

Zooplankton associations in a Mediterranean long-term time-series

MARIA GRAZIA MAZZOCCHI^{1*}, PRISCILLA LICANDRO², LAURENT DUBROCA^{1†}, IOLE DI CAPUA¹ AND VINCENZO SAGGIOMO¹

¹STAZIONE ZOOLOGICA ANTON DOHRN, VILLA COMUNALE, 80121 NAPOLI, ITALY AND ²SIR ALISTER HARDY FOUNDATION FOR OCEAN SCIENCE (SAHFOS), THE LABORATORY CITADEL HILL, PLYMOUTH PL1 2PB, UK

[†]PRESENT ADDRESS: IFREMER, LABORATOIRE ENVIRONNEMENT RESSOURCES DU LANGUEDOC-ROUSSILLON (LER/LR), AVENUE JEAN MONNET BP 171, 34203 SETE CEDEX, FRANCE

*CORRESPONDING AUTHOR: grazia.mazzocchi@szn.it

Received May 11, 2010; accepted in principle January 28, 2011; accepted for publication February 3, 2011

Corresponding editor: Mark J. Gibbons

Coastal zooplankton have been investigated since 1984 at a Long Term Ecological Research station MC (LTER-MC) in the inner Gulf of Naples (Tyrrhenian Sea, Western Mediterranean). The sampling site, located between the littoral and the open sea systems, has very active hydrography that affects plankton communities. The present work was aimed at establishing whether, in such a dynamic and variable environment, species associations and homogeneous periods could be identified as characteristic and stable features of the mesozooplankton over the period 1984–2006. Hierarchical clustering was applied to assess species associations based on a matrix of similarities between species (R-mode), and homogeneous periods based on a matrix of similarities between observations (Q-mode). The Indicator Value index [IndVal, Dufrene and Legendre (1997) Species assemblages and indicator species: the need for a flexible asymmetrical approach. *Ecol. Monogr.*, **67**, 345–366] was calculated to identify species characterizing each period. Five taxonomic groups with well-defined composition and abundance were identified as robust associations that likely reflect different modes of community functioning. The temporal course of these associations was largely shaped by strong seasonal forcing comprising both physical and biological (e.g. trophic) signals. These associations persisted over the long term, thus indicating some stable characters in the Naples zooplankton time-series, providing evidence of resilience in communities in highly variable coastal conditions.

KEYWORDS: zooplankton; associations; time-series; Mediterranean; Tyrrhenian Sea

INTRODUCTION

Species are the key biological elements in the functioning of marine pelagic systems. In fact, a species-level approach has been recommended for plankton studies aimed at better understanding the mechanisms of community dynamics and biological interactions, and developing predictive modeling capability (Marine Zooplankton Colloquium 2, 2001). In most marine

environments, plankton communities are highly diversified and numerous species, even congeners, generally co-occur. The co-occurrence of species in pelagic systems has been considered to be determined at random (Rojo *et al.*, 2000). However, the persistence of recurrent patterns in species distribution makes it very unlikely that a purely stochastic process drives plankton community structure (Kruk *et al.*, 2002).

Species can be grouped in associations because they co-occur having similar or complementary ecological needs and responses to certain environmental conditions. Plankton associations have been frequently reported in the space domain, in both freshwater and marine environments, especially where distinct hydrographic features determine strong gradients or discontinuities that clearly shape community composition and distribution (e.g. Castel and Courties, 1982; Wishner and Allison, 1986; Pinel-Alloul *et al.*, 1990; Berasategui *et al.*, 2006). For the first time on an oceanic basin scale and at a spatial resolution approaching the mesoscale (ca. 200 km), species associations of planktonic copepods were identified in the North Atlantic, with strong relationships to currents and water masses (Beaugrand *et al.*, 2002a). However, even well-defined environmental forcing sometimes fails to completely account for observed species distribution (e.g. Pinel-Alloul *et al.*, 1990). In these cases, weak relationships between species occurrence and abiotic environmental factors seem to suggest that biotic factors and interactions also play fundamental roles in structuring planktonic communities in terms of species composition. The spatial and temporal uniformity of species assemblages suggests a stable community actively structured and maintained by interactions between its members (McGowan and Walker, 1979). Species associations have long been considered sounder elements than single species in monitoring ecosystem dynamics (e.g. Colebrook *et al.*, 1961). Associations have been shown to represent a valuable tool to record environmental changes, such as episodic events and regime shifts, in relation to climate variability throughout extensive observations in space and time (Beaugrand *et al.*, 2002b; Beaugrand, 2004).

To capture the modes of population and community structure and evolution at different temporal scales, long-term time-series are the only appropriate tools to depict average patterns and estimate the extension of their variance. In the Mediterranean Sea, four ongoing time-series are monitoring the pelagic system in neritic zones, i.e. in the Balearic Sea (Fernandez de Puelles *et al.*, 2003), Ligurian Sea (Molinero *et al.*, 2005; Licandro *et al.*, 2006), Tyrrhenian Sea (Ribera d'Alcalà *et al.*, 2004; Zingone *et al.*, 2010) and in the Adriatic Sea (Cataletto *et al.*, 1995; Kamburska and Fonda-Umani, 2006). In the Gulf of Naples (Tyrrhenian Sea), plankton communities have been investigated at a fixed station (stn MC) since 1984, and since 2006 the *MareChiara* time-series has been part of the international network Long Term Ecological Research (LTER). This long-term study is aimed at characterizing the structure of coastal plankton communities in terms of standing stocks and species composition and at following their

variability at different temporal scales in relation to environmental conditions. The sampling site is located in a coastal eutrophic area that is characterized by high temporal variability of physical and chemical properties and is influenced by land runoff from a very densely populated region (Ribera d'Alcalà *et al.*, 2004). However, due to the general geomorphology and bottom topography of the Gulf, the inner shelf is also coupled with the offshore oligotrophic waters of the Tyrrhenian Sea (Carrada *et al.*, 1980).

During the first 7 years of LTER-MC, the mesozooplankton communities showed notable recurrent patterns in species structure and succession and a strong tendency to maintain a stable standing stock, even in the presence of variable environmental conditions (Mazzocchi and Ribera d'Alcalà, 1995). The analysis of plankton dynamics at seasonal scale from 1984 to 2000 showed that the different phases of the annual cycle and the occurrence of several phyto- and zooplankton species were remarkably regular in spite of interannual variations in autotrophic biomass (Ribera d'Alcalà *et al.*, 2004).

Given the previous detection of regular seasonal patterns and succession in mesozooplankton species at stn MC, in the present study, we investigated more deeply the extent of the stability of seasonal patterns and succession at the community level. Our specific aims were: (i) identifying zooplankton associations and their characteristic features, and (ii) detecting homogeneous periods both at both seasonal and inter-annual scales based on species associations. Species associations that can be identified as robust elements in zooplankton community structure, notwithstanding environmental variability, can represent useful sentinels for the early detection of variations in the system that can be related to major disturbance at local or larger scales (Beaugrand *et al.*, 2002a).

METHOD

Zooplankton sampling and analysis

The sampling site stn MC (40°48.5'N, 14°15'E) is located close to the 75 m isobath, two nautical miles from the coastline facing the city of Naples (Fig. 1). Physical and chemical parameters and plankton communities have been monitored since January 1984, with a major interruption from 1991 through February 1995. The sampling frequency was biweekly until 1990 and has been weekly since 1995 (see Ribera d'Alcalà *et al.*, 2004 for details). Here we report on mesozooplankton composition and abundance (ind. m⁻³) from the beginning of the series until 2006.

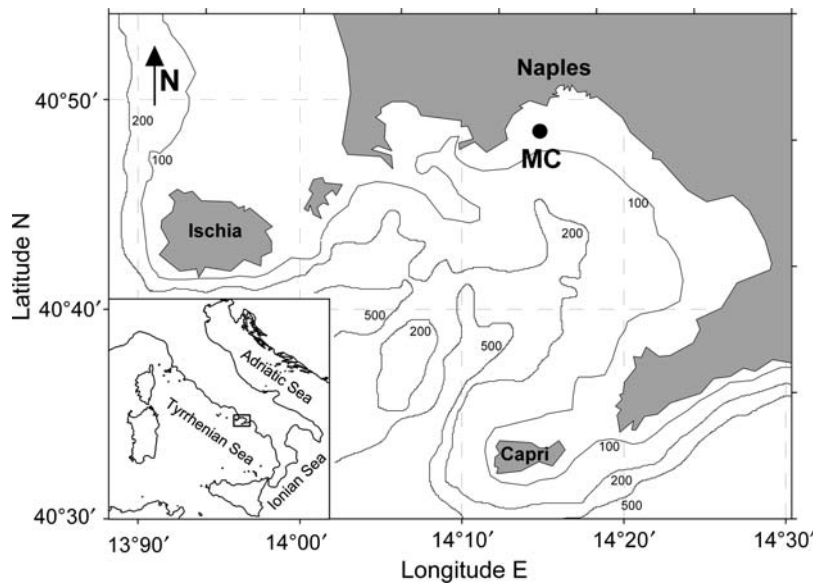


Fig. 1. Map of the Gulf of Naples (Tyrrhenian Sea, Western Mediterranean) with the location of stn MC, site of the LTER-MC time-series.

Zooplankton samples were collected by vertical tows in the upper 50 m with a Nansen net (200 μm mesh, 113 cm mouth diameter) and fixed with buffered formaldehyde (2–4% final concentration). Before microscopic analysis, the fixed sample was concentrated and re-suspended in filtered sea water up to 200 ml volume. Aliquots ranging from 1/4 to 1/32, according to sample density, were taken by using the Huntsman beaker subsampling technique (Van Guelpen *et al.*, 1982) or, more recently, by using a large-bore graduated pipette. In these aliquots, all zooplankters were identified and counted, while the rest of the sample was checked for the presence of rare species. Adult copepods were separated by gender for most species; males were identified at genus level for *Clausocalanus*, *Calocalanus* and *Oithona*, and at family level for Oncaeidae and Corycaidae. Juveniles (from CII or CIII according to species were efficiently retained by 200 μm mesh) were identified at the species level in most cases, or grouped at genus or family level (e.g. *Calocalanus*, *Oithona*, Oncaeidae, Corycaidae). Juveniles of *Clausocalanus* spp. and *Paracalanus parvus* were pooled together for the present work because of uncertainties in distinguishing them during the first years of this series. The species abundance reported here corresponds to the whole population (adult females and males, and copepodites) unless otherwise indicated. Among other zooplankters, some groups were identified at the species level during most years of the series (e.g. cladocerans, chaetognaths, siphonophores), while others were always identified at a higher taxonomic level.

Temperature, salinity and chlorophyll-*a* (chl-*a*) depth-averaged over the 0–70 m water column are reported as basic parameters to depict the environmental characteristics at the sampling site at both the seasonal and long-term scales. The hydrocast was performed using Niskin bottles equipped with reversing thermometers during the 1984–1990 period and on a few occasions during the following 1995–2000 period. Starting from 1995, CTD and fluorescence profiles were obtained with a SBE911 mounted on a Rosette sampler equipped with twelve 5 L Niskin bottles. Total chl-*a* concentration was determined at seven selected depths using a spectrophotometer until 1990 and a spectrofluorometer from 1995 onwards. Further details on sampling and analytical procedures are reported in Ribera d'Alcalà *et al.* (Ribera d'Alcalà *et al.*, 2004). To represent the structure of the water column, data were binned with 5 m resolution. The mixed layer depth Z_{ml} was chosen as the first depth where $\Delta\gamma_{\theta} \geq 0.05$ over a 5 m depth interval (Mitchel and Holm-Hansen, 1991). When $\Delta z > 5$ m (discrete Niskin samples), Z_{ml} was chosen as the first depth where $\Delta\gamma_{\theta} \geq 0.05$ when compared with the previous sampled depth (max $\Delta z = 10$ m).

