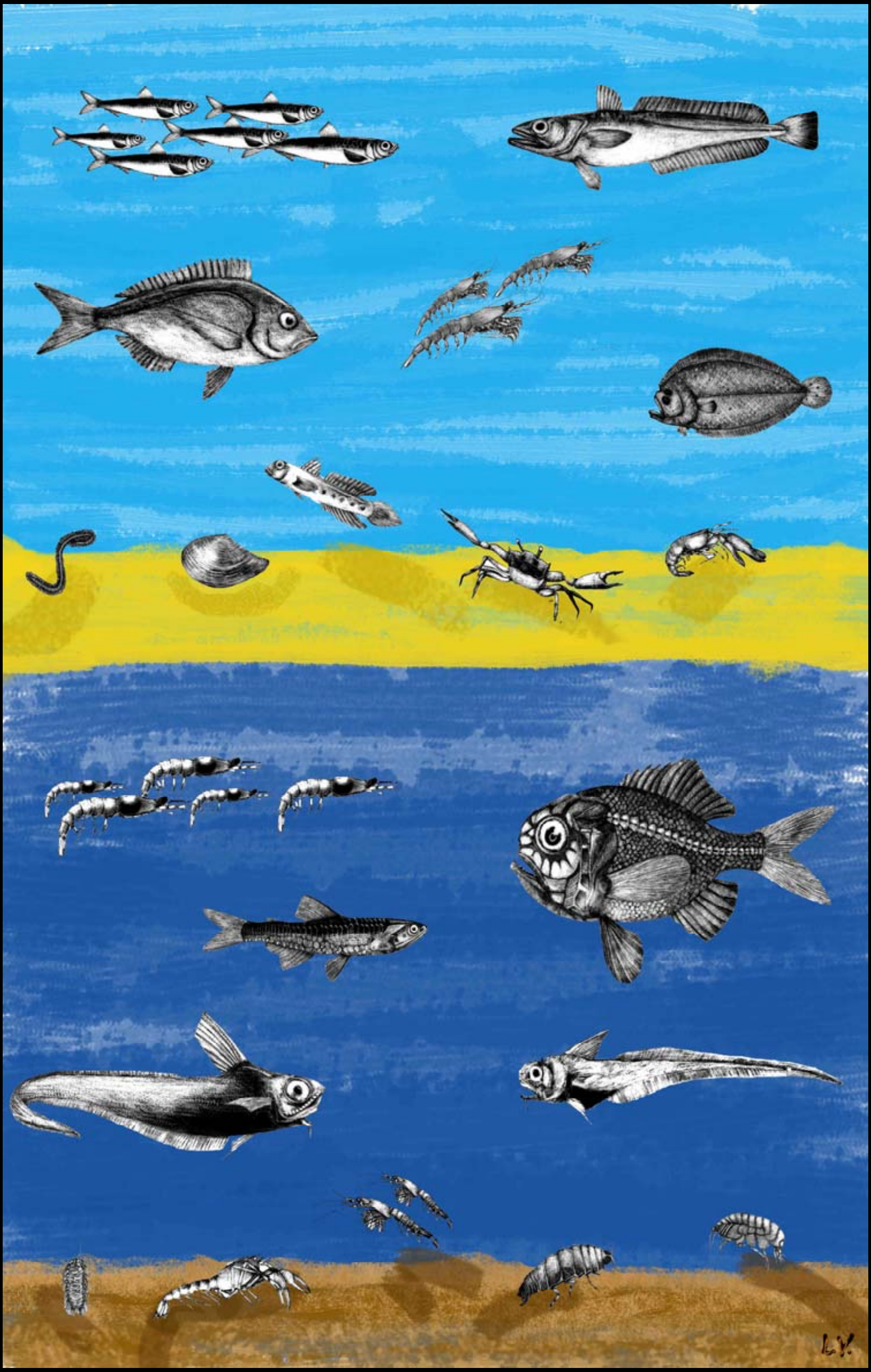
Trophic relationships in demersal communities of western Mediterranean: case studies from coastal and deep-sea ecosystems

PhD Thesis



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TROPHIC RELATIONSHIPS IN DEMERSAL COMMUNITIES OF WESTERN MEDITERRANEAN: CASE STUDIES FROM COASTAL AND DEEP-SEA ECOSYSTEMS

PHD THESIS

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L’utopia è come l’orizzonte:
cammino due passi, e si allontana di due passi.
Cammino dieci passi, e si allontana di dieci passi.
L’orizzonte è irraggiungibile.
E allora, a cosa serve l’utopia?
A questo: serve per continuare a camminare
Eduardo Galeano

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SUMMARY

The present thesis deals with the analysis of the trophic web structure and spatio-temporal variation in trophic levels of prey and predator in two different ecosystems: a coastal environment up to 80 m in the northern Sicily (Italy) and a deep-sea environment, the middle slope (450-750 m of depth) of the Balearic basin (Cabrera Archipelago - Spain). As trophic relationships are fundamental to understand biological interaction, a detailed study of the food and feeding ecology of some, coastal and deep-sea demersal fish species is attempted. Moreover to define the trophic structure of coastal and deep-sea communities a complete examination of prey (i.e. zooplankton, suprabenthos and benthos where possible) and predator, based on stable isotope analysis was carried out. Particularly suprabenthic resources were in general ignored or scarcely considered in trophic web studies, we explore the role of suprabenthos as a link between benthic and pelagic environments and its importance as forage for juveniles of coastal fish species and for adult of deep-sea fish.

Data used for the part of the thesis regarding coastal ecosystem were collected within the framework of a project where sampling was performed in four gulfs of Northern Sicily. Among these four areas the Gulf of Castellammare and the Gulf of Patti are two fishery exclusion zone, where trawl fishery was banned in 1990 (Regional Act No. 25/90) while the other two areas were submitted to an intensive trawl fishery.

In **chapter 2** *the dynamic of suprabenthic communities in the Gulf of Castellammare (NW Sicily, Italy) at a seasonal scale (from November 2004 to June 2005) was analyzed. In addition changes in species composition and assemblage structure were studied; by means of stable isotopes analysis of N and C we analyzed seasonal variations in trophic levels and source of carbon of some dominant suprabenthic species. Suprabenthos showed maximum biomass from late winter (March) to summer (June), while minimum biomass was found in autumn (November). Among suprabenthic community the highest biomasses of mysids and copepods occurred in March, one month after the peak of primary production, while amphipod biomass was higher in summer, coupled with the increase of the percentage of organic matter (%OM) recorded in spring-summer in the area. In nMDS analyses suprabenthos abundance showed a sample aggregation as a partial function of season: 3 defined groups corresponding to autumn, winter-spring and summer occurred. The increase in suprabenthos biomass was correlated (Spearman correlation) with the peak of Chl* a *on surface one and three months before sampling. Our preliminary analysis using*

stable isotopes suggests a relatively complex food web in the Gulf of Castellammare in which different food sources were observed.

The suprabenthic assemblages of four Gulfs of Northern Sicily were defined in **chapter 3**, *moreover an attempt to distinguish different trophic levels within suprabenthos and to determine spatial changes in trophic levels and source of carbon was given. The suprabenthic community was more diversified in the Gulf of Castellammare (77 taxa), but also in the Gulf of Termini Imerese and S. Agata, while a poor community occurred in Patti (40 taxa). nMDS analyses performed on suprabenthos abundance showed a sample aggregation as a function of area, but no protection effect was evident. A significant correlation was found between total suprabenthos biomass, peracarid, mysids and amphipods biomass and some environmental variables such as Chl*a *on surface three, two and one months before sampling period and with lipids, proteins and carbohydrates in the sediment organic matter suggesting that suprabenthic fauna relies on trophic resources other than those derived directly from the water column.*

In chapter 4 *the spatial variations in feeding habits and trophic level of* Pagellus erythrinus*,* Arnoglossus laterna *and* Merluccius merluccius*, three coastal demersal fish inhabiting muddy bottoms of Northern Sicily, were studied. Polychaetes and decapods were the preferred prey of* P. erythrinus *in the four gulfs. Differences in diet of samples from protected and unprotected areas were evident, with a more specialist diet in the protected gulfs.* A. laterna *fed mainly on decapods and gobiid fish. Ontogenetic changes were evident with juveniles mainly preying on suprabenthic species, e.g. mysids and small decapods. Suprabenthic species, natantian decapods and fish were dominant in the diet of juveniles of European hake,* M. merluccius*. Juveniles smaller than 160 mm of total length, mainly fed on crustaceans while a shift to a strongly pelagic diet occurred in larger specimens. Stable isotopes analysis evidenced a complex food web in coastal demersal community with at least two trophic levels among suprabenthic species and with fish as top-predators.*

In **chapter 5** *the dynamics of suprabenthos and zooplankton was analyzed off Cabrera Archipelago (Balearic Islands, Western Mediterranean). Four stations situated at 150 m (shelf-slope break), and at bathyal depths on 350, 650 and 750 m were sampled at bi-monthly intervals during six cruises performed between August, and June 2004. Suprabenthos showed maximum biomass from late spring to summer, while minimum biomass was found in autumn. Though variable, temporal dynamics of zooplankton showed peaks of biomass in late winter and summer, while minima occurred in autumn (and, at bathyal depths, in April. In MDS analyses performed* *on suprabenthos and zooplankton, suprabenthos abundance showed a sample aggregation as a function of depth, by contrast, zooplankton samples were separated by season and not by depth. Three seasonal groups corresponding to summer (June2004-August2003), autumn-winter (September and November 2003, February 2004), and spring (April 2004) were evidenced. In general suprabenthos was positively significant correlated with the sediment variables whereas zooplankton was almost exclusively dependent of Chla on surface, which suggests two different food sources for suprabenthos and zooplankton. The increase of suprabenthos abundance in April-June was also parallel to a sharp increase (ca. 2.8 times) of %OM on the sediment in the same period coupled ca. with 1-2 months of delay with the peak of surface primary production recorded in February-March (from Chla satellite imagery data) in the same area. Suprabenthos biomass was also correlated with salinity close to the bottom, suggesting a link between suprabenthos abundance and changes in the oceanographic condition of water masses close to the bottom. The stable isotope ratios of suprabenthos fauna displayed a continuum of values and confirmed a wide spectrum of feeding types (from filter-feeders to predators): three trophic levels were identified within the suprabenthic community and two among zooplankton.*

In **chapter 6** *we analyzed temporal variations in the diet of three deep-sea demersal fish on the continental slope of the Balearic Islands. Samples of* Hoplostethus mediterraneus*,* Hymenocephalus italicus *and* Nezumia aequalis *were collected within the framework of the Project IDEA, at bi-monthly intervals, between 550 and 750 m of depth. Classical stomachs contents and stable isotopes analyses were contemporarily applied in order to detect temporal variations of diet. An experiment using formalin preserved specimens and frozen specimens as control were also performed in order to verify if preserved material can be used for stable isotopy.* H. mediterraneus *mainly preyed on isopod and on lesser extent on amphipods and mysids.* H. italicus *fed on mysids and amphipods, captured in the Benthic Boundary Layer, while* N. aequalis *seemed to exhibit a more benthic behaviour with benthopelagic and benthic prey in its diet. Strong temporal variations were observed in the diet of* H. mediterraneus*, with February as a period of change in feeding habits. Temporal changes were less evident in the two macrourids and depth seemed to be a more structuring factor of diet than month. The experiment on frozen and fixed-preserved specimens showed that formalin did not affect* ^{15}N *signatures, while time of preserving strongly influenced* ^{13}C *values, with different pattern among the three species. Temporal variations of* $\delta^{15}N$ *values were evident in the three species: the lowest values were observed in September and the highest in April-June. Among the three species N. aequalis* *had the highest trophic level (4,47), occupying a high position in the deep-sea trophic web. In general deep sea trophic webs were complex, with at least three trophic levels among benthopelagic resources and with fish as top-predators: marine snow seemed to be the only one primary source of material.*

RIASSUNTO

La presente tesi analizza la struttura della rete trofica e le variazioni spazio-temporali nei livelli trofici di prede e predatori in due diversi ecosistemi: un ambiente costiero tra 40 e 80 m di profondità nella Sicilia settentrionale (Italia) e un ambiente profondo, la porzione intermedia della scarpata continentale (tra 450 e 750 m di profondità) nel bacino delle Isole Baleari (Arcipelago di Cabrera - Spagna). Poiché le relazioni trofiche sono di fondamentale importanza nella comprensione delle interazioni biologiche, è stato realizzato uno studio dettagliato dell'ecologia trofica di alcune specie di pesci demersali, di ambienti costieri e profondi. Inoltre per definire la struttura trofica delle comunità costiere e di profondità, è stato realizzato un esame completo delle prede (zooplancton, suprabenthos e benthos ove possibile) e dei predatori, basato sull'analisi degli isotopi stabili dell'azoto e del carbonio.

I dati utilizzati per la parte della tesi inerente l'ecosistema costiero, sono stati ottenuti nell'ambito di un progetto, in cui i campionamenti sono stati effettuati in quattro Golfi della Sicilia Settentrionale. Delle quattro aree, i Golfi di Castellammare e Patti sono due aree dove la pesca a strascico è stata vietata da un Decreto Regionale dal 1990 (DR. 25/90), mentre le altre due sono sottoposte ad un intenso sforzo di pesca.

Nel **capitolo 2** è stata analizzata la dinamica della comunità suprabentonica del Golfo di Castellammare (Sicilia Nord-Occidentale, Italia) su scala stagionale (dal novembre 2004 a giugno 2005). In seguito si è analizzata la composizione specifica della comunità suprabentonica e la struttura dell'associazione. Attraverso lo studio degli isotopi stabili di N e C, sono state analizzate le variazioni dei livelli trofici all'interno della comunità e della fonte di carbonio. Il suprabenthos presenta valori massimi di biomassa dal tardo inverno (marzo) all'estate (giugno), mentre i valori più bassi si osservano in autunno (novembre). All'interno della comunità suprabentonica i valori maggiori di biomassa di misidacei e copepodi sono presenti in marzo, un mese dopo il picco di produzione primaria, mentre la biomassa degli anfipodi è maggiore in estate, correlata con l'incremento della percentuale di materia organica (%OM) registrata nell'area, in primavera-estate. L'analisi nMDS mostra come l'abbondanza del suprabenthos sia funzione della stagione: infatti si osservano tre gruppi ben definiti corrispondenti all'autunno, al tardo inverno-primavera e all'estate. L'incremento nella biomassa del suprabenthos è correlato (correlazione di Spearman) con il picco di Chl*a* registrato in superficie uno e tre mesi prima del campionamento. I nostri

risultati preliminari, ottenuti dall'analisi degli isotopi stabili, suggeriscono una complessa rete trofica, in cui si osservano differenti fonti di cibo.

Le associazioni suprabentoniche di 4 Golfi della Sicilia settentrionale sono studiate nel **capitolo 3**, inoltre sono stati definiti i diversi livelli trofici esistenti all'interno della comunità suprabentonica. Infine sono state analizzate le variazioni spaziali nei livelli trofici e nella fonte di carbonio tra le 4 aeree. La comunità suprabentonica del Golfo di Castellammare è la più diversa (77 taxa), mentre quella di Patti presenta il più basso numero di taxa (40). I valori più alti di biomassa si osservano nel Golfo di Castellammare, mentre I valori più bassi sono stati riscontrati nel Golfo di Patti. L'analisi nMDS eseguita sulla matrice di abbondanza delle specie del suprabenthos mostra un'aggregazione dei campioni in funzione dell'area, non si evidenzia nessun effetto del regime di protezione. Una correlazione significativa è stata osservata tra la biomassa totale del suprabenthos, la biomassa dei peracaridi, dei misidacei e degli anfipodi e alcune variabili ambientali come la *Chl* a in superficie tre, due e un mese prima del campionamento e i lipidi, le proteine e i carboidrati presenti nella materia organica sedimentaria, suggerendo che la fauna suprabentonica si alimenta su risorse trofiche che non provengono direttamente dalla colonna d'acqua.

Nel **capitolo 4** sono analizzate le variazioni spaziali nelle abitudini alimentari e nel livello trofico di *Pagellus erythrinus*, *Arnoglossus laterna* e *Merluccius merluccius*, tre pesci costieri demersali che vivono sui fondi fangosi della Sicilia settentrionale. Nei quattro golfi *P. erythrinus* si alimenta principalmente di policheti e decapodi. Sono state evidenziate differenze nella dieta in aree protette e non protette, il fragolino presenta una dieta più specialistica nelle aree protette. *A. laterna* preda prevalentemente su decapodi e gobidi. Sono evidenti variazioni ontogenetiche nella dieta: i giovanili si alimentano su specie suprabentoniche come misidacei e piccoli decapodi. Specie suprabentoniche, decapodi natanti e pesci sono dominanti nella dieta dei giovanili del nasello, *M. merluccius.* Animali più piccoli di 160 mm di lunghezza, mangiano maggiormente crostacei mentre quelli più grandi presentano una dieta più pelagica. Nel Golfo di Castellammare, I giovanili di nasello predano principalmente su Clupeiformes, probabilmente ciò può esser dovuto ad una maggior produzione del Golfo che può sostenere una più abbondante comunità zooplanctonica (in confronto con gli altri golfi più oligotrofici) o ad un'eccezionale abbondanza di queste specie pelagiche all'epoca del campionamento.

Nel **capitolo 5** abbiamo analizzato la dinamica del suprabenthos e dello zooplancton dell'arcipelago di Cabrera (Isole Baleari, Mediterraneo Occidentale). La fauna suprabentonica e zooplanctonica è stata campionata in quattro stazioni situate a differenti profondità: a 150 m (nell'intervallo tra piattaforma e scarpata), e a 350, 650 e 750 m, ogni due mesi e con 6 campagne realizzate dall'agosto del 2003 al giugno 2004. Il suprabenthos presenta i valori massimi di biomassa dalla tarda primavera all'estate, mentre i valori minimi sono stati osservarti in autunno. Sebbene variabile, la dinamica temporale dello zooplancton mostra picchi di biomassa in inverno ed estate, mentre i valori più bassi sono stati osservati in autunno (e, in profondità anche in aprile). L'analisi nMDS evidenzia un aggregazione dell'abbondanza delle specie del suprabenthos in funzione della profondità mentre i campioni dello zooplancton sono separati in funzione della stagione. Si rilevano tre gruppi stagionali corrispondenti all'estate (giugno2004-agosto2003), all'autunno-inverno (settembre e novembre2003, febbraio2004) e alla primavera (aprile2004). In generale il suprabenthos è positivamente correlato con le variabili del sedimento, mentre lo zoopalncton dipende esclusivamente dal valore della clorofilla *a* in superficie. Ciò suggerisce due differenti fonti di cibo per il suprabenthos e lo zooplancton. L'incremento nell'abbondanza del suprabenthos osservato in aprile-giugno è inoltre correlato ad un brusco incremento (circa 2,8 volte) con la percentuale di materia organica nel sedimento, con un ritardo di circa 1-2 mesi rispetto al picco di produzione primaria in superficie registrato in febbraio-marzo (da dati di rilevamento satellitare di *Chla*). La biomassa del suprabenthos è inoltre correlata con I valori di salinità in prossimità del fondo, suggerendo un legame tra il suprabenthos e le variazioni delle condizioni oceanografiche delle masse d'acqua in prossimità del fondale. Il rapporto degli isotopi stabili della fauna suprabentonica mostra un *continuum* di valori e conferma la presenza di un ampio spettro di strategie alimentari (dai filtratori ai predatori): tre livelli trofici sono stati rilevati tra le specie del suprabenthos e due nello zooplancton.

Nel **capitolo 6** sono state analizzate le variazioni temporali della dieta di tre specie demersali di pesci di profondità sulla scarpata continentale delle isole Baleari, a tal fine sono state effettuate analisi dei contenuti stomacali e degli isotopi stabili. Campioni di *Hoplostethus mediterraneus*, *Hymenocephalus italicus* e *Nezumia aequalis*) sono stati raccolti grazie al progetto IDEA, ogni due mesi tra 550 e 750 m di profondità. E' stato eseguito un esperimento utilizzando campioni preservati in formalina e campioni congelati, per verificare la possibilità di utlizzare materiale preservato per l'isotopia stabile. *H. mediterraneus* preda principalmente su isopodi e in misura minore su antipodi e misidacei. *H. italicus* si ciba di misidacei e antipodi catturati nel Benthic Boundary Layer, mentre *N. aequali*s sembra mostare un comportamento più bentonico, preferendo prede bentopelagiche e bentoniche come tanaidacei e policheti. Ampie variazioni temporali si seervano nella dieta di *H. mediterraneus*: febbraio sembra essere un periodo di cambiamento nelle abitudini alimentari della specie. Variazioni temporali sono meno evidenti nei due macruridi e la profondità sembra essere un fattore maggiormente importante. Variazioni temporali dei valori di $\delta^{15}N$ sono evidenti nelle 3 specie: i valori minori si osservano in settembre e i più alti in aprile-giugno. Fra le 3 specie *N. aequalis* presenta il più alto livello trofico (4,47). In generale le reti trofiche profonde sembrano essere complesse con almeno 3 livelli trofici tra le specie suprabentoniche e con i pesci come predatori all'apice della rete trofica: la neve marina sembra essere l'unica fonte di carbonio nei sistemi profondi.

Chapter 1

1.1 Current state of the art

An increasing interest towards the study of fauna occupying the water layer immediately adjacent to the sea bottom (the so-called suprabenthos, hyperbenthos or Benthic Boundary Layer macrofauna: Mees and Jones, 1997) has been produced in recent years. This type of fauna is still poorly known even at descriptive levels such as its taxonomic and assemblage composition, and this is particularly true for deep-water suprabenthos.

Suprabenthos is a complex compartment composed by taxa (e.g. mysids, amphipods, cumaceans, isopods…) which constitute the permanent suprabenthos, and other taxa (e.g. euphausiids, natantian decapods) which by means of vertical or ontogenetic migrations have a more eventual relation with the water-sediment interface. Some authors distinguish between the permanent suprabenthos (=holohyperbenthos) and the eventual suprabenthos (=merohyperbenthos) (Mees and Jones, 1997). In fact, merohyperbenthos partially overlaps in terms of taxon composition with macro-mesozooplankton. The main adaptive biological feature of the permanent suprabenthos is the direct development of embryos in marsupial bags (oosteguites) developed by adult females of peracarid crustaceans. Studies on suprabenthos assemblages were first focused to the distribution of dominant taxa and assemblages in the water column (Bieri and Tokioka, 1968), as a function of depth (Sainte Marie and Brunel, 1985; Bull Jensen, 1986), and to a lesser extent to seasonal changes in assemblage composition (Boysen, 1976) and population dynamics.

In addition to this, most suprabenthic groups (e.g. mysids, amphipods), constitute the base of the diet of littoral and bathyal top predators (e.g. flatfish: Wildsih et al., 1992; hake: Cartes et al., 2004; the deep sea red shrimp *Aristeus antennatus*: Cartes, 1994; Sardà and Cartes, 1997), particularly for their smallest sizes or recruits and hence its importance in food webs dynamics. In spite its obvious ecological interest (Mees and Jones, 1997; Cartes et al, 2002), information on dynamics of suprabenthic communities is still scarce (Cartes and Sorbe, 1999; Cartes et al., 2002). In the diet of bathyal species, the smallest sizes of some important ecological (and commercial) species (e.g. the red shrimp, *A. antennatus*, CL <20 mm) and hake (TL < 10 cm) consume suprabenthos (mysids, cumaceans, and amphipods; Cartes, 1994; Sardà and Cartes, 1996; Bozzano et al. 1997), while largest juveniles increasingly consume merohyperbenthos-zooplankton (e.g. decapods, small Myctophids; Cartes et al, 2004).

Both in coastal and deep-sea environment the suprabenthos play an important role in the trophodynamics (Sorbe, 1981, 1987; Harmelink et al., 1990; Mees and Hamerlynck; 1992). A clear understanding of the energy and material fluxes in coastal and deep sea communities should always take into consideration the suprabenthos well established importance in those ecosystems (Boysen, 1975; Buhl-Jensen and Fossa, 1991; Hamerlynck and Mees, 1991; Wooldridge, 1989).

In this context different and integrated approaches can be used to analyse and understand trophic webs structure and the role of suprabenthos as an important link in the detritus-based food chains (Mees et al., 1995).

The food web is one of the earliest and most fundamental concepts in ecology. Darwin (1845) recognized the existence of a pelagic food chain. Elton is credited with first appreciating the importance of food chain and food web concepts (Lawton 1989), but major antecedents include Petersen's (1918) quantitative conceptual models of the food web that is supported by eel grass and Hardy's (1924) conceptual model of the herring food web.

Food chain and food web are two main conceptual approaches used to represent trophic structure and feeding relationships at the whole community level. Food chain studies assign species or populations to one of several discrete trophic levels. However, a shortcoming of the food chain approach is the failure to incorporate the complexity and omnivory that characterizes natural ecosystems (Polis and Strong 1996, Vander Zanden and Rasmussen, 1996). Alternatively, classical food web studies rely on species lists and the presence or absence of feeding links, and they search for across-system patterns in trophic structure (Cohen et al. 1990). Although food webs do recognize the complexity of natural systems, food webs do not weight feeding links according to their energetic or functional importance (Polis 1991, Vander Zanden and Rasmusssen 1996).

Another approach to the study of food-web is represented by ECOPATH modelling, which has been developed to understand the energetic basis for fisheries and other production systems (Polovina, 1984; Christensen and Pauly, 1993; Pauly and Christensen, 1995) and to describe trophic webs. Ecosystems however are complex and therefore all modelling methods require simplification of the ecosystem components, processes and interactions (Walters, 1986; Holling, 1987; Christensen and Pauly, 1993). One potential limit of these meta-models is that they do not consider the partitioning of lower trophic levels such as macrobenthos, infauna or suprabenthos which include several trophic guilds and strategies. Moreover, among the lowest trophic levels, suprabenthos is completely ignored in ECOPATH modelling, despite recent studies indicate higher P/B ratios for suprabenthos than for infauna (Cartes and Sorbe, 1999;

Cartes et al., 2000; Cartes et al., 2002) and how this compartment may channel a non-trivial part of the energy flow in the deep Benthic Boundary Layer suprabenthos (Cartes and Maynou, 1998).

Food webs are the representation of the feeding relationships within whole communities of organism based principally on data obtained with dietary analysis. This method is quali-quantitative and weight average formulas to assign organisms a continuous measure of trophic position, which represents the energy weighted mean path length leading to a consumer. Although numerous authors recognize that a trophic position-based approach incorporates energy flow and omnivory (Levine, 1980; Adams et al., 1983….) more general application of this approach hinges upon the ability of investigators to estimate the trophic position of organisms in the field. Indeed greater consideration of trophic position has been limited by the difficulty in collecting the required quantitative dietary data for the many species interacting in a typical food web.

However gut contents analyses have a number of limitations (Deb, 1997), for example they tend to provide mere snapshots of diets at particular point of time and space. Moreover, the durations of the snapshots vary amongst species, given variation in gut turnover (Jobling, 1993; 1994), and neglect certain types of dietary materials, such as gelatinous plankton and detritus, that may nevertheless be very important. The significance of this point is emphasized by Righton et al. (2001) who show that feeding behaviour of cod (*Gadus morhua*) varies temporally in the North Sea and Irish Sea. Organisms at the base of food webs tend to be particularly poorly characterized (Hall and Raffaelli, 1993) and excessive lumping together of species may greatly affect the apparent properties of food webs (Goldwasser and Roughgarden, 1997). Gut contents therefore offer, alone, a partial basis for elucidating the detailed interactions which must exist (Paine 1988). There are additional tools for elucidating food-web structure. A principal method involves the use of stable isotopes in particular those of Carbon and Nitrogen (Pinnegar and Polunin 2000).

Stable isotopes have become popular in recent years, for gaining extra insights into the functioning of marine and estuarine food webs (Cabana and Rasmussen 1996; Pinnegar and Polunin 2000; Fisher et al. 2001) and for providing time-integrated information about feeding relationships and energy flow through food webs (Peterson and Fry, 1987; Kling et al. 1992; Cabana and Rasmussen 1994). There is considerable interest in using stable isotopes, particularly those of nitrogen and carbon, to evaluate the structure and dynamics of ecological communities (e.g., Peterson and Fry 1987, Kling et al. 1992, France 1995, Vander Zanden et al. 1999, Post et al. 2000). One advantage of stable isotope techniques is that they combine benefits of both the trophic-level and food web paradigms in food web ecology. Many studies use trophic levels because they are simple to define, characterize the functional role of organisms, and give an idea of energy or mass flow through ecological communities (e.g., Hairston and Hairston 1993). The trophic level concept, however, is limited by the strict use of discrete trophic levels and its limited ability to capture the complex interactions and trophic omnivory that are prevalent in many ecosystems (Paine 1988, Polis and Strong 1996, Persson 1999, Vander Zanden and Rasmussen 1999). In contrast, food webs capture the complexity of trophic interactions in ecological communities, but are time-consuming to construct, often subjective in their resolution and scope (Paine 1988), and typically hold all trophic links to be of equal importance, which makes them ineffectual for tracking energy or mass flow through ecological communities (Paine 1988, Hairston and Hairston 1993, Polis and Strong 1996, Persson 1999, Vander Zanden and Rasmussen 1999). Stable isotope techniques can provide a continuous measure of trophic position that integrates the assimilation of energy or mass flow through all the different trophic pathways leading to an organism. Stable isotopes have the potential to simultaneously capture complex interactions, including trophic omnivory, and to track energy or mass flow through ecological communities (Peterson and Fry 1987, Kling et al. 1992, Cabana and Rasmussen 1996). The ratio of stable isotopes of nitrogen ($\delta^{15}N$) can be used to estimate trophic position because the $\delta^{15}N$ of a consumer is typically enriched by 3–4‰ relative to its diet (DeNiro and Epstein 1981, Minagawa and Wada 1984, Peterson and Fry 1987). In contrast, the ratio of carbon isotopes ($\delta^{13}C$) changes little as carbon moves through food webs (Rounick and Winterbourn 1986, Peterson and Fry 1987, France and Peters 1997) and, therefore, typically can be used to evaluate the ultimate sources of carbon for an organism when the isotopic signature of the sources are different. In terrestrial ecosystems, $\delta^{13}C$ is often used to differentiate between diets based on plants with different photosynthetic pathways (e.g., C_3 vs. C_4; Rounick and Winterbourn 1986, Peterson and Fry 1987, O'Leary et al. 1992). In lakes, $\delta^{13}C$ is useful for differentiating between two major sources of available energy, littoral (near shore) production from attached algae and detritus, and pelagic (open water) production from phytoplankton, because the $\delta^{13}C$ of the base of the littoral food web tends to be enriched in ^{13}C (less negative $\delta^{13}C$) relative to the base of the pelagic food web (France 1995). Variability in $\delta^{15}N_{base}$ and $\delta^{13}C_{base}$ derives from differences in the isotopic ratios of carbon and nitrogen available for uptake by organisms at the base of the food web, and through variable expression of fractionation during uptake. In aquatic systems, most primary producers and detrital energy sources have high temporal variation in $\delta^{15}N$ and $\delta^{13}C$, complicating their direct use as indicators of $\delta^{13}C_{base}$ and $\delta^{15}N_{base}$ for secondary consumers that integrate $\delta^{15}N$ and

$\delta^{13}C$ over much longer time periods (Cabana and Rasmussen 1996). Furthermore, $\delta^{15}N_{base}$ and $\delta^{13}C_{base}$ are spatially variable both within a lake (France 1995, Vander Zanden and Rasmussen 1999) and among lakes (Cabana and Rasmussen 1996, del Giorgio and France 1996). Cabana and Rasmussen (1996) and Vander Zanden and Rasmussen (1999) have suggested using long-lived primary consumers to quantify $\delta^{15}N_{base}$ and $\delta^{13}C_{base}$ in aquatic food webs because the temporal variance of their isotopic signature is much lower than that of primary producers (Cabana and Rasmussen 1996), and because they should reflect the spatial variation within and among lakes (Cabana and Rasmussen 1996, Vander Zanden and Rasmussen 1999). Although these are reasonable expectations, there is no empirical evidence that long-lived primary consumers actually do provide the hypothesized temporal integration of, or reflect the spatial variability in primary producers and detrital energy sources in aquatic food webs.

Few studies on trophic web structure used these two approaches simultaneously, especially when temporal differences can be detected. The techniques of stable isotopes analysis to lower trophic level (suprabenthos, zooplankton or benthos) were never applied at species level, considering the dominant species of suprabenthic or zooplanktonic communities; some studies analysed these compartments at a order level (Darnaude et al., 2004) or functional groups (Polunin et al., 2001) level, completely ignoring the high complexity of lower trophic level that can be include in the same family, carnivorous, deposit-feeders, filter feeders or herbivorous species. Another important focus often masked in meta-analysis is the seasonal variation in trophic levels of preys and predators, notwithstanding meta-models work with annual production data. Indeed several studies showed how diet (Cartes, 1994; Cartes and Maynou, 1998) and food consumption (Madurell and Cartes, 2005a, b; 2006) of fish and decapods vary on a seasonal scale both in coastal and deep-sea ecosystems.

1.2 The case of the trawling ban in Sicily: trophodynamic effects

Data used for the part of the thesis regarding coastal ecosystem were collected within the framework of a project where sampling were performed in four gulfs of Northern Sicily. Among these four areas the Gulf of Castellammare and the Gulf of Patti are two *fishery exclusion zones*, where trawl fishery was banned in 1990 (Regional Act No. 25/90), while the other two areas were submitted to an intensive trawl fishery. The most evident effect of the ban was, as regards the gulf of Castellammare (Pipitone et al., 2000), a dramatic increase in biomass from 1987-1989 (before the closure) to 1994 (after 4 years). Although, a biomass increase resulting from a partial or total fishing ban is to be expected (e.g., Dugan and Davis, 1993) and has been recorded also for crustacean species (Yamasaki and Kuwahara, 1989; Roa and Bahamonde, 1993), the increases observed in the Gulf of Castellammare far exceed those reported in the scientific literature for areas closed for similar or longer periods of time.

Commercial fishing is thought to have many consequences for marine ecosystems (Jennings and Kaiser 1998), one direct impact being the depletion of large predatory fishes, with implications for the prey they consume (e.g. Pinnegar et al. 2000). Analysis of catch data from the United Nations Food and Agriculture Organization (FAO), together with trophic level estimates taken from simple ecosystem models, has revealed that the mean trophic level of North Atlantic (including the Mediterranean) fisheries has declined over the last 50 years (Pauly et al. 1998a).

However, given that the body mass of fishes can increase by 5 orders of magnitude during their lifetimes, and that trophic level is expected to increase with body size (Jennings et al., 2002), a reduction in mean size associated with fishing could accentuate the apparent decline in mean trophic level, as well (Pauly et al. 2000). The feeding habits of fishes are commonly size dependent, such that as a fish increases in size within a population (e.g. Frid et al. 1999), or as mean size declines in a population subject to increased fishing mortality (Pope and Knights 1982), so the diet and trophic level will tend to change. Areas closed to fishing provide an opportunity for fishery-target species to increase in size and biomass (e.g. Roberts and Polunin 1991).

Particularly, as concerns red Pandora *Pagellus erythrinus* a dramatic increase in biomass was detected both in the Gulf of Patti (Potoschi et al., 2006) and the Gulf of Castellammare (Pipitone et al., 2000; Badalamenti et al., *submitted*; our unpublish. data) after the ban. In the Gulf of Patti, the biomass of European hake *Merluccius merluccius* increased from 1993 to 1999, then it decreased and reached the values recorded before the ban (Potoschi et al., 2006);

whilst hake biomass largely increased in the Gulf of Castellammare after the ban (Badalamenti et al., *submitted*).

Clearly this increase may have effects in ecosystem structure or trophodynamics of individual species and it can be assessed using C and N stable isotopes (Gu et al. 1997; Jennings et al. 1997). In spite of this considerations, few studies have explored trophodynamic changes among component species (St John et al. 2001; Graham et al. 2003). Such changes are critical in predicting the responses of food webs to protection or exploitation.

In this context, the classical analysis of stomach contents was used together with stable isotopes ecology in order to detect changes in trophic levels of demersal fish species and their resources. Stable isotopes of nitrogen and carbon can reveal age- or size related dietary changes in fish (e.g. Rau et al. 1981; Wainright et al. 1993; Lindsay et al. 1998; Jennings et al. 2002) and have been used to examine whether such changes occur earlier or later under differing fishing regimes (Persson and Hansson 1999).

Badalamenti et al. (2002) found a higher $\delta^{15}N$, after the ban than before in *Lophius budegassa*, but no significant trends were detectable in either *Mullus barbatus* or *Merluccius merluccius*. Moreover Jennings et al. (2001) found that intensive trawling disturbance led to reductions in the biomass of infauna and epifauna, and dramatic changes in the composition of the infauna. However, these changes are not reflected in the mean trophic level of the community, or the relationships between the trophic levels of different sizes of infauna. Studies on the influence of trawling disturbance on suprabenthic communities' structure showed that differences were mainly due to natural variability than to a protection effect (Cartes et al., *submitted*; Ligas, 2005).

Thus after 15 years from the ban, we explored the effect of trawling and protection on diet and on trophodynamic variables (trophic level and source of production) of three demersal marine species (*Merluccius merluccius*, *Arnoglossus laterna* and *Pagellus erythrinus*), chosen as representative of three trophic guilds (planktophagous, suprabenthophagous and benthophagous) and in the suprabenthic communities, .by comparison between the Gulfs of Castellammare and Patti (no-trawl areas) and the Gulfs and Termini Imerese and Patti (trawl-areas).

1.3 Aims of the study

The primary aim of the study is to analyze the trophic web structure and spatio-temporal variation in trophic levels of prey and predator in two different ecosystems: a coastal environment up to 80 m in the northern Sicily (Italy) and a deep-sea environment, the middle slope (450-750 m of depth) of the Balearic basin (Cabrera Archipelago - Spain). As trophic relationships are fundamental to understand biological interaction, a detailed study of the food and feeding ecology of some, coastal and deep-sea demersal fish species is attempted.

Moreover to define the trophic structure of coastal and deep-sea communities a complete examination of prey (i.e. zooplankton, suprabenthos and benthos where possible) and predator, based on stable isotope analysis was carried out.

The detailed objectives of the present study are:

- To analyze the dynamic of suprabenthic communities in the Gulf of Castellammare (NW Sicily, Italy) at a seasonal scale and to define changes in species composition, assemblage structure and trophic levels (Chapter 2);
- To define the suprabenthic assemblages of four Gulfs of Northern Sicily, to distinguish different trophic levels within suprabenthos and to determine spatial changes in trophic levels and source of carbon (Chapter 3);
- To describe the diet of three coastal fish species representative of different trophic guilds in four Gulfs of Northern Sicily (Chapter 4);
- To determine spatial variations in fish diet and in trophic level, elucidated by stable isotope analysis (Chapter 4) ;
- To analyze the trophodynamics of suprabenthic and zooplanktonic assemblages in a deep-sea environment (Archipelago of Cabrera, Balearic islands, Spain) and to distinguish different trophic levels among these group and their variation on a temporal (bi-monthly) scale (Chapter 5);
- To describe the diet of three deep-sea fish species off the Balearic basin and to determine temporal variations in fish diet and trophic level (Chapter 6);
- To investigate the relationships between food-available resources (zooplankton-suprabenthos) and the abundance, stomach fullness of fish along time (e.g seasonal scale) (Chapter 6);
- To describe the trophic web structure of a coastal and a deep-sea ecosystem (Chapter 4 and 6, respectively);

Data used for the part of the thesis regarding coastal ecosystem were collected within the framework of a project where sampling was performed in four gulfs of Northern Sicily. As previously mentioned, among these four areas the Gulf of Castellammare and the Gulf of Patti are two *fishery exclusion zone*, where trawl fishery was banned in 1990 (Regional Act No. 25/90), while the other two areas were submitted to an intensive trawl fishery.

Thus another important aim focused in this thesis deals with differences in trophic level of fish and their resources in protected and unprotected areas, as the case of the Gulf of Castellammare and Patti tackled in chapter 3 (suprabenthic communities) and 4 (fish).

1.4 Description of the study areas

1.4.1 The Northern Sicily

The first part of the thesis (chapters 2, 3 and 4) present data from four gulfs of Northern Sicily (Italy) (fig.1.1).

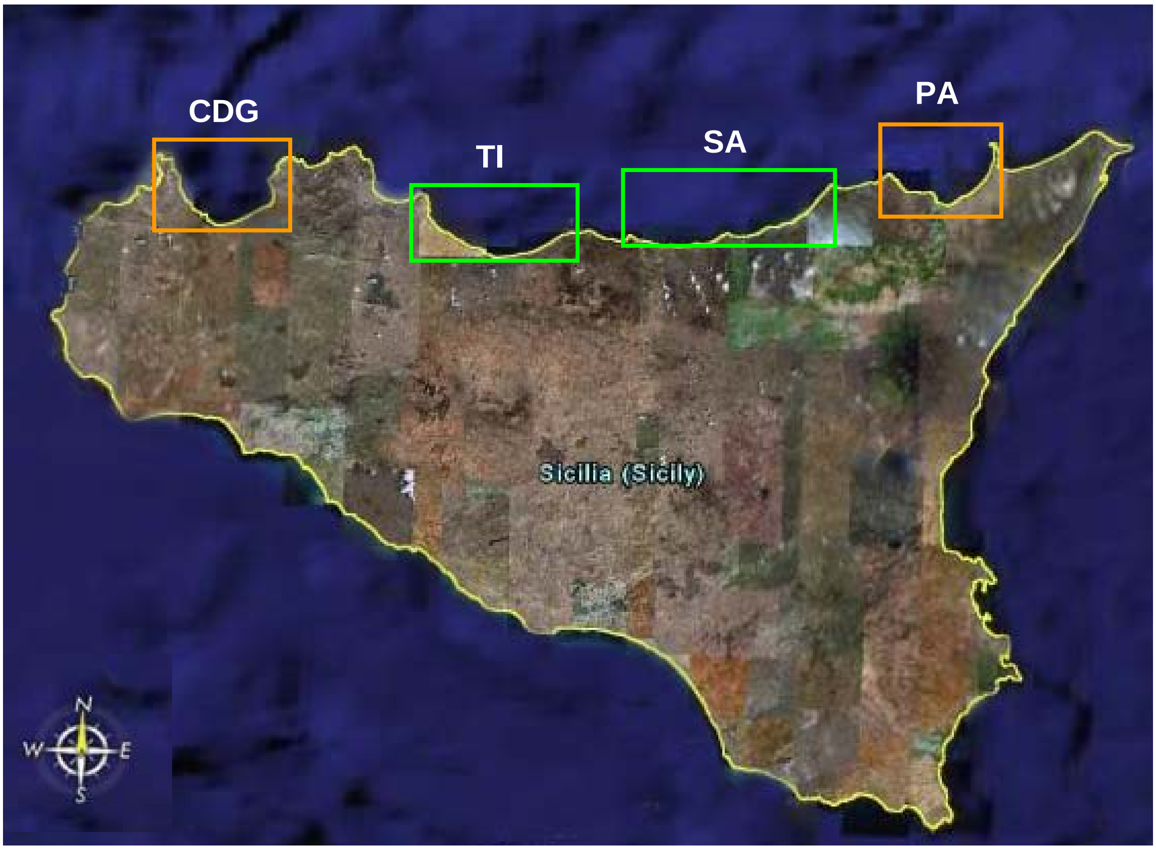

Figure 1.1 Sicily island with the four studied areas. CDG: Gulf of Castellammare; TI: Gulf of Termini Imerese; SA: Gulf of S.Agata di Militello; PA: Gulf of Patti.

The Gulf of Castellammare

The Gulf of Castellammare (38°03’N 12°54’ E) is located on the northwestern coast of Sicily (Southern Tyrrhenian Sea, Italy: Fig. 1-1). Its coastline is over 70 Km long and covers an area of about 300 Km^2.

Different rivers with irregular, transient discharge flow in the gulf: San Bartolomeo and Jato rivers and Nocella stream. They determine a very high sedimentation rate in the adjacent coastal areas, due to terrigenous sediments transport as well as local phenomena of pollution caused by sewage of urban runoff and industrial waste (Calvo & Genchi, 1989; D’Anna *et al.*, 1985, 1990; Riggio *et al*., 1992). The amount of fine sediments and detritus transported by rivers during flood times is very high and determine high turbidity of coastal sea water (Cangelosi, 1991).

During winter winds mainly blow from the 4th quadrant forcing the waters to the coast and generating centrifugal currents. During summer, winds mainly blow from the 2nd and 3rd quadrants generating drift currents towards off-shore and causing bottom currents as small up-welling.

Only regarding the bathymetric range chosen for this study (ca. 40-80 m of depth), temperature on the bottom (at 50 m of depth) vary from 14,3°C in March, to 15,5°C in August. On the bottom salinity do not change during the year (37.7 p.s.u.) (Mazzola, 1996). Chl *a* showed the lowest values in June and August (0,01 µg/l) and the highest in February and October (0,08 µg/l) corresponding to algal blooms in spring and autumn (Mazzola, 1996).

The eastern and western sides are characterized by steep cliffs, while the narrow sandy beaches occurred in the central side. Coastal bottoms from Castellammare to Nocella mouth are characterised by a Surface Fine Sand Assemblage (SFHN, *sensu* Pérès and Picard, 1964). Bottoms up to 10 m of depth are dominated by a Fine Well Sorted Sand assemblage (SFBC) with *Cymodocea nodosa* in some parts. A terrigenous-mud shelf assemblage (VTC) occurs from 25 m up to 100 m of depth, followed by a muddy slope assemblage (Arena & Bombace, 1970; Arculeo et al., 1988).

Since 1990 the Gulf is a fishery exclusion zone (regional law n. 25/1990) where trawl fishery was banned along continental shelf and part of the slope for a total of ca. 200 km^2.

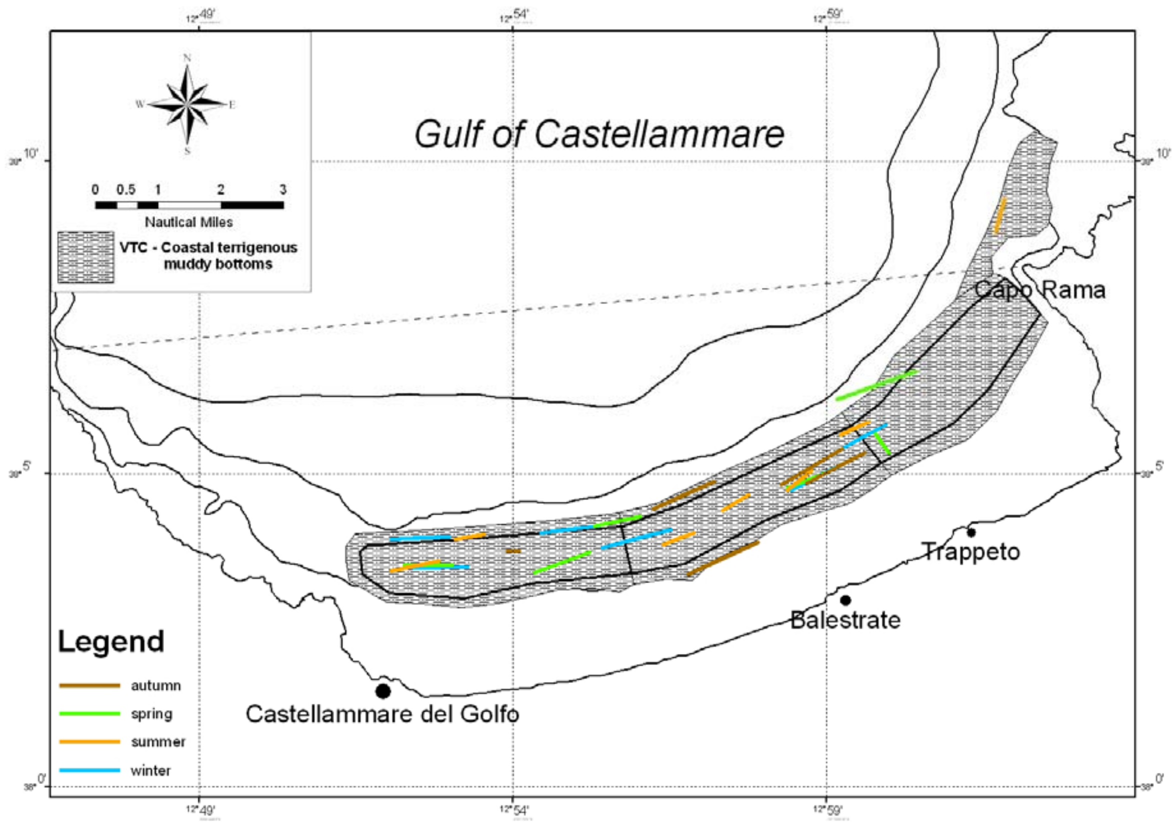

Figure 1.2 Detailed sampling area of the Gulf of Castellammare, lines indicated MACER-GIROQ samplings during four seasons from autumn 2004 to summer 2005. The grey area indicated the Terrigenous muddy bottoms (VTC, *sensu* Pérès and Picard, 1964) assemblages.

Gulfs of Termini Imerese and S.Agata di Militello

The whole area, comprised between Cape Gallo on the west and Cape Calavà on the east, includes two gulfs: Termini Imerese and S.Agata; the total extension is ca. 1900 km^2.

The gulf of Termini Imerese (fig. 1-3) is comprised between Cape Zafferano on the west and Cefalù on the east side. It is 40 km long and in the middle there is the town of Termini Imerese.

Coasts area high and steep close to Cape Zafferano and in the area between Cape Plaia and Cape Cefalù, whilst in the central part a large beach of alluvial origin. Two rivers flow in the gulf (Torto and Imera) they highly contribute to nourish the beach and to create sedimentary *facies* in the sea bottoms.

Up to 10 m sandy bottoms occur, while sandy and muddy sediments characterize the sea bottoms between 10 to 35 m of depth. From 50 m of depth the percentage of fine sediments, from silt to (pelite) mud, increases by increasing depth up to 100 m, where a typical deep muddy bottoms assemblage (VB, *sensu* Pérès and Picard, 1964) occur. The coastal zone is characterized by a long beach, with a SFBC assemblage. In front of the beach some artificial reefs occur: a pseudo-salty marsh algal community, dominated by *Cymodocea nodosa*, *Caulerpa prolifera* and *Zostera noltii* occurs between the artificial structures. Off-shore from the industrial harbour of Termini Imerese at 40 m depths, soft bottoms area composed by gravels and then by detritic sediments (SGFC and DC respectively). In succession a VTC assemblage with *Turritella communis facies* occurs up to 100m and finally a VB assemblages with *Aporrhais pes-pelecani* and *A. serrasianus* assemblages dominate the bottoms.

Wave’s movements can locally affect the streams in the gulf of Termini. All the areas seem to be influenced by a main WE current becoming from the Sardinian channel: a secondary component of this current flows in the gulfs with a W-NW/E-SE direction and generates anticlockwise micro-currents.

The gulf of S.Agata Militello (fig. 1-3) is shallow (0-8 m in the harbour) and reaches 50 m of depth at 3 km from the coast. Sediments area mainly composed of sand and mud. Biotic components are very scarce due to the high degree of the human impacts in the whole area (sewages, coastal buildings, etc.).

In front of Cefalù, bottoms are characterized by a SFGC assemblage (Fine and Gravel Sand). The remaining coastal bottoms of the gulf of S. Agata belong to DC assemblage. From 50 to 100 m of depth a transition DC-DL (shelf-edge detritic assemblage) occurs, then below 100 m of depth a transition VTC-VP is present (Cavaliere et al., 1988).

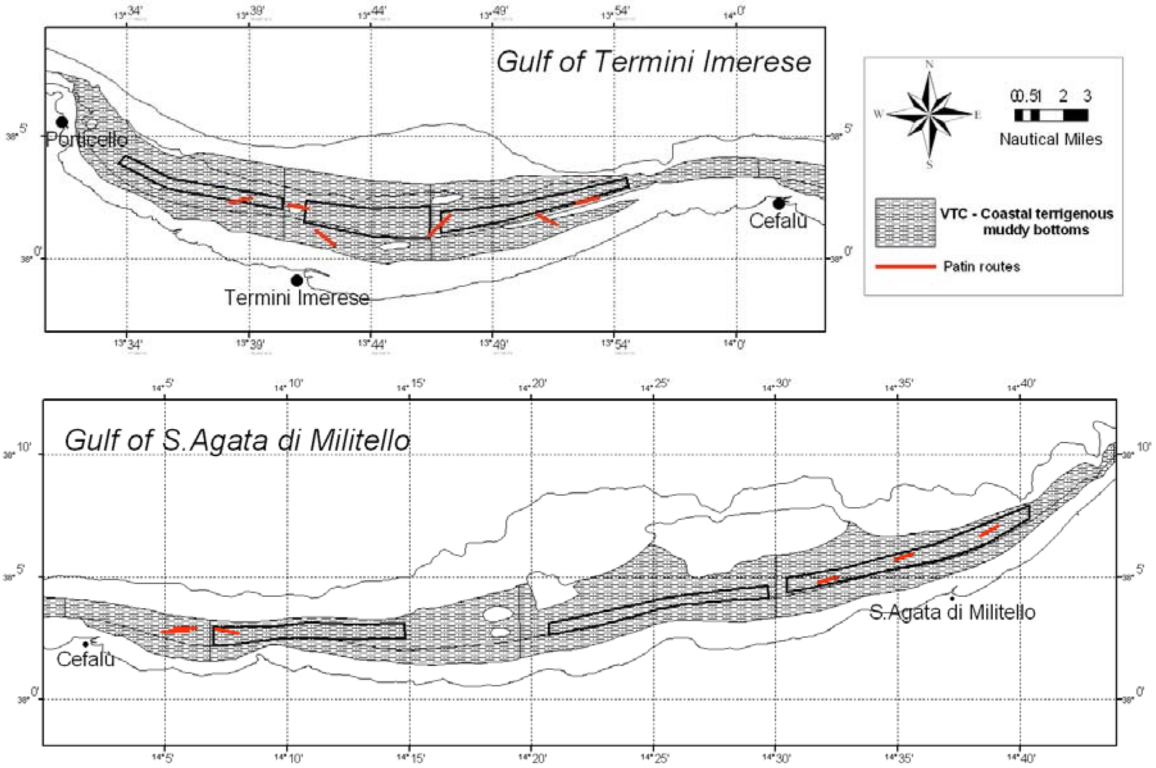

Figure 1-3. Detailed sampling areas of the Gulfs of Termini Imerese and S.Agata di Militello, lines indicated MACER-GIROQ samplings during June.

The Gulf of Patti

The Gulf of Patti (North-eastern Sicily) is comprised between Cape Milazzo on the east and Cape Calavà on the west. The eastern and western sides are characterized by steep cliffs as well as the central part where Cape Tindari is present, delimiting another sub-gulf: the gulf of Oliveri. The remaining areas are characterized by narrow sandy beaches. Bottoms from 50 to 100 m of depth are characterized by detritic assemblages (coastal detritic bottoms, off-shore detritic bottoms and muddy detritic bottoms respectively DC-DL-DE sensu Pérès and Picard, 1964) spaced out with muddy bottoms (VTC, grey areas in fig.) and rocky areas with coralligenous assemblages (“Scoglio della Secca”, “Scoglio del Cefalo” , “Scoglio Carmine” and “Scoglio di Falcone”). From 100 to 200m of depth DL assemblages occur and below 200 m VP assemblages dominate. In the gulf flow several rivers with irregular regime. Currently the bringing in of materials by these rivers is not insufficient to maintain the coastal equilibrium in terms of sedimentary balance and the effects of the phenomenon are more evident in correspondence of rivers delta where a dramatic moving back is recorded. Thus the major percentage of material comes from Montagnareale and Timeto torrents.

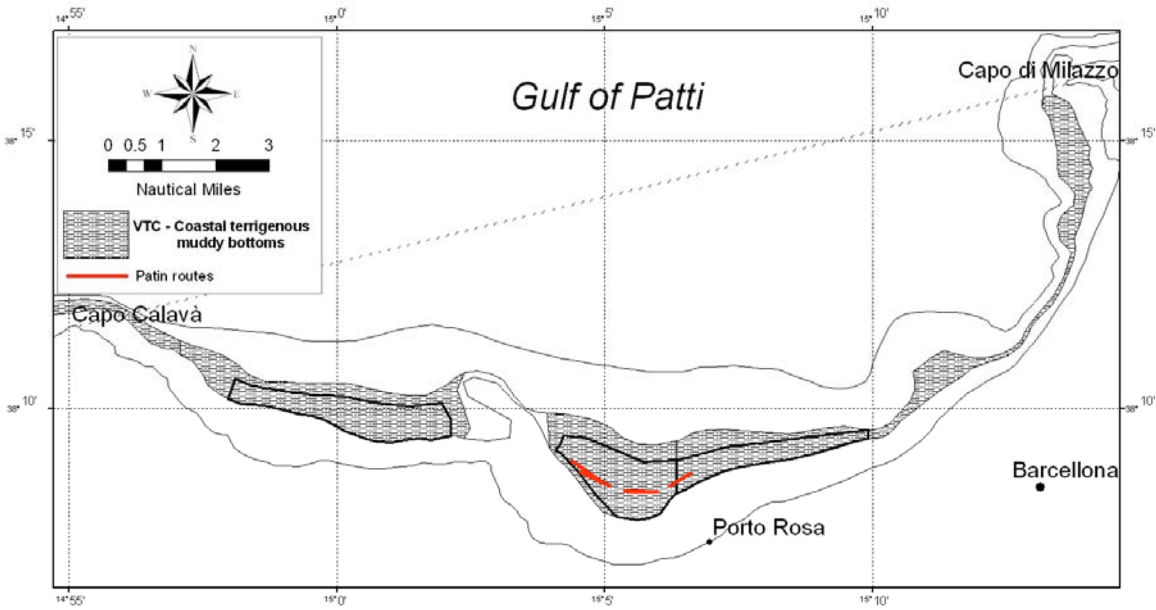

Figure 1-4 Detailed sampling area of the Gulf of Patti, lines indicated MACER-GIROQ samplings during June

1.3.2 Cabrera Archipelago

Cabrera is a small Archipelago of the Balearic islands, located on the south-west of Mallorca (Spain North-Western Mediterranean) (fig.1-5) included in the Algerian basin (39º 68'N- 2º 18'E; 39º 81'N- 2º 37'E)

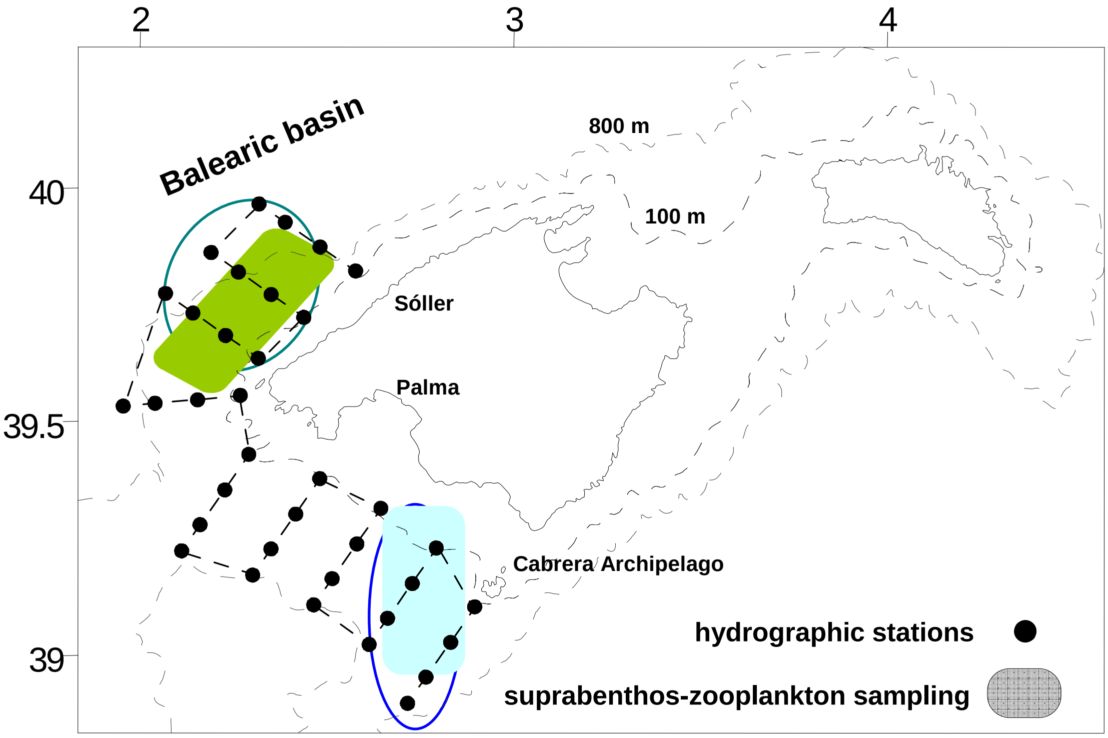

Figure 1-5. Sampling area off Mallorca Island. Grid area (with blue circle) indicates the area covered to obtain the 18 trawl samples and the 24 suprabenthos samples from shelf-slope edge, upper and middle slope (650-750 m depth).

The area was characterized by muddy bathyal bottoms (VP), where the percentage of fine particles varied from ca. 88% (at 650 m depth) to 98% (at 750 m depth). The organic matter varied from 5,6 to 6,8% at 650 and 750 m respectively all over the year.

The Ligurian-Balear basin is a high productivity area (fig. 1-6), with a unique peak of Primary Production detected from December to April peaking at February-March.

In a wide sense, the general circulation of the Western Mediterranean follows with a cyclonic pattern, and shows two well-known permanent currents:

- Algerian current (AC) in the south flowing along the Algerian coast and driving waters of recent Atlantic origin.
- Northern current (NC) comes from Ligurian Sea carrying northern waters southward along the continental slope to the Ibiza channel.

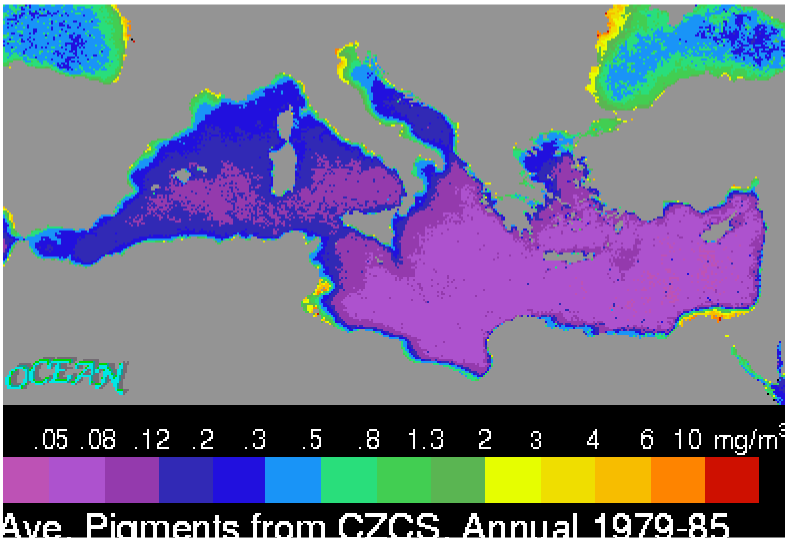

Figure 1-6. Surface pigment concentration of Mediterranean basin

It is clear that the influence of these two currents reaches the Balearic Channel. The first from the instability of the Almeria - Oran front and from the eddies detached by itself. And the second usually reaches the Ibiza channel. The seasonal variability of the NC is related with the atmospheric forcing, its transport decreases from winter (1 - 1.5 Sv) to summer (0.5 Sv). This circumstance helps to the northward progress of Mediterranean-Atlantic Waters (MAW) through the Ibiza and Majorca channels. The NC also drives southward intermediate (Winter Intermediate Waters, Levantine Intermediate Waters - WIW and LIW respectively) and deep waters (DW), and helped by the topography can generate eddies, (few tens - more a hundred Km). They sometimes cross the Ibiza channel depending of their size and depth. When it does not happen, they are topographic trapped in the Valencia gulf. It seems that there is a closed connection between the climatological factors and the circulation regime in the Balearic channels and it is possible to relate severe winters with large WIW formation and presence and mild winter with small WIW formation and presence. The mesoscale structures at north and south parts of channels (Mallorca and Ibiza channels) disturb the surface and intermediate circulation. The mesoscale structures into the Valencia Gulf, associated to WIW, play an important role on the regional circulation. Cabrera area is affected too by the big gyres of the Algerian basin.

It seems that during August and September 2003 an anticyclonic gyre was present into Valencia Gulf, which provoked a branch of Surface current flowed inside of Mallorca channel, with sharp WIW and LIW (low intermediate waters) values (Jurado-Lopez et al, submitted). During winter the wind forcing moved this gyre to the western part of Valencia Gulf, recovering the circulation through the Ibiza channel and low WIW and LIW values were observed at Mallorca channel. During spring and summer appears again a gyre, with flow inside Mallorca Channel and recovered WIW and LIW values.

1.5 Sampling methodology

1.5.1 Northern Sicily

In this study, benthopelagic macrofauna (using sledges) and megafauna (using bottom trawls) were sampled in a practically simultaneous way at the level of the Benthic Boundary Layer (BBL) at 40-80 m of depth. Benthopelagic macrofauna was sampled by means of a Macer-GIROQ suprabenthic sledge (Dauvin and Lorgére, 1989). The sledge used in this project, consists of one rectangular 40x 80 cm mouth opening, with a mechanical open–close system (fig. 1-7) that minimizes the contamination of the samples during the ascent/descent of the gear. A net of 500 µm were attached to the mouth. The gear samples the water column between 0.1–0.5 m above the bottom (Cartes et al., 1994). The area covered by the sledges for each haul was calculated from towed distance given by flowmeter located in the mouth of the net.

Fig. 1-7. One mouth modified MACER-GIROQ used during sampling in Northern Sicily.

The sledge was armed with 500 µm mesh size and was trawled to a similar speed (ca. 1.5 knots). The duration of sledge hauls over the bottom were of ca. 10 min. All samples were performed at daytime

A total of 24 samples were obtained for the Gulf of Castellammare, covering the four annual seasons between November 2004 to July 2005, within the framework of two different scientific projects (MIUR 63 and MIPAF).

In addition, only for the summer season (June-July 2005), a total of 18 samples were collected in the other three Gulfs (6 for each area, from Termini Imerese to Patti).

Suprabenthos (BBL macrofauna) and benthos fauna were collected to evaluate biomass (food availability) of prey consumed by fish and to distinguish different trophic levels among this two groups.

All samples were frozen and then at laboratory identified to lowest taxonomic level where possible, counted, weighted and, in some cases (e.g some target species), measured. Afterwards samples were dried to constant weight at 60 °C, then ground to a fine powder. One sub-sample, for carbon isotope analysis, was acidified with 1% HCl solution to remove carbonates, rinsed with distilled water and oven-dried at 40°C for 24 h, as carbonates present a less negative $\delta^{13}C$ than organic carbon (De Niro and Epstein 1978). The other, for nitrogen isotope analysis, was not acidified, as acidification results in enrichment in $\delta\,^{15}N$ (Pinnegar and Polunin 1999).

Using continuous flow, the samples were introduced into a Finnigan Delta XP plus isotope ratio mass spectrometer for isotopic analysis. Three capsules of an internal reference, international certified urea, were analysed at the beginning of each sequence and one every six samples to compensate for machine drift and as a quality control measure. Experimental precision (based on the standard deviation of replicates of the internal standard) was <0·2‰ for $\delta^{15}N$ and <0·1‰ for $\delta^{13}C$. $\delta^{13}C$ and $\delta^{15}N$ values were obtained in parts per thousand (‰) relative to Vienna Pee Dee Belemnite (vPDB) and atmospheric N_2 standards, respectively, according to the following formula:

$\delta^{13}C$ or $\delta^{15}N$ = [(Rsample/Rstandard) – 1] x 10^3 where

R = $^{13}C/^{12}C$ or $^{15}N/^{14}N$.

Three sampling cruises were carried out in the Gulf of Castellammare in November 2004, March 2005 and May-June 2005. During May 2005 survey a total of 30 hauls (10 for each depth stratum: 10-50; 51-100 and 101-200m) were carried out in four gulfs of Northern Sicily (from Castellammare to Patti).

We collected fishes from those hauls carried out at the same sites where suprabenthic samples were taken. In general we chose to collect samples from 40-80 m of depth where Coastal Muddy Bottoms (VTC, *sensu* Pérès and Picard, 1964) occurred.

Fish samples were frozen, than at laboratory identified to species level, counted, weighted and measured (Total Length, TL, to the lowest mm). From three selected species (*Pagellus erythrinus*, *Arnoglossus laterna*, *Merluccius merluccius*) stomachs and a muscle tissues were taken for stomach contents and stable isotopes analyses.

Usually, for stomach contents analysis a random sub-sample from the hauls on VTC were processed in order to obtain a number of 40 specimens for each gulf (the minimum representative samples for stomach contents analysis obtained from cumulative curves of trophic diversity, see Cartes, 1991 for further details). A number of 3 muscle tissues from each species of the same size (or similar size with no statistical differences), was prepared for stable isotope analysis as mentioned above.

1.5.2 Cabrera Archipelago

The project IDEA (*Influencia de la estructura y dinámica oceanográfica sobre poblaciones DEmersales en aguas de las Islas BAleares*) was carried out in two area off Mallorca island in two sites located at the NW and SE of the island (Soller and Cabrera respectively, see figure 1.4) at depths comprise from the shelf-slope edge (150 m) to the middle slope (750 m). During the project a total of six surveys, from August 2003 to June 2004, were carried out ((IDEA0803: 3-7 August 2003; IDEA0903: 25 September-1 October 2003; IDEA1103: 13-21 November 2003; IDEA0204: 14-20 February 2004; IDEA0404: 7-13 April 2004; IDEA0604 23-28 June 2004). To the aim of this study we considered only the data from Cabrera hauls.

A total of 24 Macer-GIROQ sledges were performed corresponding to six cruises. In each cruise, four stations (on depths at ca. the 150 m, 350 m, 650 m and 750 m isobaths) off Cabrera were sampled Distance between stations ranged from *ca.* 3 to 10 miles.

Benthopelagic macrofauna was sampled by means of a Macer-GIROQ suprabenthic sledge (see 1.4.1.for details); in this case the sledge has two superimposed nets (Cartes et al., 1994) and samples the near-bottom water column up to 1.5m above the bottom. The volumes of filtered water were estimated by means of a 2030R standard flowmeter (General Oceanics Inc.) positioned beside the central aperture of the net mouth.

One replicate by station was performed because previous studies on replicability made using suprabenthos samplers demonstrate one haul is enough to characterize the community of suprabenthos in a concrete area/time (Brattegard and Fossa, 1991).

Meso-macrozooplankton in the water column was sampled using a non-closing WP2 net with a mouth area of 1 m2, in horizontal-oblique hauls performed from ca. a few m above the sea bottom (closest estimated distance to the bottom between 13-90 m by means of an inclinometer) to the sea surface.

Both WP2 and sledges were armed with 500 µm mesh size and were trawled to a similar speed (ca. 1.5 knots). The duration of sledge hauls over the bottom were of ca. 10 min; the duration of WP2 was 10 min close to the bottom (horizontal haul) and varied between 3-15 min during the recovery of the net throughout the entire water column. Standard 2030 flowmeters were mounted on the mouth of nets to measure the amount of filtered water and/or the distance/area covered in each haul. All samples were performed at daytime.

Specimens were immediately frozen on board at -20 ºC (for posterior stable isotope analyses) or fixed (some jellyfish) in buffered formaldehyde (4%) for further detailed taxonomic identification, sorted (by aliquots ranging from 1/2 to 1/8 of samples from the small fraction of organisms in some cases), and identified to high taxa level under a stereomicroscope (at x10-

x40). Peracarids and Eucarids (Decapods) are identified to species level only for IDEA0803 (authors' unp. data). All taxa were counted and weighed (wet weight after eliminating blotting water; dry weight –DW- were not considered to avoid destruction of samples).

At laboratory, all samples were identified to the lowest taxonomic level as possible, counted, weighted and, in the case of some species, measured. At last samples were prepared for isotope analysis as mentioned above.

Fishes were sampled with a bottom trawl. The horizontal aperture of the gear was *ca.* 25,5 m (DS=1,76 m), and the vertical opening was *ca.* 2,2 m. Cod-end mesh size was 40 mm. Haul duration was always 1 h except in some particular conditions where duration is reduced to 45 minutes.

Abundance (N ind./h) and biomass (g/h) were calculated after standardizing catches to 1 hour of trawling. Distance and mouth trawl width were recorded using a SCANMAR system attached to the trawl for each haul. Fishes for stomach contents and stable isotope analyses were collected from three hauls carried out at ca. 580m, 650m and 750 m from each survey for a total of 18 hauls.

Fish samples were fixed in buffered formaldehyde, than once at laboratory, identified to species level, counted, weighted and measured (total length for all the species and in addition anal length for macrourids). From three selected species (*Hoplosthetus mediterraneus*, *Hymenocephalus italicus* and *Nezumia aequalis*) stomachs and muscle tissues were taken for stomach contents and stable isotope analyses respectively. These three species were chosen *a priori* as representative of three different feeding guilds: *H. mediterraneus* is a zooplanktonic feeder according to Pais, 2002; Madurell & Cartes, 2005a; *H. italicus* as a suprabenthic feeder and *N. aequalis* is the most benthophagous (it preys mostly on infauna) fish in the investigated demersal communities (Macpherson 1979; Mauchline and Gordon 1984, Madurell and Cartes, 2005b).

Formalin fixation systematically affected isotope signature, causing an average depletion of 1.65‰ in $\delta^{13}C$, a bias that can easily be corrected for prior to interpreting data. Preservation affected mean $\delta^{15}N$ values with far lower frequency and magnitude (Sarakinos et al., 2002; Arrington and Winemiller , 2002). To correct the bias some specimens of the same species were collected from the same area the following year (2005) during the MEDITS-ES project. Portions of white muscle were dissected from the fishes. Tissue samples from the same specimens were freeze-dried and stored in a dissecator at room temperature and used as controls. Tissue cubes were removed from the treatment after 6mo and 11mo. These were rinsed in distilled deionised water for 1 minute before freeze drying for isotope analysis.

Finally tissues were prepared and analysed for $\delta^{15}N$ and $\delta^{13}C$ as described above.

1.6 Fish species description

In the framework of three different projects (MIPAF and MIUR63 in Sicily and IDEA in the Balearic basin) we chose three species in the Northern Sicily and three species for the Balearic basin, as representative of different trophic guilds.

Two out of three coastal species chosen (*Pagellus erythrinus*, *Merluccius merluccius*) are very abundant in the Gulf of Castellammare (Pipitone et al., 2000). Juveniles of *Merluccius merluccius* were selected as benthopelagic feeder (Cartes et al., 2004; Carpentieri et al., 2005) as well as *Arnoglossus laterna* (Asvar, 1994; Darnaude et al. 2001). Adult of *A. laterna* was chosen as an epibenthic-feeder (Amezcua et al., 2003).

Within the framework of IDEA project three species were selected, on the basis of their relative abundance but also as representative of a specific trophic guild. *Hoplostethetus mediterraneus* is known as a planktonic feeder (Pais, 2002), but it also feeds on suprabenthic prey (Madurell and Cartes, 2005a). Among macrourids *Hymenocephalus italicus* and *Nezumia aequalis* are two benthopelagic feeders (Macpherson, 1979; Mauchline and Gordon, 1984), but the first is found to prey mainly on suprabenthos and the second showed more benthic preys in its diet (Madurell and Cartes, 2005b).

Description of above mentioned species is briefly presented below (Fisher et al., 1987; Whitehead et al., 1986; *www.fishbase.org*).

Pictures were downloaded from *http://free.imd.it/Colapesce/PescItalia/PaginePrincipali/Indice.htm*):

Osteichthyes:

Coastal fish

Sparidae

Pagellus erythrinus (Linnaeus, 1758) (Common Pandora)

It is a benthopelagic marine species with an oval red body (to at least 60 cm SL) without stripes or bars. The snout is at least twice as long as the eye diameter. This species is a protogynic hermaphrodite; females become males first in their third year with sizes of about 17 cm. It is found on inshore waters, on various bottom (rock, gravel, sand and mud) to 200 m (Mediterranean) or 300 m (Atlantic) and move to deeper waters during winter. It is distributed in the Eastern Atlantic from Norway to Guinea-Bissau, including Cape Verde, Madeira and the Canary Islands and the Mediterranean. Rarely recorded in Scandinavia. It is an important food fish.

Merlucciidae

Merluccius merluccius (Linnaeus, 1758) (European hake)

It is a demersal, marine species with elongated body (up to 140 cm TL); the second dorsal and anal fins are notched and inside of mouth, branchial cavity is black. The first vertebra and neural spine are attached to skull. It is found usually between 70 and 370 m of depth; it lives close to the bottom during day-time, but moves off-bottom at night. It is distributed in the eastern Atlantic from Norway and Iceland southward to Mauritania. It also occurs in the Mediterranean Sea and along the southern coast of the Black Sea. It is very commercially important.

Bothidae

Arnoglossus laterna (Walbaum, 1792) (Scaldfish)

It is a demersal, marine species with a slender body translucent and shape like a sole (maximum 25 cm SL). The males are distinguished by the elongation of certain fin rays which gives the fish a quite unusual appearance The lateral line has a marked curve over the pectoral fin, behind the pelvic fins there are two backward curved thorns. It lives on mixed or muddy bottoms, from 10 to 200 m of depth. It is distributed in the Eastern Atlantic from Norway to Angola and it is also known from the Mediterranean and Black Sea. It is of no interest for fishery.

Deep-sea fish

Trachichthyidae

Hoplostethus mediterraneus Cuvier, 1829 (Mediterranean slimehead)

It is a benthopelagic, marine species. Its body and head are deep and compressed, with large head (37-43%of SL) and eyes (about 2,5-3 in head). The body (up to 40 cm TL) is dusky pink above and silvery below. It lives in muddy bottoms up to 1175 m of depth. It is generally cosmopolitan, distributed in the Eastern Atlantic from Ireland to Senegal, Canary Islands, and the Gulf of Guinea, including the Mediterranean; it is also found in Namibia and South Africa. Also reported from Iceland. It is reported in Red Sea and in the Western Atlantic, from Georges Bank to northern Gulf of Mexico, Great Antilles, and from Venezuela to southern Brazil. It is no important for fishery.

Macrouridae

Hymenocephalus italicus Giglioli, 1884 (Italian grenadier)

It is a benthopelagic species with a slender and quite cylindrical body (to at least 25 cm TL). The head is as wide and deep and the snout is rather blunt. The mouth is large (upper jaw reaching back beyond the eye), oblique and subterminal. It has light organs small but distinct externally. It is most common at depths less than 500 m It is distributed in the Eastern Atlantic (from Portugal to Angola, including the Mediterranean), in the Western Atlantic (from Florida Straits through the Gulf of Mexico and Caribbean Sea to northern Brazil) and in the Indian Ocean (Gulf of Aden, Zanzibar and Maldives).

Nezumia aequalis (Günther, 1878) (Common Atlantic grenadier)

It is a benthopelagic species with a tapering body (to at least 36 cm TL) fairly evenly to pointed tail. The head is relatively short (15% of the body length) and compressed and the snout is blunt and high. The mouth is inferior, small and the upper jaw less than one third of the head length; the eyes are relatively large, 29 to 42% of head length. It inhabits depths from 200 to 2320 m and lives in the Eastern Atlantic (from Faroe Bank to northern Angola), in the Mediterranean Sea and in the Western Atlantic (from Davis Straits to northern Brazil).

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Chapter 2

Seasonal changes in suprabenthic community of Castellammare Gulf (North-western Sicily)

Abstract

Dynamics of suprabenthos was analyzed in the Gulf of Castellammare (North-Western Sicily-Italy) at depths ranging between 40 and 80 m. Six stations situated on muddy terrigenous bottoms (*sensu* Pérès and Picard, 1964) were sampled at season intervals during four cruises performed between November 2004 and June 2005. Suprabenthos showed maximum biomass from late winter (March) to summer (June), while minimum biomass was found in autumn (November).

Among suprabenthic community the highest biomasses of mysids and copepods occurred in March, one month after the peak of primary production, while amphipod biomass was higher in summer, coupled with the increase of the percentage of organic matter (%OM) recorded in spring-summer in the area.

In nMDS analyses suprabenthos abundance showed a sample aggregation as a partial function of season: 3 defined groups corresponding to autumn, winter-spring and summer occurred, better evidenced by the constrained Canonical Analysis of Principal Coordinates (CAP) analysis.

The increase in suprabenthos biomass was correlated (Spearman correlation) with the peak of Chl*a* on surface one and three months before sampling.

Our preliminary analysis using stable isotopes suggests a relatively complex food web in the gulf of Castellammare in which different food sources were observed. Some suprabenthic species (i.e. mysids and copepods) exhibited depleted values of $\delta^{13}C$, indicated a planktonic source of material; cumaceans and amphipods displayed more enriched $\delta^{13}C$ values, confirming this detritivorous habits. A third group with intermediate $\delta^{13}C$ values comprised species with different trophic guilds (e.g. *Ampelisca* spp., *Apherusa vexatrix*. and *Harpinia* spp.).

Assuming a conservative trophic enrichment of *ca* 3.0 ‰ between consumers and their diet, at least three trophic levels were identified within the suprabenthic community of the gulf of Castellammare: the first was composed by deposit, herbivorous and suspension feeders (*Ampelisca* spp., *Apherusa vexatrix*, *Goneplax rhomboids*); the second mainly comprised filter-feeders (mysids and Copepods) and also deposit feeders who probably fed on enriched nitrogen detritus (cumaceans, *Harpinia* spp., Gammaridea). The third group was composed by

two carnivores species the gobiid fish *Lesuerigobius sueri* and the infaunal-feeder *Philocheras bispinosus*.

Changes in isotopic composition occurred for the dominant suprabenthic species Seasonal differences in the isotopic composition of suprabenthic fauna may thus be a response to diet shifts associated with changes in the availability of different prey throughout the year and/or to change in the source of organic matter, particularly variable in coastal environments.

2.1 Introduction

The Benthic Boundary Layer (BBL) extends a few tens of metres into the water column immediately overlying the sea bed, and it is an environment of great complexity both from a physical (Gage and Tyler, 1991) and biological (Smith and Hinga, 1983) perspective. One of the most important biological characteristics of the BBL is the progressive increase of planktonic and nektonic biomass near the bottom (from around 100 m above the sea bed) within this layer (Angel, 1990), in contrast to the general exponential decline of pelagic biomass with depth in the water column (Vinogradov and Tseitlin, 1983)

Benthopelagic organisms, inhabiting the near bottom environment, are a fundamental part of the BBL (Marshall, 1965). These communities of organisms living above the sea bed have been included in distinct concepts, such as suprabenthos (Brunel et al., 1979) and hypoplankton (Mauchline, 1991).

The term suprabenthos is linked to the specific sampling methodology of the suprabenthic sledges, and refers to the macrofauna with a natatory capability that inhabits the interface closest to the sea bed (Sainte-Marie and Brunel, 1985; Elizalde et al., 1999). Because of this natatory capability, this fauna is hardly captured using dredges or corers (Elizalde et al., 1999). Some authors (Wang y Dauvin, 1994; Wang et al., 1994) distinguish between a "permanent" suprabenthos (mysids, cumaceans, decapods, amphipods, isopods and pycnogonids) and zooplankton species that live close to the bottom. As a function of size, zooplankton was subdivided in mesozooplankton (e.g. copepods, crustacean larvae, chaethognats, and polychaete larvae), macrozooplankton (ctenophores and fish post-larvae), or micronekton (large decapods and fish).

Mees & Hamerlynck (1992) and Mees & Jones (1997) differentiate the hyperbenthos in holohyperbenthos, including small fauna who spend all its life in the BBL and in merohyperbenthos, formed by larvae of nektonic and benthic adults.

In this thesis the term suprabenthos *sensu* Brunel et al. (1978) is used, including species who live in the BBL and some planktonic, epibenthic and infaunal species who spend only a part of its life (development or adult stage) in the suprabenthos (Mees y Jones, 1997).

Thus the suprabenthic fauna includes all bottom-dependent animals, which perform with variable regularity, daily or seasonal vertical migrations (Brunel et al., 1978). For some years, there has been an increase in the number of suprabenthic studies because of the perceived interactions of such species with demersal or pelagic fishes (trophic chains) and as interest has grown in benthic-pelagic coupling (Dauvin et al. 1994; Wang and Dauvin, 1994).

In coastal environment the suprabenthos play an important role in the trophodynamics (Sorbe, 1981, 1987; Harmelink et al., 1990; Mees and Hamerlynck; 1992). A clear understanding of the energy and material fluxes in coastal environment should always take into consideration the well established importance of suprabenthos' assemblages in those ecosystems (Boysen, 1975; Buhl-Jensen and Fossa, 1991; Hamerlynck and Mees, 1991; Wooldridge, 1989). Suprabenthic species, especially mysids, are an important component of the biomass of coastal regions (Williams and Collins, 1984; Marquiegui and Sorbe, 1999), structuring zooplankton communities by predation (Fulton, 1982; Wooldridg and Bailey, 1982), contributing to the diet of demersal fishes (Mauchline, 1980; Sorbe, 1981) and shrimps (Sitts and Knight, 1979), and also as important grazers of organic matter (Mees and Hamerlynck; 1992). They successfully exploit the diversity of food resources and are an important link in the detritus-based food chains (Mees et al., 1995).

Many suprabenthic species are detritivorous, feeding on organic matter from the sediment, thus constituting intermediate elements in demersal food chains (Mauchline, 1980; Sorbe, 1984; Dauvin et al., 1994).

A relative high number of suprabenthic studies have been carried out in the infralittoral and circalittoral bottoms see Mees and Jones, 1997 for a review). Nevertheless, few studies described the suprabenthic communities inhabiting coastal muddy bottoms. In Mediterranean many studies focused on surf-zone or deep-sea environments (Cartes 1998; Cartes & Sorbe, 1999; San Vicente & Sorbe, 1999; Cartes *et al.*, 2001), only recently some studies stressed on shelf suprabenthic communities of Ebro and Po rivers delta (Cartes et al, submitted, Ligas 2005). As far as the Tyrrhenian sea is concerned, there is total absence of information on suprabenthic communities.

Coastal environment is not stable: there are some important temporal fluctuations associated to changes in the pulses of organic matter derived from primary production of macrobenthic vegetal, from rivers outflow and from vertical flux.

This has an influence on the biology (e.g. recruitment) and density of benthic and benthopelagic organisms (suprabenthos) which constitute the basis of the diet of demersal fish both coastal and bathyal (Mees and Jones, 1997; Cartes & Maynou, 1998). Despite, several studies exist on seasonal variation of suprabenthic communities outside the Mediterranean, in the Bay of Biscay (Sorbe, 1984, 1989); in the Westerschelde Estuary (Mees et al., 1993); in western Sweden (Buhl-Jensen and Fosså, 1991), only recently an accurate investigation was carried out on deep sea BBL fauna off Mallorca (Cartes et al., *in press*; Madurell et al., submitted; Chapter 5 of this thesis). Studies on trophodynamics of suprabenthic communities of coastal Mediterranean environments completely lack.

Particularly the studied area, the gulf of Castellammare, is a semi-enclosed area (see Chapter 1), where rivers, with different seasonal capacity, flow. Thus the amount of organic matter from land runoff changes all over the year and can cause severe seasonal changes in suprabenthic communities structure and abundance. This can have an important role in the diet of juveniles of some important species of the gulf, e.g. the European hake *Merluccius merluccius* or the scaldfish *Arnoglossus laterna.*

In this study we used the stable isotope analysis approach in order to clarify the trophic relationships among suprabenthic species and to identify changes in trophic levels and source of carbon of dominant species. Studies of seasonality of isotopic signatures in aquatic systems are still relatively rare, (e.g., Gearing et al. 1984; Madurell et al., submitted, and add more) but they are crucial when considering organisms of small size with the potential to turn over assimilated isotopes quickly and thus exhibit differing isotopic signatures over a relatively short temporal scale (Grey in press), i.e. large seasonal variation in $\delta^{15}N$ was exhibited by individual zooplankton species (Graham 1997 and Leggett et al. 2000).

Based on these previous considerations, the aims of this chapter are:

- to determine the taxonomic composition of suprabenthic community
- to analyse seasonal changes in suprabenthos biomass, assemblage structure both qualitative and quantitative;
- to evaluate the trophic level of different suprabenthic taxa;
- to determine if any change in trophic level occurs along the four sampled seasons

Among the objectives the trophodynamics of suprabenthic communities by stable isotopes analyses at a species level was completely new and provides information about the feeding behaviour of different species and their relationships.

2.2 Materials and methods

2.2.1 Data collection

More information about the study area, the sampling methodology and processing are available in chapter 1.

Six samples of suprabenthos were collected during four seasons (total samples = 24), from November 2004 to July 2005 at depths comprised between 40 and 80 m corresponding to terrigenous-mud shelf assemblages (VTC, *sensu* Peres and Picard, 1964). The sledge used is showed in figure 2.1

Figure 2-1. One-mouth sledge modified from Macer-Giroq model (Dauvin and Lorgere, 1999) for suprabenthos sampling

2.2.2 Environmental variables

Environmental variables such as Chlorophyll *a* and water surface temperature were obtained by satellite imagery free available at: http://reason.gsfc.nasa.gov/OPS/Giovanni/. Data on sediment organic matter (SOM) only for May 2005 were courteously obtained by "Laboratoriodi Biologia Marina e Risorse, Dipartimento di Biologia Animale, Università di Palermo".

2.2.3 Data treatment

Data on abundance of fauna collected were analyzed by univariate and multivariate techniques. We performed analyses for suprabenthos and for zooplankton including the following taxa : Decapoda Natantia, Mysidacea, Amphipoda Gammaridea, Caprellidea and Hyperidea, Isopoda, Cumacea, Copepoda and Osteychthyes.

Some gelatinous zooplankton groups (salps, cnidarians) were excluded from analyses due to difficulties in their sorting and quantification. Species/taxa appearing less than twice in all samples were removed from data matrices. Some taxa, poorly represented in sledge samples belonging to the (non-swimming) epifauna-infauna (Polychaeta, Decapoda, Amphipoda, Caprellidea, Tanaidacea, and Echinodermata, Mollusca) were also removed from the data matrix.

