Changes in the planktonic community structure related to trophic conditions: the case study of the northern Adriatic Sea

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Abstract

During the 2003-2008 period differentiation in the plankton food web structure and the carbon flow within it was studied in situation when the system of the northern Adriatic Sea, one of the most productive area in the Mediterranean, switched from low nutrient to higher nutrient regime. The biomasses distribution between autotrophs, bacteria, protozoans and metazoans showed that within the upper part of the water column the microbial food web was developed during the stratification period (May-October) in oligotrophic conditions, with a larger heterotrophic/autotrophic (H/A) ratio in the western (1.25-1.88) than in the eastern part (1.23-1.45). The classical food web (H/A 0.36-0.63) was observed in the stratification period during nutrient supply by freshwater or by mixing throughout the water column (November-March). However, while the stratification period with freshets was characterized by an enhanced biomass of autotrophs and heterotrophs, during the mixing period reduction in biomass between consecutive trophic levels indicated increased carbon export from the area. In the lower part of the water column the multivorous food web was observed with greater export of the primary produced organic matter in the western area irrespective of nutrient regime.

Key words: biomass pyramid, chl *a*, bacteria, heterotrophic flagellates, ciliated protozoans, metazoans, northern Adriatic Sea

1. Introduction

One of the important aims of the community ecology is the understanding of carbon flux through the trophic levels. There is evidence that the transfer of organic carbon through the trophic levels differs between regions with diverse productivity. The plankton from unproductive regions is characterized by high relative heterotrophic biomasses resulting in an inverted biomass pyramid, whereas the plankton from productive areas is characterized by a smaller contribution of heterotrophs and a broad autotrophic base (Gasol et al., 1997). The Northern Adriatic is one of the most productive regions of the Mediterranean Sea at several trophic levels, from phytoplankton to fish (Vollenweider et al., 1992). Particularly, high but variable plankton standing crop and production was quantified off the Po River delta

and related to the spreading of its plume (Gilmartin and Revelante, 1981). All plankton biomasses show a decreasing gradient eastward from the Po River and southward from the northern Adriatic (Fonda Umani 1996). The microzooplankton composition is characterized by the dominance of ciliates other than tintinnids (Revelante and Gilmartin, 1990), while the mesozooplankton by strictly neritic copepod and cladoceran species (Fonda Umani 1996; Fonda Umani et al., 2005). Copepod nauplii of the smallest size fractions were revealed as the major mediators of material transfer between primary producers and higher trophic levels (Lučić et al., 2003; Kršinić et al., 2007).

As in many coastal systems worldwide influenced by external nutrient load, the productivity level in the northern Adriatic presents marked spatial and seasonal changes related to the alternating influence of freshwater from the western coast and advection of central Adriatic water (CAW) along the eastern coast. The prevalence of cyclonic circulation during winter causes the inflow of oligotrophic CAW into the region and the outflow of more eutrophic riverine waters along the western coast ("open" circulation), while in the late spring and summer the formation of gyres causes a lower exchange with the remainder of the Adriatic ("closed" circulation) (Hopkins et al., 1999). During the winter enrichment of upper waters with nutrients regenerated in deeper waters is superimposed to the CAW inflow. In contrast, in late spring and summer months a stable pycnocline does not permit to nutrients from deeper waters to reach upper waters where nutrient pools are consumed sustaining phytoplankton growth, thus creating oligotrophic conditions. These conditions are occasionally interrupted by the freshwater supply of new nutrients.

Since the relative biomass distribution between heterotrophs and autotrophs is regulated by nutrient supply (Duarte et al., 2000), the objectives of the study were to evaluate the functioning of the microbial food web in situation when the freshwater supply of nutrients caused the production of new organic matter (mainly eutrophic conditions) and the situation without a new input of nutrients (mainly oligotrophic conditions) in which microbial growth was based on a constant recycling of the once produced organic matter. Furthermore, the periods in which the produced organic matter was retained in the system, and the periods in which it was mainly exported from the system of the northern Adriatic were confronted. One of the objectives was also to establish if there were differences in the transfer through the trophic pyramid

between the mainly oligotrophic eastern area and the more eutrophic western area. Food web characterisation was allowed by an appropriate data set collected monthly from 2003 to 2008 that included broad changes in trophic state.

