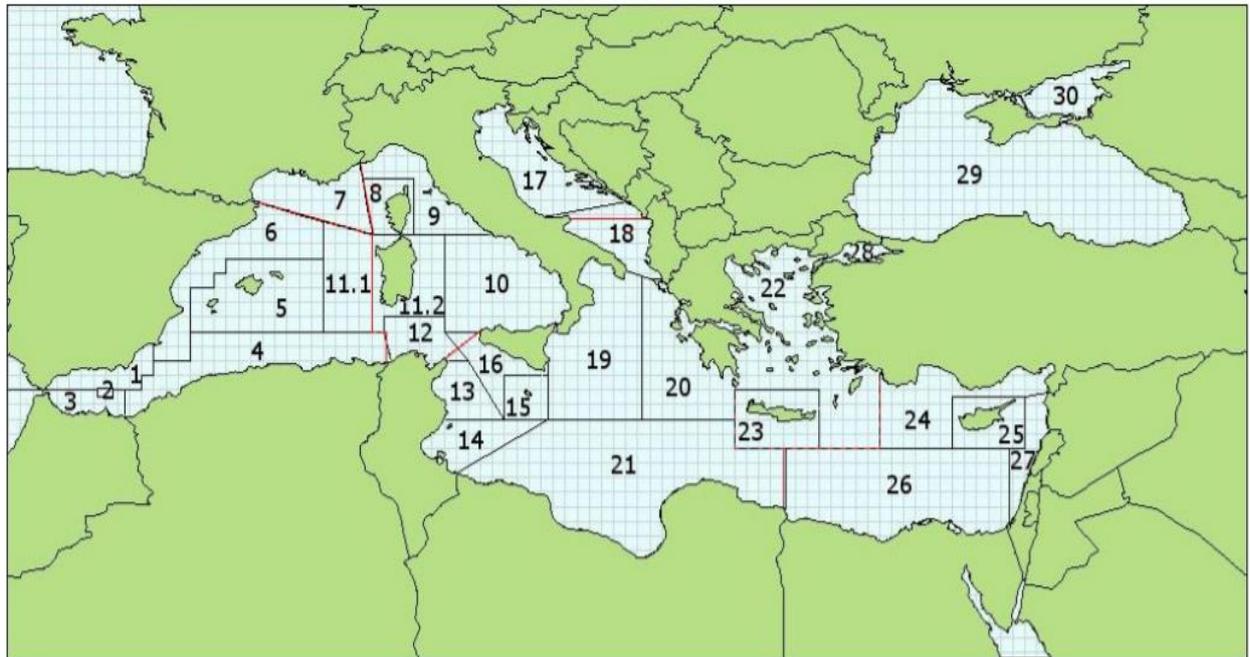


Fig. 2.3 Superficial circulation and vortices in GSA 17 (from Cataudella et al., 2011).

## *Fishery management of the Adriatic Sea*

The General Fishery Commission for Mediterranean (GFCM), in 2001, has set up 30 geographical sub-areas (GSAs) for stock assessment (figure 2.4).

GFCM geographical subareas (GSAs) (GFCM, 2009)



--- FAO Statistical Divisions (red)      ---- GFCM Geographical Subareas (black)

01 – Northern Alboran Sea	07 – Gulf of Lion	13 – Gulf of Hammamet	19 – Western Ionian Sea	25 – Cyprus
02 – Alboran island	08 – Corsica	14 – Gulf of Gabès	20 – Eastern Ionian Sea	26 – Southern Levant Sea
03 – Southern Alboran Sea	09 – Ligurian Sea and northern Tyrrhenian Sea	15 – Malta	21 – Southern Ionian Sea	27 – Eastern Levant Sea
04 – Algeria	10 – Southern and central Tyrrhenian Sea	16 – Southern Sicily	22 – Aegean Sea	28 – Marmara Sea
05 – Balearic Islands	11.1 – Western Sardinia    11.2 – Eastern Sardinia	17 – Northern Adriatic Sea	23 – Crete	29 – Black Sea
06 – Northern Spain	12 – Northern Tunisia	18 – Southern Adriatic Sea	24 – Northern Levant Sea	30 – Azov Sea

Fig. 2.4 GFCM geographical subareas (GSAs) (from GFCM, 2009).

The GSA of interest for this work is the GSA 17, it covers 92.660 km<sup>2</sup> and includes the Northern and the Central Adriatic Sea, from the Gulf of Trieste to the Gargano. This area is characterized by a wide continental shelf, northern

part having low depths that gradually increase southwards, up to a maximum of 70 meters. The west coast is predominantly low, flat and sandy and the only two rocky areas of GSA17 are Monte Conero and Gargano promontory (figure 2.5).

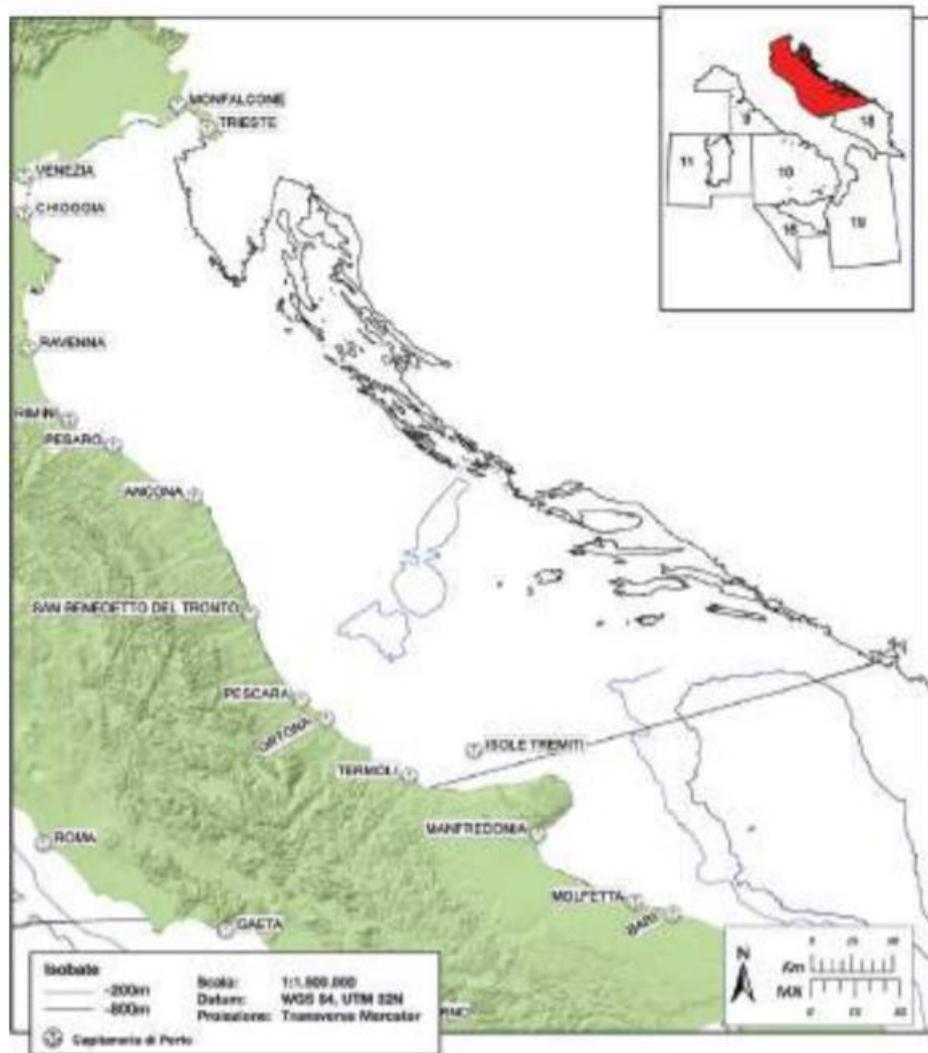


Fig. 2.5 Geographical delineation of the GSA 17 (from Cataudella et al., 2011).

The central Adriatic Sea is a transition zone between the Northern and the Southern sections (Cataudella et al., 2011).

## 2.2 - Pomo/Jabuka pits

The Pomo/Jabuka pits, in the central Adriatic Sea, is an area of approximately 4800 km<sup>2</sup>, characterised by three depressions with maximum depths of 225 m, 270 m and 240 m, respectively (figure 2.6).

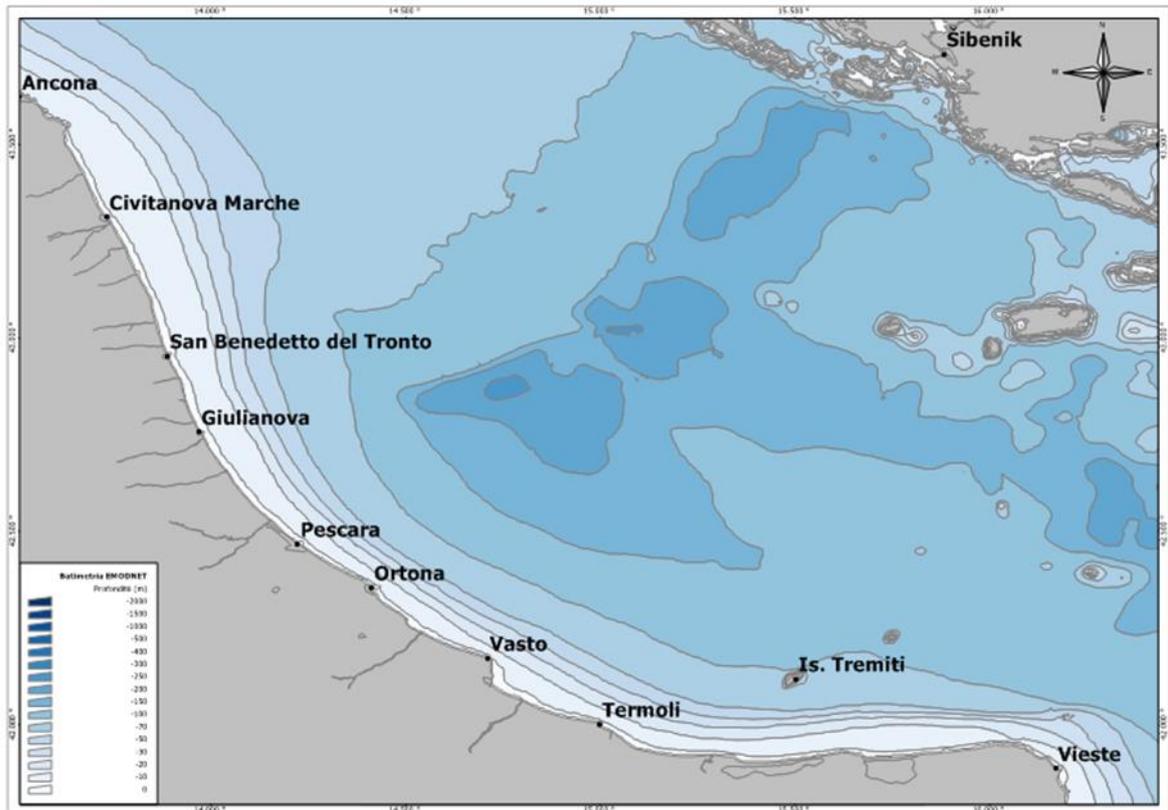


Fig. 2.6 The three Pomo's depression and the territorial division (obtained from the European Marine Observation Data Network [EMODnet] Seabed Habitats project [<http://www.emodnet-seabedhabitats.eu/>]).

This area is a very important fishing ground in the Adriatic Sea and because of its topography and the sediment composition, is a nursery for both the European hake, *Merluccius merluccius* and the Norway lobster, *Nephrops norvegicus* (Martinelli et al., 2013). The Pomo/Jabuka Pit also hosts a distinctive

subpopulation of *Nephrops norvegicus*, with high density of small-sized and slow-growing individuals (Frogliia et al., 1982, Angelini et al., 2020).

In April 2018, the General Fisheries Commission for the Mediterranean (GFCM) established a Fishery Restricted Area (FRA, spatial management measures that regulate fishing activities in the area of application) in the Pomo/Jabuka pits to protect vulnerable marine ecosystems and essential fish habitats (GFCM/41/2017/3) (Martinelli et al., 2019).

### **2.3 – Samples collection**

#### *Monthly sampling*

The monthly sampling of Norway lobster off Ancona was carried out between January and December 2019. Samples were collected by a commercial fishing vessel, within the framework of CAMPBIOL project (Data Collection Framework – Italian National Program) funded by the European Union and Italian Ministry of Agricultural, Food, Forestry and Tourism Policies (MIPAFF), carried out by CNR- IRBIM (National Research Council of Italy– Institute of Biological Resources and Marine Biotechnologies) of Ancona for the Adriatic Region. All samples were collected off Ancona harbour, approximately between 50 and 100 m of depth. A commercial vessel used has

a length of 25 m, tonnage of 98.5 t and an engine power of 480 KW. The fishing net has rhomboid meshes of 50 mm, according to the Italian legislation.

### *Sampling at Pomo/Jabuka Pit*

Pomo sampling took place by means of an experimental trawl net onboard the R/V Dallaporta, during the CNR-IRBIM “UWTV” and “ScamPo” surveys, carried out in May 2019 and October 2019, respectively (figure 2.7).

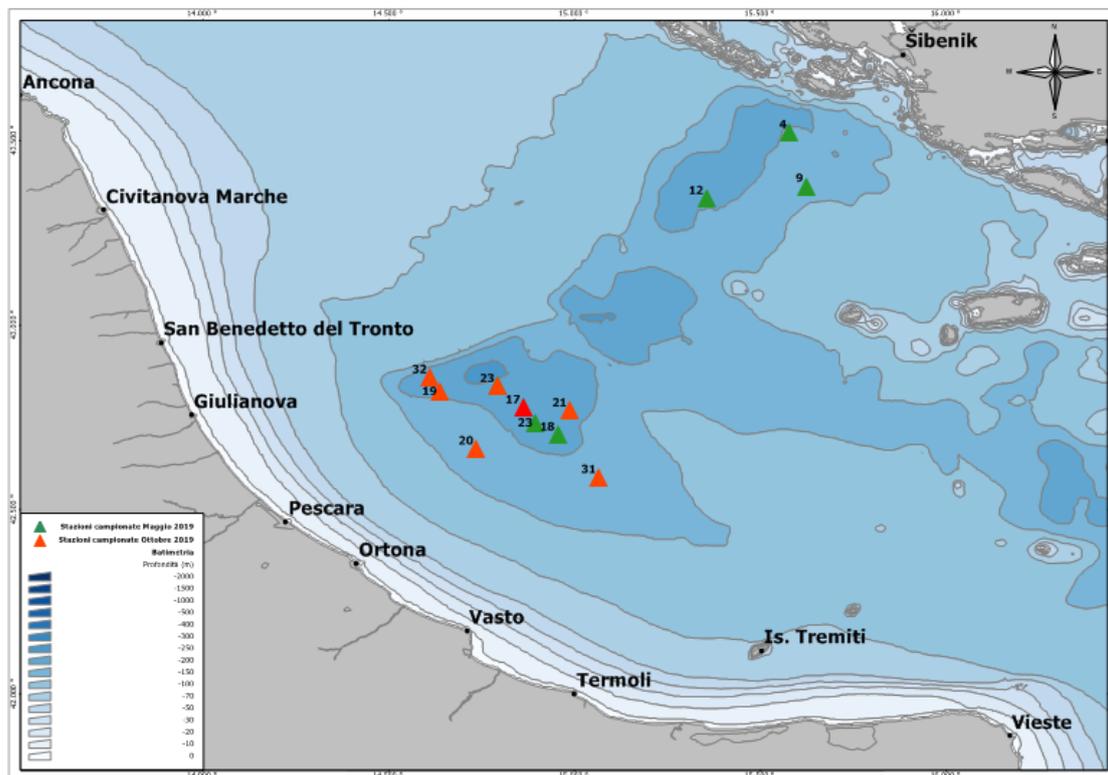


Fig. 2.7 Hauls carried in the Pomo area in May and October 2019 from whom samples for this study were taken.