Statistical differences in main taxa abundance as a function of season were established by applying a chi-squared test (Yates' correction) to the numerically dominant group. Chi-squared tests were calculated between contiguous periods. One-way ANOVAs were used to compare differences among biomass of main taxa for seasons. Data were log transformed to remove the dependency of the variance on the mean (Zar, 1984). The assumption of normality was tested using the Kolmogorov–Smirnov test and the assumption of homogeneity was tested by Cochran test. All analysis' inferences were based on the 0,05 significance level.

Diversity indices (Species richness, Shannon-Wiener, Simpson and Pielou's eveness indices) were calculated to provide information on changes in suprabenthic species among seasons.

A nMDS was first performed on Bray Curtis similarity of 4th root transformed abundance data (Clarke and Warwick, 2001). A one-way ANOSIM (Analysis of similarities; Clarke and Green, 1988) test was performed in order to detect for significant differences in suprabenthos abundance among seasons. A MVDIPSP routine (Multivariate dispersion indices) was computed to identify which group (season) was more dispersed.

Based on the null hypothesis that no differences existed among seasons and period of low and high primary production (established on satellite imagery data) a PERMANOVA (Anderson, 2001) design was created based on two factors (production with 2 levels and season, nested in production with 4 levels). On the *a priori* hypothesis that there were seasonal differences a CAP (Canonical Analysis of Principal Coordinates) analysis was then performed.

SIMPER routine was used to identify those species that most typify each season.

The correlation of biota (suprabenthos abundance) to environmental patterns (temperature, phytoplankton pigment concentration recorded simultaneously, 1, 2, 3 and 4 months before sampling) was analysed by the BIO-ENV procedure (Clarke and Ainsworth, 1993; Clarke, 1993; Somerfield et al., 1994).

All the analyses were carried out using the software PRIMER 6 plus PERMANOVA (Clarke and Warwick, 2001; Anderson, 2006) and the software STATISTICA 6.

2.2.4 Stable isotopes analysis

Once collected, samples were immediately frozen at -20°C and sorted in the laboratory. All specimens were sorted as quick as possible, identified to species level and then they were freeze-dried and stored until their preparation for analyses. Species selected for isotopic analysis (only large sizes for each species) were those that were dominant in both abundance and biomass, hence reaching a whole view of variations in the suprabenthic assemblage.

For $\delta^{13}C$ analyses, samples were decalcified to remove inorganic carbonates from the exoskeleton (Jacob et al., 2005). A small quantity of 1M hydrochloric acid (HCl) was added to the samples and left for 3 hours. The samples were then dried at 60°C for 12 h and ground to a fine powder using a pestle and a mortar. Composite samples (Table 2-1) were analysed for all suprabenthic species to minimise infra-individual differences in the isotopic composition and, to meet the minimum mass requirement for the isotopic analyses. For all the species four replicates were performed, when it was possible one replicate was represented by one individuals, in order to reduce pseudo-replication (Hulbert, 1984), when the biomass was not enough more specimens were pooled together to obtain sufficient mass for the isotope measurement (as the case of Calanoid copepods for all seasons, Cumaceans and small amphipods in autumn).

Table 2-1. Species analyzed for stable carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) isotopes. Legend: AMP (Amphipods), COP (Copepods), CUM (Cumaceans), DEC (Decapods), EUP (Euphausiids), ISO (Isopods), MYS (Mysids). [a] Species analyzed on a seasonal basis.

Group	Acronym	Species	Nov04	Mar05	May05	Jun05
AMP	Amp	*Ampelisca* spp.[a]		*	*	*
AMP	Aph	*Apherusa* spp. [a]		*	*	*
AMP	Harp	*Harpinia* spp. [a]		*	*	*
AMP	Wrec	*Westwoodilla rectirostris*		*	*	*
AMP	Gamm	Gammaridea	*			
CUM	Cum	Cumacea	*			
CUM	Lmed	*Leucon mediterraneus* [a]		*	*	*
COP	Cop	Copepoda [a]	*	*	*	*
FISH	Lsue	*Lesuerigobius sueri*	*	*		
MYS	Lgra	*Leptomysis gracilis* [a]	*	*	*	*
MYS	Eele	*Erythrops elegans* [a]		*	*	*
DEC	Pbis	*Philocheras bispinosus**	*	*	*	*
DEC	Agla	*Alpheus glaber* [a]		*	*	*
DEC	Grho	*Goneplax rhomboides*[a]		*	*	*
MYS	Aagi	*Anchialina agilis*	*			

Based on the information on its diet, when it was available, the species were classified into feeding types (Table 2-2). Correlations between δ^{13}C and δ^{15}N were calculated for each sampling period.

Seasonal variations in δ^{13}C and δ^{15}N values were analyzed only for species where data from at least 3 seasons were available. Correlations between δ^{13}C and δ^{15}N were calculated for the bulk of BBL fauna on a month scale.

Table 2-2 Feeding habits and type of prey of suprabenthos fauna based on literature data. Acronyms as in table 2-1.

Group	Species	feeding habits	prey	source
AMP	*Ampelisca* spp.	Deposit and filter feeder	detritus	Franz, Tanacredi 1992
AMP	*Apherusa* spp.	Detritivorous (herbivorous)	phytoplankton-detritus	Portland 2001
AMP	*Harpinia* spp.	deposit-feeder		
AMP	*Westwoodilla rectirostris*	deposit-feeder		
CUM	Cumacea			
CUM	*Leucon mediterraneus*	deposit-feeder		Cartes et al., 2003
COP	Copepoda	filter-feeder	phytoplankton-detritus…	
FISH	*Lesuerigobius sueri*	carnivore	zoobenthos	Costa, 1991
MYS	*Leptomysis gracilis*	Omnivore	phytoplankton-zooplankton	Maucline, 1980
MYS	*Erythrops sp*	Carnivore-deposit feeder	Copepods, hyperiids, detritus	Cartes and Sorbe 1998
DEC	*Philocheras bispinosus*	Infaunal feeder		Cartes et al. 2002
DEC	*Alpheus glaber*	deposit feeder		Cartes et al. 2002
DEC	*Goneplax rhomboides*	Infaunal feeder		Cartes et al. 2002

One-way ANOVA was performed to detect if apparent changes in stable isotopes signatures were significant.

2.3 Results

2.3.1 Species composition

A total of 22808 specimens, belonging to Mollusca (Cephalopoda), Cnidaria, Crustacea, Osteichthyes, Sipunculida and Chaethognatha was collected through the experimental samplings in the study area in the four season explored; strictly benthic species such as gastropods and bivalves among molluscs, polychetes and echinoderms were not included (Annex 2-1). All the species belong to 108 taxa; the most abundant were gammarids (mean number of individuals/100m^2=780), mysids (548) and cumaceans (239). At species level the most abundant species during the whole period were *Apherusa vexatrix*, *Harpinia* spp. and *Westwoodilla rectirostris* among gammarids, *Erythrops elegans* and *Leptomysis gracilis* among mysids and *Dyastiloides serrata* and *Leucon mediterraneus* among cumaceans.

The abundance of copepods varied significantly among contiguous seasons (autumn-winter χ^2=136, p<10^{-5}; winter-spring χ^2=129; p<10^{-5} and summer-autumn χ^2=4,2; p<0,05). Also decapods abundance differed significantly among contiguous seasons (autumn-winter χ^2=43, p<10^{-5} and winter-spring χ^2=35; p<10^{-5}). Amphipods abundance varied significantly between autumn and winter (χ^2=11,2, p<10^{-4}) and between summer and autumn (χ^2=22,5; p<10^{-5}). Mysids abundance changed significantly between autumn and winter (χ^2=12, p<10^{-4}) and between spring and summer (χ^2=11,5; p<10^{-4}). Finally cumaceans abundance varied significantly between autumn and winter (χ^2=15, p<10^{-4}), winter and spring (χ^2=12; p<10^{-4}), spring and summer (χ^2=14; p<10^{-4}) and summer and autumn (χ^2=13,9; p<10^{-4}).

Generally important variations were observed in all the most abundant taxa between autumn and winter.

2.3.2. Trend in biomass

Suprabenthos biomass changed seasonally in the Gulf of Castellammare with maximum and minimum values observed respectively in winter (March 2005-0,94 gWW/100m^2) and autumn (November 2004-0,36 gWW/100m^2) (Figure 2-2).

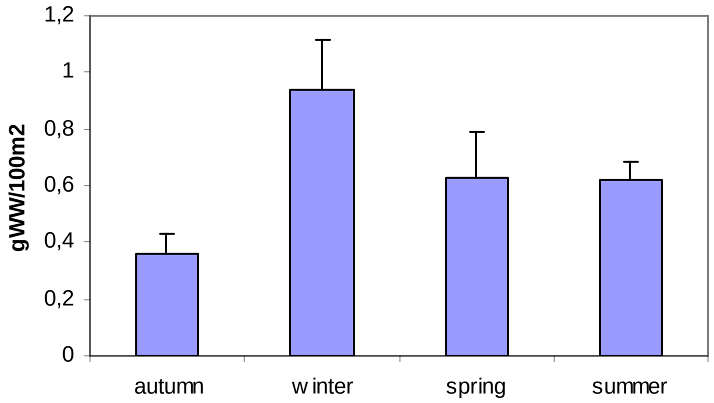

Figure 2-2. Seasonal changes in suprabenthos biomass in the Gulf of Castellammare (S.D. is also given)

As concerns peracarid crustaceans, that generally were dominant in suprabenthos, maximum biomass was recorded in March (0,459 gWW/100m^2) minimum values were recorded in November and May (0,218 and 0,189 gWW/100m^2 respectively) (fig. 2-3).

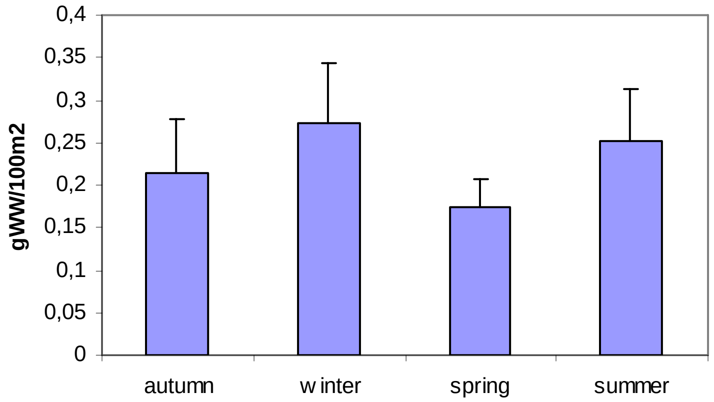

Figure 2-3. Seasonal changes in peracarid biomass in the Gulf of Castellammare (S.D. is also given)

Mysids showed a different pattern of peracarid crustaceans (fig.2-4): a trend of increasing biomass was observed from November to March (from 0,148 to 0,172 gWW/100m^2), then an abrupt decrease was observed in spring-summer (mean value 0,083 g WW/100m^2).

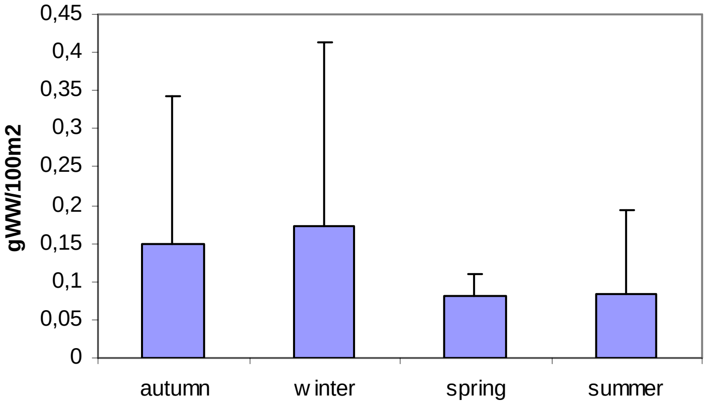

Figure 2-4. Seasonal changes in mysids biomass in the Gulf of Castellammare (S.D. is also given)

Maximum value of amphipod biomass was observed in June (0,144 gWW/100m^2), while minima occurred in autumn and spring (0,061 and 0,054 gWW/100m^2 respectively) (fig. 2-5).

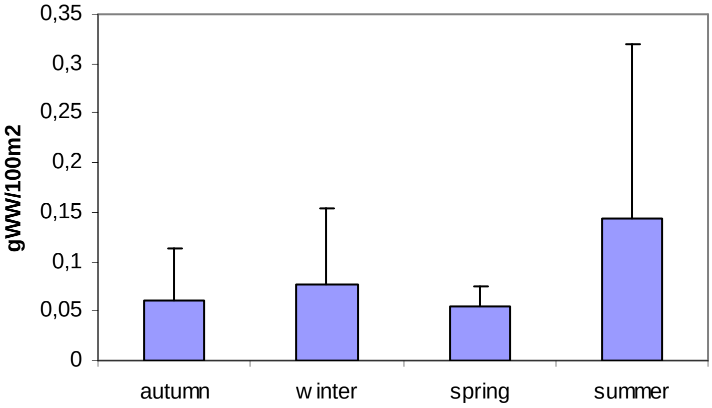

Figure 2-5. Seasonal changes in amphipods biomass (gWW/100m^2±SD) in the Gulf of Castellammare (S.D. is also given)

Maximum value of copepods biomass was observed in March (0,187 gWW/100m^2), while minima occurred in autumn and spring (0,004 and 0,015 gWW/100m^2 respectively) (fig. 2-6).

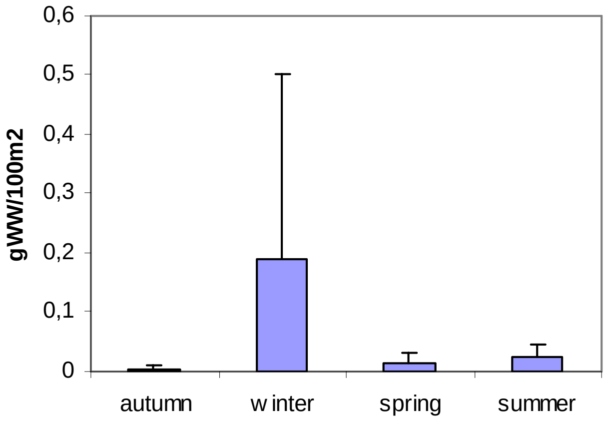

Figure 2-6. Seasonal changes in copepods biomass (gWW/100m^2±SD) in the Gulf of Castellammare (S.D. is also given)

All temporal differences in biomass were not significant.

2.3.3 Multivariate analysis

Suprabenthos varied as a function of season (Figure 2-7; stress 0,1).

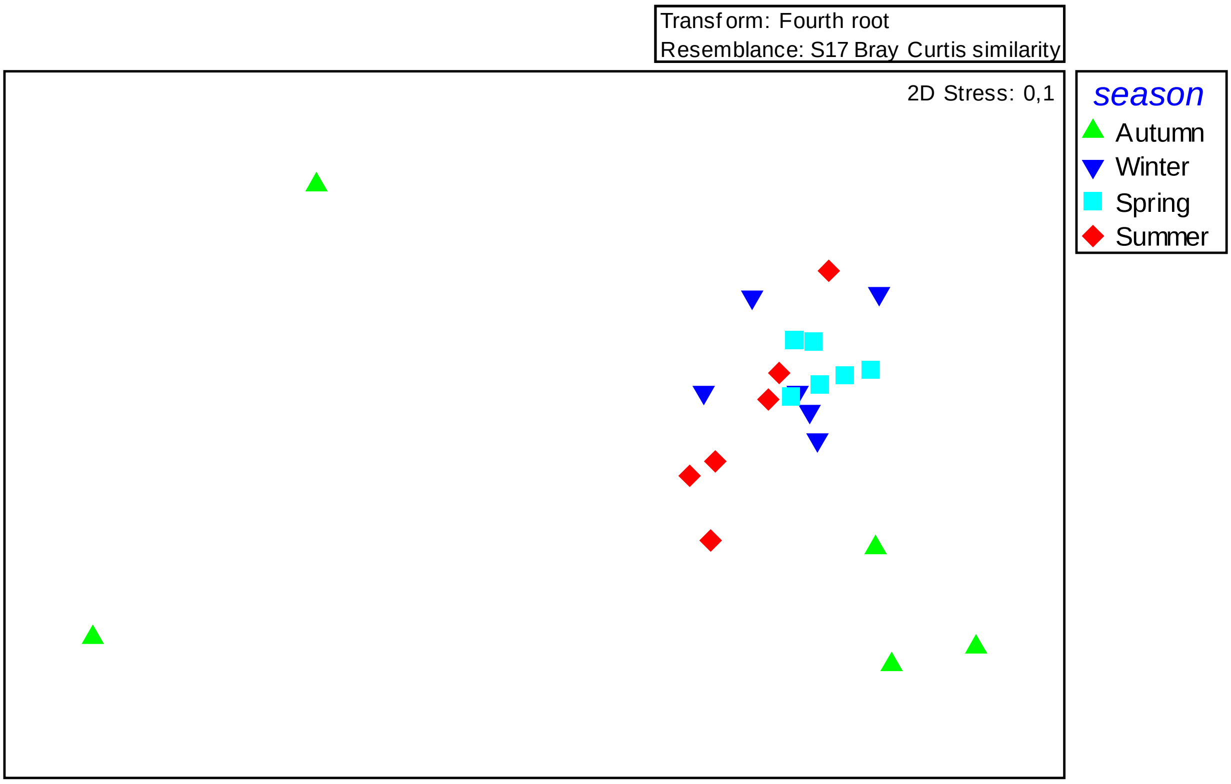

Fig. 2-7. nMDS ordination plot of average abundance data of suprabenthic species per season.

In the Gulf of Castellammare, nMDS analyses showed sampling aggregation for those hauls taken from March to June (winter-spring and summer), while autumn samples seemed to be more dispersed. Also summer samples seemed to be more separated from those collected in winter-spring.

ANOSIM test showed that seasonal differences were significant though the R value is not very high (R=0,396 p<0,05); pair-wise comparisons evidenced significant differences between all the seasons (table 2-3, 462 permutations for all pair-wise comparisons).

Table 2-3. Analysis of similarities (ANOSIM) performed on seasons. Sample statistic (Global R): 0,396; Significance level of samples statistic: 0.1%; Number of permutations: 999 (Random sample from a large number).

Groups	R-Statistic	p Level %
Autumn, Winter	0,445	0,2
Autumn, Spring	0,504	0,2
Autumn, Summer	0,48	0,2
Winter, Spring	0,25	0,4
Winter, Summer	0,324	0,6
Spring, Summer	0,431	0,2

The highest indices of dispersion (by MVDISP routine) occurred in Autumn (1,707) and in Summer (1,162), while the lowest in Spring (0,436) and Winter (0,931). Symmetrical PERMANOVA (table 2-4) provided evidence for separation of samples among seasons but no significant effect of production (high in winter and low in the other seasons) occurred.

Table 2-4. Symmetrical PERMANOVA based on the Bray–Curtis dissimilarities of the 4th root transformed multivariate data (55 species)

Source	df	SS	MS	Pseudo-F	P(perm)	Unique perms
production	1	1123,9	1123,9	0,37911	0,967	998
season(production)	2	5889,4	2944,7	3,1018	**0,001**	997
Res	19	18038	949,35			
Total	22	24987				

Based on the significant effect of PERMANOVA, a constrained CAP analysis was then performed. The horse-shoe effect obtained in the CAP analysis (fig. 2-8), showed a seasonal gradient in suprabenthos abundance from autumn to summer, probably related to the peak of primary production (see below). Winter and spring samples partially overlapped.

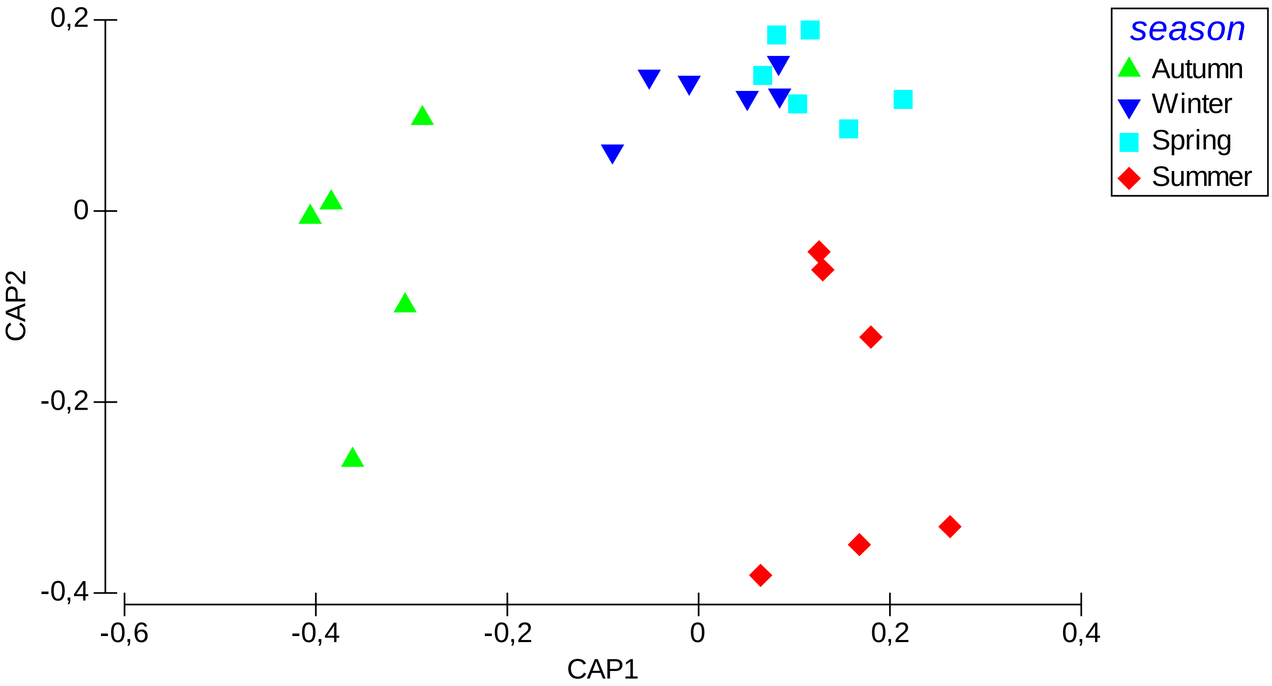

Fig. 2-8. CAP analysis based on factor season for the Gulf of Castellammare.

SIMPER analysis evidenced those species that typifying each season (table 2-5; cut off for low contributions: 60%). In autumn the most abundant *taxa* were mysids (mainly *L. gracilis*), in winter and spring copepods and, among mysids, *E. elegans* dominated the suprabenthic community, whereas in summer gammarids (e.g *Harpinia* spp and *Apherusa vexatrix*) and copepods were the most typifying species/taxa. Similarity was particularly low in autumn

Table 2-5. Percentage contribution of typifying species (SIMPER analysis) to within-group (season) similarity for the Gulf of Castellammare. Cut-off for low contribution: 60%.

Group Autumn

Average similarity: 32,00%

Taxa	Av.Abund	Av.Sim	Contrib%	Cum.%
L. gracilis	1,38	3,17	9,92	9,92
Gnathia sp. larvae	1,02	2,72	8,51	18,42
Mysidacea unid.	0,98	2,48	7,74	26,16
Processa spp.	0,81	2,12	6,63	32,79
Gammaridea unid.	1,36	1,89	5,91	38,70
Harpinia spp	1,25	1,78	5,57	44,28
A. vexatrix	1,27	1,71	5,36	49,64
A. agilis	1,38	1,52	4,75	54,39
Copepoda	0,90	1,33	4,14	58,53
Ampelisca spp	0,90	1,29	4,04	62,57

Group Winter

Average similarity: 67,58%

Taxa	Av.Abund	Av.Sim	Contrib%	Cum.%
Copepoda	4,79	6,19	9,16	9,16
E. elegans	2,82	4,20	6,22	15,38
Harpinia spp	2,46	3,94	5,83	21,21
A. vexatrix	2,32	3,47	5,14	26,35
W. rectirostris	2,14	3,27	4,84	31,19
Gnathia sp. larvae	2,17	3,18	4,71	35,89
Mysidacea unid.	2,11	2,97	4,40	40,29
M. griseus	1,95	2,83	4,19	44,48
D. serrata	1,81	2,81	4,16	48,64
L. gracilis	1,92	2,73	4,04	52,68
L. mediterraneus	1,76	2,55	3,78	56,45
Gammaridea unid.	1,97	2,36	3,50	59,95
P. marina	1,37	2,16	3,19	63,14

Group Spring

Average similarity: 75,47

Taxa	Av.Abund	Av.Sim	Contrib%	Cum.%
Copepoda	2,90	4,74	6,28	6,28
E. elegans	3,17	4,68	6,20	12,48
W. rectirostris	2,58	4,16	5,51	17,99
Harpinia spp	2,51	4,01	5,31	23,31
M. griseus	2,22	3,67	4,86	28,17
Gammaridea unid.	2,29	3,50	4,64	32,81
Cumacea unid.	2,14	3,38	4,48	37,29
D. serrata	2,39	3,33	4,42	41,70
L. mediterraneus	2,07	3,22	4,27	45,98
P. longimanus	1,87	3,04	4,03	50,01
A. longicornis	1,78	2,82	3,73	53,74
Gnathia sp. larvae	1,83	2,59	3,43	57,16
P. oculatus	1,61	2,56	3,40	60,56

Group Summer

Average similarity: 63,18%

Taxa	Av.Abund	Av.Sim	Contrib%	Cum.%
Harpinia spp	3,19	5,95	9,41	9,41
Copepoda	3,17	5,86	9,28	18,69
A. vexatrix	2,63	4,95	7,83	26,53
Gammaridea unid.	2,51	4,87	7,71	34,23
L. gracilis	2,10	3,44	5,44	39,67
Ampelisca spp	1,88	3,43	5,43	45,10
E. elegans	1,77	3,16	5,00	50,10
Gnathia sp. larvae	1,89	3,12	4,93	55,03
W. rectirostris	1,79	3,07	4,87	59,89
P. oculatus	1,67	3,04	4,81	64,71

Diversity indices (table 2-6) based on the average abundance of samples per season, showed the maximum values of diversity (H') in spring and summer and the lowest in winter. Although the highest value of specimens was recorded in winter, a dominance of only few *taxa* (i.e. copepods and the mysid *E. serrata*) was observed.

Table 2-6. Diversity indices based on the average abundance per season of suprabenthic species.

Sample	S	N	d	J'	H'(loge)	1-λ'
Autumn	52	178	9,85	0,70	2,76	0,88
Winter	76	2191	9,75	0,43	1,86	0,61
Spring	74	755	11,02	0,72	3,11	0,93
Summer	75	706	11,28	0,70	3,03	0,92

2.3.4 Correlation with environmental variables

Chl *a* derived from satellite imagery are shown in figure 2-9. In the Gulf of Castellammare there was a unique peak of Chl *a* on surface in February 2005 with a period of low production between April and October. A similar trend was observed one year before when a peak was detected in January-February.

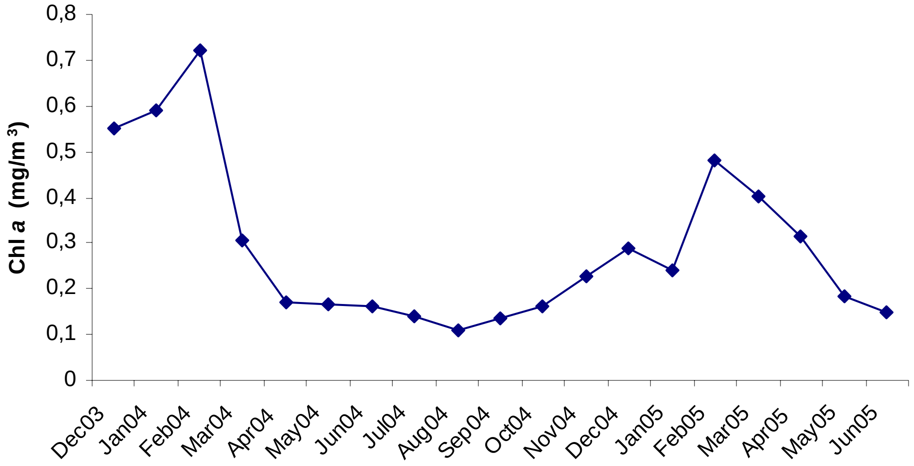

Figure 2-9. Monthly *Chl a* derived from satellite imagery (*http://reason.gsfc.nasa.gov/Giovanni*) in the Gulf of Castellammare from December 2003 to June 2005.

Surface temperature in the Gulf of Castellammare ranged between 13,6 ºC in winter (February) to 28,4 ºC in summer (August); during the sampling period temperature ranged from 19°C in November to 14°C in March and from 17°C in May to 23°C at the end of June (fig.2-10).

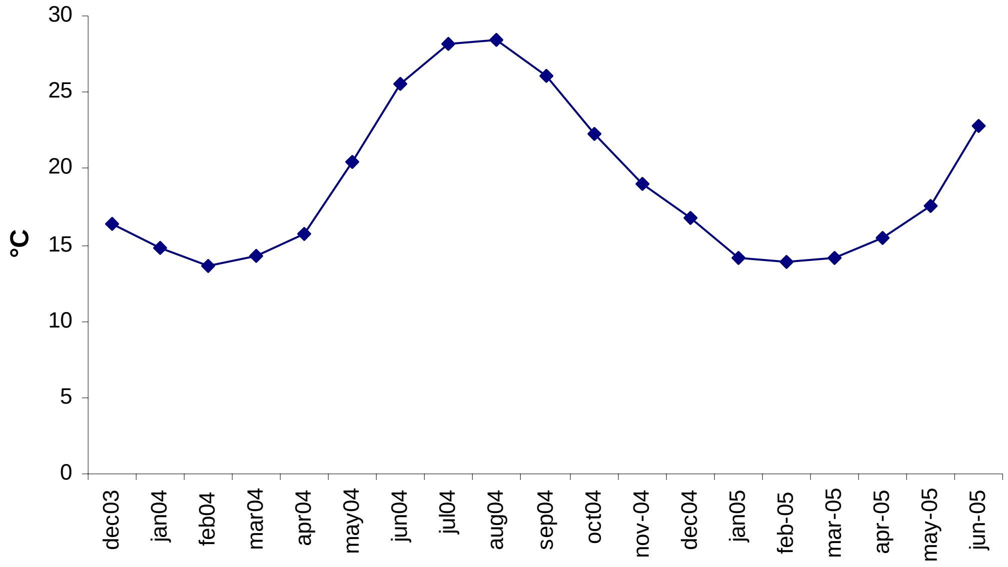

Figure 2-10. Monthly surface temperature derived from satellite imagery (*http://reason.gsfc.nasa.gov/Giovanni*) in the Gulf of Castellammare.

The BEST (BIO-ENV) routine showed a significant correlation (Spearman r=0,649; p<0,05) between suprabenthos samples and chlorophyll-*a* concentration 1 and 3 months before sampling.

2.3.5 Stable isotopes analysis

Fifteen taxa [3 mysid, 4 amphipods (and the whole taxa gammaridea in autumn), 1 cumacean (and the whole *taxon* Cumacea in autumn), 3 decapods and 1 fish] and copepods were analyzed on an annual basis for stable carbon (δ^{13}C) and nitrogen (δ^{15}N) isotopes (Table 2-1). 9 species (3 amphipods, 3 decapods, 2 mysids and 1 cumacean) and copepods were analyzed on a seasonal basis (table 2-1, species marked with ^{a}).

The ranges of δ^{15}N and δ^{13}C isotope ratios for each species are presented in Figure 2-11 and Figure 2-12 respectively.

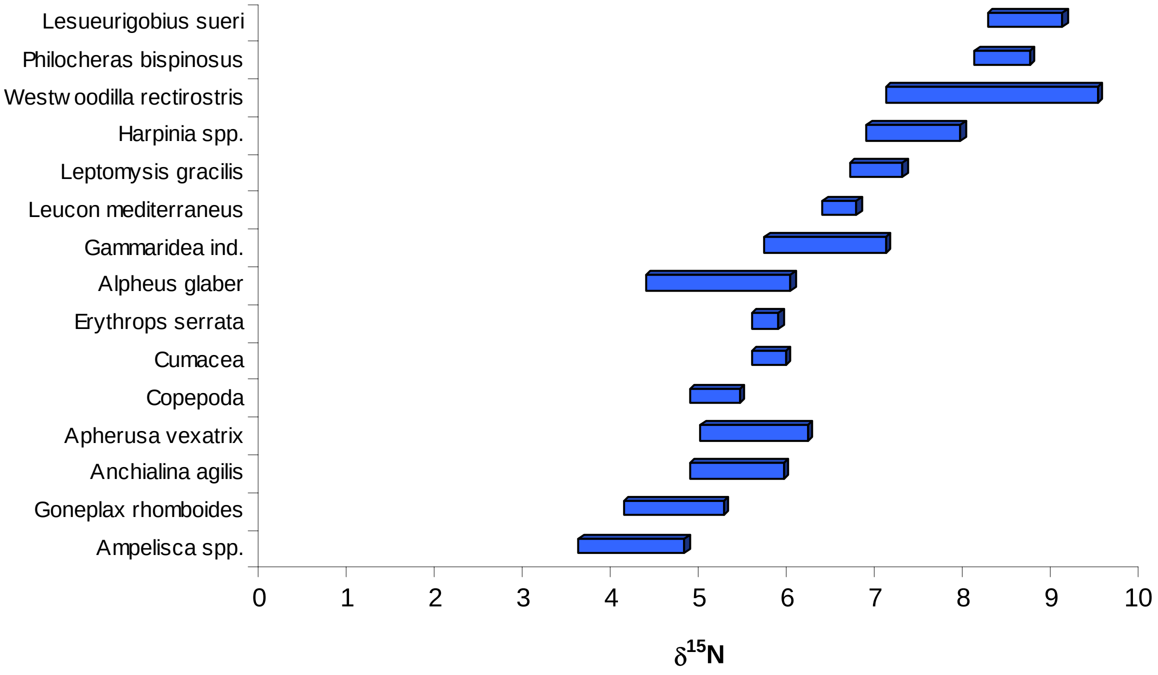

Fig. 2-11 Range of δ^{15}N values (‰) (mean ± standard deviation) for suprabenthic species

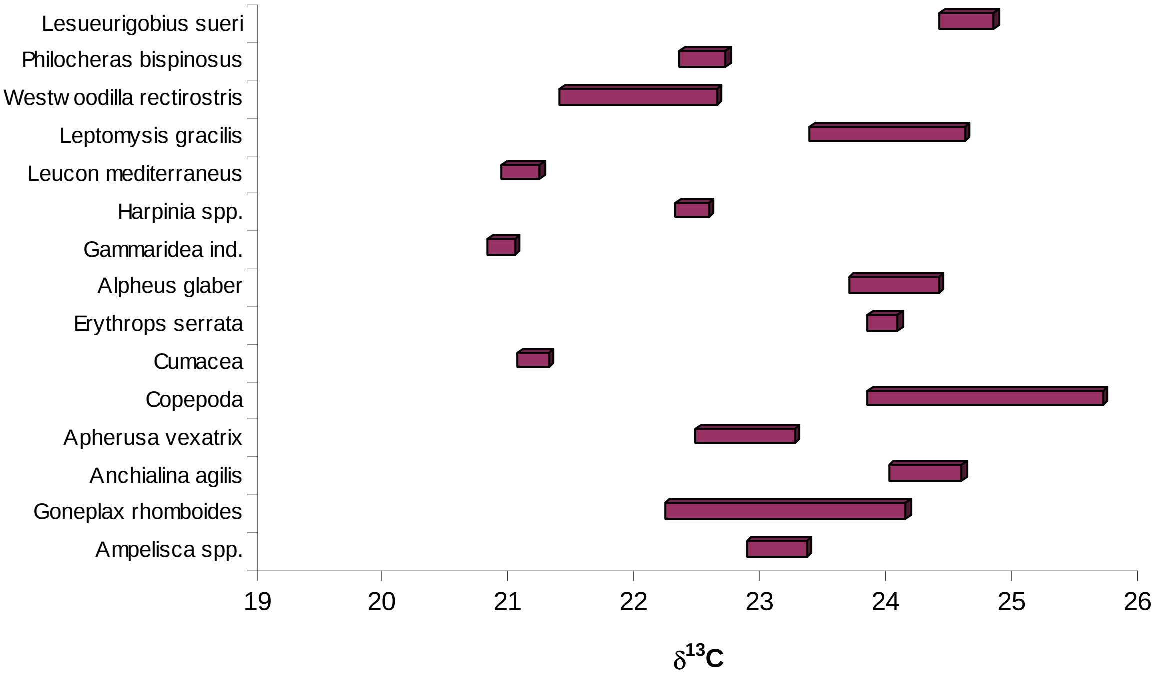

Fig. 2-12 Range of δ^{13}C values (‰) (mean ± standard deviation) for suprabenthic species (all the values were negative).

Our isotopic analysis revealed a considerable range of δ^{13}C and δ^{15}N values for suprabenthic species. Low overlap for δ^{15}N and δ^{13}C values was observed, though δ^{15}N values were less variable than δ^{13}C.

δ^{13}C values of suprabenthos taxa ranged from -24,7‰ (*Leptomysis gracilis* in summer 2005) to -20,1‰ (*Goneplax rhomboides* in Spring 2005). Stable nitrogen isotope (δ^{15}N) ranged from 2,5‰ (*Ampelisca* spp. in summer 2005) to 8,9‰ (*Lesueurigobius suerii* in winter 2005). Assuming a trophic enrichment of 3‰ (Post 2002), the overall range of δ^{15}N is indicative of three main trophic levels.

The wide range of isotopic values obtained is in accordance with many different trophic strategies, and corresponded quite well with the trophic classification based on gut contents analysis-isotopic analyses (data from the literature, Table 2-1). Thus, species with more enriched δ^{15}N values are carnivorous and scavenger species (e.g. the decapod *Philocheras bispinosus* and the fish *Lesueurigobius sueri*), while low δ^{15}N values identify mainly filter-feeders (more closely associated to plankton) (e.g. mysids *Anchialina agilis* and *Ampelisca* spp.) or deposit-feeders (some amphipods such as *Apherusa vexatrix* or *Ampelisca* spp.). The δ^{13}C values were generally higher (less negative) for carnivorous species (e.g. *L. sueri*) than for deposit-feeders (e.g. cumaceans, *Harpinia* spp.).

Plotting the mean δ^{15}N and δ^{13}C values of suprabenthic species (fig. 2-13, see table 2.1 for species list), the highest δ^{15}N value was observed in winter (March 2005) and the lowest in Summer (June 2005). The most enriched δ^{13}C value of the bulk of suprabenthos was observed in spring and the most depleted in summer. Seasonal differences of δ^{15}N and δ^{13}C values were not significant (ANOVA p>0,05).

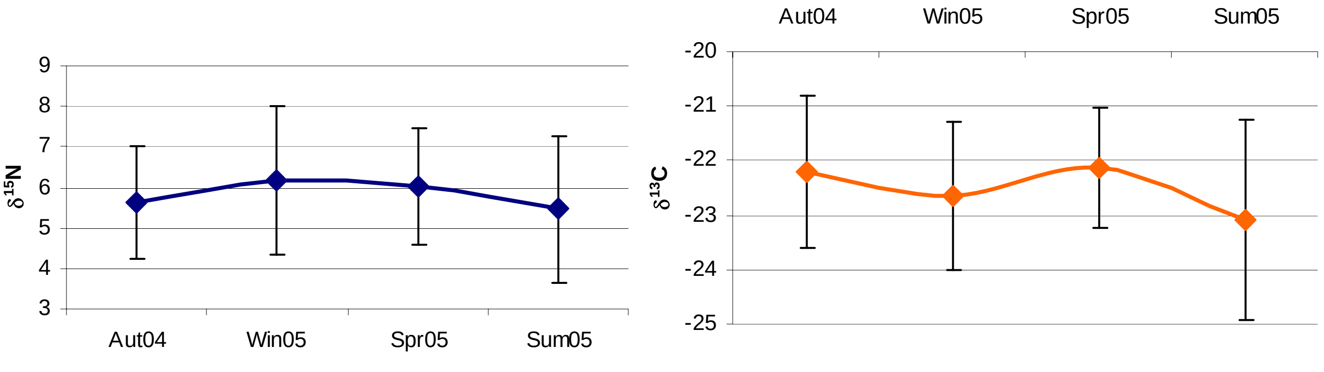

Fig. 2-13 Seasonal variation of mean δ^{15}N and δ^{13}C values of suprabenthic species

Seasonal variation of dominant prey of BBL fauna

Within suprabenthic community 8 species, dominant in terms of abundance, were analysed to detect seasonal variation in stable isotopes values: 3 amphipods (*Apherusa vexatrix, Ampelisca* spp. and *Harpinia* spp.), 2 mysids (*Leptomysis gracilis* and *Erythrops elegans*), 2 decapods (*Alpheus glaber* and *Philocheras bispinosus*), 1 cumacean (*Leucon mediterraneus*), and calanoid copepods. Moreover among epibenthic species the brachyuran crab *Goneplax rhomboides* was selected as one of the most abundant prey both for *Pagellus eryhtrinus* and *Arnoglossus laterna.*

Calanoid copepods showed significant differences observed both in δ^{15}N and δ^{13}C values throughout the year (fig. 2-14 and table 2-7). Highest δ^{15}N values were observed in the second part of he sampling period. The most enriched δ^{13}C values occurred in winter, while the most depleted in autumn.

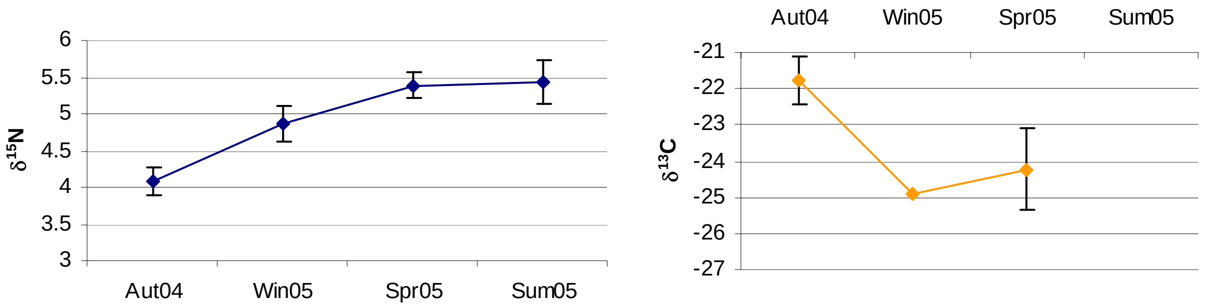

Fig. 2-14 Seasonal variation in δ^{15}N and δ^{13}C values (±SD) of Calanoid copepods. Acronyms as in table 2-1

Leptomysis gracilis showed slight seasonal variation throughout the sampling period (fig. 2-15), the lowest δ^{15}N value was observed in Autumn (6,2‰) and the highest in Summer (7‰). δ^{15}N differences were not significant. δ^{13}C values (fig. 2-15) were more enriched from autumn to spring, while the most depleted value was observed in summer. δ^{15}N and δ^{13}C differences were not significant. For *Erytrops elegans* only δ^{15}N values were available, a significant decrease was observed from winter to summer (see table 2-7).

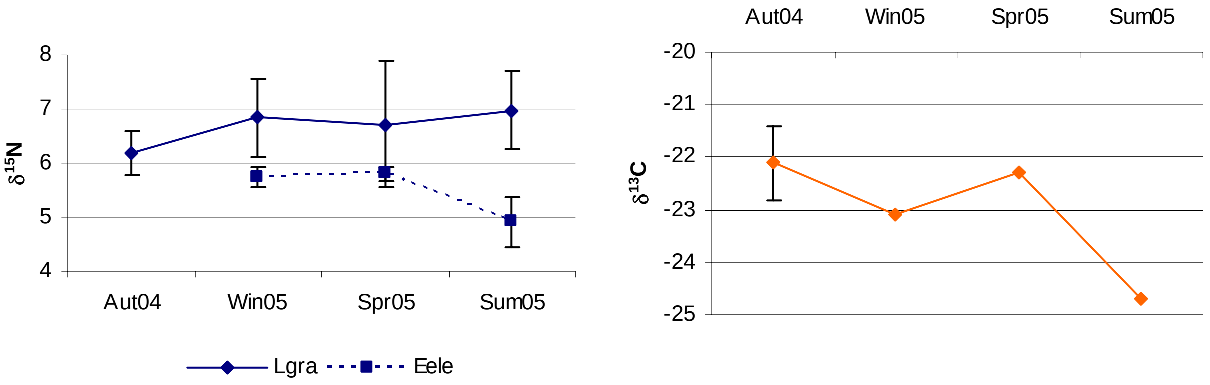

Fig. 2-15 Seasonal variation in δ^{15}N and δ^{13}C values (±SD) of *Leptomysis gracilis* and *Erytrops elegans.* Acronyms as in table 2-1

δ^{15}N values changed in *P. bispinosus* (fig. 2-16) with the highest values recorded in spring and the lowest in autumn (6,8‰ and 8,6‰ respectively); δ^{13}C values decreased from autumn to spring with the most enriched value of -21,9‰ observed in autumn and the most depleted in spring (-22,9‰). Differences were not significant. δ^{15}N and δ^{13}C values of *A glaber* did not changed significantly among seasons (fig. 2-16).

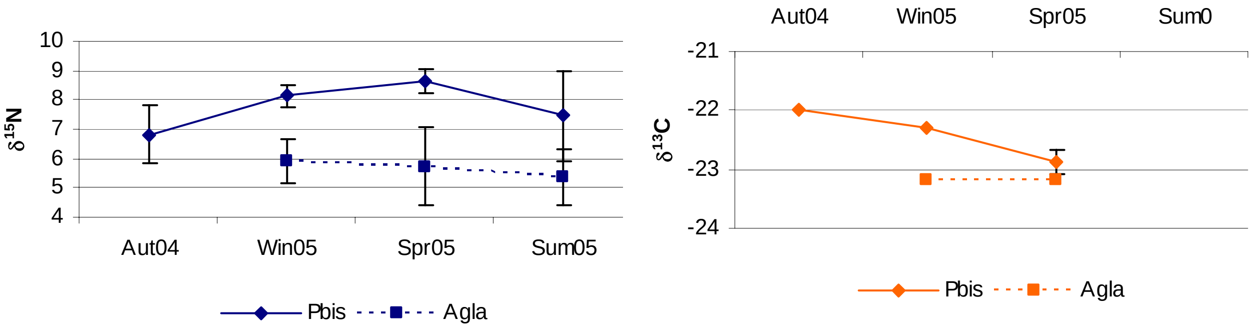

Fig. 2-16 Seasonal variation in δ^{15}N and δ^{13}C values (±SD) of *Philocheras bispinosus* and *Alpheus glaber*

A seasonal pattern was detected in δ^{13}C values of the cumacean *L. mediterraneus* (fig. 2-17). δ^{13}C values decreased (more depleted) from winter to summer. Differences were not significant for δ^{15}N, while it was not possible to test differences in δ^{13}C values because no replicates of winter and summer samples were available.

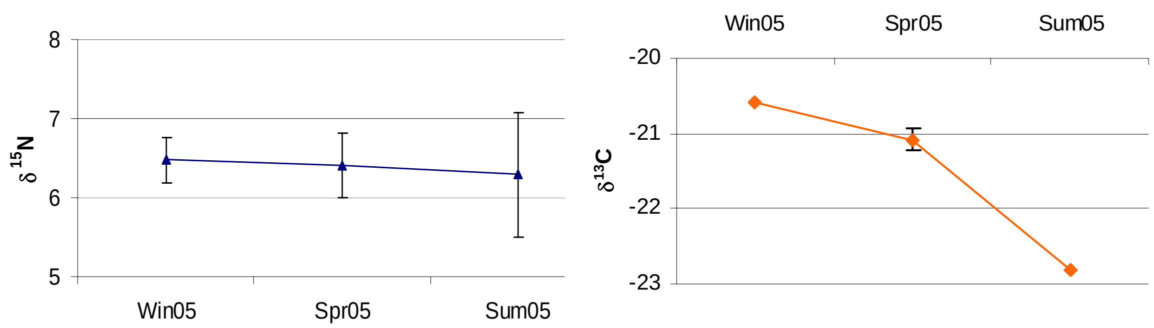

Fig. 2-17 Seasonal variation in δ^{15}N and δ^{13}C values (±SD) of *Leucon mediterraneus*.

Slight variations were observed in δ^{15}N values of the three taxa of gammarids *A. vexatrix*, *Ampelisca* spp and *Harpinia* spp (fig. 2-18). The most benthic taxa *Ampelisca* spp and *Harpinia* spp showed a similar pattern with the highest δ^{15}N values in winter and the lowest in summer, while the suprabenthic *Apherusa vexatrix* evidenced an increase in δ^{15}N value in summer. Differences were significant only for *Ampelisca* spp. (table 2-7).

δ^{13}C values markedly changed in *A. vexatrix* with the most enriched value in spring and the most depleted in summer; differences were significant (table 2-7). Slight but no significant changes were observed in *Ampelisca* spp and *Harpinia* spp δ^{13}C values.

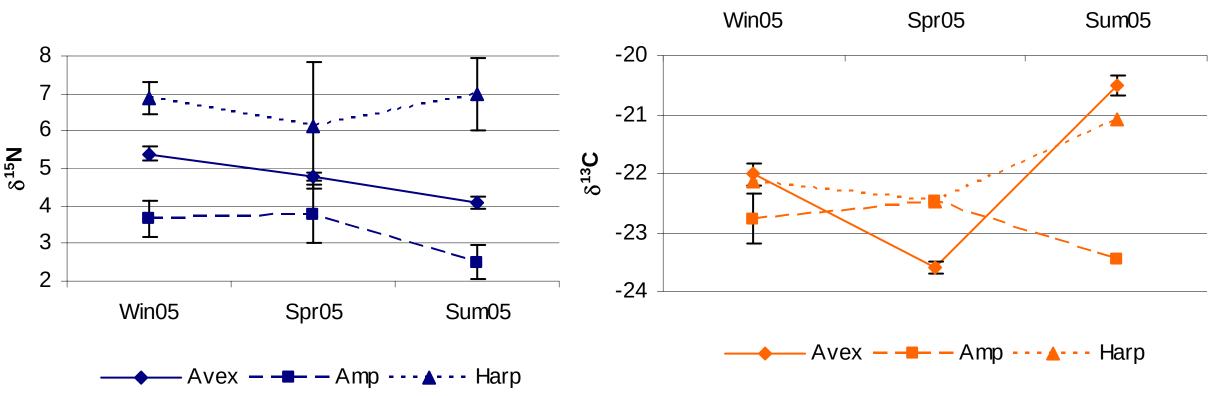

Fig. 2-18 Seasonal variation in δ^{15}N and δ^{13}C values (±SD) of *Apherusa vexatrix* (Avex), *Ampelisca* spp. (Amp) and *Harpinia* spp. (Harp)

Large differences in δ^{15}N and δ^{13}C values were observed for *G. rhomboides* (fig. 2-19). The highest δ^{15}N values was detected in November (7,4‰), with a decrease in February and April. Differences in δ^{15}N values were significant (table 2-7).

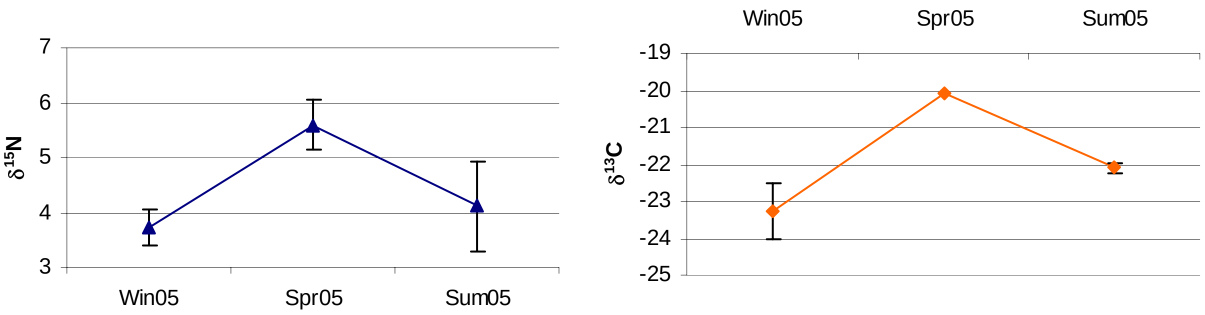

Fig. 2-19 Seasonal variation in δ^{15}N and δ^{13}C values (±SD) of *Goneplax rhomboides*

Table 2-7 ANOVA results for seasonal trends in δ^{15}N and δ^{13}C values of dominant suprabenthic species. Only significant relationships were reported: p<0,05=*; p<0,005=**; p<0,0005=***

Species	factors	*F*	*p*
E. serrata	season×δ^{15}N	7,78	*
Copepoda	season×δ^{15}C	8,95	*
Copepoda	season×δ^{15}N	28,9	***
A. vexatrix	season×δ^{13}C	30,76	*
Ampelisca spp.	season×δ^{15}N	5,89	*

Trophic level of BBL fauna

We estimated the trophic level (TL) of the BBL fauna (excepting those species where δ^{15}N values was lower than δ^{15}N of calanoid copepods) based on their δ^{15}N data and using copepods as reference material (table 2-8). δ^{15}N values were converted to trophic level based on the assumption that there was a fractionation of 3‰ per trophic level (Minagawa and Wada 1984; Post 2002) and that the base material (copepods) had a trophic level of 2:

$$TL_i=\left(\frac{\delta^{15}N_i-\delta^{15}N_{ref}}{3}\right)+2$$

where TL$_i$ is the trophic level of species *i*, $\delta^{15}N_i$ is the mean d15N of species $_{i,}$ and $\delta^{15}N_{ref}$ is the mean δ^{15}N of the copepods for the four seasons combined.

Table 2-8 Estimated TL of BBL fauna species

Taxon	TL
Copepoda	2
Cumacea	2,0
Erythrops serrata	2,2
Gammaridea	2,3
Westwoodilla rectirostris	2,4
Leucon mediterraneus	2,5
Harpinia spp.	2,6
Leptomysis gracilis	2,6
Lesueurigobius sueri	2,7
Philocheras bispinosus	2,9

Trophic web structure of BBL fauna

Plotting the annual average values of $\delta^{15}N$ and $\delta^{13}C$ of the whole BBL fauna community more than one source of carbon was evident. Three main trophic groups were evidenced by stable isotopes analysis (fig. 2-20): one group formed by filter feeders such as mysids (*Anchialina agilis* and *Erythtops serrata*) and copepods; a second group constituted by deposit feeders (i.e. some amphipods, *W. rectirostris* and cumaceans). A third group comprised species belonged to different trophic guilds (i.e. omnivores and also some deposit and/or filter-feeders). *Ampelisca* spp. that comprised filter and deposit feeders species, belonged to the "intermediate" group, seemed to be closer to filter-feeders group than the other amphipods. A strong relationship (R=0,98) was detected between *L. sueri* and the values of filter feeders indicating a predation of the gobiid fish on this group. *Philocheras bispinosus*, an infaunal feeder, probably preyed on deposit feeder such as *Ampelisca* spp.

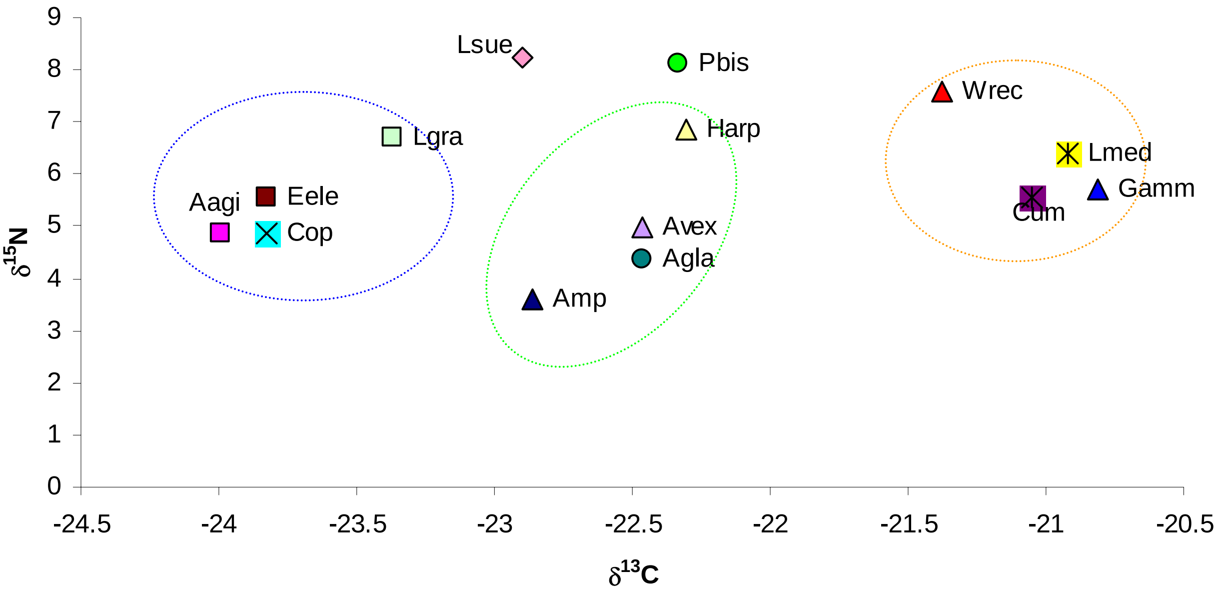

Fig. 2-20. $\delta^{15}N$ and $\delta^{13}C$ values of suprabenthos. Legend: squares= mysids; circles=decapods; triangles=amphipods; squares with cross=copepods; squares with asterisk=cumaceans. Blue circle identifies filter feeders, green circle indicates species belonged to different trophic guilds and red circle groups deposit feeders. Acronyms as in table 2-1).

2.4 Discussion

2.4.1 Fauna composition

The suprabenthos of the gulf of Castellammare consists almost entirely of crustaceans, dominated by mysids, amphipods, cumaceans and decapods. These taxonomic groups were also dominant in other suprabenthic communities from European coasts (Boysen, 1975; Buhl-Jensen, 1986; Buhl-Jensen and Fossa, 1991; Hesthagen, 1973; Kaartvedt 1985, 1989; Wang and Dauvin, 1984) and Mediterranean sea (Sorbe 1981, 1999, Cartes & Sorbe 1999, Cartes et al. 2001, Madurell & Cartes 2003).

The suprabenthic fauna recorded at the Gulf of Castellammare was diverse (86 species excluding decapod larvae); the richest group was gammaridean amphipods with 29 species, mysids and cumaceans accounting for 9 and 10 species respectively. This species richness was considerable high, comparing with Ligas (2005) findings in the Northern Adriatic continental shelf. The most abundant species during the whole period were *Apherusa vexatrix*, *Harpinia* spp. and *Westwoodilla rectirostris* among gammarids, *Erythrops elegans* and *Leptomysis gracilis* among mysids and *Dyastiloides serrata* and *Leucon mediterraneus* among cumaceans. *Philocheras bispinosus* was the most abundant decapod. A similar species dominance was observed by Ligas (2005) in the Northern Adriatic Sea, at similar depths.

2.4.2 Dynamics of BBL fauna

In open waters, temporal dynamics of meso and macrozooplankton have been the topic of a number of studies performed both in Mediterranean (Franqueville, 1971; Sabatés et al., 1989; Sardou et al. 1996), and in neighbouring areas (Bode and Alvarez-Ossorio, 2004). However, studies on the dynamics of suprabenthos (hyperbenthos, BBL macrofauna) assemblages are practically non existent in coastal environments, except in the surf-zones (Sorbe, 1981 and references cited therein) or in the estuaries (Azeteiro and Marques, 1999).

The observed pattern of biomass variability, with a peak in winter (March 2005) and a sharp decrease in autumn (November 2004) does not totally agree, at the level of the dynamics of dominant species, with previous studies performed at similar latitudes, not only on the slope (e.g. Bay of Biscay: Cartes et al., 2002), but also in muddy shelf bottoms (off Ebro Delta: Cartes et al, submitted; N Adriatic Sea: Ligas 2005).

In the coastal-BBL of the Gulf of Castellammare, the maximum densities of benthopelagic macrofauna were observed during winter (March 2005), one month after the peak of primary production recorded in the area, and also in summer (June 2005). These dynamics are probably related, among other factors, with the own population dynamics of suprabenthic species (e.g. mysids). The decrease of biomass in autumn is probably attributable to post-reproductive mortality of the spring-summer generation for most species, according for instance to the biology of mysids described by Mauchline (1980), also combined with a period of low productivity in the water column as deduced from studies on mass fluxes (e.g. Miquel et al, 1994) and satellite data in the western Mediterranean (Figure 2-7).

In other Mediterranean areas maximum zooplankton biomasses were recorded in May–June (Razouls and Kouwnberg, 1993) or in the early spring (Fernández de Puelles et al., 1995), and a maximum was also detected in April- May on the shelf-slope break in the Catalan Sea (Sabatés et al., 1989) associated with a swarm of gelatinous plankton. The peak of copepods biomass found in March in the Gulf of Castellammare was consistent with previous studies (Sardou et al, 1996) that found maximum values of zooplankton biomass in spring. The dominance of grazers observed in March 2005 (particularly small larvae of fish and decapods) could probably be a response to the peak of primary production (Chl *a* in surface) in February. This is accordance to the idea of zooplakton-phytoplankton coupling in periods of high productivity (e.g. April in Nival et al., 1975), when grazers have a limiting effect on the growth of phytoplankton.

A link between suprabenthos abundance and food supply has been documented and food sources of some suprabenthic species depend on the arrival of particulate organic matter throughout the water column (Cartes, 1998). Although data on seasonal availability of fresh OM (e.g. lipids, aminoacids) in sediments, were not taken in our study, in the Gulf of Castellammare, previous studies showed that the TSM (Total Suspended Matter) reaches the highest value (45 mg/l) in February (Mazzola, 1996). The POC (Particulate Organic Carbon) and PON (Particulate Organic Nitrogen) increased from autumn-winter and showed a maximum in spring (Mazzola, 1996). Concentration of lipids in POM was high below -15 m depth all over the year, with the highest values occurring in spring probably related with algal bloom phenomena (Mazzola, 1996). Moreover the relationship between POC and Chl *a* indicated that detritus was the only source of available resources, being the autotrophic contribution minimum; minimum values occurred during algal blooms (Mazzola, 1996). This information agrees with the maximum abundance of deposit feeders (e.g. amphipods, cumaceans and among decapods *A. glaber*) found in spring-summer.

Two different patterns were observed in our study: filter-feeders biomass (e.g. mysids, copepods, fish and decapods larvae) increased after the peak of surface Chl-*a* recorded in February, showing maximum values in winter, while deposit and infaunal feeders biomass (i.e. cumaceans, amphipods and some decapods) was higher in spring-summer corresponding to the highest values of POM.

2.4.3 Stable isotopes analysis

Our preliminary analysis using stable isotopes suggests a relatively complex food web in the gulf of Castellammare in which different food sources were observed. $\delta^{13}C$ was a good indicator of benthic and planktonic source of carbon because invertebrates associated with benthic food chains were relatively enriched with ^{13}C and easily distinguished from the plankton (Jennings et al., 1997). Some suprabenthic species (i.e. mysids and copepods) exhibited depleted values of $\delta^{13}C$, indicating a planktonic source of material for these species; cumaceans and amphipods displayed more enriched $\delta^{13}C$ values, confirming the detritivorous habits. A third group showed $\delta^{13}C$ intermediate values between the others two, and comprises species with different trophic guilds (e.g. *Ampelisca* spp., *Apherusa vexatrix*. and *Harpinia* spp.). The genus *Ampelisca* comprises deposit and suspension feeders that can contemporary use both the two trophic habits, depending on sediment type and/or on how far is its tube above the bottom (Scipione, 1989). The genus *Apherusa* comprises herbivorus and deposit feeders, that contemporary ingest organic detritus and vegetal remains.

Because no previous studies on isotopic composition of suprabenthic species in coastal environments exist, a comparison is not possible, although Darnaude et al. (2004) found more enriched $\delta^{13}C$ values for mysids off the Rhone river delta, due to the terrestrial enriched signature of POM in this area.

Recent revisions suggest that the fractionation from a consumer and its diet depends on trophic strategies, food biochemical compositions, and different metabolic pathways (Vander Zanden and Rasmussen, 2001; Vanderklift and Ponsard, 2003; McCutchan et al., 2003). Assuming a conservative trophic enrichment of *ca* 3.0 ‰ (Post, 2002) between consumers and their diet, at least three trophic levels were identified within the suprabenthic community of the gulf of Castellammare: the first was composed by deposit, herbivorous and suspension feeders (*Ampelisca* spp., *Apherusa vexatrix*, *Goneplax rhomboides*); the second mainly comprised filter-feeders (mysids and Copepods) and also deposit feeders who probably fed on enriched nitrogen detritus (cumaceans, *Harpinia* spp., Gammaridea). The third group was composed by two carnivores species the gobiid fish *L. sueri* and the infaunal-feeder *P. bispinosus*. The amphipod *W. rectirostris* exhibited a high variability in $\delta^{15}N$ signatures, with very high values in winter followed by an abrupt decrease in spring and summer, thus no clear pattern can be attributed to this species.

The suprabenthic fauna undergoes noticeable seasonal variations in abundance and biomass in the study area. Hence, the dynamics of peracarid assemblages showed that there was a peak of abundance and biomass in winter (March 2005) and in summer (June 2005). The first event was coupled to the peak of surface primary production recorded in February-March (Fig. 2-7) and regards mainly filter feeders. The second event could be parallel to the increase in organic matter in the sediment as observed in late spring-summer by Mazzola (1996) could be related to the increase of amphipod biomass. Seasonal differences in the isotopic composition of suprabenthic fauna may thus be a response to diet shifts associated with changes in the availability of different prey throughout the year. There is little information available on seasonal variability of isotopic composition in consumers and particularly for suprabenthos fauna.

In our case, suprabenthos taxa appear to have a different response to periods of low and high surface production (e.g. to the seasonal changes in food input). In general for all the species the highest $\delta^{15}N$ values were observed in spring-summer and the lowest in autumn; a different pattern was observed for $\delta^{13}C$ with more depleted values in summer and more enriched in autumn.

A similar variation in $\delta^{15}N$ and $\delta^{13}C$ values was observed for mixed crustacean zooplankton in an up-welling ecosystem off Galicia (Bode and Alvarez-Ossorio, 2004). Bode and Alvarez-Ossorio (2004) suggested that the increase of $\delta^{15}N$ values, found during the spring, coinciding with a decrease of surface nitrate concentrations. $\delta^{15}N$ signatures of zooplankton are frequently influenced by changes in nitrogen cycling (Leggett et al. 2000; O'Reilly et al. 2002).

The gulf of Castellammare, because of the particular chemical and physical characteristics of its waters, is described as a pelagic environment, due to the occurrence of different water masses as Atlantic waters, Tyrrhenic surface waters and intermediate layer waters (Mazzola, 1996). Thus the pattern described for pelagic ecosystems can be extended to our area.

Moreover a similar variation in $\delta^{15}N$ and $\delta^{13}C$ values was observed in freshwater system (Grey et al., 2001) and attributed to the utilization of microbial fraction rather than the assimilation the POM *per se* by zooplankton. Grey et al. (2001) found that, during spring, the zooplankton community comprised mainly nauplii, mixed copepod and copepodites, so a large proportion of the assemblage consisted of rapidly growing and moulting organisms. The moults would provide an ideal substrate for bacterial production and associated protozoan grazers, and coprophagy of relatively $\delta^{15}N$-rich fecal pellets has been suggested in Lake Baikal (Yoshii et al. 1999).

Changes in the isotopic composition of consumers can occur both as a result of growth and rate of tissue turnover (Tieszen et al., 1983; Phillips and Eldridge, 2006) however, juveniles and small individuals were not analyzed in this study. The potential sources of the temporal variability for consumers are then attributed mainly to the temporal changes in the isotopic values among food sources (Minagawa and Wada, 1984), though they can also be related to feeding behaviour, lipid accumulation and habitat selection (del Giorgio and France, 1996).

Madurell et al. (submitted) observed that is possible that changes in suprabenthos $\delta^{13}C$ reflect seasonally correlated changes in lipid concentration. Seasonal changes in body composition (C:N) can significantly affect the $\delta^{13}C$ signatures of suprabenthos (Matthews and Mazumder, 2005). As lipids are primarily from diet origin, accumulation of dietary lipids that have a low $\delta^{13}C$, coupled to increasing Chl *a* levels from November to March, suggest that the suprabenthos species store food reserves during the more productive periods. This stored energy is probably channelled into reproduction and growth. Synchronization between juveniles release and maximum food availability derived from spring phytoplankton_blooms has been recently reported in hyperbenthic peracarids (e.g. *Mysis mixta* and *Acanthostepheia malmgreni*) off Newfoundland (Richoux et al., 2004a, b). Overall, the enriched $\delta^{13}C$ values observed in Autumn, can also due to the high contribution of microphytobenthos, macrophytobenthos, and drifting macroalgae and seagrass fragments, as source of material (i.e. phytodetritus) that probably occur after summer.

Annex 2-1. List of the species found in the four season explored (N/100m^2), based n the average abundance per season. *Anapagurus* spp. include *A. petiti*, *A. laevis* and *Anapagurus* sp.; *Liocarcinus* spp. include *L. maculatus*, *L. depurator* and *L. pusillus*; *Ampelisca* spp. include *A. typica*, *A. diadema* and *Ampelisca* sp.; *Harpinia* spp. include *H. dellavallei*, *H. truncata* and *Harpinia* sp., *Rocinela* spp. include *R. orientalis*, *R. dumerilii* and *R. granulosa*.

Taxa	**Autumn**	**Winter**	**Spring**	**Summer**
CNIDARIA				
Cephalophyes appendiculata	-	-	0,36	1,94
CRUSTACEA				
Copepoda				
Copepoda unid.	12,02	1353,91	78,17	125,53
Decapoda				
larvae				
Decapoda larvae	-	15,50	40,10	-
Natantia larvae	-	0,65	-	-
adult				
Alpheus glaber	0,84	3,09	2,91	1,60
Athanas nitescens	-	0,53	-	0,16
Calocaris macandrae	0,19	-	-	-
Philocheras bispinosus	3,35	15,20	7,14	2,79
Parapenaeus longirostris	0,19	1,71	0,33	1,31
Processa sp.	0,88	6,20	-	-
Crangonidae juveniles unid.	0,50	-	0,73	0,70
Natantia juveniles unid.	-	21,90	-	3,31
Anapagurus spp.	-	2,47	0,75	1,01
Ebalia tuberosa	0,14	0,85	0,71	0,81
Galathea intermedia	0,17	4,68	0,40	1,03
Goneplax rhomboides	-	6,83	4,78	7,23
Macropodia rostrata	0,19	1,07	0,19	0,48
Liocarcinus spp.	-	1,67	0,20	0,00
Periclimenes scriptus	1,63	6,82	0,60	2,64
Pilumnus spinifer	-	0,33	-	-
Inachinae unid.	-	-	0,20	0,17
Majidae unid.	-	-	0,20	-
Paguridae unid.	5,31	-	-	0,16
Brachyura unid.	0,27	0,47	-	0,34
Decapoda unid.	1,45	-	-	-
Amphipoda Gammaridea				
Acidostoma sarsi	0,52	-	-	0,12
Ampelisca spp	3,42	9,32	6,12	14,98
Apherusa vexatrix	14,84	39,06	25,04	54,00
Corophium spp	-	1,00	0,35	0,48
Eriopisa elongata	0,40	0,75	0,16	0,12
Eusirus longipes	-	-	-	0,53
Gammaropsis maculatus	-	-	-	0,34
Harpinia spp	12,48	43,91	44,80	122,83
Hippomedon bidentatus	0,52	0,53	-	2,09
Iphimedia minuta	-	0,47	0,33	0,17
Leptocheirus mariae	-	-	0,99	-
Leucothoe lilleborgi	-	0,68	-	0,48
Liljeborgia dellavallei	-	-	0,51	-
Maera schmidti	0,74	0,93	0,99	1,17
Megamphopus cornutus	-	0,42	-	-
Monoculodes griseus	2,18	25,88	25,82	8,44
Orchomenella nana	-	-	0,35	0,12
Paraphoxus oculatus	1,33	6,80	7,88	9,74

Taxa	Autumn	Winter	Spring	Summer
Amphipoda Gammaridea				
Perioculodes longimanus	0,74	13,82	13,54	10,12
Photis longicaudata	-	-	0,16	-
Rhacotropis integricauda	0,88	5,18	2,42	0,87
Scopelocheirus hopei	0,33	-	-	-
Stenothoe sp.	-	-	0,18	0,17
Synchelidium maculatum	-	5,50	5,56	0,16
Tryphosites longipes	-	1,53	1,02	1,22
Trischizostoma nicanse	-	0,36	0,60	-
Westwodilla rectirostris	2,98	26,56	49,37	14,81
Amphilochidae	-	-	-	0,34
Calliopidae unid.	-	-	-	0,17
Lyssianassidae unid.	1,31	0,11	0,66	-
Oedicerotidae unid.	-	-	0,35	-
Phoxocephalidae unid.	0,33	-	-	-
Stegocephalidae unid.	-	-	0,40	-
Gammaridea unid.	17,03	29,80	33,54	43,18
Amphipoda Caprellidea				
Phtisica marina	-	4,08	1,93	1,31
Caprellidae unid.	-	-	0,55	-
Amphipoda Hyperiidea				
Euprimno macropus	-	0,79	-	-
Hyperia latissima	0,22	-	-	-
Hyperia schizogeneios	2,73	0,53	0,55	-
Mysidacea				
Acanthomysis longicornis	-	18,66	11,78	17,17
Anchialina agilis	54,90	8,86	0,92	0,91
Erythrops elegans	2,78	91,45	147,43	12,70
Haplostylus normani	0,68	9,35	1,60	0,64
Leptomysis gracilis	8,76	24,38	27,07	33,99
Mysidopsis angusta	-	2,01	4,13	0,52
Mysidopsis gibbosa	0,44	5,54	1,54	0,35
Paramblyops sp.	-	0,14	-	-
Siriella norvegica	-	-	-	0,17
Mysidacea unid.	4,31	33,28	7,23	14,62
Isopoda				
Aega gracilipes	-	0,53	-	-
Gnathia sp. Larvae	3,77	33,23	19,53	23,39
Rocinela spp.	1,26	1,48	2,72	0,78
Tanaidacea				
Apseudes latreilli	0,75	4,55	9,56	15,92
Apseudes spinosus	0,33	4,13	2,57	10,45
Leptognathia sp.	-	-	0,87	4,72
Leptochelia savinyi	-	0,17	-	1,04
Pseudoparatanais sp.	-	0,53	1,79	3,96
Cumacea				
Bodotria gibba	0,19	-	-	-
Campylaspis glabra	-	0,65	1,63	1,44
Dyastilis doriphora	0,14	9,54	5,46	2,76
Dyastilis rugosa	-	-	0,35	-
Dyastiloides serrata	3,89	13,46	51,25	19,05
Dyastloides bacescoi	-	-	0,20	-
Leucon affinis	0,19	5,61	9,48	2,95
Leucon longirostris	-	-	0,16	-

Taxa	**Autumn**	**Winter**	**Spring**	**Summer**
Cumacea				
Leucon mediterraneus	0,75	17,22	21,77	13,93
Leucon siphonatus	1,44	4,92	3,88	8,85
Cumacea ind.	1,95	5,57	23,80	6,72
Euphausiacea	-	-	0,16	-
Nicthyphanes couchi	-	207,21	-	-
Pycnogonida	-	0,74	0,58	-
MOLLUSCA				
Cephalopoda				
Sepiola sp.	-	-	-	0,35
CHAETOGNATHA	-	-	-	26,11
SIPUNCULIDA	-	16,45	14,46	39,10
Osteychthyes				
fish larvae	-	3,42	18,29	-
Arnoglossus laterna	0,09	0,71	0,53	1,00
Capros aper	-	0,11	-	-
Deltentosteus quadrimaculatus	0,56	-	-	-
Gaidropsaurus biscayensis	-	0,11	-	0,16
Gobius niger	-	0,53	0,18	0,17
Lesueurigobius friesi	-	0,11	-	-
Lesueurigobius sueri	-	1,38	0,53	1,87
Pomatochistus minutus	0,22	1,10	0,35	0,17
Serranus hepatus	-	-	-	0,52
Symphurus nigrescens	-	1,93	1,68	0,69

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Chapter 3

Trophic structure of suprabenthic communities in the Northern Sicily

Abstract

Dynamics of suprabenthos was analyzed in four areas of the Northern Sicily (Western Mediterranean) at depths ranging between 40 and 80 m. Six stations in each Gulf were sampled during one cruise performed in May-June 2005. Two of the four Gulfs (Castellammare and Patti) were declared *"fishing exclusion zones"* in 1990, when trawl fishery was banned, whilst the others two still are suffering from a strong fishing effort. The suprabenthic community was more diversified in the Gulf of Castellammare (77 taxa), but also in the Gulf of Termini Imerese and S. Agata, while a poor community occurred in Patti (40 taxa). Furthermore suprabenthos showed maximum biomass in the Gulf of Castellammare, while minimum biomass was found in the Gulf of Patti. nMDS analyses performed on suprabenthos abundance showed a sample aggregation as a function of area, but no protection effect was evident (3 groups corresponding to Castellammare, Patti and S. Agata-Termini). PERMANOVA routine also provided evidence for significant differences within sites (Gulfs), but not among protected and unprotected areas. Termini Imerese and S. Agata did not differ substantially among each other. In the whole area the most typifying species of suprabenthic assemblages were mysids (*Erythrops elegans* and *Leptomysis gracilis*), amphipods (*Westwoodilla rectirostris*, *Apherusa vexatrix* and *Harpinia* spp.) and decapods (*Philocheras bispinosus*). A significant correlation (Spearman *r*) was found between total suprabenthos biomass, peracarid, mysids and amphipods biomass and some environmental variables such as *Chla* on surface three, two and one months before sampling period and with the biopolimeric fractions of carbon in the sediment organic matter (e.g. lipids, proteins and carbohydrates) suggesting that suprabenthic fauna relies on trophic resources other than those derived directly from the water column. Our preliminary analysis using stable isotopes suggests a relatively complex food web in the whole investigated area in which different food sources were observed. Some suprabenthic species (i.e. mysids and copepods) exhibited depleted values of $\delta^{13}C$, indicated a planktonic source of material; cumaceans and amphipods displayed more enriched $\delta^{13}C$ values, confirming this detritivorous habits. A third group with intermediate $\delta^{13}C$ values comprised species with different trophic guilds (e.g. *Ampelisca* spp., *Apherusa vexatrix*. and *Harpinia* spp.).

Assuming a conservative trophic enrichment of *ca* 3.0 ‰ between consumers and their diet, at least two main trophic levels were identified within the suprabenthic community of the four

Gulfs: 1) comprised carnivorous species such as the gobiid fish *Lesuerigobius sueri* and the infaunal-feeder *Philocheras bispinosus*; 2) included deposit-feeders, but it can be divided into two sub-groups, the first composed by deposit-feeders and herbivorous (*Ampelisca* spp., *Apherusa vexatrix*, *Goneplax rhomboides*), whilst the second mainly comprised filter-feeders (mysids and Copepods) and also deposit-feeders who probably fed on enriched nitrogen detritus (cumaceans, *Harpinia* spp., Gammaridea). Local changes in isotopic signatures was observed, with highest, but not signicant, δ^{15}N values observed in the Gulf of Castellammare. As a conclusion, spatial differences observed both in suprabenthic biomass, assemblages structure and stable isotopes signatures may be mainly due to natural variability (i.e. higher rivers contribution, higher values of primary production) than to the protection measure.

3.1 Introduction

Trawl-fishing have several effects on marine ecosystem: they include changes in predator-prey relationships leading to shifts in food-web structure that are not necessarily reversed by the reduction of fishing pressure (alternative stable states; Kaiser et al., 2002); effects on abundance and body-size distributions that can result in a fauna dominated by small-size individuals (Gislason & Rice 1998, Jennings et al. 2001; Kaiser et al. 2002; Hermsen et al. 2003, Daan et al. 2005); genetic selection for different physical characteristics and reproductive traits (e.g. higher growth rate, earlier age-at-maturity, etc.) (Fromentin & Fonteneau 2001); effects on population of non-target species (e.g. cetaceans, sea birds, sea turtles, elasmobranches, etc.), as a result of by-catches (Hall 1999, Kaiser & De Groot 2000, Pranovi et al. 2001); re-suspension of superficial sediments (Palanques et al. 2001, Smith et al. 2003), reduction of habitat complexity and alteration of benthic community structure (Turner et al. 1999; Kaiser et al. 2000b, Callaway et al. 2002, Kaiser et al. 2002). The last two effects can also affect suprabenthic communities.

The northern Sicilian continental shelf was subject to an intense fishing effort, as suggested by the results of experimental trawl surveys (Arena and Bombace, 1970; Arculeo et al., 1988) and landing data (Arculeo et al., 1990). In 1990 the Sicilian Regional Government created two “fishery reserves” in the Gulfs of Castellammare, Patti and Catania with Regional Act No. 25/90 whereby bottom trawling was banned, due to its heavy impact on demersal stocks and on the substrate (Dayton et al., 1995; Auster et al., 1996). After four years the closure to fishing in the Gulf of Castellammare, Pipitone et al. (1990) found a dramatic increase in biomass. Although, a biomass increase resulting from a partial or total fishing ban is to be expected (e.g., Dugan and Davis, 1993) and has been recorded also for crustacean species (Yamasaki and Kuwahara, 1989; Roa and Bahamonde, 1993), the increases observed in Castellammare far exceed those reported in the scientific literature for areas closed for similar or longer periods of time.

Because suprabenthic species contribute substantially to the diet of demersal fish (Mauchline, 1980; Sorbe, 1981) and shrimps (Sitts and Knight, 1979), the spatial variation of suprabenthic communities in fishing exclusion zones (e.g. Castellammare and Patti) and fished areas (Termini and S. Agata) can contribute to understand the trophodynamics of these regions. In addition suprabenthic species successfully exploit the diversity of food resources and are an important link in the detritus-based food chains (Mees et al., 1995), thus the study of suprabenthos is fundamental to define the trophic web structure of demersal communities. In this context stable isotopes analyses were carried out on dominant suprabenthic species (see chapter 2 for details) in order to define the trophic position of BBL fauna in demersal food webs.

A relative high number of suprabenthic studies have been carried out in the infralittoral and circalittoral bottoms see Mees and Jones, 1997 for a review). Nevertheless, few studies described the suprabenthic communities inhabiting coastal muddy bottoms. In Mediterranean many studies focused on surf-zone or deep-sea environments (Cartes 1998; Cartes & Sorbe, 1999; San Vicente & Sorbe, 1999; Cartes *et al.*, 2001), only recently some studies stressed on coastal suprabenthic communities of Ebro and Po rivers delta (Cartes et al, submitted, Ligas, 2005). As far as the Tyrrhenian Sea is concerned, there is total absence of information on suprabenthic communities. In this paper, the distribution of the suprabenthos in four areas of the coastal muddy bottoms assemblages of Northern Sicily was studied.

Community parameters such as species richness, abundance, biomass and diversity were calculated. Such a descriptive baseline study is a first step in the attempt to clarify the role of the suprabenthos in the coastal muddy bottoms assemblages.

Thus the aims of this chapter are:

1) to describe the qualitative and quantitative structure of the suprabenthic communities of four Gulfs of Northern Sicily;
2) to define the trophic structure of surabenthic communities by means of stable isotopes analysis;
3) to analyze spatial changes in suprabenthos biomass and assemblages structure;
4) to evaluate spatial variation in the source of carbon (by means of ^{13}C) and in the trophic levels (^{15}N) of suprabenthic dominant taxa;
5) to assess if spatial differences were related to protection effect or to natural variability of the environment.

Data on stable isotopic signature of suprabenthic species were then coupled with stable isotopes data on fish, as predator of suprabenthos resources to describe the trophic web structure of demersal communities in Northern Sicily (see chapter 4 for further details).

3.2 Materials and methods

For a detailed description of study areas and sampling methodology see chapter 1 and chapter 2.

3.2.1 Data collection

Suprabenthos

A total of six samples of suprabenthos for each area were collected during one surveys carried out in June 2005 at depths from 40 to 80 m on muddy bottoms (VTC, *sensu* Peres and Picard, 1964), in four Gulfs of Northern Sicily (Castellammare, Termini Imerese, S. Agata di Militello and Patti, see fig.1-1, chapter 1) . The sampling methodology to collect, preserve and analyze suprabenthic fauna is exposed in chapter 1. Data was standardized (abundance - ind/100 m^2; wet weight - g/100 m^2) for each haul performed.

Environmetal variables

Environmental variables such as Chlorophyll *a* and surface temperature were obtained by satellite imagery at: *http://reason.gsfc.nasa.gov/OPS/Giovanni/.* Data on sediment organic matter (SOM) regarding May 2005 were courteously obtained by "Laboratorio di Biologia Marina e Risorse, Dipartimento di Biologia Animale, Università di Palermo". The sediment organic matter is constituted by refractory and instable organic compounds. The instable fraction of SOM is composed by proteins, nucleic acids, which, together with carbohydrates and lipids can be considered the organic carbon fraction easily available by benthic consumers (Dell'Anno et al., 2000). Data available include values on equivalent chloroplastic pigments (CPE) as the sum of Chl-*a* and phaeopygments concentration (Pfannkuche, 1993). In addition each compound (lipids, carbohydrates and proteins) of SOM was estimated. The values of protein (PRT) and total carbohydrates (CHO) concentrations were integrate in order to calculate the trophic index PRT/CHO, that allows to evacuate the balance between the total carbohydrates and proteins concentrations. This index describes the property and the nature of trophic resources (Danovaro *et al.*, 2000; Fabiano *et al.*, 2001).

3.2.2 Data processing

Data treatment.

Data on abundance of fauna collected was analyzed by multivariate techniques. We performed analyses for suprabenthos including 11 taxonomic groups: Decapoda Natantia, Mysidacea, Amphipoda Gammaridea and Hyperiidea, Isopoda, Cumacea, Copepoda, Pycnogonida, Cephalopoda, Chaetognatha, and Osteychthyes. Some gelatinous zooplankton groups (salps, cnidarians) were excluded from some analyses due to difficulties in their sorting and quantification. Species/taxa appearing less than twice in all samples were removed from data matrices. Some taxa, poorly represented in sledge samples belonging to the (non-swimming) epifauna-infauna (Polychaeta, Amphipoda Caprellidea, Tanaidacea, and Ophiuroidea) were also removed from the data matrix.

Statistical differences in main taxa abundance as a function of area were established by applying a chi-squared test (Yates' correction) to the numerically dominant group. One-way ANOVAs were used to compare differences among biomass of main taxa for areas. Data were square root transformed to remove the dependency of the variance on the mean (Zar, 1984) and to normalize and homogenize the variance of data (Underwood, 1997). The assumption of normality was tested using the Kolmogorov–Smirnov test and the assumption of homogeneity was tested by Cochran test. All analysis inferences were based on the 0,05 significance level.

Diversity indices (Species richness, Shannon-Wiener, Simpson and Pielou's eveness indices) were calculated to provide information on changes in suprabenthic species among seasons. An nMDS was first performed on Bray Curtis similarity of 4th root transformed abundance data (Clarke and Warwick, 1995).

Based on the null hypothesis that no differences exist among protected and unprotected areas and secondarily among areas a PERMANOVA (Anderson, 2001) design was created based on two factors (protection with 2 levels and area, nested in protection with 4 levels). PERMDISP routine (Distance-based test for homogeneity of multivariate dispersions) was the performed on significant factors evidenced by PERMANOVA. Finally the SIMPER routine was perfomed in order to identify those species that most typify each assemblages.

In order to establish possible relationships between assemblage's dynamics/structure and existing environmental gradients, we tested possible relationships (correlations) between the abundance of suprabenthos and some environmental variables (e.g. temperature, surface Chl *a* and Chl *a*, phaeopigments, biopolimeric carbon fraction in sediments) by the BIOENV procedure, based on non parametric Spearman rank correlations..

In addition, in order to correlate the biomass data of total suprabenthos, peracarids, amphipods and mysids with environmental variables available a non parametric Spearman correlation was performed with STATISTICA 6. All the univariate and multivariate analyses were carried out using the software packages PRIMER 6 plus PERMANOVA (Clarke and Warwick, 2001; Anderson, 2006) and STATISTICA 6.

3.2.3 Stable isotopes analyses

Once collected, samples were immediately frozen at -20^{o}C and sorted in the laboratory. All specimens were sorted as quick as possible, identified to species level and then they were freeze-dried and stored until their preparation for analyses. Species selected for isotopic analysis (only large sizes for each species) were those that were dominant in both abundance and biomass, hence reaching a whole view of variations in the suprabenthic assemblage.

For δ^{13}C analyses, samples were decalcified to remove inorganic carbonates from the exoskeleton (Jacob et al., 2005). A small quantity of 1M hydrochloric acid (HCl) was added to the samples and left for 3 hours. The samples were then dried at 60^{o}C for 12 h and ground to a fine powder using a pestle and a mortar. Composite samples (Table 3-1) were analysed for all suprabenthic species to minimise infra-individual differences in the isotopic composition and, to meet the minimum mass requirement for the isotopic analyses. For all the species four replicates were performed, when it was possible one replicate was represented by one individuals, in order to reduce pseudo-replication (Hulbert, 1984), when the biomass was not enough more specimens were pooled together to obtain sufficient mass for the isotope measurement (as the case of Calanoid copepods, Cumaceans and small amphipods).

Table 3-1. Species analyzed for stable carbon (δ^{13}C) and nitrogen (δ^{15}N) isotopes. Legend: AMP (Amphipods), COP (Copepods), CUM (Cumaceans), DEC (Decapods), ISO (Isopods), MYS (Mysids).

Group	Acronym	Species	CDG	TI	SA	PA
AMP	Ampe	*Ampelisca* spp.	*	*	*	
AMP	Apher	*Apherusa vexatrix*	*			
AMP	Harp	*Harpinia* spp.	*	*	*	*
AMP	Wrec	*Westwoodilla rectirostris*	*	*	*	*
CUM	Cum	Cumacea				*
CUM	Lmed	*Leucon mediterraneus*	*	*	*	
COP	Cop	Copepoda	*	*	*	*
FISH	Lsue	*Lesuerigobius sueri*		*	*	
MYS	Lgra	*Leptomysis gracilis*	*	*	*	*
MYS	Eser	*Erythrops serrata*	*	*	*	
DEC	Pbis	*Philocheras bispinosus*	*	*	*	*
DEC	Agla	*Alpheus glaber*	*	*	*	*
DEC	Grho	*Goneplax rhomboides*	*	*	*	*
MYS	Aagi	*Anchialina agilis*		*	*	

Based on the information on its diet, when it was available, the species were classified into feeding types (Table 3-2). Correlations between δ^{13}C and δ^{15}N were calculated for each sampling period. The post-hoc Tukey test was used to compare isotopic signatures within suprabenthic and zooplanktonic species (Sokal & Rohlf 1995). Spatial variations in δ^{13}C and

$\delta^{15}N$ values were analyzed only for species where data from the four areas were available. Correlations between $\delta^{13}C$ and $\delta^{15}N$ were calculated for the bulk of BBL fauna for each area.

