Analysis of time series of planktonic communities in the Adriatic Sea: distinguishing between natural and man-induced changes

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ABSTRACT

Time series (>20 years) of bacterioplankton, phytoplankton and zooplankton abundances and of phytoplankton production in the Adriatic Sea were analysed. Multivariate methods were used to extract the main patterns of year-to-year changes in abundances (Principal Component Analysis), and to discriminate between years and sites (Multidimensional Scaling). Increasing long-term trends were established for planktonic abundances and phytoplankton production in both coastal and open sea areas, presumably as a result of eutrophication processes. In the open sea, natural factors (water exchange between the Mediterranean and Adriatic Seas, and temperature) were dominant in controlling year-to-year fluctuations of plankton. On the other hand, long-term fluctuations of plankton in the coastal area were chiefly controlled by man-induced factors. Thus, besides natural factors, the dominant pattern of planktonic fluctuation was correlated with the fluctuation of nutrients coming from the land as a result of human activities.

RÉSUMÉ

Traitement des séries temporelles des communautés planctoniques en Adriatique: variations d’origines naturelle et anthropique.

Des séries temporelles (>20 ans) de données ont été analysées : abondance du bactérioplancton, du phytoplancton et du zooplancton et production phytoplantonique en Adriatique. Des méthodes statistiques (analyses multivariées) ont été utilisées pour extraire les principales variations interannuelles des paramètres étudiés (méthode en composantes principales) et pour distinguer les années et les sites (échelle multi-dimensionnelle). Des tendances d’augmentation à long terme ont été constatées pour tous les paramètres étudiés, dans la zone côtière et au large, probablement comme résultat des processus d’eutrophisation. Au large, les facteurs naturels de l’environnement (échange d’eau entre la Méditerranée et l’Adriatique, température de la mer) ont été prédominants, contrôlant les fluctuations planctoniques interannuelles, tandis que dans la zone côtière les facteurs d’origine anthropique ont été plus importants. Ainsi, en plus des facteurs naturels, les types prédominants de fluctuations planctoniques sont corrélés aux variations en nutriments d’origine terrestre.

INTRODUCTION

Data have been collected from coastal and open areas of the Adriatic Sea, by the Institute of Oceanography and Fisheries, Split, for about half a century. Basic hydrographic parameters have been followed since 1948, and a number of other chemical and biological parameters subsequently included. Long-term fluctuations of phytoplankton and primary production have been subsequently included. Long-term fluctuations of phytoplankton and primary production have been observed since 1960, and have contributed to the characterization of dynamic processes in the Adriatic ecosystem (Pucher-Petković, 1968; Pucher-Petković et al., 1971). Zooplankton fluctuations have been observed since 1957, and have been discussed in connection with variations in predator abundance (Vučetić, 1971, 1973). Bacterial fluctuations have been studied since 1968, and increasing trends have been observed in both coastal and open sea areas (Krstulović and Šolić 1990; Šolić and Krstulović, 1991a, b).

In this study, multivariate methods were used to extract the main patterns of year-to-year changes in the quantitative and qualitative composition of planktonic communities sampled at two stations, one located in the coastal area and one in the open area of the central Adriatic Sea. Interactions between planktonic fluctuations and a number of environmental factors were analysed in an effort to distinguish “baseline” variability from the effects of man-induced changes.

MATERIALS AND METHODS

Sampling sites

Coastal sea station

The coastal sea station is located in an enclosed, shallow basin, Kaštel Bay (43° 31' N; 16° 22' E). The bay, with a surface area of 61 km² (15 km long and 6 km wide) and an average depth of 23 m, communicates with the adjacent open sea through an inlet 1.8 km wide and 40 m deep. The river Jadro, which discharges into the eastern part of the bay, is the most important fresh-water source, with an average annual inflow of 10 m³ s⁻¹. The eastern part of the bay also receives municipal and industrial effluents. An agricultural area extends along the northern coast of the bay. Wide oscillations of chemical and physical parameters in this area are the result of strong land influence (Zore-Armanda, 1980). Water circulation in the bay is generated mostly by the local wind, which is related to the passage of mid-latitude cyclones over the area (Gačić, 1982; Gačić et al., 1987). Cyclones are more frequent during the winter and therefore water circulation and exchanges with the open sea are more intense in winter than in summer.