These cruises are part of a multi-year monitoring program for the evaluation of the effects of the protection measures implemented in the Pomo area and funded by the MIPAFF. The oceanographic vessel Dallaporta has a length of 35.7 m, width 7.67 m and a draft between 3 and 3.5 m. Hauls were carried out

through an experimental trawl-net, characterized by rhomboid meshes with a size of 22 mm in the body of the net and 12 mm in the cod end(Martinelli et al., 2019).

Once collected, samples were frozen on board, then transported to the IRBIM laboratories for macroscopic, biometric and stomach content analyses.

#### **2.4 – Macroscopic and biometric evaluation**

The number of analysed Norway lobster from CAMPBIOL sampling (specimens collected off Ancona) was 421 (249 males and 172 females), while for Pomo, the number of analysed specimens were 257 (146 males and 111 females) (table 2.1).

Tab. 2.1 Total number of *Nephrops norvegicus* analysed by site and sex

	<b>Males</b>	<b>Females</b>	<b>Tot</b>
<b>Off Ancona</b>	249	172	421
<b>Pomo</b>	146	111	257

Sex determination was carried out by evaluating the presence, in the male, of the copulatory organ, absent in female individuals. For each individual, the total wet weight was taken using a Precision Scale Radwag WLC 6/ F1/K balance (accuracy 0.1 g), while gonads and hepatopancreas were weighed using Mettler –Toledo XP204 (accuracy 10<sup>-4</sup> g), the carapace length (CL) was measured at the lowest mm using a caliper. The

hepatopancreas, stomach and gonads were extracted by dissecting the animal, using laboratory scissors and tweezers (figures 2.8 and 2.9).



Fig.2.8 Carapace content of *Nephrops norvegicus*.

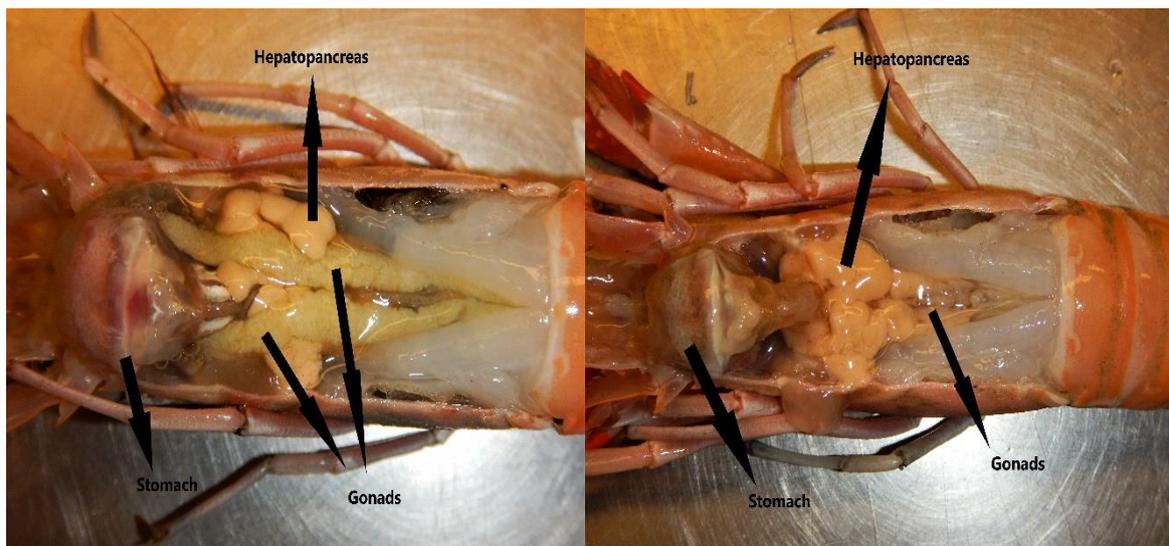


Fig. 2.9 Details of the contents of the carapace of an individual male on the right and one female on the left of *Nephrops norvegicus*.

During this last step the sexual maturity of the female individuals was annotated by using a 5-stage scale according to ICES protocol (International Council for the Exploitation of the Sea, 2010) (figure 2.10).

PROPOSED STAGES	NAME	MACROSCOPIC DESCRIPTION	COLOUR	REMARKS
1	Immature	Ovaries not visible without dissection. The ovaries are translucent, thin and threadlike.	Uncoloured	No picture available
2	Developing/ /Recovering	Ovaries barely visible without dissection. The gonads extends up to the 1st somite of the abdomen and have a granular appearance.	White to cream	The histological analyses do not differentiate the developing gonads in the first maturation from the recovering ones. The cut-size length shall be defined by area/stock, after analyses of lengths vs. maturity data.
3	Maturing	Ovaries are clearly visible through integument. The gonad occupies one third of the cephalotoracic space. The gonads extends up to the 1st somite of the abdomen.	Light green	
4	Mature	Turgid ovaries occupying the whole dorsal cephalotoracic space and extending up to the 2nd somite. Eggs visible.	Dark green	Reabsortion of the ovarian material can be observed in any of the stages 3 to 5.
5	Spent	Ovaries flaccid with green spots. Re-absorption of ovarian material. Most likely with green eggs on pleopods.	Ivory with green spots	
		Berried females		Berried female shall be recorded as a remark.

Fig 2.10 The maturity scale for *Nephrops norvegicus*, (from ICES International Council for the Exploitation of the Sea, 2010)

Once extracted, stomachs were put into 14 ml falcons filled with 70% alcohol and kept in the refrigerator until the contents were analyzed.

## 2.5 - Estimation of biological indices

The gonadosomatic index (%GSI) is an indicator of a species sexual maturity, it is estimated by the ratio of gonads weight to the weight of the organism \* 100.

$$GSI = \frac{\textit{Weight of gonad}}{\textit{Weight of total body}} \times 100$$

The hepatosomatic index (%HSI) is a proxy of the metabolic activity, and it is calculated as the ratio of the hepatopancreas weight to the weight of the organism \*100.

$$HSI = \frac{\textit{Weight of hepatopancreas}}{\textit{Weight of total body}} \times 100$$

## 2.6 - Stomach content analysis

All the stomachs were opened under a stereomicroscope.

Stomachs were firstly weighed full and then after the contents was put in a petri dish, the stomach wall was weighed, in this way by subtracting the two weights it was possible to determine the content weight and thus to evaluate the stomach fullness, as follows

$$Fulness = \frac{Weight\ of\ stomach\ contents * 100}{Weight\ of\ total\ body}$$

Afterthat, all preys were identified to the lowest taxonomic level (Fanelli et al., 2009). The percentage of volume of stomach contents was estimated using the subjective points methods (Swynnerton & Worthington 1940) for the different prey-species or prey-groups.

The following indexes were calculated (Hyslop et al., 1980):

(1) frequency of occurrence (%F) of prey,

$$\%F = \frac{Number\ of\ stomachs\ containing\ prey\ i}{Total\ number\ of\ stomachs\ containing\ prey} X100$$

(2) percentage of numeric abundance of prey (%N):

$$\%N = \frac{Number\ of\ prey\ i}{Total\ number\ of\ prey} X100$$

(3) percentage of wet weight of prey (%W):

$$\%W = \frac{Weight\ of\ prey\ i}{Total\ weight\ of\ all\ prey} X100$$

These values were used to calculate the index of relative importance in according (Pianka, 1973) (IRI) for each taxonomic category:

$$IRI = (\%N + \%W) * (\%F)$$

Trophic diversity was calculated on the diet of specimens from the two areas using the Shannon-Wiener index,  $H'$  (Shannon et al., 1949)

## 2.7 – Data analysis

Data from the sampled *Nephrops norvegicus* were analyzed by univariate and multivariate analyses. For the multivariate analysis the preys were grouped by major taxonomic group (Table 2.2).

Tab. 2.2 Total preys founded during stomach content analysis and the relative Trophic groups, ate the left the table for Off Ancona while at the right for Pomo area.

Taxa	Trophic group	Taxa	Trophic group
Bivalvia	Mollusca	Bivalvia	Mollusca
Brachyura	Decapoda	Cefalopode	Mollusca
Cefalopode	Mollusca	Decapoda	Decapoda
Decapoda	Decapoda	Ditrupa	Polychaetaha
<i>Goneplax rhomboides</i>	Decapoda	Echinoidea	Echinodermata
<i>Kurtiella bidentata</i>	Mollusca	<i>Elphidium spp.</i>	Foraminifera
<i>Liocarcinus spp.</i>	Decapoda	<i>Munida rutilanti</i>	Decapoda
Mytilidae	Mollusca	<i>Operculina spp.</i>	Foraminifera
Natantia	Decapoda	Polychaete	Polychaetaha
Pectinidae	Mollusca	Fish remanins	Osteichthyes
Penaeidae	Decapoda		
Polychaete	Polychaetha		
Porifera	Others		
Fish remanins	Osteichthyes		
Vegetables remains	Vegetables		
Sepiolidae	Mollusca		
<i>Sternapsis scutata</i>	Polychaetha		
<i>Turritella spp.</i>	Mollusca		

For samples collected off Ancona, the analysis of *N. norvegicus* specimens was done by month and by season, while for Pomo/Jabuka pits samples, the analysis was carried out taking into account the factor depth, i.e. samples from hauls above and below 200 m depth, which means also outside and inside and the pits (< 200 m outside pits and > 200 m inside pits). Off Ancona specimens were not collected in August, due to the trawl ban established in the area by National Regulation. On the other hand, in November specimens were not collected due to the low number of days-at-sea caused by bad weather conditions. Thus, the monthly analysis encompassed only 10 months and the summer season included only specimens from June and July, while autumn only September and October. For the comparison of the diets of Ancona and Pomo, the *N. norvegicus* were also grouped by size (small, medium and large respectively <30 / 31-45 / > 46 mm).

Size classes, sex ratio, and others variables (GSI, HSI, Fullness) were analysed by means of univariate analysis and specifically by ANOVA, using the software R i386 3.6.2 (R Core Team, 2019). Before performing the ANOVA tests, data were tested for homogeneity of variance by the Levene test. If the test provided a P-value <0.05, the appropriate transformations were carried out on the data. For the analysis of the probability distribution between two variables, the Welch's *t*-test was used. This is a two-sample location test, which

is used to test the hypothesis that two populations have equal means. Chi-square tests were used to verify that the frequencies of the observed values adapt to the theoretical frequencies of a predetermined probability distribution (Hatfield, 1998).

In order to calculate the trophic diversity of the preys in stomachs contents, the Shannon index was calculated, as follows:

$$H' = - \sum_{j=1}^s p_j \log_e p_j$$

Multivariate techniques were used to analyze the similarities between the samples, which consider multiple variables simultaneously. First, a non-metric nMDS (Multi Dimensional Scaling) was carried out on the Bray-Curtis resemblance matrix of 4<sup>th</sup> root-transformed prey biomass data. The coefficient of Bray- Curtis quantifies the similarity of the samples based on the counts of each variable (Gorley, 2006) and the nMDS represents very similar samples as the closest points, while further points indicate that samples are different (Gorley, 2006). Afterthat, a Permutational Multivariate Analysis of Variance (PERMANOVA) was carried out to test for differences among different levels of a factor, by using permutation methods and by calculating p-values through

Monte Carlo test (Anderson et al., 2008). The Pair-wise test was carried out on the Modified Gower resemblance to test the comparisons among the seasons. To test PERMANOVA tests were run considering two different sampling designs. In the case of specimens from Ancona, the sampling design had two factors: season, fixed, with four levels and sex, fixed, with two levels, crossed within each others. For specimens from Pomo, sampling design had, again, two factors, depth, fixed with two levels, as specified above, and sex, fixed with two levels, crossed within each others. SIMPER analysis was used to evaluate the taxa that mostly contributed to the similarity between the various groups. Canonical Analysis of principal coordinates (CAP, Anderson et al., 2008) was run, on the factor found to be significant in PERMANOVA in order to visualize separation among samples on the basis of putative factors (along the two axes). Finally the Group Average was done, the output is a dendrogram, which shows the grouping of samples into progressively smaller numbers of clusters, with a progressive increase in similarity between the clusters (Gorley et al., 2006).

Finally in order to investigate the differences in the diet of specimens collected from Ancona and Pomo, a cluster analysis was run on May and October samples, as these are the two months in common between the two sampling design. The analysis was based on averaged data (i.e. stomach contents) by month per area. The output is a dendrogram, which shows the grouping of

samples into progressively smaller numbers of clusters, with a progressive increase in similarity between the clusters (Gorley et al., 2006)

All the multivariate analyses were carried out with PRIMER6 & PERMANOVA+ (Clarke et al., 2008; Anderson et al., 2008).

## Chapter 3 - Results

### 3.1 – Biological characteristics of Norway lobster collected off Ancona

The frequency length distribution showed a unimodal distribution, with a similar size range for both sexes, between 15 and 75 mm CL for males, and between 20 and 75 mm CL for females, with very few female individuals larger than 75 mm (figure 3.1). However, length distribution significantly differed between the two sexes (Welch t-test,  $t=4.4$ ,  $df=266.5$ ,  $p\text{-value} < 0.001$ ), being the mean CL of males = 47.3 mm, while for females this was 42.6 mm.

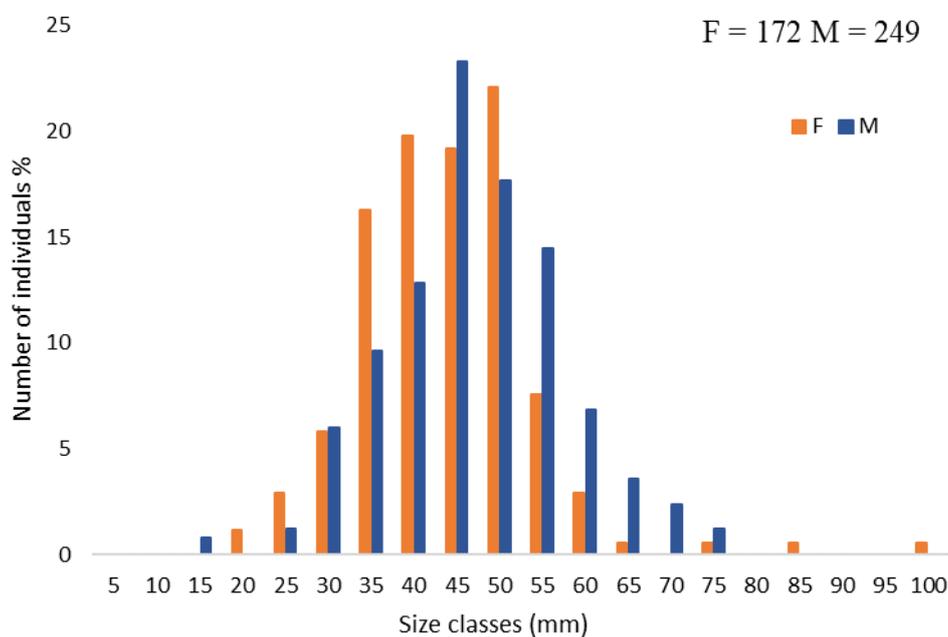


Fig. 3.1 Frequency distribution of the sampled *Nephrops norvegicus*.