Table 3-2 Feeding habits and type of prey of suprabenthos and zooplankton fauna based on literature data AMPH (Amphipods), COP (Copepods), CUM (Cumaceans), DEC (Decapods), MYS (Mysids)

Group	**Species**	**feeding habits**	**prey**	**source**
AMP	*Ampelisca* spp.	deposit-feeder	detritus	Franz, Tanacredi 1992
AMP	*Apherusa* spp.	Detritivorous (herbivorous)	phytoplankton-detritus	Portland 2001
AMP	*Harpinia* spp.	deposit-feeder		
AMP	*Westwoodilla rectirostris*	deposit-feeder		Beare, 2004
CUM	Cumacea			
CUM	*Leucon mediterraneus*	deposit-feeder		Cartes et al., 2003
COP	Copepoda	filter-feeder	phytoplankton-detritus…	
FISH	*Lesuerigobius sueri*	carnivore	zoobenthos	Costa, 1991
MYS	*Leptomysis gracilis*	Omnivore	phytoplankton-zooplankton	Maucline, 1980
MYS	*Erythrops sp*	Carnivore-deposit feeder	Copepods, hyperiids, detritus	Cartes and Sorbe 1998
DEC	*Philocheras bispinosus*	Infaunal feeder		Cartes et al. 2002
DEC	*Alpheus glaber*	Deposit feeder		Cartes et al. 2002
DEC	*Goneplax rhomboides*	Infaunal feeder		Cartes et al. 2002
MYS	*Anchialina agilis*			
MYS	*Acanthomysis longicornis*			

For stable isotopy only few dominant species were selected for analyses.

3.3 Results

3.3.1 Species composition

A total of 109 (Annex 3-1) taxa were identified in May 2005 in the four areas. At genus and species level 17 decapods, 8 mysids, 27 amphipods, 1 caprellid, 1 hyperiid, 4 isopods, 4 tanaids, 14 cumaceans, 8 fish) were recorded.

In all the Gulfs the most abundant *taxa* were mysids, gammarids and cumaceans. Calanoid copepods, natantian decapods larvae and fish larvae were also abundant, but the further two taxa belong to merosuprabenthos. Decapods abundance was significantly higher in the Gulf of Castellammare than in the other three areas (χ^2=41,69; p<10^{-5} between Castellammare and Patti; χ^2=11,35; p<10^{-4} between Castellammare and Termini; χ^2=18,74; p<10^{-5} between Castellammare and S. Agata). Also decapods abundance was higher in Termini (χ^2=10,75; p<10^{-3}) and S. Agata (χ^2=5,35; p=0,02) than in Patti.

The abundance of mysids was very low in Patti (χ^2=38,28; p<10^{-5} CDG-PA; χ^2=21,97; p<10^{-5} TI-PA; χ^2=31,17; p<10^{-5} SA-PA). The mean number of cumaceans was significantly higher in Castllammare than in the other three Gulfs (χ^2=118,96; p<10^{-5} CDG-PA; χ^2=105,46; p<10^{-5} CGD-TI; χ^2=102,29; p<10^{-5} CDG-SA). The abundance of amphipods was significantly higher in Castellammare than in the other three Gulfs (χ^2=53,4; p<10^{-5} CDG-PA; χ^2=36,95; p<10^{-5} CGD-TI; χ^2=21,42; p<10^{-5} CDG-SA).

At a species level, the decapod *Philocheras bispinosus* was the most abundant in the whole sampled area, while the epibenthic brachyuran *Goneplax rhomboides* was dominant in the Gulf of Castellammare. Among amphipods *Apherusa vexatrix* was more abundant in Castellammare and Patti; *Harpinia* spp. in Castellammare and S. Agata and *Westwoodilla rectirostris* in the whole area except Patti. The isopod *Gnathia* spp. and the cumaceans *Diastyloides serrata* and *Leucon mediterraneus* were abundant only in the Gulf of Castellammare. Among mysids, *Leptomysis gracilis* was more abundant in the Gulfs of Termini and S. Agata, while *Erythrops elegans* was more abundant in Castellammare.

The Gulf of Castellammare hosted the more diverse suprabenthic assemblage (table 3-3) with a total of 77 taxa collected and 70 species, while only 40 taxa were recorded in the Gulf of Patti (H'=1,96).

Table 3-3. Mean number of species (S), mean number of individuals (N), Species richness (d), Eveness (J'), diversity (H') and dominance (1-λ') for suprabenthic species in May 2005. Standard deviation (SD) is also given.

Area	S	SD	N	SD	d	SD	J'	SD	H'(loge)	SD	1-λ'	SD
CDG	36,50	2,07	63,67	5,39	8,55	0,43	0,98	0,00	3,53	0,05	0,98	0,00
PA	14,83	3,25	22,00	4,29	4,46	0,80	0,97	0,01	2,61	0,23	0,96	0,01
TI	27,33	8,26	40,50	13,62	7,10	1,60	0,98	0,01	3,19	0,33	0,98	0,01
SA	21,00	10,64	36,50	17,81	5,45	2,37	0,97	0,01	2,78	0,78	0,95	0,06

3.3.2 Spatial variations in biomass

Suprabenthos biomass varied spatially (Fig. 3-2): the highest total suprabenthos biomass (excluded large decapods, fish and temporal suprabenthos) was observed in Castellammare and S. Agata. Differences were significant (ANOVA $F_{1,20}$=5,71; P=0,005), particularly total suprabenthos biomass in Patti differed from the other three Gulfs (Fig. 3-2).

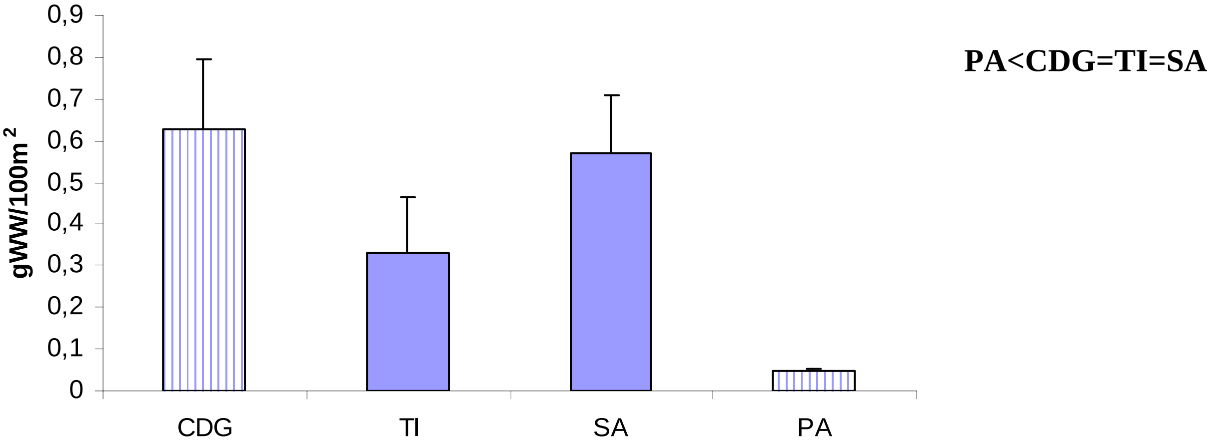


Figure 3-2. Seasonal changes in suprabenthos biomass (ggWW/100m^2) by area (large decapods excluded). Results of post-hoc HSD Tukey test was also showed. Bars indicate protected areas, full colours indicate unprotected areas.

Peracarids biomass also differed significantly (ANOVA $F_{1,20}$= 3,33; p=0,04) among areas, the highest values were observed in S. Agata and Termini Imerese.

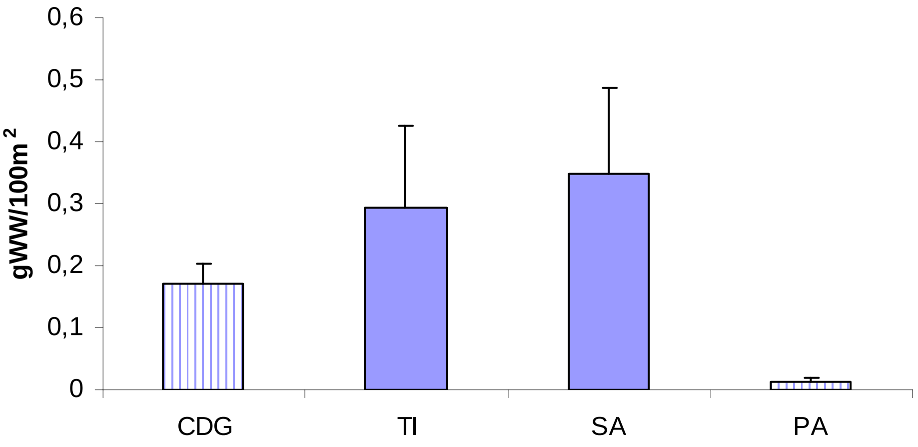


Figure 3-3. Spatial changes in peracarids biomass (ggWW/100m^2) by area.

Amphipods biomass changed spatially (ANOVA $F_{1,20}$=10,796; p<10^{-3}). The highest biomass occurred in Castellammare (fig. 3-4).

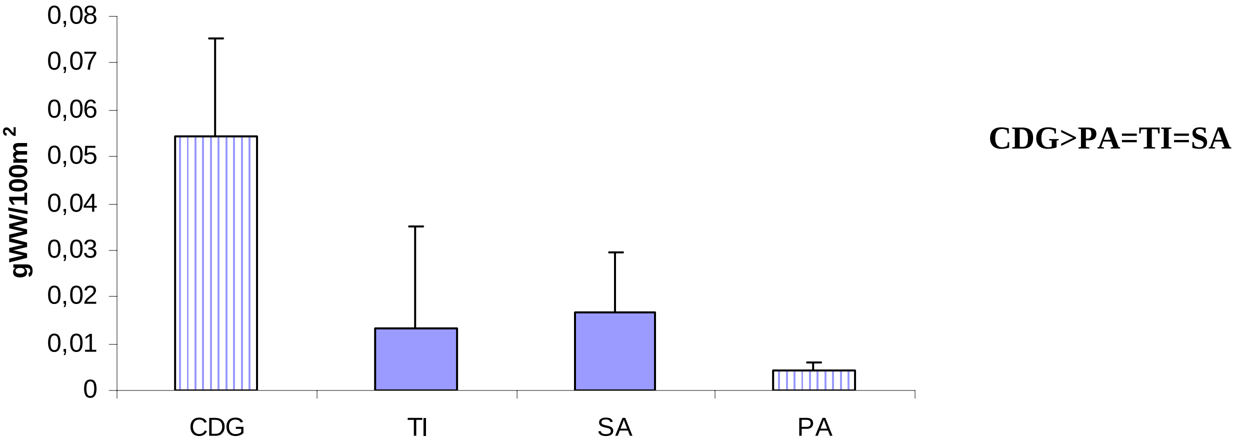


Figure 3-4. Seasonal changes in amphipods biomass (ggWW/100m^2) by area. Results of post-hoc HSD Tukey test was also showed

The highest values of mysids biomass were recorded in the Gulfs of Termini and S.Agata (fig. 3-5). Differences were significant (ANOVA $F_{1,20}$=3,75; p=0,027).

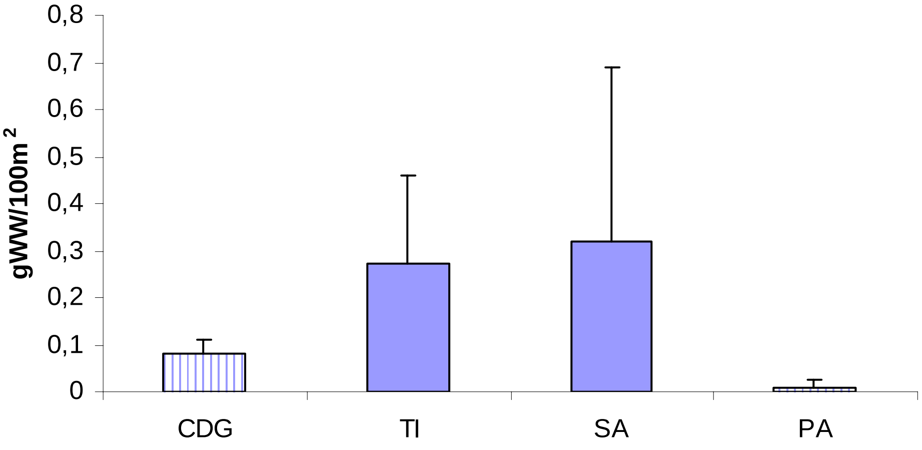


Figure 3-5. Seasonal changes in mysids biomass (ggWW/100m^2) by area

Copepods biomass was low in the four Gulfs and did not differ significantly (fig. 3-6).

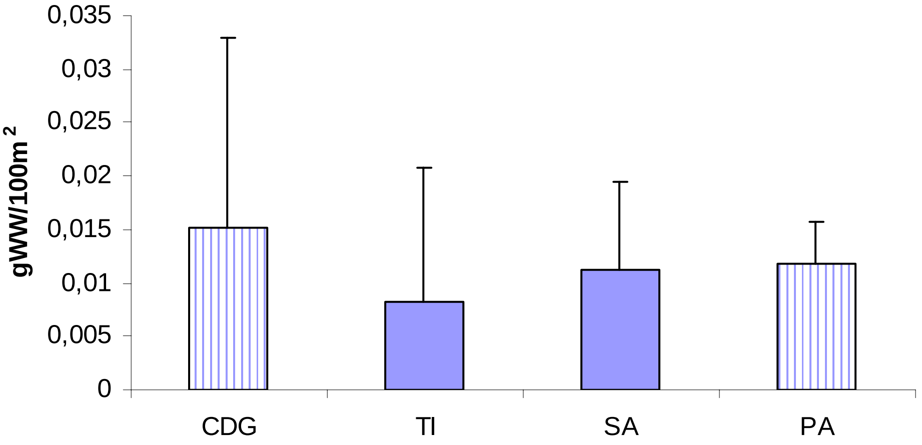


Figure 3-6. Seasonal changes in calanoid copepods biomass (ggWW/100m^2) by area

Cumaceans biomass also differed spatially (ANOVA $F_{1,20}$=4,848; p=0,010), with the highest value recorded in Castellammare (0,06 gWW/100m^2; CDG>PA=TI=SA).

3.3.3 Suprabenthic assemblages

NMDS analysis (based on Bray-Curtis distance of 4th root transformed abundance data) showed a clear separation among areas (Fig. 3-7). Castellammare samples were more aggregated, while Patti more separated, both were well-separated from the other two areas.

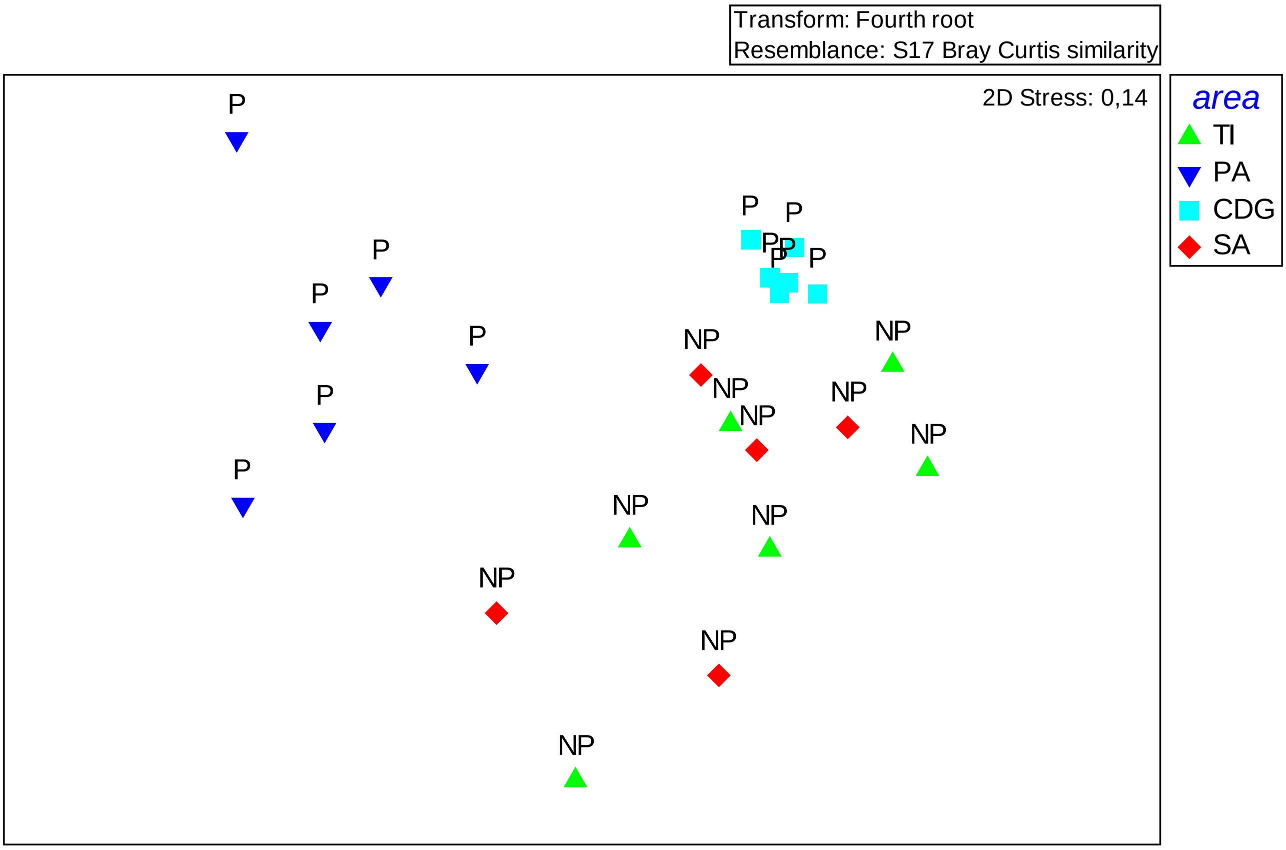


Fig. 3-7 nMDS performed on abundance data of suprabenthic assemblages of four Gulfs of Northern Sicily in May 2005; symbols indicate the areas and labels mean protected (P) and unprotected (NP) area.

Symmetrical PERMANOVA provided evidence (table 3-4) for separation of samples among areas but no significant effect of protection occurred.

Table 3-4 Symmetrical PERMANOVA based on the Bray–Curtis dissimilarities of the 4th root transformed multivariate data (81 species)

Source	df	SS	MS	Pseudo-F	P(perm)	perms
protection	1	4130,2	4130,2	0,81546	0,628	999
area(protection)	2	10130	5064,9	4,8212	**0,001**	998
Res	20	21011	1050,6			
Total	23	35271				

A pairwise comparison (table 3-5) showed significant differences among all the areas, except between Termini and S. Agata assemblages.

Table 3-5 Pair-wise test per factor “area”

Groups	t	P(perm)	perms
TI, PA	2,4767	**0,003**	417
TI, CDG	2,2066	**0,001**	404
TI, SA	1,2196	0,117	417
PA, CDG	3,4177	**0,004**	405
PA, SA	1,6907	**0,028**	407
CDG, SA	2,0355	**0,004**	389

Moreover the PERMDISP routine was significant for group factor area (*F*= 8,87; p=0,002). The highest dispersion of abundance data occurred in S. Agata (Average 36,11; standard error 4,28) and Termini (Av 30,56; SE 2,31), it indicated a higher variability of suprabenthic assemblages in these two Gulfs than in Patti (Av 28,76; SE 2,10) and Castellammare (Av 17,19; SE 0,82).

SYMPER analysis (table 3-6, cut-off for low contribution 60%) showed the most typifying species/taxa for each area.

Because the nMDS ordination showed an overlap between Termini and S. Agata samples, a SIMPER analysis was performed grouping TI and SA samples and comparing first with Castellammare and secondarily with Patti (table 3-7) in order to detect dissimilarity among areas.

L. gracilis, *W. rectirostris*, *A. vexatrix* and unidentified mysids were the taxa that most contributed to dissimilarity among Termini and S. Agata samples and Patti. *E. elegans*, *A. vexatrix*, *Harpinia* spp. and *M. griseus* were more abundant in the Gulf of Castellammare than in the others two Gulfs.

Table 3-6 SIMPER analysis (cut-off for low contribution 60%) within areas and between areas

Group CDG
Average similarity: 73,23

Taxa	Av.Abund	Av.Sim	Sim/SD	Contrib%	Cum.%
Copepoda	2,9	4,49	9,39	6,13	6,13
Erythrops elegans	3,17	4,41	2,27	6,02	12,15
Westwodilla rectirostris	2,58	3,93	7,13	5,37	17,52
Harpinia spp.	2,51	3,79	10,73	5,18	22,7
Monoculodes griseus	2,22	3,47	7,33	4,74	27,44
Cumacea unid.	2,12	3,17	7,94	4,33	31,77
Dyastiloides serrata	2,39	3,14	2,98	4,29	36,06
Leucon mediterraneus	2,07	3,04	5,09	4,15	40,21
Perioculodes longimanus	1,87	2,87	16,63	3,92	44,13
Acanthomysis longicornis	1,78	2,66	6,29	3,64	47,77
Gammaridea unid.	2,08	2,63	1,36	3,59	51,36
Gnathia sp. larvae	1,83	2,45	3,91	3,34	54,7
Paraphoxus oculatus	1,61	2,42	13,1	3,31	58,01
Leucon affinis	1,64	2,36	6,55	3,22	61,23

Group PA
Average similarity: 51,05

Taxa	Av.Abund	Av.Sim	Sim/SD	Contrib%	Cum.%
Copepoda	3,06	15,68	8,8	30,71	30,71
Apherusa vexatrix	1,72	8,51	6,87	16,67	47,38
Gammaridea unid.	1,24	5,1	1,32	9,99	57,37
Erythrops elegans	1,27	4,45	1,3	8,71	66,08

Group TI
Average similarity: 49,72

Taxa	Av.Abund	Av.Sim	Sim/SD	Contrib%	Cum.%
Leptomysis gracilis	2,73	7,05	4,41	14,19	14,19
Westwodilla rectirostris	1,97	4,13	4,89	8,31	22,49
Mysidacea unid.	1,92	3,37	1,32	6,78	29,28
Erythrops elegans	1,94	3,09	1,22	6,21	35,49
Dyastiloides serrata	1,28	3,07	4,42	6,17	41,66
Philocheras bispinosus	1,34	3,05	7,33	6,13	47,79
Acanthomysis longicornis	1,37	2,98	1,36	6	53,79
Copepoda	1,86	2,59	1,12	5,22	59,01
Harpinia sp.	1,34	2,59	1,33	5,21	64,22

Group SA
Average similarity: 38,18

Taxa	Av.Abund	Av.Sim	Sim/SD	Contrib%	Cum.%
Copepoda	3,35	10,62	2,73	27,81	27,81
Leptomysis gracilis	2,72	4,9	1,34	12,83	40,64
Dyastiloides serrata	1,3	2,67	1,23	6,99	47,63
Anchialina agilis	1,19	2,41	1,33	6,31	53,95
Erythrops elegans	1,26	2,2	1,32	5,77	59,72
Westwodilla rectirostris	1,57	2,15	0,79	5,63	65,35

Table 3-7 SIMPER analysis performed grouping TI and SA samples (cut-off for low contrinbution:50%)

Groups TI-SA & PA
Average dissimilarity = 60,97

Taxon	Group TI&SA Av.Abund	Group PA Av.Abund	Av.Diss	Diss/SD	Contrib%	Cum.%
Leptomysis gracilis	2,72	0,51	3,75	2,06	6,16	6,16
Apherusa vexatrix	0,27	1,72	2,54	2,49	4,17	10,32
Westwodilla rectirostris	1,77	0,71	2,14	1,85	3,51	13,83
Unid. Mysidacea	1,43	0	2,11	1,13	3,47	17,3
fish larvae	1,86	1,07	1,95	1,11	3,2	20,5
Copepoda	2,61	3,06	1,83	0,95	3	23,5
Erythrops elegans	1,6	1,27	1,81	1,22	2,97	26,47
Unid. Amphipoda	0,9	1,24	1,77	1,21	2,91	29,38
Philocheras bispinosus	1,16	0,17	1,71	1,68	2,81	32,19
Chaethognatha	0,61	0,78	1,71	0,86	2,8	34,99
Acanthomysislongicorne	1,1	0,16	1,7	1,33	2,8	37,79
Harpinia spp.	1,2	0,6	1,66	1,34	2,72	40,5
Phtisica marina	0,92	0	1,48	0,92	2,43	42,94
Dyastiloides serrata	1,29	0,59	1,48	1,27	2,42	45,36
Leptocheirus mariae	0,44	0,62	1,44	0,73	2,36	47,72
Anchialina agilis	0,89	0	1,38	1,34	2,26	49,98

Groups TI-SA & CDG
Average dissimilarity = 51,30

Taxon	Group TI&SA Av.Abund	Group CDG Av.Abund	Av.Diss	Diss/SD	Contrib%	Cum.%
Erythrops elegans	1,6	3,17	1,93	1,36	3,75	3,75
Apherusa vexatrix	0,27	1,89	1,69	1,97	3,29	7,04
Unid. Cumacea	0,53	2,12	1,6	1,97	3,12	10,17
Unid. Amphipoda	0,9	2,08	1,54	1,39	3	13,17
Dyastilis doriphora	0	1,5	1,51	5,16	2,94	16,1
Gnathia spp. larvae	0,36	1,83	1,51	1,72	2,94	19,04
Harpinia spp.	1,2	2,51	1,44	1,3	2,82	21,86
Monoculodes griseus	0,89	2,22	1,43	1,41	2,79	24,65
Leptomysis gracilis	2,72	1,78	1,41	1,91	2,75	27,4
Leucon affinis	0,34	1,64	1,3	1,97	2,54	29,93
Perioculodes longimanus	0,66	1,87	1,29	1,54	2,52	32,45
Goneplax rhomboides	0,17	1,44	1,28	2,66	2,49	34,94
Dyastiloides serrata	1,29	2,39	1,24	1,37	2,43	37,37
Unid. Mysidacea	1,43	1,2	1,23	1,33	2,41	39,77
Leucon mediterraneus	0,95	2,07	1,22	1,31	2,37	42,14
Paraphoxus oculatus	0,46	1,61	1,18	1,74	2,3	44,45
Leucon siphonatus	0,19	1,3	1,15	2,18	2,23	46,68
Mysidopsis angusta	0	1,14	1,13	1,87	2,21	48,89

3.3.4 Environmental variables

Chl *a* derived from satellite imagery are shown in figures 3-8÷3-11. In the Gulf of Castellammare there was a unique peak of Chl *a* on surface in February 2005 with a period of low production in summer-late autumn 2004 and from spring to early summer 2005.

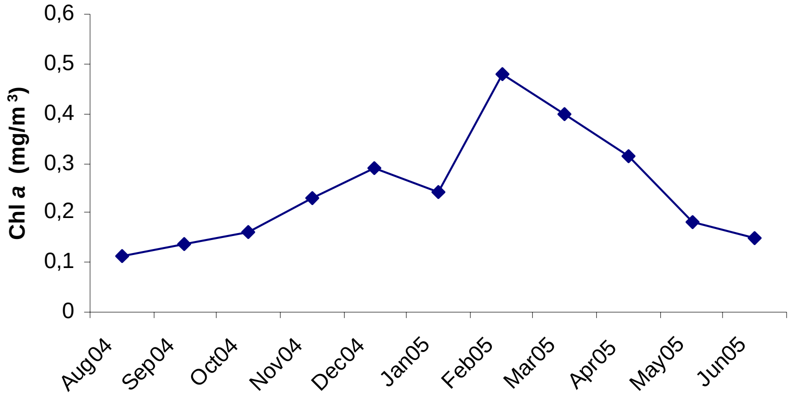


Figure 3-8. Monthly *Chl a* derived from satellite imagery (*http://reason.gsfc.nasa.gov/Giovanni*) in the Gulf of Castellammare from August 2004 to June 2005.

In the Gulf of Patti there was more than one peak of Chl *a* on surface, the first arose from November to December 2004, then two other peaks occurred respectively in February and April 2005. It seemed there was a period of high production from the end of autumn to early spring. A period of low production was detected in summer-early autumn 2004 and late spring 2005.

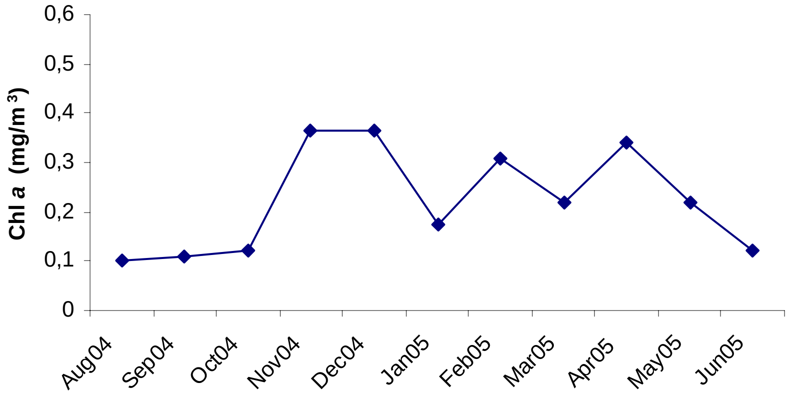


Figure 3-9. Monthly *Chl a* derived from satellite imagery (*http://reason.gsfc.nasa.gov/Giovanni*) in the Gulf of Patti from August 2004 to June 2005.

In the Gulf of Termini Imerese there was a unique peak of Chl *a* on surface in February 2004 with a period of increasing chlorophyll *a* surface concentration from November to February.

After the peak production was high until April; a period of low production was detected in summer.

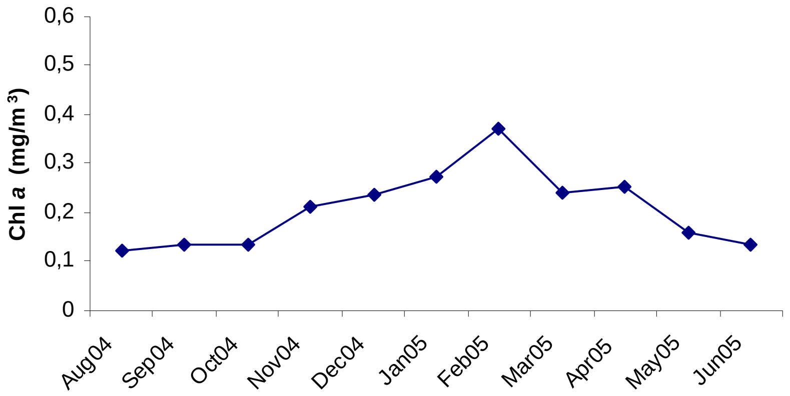


Figure 3-10. Monthly *Chl a* derived from satellite imagery (*http://reason.gsfc.nasa.gov/Giovanni*) in the Gulf of Termini Imerese from August 2004 to June 2005.

In the Gulf of S. Agata one prolonged peak of Chl *a* on surface occurred from January to April 2004. A period of low production was detected from August to December 2004 and from May to June 2005.

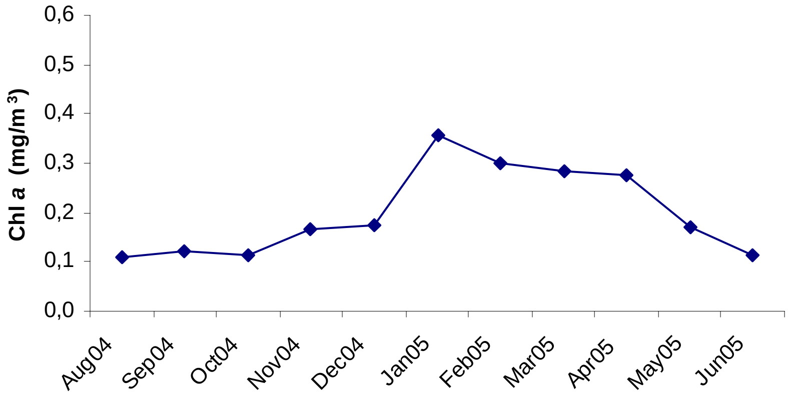


Figure 3-11. Monthly *Chl a* derived from satellite imagery (*http://reason.gsfc.nasa.gov/Giovanni*) in the Gulf of S. Agata from August 2004 to June 2005.

In general in all the Gulfs the peak of production was detected in January-February but the highest values of surface chlorophyll *a* concentration was recorded in the Gulf of Castellammare (0,48 mg/mm^3).

Surface temperature in the Gulf of Castellammare from May to June 2005 ranged between 17,54 °C in May and 22,73 °C in June. In May 2005 surface temperatures were 20,27 °C (± 0,89) in the Gulf of Termini Imerese, 20,68 °C (±0,42) in the Gulf of S. Agata and 19,75 °C (±0,04) in the Gulf of Patti. In June 2005 temperatures quickly increased of 4-5 degrees reaching a value of 24,6 °C (±0,05) in Termini, of 24,96 °C (±0,04) in S. Agata and of 24,24 °C (±0,33) in Patti.

The concentration of Chl *a* in sediments was significantly higher in Patti than in Castellammare (mean values 0,59 µg/g and 0,31 µg/g respectively), while the concentration of phaeopigments significantly differed between Castellammare (3,9 µg/g) and Patti (2,9 µg/g) and between Termini (5,1 µg/g) and S. Agata (3,8 µg/g). The biopolymeric fraction of sedimentary carbon (C-BPF), obtained as the sum of lipids (C-LIP), proteins (C-PRT) and carbohydrates (C-CHO) equivalent carbon, significantly varied between Castellammare (3576 µg/g) and Patti (2178 µg/g), and between Termini (3126 µg/g) and S. Agata (2711 µg/g)

3.3.5 Correlation with environmental variables

The BEST (BIOENV) routine (Clarke and Warwick, 2001) evidenced which combination of variables (PP concentration recorded 4, 3, 2 and 1 month before and simultaneously to the sampling, surface temperature and SOM parameters such as Chl *a*, phaeopigments, lipids, proteins, carbohydrates) generated the best match to the biotic data. Among the explored variables the best match (Spearman r=0,546; p<0,05) was obtained with 2, 3 and 4 variables. Within them chlorophyll *a* recorded one and two months before the sampling, phaeopigments and the biopolymeric fraction of sedimentary carbon (C-BPF) were the variables that best matched with biotic data, being PP2 and PP1 correlated each other, as resulted by Draftsman plots of all the explored variables..

The spatial biomass pattern of total suprabenthos, peracarids and mysids and amhipods was mainly explained by few environmental variables (table 3-8). In general surface temperature did not affect spatial variability, while Chl *a* recorded 3 months before sampling was positively correlated with total suprabenthos, total peracarids and mysids biomass. Total suprabenthos, total peracarids and amphipods biomass were negatively correlated with Chl *a* recorded 2 and 1 month before sampling and with Chl *a* in SOM. Phaeopigments showed a positive correlation with some taxa; among C-BPF, proteins and lipids were the most explaining variables of suprabenthic biomass' patterns.

Table 3-8. Spearman correlation of suprabenthic taxa with environmental variables. Only significant ($p<0,05$) correlations are shown.

Total suprabenthos	**Valid**	**Spearman**	**t(N-2)**	**p-level**
PP3mo	24	0,485	2,598	0,016
PP2mo	24	-0,549	-3,082	0,005
PP1mo	24	-0,549	-3,082	0,005
chla sediment	24	-0,549	-3,082	0,005
phaeopigments	24	0,581	3,352	0,003
C-BPF	24	0,549	3,082	0,005
proteins	24	0,549	3,082	0,005
Total peracarids	**Valid**	**Spearman**	**t(N-2)**	**p-level**
PP3mo	24	0,533	2,955	0,007
PP2mo	24	-0,501	-2,713	0,013
PP1mo	24	-0,501	-2,713	0,013
chla sediment	24	-0,565	-3,214	0,004
phaeopigments	24	0,549	3,082	0,005
C-BPF	24	0,565	3,214	0,004
proteins	24	0,565	3,214	0,004
Mysids	**Valid**	**Spearman**	**t(N-2)**	**p-level**
PP3mo	24	0,705	4,666	0,000
chla sediment	24	-0,571	-3,260	0,004
C-BPF	24	0,571	3,260	0,004
proteins	24	0,571	3,260	0,004
Amphipods	**Valid**	**Spearman**	**t(N-2)**	**p-level**
PP2mo	24	-0,635	-3,858	0,001
PP1mo	24	-0,635	-3,858	0,001
phaeopigments	24	0,608	3,595	0,002
lipids	24	0,501	2,713	0,013

3.3.6. Stable isotope ratios of Carbon and Nitrogen ($\delta^{13}C$ and $\delta^{15}N$)

Ten taxa (2 mysids, 4 amphipods, 1 cumacean and 3 decapods) and copepods were analyzed for Castellammare for stable carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) isotopes (see also Table 3-1).

Maximum and minimum $\delta^{15}N$ values were consistent, ranged from 3,8‰ (*Ampelisca* spp.) to 8,6‰ (*Philocheras bispinosus*). However the intermediate values were difficult to interpret, especially as concerns the cumacean *L. mediterraneus* that showed a high trophic level. Assuming a trophic enrichment of 3‰ (Post 2002), the overall range of $\delta^{15}N$ is indicative of two main trophic levels for the Castellammare suprabenthic assemblage in May 2005 (Fig. 3-13).

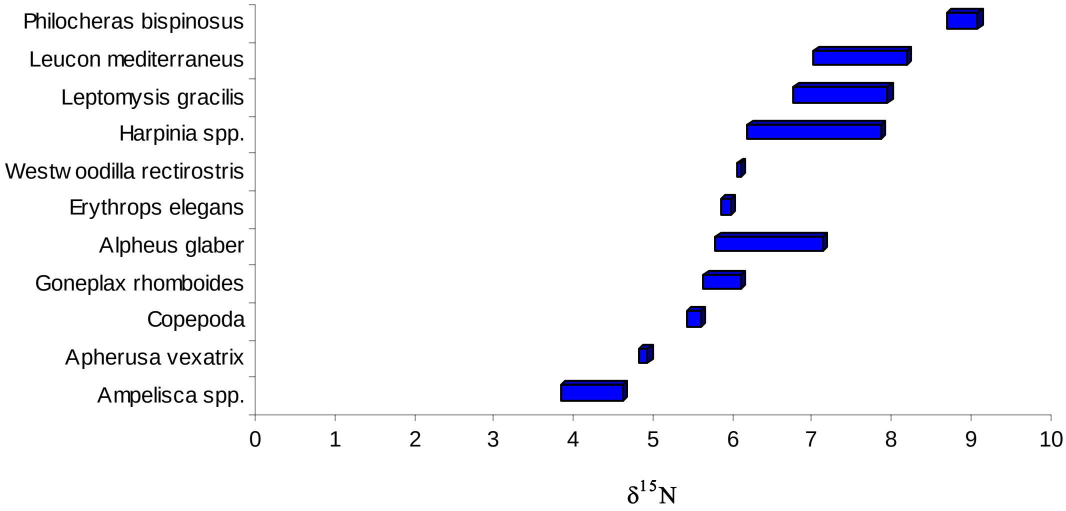


Fig. 3-13 Range of $\delta^{15}N$ values (‰) (mean ± standard deviation) for suprabenthic species in the Gulf of Castellammare.

Nine taxa (2 mysids, 3 amphipods, 3 decapods and 1 fish), the whole *taxon* Cumacea and copepods were analyzed to analyze stable carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) isotopes in the Gulf of Patti (see also table 3-1). In this area $\delta^{15}N$ values ranged from 2,3‰ (*Westwoodilla rectirostris*) to 8,65‰ (*Lesueurigobius sueri*).

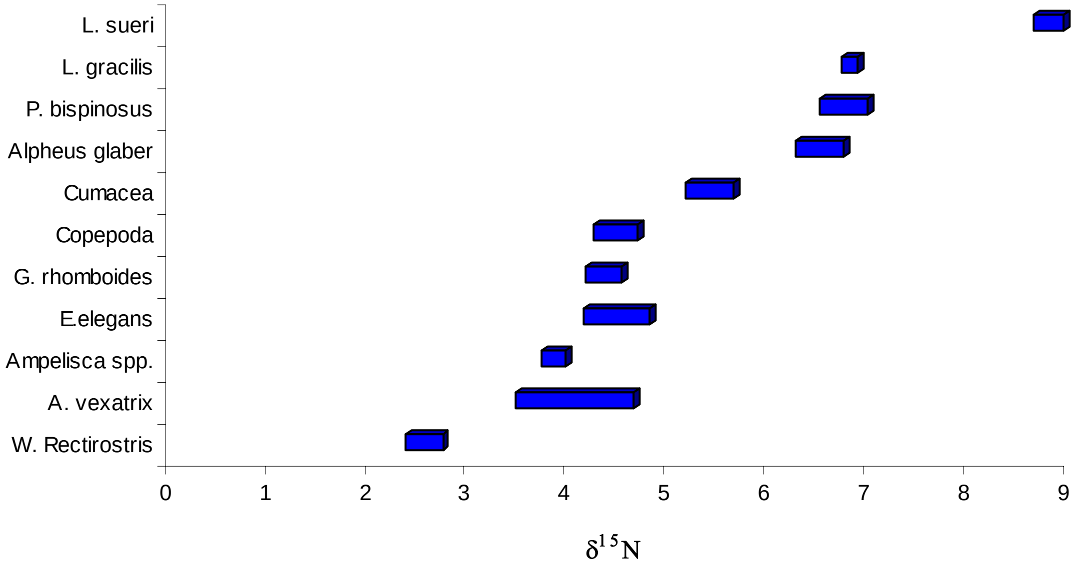


Fig. 3-14 Range of $\delta^{15}N$ values (‰) (mean ± standard deviation) for suprabenthic species in the Gulf of Patti.

Nine taxa (3 mysids, 1 amphipod, 3 decapods, 1 cumacean and 1 fish) and copepods were analyzed to analyze stable carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) isotopes in the Gulf of Termini Imerese (see also table 3-15). In this area $\delta^{15}N$ values ranged from 2,9‰ (*Goneplax rhomboides*) to 8,27‰ (*Lesueurigobius sueri*).

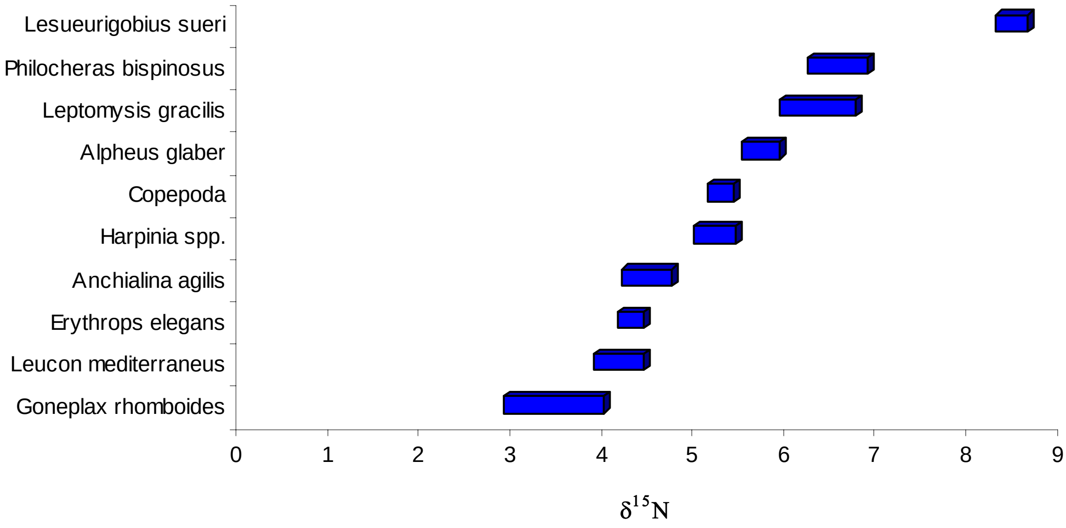


Fig. 3-15 Range of δ^{15}N values (‰) (mean ± standard deviation) for suprabenthic species in the Gulf of Termini Imerese.

Ten taxa (3 mysids, 3 amphipods, 3 decapods and 1 fish), the whole taxon of cumaceans and copepods were analyzed in the Gulf of S. Agata (see also table 3-XX). In this area δ^{15}N values ranged from 1,68‰ and 1,72‰ (Cumacea and *Westwoodilla rectirostris*, respectively) to 7,42‰ (*Lesueurigobius sueri*) (Fig. 3-16).

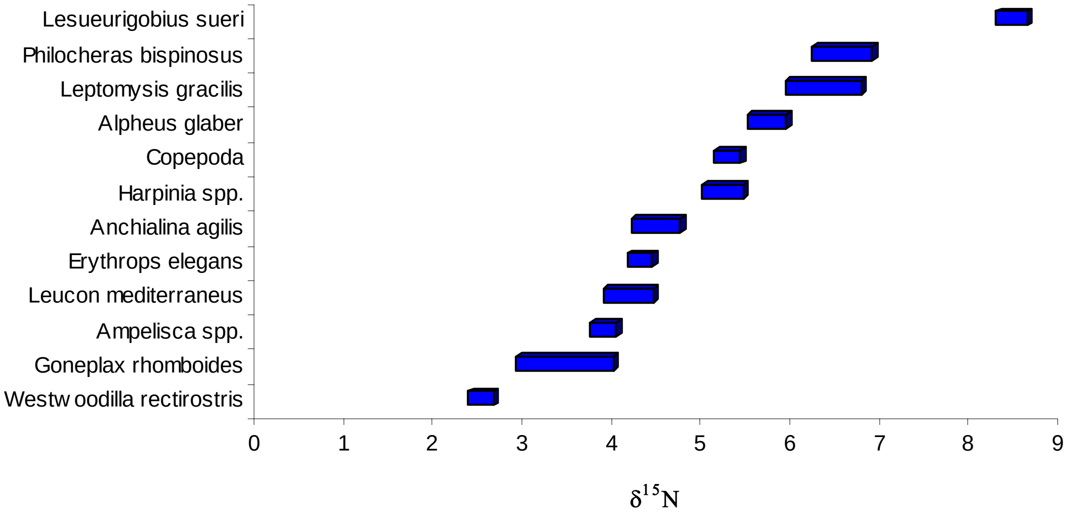


Fig. 3-16 Range of δ^{15}N values (‰) (mean ± standard deviation) for suprabenthic species in the Gulf of S. Agata.

Due to the low biomass obtained after samples hydrolysis few measurement of δ^{13}C were performed.

$\delta^{13}C$ values of suprabenthos taxa of the Gulf of Castellammare ranged from -24,2‰ (Copepoda) to -20,1‰ (*Goneplax rhomboides*) (Fig. 3-17).

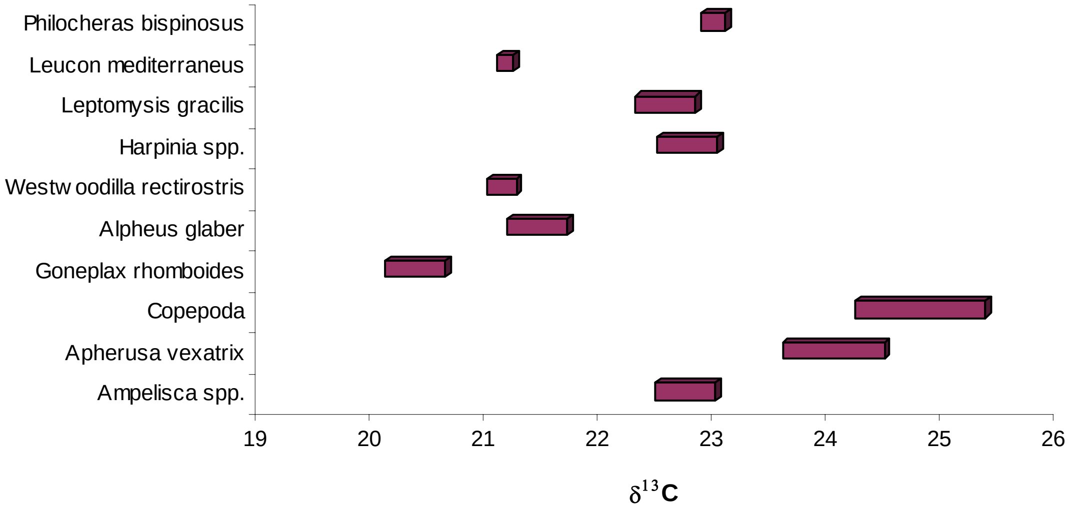


Fig. 3-17 Range of $\delta^{13}C$ values (‰) (mean ± standard deviation) for suprabenthic species in the Gulf of Castellammare, values were negative.

In the Gulf of Patti $\delta^{13}C$ values ranged from -22,6‰ (Copepoda) to -18,74‰ (*Ampelisca* spp.) (Fig. 3-18).

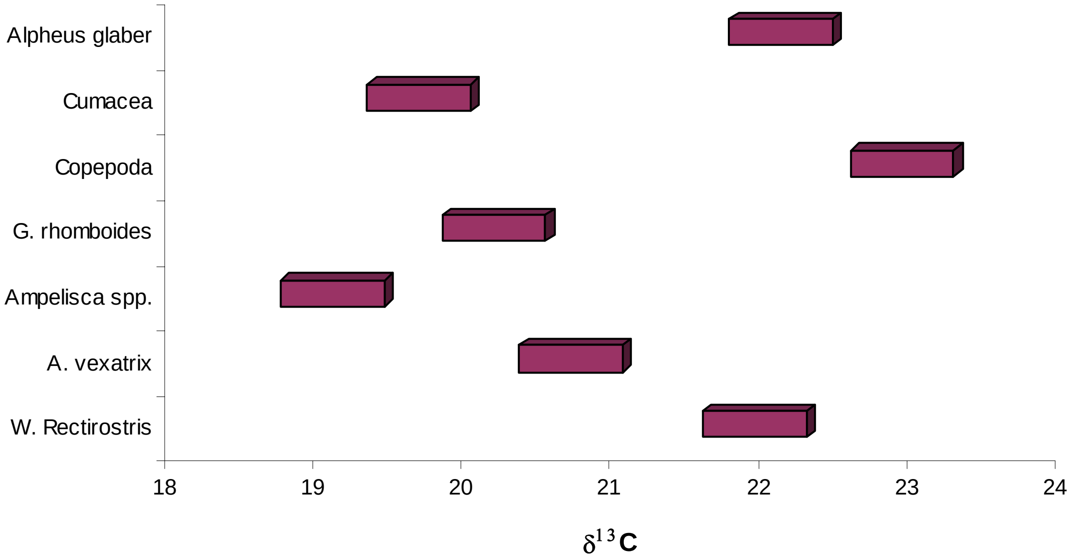


Fig. 3-18 Range of $\delta^{13}C$ values (‰) (mean ± standard deviation) for suprabenthic species in the Gulf of Patti, values were negative.

$\delta^{13}C$ values of Termini Imerese suprabenthos taxa ranged from -24,4‰ (*Erythrops serrata*) to -21,3‰ (*Westwoodilla rectirostris*) (Fig. 3-19).

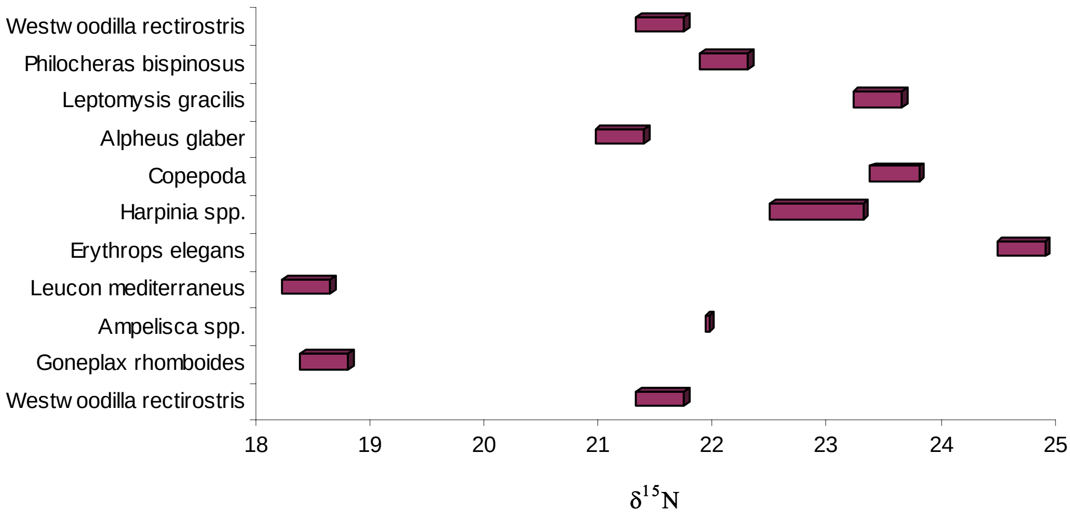


Fig. 3-19 Range of $\delta^{13}C$ values (‰) (mean ± standard deviation) for suprabenthic species in the Gulf of Termini Imerese, values were negative.

$\delta^{13}C$ values of S. Agata suprabenthic species ranged from -24,4‰ (*Erythrops elegans*) to -18,2‰ (Cumacea) (Fig. 3-20).

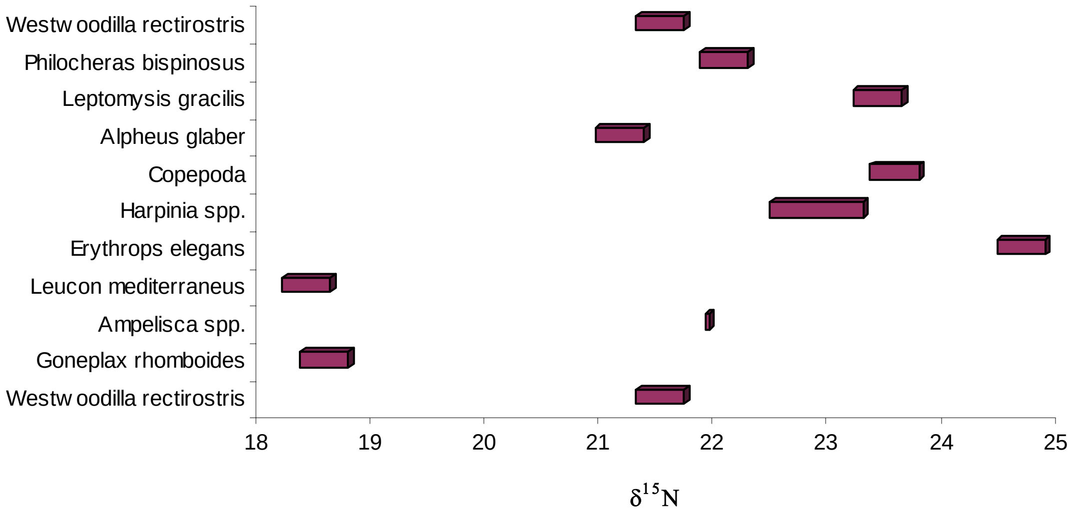


Fig. 3-20 Range of $\delta^{13}C$ values (‰) (mean ± standard deviation) for suprabenthic species in the Gulf of S. Agata, values were negative.

The wide range of isotopic values obtained is in accordance with many different trophic strategies, and corresponded quite well with the trophic classification based on gut contents analysis-isotopic analyses (data from the literature, Table 2). In all the Gulfs the species with more enriched $\delta^{15}N$ values were *P. bispinosus*, *L. sueri*, *L. gracilis* and only in the Gulf of Castellamamre *L. mediterraneus*; all of them are carnivorous and scavenger species. In contrast low δ^{15}N values identify mainly filter-feeders (more closely associated to plankton) (e.g. mysids *Erythrops serrata*) or deposit-feeders (the amphipods *A. vexatrix* and *Harpinia* spp.). The δ^{13}C values were generally enriched (less negative) for deposit feeders (e.g. amphipods and cumaceans) than for filter-feeders (e.g. copepods and mysids).

3.3.6.1 Relationship between δ^{15}N and δ^{13}C

In the Gulf of Castellammare, the C-N correlation of suprabenthic species in May 2005, showed three distinct groups (see also chapter 2, fig. 2-20). One group was characterized by species with depleted δ^{13}C values, indicating mainly filter feeders (i.e. copepods and *L. gracilis*), the second included suspension and deposit- feeders (i.e. *Harpinia* spp. and *A. vexatrix*): The third group was mainly composed by deposit and infaunal feeders, relationship between δ^{15}N and of suprabenthos fauna by each area was generally weak (Fig. 3-21).

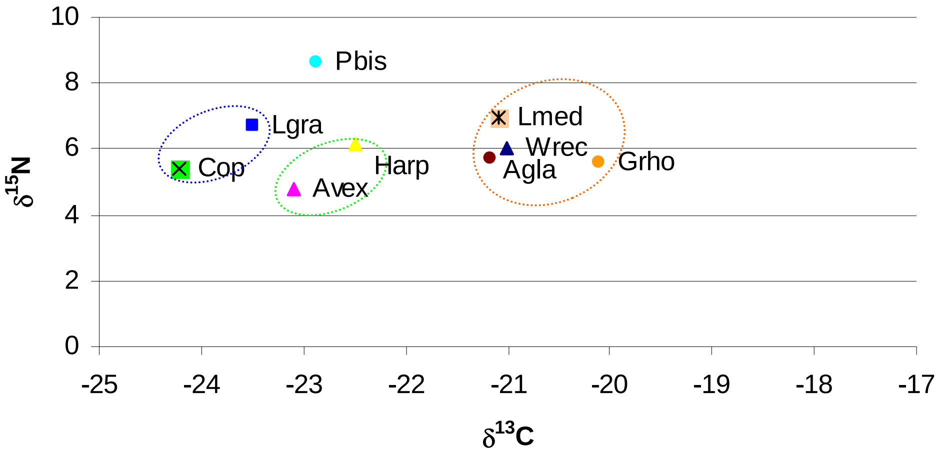


Fig 3-21 C-N correlation of δ^{15}N and δ^{13}C values of suprabenthic species in May-June 2005 in the Gulf of Castellammare.
(Legend: squares= mysids; circles=decapods; triangles=amphipods; squares with cross=copepods; squares with asterisk=cumaceans. Blue circle identifies filter feeders, green circle indicates species belonged to different trophic guilds and red circle groups deposit feeders. Acronyms as in table 3-1).

In the Gulf of Patti (fig. 3-22), due to low biomass recorded and the further reduction caused by samples hydrolysis for ^{13}C, few analysis were performed. Notwithstanding the same three groups, evidenced in Castellammare occurred, even if δ^{13}C values of the third groups were more enriched, probably indicating a more terrestrial source of carbon.

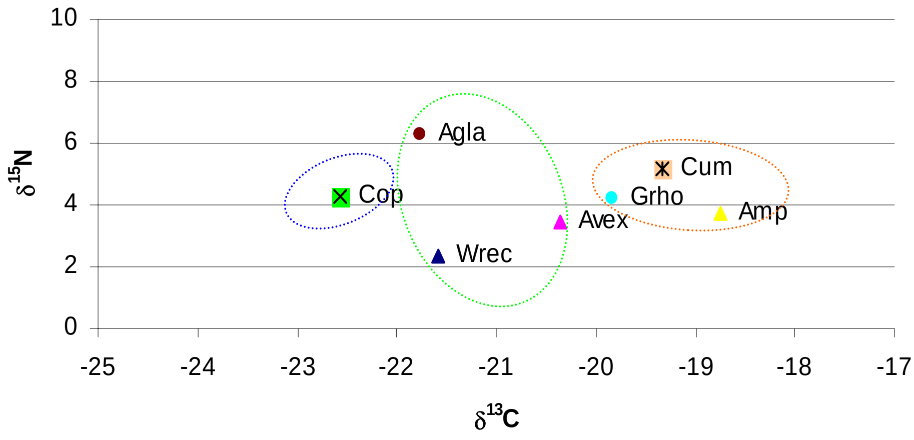


Fig 3-22 C-N correlation of $\delta^{15}N$ and $\delta^{13}C$ values of suprabenthic species in May-June 2005 in the Gulf of Patti. (Legend: see figure 3-21)

In the Gulf of Termini Imerese (fig. 3-23), *Ampelisca* spp. occurred in the second group. This is probably due to the large spectrum of resources utilized by *Ampelisca* species, being some taxa deposit feeders, while others suspension feeders.

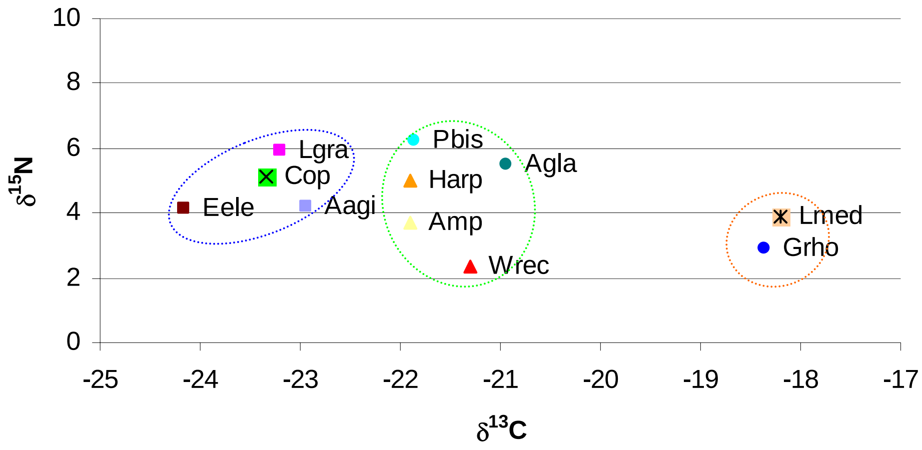


Fig 3-23 C-N correlation of $\delta^{15}N$ and $\delta^{13}C$ values of suprabenthic species in May-June 2005 in the Gulf of Termini Imerese.
(Legend: see figure 3-21)

Also in the Gulf of S. Agata (fig. 3-24), the same three groups were evident. The trophic level of the filter feeders group seemed to be higher than in the other areas, particularly *L. gracilis* showed a $\delta^{15}N$ value of 6,7, but this is probably due to the larger size specimens occurred in this Gulf.

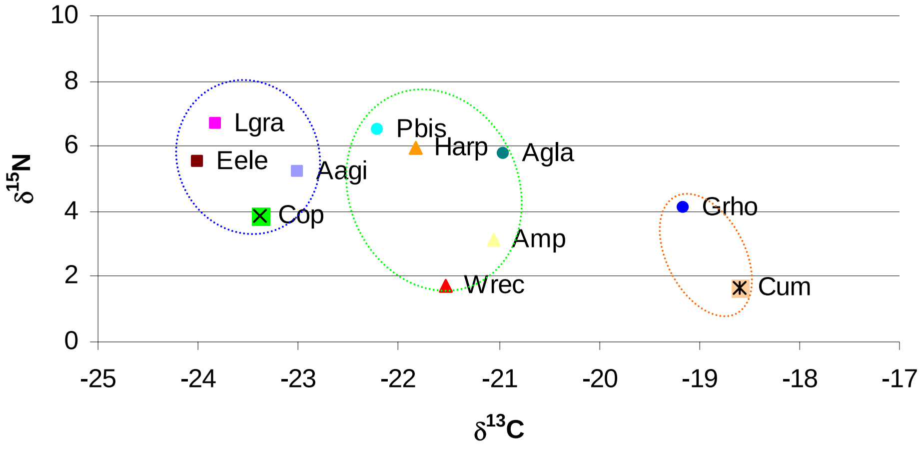


Fig 3-24 C-N correlation of $\delta^{15}N$ and $\delta^{13}C$ values of suprabenthic species in May-June 2005 in the Gulf of S. Agata.
(Legend: see figure 3-21)

Plotting the mean $\delta^{15}N$ and $\delta^{13}C$ values of suprabenthic species (fig. 3-25), the mean trophic level did not change significantly (ANOVA p>0,05) among areas, although $\delta^{15}N$ values for the Castellammare suprabenthic assemblage were slightly higher than the other areas.

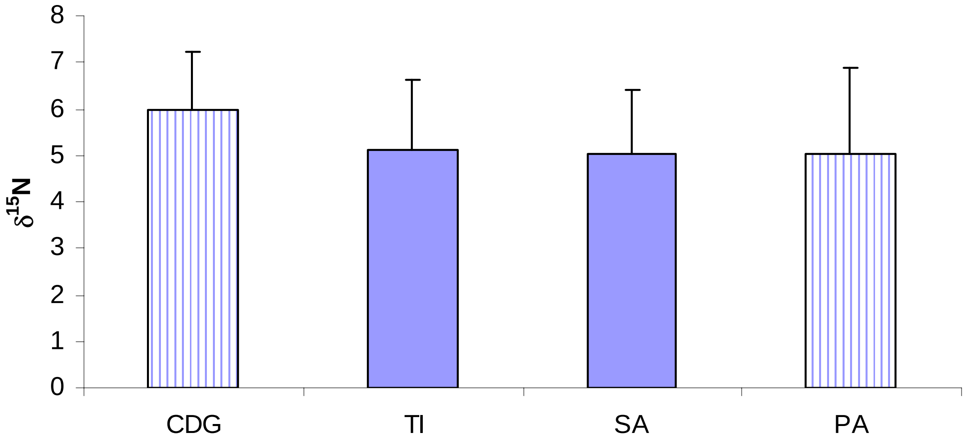


Fig. 3-25. Mean trophic level of the bulk of suprabenthic species per area (+SD); vertical bars indicated protected areas; full colour indicated unprotected areas.

The mean $\delta^{13}C$ values did not change significantly (ANOVA p>0,05) among areas (fig. 3-26), although $\delta^{13}C$ values of Patti species were more enriched than the other areas.

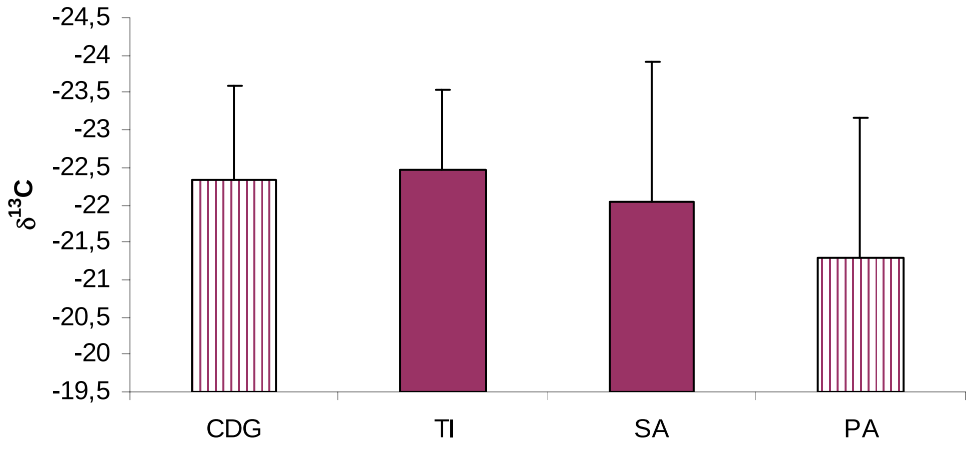


Fig. 3-26. Mean $\delta^{13}C$ value of the bulk of suprabenthic species per area (+SD); vertical bars indicated protected areas; full colour indicated unprotected areas.

3.3.6.2 Spatial variation of dominant suprabenthic species

Among suprabenthic community 5 species, dominant in terms of abundance, were analysed to detect spatial variation in stable isotopes values: 2 mysids (*L. gracilis* and *E. serrata*), 2 decapods (*A. glaber and P.bispinosus*) and 1 fish (*L. sueri*). Moreover Calanoid copepoda and, among epibenthic species collected by MG-sledge and abundant in the diet of *P. erythrinus* and *A. laterna*, the brachyuran crab *G. rhomboides* were processed. Due to the low biomass obtained after samples hydrolysis for $\delta^{13}C$ analyses, only spatial variations of $\delta^{15}N$ values were presented in this study.

$\delta^{15}N$ values changed markedly in Copepoda by area (fig. 3-27) with the highest values recorded in Castellammare and the lowest in Patti (5,4‰ and 4,2‰ respectively); differences were significant (ANOVA F=18,65; $p<10^{-5}$). A post-hoc Tukey HSD test evidenced the source of variations (fig- 3-27).

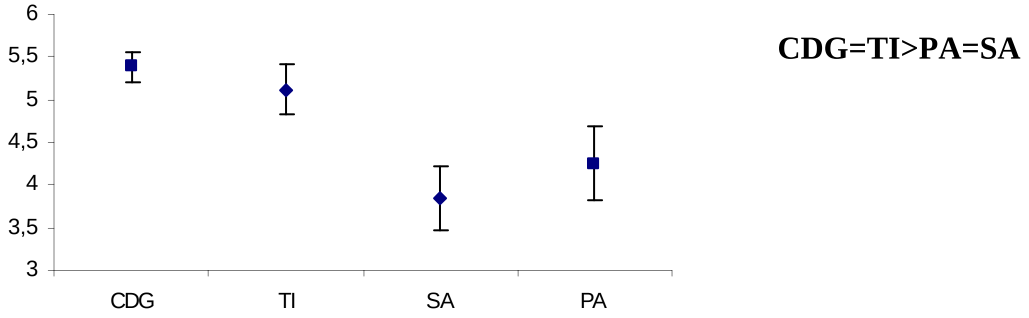
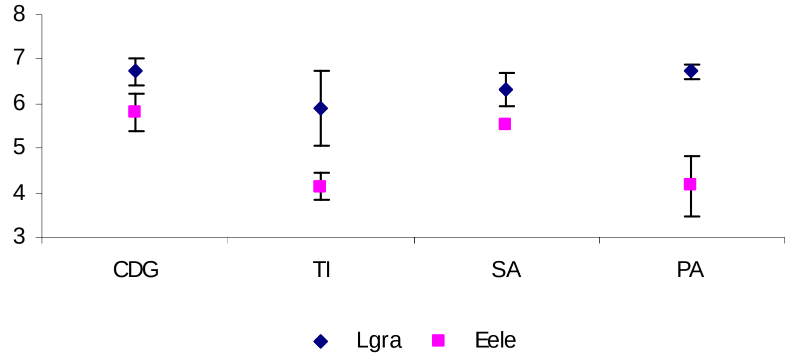


Fig. 3-27 $\delta^{15}N$ values of Copepoda (±SD) in the four Gulfs. *Post hoc* Tukey HSD test was also given. Squares indicate protected areas; rhomboids indicate unprotected areas.

δ^{15}N values varied significantly for *E. elegans* by area (fig. 3-28; ANOVA F=9,0; p=0,01) with the highest values recorded in Castellammare and the lowest in Patti and Termini (5,8‰ and 4,1‰ respectively). A post-hoc Tukey HSD test evidenced the source of variations (fig. 3-28). δ^{15}N values of *L. gracilis* did not showed a spatial trend among the four areas.



SA = CDG >PA=TI

Fig. 3-28 δ^{15}N values of *Leptomysis gracilis* and *Erytrops elegans* (±SD) in the four Gulfs.

δ^{15}N values did not differ significantly in *A. glaber* by area (fig. 3-29) though high, but more variables values were recorded in Castellammare.

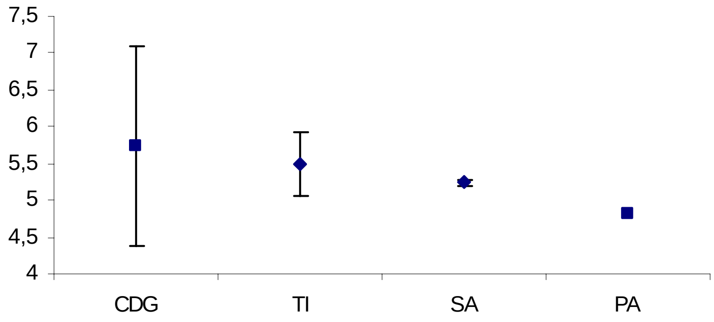


Fig. 3-29 δ^{15}N values of *Alpheus glaber* (±SD) in the four Gulfs. Squares indicate protected areas; rhomboids indicate unprotected areas.

δ^{15}N values changed significantly in *P. bispinosus* by area (fig. 3-30) (ANOVA F=17,96; $p<10^{-4}$), the highest values occurring in Castellammare. Castellammare samples differed from Termini and S. Agata ones (fig. 3-30).

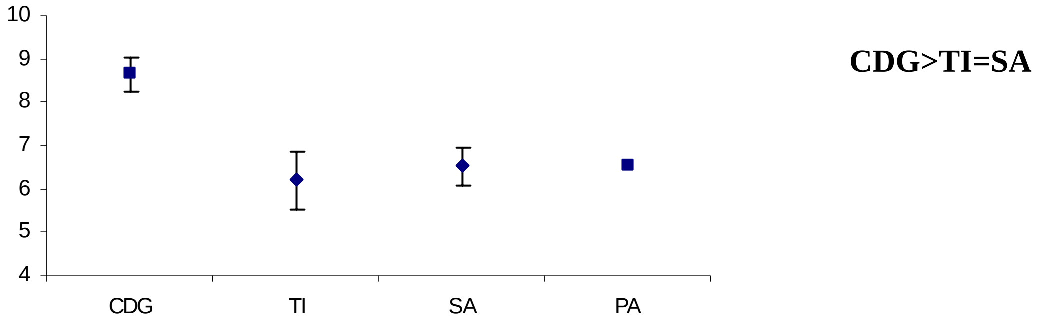


Fig. 3-30 δ^{15}N values of *Philocheras bispinosus* (±SD) in the four Gulfs. *Post hoc* test for *P. bispinosus* among areas was also given

δ^{15}N values changed significantly in *L. sueri* by area (fig. 3-31) (ANOVA F=6; p=0,02), the highest values occurring in Castellammare. Castellammare and Patti samples differed from S. Agata (fig. 3-31).

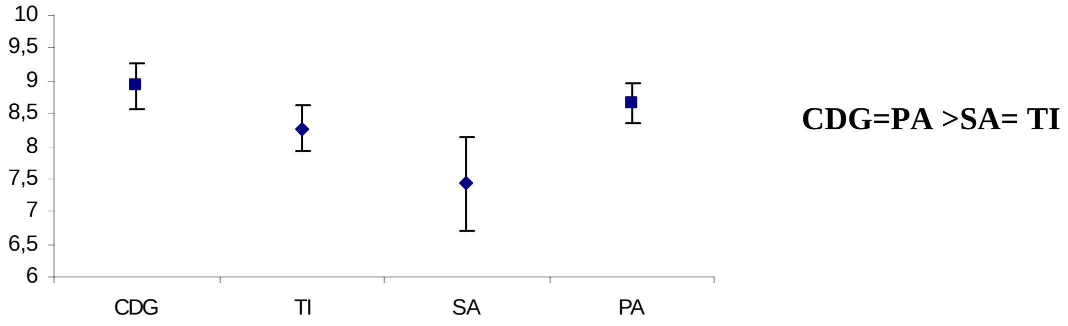


Fig. 3-31 δ^{15}N values of *Lesueurigobius sueri* (±SD) in the four Gulfs. Squares indicate protected areas; rhomboids indicate unprotected areas.

δ^{15}N values varied significantly in *G. rhomboides* by area (fig. 3-32) (ANOVA F=6,05; p=0,02), with the highest values occurred in Castellammare and the lowest in Termini, even if Castellammare samples only differed significantly from Patti (fig. 3-32).

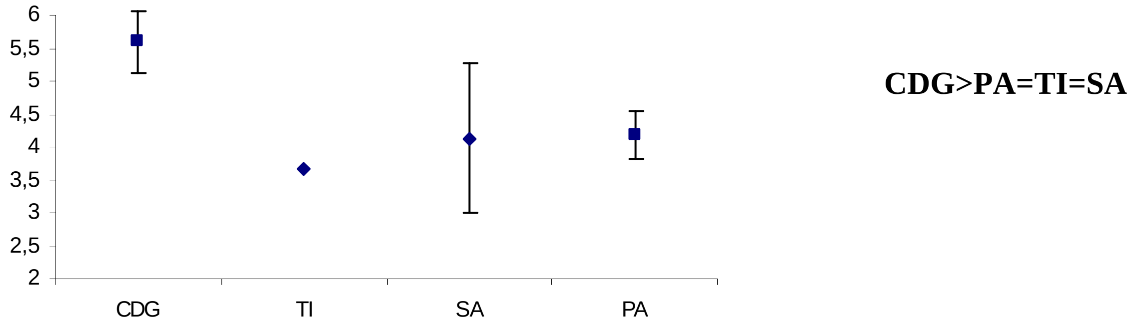


Fig. 3-32 δ^{15}N values of *Goneplax rhomboides* (±SD) in the four Gulfs. Squares indicate protected areas; rhomboids indicate unprotected areas.

3.3.6.3 Trophic level of BBL fauna

We estimated the trophic level (TL) of the BBL fauna (excepting those species where $\delta^{15}N$ values was lower than $\delta^{15}N$ of calanoid copepods) based on their $\delta^{15}N$ data and using copepods as reference material (table 5-9). $\delta^{15}N$ values were converted to trophic level based on the assumption that there was a fractionation of 3‰ per trophic level (Vander Zanden & Rasmussen, 2001; Post 2002) and that the base material (copepods) had a trophic level of 2:

$$TL_i = \left(\frac{\delta^{15}N_i - \delta^{15}N_{ref}}{3}\right) + 2$$

where TL_i is the trophic level of species *i*, $\delta^{15}N_i$ is the mean $\delta\ ^{15}N$ of species $_{i,}$ and $\delta^{15}N_{ref}$ is the mean $\delta^{15}N$ of the copepods recorded annual in the Gulf of Castellammare and in each area.

In general the trophic levels of suprabenthic species were consistent among areas, although the highest values were observed in the Gulf of Castellammare (table 3-9). This is particularly evident for species that occupied the higher trophic level, such as *P.bispinosus* and *L. sueri*.

Table 3-9. Estimated TL of BBL fauna (suprabenthos) species

	CDG	PA	TI	SA
Copepoda	2	2	2	2
G. rhomboides	2,29	-	-	-
A.glaber	2,34	2,52	2,26	2,35
E. elegans	2,36	-	-	2,26
W. rectirostris	2,43	-	-	-
Harpinia spp.	2,48	-	-	2,40
Cumacea	-	2,15	-	-
L. gracilis	2,67	2,67	2,40	2,56
A. agilis	-	-	-	2,17
L. mediterraneus	2,75	-	-	-
P. bispinosus	3,31	2,60	2,51	2,60
L. sueri	3,40	3,31	3,18	2,91

3.4 Discussion

3.4.1 Fauna composition

The present study is the first source of information about suprabenthic communities in the southern Tyrrhenian Sea. Suprabenthos of the northern Sicily consists almost entirely of crustaceans, dominated by mysids, amphipods, cumaceans and decapods. These taxonomic groups were also dominant in other suprabenthic communities from European coasts (Boysen, 1975; Buhl-Jensen, 1986; Buhl-Jensen and Fossa, 1991; Hesthagen, 1973; Kaartvedt S., 1985, 1989; Wang and Dauvin, 1984) and Mediterranean sea (Sorbe 1981, 1999, Cartes & Sorbe 1999, Cartes et al. 2001, Madurell & Cartes 2003).

Despite the oligotrophic character of this part of the western Mediterranean, the present results suggest that the suprabenthos there is not as impoverished as regards numbers of species as compared to the western basin (e.g. Catalan Sea, Cartes et al. *submitted*), as one would expect. The suprabenthic fauna recorded at the whole sampled area was diverse (86 species excluding decapod larvae); the richest group was gammaridean amphipods with 27 species, decapods, mysids and cumaceans accounting for 17, 8 and 13 species respectively. This species richness was considerable high, comparing with Ligas (2005) findings in the Northern Adriatic continental shelf. The most abundant species in the whole area were *Apherusa vexatrix*, *Harpinia* spp., *Westwoodilla rectirostris* and *Monoculodes griseus* among gammarids, *Erythrops elegans*, *Leptomysis gracilis* and *Acanthomysis longicornis* among mysids and *Dyastiloides serrata* and *Leucon mediterraneus* among cumaceans. *Philocheras bispinosus* was the most abundant decapod and the small flatfish *Symphurus nigrescens* was the most abundant suprabenthic fish. A similar species dominance was observed by Ligas (2005) in the Northern Adriatic Sea, at similar depths.

3.4.2 Local changes in composition and community structure of BBL fauna

The suprabenthic assemblages of the four Gulfs of northern Sicily appear quite similar in species composition. The average similarity in species abundance among the four Gulfs accounted between 51 and 68%. The most remarkable feature arising after comparison of the suprabenthic assemblages of the four areas is the impoverished condition of the Gulf of Patti. This area showed very low values of species richness and abundance and it could be attributed to the higher oligotrophy of this area compared with the other three Gulfs (on *http://www.regione.sicilia.it/presidenza/ucomrifiuti/Acque/DOCUMENTI/DOCUMENTI_B/B4.pdf*). The inland of the Gulf is a scarcely cultivated area and the torrents present irregular regimes that do not largely contribute to waters enrichment; these conditions result in very oligotrophic waters.

The other three areas were locally characterized by a larger hydrographic basin and several rivers flow in the three coastal areas: particularly the Gulf of Castellammare has a 1067 km^2 hydrographic basin extension that results in a large contribution of inorganic and organic compounds in the opposite coastal waters. In fact the enhancement of the productivity of coastal benthic ecosystems by land-based run-off has been demonstrated in various areas (e.g. Cloern 2001, Maslowski 2003) and has been linked mainly to river input of nutrients and subsequent increases in plankton production and deposition in the benthos (Ardisson & Bourget 1997, Josefson & Conley 1997).

This area showed the richest suprabenthic assemblages, accounting with 64 species and a total of 77 taxa (for a total of 757 ind/100m^2) in May 2005. The other two areas showed high values of diversity, thus an increase in species abundance and/or diversity due to the effects of the protection (e.g. *fishing exclusion zones*) in the Gulfs of Castellammare and Patti can be excluded. Trawling activity can largely influence benthic communities by reduction of habitat complexity and alteration of their structure (Turner et al. 1999; Kaiser et al. 2000b, Callaway et al. 2002, Kaiser et al. 2002), causing a decrease in diversity and/or production for infauna and meiofauna (Jennings et al., 2001; Schratzeberger et al., 2002). As previously observed by Cartes et al. (submitted) off the Ebro delta and Ligas, (2005) off the Po delta, no influences were evidenced for BBL fauna.

Local changes in faunal composition can be attributed to natural variability of the four areas, though all the samples were collected from muddy terrigenous bottoms. In general in the whole area the trophic index PRT/CHO (average value 4.06 ± 1.60) indicated an accumulation of proteic material and then the occurrence of live organic matter or fresh detritus (Danovaro et al., 1993). The index is particularly high in Termini, S. Agata and Castellammare.

In general suprabenthos biomass was positively correlated with primary production three months before the sampling and negatively with PP recorded two and one months before, suggesting that suprabenthic fauna relies on trophic resources other than those derived directly from the water column, probably linked to the sediment or the sediment-water interface (a lack of correlation with surface phytoplankton concentration simultaneously recorded at the sampling time occurred). Moreover the positive correlation probably indicated that the increase in biomass represented the peak of adults and not of recruits. As in the case of community composition, the feeding guilds also showed important differences between the four areas. Differences, however, affected mainly the Patti assemblages, with lower abundance of deposit feeders compared to the other three areas. This is probably linked to differences in sediment composition in the four areas, as pointed out by Cartes et al. (2001) when comparing the trophic structure of peracarid assemblages of the Catalan Sea and the SW Bay of Biscay. The low contribution of deposit feeders in the Gulf of Patti (among amphipods, *Apherusa* spp. a phytoplankton feeder species was the most abundant) could be a result of the lack of river discharges in the area, as previously observed. However a positive correlation was observed between some fractions of SOM and suprabenthos biomass, such as proteins, lipids and the biopolimeric fraction of carbon (C-BPF). Particularly the Gulf of Castellammare presented a higher concentration of C-BPF, proteins and total carbohydrates in the sediment (Lab. Ecologia Marina-Univ. of Palermo, 2006) and it could be explained the higher percentage of infaunal and deposit feeders (i.e cumaceans, *W. rectirostris* and *Monoculodes griseus* among amphipods, *P. bispinosus* and *G. rhomboides* among decapods) in this area. The protein concentration in sediments defines the trophic characteristic of a system and the organic nitrogen is considered as the main limiting factor for deposit-feeders (Danovaro et al., 1999b). Therefore the enrichment of sediment OM positively influenced benthic communities (Manini et al., 2000) and could be also enhance a more structured and abundant suprabenthic community (Cartes et al., submitted).

3.4.3 Stable isotopes analysis

Our preliminary analysis using stable isotopes suggests a relatively complex food web in the whole area in which different food sources were observed. $\delta^{13}C$ was a good indicator of benthic and planktonic source of carbon because invertebrates associated with benthic food chains were relatively enriched with ^{13}C and easily distinguished from the plankton (Jennings et al., 1997). As previously observed in chapter 2, some suprabenthic species (i.e. mysids and copepods) exhibited depleted values of $\delta^{13}C$, indicating a planktonic source of material for these species; cumaceans and amphipods displayed more enriched $\delta^{13}C$ values, confirming the detritivorous habits. In general, as concern the results of the whole area, they confirm the pattern yet observed in the Gulf of Castellammare, with at least two clear trophic guilds (see chapter 2 for details). Within sites, the data suggested that individuals of the same species were adopting similar feeding strategies, as previously observed by Jennings et al. (1997) for higher trophic levels off Mallorca coasts. However a certain degree of spatial variability was observed in stable isotopes signatures within areas.

Although the mean trophic level of the bulk of suprabenthic species was higher in Castellammare, this difference was not significant and seemed to be related with a variety of environmental variables. The values obtained for Patti samples was comparable with the two unprotected areas, therefore no effect of protection on suprabenthic communities was evidenced using stable isotopes ecology.

The number of trophic levels identified within the suprabenthic community of the four Gulfs, assuming a conservative trophic enrichment of *ca* 3.0 ‰ (Post, 2002) between consumers and their diet, were at least three, as previously observed only for the Gulf of Castellammare (chapter 2). Some local differences occurred at species level: *P. bispinosus*, *L. sueri*, *G. rhomboides* and *E. elegans* showed more enriched $\delta^{15}N$ values in the Gulf of Castellammare. This can be due to multiple reasons: concerning filter-feeders such as *E. elegans* it can be attributed to the higher values of primary production recorded in the Gulf, which can sustain a more abundant and diversified plankton communities (Kivi et al. 1996). As regards infaunal and deposit feeders (e.g. *G. rhomboides* and *P. bispinosus*) the higher value could be due to the higher trophic conditions of SOM in this area, particularly enriched of proteins and carbohydrates, more available for benthic consumers (Mannini et al., 2000) and to the higher terrestrial POM inputs into the benthos due to river load. In fact Darnaude et al. (2004) found that terrestrial influence in deltaic areas may be greatest at intermediate depths (30–50 m). *Lesueurigobius sanzoi* was found to feed mainly on crustaceans, *P. bispinosus*, mysids and *G. rhomboides* being among its prey (Paulo-Martins et al., 2004). Therefore the higher $\delta^{15}N$ value observed for *L. sueri* is a consequence of the higher trophic level of its prey.