2. Material and methods

Measurements were performed at three stations at the Po River delta - Rovinj transect located in the northern Adriatic (Fig. 1) between 2003 and 2008 on a monthly scale. Temperature and salinity were measured continuously throughout the water column during the downcasts of a SEABIRD SBE 25 CTD probe, while nutrients, chl *a*, heterotrophic bacteria (HB) and heterotrophic flagellates (HF) were measured at five depths (surface, 5 m, 10 m, 20 m, and 1 m above the bottom: 30-35 m). Microzooplankton was taken at surface, 10 m, 20 m and near the bottom.

Water samples were collected with 5 l PVC Niskin samplers. Inorganic nutrients were analyzed in unfiltered water immediately after collection (Parsons et al., 1984; Ivančić and Degobbis, 1984). Dissolved inorganic nitrogen (DIN) was calculated as the sum of nitrate, nitrite and ammonia. Total chlorophyll *a* concentrations (chl *a*) were determined by filtration of 500 ml on Whatman GF/C filters. Filters were frozen (–18 ºC) and analyzed within a few days by the fluorometric procedure after Parsons et al. (1984). Samples for heterotrophic bacteria (HB), heterotrophic pico- and nanoflagellates (HF) abundances were preserved with formaldehyde (2% final concentration) and stored at 4 °C. HB and HF were estimated by epifluorescence microscopy (Leitz Laborlux D and Nikon Microphot-SA at a magnification of 1000x). HB was determined after staining with 4', 6-diamidino-2-phenylindole (DAPI; 1 µg ml^{-1}, final conc.) following a modification of the method of Porter and Feig (1980). HF was obtained by the primuline staining technique (Caron, 1983). Microzooplankton samples were preserved with formaldehyde (2.5% final concentration) neutralized with $CaCO_3$. In the laboratory, samples were sedimented until the original volume of 5 L was reduced to 10 mL, which took 72 hours (Kršinić, 1980). The organisms were counted with an Olympus inverted microscope at magnifications of 100x and 400x. The microzooplankton was separated in two categories: ciliated protozoans (nonloricate ciliates and tintinnids), and metazoans (copepods nauplii, copepodites and small adult copepods).

105 The autotrophic carbon content was obtained by converting chl *a* using a factor of
106 47 µg C/µg chl *a* (Latasa et al., 2005) and HB by a conversion factor of 20 fg C/cell
107 (Lee and Fuhrman, 1987). The carbon content in HF was obtained summing the
108 content in heterotrophic pico- and nanoflagellates. Heterotrophic picoflagellates'
109 abundance was converted to carbon content by a factor of 1500 fg C/cell (Zubkov et
110 al., 1998). Heterotrophic nanoflagellates cell volumes were estimated using the
111 spherical or cylindrical equation (Norland, 1993) and volumes were converted to
112 carbon content by the factor 0.22 pg C μm^{-3} (Børsheim and Bratbak, 1987). The
113 biovolume of nonloricate ciliates was calculated by comparing the shape of the
114 plasmatic body of each organism to one or more geometrical bodies (Edler, 1979).
115 The tintinnids biovolume was estimated for each species by measuring the linear
116 dimensions of the lorica. The geometrical method was also applied to determine the
117 biovolume of copepod nauplii and postnaupliar copepods. The biovolume of nauplii
118 was calculated according to the modified formula for the biovolume of rotifers
119 (Ruttner-Kolisko, 1977). The body of almost all copepods may be equated to two
120 geometrical forms: cephalothorax to the ellipsoid, and abdomen to the cylinder
121 (Shmeleva, 1965). The following conversion factors were used to transform
122 biovolumes into carbon biomass: for nonloricate ciliates 0.14 pgC μm^{-3} (Putt and
123 Stoecker, 1989), for tintinnids 444.5 pgC + (lorica volume in μm^{-3} x 0.053 pgC)
124 according to Verity and Langdon (1984), for copepod nauplii and postnaupliar
125 copepods 0.08 pgC μm^{-3} (Beers and Stewart, 1970; Monti and Fonda Umani, 1999).