For the remaining analyses, the samples were grouped by months and seasons.

The sex ratio showed the relationship between the number of male and female

individuals, the number of males is higher in all months except June and July, where females are higher only in summer (figures 3.2 and 3.3).

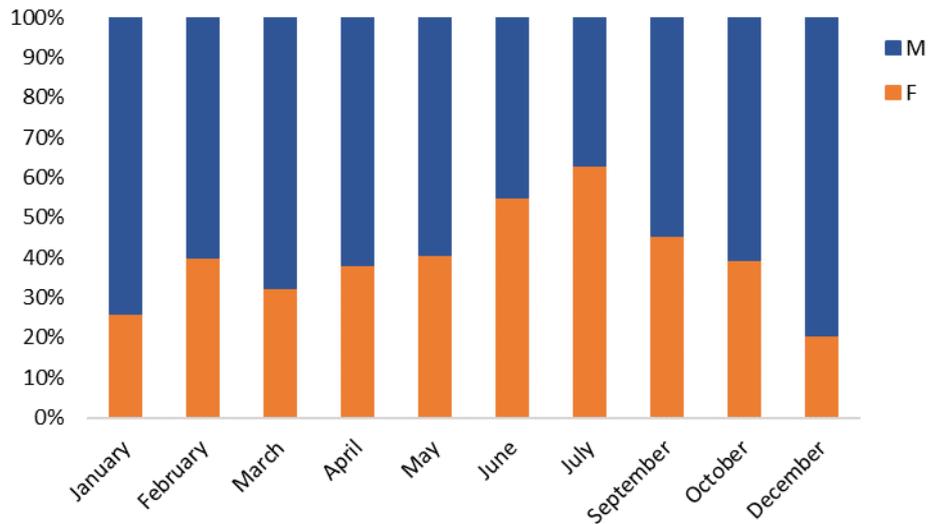


Fig. 3.2 Temporal trend of the number *Nephrops norvegicus* divided by sex per month.

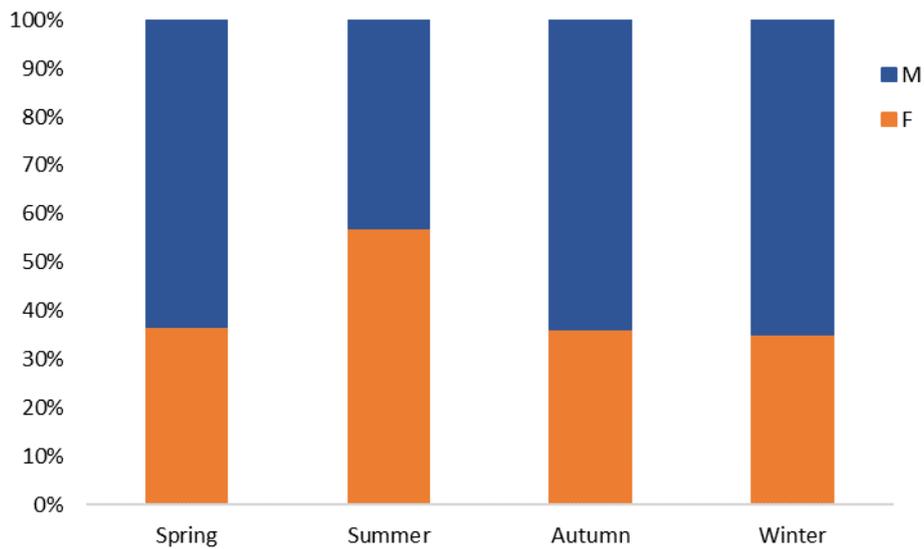


Fig. 3.3 Temporal trend of the number of *Nephrops norvegicus* divided by sex per season.

The gonadosomatic index (%GSI) has been calculated for both sex and related to months and season (figures 3.4 and 3.5). For females, the highest %GSI

values were recorded in summer. For males the value remains constant throughout the year.

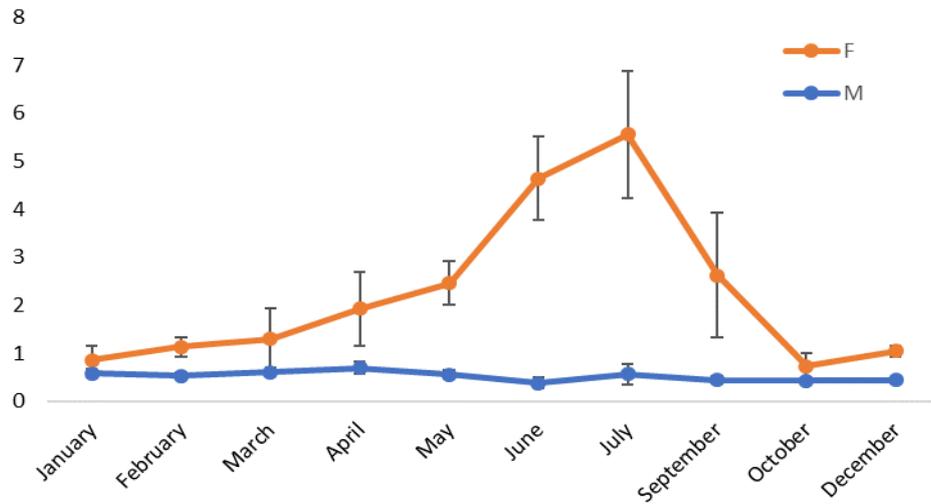


Fig. 3.4 Temporal trend of the %GSI of *Nephrops norvegicus* divided by sex and month.

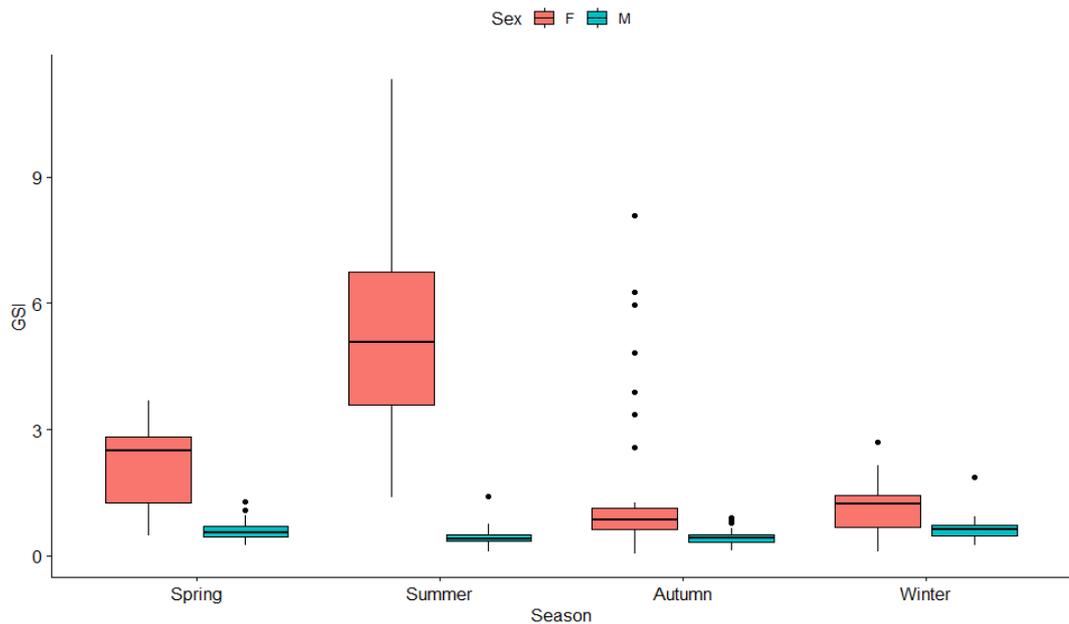


Fig. 3.5 Temporal trend of the %GSI of *Nephrops norvegicus* divided by sex and season. However, differences were not significant for any of the factors tested (table 3.1).

Tab. 3.1 Results of non parametric ANOVA for %GSI by sex and season.

	<b>Df</b>	<b>MS</b>	<b>F</b>	<b>Pr(&gt;F)</b>
<b>Sex</b>	1	0.03	0.07	0.79
<b>Season</b>	3	0.29	0.60	0.62
<b>Sex:Season</b>	3	0.73	1497	0.21
<b>Residuals</b>	317	0.49		

The hepatosomatic index (%HSI) has been calculated for both sex and related to months and seasons. For females, %HSI values remained fairly constant except for an increase in September and October. The males showed constant %HSI values, with the lowest values observed in the winter period (figures 3.6 and 3.7).

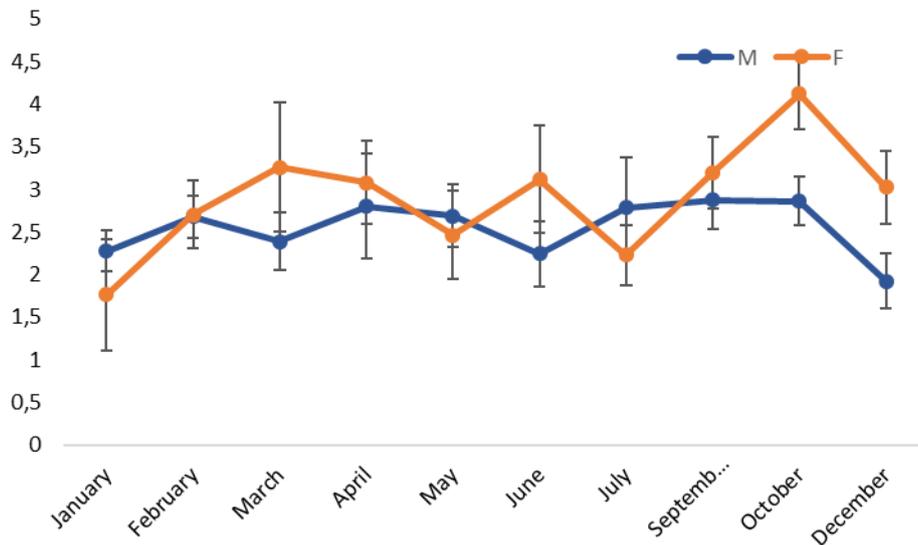


Fig. 3.6 Temporal trend of the %HSI of sampled *Nephrops norvegicus* divided by sex and month.

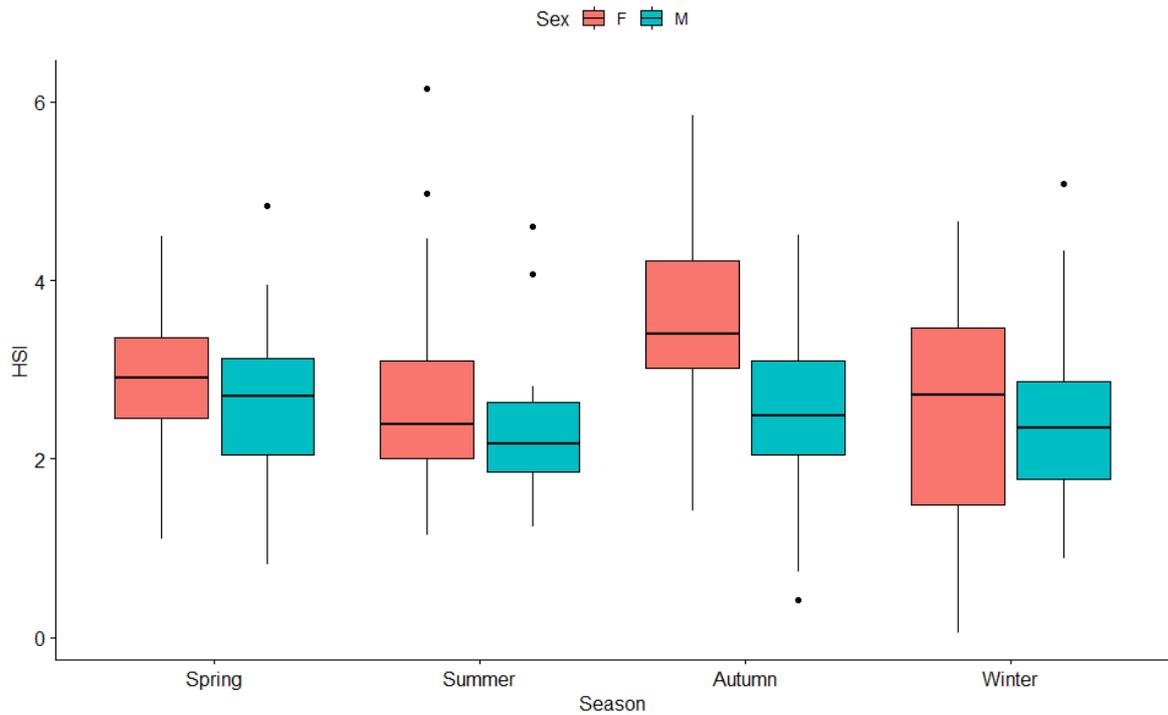


Fig. 3.7 Temporal trend of the %HSI of sampled *Nephrops norvegicus* divided by sex and season.

The differences in %HSI were highly significant for all factors tested (table 3.2).

Tab. 3.2 Result of ANOVA for HSI by sex and season.

	<b>Df</b>	<b>MS</b>	<b>F</b>	<b>Pr(&gt;F)</b>
<b>Sex</b>	1	14092	15888	8.34e-05 ***
<b>Season</b>	3	4634	5224	0.00156 **
<b>Sex:Season</b>	3	3856	4347	0.00509 **
<b>Residuals</b>	317	0.887		

The fullness index slightly varied across seasons (figure 3.8).

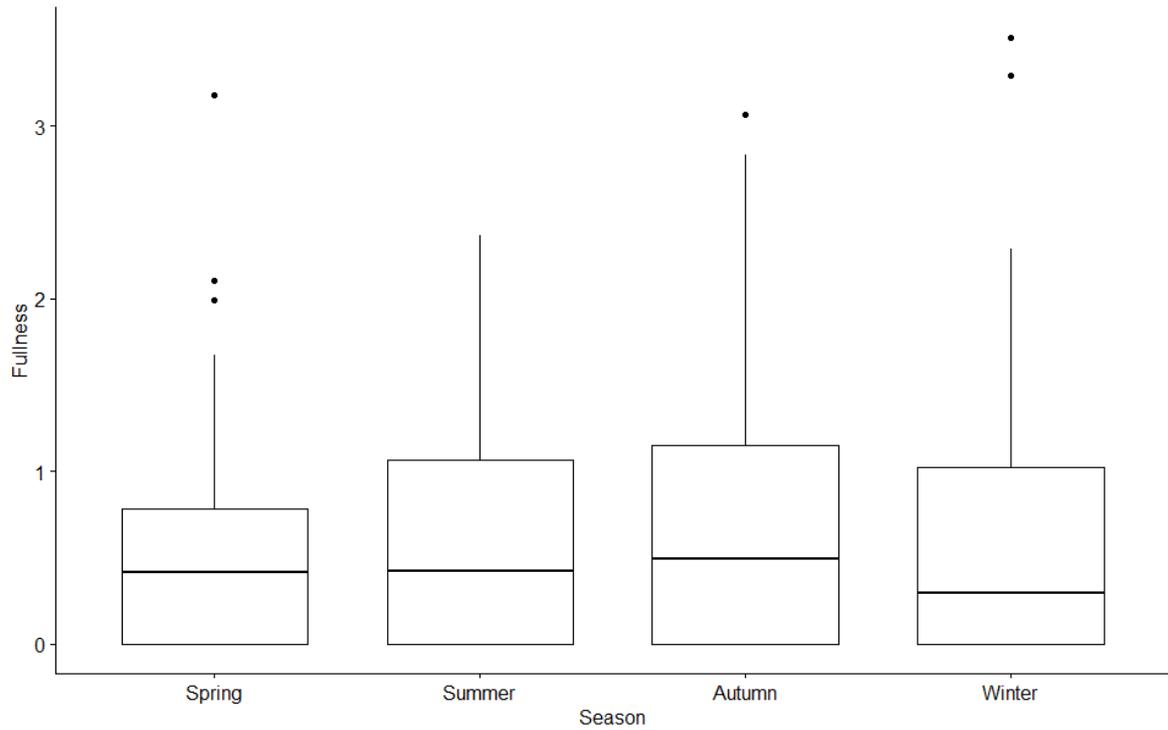


Fig. 3.8 Temporal trend of the Fullness index of *Nephrops norvegicus* by season.