Annex 3-1. Mean abundance (N/100m^2) of species collected by MG sledge in four Gulfs of Northern Sicily. CDG: Castellammare; PA: Patti; TI: Termini Imprese; SA: S. Agata

Area	**CDG**	**PA**	**TI**	**SA**
Taxon				
CNIDARIA				
Celophyes appendiculata	0,36	0,48	-	-
CRUSTACEA				
Decapoda				
larvae	40,10	21,73	25,40	72,62
adult				
Alpheus glaber	2,91	0,55	1,02	0,21
Athanas nitescens	-	-	0,11	0,11
Chlorotocus crassicornis	-	-	0,32	-
Hippolite holthuisi	-	-	0,13	-
Parapenaeus longirostris	0,33	0,15	0,88	2,52
Periclimenes sp.	0,60	0,58	-	-
Philocheras bispinosus	7,14	0,18	5,82	3,55
Pontocaris lacazei	0,35	-	0,11	0,41
Processa sp.	-	-	0,16	-
Crangonidae unid.	0,73	-	-	-
Anapagurus sp.	0,75	-	0,11	-
Ebalia tuberosa	0,71	-	0,11	-
Galathea intermedia	0,40	-	-	0,43
Goneplax rhomboides	4,78	0,18	0,32	0,11
Liocarcinus spp.	0,20	-	-	-
Macropodia rostrata	0,19	-	-	0,21
Pilumnus spinifer	-	-	-	-
Upogebia typica	-	-	-	0,11
Inachinae unid	0,20	-	0,11	-
Majidae unid	0,20	-	-	-
Paguridae unid	-	-	0,45	-
Brachyura unid	-	-	0,11	-
Decapoda unid	-	1,22	-	-
Amphipoda Gammaridea				
Ampelisca spp.	6,12	0,75	1,78	6,63
Amphilochus sp.	-	0,14	-	-
Apherusa vexatrix	25,04	9,37	0,13	0,61
Corophium sp.	0,35	0,15	-	1,35
Eriopisa elongata	0,16	-	0,16	0,53
Eusirus longipes	-	-	0,13	-
Harpinia dellavallei	-	-	0,51	-
Harpinia sp.	44,80	1,29	6,55	12,53
Iphimedia eblanae	-	0,19	-	-
Iphimedia minuta	0,33	-	-	-
Leptocheirus mariae	0,99	7,64	-	1,44
Leucothoe lilljeborgi	-	-	0,11	0,30
Liljeborgia dellavallei	0,51	-	-	-
Lyssianassidae unid.	0,66	-	-	-
Maera grossimana	-	-	0,16	-
Maera schmidti	0,99	-	0,11	0,21
Maera sodalis	-	-	0,13	0,91
Monoculodes griseus	25,82	0,43	4,15	4,22
Oedicerotidae	0,35	-	-	-
Orchomenella nana	0,35	-	0,64	-
Paraphoxus oculatus	7,88	0,00	0,70	0,89

Area	**CDG**	**PA**	**TI**	**SA**
Taxon				
Amphipoda Gammaridea				
Perioculodes longimanus	13,54	1,55	1,19	3,30
Photis longicaudata	0,16	-	-	-
Rhachotropis integricauda	2,42	-	5,26	1,07
Stegocephalidae	0,40	-	-	-
Stenothoe sp	0,18	-	-	-
Synchelidium maculatum	5,56	0,34	0,46	6,84
Tryphosites longipes	1,02	-	0,16	0,23
Trischizostoma nicanse	0,60	-	-	-
Westwodilla rectirostris	49,37	0,95	23,67	21,74
Gammaridea unid.	33,54	3,58	6,41	8,06
Amhipoda Hyperiidea				
Hyperia schizogeneios	0,55	0,15	-	-
Amphipoda Caprellidea				
Phtisica marina	1,93	-	0,93	23,78
Caprellidae ind	0,55	-	1,49	0,85
Isopoda				
Idotea linearis	-	-	-	0,23
Gnathia sp. (Larvae)	19,53	0,89	0,11	1,05
Rocinela dumerilii	2,72	-	0,32	-
Rocinela granulosa	-	-	2,07	0,41
Cymothoidae	-	-	0,16	0,30
Tanaidacea				
Apseudes latreilli	9,56	-	0,34	-
Apseudes spinosus	2,57	0,15	0,24	-
Leptognathia sp.	0,87	-	-	-
Pseudoparatanais sp.	1,79	-	-	-
Cumacea				
Bodotria scorpiodes	-	-	-	0,11
Campylaspis glabra	1,63	-	-	0,23
Diastylis doriphora	5,46	-	-	-
Diastylis neapolitana	-	-	0,95	0,46
Diastylis rugosa	0,35	0,15	-	-
Diastyloides serrata	51,25	1,02	3,70	5,48
Diastyloides bacescoi	0,20	-	-	-
Eudorella nana	-	1,24	-	-
Eudorella truncata	-	-	0,45	-
Iphinoe serrata	-	0,15	0,75	0,23
Leucon affinis	9,48	-	0,35	0,42
Leucon mediterraneus	21,93	1,86	3,42	3,84
Leucon siphonatus	3,88	-	0,53	-
Cumacea ind	23,80	0,31	1,06	1,29
Mysidacea				
Acanthomysis longicornis	11,78	0,15	6,99	5,25
Anchialina agilis	0,92	-	1,07	3,69
Erythrops elegans	147,43	5,99	40,35	6,25
Haplostylus normanni	1,60	-	1,34	0,23
Leptomysis gracilis	27,07	3,09	61,70	135,55
Lophogaster typicus	-	-	0,16	-
Mysidopsis angusta	4,13	0,65	-	-
Mysidopsis gibbosa	1,54	-	0,36	-
Mysidacea unid.	7,23	-	26,92	24,75

Area	**CDG**	**PA**	**TI**	**SA**
Taxon				
Copepoda				
Copepoda unid	78,17	91,66	55,91	140,01
CHELICERATA				
Pycnogonida	0,58	0,15	0,11	0,72
SIPUNCULIDA				
Sipunculida unid.	14,46	0,54	0,64	3,37
MOLLUSCA Cephalopoda				
Sepieta oweniana	-	-	0,16	-
Sepieta sp	-	-	0,16	-
Sepiolidae	0,18	0,48	0,96	0,22
Cephalopoda	-	0,15	0,11	-
CHAETHOGNATA				
Chaethognatha unid.	-	10,70	6,80	5,00
Osteychtyes				
larvae	18,29	5,72	13,99	49,86
adult				
Arnoglossus laterna	0,53	-	-	-
Gaidropsaurus biscayensis	-	-	0,48	-
Gobius niger	0,18	-	0,00	-
Leseurogobius friesi	-	-	1,64	-
Leseurogobius sueri	0,53	-	2,32	0,46
Pomatochistus sp.	-	-	-	0,46
Serranus hepatus	1,68	-	0,13	-
Symphurus nigrescens	1,68	-	5,37	2,82

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Chapter 4

Spatial variations of feeding habits and trophic level of three coastal demersal fish inhabiting muddy bottoms of Northern Sicily. A gut-contents and stable-isotopes analysis.

Abstract

The feeding intensity and the diet of *Pagellus erythrinus*, *Arnoglossus laterna* and *Merluccius merluccius* were studied along four gulfs of northern Sicily (Western Mediterranean) in spring 2005. All the specimens were collected from hauls carried out on muddy terrigenous bottoms (VTC assemblage, *sensu* Pérès and Picard, 1964) from 40 to 80 m of depth. Among the four gulfs (Castellammare, Termini Imerese, S.Agata and Patti) two (Castellammare and Patti) were declared "*fishing exclusion zone*" and trawl fishery was banned since 1990, by a Regional Act.

Polychaetes and decapods (mainly the brachyuran crab *Goneplax rhomboides* and the alpheid *Alpheus glaber*) were the preferred prey of *P. erythrinus* in the four gulfs. Differences in diet of samples from protected and unprotected areas were evident. *P. erythrinus* exhibited a more specialist diet in the protected gulfs. *A. laterna* fed mainly on decapods (*G. rhomboides*) and gobiid fish (*Lesueurigobius sueri*). Ontogenetic changes were evident with juveniles mainly preying on suprabenthic species, e.g. mysids and small decapods. Spatial differences occurred in scald fish diet, but no protection effect was evident. Suprabenthic species, natantian decapods and fish were dominant in the diet of juveniles of European hake, *M. merluccius.* Juveniles smaller than 160 mm of total length mainly fed on crustaceans while a shift to a strongly pelagic diet occurred in larger specimens. In the Gulf of Castellammare, juveniles of hake mainly preyed on Clupeiformes, probably due to the higher production of this Gulf that may sustain a more abundant zooplankton community, compared with the other gulfs or to an occasional and super-abundant presence of pelagic fish at the time of sampling. Low dietary overlap values were generally recorded implying high resource partitioning, the three species pertained to three well differentiated trophic guilds. A high selectivity for crustacean decapods is a widespread feeding behaviour in the three fish, although they differed in the percentage of consumption and partially in the species selected. Diets and food partitioning among the three species vary in time directionally (ontogenetic development) and spatially (along a longitude scale). Juveniles of *A. laterna* mainly exploited suprabenthic species, while juveniles of *M. merluccius*, in our study preyed on natantian decapods, thus no overlap existed. Although adult specimens of *A. laterna* and *P. erythrinus* (the only considered in our study) seemed to feed on the same resources (e.g. *Goneplax rhomboides* and *A. glaber*), the degree of overlap was low,

because other prey items were differently exploited by the two species: polychaetes by *P. erythrinus* and *L. sueri* by *A. laterna*.

δ^{15}N values consumers varied from 11,87 (*P. erythrinus*) to 9,2 (juveniles of *M. merluccius*). *P. erythrinus* exhibited a trophic level (TL) of 4,85 and *A. laterna* of 4,25; specimens of *M. merluccius* smaller than 160 mm had a TL=4 while hakes larger than 160mm exhibited a higher TL of 4,18. The strong correlation obtained for fish and suprabenthos of the Gulf of Castellammare is indicative of a single type of primary source material i.e. detritus supporting this trophic web.

4.1 Introduction

An important aspect of the ecology of fish populations is their diet (Ortega and Salas 1988). Feeding is one of the main activities that influences the fitness of the species (Rincon-Cinca, 1993) since growth depend on food supply (Beverton and Holt, 1954). The productivity of individuals and populations is influenced by the quality and quantity of the food they are able to obtain (Bowen, 1996).

Partitioning of resources between coexisting species has been suggested as one of the processes responsible for structuring populations and, consequently, communities. Data compiled by Ross (1986) showed that, contrary to terrestrial organisms for which habitat segregation is more important, for assemblages of fishes and other aquatic animals, trophic separation is much more meaningful. According to Gerking (1994), partitioning of food resources is the main factor in the development of fish communities in nearly all aquatic habitats. Although there have been many detailed studies of dietary composition and stomach contents in Mediterranean littoral fishes (Bell and Harmelin-Vivien 1983; Khoury 1987; Rosecchi and Nouaze 1987), the functioning of whole littoral ecosystems and ultimate sources of materials for their subsistence have rarely been considered (Ros et al. 1985).

In this chapter we studied the feeding ecology of three littoral fish species at a large spatial scale, comprising four gulfs of the northern Sicily: *Pagellus erythrinus*, *Arnoglossus laterna* and juveniles of *Merluccius merluccius* were chosen as representatives of main feeding strategies or guilds found in fish communities.

Knowledge of the feeding ecology of non commercial, as well as commercial species is essential for implementing a multi-species approach to fishery management (Gulland, 1977).

The common pandora (*Pagellus erythrinus*) (Linnaeus,1758) is a demersal marine fish distributed along the European and African coasts of the Atlantic Ocean, from Norway to Angola, and around Canary Islands. It is also present in the Mediterranean and Black seas (Bauchot and Hureau, 1986; Mytilineaou, 1989). Its diet has been widely investigated in the Mediterranean sea (Ardizzone and Messina, 1983; Rosecchi, 1983; Caragitsou and Papaconstantinou, 1988; Andaloro and Giarritta, 1983; Benli et al., 2001). While previous investigations provided information on the general or seasonal diet composition of red Pandora, this study documented spatial variation in its diet and explored the possible links to protection effects.

Scaldfish (*Arnoglossus laterna*) commonly inhabits sandy and muddy bottoms at depths between 10 and 200m (Nielsen, 1973) and spawns between March and November in the Mediterranean (Tortonese 1975). *A. laterna* (Walbaum, 1792) grows to a maximum of 19 cm in the Mediterranean waters (Fisher, 1987). Remarkably few investigations are presented on the food items and the diurnal feeding behaviour of *A. laterna* (Gibson and Ezzi, 1980; Avsar, 1993; Cabral et al, 2002). In general all these studies did not take in account the ontogenetic shift occurred in *A. laterna*, with juveniles mostly preying on suprabenthic resources. Only Darnaude et al. (2001) examined the diet of juveniles of *A. laterna* in a shallow sandy area. These authors found a high predation on bivalves and amphipods by juveniles of scaldfish.

Because of its economic interest, the diet of the European hake *Merluccius merluccius* has been widely studied (e.g. Froglia, 1973; Velasco & Olaso, 1998). Prey comprises benthopelagic organisms with ontogenic changes in the diet. Small juveniles prey on small crustaceans (euphausiids, natantian decapods and mysids), while fish assume an increasing importance in the diet with increasing size of hake. The smallest juveniles seem to move up the water column at night (Orsi-Relini et al., 1997) probably to feed. Food intake peaks at sunset and morning in the Adriatic Sea (Froglia, 1973). Studies on hake feeding have mainly been focused on the description of ontogenic, seasonal and bathymetric changes in the diet at a local scale, with little attention to variables affecting the feeding and food at wide spatial scales (Cartes et al., 2004).

The use of stable isotopes of nitrogen and carbon for the study of trophic interactions is now common in aquatic ecosystems (Pinnegar and Polunin, 2000). Analysis of stable isotope composition provides an indication of the origin and transformations of organic matter. Stable isotopes integrate short-term variation in diet and thus are less subject to temporal bias. In fish, tissue turnover times may be as slow as 0.1–0.2% day^{-1} (Hesslein et al. 1993). The δ^{15}N in tissues of consumers is typically greater by 3‰ relative to their prey, and stable N isotope data have been used to define the trophic levels of organisms (Owens 1987). In contrast, tissues tend to be rather weakly enriched in ^{13}C as trophic level increase (less than 1‰), and ^{13}C may thus act as a useful indicator of material sources of production. This method was also applied to study the effects of bottom trawling on the trophic structure of infaunal and epifaunal benthic communities (Jennings et al., 2001) and to detect if differences occurred in target species trophic levels before and after the closure to fishing (Badalamenti et al., 2002). Despite of this, results of stable isotope often need the knowledge of dietary composition by gut content analyses to be correctly interpreted. Hence, the importance of the simultaneous study of gut contents and stable isotopic composition.

Therefore, the present chapter describes the feeding habits of three fish species inhabiting coastal muddy bottoms of Northern Sicily. These three species are presumably representative of three different trophic guilds: *Pagellus erythinus* actively forages on benthic prey

(Ardizzone and Messina, 1983; Rosecchi, 1983; Caragitsou and Papaconstantinou, 1988; Andaloro and Giarritta, 1983; Benli et al., 2001), juveniles of *Arnoglossus laterna* preyed mainly on suprabenthic prey while adults on epibenthic species (Gibson and Ezzi, 1980; Avsar, 1993; Cabral et al, 2002; Darnaude et al., 2001) and juveniles of *Merluccius merluccius* also on suprabenthos and pelagic prey (Maynou et al, 2003;Cartes et al., 2004; Carpentieri et al, 2005). The importance of spatial changes in food resources available for fish and the determination of changes in trophic level and in source of carbon elucidated by stable isotopes analysis of $\delta^{13}C$ and $\delta^{15}N$ are important aspects of this chapter hardly considered in previous similar approaches.

Diet description was useful to highlight two other important aims of this chapter.

First we investigated possible effect of fishing exclusion zones on diets of three fish species considering their particular feeding behaviour. Trawl fishing has several and severe effects on marine ecosystems: changes in predator-prey relationships and the consequent change of food-web structure (Jones, 1992; Lindeboom, 1995; Kaiser et al., 1996); re-suspension of superficial sediments (Palanques et al. 2001, Smith et al. 2003); reduction of habitat complexity and alteration of benthic community structure (Turner et al. 1999; Kaiser et al. 2000b, Callaway et al. 2002, Kaiser et al. 2002). We expected that, if a protection effect exists, it should be more evident on fish species that prey on benthic organisms (e.g. *P. eryhtrinus*) than on species with a different feeding habit (e.g. *A. laterna* and *M. merluccius*).

Finally, after coupling stomach contents and stable isotopes data with biomass, abundance and stable isotopes signatures of available resources, an attempt to describe the whole trophic web of a coastal muddy bottoms community off the Northern coasts of Sicily is given.

4.2 Materials and methods

A detailed description of materials and methods is in Chapter 1.

As far *Merluccius merluccius* is concerned, the diet of juveniles was compared with the availability of trophic resources using data from suprabenthos samples (see chapter 2). Only hakes with less than 250 mm of total length and inhabiting 40-80 m depths were analyzed. This because larger fish were considered adults (250 mm is the smallest size at which mature females were found - Lleonart, 2001) and potential predators of recruits (Bozzano et al., 1997). The trophic resource of juvenile hake consists of small vagile macrofauna (the so-called suprabenthos or hyperbenthos: Mees and Jones, 1997), which was collected with a Macer-GIROQ sledge fitted with one 500 µm-mesh net.

4.2.1 Stomach contents analysis

Immediately after capture, specimens were fixed frozen. Once in the laboratory, they were measured to the nearest mm: total length (TL) for *Pagellus erythrinus*, *Merluccius merluccius* and *Arnoglossus laterna*.

All individuals were weighted to the nearest 0,1 g and dissected for stomach contents analysis. A total of 660 fish from the three species under study were analysed (Table 6.1)

Table 4-1 Number of individuals analysed by area. Length range also included

	CDG	TI	SA	PA	Length range (mm)
Pagellus erythrinus	52	43	51	89	95-270
Arnoglossus laterna	102	91	46	71	50-155
Merluccius merluccius	26	36	48	-	65-250

All prey items were identified to the lowest possible taxonomic level, counted and weighed (precision 0.0001g) after excess moisture had been removed by blotting on tissue paper and, where possible, measured using a micrometer.

4.2.2 Stable isotopes analysis

Detailed description of stable isotopes analysis in Chapter 1.

4.2.3 Data analysis

4.2.3.1 Indices used for stomachs contents analysis

Numerous indices have been described to quantitatively express the importance of different prey in the diets of fish (see Hyslop, 1980; Pianka 1988; Cortés 1997, for a review):

1. Vacuity coefficient (%V): number of empty stomachs expressed as a percentage the total number of stomachs analyzed;
2. Percentage frequency of occurrence (%F): number of stomach in which a food item was found, expressed as a percentage of the total number of non-empty stomachs;
3. Percentage numerical abundance (%N): number of each prey item in all non-empty stomachs in a sample, expressed as the percentage of the total;
4. Percentage gravimetric composition (%W): wet weight of each prey group, expressed as the percentage of the total weight of the stomach contents
5. Index of relative Importance (IRI) (Pinkas et al., 1971) was calculated for each prey group and used to assess preferred diet prey:

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

This index has been expressed as %IRI=(IRI/ΣIRI) x 100. Prey items were sorted in decreasing order according to their %IRI contribution, and then cumulative %IRI was calculated.

6. Stomach fullness (stomach weight/shrimp weight x100);
7. Standard diversity indices calculated for each prey item:
   (i) Trophic diversity (H’): calculated used Shannon-Wiener equation (Shannon & Weaver, 1949):

$$H' = \sum_{i=1}^{n} pi \ln(pi)$$

Where p_i is the proportion of a specific prey category for the n categories of prey listed

(ii) Pielou’s eveness (J’; Pielou, 1966) was calculated according to:

$$J' = H' / \log(S)$$

Where S is the number of species

(iii) The inverse of Dominance (Simpson’s index) was determined as follows:

$$\lambda = 1 - \sum_{i=1}^{n} p_i^2$$

When it is not possible to weight separately all the prey found, percentage volume of stomach contents was estimated using the subjective points’ methods (Swynnerton & Worthington, 1940) for the different prey-species or prey-groups.

Diet was characterised for each species by area.

4.2.3.2 Intraspecific relationships

An nMDS ordination was performed on a Bray-Curtis or modified Gower resemblance matrix based on 4th root transformed and standardized biomass data of stomach contents. Based on the null-hypothesis that there is no differences in the diet of the three species in protected (*fishing* *exclusion zone*) and unprotected areas, a PERMANOVA (Anderson, 2001) design was created on two factors (protection, fixed with 2 levels and areas random with 4 levels, nested in protection). In the case of *M. merluccius*, where no sufficient specimens from Patti were available, an asymmetric design (2 unprotected areas VS one protected area) was made. A PERMDISP test (Anderson, 2006) was then performed on factor resulted significant by PERMANOVA. A SIMPER analysis was applied to each species to determine the most typifying prey species in each area. One-way ANOVAs were used to compare the mean prey number, mean prey weight and fullness among areas. Data were *log* transformed to remove the dependency of the variance on the mean, and in the case of fullness were *arc-sin* transformed (Underwood, 1997). The assumptions of normality and homogeneity were tested using the Kolmogorov–Smirnov test and Cochran's test respectively. Statistical differences in prey occurrence as a function of area were established by applying a chi-squared test (Yates' correction) to the numerically dominant prey (in terms of %N).

4.2.3.3 Interspecific relationships

Dietary overlap between species in the same area was also measured for each species by applying the percentage of similarity used by Schoener (1970):

$$\alpha = 1 - 0.5(\sum|p_{ij}-p_{ik}|)$$

where α is the similarity between diets of species x and y

p_{xi} is the proportion of food category i in the diet of species x

p_{yi} is the proportion of food category i in the diet of species y

Diet overlap increases from 0 to 1 and it is generally considered to be biologically significant when it exceeds 0.60 (Keast, 1978; Macpherson, 1981; Wallace, 1981). This threshold value is rather arbitrary, and other authors assume significant overlap values of 0.80 (Cartes & Sardà, 1989) for inter-specific food overlap. Overlap was calculated using %W values.

Trophic interactions were analysed according to the four areas sampled.

nMDS analysis using Bray-Curtis similarity index (Field et al., 1982) was performed on the standardized %W values of the different groups of prey items to describe overall inter-specific variations in food habits. SIMPER analysis was performed to identify the most typifying prey for each species and the degree of similarity/dissimilarity among them.

A PERMANOVA design was created based on three factors (species fixed with 3 levels; protection fixed with 2 levels and area, random nested in protection, with 3 levels). Since no specimens of *M. merluccius* were available from Patti, the design was asymmetric and based on three areas.

4.2.3.4 Changes in trophic levels

ANOVA was performed to evaluate possible significant changes in δ^{15}N and δ^{13}C values of each species by area. The trophic level (TL) of each species was calculated based on their δ^{15}N data and using copepods as reference material. δ^{15}N values were converted to trophic level based on the assumption that there was a fractionation of 3‰ per trophic level (Minagawa and Wada 1984; Post 2002) and that the base material (copepods) had a trophic level of 2:

$$\mathrm{TL_i}=(\frac{\delta^{15}\mathrm{N}_i-\delta^{15}\mathrm{N}_{\mathrm{ref}}}{3})+2$$

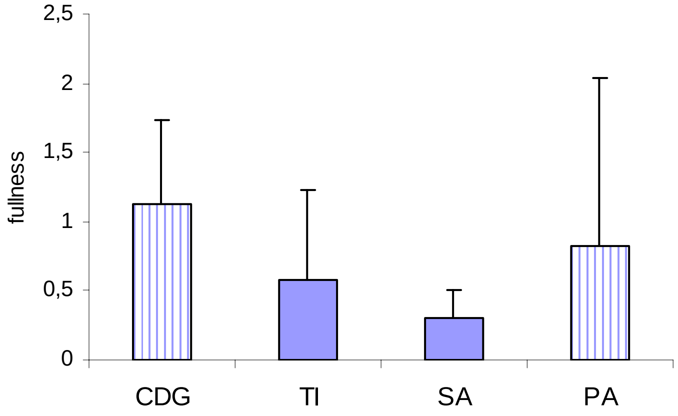
where $\mathrm{TL_i}$ is the trophic level of species *i,* $\delta^{15}\mathrm{N_i}$ is the mean d15N of species *i*, and $\delta^{15}\mathrm{N_{ref}}$ is the mean δ^{15}N of the copepods.

4.3 Results

4.3.1. Diet composition and spatial variation

Pagellus erythrinus

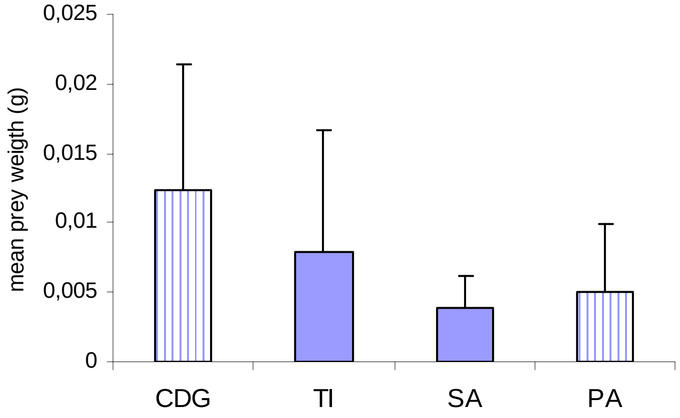
A total of 235 individuals from May 2005 were analyzed, among these 126 (53,6%) had the stomachs empty. From 109 stomachs containing food, 60 categories of prey items were identified (Annex 4-1). Diet chiefly consisted of decapods (63% IRI). In terms of %IRI the brachyuran *Goneplax rhomboides* was the dominant prey. Accordingly, polychaetes (2% IRI), molluscs (1,5% IRI) and fish (6,5% IRI) were the preferential prey items ingested. At a species level the decapods *Goneplax rhomboides* and *Alpheus glaber*, the fish *Leseurogobius sueri* and among polychaetes *Glycera* sp. were the main prey items ingested in terms of %F and %N (Annex 4-1). According to the different indices used to determine the diet, polychaetes, decapods, fish and to a lesser extent molluscs, were the most important prey ingested by *P. erythrinus* in the four areas (Annex 4-1). Decapods were dominant in the four areas, particularly in the Gulf of Termini Imerese (74.69% IRI) and in the Gulf of Patti (66,19% IRI), whereas fish were dominant in the Gulf of S. Agata (15,86 % IRI) (Annex 4-1). The highest number of empty stomachs was found in the Gulf of Patti and the lowest in the Gulf of S. Agata (χ^2=8,00; $p<10^{-3}$). Significant differences were found in mean prey number (K-W= 87.75; $p<10^{-4}$) between the areas, in fullness (ANOVA $F_{1,108}$=12,4 $p<10^{-7}$) and in the mean prey weight (ANOVA $F_{1,108}$=9,97 $p>10^{-6}$). A *post-hoc* comparison evidenced significant differences between fullness of specimens from Castellammare and those from Termini and S. Agata; also the fullness of Patti fish was different from S. Agata (fig. 4-1).



CDG=PA>TI=SA

Figure 4-1 Mean values of fullness among areas (±SD). Post-hoc Tukey test for differences in fullness (on the right). Bars indicated protected areas, full colour indicated unprotected areas.

Moreover the mean weight of prey of samples from Castellammare significantly differed from Patti and S.Agata. (*Post hoc* Tukey test for unequal number of samples) (Fig. 4-2).



CDG=TI>PA=SA

Figure 4-2 Mean values of weight of prey per stomachs among areas (±SD). Post-hoc Tukey test for differences in fullness (on the right). Bars indicated protected areas, full colour indicated unprotected areas.

nMDS showed separation between samples from protected and non protected areas (fig. 4-3).

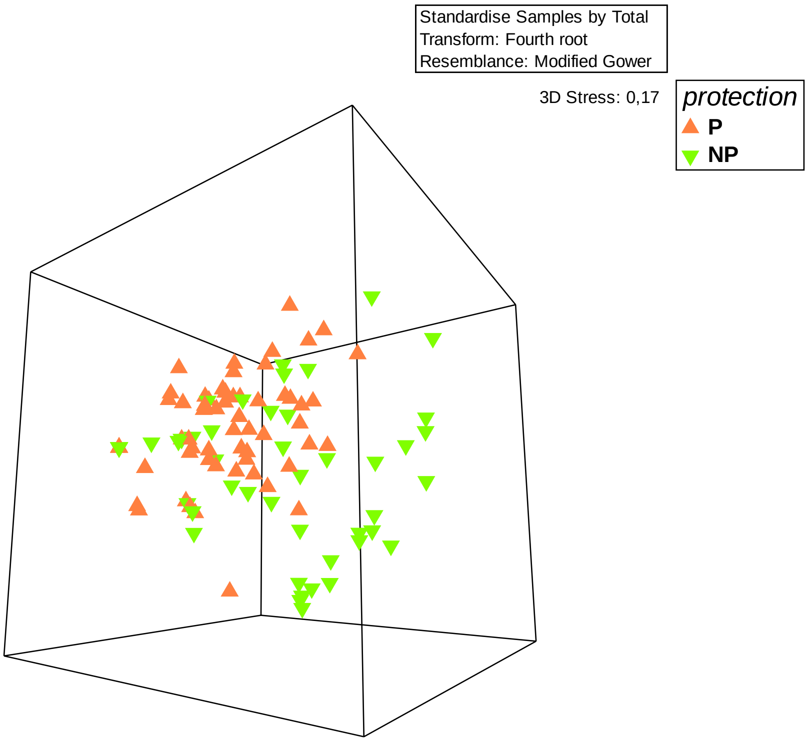


Fig. 4-3. A tri-dimensional nMDS ordination of samples of *P. erythrinus* from protected and unprotectd areas (Stress=0,17)

Symmetrical PERMANOVA provided evidence for protection effect (table 4-2).

Table 4-2. PERMANOVA based on the modified Gower dissimilarities of standardized and 4th root transformed data (71 taxa).

Source	df	SS	MS	Pseudo-F	P(perm)	perms
protection	1	2,762	2,762	3,0755	0,0061	9930
area(protection)	2	1,8066	0,90328	1,4688	0,0632	9902
Res	105	64,575	0,615			
Total	108	69,039				

A PERMDISP test for the homogeneity of dispersion (table 4-4) evidenced higher dispersion for non protected areas samples than for protected. The same test performed on areas evidenced a gradient of increasing dispersion from Castellammare to Termini samples.

Table 4-4 PERMDISP test for samples from protected and unprotected areas (above) and among areas (below). For factor “area” also pair-wise comparisons were given.

DEVIATIONS FROM CENTROID		
Factor Protection		
F: 31,466		
P(perm): 0,001		
MEANS AND STANDARD ERRORS		
Group	Average	SE
P	0,696	1,971E-2
NP	0,849	1,8168E-2
DEVIATIONS FROM CENTROID		
Factor Area		
F: 15,264		
P(perm): 0,001		
PAIRWISE COMPARISONS		
Groups	t	P(perm)
(CDG,PA)	3,572	4E-3
(CDG,TI)	5,417	1E-3
(PA,SA)	2,194	3,7E-2
(PA,TI)	2,91	1E-2
(SA,TI)	1,225	0,239
MEANS AND STANDARD ERRORS		
Group	Average	SE
CDG	0,612	3,05E-2
PA	0,746	2,33E-2
SA	0,816	2,15E-2
TI	0,864	3,45E-2

The feeding habits of *P. eythrinus* varied spatially (fig.4-4). In the Gulf of Castellammare it mainly preyed on polychaetes and decapods, while in the Gulfs of Termini and S. Agata fish were also important in its diet. In the Gulf of Patti molluscs together with decapods were important prey items. In all the gulfs vegetal remains, *Cymodocea nodosa* leaves, foraminiferans and detritus frequently occurred (indicated as “other” in fig 4-4).

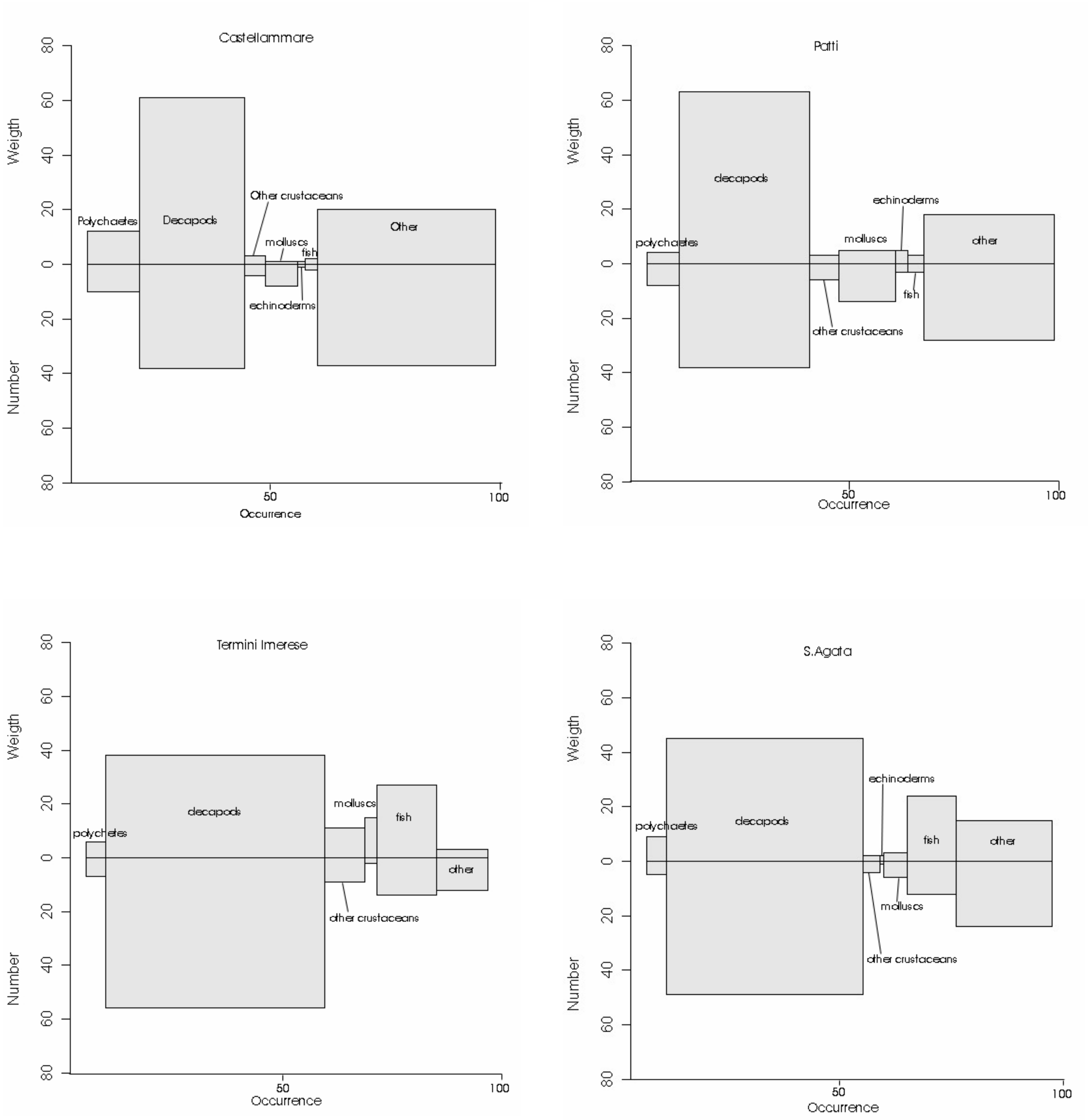


Fig. 4-4. Relative importance of prey groups (IRI >1%) of *P. erythrinus* diet. Areas of rectangles are proportional to the %IRI. %F: percentage contribution by frequency of occurrence; %N: by number; %W: by weight.

The consume (%N) of the brachyuran *G. rhomboides* differed significantly between Termini and Castellammare (χ^2=9,12; *p* 0,003), S.Agata (χ^2=19,4; *p* 10^{-5}), and Patti (χ^2=15,47; *p* 10^{-4}). Chi-squared test revealed significant differences between the ingestion of the decapod *A. glaber*: Castellammare differed from Termini (χ^2=25,81; $p<10^{-5}$) and S. Agata (χ^2=13,62;

p<10^{-4}), and Patti varied from Termini (χ^2=20,9; *p*<10^{-5}) and S.Agata (χ^2=9,63; *p*<10^{-4}). Also the consume of fish significantly differed between the four areas: Castellammare differed from Termini (χ^2=25,81; *p*<10^{-5}) and S. Agata (χ^2=15,92; *p*<10^{-4}), and Patti from Termini (χ^2=23,25; *p*<10^{-5}) and S.Agata (χ^2=13,68; *p*<10^{-4}).

Trophic diversity did not vary significantly in the four areas (mean value 2,71±0,22).

Relatively low overlap was observed between all the areas, except between Castellammare and Patti (Table 4–4).

Table 4-4. Spatial dietary overlap of *Pagellus erythrinus.* Acronyms as in table 4-6.

	CDG	PA	SA	TI
CDG	1	0,75	0,37	0,49
PA		1	0,41	0,42
SA			1	0,48
TI				1

SIMPER analysis showed low values of similarity (table 4-6) between samples per area, ranging from 20,6 to 26,4% in Patti and Castellammare respectively.

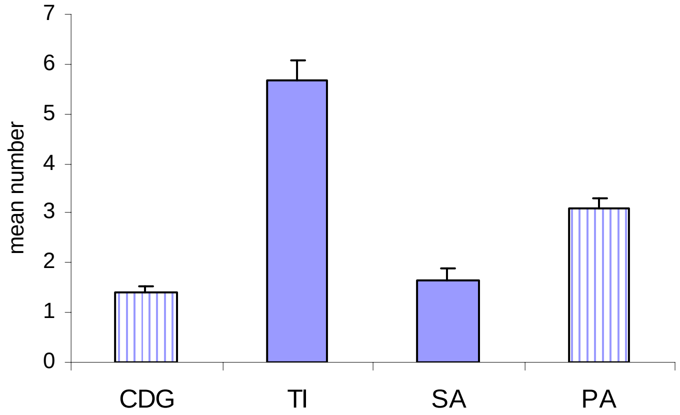
In general *G. rhomboides* was the most typifying species in all the gulfs, its contribution to the similarity ranged from 36,4% in the Gulf of S.Agata to 86,2% in the Gulf of Castellammare. *G. rhomboides* reached the highest contribution in the two protected Gulfs. In the Gulfs of S. Agata and Termini *A. glaber* largely contributed to similarity (28,5 and 25,9 respectively).

Table 4-6. SIMPER for *P. erythrinus* foregut samples from 4 areas of Northern Sicily (cut-off for low contribution 90%)

Castellammare			
Average similarity: 26,43			
	Av.Abund	Av.Sim	Contrib%
Goneplax rhomboides	0,47	22,77	86,15
Termini			
Average similarity: 24,11			
	Av.Abund	Av.Sim	Contrib%
Goneplax rhomboides	0,26	13,61	56,46
Alpheidae	0,16	6,23	25,86
Conger conger	0,18	2,81	11,64
S.Agata			
Average similarity: 20,78			
	Av.Abund	Av.Sim	Contrib%
Goneplax rhomboides	0,15	7,56	36,36
Alpheidae	0,15	5,92	28,46
Unid. Fish	0,14	3,54	17,05
Unid. Decapoda	0,06	1,93	9,27
Patti			
Average similarity: 20,63			
	Av.Abund	Av.Sim	Contrib%
Goneplax rhomboides	0,29	16,48	79,9
bivalves	0,05	1,25	6,07

Arnoglossus laterna

310 individuals were examined, among these and 135 (43,5%) were empty. The percentage of empty stomach significantly varied (p<0,05) between S. Agata and Patti (χ^2=13,63), Patti and Castellammare (χ^2=30,47), Patti and Termini (χ^2=10,73) and between Castellammare and Termini (χ^2=4,88). A total of 175 stomachs were analyzed for diet composition and 32 prey items were identified Annex 4-2). Diet mainly consisted of decapods (28 %IRI) and fish (65 %IRI). Mean prey weight and fullness did not significantly vary among areas, while mean number of prey did (K-W$_{3,134}$=14,41; p<0,05; fig. 4-5).



TI=PA>SA=CDG

Fig. 4-5. Mean number of prey in stomach contents per area (±SD) (Mann-Whitney post-hoc test showed on the right)

A.laterna preyed mainly on decapod and fish in the four areas (fig. 4-6). The proportion (in terms of %IRI) of decapod ingested was higher in Castellammare and S.Agata, while fish were dominant in the diet in Patti; mysids and amphipods were also important prey item in Termini samples.

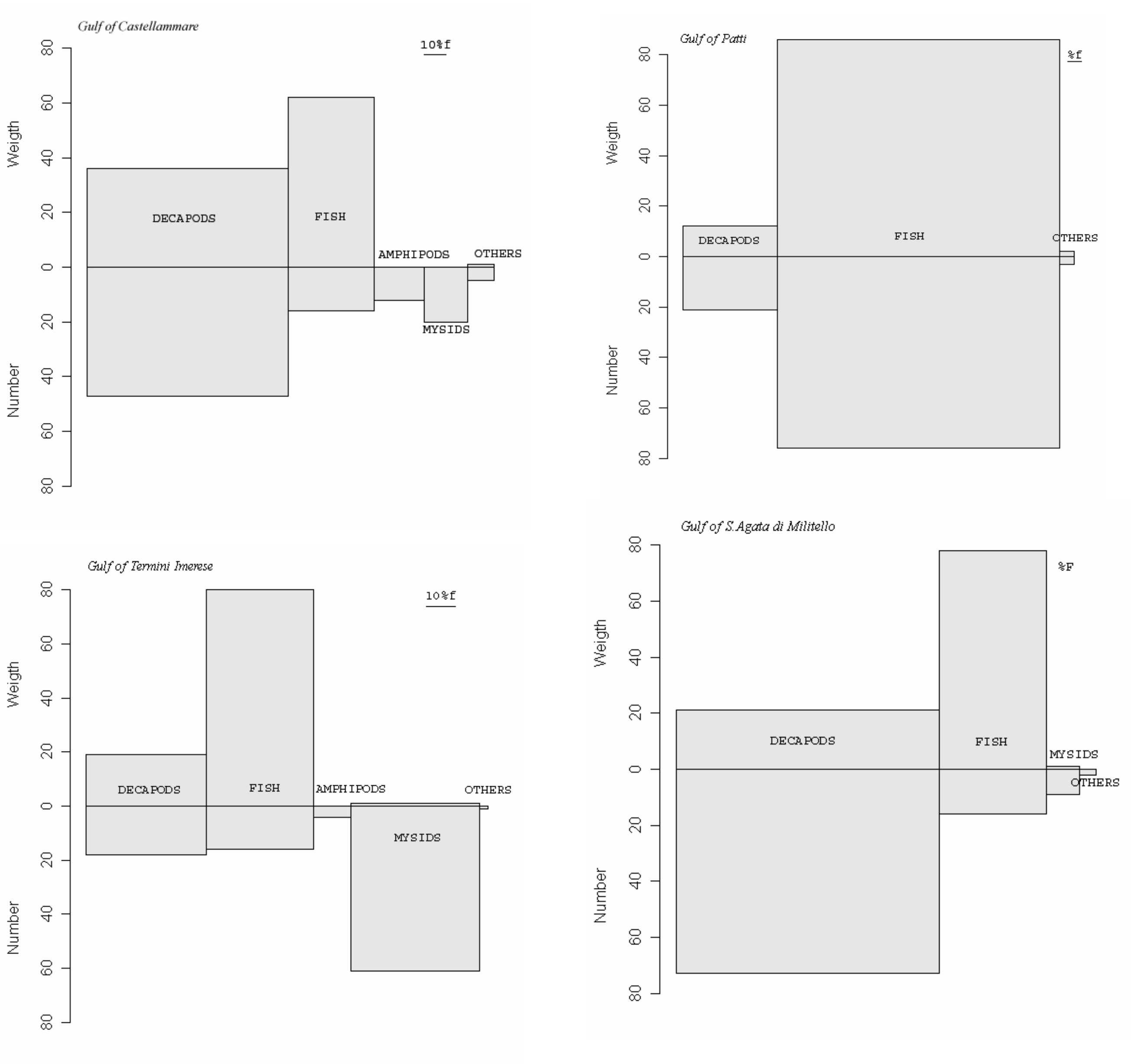


Fig. 4-6. Relative importance of prey groups (IRI >1%) of *A. laterna* diet. Areas of rectangles are proportional to the %IRI. %F: percentage contribution by frequency of occurrence; %N: by number; %W: by weight are given.

nMDS showed slight differences in the diet of *A.laterna* among areas (fig. 4-7). Samples from Castellammare and Termini seemed to be more aggregated with respect to Patti and S.Agata.

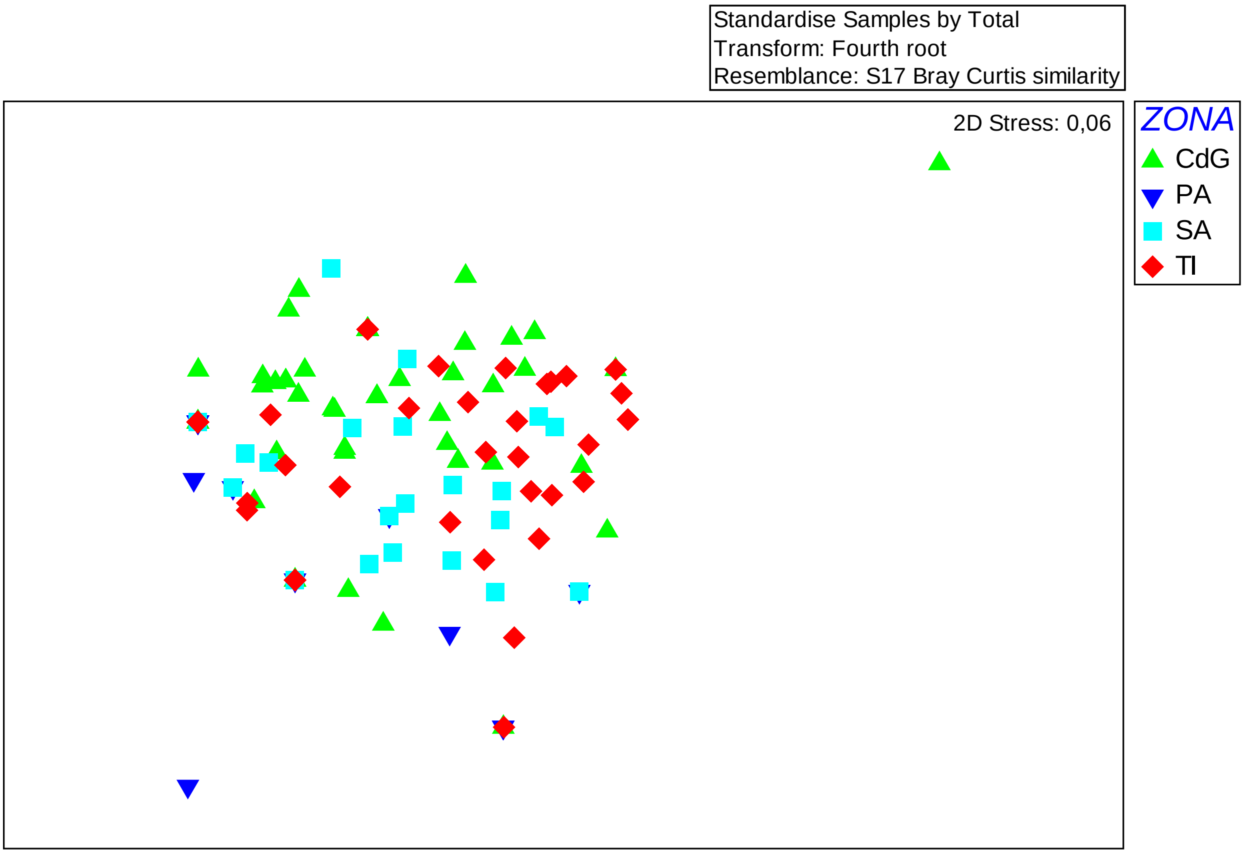


Fig. 4.7 nMDS ordination of foregut samples of *A. laterna* from four gulfs of Norther Sicily.

A MVDISP test proved that samples from Castellammare and Patti were less dispersed (dispersion 0,95 and 0,96 respectively) than samples from Termini and S. Agata (1,07 and 1,12 respectively). A PERMANOVA provided evidence for separation of samples (diets) among areas, but protection effect did not result significant (table 4-7).

Table 4-7. PERMANOVA based on the Bay-Curtis dissimilarities of standardized and 4th root transformed data (32 taxa).

Source	df	SS	MS	Pseudo-F	P(perm)	perms
protection	1	13075	13075	0,60922	0,7218	9956
area(protection)	2	42925	21462	6,0563	0,0001	9938
Res	171	6,0599E5	3543,8			
Total	174	6,7553E5				

SIMPER analysis evidenced the species that more typify *A. laterna* diets in the four areas (table 4-8). In all the areas the preferred prey was the gobiid fish *L. sueri*, it contributed for more than 90% to the diet of *A. laterna* in the Gulf of Patti, for 44% and 60% respectively in the gulf of S. Agata and Termini. In the Gulf of Castellammare *G. rhomboides* was the most typifying species in the diet of scaldfish. MVDISP and SIMPER indicated a more specialist diet for samples from protected areas comparing with samples from non protected areas.

Table 4-8. Simper analysis for *A.laterna* foregut samples from 4 areas of Northern Sicily (cut-off for low contribution 90%)

Group CdG

Average similarity: 27,88

Taxa	Av.Abund	Av.Sim	Contrib%	Cum.%
G. rhomboides	1,61	20,76	74,47	74,47
L. sueri	0,86	5,51	19,75	94,22

Group PA

Average similarity: 31,20

Taxa	Av.Abund	Av.Sim	Contrib%	Cum.%
L. sueri	1,78	28,99	92,93	92,93

Group SA

Average similarity: 15,53

Taxa	Av.Abund	Av.Sim	Contrib%	Cum.%
L. sueri	0,93	6,86	44,16	44,16
A. glaber	0,69	3,27	21,08	65,24
Crangonidae	0,59	1,89	12,15	77,39
P. bispinosus	0,44	1,14	7,35	84,74
G. rhomboides	0,44	0,93	5,98	90,72

Group TI

Average similarity: 19,59

Taxa	Av.Abund	Av.Sim	Contrib%	Cum.%
L. sueri	1,14	11,62	59,29	59,29
Erythrops sp.	0,85	4,03	20,58	79,87
A. glaber	0,48	1,84	9,37	89,24
P. bispinosus	0,31	0,48	2,45	91,69

Thus significant differences (ANOSIM one way factor R=0,253; p<0,05) were evidenced among diets of different size specimens: a clear ontogenetic shift in the diet occurred between small (55-100 mm TL) and large (>105) size specimens (see fig. 4-7).

SIMPER analyses (table 4-9) evidenced those species that most contribute to diet of small and large individuals. Juveniles of *A. laterna* fed on suprabenthic species, i.e. mysids (*Leptomysis gracilis* and *Erythrops* sp.), amphipods (mainly *Ampelisca* sp.) and small decapods (mainly *Philocheras bispinosus*). However small specimens of *G. rhomboides* (carapace length – CL - ranged from 3,3 to 3,8mm; carapace width – CW - ranged from 4,9 to 5,8mm) and *L. sueri* (mean TL=30mm) occurred in stomach contents. Large size specimens preyed mainly on large individuals of *Goneplax rhomboides* (CL ranged from 4,5 to 5,2mm; CW ranged from 6,1 to 9,2mm) and *L. sueri* (mean TL=45mm).

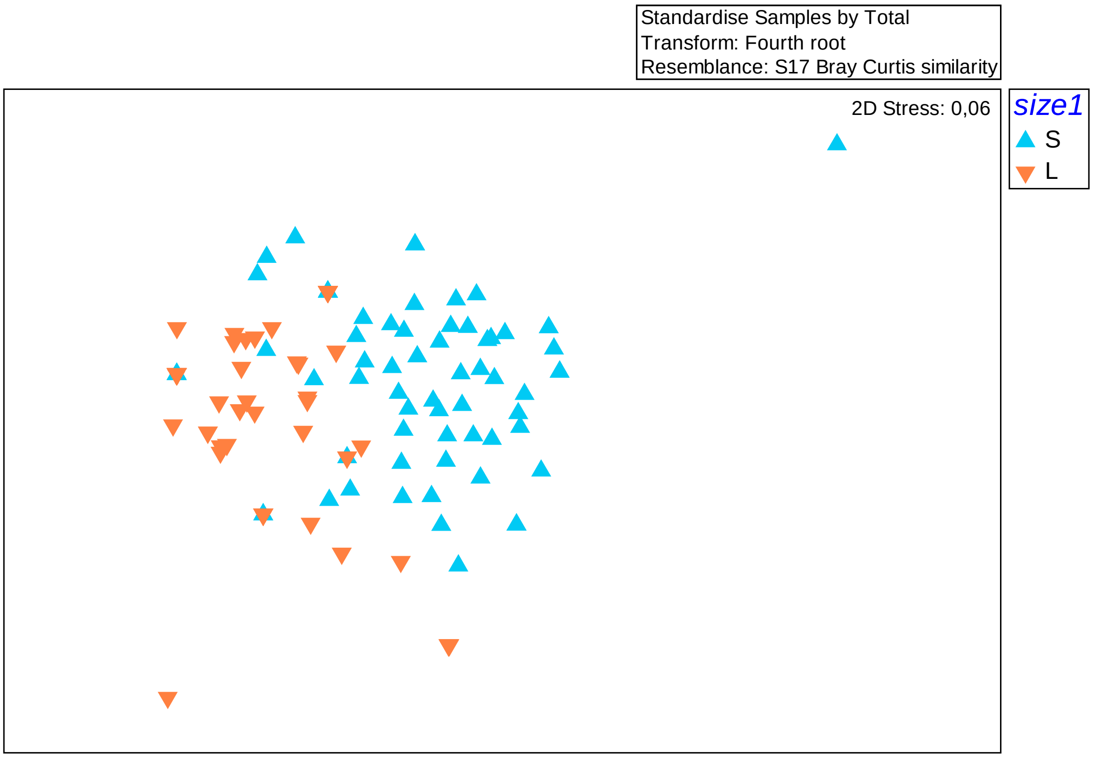


Fig. 4.7 nMDS ordination of foregut samples of *A. laterna* based on two size. S=juveniles (TL<100mm) and L=adults (TL>105 mm).

Table 4-9. Simper analysis of two size classes of *A. laterna* foregut samples (cut-off for low contribution 90%)

Group S

Average similarity: 14,09

Taxon	Av.Abund	Av.Sim	Sim/SD	Contrib%	Cum.%
Erythrops sp.	0,93	4,80	0,40	34,06	34,06
G. rhomboides	0,72	3,32	0,24	23,57	57,63
P. bispinosus	0,61	2,02	0,25	14,32	71,95
Crangonidae	0,46	1,21	0,17	8,59	80,54
L. gracilis	0,37	0,71	0,15	5,01	85,56
Ampelisca sp.	0,34	0,61	0,14	4,30	89,85
L. sueri	0,23	0,36	0,06	2,55	92,40

Group L

Average similarity: 31,89

Taxon	Av.Abund	Av.Sim	Sim/SD	Contrib%	Cum.%
L. sueri	1,63	22,70	0,60	71,19	71,19
G. rhomboides	0,89	6,49	0,30	20,36	91,55

Merluccius merluccius

116 individuals with food in the stomachs, between 65 and 245 mm of total length, were examined for diet composition and 46 prey items were identified (Annex 4-3). Diet mainly consisted of fish (84 %IRI) and decapods (9%IRI).

Fullness varied significantly among areas (ANOVA $F_{1,105}$=3,17; p<0,05 – fig. 4-8), while mean prey weight and number did not .

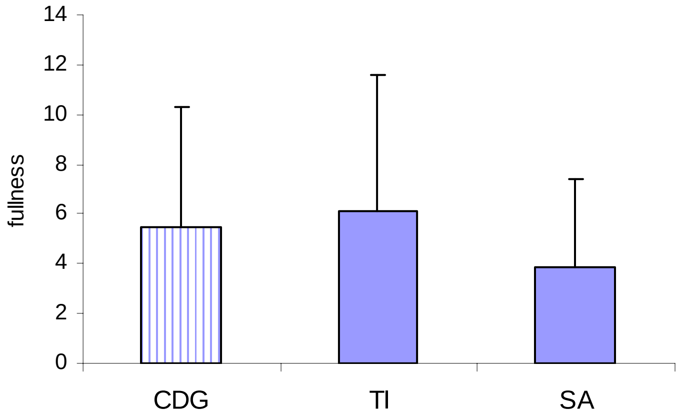


Figure 4-8 Mean values of fullness among areas.

M. merluccius preyed mainly on fish and decapods in the three areas (fig. 4-9). The proportion (in terms of %IRI) of decapod ingested was higher in S. Agata, while fish were dominant in the diet in Castellammare; other crustaceans (e.g. mysids and amphipods) and cephalopods (small sepioliids) were also important prey item in Termini samples.

The consume (%N) of decapods significantly differed between Castellammare and S. Agata (χ^2=8,16; p=0,004). Fish (%N) were mainly predated in Castellammare than in Termini and S. Agata (χ^2=20,83; $p<10^{-5}$ and χ^2=12,05; $p<10^{-4}$ respectively).

Trophic diversity indices indicated a more diverse diet in Termini Imerese and S. Agata than in Castellammare (Table 4-10).

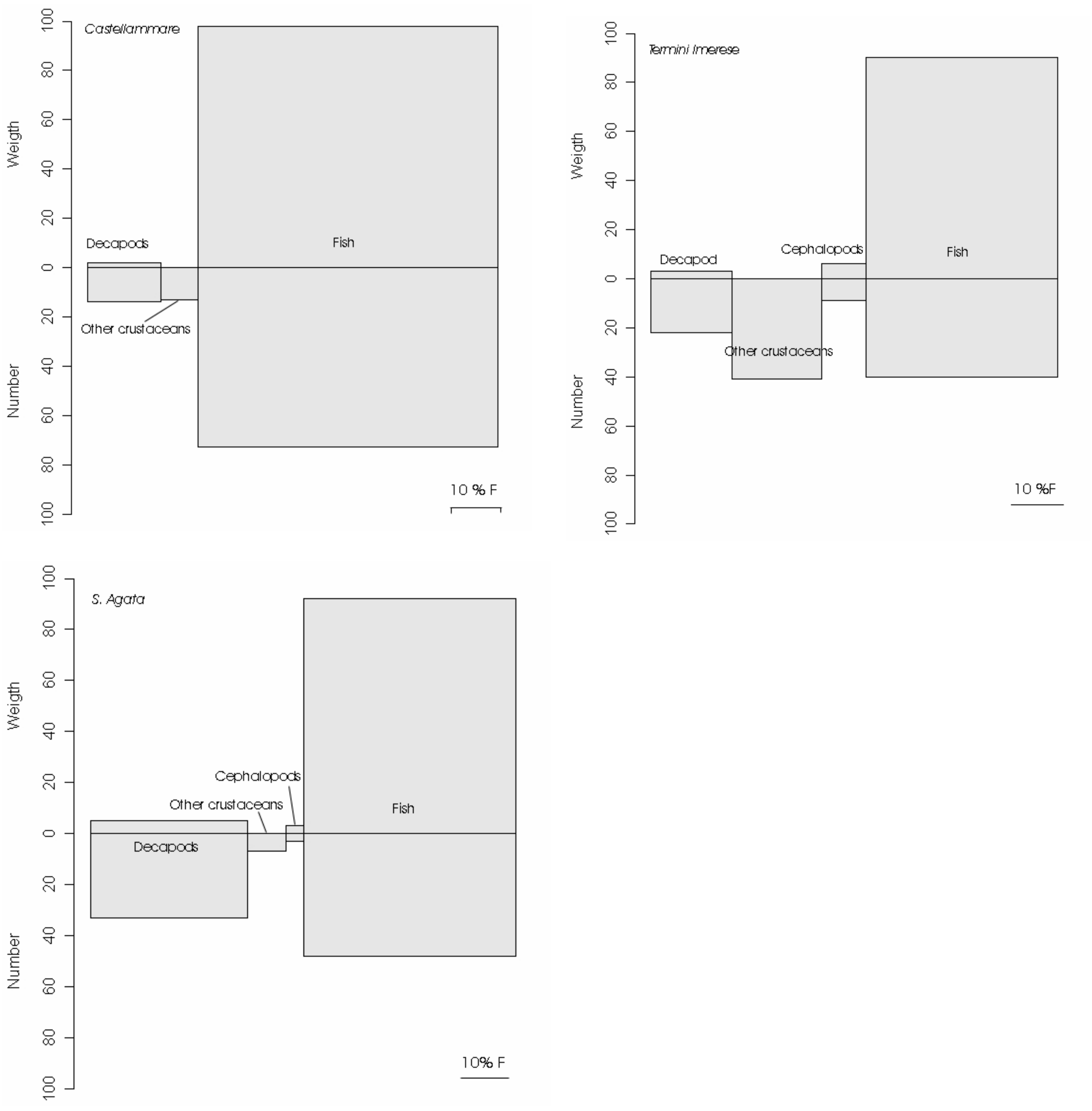


Fig. 4-9. Frequency of occurrence of prey combined with % weight and % number for *Merluccius merluccius* by area (grey areas represent %IRI values)

Table 4-10. Number of species (S), species richness (d), Eveness (J'), Diversity (H') and Dominance (1-λ') of gut contents of *M. merluccius* from three gulfs of Northern Sicily.

Sample	S	N	d	J'	H'	1-λ'
CDG	17,00	100,00	3,47	0,78	2,22	0,84
TI	30,00	100,00	6,30	0,87	2,97	0,94
SA	30,00	100,00	6,30	0,90	3,06	0,95

Differences in the diet of *M. merluccius* among areas (fig. 4-10) were not evident in nMDS analysis.

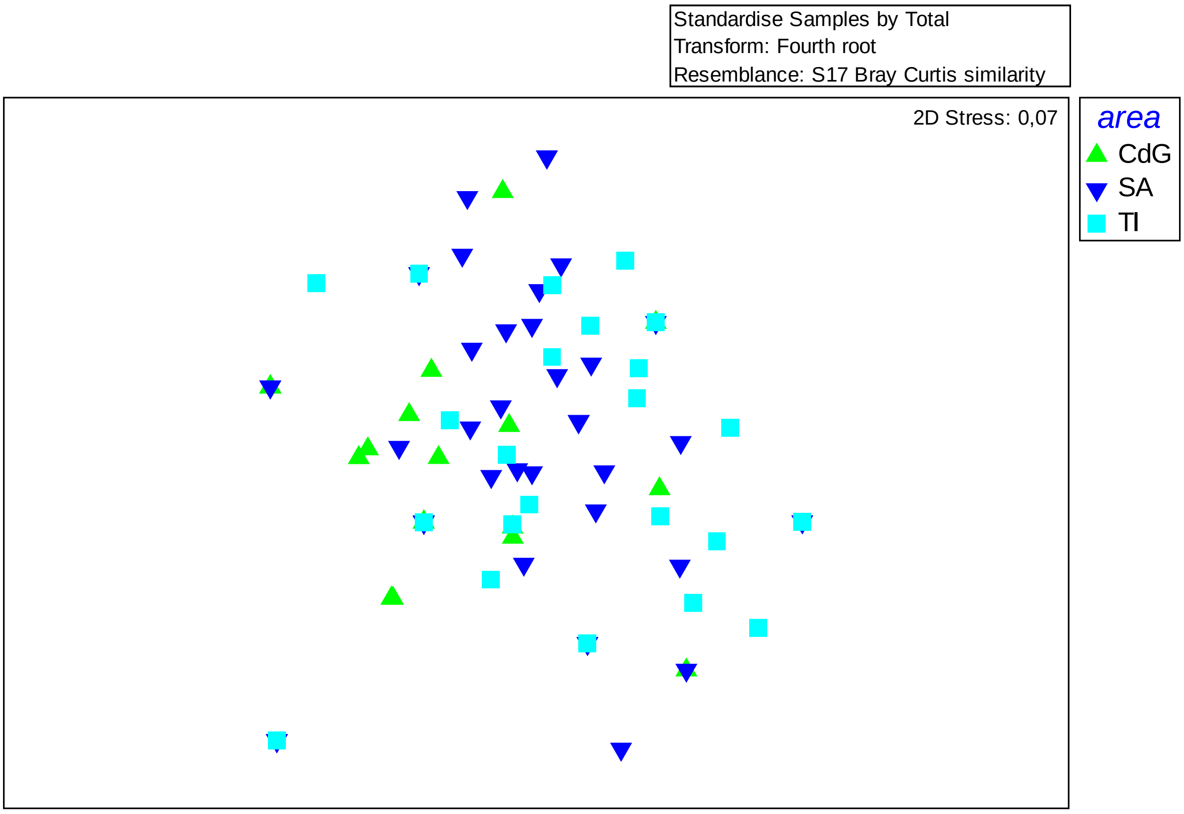


Figure 4-10 nMDS ordination of foregut samples of *M. merluccius* from three gulfs of Northern Sicily.

Asymmetrical PERMANOVA (two factors: protection, fixed with 2 levels and area, random with 3 levels nested in protection) provided evidence for significant differences among diets of European hake (Table 4-11) from Castellammare and from the other two unprotected gulfs, since no area effect was apparent, probably due to a similar diet of *M. merluccius* in the two non protected areas.

Table 4-11. PERMANOVA based on the Bay-Curtis dissimilarities of standardized and 4th root transformed data (46 taxa).

Source	df	SS	MS	Pseudo-F	P(perm)	perms
protection	1	18087	18087	3,451	**0,01**	997
area(protection)	1	5645	5645	1,3684	0,17	998
Res	105	4,3316E5	4125,4			
Total	107	4,5687E5				

This consideration was supported by SIMPER analysis (table 4-12) based on factor area: in the Gulf of Castellammare European hake fed mainly on *Clupea pilchardus* that contributed for 45% to similarity among samples, while in the other two gulfs it preyed mostly on other fish including benthic fish such as *L. sueri* (its contribution ranged from 14% in S. Agata to 50% in Termini), large decapods (e.g. the natantian shrimps *Chlorotocus crassicornis* and *Processa* spp) and sepiolids.

Table 4-12. Simper analysis of of *M. merluccius* foregut samples for factor "area" (cut-off for low contribution 90%)

Group CdG					
Average similarity: 22,56					
Taxon	Av.Abund	Av.Sim	Sim/SD	Contrib%	Cum.%
fish unid.	1,22	11,34	0,48	50,28	50,28
Clupea pilchardus sardina	1,18	10,17	0,38	45,11	95,39
Group TI					
Average similarity: 12,30					
Taxon	Av.Abund	Av.Sim	Sim/SD	Contrib%	Cum.%
fish unid.	0,93	6,63	0,3	53,95	53,95
Lesueurigobius suerii	0,67	2,95	0,21	23,97	77,92
Sepieta sp	0,56	1,95	0,17	15,86	93,79
Group SA					
Average similarity: 9,19					
Taxon	Av.Abund	Av.Sim	Sim/SD	Contrib%	Cum.%
fish unid.	0,74	3,89	0,25	42,32	42,32
Chlorotocus crassicornis	0,52	1,69	0,19	18,4	60,72
Lesueurigobius suerii	0,37	0,72	0,13	7,86	68,59
Processa sp	0,32	0,56	0,11	6,13	74,72
Cepola rubescens	0,33	0,56	0,09	6,06	80,77
Trachurus trachurus	0,27	0,45	0,07	4,91	85,68
Sepieta sp	0,24	0,35	0,07	3,76	89,45
Engraulis encrasicolus	0,2	0,28	0,05	3,02	92,47

The PERMDISP test ($F_{2,108}$=15,95; p=0,006) revealed a high degree of dispersion for samples from unprotected areas (average distance from centroids 64,97) than from protected areas (56,84).

Because of the high consumption on pelagic resources (i.e Clupeiformes) recorded in the Gulf of Castellammare, a non parametric Spearman correlation was performed looking for correlation between fullness of hake and geographical (latitude, longitude, depth and hour of sampling), biological (total length of hakes) and environmental data (surface phytoplankton concentration 4, 3, 2, 1 month before sampling and simultaneously - PP4, PP3, PP2, PP1 and PPsim respectively). Fullness was significantly correlated only with longitude. Spearman correlation was then performed for each gulf separately. No significant correlation was found between fullness of hakes from S. Agata and Termini and all the possible explanatory variables. Whilst, in the gulf of Castellammare fullness of hakes was significantly correlated (Spearman correlation) with geographical coordinates of sampling haul (latitude and longitude; R=0,48 and R=0,44 respectively; p<0,05), depth (R=0,45; p<0,05) and all the measures of primary production (p<0,05). The correlation was negative in the case of PP4, PP3 and PPsim (R=-0,47; p<0,05), while it was positive in the case of PP2 and PP1 (R=0,47; p<0,05).

Though specimens below 250 mm were considered juveniles (Lleonart, 2001), Carpentieri et al. (2005) suggested an ontogenetic shift from a diet dominated by crustaceans (mysids, euphausiids and decapods) towards a fully piscivorous diet around 160 mm TL. Based on these considerations a SIMPER analysis was performed for two size ranges: size 1 included specimens smaller than 160 mm TL and size 2 comprised those larger than 160 mm TL. As a large part of diet consisted of unidentified fish, that did not give any useful information, they were not considered in further analysis.

The gobid fish *L sueri*, natantian decapods and sepiolids were those *taxa* that most typifying the diet of smaller specimens (Table 4-13); pelagic fish (Clupeiformes and *Trachurus trachurus*) but also *L. sueri* were the species that most contributed to similarity of larger specimens. Average dissimilarity between smaller and larger specimens' diets was 94,9%.

Table 4-13. Simper analysis of two size classes of *M. merluccius* foregut samples (cut-off for low contribution 90%)

Group S					
Average similarity: 9,62					
Species	Av.Abund	Av.Sim	Sim/SD	Contrib%	Cum.%
Lesueurigobius suerii	0,74	3,63	0,24	37,75	37,75
Chlorotocus crassicornis	0,53	1,95	0,18	20,25	58,00
Processa sp	0,48	1,57	0,15	16,34	74,33
Sepieta sp	0,36	0,69	0,10	7,15	81,49
Group M					
Average similarity: 8,08					
Species	Av.Abund	Av.Sim	Sim/SD	Contrib%	Cum.%
Clupea pilchardus sardina	0,65	3,49	0,20	43,23	43,23
Engraulis encrasicolus	0,33	0,94	0,10	11,60	54,83
Lesueurigobius suerii	0,36	0,86	0,12	10,66	65,48
Trachurus trachurus	0,33	0,77	0,10	9,51	74,99
Sepieta sp	0,32	0,69	0,10	8,58	83,58

ANOSIM test performed on factor size, considering the two size-classes as below, showed significant (R=0,04; p=0,037), but very low, differences among the diet of smaller than 160 mm LT and larger hakes.

4.3.4 Overall species analysis

Overall, dietary overlap based on Schoener's index revealed a very low dietary affinity between *M. merluccius* and the other two species and also between *P. erythrinus* and *A. laterna* (table 4-14).

Table 4–14. Diet overlap for the three demersal fish species using Schoener's Index. Pery: *P. erythrinus*; Alat: *A. laterna*; Mmer: *M. merluccius*

	Pery	Alat	Mmer
Pery	1	0,204	0,098
Alat		1	0,048
Mmer			1

The degree of overlap between the three species was very low among the studied areas. The diet of *M .merluccius* was clearly separated from the other two species (the index was always lower than 0,2). The overlap was also low between *A. laterna* and *P. erythrinus* particularly in the gulf of Patti (Table 4-15); the index was slightly higher when samples of *P. erythrinus* and *A. laterna* from the Gulf of Castellammare were compared (0,36).

Table 4–15. Diet overlap for the three demersal fish species using Schoener's Index. Pery: *P. erythrinus*; Alat: *A. laterna*; Mmer: *M. merluccius*. Indices were calculated for each area separately. CDG. Castellammare; PA. Patti; SA: S. Agata; TI: Termini.

	Pery-CDG	Alat-CDG	Mmer-CDG
Pery-CDG	1	0,363	0,016
Alat-CDG		1	0,010
Mmer-CDG			1
	Pery-PA	**Alat-PA**	**Mmer-PA**
Pery-PA	1	0,076	-
Alat-PA		1	-
Mmer-PA			1
	Pery-SA	**Alat-SA**	**Mmer-SA**
Pery-SA	1	0,213	0,173
Alat-SA		1	0,074
Mmer-SA			1
	Pery-TI	**Alat-TI**	**Mmer-TI**
Pery-TI	1	0,215	0,136
Alat-TI		1	0,060
Mmer-TI			1

SIMPER analysis showed the more characteristic prey for each species (table 4-16): the brachyuran crab *G. rhomboides*, that contributed for 79,8%, and the alpheid *A. glaber* were the most typifying species of *P. erythrinus* diet. Scald fish diet was dominated by the fish *L. sueri* (65,3%) but also by *G. rhomboides* and *A. glaber*, though they contributed to a lesser extent (14,5 and 9,6 respectively). The similarity degree is very low in *M. merluccius* (11,1%), its diet was mainly composed of fish (both benthic and pelagic) and natantian decapods.

Table 4-16a. SIMPER analysis within species (cut off for low contributions 90% in the case of single species; for the pair-wise comparison only those species who contributed for at least 3% to dissimilarity were given)

Group *P. erythrinus*					
Average similarity: 19,55					
Species	Av.Abund	Av.Sim	Sim/SD	Contrib%	Cum.%
G. rhomboides	1,52	15,59	0,58	79,76	79,76
A. glaber	0,53	1,73	0,21	8,84	88,6
Unid. Fish	0,27	0,38	0,09	1,92	90,52
Group A. laterna					
Average similarity: 19,78					
Species	Av.Abund	Av.Sim	Sim/SD	Contrib%	Cum.%
L. sueri	1,21	12,91	0,42	65,27	65,27
G. rhomboides	0,61	2,88	0,2	14,55	79,82
A. glaber .	0,5	1,9	0,16	9,59	89,4
Erythrops sp.	0,36	0,71	0,13	3,61	93,02
Group *M. merluccius*					
Average similarity: 11,11					
Species	Av.Abund	Av.Sim	Sim/SD	Contrib%	Cum.%
Unid. Fish	0,92	6,54	0,32	58,89	58,89
L. sueri	0,43	1,21	0,14	10,89	69,78
C. pilchardus	0,34	0,89	0,1	7,97	77,76
Sepieta sp.	0,28	0,56	0,09	5,03	82,79
C. crassicornis	0,28	0,49	0,11	4,43	87,22
Processa spp	0,22	0,29	0,08	2,58	89,8
E. encrasicolus	0,18	0,23	0,05	2,07	91,87

Table 4-16b. SIMPER analysis between species (cut off for low contributions 90% in the case of single species; for the pair-wise comparison only those species who contributed for at least 3% to dissimilarity were given)

Groups Pery & Alat

Average dissimilarity = 90,57

	Group Pery	Group Alat				
Species	Av.Abund	Av.Abund	Av.Diss	Diss/SD	Contrib%	Cum.%
G. rhomboides	**1,52**	0,61	17,29	0,98	19,09	19,09
L. sueri	0,03	**1,21**	14,21	0,77	15,69	34,78
A. glaber	0,53	0,5	9,11	0,64	10,06	44,84
Unid. Fish	0,27	0,19	4,33	0,41	4,79	49,63
Erythrops sp.	0	0,36	3,25	0,38	3,59	53,22

Groups Pery & Mmerl

Average dissimilarity = 97,57

	Group Pery	Group Mmerl				
Species	Av.Abund	Av.Abund	Av.Diss	Diss/SD	Contrib%	Cum.%
G. rhomboides	**1,52**	0	17,3	0,96	17,73	17,73
Unid. Fish	0,27	**0,92**	11,17	0,7	11,45	29,17
A. glaber	0,53	0,09	5,86	0,53	6,01	35,18
L. sueri	0,03	0,43	4,71	0,41	4,83	40,01
C. pilchardus sardina	0	0,34	3,85	0,34	3,95	43,96
Decapoda Natantia	0,26	0,13	3,56	0,41	3,65	47,6
S. oweniana	0,03	0,28	3,42	0,32	3,5	51,11

Groups Alat & Mmerl

Average dissimilarity = 94,12

	Group Alat	Group Mmerl				
Species	Av.Abund	Av.Abund	Av.Diss	Diss/SD	Contrib%	Cum.%
L. sueri	**1,21**	0,43	16,83	0,83	17,88	17,88
Unid. Fish	0,19	**0,92**	12,19	0,69	12,95	30,83
G. rhomboides	0,61	0	7,59	0,49	8,06	38,89
A. glaber	**0,5**	0,09	6,68	0,47	7,1	45,99
C. pilchardus sardina	0	**0,34**	4,34	0,34	4,61	50,61

Groups Alat & Mmerl

Average dissimilarity = 94,12

Erythrops sp.	0,36	0	3,59	0,38	3,82	54,42
S. oweniana	0,02	0,28	3,55	0,32	3,77	58,2
Gobidae	0,16	0,14	3,44	0,32	3,65	61,85
C. crassicornis	0	0,28	3,06	0,33	3,25	65,1

nMDS analysis (stress 0,13) showed a certain degree of segregation between the three species' diets (fig. 4-11). A gradient of partial overlap was detected, from *P. erythrinus* to *M. merluccius*.

Though the ANOSIM routine provided evidence for separation between the diets of the three species (p<0,05) the R value is very low (R=0,179). The pair-wise comparison among species showed lower R values between *P. erythrinus* and *A. laterna* (R=0,161) and between *A. laterna* and *M. merluccius* (R=0,149) than between *P. erythrinus* and *M. merluccius* (R=0,245).

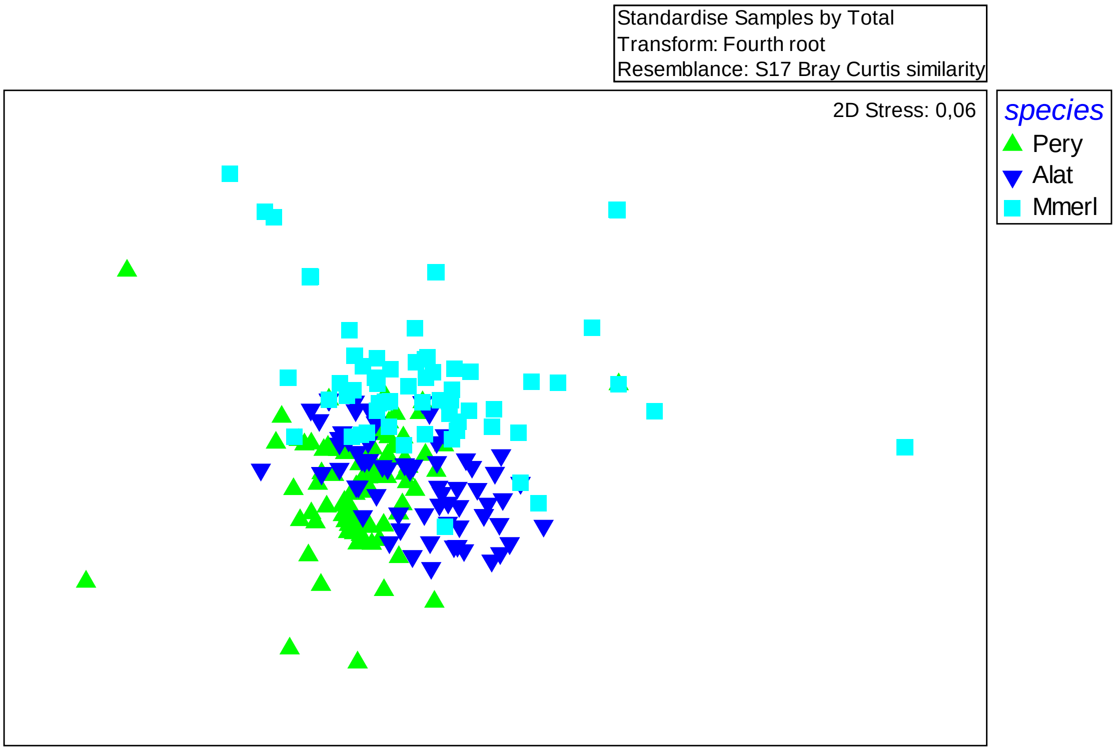


Fig. 4-11. nMDS of of gut contents of *P. erythrinus*, *A .laterna* and *M. merluccius*

Asymmetrical PERMANOVA provided evidence for significant differences among species diets, among species diets in protected and unprotected areas and for the interactions (table 4-17).

Table 4-17. PERMANOVA based on the Bay-Curtis dissimilarities of standardized and 4th root transformed data (88 taxa).

Source	df	SS	MS	Pseudo-F	P(perm)	perms
species	2	1,2262E5	61309	9,3912	0,001	998
protection	1	28474	28474	3,6993	0,009	997
area(protection)	1	7894,5	7894,5	2,1041	0,026	998
speciesxprotection	2	30968	15484	2,3718	0,019	998
speciesxarea(protection)	2	13474	6737	1,7956	0,011	998
Res	287	1,0768E6	3752			
Total	295	1,2915E6				

The PERMDISP routine was then performed on significant terms (effects) of PERMANOVA; the test performed on “species” was significant ($F_{3,296}$=6,85; p=0,004) and showed higher dispersion for *M. merluccius* samples (Average deviation from centroids: 64,52) than for *P. erythrinus* (58,92). Samples from protected areas resulted less dispersed (60,28) than samples from unprotected areas (66,31) ($F_{2,296}$=32,78; p=0,001). Finally the PERMDISP performed on factor “area” evidenced significant differences ($F_{3,296}$=12,48; p=0,001), with samples from Castellammare (60,28) less dispersed than samples from S. Agata (66,1) and Termini (65,37).

4.3.5 Stable isotopes analysis

Due to a delay in samples processing caused by the workgroup of Newcastle (responsible of stable isotopes analyses for the project), only some preliminary data from the Gulf of Castellammare are available to date.

These data regard to samples of *P. erytrhinus* of 195 mm TL (±0,00); *A. laterna* between 80 and 132 mm TL and *M. merluccius* <160 mm TL (120-140mm) and > 160 mm TL (160-240 mm).

$\delta^{15}N$ values of infaunal and epibenthic feeders (*P. erythrinus* and *A. laterna*) were higher than those of the pelagic-feeder *M. merluccius* (table 4-18). Assuming a fractionation of 3‰ per trophic level (Minagawa and Wada 1984; Post 2002) *P. erythrinus* exhibited a trophic level (TL) of 4,85 and *A. laterna* of 4,25; specimens of *M. merluccius* smaller than 160 mm had a TL=4 while hakes larger than 160mm exhibited a higher TL of 4,18. The most depleted $\delta^{13}C$ values were shown by smaller hakes (table 4-18).

Table 4-18. $\delta^{15}N$ and $\delta^{13}C$ values (mean ‰ ± SD) of fish in the Gulf of Castellammare

	$\delta^{15}N$	SD	**$\delta^{13}C$**	SD
P. erythrinus	11,84	0,54	-18,00	
A. laterna	10,47	1,10	-18,77	0,73
M. merluccius <160 mm TL	9,29	0,86	-19,02	0,47
M. merluccius >160 mm TL	9,83	0,40	-18,16	0,32

4.3.5.1 Trophic web structure

Ascending the food web from the suprabenthos, via decapod crustaceans and gobiid fish to the demersal fish considered there was a general increase in $\delta^{15}N$ and $\delta^{13}C$ of consumers (fig. 4-12). The correlation between $\delta^{15}N$ and $\delta^{13}C$ of all groups in May 2005, in the Gulf of Castellammare was highly significant (R=0,78; p<0,0001).

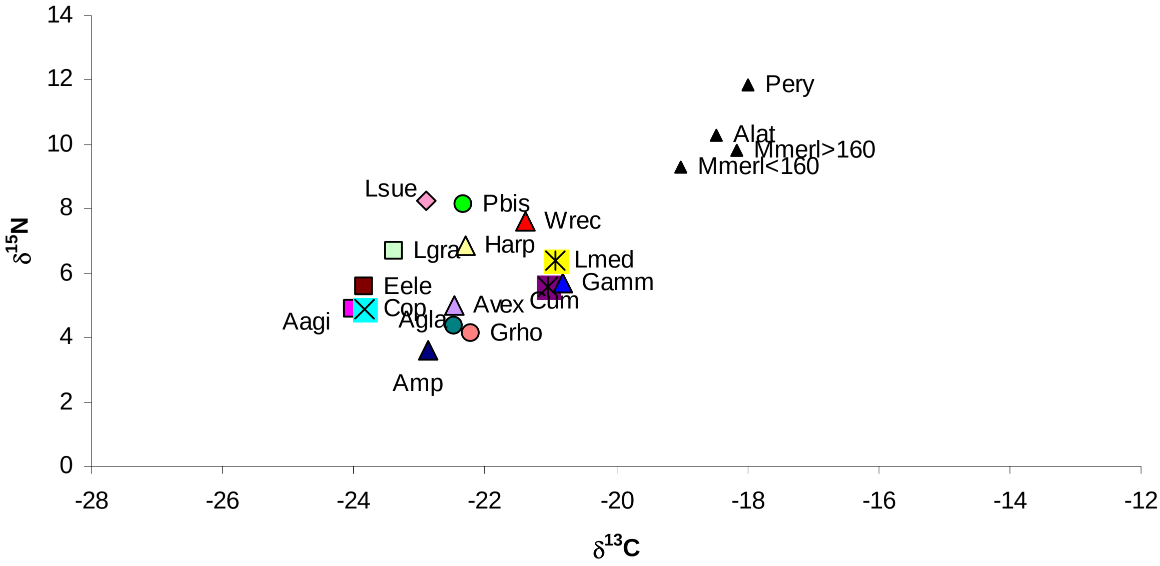


Fig. 4-9. δ^{15}N and δ^{13}C of fish (black triangles) and suprabenthos (symbols and acronyms as in chapters 2 and 3). Pery: *P. erythrinus*; Alat: *A. laterna*; Mmerl<160: *M. merluccius* smaller than 160 mm TL; Mmerl>160mm: *M. merluccius* larger than 160 mm TL.

4.4 Discussion

4.4.1 Diet composition and spatial variation

Pagellus erytrinus

The presence of many common benthic and epibenthic organisms, such a decapods, polychaetes, bivalves, gastropods, benthic fish (e.g. gobiids) and amphipods further implies that red pandora normally feeds on zoobenthos. Some large nektonic cephalopods (*Sepia elegans*, *Sepietta oweniana*) were eaten, but rather seldom. The food items and stomach contents of red Pandora observed in the present study are in general agreement with the findings of other authors (Ardizzone and Messina, 1983; Rosecchi, 1983; Caragitsou and Papaconstantinou, 1988; Andaloro and Giarritta, 1983; Benli et al., 2001). Food items may vary from area to area and even from gear to gear, but certain key features are common. In all the studies red Pandora seemed to feed mainly on crustaceans and polychaetes, while in our study fish (e.g. *Lesuerigobius sueri*, *Conger conger* and unidentified Ophychtydae) were also important food item. The low trophic similarity found in the study areas, in relation with the availability of food supply suggests that in a food abundant environment, *P. erythrinus* would experience limited competition. The frequent findings of inorganic material (e.g. mud) in the stomach contents suggest the ability of red Pandora to capture organisms that are buried in the mud, as previously observed by Ardizzone and Messina (1983). The percentage of empty stomachs observed in our study was higher than in other areas (Caragitsou and Papaconstantinou, 1988) but almost the same as that in the middle Tyrrhenian Sea (Ardizzone and Messina, 1983), in the gulf of Lion (Rosecchi, 1983) and in the Adriatic Sea (Rijavec and Zupanovic, 1965).

Significant differences occurred in red Pandora diet from protected and unprotected areas: the higher dispersion in the data from non protected areas could be correlated with a more generalist diet, comparing with a more specialist behavior in the protected areas. *P. erythrinus* seemed to prefer the brachyuran crab *G. rhomboides* in all the studied areas, but it was the most typifying species in the diet of the specimens from protected areas, contributing more than 70% to similarity. Our data on suprabenthos (see chapter 3) suggests that *G. rhomboides* was more abundant at least in the Gulf of Castellammare (5 ind/100m^2) than in Termini and S. Agata (0,2 ind/100m^2). Since no previous data on macrobenthic communities of the four gulfs before this study exists, it is not possible to attribute the highest abundance of this species to a protection effect. Trawling disturbance affects the structure and diversity of benthic communities (Jennings and Kaiser, 1998; Hall, 1999; Kaiser and de Groot, 2000). Large, slow-growing species are particularly vulnerable to trawling disturbance (Engel and Kvitek, 1998;

Kaiser et al., 2000; Bergman and van Santbrink, 2000a), while smaller individuals and species suffer lower mortality rates (Gilkinson et al., 1998).

Species with large body sizes are therefore less able to compensate for high mortality rates inflicted by bottom trawling than smaller sized species that have faster life cycle rates and higher reproductive output. As a result, benthic communities in trawled areas are dominated by smaller species with higher productivity per unit biomass or by highly mobile epibiota (Kaiser et al. 2000, Jennings et al. 2001). In the case of *G. rhomboides*, a small size organism, trawling could be not responsible for lower biomass in unprotected areas, since a very low biomass and abundance was also observed in Patti. However it is possible that burrowing organism such as *G. rhomboides* (Atkinson and Taylor, 1988) are better preserved from the effects of trawling, thus the highest abundance of this species in the Gulf of Castellammare and in gut contents in that area.

Arnoglossus laterna

The scaldfish, *A. laterna* was found to prey manly on decapods and fish in the whole study area. This species, as other Bothidae, is an active predator, responding primarily to moving prey and thus feeding mainly on active rather than on sedentary prey (De Groot, 1971; Braber and De Groot, 1973). Thus *A. laterna* should be able to capture large and more resistant prey that move quickly such as fish and crustaceans (Darnaude et al., 2001). For example small gobid fish, made the second abundant prey in the diet due to their slow motion capacity and visibility which characteristics attract scald fish, as invoked by Gibson and Ezzi (1980).

Our findings partially agree with other authors. Avsar (1993) found that *A. laterna* fed mainly on crustaceans (Amphipoda, Euphausiacea, Decapoda, Mysidacea and Copepoda) in Turkey, with fish and polychaetes as a secondary prey items; similar feeding habits were described by Gibson and Ezzi (1980) off Scottish coasts and by Cabral et al. (2002) off Portugal coasts, although in these two areas it seemed to preyed almost exclusively on crustaceans. Shrimps were the main food item of scaldfish in the Irish sea (Amezcua et al., 2003); while amphipods, mysids, pagurids, shrimps and occasionally polychaetes were the preferential prey of juveniles of *A. laterna* in the gulf of Fos (NW Mediterranean) (Darnaude et al. (2001).

It is interesting to note differences between size-classes that could be a strategy to reduce intra-specific competition. Small specimens (smaller than 100mm of TL) mainly preyed on suprabenthic species such as mysids, amphipods and small decapods (i.e. *Philocheras bispinosus*), as observed by Darnaude et al. (2001), while adult (larger than 105mm of TL) fed almost exclusively on the brachyuran crab *G. rhomboides* and the gobid fish *L. sueri*. Even

though these two species occurred in juveniles stomach contents, they were smaller than that found in adult scald fish, and thus competition was avoided by exploiting on different size-classes of the same prey, less mobile on average for adult (large specimens).
Spatial difference occurred in scald fish diet; notwithstanding *G. rhomboides* and *L. sueri* were the preferential prey in the four gulfs. Diet of *A. laterna* seemed to be more specialist in the Gulfs of Patti and Castellammare, although no clear protection effect was detected.

Merluccius merluccius

Juveniles of hake manly fed on fish and small natantian decapods. *M. merluccius* diet shifted from natantian decapods and small gobid fish (i.e. *L sueri*), consumed by the smaller hakes (<160mm), to pelagic fishes (i.e. Clupeiformes) consumed by larger hakes. Before the transition to the complete icthyophagous phase, hake showed more generalized feeding habits where natantian decapods (*Chlorotocus crassicornis*, *Processa* spp.), euphausiids, mysids, small cephalopods (Sepiolidae) and benthic fish dominated the diet, as observed by Carpentieri et al. (2005) in the central Tyrrhenian Sea and by Bozzano et al. (2005) in the Catalan Sea, although the depth range explored in these studies was wider. Specific size-related differences in prey spectrum seem to be associated with different spatial distributions or genetic needs (or with both) (Flamigni, 1984; Jukic and Arneri, 1984; Velasco and Olaso, 1998). The patterns observed in the present study did not indicate a strong partitioning among the two considered size classes, differently from Carpentieri et al. (2005) findings.

Spatial variations were observed in diet of *M. merluccius*, though a significant protection effect could be deduced from our data, other considerations allow refusing this result. In the case of *M. merlucciu*s an asymmetrical design was created, with two unprotected areas and only the Gulf of Castellammare as protected area. Our results indicated that the diet of hakes in the gulfs of S. Agata and Termini were very similar and strongly differed from Castellammare as concerns the high consumption of Clupeiformes in this area. Clupeiformes are pelagic resources, clearly not influenced by trawling activities, but strongly correlated, with a delay, to peaks of primary production. In fact the study of correlations between fullness and ppc resulted significant only in the gulf of Castellammare, confirming this consideration. Most hake prey in this study were pelagic in origin (euphausiids, clupeiforms, natantian decapods) and their higher concentrations probably may depend (with a logic time delay of 1-2 month) on local increases in primary production (see also chapter 2 and 3). Euphausiids and *Sardina pilchardus* can directly eat phytoplankton, and small euphausiids showed a peak of abundance in April in the Ligurian Sea (Sardou et al., 1996), situated in the north of the study area.