Statistical analysis

Zooplankton abundance data were organized in a matrix of 93 taxonomic categories \times 609 raw observations for the whole period 1984–2006. Only 16 taxa that occurred in our samples with frequency $< 2\%$ were eliminated from the analysis, since their presence was

considered occasional. The zooplankton categories are heterogeneous in terms of taxonomic composition, from species to families for the most abundant groups (e.g. copepods, cladocerans), from genus to class level for the less abundant or rare groups. In some cases (e.g. appendicularians, salps, chaetognaths, siphonophores, meroplankton larvae and a few copepod genera), our identification was limited to higher-than-species level during the first years of the time-series and, for the present analysis, we considered this level in order to maintain homogeneity throughout the whole period. In other cases, when species belonging to the same genus (e.g. *Candacia*, *Euchaeta*, *Lucicutia*, *Vetoria*, etc.) or the same family (e.g. Eucalanidae for *Eucalanus*, *Subeucalanus*; Pontellidae for *Pontella*, *Anomalocera*, *Labidocera*) were all rare and with negligible abundance, they were grouped at the genus or family levels in order that they did not disappear in the analysis. This choice of considering heterogeneous taxa as primary indicators has limits, but allowed us to maintain more complete information on community composition. Obviously, finer identification produces more accurate information and therefore this would be preferable to identification at a broader taxonomic level. On the other hand, producing a time-series with counts of every single plankton species is something hard to achieve as the identification of some taxonomic groups (e.g. meroplankton larvae) is difficult and needs to be verified by (a very few available) taxonomic experts. To our knowledge, no plankton time-series provides information at the species level for all taxonomic groups. Nevertheless, different ordination techniques (including clustering) have been applied to time-series of heterogeneous plankton taxa (e.g. Licandro and Ibanez, 2000; Beaugrand *et al.*, 2002a, among others). Although relationships cannot be always explored at a fine taxonomic level such as that of the species, still a large taxonomic group can be associated with well-defined biological and ecological characteristics that can be used to clarify the interactions and changes observed within the zooplankton community in the Gulf of Naples.

The zooplankton dataset was analyzed with two different approaches (Legendre and Legendre, 1998): (i) R-mode classification was used based on a matrix of similarities between species to discern associations in the whole zooplankton community; (ii) Q-mode classification was performed based on a matrix of similarities between observations to find out homogeneous periods in the whole time-series.

To calculate the similarity between two species or two observations (dates), the Hellinger distance (Rao, 1995) was applied to the log-transformed zooplankton

abundance. This metric distance gives low weights to rare species and is well adapted to abundance data (see Legendre and Legendre, 1998 for details).

Considering the species abundance table $Y = [y_{ij}]$ of size $(n \times p)$ with dates (rows) $i = \{1, \dots, n\}$ and species (columns) $j = \{1, \dots, p\}$, the row sums are y_{i+} and the column sums are y_{+j} .

The Hellinger distance between species a and b was calculated according to Legendre and Gallagher (Legendre and Gallagher, 2001) as follows:

$$D(\text{species}_a, \text{species}_b) = \sqrt{\sum_{i=1}^n \left[\sqrt{\frac{y_{ai}}{y_{+a}}} - \sqrt{\frac{y_{bi}}{y_{+b}}} \right]^2}$$

and the Hellinger distance between dates c and d was calculated as follows:

$$D(\text{date}_c, \text{date}_d) = \sqrt{\sum_{j=1}^p \left[\sqrt{\frac{y_{cj}}{y_{c+}}} - \sqrt{\frac{y_{dj}}{y_{d+}}} \right]^2}$$

On the basis of the similarity matrices, flexible agglomerative hierarchical clustering was used for the R-mode and the Q-mode classifications, with beta set at the value of -0.25 as an intermediate solution between single and complete linkage (Lance and Williams, 1967).

The optimal number of clusters in the dendrograms resulting from the R-mode and Q-mode analyses was computed with the “gap statistic”, a statistical procedure proposed for estimating the appropriate number of groups in a set of data that is applicable to any clustering method (Tibshirani *et al.*, 2001). This method compares the change in within-cluster dispersion with that expected under an appropriate reference null distribution of the data. The rationale behind the gap statistic and the detailed procedure for the step by step computation are reported in Tibshirani *et al.* (Tibshirani *et al.*, 2001).

Clustering of species associations and observations were both represented in “heat map” plots of the raw and frequency transformed dataset (e.g. Eisen *et al.*, 1998). A heat map is a graphical representation of data where the values taken by a variable in a two-dimensional map are represented by squares with color gradients, i.e. darker squares for higher values and lighter squares for lower values.

To identify species assemblages that characterize each period, the Indicator Value index (IndVal, Dufrene and Legendre *et al.*, 1997) was calculated for each species based on the observation classification. The IndVal index combines the species relative abundance (the so-called specificity, A_{jk}) with the species relative

frequency of occurrence in a given group of observations (the so-called fidelity, B_{jk}):

$$\text{IndVal}_{jk} = A_{jk} \times B_{jk} \times 100$$

A_{jk} is the ratio between the mean abundance of the species j in the observations of the group k and the sum of the mean abundance of the species j in all groups:

$$A_{jk} = \frac{N_{sp_{jk}}}{N_{sp_{+k}}}$$

B_{jk} is the ratio between the number of observations in the group k where the taxon j is present and the total number of observations in k :

$$B_{jk} = \frac{N_{obs_{jk}}}{N_{obs_{+k}}}$$

The IndVal analysis identifies the most characteristic species in each period not only on the basis of their highest abundance but also on their regular occurrence in that period. Therefore, the IndVal index is maximum when all individuals of a species are found in a single group of observations and when the species occurs in all observations of that group. Following Dufrene and Legendre (Dufrene and Legendre, 1997), only indicator values $\geq 25\%$ were retained.

The groups of species assembled by the R-mode classification were validated by comparison with the zooplankton associations identified by the IndVal analysis, as the two methods provide a different type of information: while the first method implies a hierarchical link between the different taxa, the second one allows identification of the most characteristic species independently from other taxa in the assemblage.

Interannual changes in zooplankton associations were represented by annual normalized anomalies (A_i). For each association, the annual means for the 19 years of the series were calculated (\bar{T}_i), the average of these annual means (\bar{T}) was subtracted from each of them and the differences obtained (anomalies) were normalized by the standard deviation of the annual mean values to allow comparison among associations: $A_i = (\bar{T}_i - \bar{T}) / \sigma(\bar{T}_i)$.

To highlight possible long-term changes in the annual cycles of zooplankton associations, we analyzed their phenology with the method of Greve *et al.* (Greve *et al.*, 2005). From the weekly abundances of each association, a cumulative sum of values was calculated. The annual sum was set at 100%, and the week of the passage of the 15, 50 and 85% cumulative abundance levels for each year was determined. These levels are

defined as the “start of the season”, “middle of the season” and “end of the season”, respectively.

RESULTS

Environmental variability

The average annual cycles of depth-averaged temperature, salinity and chl-*a* show that the water column at stn MC was subjected to seasonal forcing (Fig. 2). Temperature ranged from 14°C in March to 19°C in September–October, with the largest interannual variability recorded in the summer months. Salinity was highest in November–December (37.98 psu) and lowest in May (37.84 psu). The mean annual cycle of chl-*a* was characterized by three successive peaks, the major one in March (0.97 µg L⁻¹) and the others in May (0.77 µg L⁻¹) and October (0.65 µg L⁻¹), while the minima were recorded in January and December (~0.4 µg L⁻¹).

In the long term, the seasonal values of the environmental parameters showed a pronounced interannual variability (Fig. 3). The most notable patterns were the increasing trend of summer temperature and the general decreasing trend of chl-*a*. Salinity showed multi-year oscillations without clear trends in any of the four seasons.

The average annual cycle of the water column structure showed that fully mixed conditions down to 60–70 m depth occurred from December to mid-March, while the seasonal stratification started in late March and lasted until October, with the upper 10 m layer being stable in the period May–August (Fig. 4A). A remarkable interannual variability was observed in the mean annual depth of the mixed layer, with very similar patterns at the beginning (1985–1990) and at the end (2001–2006) of the study period (Fig. 4B). Those patterns were characterized by an increasing trend of the extension of the mixed layer and differed from what observed during the central years (1996–1999), when the mean depths of the mixed layer were more similar.

General features of zooplankton abundance and composition

Mesozooplankton abundance at stn MC averaged 1708 ind. m⁻³ over the whole series; it showed less variability in 1984–1990 than in 1995–2006 (Fig. 5), although interannual averages were similar in the two periods (1799 ± 264 and 1655 ± 431 ind. m⁻³, respectively). The highest values were recorded at the beginning (1984–1985) and at the end (2004–2006) of the

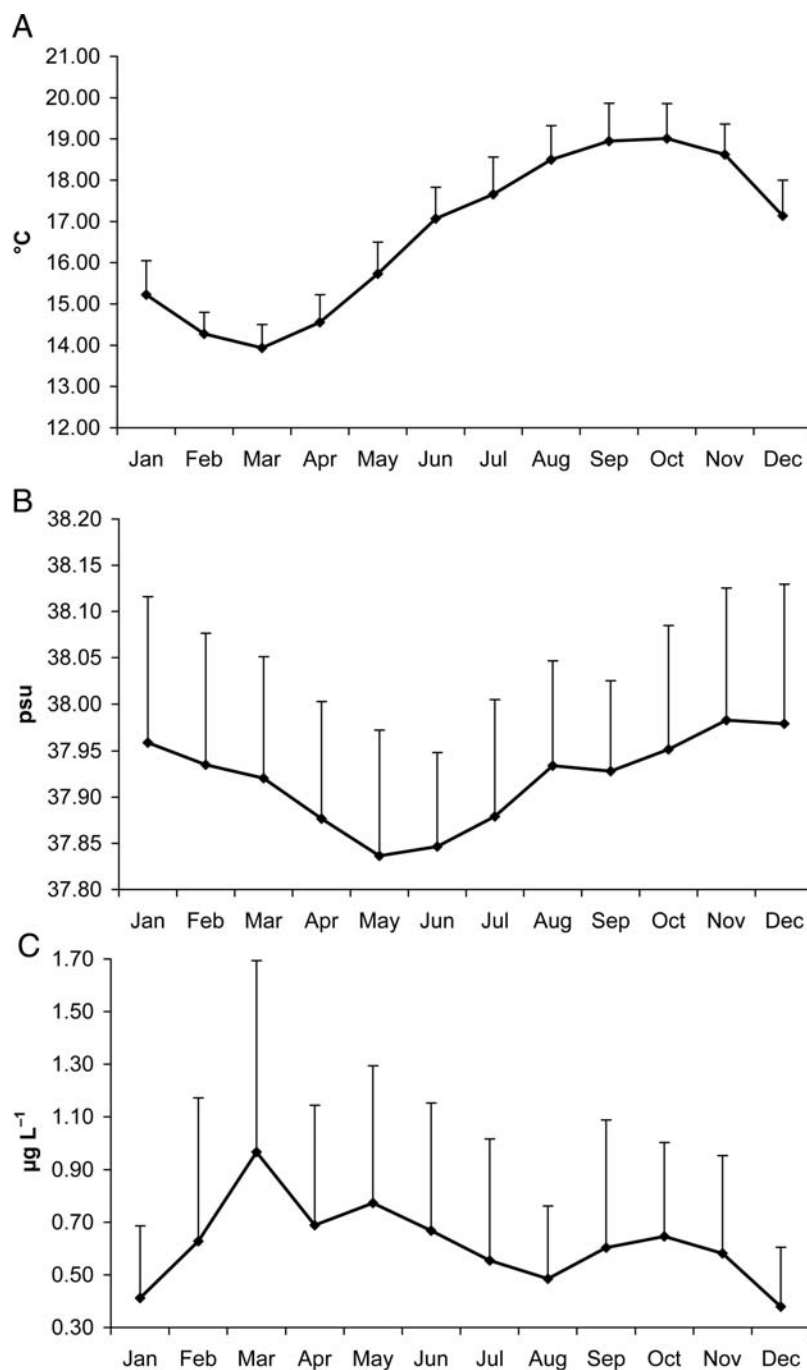


Fig. 2. Mean annual cycle of depth-averaged (0–70 m) environmental parameters at stn MC for the period 1984–2006; **(A)** temperature, °C; **(B)** salinity, psu; **(C)** chl-*a*, µg L⁻¹. Vertical lines represent +1 standard deviation.

series; the minima occurred in 1995–1996 and 2001–2002.