126 The biomass pyramid was constructed taking the autotrophic biomass as 1. The
127 bacteria, protozoans (heterotrophic flagellates and ciliated protozoans) and
128 metazoans contribution in community biomass was obtained by dividing their
129 respective biomass by the autotrophic biomass.

130

131 **3. Results**

132 From May to September chl *a* concentrations were related to the freshwater input.
133 In absence of new freshwater input minimal chl *a* concentrations were observed in
134 the whole area (Fig. 2). In these months with "closed" circulation and stratified water
135 column, even low impulses of freshwater discharge can extend to the west (stations
136 SJ101 and SJ108) as indicated by lowering of the surface salinity. Concomitantly,

increased chl *a* values were observed, more frequently at the western stations, while at the eastern station (SJ107) this was the case only after large impulses of freshwater discharge, as observed in May 2004 and May-June 2008 (Fig. 2).

From November to March chl *a* concentrations were generally higher than during the stratification without freshets and often not related to the freshwater input. For instance during the winter of 2004 the freshwater input was markedly higher than during the winter of 2007. Winter chl *a* concentrations of these two years did not differ markedly (Fig. 2). Most often during the winter river waters did not influence the area, as indicated by generally high surface salinity in these months.

Results for April and October are not included in the data elaboration, since in these transient months progressive heating or cooling of the water surface starts the stratification or mixing through the water column, but usually these processes are not completed yet. The circulation in the area turns from “open” to “closed” type (April) and vice versa (October). In these months freshwater could be retained in the area and stimulate phytoplankton growth (case in November 2005 when impulses of 950-1500 m^3 s^{-1} lowered surface salinity at SJ108 for 10 units; Fig. 2), or could be mainly exported from the area not influencing phytoplankton biomass (case in November 2006 when similar impulses did not lower surface salinity). All values given in brackets throughout the Results section are medians, if not reported otherwise.

3.1. Nutrient status, autotrophic biomass and heterotrophic abundance

During the May-September period DIN concentrations in the water column of the eastern station were minimal (0.67-0.82 µmol l^{-1}) and PO_4 was practically exhausted (Fig. 3). Comparably low nutrient concentrations were found in the water column of the western stations in occasions when there was no alimentation with freshwater (steady conditions; Fig. 3). Higher nutrient concentrations, at all stations were observed only in bottom waters. Consequently, chl *a* values in the water column were minimal and similar in the entire area (0.22-0.31 µg l^{-1}), and higher values were observed only in bottom waters (Fig. 4). In conditions of similar chl *a* values, larger abundance of heterotrophic organisms was observed at the western stations, especially in the upper parts of the water column (Fig. 4, 5). The difference in abundances increased with the trophic level: in the upper 5 m at the western stations abundances of HB (61-63 $\cdot 10^7$ cell l^{-1}) were for about ¼, for HF (1.1-1.2$\cdot 10^6$ cell l^{-1})

169 twice, for ciliates (77 ind. l^{-1}) more than twice and for metazoans (47 ind. l^{-1}) several
170 times higher than at the eastern station.

171 While chl *a* minimum at the eastern station generally persisted during the whole
172 summer, at the western stations episodic inputs of freshwater nutrients, particularly at
173 the surface (DIN 3.7 µmol l^{-1}, PO_4 0.08 µmol l^{-1}; Fig. 3), increased chl *a* values
174 interrupting the summer minimum ("freshets"; Fig. 4). During these episodes chl *a*
175 concentrations, HB, HF and ciliates abundances were generally maximal during the
176 year practically down to 20 m (Fig 4, 5). Maximal values were found at the surface
177 (chl *a* 2.12µg l^{-1}, HB $124 \cdot 10^7$ cell l^{-l}, HF $1.6 \cdot 10^6$ cell l^{-l}, ciliates 308 ind. l^{-1}) and
178 gradually decreased through the water column. Only the abundances of metazoans
179 at the surface (34 ind. l^{-1}) were generally lower than in the absence of freshwater
180 (Fig. 5). On the contrary, in bottom waters chl *a* concentrations were lower and all
181 microbes were less abundant than in conditions without freshwater influence.