Relationships between some parameters of fish communities such as the diversity and regional surface phytoplankton concentrations has previously been reported (McClatchie et al., 1996; Cartes et al, 2004), with striking correspondence between hot spots of diversity and regions of high productivity probably associated with areas of geostrophic current intensification. Our results would suggest a (delayed) trophic link in these relationships. Fullness of hake was also correlated with latitude; therefore a possible relation between food intake and primary production would follow more a spotted model. As observed in chapter 3, the gulf of Castellammare has a 1067 km^2 hydrographic basin extension that results in a large contribution of inorganic and organic compounds in the opposite coastal waters. In fact the enhancement of the productivity of coastal benthic ecosystems by land-based run-off has been demonstrated in various areas (e.g. Cloern 2001, Maslowski 2003) and has been linked mainly to river input of nutrients and subsequent increases in plankton production and deposition in the benthos (Ardisson & Bourget 1997, Josefson & Conley 1997). Moreover the mean value of PP was higher in the gulf of Castellammare than in the other two gulfs and it could be enhance a more abundant and diversified plankton communities (Kivi et al. 1996), which can sustain a higher biomass of planktophagous species such as Clupeiformes.

4.4.2 Overall species analysis

All discussion on the possible influence of resource partitioning on competition is often controversial and even speculative. High overlap values between species were uncommon in marine communities, indicating that on the whole species did partition the available resources (Grassle & Sanders 1973, Macpherson 1981, Blaber & Bulman 1987, Cartes 1998a).

In addition, as explained in chapter one the choice of the three species was based on differences in their diet by literature data. The three selected species are presumably representative of three different trophic guilds: *Pagellus erythinus* actively forages on benthic prey (Ardizzone and Messina, 1983; Rosecchi, 1983; Caragitsou and Papaconstantinou, 1988; Andaloro and Giarritta, 1983; Benli et al., 2001), juveniles of *Arnoglossus laterna* preyed mainly on suprabenthic prey while adults on epibenthic species (Gibson and Ezzi, 1980; Avsar, 1993; Cabral et al, 2002; Darnaude et al., 2001; 2004) and juveniles of *Merluccius merluccius* also on suprabenthos and pelagic prey (Maynou et al, 2003; Cartes et al., 2004; Bozzano et al., 2005; Carpentieri et al., 2005).where juveniles of *M. merluccius* was supposed to show a stronger pelagic habit and to prey on pelagic-benthopelagic organisms, *A. laterna* was representative of a "benthopelagic-epibenthic feeder" guild and *P. erythrinus* was chosen as an "epibenthic-infaunal feeder". In the present study, low dietary overlap values were generally recorded, implying exploitation of different food by species situated in different trophic guilds. A high selectivity for crustacean decapods is a widespread feeding behaviour in the three fish, although they differed in the percentage of consumption and partially in the species selected. Diets and food partitioning among the three species vary in time directionally (ontogenetic development) and spatially (along a longitude scale). Juveniles of *A. laterna* mainly exploited suprabenthic species, while juveniles of *M. merluccius*, in our study preyed on natantian decapods, thus no overlap existed. The prey exploited by fish is a function, among other factors, of its foraging methods and the morphology of its alimentary tract (De Groot, 1971). The three species differed for both these features, although adult specimens of *A. laterna* and *P. erythrinus* (the only considered in our study) seemed to feed on the same resources (e.g. *Goneplax rhomboides* and *A. glaber*), the degree of overlap was low, because other prey was differently exploited by the two species: polychaetes for *P. erythrinus* and *L. sueri* for scald fish. In general these two species on mud have a similar diet, probably indicating that during this period (May 2005) this sediment type had enough food resources to allow different species to eat similar things (Amezcua et al., 2003). Some prey, such as *L. sueri* and *A. glaber*, among others, were exploited in a different proportion by the three species, and this could explain the good degree of overlap observed in the nMDS ordination and also revealed by SIMPER analysis. In general the diet of the three species seemed to be more specialist in the gulf of Castellammare than in the other two gulfs, while among the three species the diet of *M. merluccius* was more generalist.

In addition the degree of overlap increased in the Gulf of Castellammare, probably due to the higher abundance of suprabenthic and to a lessee extent of epibenthic resources (see chapter 3) occurred in this area.

4.4.3 Trophic web structure

Our analyses highlighted many apparent similarities in the information provided by gut-contents analysis and stable isotopes. The enrichment in ^{15}N across invertebrate and fish was consistent with the widespread recognition that ^{15}N provides a measure of trophic level (Owens, 1987).

The strong correlation obtained for fish and suprabenthos of the Gulf of Castellammare is indicative of a single type of primary source material supporting, at least the selected demersal species.

The correlation between δ^{15}N and δ^{13}C was higher than that in communities of Mediterranean littoral (e.g. cfr. Lepoint et al, 2000; Pinnegar and Polunin, 2000) when an array of possible sources of production, including plankton, macroalgae and seagrasses, and the relationship between $\delta^{15}N$ and $\delta^{13}C$ is much weaker (e.g. consumers in Pinnegar and Polunin, 2000 *r*=0,46; non-epiphytic consumers in Lepoint et al., 2000 *r*=0,24). In our case the high value of correlation may indicate that this trophic web was mainly based on detritus. If macrophytes such as phanerogames had been significant contributors, then higher and more variables values of $\delta^{13}C$ would have been indicated for the source material of costal muddy bottoms food webs than were found (Polunin et al., 2001). In the case of *M. merluccius* clupeoids were important prey items, not explored by stable isotopes analysis in our study, although Pinnegar et al. (2002) found a value of $\delta^{15}N$ of 7,86 and 7,87 for *Sardina pilchardus* and *Engraulis encrasicolus* in the Gulf of Castellammare.

However, juveniles of hake were more depleted in ^{15}N than would be expected (>3‰ trophic step) if they were mainly consuming clupeoids as observed in the Gulf of Castellammare. Therefore it is possible that the high contribution of clupeifomes found in stomach contents of hakes in May 2005 in the Gulf of Castellammare is due to a particular high abundance of this resource in this period, but probably clupeoids could not be exploited throughout the year in the same way and suprabenthic resources could be preferred.

In contrast the higher enrichment in ^{15}N observed for *P. erythrinus* than its preferred prey *G. rhomboides* (4,11‰±1,13) and *A. glaber* (4,36‰±0,92) indicate that this species may be obtaining much of their body N from sources such as carnivorous polychaetes. Although no data on isotopes signatures for polychaetes were yet available in our study, Darnaude et al. (2004) found $\delta^{15}N$ values for surface and subsurface deposit polychaetes inhabiting muddy bottoms off Rhone River delta between 30 and 100 m of depth from 6,09‰ to 7,17‰ and 9,12-9,17‰ Notwithstanding that study was carried out in a more eutrophic ecosystem than the Gulf of Castellammare, these values were consistent with an enrichment of 3‰ between polychetes and red Pandora.

The calculation of trophic level for top predators indicated a high TL for *P. erythrinus*, *A. laterna* (despite its small size) and *M. merluccius*. This generally agrees with the suggestion that aquatic food chains typically have no more than 4 or 5 trophic steps (Hall and Raffaelli, 1993). Coastal fish inhabiting muddy bottoms off the Gulf of Castellammare appeared to be foraging over 2 to 3 full trophic levels.

Annex 4-1 List of taxa identified in stomach contents of *P.eythrinus* in May 2005 in four areas of Northern Sicily. CDG: Castellammare del Golfo; TI: Termini Imerese; SA: S.Agata; PA: Patti.

	Castellammare del Golfo				**Termini Imerese**			
	%W	**%N**	**%F**	**%IRI**	**%W**	**%N**	**%F**	**%IRI**
PORIFERA								
Unid. Porifera	-	-	-	-	-	-	-	-
POLYCHAETA								
Glycera sp.	0,61	1,08	7,14	0,04	-	-	-	-
Eunicidae	7,12	0,54	3,57	0,27	-	-	-	-
Nephtydae	0,83	2,16	10,71	0,32	-	-	-	-
Phyllodocidae	0,93	1,62	7,14	0,18	-	-	-	-
Sternaspis scutata	0,61	0,54	3,57	0,04	-	-	-	-
Detritivorous polychaetes	0,07	0,54	3,57	0,02	-	-	-	-
Unid. Polychaetha	1,47	3,24	17,86	0,83	5,96	14,29	10,00	3,18
CRUSTACEA								
Decapoda								
Alpheus glaber	5,32	1,62	10,71	0,73	18,11	28,57	30,00	21,98
Anapagurus petiti	-	-	-	-	-	-	-	-
Anapagurus sp.	-	-	-	-	0,13	4,76	5,00	0,38
Athanas nitescens	-	-	-	-	-	-	-	-
Callianassa tyrrenia	-	-	-	-	-	-	-	-
Goneplax rhomboides	46,70	29,73	78,57	59,15	17,10	52,38	45,00	49,08
Jaxea nocturna	-	-	-	-	-	-	-	-
Philocheras bispinosus	-	-	-	-	0,23	4,76	5,00	0,39
Processa macrophthalma	-	-	-	-	-	-	-	-
Processa sp.	-	-	-	-	0,38	4,76	5,00	0,40
Upogebia sp.	-	-	-	-	1,19	4,76	5,00	0,47
Solenocera membranacea	6,25	0,54	3,57	0,24	-	-	-	-
Alpheidae	-	-	-	-	-	-	-	-
Portunidae	-	-	-	-	0,53	9,52	10,00	1,58
Thalassinidae	-	-	-	-	-	-	-	-
Unid. Caridea	-	-	-	-	-	-	-	-
Unid. Natantia	2,85	5,41	14,29	1,16	0,34	4,76	5,00	0,40
Unid. Brachyura	-	-	-	-	-	-	-	-
Unid. Decapoda	-	-	-	-	-	-	-	-
Decapoda larvae	0,04	1,08	3,57	0,04	-	-	-	-
Amphipoda								
Phoxocephalidae	0,05	1,08	3,57	0,04	-	-	-	-
Gammaridea	-	-	-	-	-	-	-	-
Unid. Amphipoda	0,15	1,08	7,14	0,09	-	-	-	-
Isopoda								
Idotea sp.	-	-	-	-	-	-	-	-
Natatolana borealis	-	-	-	-	-	-	-	-
Cymothoidae	-	-	-	-	0,03	4,76	5,00	0,38
Cumacea								
Unid. Cumacea	0,02	0,54	3,57	0,02	-	-	-	-
Mysidacea								
Unid. Mysidacea	0,00	0,00	0,00	0,00	-	-	-	-
Tanaidacea								
Leptognathia sp.	2,77	1,08	7,14	0,27	11,14	14,29	15,00	5,99
Ostracoda								
Unid. Ostracoda	-	-	-	-	-	-	-	-
MOLLUSCA								
Dentalium sp.	0,41	3,24	7,14	0,26	-	-	-	-
Nuculana sp.	-	-	-	-	-	-	-	-

	Castellammare del Golfo				**Termini Imerese**			
	%W	**%N**	**%F**	**%IRI**	**%W**	**%N**	**%F**	**%IRI**
Turritella turbona	-	-	-	-	-	-	-	-
Turritella sp.	0,03	0,54	3,57	0,02	-	-	-	-
Cardiidae	-	-	-	-	-	-	-	-
Veneridae	-	-	-	-	-	-	-	-
Sepia elegans	-	-	-	-	-	-	-	-
Sepieta oweniana	-	-	-	-	14,95	4,76	5,00	1,55
Unid. Gastropoda	-	-	-	-	-	-	-	-
Unid. Bivalvia	0,76	3,78	21,43	0,96	-	-	-	-
ECHINODERMATA	0,03	0,54	3,57	0,02	-	-	-	-
Amphiura filiformis	-	-	-	-	-	-	-	-
Ophiuroidea	-	-	-	-	-	-	-	-
Echinoidea	-	-	-	-	-	-	-	-
Holoturia sp.	0,72	0,54	3,57	0,04	-	-	-	-
TUNICATA								
Unid. Tunicata	-	-	-	-	0,62	4,76	5,00	0,42
OSTEYCHTHYES								
Fish larvae	-	-	-	-	1,13	4,76	5,00	0,46
Conger conger	1,95	1,08	7,14	0,21	18,86	14,29	15,00	7,81
Leseurogobius sueri	-	-	-	-	4,76	4,76	5,00	0,75
Ophicthydae	-	-	-	-	-	-	-	-
Unid. Fish	-	-	-	-	2,47	4,76	5,00	0,57
scales	0,05	1,08	7,14	0,08	-	-	-	-
OTHER								
Cymodocea nodosa	0,54	5,41	32,14	1,88	-	-	-	-
Vegetal rests	-	-	-	-	0,07	4,76	5,00	0,38
Nematoda	0,19	4,32	7,14	0,32	-	-	-	-
Foraminifera	0,04	0,54	3,57	0,02	-	-	-	-
Detritus	10,13	10,81	71,43	14,73	-	-	-	-
Digested material	9,38	16,22	71,43	18,01	2,01	14,29	15,00	3,84

	S.Agata				**Patti**			
	%W	**%N**	**%F**	**%IRI**	**%W**	**%N**	**%F**	**%IRI**
PORIFERA								
Unid. Porifera	-	-	-	-	0,01	0,66	2,94	0,03
POLYCHAETA								
Glycera sp.	1,48	2,13	3,23	0,18	1,19	2,65	5,88	0,30
Eunicidae	-	-	-	-	-	-	-	-
Nephtydae	-	-	-	-	1,32	0,66	2,94	0,08
Phyllodocidae	-	-	-	-	-	-	-	-
Sternaspis scutata	-	-	-	-	-	-	-	-
Detritivorous polychaetes	6,53	4,26	6,45	1,10	0,22	1,32	5,88	0,12
Unid. Polychaetha	0,52	2,13	3,23	0,13	1,20	3,31	14,71	0,87
CRUSTACEA								
Decapoda								
Alpheus glaber	15,18	19,15	29,03	15,76	6,45	3,97	17,65	2,41
Anapagurus petiti	-	-	-	-	0,02	0,66	2,94	0,03
Anapagurus sp.	-	-	-	-	0,13	1,32	5,88	0,11
Athanas nitescens	0,48	2,13	3,23	0,13	-	-	-	-
Callianassa tyrrenia	-	-	-	-	-	-	-	-
Goneplax rhomboides	19,46	21,28	32,26	20,77	53,94	23,84	61,76	62,91
Jaxea nocturna	0,07	2,13	3,23	0,11	-	-	-	-
Philocheras bispinosus	-	-	-	-	-	-	-	-
Processa macrophtalma	-	-	-	-	0,44	1,32	5,88	0,14

	S.Agata				**Patti**			
	%W	**%N**	**%F**	**%IRI**	**%W**	**%N**	**%F**	**%IRI**
Processa sp.	0,44	2,13	3,23	0,13	-	-	-	-
Upogebia sp.	1,75	2,13	3,23	0,20	0,20	0,66	2,94	0,03
Solenocera membranacea	-	-	-	-	-	-	-	-
Alpheidae	-	-	-	-	0,11	0,66	2,94	0,03
Portunidae	-	-	-	-	-	-	-	-
Thalassinidae	0,80	2,13	3,23	0,15	-	-	-	-
Unid. Caridea	0,52	2,13	3,23	0,13	0,05	0,66	2,94	0,03
Unid. Natantia	2,88	10,64	16,13	3,45	1,24	2,65	8,82	0,45
Unid. Brachyura	-	-	-	-	0,11	0,66	2,94	0,03
Unid. Decapoda	2,74	10,64	16,13	3,41	-	-	-	-
Decapoda larvae	0,66	12,77	19,35	4,11	0,01	0,66	2,94	0,03
Amphipoda								
Phoxocephalidae	-	-	-	-	-	-	-	-
Gammaridea	-	-	-	-	0,14	1,32	5,88	0,11
Unid. Amphipoda	-	-	-	-	0,08	0,66	2,94	0,03
Isopoda								
Idotea sp.	-	-	-	-	0,49	0,66	2,94	0,04
Natatolana borealis	-	-	-	-	0,56	0,66	2,94	0,05
Cymothoidae	0,17	2,13	3,23	0,12	-	-	-	-
Cumacea								
Unid. Cumacea	-	-	-	-	-	-	-	-
Mysidacea								
Unid. Mysidacea	-	-	-	-	0,01	0,66	2,94	0,03
Tanaidacea								
Leptognathia sp.	2,18	2,13	3,23	0,22	1,58	1,32	5,88	0,22
Ostracoda								
Unid. Ostracoda	0,00	2,13	3,23	0,11	0,01	0,66	2,94	0,03
MOLLUSCA								
Dentalium sp.	-	-	-	-	-	-	-	-
Nuculana sp.	-	-	-	-	0,42	0,66	2,94	0,04
Turritella turbona	-	-	-	-	0,14	1,32	5,88	0,11
Turritella sp.	-	-	-	-	0,39	1,32	5,88	0,13
Cardiidae	-	-	-	-	0,17	0,66	2,94	0,03
Veneridae	-	-	-	-	0,27	1,32	5,88	0,12
Sepia elegans	1,85	2,13	3,23	0,20	-	-	-	-
Sepieta oweniana	-	-	-	-	-	-	-	-
Unid. Gastropoda	-	-	-	-	0,92	1,99	5,88	0,22
Unid. Bivalvia	-	-	-	-	2,25	5,96	20,59	2,21
ECHINODERMATA	0,16	2,13	3,23	0,12	3,27	0,66	2,94	0,15
Amphiura filiformis	-	-	-	-	1,06	0,66	2,94	0,07
Ophiuroidea	-	-	-	-	0,22	0,66	2,94	0,03
Echinoidea	0,01	2,13	3,23	0,11	-	-	-	-
Holoturia sp.	3,12	6,38	9,68	1,45	0,48	0,66	2,94	0,04
TUNICATA								
Unid. Tunicata	-	-	-	-	-	-	-	-
OSTEYCHTHYES								
Fish larvae	-	-	-	-	0,20	1,32	5,88	0,12
Conger conger	-	-	-	-	-	-	-	-
Leseurogobius sueri	-	-	-	-	-	-	-	-
Ophicthydae	4,41	4,26	6,45	0,88	0,94	0,66	2,94	0,06
Unid. Fish	19,69	17,02	25,81	14,98	1,48	1,32	5,88	0,22
scales	-	-	-	-	-	-	-	-
OTHER								

	S.Agata				Patti			
	%W	**%N**	**%F**	**%IRI**	**%W**	**%N**	**%F**	**%IRI**
Cymodocea nodosa	-	-	-	-	-	-	-	-
Vegetal rests	0,19	2,13	3,23	0,12	0,21	0,66	2,94	0,03
Nematoda	0,02	2,13	3,23	0,11	0,01	2,65	5,88	0,20
Foraminifera	-	-	-	-	0,01	0,66	2,94	0,03
Detritus	3,27	8,51	12,90	2,40	7,40	10,60	47,06	11,09
Digested material	11,40	29,79	45,16	29,40	10,64	12,58	55,88	17,00

	CDG	**TI**	**SA**	**PA**
No of individuals	52	43	51	89
%V	53,85	53,49	39,22	61,80
mean fullness	1,13	0,59	0,31	0,83
mean N of prey	6,61	2,15	2,71	4,44
mean W of prey	0,85	0,49	0,25	0,33
Eveness	0,75	0,87	0,86	0,79
Diversity	2,51	2,56	2,83	2,96
Dominance (1-λ')	0,87	0,89	0,92	0,91

Annex 4-2. List of taxa identified in stomach contents of *A. laterna* in May 2005 in four areas of Northern Sicily. CDG: Castellammare del Golfo; TI: Termini Imerese; SA: S.Agata; PA: Patti.

	Castellammare				*Termini Imerese*			
Taxon	**W%**	**N%**	**F%**	**IRI%**	**W%**	**N%**	**F%**	**IRI%**
CTENOPHORA								
Cephalyes appendiculata	-	-	-	-	-	-	-	-
PLATYHELMINTHES	-	-	-	-	-	-	-	-
POLYCHAETA								
Ampharetidae	0,1	1,1	2,7	0,1	-	-	-	-
CRUSTACEA								
Ostracoda	0,0	0,5	1,4	0,0	-	-	-	-
Copepoda	0,0	1,1	2,7	0,0	0,0	1,0	3,9	0,1
Decapoda								
Natantia								
Alpheus glaber	3,8	3,2	8,1	0,9	15,5	4,4	15,7	5,8
Philocheras bispinosus	0,7	4,3	10,8	0,9	0,1	4,4	13,7	1,1
Crangon crangon	-	-	-	-	-	-	-	-
Crangonidae	0,0	0,5	1,4	0,0	0,1	4,4	9,8	0,8
Processa sp.	0,4	1,1	2,7	0,1	2,4	1,5	5,9	0,4
Natantia post-larvae	0,0	1,6	2,7	0,1	0,0	0,0	0,0	0,0
Reptantia								
Upogebia typica	3,2	0,5	1,4	0,1	-	-	-	-
Brachyura								
Goneplax rhomboides	28,0	33,0	58,1	57,3	1,0	2,4	7,8	0,5
Unid. Brachyura	-	-	-	-	0,0	0,5	2,0	0,0
Anomura								
Anapagurus sp. Juv.	0,1	1,1	1,4	0,0	-	-	-	-
Galathea sp. juv.	0,0	1,6	4,1	0,1	-	-	-	-
Unid. Decapoda	0,1	0,5	1,4	0,0	0,0	0,5	2,0	0,0
Mysidacea								
Anchialina agilis	-	-	-	-	0,1	7,3	13,7	1,9
Acantomysis longicorne	0,1	1,1	1,4	0,0	0,0	0,0	0,0	0,0
Erythrops elegans	0,1	14,4	12,2	2,8	0,4	44,7	35,3	29,5
Leptomysis gracilis	0,1	4,3	6,8	0,5	0,1	6,8	7,8	1,0
Lophogaster typicus	-	-	-	-	-	-	-	-
Siriella sp.	-	-	-	-	-	-	-	-
Unid. Mysidacea	-	-	-	-	0,0	2,4	3,9	0,2
Cumacea								
Dyastiloides serrata	0,0	0,5	1,4	0,0	-	-	-	-
Isopoda								
Unid. Isopoda	0,4	1,1	2,7	0,1	-	-	-	-
Amphipoda								
Ampelisca typica	0,0	0,5	1,4	0,0	-	-	-	-
Ampelisca sp.	0,2	5,3	12,2	1,1	0,0	1,5	5,9	0,2
Apherusa sp	0,0	2,1	2,7	0,1	-	-	-	-
Corophium sp.	0,0	0,5	1,4	0,0	-	-	-	-
Leptocheirus mariae	0,0	0,5	1,4	0,0	-	-	-	-
Monoculodes griseus	-	-	-	-	0,0	1,0	3,9	0,1
Westwodilla rectirostris	0,0	2,1	2,7	0,1	-	-	-	-
Leptocheirus mariae	0,0	0,5	1,4	0,0	-	-	-	-
Unid. Gammaridea	0,1	0,5	1,4	0,0	0,0	1,9	7,8	0,3
OSTHEYCHTHYES								
Leseurogobius sueri	61,4	12,2	29,7	35,4	77,5	10,2	35,3	57,3
Unid. Gobidae	0,7	0,5	1,4	0,0	2,5	1,9	7,8	0,6
Fish larvae	0,1	1,1	2,7	0,1	-	-	-	-

	Castellammare				*Termini Imerese*			
Taxon	**W%**	**N%**	**F%**	**IRI%**	**W%**	**N%**	**F%**	**IRI%**
OSTHEYCHTHYES								
scales	0,0	1,1	1,4	0,0	0,1	2,4	3,9	0,2
Unid. Fish	0,1	1,6	4,1	0,1	0,1	1,0	3,9	0,1
Others								
Vegetal remains	0,0	0,5	1,4	0,0	-	-	-	-

	S.Agata				*Patti*			
Taxon	**W%**	**N%**	**F%**	**IRI%**	**W%**	**N%**	**F%**	**IRI%**
CTENOPHORA								
Cephalyes appendiculata	0,1	1,1	4,0	0,1	-	-	-	-
PLATYHELMINTHES	-	-	-	-	2,3	3,4	4,3	0,3
POLYCHAETA								
Ampharetidae	-	-	-	-	-	-	-	-
CRUSTACEA								
Ostracoda	-	-	-	-	-	-	-	-
Copepoda	0,0	1,1	4,0	0,1	-	-	-	-
Decapoda								
Natantia								
Alpheus glaber	16,7	8,9	28,0	14,4	5,4	10,3	13,0	2,3
Philocheras bispinosus	0,5	13,3	20,0	5,6	-	-	-	-
Crangon crangon	-	-	-	-	0,5	3,4	4,3	0,2
Crangonidae	1,0	34,4	24,0	17,1	-	-	-	-
Processa sp.	0,2	3,3	12,0	0,9	-	-	-	-
Natantia post-larvae	0,5	1,1	4,0	0,1	-	-	-	-
Reptantia								
Upogebia typica	-	-	-	-	5,4	3,4	4,3	0,4
Brachyura								
Goneplax rhomboides	1,8	5,6	20,0	3,0	-	-	-	-
Unid. Brachyura	0,1	4,4	12,0	1,1	-	-	-	-
Anomura								
Anapagurus sp. Juv.	0,5	1,1	4,0	0,1	-	-	-	-
Galathea sp. juv.	0,0	1,1	4,0	0,1	-	-	-	-
Unid. Decapoda	-	-	-	-	0,4	3,4	4,3	0,2
Mysidacea								
Anchialina agilis	-	-	-	-	-	-	-	-
Acantomysis longicorne	-	-	-	-	-	-	-	-
Erythrops elegans	0,1	5,6	4,0	0,5	-	-	-	-
Leptomysis gracilis	0,4	1,1	4,0	0,1	-	-	-	-
Lophogaster typicus	0,4	1,1	4,0	0,1	-	-	-	-
Siriella sp.	0,1	1,1	4,0	0,1	-	-	-	-
Unid. Mysidacea	-	-	-	-	-	-	-	-
Cumacea								
Dyastiloides serrata	-	-	-	-	-	-	-	-
Isopoda								
Unid. Isopoda	-	-	-	-	-	-	-	-
Amphipoda								
Ampelisca typica	-	-	-	-	-	-	-	-
Ampelisca sp.	-	-	-	-	-	-	-	-
Apherusa sp	-	-	-	-	-	-	-	-
Corophium sp.	-	-	-	-	-	-	-	-
Leptocheirus mariae	-	-	-	-	-	-	-	-
Monoculodes griseus	-	-	-	-	-	-	-	-
Westwodilla rectirostris	-	-	-	-	-	-	-	-
Leptocheirus mariae	-	-	-	-	-	-	-	-

	S.Agata				*Patti*			
Taxon	**W%**	**N%**	**F%**	**IRI%**	**W%**	**N%**	**F%**	**IRI%**
Amphipoda Gammaridea								
Unid. Gammaridea	-	-	-	-	-	-	-	-
OSTHEYCHTHYES								
Leseurogobius sueri	73,6	10,0	32,0	53,8	79,0	62,1	60,9	94,5
Unid. Gobidae	-	-	-	-	4,6	3,4	4,3	0,4
Fish larvae	-	-	-	-	-	-	-	-
scales	0,0	1,1	4,0	0,1	-	-	-	-
Unid. Fish	4,1	4,4	16,0	2,7	2,2	10,3	13,0	1,8
Others								
Vegetal remains	-	-	-	-	-	-	-	-

	CDG	**TI**	**SA**	**PA**
No of individuals	102	91	46	71
Vacuity index (%V)	27,5	44,0	41,3	67,6
Mean fullness	0,7	1,1	0,9	0,9
Mean No. of prey	2,5	4,0	3,2	1,3
Mean prey weight (g)	0,1	0,2	0,1	0,1
Eveness (J')	0,7	0,7	0,8	0,6
Diversity (H')	2,5	2,1	2,2	1,3
Dominance (1-λ')	0,9	0,8	0,8	0,6

Annex 4-3. List of taxa identified in stomach contents of *M. merluccius* in May 2005 in four areas of Northern Sicily.

	Castellammare del Golfo				**Termini Imerese**				**S. Agata**			
Taxon	**%W**	**%N**	**%F**	**%IRI**	**%W**	**%N**	**%F**	**%IRI**	**%W**	**%N**	**%F**	**%IRI**
CRUSTACEA												
Decapoda												
Solenocera membranacea	1,48	1,79	3,85	0,24	1,00	1,11	2,78	0,25	0,20	1,02	2,08	0,13
Alpheus glaber	0,05	1,79	3,85	0,13	0,06	1,11	2,78	0,14	0,48	4,08	6,25	1,42
Chlorotocus crassicornis	0,36	3,57	7,69	0,57	0,83	2,22	5,56	0,73	1,75	9,18	16,67	9,05
Pontocaris lacazei	-	-	-	-	0,88	2,22	5,56	0,75	0,65	3,06	6,25	1,15
Processa cfr nouveli	0,12	1,79	3,85	0,14	-	-	-	-	-	-	-	-
Processa sp	0,06	3,57	7,69	0,53	0,36	10,00	8,33	3,73	0,43	7,14	12,50	4,71
Pandalidae unid.	-	-	-	-	-	-	-	-	0,40	2,04	4,17	0,51
Crangonidae unid.	-	-	-	-	0,09	2,22	2,78	0,28	0,16	1,02	2,08	0,12
Caridea unid.	-	-	-	-	-	-	-	-	0,13	1,02	2,08	0,12
Upogebia typica	-	-	-	-	-	-	-	-	0,13	1,02	2,08	0,12
Scyllarus cfr arctus	-	-	-	-	0,15	1,11	2,78	0,15	-	-	-	-
Decapoda Natantia	0,03	1,79	3,85	0,13	0,07	2,22	5,56	0,55	0,36	3,06	6,25	1,06
Amphipoda Gammaridea												
Ampelisca typica	-	-	-	-	0,03	10,00	2,78	1,20	-	-	-	-
Ampelisca sp	-	-	-	-	0,01	4,44	2,78	0,54	-	-	-	-
Monoculodes griseus	-	-	-	-	0,01	1,11	2,78	0,13	-	-	-	-
Gammaridea Unid.	-	-	-	-	-	-	-	-	0,00	1,02	2,08	0,11
Mysidacea												
Lophogaster typicus	-	-	-	-	0,03	1,11	2,78	0,14	0,02	1,02	2,08	0,11
Mysidacea unid.	-	-	-	-	0,01	1,11	2,78	0,13	0,00	1,02	2,08	0,11
Cumacea												
Dyastilis sp	-	-	-	-	0,01	1,11	2,78	0,13	-	-	-	-
Isopoda												
Rocinela dumerili	0,02	3,57	7,69	0,52	-	-	-	-	0,14	2,04	4,17	0,45
Rocinela orientalis	-	-	-	-	-	-	-	-	0,08	1,02	2,08	0,11
Euphausiacea												
Euphausiacea unid.	-	-	-	-	0,04	4,44	8,33	1,62	-	-	-	-
Crustacea unid.	0,04	8,93	7,69	1,31	0,03	2,22	5,56	0,54	-	-	-	-
MOLLUSCA												
Cephalopoda												
Sepieta sp	-	-	-	-	6,06	5,56	13,89	6,98	2,68	3,06	6,25	1,78
Sepiolidae	-	-	-	-	0,22	3,33	5,56	0,85	-	-	-	-
Gastropoda												
Turritella sp	-	-	-	-	0,41	2,22	5,56	0,63	-	-	-	-
OSTEYCHTHYES												
Larvae	-	-	-	-	-	-	-	-	-	2,04	2,08	0,21
Adult												
Antonogadus megalokydon	-	-	-	-	0,31	1,11	2,78	0,17	-	-	-	-
Cepola rubescens	5,26	1,79	3,85	0,51	-	-	-	-	7,70	6,12	10,42	7,16
Clupea pilchardus sardina	55,77	28,57	38,46	61,60	-	-	-	-	11,90	2,04	4,17	2,89
Clupeiformes	-	-	-	-	6,51	2,22	5,56	2,10	1,99	1,02	2,08	0,31
Deltentosteus quadrimaculatus	-	-	-	-	1,33	1,11	2,78	0,29	-	-	-	-
Engraulis encrasicolus	10,51	3,57	7,69	2,06	15,93	1,11	2,78	2,05	27,01	3,06	6,25	9,34
Gadidae unid.	0,81	3,57	7,69	0,64	9,97	1,11	2,78	1,33	-	-	-	-
Gobidae unid.	0,66	1,79	3,85	0,18	1,50	2,22	5,56	0,89	1,25	5,10	6,25	1,97
Gobius niger jozo	-	-	-	-	-	-	-	-	7,26	1,02	2,08	0,86
Lesueruigobius suerii	0,28	3,57	7,69	0,56	4,30	14,44	22,22	18,02	2,59	11,22	14,58	10,01
Mullus barbatus	6,84	1,79	3,85	0,63	-	-	-	-	-	-	-	-
Sardinella aurita	11,07	1,79	3,85	0,94	-	-	-	-	-	-	-	-

Scorpaenidae unid.	-	-	-	-	5,15	1,11	2,78	0,75	-	-	-	-
	Castellammare del Golfo				**Termini Imerese**				**S. Agata**			
Taxon	**%W**	**%N**	**%F**	**%IRI**	**%W**	**%N**	**%F**	**%IRI**	**%W**	**%N**	**%F**	**%IRI**
Spicara sp	-	-	-	-	-	-	-	-	3,23	1,02	2,08	0,44
Trachurus trachurus	-	-	-	-	20,73	2,22	5,56	5,51	13,40	4,08	8,33	7,24
Fish unid	6,64	26,79	46,15	29,30	23,94	13,33	30,56	49,25	15,82	13,27	25,00	36,13
OTHERS												
Elphidium sp.	-	-	-	-	-	-	-	-	0,00	3,06	6,25	0,95
Foraminifera	-	-	-	-	-	-	-	-	0,00	2,04	4,17	0,42
Unid. digested material	-	-	-	-	0,06	1,11	2,78	0,14	0,18	3,06	6,25	1,01

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Chapter 5

Trophic structure of a benthopelagic boundary layer community off Cabrera (Balearic Archipelago)[1]

Abstract

Dynamics of suprabenthos and zooplankton was analyzed in SE (off Cabrera Archipelago) of Mallorca (Balearic Islands, Western Mediterranean) at depths ranging between 135-780 m. Four stations situated at 150 m (shelf-slope break), and at bathyal depths on 350, 650 and 750 m were sampled at bi-monthly intervals during six cruises performed between August 2003, and June 2004. Suprabenthos showed maximum biomass from late spring to summer (April to August), while minimum biomass was found in autumn (September-November). Though variable, temporal dynamics of zooplankton showed peaks of biomass in late winter and summer (February and June), while minima occurred in autumn (August-September) and, at bathyal depths, in April. In MDS analyses performed on suprabenthos and zooplankton, suprabenthos abundance showed a sample aggregation as a function of depth (3 groups corresponding to the shelf-slope break, upper slope – over 350 m; and the middle, deeper part of the slope – over 650-750 m), without any separation of hauls by season. By contrast, zooplankton samples were separated by season and not by depth. Three seasonal groups corresponding to summer (June2004-August2003), autumn-winter (September and November 2003, February 2004), and spring (April 2004) were evidenced. In general suprabenthos was positively significant correlated with the sediment variables (e.g. %OM, potential REDOX), whereas zooplankton was almost exclusively dependent of *Chla* on surface, which suggests two different food sources for suprabenthos and zooplankton. The increase of suprabenthos abundance in April-June was also parallel to a sharp increase (*ca.* 2.8 times) of %OM on the sediment in the same period coupled *ca.* with 1-2 months of delay with the peak of surface primary production recorded in February-March (from *Chla* satellite imagery data) in the same area. Suprabenthos biomass was also correlated with salinity close to the bottom, suggesting a link between suprabenthos abundance and changes in the oceanographic condition of water masses close to the bottom.

In addition stable isotope (δ^{13}C and δ^{15}N) analyses were performed on suprabenthic and zooplanktonic fauna in the same area at depths ranging between 350 and 780 m. For isotope

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analyses Twenty species (1 mysid, 11 amphipods, 4 cumaceans [and Cumacea as a whole *taxon*], 3 isopods and 1 decapod) for suprabenthos and twelve species (3 euphausiids, 3 hyperiids, 4 decapods and 2 fish) for zooplankton were analyzed on a seasonal basis for stable carbon and nitrogen isotopes. Copepods were also analyzed to taxa level.
$\delta^{13}C$ values of suprabenthos taxa ranged from -23,2 (*Andaniexis mimonectes* in February 2004) to -14,9 (*Cyclaspis longicaudata* in February 2004). Stable nitrogen isotope ($\delta^{15}N$) ranged from 1,3‰ (*Lepechinella manco* in February 2004) to 10,5‰ (*Gnathia* sp. in November 2003). Both $\delta^{13}C$ and $\delta^{15}N$ suggest that there are three trophic levels within the suprabenthic community

Our isotopic analyses revealed a narrower and on average lower range of $\delta^{13}C$ and $\delta^{15}N$ values for zooplanktonic species, comparing with suprabenthic species, especially for $\delta^{13}C$. $\delta^{13}C$ values of zooplanktonic *taxa* ranged from -19,4‰ (*Cyclothone pygmaeus* in February 2004) to -23,7‰ (copepods in April 2004). Stable nitrogen isotopes ($\delta^{15}N$) ranged from 3,4‰ (*Phrosina semilunata* in February 2004) to 6,4‰ (*Pasiphaea multidentata* in August 2003). The overall range of $\delta^{15}N$ is indicative of only two trophic levels.

The stable isotope ratios of suprabenthos fauna displayed a continuum of values and confirmed a wide spectrum of feeding types (from filter-feeders to predators). In general, and in spite the poor knowledge about diets available for most suprabenthic species, higher $\delta^{15}N$ were found for parasite isopods (*Gnathia* sp.) and carnivore amphipods (e.g. *Rhachotropis* spp., *Nicippe tumida*) consuming copepods. Low overlap for $\delta^{13}C$ and $\delta^{15}N$ values was observed, though $\delta^{15}N$ values were less variable than $\delta^{13}C$, which suggest high resource partitioning in this community. $\delta^{13}C$ values point to one primary source of carbon throughout the sampling year.

Seasonal variations in isotopic composition for both $\delta^{13}C$ and $\delta^{15}N$ were low (less than 1‰ and 3‰, respectively) and conditional on each species. Low correlation between $\delta^{13}C$ and $\delta^{15}N$ of suprabenthic fauna were found for all periods studied, though increasing from February 2004 to June 2004 (after the main peak of Chl *a* in surface). C:N ratio (indicator of lipid content) showed higher values in summer than in winter. This suggests that lipid content may explain the seasonal patterns of $\delta^{13}C$ variability, and due the lipidic nature of Chl *a*, it possibly indicates the peak of primary production on surface.

5.1 Introduction

An increasing interest towards the study of fauna occupying the water layer immediately adjacent to the sea bottom (the called suprabenthos, hyperbenthos or Benthic Boundary Layer macrofauna: Mees and Jones, 1997) has been produced in recent years. This type of fauna is still poorly known even at decriptive levels such as its taxonomic and assemblage composition, and this is particularly true for deep-water suprabenthos.

Suprabenthos is a complex compartment composed by taxa (e.g. mysids, amphipods…) which constitute the permanent suprabenthos, and other taxa (e.g. euphausiids, natantian decapods) which by means of vertical or ontogenic migrations have a more eventual relation with the water-sediment interface. Some authors distingush between the permanent suprabenthos (=holohyperbenthos) and the eventual suprabenthos (=merohyperbenthos) (Mees and Jones, 1997). In fact, merohyperbenthos partially overlaps in terms of taxon composition with macro-mesozooplankton. The main adaptative biological feature of the permanent suprabenthos is the direct development of embryos in marsupial bags (oosteguites) developed by adult females of peracarid crustaceans.

Studies on suprabenthos assemblages were first focused to the distribution of dominant taxa and assemblages in the water column (Bieri and Tokioka, 1968), as a function of depth (Sainte Marie and Brunel, 1985; Bull Jensen, 1986), and to a lesser extend to seasonal changes in assemblage composition (Boysen, 1976) and population dynamics. Recently, studies on plankton-suprabenthos coupling (Richoux et al., 2004a, b) evidenced synchronism between juveniles relase and maximum food availability derived from spring phytoplankton blooms in the mysid *Mysis mixta* and the amphipod *Acanthostepheia malmgreni* inhabiting the shallowest part of the slope (at 240 m depth). The influence of environmental variability on suprabenthos dynamics has been rarely checked particularly at bathyal depths. Changes in sediment type associated to hydrodynamism (Cartes et al., 2003), or the arrival of fresh organic matter to the bottom have been suggested as posiible causes of this variability (Cartes et al, 2002; Richoux et al., 2004a, b).

In addition to this, most suprabenthic groups (e.g. mysids, amphipods), constitute the base of the diet of litoral and bathyal top predators (e.g. flatfish: Wildsih et al., 1992; hake: Cartes et al., 2004; the deep sea red shrimp *Aristeus antennatus*: Cartes, 1994; Sardà and Cartes, 1997), particularly for their smallest sizes or recruits and hence its importance in food webs dynamics. In spite its obvious ecological interest (Mees and Jones, 1997; Cartes et al, 2002), information on dynamics of suprabenthic communities is still scarce (Cartes and Sorbe, 1999;

Cartes et al., 2002). In the diet of bathyal species, the smallest sizes of some important ecological (and commercial) species (e.g. the red shrimp, *A. antennatus*, CL <20 mm) and hake (TL < 10 cm) consume suprabenthos (mysids, cumaceans, and amphipods; Cartes, 1994; Sardá and Cartes, 1997; Bozzano et al. 1997), while largest juveniles increasingly consume merohyperbenthos-zooplankton (e.g. decapods, small Myctophids; Cartes et al, 2004).

Temporal dynamics of open-water zooplankton communities has been the focus of a number of studies in the western Mediterranean (Franqueville, 1971; Sabatés et al., 1989; Sardou et al., 1996). The variety of gears (e.g. Bongos, IKMT), difference in mesh-size used, and sampling design are important limitations for detailed comparisons. However, maximum zooplankton biomass has been regularly reported in spring (Sabatés et al. 1989; Sardou et al, 1996), sometimes along the continental shelf edge in association with hydrographic fronts (Sabatés et al., 1989), which act as nursery areas for some zooplankton stages (Bouchet et al., 1987). Another regular pattern in the western Mediterranean is the dominance of jellyfish (e.g. salps, cnidarians) among deep macroplankton in winter (January to March period) (Franqueville, 1971). Because studies on the dynamics of suprabenthos are practically inexistent at bathyal depths (Cartes et al., 2002; Richoux et al., 2004a, b), this is the first comparative study focused to the simultaneous dynamics of both compartments.

Furthermore among the different aspects still poorly known for suprabenthos fauna, their position in trophic webs (e.g. the type of food exploited, and the trophic level of species) is of particular importance. Different functional guilds or feeding habits have been determined for some taxa based on limited information on diets (Cartes et al., 2002), and on behaviour or morphological features (e.g. Lysianassidae are in general considered as scavengers: Sainte-Marie, 1992; Eusiridae as carnivores by the possession of large gnatopods: Enequist, 1949).

In recent decades, stable isotope ratios of nitrogen and carbon have been used to elucidate patterns in food webs. The combined analysis of carbon and nitrogen stable isotopes is a powerful tool for identifying the ultimate organic matter sources and trophic position of consumers. The use of stable isotopes relies in the fact that the carbon isotope ($^{13}C/^{12}C$ or $\delta^{13}C$) of consumers reflects that of their food sources, with minimal enrichment or fractionation (0-1‰, Fry and Sherr, 1984; Michener and Schell, 1994; McCutchan et al., 2003), while, the nitrogen isotope ($^{15}N/^{14}N$ or $\delta^{15}N$) displays a stepwise enrichment of about 3-4‰ with each trophic level (Minagawa and Wada, 1984; Owens, 1987; Post, 2002).

In deep-sea, stable isotope analysis has been successfully carried out to analyse the trophic links in deep-sea corals (e.g. Kiriakoulakis et al 2005), in foraminifera (e.g. Corliss et al., 2002), and in polychaetes from hydrothermal vents (e.g. Levesque et al., 2003). However, in the study of macrofauna this approach has been hardly adopted, especially in deep sea environments (Iken et al., 2001, 2005, Polunin et al., 2001) and the application of isotopic analysis on suprabenthos fauna at species level has been limited to the Antarctic Ocean (Nyssen et al, 2002, 2005). Additionally, most of these studies have focused on the spatial variability of trophic relationships and on food web structure, with little consideration on temporal variation. Studies on seasonality of isotopic signatures in deep-sea are still relatively rare (Corliss et al., 2002) even in freshwater systems and coastal-shelf areas (e.g., Zohary et al., 1994; Vizzini and Mazzola, 2003; Bode and Alvarez-Ossorio, 2004).

Food sources in deep-sea tend to vary temporally in response to changes in primary production (Billett et al., 2001). The seasonal input to bathyal communities has an effect on suprabenthos and zooplankton abundance and biomass in the study area (Cartes et al., *in press*). In deep-sea, evidence for seasonal isotopic differences in consumers has been reported for benthic foraminifera as a response to the spring phytoplankton bloom (Corliss et al., 2002). Suprabenthos fauna, because they possess rapid tissue turnover times (Cartes et al., 2002), might exhibit isotopic compositions which follow these seasonal patterns in food sources.

In this context this chapter focused on two different aims: the objective of the first part of this study is to describe the dynamics of suprabenthos and zooplankton in one region situated around the island of Mallorca (Balearic Islands) and to explore possible environmental variables which could explain this dynamics. Winter winds forcing providing from the Gulf of Lyons acts on the oceanographic dynamics of Western Mediterranean water masses, controlling the south-north flux of Atlantic waters at surface around the Balearic Island (Font, 1987). This is combined with other oceanographic processes (e.g. dynamics of levantine intermediate -LIW, and winter intermediate –WIW- waters) occurring in sub-surface layers (Font, 1987; López-Jurado, et al., *submitted*). As a consequence differences in annual ocean dynamics and in the biological response of trophic webs are expected in the study site.

In the second part trophic relationships of deep BBL fauna were elucidated by stable isotopes analyses in order: (1) to determine the complexity of suprabenhic and zooplankton communities by establishing the number of trophic level among BBL fauna; (2) to assess temporal variations in the mean trophic level both of the bulk of suprabenthos and zooplankton and of the dominant species, and (3) to investigate possible seasonal changes in food sources of BBL fauna exploring temporal changes in $\delta^{13}C$ for the whole suprabenthic assemblage and in the dominant species.

5.2 Materials and methods

5.2.1 Data collection

Suprabenthos and zooplankton

A total of four samples of suprabenthos were collected during six surveys carried out from August 2003 to June 2004 at depths from 150 to 750 m, distance between stations ranged from ca. 3 to 10 miles. The sampling methodology to collect, preserve and analyze suprabenthic and zooplanktonic fauna is exposed in chapter 1.

Data was standardized (abundance - ind/100 m2; wet weight - g/100 m2) for each haul performed.

Environmental variables

CTD transects were completed using a SBE911 and SBE25 profiler furnished with a fluorometer (only in IDEA04 and IDEA06) and from a SB37-SM mounted on the mouth of the trawl used from the catch of megabenthic fauna (fish and large invertebrates) in simultaneous hauls performed in the same stations (López-Jurado et al., *submitted*). From each profile we obtained as a measure the temperature and salinity 5 m above the bottom ($T_{5\ mab}$; $S_{5\ mab}$), and during IDEA04 and IDEA06 fluorometry along all the water column. Maximum fluorometry, the depth where maximum flurometry was recorded, and the sum of flurometer reading by each m from surface to 200 m depth have been calculated from raw data.

Sediment for granulometric and organic analyses were collected using a Shipeck grab per each station and cruise. Sediment variables obtained from sediment analyses were D_{50} (mean grain size), percentage of gravels, sand and mud (silt+clay) using Sedigraph techniques (see Massutí et al., *submitted*). We obtained also total organic matter (%OM) of collected sediment, calculated by the difference between dry weight (DW: 80ºC for 24 h to constant weight) and ash weight (500 ºC in a furnace for 2 h). REDOX was also obtained from sediments in grabs (using a ThermoOrion 250A sensor) reading voltage at surface and at 5 cm deep in the mud.

5.2.3 Data processing

Data treatment.

Data on abundance of fauna collected was analyzed by clustering analysis and MDS techniques. Based on the correlation matrix generated by clustering, Multi-Dimensional

Scaling (MDS) were applied. We performed analyses for suprabenthos and for zooplankton including the following taxa:

1) suprabenthos, 15 taxonomic groups (Siphonophora, Decapoda Natantia, Euphausiacea, Mysidacea, Amphipoda Gammaridea and Hyperiidea, Isopoda, Cumacea, Ostracoda, Copepoda, Nebaliacea, Pycnogonida, Cephalopoda, Chaetognatha, and Osteychthyes).

2) zooplankton, comprising 24 taxa (Scyphozoa, Siphonophora, Ctenophora, Polychaeta, Decapoda Natantia, Decapoda larvae, Euphausiacea, Euphausiacea larvae, Mysidacea, Amphipoda Gammaridea and Hyperiidea, Isopoda, Cumacea, Ostracoda, Calanoidea, Cyclopoidea, Gastropoda Prosobranchia, Pteropoda, Cephalopoda, Chaetognatha, Thaliacea, Pyrosomida and Osteychthyes, adult and larvae).

For certain analyses (e.g. Multiple Regression models), we considered only some of these taxa (see below). The benthic boundary layer macrofauna comprises, in fact, a mixture of taxa with different ecological habits and distribution in the water column-sediment interface (see Mees and Jones, 1997) and it is often impossible to separate suprabenthos and zooplankton species unless working at species level. Some gelatinous zooplankton (salps, cnidarians) were excluded from some analyses due to difficulties in their sorting and quantification. Species/taxa appearing less than twice in all samples were removed from data matrices. Some taxa, poorly represented in sledge samples belonging to the (non-swimming) epifauna-infauna (Polychaeta, Amphipoda Caprellidea, Tanaidacea, and Ophiuroidea) were also removed from the data matrix.

The similarity index used in both cluster and MDS ordination was 1-r (correlation coefficient of Pearson, after log-transformation of data). The aggregation algorithm was the U.P.G.M.A. (Unweighted-paired group method average). The software STATISTICA 5.0 was used.

In order to establish possible relationships between assemblage's dynamics/structure and existing environmental gradients, we tested possible relationships (correlations) between the abundance and MDS dimensions of suprabenthos-zooplankton and some environmental variables (e.g. temperature, %OM in sediments) simultaneously recorded. We searched for possible correlations at two levels: 1) simple non parametric Spearman rank correlations; and 2) Multi-linear regression models (MLR; after log transformation of data), to identify the main explanatory variables of the dynamics of suprabenthos/zooplankton.

5.2.2 Stable isotope analyses

For stable isotopes analyses see details in chapter1.

Once collected, samples were immediately frozen at -20°C and sorted in the laboratory. All specimens were sorted as quick as possible, identified to species level and then they were freeze-dried and stored until their preparation for analyses. Species selected for isotopic analysis (only large sizes for each species) were those that were dominant in both abundance and biomass, hence reaching a whole view of variations in the suprabenthic and zooplankton assemblage.

For $\delta^{13}C$ analyses, samples were decalcified to remove inorganic carbonates from the exoskeleton (Jacob et al., 2005). A small quantity of 1M hydrochloric acid (HCl) was added to the samples and left for 3 hours. The samples were then dried at 60°C for 12 h and ground to a fine powder using a pestle and a mortar. Composite samples (Table 5-1) were analysed for all suprabenthic and zooplantonic species to minimise infra-individual differences in the isotopic composition and, to meet the minimum mass requirement for the isotopic analyses.

For all the species four replicates were performed, when it was possible one replicate was represented by one individuals, in order to reduce pseudo-replication (Hulbert, 1984), when the biomass was not enough more specimens were pooled together to obtain sufficient mass for the isotope measurement (as the case of Calanoid copepods, Cumaceans and small amphipods). All processed material was from 650 and 750 m samples.

Based on the information on its diet, when it was available, the species were classified into feeding types (Table 5-2). Correlations between $\delta^{13}C$ and $\delta^{15}N$ were calculated for each sampling period. The post-hoc Tukey test was used to compare isotopic signatures within suprabenthic and zooplanktonic species (Sokal & Rohlf 1995). Seasonal variations in $\delta^{13}C$ and $\delta^{15}N$ values were analyzed only for species where data from at least 4 months were available. Correlations between $\delta^{13}C$ and $\delta^{15}N$ were calculated for the bulk of BBL fauna on a month scale.

Table 5-1. Species analyzed for stable carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) isotopes. Legend: AMP (Amphipods), COP (Copepods), CUM (Cumaceans), DEC (Decapods), EUP (Euphausiids), ISO (Isopods), MYS (Mysids).

Group	**Acronym**	**Species**	**Aug03**	**Sep03**	**Nov03**	**Feb04**	**Apr04**	**Jun04**
AMP	Amim	*Andaniexis mimonectes*				*	*	
MYS	Barc	*Boreomysis arctica*	*	*	*	*	*	*
AMP	Btyp	*Bruzelia typica*				*	*	
DEC	Cmac	*Calocaris macandrae*	*	*		*	*	*
CUM	Cverr	*Campylaspis verrucosa*		*			*	
COP	Cop	Copepoda	*	*	*	*	*	*
CUM	Cum	Cumacea	*	*		*		
CUM	Clon	*Cyclaspis longicaudata*		*		*	*	
FISH	Cbra	*Cyclothone braueri*	*	*	*	*	*	*
FISH	Cpyg	*Cyclothone pygmaeus*				*		
AMP	Epar	*Epimeria parasitica*				*	*	
EUP	Ekro	*Euphausia kronhi*				*		
DEC	Gele	*Gennadas elegans*	*			*	*	
ISO	Gnat	*Gnathia* sp. (larvae)	*	*	*	*	*	
AMP	Lman	*Lepechinella manco*				*	*	
EUP	Mnor	*Meganyctiphanes norvegica*	*	*	*		*	
ISO	Matl	*Munnopsurus atlanticus*	*	*	*	*	*	
ISO	Nbor	*Natatolana borealis*	*	*	*	*	*	
EUP	Nmeg	*Nematoscelis megalops*	*	*	*	*	*	
AMP	Ntum	*Nicippe tumida*				*	*	
DEC	Pmul	*Pasiphaea multidentata*	*					
AMP	Psed	*Phronima sedentaria*		*	*	*		
AMP	Psem	*Phrosina semilunata*		*		*		
CUM	Psym	*Platisympus typicus*		*		*	*	
CUM	Parm	*Procampylaspis armata*					*	
AMP	Rcae	*Rhachotropis caeca*	*	*	*	*	*	*
AMP	Rgri	*Rhachotropis grimaldii*	*	*		*	*	
AMP	Rros	*Rhachotropis rostrata*	*	*	*	*	*	*
DEC	Sarc	*Sergestes arcticus*				*	*	
DEC	Srob	*Sergia robusta*		*				
AMP	Scri	*Stegocephaloides christianensis*				*	*	
AMP	Saff	*Syrrhoe affinis*				*	*	
AMP	Tlon	*Tryphosites longipes*	*	*	*	*	*	
AMP	Varm	*Vibilia armata*	*	*				

Table 5-2. Feeding habits and type of prey of suprabenthos and zooplankton fauna based on literature data (AMPH (Amphipods), COP (Copepods), DEC (Decapods), EUPH (Euphausiids), ISOP (Isopods), MYS (Mysids)

Group	**Species**	**feeding habits**	**prey**	**source**
MYS	*Boreomysis arctica*	filter-feeder	phytodetritus, copepods, cnidarians	Cartes, Sorbe 1996
COP	Copepods	filter-feeders	phytoplankton-detritus…	
FISH	*Cyclothone braueri*	carnivore	copepods, ostracods	Mauchline & Gordon, 1983, 1985, Palma, 1990
DEC	*Gennadas elegans*	carnivore	copepods	Cartes et al. 2002
DEC	*Sergestes arcticus*	carnivore	migratory mesozoplankton	Lagardére, 1976
DEC	*Pasiphaea multidentata*	carnivore	gammarids, hyperiids, macruran decapods	Cartes 1993
ISOP	*Gnathia* sp.	parasite	hematophagous	Naylor, 1972
ISOP	*Munnopsurus atlanticus*	deposit-carnivore	detritus, foraminiferans	Cartes et al. 2000
ISOP	*Natatolana borealis*	omnivorous-scavenger	carrion, polychaethes, crustaceans	Wong & Moore 1995
AMPH	*Nicippe tumida*	carnivore	copepods	Cartes, unpubl.
AMPH	*Rhachotropis caeca*	carnivore	copepods	Cartes et al. 2001
AMPH	*Rhachotropis grimaldii*	carnivore	copepods	Cartes et al. 2001
AMPH	*Rhachotropis rostrata*	carnivore	copepods	Cartes et al. 2001
AMPH	*Syrrhoe affinis*	probably carnivore	copepods, gelatinous	Cartes, unpubl..
AMPH	*Tryphosites* sp.	probably parasite	hematophagous (?)	
AMPH	*Phronima sedentaria*	carnivore	salps	Madison & Harbison 1977
EUPH	*Euphausia kronii*	carnivore, detritus	phyto-zooplankton	Mauchline, 1980
EUPH	*Nematoscelis magelops*	carnivore	copepods	Barange et al. 1991
EUPH	*Meganycthiphanes norvegica*	omnivorous	phyto-zooplankton	Mauchline & Fisher 1969 Casanova-Solier 1974

5.3 Results

5.3.1 Species composition

Suprabenthos

A total of 117 (Annex 5-1) species were identified in August 2003 (11 decapods, 2 euphausiids, 14 mysids, 44 amphipods, 1 caprellid, 6 hyperiids, 13 isopods, 1 tanaid, 23 cumaceans, 2 fish); the abundance of each specimen (N /100 m^2) was also given only for August 2003, where samples were identified to species level. The most abundant *taxa* were mysids (844 specimens/100m^2), amphipods (772), isopods (393) and cumaceans (258). Calanoid copepods and natantian decapods larvae were also abundant. The deepest (650 and 750 m) stations were richer in species than the shelf (150 m) and shelf-slope edge (350 m) stations. Among amphipods the most abundant species were *Rhachotropis* spp., among isopods *Munnopsurus atlanticus* and among mysids *Boreomysis megalops* at 350 m *and B. arctica* at the deepest stations. The number and diversity of species increased with depth, reaching a maximum (S=91, *H'*= 4,15) at the station located at 750 m (table 5-3).

Zooplankton

18 species (Annex 5-1) were collected in August 2003 (2 decapods, 4 euphausiids, 2 mysids, 11 hyperiids, 1 ostracod and 1 fish). Hyperiids (mainly *Hyperia schizogeneios* and *Primno cf. macropa*), euphausiids (mainly *Nematoscelis megalops*) and mysids (mainly *Leptomysis gracilis* at 166 m) were the most abundant groups. Also euphausiids and calanoid copepods dominated zooplanktonic community. The fish *Cyclothone braueri* was abundant (50 specimens/100m^2) in the deepest samples (750 m). The highest values in the number and diversity of species were observed in the "most inshore" (at 166 m) and in the "most offshore" station (at 750 m) (see table 5-3).

Table 5-3 Number of species (S), number of individuals (N), Species richness (d), Eveness (J'), diversity (H') and dominance (1-λ') for zooplanktonic (M1-M4) and suprabenthic (P1-P4) in August 2003. Depth f each sample was also reported

Sample	S	N	d	J'	H'	1-λ'
M1-166m	15	101	3,04	0,76	2,06	0,82
M3-347m	13	171	2,34	0,50	1,30	0,57
M4-760m	20	165	3,72	0,65	1,95	0,72
P1-155m	37	132	7,38	0,72	2,60	0,82
P2-670m	77	206	14,27	0,91	3,95	0,97
P3-340m	64	187	12,04	0,90	3,73	0,96
P4-747m	91	231	16,53	0,92	4,15	0,98

5.3.2 Trends in biomass

Suprabenthos

Suprabenthos biomass changed seasonally and as a function of depth gradients off Cabrera (Fig. 5-1).

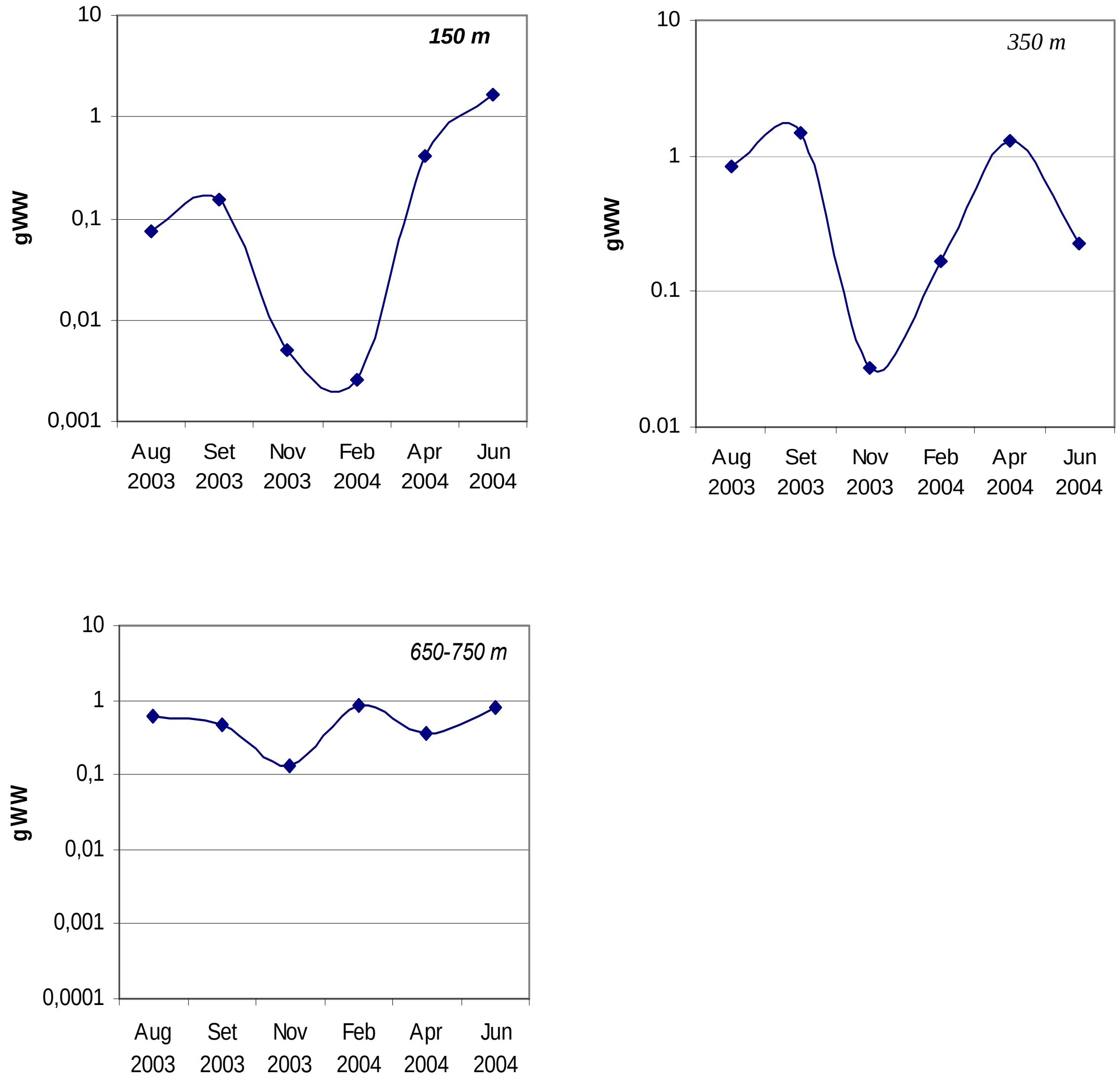


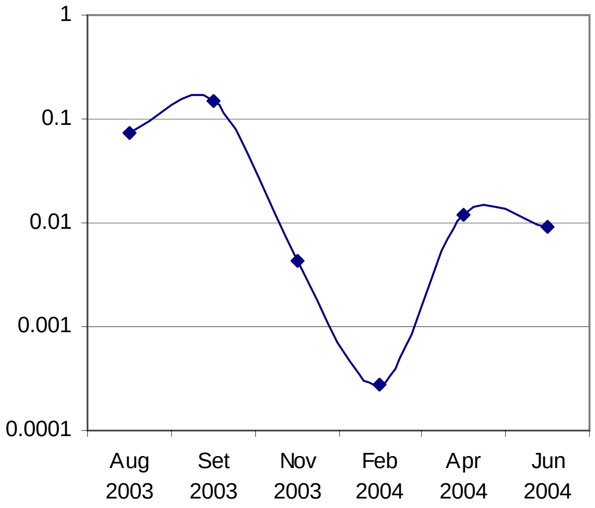
Figure 5-1. Seasonal changes in suprabenthos biomass by depth (150, 350 and 650-750m) off Cabrera

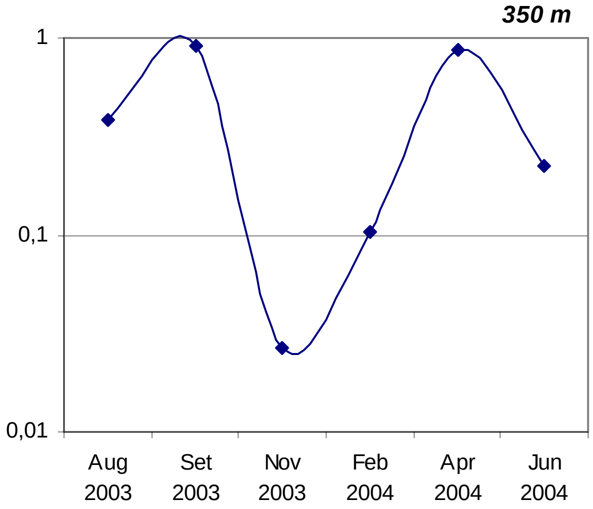
Mean annual biomass was on average an order of magnitude higher on upper-middle slope depths (between 350-750m: between 0.493 gWW/100 m^2 at 750m and 0.671 gWW/100 m^2 at 350m) than on the shelf-slope break (150m: between 0.003-0.388 gWW/100 m^2).

Off Cabrera, maximum biomass was recorded in April-June at 150m, in August-September and April at 350m, and in February and June-August at 650-750 m (Fig. 5-1). Minimum values were recorded in November-February at 150m and in November at 350m-750m, with a decrease to 0.027 gWW/100 m^2 at 650m.

Some regularities in the temporal dynamics of suprabenthos were found, indicating peaks of maximum biomass occurring from late spring to summer (April to August) and never or rarely in autumn-winter (September-November and February). By contrast, minima were regularly found in autumn (in September -November).

As concerns peracarid crustaceans different patterns of maximum biomass occurred at different depths (Fig. 5-2): At the shelf-slope edge and on the upper slope two peaks were detected in September and in April, while on the middle slope in August and in February. Maximum biomass was recorded in April-June at 150m, in September and April at 350 m and in February at 650-750 m (Fig. 5-2). Low values were recorded at 150 m during all year (mean value 0,042 gWW/100m^2).





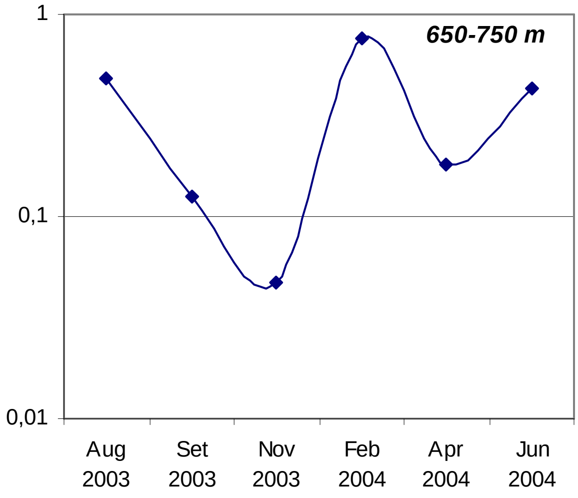
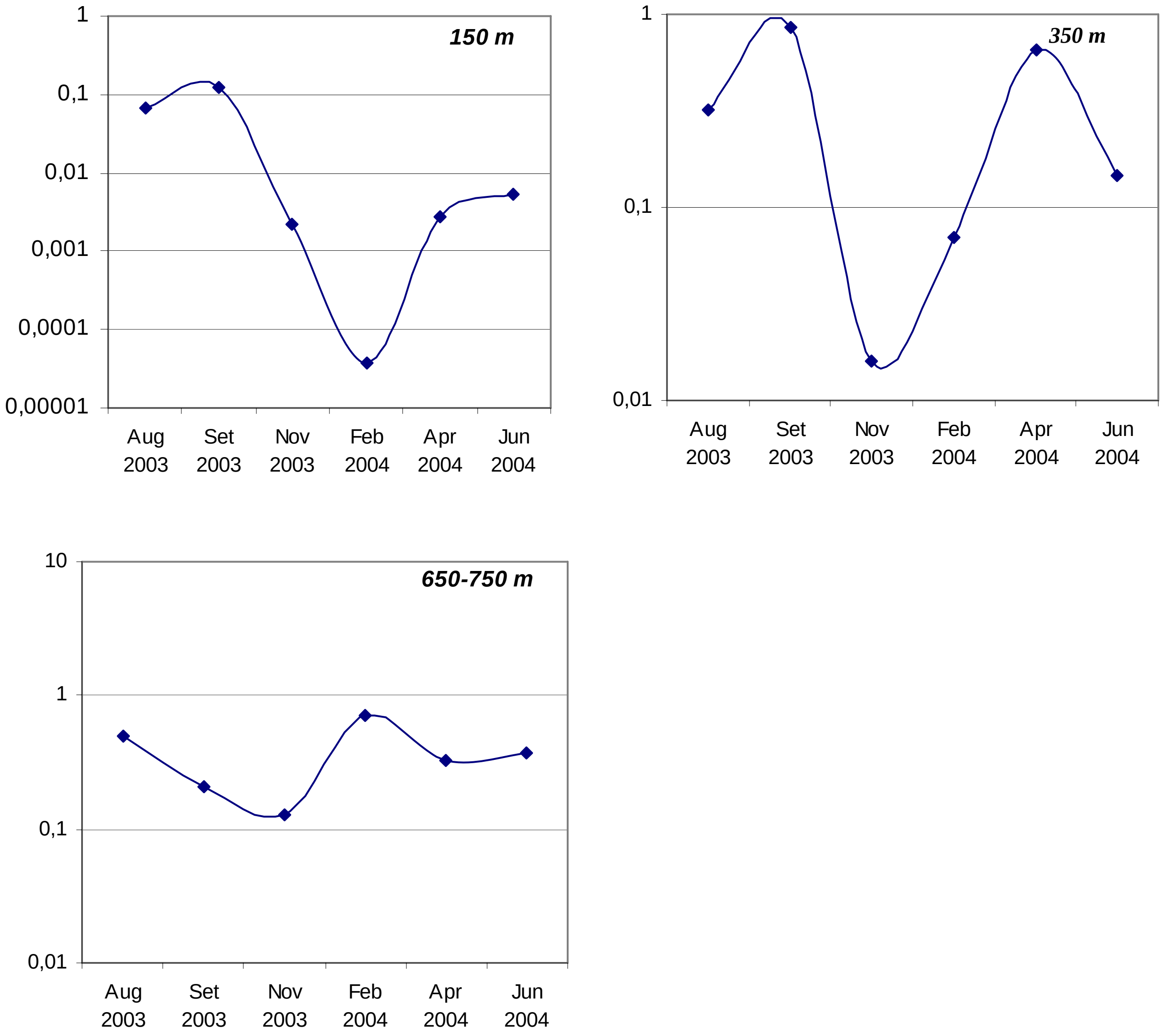


Figure 5-2. Seasonal changes in peracarid biomass by depth off Cabrera

Mysids showed a similar pattern than the whole peracarid crustaceans (fig. 5-3): a trend of increasing biomass was observed from August to September and from and from April to June at shelf-slope edge, on the upper slope two peaks were evident in September and April, on the middle slope biomass trend was more constant along the year (0,13-0,72 gWW/100m^2), slightly increasing in August and November. Maximum values were recorded in September and April at 350 m and in February at 650-750 m.

Figure 5-3. Seasonal changes in mysids biomass by depth off Cabrera

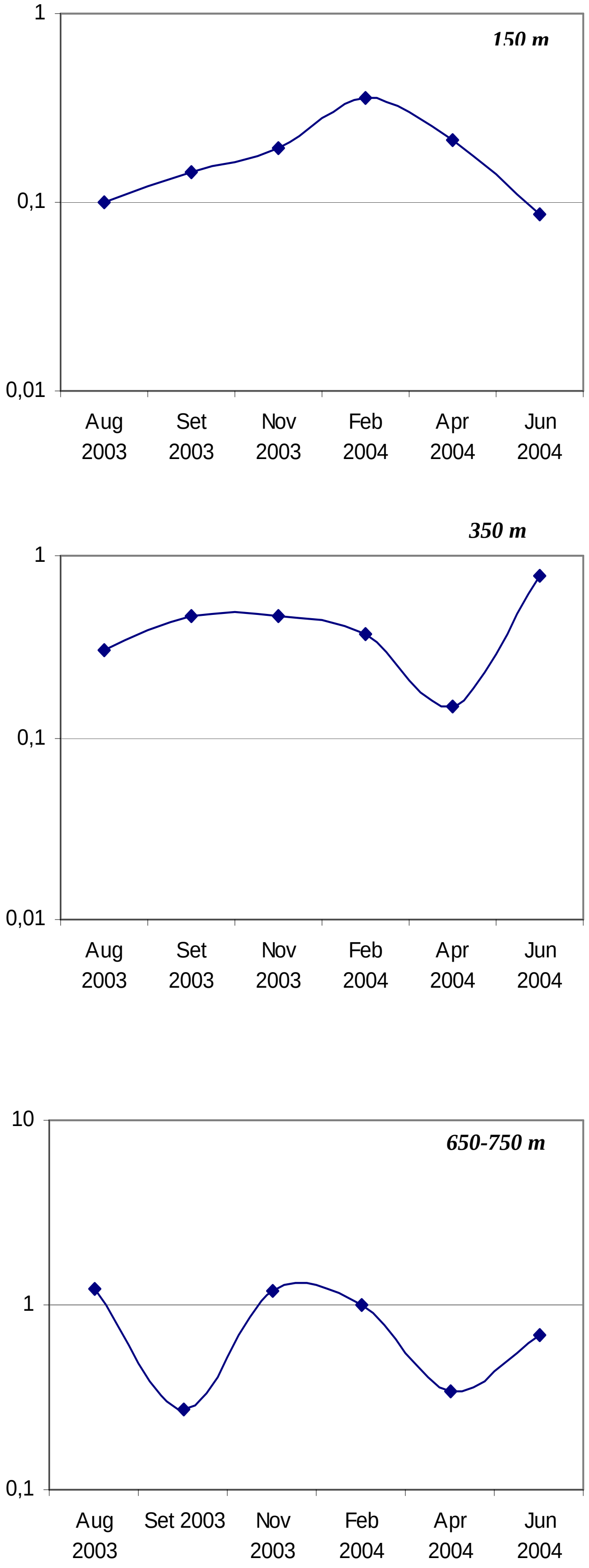


Zooplankton.

Though zooplankton showed spatial and temporal changes in our sampling, these fluctuations were not as important as in the case of suprabenthos.

Mean annual biomass increased offshore, ranging between 0.183-0.330 gWW/100 m^2 on the 150m isobath, increasing to 0.397-0.423 gWW/100 m^2 at 350m and to 0.657-0.879 gWW/100 m^2 offshore (on 650-750m) (Fig. 5-4).

Figure 5-4. Seasonal changes in zooplankton biomass by depth off Cabrera.



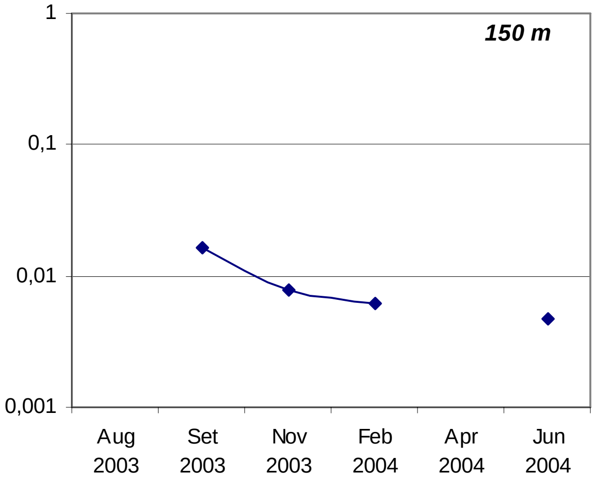
Off Cabrera, maximum biomass was recorded in February (0.360 gWW/100 m^2) at 150m, in June (0.784 gWW/100 m^2) at 350m, and in a virtual year later, in August, offshore at 650-750 m (1.211 gWW/100 m^2; Fig. 5-4). Minimum values were recorded in June-August at 150m (0.085 gWW/100 m^2), in April at 350m (0.148 gWW/100 m^2) and in April and September in 650-750m (0.275-0.345 gWW/100 m^2).

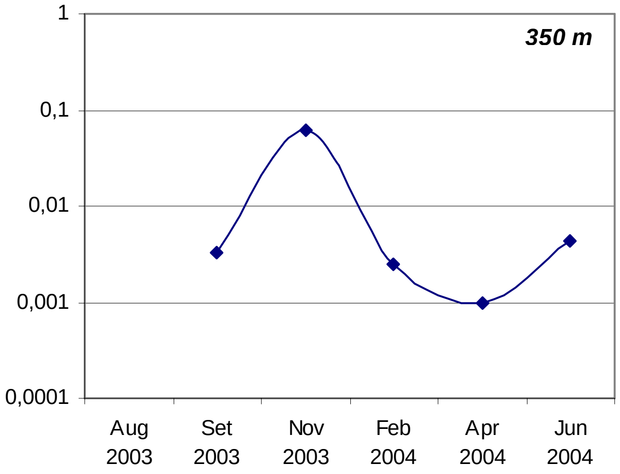
It is difficult to identify regularities in the temporal dynamics of zooplankton. Peaks of maximum biomass regularly occurred in late winter and summer (February and June) and never in autumn (September-November), while some minima occurred in autumn (August-September) and also in April.

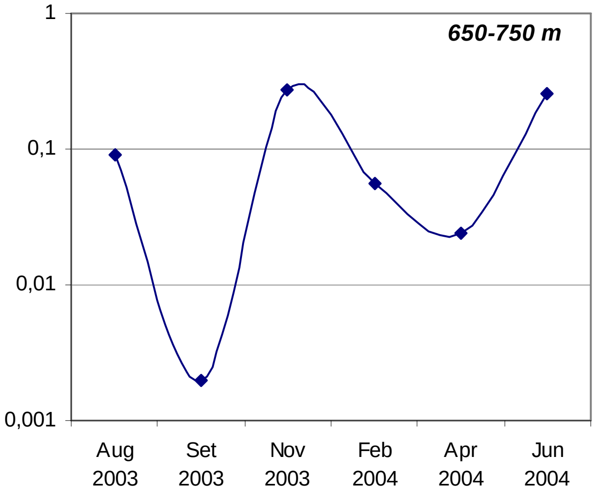
Maximum euphausiids biomass was recorded at 350 and 650-750 m in November and in June. At 650-750 m another peak was detected in August (Fig. 5-5).

Mean euphausiids biomass increased off-shore ranging between 0-0.0165 gWW/100 m^2 on the 150m isobath, increasing to 0-0.061 gWW/100 m^2 at 350m and to 0.001-0.269 gWW/100 m^2 offshore (on 650-750m) (Fig. 5.5).

Figure 5-5. Seasonal changes in euphausiids biomass by depth off Cabrera







5.3.3 Multivariate analysis

Suprabenthos

Suprabenthos varied as a function of depth gradients (Fig.5-6).

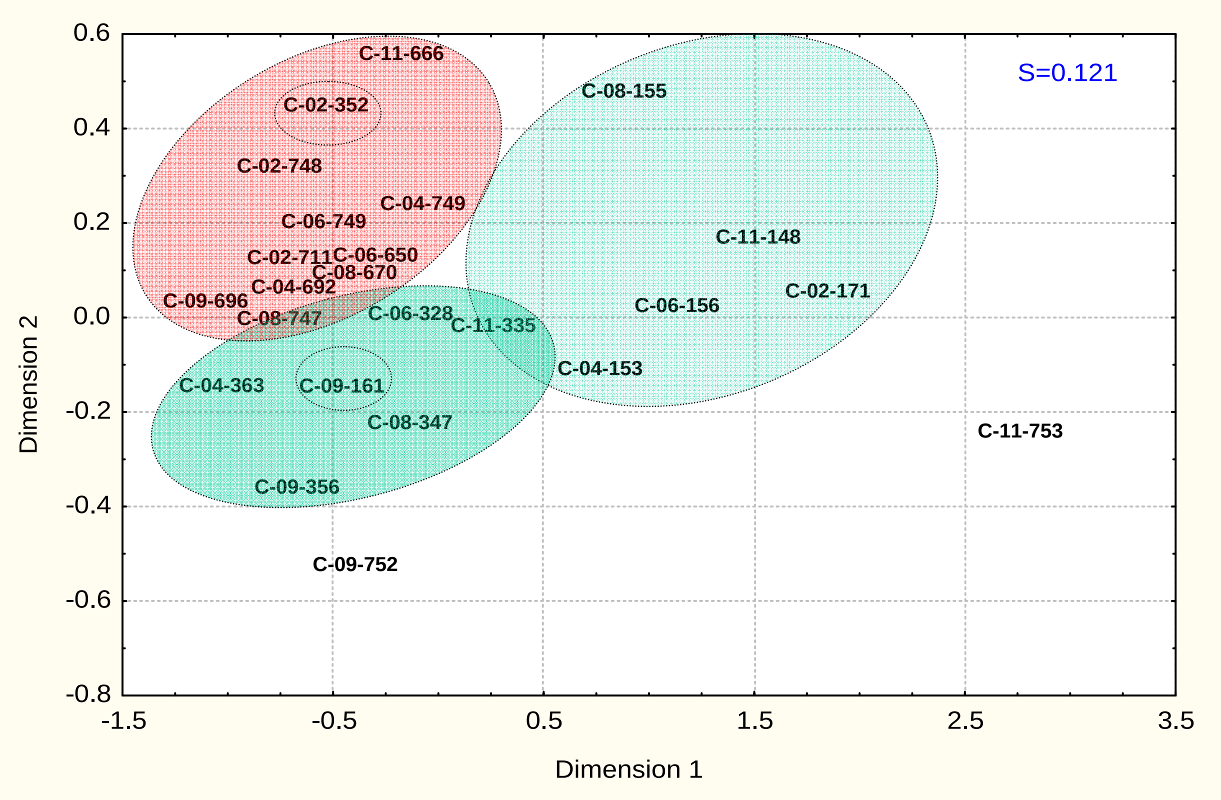


Figure 5-6. MDS performed on suprabenthos abundance off Cabrera.

Off Cabrera, MDS analyses showed sampling aggregation for those hauls taken around 150m, and 350m which appeared segregated from the deeper samples (650 and 760m). Samples from the deepest part of the slope (650-750m) were not separated between them. The haul C11-753m, characterized by a very low abundance of fauna, was more related with hauls of the shelf-slope break, probably for this reason.

Within each depth group we did not find any trend related with seasonality, for instance aggregation of samples from summer, or winter, as we found among zooplankton (see below),

The taxonomic composition of suprabenthos by depth strata was showed in Table 5-4.

Table 5-4. Composition of suprabenthos (abundance: ind/100 m^2) collected off Cabrera by depth strata and area. (**) taxa exclusively or mainly composed by permanent suprabenthos; (*) taxa composed both by suprabenthic and meso-bathypelagic species, or infauna stages.

	Cabrera		
	150m	***350m***	***650-750m***
Medusae (plus Scyphozoa)*	0	0	0,2
Siphonophora	0,6	2,5	1,0
Natantia*	0,9	3,1	1,2
Euphausiacea	0,8	1,1	2,0
Mysidacea **	21,4	**85,2**	56,7
Amphipoda Gammaridea**	8,1	**94,1**	72,3
Amphipoda Hyperiidea	0,5	0,3	1,4
Amphipoda Caprellidea	0	0,5	0
Isopoda**	4,2	39,1	**67,6**
Tanaidacea	0	0,2	0,1
Cumacea*	3,4	**57,2**	19,9
Ostracoda*	7,1	6,2	8,9
Copepoda Calanoidea*	267,1	**342,4**	140,9
Cyclopoidea	3,2	0,1	0,9
Nebaliacea**	0	0	0
PYCNOGONIDA*	0	0,04	0
Gastropoda (Cymbulia peroni)	0,1	0	0
Pteropoda	0,4	0,3	0,1
Cephalopoda	0,2	0,1	0
CHAETOGNATHA	4,5	13,8	2,5
THALIACEA	0	0,1	0,02
Pyrosomidae	0	0,03	0,02
Osteichthyes*	0,6	0,6	2,6

Apart of copepods, which were always dominant in number, peracarids (belonging to permanent suprabenthos: mysids, gammaridean amphipods, isopods and cumaceans) were the dominant taxa. These same taxa increased their densities at bathyal stations in comparison to the shelf-slope break. Most taxa showed peaks at intermediate depths (350 m).

Zooplankton.

In contrast to suprabenthos dynamics, changes in the abundance of zooplankton showed a clear seasonal pattern (Figure 5-6).

A seasonal pattern occurred off Cabrera. Most samples of June2003 and August2004 (summer) were grouped between them in the top of the plot (Fig. 5-6). By contrast, samples performed in September-November2003 and February2004 (autumn-winter) were grouped in the opposite site of the plot. April2004 samples appeared grouped between them thus suggesting spring is a period of change between two extreme situations regarding taxonomic composition of zooplankton.

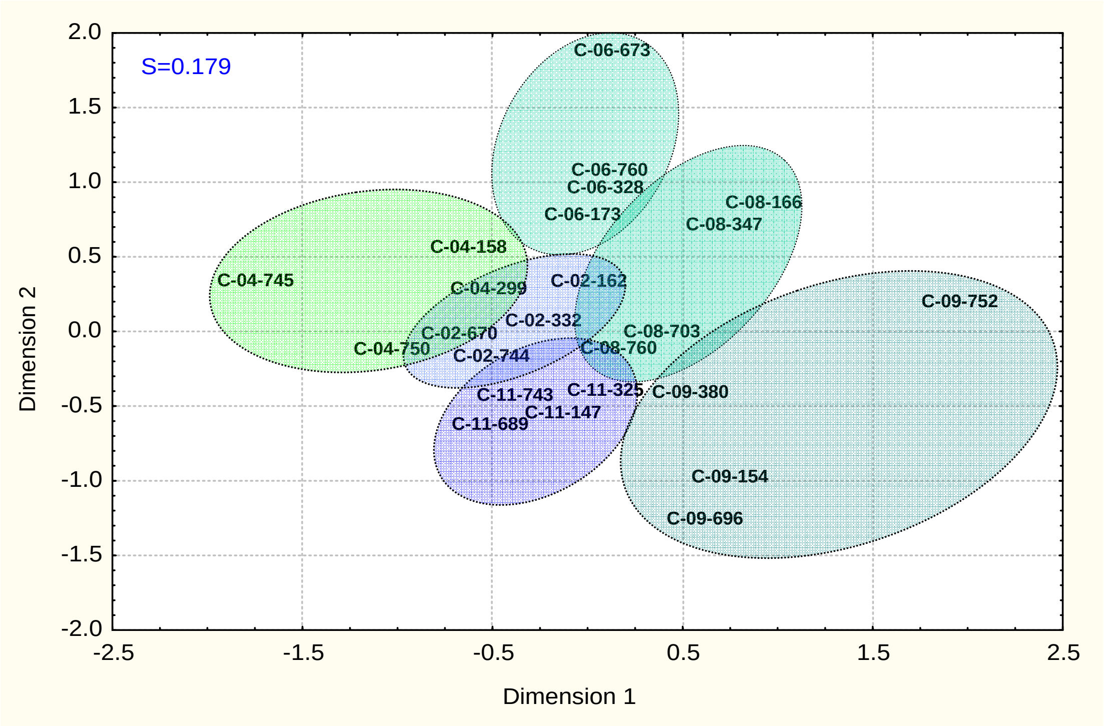


Figure 5-6. MDS performed on zooplankton biomass off Cabrera.

The taxonomic composition of zooplankton is given in Table 5-5 as a function of season.

Table 5-5. Seasonal composition (abundance: ind/100 m^2; biomass: gWW/100 m^2) of zooplankton collected off Cabrera. (**) suprabenthic taxa collected in the water column (15 mab to surface); (-) < 0.001 gWW/100 m^2.