The fullness index calculated separately for females and males, by month show a quite constant trend (figure 3.9), however an increase was observed for males in September and October. This trend is also confirmed by season (figure 3.10).

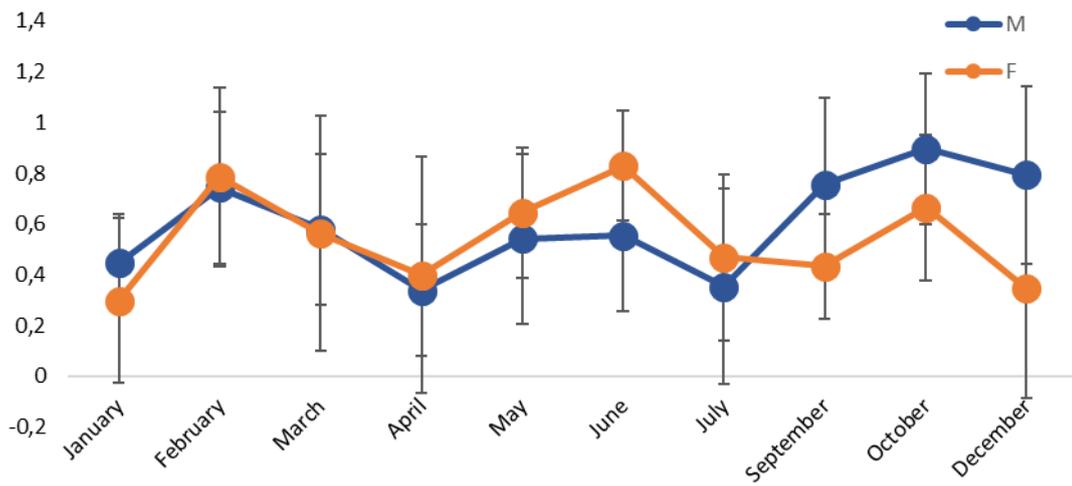


Fig. 3.9 Temporal trend of the Fullness index of *Nephrops norvegicus* by sex and month.

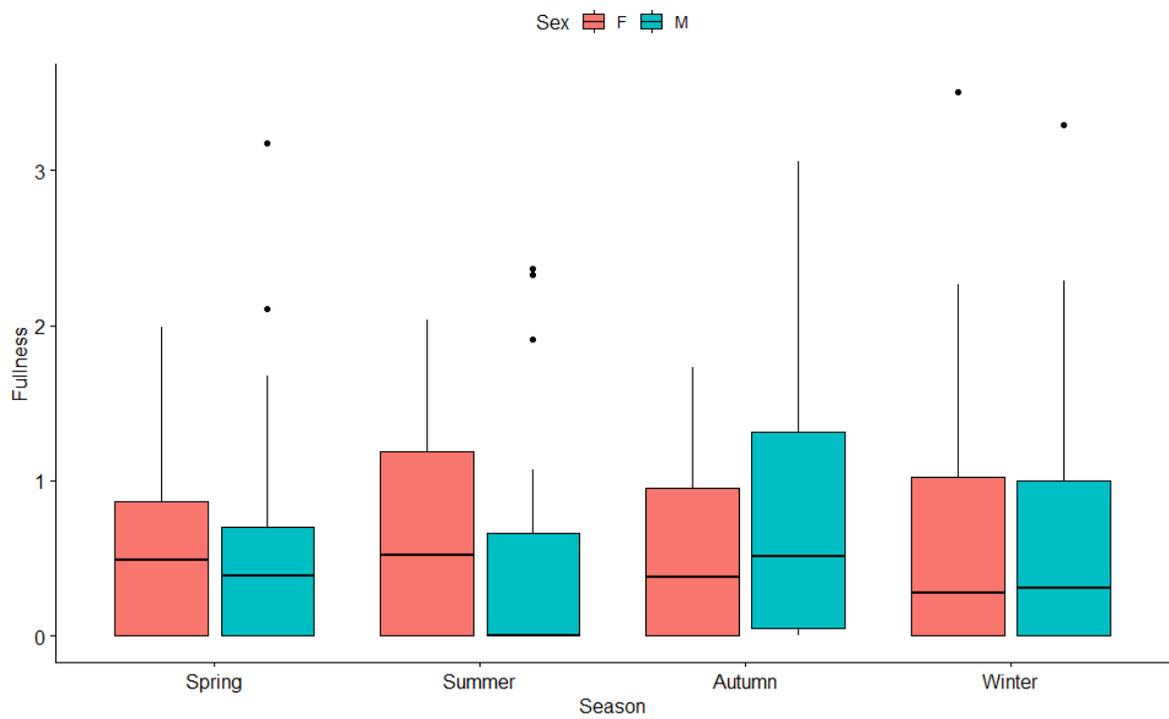


Fig. 3.10 Temporal trend of the Fullness index of *Nephrops norvegicus* by sex and season.

However, differences were not significant for any of the factors tested (table 3.3).

Tab. 3.3 Result of ANOVA for fullness by sex and season.

	Df	MS	F	Pr(>F)
<b>Sex</b>	1	0.03	0.07	0.79
<b>Season</b>	3	0.29	0.596	0.62
<b>Sex:Season</b>	3	0.73	1497	0.21
<b>Residuals</b>	317	0.49		

When %GSI and %HSI of females were plotted together (figure 3.11), they show an almost opposite trend: while %HSI increased in autumn, the maximum value of %GSI was in summer.

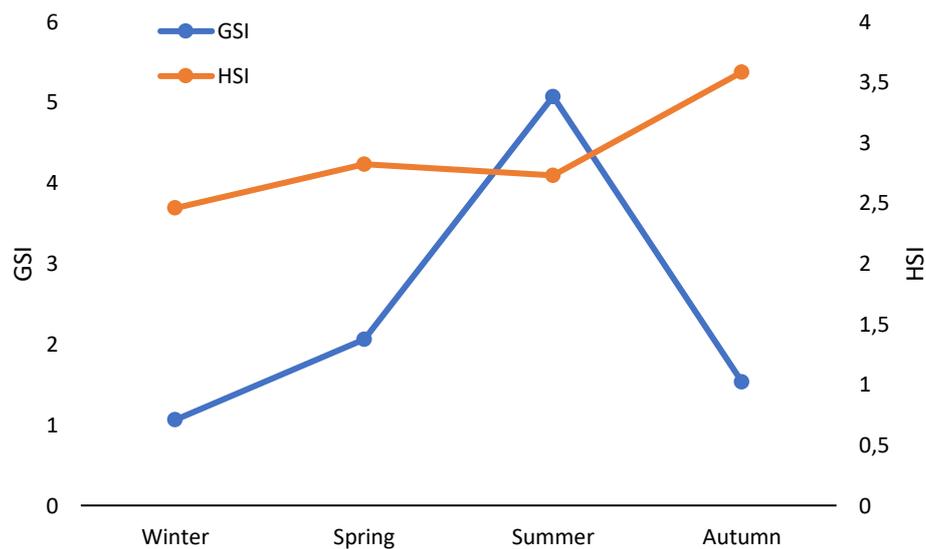


Fig. 3.11 Temporal trend of %GSI, %HSI and Fullness of *Nephrops norvegicus* by season.

### 3.2 - Diet composition of specimens collected off Ancona

The total number of full stomachs analysed were 235. A total of 18 taxa were identified, mainly decapods and fishes (see Annex 1). Among decapods, the species *Goneplax rhomboides* and the genus *Liocarcinus* sp. represented the most abundant Decapoda found in the stomach contents. The analysis of diet composition in terms of %IRI showed that *N. norvegicus* mostly fed on Decapoda, Osteichthyes and Polychaeta, throughout the year (figure 3.12).

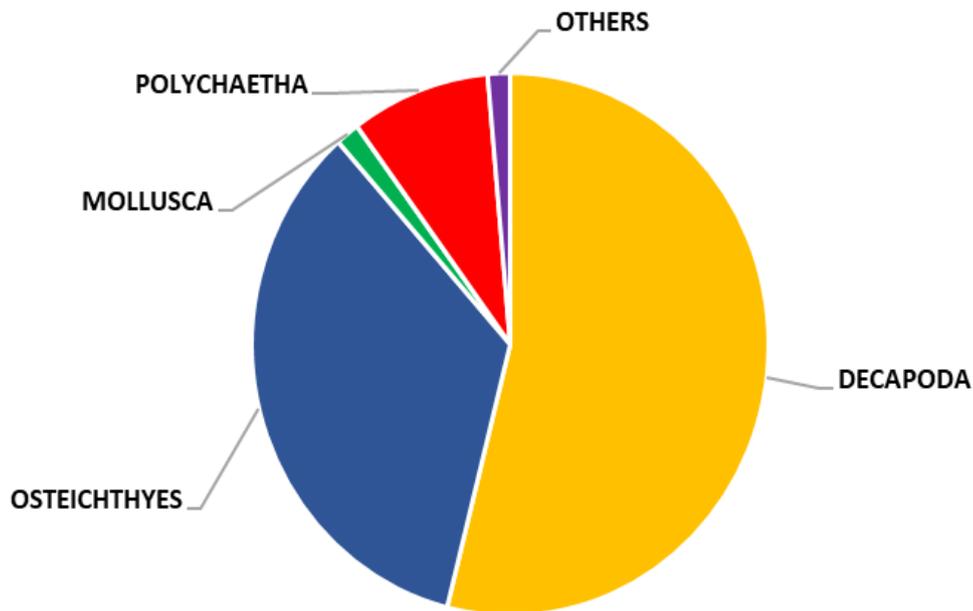


Fig. 3.12 Diet composition (in terms of %IRI) of *Nephrops norvegicus* during the year.

At seasonal level, the most abundant prey were always Decapoda and Osteichthyes, with an increase in the consumption of Polychaeta in autumn (figure 3.13).

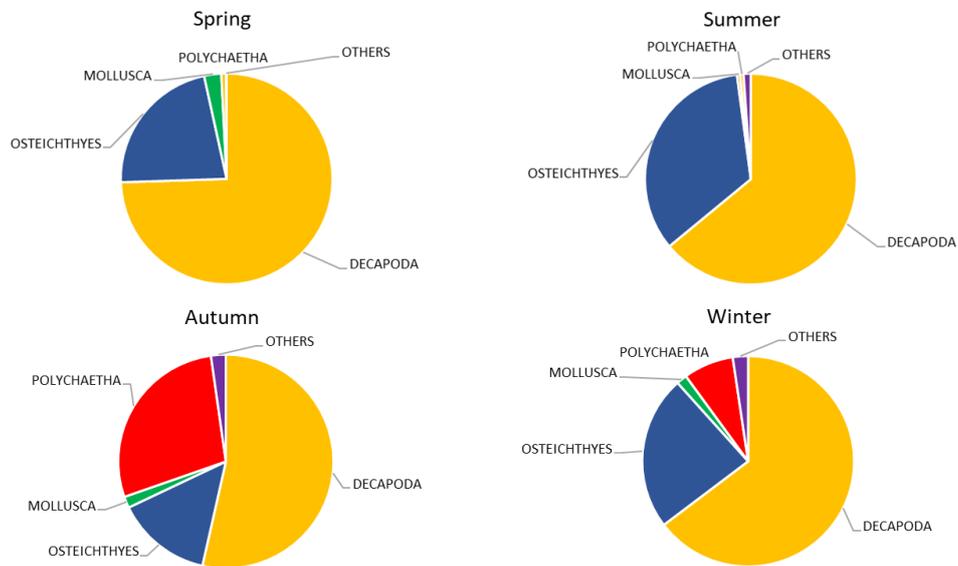


Fig. 3.13 Diet composition (in term of %IRI) of *Nephrops norvegicus* by season.

The nMDS plot showed division by season, in particular, a grouping of summer and spring can be seen. (figure 3.14).

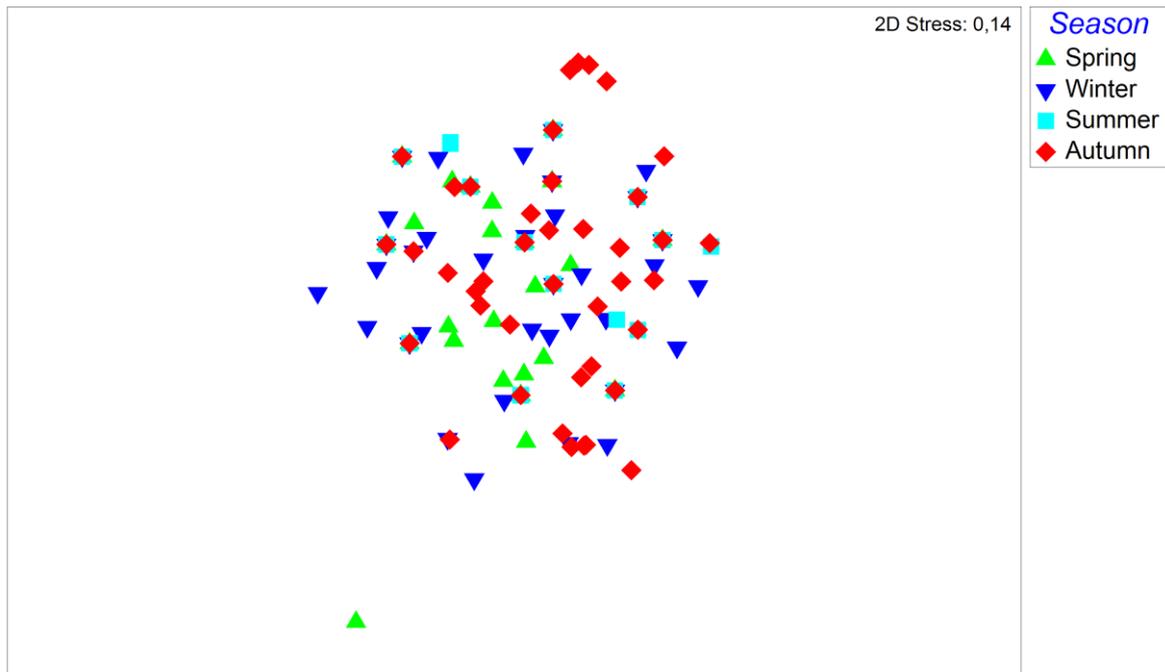


Fig. 3.14 nMDS plot by season.