182 From November to March mixing processes homogenized the entire water
183 column. Nutrient concentrations were markedly higher than during the stratification,
184 except during freshets (Fig. 3). At the eastern station higher nutrient concentrations
185 (0.02-0.03 and 1.87-2.12 µmol l^{-1} for PO_4 and DIN, respectively; Fig. 3) were related
186 to about twice higher chl *a* concentrations (0.46-0.54 µg l^{-1}) than during the
187 stratification (Fig. 4). Yet, abundances of HB ($35\text{-}39 \cdot 10^7$ cell l^{-l}; Fig 4b), and
188 particularly HF ($0.29\text{-}0.36 \cdot 10^6$ cell l^{-l}; Fig 4) in upper waters were lower than during
189 the stratification. In contrast, ciliates abundances (46-63 ind. l^{-1}) were generally about
190 twice higher, while metazoans abundances (12-20 ind. l^{-1}) were comparable to the
191 ones during the stratification (Fig. 5). At the western stations during the mixing period
192 nutrient (0.05-0.15 µmol $l^{-1}$and 2.95-5.24 µmol l^{-1}; for PO_4 and DIN, respectively; Fig.
193 3) and chl *a* concentrations (0.54-0.96 µg l^{-1}; Fig. 4) were higher than at the eastern
194 station. Yet, heterotrophs abundances were similar as at the east and lower than
195 during the stratification, with and without freshets (Fig. 4,5). Only ciliates abundances
196 (48-74 ind. l^{-1}) were comparable to the one during the stratification without freshets.

197 During all periods down to 20 m nonloricate ciliates predominated (60-80% of
198 ciliated protozoans abundance) over tintinnids, with the lowest contribution during the
199 stratification with freshets (Table 1). In bottom waters nonloricate ciliates dominated
200 (about 62-65%) over tintinnids during the mixing period, while during the stratification
201 their contribution was nearly equal. Among the metazoan, copepods nauplii

dominated in the upper 20 m during the stratification and in the upper 10 m during the mixing period (60-78% of metazoans abundance). In bottom waters copepods nauplii accounted for about half of metazoans abundance (Table 1).

3.2. Transfer of matter through the food web

During the stratification the biomass distribution at the eastern station was similar as at the western stations in steady conditions (Fig. 6). The biomass of heterotrophs largely exceeded the autotrophic one in the water column down to 20 m (heterotrophic/autotrophic biomass ratios -H/A-1.23-1.88; Table 1). H/A ratios were higher at the western than at the eastern stations. At the surface and 10 m, the biomass was about evenly distributed between heterotrophic bacteria and autotrophs, and the transfer to the protozoans was maximal, compared to other depths (Fig. 6). The transfer to the metazoans was higher at 10 and 20 m than at the surface. As opposed to the water column, the bottom layer of both areas was characterized by a smaller contribution of heterotrophs (H/A ratios 0.44-0.58) and a broad autotrophic base, approaching a “normal” pyramidal distribution, otherwise the metazoans contribution exceeded that of protozoans (Fig. 6). The upward transfer was lower than in the water column. The upward transfer in the water column was always higher at the western stations, while it was contrary at the bottom (Fig. 6).

During the stratification with freshets, the biomass pyramid was characterized with a broad autotrophic base (H/A 0.38-0.63; Table 1) and a stepwise upward decrease in biomass (Fig. 6). Generally, during freshets, the transfer from autotrophs to higher trophic levels was markedly lower than in steady conditions (Fig 6).

During the mixing period the biomass pyramid at eastern and western stations was similar (Fig. 7). In the whole water column the pyramid was characterized by a broad autotrophic base (0.36-0.61, Table 1), and a generally stepwise decrease in biomass upward, although metazoans biomass exceeded that of protozoans (Fig 7). H/A ratios were lower at the western than at the eastern stations. At the western stations the “strength” of upward transfer was lower to that during the stratification in steady conditions (Fig. 6 and 7). At the eastern station the transfer to the heterotrophic bacteria and metazoans was generally higher than at the western stations while the transfer to the protozoans was somewhat lower (Fig. 7).