Abundance (ind/100 m2)	***Ago***	***Sep***	***Nov***	***Feb***	***Apr***	***Jun***
Medusae (plus Scyphozoa)	0,4	0,1	**0,8**	**0,7**	0,2	0,5
Siphonophora	**28,1**	17,9	16,5	13,0	4,2	10,1
Ctenophora	0,03	0	0,1	0,03	0	0,1
POLYCHAETA	0	0	0	0,2	0,4	0,1
Natantia	0,4	**1,4**	0,7	0,8	0,6	0,9
larvae	**37,5**	4,1	11,9	**27,4**	17,7	1,1
Euphausiacea	5,9	**19,2**	**21,8**	6,9	1,0	**19,0**
larvae	**83,2**	3,7	10,5	32,7	54,9	44,0
*Mysidacea***	2,0	**9,5**	2,9	2,4	1,2	0
*Amphipoda Gammaridea***	0,4	0,1	0,1	0,5	**2,0**	0
Amphipoda Hyperiidea	**25,9**	11,7	11,5	19,4	6,0	18,7
*Isopoda***	0,2	0,1	0,1	1,0	**2,6**	0,4
*Cumacea***	0	0,1	0,03	0	0,1	0
Ostracoda	**160,8**	18,3	**120,6**	**134,6**	95,0	69,9
Copepoda Calanoidea	**2153,2**	397,6	1438,5	**2772,0**	1646,5	1593,6
Cyclopoidea	9,2	18,5	39,0	**63,9**	**53,6**	0
Cladocera	0	0	0,5	0,6	0	0
Gastropoda	**10,8**	**7,1**	**9,8**	0	0,7	0,2
Pteropoda	**86,8**	13,0	11,9	5,7	0,3	0
Cephalopoda	0,03	0	0,1	0,03	0,01	0,1
CHAETOGNATHA	**123,1**	16,3	52,2	18,0	17,4	**71,7**
THALIACEA	1,8	**3,9**	0,4	**4,2**	2,5	0,1
Pyrosomidae	0,3	0,1	0,3	**2,5**	0,7	0,1
Osteichthyes	4,5	1,7	**14,4**	**10,7**	5,1	0,6
larvae	**25,2**	0,1	0,8	0,9	1,4	**14,3**
OTHER taxa	0	0	0	0,1	0,1	0
TOTAL	2759,8	544,3	1765,4	3118,3	1914,1	1845,5

Biomass (gWW/100 m2)						
Medusae (plus Scyphozoa)	**2,543**	0,262	0,056	1,225	0,695	**2,845**
Siphonophora	0,771	0,883	**1,051**	**1,189**	0,427	0,893
Ctenophora	0,001	0	**0,058**	**1,685**	0	-
POLYCHAETA	0	0	0	0,002	**0,048**	-
Natantia	0,559	0,627	**0,851**	0,424	0,396	**0,657**
larvae	0,160	**0,477**	0,030	0,127	0,062	0,004
Euphausiacea	0,955	0,505	**2,478**	0,952	0,218	**2,677**
larvae	**0,221**	0,015	0,062	0,045	**0,134**	0,058
*Mysidacea***	0,131	**0,784**	0,046	0,313	0,141	0,031
*Amphipoda Gammaridea***	0,005	-	-	0,003	0,014	0
Amphipoda Hyperiidea	0,259	0,150	0,250	**1,208**	0,105	**0,813**
*Isopoda***	0,005	0,001	0,000	0,008	**0,018**	0,003
*Cumacea***	0	-	-	0	0,001	0,004
Ostracoda	0,094	0,009	0,084	**0,117**	0,085	0,050
Copepoda Calanoidea	**3,989**	0,434	**2,543**	**3,632**	1,926	2,107
Cyclopoidea	0,003	0,008	**0,021**	**0,032**	0,022	0
Cladocera	0	0	-	-	0	0
Gastropoda	**0,426**	0,038	0,011	0	-	0,025
Pteropoda	0,116	0,086	0,097	**0,262**	0,005	0
Cephalopoda	0,001	0	0,002	**0,017**	0,000	0,001
CHAETOGNATHA	**0,858**	0,140	**0,765**	0,489	0,471	0,470
THALIACEA	0,733	0,655	0,703	**1,585**	0,170	0,157
Pyrosomidae	0,008	0,001	0,887	0,397	0,001	**2,504**
Osteichthyes	1,264	0,142	**2,141**	**1,975**	1,163	0,058
larvae	0,103	0,017	0,033	0,034	0,027	**1,537**
OTHER taxa	0,054	0	0	0,346	0,001	0,447
TOTAL	13,260	5,234	12,614	16,377	6,156	15,341

Apart of copepods, the dominant taxa in terms of abundance (by decreasing order) were ostracods, chaetognaths, pteropods, euphausiids (adults and larvae) and decapod larvae. Some suprabethic taxa (mysids, gammaridean amphipods, isopods, and cumaceans) also appeared in the water column though in low numbers (to 1-2 ind/100 m^2). Maximum densities of zooplankton were recorded in February2004, whereas minima were found after this peak (in April-June) and also in September2003. By number, differences in the proportion of taxa between summer (June-August) and autumn-winter (September-November and February) were low, and only chaetognaths and fish larvae had higher densities in summer.

In terms of biomass, most taxa showed peaks in February. Bearing in mind seasonal groups derived from MDS analyses, jellyfish, gastropods (*Cymbulia peroni*) and Pyrosomids were abundant in summer (June-August). By contrast, groups mainly dominating in autumn-winter zooplankton were siphonophores, ctenopohores, Natantian decapods (depending of species) and decapod larvae, cyclopoids, pteropods, salps (in February) and adult meso-bathypelagic fish. Other taxa presented strong oscillations in different periods of the year.

5.3.4 Environmental variables

Salinity (S) 5mab ranged from 38.28 ppm at 150 m (Nov2003) to 38.51 ppm at 350 m (June2004). In general S increased from the shelf-slope break to bathyal stations (Fig. 5-7a).

Seasonal pattern was different depending of depth. Maximum S was regularly recorded in June2004 both at 150m and deeper on the slope. However, at 150m maxima were also recorded in September-November 2004.

Temperature (T) 5mab, showed different pictures depending of depth and ranged between 12.94 ºC at 650 m to 13.47 ºC at 150 m. On average there was an increase of T close to the bottom of 0.2-0.3 ºC from the shelf slope break to 650-750m on the slope. Seasonal pattern depended on depth. On the shelf slope break maximum T was recorded in February decreasing in April-June. Deeper there was an increase of T in June.

Trends in %OM and in potential REDOX by depth are in Figure 5-8.

Figure 5-7. Trends in Temperature (T) (on the right) and Salinity (ppm) (on the left) close to the bottom on 150, 350, and 650-750 m depths off Cabrera Archipelago.

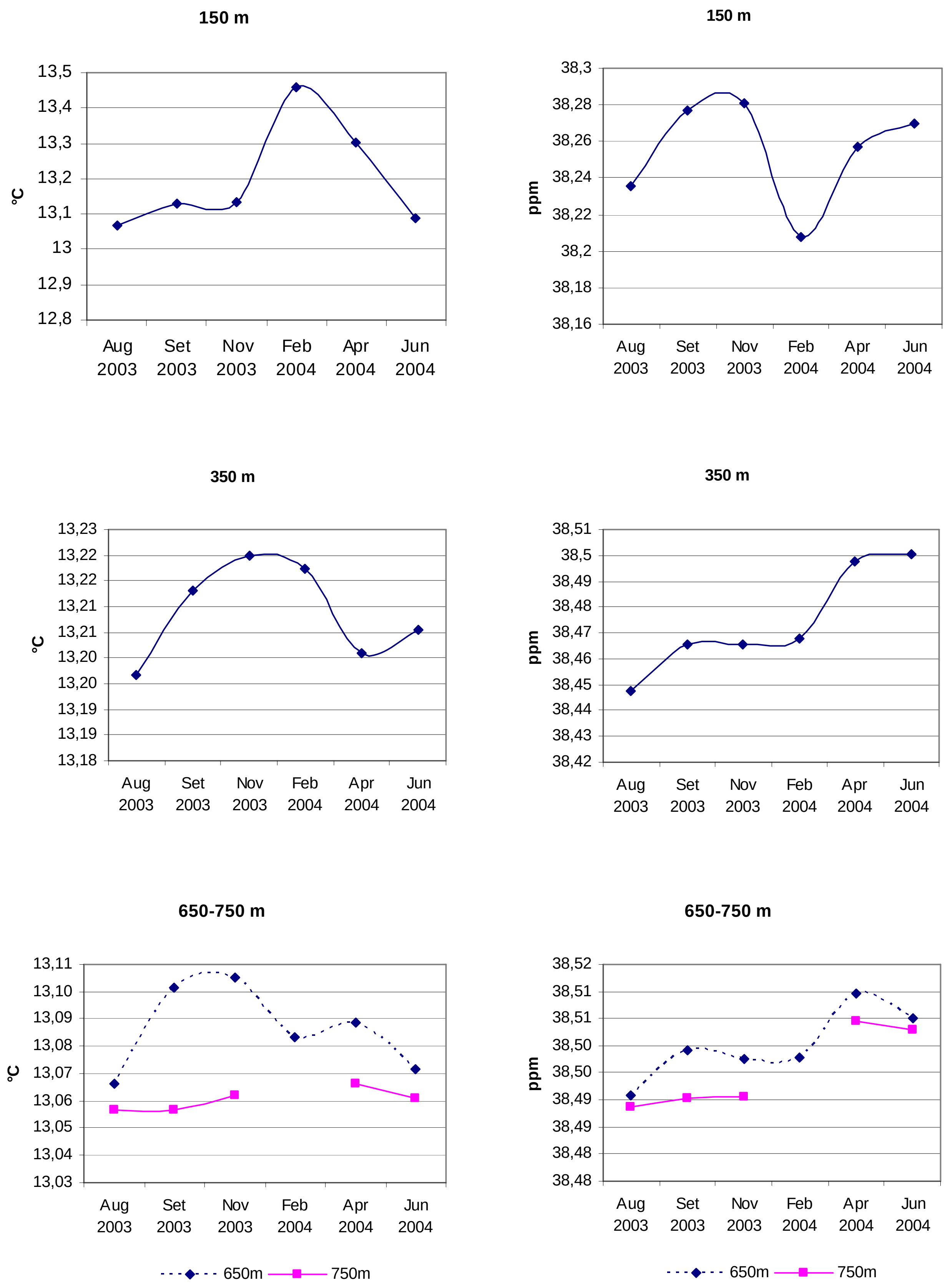
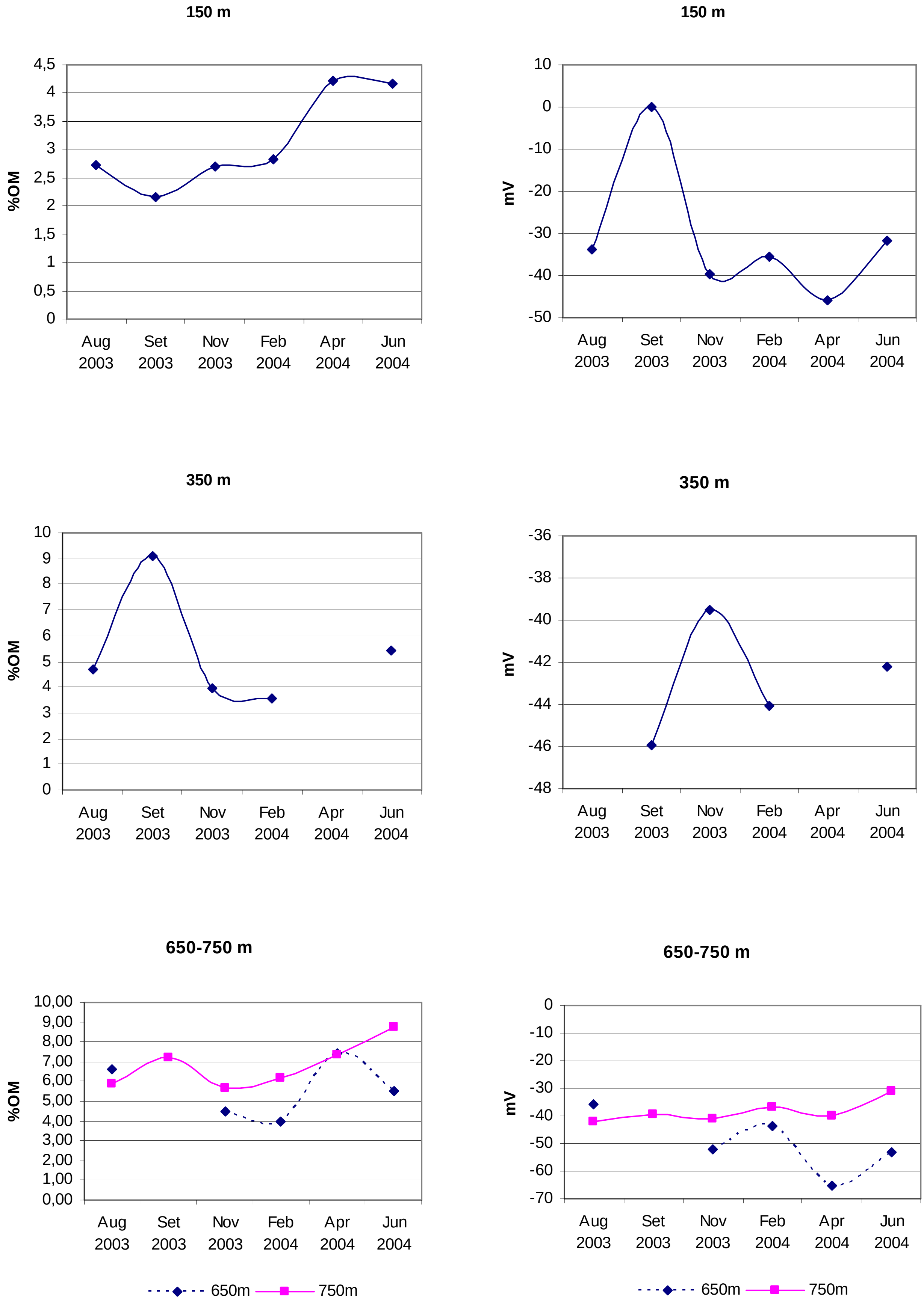


Figure 5-8. Trends in %OM (on the right) and potential REDOX (mV)(on the left) in sediments close to the bottom on 150, 350, and 650-750 m depths off Cabrera Archipelago.



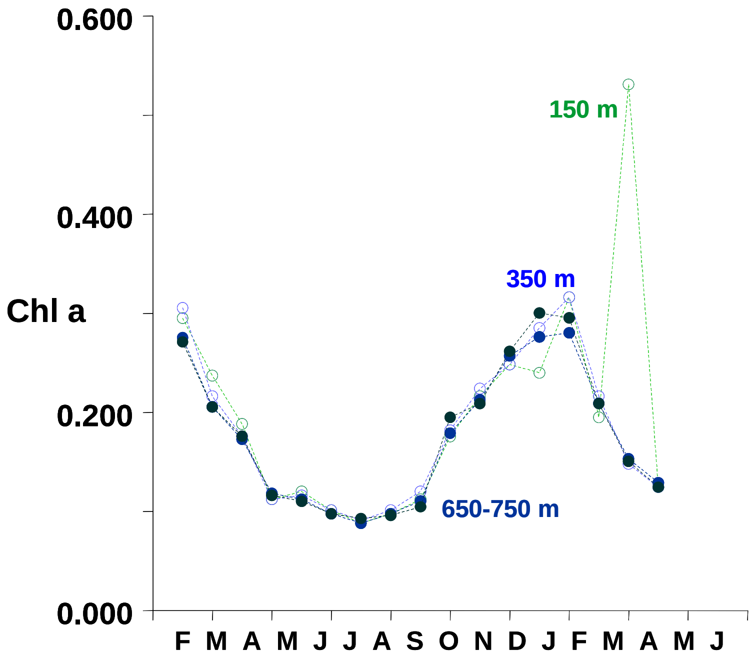
The %OM increased with depth, from the shelf-slope break (1.6-4.5%) to bathyal stations (2.5-9%), and this increment was, in general, parallel to the increase in the proportion of mud on sediments (data not included).

The most regular temporal trend was the increase of OM from February2004 to April and June recorded in both areas and at all depths. In February2004 it was recorded the minimal value of OM in sediments at bathyal stations below 350 m depth.

Records of potential REDOX (Figure 5-8 on the left) showed less variability (a narrower range of values) with minima recorded in November and April at 150 m (range -46 to –39 mV) and at 650 m (range -65 to -52 mV), a more constant trend was detected deepest at 750 m (range -42 to -31 mV).

Chl *a* derived from satellite imagery are shown in Figure 5-9. Off Cabrera there was a unique peak of Chl *a* on surface in February-March 2004 with a period of low production between May-October. The accentuate peak observed at 150 m in March-April could be derived from an occasional phytoplanktonic bloom not observed the year before.

Figure 5-9. Monthly *Chl a* derived from satellite imagery (http://reason.gsfc.nasa.gov/Giovanni) off Cabrera.



5.3.5 Possible explanatory variables of dynamics (MDS dimensions and biomass).

5.3.5.1 MDS dimensions

Suprabenthos.

The ordination of hauls in inertia axes of suprabenthos was correlated off Cabrera (D1) with salinity (*S*), *REDOX* on surface of sediments, and with Chla recorded simultaneously to 2 months before ($Chla_{sim}$, $Chla_{1mo}$, $Chla_{2mo}$) (table 5.7) .D2 was correlated with depth, with *%OM*, and with textural parameters of sediments (% of gravels, sands, inversely with finer – mud – sediments, median size) and with *REDOX* potential (difference from surface to 5 cm into the sediment).

Table 5-6. Correlations between MDS dimensions (abundance) and environmental (physical, and geochemical) variables of suprabenthos;

CABRERA							
		DIM. 1			**DIM. 2**		
		n	***r***	***p-level***	***n***	***r***	***p-level***
Depth (m)		24	-0,456	0,025	24	-0,0457	0,832
T(5mab)		23	0,1877	0,391	23	-0,0217	0,922
S(5mab)		23	-0,541	0,008	23	0,17	0,438
OM (%)		22	-0,467	0,028	22	-0,2332	0,296
gravels	*(%)*	21	0,6431	0,002	21	0,0114	0,961
sand	*(%)*	21	0,7416	1E-04	21	-0,0805	0,729
mud	*(%)*	21	-0,742	1E-04	21	0,0805	0,729
median phi	*mm*	21	0,713	3E-04	21	-0,2701	0,236
REDOX	*mV (surf)*	24	-0,001	0,995	24	-0,1724	0,421
	mV (5 cm)	18	0,0588	0,817	18	-0,0114	0,964
Chla satellite	*4 month*	24	-0,15	0,485	24	0,0218	0,92
	3 month	24	-0,186	0,383	24	0,1603	0,454
	2 month	24	-0,144	0,501	24	0,2871	0,174
	1 month	24	0,1071	0,618	24	0,3513	0,092
	simult.	24	-0,037	0,862	24	0,3584	0,086
Fluor	*Max depth Chl*	10	0,1281	0,724	10	0,0671	0,854
	Fluorometry	10	-0,079	0,828	10	-0,1037	0,776
	ΣFlu200m	10	-0,506	0,136	10	0,0549	0,88

Zooplankton.

Off Cabrera, dimension 1 (D1) of MDS (i.e. the ordination of hauls in inertia axes) performed on zooplankton was significantly correlated with salinity (*S*), *REDOX* on surface of sediments, and with Chla recorded simultaneously to 2 months before ($Chla_{sim}$, $Chla_{1mo}$, $Chla_{2mo}$). D2 was correlated with Chla recorded between 1 to 4 months before hauls ($Chla_{1mo}$, $Chla_{2mo}$, $Chla_{3}$, $Chla_{4mo}$) (table 5-7).

In general, changes in the affinity between hauls off Cabrera seem to be related with *Chla* on surface.

Table 5-7. Correlations between MDS dimensions (abundance) and environmental (physical, and geochemical) variables of zooplankton.

CABRERA

		DIM. 1			**DIM. 2**		
		n	***r***	***p-level***	***n***	***r***	***p-level***
Depth (m)		24	-0,124	0,56394	24	-0,174	0,4163
T(5mab)		23	-0,198	0,36605	23	-0,042	0,8509
S(5mab)		23	-0,304	0,15796	23	0,0751	0,7334
M.O.(%)		22	-0,033	0,88299	22	0,1237	0,5835
gravels	*(%)*	21	0,091	0,6943	21	-0,013	0,9553
sand	*(%)*	21	0,118	0,60991	21	-0,074	0,7498
mud	*(%)*	21	-0,118	0,60991	21	0,074	0,7498
median phi	*mm*	21	0,235	0,30503	21	0,0779	0,7371
REDOX	*mV (sup)*	24	0,464	0,02224	24	-0,054	0,8038
	mV (5 cm)	18	0,325	0,18808	18	0,2755	0,2684
Chla satèl.lit	*4 month*	24	0,035	0,87006	24	0,7954	3E-06
	3 month	24	-0,321	0,12671	24	0,6376	0,0008
	2 month	24	-0,537	0,00686	24	0,586	0,0026
	1 month	24	-0,8	2,6E-06	24	0,4466	0,0287
	simult.	24	-0,841	2,7E-07	24	-0,065	0,7617
Fluor	*Prof.max Cl*	10	0,165	0,64946	10	0,0976	0,7886
	Fluorometry	10	0,75	0,01248	10	-0,061	0,8671
	ΣFlu200m	10	0,421	0,22598	10	-0,402	0,2489

A comparison between results from suprabenthos and zooplankton suggest that suprabenthos was related with a number of sediment features (grain size, nature of sediments, %OM…) and to a lesser extend with Chl *a* on surface while zooplankton was mainly related with records of Chl *a* on surface, especially with simultaneous or recent (< 2months) readings.

5.3.5.2 Abundance

Suprabenthos

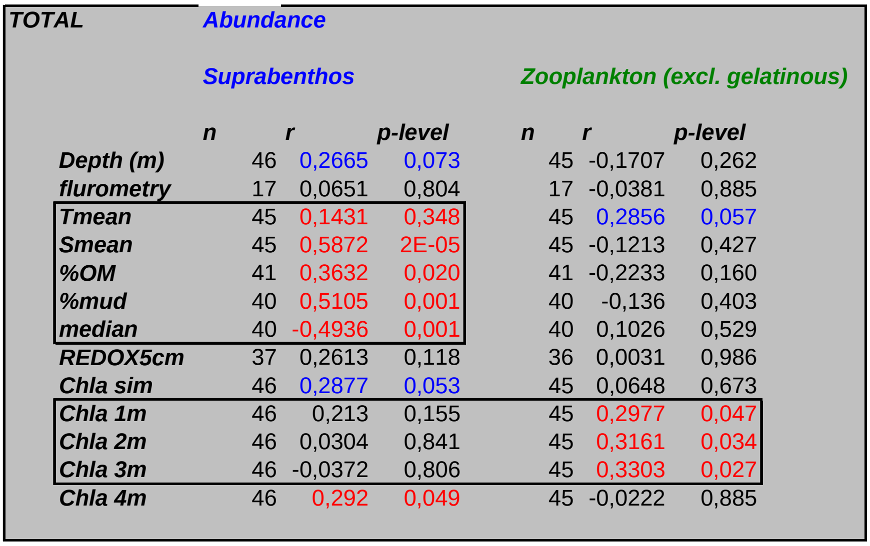
Off Cabrera, both suprabenthos and peracarid abundances were significantly higher with higher *S* 5mab, with % of mud (and smaller median grain size), %OM and with more oxidized sediments (5cm). In addition to these variables, *Chla* taken simultaneously and 4months before samplings (only for peracarids) also were higher with higher abundance (table 5-8).

Zooplankton.

Off Cabrera, abundance of zoooplankton was significantly higher with higher *Chla* on surface 3 months before sampling, and higher *T*.

Comparing trends between suprabenthos and zooplankton, and as it happened in the case of MDS dimensions, intra-annual fluctuations in suprabenthos abundance were more dependent of variables related with sediment nature and conditions (e.g. OM, REDOX), and with salinity close to the bottom. By contrast, zooplankton was almost exclusively dependent of *Chla* on surface (table 5-8).

Table 5-8. Correlations between abundance and environmental (physical, and geochemical) variables: (a) suprabenthos; (b) zooplankton.



TOTAL	**Abundance**					
	Suprabenthos			**Zooplankton (excl. gelatinous)**		
	n	**r**	**p-level**	**n**	**r**	**p-level**
Depth (m)	46	0,2665	0,073	45	-0,1707	0,262
flurometry	17	0,0651	0,804	17	-0,0381	0,885
Tmean	45	0,1431	0,348	45	0,2856	0,057
Smean	45	0,5872	2E-05	45	-0,1213	0,427
%OM	41	0,3632	0,020	41	-0,2233	0,160
%mud	40	0,5105	0,001	40	-0,136	0,403
median	40	-0,4936	0,001	40	0,1026	0,529
REDOX5cm	37	0,2613	0,118	36	0,0031	0,986
Chla sim	46	0,2877	0,053	45	0,0648	0,673
Chla 1m	46	0,213	0,155	45	0,2977	0,047
Chla 2m	46	0,0304	0,841	45	0,3161	0,034
Chla 3m	46	-0,0372	0,806	45	0,3303	0,027
Chla 4m	46	0,292	0,049	45	-0,0222	0,885

5.3.6. Stable isotope ratios of Carbon and Nitrogen (δ^{13}C and δ^{15}N)

Suprabenthos

Twenty species (1 mysid, 11 amphipods, 4 cumaceans (and the whole *taxon* Cumacea), 3 isopods and 1 decapod) and copepods were analyzed on a annual basis for stable carbon (δ^{13}C) and nitrogen (δ^{15}N) isotopes (Table 5-1). 6 species (3 amphipods, 1 decapod, 1 isopod and 1 mysid) and copepods and cumaceans were analyzed on a seasonal basis (table 1, species with *).

The ranges of δ^{15}N and δ^{13}C isotope ratios for each species are presented in Figure 5-10 and Figure 5-11 respectively.

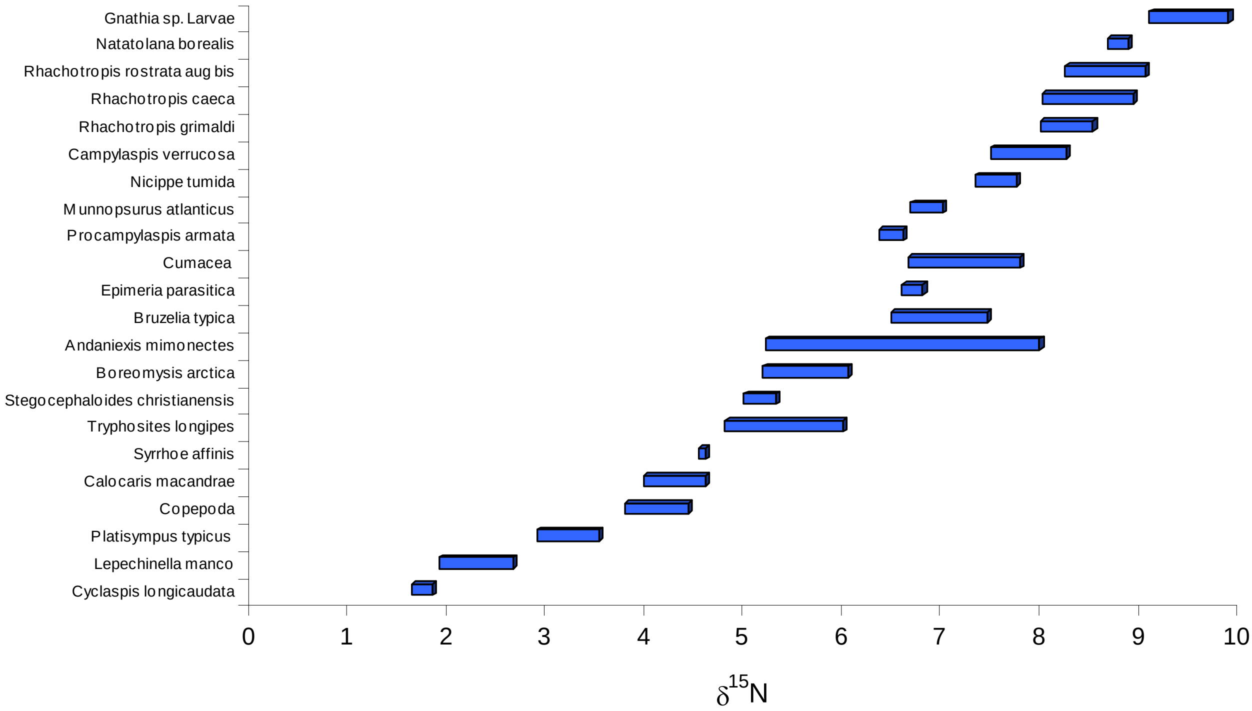


Fig. 5-10 Range of δ^{15}N values (‰) (mean ± standard deviation) for suprabenthic species

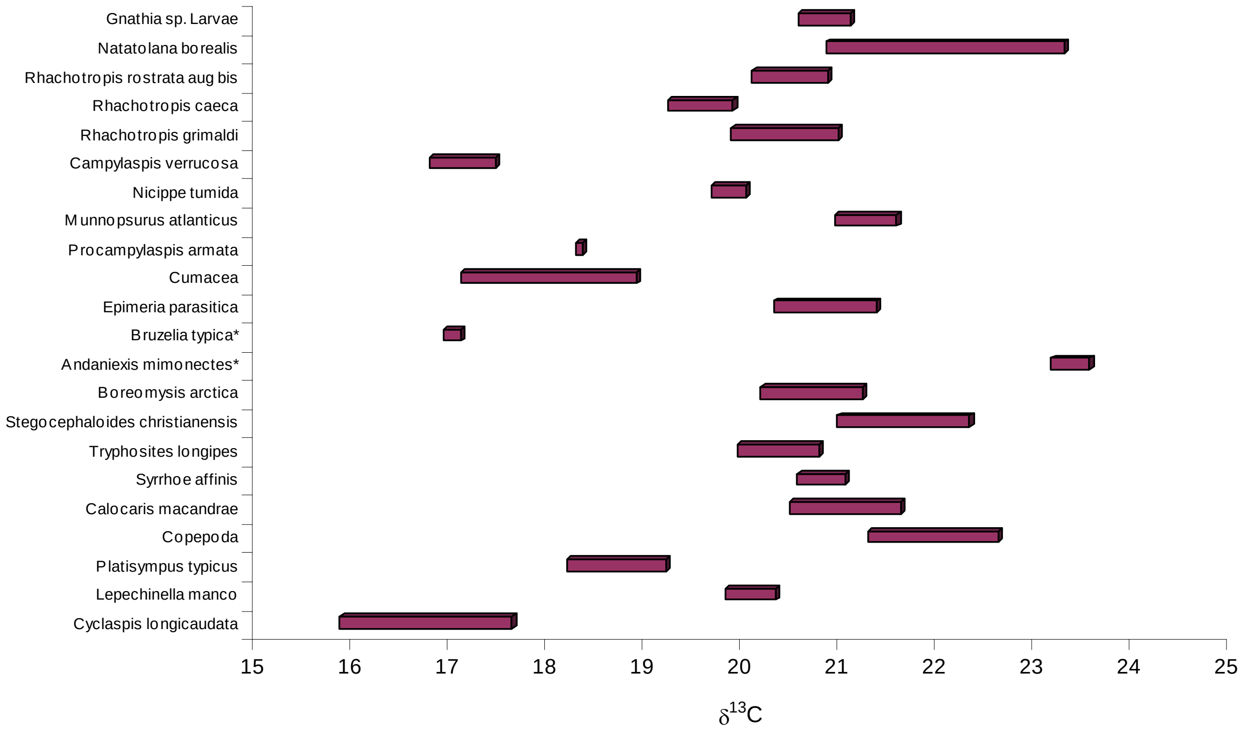


Fig. 5-11 Range of $\delta^{13}C$ values (‰) (mean ± standard deviation) for suprabenthic species (all the values were negative).

Our isotopic analysis revealed a considerable range of $\delta^{13}C$ and $\delta^{15}N$ values for suprabenthic species. Low overlap for $\delta^{15}N$ and $\delta^{13}C$ values was observed, though $\delta^{15}N$ values were less variable than $\delta^{13}C$. $\delta^{13}C$ values of suprabenthos taxa ranged from -23,2‰ (*Andaniexis mimonectes* in February 2004) to -14,9‰ (*Cyclaspis longicaudata* in February 2004). Stable nitrogen isotope ($\delta^{15}N$) ranged from 1,3‰ (*Lepechinella manco* in February 2004) to 10,5‰ (*Gnathia* sp. in November 2003). Assuming a trophic enrichment of 3‰ (Post 2002), the overall range of $\delta^{15}N$ is indicative of three main trophic levels. The wide range of isotopic values obtained is in accordance with many different trophic strategies, and corresponded quite well with the trophic classification based on gut contents analysis-isotopic analyses (data from the literature, Table 2). Thus, species with more enriched $\delta^{15}N$ values are carnivorous and scavenger species (e.g. the amphipods *Rhachotropis* spp., *Natatolana borealis*), while low $\delta^{15}N$ values identify mainly filter-feeders (more closely associated to plankton) (e.g. mysids *Boreomysis* spp.) or deposit-feeders (some species of cumaceans and maybe the amphipod *L. manco*). The $\delta^{13}C$ values were generally higher (less negative) for carnivorous species (e.g. *Rhachotropis* spp.) than for filter-feeders (e.g. Copepods, *Boreomysis arctica*).

Zooplankton

Twelve species (3 euphausiids, 3 hyperiids, 4 decapods and 2 fish) and copepods were analyzed on an annual basis for stable carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) isotopes (Table 5-1) The ranges of $\delta^{15}N$ and $\delta^{13}C$ isotope ratios for each species are presented in Figure 5-12 and Figure 5-13 respectively.

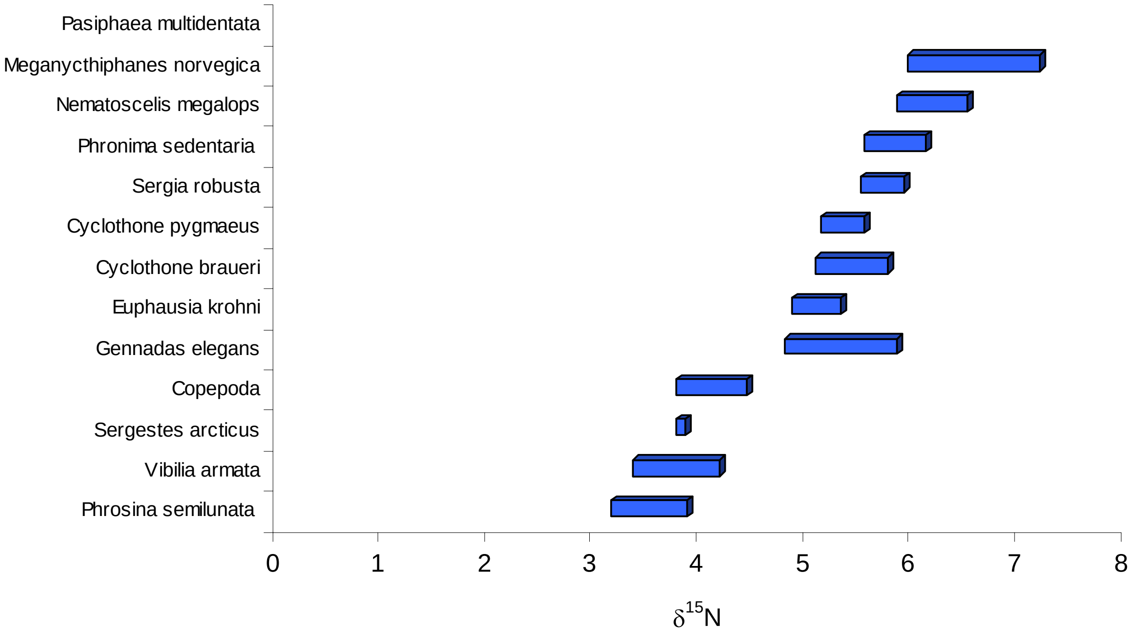


Fig. 5-12 Range of $\delta^{15}N$ values (‰) (mean ± standard deviation) for zooplanktonic species

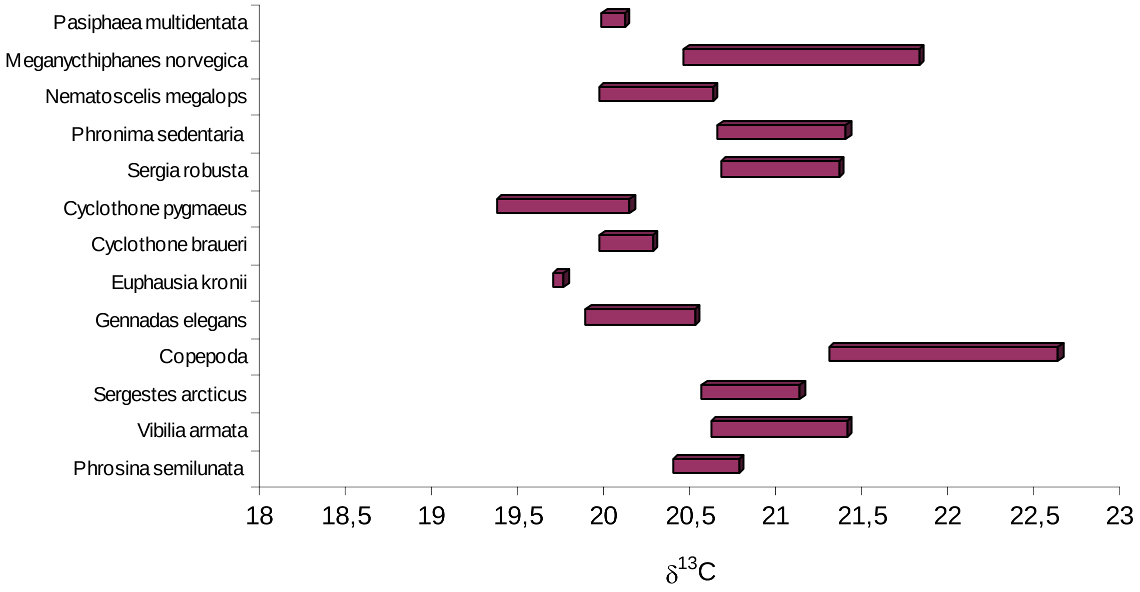


Fig. 5-13 Range of $\delta^{13}C$ values (‰) (mean ± standard deviation) for zooplanktonic species

Our isotopic analysis revealed a narrower range of $\delta^{13}C$ and $\delta^{15}N$ values for zooplanktonic species, comparing with suprabenthic species, especially for $\delta^{13}C$. $\delta^{13}C$ values of zooplanktonic *taxa* ranged from -19,4‰ (*Cyclothone pygmaea* in February 2004) to -23,7‰ (copepods in April 2004). Stable nitrogen isotopes ($\delta^{15}N$) ranged from 3,4‰ (*Phrosina semilunata* in February 2004) to 6,4‰ (*Pasiphaea multidentata* in August 2003). The overall range of $\delta^{15}N$ is indicative of only two trophic levels.

The narrow range of isotopic values obtained is in accordance with few trophic strategies, and corresponded quite well with the trophic classification based on gut contents analysis-isotopic analyses (data from the literature, Table 5-2). Thus, species with more enriched $\delta^{15}N$ values are carnivorous (e.g. the decapod *P. multidentata*, the euphausiids *M. norvegica* and *N. megalops*) which preyed on other decapods, hyperiids, gammariids or other euphausiids, while low $\delta^{15}N$ values identify mainly filter-feeders (more closely associated to plankton) or small carnivores which preyed on copepods (e.g. small decapods and hyperiids). In general more enriched $\delta^{13}C$ values were related to those taxa that exhibit omnivory (copepods and *M. norvegica*) which by means of vertical migrations can feed on the photic zone (phytoplankton included). A more depleted signature corresponds to strictly carnivores species (i.e. *Cyclothone* spp.) and to bathypelagic-non migratory species (i.e. *N. megalops*, *G. elegans*).

5.3.6.1 Relationship between δ^{15}N and δ^{13}C

The relationship between δ^{15}N and δ^{13}C of suprabenthos fauna by each period was generally strong, (Fig. 5-14). The suprabenthos δ^{13}C-δ^{15}N relationships were higher in August (r=0,522); November (r=0,636) and in April (r=0,565), in September and February they were nearly to 0,35. In June the r-value of correlation is 0,825 though in this month very few measurements were performed.

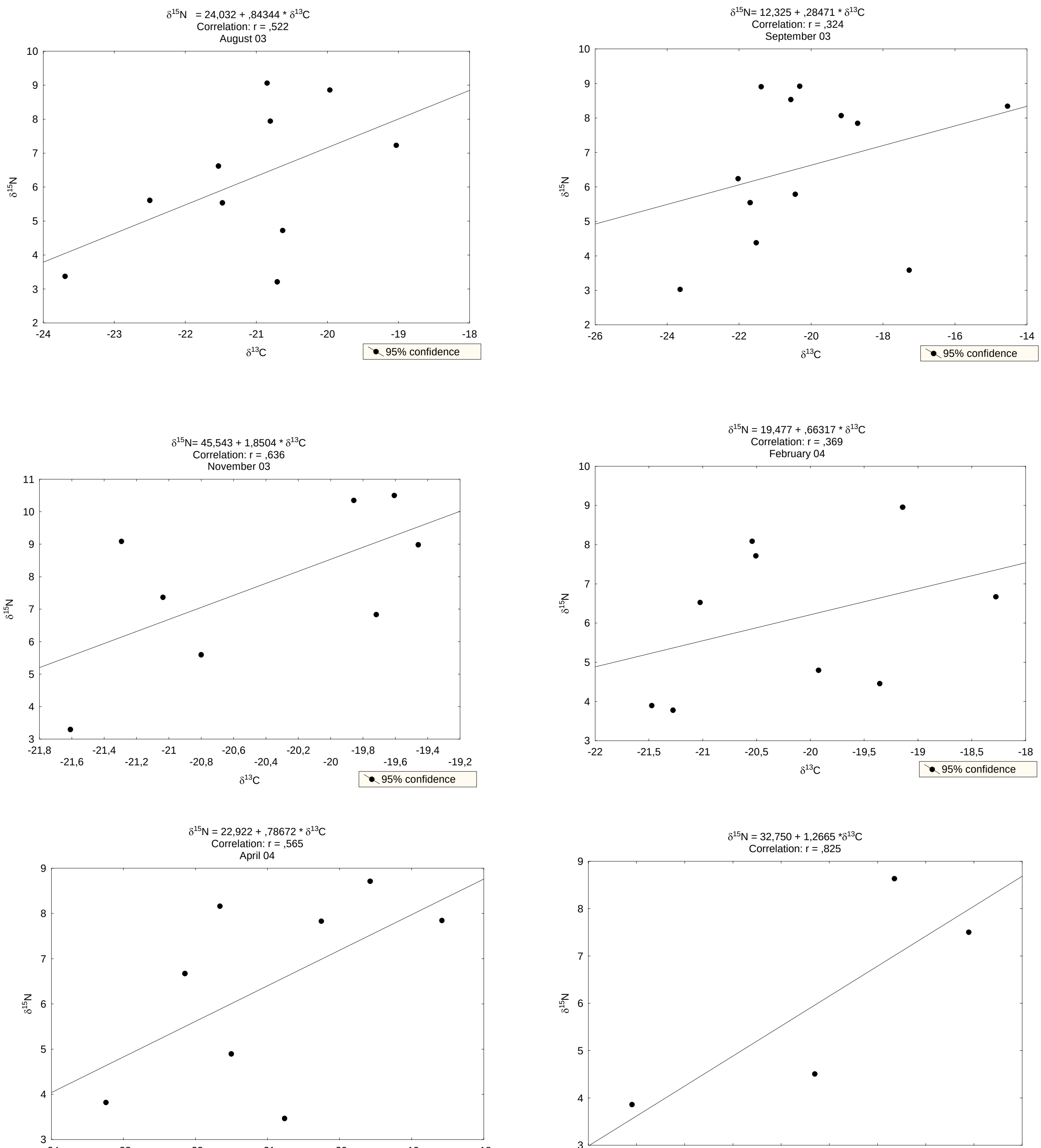


fig.5-14 δ^{15}N and δ^{13}C of bulk suprabenthos fauna for the different periods studied

Plotting the mean δ^{15}N and δ^{13}C values of suprabenthic species (fig. 5-15, see table 5-1 for species list), the highest δ^{15}N value was observed in November and the lowest in February. The most enriched δ^{13}C value of the bulk of suprabenthos was observed in February.

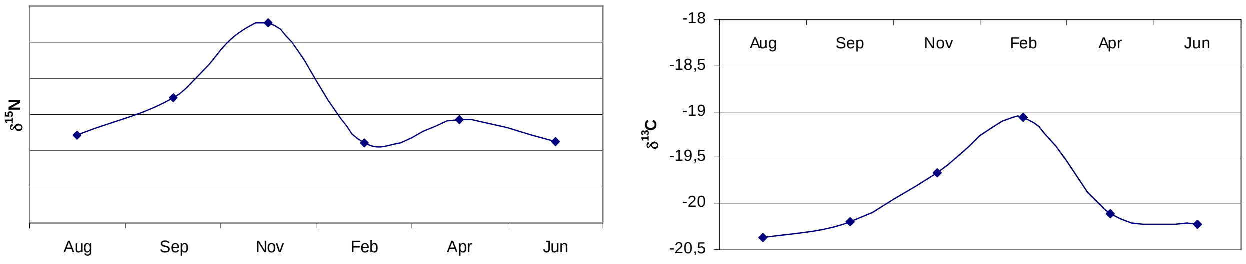


Fig. 5-15 Seasonal variation of mean δ^{15}N and δ^{13}C values of dominant (suprabenthic) species

There was a correlation between the increase in the mean TL of the whole community in February and the enrichment of ^{13}C, thus a change in the food source for suprabenthos probably occurred.

5.3.6.2 Seasonal variation of dominant prey of BBL fauna

Suprabenthos

Among suprabenthic community 7 species, dominant in terms of abundance, were analysed to detect seasonal variation in stable isotopes values: 3 amphipods (*R. rostrata*, *R. caeca* and *T. longipes*), 1 mysid (*B.arctica*) and 3 isopods (*Gnathia* sp., *N. borealis* and *M. atlanticus*).

Rhachotropis rostrata and *R.caeca* showed slight seasonal variation throughout the sampling period (fig. 5-16), the lowest δ^{15}N value was observed for both the species in February (8,1‰ and 7,7‰ respectively) and the highest in November (9,1‰ and 10,3‰ respectively). δ^{15}N differences were significant for both the species (see table 5-8). The δ^{13}C values (fig. 5-16) showed the most enriched values in September and June for *R.caeca* and in the second part of the sampling period in *R.rostrata*.

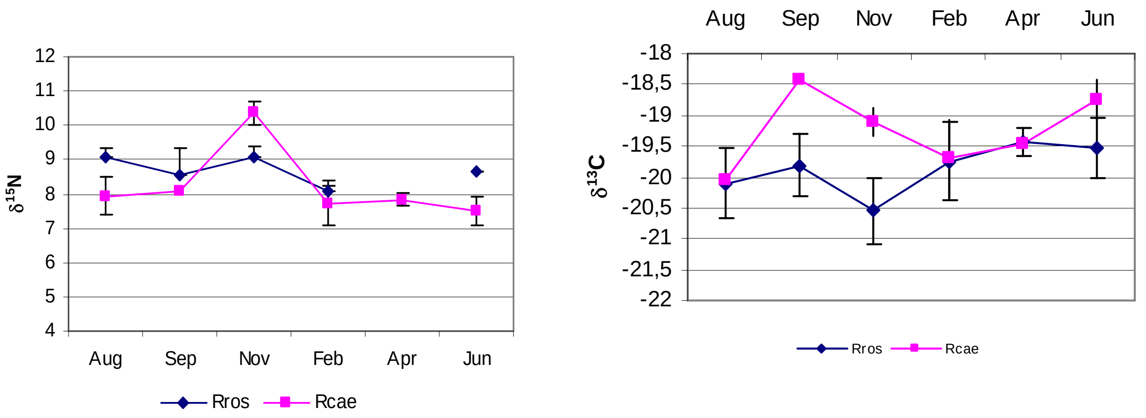


Fig. 5-16 Seasonal variation in δ^{15}N and δ^{13}C values (±SD) of co-generic species of genus *Rhachotropis.* Acronyms as in table 1

δ^{15}N values changed markedly in *T. longipes* (fig. 5-17) with the highest values recorded in November and the lowest in April (5,6‰ and 3,5‰ respectively); δ^{13}C (fig. 5-17) values showed a significant seasonal trend (see table 5-9) with the most enriched value of -18,6‰ observed in February and the most depleted in September.

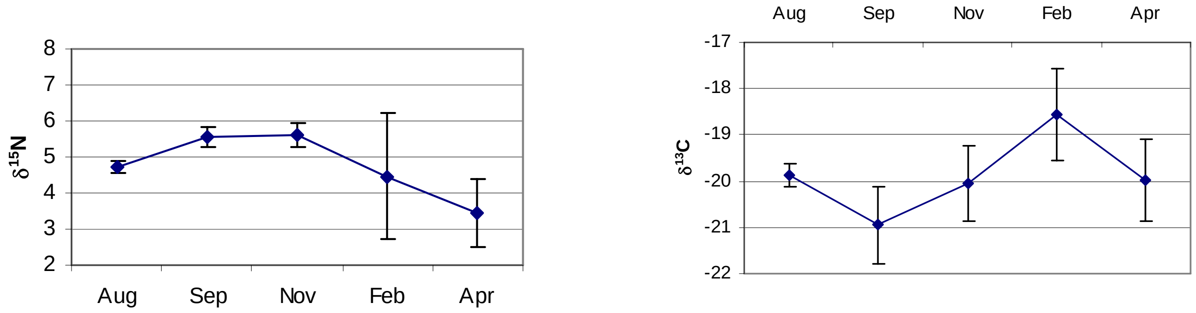


Fig. 5-17 Seasonal variation in δ^{15}N and δ^{13}C values (±SD) of *Tryphosites longipes*

A seasonal pattern was detected both in δ^{15}N and δ^{13}C values of the mysid *B. arctica* (fig. 5-18). The highest value of δ^{15}N was observed in November and the lowest in June, after a decrease already in February. δ^{13}C values increased (more enriched) from August to November and then decreased in April and June. Differences were significant both for δ^{15}N and δ^{13}C values (table 5-9).

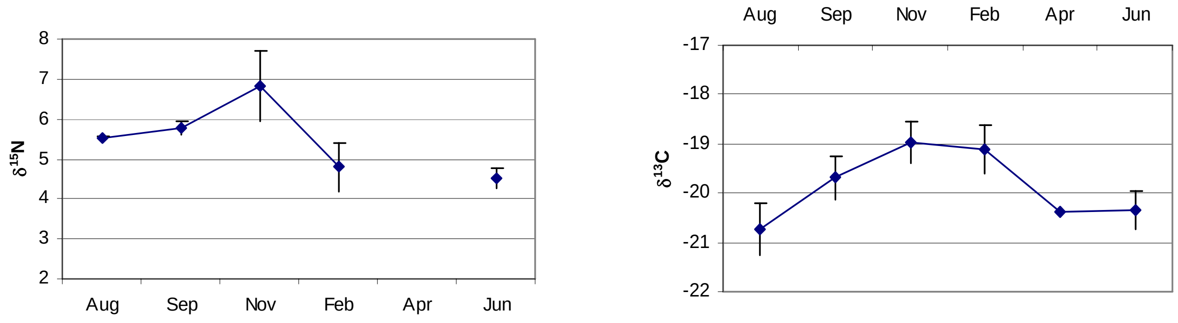


Fig. 5-18 Seasonal variation in δ^{15}N and δ^{13}C values (±SD) of *Boreomysis arctica*

Small differences in δ^{15}N and δ^{13}C values were observed for *M. atlanticus* (fig. 5-19), though δ^{13}C values were less variable than δ^{15}N. The highest δ^{15}N values was detected in November (7,4‰), with a decrease in February and April Differences in δ^{15}N values were significant (table 5-9).

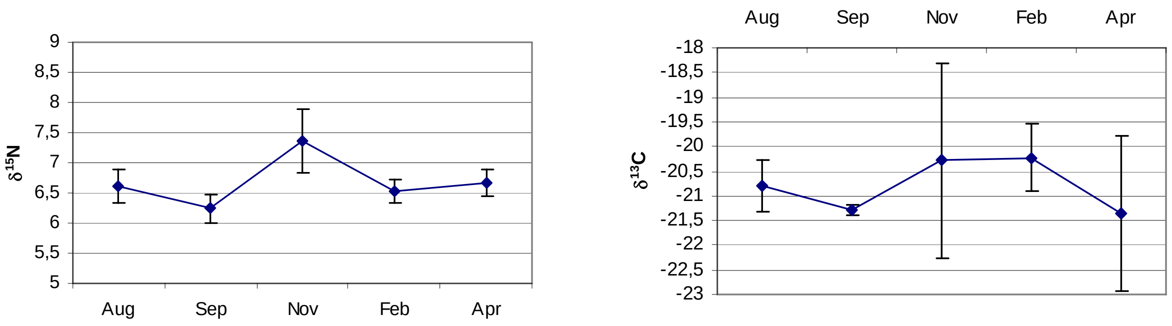


Fig. 5-19 Seasonal variation in δ^{15}N and δ^{13}C values (±SD) of *Munnoupsurus atlanticus*

A clear seasonal trend was also detected for *Gnathia* sp. (Fig. 5-20), for this species the highest δ^{15}N value was observed in November (10,5‰); the most enriched δ^{13}C value was examined in February (-18,3‰) and the most depleted in September (-19,6‰), these difference were significant (table 5-9).

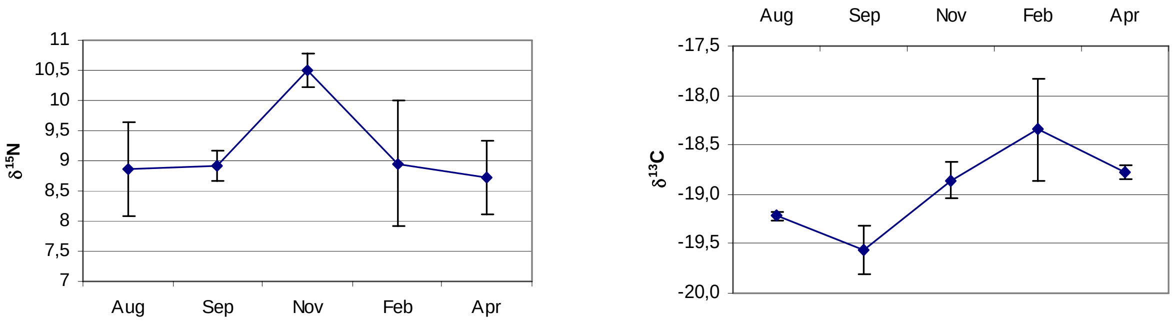


Fig. 5-20 Seasonal variation in δ^{15}N and δ^{13}C values (±SD) of *Gnathia* sp.

δ^{15}N and δ^{13}C values significantly changed in *N. borealis* (table 5-9): the highest values of δ^{15}N were recorded from September to November and in April, the lowest values were observed in August and February. δ^{13}C values became more enriched from August to February, the most depleted values were observed in summer and spring (Fig. 5-21).

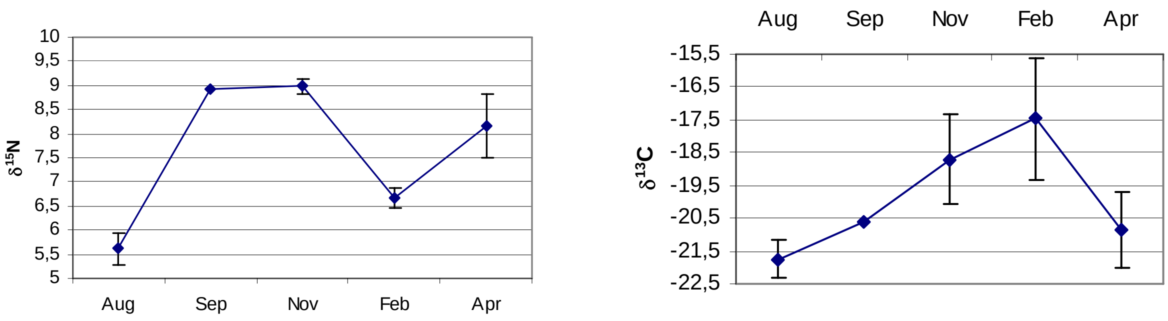


Fig. 5-21 Seasonal variation in δ^{15}N and δ^{13}C values (±SD) of *Natatolana borealis*

Zooplankton

Among zooplankton 3 species (2 euphausiids *M. norvegica* and *N. megalops* and 1 fish *C. braueri*) and the whole *taxon* of calanoid copepods were analysed to detect seasonal variation in stable isotopes values.

The seasonal pattern of the two euphausiids was different. In *M. norvegica* the highest δ^{15}N value was observed in November (7,85‰), while in *N. megalops* in April (6.77‰). δ^{15}N values were more variable (and significant, see table 5-10) in the first species (Fig.5-22). In both species δ^{13}C values became more enriched from August to February, for *N. megalops* these differences were significant (Tab. 5-9).

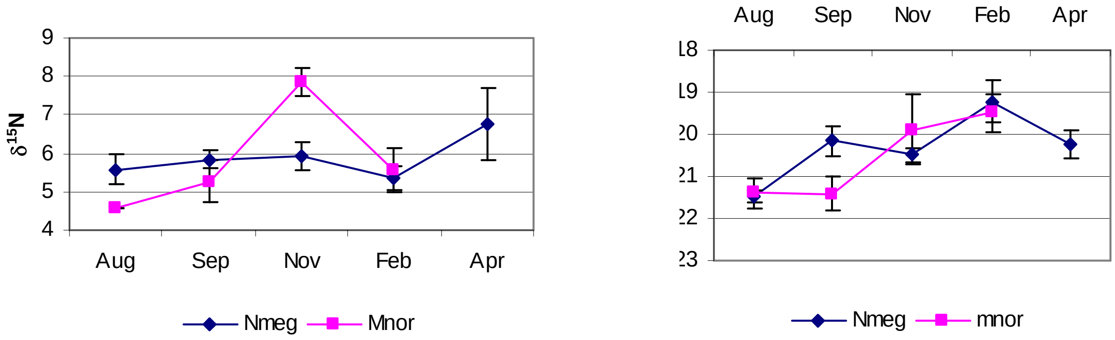


Fig. 5-22 Seasonal variation in δ^{15}N and δ^{13}C values (±SD) of *Nematoscelis megalops* and *Meganycthiphanes norvegica*. Acronyms as in table 5-1.

In *C.braueri* the highest δ^{15}N value was observed in September and November (5,3‰) with the lowest in April. The most depleted δ^{13}C value was detected in September and the most enriched in November - February (Fig. 5-23). Only differences in δ^{15}N values were significant (Tab. 5-9).

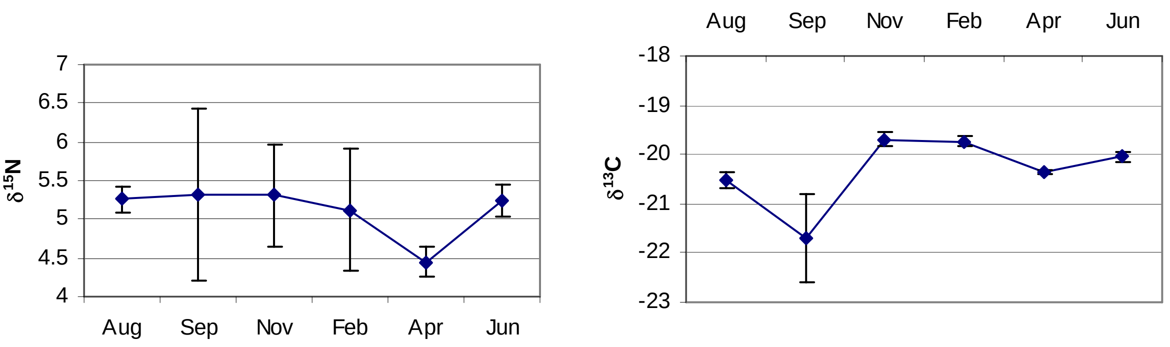


Fig. 5-23 Seasonal variation in δ^{15}N and δ^{13}C values (±SD) of *Cyclothone braueri*

Calanoid copepods showed significant differences observed both in δ^{15}N and δ^{13}C values throughout the year (fig. 5-24 and tab. 5-9). Highest δ^{15}N values were observed in the second part of he sampling period. The most enriched δ^{13}C values occurred in November and February, while the most depleted in Summer (August-September) and Spring (April-June).

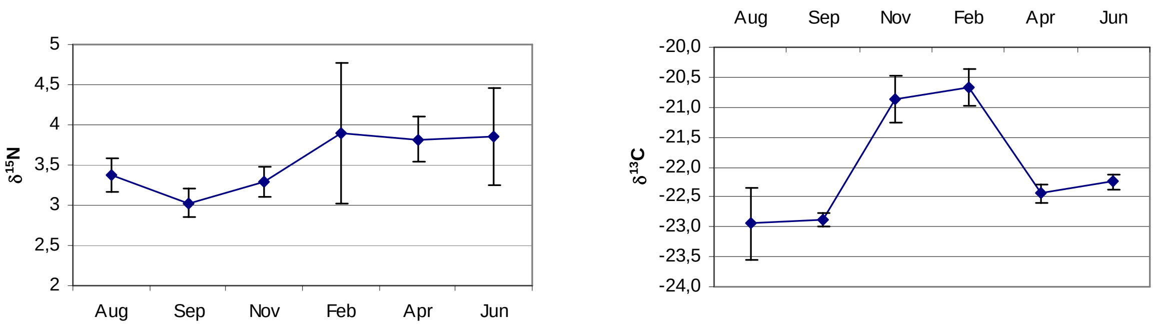


Fig. 5-24 Seasonal variation in δ^{15}N and δ^{13}C values (±SD) of Calanoid copepods.

Table 5-9. ANOVA results for seasonal trends in δ^{15}N and δ^{13}C values of dominant suprabenthic and zooplanktonic species. Only significant relationships were reported: $p<0,05$=*; $p<0,005$=**; $p<0,0005$=***

Species	factors	*F*	*p*
R.rostrata	monthxδ^{15}N	6,56	*
R.caeca	monthxδ^{15}N	9,88	**
B.arctica	monthxδ^{15}N	7,68	*
B.arctica	monthxδ^{13}C	6,71	*
T.longipes	monthxδ^{13}C	4,95	*
M.atlanticus	monthxδ^{15}N	3,83	*
Gnathia sp.	monthxδ^{15}N	4,62	*
Gnathia sp.	monthxδ^{13}C	7,85	*
N. borealis	monthxδ^{15}N	99,34	***
N. borealis	monthxδ^{13}C	10,56	*
C.braueri	monthxδ^{13}C	10,45	**
N.megalops	monthxδ^{13}C	12,49	**
M.norvegica	monthxδ^{15}N	45,03	***
Copepoda	monthxδ^{15}N	8,78	**
Copepoda	monthxδ^{13}C	14,72	***

5.3.6.3 Trophic level of BBL fauna

We estimated the trophic level (TL) of the BBL fauna (excepting those species where δ^{15}N values were lower than δ^{15}N of calanoid copepods) based on their δ^{15}N data and using copepods as reference material (table 5-10). δ^{15}N values were converted to trophic level based on the assumption that there was a fractionation of 3‰ per trophic level (Vander Zanden & Rasmussen, 2001; Post 2002) and that the base material (copepods) had a trophic level of 2:

$$TL_i = \left(\frac{\delta^{15}N_i - \delta^{15}N_{ref}}{3}\right) + 2$$

Where TL_i is the trophic level of species *i,* $\delta^{15}N_i$ is the mean $\delta\ ^{15}$N of species $_{i,}$ and $\delta^{15}N_{ref}$ is the mean δ^{15}N of the copepods.

Table 5-10 Estimated TL of BBL fauna (suprabenthos* and zooplankton**) species

Taxon	TL	Taxon	TL
Copepoda	2,0	*Procampylaspis armata**	2,9
*Calocaris macandrae**	2,1	*Pasiphaea multidentata*	2,9
*Syrrhoe affinis**	2,3	*Bruzelia typica**	2,9
*Gennadas elegans***	2,3	*Epimeria parasitica**	2,9
*Tryphosites longipes**	2,3	Cumacea *	3,0
*Stegocephaloides christianensis**	2,4	*Munnopsurus atlanticus**	3,0
*Cyclothone braueri***	2,4	*Nicippe tumida**	3,2
*Cyclothone pygmaea***	2,5	*Campylaspis verrucosa**	3,2
*Boreomysis arctica**	2,5	*Rhachotropis grimaldi**	3,4
*Andaniexis mimonectes**	2,5	*Rhachotropis caeca**	3,4
*Sergia robusta***	2,6	*Rhachotropis rostrata**	3,5
Phronima sedentaria **	2,6	*Cirolana borealis**	3,6
*Nematoscelis megalops***	2,7	*Gnathia**	3,8
*Meganycthiphanes norvegica***	2,7		

Plotting the annual average values of δ^{15}N and δ^{13}C of the whole BBL fauna community (comprising suprabenthic and zooplanktonic species, fig. 5-25) more than one source of carbon was evident (see subsection 6.4.3 for a detailed description about trophic web structure).

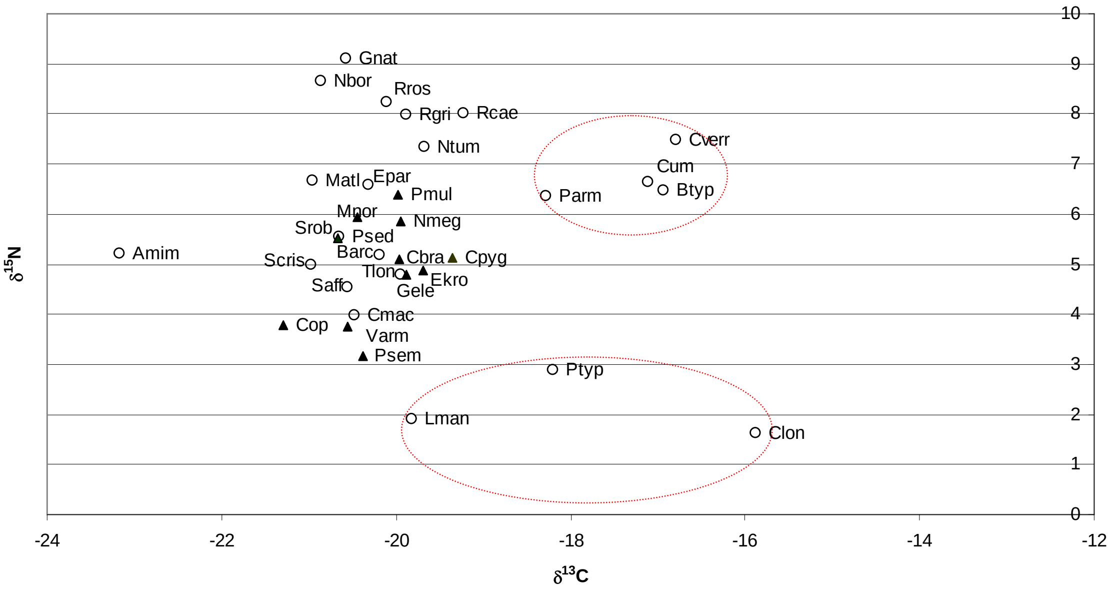


Fig. 5-25 δ^{15}N and δ^{13}C values of suprabenthos (circles) and zooplankton (black triangles) (see legend in table 5-1).

5.4 Discussion

5.4.1 Dynamics of BBL fauna

In open waters, temporal dynamics of meso and macrozooplankton have been the topic of a number of studies performed both in Mediterranean (Franqueville, 1971; Sabatés et al., 1989; Sardou et al. 1996), and in neighbouring areas (Bode and Alvarez-Ossorio, 2004). However, studies on the dynamics of suprabenthos (hyperbenthos, BBL macrofauna) assemblages are practically non existent at bathyal depths (Cartes et al., 2002). Therefore, this is the first comparative study focused on the simultaneous dynamics of both compartments. In addition, most studies performed in deep waters, lack the environmental data simultaneously recorded in our area.

Different marine taxa inhabit both the water column and the sediment-near bottom water interface, therefore belonging to suprabenthos and zooplankton. Most of these taxa have planktonic (meso or bathypelagic) and suprabenthic species. This is the case of copepods (Jaume et al., 2000; Ohtsuka et al., 2005), chaetognaths (hyperbenthic species described by Casanova, 1993; abyssal species recorded by Christiansen et al., 1999), decapods and mysids, (Vereshchaka, 1995) among others. In our case, the comparison of fauna belonging to the suprabenthic and the midwater habitats gave a low similarity (0.5–8%), between suprabenthic and zooplankton crustaceans (16 species of Eucarids and 98 of Peracarids: based on results from IDEA0803 survey). Only at the shallowest station of SE Mallorca, on the shelf-slope break at 155 m, similarity increased (to 36%) because of the simultaneous occurrence of the neritic mysid *Leptomysis gracilis* in the water column and close to the bottom (author's unpublished data). These results also suggest that, excluding the fauna of the shelf-slope break, bathyal suprabenthic species rarely move up into the water column more than 15 m above bottom (Cartes, 1998).

Dynamics of peracarid assemblages off Cabrera showed a seasonal trend for suprabenthic communities in the SE of Mallorca. There was a peak of biomass regularly appearing in summer (June 2004, August 2003), with a sharp decrease of biomass, simultaneously occurring at all depths, in autumn (September-November 2003). This pattern in the dynamics of suprabenthos agrees, at the level of the dynamics of dominant species, with that found in previous studies performed at similar latitudes, not only on the slope (e.g. Bay of Biscay: Cartes et al., 2002), but also in muddy shelf bottoms (off Ebro Delta: Cartes et al, submitted; N Adriatic Sea: Ligas, 2005; S Tyrrhenian sea: chapter 2 of this thesis). These dynamics are probably related, among other factors, with the own population dynamics of suprabenthic species (e.g. mysids). The decrease of biomass in autumn is probably attributable to post-reproductive mortality of the spring-summer generation for most species, according for instance to the biology of mysids described by Mauchline (1980), also combined with a period of low productivity in the water column as deduced from studies on mass fluxes (e.g. Miquel et al, 1994) and satellite data in the western Mediterranean (Fig. 5-9).

In contrast to suprabenthos, zooplankton dynamics off the Balearic Islands agreed only in part with previous studies performed in open waters in the western Mediterranean (Franqueville, 1971; Sabatés et al., 1989; Sardou et al., 1996). The variety of gears (e.g. Bongos, IKMT) used, difference in mesh-size, and sampling design are important limitations for comparisons. As an example, abundance of Natantian decapods (e.g. Pasiphaeidae, Sergestidae) in our sampling was an order of magnitude below abundances obtained by Sardou et al. (1996) in the Ligurian Sea, probably because they used an IKMT net, with larger mouth and mesh sizes than our WP2. The dynamics of maximum biomass of zooplankton depend on the distance from the coast. Previous studies (Sabatés et al. 1989; Sardou et al, 1996) found maximum values of zooplankton biomass in spring. In the Catalan coast, higher densities of mesozooplankton were recorded in April-May along the continental shelf edge in association with a permanent hydrographic front (Sabatés et al., 1989). Frontal systems act somehow as nursery areas, where species are localized during their more intense growing phases (e.g. copepodites: Boucher et al., 1987). Peaks of biomass might be related with increases of total mass and organic carbon fluxes in the water column (Miquel et al. 1994) recorded for instance off the Ligurian Sea in late winter-early spring, linked to phytoplankton blooms.

Over the shelf-slope break (at 150 m depths) off Mallorca, we found high densities of zooplakton in spring (April), though the highest densities were displaced to June-August or February. The highest average biomass was recorded on the shelf-slope break in comparison to bathyal stations, probably a consequence of the occurrence of frontal systems along the shelf edge (Font, 1987; López Garcia et al., 1994) over depths of 100-150 m (Font et al., 1988). Frontal systems are present around the islands along the year showing seasonal and inter-annual variations (García-Ladona et al., 1996). These oceanographic structures separate two different surface waters masses, the Modified Atlantic Water (MAW) progressing northward from the Algerian basin and the Local Atlantic Water (LAW),a resident water in the Balearic basin. The Mallorca channel acts as meeting point of these water masses and the progress northward of MAW begins after the winter north-wind forcing relax.

Over bathyal depths (350 to 750 m), however, peaks of biomass were never recorded in spring (April), but in late winter (February) and early summer (particularly in June) while the minimum also occurred in autumn (September). According to this pattern, Franqueville (1971) reported maximum abundance of deep macroplankton in winter (January to March period) in the Gulf of Lyons, characterized by the dominance of jellyfish (e.g. *Pelagia noctiluca*, *Salpa fusiformis*). However, Sardou et al. (1996) found the same dominant species (e.g. salps and *Cymbulia peroni*, siphonophores and pyrosimids, and among crustaceans *Gennadas elegans*) for macrozooplankton, though reaching maximum biomass during spring (from March to May) in the Ligurian Sea. In our case, spring minimum offshore was associated with the dominance of jellyfish (salps and cnidarians) and copepods, with mean size of macroplankton reaching its minimum (0.0004 g/ind). The dominance of grazers in this period (particularly salps, though including also small larvae of euphausiids and decapods) could probably be a response to the peak of primary production (Chl *a* in surface) from February-March. This is accordance to the idea of zooplakton-phytoplankton coupling in periods of high productivity (e.g. April in Nival et al., 1975), when grazers have a limiting effect on the growth of phytoplankton.

Zooplankton succession, e.g. its trophic dynamics, depends on food source variability, for instance of the concentration of phytoplankton in the photic zone. In our case, the increase of grazers in April may imply a decrease in the trophic level of the whole zooplankton communities. In the NW of Spain consumption of nitrate in spring explained the almost simultaneous increase in heavy N isotopes ($\delta^{15}N$) by mesozooplankton in May-June (Bode and Alvarez-Ossorio, 2004), which was correlated with the abundance of carnivore zooplankton.

In short, the expected spring peak in the western Mediterranean was not clearly observed in our study, particularly over bathyal depths. Besides the local variability (e.g. distance to coast), and oceanographic and climatic conditions (e.g. temperature, salinity, wind regime), which have an influence on zooplankton dynamics, long-term studies on primary production deduced from satellite imagery evidenced inter-annual variability in the NW Mediterranean. A unique peak of Chl *a* in surface (Morales-Blake, 2006; own data downloaded from *http://reason.gsfc.nasa.gov/Giovanni*) regularly appears during winter in open waters. Some long-term variability in the peak of Chl *a* could also explain the peak of biomass recorded in February, instead of April, in our study.

Suprabenthos and zooplankton were related to a number of environmental variables. Comparing trends for both compartments (both taxonomic changes and intra-annual fluctuations in biomass), suprabenthos was mainly related to the sediment nature (e.g. grain size) and its *trophic* condition (e.g. %OM, potential REDOX), whereas zooplankton was almost exclusively dependent on Chl *a* in surface.

A link between suprabenthos abundance and food supply has been documented and food sources of some suprabenthic species depend on the arrival of particulate OM throughout the water column. Hence, in the neritic domain, mysids such as *Anchialina agilis* can feed on phytoplankton in the water column (Ribera-Maycas, 1997), far away from the sediment-bottom interface. In the bathyal domains of the western Mediterranean and the Bay of Biscay it has been suggested a coupling between the main peak of abundance recorded in June-July and food inputs derived from surface primary production (Cartes and Sorbe, 1996; Cartes et al., 2002). Bathyal mysids, the case of *Boreomysis arctica*, consumed phytodetritus at 700-1200 m during summer in the Catalan Sea. Synchronism between juveniles release and maximum food availability derived from spring phytoplankton blooms has been recently reported for hyperbenthic peracarids of different taxa (*Mysis mixta* and *Acanthostepheia malmgreni*) off Newfoundland (Richoux et al., 2004a, b). Although it was not possible to perform detailed analyses on fresh OM (e.g. lipids, aminoacids) in sediments, the increase of %OM in April-June 2003 (2-2.6 times higher than in February2004 at 350-750 m) suggest an arrival of new organic matter at bathyal depths 1-2 months after the peak of surface primary production during February-March (Chl *a* readings by satellite imagery). The speed of response by macrofaunal species to these inputs might depend on of the trophic level of species. Although carnivore suprabenthos has probably a more delayed response than filter-feeder to the main input of surface production (Cartes et al., 2002), the main component of the biomass corresponded to mysids, which are mainly filter feeders. This would explain the positive correlations existing between the whole suprabenthos biomass and OM.

Both suprabenthos and zooplankton have marine snow, e.g., detritus derived from primary production reaching bathyal depths, as main food source, as it has been reported by the use of stable isotopy both in Sòller (NW of Mallorca, Balearic basin Madurell et al., submitted) and previously in the Algerian Basin (Polunin et al., 2001). Our results suggest, in addition, that this food source is exploited in two different ways by both compartments, zooplankton feeding directly on phytoplankton by vertical daily movements to the photic zone, and suprabenthos linked to deposited and/or re-suspended detritus in the BBL. The biomass of suprabenthos increased from autumn-winter (Sep-Nov-Feb) to spring-early summer (April-

June), with a parallel increase in the %OM in sediments (from a range of 2.69-7.62% in Nov-Feb to a range of 4.17-9.43% in April-June), which in some cases represented an increase of *ca* 3% OM. In spite peaks of Chl *a* were nearly simultaneous (in February-March, beginning in December), there was a sharp peak of Chl *a* in February. The main peak of suprabenthos biomass at slope depths (350-750m) occurred in February and April and probably in relation to this, the %OM had also maximum values in April.

Despite the narrower range of variation in salinity (from 38.18-38.52 ppm between 150-750m, and 38.44-38.52 ppm deeper at 350-750m) in comparison to other variables (e.g. OM), *S* was also correlated with suprabenthos-zooplankton biomass. At slope depths, only Ghidalia and Bourgois (1961) hypothesized on the distribution of some deepwater shrimps (*Aristeus antennatus* and *Aristaeomorpha foliacea*), which would be linked to intermediate and deep-water masses in the Mediterranean, without any data supporting this hypothesis. Correlations with salinity close to the bottom suggest a link between suprabenthos abundance (increment of biomass in June-August) and changes in the oceanographic conditions close to the bottom (López-Jurado et al., *submitted*). In spite of this possible coupling with salinity, OM showed a stronger increase in April-June after the peak of Chl *a* in surface. It is possible, in addition, that *S* recorded by CTDs are in fact reflecting the increase in OM in the environment, because salinity is measuring the increase of dissolved particles in the water mass, disolved organic matter included. The input of LIW can also contribute of a higher OM re-suspension by increase in bottom currents by contact of water masses with slope depths. Probably a combination of all these factors may explain the positive relation between *S* and suprabenthos biomass.

5.4.2 Trophodynamics of BBL fauna elucidated by stable isotope analyses

Stable carbon and nitrogen isotope mean values for suprabenthic and zooplanktonic species are consistent with previously reported values in the deep Mediterranean for suprabenthos as a whole group (Polunin et al., 2001). Although the feeding habits and strategies of the suprabenthos and zooplankton fauna have hardly been studied, the isotopic signatures of the suprabenthic and zoooplanktonic species studied here agree with findings in the literature on the feeding ecology of these species, based on gut content analysis (Cartes et al., 2002; Table 5-2). The wide range of the isotopic composition of carbon and nitrogen (δ^{13}C and δ^{15}N) displayed by suprabenthos species, and in less extent by zooplankton species, suggested high diversity in their trophic strategies (i.e. filter feeders, deposit feeders, detritus feeders, scavengers and carnivores). The differences observed in the isotopic signatures of carbon and nitrogen among the different suprabenthic species also suggests food resource partitioning within this community. In a food limited system like the deep-sea, diversification of feeding strategies is necessary for coexisting species. According to the Stability–Time Hypothesis (Sanders, 1968), a high degree of trophic specialization is expected in oligotrophic deep-sea sites by long-term adaptation of deep sea species due to limited and predictable food sources (i.e. seasonal pulses) (Iken et al., 2001). Comparing zooplankton and suprabenthos species there was a certain overlap between species (Fig. 5-25), i.e *Tryphosites longipes* and *Gennadas elegans*, *Calocaris macandrae* and *Vibilia armata*, *Boreomysis arctica* and *Cyclothone braueri*, but food partitioning in this case was guaranteed by the different position along the water column of suprabenthic and zooplanktonic species.

Several studies have shown a nitrogen isotopic enrichment (or fractionation) of about 3.4 ‰ with increasing trophic position (e.g., DeNiro and Epstein, 1981; Minawaga and Wada, 1984; Cabana and Rasmussen, 1994). In the deep-sea benthic ecosystem off the NE Atlantic Ocean (Porcupine Abyssal Plain), Iken et al. (2001) showed that there is significant overlap in nitrogen isotopic values between trophic levels, reducing the conventional 3‰ stepwise enrichment. However, recent revisions suggest that the fractionation from a consumer and its diet depends on trophic strategies, food biochemical compositions, and different metabolic pathways (Vander Zanden and Rasmussen, 2001; Vanderklift and Ponsard, 2003; McCutchan et al., 2003). Assuming a conservative trophic enrichment of *ca* 3.0 ‰ (Post, 2002) between consumers and their diet, at least three trophic levels were identified within our suprabenthic community and two for zooplankton. Considerable similar wide range of δ^{15}N values was observed for amphipods sampled in the eastern Weddell Sea, where three levels of the food web were covered by the 8 species examined (Nyssen et al., 2002). In the Catalano-Balearic Basin depth effect can be excluded for our data because all the specimens provided from 650 and 750m depth. The isotopic signature, however, depends of the origin of C and N source (Polunin et al., 2001), and depth is an important gradient determining the food sources. Previous studies performed along wide bathymetric ranges already showed how the δ^{13}C and δ^{15}N signature of a species can change with depth (e.g. the shrimps *Acanthephyra eximia* and *Aristeus antennatus*, Polunin et al., 2001). In addition both stations in the Catalano-Balearic Basin were included in two different assemblages. An important boundary for species renewal situated at *ca*. 500 m was evidenced for different taxa, from fish (Moranta et al., 1998) and decapods (Cartes et al., 1994), to the own suprabenthic peracarids (Bellan-Santini, 1990; Cartes et al., 2003). Similar temporal and/or spatial heterogeneity in sampling could explain the wide range found by Nyssen et al (2002) in the Antarctic Ocean, where different compartments were sampled. On the other hand, $\delta^{13}C$ enrichment per trophic level was not as consistent as $\delta^{15}N$ fractionation, though the stable carbon isotope $\delta^{13}C$ is commonly used to quantify energy flow. Thus, since there is little trophic enrichment between an organism and its diet (Vander Zanden and Rasmussen, 2001; Post, 2002), the isotope ^{13}C indicates dietary sources of carbon.

The suprabenthic fauna undergoes noticeable seasonal variations in abundance and biomass in the study area (Cartes et al., *in press*). Hence, the dynamics of peracarid assemblages showed that there was a peak of abundance and biomass in summer and parallel to an increase in organic matter in the sediment during the same period, which in turn is coupled to the peak of surface primary production recorded in February-March (Fig. 5-9). Seasonal differences in the isotopic composition of suprabenthic fauna may thus be a response to diet shifts associated with changes in the availability of different prey throughout the year. There is little information available on seasonal variability of isotopic composition in consumers and particularly for suprabenthos fauna. In deep-sea, the $\delta^{13}C$ isotopic composition of benthic foraminifera decreases after a seasonal phytodetritus input (Corliss et al., 2002). In our case, suprabenthos taxa appear to have a different response to periods of low and high surface production (e.g. to the seasonal changes in food input). The $\delta^{13}C$-$\delta^{15}N$ correlations increased from February to June, after the peak of primary production of February-March (see Fig. 5-9). Accordingly, we observed weak $\delta^{13}C$-$\delta^{15}N$ correlations in periods of low surface productivity (September). A strong correlation was noticed in November, when surface productivity was also low. This trend is probably indicative of more than one food source. For the bulk of the suprabenthic species and the whole zooplankton assemblage (see also discussion of chapter 6) the main food source is the so-called marine snow, coupled to the peak of Chl *a* and surface production. According to Polunin et al. (2001) considerations about primary sources of material, marine snow almost certainly constitutes the main supply of food materials to the sea floor in the study area. The correlation between $\delta^{15}N$ and $\delta^{13}C$ for cumaceans (except *Cyclaspis longicaudata*), *B. typica* and *L. manco* is also strong (r=0,86 and p=0,027). These species had more enriched $\delta^{13}C$ values; probably macrophytae remainssuch as large algae were significant contributors (Polunin et al., 2001). Large macrophytae pieces (e.g. *Posidonia oceanica* fragments) arrive to bathyal depths, though no data on seasonal fluctuations in the arrivals of this POM exist. Probably it is more intense after late summer-early autumn storms, which in general coincide with the period where C-N correlations were the lowest. In addition *C. longicaudata* and *P. typicus* normally inhabit lower slope bottoms below 1000 m of depth (Cartes and Sorbe, 1997) and migrate upper on the middle slope in some period of the year.

Polunin et al. (2001) found a depth changes in isotopic signatures, with more enriched $\delta^{13}C$ values found in the lower slope and it could explain the more enriched value observed for these species. Finally we can conclude that there was one primary source of material, the marine snow according Polunin et al. (2001) findings.

Changes in the isotopic composition of consumers can occur both as a result of growth and rate of tissue turnover (Tieszen et al., 1983; Phillips and Eldridge, 2006) however, juveniles and small individuals were not analyzed in this study. The potential sources of the temporal variability for consumers are then attributed mainly to the temporal changes in the isotopic values among food sources (Minagawa and Wada, 1984), though they can also be related to feeding behaviour, lipid accumulation and habitat selection (del Giorgio and France, 1996).

Madurell et al. (submitted) observed that is possible that changes in suprabenthos $\delta^{13}C$ reflect seasonally correlated changes in lipid concentration. The negative relationship between C:N and $\delta^{13}C$ among suprabenthos taxa is consistent with the hypothesis that seasonal changes in body composition (C:N) can significantly affect the $\delta^{13}C$ signatures of suprabenthos (Matthews and Mazumder, 2005). As lipids are primarily from diet origin, accumulation of dietary lipids that have a low $\delta^{13}C$, coupled to increasing Chl*a* levels from November to June, suggest that the suprabenthos species store food reserves during the more productive periods. This stored energy is probably channelled into reproduction and growth. Synchronization between juveniles release and maximum food availability derived from spring phytoplankton blooms has been recently reported in hyperbenthic peracarids (e.g. *Mysis mixta* and *Acanthostepheia malmgreni*) off Newfoundland (Richoux et al., 2004a, b).

We observed significant seasonal changes in $\delta^{13}C$ and $\delta^{15}N$ values for the dominant species of BBL fauna. For all the examined species there was an enrichment in $\delta^{13}C$ values in February (and November, see from Figg.5-16 to 5-24), as observed for *Plesionika martia* (Fanelli and Cartes, submitted). This trend occurred both for small (i.e. *B. arctica*) and large (i.e. *Rhachotropis* spp.), confirming a change in the origin of food sources for all the species and not linked to the size. As previously observed, both suprabenthos and zooplankton have marine snow, e.g., detritus derived from primary production reaching bathyal depths, as main food source, as it has been reported by the use of stable isotopy both in Sóller (Madurell et al., submitted) and previously in the Algerian Basin (Polunin et al., 2001). More over a unique peak of Chl *a* in surface (see references cited therein) regularly appears during winter in open waters. Marine snow in winter could be derived more by large carrion degradation (more enriched in ^{13}C, Polunin et al., 2001) than from phytodetritus. These considerations are consistent with the degradation of marine snow leaving residues of more refractory materials

(Williams and Gordon 1970; Mullerniklas et al. 1994), such as inorganic carbonates and chitin, which are known to be enriched in ^{13}C compared to other biochemical fractions (Bunn et al 1995).

Annex 5-1. List of suprabenthic and zooplanktonic species collected in August 2003 (N/100m^2). **M**: samples from WP2; **P**: samples from MG-sledge.