The communities were numerically dominated by copepods, followed by cladocerans, tunicates, meroplankton, cnidarians, chaetognaths and ostracods (Table I). A few other taxonomic groups (e.g. amphipods, pteropods, fish eggs and larvae) were much less abundant and

frequent, thus contributing with negligible percentages (<0.1%) to total zooplankton abundance. Tunicates and meroplankton, which were mainly represented by appendicularians and decapod larvae, respectively, contributed more in the second than in the first period. The bulk of the copepod assemblage was made up of small individuals (≤ 1 mm), namely juveniles of *Clausocalanus* spp. and

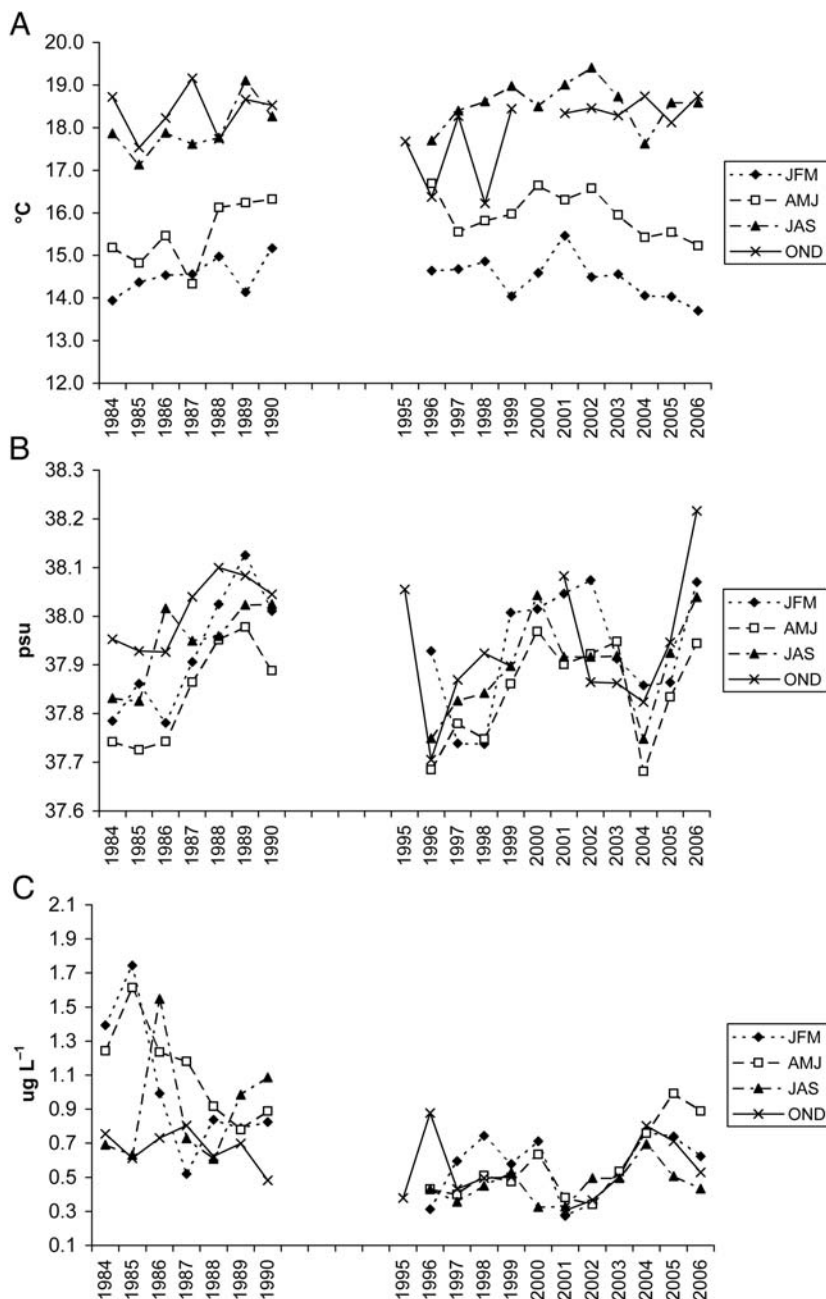


Fig. 3. Interannual variability of depth-averaged (0–70 m) environmental parameters at stn MC, separately shown for winter (JFM), spring (AMJ), summer (JAS) and autumn (OND). (A) temperature, °C; (B) salinity, psu; (C) chl-*a*, $\mu\text{g L}^{-1}$.

P. parvus, and ten other species or genera that ranked very similarly in the two periods (Table I). No major differences were observed in the percentage contribution of the most abundant species, with the exception of *Centropages typicus*, which was relatively more important in the first than in the second period. In contrast, *Calocalanus* spp. and *Ctenocalanus vanus* were slightly more important in the second than in the first period.

Species associations

The dendrogram resulting from the R-mode classification applied to the whole dataset (1984–2006) shows that mesozooplankton communities at stn MC can be partitioned into different species associations (Fig. 6). Five clusters are well separated, as indicated by the gap statistic (Fig. 7). The first cluster (A1) gathers together the very abundant copepods *Acartia clausi* and *C. typicus*

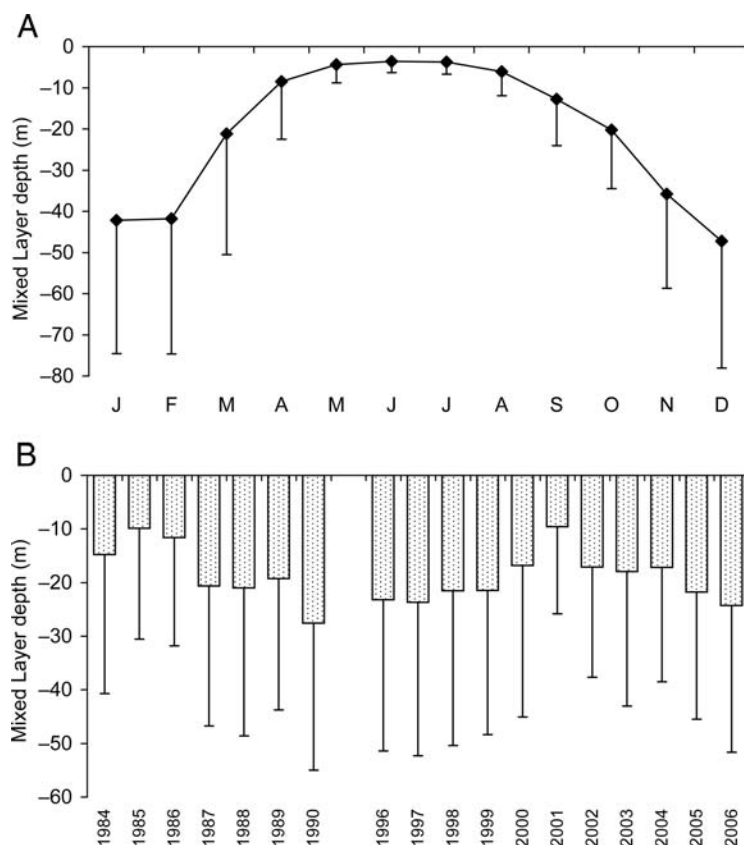


Fig. 4. Seasonal (A) and interannual (B) variability of the depth of the mixed layer at stn MC. The year 1995 was excluded from the computation of the monthly and annual means because of numerous missing values. Vertical lines represent -1 standard deviation.

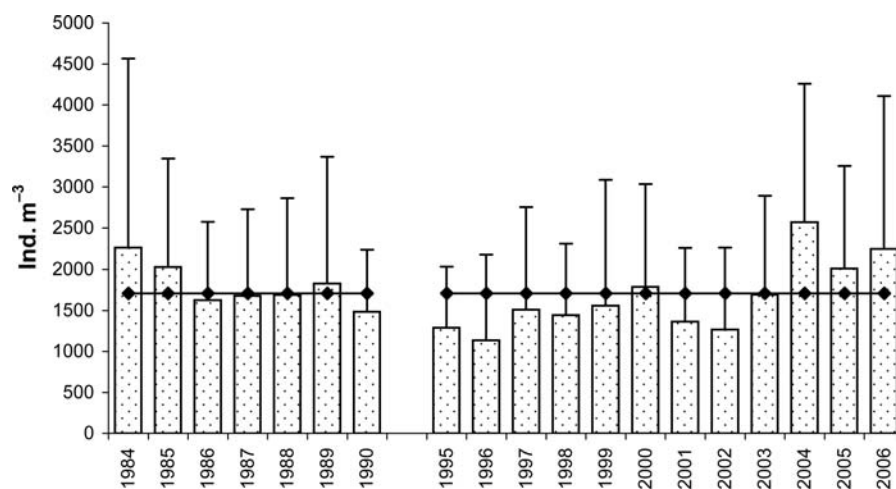


Fig. 5. Mesozooplankton abundance (Ind. m^{-3} ± 1 standard deviation) at stn MC; annual means (columns) and interannual mean for the whole period (horizontal line).

that peak in spring–early summer, the common spring copepods *Clausocalanus pargens*, *Oithona similis*, *Isias clavipes* and *Oithona nana*, and some less common spring taxa (the cladocerans *Podon* and *Pleopis*, bivalve larvae, the

copepods *Oithona atlantica*, *Acartia discaudata*, *Calanus helgolandicus*, Eucalanidae). The second cluster (A2) groups the summer zooplankton, which comprise very abundant cladocerans (*Penilia avirostris*, *Evadne spinifera* and

Table I: Percentage contribution of major groups and species to, respectively, total mesozooplankton and total copepod abundances in the two periods of the time-series at stn MC

	1984–1990 (%)	1995–2006 (%)
Mesozooplankton		
Copepods	69.4	62.1
Cladocerans	18.8	21.2
Tunicates	8.2	11.8
Meroplankton	3.0	3.7
Cnidarians	0.4	0.3
Chaetognaths	0.2	0.6
Ostracods	0.1	0.1
Sum	100.0	99.8
Copepod		
(<i>Clausocalanus</i> spp. + <i>P. parvus</i>)	28.5	28.3
copepodites		
<i>Acartia clausi</i>	15.1	14.7
<i>Paracalanus parvus</i> adults	12.5	12.3
<i>Centropages typicus</i>	11.2	7.5
<i>Clausocalanus</i> spp. adults	6.8	7.4
<i>Temora stylifera</i>	5.4	6.8
<i>Oithona</i> spp. copepodites	4.9	5.8
Oncaeids	3.5	3.1
<i>Calocalanus</i> spp.	2.0	3.3
<i>Oithona similis</i>	1.9	1.2
<i>Ctenocalanus vanus</i>	1.1	1.6
Sum	92.9	92.0

Taxa are listed according to a decreasing rank order. Copepod populations include both adults and copepodites, when not differently specified.