4. Discussion

The most prominent shift from oligotrophic to eutrophic conditions was observed during the stratification period, when a clear change from microbial to classical food web occurred. During the stratification without freshets, characterised with mainly oligotrophic conditions, the shape of the biomass pyramid in upper waters of the entire area was similar and characterized with the heterotrophic dominance of biomass. Such biomass distribution implies a fast turnover of autotrophs which allows them to support high relative biomasses of heterotrophs (Gasol et al., 1997). In the conditions of recycled production microbial food webs play an important role in nutrient recycling (Azam et al., 1983) and bacteria use a higher portion of primary production exerting control over them. The whole study area was dominated by small autotrophs (Socal et al., 2008; CMR, unpubl. data), which were not grazed by metazoans but rather consumed by nano- and microheterotrophs, and most of the fixed carbon flows through the microbial food web as observed in similar conditions (Legendre and Rassoulzadegan, 1995). The small size of the phytoplankton was most probably the reason of the low grazing pressure of herbivorous metazoans during that period, since copepods feed better on larger sized (>10μm) particles (Nielsen and Hansen, 1995).

The leading role of heterotrophs in the northern Adriatic during the stratification without freshets can be compared to the regular situation in the Mediterranean Sea, but with an opposite spatial trend. In the Mediterranean Sea, higher values of H/A ratios (0.9 to 3.9) were more frequent in oligotrophic regions during stratification (Pedrós-Alió et al., 1999, Siokou-Frangou et al., 2002) and in the most oligotrophic oceanic ecosystems in general (Cho and Azam, 1990, Caron et al., 1995). On the contrary, higher H/A ratios in the northern Adriatic were found at the western, more eutrophic part. In the water column down to 20 m, the overall trend of H/A ratios >1 was observed during the stratification without freshets, indicating the high efficiency in keeping resources, that is a pattern more typical for oligotrophic ecosystems (Siokou-Frangou et al., 2010). Although the production of fresh organic matter was low and similar at both areas, a quite higher abundance of bacteria at the west was probably supported by the excess of organic matter retained after freshets or/and related to higher PO_4 input in this area under direct riverine influence. Since PO_4 is

confirmed as the limiting element for microbial growth in the northern Adriatic (Ivančić et al., 2009), it could be that P addition had been exploited only by bacteria which can utilize dissolved organic nitrogen thus outcompeting autotrophs, or added P was accumulated in bacteria and picophytoplankton, forming a P enriched diet for grazers. The resulting heterotrophic biomass would then have been quickly channelled toward larger consumers, as observed for other areas (Thingstad et al., 2005; Siokou-Frangou et al., 2010).

In contrast, a clear west to east gradient in terms of abundances and biomass transfer was evidenced during the freshets. H/A ratios were lower (0.4-0.6) approaching the values regularly found in eutrophic and coastal zones (~0.2; Ducklow and Carlson, 1992). During intermittent periods of the freshwater nutrient supply, the clear change from microbial to “classical food web” occurred. Increased nutrients led to an enhanced biomass of autotrophs, both nano- and microphytoplankton. The biomass of bacteria, protozoans (except heterotrophic flagellates) and metazoans also increased, matching the changes in autotrophic biomass. Besides the considerably higher ciliated protozoan densities, the most abundant component of the northern Adriatic microzooplankton (Kršinić, 1995), the contribution of tintinnids to the total protozoan biomass also increased. This change in the community structure occurred throughout the entire water column. Similar changes in the structure of protozoan population were recorded in the eutrophicated part of the northern and central Adriatic (Revelante and Gilmartin, 1983; Bojanić et al., 2005). During freshets ciliated protozoans might act as important direct or indirect factor of “top-down” control by cropping on bacteria or their potential consumers, heterotrophic flagellates, respectively. The biomass of metazoans, in which copepod nauplii prevailed over copepods, also increased and exceeded that of protozoans. Since the food availability rapidly increased, the metazoans grazing became very intensive. Although the protozoans are always present in the diet of metazoans, large nauplii and copepods were recognized to be important grazers of microphytoplankton in the northern Adriatic when ephemeral increases of autotrophic biomass occur (Lučić et al., 2003; Fonda Umani et al., 2005; Kršinić et al., 2007). Thus, during the freshets, a fraction of microalgal production would be channelled by herbivorous mesograzers to higher consumers. A shift in biomass distribution, from a dominance of heterotrophs at low to that of autotrophs at high nutrient inputs was experimentally

confirmed (Duarte et al., 2000). From these connections between the trophic levels in the northern Adriatic, it derives that during the freshets heterotrophic nutrition (i.e. predation) prevailed, while in steady conditions “bottom-up” control dominated.