IDEA0803	**M1**	**M3**	**M4**	**P1**	**P2**	**P3**	**P4**
Depth	166m	347m	760m	155m	670m	340m	747m
Taxon							
Decapoda							
Decapoda larvae	153	294	56	96	3	13	9
Adult							
Calocaris macandreae	0	0	0	0	5	0	2
Galathea sp.	0	0	0	1	0	0	1
Funchalia woodwardi	0	0	0	0	1	0	0
*Gennadas elegans**	0	7	7	0	0	0	0
Pandalina profunda	0	0	0	0	0	12	1
Pasiphaea multidentata	0	0	4	0	0	0	0
Philocheras bispinosus.	0	0	0	7	0	0	0
Philocheras echinulatus	0	0	0	0	0	1	0
Plesionika gigliolii	0	0	0	0	0	2	0
Plesionika martia	0	0	0	0	1	0	1
Pontocaris lacazei	0	0	0	1	3	0	1
Processa nouveli	0	0	0	15	0	3	0
Sergestes arcticus	0	0	0	0	1	0	0
Stomatopoda							
Squilla mantis (larvae)	1	1	0	0	0	0	0
Euphausiacea							
larvae+post-larvae	123	0	133	1	3	4	3
Euphausia krohni	0	0	7	0	0	1	2
Meganyctiphanes norvegica	0	0	3	0	0	0	0
*Nematoscelis megalops***	0	0	34	0	0	2	0
Stylocheiron sp.	0	4	1	0	0	0	0
Mysidacea							
Anchialina agilis	0	0	0	28	0	0	0
*Gastrossacus sp. **	0	0	0	0	1	0	0
*Boreomysis arctica***	0	0	0	0	187	0	229
Boreomysis megalops	0	0	0	1	0	174	0
*Calyptomma puritani***	0	0	0	0	0	0	1
Erythrops neapolitana	0	0	0	1	2	17	0
Eucopia hanseni	0	0	1	0	0	0	0
Leptomysis gracilis	63	0	0	68	0	12	0
Lophogaster typicus	0	0	0	1	0	21	0
Mysideis parva	0	0	0	0	23	10	0
Paramblyops rostrata	0	0	0	0	22	1	7
Parapseudomma calloplura	0	0	0	0	7	0	4
Parerythrops lobiancoi	0	0	0	0	2	0	22
Pseudomma sp.	0	0	0	0	0	2	0
Siriella norvegica	0	0	0	0	1	0	0
Amphipoda gammaridea							
Ampelisca (dalmatina)	0	0	0	10	0	0	0
Amphilochus brunneus	0	0	0	1	1	5	1
*Andaniexis mimonectes***	0	0	0	1	0	2	52
Arrhis mediterraneus	0	0	0	0	0	1	8
Bathymedon acutifrons	0	0	0	0	0	0	4
*Bathymedon banyulsensis***	0	0	0	0	0	0	4
Amphipoda gammaridea							
Arculfia trago mediterranea	0	0	0	0	2	0	0
Aoridae unid.	0	0	0	0	1	0	0
Bathymedon longirostris	0	0	0	0	5	14	27

IDEA0803	**M1**	**M3**	**M4**	**P1**	**P2**	**P3**	**P4**
Depth	166m	347m	760m	155m	670m	340m	747m
Amphipoda gammaridea							
Bathymedon monoculodiformis	0	0	0	0	1	0	1
*Bruzelia typica***	0	0	0	0	11	0	8
Corophium sp.	0	0	0	0	0	0	1
Epimeria parasitica	0	0	0	0	6	0	5
Eusirus leptocarpus	0	0	0	0	0	0	1
Eusirus longipes	0	0	0	0	0	1	1
Gammaridea unid. (D)	0	0	0	2	0	0	0
Halice walkeri	0	0	0	0	2	0	1
Halicreion aequicornis	0	0	0	0	0	0	1
Harpinia sp.	0	0	0	2	0	0	2
Idunella pirata	0	0	0	0	0	0	1
*Ileraustroe ilergetes***	0	0	0	0	21	0	37
Lepechinella manco	0	0	0	0	7	5	1
Lepidecreum subclypeatum	0	0	0	0	5	19	3
Leucothoe lilljeborgi	0	0	0	1	1	0	0
Lysianassa plumosa	0	0	0	0	1	0	2
Lysianassidae	0	0	0	1	0	3	0
Melphidipella macra	0	0	0	2	0	0	0
Metaphoxus simplex	0	0	0	0	1	0	1
Monoculodes packardi	0	0	0	1	3	7	0
Monoculodes acutipes	0	0	0	0	1	0	4
Monoculodes sp.	0	0	0	1	1	0	2
Nicippe tumida	0	0	0	0	24	1	4
Oediceropsis brevicornis	0	0	0	0	2	7	1
Oediceroides pilosa	0	0	0	0	0	0	2
Oedicerotidae	0	0	0	1	0	0	1
Orchomenella nana	0	0	0	0	7	2	20
Pardalisca mediterranea	0	0	0	0	0	0	3
Perioculodes longimanus	0	0	0	15	1	1	0
Pleusymtes sp.	0	0	0	1	0	0	0
Podoprion bolivari	0	0	0	0	0	0	1
*Pseudotiron bouvieri**	0	0	0	0	4	0	5
Phoxocephalidae sp. 1	0	0	0	0	0	1	0
*Rhachotopis caeca***	0	0	0	0	25	0	76
Rhachotropis grimaldii	0	0	0	0	15	0	3
Rhachotropis integricauda	0	0	0	3	0	59	1
*Rhachotropis rostrata**	0	0	0	0	13	0	7
*Scopelocheirus hopei***	0	0	0	0	0	10	1
Sophrosyne hispana	0	0	0	1	0	0	0
Stegocephaloides christianensis	0	0	0	0	21	4	7
Synchelidium maculatum	0	0	0	0	0	1	1
Syrrhoe affinis	0	0	0	0	31	1	8
Syrrhoites pusilla	0	0	0	0	0	12	4
Tmetonyx similis	0	0	0	0	0	1	0
Tryphosites alleni	0	0	0	0	7	0	2
Tryphosites longipes	0	0	0	3	11	3	6
Tryphosella sp.	0	0	0	0	1	0	0
Urothoe corsica	0	0	0	0	1	1	2
Westwoodilla rectirostris	0	0	0	7	0	9	2
Amphipoda Caprellidea							
Parvipalpus major	0	0	0	0	0	1	0

IDEA0803	**M1**	**M3**	**M4**	**P1**	**P2**	**P3**	**P4**
Taxon							
Amphipoda Hyperiidea							
Anchylomera blossevillei	0	1	0	0	0	0	0
Hyperia latissima	2	0	0	0	0	0	0
Brachyschelus crusculum	0	2	0	0	0	0	0
Hyperoides longipes	0	0	2	0	0	0	0
Hyperia schizogeneios	71	15	7	3	1	1	0
Hyperoche kroyeri	3	0	0	0	0	0	0
Phronima sedentaria	0	1	0	0	0	0	0
Phrosina semilunata	1	1	0	0	0	0	0
Primno cf.macropa	18	9	2	0	0	0	0
Scina borealis	1	0	4	0	4	0	0
Scina crassicornis	3	0	6	0	1	0	0
Vibilia armata	0	0	7	0	3	0	0
Vibilia cultripes	0	0	0	0	0	0	1
Typhidae unid.	0	0	1	0	0	0	0
Hyperiidea unid.	0	0	0	1	0	0	0
Isopoda							
Anthuridae							
*Belonectes parvus***	0	0	0	0	24	0	32
Bopyridae							
Chelator chelatus	0	0	0	0	0	2	13
Natalolana borealis	0	0	0	0	3	1	2
Desmosomatidae							
Desmosoma lineare	0	0	0	0	0	0	3
Disconectes furcatum	0	0	0	6	1	30	1
Disconectes cf. phallangium	0	0	0	0	0	0	1
*Disconectes sp.**	0	0	0	0	7	13	14
Eurycopidae**	0	0	0	0	0	0	4
Eurydice sp.	0	0	0	2	0	0	1
Gnathia sp.larvae**	0	0	0	3	31	7	18
*Ilyarachna longicornis**	0	0	0	1	31	6	17
Ischnomesus bispinosum	0	0	0	0	0	0	2
*Munnopsurus atlanticus***	0	0	0	0	34	0	83
Tanaidacea							
Apseudes sp.	0	0	0	0	1	0	1
Cumacea							
*Bathycuma brevirostris**	0	0	0	0	0	0	1
Campylaspis glabra	0	0	0	2	4	16	10
Campylaspis sulcata	0	0	0	0	0	41	0
Campylaspis vitrea	0	0	0	0	1	0	0
Camylaspis verrucosa	0	0	0	0	24	3	3
Campylaspis sp.	0	0	0	0	1	0	0
Campylaspis cf. macrophthalma	0	0	0	0	0	1	1
Cumella spinoculata	0	0	0	0	0	0	1
Cumellopsis puritani	0	0	0	0	0	1	0
*Cyclaspis longicaudata***	0	0	0	0	8	0	3
Diatyloides serrata	0	0	0	0	0	5	2
Hemilamprops normani	0	0	0	0	5	26	0
Leptostylis macrura	0	0	0	0	1	3	1
Leptostylis sp.	0	0	0	0	0	0	1
Leucon affinis	0	0	0	0	1	2	0
Leucon longirostris	0	0	0	0	0	0	4
Makrokylindrus stebbingi	0	0	0	0	1	10	0

IDEA0803 ***Taxon***	**M1**	**M3**	**M4**	**P1**	**P2**	**P3**	**P4**
Cumacea							
Makrokylindrus longipes	0	0	0	0	0	1	0
Nannastacus unguiculatus	0	0	0	0	0	1	0
*Platysympus typicus***	0	0	0	0	9	0	10
*Procampylaspis bonnieri**	0	0	0	0	1	1	5
Procampylaspis sp.*	0	0	0	0	1	1	2
Procampylaspis armata	0	0	0	0	4	26	15
Ostracoda	66	736	359	20	46	30	61
Copepoda							
Calanoidea	1457	11574	6987	2952	569	781	378
Cyclopoidea	0	0	40	0	0	0	0
Ostheichthyes							
Cyclothone braueri	0	3	47	0	2	0	4
Cyclothone pygmaea	0	0	0	0	0	0	1

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Chapter 6

Temporal variations in the diet of three deep-sea demersal fish on the continental slope of the Balearic Islands elucidated by stomach contents and stable isotope analyses

Abstract

The temporal variations in feeding habits and trophic levels of three deep-sea species (*Hoplostethus mediterraneus*, *Hymenocephalus italicus* and *Nezumia aequalis*) off the Archipelago of Cabrera (Balearic Basin) were investigated. Samples were collected within the framework of the Project IDEA, during six scientific trawl surveys from August 2003 to June 2004, at bi-monthly intervals, between 550 and 750 m of depth. Classical stomachs contents and stable isotopes analyses were contemporarily applied in order to detect temporal variations of diet. An experiment using formalin preserved specimens and frozen specimens as control were also performed in order to verify if preserved material can be used for stable isotopy.

The slime-head *H. mediterraneus* mainly preyed on isopod (e.g. *Natatolana borealis*) and on lesser extent on amphipods and mysids. *H. italicus* fed on mysids (mainly *B. arctica*) and amphipods, captured in the Benthic Boundary Layer, while *N. aequalis* seemed to exhibit a more benthic behaviour with benthopelagic and benthic prey (e.g tanaids and polychaetes) in its diet.

Strong temporal variations were observed in the diet of *H. mediterraneus*, with February as a period of change in feeding habits. Temporal changes were less evident in the two macrourids and depth seemed to be a more structuring factor of diet than month.

Low levels of dietary overlap occurred throughout the year among the three species, implying high resource partitioning and probably low levels of competition between coexisting species.

The experiment on frozen and fixed-preserved specimens showed that formalin did not affect ^{15}N signatures, while time of preserving strongly incluenced ^{13}C values, with different pattern among the three species.

Temporal variations of $\delta^{15}N$ values were evident in the three species: the lowest values were observed in September and the highest in April-June. Among the three species *N. aequalis* had the highest trophic level (4,47), occupying a high position in the deep-sea trophic web.

In general deep sea trophic webs were complex, with at least three trophic levels among benthopelagic resources and with fish as top-predators: marine snow seemed to be the only one primary source of material. Correlation between $\delta^{15}N$ and $\delta^{13}C$ on a month scale became

strong from November to April and then decreased from June to August, the strongest correlation (R=0,85) was found in April, one month after the highest value obtained for the bulk of benthopelagic species. The strong correlation found for benthopelagic species occurred about one month later the peak of primary production recorded in the area (by satellite images). It was reasonable to expect that there was a delay of more than one month in the higher trophic level as in fish; these results are consistent with other findings in the same area.

6.1 Introduction

The study of dietary composition and feeding habits of fish has been considered as one of the most important topics in the ecology of marine communities. Both the biology of species (at a population level) and their interaction with biological and environmental factors are influenced by food resources exploited by fish. At bathyal depths most of our knowledge on deep-sea demersal communities comes principally from areas where deep-water fisheries are commercially developed. This is true both for fish and decapod crustaceans communities. In terms of dietary studies many deep-water fish populations of Mediterranean have been the subject of intensive study, particularly the Catalan sea (e.g Macpherson, 1981; Carassón et al, 1992; Carassón and Matallanas 1998, 2001) and the Balearic Basin, where a great deal of information exist also on decapod crustaceans and recently in the Ionian sea (eastern Mediterranean) (Madurell et al., 2004; Madurell and Cartes, 2005a, 2005b, 2006). Out of the Mediterranean, detailed information exists for istance on Rockall trough in the north-eastern Atlantic (Mauchline and Gordon, 1990) the NE Pacific (Pearcy and Ambler, 1974 etc.).

The bathyal communities of the deep Mediterranean are characterized by fishery exploitation directed to large Penaeoidean shrimps *Aristeus antennatus* and *Aristaeomorpha foliacea* (Cartes and Sardà, 1992; Sardà et al., 1994).

Most deep-sea demersal fish are active predators, regarded as generalist (i.e. highly diversified diets) and depend mainly on benthopelagic and mesopelagic prey (Mauchline and Gordon, 1986). Many deep sea fish show diel and seasonal feeding patterns (e.g. Macpherson, 1980; Mauchline and Gordon, 1984; Blaber and Bulman, 1987; Priede et al, 1994; Atkinson, 1995), which have been often related to the temporal vertical migrations of their mesopelagic prey (Bulman and Blaber, 1987; Atkinson, 1995, Gartener et al., 1997). It is crucial the simultaneous sampling of the trophic resources exploited by fish to fully understand temporal (e.g. seasonal) and spatial changes in the diet, trophic habits and trophic level (elucidated by stable isotope analysis) of bathyal fish. The Benthic Boundary Layer (BBL) macrofauna, also known as suprabenthos or hyperbenthos (mainly peracarid and decapod crustaceans), are a key compartment linking lower trophic levels and top predators such as fish (Cartes 1998b; Carassón and Cartes, 2002). However, despite the accepted role of this fauna in the bathyal food webs little is known about their interactions and their dynamics. To date, studies on the feeding habits of deep-sea fish have focused mainly on depth related changes, whereas only few studies have addressed aspects of seasonal feeding cycles, primarily because of the difficulty of samples collection at these depths.

The deep Mediterranean is a zone with a high environmental stability with respect to temperature and salinity (Margalef, 1985), while other basic physiographic parameters such as the proportion of mud, and the organic matter in sediments, and seem also to vary little (Carpine, 1970; Cartes et al., 2002b). Therefore, this would be a good ecosystem in which to study trophic relationships among fauna. Deep sea fauna of western basin is well-known, but hardly information exists on seasonal changes in diets and trophic levels.

In general studies on feeding relationships of deep-sea organisms are very limited and they were concentrated on gut contents data. These data, however, have substantial limitations. For example gut contents reflect diets at particular points in time and space, and severely neglect certain types of dietary materials such as gelatinous plankton and detritus that may nevertheless be very important in the sustenance of marine food-webs. Other limitations include problems associated with the voiding of gut-contents upon capture. Furthermore many animals (e.g. decapod crustaceans) crush or grind their food, such that identification of prey becomes very difficult. These difficulties are especially great in the study of food-webs in the deep-ocean.

The use of stable isotopes of nitrogen and carbon for the study of trophic interactions is now common in aquatic ecosystems (Pinnegar and Polunin, 2000). Analysis of stable isotope composition provides an indication of the origin and transformations of organic matter. Stable isotopes integrate short-term variation in diet and thus are less subject to temporal bias. In fish, tissue turnover times may be as slow as 0.1–0.2% day–1 (Hesslein et al. 1993). The $\delta^{15}N$ in tissues of consumers is typically greater by 3‰ relative to their prey, and stable N isotope data have been used to define the trophic levels of organisms (Owens 1987). In contrast, tissues tend to be rather weakly enriched in ^{13}C as trophic level increase (less than 1‰), and ^{13}C may thus act as a useful indicator of material sources of production. This method was rarely applied to detect seasonal changes in fish diet (Polunin et al., 2001; Mac Neil et al., 2005), and studies on the application of stable isotopy in deep-sea ecosystems to elucidate temporal changes in trophic levels completely lack. In spite of this, results of stable isotopy often need the knowledge of dietary composition by gut content analyses to be correctly interpreted. Hence, the importance of the simultaneous study of gut contents and stable isotopic composition.

Therefore, the present chapter describes the feeding habits of three fish species in a bathyal site of the Balearic basin. These three species are presumably representative of the three main trophic guilds: *Hoplostethus mediterraneus* actively forages on natatory benthopelagic prey (Pais, 2002; Madurell & Cartes, 2005a), *Hymenocephalus italicus* preyed mainly on suprabenthic prey while *Nezumia aequalis* also on infauna (i.e. polychaetes) (Macpherson 1979; Mauchline and Gordon 1984, Madurell and Cartes, 2006). The importance of temporal changes in food resources available for fish and the determination of changes in trophic level and in source of carbon elucidated by stable isotopes analysis of $\delta^{13}C$ and $\delta^{15}N$ are important aspects of this chapter hardly considered in previous similar approaches.

Finally, after coupling stomach contents and stable isotopes data with biomass, abundance and stable isotopes signatures of available resources, an attempt to describe trophic web structure of a bathyal benthopelagic boundary layer community off the Cabrera Archipelago is given.

6.2 Material and methods

Detailed description of the study area and sampling procedure in Chapter 1

6.2.1 Stomach contents analysis

Immediately after capture, specimens were fixed in 10% buffered formaldehyde. Once in the laboratory, they were measured to the nearest mm: total length (TL) for *Hoplostethus mediterraneus* and pre-anal (PAL) length for *Hymenocephalus italicus* and *Nezumia aequalis*. All specimens were weighted to the nearest 0,1 g and dissected for stomach contents analysis. A total of 607 individuals from the three species under study were analysed (Table 6-1)

Table 6-1 Number of specimens dissected (number of fishes with food in stomachs) by month. Length range (TL) is also included

	Aug 03	Sep 03	Nov 03	Feb 04	Apr 04	Jun 04	Length range (mm)
Hoplostethus mediterraneus	37(30)	33(22)	28(18)	11(6)	2(2)	11(7)	67-214
Hymenocephalus italicus	41(30)	31(17)	60(40)	36(23)	27(22)	32(24)	50-182
Nezumia aequalis	45(35)	21(11)	56(35)	69(48)	28(22)	39(33)	105-271

All prey items were identified to the lowest possible taxonomic level, counted and weighed (precision 0.0001g) after excess moisture had been removed by blotting on tissue paper and, where possible, measured using a micrometer.

6.2.2 Stable isotopes analysis

A detailed description of stable isotopes analysis procedure was given in chapter 1.

For fish, three specimens for each species and survey of the same size (table 6-2, ANOVA p>0,05) were chosen to ensure that eventual changes in stable isotopes signatures were due to a season effect and not to a size effect.

Table 6-2. Size range of the specimens analysed by month. For *H.mediterrane*us TL (total length) was reported, while for *H.italicus* and *N.aequalis* PAL (pre-anal length) was given. ANOVA results was also showed

	Aug 03	Sep 03	Nov 03	Feb 04	Apr 04	Jun 04	*F*	p
Hoplostethus mediterraneus	160-195	157-165	156-159	164-214	133-167	158-173	1,18	0,18
Hymenocephalus italicus	33-42	35	34-35	31-41	34-43	31-33	1,48	0,26
Nezumia aequalis	40-47	41-44	42-45	44-46	42-45	39-49	0,33	0,88

Statistical differences in isotopes signatures as a function of season were tested by one-way ANOVA. Where interaction proved significant, these were investigated by *a posteriori* comparisons using Tukey's HSD (Underwood 1997).

To assess the fixative dependant shift of δ^{13}C and δ^{15}N (see chapter 1 for a detailed description of the experiment) in preserved material, three samples for each species from freeze-dried and preserved specimens were processed and then differences were tested by one-way ANOVA.

Table 6-3. Size range of the specimens used to assess the fixative dependant shift of $\delta^{13}C$ and $\delta^{15}N$ in preserved material. For the three species TL (total length) was reported. P values of ANOVA were also showed

	May05	*p*
Hoplostethus mediterraneus	160	-
Hymenocephalus italicus	150-160	>0,05
Nezumia aequalis	180-190	>0,05

6.2.3 Data analysis

6.2.3.1 Indices used for stomachs contents analysis

Numerous indices have been described to quantitatively express the importance of different prey in the diets of fish (see Hyslop, 1980; Piankas et al., 1971; Cortés 1997, 1998 for a review):

1. Vacuity coefficient (V%): number f empty stomachs expressed as a percentage the total number of stomachs analysed;
2. Percentage frequency of occurrence (%F): number of stomach in which a food item was found, expressed as a percentage of the total number of non-empty stomachs;
3. Percentage numerical abundance (%N): number of each prey item in all non-empty stomachs in a sample, expressed as the percentage of the total;
4. Percentage gravimetric composition (%W): wet weight of each prey group, expressed as the percentage of the total weight of the stomach contents
5. Index of relative Importance (IRI) (Piankas et al., 1971) was calculated for each prey group and used to assess preferred diet prey:

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

This index has been expressed as $\%IRI=(IRI/\Sigma IRI) \times 100$. Prey items were sorted in decreasing order according to their %IRI contribution, and then cumulative %IRI was calculated. Food items were grouped into three categories of preference according their contribution to the cumulative %IRI (Rosecchi & Nouaze, 1987): preferential or dominant prey (when cumulative %IRI attains at least 50% of the total %IRI); secondary prey (prey whose cumulative %IRI, when added to that of preferential prey, attains at least 75% of the total %IRI): and occasional or accidental prey (the remaining prey).

6. Stomach fullness (stomach weight/fish weight x100);
7. Standard diversity indices calculated for each prey item:

(i) Trophic diversity (H'): calculated used Shannon.Wiener equation (Shannon & Weaver, 1963):

$$H' = \sum_{i=1}^{n} p_i \ln(p_i)$$

Where p_i is the proportion of a specific prey category for the n categories of prey listed

(ii) Pielou's eveness (J') was calculated according to:

$$J' = H' / \log(S)$$

Where S is the number of species

(iii) The inverse of Dominance (Simpson's index) was determined as follows:

$$\lambda = 1 - \sum_{i=1}^{n} p_i^2$$

When it is no possible to weight separately all the preys found, percentage volume of stomach contents was estimated using the subjective points methods (Swynnerton & Worthington, 1940) for the different prey-species or prey-groups. Based on this method a predetermined number of points was given to each prey as a function of their combination to the total volume of gut content (e.g. 100 points for the most dominant prey etc.).

Diet was characterised for each species by seasons.

6.2.3.2 Intraspecific relationships

Statistical differences in prey dominance as a function of season were established by applying a chi-squared test (Yates' correction) to the numerically dominant prey (in terms of %IRI). Chi-squared tests were calculated between contiguous periods. One-way ANOVAs were used to compare the mean prey number, mean prey weight and fullness among seasons. Data were log or square root transformed to remove the dependency of the variance on the mean (Zar, 1984), and in the case of fullness were arc-sen transformed (Underwood, 1997). The assumption of normality was tested using the Kolmogorov–Smirnov test and the assumption of homogeneity was checked by Cochran test. In default of normality of the data, the Kruskall–Wallis (K–W) test was applied on the raw data and *a posteriori* Mann-Whitney *U* test was performed on each pair of median values to test for significant differences. All analysis inferences were based on the 0,05 significance level.

Dietary overlap between seasons was also measured for each species by applying the percentage of similarity used by Schoener (1970):

$$\alpha = 1 - 0.5(\sum|p_{ij} - p_{ik}|)$$

where α is the similarity between diets of species *x* and *y*

p_{xi} is the proportion of food category *i* in the diet of species *x*

p_{yi} is the proportion of food category *i* in the diet of species *y*

Diet overlap ranges from 0 to 1 and it is generally considered to be biologically significant when it exceeds 0.60 (Keast, 1978; Macpherson, 1981; Wallace, 1981). This threshold value is rather arbitrary, and other authors assume significant overlap values of 0.80 (Cartes & Sardà, 1989) for intraspecific food overlap. Overlap was calculated using %W values, weight is the most appropriate parameter to consider in mass balance relationships or models.

A SIMPER analysis was applied to each species to determine the most typifying prey species for season. The Schoener index was applied to assess the overlap degree between samples per month.

An experimental design were therefore created with two factors: months (six levels, fixed), and depth (three levels, random, nested n month).

The fish gut contents variables were highly skewed and contained a great many zeros, making traditional analyses (which assume normality of errors) unsuitable, so nonparametric approaches were used. PERMANOVA (Anderson, 2001; McArdle and Anderson, 2001) was used to analyse the multivariate data set in response to the complete experimental design (including interactions). This method allows multivariate data to be analysed on the basis of any distance or dissimilarity measure of choice, in response to any complex experimental design, with P-values obtained using permutations. We used Bray-Curtis or Modified Gower (when raw data have many zeros) as a dissimilarity measure for the PERMANOVA.

Non metric multi-dimensional scaling (nMDS) on the basis of the Bray-Curtis dissimilarity measure was used as an unconstrained ordination method to visualize multivariate patterns. In addition, canonical analysis of principal coordinates (CAP, Anderson and Willis, 2003; Anderson and Robinson, 2003) was used as a constrained ordination procedure to visualize patterns by reference to particular hypotheses. The CAP analyses were only done on appropriate terms found to be significant using PERMANOVA.

A PERMANOVA one factor design was also created (factor month fixed, 6 level) in order to detect significant changes among months.

6.2.3.3 Interspecific relationships

In order to study the inter-specific trophic relationships between the three slope dwelling fish species, dietary overlap was calculated using Schoener's index (see subsection 6.2.3.2).

Trophic interactions were analysed according to the six months sampled.

Non parametric MDS were performed on Bray-Curtis resemblance matrix based on standardized and log(x+1) transformed biomass data (Field et al., 1982) of the different groups of prey items to describe overall inter-specific variations in food habits. SIMPER analysis was performed to identify the most typifying prey for each species and the degree of similarity/dissimilarity among them.

All the analyses were performed with PRIMER 6 and PERMANOVA plus software (Clark and Warwick, 1995; Anderson 2006), STATISTICA 6 and R-plus.

6.3 Results

6.3.1. Overall diet species by species

Hoplostethus mediterraneus

122 individuals were examined, among these 12 (9,8%) were everted and 34 (27,9%) were empty. A total of 76 stomachs were analyzed for diet composition. 32 prey items were identified (Annex 6-1). The diet of *H.mediterraneus* consisted mainly of crustaceans and fish (fig. 6-1). Isopods (*Natatolana borealis*) were the dominant prey ingested. Among other crustaceans amphipods (mainly *Tryphosites* spp., *Rhacotropis rostrata* and Lissianassidae) and mysids (mainly *Boreomysis arctica*) were the most frequently ingested. The isopod *N. borealis* was the most abundant prey, it occurred in large number, up to 32 specimens were found in a single stomach. All the individuals were in a good state and sizes ranged from 6,3 to 21,2 mm of total length.

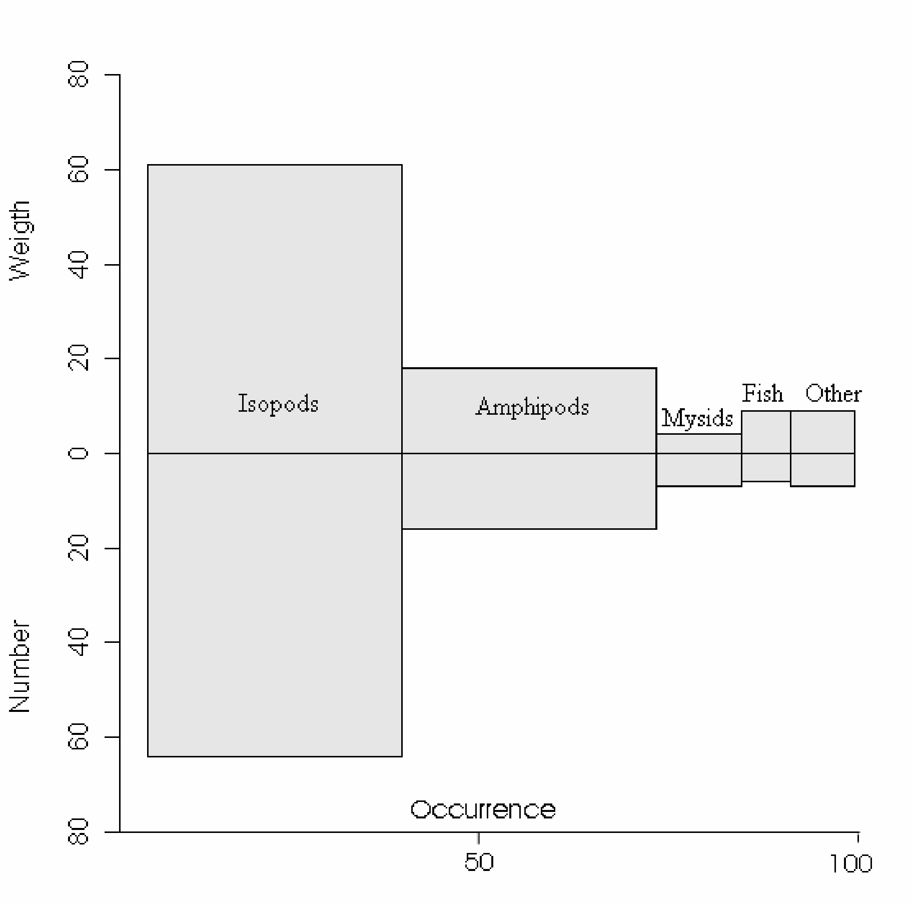


Fig. 6-1. Relative importance of prey groups (IRI >1%) to *H.mediterraneus* diet. Areas of rectangles are proportional to the %IRI. %F: percentage contribution by frequency of occurrence; %N: by number; %W: by weight are given.

Hymenocephalus italicus

A total of 224 individuals from August 2003 to June 2004 were analyzed, among these 14 (6%) had the stomachs everted and of the remaining 210, 48 (21%) were empty. From 162 stomachs containing food, 52 categories of prey items were identified (Annex 6-2). Diet chiefly consisted of crustaceans. In terms of %IRI not a single dominant prey item was identified. Accordingly, mysids (58,04% IRI), copepods (14,16% IRI) and isopods (11.12% IRI) were the preferential prey items ingested (Figure 6–2).

Other important prey items were ostracods (9.95% IRI) and amphipods (4.97% IRI), whereas polychaetes, fish and other crustaceans (e.g. cumaceans and decapods) were ingested occasionally. At a species level *Boreomysis arctica*, *Natatolana borealis* and *Munnopsurus atlanticus* were the main prey items ingested in terms of %F and %N (Annex 6-2).

Trophic diversity values (H': 2,42±0,18; see also Annex 6-2) had approximately the same value during all the sampling periods.

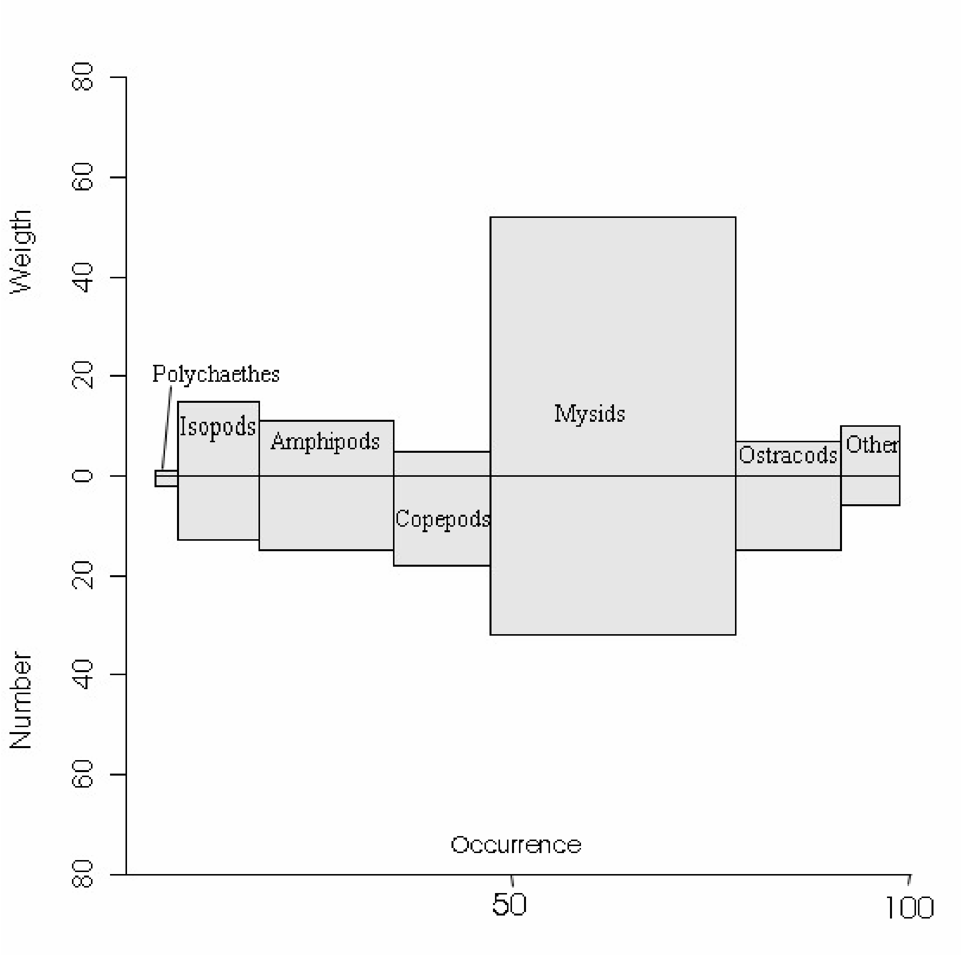


Fig. 6-2. Relative importance of prey groups (IRI >1%) to *H.italicus* diet. Areas of rectangles are proportional to the %IRI. %F: percentage contribution by frequency of occurrence; %N: by number; %W: by weight are given.

Nezumia aequalis

A total of 258 individuals were examined, however among these 50 (19,8%) individuals were everted and 23 (9,1%) were empty. A total of 185 stomachs were analyzed for diet composition and 75 prey items were identified (Annex 6-3). Amphipods (31.71% IRI) were the dominant prey ingested, followed by isopods (22.37% IRI), polychaethes (22.04% IRI) and mysids (16.07% IRI) as secondary prey (Fig. 6-3, Annex 6-3). Other crustaceans (e.g. cumaceans, decapods, tanaids and ostracods) were ingested in a more irregular way (Annex 6-3).

At a species level, *Munnopsurus atlanticus*, *Boreomysis arctica* and *Maera schmidti* were the main prey items ingested in terms of %F and %N (Annex 6-3). Ostracods occurred frequently (%F=10) in stomach contents but their contribution in terms of weight is low, probably they are accidentally ingested.

Trophic diversity values is higher than in the other examined species (*H'*: 3,09±0,31; see also Annex 6-3), values changed little during all the sampling periods.

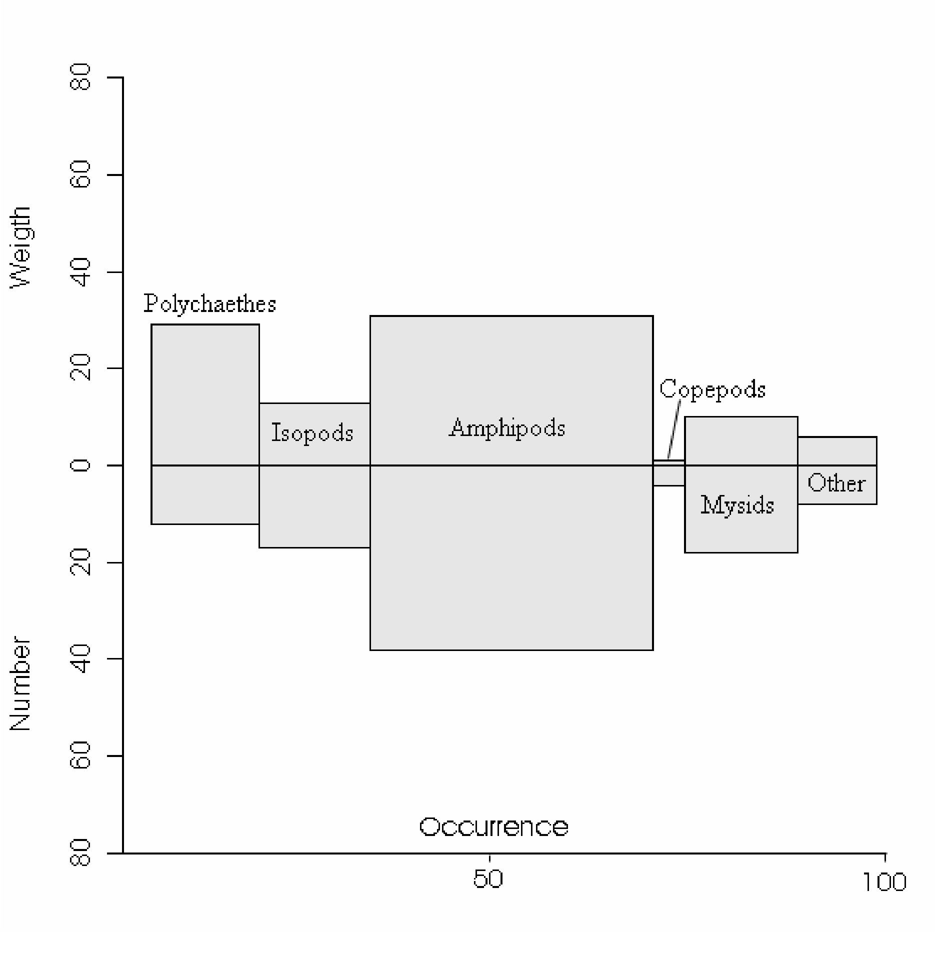


Fig. 6-3. Relative importance of prey groups (IRI >1%) to *N.aequalis* diet. Areas of rectangles are proportional to the %IRI. %F: percentage contribution by frequency of occurrence; %N: by number; %W: by weight are given.

6.3.2 Temporal variation in diet composition

Hoplostehus mediterraneus

The dominant food items were the same throughout the whole period studied (i.e. the isopod *Natatolana borealis*) however significant variations of their proportions between seasons were observed (Annex 6-1). *N. borealis* was the most abundant prey from August to November (%IRI values ranged from 97,5 to 98,8) and in June (%IRI 85,5), while in February it was replaced by *Tryphosites* spp (%IRI 51,2), and *B. arctica* (%IRI 24).

Chi-squared test revealed significant differences between the ingestion of isopods among contiguous periods (χ^2=79,83, p<10^{-5} between November and February); mysids (χ^2=45,65, p<10^{-5} between November and February; χ^2=39,24, p<10^{-5} between February and June) and amphipods (χ^2=19,5, p<10^{-5} between November and February; χ^2=8,37; p<10^{-3} between February and June).

The lowest values of diversity were found in September and November due to the complete dominance of *N. borealis* in the diet (Annex 6-1). A significant decrease in the mean number of prey ingested (ANOVA $F_{1,80}$=3,09; p =0,01) was observed in April-June (1,50 and 2,18 in April and June respectively), whereas higher values were found in summer (8,83 and 10,18 in August and September respectively). No significant differences were found in mean prey weight and fullness (ANOVA p >0,05) by month. The lowest percentage of empty stomachs was found in August (χ^2 test; p<0,05 between August and all months). Fullness was significantly correlated (Spearman correlation) with month (R=-0,305; p<0,005) and depth (R=-0,407; p<0,005), but did not with any of the possible environmental explanatory variables (see also chapter 5). Fullness reached its minimum values in September 2003.

Overlap values were very high throughout all the sampling period, only February showed very low values of overlap in comparison with the other months (Table 6–1). February 2004 coincided also with the period of maximum change in the diet.

Table 6–1. Seasonal dietary overlap of *Hoplostethus mediterraneus*

	Aug	Sep	Nov	Feb	Jun
Aug	1	0,88	0,88	0,21	0,88
Sep		1	0,95	0,19	0,94
Nov			1	0,20	0,94
Feb				1	0,21
Jun					1

SIMPER analysis evidenced that the most typifying species in the first part of the year (from August to November) is *N. borealis* (Table 6-2). Its contribution to the similarity between samples per month ranged between 96,53% in August to 99,23% in November. The species that most contributes to February samples similarity were *Tryphosites* spp (65,18%) and *B.arctica* (22,28%). The highest values of similarity between samples were observed from August to November (more than 50%). The highest values of dissimilarity among contiguous months was observed between November and February (average dissimilarity: 74,7%).

Table 6-2 SIMPER analysis of gut contents of *H. mediterraneus* from August 2003 to June 2004 (cut-off for low contribution 90%).

Group August

Average similarity: 50,39

Species	Av.Abund	Av.Sim	Sim/SD	Contrib%	Cum.%
N. borealis	2,57	46,15	1,37	91,58	91,58

Group September

Average similarity: 58,88

Species	Av.Abund	Av.Sim	Sim/SD	Contrib%	Cum.%
N. borealis	2,67	57,06	1,5	96,91	96,91

Group November

Average similarity: 61,43

Species	Av.Abund	Av.Sim	Sim/SD	Contrib%	Cum.%
N. borealis	2,63	60,74	1,45	98,87	98,87

Group February

Average similarity: 31,85

Species	Av.Abund	Av.Sim	Sim/SD	Contrib%	Cum.%
T. longipes	1,62	13,82	0,73	43,38	43,38
N. borealis	1,18	6,59	0,48	20,68	64,07
B. arctica	1,25	6,01	0,48	18,87	82,94
Tryphosites sp.	1,16	5,44	0,48	17,06	100

Group June

Average similarity: 31,55

Species	Av.Abund	Av.Sim	Sim/SD	Contrib%	Cum.%
N. borealis	1,85	26,57	0,69	84,21	84,21
T. longipes	0,73	1,67	0,19	5,29	89,51
Gammaridea	0,63	1,47	0,19	4,67	94,17

A seasonal trend in the diet was observed during the year (fig. 6-4) by nMDS analysis, performed on standardized and 4th root transformed biomass data of prey per each individual. Two groups were detected, one grouped samples of late summer-autumn (August, September and November) and the second grouped samples of winter-spring (February, April and June) with some specimens from late summer-autumn.

This segregation was clearly due to the dominance of the isopod *N. borealis* in the diet in the first part of the sampling period (fig. 6-5). February did not appear segregated from the rest of periods in the nMDS, in spite it was the month where diet changes were more evident. Mean size of specimens analyzed in February was 163,5 mm (±20,7), instead from August to November mean total length ranged from 140 to 146 mm and in June it was 145,6 mm (±33).

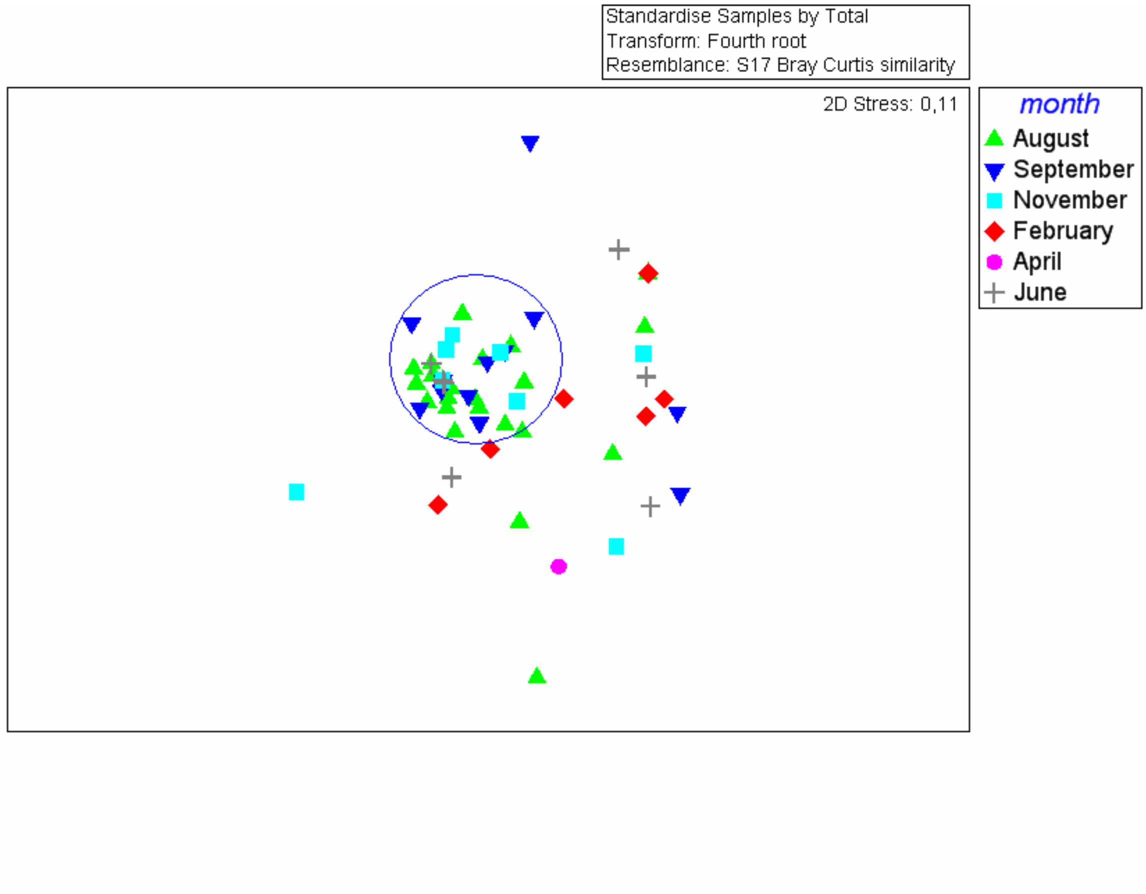


Fig. 6-4 nMDS ordination of foreguts samples of *H. mediterraneus* based on the Bray-Curtis dissimilarities of standardized and 4th root transformed data. Symbols indicate months

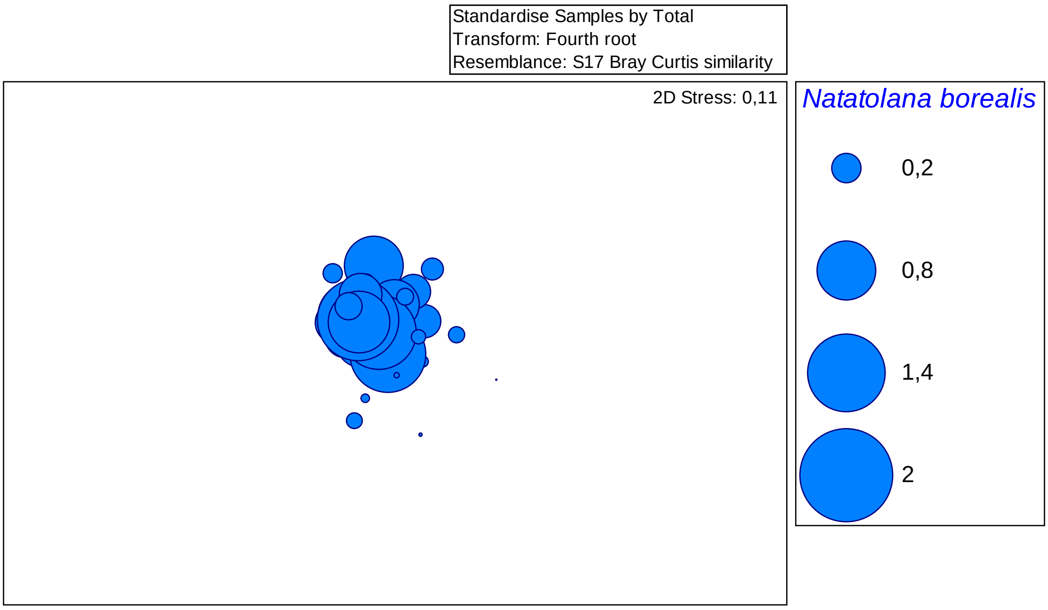


Figure 6-5. 2D Bubble plot of nMDS ordination of fig. 6-4: bubbles size indicates the weight of *Natatolana borealis* in stomach contents.

Symmetrical PERMANOVA (one factor, month with 6 levels) provided evidence for statistical differences in diet by month ($F_{5,85}$=2,39 p<0,05). A pair-wise comparison (only pair-wise comparisons among contiguous months were given) showed significant differences only between November and February (table 6-3).

Table 6-3 PERMANOVA pair-wise test on term "month" (only values among contiguous months were given).

Groups	t	P(perm)	Unique perms
August, September	0,79202	0,6514	9944
August, June	1,0448	0,3342	9875
September, November	Not a real number	0,9971	9723
November, February	2,4678	**0,0053**	1788
February, April	1,1129	0,4278	7
February, June	1,1012	0,2876	2149
April, June	1,3965	0,119	8

Hymenocephalus italicus

According to the different indices used to determine the diet, mysids, copepods, isopods and to a lesser extent amphipods, were the most important prey ingested by *H.italicus* throughout the whole period studied (Table 6–6). Mysids were dominant throughout the year, particularly in August (80.98% IRI) and in February (66,39% IRI), whereas isopods (mainly *N .borealis*) were dominant in February and April (28,71 and 18,44 % IRI respectively) (Table 6-6).

The highest number of empty stomachs was found in September and the lowest in April (χ^2 test $p < 0.05$).

Mean prey number and fullness did not vary significantly among months, while mean prey weight did (K-W 13,72; p=0.02),

The consume (%N) of isopods differed significantly between contiguous months: August and September (χ^2=4,34; p=0,03), November and February (χ^2=8,37; p= 0,004) and between April and June (χ^2=10,32; $p = 10^{-3}$).

Trophic diversity values were the same among all the months (2,42±0,18).

Relatively low overlap was observed between all the sampled months, except between September- November and November-February (Table 6–4). Changes in diet were observed from September to November and from February to April.

Table 6–4. Seasonal dietary overlap of *Hymenocephalus italicus*

	aug	sep	nov	feb	apr	jun
aug	1	0,42	0,59	0,57	0,46	0,74
sep		1	0,56	0,52	0,44	0,50
nov			1	0,59	0,67	0,61
feb				1	0,46	0,66
apr					1	0,49
jun						1

SIMPER analysis showed low values of similarity between samples per month, ranging from 18 in June and Febraury to 27% in August and September (table 6-5).

In general *Boreomysis arctica* and other mysids were the most typifying species throughout the year, their contribution to the similarity ranged from 12% in June to 71% in August. Ostracods (mainly *Philomedes* sp. and Cyprinidae in general) highly contributed to similarity especially in September and November (27% and 57% respectively), the isopod *Munnopsurus atlanticus* is one of the most typifying species in April (5%) and June (9%) diets.

Table 6-5 SIMPER analysis of standardized and 4th root transformed data of *H. italicus* from August 2003 to June 2004 (cut-off for low contribution 90%).

Group August

Average similarity: 27,19

Species	Av.Abund	Av.Sim	Sim/SD	Contrib%	Cum.%
Boreomysis arctica	1,86	19,48	0,75	71,62	71,62
Cyprinidae	0,59	2,58	0,31	9,49	81,11
Copepoda	0,41	1,29	0,25	4,73	85,84
Tryphosites spp	0,46	1,05	0,15	3,87	89,7
scales and bone remains	0,35	0,75	0,18	2,76	92,47

Group September

Average similarity: 22,76

Species	Av.Abund	Av.Sim	Sim/SD	Contrib%	Cum.%
Boreomysis arctica	1,07	7,35	0,34	32,27	32,27
Cyprinidae	1,09	6,32	0,49	27,78	60,06
Copepoda	0,79	3,87	0,41	17	77,06
Mysidacea	0,83	3,5	0,33	15,35	92,41

Group November

Average similarity: 22,94

Species	Av.Abund	Av.Sim	Sim/SD	Contrib%	Cum.%
Cyprinidae	1,46	13,15	0,64	57,31	57,31
Boreomysis arctica	1,03	5,48	0,36	23,88	81,2
Mysidacea	0,54	1,71	0,19	7,44	88,64
Gammaridae	0,39	0,82	0,13	3,57	92,21

Group February

Average similarity: 18,33

Species	Av.Abund	Av.Sim	Sim/SD	Contrib%	Cum.%
Boreomysis arctica	1,41	11,96	0,52	65,23	65,23
Andaniexis mimonectes	0,52	1,74	0,18	9,5	74,73
Natatolana borealis	0,58	1,47	0,19	8,02	82,75
Copepoda	0,48	1,27	0,23	6,91	89,66
Mysidacea	0,49	1,18	0,18	6,45	96,11

Group April

Average similarity: 24,89

Species	Av.Abund	Av.Sim	Sim/SD	Contrib%	Cum.%
Natatolana borealis	1,11	7,01	0,4	28,17	28,17
Copepoda	1	5,9	0,59	23,7	51,87
Mysidacea	0,92	4,78	0,46	19,2	71,08
Boreomysis arctica	1	4,42	0,36	17,74	88,82
Munnopsurus atlanticus	0,55	1,31	0,2	5,25	94,06

Group June

Average similarity: 18,14

Species	Av.Abund	Av.Sim	Sim/SD	Contrib%	Cum.%
Mysidacea	1,04	6,91	0,35	38,1	38,1
Cyprinidae	0,8	3,98	0,35	21,97	60,07
Boreomysis arctica	0,73	2,23	0,23	12,27	72,34
Copepoda	0,59	2,05	0,27	11,3	83,64
Munnopsurus atlanticus	0,55	1,65	0,17	9,12	92,76

Seasonal variations were not evident in *H. italicus* diet (fig. 6-6). NMDS (performed on modified Gower resemblance matrix of standardized and 4th root transformed biomass data) showed a clear separation only between November and April.

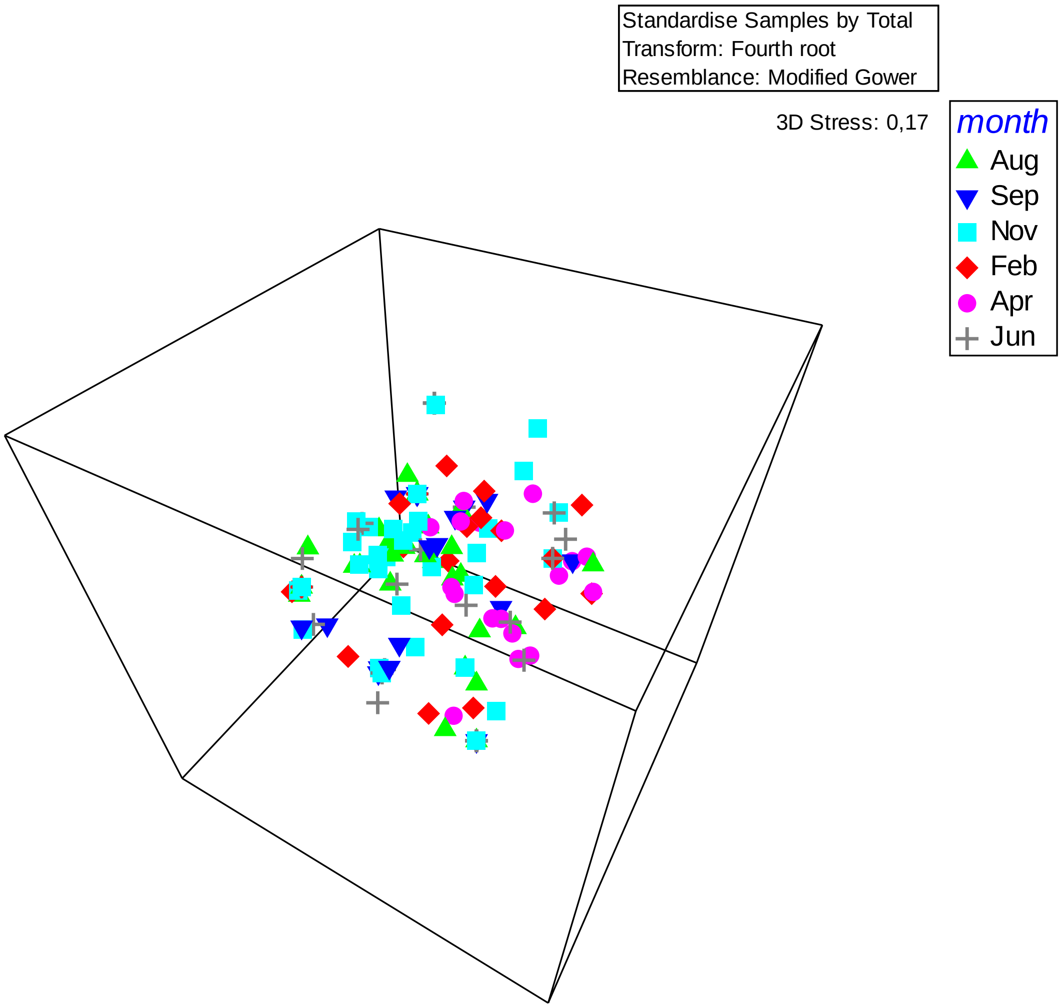


Figure 6-6 3D nMDS ordination based on Modified Gower dissimilarities of standardized and 4th root transformed data of *H. italicus* from August 2003 to June 2004. Symbols represent months as in figure legend.

It seemed there were two structuring factors in separating samples: month and depth. Based on this hypothesis a PERMANOVA design was created on two factors (month fixed 6 levels, depth random 3 levels, nested in month). Symmetrical PERMANOVA analysis provided evidence for significant differences among months and mainly for the interaction between months and depth (table 6-5).

Table 6-5. Results of PERMANOVA analysis for *H .italicus* (28 taxa).

Source	df	SS	MS	Pseudo-F	P(perm)	Unique perms
month	5	8,21	1,64	1,53	**0,023**	9873
depth(month)	9	9,19	1,02	1,49	**0,004**	9796
Res	142	97,55	0,68			
Total	156	114,71				

Grouping samples per haul (depth), foreguts were separated mainly as a function of depth than of month (Fig. 6-7).

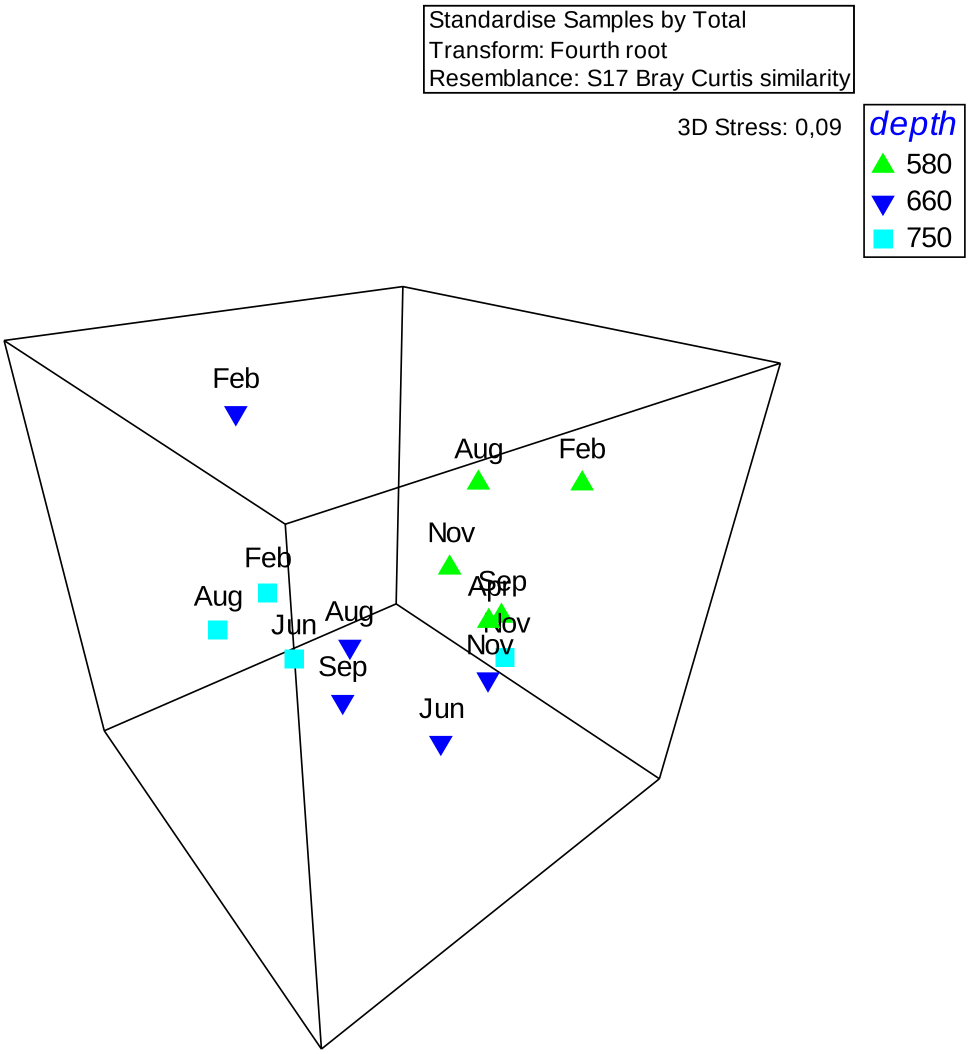


Figure 6-7 3D nMDS ordination based on Modified Gower dissimilarities of standardized and 4th root transformed data of *H. italicus* from August 2003 to June 2004. Symbols represent depths, as in figure legend, and labels indicate month

CAP analysis was performed on the appropriate terms found to be significant using PERMANOVA. CAP done on depth showed a gradient in the ordination of gut contents by depth (fig. 6-8), while CAP analysis based on months (fig. 6-9) provided evidence for a separation of samples along the two axes: axis one separates August and part of November samples from the other months, probably due to the high consumption on *B. arctica* observed at least in August; the second axis divided February and April samples from September and November. This could be attributed to the predation on cyprinids and *B. arctica* in September-November contrasting with the higher consumption of mysids, copepods, *M. atlanticus* and *N. borealis* in February-April.

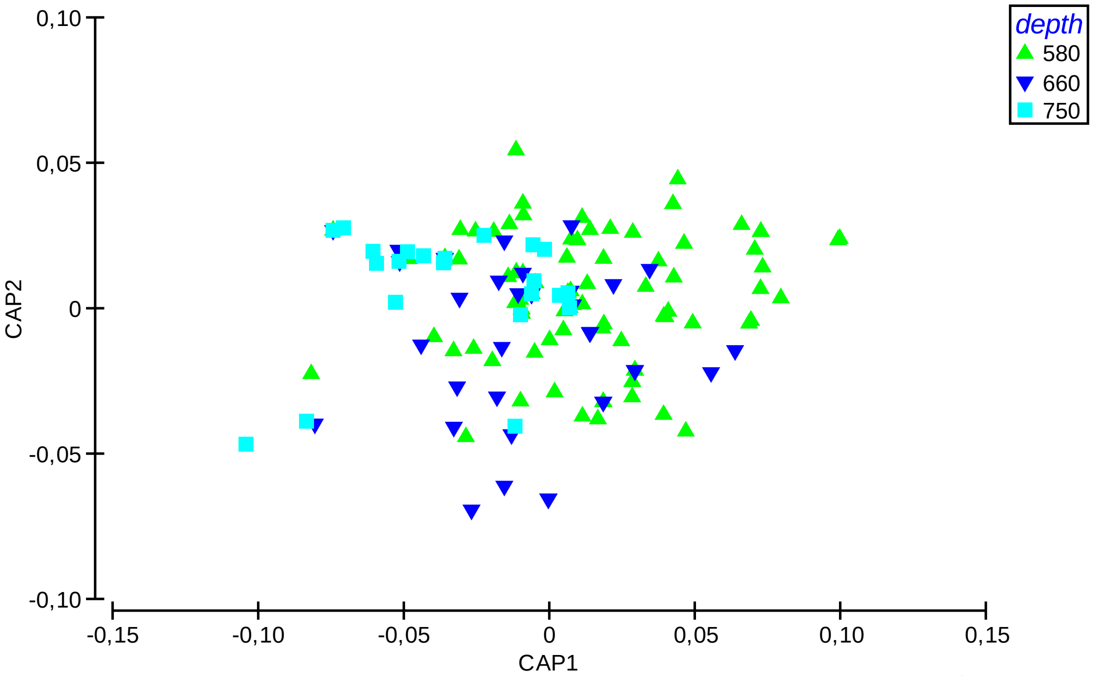


Figure 6-8 CAP analysis based on Modified Gower dissimilarities of standardized and 4th root transformed data of *H. italicus* from August 2003 to June 2004, and performed on factor "depth". Symbols represent depths.

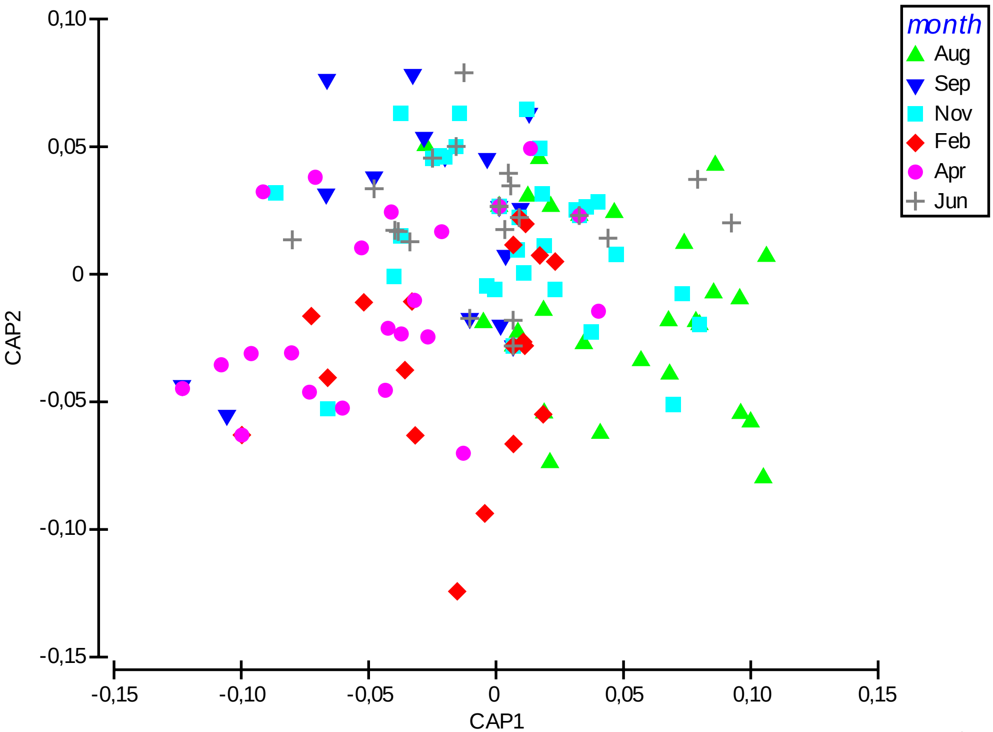


Figure 6-9 CAP analysis based on Modified Gower dissimilarities of standardized and 4th root transformed data of *H. italicus* from August 2003 to June 2004, and performed on factor "month". Symbols represent months.

Nezumia aequalis

Seasonal variations in the diet of *N. aequalis* are shown in Annex 6-3. Poychaetes and amphipods were the main prey items ingested in all seasons; however the ingestion of polychaetes (mainly Aphroditidae and Eunicidae) increased gradually from August to February and dominated the species' diet. Isopods (i.e *Munnopsurus atlanticus*) were abundant throughout the year but mainly in April. Mysids were consumed mainly in April and June (30 and 40% IRI respectively).

Chi-squared test revealed significant differences between the ingestion of isopods among contiguous months (χ^2=8,39; *p* 0,004 in November-February), amphipods (χ^2=4,4; *p* 0,04 in August-June; χ^2=6,27; *p* 0,01 in September-November; χ^2=4,88 *p* 0,03 in February-April).

Significant differences in mean weight of prey were found between February and April (*post-hoc* Tukey NHSD test p<0,001). Mean fullness and mean number of prey in stomachs did not differ significantly (ANOVA p>0,05). The lowest percentage of empty stomach was found in June and the highest in September (χ^2 test *p*<0,05 between August and September-November; September and April-June; November and April-June; February and June)

Relatively high overlap was observed between samples from August, September, November and February, maybe due to a similar diet in the first part of the sampling period (Table 6–6).

Table 6–6. Seasonal dietary overlap of *Nezumia aequalis*

	Aug	Sep	Nov	Feb	Apr	Jun
Aug	1	0,61	0,69	0,50	0,56	0,43
Sep		1	0,61	0,40	0,63	0,31
Nov			1	0,58	0,58	0,45
Feb				1	0,49	0,55
Apr					1	0,43
Jun						1

The similarity (SIMPER analysis) within months was very low, ranging from 21 to 31% (table 6-7). The most typifying taxa from August to February were polychaetes (mainly Aphroditidae and Eunicidae) and gammarids. The contribution of Aphroditidae to diet was 14% in August, 30% in September, 23% in November and 6% in February, when Eunicidae were more abundant (20%). Amphipods (mainly *Rhacotropis* spp), mysids (*B. arctica*, *Parapseudomma calloplura* and *Mysideis parva*) and isopods (*M. atlanticus*) largely contributed to diet throughout the year, but their contribution was higher in April and June.

Table 6-7. SIMPER analysis based on tandardized and log(x+1) transformed data of *N. aequalis* from August 2003 to June 2004 (cut-off for low contribution 80%)

Group August

Average similarity: 21,00

Species	Av.Abund	Av.Sim	Sim/SD	Contrib%	Cum.%
Gammaridea	0,91	3,33	0,55	15,87	15,87
Aphroditidae	0,92	2,93	0,38	13,98	29,84
Maera schmidti	0,88	2,37	0,42	11,31	41,15
Ostracoda	0,55	1,42	0,31	6,75	47,9
Rhacotropis spp	0,6	1,25	0,31	5,97	53,87
Polichaetha ind.	0,62	1,24	0,3	5,92	59,79
Tryphosites spp	0,62	1,2	0,26	5,72	65,51
Munnopsurus atlanticus	0,56	1,16	0,32	5,53	71,04
Suprabenthic isopods	0,53	1,15	0,26	5,45	76,49
Copepoda	0,4	0,8	0,33	3,81	80,3

Group September

Average similarity: 22,84

Species	Av.Abund	Av.Sim	Sim/SD	Contrib%	Cum.%
Aphroditidae	1,46	6,89	0,64	30,16	30,16
Gammaridea ind	0,87	3,52	0,52	15,4	45,56
Boreomysis arctica	0,88	2,77	0,38	12,14	57,69
Rhacotropis spp	0,76	2,32	0,38	10,16	67,85
Munnopsurus atlanticus	0,77	2,04	0,38	8,92	76,78

Group November

Average similarity: 30,69

Species	Av.Abund	Av.Sim	Sim/SD	Contrib%	Cum.%
Munnopsurus atlanticus	1,26	7,39	1,17	24,09	24,09
Aphroditidae	1,43	6,99	0,64	22,76	46,85
Gammaridea ind	0,88	3,27	0,55	10,64	57,49
Rhacotropis spp	0,95	3,06	0,43	9,98	67,47
Boreomysis arctica	0,82	2,63	0,51	8,57	76,05
Polychaetha ind.	0,6	1,27	0,28	4,13	80,18

Group February

Average similarity: 22,45

Species	Av.Abund	Av.Sim	Sim/SD	Contrib%	Cum.%
Eunicidae	1,16	4,39	0,47	19,57	19,57
Gammaridea ind	0,99	4,36	0,58	19,44	39,02
Munnopsurus atlanticus	0,79	3,11	0,51	13,84	52,86
Polichaetha ind.	0,66	1,55	0,27	6,89	59,74
Aphroditidae	0,65	1,39	0,25	6,17	65,92
Boreomysis arctica	0,54	1,22	0,3	5,43	71,35
Rhacotropis spp	0,5	0,98	0,27	4,38	75,73
Ostracoda	0,38	0,74	0,26	3,31	79,04

Group April

Average similarity: 29,08

Species	Av.Abund	Av.Sim	Sim/SD	Contrib%	Cum.%
Munnopsurus atlanticus	1,49	9,6	1,01	33,02	33,02
Mysidacea ind	1,26	6,35	0,73	21,86	54,88
Rhacotropis spp	0,81	2,81	0,45	9,65	64,53
Parapseudomma calloplura	0,71	2,14	0,45	7,36	71,89
Gammaridea ind	0,76	2,08	0,35	7,17	79,06

Group June
Average similarity: 27,35

Species	Av.Abund	Av.Sim	Sim/SD	Contrib%	Cum.%
Mysidacea ind	1,23	6,9	0,87	25,23	25,23
Munnopsurus atlanticus	1,09	6,18	0,97	22,6	47,83
Parapseudomma calloplura	0,98	4,12	0,59	15,05	62,88
Gammaridea ind	0,7	2,41	0,43	8,79	71,68
Polichaetha ind.	0,77	2,06	0,35	7,54	79,21

By nMDS analysis it could be delineated a partial seasonal trend (stress=0,17). Two main groups were obtained (fig. 6-10): one group formed by early and late summer samples (from June to August) and the second by autumn-winter samples (from November to February). April samples were located in both the groups but it seems they segregated more with the summer group (fig. 6-10). April seemed to be period of change in trophic habits of *N.aequalis*, also detected by the low value of overlap (table 6-6) between February and April.

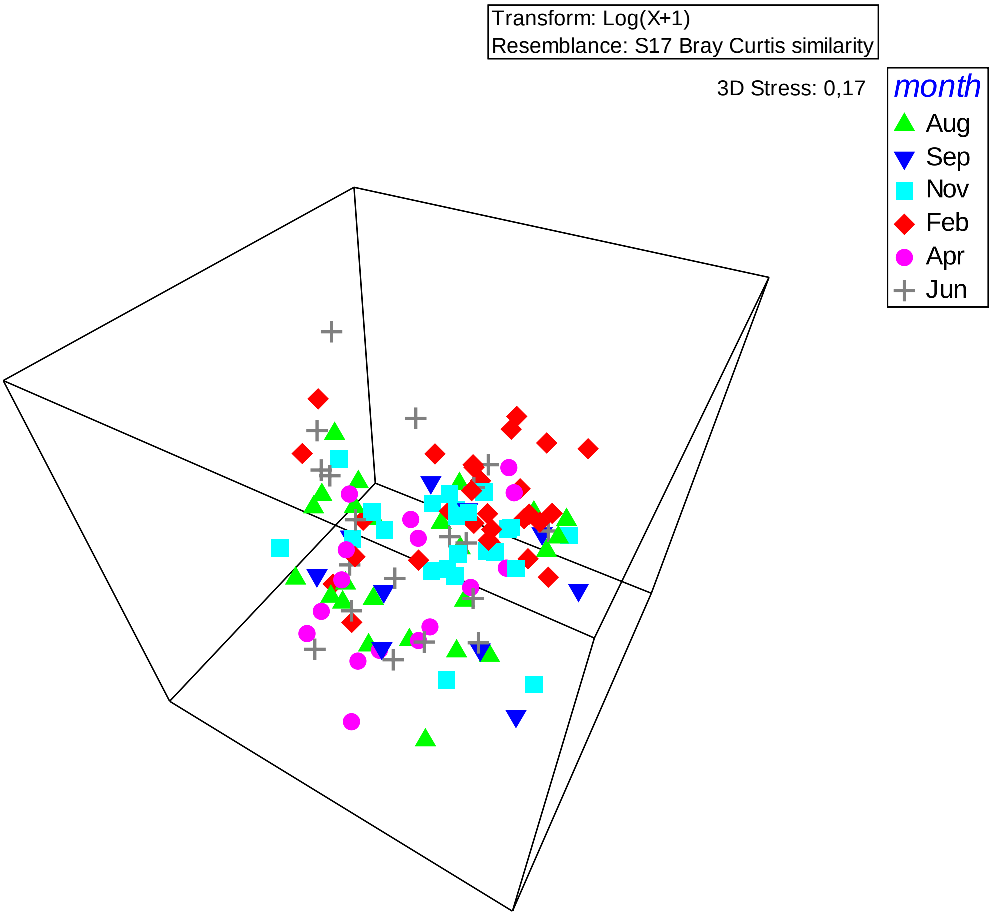


Fig. 6-10. 3-D nMDS ordination of foreguts of *N. aequalis* based on standardized and log(x+1) transformed data from August 2003 to June 2004.

The MVDISP routine performed on month, showed high values of dispersion for summer samples (June, August, September, values ranged from 1,19 to 1,26), instead lower values were detected for November and February, that resulted low dispersed (see also MDS).

Symmetrical PERMANOVA provided evidence for significant difference both for factor month and depth (table 6-8).

Table 6-8. PERMANOVA based on Bray-Curtis dissimilarities of standardized and log(x+1) transformed data for *N. aequalis*.

Source	df	SS	MS	Pseudo-F	P(perm)	Unique perms
month	5	31315	6263	1,8582	**0,0101**	9903
depth(month)	9	31258	3473,1	1,5817	**0,004**	9833
Res	106	2,3275E5	2195,8			
Total	120	2,9522E5				

The pair-wise comparison performed on factor "month" (table 6-9a) evidenced two period of change in diet: between June and August and between February and April It is interesting to note that the major discontinuity in diet seemed to be between 580 and 660 m of depth (table 6-8b) and less between 580-750 m. This consideration was confirmed also by CAP ordination (Fig. 6-11).

Table 6-8. a) PERMANOVA pair-wise test performed on term 'month'. Only pair-wise comparisons among contiguous months were given; b) PERMANOVA pair-wise test performed on Term 'depth'

a)

Groups	t	P(perm)	Unique perms
Aug, Sep	0,47878	0,973	998
Aug, Jun	1,4773	**0,036**	998
Sep, Nov	1,0265	0,406	999
Nov, Feb	1,0509	0,382	998
Feb, Apr	1,7426	**0,022**	999
Apr, Jun	1,1799	0,192	999

b)

Groups	t	P(perm)	perms
August			
750, 580	1,6871	**0,007**	999
September			
580, 660	1,054	0,364	120
November			
750, 580	0,90674	0,599	993
750, 660	0,76743	0,66	278
580, 660	0,87981	0,619	276
February			
750, 580	1,4052	0,061	982
750, 660	0,96415	0,523	998
580, 660	1,5633	**0,035**	990
April			
580, 660	1,2486	0,153	941
June			
750, 580	0,8719	0,689	970
750, 660	1,3228	0,114	36
580, 660	1,6713	**0,016**	66

A CAP analysis performed on depth (as found significant by PERMANOVA) showed a partially separation of samples as function of depth. Depth seemed to be the more structuring factor than month in the ordination of *N. aequalis* gut contents, although a certain degree of overlap was observed particularly between samples from 580 and 750 m. The first axis seems to separate samples from 580 m to deepest samples, while the second axis could be linked to some environmental variables such as grain size of sediments.

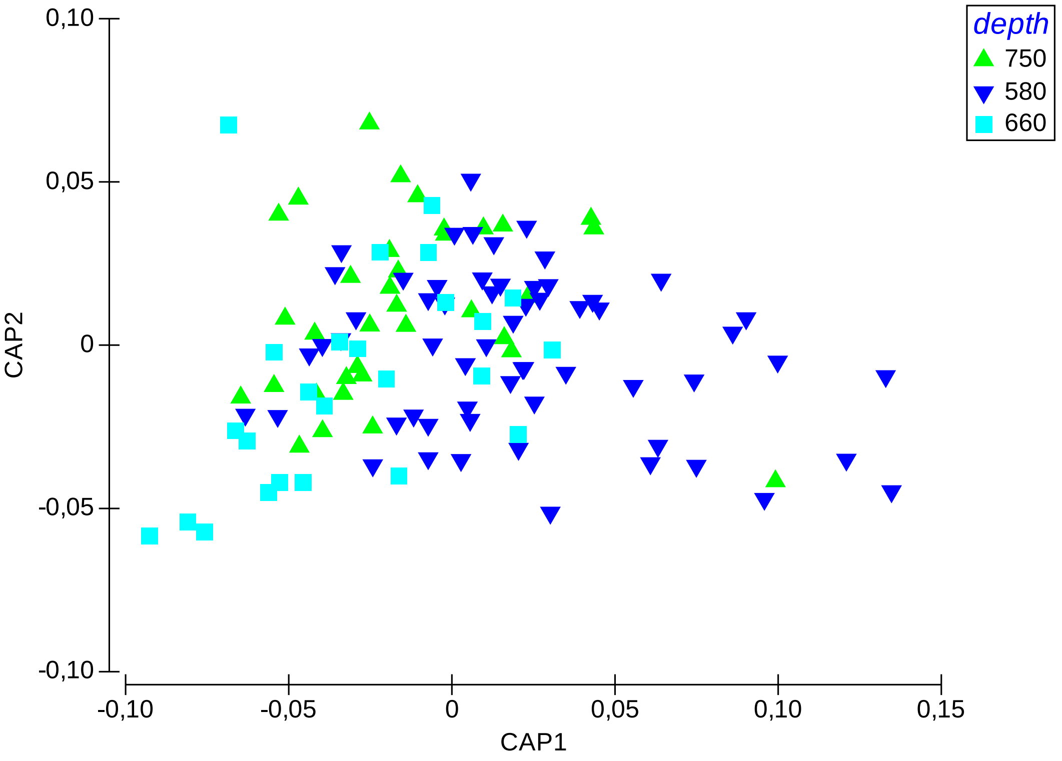


Figure 6-11 CAP analysis based on Modified Gower dissimilarities of standardized and 4th root transformed data of *N. aequalis* from August 2003 to June 2004, and performed on factor “month”. Symbols represent months.

6.3.4 Overall species analysis

Overall, dietary overlap based on Schoener's index revealed a very low dietary affinities between *H. mediterraneus* and two macrourids and also between *H. italicus* and *N. aequalis* (table 6-9).

Table 6-9. Diet overlap for the three demersal fish species using Schoener's Index. Hmed: *H.mediterraneus*; Hita: *H.italicus*; Naeq: *N.aequalis*.

	Hmed	Hita	Naeq
Hmed	1	0,13	0,10
Hita		1	0,28
Naeq			1

The degree of overlap between the three species was very low throughout the whole sampling period between August and September, increasing later in November and February (table 6-10; in April no *H. mediterraneus* samples were available) and decreasing finally again in June. The value was particularly low between *H.mediterraneus* and *N.aequalis* (ranged from 0,02 to 0,19), whilst between the two macrourids it ranged from 0,18 to 0,35.

Table 6–10. Diet overlap for the three demersal fish species using Schoener's Index. Hmed: *H.mediterraneus*; Hita: *H.italicus*; Naeq: *N.aequalis*. Indices were calculated for each month separately.

	augHM	augHI	augNA		febHM	febHI	febNA
augHM	1	0,04	0,19	febHM	1	0,36	0,08
augHI		1	0,23	febHI		1	0,18
augNA			1	febNA			1
	sepHM	sepHI	sepNA		aprHM	aprHI	aprNA
sepHM	1	0,06	0,04	aprHM	1	-	-
sepHI		1	0,22	aprHI		1	0,35
sepNA			1	aprNA			1
	novHM	novHI	novNA		junHM	junHI	junNA
novHM	1	0,21	0,08	junHM	1	0,02	0,02
novHI		1	0,29	junHI		1	0,26
novNA			1	junNA			1

SIMPER analysis showed the most typifying preys for each species (table 6-11): The isopod *N. borealis* and the *Tryphosites* spp. characterized *H. mediterraneus* diet. *H. italicus* was dominated by mysids (mainly *B. arctica* and *P. calloplura*), gammarids and isopods (*N. borealis*). The similarity degree is higher in *N. aequalis* diet than in the other two species (51,1%), the benthic amphipod *Maera schmidti* and among polychaethes, Aphroditidae typified its diet. The high degree of dissimilarity between *H. mediterraneus* and *H.italicus* and between the first and *N. aequalis* (93,2% and 97,7% respectively) was due to the high abundance of *N. borealis*, the decapod *Calocaris macandrae* and *Tryphosites* spp. in the Mediterranean slime-head diet. Average dissimilarity between the two macrourids was 78% and it was attributable to the high abundance of *B. megalops* in *H. italicus* diet and of *Maera schmidti* and polychaetes, two benthic taxa, in *N. aequalis* diet.

Table 6-11. SIMPER analysis within and between species (preys with less than 5% of contribution were cut off)

H.mediterraneus

Average similarity: 39,84

Taxa	Av.Abund	Av.Sim	Sim/SD	Contrib%
N. borealis	1,4	32,44	1,34	81,43
Tryphosites sp.	0,12	4,44	2,48	11,14

H.italicus

Average similarity: 35,33

Taxa	Av.Abund	Av.Sim	Sim/SD	Contrib%
B.arctica	0,06	17,49	1,36	49,51
Mysidacea	0,01	3,79	1,32	10,74
Gammaridea	0,01	2,27	1,12	6,42
N. borealis	0,01	2,24	0,63	6,33
P. calloplura	0,01	1,98	0,61	5,6

N.aequalis

Average similarity: 51,09

Taxa	Av.Abund	Av.Sim	Sim/SD	Contrib%
M. schmidti	0,02	13,31	2,95	26,06
Aphroditidae	0,01	5,52	0,83	10,81
M. atlanticus	0,01	4,37	2,02	8,56
R. rostrata	0,01	4,03	3,91	7,88
Gammaridea ind	0,01	3,23	3,71	6,32
B. arctica	0,01	2,91	2,29	5,7
Polychaeta	0,01	2,72	1,71	5,32

Groups Hmed & Hita

Average dissimilarity = 93,17

	Group Hmed	Group Hita			
Taxa	Av.Abund	Av.Abund	Av.Diss	Diss/SD	Contrib%
N. borealis	1,4	0,01	56,07	2,17	60,18
C. macandrae	0,13	0	7,82	0,49	8,39
Tryphosites sp.	0,12	0	7,42	1,11	7,97
Myctophidae	0,06	0	6,25	0,53	6,71

Groups Hmed & Naeq

Average dissimilarity = 97,67

	Group Hmed	Group Naeq			
Taxa	Av.Abund	Av.Abund	Av.Diss	Diss/SD	Contrib%
N. borealis	1,4	0	56,69	2,22	58,04
C. macandrae	0,13	0,01	8,12	0,52	8,32
Tryphosites sp.	0,12	0	7,33	1,11	7,5
Myctophidae	0,06	0	6,28	0,54	6,43
B. megalops	0,08	0,01	4,98	0,8	5,1

Groups Hita & Naeq

Average dissimilarity = 77,71

	Group Hita	Group Naeq			
Taxa	Av.Abund	Av.Abund	Av.Diss	Diss/SD	Contrib%
B. megalops	0,06	0,01	19,65	1,31	25,29
M. schmidti	0	0,02	7,9	1,68	10,17
Aphroditidae	0	0,01	5,37	1,1	6,91

nMDS analysis (stress 0,13) showed a well-defined segregation between the three species diets (fig. 6-12). ANOSIM routine provided evidence of a good separation beween the diets of these three species (R=0,808; p<0,05).

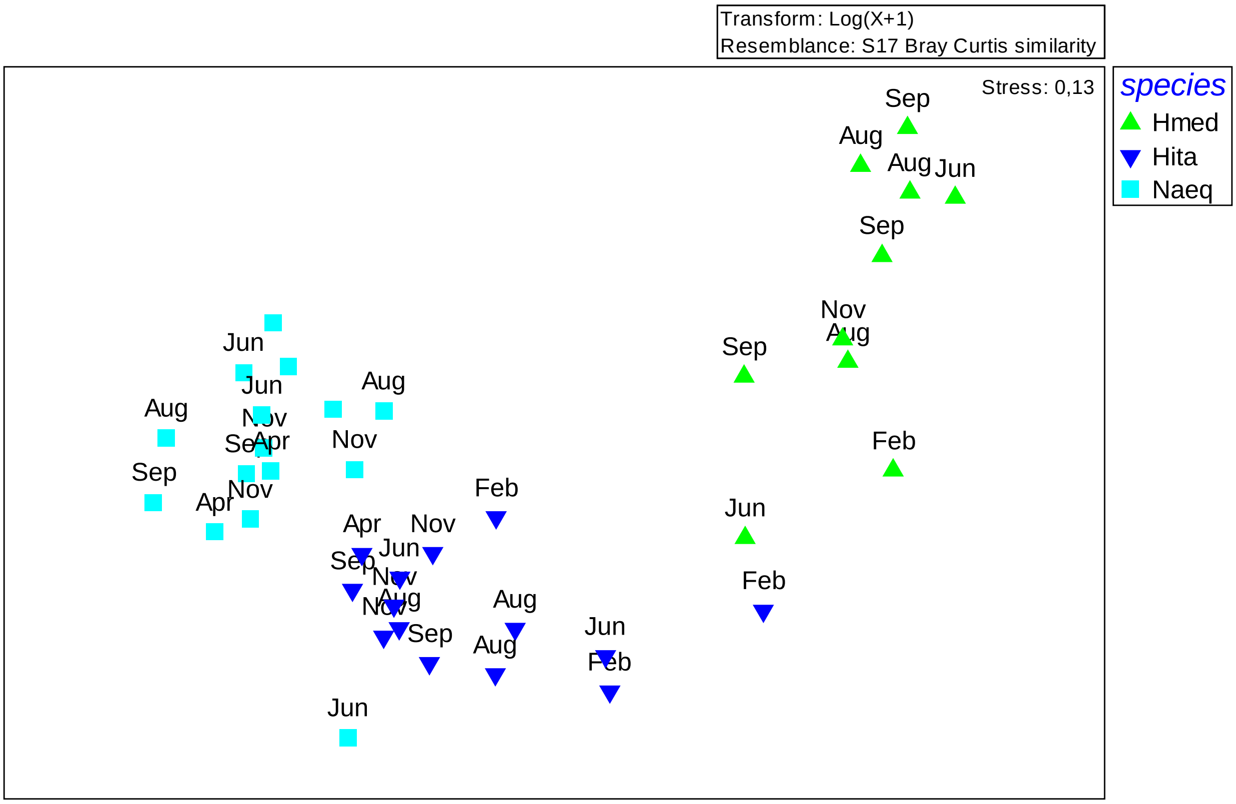


Fig. 6-12. nMDS of of gut contents of *N. aequalis* (Naeq), *H. italicus* (Hita) and *H. mediterraneus* (Hmed)

The Multivariate dispersion index (MVDISP) indicated high dispersion for *N. aequalis* samples (1,065), whereas *H. mediterraneus* seemed to be the most specialist (0,898) among the three species.

Even nMDS performed only for the two macrourids revealed a good separation between their diets maintained along temporal scale (fig. 6-13). ANOSIM test also provided evidence for segregation between macrourids feeding habits (R=0,612; p<0,05).