Open sea station

The open sea station, located southeast of Cape Stončica on Vis Island (43° 00' N; 16° 20' E), is about 100 m deep and about 50 km offshore. This station is typical of the open middle Adriatic, characterized by smaller oscillations of chemical and physical parameters than the station previously mentioned (Buljan and Zore-Armanda, 1979).

Data sets

Time series listed in Table 1 consist of monthly values detrended for seasonal patterns. The seasonal component removal is based on the additive difference from the moving average method, using a 12-month moving average. Therefore, time series of bacterioplankton data contained 241 values, whereas all other time series contained 265 values. The same methods of sampling and analyses were applied throughout the study period.

Table 1

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Abbreviation</th>
<th>Period</th>
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<tbody>
<tr>
<td>Bacterioplankton</td>
<td>B</td>
<td>1968-1990</td>
</tr>
<tr>
<td>Phytoplankton</td>
<td>P</td>
<td>1962-1982</td>
</tr>
<tr>
<td>Diatoms</td>
<td>DIA</td>
<td>1962-1982</td>
</tr>
<tr>
<td>Dinoflagellates</td>
<td>DIN</td>
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</tr>
<tr>
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<td>Primary production</td>
<td>PP</td>
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<tr>
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<td>CL</td>
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<td>Appendicularia</td>
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</tr>
<tr>
<td>Thaliacea</td>
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<td>1962-1982</td>
</tr>
<tr>
<td>Medusae/Siphonophora</td>
<td>MS</td>
<td>1962-1982</td>
</tr>
<tr>
<td>Mollusca</td>
<td>MO</td>
<td>1962-1982</td>
</tr>
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<td>Polychaeta</td>
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</tr>
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</table>

† since investigations were first begun, two groups, Medusae and Siphonophora, have been counted together and will be referred to as Medusae/Siphonophora in this paper.

Data collection

Bacterioplankton

Samples were collected at depths of 0, 10, 20 and 35 m at the coastal sea station, and 0, 10, 20, 30, 50, 75 and 100 m at the open sea station. Samples were processed at sea immediately after sampling. Mean values integrated for the entire water column were used as input data for analysing the time series. The spreading technique on solid ZoBell medium (ZoBell, 1946) was used in two replications. The number of heterotrophic bacteria was expressed as CFU ml⁻¹ (colony-forming units) after 7-day incubation at 20°C.

Phytoplankton

Samples for phytoplankton quantitative and qualitative analysis were preserved in 2% formaldehyde previously neutralized by sodium borate. Material was left to sediment for 24 h in chambers of 25 cm³ and then counted under an inverted Utermölh microscope. Samples were collected
at the same depths as the bacteria, and the mean values integrated for the entire water column were used as input data for analysing the time series. Phytoplankton density was expressed as cell number per litre of seawater.

Primary production

Primary production was measured by $^{14}$C technique following Steemann Nielsen (1952). Samples (100 ml) were incubated in situ at different depths in the euphotic zone. Samples were incubated for 6 h with the addition of $4 \mu$Ci of carrier-free NaH$^{14}$CO$_3$. Particulate material was collected by gentle vacuum filtration on to 0.45 mm Nuclepore filters before measurement of radioactivity by liquid scintillation. Primary production was integrated for the entire euphotic zone and expressed in carbon biomass per m$^2$ year$^{-1}$.

Zooplankton

Zooplankton samples were collected by vertical hauls with a Hensen net (0.42 m$^2$, 300 mm mesh size), towed at a speed of 0.3 m s$^{-1}$ from bottom to surface. The collected material was preserved in 2% formaldehyde. Subsamples amounting to 1/20 on the catch were counted for the most representative groups, and poorer represented ones were analysed as a composite sample. Only adult organisms were counted. Density is expressed as a number of individuals per m$^3$.