During the stratification in steady conditions autotrophic biomass notably increased below 20 m, due to the pool of regenerated nutrients. The excess biomass of autotrophs under conditions of nutrient availability fairly exceeded the capacity of heterotrophs to use their production. While in the upper layers bacterial biomass accumulated probably due to closed circulation, in the lower layers it could partly sink to the sediments or be exported southerly from the northern Adriatic. Although the microbial food web pathways were also important, especially on the eastern part, the grazing activity of metazoans increased, removing a considerable portion of microbial biomass. The occurrence of herbivorous and microbial grazing modes (multivorous food web; Siokou-Frangou et al., 2002) suggested more efficient energy transfer to higher trophic levels in the lower part of the water column during the stratification.

During the water column mixing (November to March) chl *a* concentrations were higher than during the stratification without freshets. Phytoplankton growth was sustained mostly by the nutrients regenerated during autumn and redistributed in the water column after the mixing (Gilmartin et al., 1990), while freshwater nutrients were a less important source. Most often the river waters were directed southward in a narrow coastal belt and did not influence the area. During this period the biomass distribution was represented by broad autotrophic base with reduction in biomass between consecutive trophic levels indicating increased carbon export, usual in these conditions (Gasol et al., 1997), being less available to planktonic heterotrophs. In the eastern part a much greater proportion of autotrophs flowed to bacteria then at the west. Consequently at the east lower autotrophic biomass caused significantly lower grazing pressure of metazoans on primary production. Thus copepod predation was probably channelled to ciliates. In contrast, on the west higher biomass of ciliates could be attributed to a weaker grazing control by metazoans. Their grazing pressure was more intensive on autotrophs due to their higher biomass. The greater availability of autotrophs on the west and similar abundance of heterotrophs at both parts, suggests that the export of primary production was larger on the western part.

The results showed a seasonal differentiation regarding the food web structure and the carbon flow within it in the northern Adriatic. Although conversion factors for

the calculation of autotrophic and heterotrophic biomass could be a source of errors, the obtained results are robust enough to support observed differences. The observation that the microbial food web has been developed in the upper part of the water column during the stratification without new input of nutrients (oligotrophic or steady conditions), whereas the classical food web was found during increased nutrients, provided by freshets and water column mixing, could support a wide range of conversion factors. During the period of microbial food web domination the carbon transfer was higher at the western part, while on the contrary, in the period of classical food web domination the transfer was higher at the eastern part. The multivorous food web was observed in the lower part of the water column with greater export of the primary produced organic matter at the west, irrespective of the season.

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Figure caption

Fig. 1. Research area and sampling stations in the northern Adriatic Sea.

Fig. 2. Daily mean of the Po River discharge rate (Q), salinity (S) and chlorophyll *a* concentrations (chl *a*) at the surface of sampling stations during the period 2003-2008.

Fig. 3. Box-whiskers plot of orthophosphate (PO_4) and dissolved inorganic nitrogen (DIN) during the stratification and mixing period at the eastern (ES) and western (WS) stations in conditions with (freshets) and without freshwater input (steady) during 2003-2008. Surface values are denoted with □, 5 m □, 10 m ■, 20 m ■, and bottom ■. Vertical bars are referred to 95% of data.

Fig. 4. Box-whiskers plot of chlorophyll *a* (chl *a*), heterotrophic bacteria (HB) and heterotrophic flagellates (HF) during the stratification and mixing period at the eastern (ES) and western (WS) stations in conditions with (freshets) and without freshwater input (steady) during 2003-2008. Surface values are denoted with □, 5 m □, 10 m ■, 20 m ■, and bottom ■. Vertical bars are referred to 95% of data.

Fig. 5. Box-whiskers plot of ciliated protozoans and metazoans during the stratification and mixing period at the eastern (ES) and western (WS) stations in conditions with (freshets) and without freshwater input (steady) during 2003-2008. Surface values are denoted with □, 10 m ■, 20 m ■, and bottom ■. Vertical bars are referred to 95% of data.