The differences in %W were significant only for season, but not by sex, than the shift in diet has occurred in spring-summer and in summer-autumn. (table 3.4).

Tab. 3.4 Result of PERMANOVA for % W by sex and season (a) and the results of pairwise test for % W by season (b).

<b>a) Source</b>	<b>Df</b>	<b>MS</b>	<b>Pseudo-F</b>	<b>P</b>
<b>Season</b>	3	8405.3	22798	0.006
<b>Sex</b>	1	1027.7	0.28	0.90
<b>SeasonxSex</b>	3	2874.1	0.78	0.68
<b>Residuals</b>	222	3686.9		
<b>Total</b>	229			
<b>b) Groups</b>	<b>t</b>	<b>P(perm)</b>		
<b>Winter, Spring</b>	0.50	0.92		
<b>Spring, Summer</b>	17279	0.02		
<b>Summer, Autumn</b>	22415	0.001		
<b>Autumn, Winter</b>	11331	0.27		

The CAP analysis showed a strong separation among seasons, summer and spring showing a more marked division, winter and autumn were well separated from the other two seasons (figure 3.15).

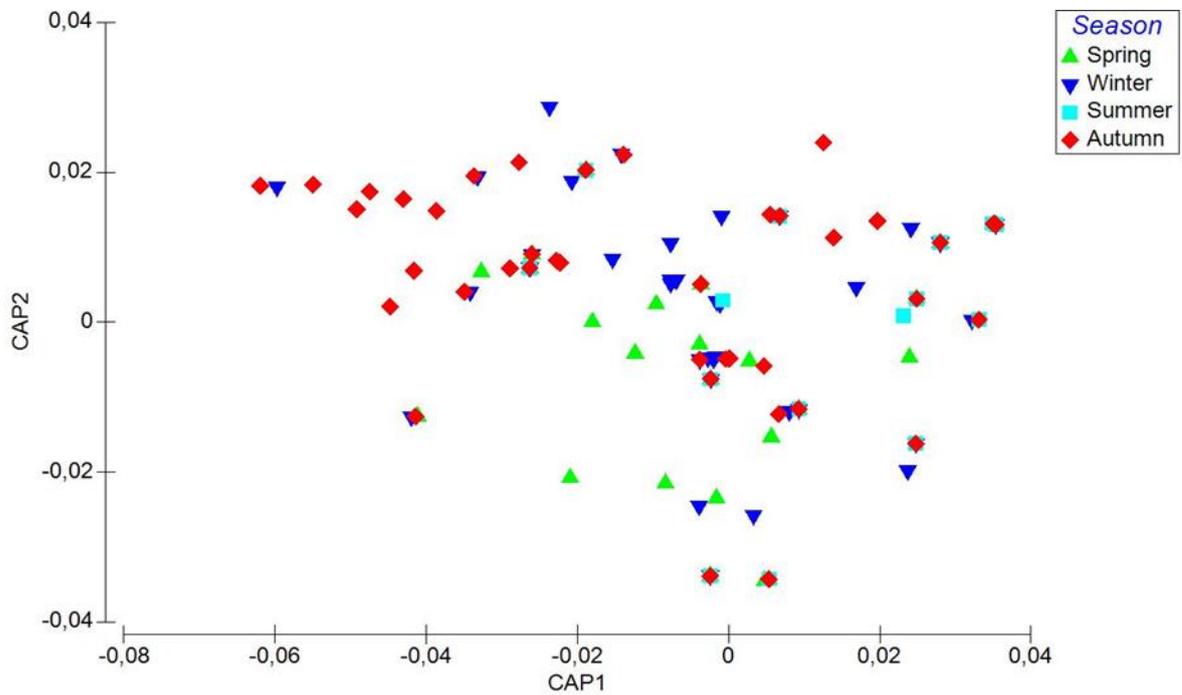


Fig. 3.15 Cap plot based on factor season.

SIMPER analysis showed a strong contribution of Decapoda and Osteichthyes in every season, in Winter other taxa contributed to similarity in the diet, as *Liocarcinus* sp., *Goneplax rhomboides*, Brachyura and Polychaeta (table 3.5).

Tab. 3.5 Similarity percentage of different prey by season.

<b>Group Spring</b>		<b>Average similarity: 17.73</b>			
Taxon	Av.Abund	Av.Sim	Sim/SD	Contrib%	Cum.%
Decapoda	0.2	4.84	0.28	27.28	27.28
Osteichthyes	0.2	4.43	0.28	25	52.28
Brachyura	0.16	2.67	0.22	15.04	67.32
<i>Goneplax rhomboides</i>	0.17	2.63	0.22	14.83	82.16
<i>Liocarcinus sp.</i>	0.18	2.62	0.22	14.8	96.96
<b>Group Winter</b>		<b>Average similarity: 14.97</b>			
Decapoda	0.18	3.75	0.24	25.03	25.03
Osteichthyes	0.2	2.87	0.25	19.2	44.23
<i>Liocarcinus sp.</i>	0.18	2.83	0.22	18.92	63.14
<i>Goneplax rhomboides</i>	0.15	2.03	0.17	13.57	76.72
Brachyura	0.14	1.97	0.19	13.16	89.87
Polychaeta	0.08	0.61	0.15	4.07	93.94
<b>Group Summer</b>		<b>Average similarity: 25.64</b>			
Osteichthyes	0.37	15.3	0.54	59.67	59.67
Decapoda	0.19	6.81	0.34	26.56	86.23
<i>Liocarcinus sp.</i>	0.14	1.94	0.19	7.57	93.8
<b>Group Autumn</b>		<b>Average similarity: 18.41</b>			
Brachyura	0.27	6.8	0.36	36.96	36.96
Decapoda	0.17	3.42	0.23	18.58	55.54
Osteichthyes	0.18	2.87	0.24	15.58	71.11
Polychaeta	0.18	2.84	0.3	15.4	86.52
<i>Liocarcinus sp.</i>	0.13	1.12	0.15	6.1	92.62

The Shannon index ( $H'$ ) displayed values ranging from 0.67 to 1.36 through the year, however seasonal differences were not significant (Univariate Permanova, P-value > 0.05) (table 3.6).

Tab 3.6 Results of Univariate Permanova test for the Shannon index .

	<b>Df</b>	<b>MS</b>	<b>Pseudo-F</b>	<b>P(perm)</b>
<b>Season</b>	3	0.14	0.81	0.49
<b>Sex</b>	1	0.003	0.02	0.89
<b>SeasonxSex</b>	3	0.29	17615	0.15
<b>Residuals</b>	227	0.17		
<b>Total</b>	234			

List of the taxa found in *Nephraps norvegicus* stomach contents collected during the seasons. Values are expressed in percentage by weight (%W), abundance (%N), frequency (%F) and index of relative importance (%IRI)

	Spring				Winter				Summer				Autumn				
	%W	%N	%F	%IRI	%W	%N	%F	%IRI	%W	%N	%F	%IRI	%W	%N	%F	%IRI	
<i>Bivalvia</i>	0,71	5,43	5,88	0,36	0,33	1,59	1,56	0,03	0,82	5,56	4,55	0,29	0,79	5,05	6,58	0,38	
<i>Brachyura</i>	12,59	15,22	23,53	6,54	10,72	11,90	21,88	4,95	6,73	8,33	13,64	2,05	23,99	15,15	36,84	14,42	
<i>Cefalopode</i>	0,96	2,17	3,92	0,12	1,20	0,79	1,56	0,03	-	-	-	-	5,54	2,02	5,26	0,40	
<i>Decapoda</i>	13,06	17,39	29,41	8,96	13,52	14,29	25,00	6,95	13,77	19,44	31,82	10,57	11,27	8,59	22,37	4,44	
<i>Goneplax ramboides</i>	12,95	13,04	23,53	6,12	15,73	9,52	18,75	4,74	4,44	5,56	9,09	0,91	4,90	3,54	9,21	0,78	
<i>Kurtiella bidentata</i>	-	-	-	-	0,21	0,79	1,56	0,02	-	-	-	-	-	-	-	-	-
<i>Liocarcinus</i> spp.	19,41	14,13	23,53	7,89	19,48	13,49	23,44	7,73	15,61	15,28	25,00	7,72	17,75	6,06	15,79	3,76	
<i>Mytilidae</i>	-	-	-	-	0,47	1,59	1,56	0,03	6,52	2,78	2,27	0,21	0,00	-	-	-	-
<i>Natantia</i>	6,29	8,70	11,76	1,76	2,49	3,97	6,25	0,40	-	-	-	-	4,51	7,58	9,21	1,11	
<i>Pectinidae</i>	-	-	-	-	0,38	0,79	1,56	0,02	-	-	-	-	-	-	-	-	-
<i>Penaeidae</i>	-	-	-	-	0,83	1,59	3,13	0,08	-	-	-	-	-	-	-	-	-
<i>Polychaeta</i>	0,34	2,17	1,96	0,05	2,18	16,67	15,63	2,94	0,42	6,94	4,55	0,33	6,54	37,88	28,95	12,86	
<i>Porifera</i>	-	-	-	-	0,97	0,79	1,56	0,03	-	-	-	-	-	-	-	-	-
<i>Fish remains</i>	15,21	16,30	29,41	9,27	20,81	13,49	26,56	9,11	49,66	30,56	50,00	40,11	16,81	9,60	25,00	6,60	
<i>Vegetables remains</i>	0,61	1,09	1,96	0,03	2,41	5,56	10,94	0,87	2,05	5,56	9,09	0,69	5,63	4,04	10,53	1,02	
<i>Sepioliidae</i>	13,52	2,17	3,92	0,62	7,67	2,38	4,69	0,47	-	-	-	-	-	-	-	-	-
<i>Sternopsis scutata</i>	4,35	2,17	3,92	0,26	-	-	-	-	-	-	-	-	2,27	0,51	1,32	0,04	
<i>Turritella</i> spp.	-	-	-	-	0,61	0,79	1,56	0,02	-	-	-	-	-	-	-	-	-

### 3.3 Biological characteristics of Norway lobster collected Pomo/Jabuka pits

The frequency length distribution showed a bimodal distribution, with a medium size between 20 and 35 mm CL for females and between 35 and 50 mm CL for males (figure 3.16). Length distribution significantly differed between the two sexes (Welch t-test,  $t=15.7$ ,  $df=253.2$ ,  $p\text{-value} < 0.001$ ), being the mean CL of males = 38.8 mm, while for females this was 27.8 mm.

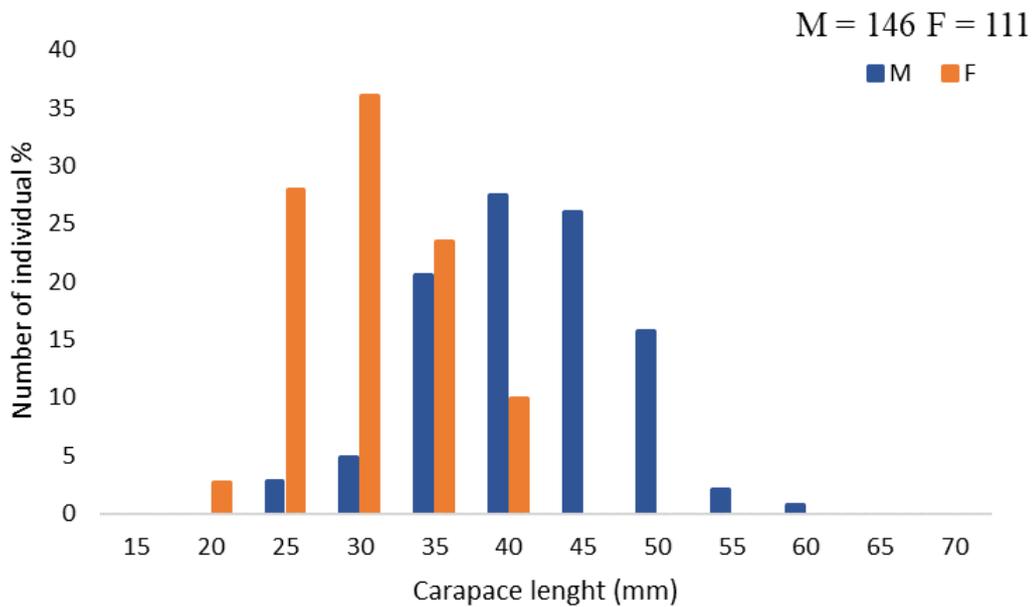


Fig. 3.16 Frequency distribution of the *Nephrops norvegicus*.

The number of males to females was higher inside and outside of the pit (figure 3.17).

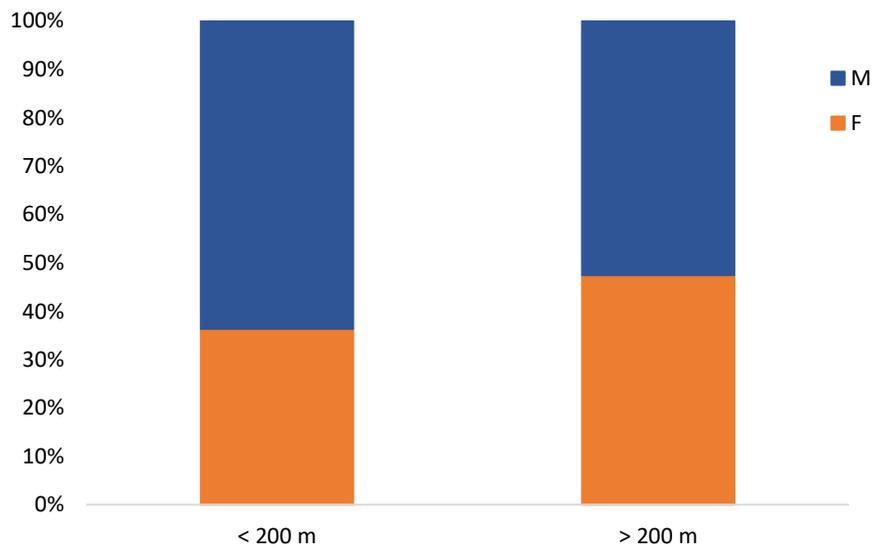


Fig. 3.17 Sex ratio of *Nephrops norvegicus* in Pomo by depth.

The higher %GSI values for females were observed inside the pit, while similar values were observed for males both outside and inside (figure 3.18).