Pseudoevadne tergestina) and less abundant taxa, such as salps, pteropods, and a few uncommon copepods (*Paracartia grani*, *Paracartia latisetosa*, *Centropages ponticus* and *Centropages kröyeri*). The third cluster (A3) includes a large number of taxa and copepod species that occur at a very low frequency or even rarely at stn MC. A subgroup of this cluster assembles a few copepod species that are typical of the open sea and occur at our site with negligible abundance and generally in winter (e.g. *Haloptilus* spp., *Heterorhabdus papilliger*, *Lucicutia* spp., *Scolecithricella* spp.). The fourth cluster (A4) associates abundant (*Temora stylifera*, *Clausocalanus furcatus*, *Oithona plumifera*), less abundant (*Centropages violaceus*, *Sapphirina* spp.) and rare (*Acartia negligens*) copepods together with chaetognaths and doliolids, all taxa with peaks in autumn. The fifth cluster (A5) is similarly large as the third one and comprises taxa whose peaks span from October until June. This cluster includes ostracods and numerous copepod species (e.g. *Euchaeta* spp., *Neocalanus gracilis*, *Pleuromamma* spp., six *Clausocalanus* species, *C. vanus*) that thrive in off-shore waters. It includes also meroplankton that peak in winter (e.g. larvae of echinoderms and cirripeds), and decapod larvae that peak in spring.

Zooplankton associations identified by the cluster analysis show very different annual cycles (Fig. 8). A1 is characterized by a peak period extended from April to July, mainly driven by the high abundances of *A. clausi* and *C. typicus*. A2 has a narrow seasonal cycle lasting from June until October, with a steep and notable peak in August that is made by the outburst of cladocerans. A3 shows by far the lowest abundance and is almost restricted to the winter months. A4 abundance starts to increase slowly from May to July, reaches a peak in October and almost disappears in December. A5 is the most abundant association and has an extended seasonal cycle. It accounts for most of the winter zooplankton and shows two distinct peak periods, in March–April and in August. The first peak is due to a diversified group of taxa (*Oithona* spp., *Clausocalanus* spp. juveniles, adults of *Clausocalanus paululus*, *C. pergens*, and *C. arcuicornis*, oncaeids, *Calocalanus* spp., *C. vanus* and meroplankton), while the second peak is due almost exclusively to *P. parvus*.

The five zooplankton associations differ also in their long-term variability, represented in Fig. 9 as annual anomalies, whose patterns were mainly shaped by the most abundant species in each cluster. The annual anomalies of A1 were positive during the first part of the time-series and alternated between negative and positive phases in the second part; the generally decreasing trend was mostly driven by *C. typicus* in the first part and by *A. clausi* in the second part. A2 anomalies had an increasing trend in the second part of the series, shaped by *P. avirostris*. The anomalies of A3 changed notably throughout the years without any clear pattern, while those of A4 remained close to zero during most of the series until a notable positive peak appeared in 2004, due to *T. stylifera*. The anomalies of A5 were positive only at the beginning and at the end of the series and close to zero or slightly negative in the rest of the years, closely resembling the long-term pattern of the abundant group composed by juveniles of *Clausocalanus* spp. + *P. parvus*. Interestingly, the patterns of A2 (summer) and A4 (autumn) were similar and phased from the year 2000 onwards.

During the period under study, a few interesting changes could be observed in the phenology of two out of the five zooplankton associations (Fig. 10). The typical season of the abundant A1 became early and shorter, from mid May–late July to April–May. For the broad and scattered season of A3, composed of rare species, the middle and end phases advanced by 2–3 months at the end of the series. Differently, A2 (summer) and A4 (autumn) maintained restricted and quite stable timing, and also A5, which spanned through a large part of the year, did not show

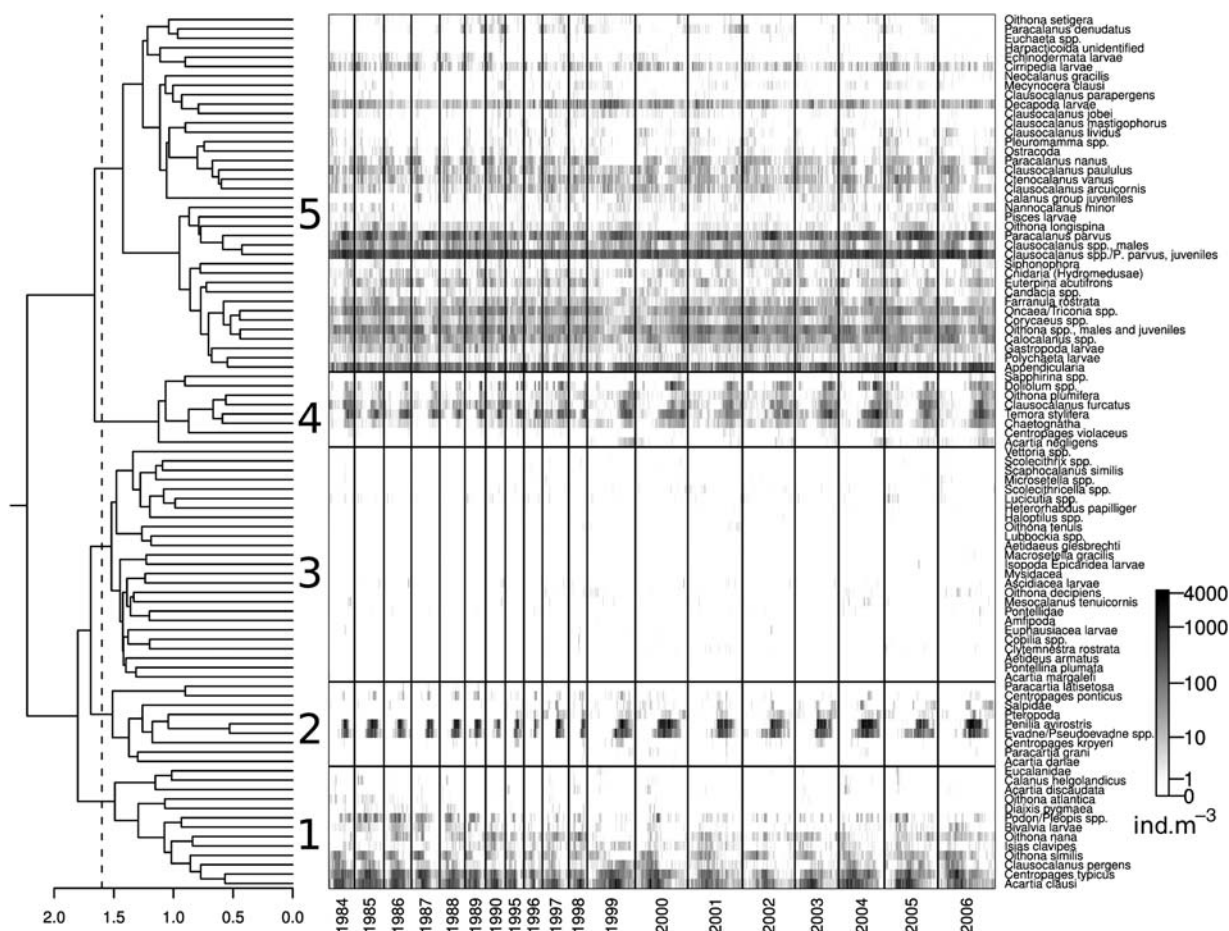


Fig. 6. Temporal distribution of the abundance (Ind. m^{-3} , $\text{Log}_{10} + 1$ transformed) of the 93 selected zooplankton taxa from the LTER-MC dataset in the period 1984–2006. Species were ordered according to the results of the hierarchical classification based on the Hellinger distance and the flexible average linkage clustering. The vertical lines separate the years; the difference in column width depends on data frequency (from weekly to biweekly or sometimes monthly). The horizontal lines mark the separation between clusters.

remarkable variability in phenology during the series, with the exception of 1984 when the season started 2 months later than usual.

Homogeneous periods

The hierarchical classification clustered the observations of our time-series in five homogeneous periods (Fig. 11), as estimated by the gap statistic (Fig. 12). Within each period, the observations displayed similar and persistent features over the years. Cluster 1 identified the winter–early spring period (P1); cluster 2 grouped mid-spring–early summer dates (P2); clusters 3 and 4 separated the mid-summer (P3) from the late summer–early autumn dates (P4); cluster 5 grouped the autumn–early winter dates (P5).

Each of the five periods is characterized by a group of zooplankton taxa as indicated by the IndVal values (Table II). Winter–early spring (P1) is denoted by nine

taxa, all contributing quite similarly, in terms of both relative abundance (A_{ij} , specificity) and frequency (B_{ij} , fidelity); most of these taxa are grouped in A5. Mid-spring–early summer (P2) is mainly characterized by *A. clausi* and *C. typicus*, which are determinant for both abundance and frequency. Another eight taxa contribute to this period more for their regular occurrence than for abundance; most of them belong to A1, and only three of them to A5. Mid-summer (P3) is identified by nine taxa that are clearly dominated by cladocerans (*P. avirostris*, *Evadne* and *Pseudoevadne*). The contribution of *P. parvus* is lower in relative abundance, but highest in relative frequency, similar to juveniles of *Clausocalanus* spp. and *P. parvus*, and the less abundant *Farranula rostrata*. This period encompasses a similar number of taxa belonging to A2 and A5. Late summer–early autumn (P4) is strongly characterized by *Doliolum* spp. and *T. stylifera*, both with high relative abundance and frequency. The other eight taxa follow with lower and similar

IndVal. All of them are good indicators of this period mainly because of their very regular occurrence ($B_{ij} = 0.94\text{--}1.00$), with the only exception of *Sapphirina* spp. ($B_{ij} = 0.73$). Six taxa in P4 are clustered in A4 and four taxa in A5. Finally, autumn–early winter (P5) is the least characterized period, with very low IndVal values, which are slightly higher than 25% for only four copepod species, i.e. *Paracalanus denudatus*, *C. violaceus*, *Nannocalanus minor*, *Calocalanus* spp. These copepods, and in particular the latter two species, are much more important for their relative frequency than for their relative abundance.

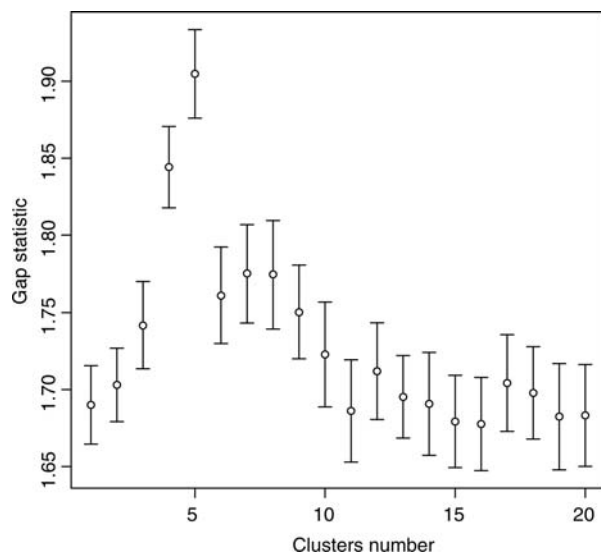


Fig. 7. Results of the “gap statistic” method (Tibshirani *et al.*, 2001) applied to the clustering of zooplankton species in the LTER-MC and indicating that the estimated numbers of clusters is 5.

For the observation clustering, the gap statistic also identified twelve clusters (Fig. 12), with a further fragmentation of winter and spring, and some interannual variability without any discernible pattern or trend (not shown).