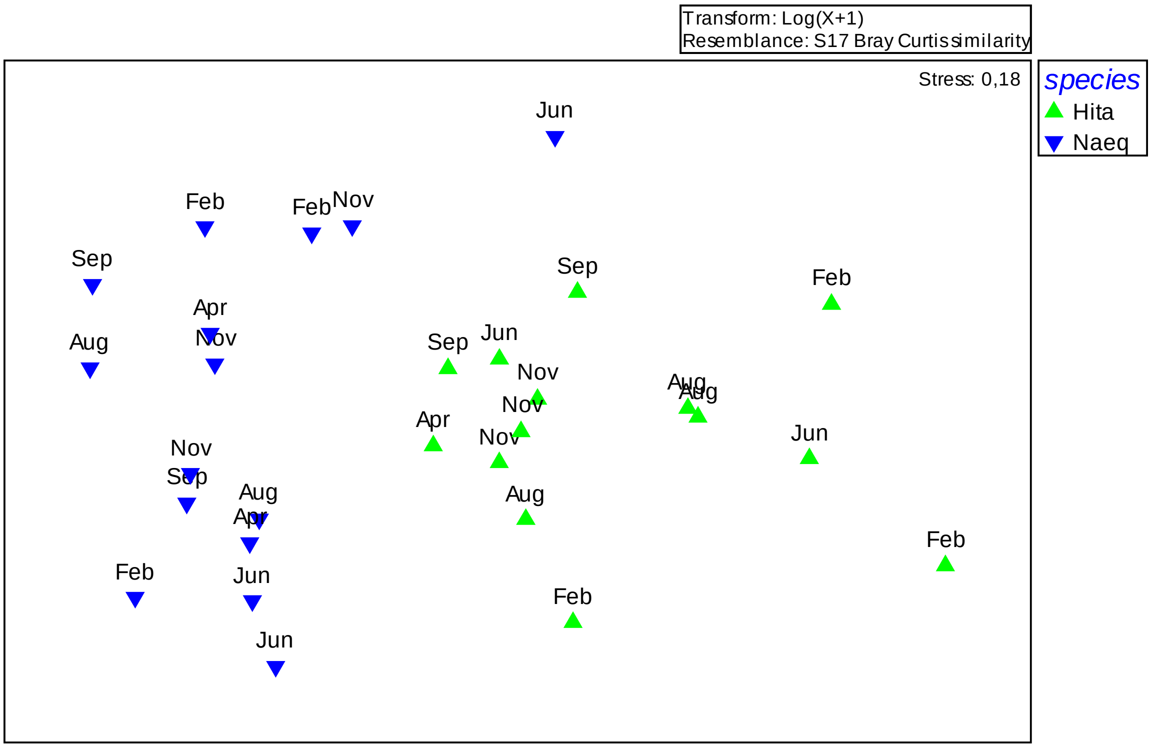
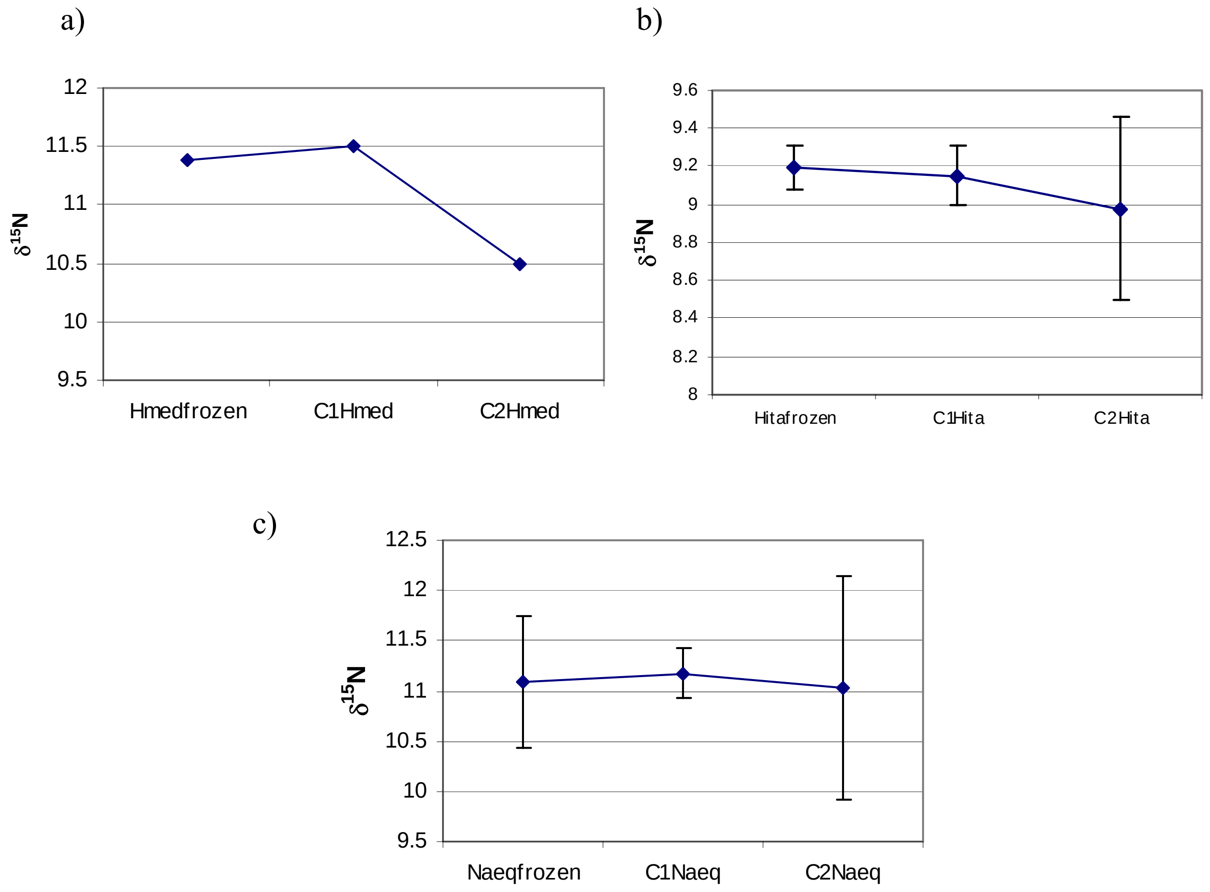


Fig. 6-13 nMDS of gut contents of *N. aequalis* and *H. italicus*

6.3.5 Fixative dependant shifts of $\delta^{13}C$ and $\delta^{15}N$ in preserved fish muscle

Little but not significant differences (ANOVA p>0,05 for all the species) were observed in $\delta^{15}N$ values between control sample (frozen) and preserved samples after 6 and 11 months for *H. italicus* and *N. aequalis* (see fig. 6-14b-c). An abrupt decrease was evident for *H. mediterraneus* (fig.6-14 a), but no replies was available for this species, so it was not possible to test if this difference was significant.

Fig.6.X. Trend in $\delta^{15}N$ values for *H.mediterraneues* (a), *H.italicus* (b) and *N. aequalis* (c), for control (frozen) and preserved samples after 6 (C1) and 11 (C2) months (±confidence range).



The duration of preservation (from 6 to 11 months) affected carbon isotopic ratios of muscle in all the analyzed species (see fig 6-15 and table 6.X). Mean isotopic values for tissue/treatment and control are provided in Table 6-12.

Table 6-12 Results of ANOVA and post-hoc Tukey test for $\delta^{13}C$ values of *H. italicus* and *N. aequalis*. Control=frozen sample; C1 after 6 months fixed in formalin, C2= after 11 months.

Hymenocephalus italicus			
ANOVA	*F*=15,42	P=0,004	
	Post-hoc HSD Tukey test		
	control	C1	C2
control		-	-
C1	**		-
C2	n.s.	**	

Nezumia aequalis			
ANOVA	*F*=76,5	$p<10^{-5}$	
	Post-hoc HSD Tukey test		
	control	C1	C2
control		-	-
C1	***		-
C2	*	***	

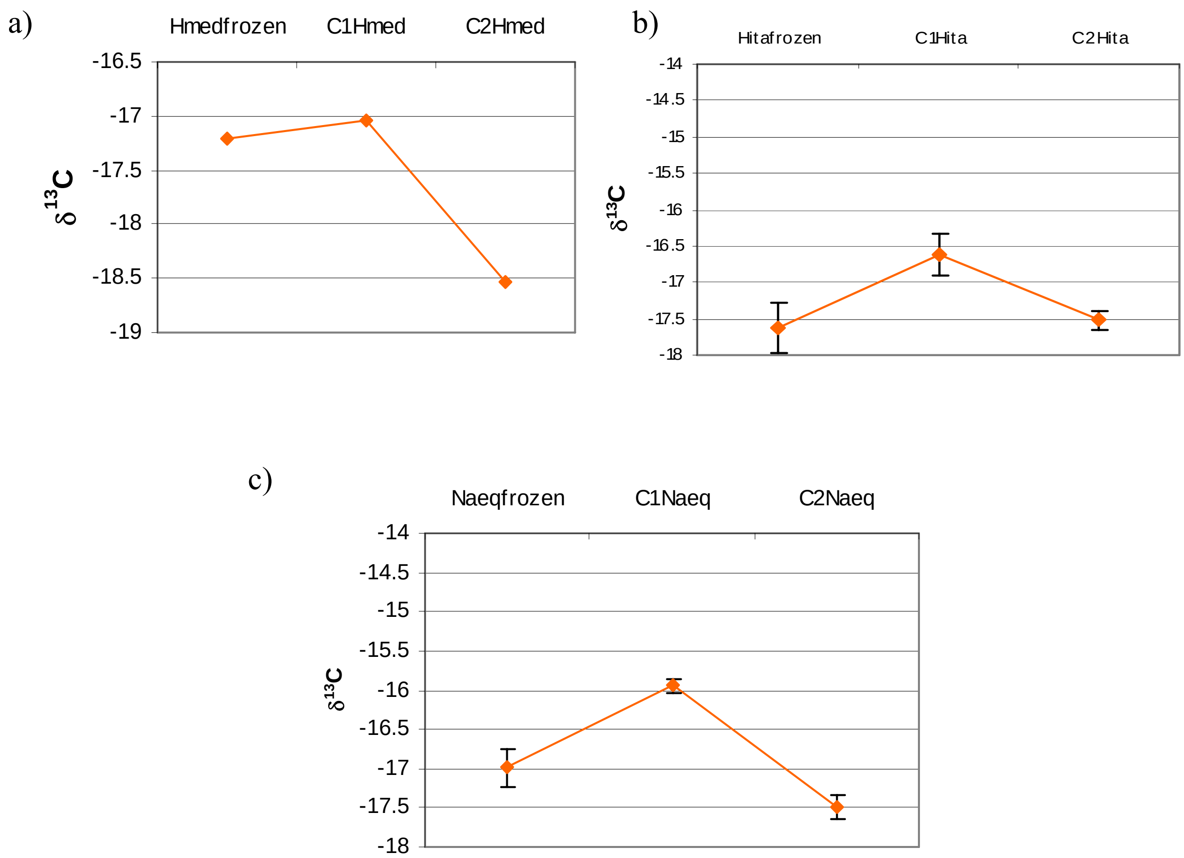


Fig.6-15. Trend in δ^{13}C values for *H.mediterraneues* (a), *H.italicus* (b) and *N. aequalis* (c), for control (frozen) and preserved samples after 6 (C1) and 11 (C2) months (±confidence range).

This experiment did not give a clear pattern for the interpretation of δ^{13}C values. Individual variance was lower in ^{13}C than in ^{15}N composition. In spite ^{13}C values of C1-C2 were significant different from frozen material (e.g. different pattern of variation for the three species between controls and frozen materials), hence it was not possible to identify any progressive trend with time.

As conclusion ^{15}N did not vary as a function of time of preservation (in 11 months) in our three target species, while no significant tendencies were observed for ^{13}C.

6.3.6 Temporal variation in stable isotopes signatures

Hoplostethus mediterraneus

Seasonal differences occurred in $\delta^{15}N$ values (fig. 6-16) with the lowest value observed in September and the highest in April. Differences were not significant (ANOVA, *F*=0,52; p=0,75).

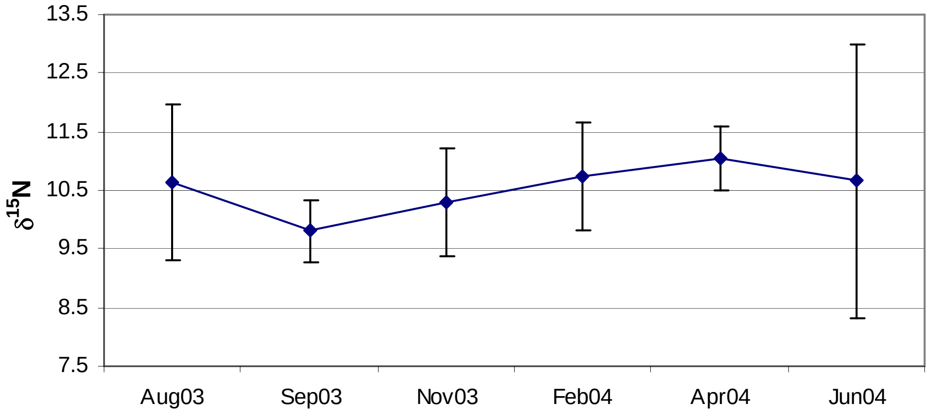


Figure 6-16. Seasonal trend of $\delta^{15}N$ values for *H.mediterraneus* (±confidence range)

Significant differences were evident in $\delta^{13}C$ signatures by season (fig. 6-17) with the most enriched value observed in February and the most depleted in June (ANOVA *F*: 39,24; $p<10^{-7}$). There was a clear depletion of ^{13}C from February to April 2004.

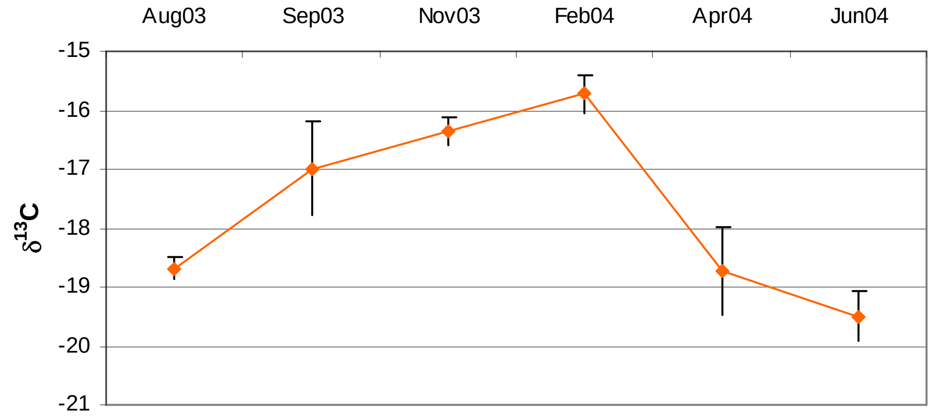


Figure 6-17. Seasonal trend of $\delta^{13}C$ values for *H.mediterraneus* (±confidence range)

A post-hoc TSD Tukey test evidenced the source of variation by month (table 6-12).

Table 6-12. Significance levels of the post-hoc Tukey tests performed to evaluate differences in the $\delta^{13}C$ composition of *H.mediterraneus*. Each cell of the table includes the results for $\delta^{13}C$. * = $P \leq 0.05$; ** = $P \leq 0.01$; ***= $P \leq 0.001$; n.s.: not-significant difference ($P > 0.05$).

Tukey HSD test; Probabilities for Post Hoc Tests Error: Between MS = ,16955, df = 11,000

month	Aug03	Sep03	Nov03	Feb04	Apr04	Jun04
Aug03		-	-	-	-	-
Sep03	**		-	-	-	-
Nov03	***	n.s		-	-	-
Feb04	***	*	n.s		-	-
Apr04	n.s	**	***	***		-
Jun04	n.s	***	***	***	n.s.	

Hymenocephalus italicus

Significant differences occurred both in $\delta^{15}N$ and $\delta^{13}C$ signatures by season (figg. 6-18 and 6-19). The most enriched value of $\delta^{15}N$ was observed in April and the most depleted in September (ANOVA *F*: 3,85; *p*: 0,026). There was a progressive increase in ^{15}N from September 2003 to April 2004.

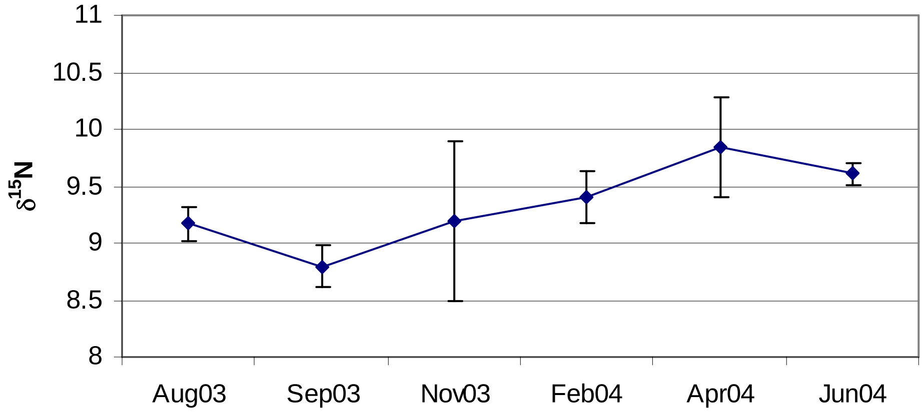


Figure 6-18. Seasonal trend of $\delta^{15}N$ values for *H.italicus* (±confidence range)

An abrupt decrease in $\delta^{13}C$ value was detected from November to February (ANOVA *F*: 74; $p<10^{-7;}$ fig. 6-19). There were two different patterns: one period corresponding to late summer (Aug-Sep) and autumn with ^{13}C enrichment and a period in winter-spring and early summer with high ^{13}C depletion in muscle.

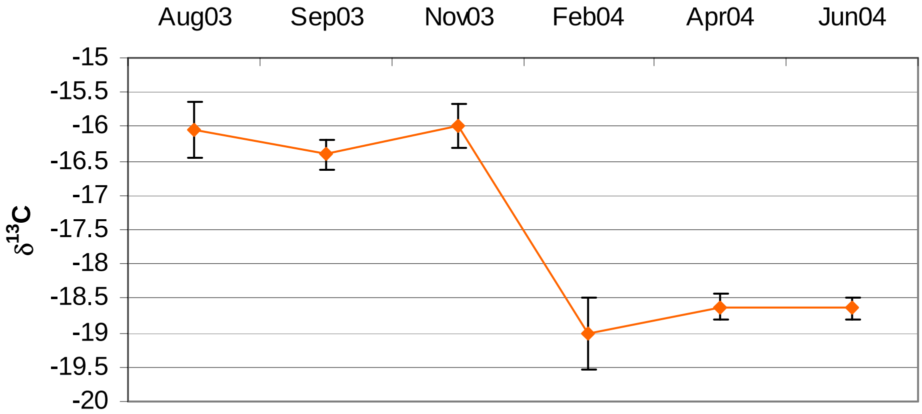


Figure 6-19. Seasonal trend of δ^{13}C values for *H.italicus* (±confidence range)

A post-hoc HSD Tukey test showed the source of variation both for δ^{15}N and δ^{13}C signatures by season (table 6-13)

Table 6-13. Significance levels of the post-hoc Tukey tests performed to evaluate differences in the isotopic composition (δ^{13}C and δ^{15}N). Each cell of the table includes the results for both δ^{13}C and δ^{15}N (δ^{13}C/δ^{15}N, respectively). * = $P \leq 0.05$; ** = $P \leq 0.01$; ***= $P \leq 0.001$; n.s.: not-significant difference ($P > 0.05$).

month	Aug03	Sep03	Nov03	Feb04	Apr04	Jun04
Aug03		-	-	-	-	-
Sep03	n.s./ n.s.		-	-	-	-
Nov03	n.s./ n.s.	n.s./ n.s.		-	-	-
Feb04	n.s./***	n.s./***	n.s./***		-	-
Apr04	n.s./***	*/***	n.s./***	n.s./n.s.		-
Jun04	n.s./***	n.s./***	n.s./***	n.s./n.s.	n.s./n.s.	

Nezumia aequalis

Seasonal differences occurred in δ^{15}N values (fig. 6-20) with the lowest value observed in September and the highest in June. Differences were not significant (ANOVA, *F*=2,82; p=0,065). As in the case of *H. italicus* there was a progressive increase in δ^{15}N values from September to June.

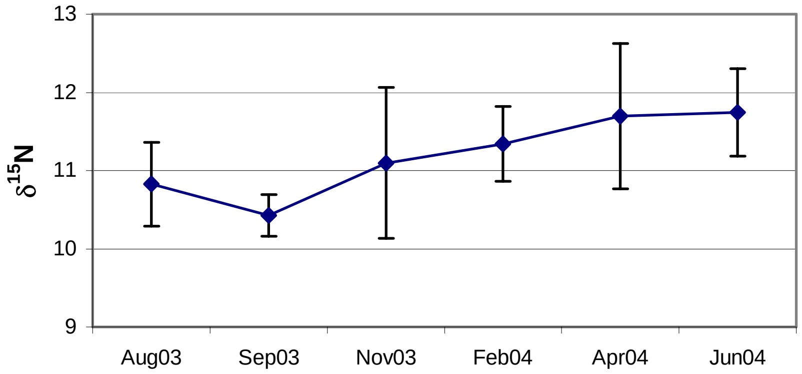


Figure 6-20. Seasonal trend of δ^{15}N values for *Nezumia aequalis* (±confidence range)

Significant differences were evident in $\delta^{13}C$ signatures by season (fig. 6-21) with the most enriched value observed in November and the most depleted in June (ANOVA *F*: 5,96; *p*: 0,005).

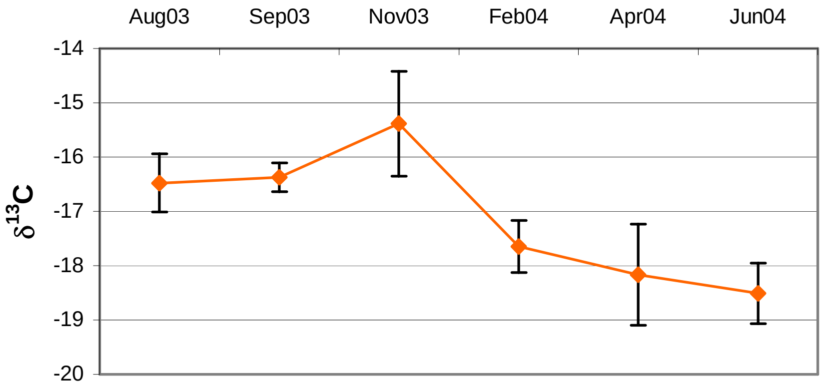


Figure 6-21. Seasonal trend of $\delta^{13}C$ values for *Nezumia aequalis* (±confidence range)

A post-hoc TSD Tukey test evidenced the source of variation (see table 6-14). April and June values showed some differences with September and November. The general pattern observed for ^{13}C was similar to that exhibited by *H. italicus*.

Table 6-14. Significance levels of the post-hoc Tukey tests performed to evaluate differences in the $\delta^{13}C$ composition of N.aequalis. Each cell of the table includes the results for $\delta^{13}C$. * = $P \leq 0.05$; ** = $P \leq 0.01$; ***= $P \leq 0.001$; n.s.: not-significant difference ($P > 0.05$).

month	Aug03	Sep03	Nov03	Feb04	Apr04	Jun04
Aug03		-	-	-	-	-
Sep03	n.s.		-	-		-
Nov03	n.s.	n.s.		-	-	-
Feb04	n.s.	n.s.	n.s.		-	-
Apr04	n.s.	n.s.	*	n.s.		-
Jun04	n.s.	*	*	n.s.	n.s.	

6.4 Discussion

6.4.1 Diet composition and temporal variation

Hoplostethus mediterraneus

The results of the present study indicate that *Hoplostethus mediterraneus* actively forage on natatory benthopelagic prey, which must be captured in the benthic boundary layer (BBL), located a few metres above the bottom (Hargreaves 1984; Angel 1990). The isopod *Natatolana borealis* was the preferred prey ingested by *H. mediterraneus i*n the bathyal Balearic basin, while crustaceans (mainly *Boreomysis arctica*, *Rhachotropis rostrata* and *Tryphosites* spp) and fish (Myctophidae) are secondary prey items. A seasonal pattern was observed by segregation of late summer-autumn samples from winter-spring.

Our results are very different from previous studies due to the high dominance of the isopod *N. borealis*, while in other studies mesopelagic migrating prey (i.e. natantian decapods and myctophids) dominated the diet, even though these studies are from different geographic areas.

In the Aegean sea *H. mediterraneus* preyed mainly on natantian decapods (i.e *Sergestes arcticus*) throughout the year, also mysids and amphipods were selected in spring and winter (Madurell and Cartes 2005a). In the south coast of Portugal (Pais, 2002) isopods and euphausiids were preferred preys. Other studies report that the species has a mixed diet but feed primarily on bethopelagic natantian decapods (Marshall and Merrett, 1977; Du Buit 1978; Merrett and Marshall, 1981). In the Porcupine Bight a diet based on decapods, mysids and amphipods has been observed (Gordon and Duncan, 1987).

In our study only adult specimens (mean TL=153±4 with the exception of few small individuals) were analyzed. The switch in food habits with increasing body size may involve a change of habitat. Smaller–shallower and bigger–deeper trends are commonly cited in the distribution patterns of upper bathyal fish (Helfman 1978; Keast 1978; Macpherson and Duarte 1991). Hence, juveniles of these bathyal species are concentrated at shallower depths than those inhabited by adults. Our sampling (between 580 and 750 m) was restricted to depths where large-sized specimens of *H. mediterraneus* were clearly dominant. Diet results indicated an increase of benthopelagic habits in larger specimens, as it has been suggested for Myctophidae (e.g. *Lampanyctus crocodilus*: Stefanescu and Cartes, 1992) or among decapods for Pasiphaeidae (Cartes, 1993x).

The overall seasonal changes observed in the relative importance of preferred prey seem related to fluctuations in available prey in the environment, as has been suggested in several studies of deep-sea fish (e.g. Gordon 1979; Blaber and Bulman 1987). Changes in the foraging behaviour of fish inhabiting abyssal depths have also been related to the influence of seasonal changes in food supply (Armstrong et al. 1991; Priede et al. 1994). As ascertained by the intraspecific overlap, the highest overall seasonal fluctuations occurred in spring and to a lesser extent in winter, whereas the diet was more similar in summer–autumn. *H. mediterraneus* consumed more mysids and amphipods in winter (February) and spring (June), but the isopod *N. borealis* completely dominated its diet in summer and autumn.

Simultaneous sampling of food resources in the study area (see chapter 5 for details) showed high abundance of peracarid and mysids in August and from February to June; euphausiids are largely available from August to November and in June and natant decapods during summer. Data on the availability of large specimens of *N. borealis* (10-20 mm TL) were not available, because they may avoid both MG sledge or WP2, armed with a 0,5 mm mesh size, due to their higher swimming capability (Wong and Moore, 1996). Our data regarded only small specimens (2-5 mm rarely 10 mm) (Kaim-Malka, 1997). Despite the high abundance of other crustaceans, positively selected in other geographic areas, *H. mediterraneus* off Cabrera preyed almost exclusively on *N. borealis*, it is possible to classify this species as a selective feeder.

Hymenocephaus italicus and *Nezumia aequalis*

The two macrourids inhabiting mid-bathyal depths in the Balearic basin (*H. italicus* and *N. aequalis*) showed very diverse diets, based mainly on suprabenthic prey (i.e. amphipods, copepods, ostracods, isopods and cumaceans) and infauna (i.e. polychaetes and tanaids). Macrourids are characteristically described as generalist feeders, with widely diversified diets (Pearcy and Ambler 1974; Macpherson 1979; Mauchline and Gordon 1984). This probably constitutes an adaptive advantage in the deep-water environments of low productivity inhabited by macrourids (Pearcy and Ambler 1974). However, though described as euryphagous, macrourids also remain selective regarding prey type and prey size (Pearcy and Ambler 1974; Geistdoerfer 1978; Macpherson 1979; Mauchline and Gordon 1984). Therefore, in many species, large sizes have more benthopelagic diets (and probably habits) and prey upon large organisms. Previous studies on our two target species (McLellan 1977; Geistdoerfer 1978; Macpherson 1979) showed that differences in the diets of *H. italicus* and *N. aequalis* are related to the morphology of these species. Accordingly, *H. italicus*, with a large apical mouth, and a rather blunt snout displayed a more pelagic diet. By contrast, species with an inferior mouth (*Nezumia* spp) may forage on slow moving prey with the snout orientated to the substrate (McLellan, 1977), they probably used the rostrum as a sensory probe (McLellan 1977; Gartner et al. 1997). Thus *N. aequalis* preyed more on infaunal organisms (e.g. *Apseudes spinosus,* polychaetes, and *Maera schmidti*) than *H. italicus*.

Contrasting with other authors' findings (Marshall and Merrett, 1977; McLellan, 1977; MacPherson, 1979, Merret and Marshall, 1981) and according to Madurell and Cartes (2006) *H. italicus* did not show a strong pelagic habit, preying mainly on BBL fauna than on mesopelagic resources (i.e. copepods and euphausiids). The diet of *N. aequalis* was similar to other geographic areas and also to the diets of other species of *Nezumia*. In the Atlantic Ocean, the diets of *N. sclerorhynchus* (Marques & Alameida, 1998), *N. aequalis* (Macpherson, 1979; Hoffmann, 1982; Mauchline & Gordon, 1984; Carrason & Matallanas, 1989; Marques & Almeida, 1998), and *N. bairdi* (Savvatimsky, 1989; Langton & Bowman, 1980) consist primarily of polychaetes, amphipods, shrimp and other epibenthic organisms. In the Ionian Sea *N. sclerorhynchus* was found to prey mainly on amphipods and copepods (Madurell and Cartes 2006). The large number and variety of amphipods consumed is probably attributable to their relative abundance, facility in its capture and small size (Coggan et al., 1999).

Seasonal changes in the diet have been reported for most macrourids in the Rockall Trough (Mauchline and Gordon 1984), whereas at mid-bathyal depths off the western Mediterranean these seasonal changes were not apparent (Macpherson 1979). Size may explain these rather controversial trends, because the Atlantic macrourids are larger than those inhabiting the deep Mediterranean. There is an increasing exploitation of benthopelagic resources by large sizes among macrourids (Haedrich and Henderson 1974; Mauchline and Gordon 1984; Drazen et al. 2001). This may imply that seasonal changes in the diet are due to larger seasonal fluctuations in pelagic prey in comparison with those of benthic prey. The benthic diet of deep-western Mediterranean macrourids on the upper-middle slope would result in no distinctive seasonal differences in the diet of fish (Macpherson 1979). In the Ionian Sea, seasonal changes in the diet were reported for *Nezumia sclerorhynchus* (Madurell and Cartes, 2006). Some but not strong seasonal changes occurred in our study mostly related to mobile prey such as mysids, mainly consumed in April and June, when they were also abundant in the environment (Cartes et al., *in press*). Seasonality in food supply could be a factor although dietary studies indicate no or slight seasonal variation in prey composition of the diet which is dominated by epibenthic invertebrates which are present throughout the year (Macpherson, 1979; Relini-Orsi & Wurts, 1979; Mauchline & Gordon, 1984; Carrasson & Matallanas, 1989). Changes in diet could be also related, a part from seasonal variation in resource availabitlity, to different energetic requirements for reproduction. In the Mediterranean spawning of *N. aequalis* occurs throughout the year, mainly in three episodes (January–February, April–May and October) (Carrasson & Matallanas, 1989; Massutì *et al*., 1995) and it could be explain diet variations occurred in September-November and February-April.

In general the diet of *N. aequalis* seemed to vary more than *H. italicu*s; in both the species the most important effect on feeding was found to be the depth.

A lack of strong seasonality in energetic processes could also reflect the relatively high trophic position of the *N. aequalis* (see below), as observed in other macrourids of the genus *Coryphaenoides* (Drazen, 2002). This is probably due to the high predation of *N. aequalis* on carnivore polychaetes as suggested by Badalamenti et al. (2002) to explain the high trophic level of red mullet *Mullus barbatus*. Despite the importance of seasonal inputs of phytodetritus to the deep-sea on a variety of sediment infauna and epifauna, it appears that these effects have little impact on the top predators, the macrourids (Drazen, 2002).

6.4.2 Interspecific relationships

As explained in chapter one the choice of the three species was based on literature data where *H. mediterraneus* was supposed to show a stronger pelagic habit and to prey on pelagic-benthopelagic organisms, *H. italicus* was representative of a “benthopelagic feeder” guild and *N.aequalis* was chosen as an “epibenthic-infaunal feeder”. So the degree of overlap, as we expected, was relatively low, coinciding with previous infaunal studies in bathyal fish communities (e.g. Macpherson, 1981; Carrassón & Cartes, 2002; Madurell and Cartes, 2005b). This suggests that food resources were well partitioned for these three co-existing species. In the Balearic basin, overall overlap was lower between macrourids and the silver slimehead *H. mediterraneus* but also within macrourids, as happened in the Ionian sea (Madurell and Cartes, 2006). This pattern was consistent along all seasons, suggesting a good resources partitioning throughout the year.

Low levels of dietary overlap can occur among macrourids (Macpherson 1979; Mauchline and Gordon 1984; Blaber and Bulman 1987; Carrassón and Matallanas 2002), implying high resource partitioning and probably low levels of competition between coexisting species. Niche separation is related to differences in the depth range inhabited (e.g. centres of gravity) by each species. However, even in the narrow depth-range studied here, dietary overlap was also very low.

The description of the diet of the two macrourids analysed (*H. italicus* and *N. aequalis*) was rather species-specific. As observed above, the differences in the diets among these two species are attributed to the different morphology and feeding modes of these species (McLellan, 1977; Geistdoerfer, 1978; Macpherson, 1979b). Consequently, food resource interactions among these co-existing macrourids are probably weak. Other studies suggest diet partitioning in macrourids related to differences in feeding habits (McLellan, 1977, Pearcy & Ambler, 1974; Macpherson, 1979b; Blaber & Bulman, 1987), though food partitioning between macrourids that exploit similar resources can also be related to differences in their centres of depth distribution (Mauchline & Gordon, 1984; Drazen et al., 2001; Carrassón & Matallanas, 2002).

H. mediterraneus, as expected, showed very low value of overlap with macrourids. In February Schoener index between *H. mediterraneus* and *H. italicus* was slightly higher (α=0,36 but not significant) due to the increasing of *B. megalops* in the diet of the first, whereas this mysid is the most typifying species of *H. italicus* diet.

At bahtyal depths high overlap values may also depend on the diversity of available prey (or main food resource exploited). Thus, data on crustacean decapods suggest that diversity is probably lower in the benthopelagic than in the benthic domain (Cartes 1998a), as confirmed by the highest value of dietary diversity in the more benthic species *N. aequalis*.

Changes in feeding guilds depending on season were also observed and linked to the seasonal fluctuations of prey. Thus, *H. mediterraneus* was closely related to benthic feeders (i.e. macrourids) in winter-spring (February and April) due to an increased consumption of suprabenthic prey and coinciding with a period of increasing availability of this type of prey in the environment (Cartes et al., *in press*; Chapter 5).

6.4.3 Stable isotopes data

Experiment

As regard our experiment (see subsection 6.3.4), formalin fixation affected $\delta^{13}C$ in all the species over the examined period. In general this resulted in an average $\delta^{13}C$ depletion of 1.35‰ (SD= 0.25‰). The pattern changes depending on the species, in both the macrourids there is an abrupt enrichment in $\delta^{13}C$ after six months, resulted in an average value of 1‰ following by a depletion (of 1,5‰ in *N. aequalis* and 0,9‰ in *H. italicus*); in *H.*

mediterraneus there is a slight enrichment (0,15‰) in $\delta^{13}C$ values after six months and then a strong depletion after 11 months (1,5‰)

Formalin may have affected preserved specimens through two very different mechanisms. The first is selective loss of material from the specimen during preservation. Formalin hydrolyzes protein (Von Endt 1994; Hobson et al. 1997; Gloutney and Hobson 1998; Bosley and Wainright 1999) the observed depletion of $\delta\ ^{13}C$ in formalin-preserved specimens is consistent with a loss of hydrolyzed proteins. However, differences in protein content among our three tested organisms, related with the swimming capacity, probably explain the observed patterns.

The second mechanism is uptake of the preservative into the tissue. Formalin is a carbon-based chemical with characteristic $\delta^{13}C$ signatures. Once preserved samples are immersed, their signature may shift toward that of the preservative (Hobson et al. 1997; Gloutney and Hobson 1998; Bosley and Wainright 1999; Ponsard and Amlou 1999). Findings from our experiments and from the literature (Mullin et al. 1984; Hobson et al. 1997; Gloutney and Hobson 1998; Ponsard and Amlou 1999, Saranikos et al., 2002) indicate a minimal effect of formalin on $\delta\ ^{15}N$ only for macrourids (mean formalin effect: 0,19 ± 0,05‰), but not for *H. mediterraneus* where a depletion of 1,01‰ after 11 mo was observed. Sarakinos et al. (2002) examined preservation effects after 6 months, we observe that this period is not enough to find a clear pattern, an abrupt decrease was found after 11 mo, so further research is needed to verify if preservation effects remain similar or to establish how they influence the signatures.

Based on our experiment we mainly considered $\delta\ ^{15}N$ results, because no significant differences in $\delta^{15}N$ values between frozen and after 6 and 11 mo preserved materials were observed. In contrast $\delta^{13}C$ values significantly differed across the three considered period (ANOVA, $p<0,05$) and trend should be interpreted with caution.

Temporal changes

Seasonal differences in $\delta^{15}N$ values were observed in the three species: *H. mediterraneus* and *H. italicus* exhibited the lowest value in September and the highest in April, these differences were not significant for the first species and significant for the second (ANOVA F: 3,85; p: 0,026).

Quasi-significant differences in $\delta^{15}N$ values (ANOVA, F=2,82; p=0,065) occurred in *N. aequalis* with the lowest value observed in September and the highest in June.

Significant temporal changes were evident in the origin of carbon sources for the three species: in general an enrichment of $\delta^{13}C$ values was observed in November (*N. aequalis* and

H. italicus) and in February (*H. mediterraneus*). Thus the signature appeared to be depleted from April to September, corresponding to high trophic resources availability in the environment. The increase of suprabenthos abundance in April-June was parallel to a sharp increase (*ca.* 2.8 times) in the %OM on sediment during the same period, coupled *ca.* 1-2 months of delay with the peak of surface primary production recorded in February-March (from *Chla* satellite imagery data) (Cartes et al., *in press*). As observed for *P. martia* (Fanelli and Cartes, submitted), the lower availability of resources in the environment occurred from late autumn to winter may be responsible of lower feeding activity. Otherwise they could feed more on the water column (i.e. marine snow) than exhibited active predation on scarce mesopelagic preys. This is consistent with the degradation of marine snow leaving residues of more refractory materials (Williams and Gordon 1970; Mullerniklas et al. 1994), such as inorganic carbonates and chitin, which are known to be enriched in ^{13}C compared to other biochemical fractions (Bunn et al 1995).

These patterns of variability in C^{13} and N^{15} signatures agreed with Polunin et al. (2001), these author found temporal changes between October and May in a bathyal community off Ibiza (Balearic islands), with highest values in May.

Seasonal variation of δ^{15}N and δ^{13}C of planktonic material occur widely (e.g. Wainwright and Fry, 1994) including the Mediterranean (Dauby et al., 1990; Madurell et al., submitted; chapter 5 of this thesis). δ^{15}N values of surface plankton collected off Corsica were found to be much higher in the spring than in the autumn, since turbulent mixing and upwelling caused by off-shore wings bring nitrate which is high in δ^{15}N to the surface where it is taken up by phytoplankton (Michener and Shell, 1994). As this mixing and upwelling ceases, the surface rapidly become oligotrophic and primary production becomes largely dependent on recycled nitrogen, especially n the form of ammonia which is generally depleted in ^{15}N. Many small invertebrates feed non-selectively on the fallen detritus or plankton and because they generally possess rapid tissue turnover times, they might be expected to exhibit isotopic composition which follows these seasonal patterns in the plankton.

Trophic level

We estimated the trophic level (TL) of the top predator based on their δ^{15}N data and using copepods as reference material. δ^{15}N values were converted to trophic level based on the assumption that there was a fractionation of 3‰ per trophic level (Minagawa and Wada 1984; Post 2002) and that the base material (copepods) had a trophic level of 2:

$$\mathrm{TL_i} = \left(\frac{\delta^{15}\mathrm{N}_i - \delta^{15}\mathrm{N}_{\mathrm{ref}}}{3}\right) + 2$$

Where TL_i is the trophic level of species *i*, $\delta^{15}N_i$ is the mean d15N of species $_{i,}$ and $\delta^{15}N_{ref}$ is the mean $\delta^{15}N$ of the copepods.

In this way the trophic level exhibited by *N. aequalis* was 4.47, 4.25 in *H. mediterraneus* and 3.85 in *H. italicus*. The value found for *N. aequalis* is equivalent to the trophic value occupied elsewhere by large sharks (Cortés, 1999) and generally agrees with the suggestion that aquatic food chains typically have no more than 4 or 5 trophic steps (Hall & Raffaelli, 1993). *H. mediterraneus* fitted with this guild, while *H.italicus* occupied a lower trophic level. In general these results were considerably higher than values calculated by Stergiou and Karpouzi (2002) with TROPH (by using Trophlab; Pauly et al., 2000) based on literature data of gut contents, whilst they are consistent with what found by Polunin et al. (2001) off Ibiza, by stable isotopes analysis.

Trophic web structure

The strong correlation observed between $\delta^{15}N$ and $\delta^{13}C$ (fig. 6-22; *r*=0,71, *p*=0,001) are indicative of a single type of a primary source material supporting deep-sea communities. Correlation was calculated by removing cumaceans and the amphipods *Bruzelia typica* and *Lepechinella manco* from the analyses (species inside the blue circles in fig.6-22).

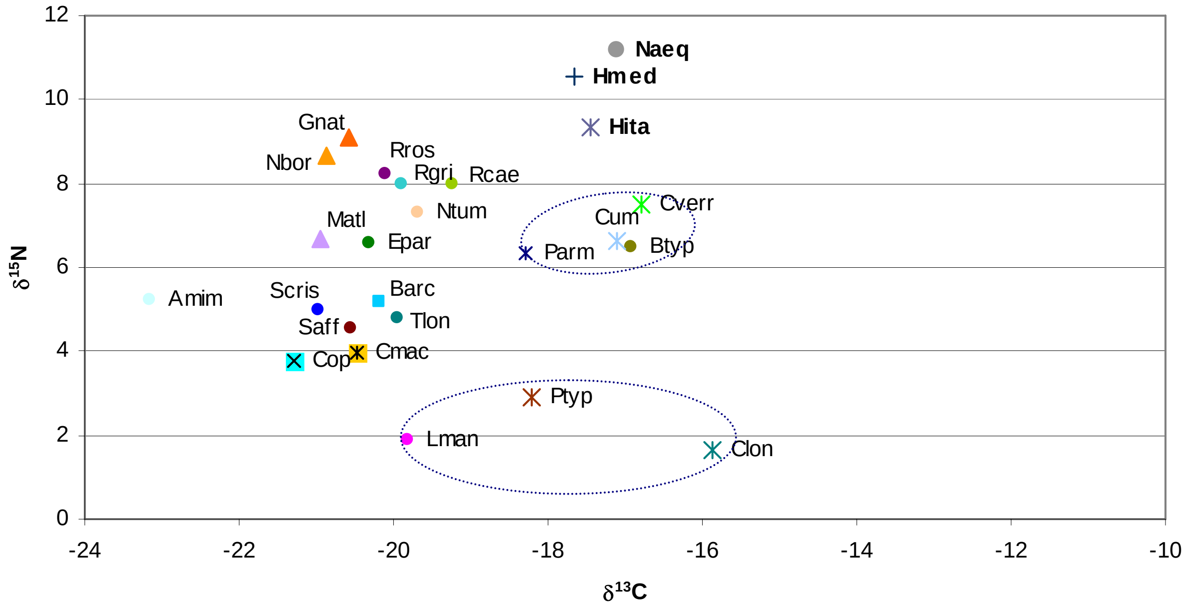


Fig. 6-22. $\delta^{15}N$ and $\delta^{13}C$ values of fish and suprabenthos (see legend in table 5-1 in chapter 5 for acronyms). Hmed: *H.mediterraneus*; Hita: *H.italicus*; Naeq: *N.aequalis*. Legend: triangles indicate isopods; circles=amphipods; squares=mysids; squares with cross=copepods; squares with asterisk=decapods; asterisks=cumaceans.

Vertical fluxes of particulate organic matter have been measured in the study region, the rates of organic carbon at 800 to 100 m depth being 1 to 5 g m^{-2} yr^{-1} (Miquel et al., 1994). As previously found by Polunin et al. (2001), the correlation between $\delta^{15}N$ and $\delta^{13}C$ contrasts with those in communities of Mediterranean littoral (e.g. cfr. Lepoint et al, 2000; Pinnegar and Polunin, 2000) when an array of possible sources of production, including plankton, macroalgae and seagrasses, and the relationship between $\delta^{15}N$ and $\delta^{13}C$ is much weaker (e.g. consumers in Pinnegar and Polunin, 2000 *r*=0,46; non-epiphytic consumers in Lepoint et al., 2000 *r*=0,24). According to Polunin et al. (2001) considerations about primary sources of material, marine snow almost certainly constitutes the main supply of food materials to the sea floor in the study area. The correlation between $\delta^{15}N$ and $\delta^{13}C$ for cumaceans (except *Cyclaspis longicaudata*), *B. typica* and *L. manco* is also strong (r=0,86 and p=0,027). These species had more enriched $\delta^{13}C$ values, probably macrophytes were significant contributors. In addition *C. longicaudata* and *P. typicus* normally inhabit lower slope bottoms below 1000 m of depth (Cartes and Sorbe, 1997) and migrate upper on the middle slope in some period of the year. Polunin et al. (2001) found a depth changes in isotopic signatures, with more enriched $\delta^{13}C$ values found in the lower slope and it could explain the more enriched value observed for these species. Finally we can conclude that there was one primary source of material, the marine snow according Polunin et al. (2001) findings who indicated only one primary source of material i.e. marine snow.

The spread of $\delta^{15}N$ and $\delta^{13}C$ found for fishes and their prey is consistent with the highly diversified diet of this species observed in this and in previously studies (see references cited therein), but do not perfectly agree with Polunin et al. (2001). There appear to be two groups, one formed by top-predators such as *N. aequalis* and *H. mediterraneus* (estimated TL=4,7 and 4,3 respectively) which consume respectively benthic/suprabenthic and pelagic/suprabenthic preys, with high $\delta^{15}N$ (i.e. *Rhachotropis* spp., *Natatolana borealis*, see chapter 5); *H. italicu*s belongs to a second group which preferentially consume suprabenthic preys, with lower $\delta^{15}N$ values (i.e. *B. arctica*).

Correlation between $\delta^{15}N$ and $\delta^{13}C$ on a month scale became strong from November to April and then decreased from June to August (fig. 6-23), the strongest correlation (R=0,85) was found in April, one month after the highest value obtained for the bulk of benthopelagic species (Madurell et al., submitted; chapter 5).

The strong correlation found for benthopelagic species occurred about one month later the peak of primary production recorded in the area (by satellite images). It was reasonable to expect that there was a delay of more than one month in the higher trophic level as in fish; these results are consistent with other findings in the same area (Madurell et al., submitted; Fanelli and Cartes, submitted).

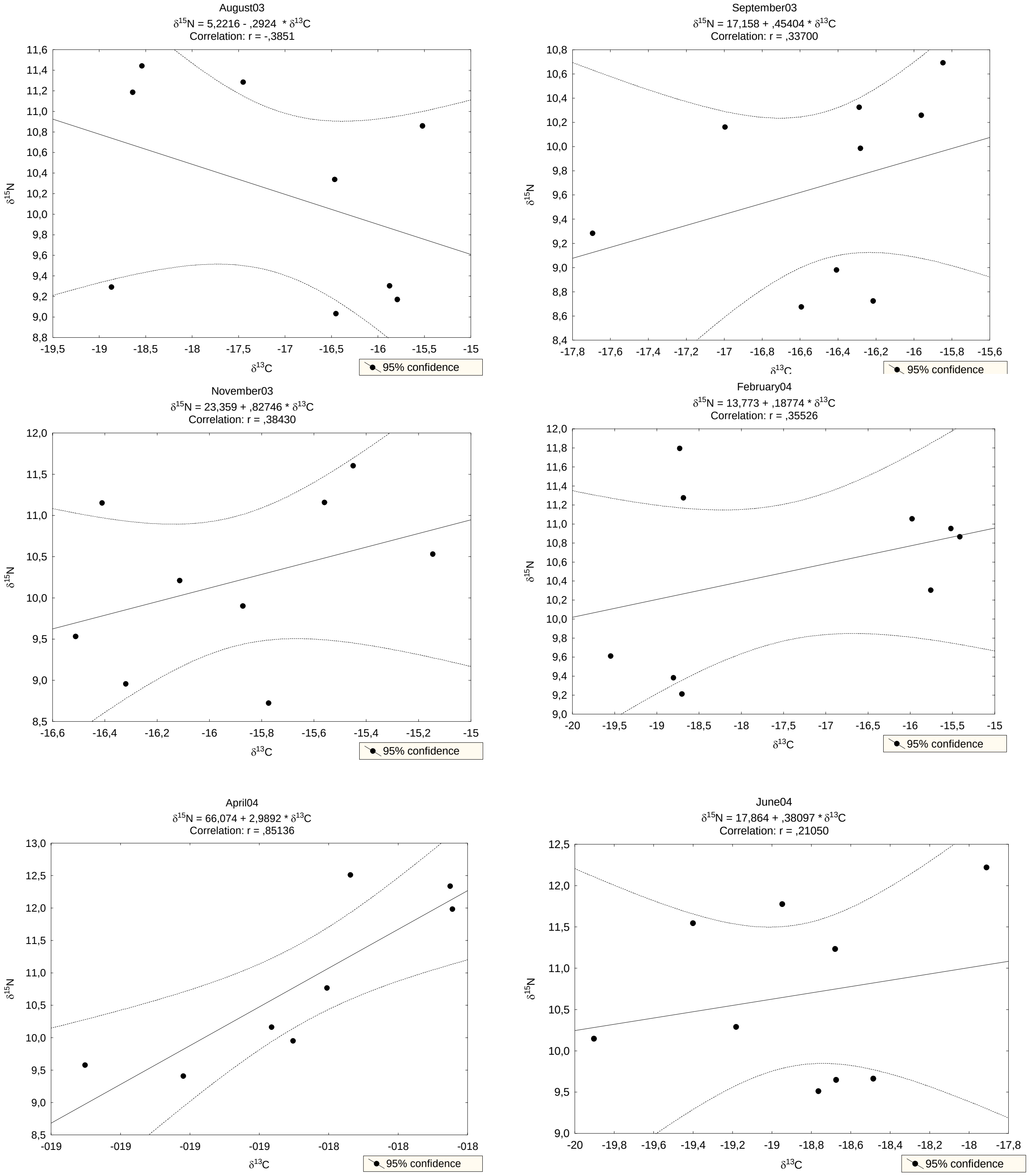


Figure 6-23. C-N plot of mean $\delta^{15}N$ and δ ^{13}C values of benthopelagic species and fish per month.

ANNEX 6-1. Dietary composition and trophic diversity indices of *H .mediterraneus* from August 2003 to June 2004. %W: percentage contribution by weight; %N: by number;.%F: by frequency of occurrence; %IRI by index of relative importance.

	August 03				September 03				November 03			
Taxa	**%W**	**%N**	**%F**	**%IRI**	**%W**	**%N**	**%F**	**%IRI**	**%W**	**%N**	**%F**	**%IRI**
CRUSTACEA												
Isopoda												
Natatolana borealis	83,17	79,09	90,00	97,50	95,97	87,95	90,91	98,80	92,35	83,52	85,00	98,66
Amphipoda												
Acidostoma sarsi	-	-	-	-	0,04	0,45	4,55	0,01	-	-	-	-
Epimeria parasitica	-	-	-	-	-	-	-	-	-	-	-	-
Monoculodes sp.	-	-	-	-	-	-	-	-	-	-	-	-
Nicippe tumida	-	-	-	-	0,04	0,45	4,55	0,01	-	-	-	-
Podoprion bolivari	0,04	0,38	3,33	0,01	0,40	0,89	9,09	0,07	0,51	1,10	5,00	0,05
Rhacotropis rostrata	0,12	0,76	6,67	0,04	0,04	0,45	4,55	0,01	-	-	-	-
Tmetonix similis	0,07	0,38	3,33	0,01	-	-	-	-	-	-	-	-
Tryphosites alleni	0,13	0,38	3,33	0,01	0,14	0,45	4,55	0,02	0,65	2,20	10,00	0,19
Tryphosites longipes	1,38	4,56	16,67	0,66	0,79	2,68	18,18	0,37	1,09	4,40	10,00	0,36
Tryphosites sp.	0,41	2,66	23,33	0,48	0,05	0,45	4,55	0,01	0,93	2,20	5,00	0,10
Trischizostoma nicaense	0,33	0,76	6,67	0,05	-	-	-	-	-	-	-	-
Lyssianassidae	0,17	1,14	10,00	0,09	0,09	1,34	13,64	0,11	0,01	1,10	5,00	0,04
Unid. Gammaridea	0,16	0,38	3,33	0,01	0,29	1,34	13,64	0,13	2,62	2,20	10,00	0,32
Mysidacea								0,00				
Boreomysis arctica	1,33	2,66	20,00	0,53	0,92	2,68	18,18	0,39	1,01	2,20	10,00	0,21
Mysideis parva	0,06	0,76	6,67	0,04	-	-	-	-	-	-	-	-
Paraseudomma calloplura	0,04	0,38	3,33	0,01	-	-	-	-	-	-	-	-
Unid. Mysidacea	0,10	1,52	10,00	0,11	-	-	-	-	-	-	-	-
Decapoda												
Pasiphaea sp.	-	-	-	-	1,07	0,45	4,55	0,04	-	-	-	-
Plesionika martia	-	-	-	-	-	-	-	-	-	-	-	-
Sergestes arcticus	0,01	0,76	6,67	0,03	-	-	-	-	-	-	-	-
Sergestidae	-	-	-	-	-	-	-	-	-	-	-	-
Unid. Natantia	-	-	-	-	-	-	-	-	-	-	-	-
Cumacea							-	-				
Campylaspis verrucosa	0,01	0,38	3,33	0,01	-	-	-	-	-	-	-	-
Copepoda	0,01	0,38	3,33	0,01	-	-	-	-	-	-	-	-
Unid. Crustacea	0,08	0,38	3,33	0,01	-	-	-	-	-	-	-	-
POLYCHAETHA	-	-	-	-	0,16	0,45	4,55	0,02	-	-	-	-
PISCES												
Lampanyctus crocodilus	11,84	0,38	3,33	0,27	-	-	-	-	-	-	-	-
Myctophidae	0,43	0,38	3,33	0,02	-	-	-	-	-	-	-	-
Unid. Fish	0,01	1,14	10,00	0,08	-	-	-	-	0,82	1,10	5,00	0,06
OTHERS												
Digested material	0,10	0,38	3,33	0,01	-	-	-	-	-	-	-	-
Gelatinous reamains	0,08	0,38	3,33	0,01	-	-	-	-	-	-	-	-

	February 04				April 04				June 04			
Taxa	**%W**	**%N**	**%F**	**%IRI**	**%W**	**%N**	**%F**	**%IRI**	**%W**	**%N**	**%F**	**%IRI**
CRUSTACEA												
Isopoda												
Natatolana borealis	17,15	13,51	50,00	16,45	-	-	-	-	48,82	67,57	62,50	85,48
Amphipoda				-								
Acidostoma sarsi	-	-	-	-	-	-	-	-	-	-	-	-
Epimeria parasitica	-	-	-	-	-	-	-	-	1,40	2,70	12,50	0,60
Monoculodes sp.	-	-	-	-	-	-	-	-	0,05	2,70	12,50	0,40
Nicippe tumida	2,34	2,70	16,67	0,90	-	-	-	-	-	-	-	-
Podoprion bolivari	-	-	-	-	-	-	-	-	-	-	-	-

	February 04				April 04				June 04			
Taxa	**%W**	**%N**	**%F**	**%IRI**	**%W**	**%N**	**%F**	**%IRI**	**%W**	**%N**	**%F**	**%IRI**
Rhacotropis rostrata	-	-	-	-	-	-	-	-	-	-	-	-
Tmetonix similis	-	-	-	-	-	-	-	-	-	-	-	-
Tryphosites alleni	-	-	-	-	-	-	-	-	-	-	-	-
Tryphosites longipes	17,18	35,14	66,67	37,42	-	-	-	-	1,47	8,11	25,00	2,81
Tryphosites sp.	6,82	18,92	50,00	13,81	77,82	33,33	50,00	55,58	0,22	2,70	12,50	0,43
Trischizostoma nicaense	-	-	-	-	-	-	-	-	-	-	-	-
Lyssianassidae	0,88	2,70	16,67	0,64	-	-	-	-	-	-	-	-
Unid. Gammaridea	-	-	-	-	-	-	-	-	-	-	-	-
Mysidacea												
Boreomysis arctica	20,38	24,32	50,00	23,98	-	-	-	-	0,26	5,41	25,00	1,66
Mysideis parva	-	-	-	-	-	-	-	-	0,01	2,70	12,50	0,40
Parapseudomma calloplura	-	-	-	-	-	-	-	-	-	-	-	-
Unid. Mysidacea	-	-	-	-	-	-	-	-	-	-	-	-
Decapoda												
Pasiphaea sp.	-	-	-	-	-	-	-	-	-	-	-	-
Plesionika martia	-	-	-	-	-	-	-	-	47,56	2,70	12,50	7,38
Sergestes arcticus	-	-	-	-	-	-	-	-	-	-	-	-
Sergestidae	-	-	-	-	-	-	-	-	0,04	2,70	12,50	0,40
Unid. Natantia	-	-	-	-	-	-	-	-	0,18	2,70	12,50	0,42
Cumacea												
Campylaspis verrucosa	-	-	-	-	-	-	-	-	-	-	-	-
Copepoda	-	-	-	-	-	-	-	-	-	-	-	-
Unid. Crustacea	-	-	-	-	13,54	33,33	50,00	23,43	-	-	-	-
PISCES												
Lampanyctus crocodilus	-	-	-	-	-	-	-	-	-	-	-	-
Myctophidae	35,24	2,70	16,67	6,79	-	-	-	-	-	-	-	-
Unid. Fish	-	-	-	-	8,65	33,33	50,00	20,99	-	-	-	-
OTHERS												
Digested material	-	-	-	-	-	-	-	-	-	-	-	-
Gelatinous reamains	-	-	-	-	-	-	-	-	-	-	-	-

	Aug	**Sep**	**Nov**	**Feb**	**Apr**	**Jun**
No of individuals	37	33	28	11	2	11
Vacuity index (%V)	18,92	33,33	35,71	45,45	0,00	36,36
Mean fullness	0,007	0,006	0,010	0,001	0,026	0,004
Mean No. of prey	7,49	7,16	3,25	3,36	1,50	2,18
Mean prey weight (g)	0,30	0,28	0,11	0,07	0,08	0,11
Eveness (J')	0,35	0,25	0,35	0,82	1,00	0,57
Diversity (H')	1,09	0,63	0,77	1,59	1,10	1,31
Dominance (1-λ’)	0,63	0,78	0,70	0,24	0,33	0,47

ANNEX 6-2. Dietary composition and trophic diversity indices of *H.italicus* from August 2003 to June 2004. %W: percentage contribution by weight; %N: by number;.%F: by frequency of occurrence; %IRI by index of relative importance

	August 03				September 03				November 03			
Taxon	**%W**	**%N**	**%F**	**%IRI**	**%W**	**%N**	**%F**	**%IRI**	**%W**	**%N**	**%F**	**%IRI**
POLYCHAETA												
Aphroditidae	0,86	2,06	6,67	0,25	3,33	1,75	5,88	0,59	0,80	2,24	7,50	0,50
Unid. Polychaetha	0,03	1,03	3,33	0,05	-	-	-	-	-	-	-	-
CRUSTACEA												
Isopoda												
Gnathia spp. (larvae)	-	-	-	-	1,94	1,75	5,88	0,43	-	-	-	-
Eurydice grimaldii	-	-	-	-	-	-	-	-	-	-	-	-
Ilyarachna longicornis	0,64	2,06	6,67	0,23	1,94	1,75	5,88	0,43	-	-	-	-
Munnopsurus atlanticus	-	-	-	-	0,81	1,75	5,88	0,30	2,45	2,99	10,00	1,19
Natatolana borealis	-	-	-	-	5,61	5,26	11,76	2,53	8,03	5,22	10,00	2,90
Amphipoda Gammaridea												
Acidostoma sarsi	-	-	-	-	-	-	-	-	0,21	0,75	2,50	0,05
Andaniexis mimonectes	1,55	6,19	23,33	2,34	0,38	1,75	5,88	0,25	1,06	5,22	12,50	1,72
Bruzelia tipica	-	-	-	-	-	-	-	-	-	-	-	-
Maera schmidti	3,73	1,03	3,33	0,21	-	-	-	-	-	-	-	-
Monoculodes acutipes	-	-	-	-	0,47	1,75	5,88	0,26	-	-	-	-
Orchomenella nana	0,06	1,03	3,33	0,05	0,38	1,75	5,88	0,25	-	-	-	-
Pardalisca sp.	-	-	-	-	-	-	-	-	-	-	-	-
Rhacotropis caeca	-	-	-	-	-	-	-	-	-	-	-	-
Rhacotropis grimaldii	0,47	1,03	3,33	0,06	-	-	-	-	1,07	0,75	2,50	0,10
Rhacotropis rostrata	-	-	-	-	-	-	-	-	1,63	0,75	5,00	0,26
Rhacotropis sp.	-	-	-	-	-	-	-	-	-	-	-	-
Syrrhoe affinis	-	-	-	-	-	-	-	-	-	-	-	-
Tryphosites longipes	6,51	4,12	13,33	1,84	-	-	-	-	-	-	-	-
Tryphosites alleni	0,62	1,03	3,33	0,07	-	-	-	-	-	-	-	-
Tryphosites sp	-	-	-	-	-	-	-	-	3,14	2,24	7,50	0,88
Lissianassidae	0,26	1,03	3,33	0,06	-	-	-	-	-	-	-	-
Stegocephalidae	-	-	-	-	0,38	1,75	5,88	0,25	-	-	-	-
Unid. Gammaridea	0,41	3,09	10,00	0,45	5,89	5,26	17,65	3,90	3,98	5,22	15,00	3,02
Amphipoda Hyperidea												
Anchilomera blossevillei	-	-	-	-	0,09	1,75	5,88	0,22	-	-	-	-
Copepoda	1,49	12,37	26,67	4,79	12,47	12,28	41,18	20,17	3,29	13,43	17,50	6,40
Mysidacea												
Boreomysis arctica	67,81	30,93	63,33	80,98	32,20	10,53	35,29	29,84	45,06	12,69	37,50	47,34
Calyptomma puritani	3,69	6,19	13,33	1,70	-	-	-	-	1,59	2,24	5,00	0,42
Dactylamblyops sp.	-	-	-	-	-	-	-	-	-	-	-	-
Mysideis parva	-	-	-	-	12,17	8,77	17,65	7,31	-	-	-	-
Parapseudomma calloplura	4,57	4,12	10,00	1,13	-	-	-	-	1,37	2,24	5,00	0,39
Unid. Mysidacea	3,53	3,09	10,00	0,86	7,90	14,04	35,29	15,32	5,63	5,97	20,00	5,07
Decapoda												
Calocaris macandrae	-	-	-	-	-	-	-	-	2,56	0,75	2,50	0,18
Pasiphaea sp.	-	-	-	-	-	-	-	-	0,60	0,75	2,50	0,07
Euphausiacea												
Euphausiacea larvae	-	-	-	-	-	-	-	-	0,55	0,75	2,50	0,07
Unid. Euphausiacea	-	-	-	-	-	-	-	-	-	-	-	-
Tanaidacea												
Leptognathia sp.	-	-	-	-	0,81	1,75	5,88	0,30	-	-	-	-
Cumacea												
Campylaspis verrucosa	-	-	-	-	-	-	-	-	-	-	-	-
Platysympus typicus	-	-	-	-	-	-	-	-	0,27	0,75	2,50	0,06

	August 03				September 03				Novembert 03			
Taxon	**%W**	**%N**	**%F**	**%IRI**	**%W**	**%N**	**%F**	**%IRI**	**%W**	**%N**	**%F**	**%IRI**
Ostracoda												
Asteropina mariae	0,03	2,06	3,33	0,09	-	-	-	-	0,12	0,75	2,50	0,05
Philomedes	0,25	4,12	6,67	0,38	9,15	10,53	23,53	9,16	9,40	18,66	35,00	21,47
Unid. Cyprinidae	2,11	6,19	23,33	2,51	3,70	14,04	23,53	8,26	4,48	8,21	25,00	6,93
Unid. Crustacea	-	-	-	-	0,38	1,75	5,88	0,25	1,77	0,75	2,50	0,14
CHAETHOGNATA	-	-	-	-	-	-	-	-	0,45	1,49	5,00	0,21
MOLLUSCA												
Unid. Cephalopoda	-	-	-	-	-	-	-	-	-	-	-	-
PISCES												
Scales	1,19	6,19	20,00	1,91	-	-	-	-	0,17	2,24	5,00	0,26
Unid. Fishes	-	-	-	-	-	-	-	-	-	-	-	-
OTHERS												
Digested material	0,17	1,03	3,33	0,05	-	-	-	-	0,81	2,24	5,00	0,33

	February 04				April 04				June 04			
Taxon	**%W**	**%N**	**%F**	**%IRI**	**%W**	**%N**	**%F**	**%IRI**	**%W**	**%N**	**%F**	**%IRI**
POLYCHAETHA												
Aphroditidae	-	-	-	-	0,76	1,16	4,55	0,13	1,65	2,74	7,69	0,85
Unid. Polychaetha	0,33	2,67	8,70	0,44	-	-	-	-	-	-	-	-
CRUSTACEA												
Isopoda												
Gnathia spp. (larvae)	0,46	1,33	4,35	0,13	-	-	-	-	-	-	-	-
Eurydice grimaldii	0,46	1,33	4,35	0,13	-	-	-	-	-	-	-	-
Ilyarachna longicornis	-	-	-	-	-	-	-	-	-	-	-	-
Munnopsurus atlanticus	-	-	-	-	8,18	4,65	18,18	3,48	5,14	6,85	19,23	5,84
Natatolana borealis	21,69	21,33	21,74	15,77	36,05	18,60	40,91	33,34	-	-	-	-
Amphipoda Gammaridea												
Acidostoma sarsi	-	-	-	-	-	-	-	-	-	-	-	-
Andaniexis mimonectes	1,88	6,67	21,74	3,13	1,86	3,49	13,64	1,09	2,63	9,59	11,54	3,57
Bruzelia tipica	0,27	1,33	4,35	0,12	-	-	-	-	-	-	-	-
Maera schmidti	-	-	-	-	-	-	-	-	-	-	-	-
Monoculodes acutipes	-	-	-	-	-	-	-	-	-	-	-	-
Orchomenella nana	1,97	1,33	4,35	0,24	-	-	-	-	-	-	-	-
Pardalisca sp.	-	-	-	-	0,09	1,16	4,55	0,08	-	-	-	-
Rhacotropis caeca	-	-	-	-	2,27	1,16	4,55	0,23	-	-	-	-
Rhacotropis grimaldii	-	-	-	-	-	-	-	-	-	-	-	-
Rhacotropis rostrata	-	-	-	-	-	-	-	-	-	-	-	-
Rhacotropis sp.	0,82	1,33	4,35	0,16	-	-	-	-	2,40	1,37	3,85	0,37
Syrrhoe affinis	-	-	-	-	0,75	1,16	4,55	0,13	-	-	-	
Tryphosites longipes	-	-	-	-	-	-	-	-	4,16	1,37	3,85	0,54
Tyiphosites alleni	-	-	-	-	-	-	-	-	-	-	-	-
Tyiphosites sp	1,39	1,33	4,35	0,20	3,17	1,16	4,55	0,29	-	-	-	-
Lissianassidae	-	-	-	-	-	-	-	-	-	-	-	-
Stegocephalidae	-	-	-	-	-	-	-	-	-	-	-	-
Unid. Gammaridea	6,11	2,67	8,70	1,29	2,64	4,65	18,18	1,98	2,68	1,37	3,85	0,39
Amphipoda Hyperidea												
Anchilomera blossevillei	-	-	-	-	-	-	-	-	-	-	-	-
Copepoda	1,36	13,33	26,09	6,46	8,28	30,23	50,00	28,71	3,77	23,29	26,92	18,44
Mysidacea												
Boreomysis arctica	48,78	26,67	52,17	66,39	23,90	9,30	36,36	18,00	49,08	16,44	23,08	38,26
Calyptomma puritani	-	-	-	-	-	-	-	-	-	-	-	-
Dactylamblyops sp.	-	-	-	-	-	-	-	-	0,83	1,37	3,85	0,21
Mysideis parva	0,65	1,33	4,35	0,15	-	-	-	-	-	-	-	-
Parapseudomma calloplura	-	-	-	-	1,84	1,16	4,55	0,20	5,72	4,11	7,69	1,91

	February 04				April 04				June 04			
	%W	**%N**	**%F**	**%IRI**	**%W**	**%N**	**%F**	**%IRI**	**%W**	**%N**	**%F**	**%IRI**
Unid. Mysidacea	2,32	6,67	21,74	3,29	5,75	11,63	40,91	10,60	9,19	12,33	34,62	18,85
Decapoda												
Calocaris macandrae	-	-	-	-	-	-	-	-	-	-	-	-
Pasiphaea sp.	-	-	-	-	-	-	-	-	-	-	-	-
Euphausiacea												
Euphausiacea larvae	-	-	-	-	-	-	-	-	-	-	-	-
Unid. Euphausiacea	4,05	1,33	4,35	0,40	-	-	-	-	3,75	2,74	7,69	1,26
Tanaidacea												
Leptognathia sp.	-	-	-	-	-	-	-	-	-	-	-	-
Cumacea												
Campylaspis verrucosa	-	-	-	-	0,21	1,16	4,55	0,09	-	-	-	-
Platysympus typicus	-	-	-	-	-	-	-	-	-	-	-	-
Ostracoda												
Asteropina mariae	-	-	-	-	-	-	-	-	-	-	-	-
Philomedes	0,92	2,67	8,70	0,53	1,29	3,49	9,09	0,65	2,76	5,48	11,54	2,41
Unid. Cyprinidae	-	-	-	-	0,78	1,16	4,55	0,13	3,08	6,85	19,23	4,83
Unid. Crustacea	1,07	1,33	4,35	0,18	-	-	-	-	3,14	4,11	11,54	2,12
CHAETHOGNATA	-	-	-	-	-	-	-	-	-	-	-	-
MOLLUSCA												
Unid. Cephalopoda	-	-	-	-	-	-	-	-	0,10	1,37	3,85	0,14
PISCES												
Scales	0,17	2,67	8,70	0,42	0,16	2,33	9,09	0,34	-	-	-	-
Unid. Fishes	-	-	-	-	-	-	-	-	-	-	-	-
OTHERS												
Digested material	1,26	2,67	8,70	0,58	1,52	2,33	9,09	0,52	-	-	-	-

	Aug	**Sep**	**Nov**	**Feb**	**Apr**	**Jun**
No of individuals	45	31	60	34	27	32
Vacuity index (%V)	26,83	**45,16**	33,33	32,35	**18,52**	25,00
Mean fullness	0,160	0,089	0,098	0,167	0,173	0,183
Mean No. of prey	3,23	3,35	3,35	3,26	3,91	2,81
Mean prey weight (g)	0,01	0,01	0,01	0,01	0,01	0,01
Eveness (J')	0,82	0,88	0,83	0,78	0,77	0,86
Diversity (H')	2,46	2,59	2,65	2,25	2,20	2,38
Dominance (1-lambda')	0,87	0,92	0,91	0,85	0,84	0,89

ANNEX 6-3. Dietary composition and trophic diversity indices of *N. aequalis*, %W: percentage contribution by weight; %N: by number;.%F: by frequency of occurrence; %IRI by index of relative importance.

	August03				September03				November03			
Taxon	**%W**	**%N**	**%F**	**%IRI**	**%W**	**%N**	**%F**	**%IRI**	**%W**	**%N**	**%F**	**%IRI**
CTENOPHORA												
Chelopyes appendiculata	-	-	-	-	-	-	-	-	-	-	-	-
NEMATODA												
Unid. Nematoda	-	-	-	-	-	-	-	-	0.26	0.54	0.02	0.05
POLYCHAETHA												
Aphroditidae	14.40	4.92	20.00	14.03	25.30	10.00	28.57	31.25	23.82	7.07	0.30	25.53
Eunicidae	2.83	2.19	8.89	1.90	-	-	-	-	5.97	2.17	0.09	2.07
Lumbrinereidae	-	-	-	-	-	-	-	-	-	-	-	-
Onuphidae	-	-	-	-	-	-	-	-	-	-	-	-
Phyllodocidae	1.52	1.09	4.44	0.50	-	-	-	-	-	-	-	-
Unid. Polychaetha	2.13	3.28	13.33	3.07	1.57	2.50	9.52	1.20	3.16	3.80	0.16	3.10
CRUSTACEA												
Isopoda												
Cirolana borealis	16.67	3.28	11.11	9.44	-	-	-	-	2.94	2.17	0.09	1.30
Iliarachna longicornis	1.84	2.19	8.89	1.52	0.49	2.50	4.76	0.44	0.37	0.54	0.02	0.06
Leptognathia sp.	0.14	0.55	2.22	0.07	-	-	-	-	-	-	-	-
Gnathia sp.	1.21	1.09	4.44	0.44	0.32	2.50	4.76	0.42	0.93	1.63	0.07	0.49
Munnopsurus atlanticus	3.21	4.37	15.56	5.02	12.28	7.50	19.05	11.67	5.16	16.30	0.41	24.57
Amphipoda Gammaridea												
Acidostoma sarsi	0.07	0.55	2.22	0.06	1.69	2.50	4.76	0.62	-	-	-	-
Andaniexis mimonectes	0.85	2.73	6.67	1.02	1.46	0.00	4.76	0.22	0.18	1.09	0.05	0.16
Bathymedon longirostris	-	-	-	-	-	-	-	-	-	-	-	-
Bruzelia typica	0.15	0.55	2.22	0.07	-	-	-	-	1.01	1.09	0.05	0.27
Epimeria parasitica	0.53	0.55	2.22	0.10	-	-	-	-	2.91	1.09	0.05	0.51
Eusirus longipes	-	-	-	-	-	-	-	-	-	-	-	-
Harpinia sp.	0.03	0.55	2.22	0.05	0.38	2.50	4.76	0.42	0.29	0.54	0.02	0.05
Hippomedon bidenattus	0.07	0.55	2.22	0.06	-	-	-	-	-	-	-	-
Lepechinella manco	0.65	1.09	4.44	0.33	0.32	2.50	4.76	0.42	-	-	-	-
Maera schmidti	12.90	8.20	22.22	19.96	15.80	10.00	14.29	11.42	5.76	2.17	0.07	1.51
Monoculodes sp.	-	-	-	-	-	-	-	-	-	-	-	-
Nicippe tumida	0.25	0.55	2.22	0.08	-	-	-	-	-	-	-	-
Orchomenella nana	-	-	-	-	-	-	-	-	-	-	-	-
Pseudotiron bouvieri	-	-	-	-	-	-	-	-	-	-	-	-
Rhacotropis caeca	0.08	0.55	2.22	0.06	-	-	-	-	0.80	1.09	0.05	0.24
Rhacotropis grimaldi	0.45	1.09	4.44	0.29	-	-	-	-	2.64	2.17	0.09	1.22
Rhacotropis rostrata	2.34	1.09	2.22	0.33	6.04	7.50	19.05	7.99	2.91	1.63	0.07	0.87
Rhacotropis sp.	3.24	2.19	8.89	2.05	-	-	-	-	3.46	4.35	0.16	3.48
Stegocephaloides cristianensis	0.66	1.09	4.44	0.33	0.25	2.50	4.76	0.41	2.02	2.72	0.09	1.20
Syrroe affinis	2.54	2.73	11.11	2.50	1.19	2.50	9.52	1.09	2.35	0.54	0.02	0.18
Tmetonix similis	-	-	-	-	-	-	-	-	-	-	-	-
Triphosites longipes	2.17	1.09	4.44	0.62	3.37	2.50	4.76	0.87	1.18	0.54	0.02	0.11
Triphosites alleni	4.08	3.83	11.11	3.74	-	-	-	-	-	-	-	-
Triphosites sp.	-	-	-	-	3.80	2.50	4.76	0.93	1.31	0.54	0.02	0.12
Unid. Lyssianassidae	0.47	2.73	4.44	0.61	-	-	-	-	-	-	-	-
Unid. Oedicerotidae	0.07	0.55	2.22	0.06	-	-	-	-	-	-	-	-
Unid. Gammaridea	3.61	9.29	22.22	12.21	6.47	10.00	23.81	12.15	5.98	7.61	0.25	9.51
Amphipoda Hyperidea												
Euprimno macropus	-	-	-	-	-	-	-	-	0.64	1.09	0.02	0.11
Phronima sedentaria	-	-	-	-	-	-	-	-	-	-	-	-
Phrosina semilunata	-	-	-	-	1.71	0.00	4.76	0.25	-	-	-	-

	August03				September03				November03			
Taxon	**%W**	**%N**	**%F**	**%IRI**	**%W**	**%N**	**%F**	**%IRI**	**%W**	**%N**	**%F**	**%IRI**
Copepoda												
Unid. Copepoda	1.00	4.37	15.56	3.56	0.45	2.50	9.52	0.87	1.53	7.07	0.18	4.37
Mysidacea												
Boreomysis arctica	2.69	4.92	13.33	4.32	6.09	12.50	19.05	10.97	5.31	7.61	0.25	9.04
Calliptoma puritani	0.01	0.55	2.22	0.05	-	-	-	-	-	-	-	-
Dactylamblyops sp.	0.55	2.73	6.67	0.93	-	-	-	-	0.15	0.54	0.02	0.04
Mysideis parva	0.23	1.64	6.67	0.53	1.73	10.00	9.52	3.46	2.41	3.80	0.14	2.37
Mysidopsis gibbosa??	-	-	-	-	-	-	-	-	0.88	1.63	0.02	0.16
Parapseudomma calloplura	1.30	4.37	13.33	3.22	-	-	-	-	3.12	4.89	0.14	3.06
Unid. Mysidacea	0.79	1.64	4.44	0.46	-	-	-	-	1.82	4.35	0.14	2.35
Decapoda												
Calocaris macandrae	4.47	0.55	2.22	0.47	-	-	-	-	2.70	0.54	0.02	0.21
Pontocaris lacazei	-	-	-	-	-	-	-	-	-	-	-	-
Processa sp	-	-	-	-	-	-	-	-	1.96	0.54	0.02	0.16
Pandalidae post larvae	0.25	1.09	4.44	0.25	-	-	-	-	-	-	-	-
Unid. Decapoda Natantia	0.44	0.55	2.22	0.09	-	-	-	-	2.75	1.09	0.05	0.49
Unid. Axiidae	0.27	0.55	2.22	0.08	-	-	-	-	-	-	-	-
Unid. Geryonidae juv	1.96	0.55	2.22	0.24	-	-	-	-	-	-	-	-
Unid. Inachinae	-	-	-	-	-	-	-	-	-	-	-	-
Euphausiacea												
Meganycthiphanes norvegica	-	-	-	-	-	-	-	-	-	-	-	-
Stilocheiron abbreviatum	0.22	1.09	4.44	0.25	-	-	-	-	-	-	-	-
Tanaidacea												
Apseudes spinosus	0.45	1.09	4.44	0.29	2.53	2.50	4.76	0.74	0.21	0.54	0.02	0.05
Unid. Tanadaicea	0.09	0.55	2.22	0.06	-	-	-	-	-	-	-	-
Cumacea												
Cyclaspis longicaudata	0.14	0.55	2.22	0.07	-	-	-	-	-	-	-	-
Leucon macrorhinus	-	-	-	-	-	-	-	-	-	-	-	-
Dyastiloides serrata	-	-	-	-	-	-	-	-	-	-	-	-
Unid. Cumacea	0.29	1.09	4.44	0.26	-	-	-	-	0.09	0.54	0.02	0.04
Ostracoda												
Asteropina mariae	0.05	0.55	2.22	0.06	-	-	-	-	-	-	-	-
Philomedes	0.76	1.09	2.22	0.18	2.17	0.00	9.52	0.64	0.58	2.17	0.09	0.70
Unid. Cyprinidae	0.05	1.09	2.22	0.11	0.84	2.50	4.76	0.49	0.15	1.09	0.02	0.08
Unid. Ostracoda	0.82	2.19	6.67	0.85	-	-	-	-	0.29	1.09	0.05	0.17
Unid. Crustacea	-	-	-	-	0.22	0.00	4.76	0.03	-	-	-	-
PISCES												
Cyclotone braueri	-	-	-	-	-	-	-	-	-	-	-	-
fish remains (bones and scales)	-	-	-	-	-	-	-	-	-	-	-	-
OTHER												
Unid. digested material	4.00	2.73	11.11	3.19	3.54	-	9.52	1.04	-	-	-	-

	February04				April04				June04			
Taxon	**%W**	**%N**	**%F**	**%IRI**	**%W**	**%N**	**%F**	**%IRI**	**%W**	**%N**	**%F**	**%IRI**
CTENOPHORA												
Cephalyes appendiculata	0.94	0.49	1.89	0.09	-	-	-	-	-	-	-	-
NEMATODA												
Unid. Nematoda	-	-	-	-	-	-	-	-	-	-	-	-
POLYCHAETHA												
Aphroditidae	12.81	4.43	15.09	6.19	13.98	4.00	14.29	3.12	5.33	3.20	10.71	2.35
Eunicidae	21.28	6.90	24.53	24.14	-	-	-	-	0.52	1.60	3.57	0.19
Lumbrinereidae	3.85	0.99	3.77	0.64	9.75	0.80	3.57	1.05	1.21	0.80	3.57	0.18
Onuphidae	3.13	1.48	3.77	0.61	-	-	-	-	-	-	-	-

	February04				April04				June04			
Taxon	**%W**	**%N**	**%F**	**%IRI**	**%W**	**%N**	**%F**	**%IRI**	**%W**	**%N**	**%F**	**%IRI**
POLYCHAETHA	-	-	-	-	-	-	-	-	-	-	-	-
Phyllodocidae	-	-	-	-	-	-	-	-	-	-	-	-
Unid. Polychaetha	4.82	3.94	15.09	4.62	6.61	3.20	14.29	3.91	11.85	5.60	25.00	11.21
CRUSTACEA												
Isopoda												
Cirolana borealis	1.81	3.45	7.55	1.39	2.34	2.40	10.71	1.42	1.26	0.80	3.57	0.19
Iliarachna longicornis	0.22	0.99	3.77	0.16	0.81	0.80	3.57	0.16	0.50	0.80	3.57	0.12
Leptognathia sp.	-	-	-	-	-	-	-	-	-	-	-	-
Gnathia sp.	0.29	0.99	3.77	0.17	0.98	1.60	7.14	0.51	-	-	-	-
Munnupsurus atlanticus	4.41	11.33	26.42	14.52	13.08	19.20	42.86	38.56	4.53	13.60	46.43	21.62
Amphipoda Gammaridea												
Acidostoma sarsi	-	-	-	-	-	-	-	-	-	-	-	-
Andaniexis mimonectes	0.40	2.96	11.32	1.33	1.75	4.80	21.43	3.91	-	-	-	-
Bathymedon longirostris	-	-	-	-	0.81	0.80	3.57	0.16	-	-	-	-
Bruzelia typica	0.14	0.49	1.89	0.04	-	-	-	-	-	-	-	-
Epimeria parasitica	0.70	0.49	1.89	0.08	2.78	2.40	10.71	1.55	-	-	-	-
Eusirus longipes	0.64	0.99	3.77	0.21	-	-	-	-	4.47	2.40	10.71	1.89
Harpinia sp.	-	-	-	-	-	-	-	-	-	-	-	-
Hippomedon bidenattus	0.56	0.49	1.89	0.07	-	-	-	-	-	-	-	-
Lepechinella manco	0.16	0.49	1.89	0.04	0.16	0.80	3.57	0.10	-	-	-	-
Maera schmidti	3.88	1.97	5.66	1.16	4.59	0.80	3.57	0.54	1.17	0.80	3.57	0.18
Monoculodes sp.	0.12	0.49	1.89	0.04	-	-	-	-	-	-	-	-
Nicippe tumida	-	-	-	-	-	-	-	-	-	-	-	-
Orchomenella nana	0.20	0.49	1.89	0.05	0.55	1.60	7.14	0.43	0.09	0.80	3.57	0.08
Pseudotiron bouvieri	-	-	-	-	0.16	1.60	3.57	0.18	-	-	-	-
Rhacotropis caeca	0.50	0.49	1.89	0.07	-	-	-	-	-	-	-	-
Rhacotropis grimaldi	0.70	0.99	3.77	0.22	1.72	3.20	10.71	1.47	3.41	3.20	10.71	1.82
Rhacotropis rostrata	0.50	0.49	1.89	0.07	-	-	-	-	-	-	-	-
Rhacotropis sp	1.78	2.96	9.43	1.56	3.01	4.00	17.86	3.49	1.90	2.40	10.71	1.18
Stegocephaloides cristianensis	0.26	0.99	3.77	0.16	0.32	1.60	3.57	0.19	0.80	1.60	7.14	0.44
Syrroe affinis	-	-	-	-	1.17	0.80	3.57	0.20	-	-	-	-
Tmetonix similis	0.79	0.49	1.89	0.08	-	-	-	-	-	-	-	-
Triphosites longipes	1.13	0.99	3.77	0.28	2.10	0.80	3.57	0.29	1.26	1.60	3.57	0.26
Triphosites alleni	-	-	-	-	-	-	-	-	-	-	-	-
Triphosites sp.	0.69	0.49	1.89	0.08	-	-	-	-	-	-	-	-
Unid. Lyssianassidae	-	-	-	-	-	-	-	-	-	-	-	-
Unid. Oedicerotidae	-	-	-	-	-	-	-	-	-	-	-	-
Unid. Gammaridea	7.29	9.85	30.19	18.08	5.04	4.80	17.86	4.90	2.73	6.40	21.43	5.03
Amphipoda Hyperidea												
Euprimno macropus	-	-	-	-	-	-	-	-	-	-	-	-
Phronima sedentaria	0.42	0.49	1.89	0.06	-	-	-	-	-	-	-	-
Phrosina semilunata	-	-	-	-	-	-	-	-	-	-	-	-
Copepoda												
Unid. Copepoda	0.70	4.43	15.09	2.71	1.94	3.20	14.29	2.05	0.59	2.40	10.71	0.82
Mysidacea												
Boreomysis arctica	4.13	5.42	16.98	5.66	4.06	4.00	10.71	2.41	3.68	4.80	10.71	2.33
Calliptoma puritani	-	-	-	-	1.16	0.80	3.57	0.20	-	-	-	-
Dactylamblyops sp.	-	-	-	-	0.62	0.80	3.57	0.14	0.67	1.60	7.14	0.42
Mysideis parva	2.46	4.43	13.21	3.18	2.57	4.00	17.86	3.27	1.01	2.40	7.14	0.63
Parapseudomma calloplura	0.81	4.93	13.21	2.64	3.18	8.00	25.00	7.79	5.67	20.00	32.14	21.19
Unid. Mysidacea	1.08	3.45	11.32	1.79	7.97	12.80	28.57	16.54	5.94	11.20	35.71	15.72

	February04				April04				June04			
Taxon	**%W**	**%N**	**%F**	**%IRI**	**%W**	**%N**	**%F**	**%IRI**	**%W**	**%N**	**%F**	**%IRI**
Decapoda												
Calocaris macandrae	9.97	1.97	7.55	3.15	-	-	-	-	16.00	3.20	14.29	7.04
Pontocaris lacazei	-	-	-	-	-	-	-	-	16.12	0.80	3.57	1.55
Processa sp	-	-	-	-	-	-	-	-	-	-	-	-
Pandalidae post larvae	-	-	-	-	-	-	-	-	-	-	-	-
Unid. Decapoda Natantia	0.94	0.49	1.89	0.09	-	-	-	-	-	-	-	-
Unid. Axiidae	-	-	-	-	-	-	-	-	-	-	-	-
Unid. Geryonidae juv	-	-	-	-	-	-	-	-	-	-	-	-
Unid. Inachinae	-	-	-	-	0.55	0.80	3.57	0.13	-	-	-	-
Euphausiacea												
Meganycthiphanes norvegica	-	-	-	-	-	-	-	-	7.29	0.80	3.57	0.74
Stilocheiron abbreviatum	-	-	-	-	-	-	-	-	-	-	-	-
Tanaidacea												
Apseudes spinosus	-	-	-	-	1.64	0.80	3.57	0.24	-	-	-	-
Unid. Tanadaicea	-	-	-	-	-	-	-	-	-	-	-	-
Cumacea												
Cyclaspis longicaudata	-	-	-	-	1.53	0.80	3.57	0.23	-	-	-	-
Leucon macrorhinus	0.14	0.49	1.89	0.04	-	-	-	-	-	-	-	-
Dyastiloides serrata	-	-	-	-	0.77	0.80	3.57	0.16	-	-	-	-
Unid. Cumacea	0.09	0.49	1.89	0.04	-	-	-	-	-	-	-	-
Ostracoda												
Asteropina mariae	-	-	-	-	-	-	-	-	-	-	-	-
Philomedes	1.27	3.94	7.55	1.37	-	-	-	-	0.83	3.20	10.71	1.11
Unid. Cyprinidae	-	-	-	-	-	-	-	-	-	-	-	-
Unid. Ostracoda	0.52	1.97	7.55	0.66	-	-	-	-	-	-	-	-
Unid. Crustacea	-	-	-	-	0.14	0.80	3.57	0.09	-	-	-	-
PISCES												
Cyclothone braueri	0.80	0.49	1.89	0.08	-	-	-	-	-	-	-	-
fish remains (bones and scales)	0.54	4.43	11.32	1.97	-	-	-	-	0.61	4.00	14.29	1.69
OTHER												
Unid. digested material	2.15	0.99	3.77	0.41	0.83	1.60	7.14	0.48	-	-	-	-

	Aug	**Sep**	**Nov**	**Feb**	**Apr**	**Jun**
No of individuals	45.00	21.00	56.00	69	28.00	39.00
Vacuity index (%V)	22.22	47.62	37.50	28.99	21.43	15.38
Mean fullness	0.09	0.06	0.08	0.117	0.09	0.10
Mean No. of prey	6.78	5.00	7.67	5.97	5.95	6.25
Mean prey weight (g)	0.04	0.03	0.05	0.05	0.02	0.04
Eveness (J')	0.90	0.92	0.87	0.88	0.85	0.85
Diversity (H')	3.56	2.77	3.15	3.29	2.99	2.78
Dominance (1-lambda')	0.97	0.93	0.95	0.96	0.93	0.92

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General remarks

This study allows highlighting some general considerations

- **It underlined the importance of suprabenthos in coastal and deep-sea ecosystems as a link between pelagic and benthic environments;**
- In coastal environments suprabenthic resources are mainly exploited by juveniles of some demersal species (i.e. *Arnoglossus laterna* and *Merluccius merluccius*);
- In deep sea environments suprabenthic resources are exploited by species belonging to different trophic guilds but they contribute differently to fish diet. This difference is probably due to the different size of suprabenthic species in coastal and deep sea environments with large size specimens inhabiting deep-sea bottoms, in contrast with juveniles and larvae living in coastal ecosystems (estuaries, surf-zones…);
- **Dynamics of suprabenthos are similar in costal and deep-sea environments, with only one peak of species' abundance. This peak is generally related (with a delay) to the peak of primary production recorded from January to March in the Western Mediterranean;**
- Peaks of abundance and biomass were observed one month after the peak of PP (observed in February) in the coastal ecosystem of the Gulf of Castellammare;
- The increase of suprabenthos abundance in April-June in the deep-sea ecosystem of the Archipelago of Cabrera was also parallel to a sharp increase (*ca.* 2.8 times) of %OM on the sediment in the same period coupled *ca.* with 1-2 months of delay with the peak of surface primary production recorded in February-March;
- **Demersal trophic webs are complex with at least 4 or 5 links, deep-sea trophic webs seemed to be more complex than coastal ones;**
- In coastal environments among suprabenthic species two trophic levels were identified by stable isotopes analysis and generally species belonged to two trophic guilds: deposit-filter feeders and carnivores;
- There is a single type of a primary source material supporting deep-sea communities, the marine snow. Three trophic levels were identified among suprabenthic species and two among zooplankton;

- Stable isotopes analyses revealed seasonal changes in trophic levels and source of carbon, both for fish and suprabenthic resources;
- **Seasonal changes in trophic levels were stronger in deep-sea than in coastal suprabenthic communities.**