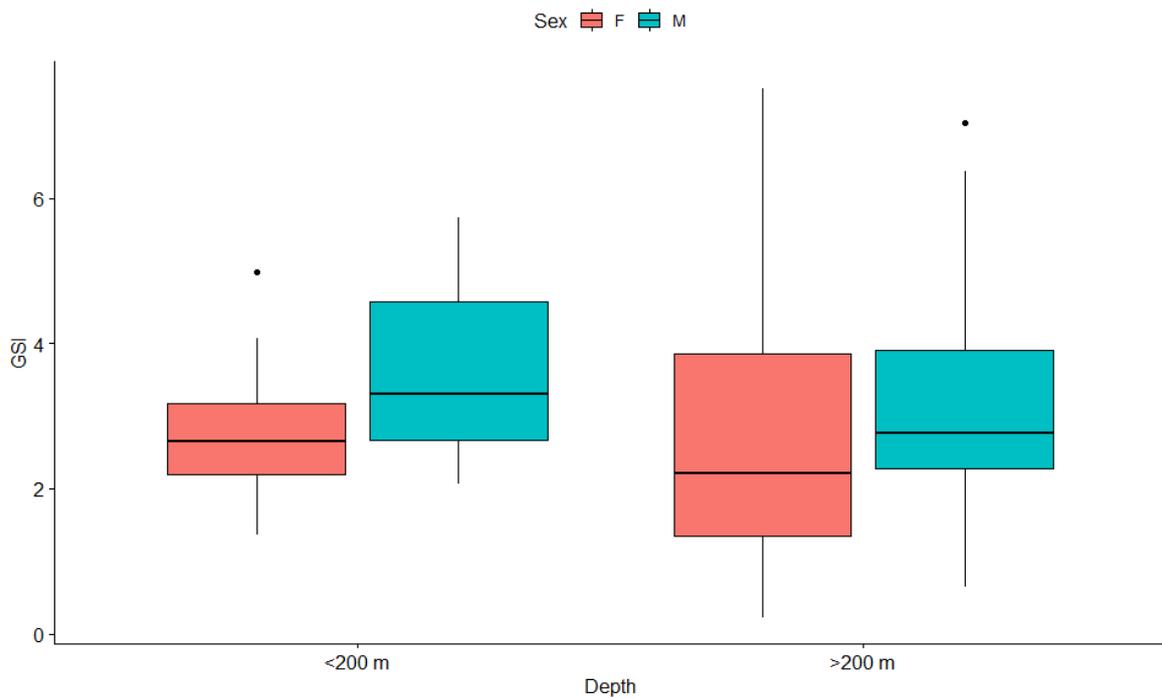


Fig. 3.18 Trend of %GSI of *Nephrops norvegicus* in Pomo area by sex and depth.

However, differences were not significant for any of the factors tested (table 3.7).

Tab. 3.7 Result of ANOVA for %GSI by sex and season.

	<b>Df</b>	<b>MS</b>	<b>F</b>	<b>Pr(&gt;F)</b>
<b>Sex</b>	1	6624	3124	0.08
<b>Depth</b>	1	4234	1997	0.16
<b>Sex:Depth</b>	1	2571	1213	0.27
<b>Residuals</b>	121	2120		

%HSI values were lower inside the pit than outside for both sexes (figure 3.19).

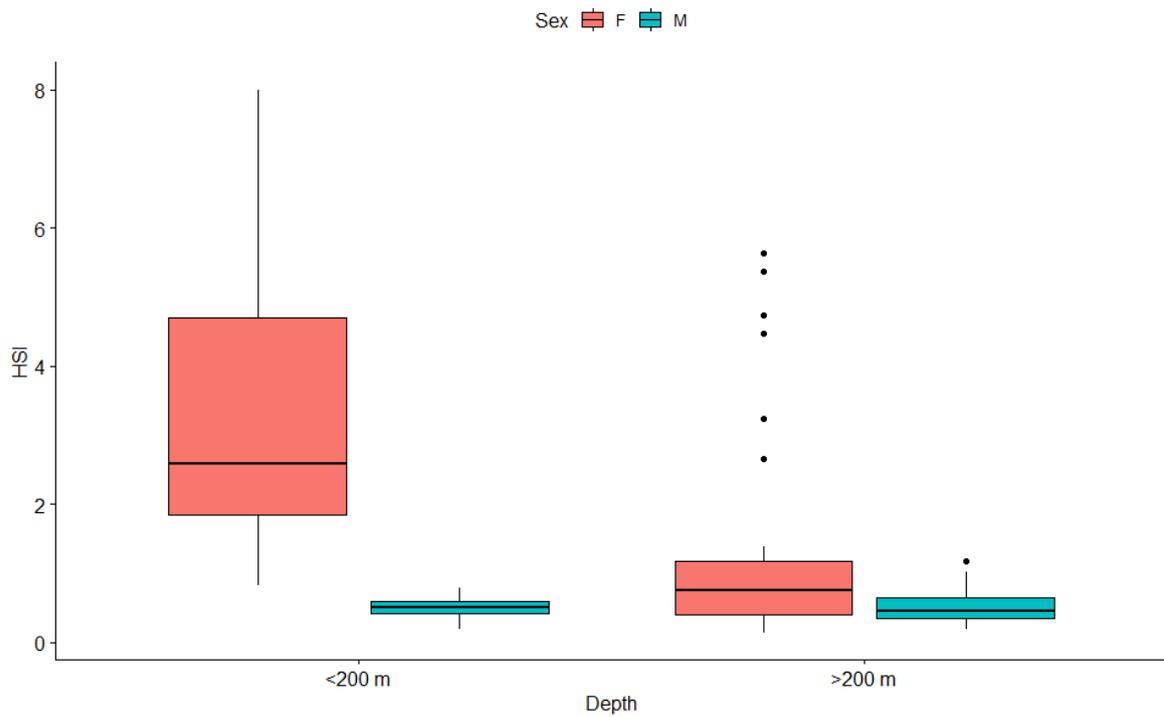


Fig. 3.19 Trend of the %HSI of *Nephrops norvegicus* in Pomo area by sex and depth.

The differences in %HSI were highly significant for all the factors tested (table 3.8)

Tab. 3.8 Result of ANOVA for HSI by sex and depth.

	<b>Df</b>	<b>MS</b>	<b>F</b>	<b>Pr(&gt;F)</b>
<b>Sex</b>	1	65.08	54180	2.42e-11 ***
<b>Depth</b>	1	11.46	9542	0.003 **
<b>Sex:Depth</b>	1	25.14	20930	1.16e-05 ***
<b>Residuals</b>	121	1.20		

Overall the fullness index was similar inside and outside the pit (figure 3.20).

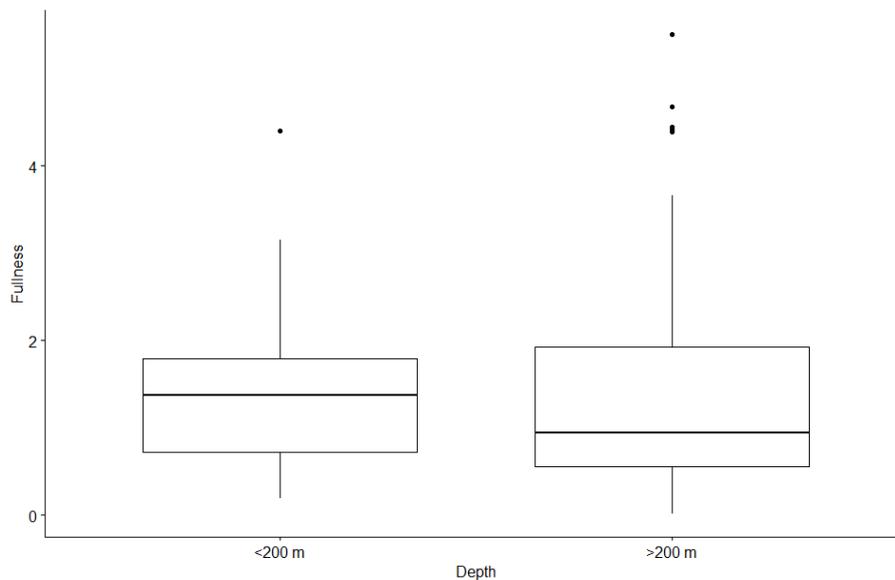


Fig. 3.20 Trend of Fullness index of *Nephrops norvegicus* in Pomo area related to depth.

The fullness index calculated separately for females and males, by depth, did not show significant differences (Table 3.10), however an increase of the index inside the pit was observed in females (figure 3.21).

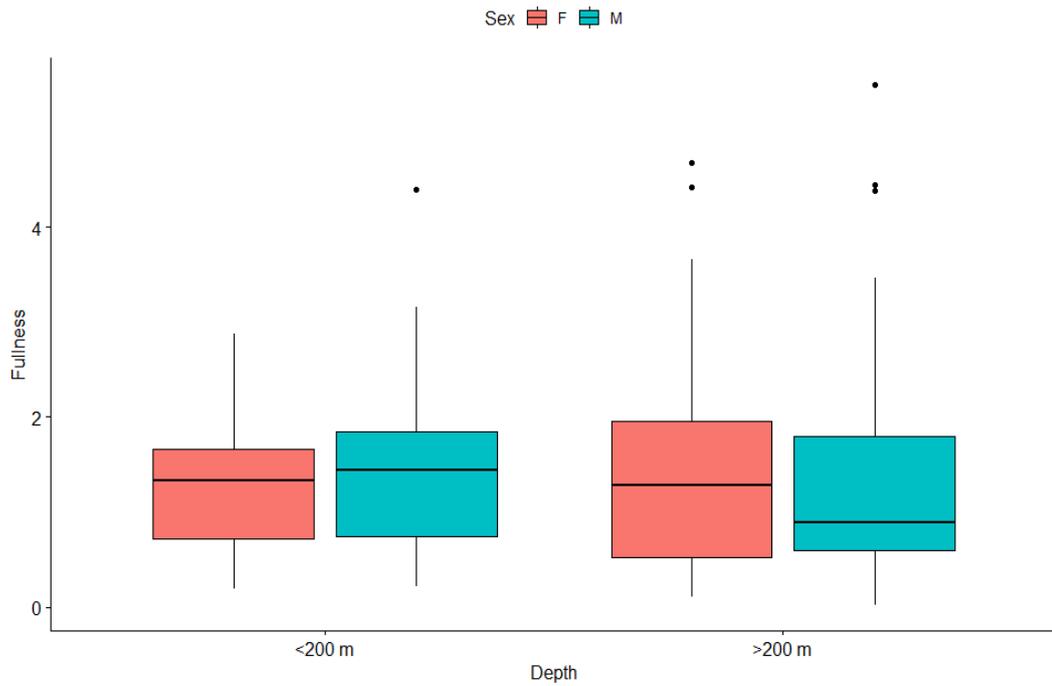


Fig. 3.21 Trend of the Fullness index of *Nephrops norvegicus* divided by sex and depth.

Tab. 3.9 Result of ANOVA for fullness by sex and depth.

	<b>Df</b>	<b>MS</b>	<b>F</b>	<b>Pr(&gt;F)</b>
<b>Sex</b>	1	0.06	0.04	0.83
<b>Depth</b>	1	0.03	0.02	0.88
<b>Sex:Depth</b>	1	0.78	0.62	0.43
<b>Residuals</b>	121	1253		

### 3.4 – Diet composition of specimens collected in Pomo

The total number of full stomachs analysed were 127. A total of 10 taxa were identified, mainly decapods and fishes (see Annex 2). Among decapods, the species *Munida ruttianti* represented the most abundant Decapoda found in the stomach contents. The analysis of diet composition in terms of %IRI showed that the species mostly fed on Decapoda, Osteichthyes and Polychaeta, throughout the year (figure 3.22).

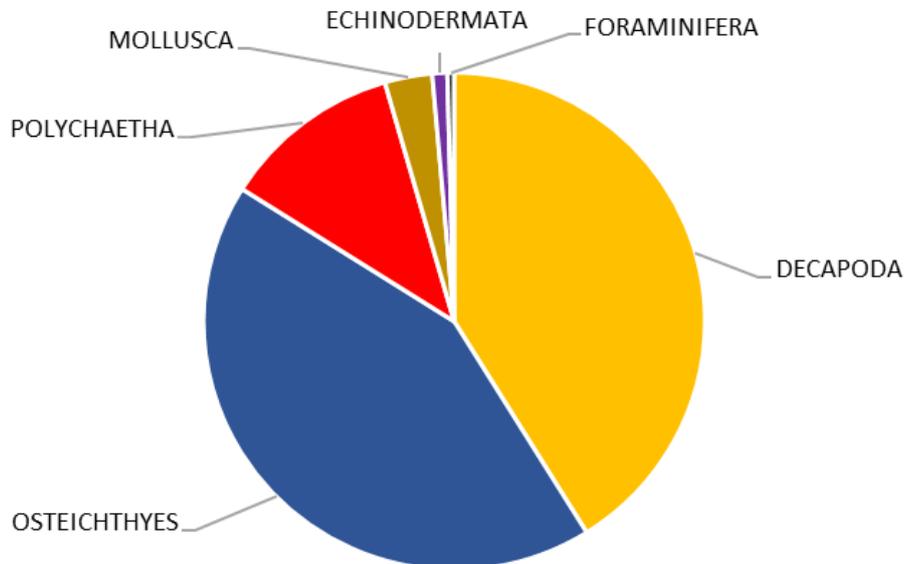


Fig. 3.22 Diet composition (in terms of %IRI) of *Nephrops norvegicus*, in Pomo area.

At depth level, the most abundant prey were Decapoda and Osteichthyes outside the pit, instead, inside the pit, the most abundant prey were Decapoda, Polychaeta and Foraminifera (figure 3.23).

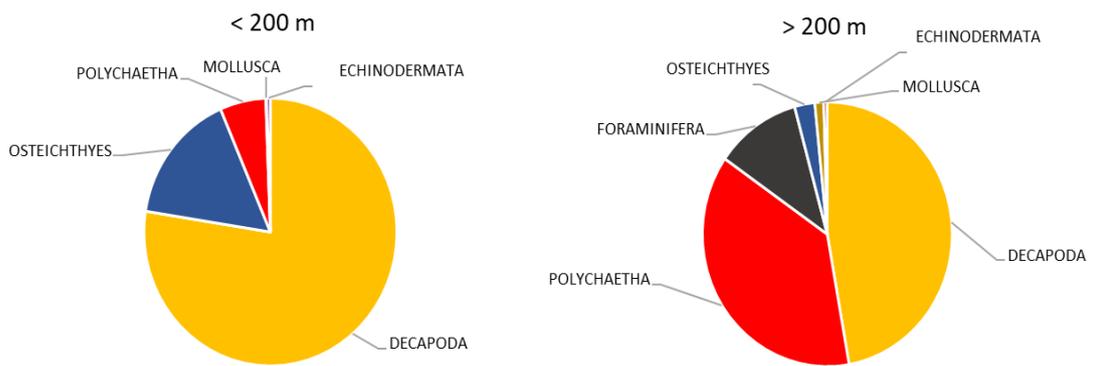


Fig. 3.23 Diet composition (in terms of %IRI) of *Nephrops norvegicus* inside and outside Pomo pits.

The nMDS plot showed a clear separation of samples collected inside and outside the pit (figure 3.24).

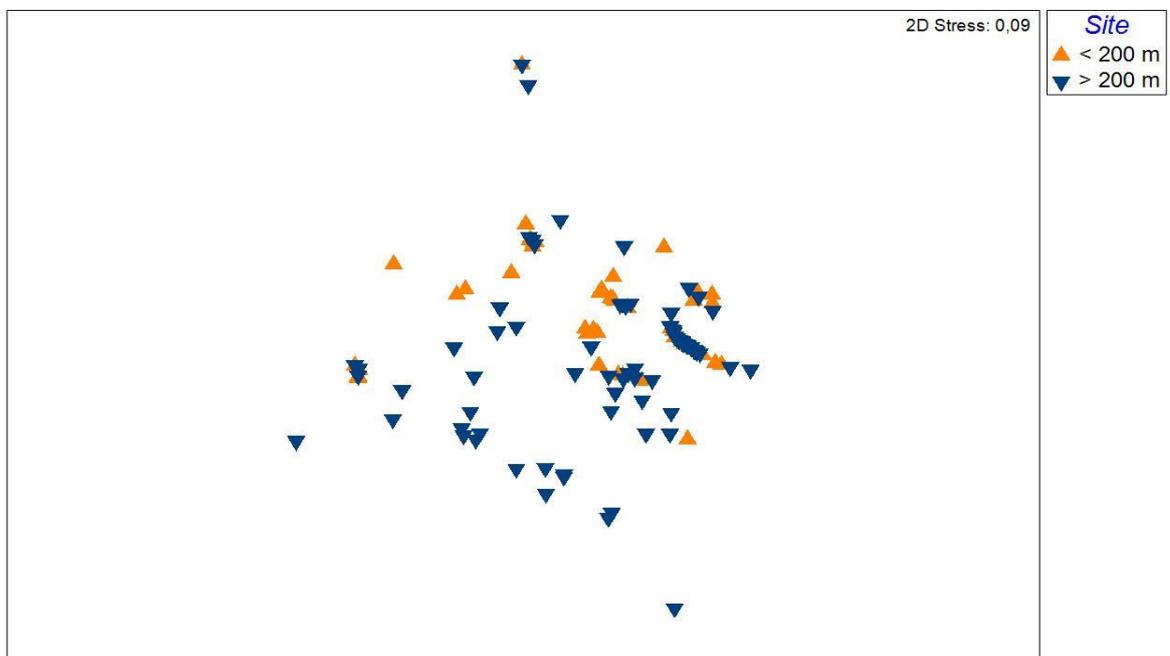


Fig 3.24 nMDS plot by site (inside and outside Pomo pits).