DISCUSSION

The present work was aimed at establishing whether species associations and homogeneous periods could be identified in the time course of mesozooplankton communities at a coastal site subjected to pronounced environmental fluctuations. The site of the LTER-MC time-series is characterized by dynamic interactions between the coastal and the open sea systems, which result in changes in the physical and chemical properties of the water column at different time-scales, from weekly to seasonal and interannual, as shown by present and previous results (Ribera d’Alcalà *et al.*, 2004; Zingone *et al.*, 2010). Clear seasonal signals appear in the patterns of basic (this study) and more numerous (Ribera d’Alcalà *et al.*, 2004) environmental parameters, and in the abundance and species composition of pelagic communities, from phytoplankton (Zingone *et al.*, 1990, 2010) to microzooplankton (Modigh and Castaldo, 2002) and mesozooplankton (Mazzocchi and Ribera d’Alcalà, 1995). The overall seasonal pattern of the water column structure at stn MC is typically driven by the seasonal cycle of heat flux, which interplays with freshwater inputs due to coastal runoff. Because of the pronounced irregularity of freshwater contributions, the mixed layer depth may display abrupt changes even between two close sampling events, as revealed by its extensive variability especially

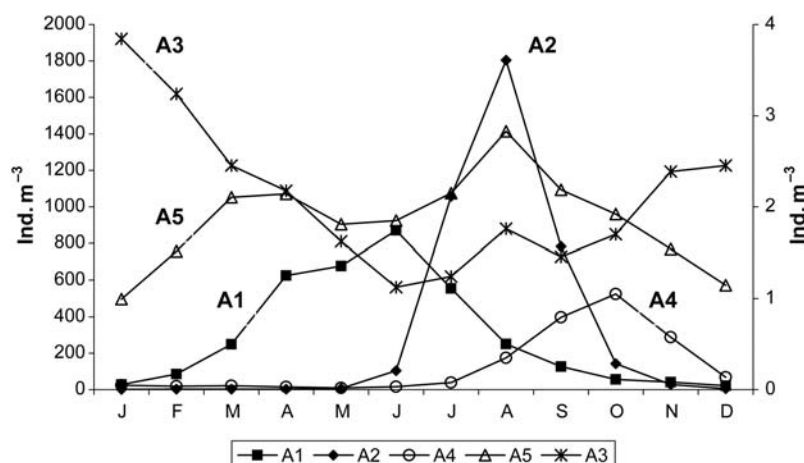


Fig. 8. Average annual cycles of abundance of the five zooplankton associations identified by the cluster analysis presented in Fig. 6. Association A3 is represented on the y -right axis because of its very low abundance.

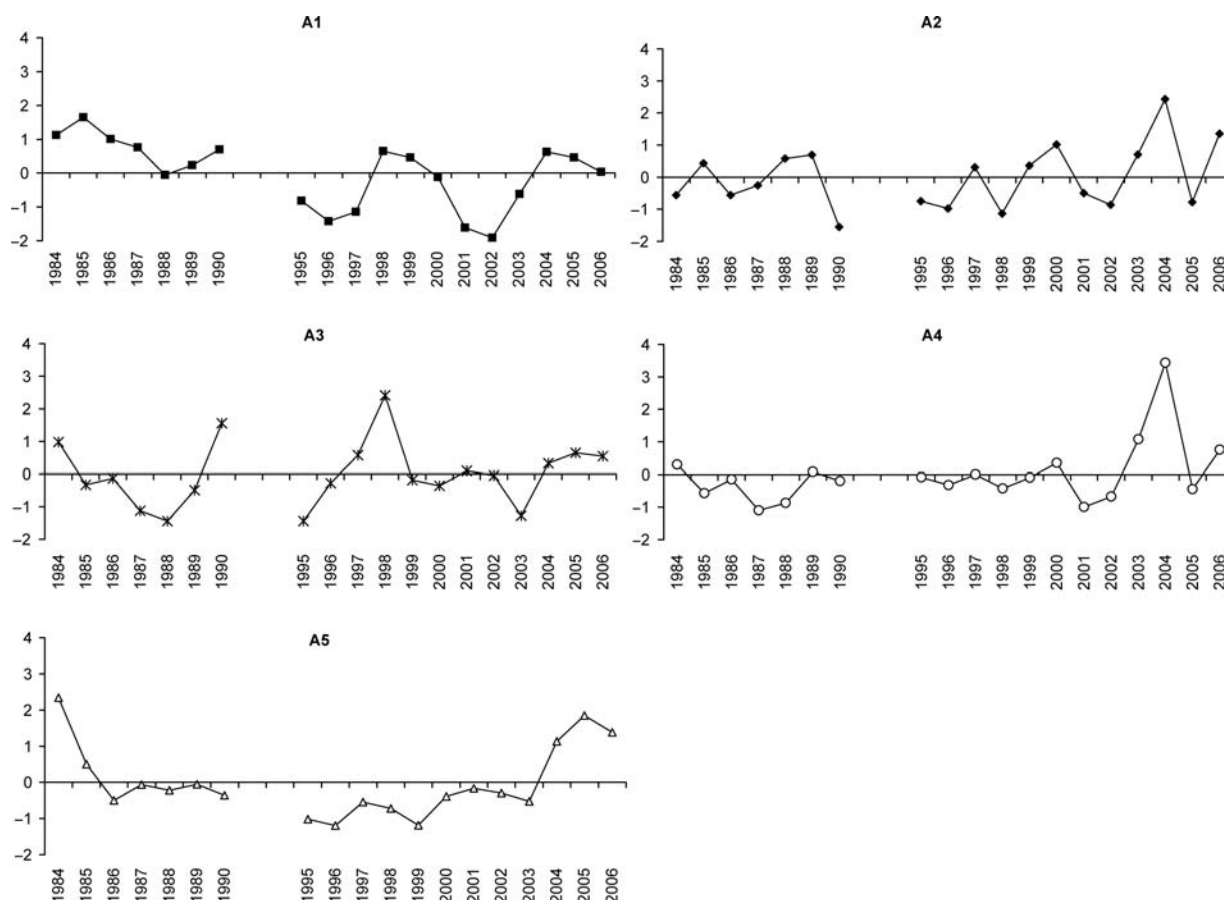


Fig. 9. Annual anomalies of the abundance of the five zooplankton associations identified by the cluster analysis presented in Fig. 6.

in the winter months. Over the long term, neither a monotonic trend nor a shift has appeared in the depth of the mixed layer during the period under study, but three multi-annual phases. The first and the last phases, lasting 6 years each, were characterized by a tendency of the mean annual mixed layer to deepen from ~ 10 to ~ 25 – 30 m, while the central phase showed a remarkable stability of the annual mean around 22 m. Analyzing in greater detail the dynamics of winter phytoplankton in the same period 1984–2006, Zingone *et al.* (Zingone *et al.*, 2010) revealed a significant trend in the frequency of fully mixed conditions in relation to the intensification of winds. This trend was accompanied by a significant decline in the late-winter peaks of chl-*a* (Zingone *et al.*, 2010; present Fig. 3C), which were frequent until 2000 and mostly caused by colonial diatoms and much lower in recent years and mainly caused by small flagellates and small non-colonial diatoms (Zingone *et al.*, 2010). It seems therefore that the environmental characteristics at stn MC are subjected to extensive variability affecting water column and autotrophic communities over a wide range

of temporal scales. However, notwithstanding the remarkable variability of local conditions, five clusters of different size and taxonomic composition have been identified in the present study as robust associations in the zooplankton communities.

Zooplankton associations have been generally identified in the space domain, as in freshwater lakes (e.g. Pinel-Alloul *et al.*, 1990), in estuarine environments (e.g. Castel and Courties, 1982) and in the sea at the large scale of biomes (Longhurst, 1998) and at smaller regional scales (e.g. Berasategui *et al.*, 2006). For spatial associations identified in neritic regions with complex hydrographic regimes (Berasategui *et al.*, 2006) or in the open ocean (Beaugrand *et al.*, 2002b), it appears that hydrodynamics is a determinant factor denoting strong relationships between association boundaries and oceanographic structures. The strong spatial autocorrelation of plankton community structure that was identified over scales of 50 km off British Columbia revealed low temporal stability, with coherence not longer than 1–10 days (Mackas and Sefton, 1982). Here we show that robust zooplankton associations can also persist

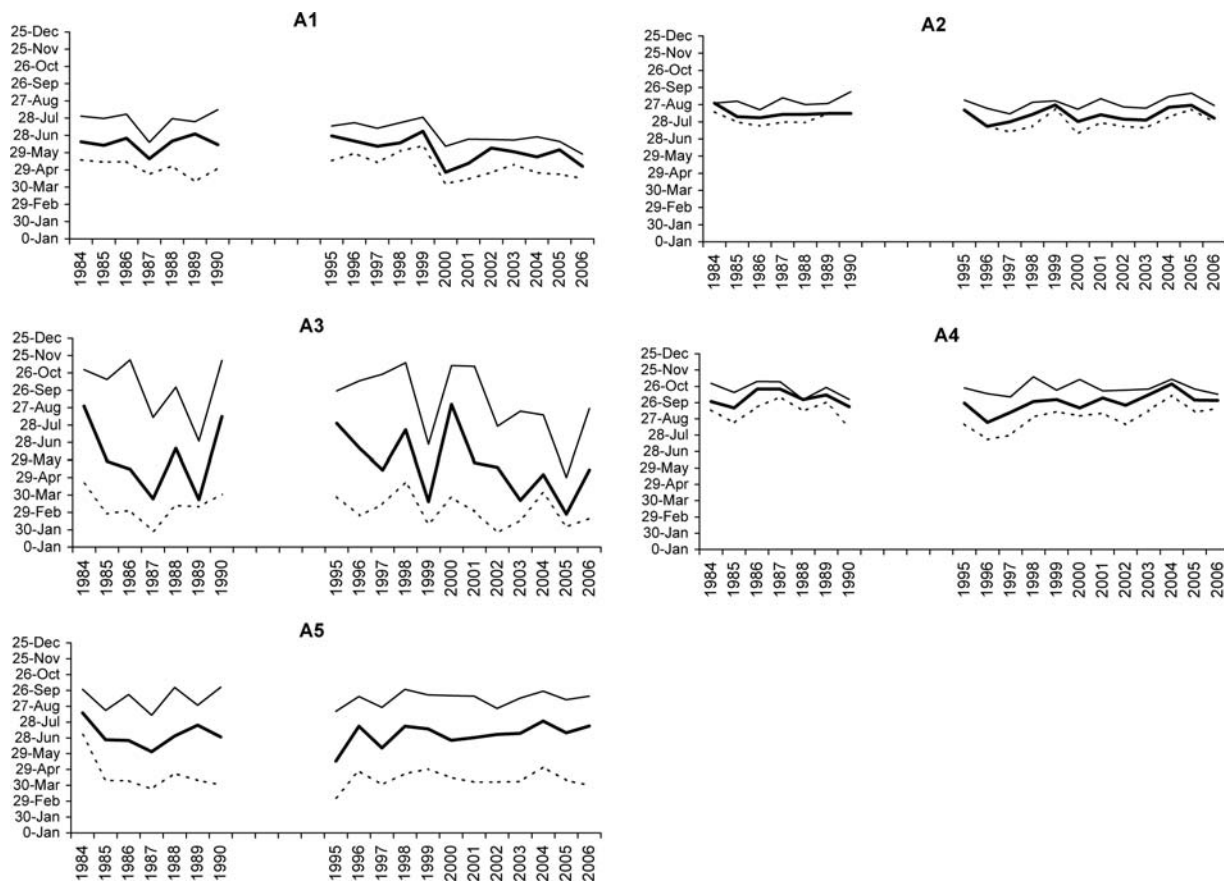


Fig. 10. Long-term variability in the seasonal cycle of the five zooplankton associations (A1–A5) recorded at stn MC. The phenology of each association is indicated by the start (dotted line), middle (continuous bold line), and end (continuous regular line) part of the season based on cumulative abundances according to Greve *et al.* (Greve *et al.*, 2005).

over the long term. Common features or tight interactions may represent strong linking factors between the elements of an association. The common feature grouping taxa within the five associations found in the LTER-MC is basically the seasonal cycle.