Data analysis

Two multivariate techniques, Principal Component Analysis (PCA) and Multidimensional Scaling (MDS), as well as spectral analysis, were used in data analysis.

Principal Component Analysis (PCA)

PCA was used to extract the main patterns of year-to-year changes in abundance. The data input to each analysis consisted of a set of variables representing annual fluctuations in abundance as a continuous time series from 1962 to 1982. The analyses were all based on correlation matrices involving the standardization of each variable to zero mean and unit variance. The purpose of this is to eliminate differences in abundance between studied groups or between areas, leaving only the relative year-to-year changes in abundance.

Multidimensional Scaling (MDS)

MDS (Kruskal and Wish, 1978) is a nonparametric method which uses the rank order of similarities between samples rather than their absolute values. The ordination procedure results in a scatter plot in which each replicate sample is represented by a point, the distances between points following the same rank order as the pairwise dissimilarities in species composition between samples. The extent to which this ideal is realized, in a two-dimensional plot for example, is indicated by a "stress" coefficient. In our study MDS ordination, based on the Bray-Curtis similarity matrix (Bray-Curtis, 1957), was used for discrimination between years.

Spectral analysis

Spectral analysis or estimation of the frequency spectrum was applied to establish periodicities in cyclical oscillations of studied groups. This procedure permits decomposition of the variance of the data into contributions over a range of frequencies. The analysis is based on a representation of the series as a sum of sinusoids at the Fourier frequencies. In our study, the spectra were based on time series of 21 annual values and calculated with a maximal lag of 11 years.

RESULTS AND DISCUSSION

Trend analysis

Fluctuations in the abundance of bacteria, total phytoplankton and total zooplankton, as well as in primary production, are shown in Figure 1. Fitted second-order polynomial curves are superimposed to emphasize long-term trends. A trend of increasing numbers of heterotrophic bacteria was observed at both stations over the period 1968-1990. At the coastal sea station, the upward trend was observed in the early 1970s and average mean values were about 400 CFU ml$^{-1}$. The maximal value of 1267 CFU ml$^{-1}$ was recorded in 1990. At the open sea station, the increasing trend was observed from the late 1970s onwards. There were no substantial changes in the number of heterotrophic bacteria at this station before 1979, and the average value was about 100 CFU ml$^{-1}$. After 1980, the changes became more apparent each year. The maximum value of 721 CFU ml$^{-1}$ recorded in 1990 was almost seven times higher than the mean value in the period 1968-1979.

Similar trends were established for total phytoplankton abundance. An upward trend spanning the whole studied period (1962-1982) was established at the coastal sea station, whereas at the open sea station, a marked increase in the abundance of phytoplankton was observed after 1976. Upward trends were established for all studied phytoplankton groups (Diatoms, Dinoflagellates and Coccolithophoridae) at both stations.

Less pronounced increasing trends in the abundance of total zooplankton were established at both stations. No regular pattern of fluctuations common to all zooplankton groups was recorded, even though some groups showed similarity in temporal distribution. Upward trends were established for Cladocera at the coastal sea station, and for Copepoda, Cladocera and Thaliacea at the open sea station. Downward trends were established for Copepoda, Appendicularia and Thaliacea at the coastal sea station, and for Decapoda and Chaetognatha at the open sea station. No trend was established for other studied groups.

An upward trend of primary production was more evident at the coastal sea station. At this station, primary production increased from about 100 g C m$^{-2}$ year$^{-1}$,
Long-term fluctuations in the abundance of plankton (bacterioplankton, phytoplankton, zooplankton) and primary production at the (A) coastal and (B) open sea station. Fitted second-order polynomials are superimposed (dashed lines).
observed in 1962, to an average value of 190 g C m⁻² year⁻¹ for the period 1968-1979, and to values of about 250 g C m⁻² year⁻¹ after 1979. The maximal value of 270 g C m⁻² year⁻¹ was established in 1980. At the open sea station in the period from 1962 to 1979, an average primary production was around 60 g C m⁻² year⁻¹. Primary production increased considerably only after 1979 (the mean value for the latter period was close to 90 g C m⁻² year⁻¹).