The differences in %W were significant for all the factors tested (table 3.10).

Tab. 3.10 Result of PERMANOVA for %W by site and size.

	<b>Df</b>	<b>MS</b>	<b>Pseudo-F</b>	<b>P(perm)</b>
<b>Site</b>	1	9241.4	34148	0.02
<b>Size</b>	2	5658.1	20907	0.05
<b>SitexSize</b>	2	2216.1	0.82	0.56
<b>Residuals</b>	121	2706.3		
<b>Total</b>	126			

SIMPER analysis showed a strong contribution of *Munida rutllanti* both inside and outside the pit (table 3.11), with a general very-high contribution to similarity (on average 70%).

Tab 3.11 Results of SIMPER analysis for factor site.

<b>Group &lt; 200 m</b>		<b>Average similarity: 36.09</b>			
Taxon	<b>Av.Abund</b>	<b>Av.Sim</b>	<b>Sim/SD</b>	<b>Contrib%</b>	<b>Cum.%</b>
<i>Munida rutllanti</i>	0.48	23.99	0.82	66.48	66.48
Osteichthyes	0.31	9.59	0.45	26.57	93.05
<b>Group &gt; 200 m</b>		<b>Average similarity: 30.50</b>			
<i>Munida rutllanti</i>	0.43	22.94	0.72	75.21	75.21
Polychaeta	0.14	3.76	0.34	12.33	87.53
Osteichthyes	0.11	1.27	0.17	4.17	91.7

The Shannon index ( $H'$ ) displayed values ranging from 0.65 to 1.37 through the year, did not vary significantly by depth (Univariate Permanova, P-value > 0.05) (table 3.12).

Tab 3.12 Results of Univariate Permanova test for the Shannon index.

	<b>Df</b>	<b>MS</b>	<b>Pseudo-F</b>	<b>P(perm)</b>
<b>Site</b>	1	0.11	0.64	0.42
<b>Month</b>	1	0.18	1054	0.31
<b>SitexMonth</b>	1	0.07	0.39	0.52
<b>Residuals</b>	123	0.17		
<b>Total</b>	126			

List of the taxa found in *Nephtys norvegicus* stomach contents outside and inside the Pomo pit.

Values are expressed in percentage by weight(%W), abundance (%N), frequency (%F) and index of relative importance (%IRI)

	< 200 m				> 200 m			
	%W	%N	%F	%IRI	%W	%N	%F	%IRI
Bivalvia	1,09	10,34	8,00	0,92	2,18	7,76	11,69	1,16
Cefalopode	8,24	2,59	6,00	0,65	9,25	2,59	3,90	0,46
Decapoda	6,55	6,90	16,00	2,15	5,79	11,21	16,88	2,87
Ditrupea	0,09	1,72	2,00	0,04	0,17	3,45	2,60	0,09
Echinoidea	0,56	0,86	2,00	0,03	5,16	4,31	6,49	0,61
<i>Elphidium spp.</i>	0,09	2,59	2,00	0,05	0,42	70,69	15,58	11,08
<i>Munida rutilanti</i>	52,75	28,45	66,00	53,59	62,85	40,52	61,04	63,10
<i>Operculina spp.</i>	0,28	3,45	2,00	0,07	0,40	42,24	10,39	4,43
Polychaeta	2,38	24,14	16,00	4,24	5,70	149,14	33,77	52,28
Fish remains	27,98	18,97	44,00	20,65	8,08	12,07	18,18	3,66

### 3.5 – Diet comparison between Ancona and Pomo/Jabuka pits

The Cluster analysis showed a clear separation between samples from Pomo and Ancona, being separation mostly function of site, but also month. For the Ancona samples, a further grouping by size can be seen, with the large ones of both months separating also for the Pomo samples can be seen the large well divided by the small and medium (figure 3.25).

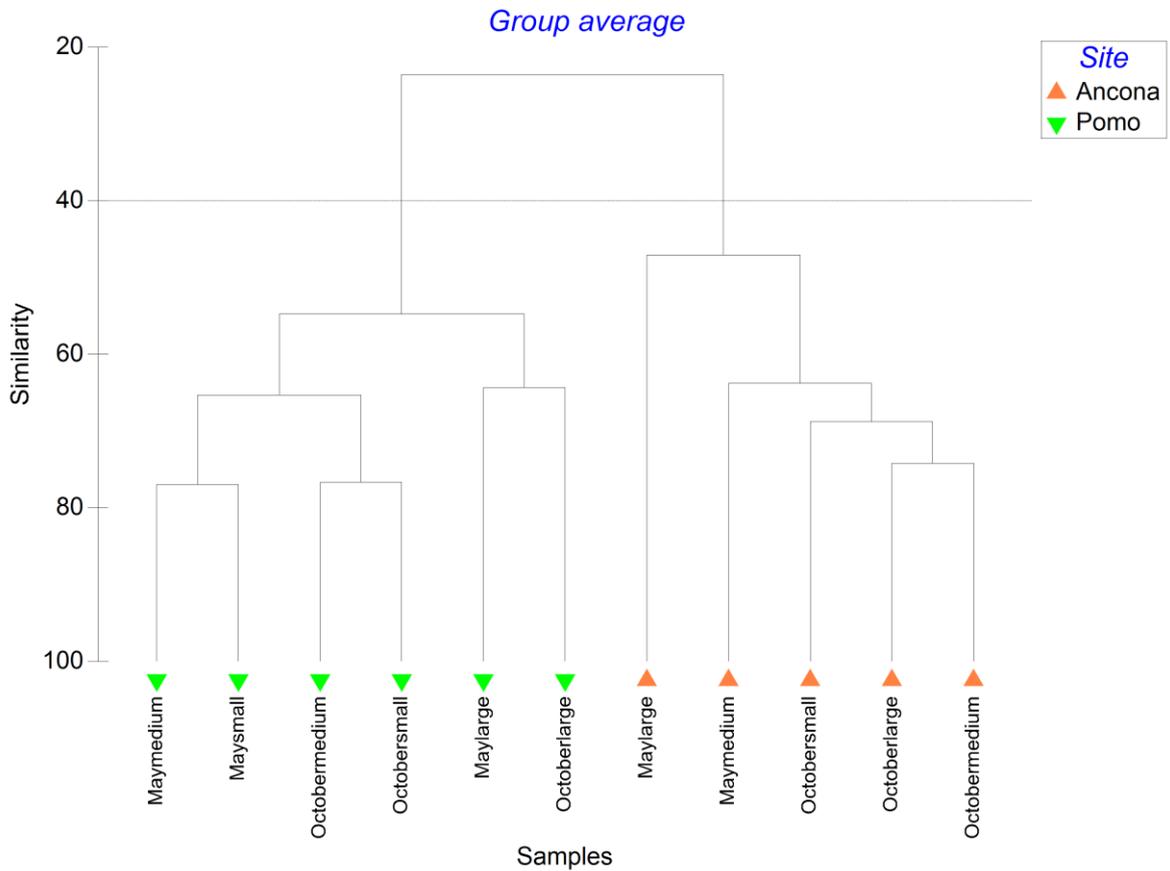


Fig. 3.25 Similarity percentage of the different prey by site, month and size.

The nMDS, with overlaid clusters at 40 and 60% of similarity, showed a clear separation of samples collected off Ancona and in the Pomo pit. The separation also occurred between months, sites and size.(figure 3.26).

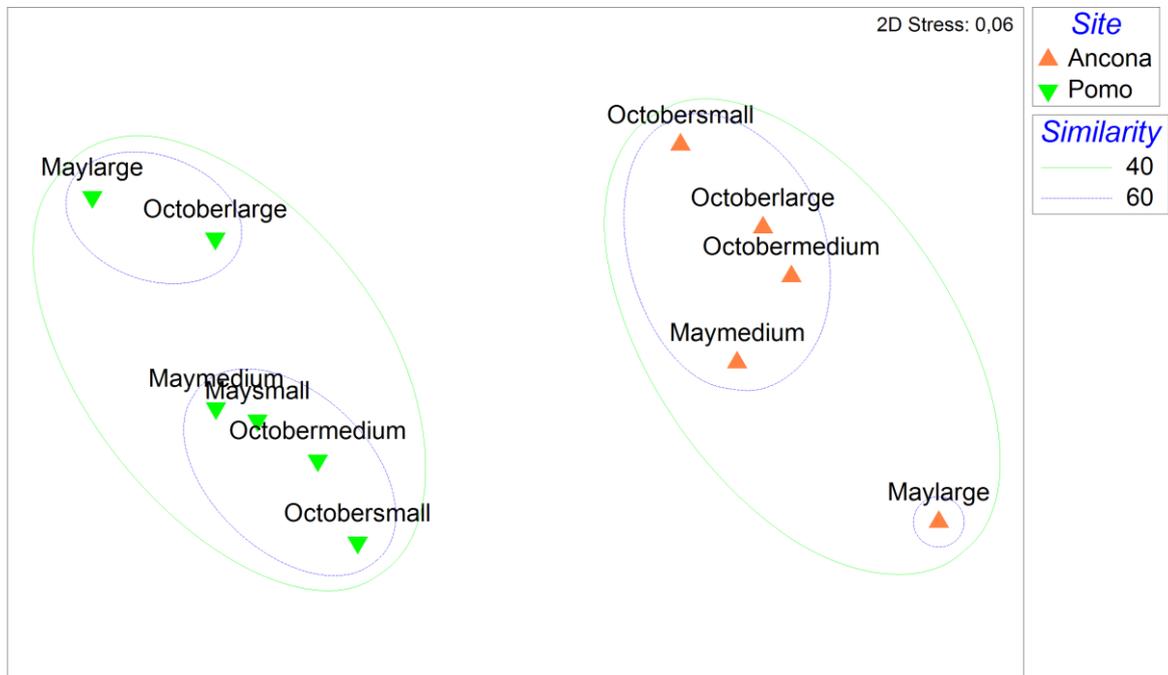


Fig 3.26 nMDS plot by site and month (Off Ancona and Pomo).

The differences in % W were significant for site and month (table 3.13).

Tab. 3.13 Result of. PERMANOVA for % W by site and month.

	<b>Df</b>	<b>MS</b>	<b>Pseudo-F</b>	<b>P(MC)</b>
<b>Site</b>	1	67761	22152	0.01
<b>Month</b>	1	16586	54224	0.01
<b>SitexMonth</b>	1	963.89	0.31	0.88
<b>Residuals</b>	123	3058.9		
<b>Total</b>	126			

The CAP analysis shows a separation among months and by site. It is possible to see a good separation between the sites, also by month (figure 3.27).

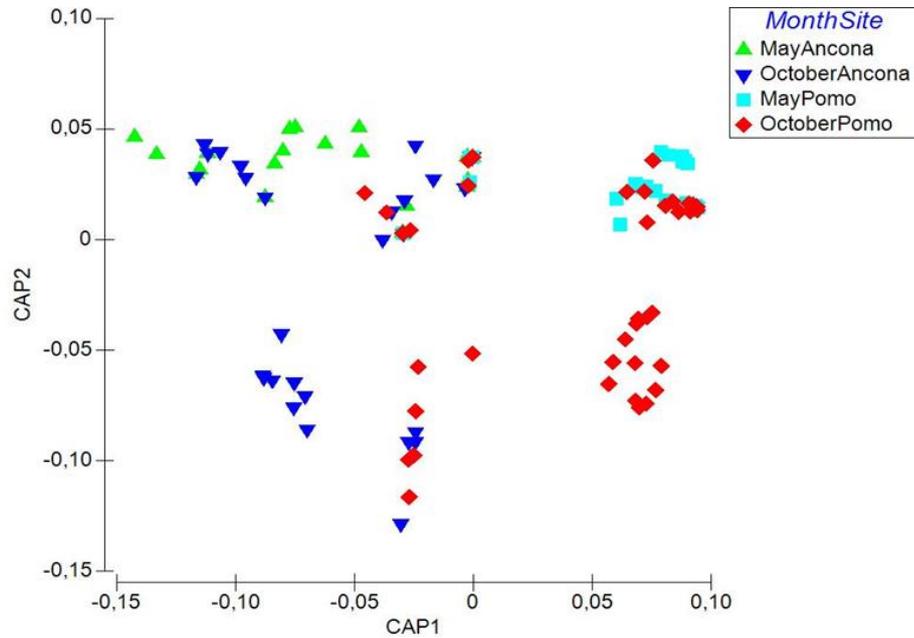


Fig. 3.27 Cap plot based on factor site and season.

The SIMPER analysis showed a strong contribution of Decapoda, Brachyura and Osteichthyes in specimens from Ancona, while in those from Pomo, the major components of the diet were *Munida ruttianti* with a very-high contribution to similarity (69%), followed by Osteichthyes and Decapoda, (table 3.14).

Tab 3.14 Results of SIMPER analysis for factor site.

<b>Group Ancona</b>		<b>Average similarity: 19.63</b>			
Taxon	Av.Abund	Av.Sim	Sim/SD	Contrib%	Cum.%
Decapoda	0.25	7.86	0.36	40.02	40.02
Brachyura	0.19	3.62	0.27	18.43	58.45
Osteichthyes	0.15	3.18	0.24	16.2	74.65
Polychaeta	0.14	1.75	0.23	8.9	83.55
<i>Liocarcinus sp.</i>	0.14	1.34	0.17	6.84	90.39
<b>Group Pomo</b>		<b>Average similarity: 32.55</b>			
<i>Munida ruttianti</i>	0.46	22.38	0.77	68.77	68.77
Osteichthyes	0.23	5.37	0.32	16.49	85.26
Decapoda	0.12	2.3	0.18	7.08	92.34

The Shannon index ( $H'$ ) displayed values ranging from 0.62 to 1.09 through the year, did not vary significantly across seasons (Univariate Permanova, P-value > 0.05) (table 3.15).

Tab 3.15 Results of Univariate Permanova test for the Shannon index.

	Df	MS	Pseudo-F	P(MC)
<b>Site</b>	1	0.11	0.64	0.42
<b>Month</b>	1	0.18	1054	0.29
<b>SitexMonth</b>	1	0.07	0.39	0.53
<b>Residuals</b>	123	0.17		
<b>Total</b>	126			

## **Chapter 4 – Discussion**

### **4.1 - Overview of the *Nephrops norvegicus* diet in the Adriatic Sea**

*Nephrops norvegicus* lives in burrows constructed in muddy sediments, spending a large part of the day inside the burrow (Abelló et al., 2002). The underground lifestyle of Norway lobster influences its behaviour, physiology and diet, feeding only when they come out of the dens as described by (Sbragaglia, 2013).