The seasonal signature

The annual cycle is very clearly shaped for both the summer (A2) and autumn (A4) associations, which include a limited number of species that have a very regular timing of occurrence and a limited period of highest abundance. The evident difference between the species characterizing the two associations is related to their life history traits. Most of the autumnal species are present and reproduce throughout the year, although they occur with few individuals out of their peak season. This is the case of the copepods *T. stylifera*, *C. furcatus*, *O. plumifera* and chaetognaths. In contrast, the summer cladocerans *P. avirostris*, *E. spinifera* and *P. tergestina* disappear completely from the water column and

overwinter as resting eggs in the bottom (Egloff *et al.*, 1997), at the end of their abundance cycle characterized at stn MC by very high and steep peaks lasting for a limited period (Mazzocchi and Ribera d'Alcalà, 1995; Ribera d'Alcalà *et al.*, 2004). The associations A1 and A5 cover a longer annual period and largely overlap in spring, though showing clear differences. A1 associates only spring-early summer taxa, the most abundant of which are the calanoids *A. clausi* and *C. typicus*, both very common and abundant in Mediterranean coastal regions. At stn MC, they rank among the dominant species and show overlapping peaks that cover an extended period from April to August (Mazzocchi and Ribera d'Alcalà, 1995). In addition, also the abundant calanoid *C. peregrinus* and the cyclopoid *O. similis* are associated in A1; these two species, which are very common in coastal environments, also thrive in open sea waters over a wide latitudinal range but preferentially in eutrophic conditions (Peralba and Mazzocchi, 2004; Castellani *et al.*, 2005; Peralba *et al.*, 2010). Interestingly, the large suspension feeders *Calanus*

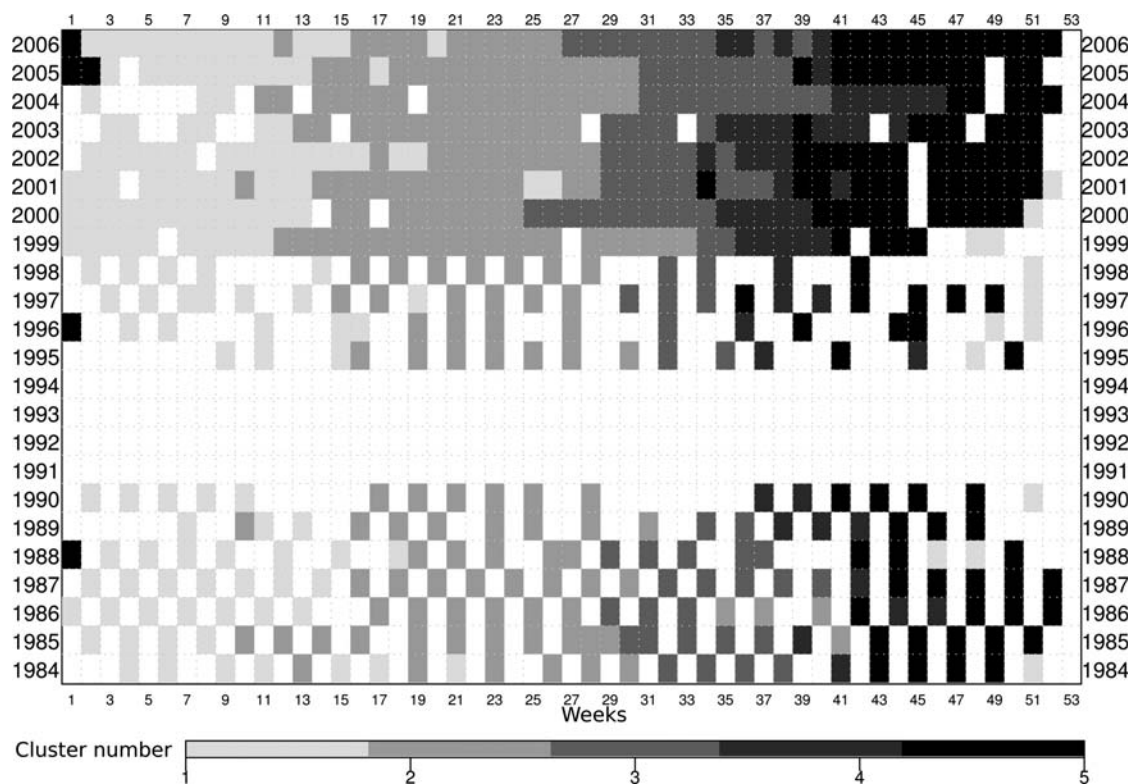


Fig. 11. Clustering of observations for the LTER-MC zooplankton dataset in 1984–2006 (weeks on the *x*-axis, years on the *y*-axis). Each cluster is represented by a different grey density level, as reported at the bottom of the panel; white squares = no data. Taxa characterizing each cluster with IndVal values > 25% are presented in Table II.

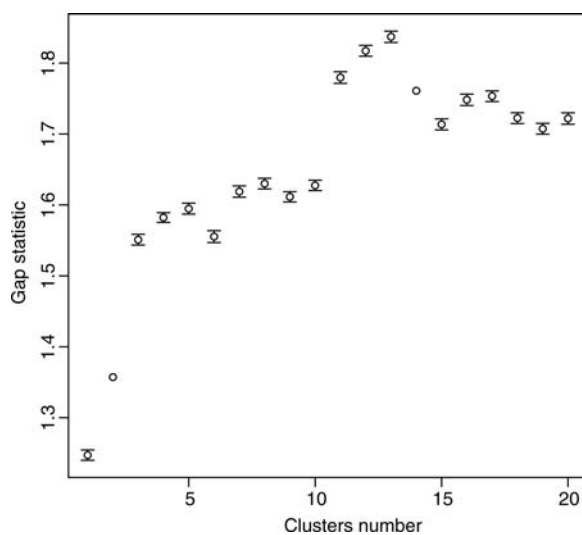


Fig. 12. Results of the “gap statistic” method (Tibshirani *et al.*, 2001) applied to the clustering of observations in the LTER-MC. The distribution of 5 clusters is reported in Fig. 11.

helgolandicus and Eucalanidae are also associated in A1, though they occur at stn MC with low frequency and abundance. The distribution of these large calanoids in the open Mediterranean indicates that they generally

occur in association with high phytoplankton concentrations (Siokou-Frangou *et al.*, 2010). It seems therefore that a common requisite for taxa grouped in A1 is the establishment of a stratified water column and availability of abundant phytoplankton, environmental conditions that occur in spring at stn MC (Ribera d’Alcalà *et al.*, 2004) and are timed with population development of the above-mentioned copepod species (M. G. Mazzocchi *et al.*, submitted for publication).

The association A5 also shows a spring peak but, differently from A1, it encompasses also winter–early spring taxa with populations that grow during the mixed phase of the water column, which lasts at stn MC from December throughout mid-March. A5 appears as a large and very heterogeneous group that includes different categories: common winter copepods (e.g. *Calocalanus* spp., *C. paululus*, *Paracalanus nanus*, among others), less frequent offshore copepods, typical neritic meroplankton and, unexpectedly, also the abundant summer *P. parvus* and the grouped juveniles of *P. parvus* and *Clausocalanus* spp. that represent the most abundant copepod category at stn MC (Table II). Copepods associated in A5 that are not abundant but are regularly present in winter–spring at stn MC (e.g. *N. gracilis*, *N. minor*, *Candacia* spp., *Euchaeta*

Table II: Zooplankton taxa that characterize the five groups identified by the cut-off level of $d=2.5$ in the dendrogram of observations (Fig. 11) for the period 1984–2006 at stn MC

Periods	Taxa	IndVal	A_{ij}	B_{ij}	Associations
P1	<i>Clausocalanus lividus</i>	50.59	0.58	0.87	A5
	<i>Oithona similis</i>	46.91	0.48	0.97	A1
	<i>Pleuromamma</i> spp.	42.69	0.48	0.89	A5
	<i>Clausocalanus paululus</i>	41.04	0.41	1.00	A5
	<i>Paracalanus nanus</i>	38.37	0.39	0.98	A5
	<i>Lucicutia</i> spp.	34.95	0.58	0.60	A3
	Echinodermata larvae	34.86	0.49	0.71	A5
	Cirripedia larvae	32.47	0.37	0.89	A5
P2	Ostracoda	30.87	0.34	0.90	A5
	<i>Acartia clausi</i>	71.64	0.72	1.00	A1
	<i>Centropages typicus</i>	61.33	0.61	1.00	A1
	Decapoda larvae	44.49	0.45	0.99	A5
	<i>Oithona nana</i>	39.26	0.47	0.84	A1
	<i>Podon/Pleopis</i>	38.48	0.50	0.77	A1
	<i>Isias clavipes</i>	36.26	0.40	0.90	A1
	<i>Ctenocalanus vanus</i>	35.80	0.37	0.98	A5
	Bivalvia larvae	35.73	0.53	0.68	A1
	<i>Clausocalanus perges</i>	32.41	0.34	0.94	A1
	<i>Clausocalanus arcuicornis</i>	30.65	0.32	0.95	A5
P3	<i>Penilia avirostris</i>	78.64	0.79	1.00	A2
	<i>Evadne/Pseudoevadne</i>	72.74	0.73	1.00	A2
	<i>Centropages ponticus</i>	49.52	0.76	0.65	A2
	<i>Paracalanus parvus</i>	48.15	0.48	1.00	A5
	<i>Oithona longispina</i>	36.29	0.43	0.85	A5
	Pteropoda	33.58	0.49	0.68	A2
	<i>Clausocalanus</i> spp./ <i>P. parvus</i> copepodites	30.45	0.30	1.00	A5
	<i>Farranula rostrata</i>	27.45	0.27	1.00	A5
	<i>Candacia</i> spp.	26.93	0.29	0.92	A5
P4	<i>Doliolum</i> spp.	71.43	0.78	0.92	A4
	<i>Temora stylifera</i>	64.68	0.65	1.00	A4
	<i>Clausocalanus furcatus</i>	49.72	0.50	1.00	A4
	<i>Oithona plumifera</i>	43.98	0.44	1.00	A4
	Chaetognatha	40.86	0.42	0.98	A4
	<i>Sapphirina</i> spp.	33.14	0.46	0.73	A4
	Siphonophora	31.25	0.33	0.96	A5
	Cnidaria	31.08	0.33	0.94	A5
	<i>Corycaeus</i>	30.44	0.30	1.00	A5
	Appendicularia	28.68	0.29	1.00	A5
P5	<i>Paracalanus denudatus</i>	37.76	0.49	0.78	A5
	<i>Centropages violaceus</i>	33.99	0.41	0.82	A4
	<i>Nannocalanus minor</i>	28.56	0.31	0.93	A5
	<i>Calocalanus</i> spp.	26.20	0.26	1.00	A5

Taxa are sorted by clusters (P) and decreasing IndVal values. The values of relative abundance (A_{ij}) and relative frequency (B_{ij}) are also reported.

spp., *Pleuromamma* spp., *Oithona setigera*, among others) do occur in open and deeper layers (50–300 m) of the Tyrrhenian Sea (Scotto di Carlo *et al.*, 1984). Their presence at stn MC is likely due to the advection of water masses from the open Tyrrhenian Sea, which is more intense in that period of the year (Carrada *et al.*, 1980; Gravili *et al.*, 2001). However, the environmental forcing assembling the other numerous taxa in A5 is not easily identifiable and it is probable that some biological and behavioural interactions play a major role in maintaining

this diversified association. The trophic link can be recognized at least in two cases, as discussed in the following.