These results suggest that the most common pattern of fluctuations of all studied parameters takes the form of an increasing long-term trend. This may in all likelihood be accounted for by eutrophication, which was first identified in the coastal area, but extended towards the open Adriatic after the late 1970s. As a result there were decreasing trends in the ratio of the bacterial and phytoplankton abundances at the coastal sea station to those of the open sea station (Fig. 2). The maximal value of this ratio for bacteria, recorded in 1974, was 6.1, but it fell below 2.0 after 1984. Moreover, this ratio for phytoplankton decreased from 20 (an average value from the period 1962-1970) to less than 5 after 1974. Therefore, it can be concluded, with regard to the eutrophication level, that the differences between coastal area and the open sea are diminishing.

The timing of the phytoplankton abundance maximum has changed during the investigated period. In recent years, phytoplankton abundance maxima, together with the peaks formerly observed in spring and autumn, have appeared in summer as well. This phenomenon is more pronounced in the coastal area (Fig. 3), indicating a disturbance of the seasonal cycle due to eutrophication. The delaying of maximal abundances throughout the warmer period (from June to October) suggests that nutrient supply was not a limiting factor for the phytoplankton bloom in Kaštela Bay, and that temperature became a primary mechanism in controlling phytoplankton abundance.

Analysis of the qualitative composition of phytoplankton and zooplankton also showed long-term trends for some groups. Diatoms, as a dominant phytoplankton group at the coastal sea station, accounted for more than 90% of the total phytoplankton in the period 1962-1974. After 1974 a downward trend was established, and contribution of Diatoms to the total number fell below 60%. At the same time, the contribution to the total phytoplankton increased from <1% to 17% and from 2% to 28% for Dinoflagellates and Coccolithophorids, respectively (Fig. 4). During the same period, at the open sea station the contribution of Coccolithophorids to total phytoplankton increased from 50% to 80%, whereas the contribution of Diatoms decreased from 50% to 20%. The contribution of Dinoflagellates to the total number was almost negligible and no trend was established.

Among zooplankton groups, at the coastal sea station, there was a marked downward trend for Copepoda and an upward trend for Cladocera (Fig. 4). The contribution of Copepoda to the total zooplankton decreased from 75% to 45%, whereas the contribution of Cladocera increased from 3% to 45%. Similar but less pronounced trends for Copepoda and Cladocera were also observed at the open sea station. At this station, the decreasing contribution of

Figure 2

Long-term fluctuations of ratios of (A) bacterial and (B) phytoplankton abundance at the coastal sea station to those at open sea station.

Figure 3

Changes in the phytoplankton maximum timing in the course of the studied period.
Appendicularia as well as an increasing contribution of Thaliacea to total zooplankton were also established.

**Multivariate analyses of detrended time series**

*Principal Component Analysis*

Principal Component Analysis (PCA) was carried out on an array consisting of the data sets for all phytoplankton and zooplankton groups and primary production for each year. All time series were previously detrended and standardized (zero mean, unit variance). The trend removal procedure permits subtraction of an estimated (best fitting) trend component from a time series. Figure 5 shows the plot for the first two component weights for the coastal and open sea station.

At the coastal sea station (Fig. 5a), the first principal component (PC1) explains 45% of variability and can be regarded as the best possible single representation of the annual fluctuations in abundance for all groups of plankton. All PC1 weight values were positive, suggesting that PC1 represented a pattern of variation more or less common to all groups. However, phytoplankton groups (with the exception of Dinoflagellates) were better represented by this pattern than zooplankton groups. Thus, high positive values of PC1 weight (coinciding with very low values of PC2 weight) were found for Diatoms, Coccolithophorids, total phytoplankton and primary production. Decapoda, Thaliacea, Medusa/Siphonophora and Appendicularia were zooplankton groups which were best represented by PC2. Negative PC2 weight values were found for Chaetognatha and Polychaeta. At the open sea station (Fig. 5b), PC1 explains 47% and PC2 explains 31% of variability. Year-to-year fluctuations of all phytoplankton
Figure 5
Principal Component Analysis of detrended and standardized (zero mean, unit variance) planktonic data sets. Ordination of first two component weights for (A) coastal and (B) open sea station. Phytoplankton parameters (individual groups, total phytoplankton and primary production) are underlined. Abbreviations as in Table 1.