Diet composition is quite diverse, but some preys are mostly represented through seasons and in the different areas. The most common preys were epibenthic species, such as Brachyura, Polychaeta and Bivalvia, in addition a large percentage of fish. In the present study, decapods (especially the brachyuran crabs *Liocarcinus sp.*, *Goneplax rhomboides*) resulted very important prey; similar feeding habits were described by (Cristo, 1998). The high abundance of fish remains found in *Nephrops norvegicus* stomach contents is consistent with its scavenging activity (Cristo et al., 1998).

### **4.2 – Diet composition and biological characteristics of Norway lobster collected from off Ancona**

Concerning the biological indices and specifically the %GSI, this was found to increase in females during summer, in agreement with the reproductive period

of the species reported by (Colella, 2012) and (Rosa, 2002). For males, any fluctuations in the index across seasons were observed, consistently with a pattern of continuous spermatogenesis described by (Farmer, 1974) and (Sardà, 1998).

In females, the %HSI index showed an increase after the reproductive period in autumn, with an opposite trend to that described by (Rosa, 2002) in the Atlantic Ocean, but consistent to other findings in the Mediterranean (Fanelli E., pers. comm.). Our results could be attributed to a shift in the diet in autumn, with a higher consumption of Polychaeta, and a recovery after the reproductive activity. For males there were no significant variations during the year, showing a constant trend. This could be due to the fact that they do not need to invest lipids in the production of eggs unlike females, which use many lipids in the formation of the egg yolk as described by (Relini, 1998) and (Dall, 1981).

Stomach fullness, used as a proxy of feeding intensity, was quite constant throughout the year, pointing to a continuous predatory behavior during all seasons in according to (Parslow-Williams, 2002) NE Atlantic Sea (Irish Sea), without a difference between the sexes.

The diet found in the samples from Ancona showed low trophic diversity with Decapoda as the main prey in all seasons. Decapoda in the diet were mostly represented by *Liocarcinus sp.* and *Goneplax rhomboides*. These two species

are particularly abundant in this area, as described by (Santelli, 2017). These findings were also in agreement with those reported by (Cristo, 1998). A secondary main item was represented by fishes, although this does not mean an active predatory behavior by the Norway lobster as they were represented mostly by remains: i.e. any otoliths have been found and this indicates that the fishes were not consumed whole. Such abundant fish remains on the seabed likely indicated they are discard of the intense fishing activity in the area, as described by (Tsagarakis, 2014) and (Santojanni, 2005). The diet composition differs during the seasons, probably due to the higher abundances of fishes, as discard spring and summer and of polychetes in autumn and winter. The greater abundance of fishes could be attributed to the higher number of days-at-sea spent by fishermen during spring and summer, characterized by general good weather conditions. The highest values of abundance of prey in the stomachs were recorded in autumn confirming the trend shown for Fullness.

#### **4.3 – Diet composition and biological characteristics of Norway lobster collected from Pomo/Jabuka pits**

The %GSI resulted similar for both sexes inside and outside the pit, highlighting no difference in sexual maturity.

%HSI index showed higher values for females outside the pit compared to females inside the pit, for males the values are similar outside and inside the

pit. The change in %HSI values can be associated with a shift in diet: Osteichthyes are richer in lipids, as described by (Watanabe, 1982) respect lipid levels in Crustacea as explained by (Sallam, 2009), and outside the pit fish were an abundant prey. This may have led to an increase in HSI as described by (Rosa, 2002) in Atlantic Ocean.

Fullness values are similar for both sex and depth, it indicates a continuous predatory behavior in according to (Parslow-Williams, 2002) in the NE Atlantic Sea (Irish Sea).

At Pomo, the diet of Norway lobster is characterized by a low number of taxa, with a clear dominance of the galatheid crab *Munida ruttianti*, both inside and outside the pit. Differences in *M. ruttianti* contribution to the diet were observed, with a slightly greater consumption inside the pit, probably due to its greatest abundance here. The species is reported to inhabit shallower waters by (Maiorano, 2013) in the NW Ionian Sea, but our findings support the idea of a wider (and deeper) distribution in the Pomo area. The large presence of *Munida ruttianti* in stomach contents is justified by a dominance of this Decapoda in the seabed of Pomo as described by (Frogliia, 2017). Finally, outside the pit fishes represented another abundant component in the diet, while in the pit polychaetes were secondary prey items, after *Munida ruttianti*.

#### **4.4 – Differences in the diet of specimens collected off Ancona and from the Pomo/Jabuka pits**

The Norway lobster diets from Ancona and Pomo were compared in order to check for differences due to the different fishery pressure in the investigated areas. Previous studies carried out in areas characterised by different fishery regimes (Fanelli et al., 2009; Castriota et al., 2005) showed differences in the diet of benthivorous fish species attributed to the fishing ban. Indeed, our results highlighted differences in the diet composition of the species in the two areas. The differences in the *N. norvegicus* diets can be linked to the presence of sensitive species, such as *Munida ruttianti*, to the trawling that in Pomo are more abundant than in Ancona, an area highly impacted by this type of fishing (figure 4.1) (Rijnsdorp et al., 2017).

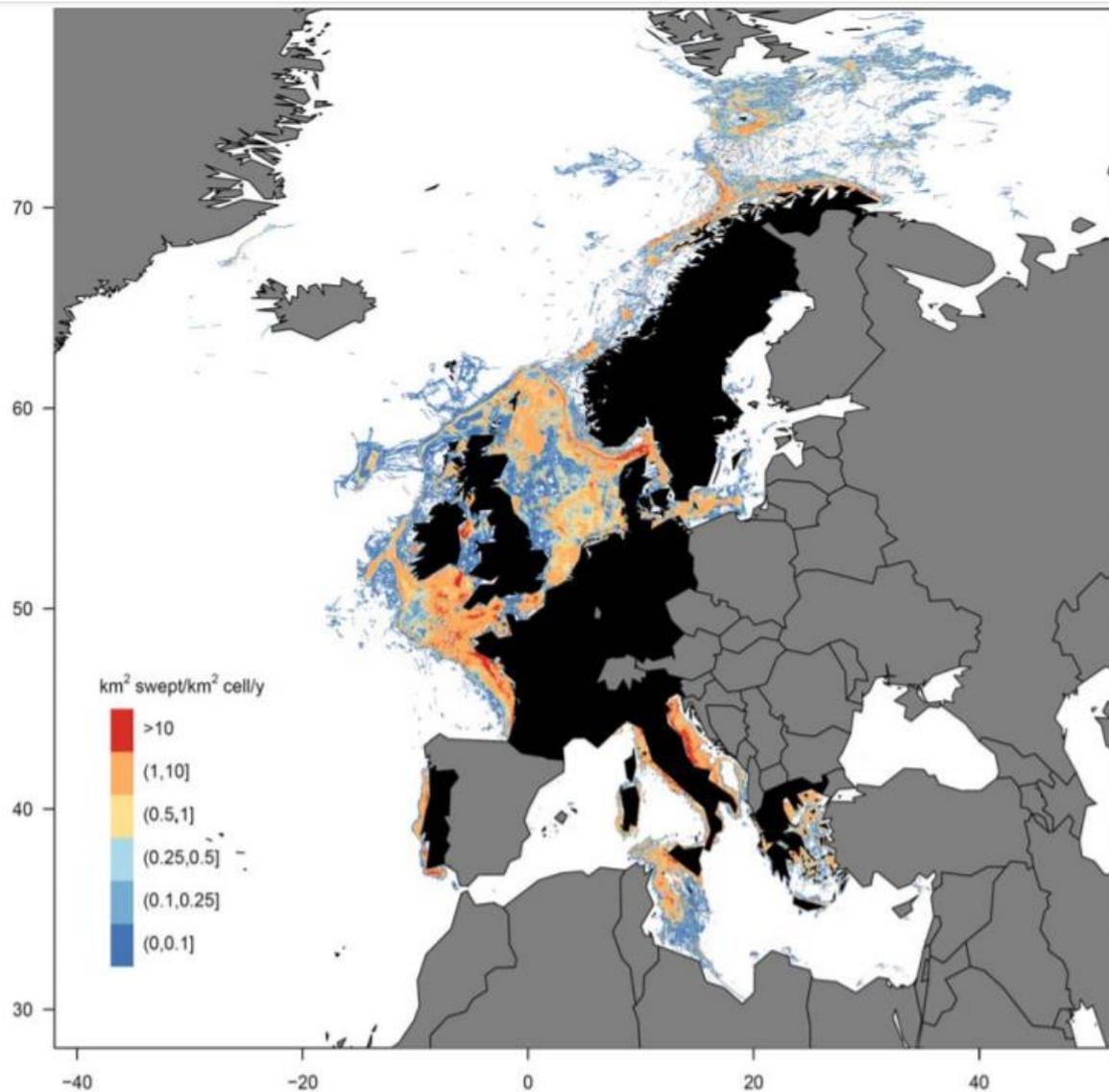


Fig. 4.1 Average annual trawling intensity on the surface of the seabed (from Rijnsdorp et al., 2017).

Bottom trawling can cause a dominance of species with short life cycle and an average decrease in the size of the organisms that will invest their energies to reproduce early according to (Kaiser, 1996).

The diet of the Norway lobsters of Ancona and Pomo were different both as regards %W and for the type of prey consumed. Such differences are most

likely due to a different structure of the benthic community that in turn influences the diets.

## Chapter 5 – Conclusion

The results of this work contributed to the knowledge on the feeding ecology of Norway lobster from the central Adriatic Sea.

Our results confirmed the scavenger behaviour of the species and the strong relationship of the diet of Norway lobster diet with the local benthic community. Indeed, being the species a scavenger, the differences found between the diet of specimens collected in Ancona and Pomo were not due to prey selection but essentially to a different prey availability. This in turn is linked to the different fishing pressure occurring in the two studied area, with a greater abundance of fish remains, probably discarded by fishermen during their activity, in specimens collected off Ancona and the almost dominance of *Munida ruttianti* in Pomo.

This study lends itself to further analysis such as isotopic analysis to determine at what trophic level the Norway lobster feeds at. Another interesting aspect could be the determination of contaminants in tissues or microplastics in stomach contents, a topic of great relevance as reported by Cau (2019) off Sardinian coasts (Western Mediterranean). The results discussed for Pomo will improve the knowledge of this particular area of the central Adriatic. Finally, data on species' size and abundance acquired before 2018 (establishment of the

FRA) can be compared to the data here analysed in order to evaluate the effectiveness of the fishery management measures adopted in the Pomo Pit.

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## **La dieta dello scampo (*Nephrops norvegicus*) nel Mar Adriatico**

Lo scampo *Nephrops norvegicus* è un crostaceo decapode presente in Mediterraneo, Mar Nero e in Oceano Atlantico a profondità comprese tra i 50 e 800 metri. È una specie con un elevato valore commerciale, in Adriatico è seconda solo alla Panocchia *Squilla mantis*. Lo scampo è una specie sedentaria che abita nelle tane costruite in sedimenti fangosi, trascorrendo gran parte della giornata all'interno della tana.

Lo scopo di questa tesi è stato valutare la dieta dello scampo in diverse aree dell'Adriatico, in particolare in campioni raccolti nelle aree di pesca antistanti Ancona e nella fossa di Pomo, area dichiarata *Fishery Restricted Area (FRA)*, dove è vietata la pesca a strascico, nel 2018.

I campioni da Ancona sono stati raccolti mensilmente nell'ambito del progetto CAMPBIOL da gennaio a dicembre 2019. I campioni provenienti da Pomo sono stati raccolti in due campagne, UWTV (Under Water TV) svolta a maggio 2019 e ScamPo realizzata nell'ottobre 2019.

Ogni individuo è stato pesato (in g) e misurato (lunghezza carapace, CL in mm), è stato determinato il sesso e la maturità sessuale, sono state estratte le gonadi, l'epatopancreas e lo stomaco e pesati. Questi dati sono stati utilizzati per calcolare diversi indici: indice gonadosomatico (%GSI), epatosomatico

(%HSI) e Fullness. I dati ottenuti sono stati analizzati mediante analisi univariate. Successivamente sono stati analizzati i contenuti stomacali per definire la dieta. Inizialmente sono stati calcolati degli indici descrittivi quali frequenza di comparsa (%F), abbondanza delle prede (%N), peso delle prede (%W) e l'indice di importanza relativa (%IRI). Successivamente si dati sono stati analizzati mediante analisi multivariata utilizzando disegni di campionamento complessi a due fattori: mese e sesso per i campioni provenienti da Ancona e profondità (<200 m fuori e >200 m dentro la fossa) e sesso per quelli provenienti da Pomo.

L'analisi del %GSI per i campioni di Ancona ha confermato il periodo riproduttivo delle femmine in estate. L'analisi del %HSI ha mostrato un aumento nel periodo autunnale (dopo la riproduzione), dato che potrebbe indicare un cambio nella dieta durante l'autunno. Il valore medio della Fullness, per maschi e femmine, non è cambiato durante l'anno, suggerendo un'attività predatoria costante durante l'anno.

L'analisi dei contenuti stomacali dei campioni provenienti da Ancona ha mostrato una bassa diversità trofica, con decapodi, pesci e policheti come prede dominanti in tutte le stagioni. È comunque evidente una certa differenza nel consumo delle diverse prede, da attribuire ad una diversa disponibilità delle risorse bentoniche durante l'anno. Tra i decapodi le prede più comuni

sono stati i granchi del genere *Liocarcinus sp.* e la specie *Goneplax rhomboides*.

Nella fossa di Pomo il %GSI ha un andamento pressoché costante sia per le femmine che per i maschi, dentro e fuori la fossa. Il %HSI ha mostrato differenze significative tra dentro e fuori la fossa, con valori molto alti per gli individui femminili fuori dalla fossa. Questi valori potrebbero essere dovuti ad un maggior consumo di pesci, prede caratterizzate da un più elevato apporto energetico rispetto a decapodi e policheti. La preda dominante, sia dentro che fuori la fossa, è risultata essere il granchio galateide *Munida rutilanti*, ma mentre all'interno della fossa la seconda preda più abbondante sono stati i policheti, fuori lo sono stati i pesci.

Il confronto della dieta di individui provenienti da Ancona con quelli campionati a Pomo ha mostrato differenze significative tra le due aree, da attribuire probabilmente alla differente composizione della comunità bentonica. Da letteratura è noto che gli scampi sono *scavenger* non selettivi, consumano qualsiasi preda sia a disposizione sul fondale. Sui fondi strascicabili, la loro dieta è dunque fortemente influenzata sia dalle attività stesse di strascico, che modificano la comunità bentonica presente, formata in prevalenza da specie detritivore, opportuniste e spazzine, sia dallo scarto del pescato stesso, specialmente piccoli pesci o resti.

Questo studio si presta ad ulteriori approfondimenti come per es. l'integrazione dell'analisi degli isotopi stabili con quella dei contenuti stomacali, per ottenere una visione più completa dell'ecologia trofica della specie. Un altro aspetto molto interessante potrebbe essere la ricerca di contaminanti nei tessuti e microplastiche nei contenuti stomacali di questi animali, considerato il loro elevato valore commerciale. Inoltre, questi risultati potranno essere utili nel valutare l'andamento della comunità di *Nephrops norvegicus* a Pomo dopo l'istituzione della FRA nel 2018.