In a subgroup of A5, appendicularians are closely associated to the copepods corycaeids, oncaeids and *F. rostrata*. Appendicularians have high growth rates (Hopcroft and Roff, 1995) and high filtration rates (Paffenhöfer, 1973) on a wide size spectrum of particles (Deibel, 1998) and they may match the winter phytoplankton bloom recurring at stn MC better than copepods (Ribera d'Alcalà *et al.*, 2004). Oncaeids are reported to feed on sinking aggregates originating from discharged appendicularian houses (Ohtsuka *et al.*, 1996), and corycaeids and *Farranula* may have the same feeding habit because of similarities in their feeding appendages and motion behaviour (Hwang and Turner, 1995; pers. obs.). Both appendicularians and *Oncaea* have their highest contribution to total abundance in winter, when the annual minimum in mesozooplankton standing stocks is always recorded at stn MC (Ribera d'Alcalà *et al.*, 2004). It seems that in the oligotrophic waters characterising the winter months, oncaeids may benefit from food items that are supplied by appendicularian discharged houses and are not accessible to calanoids. It is therefore probable that this sub-association is based on trophic interactions. Another case where trophic factors can be suggested as possible links between associated zooplankton taxa occurs in the autumnal A4. In this group, the thaliacean *Doliolum* and the copepods *Sapphirina* are closely linked likely because of feeding interactions, since Sapphirinidae have long been known to be parasites and predators of other thaliaceans, i.e. salps (Harbison, 1998).

A very distinct group is A3, which gathers together numerous and various taxa (e.g. *Scolecithrix*, *Scaphocalanus*, *Aetideus*, *Lucicutia*, *Heterorhabdus*, *Microsetella*, Amphipoda, Mysidacea, among others) that always occur at stn MC with a very small number of individuals and can be considered rare contributors to the winter community. These taxa are common in open and deeper waters (Scotto di Carlo *et al.*, 1984) and are likely advected into the inner Gulf of Naples by Tyrrhenian waters, similar to some copepods grouped in A5 and commented above, from which they differ by the remarkable lower abundance. Taxa in A3 have winter peaks as common distinguishing features, but their rarity makes A3 a quite loose community.

The correspondence between the homogeneous periods identified in the LTER-MC and zooplankton associations appear more clearly for spring, summer and autumn, periods strongly characterized by few taxa with very high IndVal values. Spring is indeed dominated by the abundant *A. clausi* and *C. typicus*; summer

by the cladocerans *P. avirostris*, *Evadne* and *Pseudoevadne*; autumn by peaks of doliolids and the copepods *T. stylifera*, *C. furcatus* and *O. plumifera*. The cladoceran *P. avirostris*, together with the small copepod *P. parvus*, are by far the dominant species in the summer communities (Mazzocchi and Ribera d'Alcalà, 1995; Ribera d'Alcalà *et al.*, 2004) and their prevalence is likely related to biological traits of their life cycles. The rapid and sharp increase of the *P. avirostris* population is favored by parthenogenetic reproduction, short developmental time and high growth rates, as reported also for another Mediterranean coastal area (Atienza *et al.*, 2008). The rapid increase of *P. parvus* might be due to the egg hatching time, which is faster than in other co-occurring copepods (Ianora, 1998). The fact that the two species belong to different associations (A2 and A5, respectively) is likely due to the temporal extension of their population cycle, very limited in the former and very extended in the latter (Mazzocchi and Ribera d'Alcalà, 1995). These small grazers, thriving in summer at stn MC, can efficiently exploit the phytoplankton blooms which are well diversified communities characterized by small-size diatoms, often in a non-colonial stage, and by unarmored small dinoflagellates (Zingone *et al.*, 1990; Ribera d'Alcalà *et al.*, 2004). During late autumn–winter, as discussed above, the community is enriched by the occurrence of larger species coming from offshore waters and appears taxonomically more diversified (Ribera d'Alcalà *et al.*, 2004), and the period is consequently less characterized by specific taxa, as reflected by the lower IndVal values. A contrasting scenario has been reported for the 20-years time-series at stn L4 in the English Channel, where zooplankton is more diverse in summer, and strongly dominated by a few species in winter (Eloire *et al.*, 2010), reflecting adaptation to different latitudinal conditions.

The strong seasonal signature of mesozooplankton at stn MC emerges clearly from different perspectives, when approached at the community level (this study) as well as at the group or the species level (Mazzocchi and Ribera d'Alcalà, 1995; Ribera d'Alcalà *et al.*, 2004; M. G. Mazzocchi *et al.*, submitted for publication). Seasonal patterns are evident in reproductive and developmental traits of some subtemperate species that are common at stn MC (Ianora, 1998). However, it is still unclear which are the crucial abiotic and biotic factors and how they interplay in shaping the cycle of population abundance. For example, a recent modeling study combining *in situ* and experimental data for *T. stylifera* has shown that dietary composition affects population growth, but it is far from being the only determinant of the cycle of abundance of this copepod at stn MC

(Mazzocchi *et al.*, 2006). The seasonal changes in zooplankton communities in the inner Gulf of Naples are a marked feature of the succession of zooplankton associations identified in the present study. The persistence of associations in our variable coastal site supports their coherence. However, the complexity of taxonomic composition makes it quite challenging to reveal the precise reasons for this higher-level succession.

Species associations in the long term

In the long term, the five associations behaved differently, indicating independent responses to interannual environmental fluctuations. The summer A2 showed a clear increasing trend in the annual anomalies in the second part of the time-series, likely in relation to the increasing trend recorded in the summer temperature. However, the phenology of A2 did not seem to have been affected by this temperature increase, since the season of this association remained stable and restricted between July and mid-September throughout the years. It is possible that temperature in warm years resulted in an increase in population numbers (most likely of cladocerans) by accelerating the reproductive cycle with shorter developmental times in *P. avirostris* (Atienza *et al.*, 2008) or with paedogenesis in podonids (*Evadne* and *Pseudoevadne*) (Egloff *et al.*, 1997) but without extending the reproductive season. The autumnal A4 was the most stable association in terms of abundance, with weak oscillations throughout the period until the highly positive anomaly of year 2004. The strong similarities observed in the interannual patterns of A2 and A4 from year 2000 onwards might indicate that the two assemblages started to phase in the last years because they were affected by a common factor (i.e. summer temperature) though still maintaining distinct taxonomic and seasonal characters.

The highly positive anomalies of A5 abundance observed only at the beginning and the end of the period examined match with the highest values of chl-*a* concentration recorded in the same years in the integrated water column at stn MC, suggesting that A5 is actually the association most sensitive to autotrophic food availability. The patterns of annual anomalies for the abundant A1 and the poor A3 both reversed in 1988, when they started to increase after 4–5 years of a decreasing trend. This common change recalls the changes reported in the same year (1988) from a zooplankton time-series in the Gulf of Trieste (Conversi *et al.*, 2009). Following changes in the winter sea surface temperature which started in 1988 in the North Adriatic, changes were recorded in the copepod abundance and composition, namely the new arrival of

Diaixis pygmaea, the rise or decline of several populations, and changes in the phenology of some species (Conversi *et al.*, 2009). An extended analysis of the same time-series in the North Adriatic in parallel with a series in the Ligurian Sea (north-western Mediterranean) pointed to an abrupt change, identifiable at the end of the 1980s, involving both the physical and biological systems in the North Mediterranean, which can be considered a regime shift (Conversi *et al.*, 2010). Changes at the end of the 1980s have also been reported in the pelagic communities in other European seas (e.g. Alheit *et al.*, 2005). Unfortunately, we could not attempt a similar analysis because we lack the data in a crucial period, i.e. the years 1991–1994. On the other hand, the oscillating patterns of anomalies from 1995 onwards seem to indicate that the inversion in A1 and A3 abundances was rather part of zooplankton interannual variability than a signal of a shift. Overall, our results show that, with the exception of A5, none of zooplankton associations at stn MC reflected the changes recorded in the autotrophic compartment with similarly striking changes in their interannual patterns. Subtle changes taking place in the zooplankton communities in the inner Gulf of Naples are suggested by the patterns of a few rare copepod species, such as the disappearance of *Acartia margalefi* and *P. latisetosa*, and the increased (although still modest) contribution of large calanoids from offshore waters, suggesting that the sampling site is acquiring less coastal characteristics (M. G. Mazzocchi *et al.*, submitted for publication).

CONCLUDING REMARKS

In conclusion, mesozooplankton at stn MC are characterized by the occurrence of five taxonomic assemblages (associations) that are well defined in composition and abundance and likely reflect different modes of community functioning. The temporal course of these associations is largely shaped by strong seasonal forcing comprising both physical and biological signals. The linking factors within associations seem also to include trophic interactions, which appear clearly in a few cases that link copepod species to pelagic tunicates. As also recently observed in the English Channel (Eloire *et al.*, 2010), the seasonal variability of zooplankton at stn MC is definitely greater than the variability on the interannual scale, which still appears weak in the course of the two decades of the present study. In the Ligurian Sea (Molinero *et al.*, 2008) and in the North Adriatic (Conversi *et al.*, 2010), which are Mediterranean basins more clearly linked to the North Atlantic

climate regime, a visible covariance between large scale climate patterns and community structure has been observed. Our data set shows either strong stability in the associations or interannual variability dissimilar from either the patterns of the mixed layer or the patterns displayed at the two sites mentioned above. This, in turn, suggests for mesozooplankton at LTER-MC that: (i) the coastal Gulf of Naples, which is at the border between the central and southern regions of the Western Mediterranean, is under the influence of a more complex climate regime than the North Mediterranean (Lionello *et al.*, 2006) and its environmental variability integrates and masks the possible effects of fluctuations at a larger scale, and (ii) most of the main species at stn MC are typical of warm-waters and in the Gulf of Naples they are not at the boundary of their latitudinal distribution as instead reported for warm-water species in the Bay of Biscay (Villate *et al.*, 1997) and in the NE Atlantic (Beaugrand *et al.*, 2002a, b). However, a further quantitative analysis is required. Fluctuations in coastal marine environments, due to variability and interplay of natural and anthropogenic factors, may induce diverse responses to external forcing in pelagic species and communities. One would expect that if species associations reflect common adaptations to certain conditions, any significant variation in the environment should produce a significant change in the associations and, therefore, in the community structure. According to the results of our study, the zooplankton associations identified persist in time and indicate some stable characters in the LTER-MC time-series, providing evidence of resilience at the community level.

ACKNOWLEDGEMENTS

We are very grateful to Augusto Passarelli, Ciro Chiaese and Ferdinando Tramontano for the regular sampling at stn MC and to the crew of the R/V *Vetoria* for their assistance. We thank all colleagues involved in the LTER-MC program for fruitful collaboration and discussions. We also thank two anonymous reviewers for their constructive comments.

FUNDING

The work at LTER-MC is financially supported by SZN. The retrospective analysis was supported by the Italian project MIUR-VECTOR, the NoE EUR-OCEANS (FP6) and the European IP SESAME (FP6).