groups and primary production were better represented by PC2, whereas fluctuations of most zooplankton groups (except Cladocera) were better represented by PC1.

Multidimensional Scaling
Multidimensional Scaling (MDS) ordination of double-square root transformed planktonic abundance data was used to discriminate between years (Fig. 6). MDS configurations showed a clear separation to the left of years with higher abundance, and to the right of years with lower abundance of the planktonic groups best represented by PC1.

At the coastal sea station, PC1 represented a pattern of fluctuation which was characterized by maximal values in the years 1975, 1979 and 1980, which are separated to the left on the MDS plot. Minimal values were observed in 1971, 1977, 1981 and 1982, and these years are separated to the right.

On the other hand, according to PC2, maximal values (1976 at the coastal, and 1970-1972, 1979-1980 at the open sea station) are separated at the top, and minimal values (1968-1971 at the coastal, and 1966, 1974-1976 and 1981-1982 at the open sea station) at the bottom of the MDS plot. All other years are clustered around the centre.

Figure 7 shows the MDS ordination of percentage presence of individual phytoplankton and zooplankton groups. The years separated from the central group are those in which the proportion of planktonic groups showed a considerable departure from the average presence for the entire period.

At the coastal sea station (Fig. 7a), in the year 1972 and during the period 1976-1982, Copepoda showed a significant decline, whereas the contribution of Cladocera to total zooplankton increased. In the years 1975 and 1981 the percentage of Appendicularia also increased. These years are separated to the left on the MDS plot.

Moreover, in the years 1973, 1976 and 1981 the contribution of Diatoms to the total phytoplankton significantly decreased, while the contribution of Dinoflagellates and Coccolithophorids increased. These years are set closer to the bottom on the MDS plot.

At the open sea station (Fig. 7b), the years separated to the left of the central group (1977-1980) were years of significant decrease in the Copepoda contribution, and increase in the Cladocera and Thaliacea (only in 1980) contribution to total zooplankton. Moreover, this period was characterized by a marked increase in the Coccolithophorids contribution to total phytoplankton. Years 1981 and 1982, separated at the top, were characterized by the increase in the proportion of Copepods and Diatoms. A marked decrease of Coccolithophorids and an increase of Diatoms and Dinoflagellates were observed in the years 1967-1969, which are set in the lower right corner of the MDS plot. Another minimum in the proportion of Copepoda was observed in the year 1975, which is set on the MDS plot near the previous group of years.

Relation to the environment
The relationship between the fluctuation in plankton (represented by PC1 and PC2 as two main patterns of fluctuations which account for 66% and 78% of variability for the coastal and open sea stations, respectively) and the fluctuations in a number of environmental factors was analysed. Significant relationships are listed in Table 2.

Open sea station
At the open sea station (Fig. 8), the dominant pattern of planktonic year-to-year fluctuations (PC1) was in good correlation with deep-water salinity (DWS). Common maxima were recorded in 1975 and 1980, and common minima in
Figure 6

(A) Multidimensional Scaling (MDS) ordination of years for double-square root transformed planktonic data sets (stress = 0.0074 at the coastal sea station; stress = 0.0046 at the open sea station). (B) First Principal Components (PC1) (45% and 47% of the variability at the coastal and open sea station, respectively). (C) Second Principal Components (PC2) (21% and 31% of the variability at the coastal and open sea station, respectively). Data sets for PCA were previously detrended and standardized (zero mean, unit variance).