REFERENCES

- Alheit, J., Möllmann, C., Dutz, J. *et al.* (2005) Synchronous ecological regime shifts in the central Baltic and the North Sea in the late 1980s. *ICES J. Mar. Sci.*, **62**, 1205–1215.
- Atienza, D., Saiz, E., Skovgaard, A. *et al.* (2008) Life history and population dynamics of the marine cladoceran *Penilia avirostris* (Branchipoda: Cladocera) in the Catalan Sea (NW Mediterranean). *J. Plankton Res.*, **30**, 345–357.
- Beaugrand, G. (2004) Monitoring marine plankton ecosystems. I: description of an ecosystem approach based on plankton indicators. *Mar. Ecol. Prog. Ser.*, **269**, 69–81.
- Beaugrand, G., Ibanez, F., Lindley, J. A. *et al.* (2002a) Diversity of calanoid copepods in the North Atlantic and adjacent seas: species associations and biogeography. *Mar. Ecol. Prog. Ser.*, **232**, 179–195.
- Beaugrand, G., Reid, P. C., Ibanez, F. *et al.* (2002b) Reorganization of North Atlantic marine copepod biodiversity and climate. *Science*, **296**, 1692–1694.
- Berasategui, A. D., Menu Marque, S., Gomez-Erache, M. *et al.* (2006) Copepod assemblages in a highly complex hydrographic region. *Estuar. Coast. Shelf Sci.*, **66**, 483–492.
- Carrada, G. C., Hopkins, T. S., Bonaduce, G. *et al.* (1980) Variability in the hydrography and biological features of the Gulf of Naples. *PS.Z.N.I.: Mar. Ecol.*, **1**: 105–120.
- Castel, J. and Courties, C. (1982) Composition and differential distribution of zooplankton in Arcachon Bay. *J. Plankton Res.*, **4**, 417–433.
- Castellani, C., Irigoien, X., Harris, R. P. *et al.* (2005) Feeding and egg production of *Oithona similis* in the North Atlantic. *Mar. Ecol. Prog. Ser.*, **288**, 173–182.
- Cataletto, B., Feoli, E., Fonda Umani, S. *et al.* (1995) Eleven years of time-series analysis on the net-zooplankton community in the Gulf of Trieste. *ICES J. Mar. Sci.*, **52**, 669–678.
- Colebrook, J. M., Glover, R. S. and Robinson, G. A. (1961) Contribution towards a plankton atlas of the North-Eastern Atlantic and the North Sea. General introduction. *Bull. Mar. Ecol.*, **5**, 67–80.
- Conversi, A., Peluso, T. and Fonda-Umani, S. (2009) Gulf of Trieste: a changing ecosystem. *J. Geophys. Res.*, **114**, C03S90, doi:10.1029/2008JC004763.
- Conversi, A., Fonda Umani, S., Peluso, T. *et al.* (2010) The Mediterranean Sea regime shift at the end of the 1980s, and intriguing parallels with other European basins. *PLoS ONE*, **5**, e10633. doi:10.1371/journal.pone.0010633.
- Deibel, D. (1998) Feeding and metabolism of Appendicularia. In Bone, Q. (ed.), *The Biology of Pelagic Tunicates*. Oxford Univ. Press, New York, pp. 140–149.
- Dufrene, M. and Legendre, P. (1997) Species assemblages and indicator species: the need for a flexible asymmetrical approach. *Ecol. Monogr.*, **67**, 345–366.
- Egloff, D. A., Fofonoff, P. W. and Onbé, T. (1997) Reproductive biology of marine cladocerans. *Adv. Mar. Biol.*, **31**, 79–167.
- Eisen, M. B., Spellman, P. T., Brown, P. O. *et al.* (1998) Cluster analysis and display of genome-wide expression patterns. *Proc. Natl. Acad. Sci.*, **95**, 14863–14868.
- Eloire, D., Somerfield, P., Conway, D. V. P. *et al.* (2010) Temporal variability and community composition of zooplankton at station L4 in the Western Channel: 20 years of sampling. *J. Plankton Res.*, **32**, 657–679.
- Fernandez de Puelles, M. L., Pinot, J.-M. and Valencia, J. (2003) Seasonal and interannual variability of zooplankton community in waters off Mallorca island (Balearic Sea, Western Mediterranean): 1994–1999. *Oceanol. Acta*, **26**, 673–686.
- Gravili, D., Napolitano, E. and Pierini, S. (2001) Barotropic aspects of the dynamics of the Gulf of Naples (Tyrrhenian Sea). *Cont. Shelf Res.*, **21**, 455–471.
- Greve, W., Prinage, S., Zidowitz, H. *et al.* (2005) On the phenology of North Sea ichthyoplankton. *ICES J. Mar. Sci.*, **62**, 1216–1223.
- Harbison, G. R. (1998) The parasites and predators of Thaliacea. In Bone, Q. (ed.), *The Biology of Pelagic Tunicates*. Oxford Univ. Press, New York, pp. 187–214.
- Hopcroft, R. R. and Roff, J. C. (1995) Zooplankton growth rates: extraordinary production by the larvacean *Oikopleura dioica* in tropical waters. *J. Plankton Res.*, **17**, 205–220.
- Hwang, J.-S. and Turner, J. T. (1995) Behaviour of cyclopoid, harpacticoid, and calanoid copepods from coastal waters of Taiwan. *PS.Z.N.I.: Mar. Ecol.*, **16**, 207–216.
- Ianora, A. (1998) Copepod life history traits in subtemperate regions. *J. Mar. Syst.*, **15**, 337–349.
- Kamburska, L. and Fonda-Umani, S. (2006) Long-term copepod dynamics in the Gulf of Trieste (Northern Adriatic Sea): recent changes and trends. *Clim. Res.*, **31**, 195–203.
- Kruk, C., Mazzeo, N., Lacerot, G. *et al.* (2002) Classification schemes for phytoplankton: a local validation of a functional approach to the analysis of species temporal replacement. *J. Plankton Res.*, **24**, 901–912.
- Lance, G. N. and Williams, W. T. (1967) A general theory of classificatory sorting strategies. I. Hierarchical systems. *Comp. J.*, **9**, 373–380.
- Legendre, P. and Gallagher, E. D. (2001) Ecologically meaningful transformations for ordination of species data. *Oecologia*, **129**, 271–280.
- Legendre, P. and Legendre, L. (eds) (1998). *Numerical Ecology. Developments in Environmental Modelling*, Vol. 20. Elsevier Science B.V., Amsterdam.
- Licandro, P. and Ibanez, F. (2000) Changes of zooplankton communities in the Gulf of Tigullio (Ligurian Sea, Western Mediterranean) from 1985 to 1995. Influence of hydroclimatic factors. *J. Plankton Res.*, **22**, 2225–2253.
- Licandro, P., Ibanez, F. and Etienne, M. (2006) Long-term fluctuations (1974–1999) of the salps *Thalia democratica* and *Salpa fusiformis* in the northwestern Mediterranean Sea: relationships with hydroclimatic variability. *Limnol. Oceanogr.*, **51**, 1832–1848.
- Lionello, P., Malanotte-Rizzoli, P. and Boscolo, R. (2006) *Mediterranean Climate Variability*. Elsevier, Amsterdam.
- Longhurst, A. (1998) *Ecological Geography of the Sea*. Academic Press, San Diego, pp. 398.
- Mackas, D. L. and Sefton, H. A. (1982) Plankton species assemblages off southern Vancouver Island: geographic pattern and temporal variability. *J. Mar. Res.*, **40**, 1173–1200.
- Marine Zooplankton Colloquium 2 (2001) Future marine zooplankton research—a perspective. *Mar. Ecol. Prog. Ser.*, **222**, 297–308.
- Mazzocchi, M. G. and Ribera d'Alcalà, M. (1995) Recurrent patterns in zooplankton structure and succession in a variable coastal environment. *ICES J. Mar. Sci.*, **52**, 679–691.

- Mazzocchi, M. G., Buffoni, G., Carotenuto, Y. *et al.* (2006) Effects of food conditions on the development of the population of *Temora stylifera*: a modeling approach. *J. Mar. Syst.*, **62**, 71–84.
- McGowan, J. A. and Walker, P. A. (1979) Structure in the copepod community on the North Pacific central gyre. *Ecol. Monogr.*, **49**, 195–226.
- Mitchel, B. G. and Holm-Hansen, O. (1991) Observations and modeling of the Antarctic phytoplankton crop in relation to mixing depth. *Deep-Sea Res., Part A*, **38**, 981–1007.
- Modigh, M. and Castaldo, S. (2002) Variability and persistence in tintinnid assemblages at a Mediterranean coastal site. *Aquat. Microb. Ecol.*, **28**, 299–311.
- Molinero, J. C., Ibanez, E., Nival, P. *et al.* (2005) North Atlantic climate and northwestern Mediterranean plankton variability. *Limnol. Oceanogr.*, **50**, 164–171.
- Molinero, J. C., Ibanez, E., Souissi, S. *et al.* (2008) Climate control on the long-term anomalous changes of zooplankton communities in the Northwestern Mediterranean. *Global Change Biol.*, **14**, 11–26.
- Ohtsuka, S., Böttger-Schnack, R., Okada, M. *et al.* (1996) *In situ* feeding habits of *Oncaea* (Copepoda: Poecilostomatoida) from the upper 250 m of the central Red Sea, with special reference to consumption of appendicularian houses. *Bull. Plankton Soc. Jap.*, **43**, 89–105.
- Paffenhöfer, G. A. (1973) The cultivation of an appendicularian through numerous generations. *Mar. Biol.*, **22**, 183–185.
- Peralba, A. and Mazzocchi, M. G. (2004) Vertical and seasonal distribution of eight *Clausocalanus* species (Copepoda: Calanoida) in oligotrophic waters. *ICES J. Mar. Sci.*, **61**, 645–653.
- Peralba, A., Mazzocchi, M. G. and Harris, R. H. (2010) Niche separation of *Clausocalanus* spp. in the Mediterranean Sea and the Atlantic Ocean. *ICES Cooperative Res. Rep.*, **300**, 51–55.
- Pinel-Alloul, B., Méthot, G., Verreault, G. *et al.* (1990) Zooplankton species associations in Quebec lakes: variation with abiotic factors, including natural and anthropogenic acidification. *Can. J. Fish. Aquat. Sci.*, **47**, 110–121.
- Rao, C. R. (1995) A review of canonical coordinates and an alternative to correspondence analysis using Hellinger distance. *Quaestio.*, **19**, 23–63.
- Ribera d'Alcalà, M., Conversano, E., Corato, F. *et al.* (2004) Seasonal patterns in plankton communities in a pluriannual time series at a coastal Mediterranean site (Gulf of Naples): an attempt to discern recurrences and trends. *Sci. Mar.*, **67**(Suppl.3), 65–83.
- Rojas, C., Ortega-Mayagoitia, E. and Alvarez-Cobelas, M. (2000) Lack of pattern among phytoplankton assemblages. Or, what does the exception to the rule mean? *Hydrobiologia*, **424**, 133–139.
- Scotto di Carlo, B., Ianora, A., Fresi, E. *et al.* (1984) Vertical zonation patterns for Mediterranean copepods, from the surface to 3000 m at a fixed station in the Tyrrhenian Sea. *J. Plankton Res.*, **6**, 1031–1056.
- Siokou-Frangou, I., Christaki, U., Mazzocchi, M. G. *et al.* (2010) Plankton in the open Mediterranean Sea: a review. *Biogeosciences*, **7**, 1543–1586.
- Tibshirani, R., Walther, G. and Hastie, T. (2001) Estimating the number of clusters in a data set via the gap statistic. *J. R. Statist. Soc. B*, **63**(Part 2), 411–423.
- Van Guelpen, L., Markle, D. F. and Duggan, D. J. (1982) An evaluation of accuracy, precision, and speed of several zooplankton subsampling techniques. *J. Cons. Int. Explor. Mer.*, **40**, 226–236.
- Villate, E., Moral, M. and Valencia, V. (1997) Mesozooplankton community indicates climate changes in a shelf area of the inner Bay of Biscay throughout 1988 to 1990. *J. Plankton Res.*, **19**, 1617–1636.
- Wishner, K. F. and Allison, S. K. (1986) The distribution and abundance of copepods in relation to the physical structure of the Gulf Stream. *Deep-Sea Res.*, **33**, 705–731.
- Zingone, A., Montresor, M. and Marino, D. (1990) Summer phytoplankton physiognomy in coastal waters of the Gulf of Naples. *PS.Z.N.I.: Mar. Ecol.*, **11**, 157–172.
- Zingone, A., Dubroca, L., Iudicone, D. *et al.* (2010) Coastal phytoplankton do not rest in winter. *Estuaries Coasts*, **33**, 342–361.