1971 and 1977. Pronounced DWS maxima recorded in 1968-1969 were not associated with PC1 to the same extent (Fig. 8a). The relationship between fluctuations of plankton and DWS may presumably be attributed to the ingression of Mediterranean water. Thus, a salinity increase in the deeper layers of the south and central
Figure 7

(A) Multidimensional Scaling (MDS) ordination of years for untransformed data sets consisting of percentage proportion of phytoplankton and zooplankton groups (stress = 0.0386 at the coastal sea station; stress = 0.0730 at the open sea station). (B) Long-term fluctuations in the contributions of individual phytoplankton groups to total phytoplankton. (C) Long-term fluctuations in the contributions of the most important zooplankton groups to total zooplankton.
Adriatic could be a good indicator of ingressions of Mediterranean water, which is characterized by higher salinity and nutrient concentrations in comparison with the open Adriatic (Buljan, 1953; Zore-Armanda, 1969, 1974). The relationship between ingressions and increased frequency of southerly-type weather (characterized by low atmospheric pressure, south-easterly wind and higher precipitation) was also reported (Baranović et al., 1993). The years 1967-1968 and 1980 were considered to be a period of marked ingressions. Increases in salinity and nutrient levels were recorded from the Adriatic (Zore-Armanda et al., 1987), resulting in a maximal phytoplankton population in 1968 (Pucher-Petković and Marasović, 1980) and maximal primary production in 1969 (Pucher-Petković et al., 1987). Maximal quantities of sardine eggs were also observed in 1968/69 and 1969/70 (Karlovac, 1973). Next, a primary production maximum was reported for the year 1980 (Pucher-Petković et al., 1987). Maximal total bacterial counts were found in the same year, as well as maximal bacterioplankton production (Krstulović et al., 1995). Two pronounced maxima in the 23-year time series of heterotrophic bacteria density were recorded in 1968 and 1980 (Šolić and Krstulović, 1991 a).

The next significant pattern of plankton fluctuations (PC2) takes the form of cyclical oscillations in phase with cyclical oscillations of temperature showing a 10 and 7.6 year periodicity (Fig. 8 b). Common maxima were observed in 1963, 1971-1972 and 1977-1979, and common minima in 1966, 1975 and 1981-1982 (maximal values of both PC1 and PC2 patterns coincided in 1980). Spectral analysis was applied to establish periodicities of cyclical oscillations of the time series, but due to the limited data (21-year time series are too short for the application of this analysis) the accuracy of established periodicities is questionable. However, the clear patterns in the spectra, reflecting different planktonic groups, give some confidence in their reality. Observed periods are very close to the data on the sardine catch along the eastern Adriatic coast, for which oscillations of 8 and 11 years have been reported (Županović, 1968; Regner and Gačić, 1974). The periods of 2-3 and 8-12 years were reported for temperature, salinity, primary production, zooplankton and different developmental stages of anchovy (Regner, 1985). Spectral analysis of sardine catches between 1873 and 1972 and solar activity from 1749 to 1972 for the eastern Adriatic, produced 11-year cycles for both data series (Regner and Gačić, 1974).
Coastal sea station

At the coastal sea station (Fig. 9), a significant relationship was found between year-to-year changes in phosphate concentrations, surface salinity, precipitation quantities and PC1 of plankton abundance (Tab. 2, Fig. 9a). Common maxima were recorded in 1968-1969 and 1974, and common minima in 1966-1967 and 1970-1973.

Figure 9
Coastal sea station. (A) First Principal Component (PC1) (detrended and standardized planktonic data sets), and annual means of phosphate concentration (detrended time series), quantity of precipitation and surface salinity. (B) Second Principal Component (PC2) (detrended and standardized planktonic data sets), and annual means of surface temperature. Fitted 6th order polynomials are superimposed (dashed lines).
Pronounced salinity maxima in 1981-1982, and minima in 1977-1980, as well as a phosphate minimum in 1976, were not associated with PC1 to the same extent (Fig. 9a). A connection between all environmental factors (phosphate, salinity and precipitation) was evidenced. Kaštelna Bay receives fresh water from the river Jadro, which carries considerable quantities of nutrients into the bay. Moreover, an agricultural area extends along the northern coast of the bay, while the southern side is bound by the Marjan peninsula covered with a pine forest, so that precipitation waters also transport appreciable amounts of nutrients. Therefore, both higher phosphate concentrations and lower surface salinity were caused by a greater quantity of precipitation.

Next in importance was the pattern of plankton fluctuations, which was in good correlation with temperature. Pronounced maximal and minimal values in 1976 and 1969 respectively were common to both time series (Fig. 9b). However, oscillations of temperature in Kaštelna Bay were not in phase with temperature oscillations in the open sea. Thus, temperature oscillations in Kaštelna Bay are chiefly controlled by local meteorological conditions. Temperature changes depend mostly on water circulation (exchange with the open sea and vertical mixing) in the bay, induced by local winds (Gačić et al., 1987).

**Between-site comparison**

MDS ordination was used in comparing fluctuations of plankton between the coastal and open sea areas. Analyses were carried out on arrays consisting of data sets for: (i) all the groups for each year; and (ii) all the years for each group (Fig. 10).

**Table 2**

<table>
<thead>
<tr>
<th></th>
<th>Open sea station</th>
<th>Coastal sea station</th>
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<tbody>
<tr>
<td></td>
<td>DWS ST</td>
<td>PH P SS ST</td>
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<tr>
<td>PC1</td>
<td>0.59**</td>
<td>-0.46*</td>
</tr>
<tr>
<td>PC2</td>
<td>-0.85**</td>
<td>-0.51*</td>
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\[ P<0.05; \quad \text{**} P<0.005; \quad \text{***} P<0.0005. \]

MDS ordination of years showed a clear separation between the coastal area and the open sea. For both sites, years set to the right are those with lower abundances of most of the plankton groups, whereas those set to the left are those with higher abundances (Fig. 10a). Therefore, MDS ordination of years represents upward long-term trends of plankton abundances at the both sites. A time shift between two clusters of years was also observed. Thus, the trophic state of the coastal sea station during the 1960s corresponded to the trophic state of the open sea during the 1970s. Increasing eutrophication, first identified in the coastal area, extended towards the open Adriatic during the last decade. MDS ordination of planktonic groups showed a clear dissimilarity of phytoplankton year-to-year fluctuations between the coastal area and the open sea. On the other hand, long term fluctuations in zooplankton groups and bacteria bore a closer resemblance at both sites, and were more or less clustered together (Fig. 10b). Moreover, fluctuations of phytoplankton and zooplankton resembled one another more in the open sea than in the coastal area.

Discrimination between sites. (A) Multidimensional Scaling (MDS) ordination of years for double-square root transformed data sets (all groups of plankton for each year) (stress = 0.0654). Years for the coastal sea station are underlined. (B) Multidimensional Scaling (MDS) ordination of planktonic groups for double-square root transformed data sets (all years for each group) (stress = 0.0721). Groups for the coastal sea station are underlined. Abbreviations as in Table 1.

**Distinguishing between natural and man-induced changes**

Fluctuations of plankton in the Adriatic Sea are due to the combined effects of a number of factors. Some of them act as a natural process, while the others are influenced
Distinguishing between natural and man-induced changes (PCI, PC2 – first two principal components)

<table>
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<td>Open Sea:</td>
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<tr>
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<td>Increasing long-term trends</td>
<td>man-induced</td>
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<tr>
<td>2. Ingression of Mediterranean water</td>
<td>PC1 (47% of variability)</td>
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</tr>
<tr>
<td>3. Temperature</td>
<td>PC2 (21% of variability)</td>
<td>natural</td>
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<td>Coastal sea:</td>
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<tr>
<td>1. Eutrophication</td>
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<td>2. Input of nutrients as a result of human activities</td>
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</tr>
<tr>
<td>3. Temperature</td>
<td>PC2 (21% of variability)</td>
<td>natural</td>
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</tbody>
</table>

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