Fig. 6. Mean (plus standard error) biomasses of metazoans, protozoans and bacteria relative to that of autotrophic biomass for the stratification period at the eastern and western stations in conditions with (freshets) and without freshwater input (steady) during 2003-2008.

Fig. 7. Mean (plus standard error) biomasses of metazoans, protozoans and bacteria relative to that of autotrophic biomass for the mixing period at the eastern and western stations during 2003-2008.

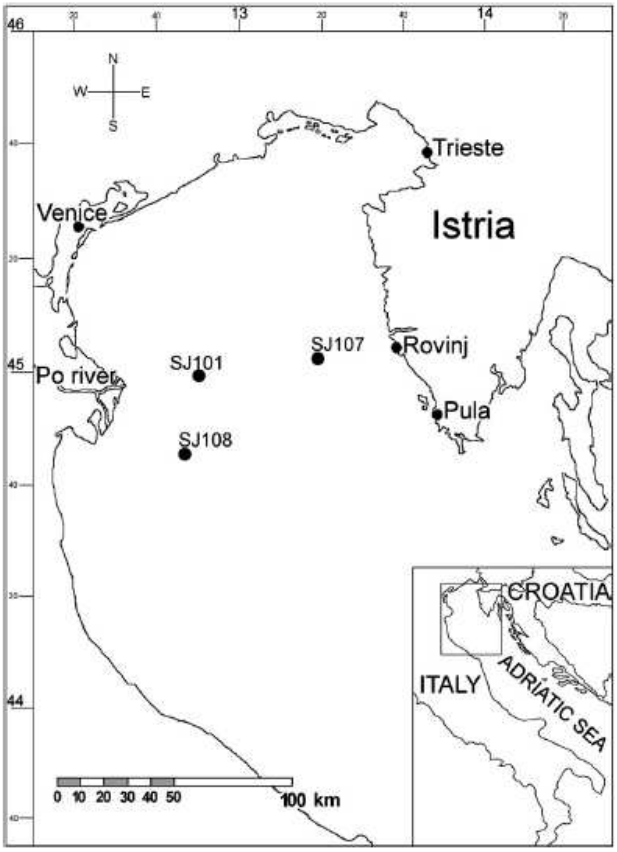

Fig. 1. Research area and sampling stations in the northern Adriatic Sea.

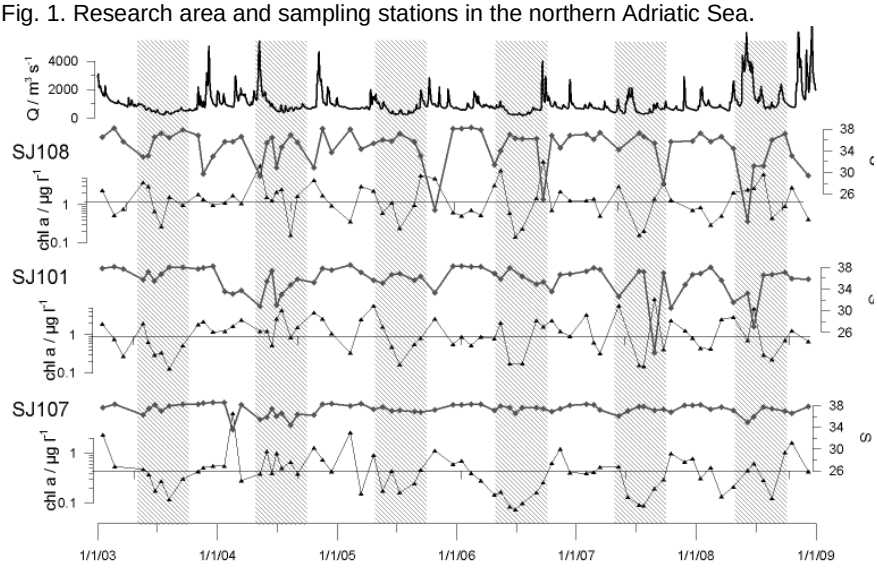

Fig. 2. Daily mean of the Po River discharge rate (Q), salinity (S) and chlorophyll a concentrations (chl a) at the surface of sampling stations during the period 2003–2008. Stratification periods (May–September) are shaded in gray.

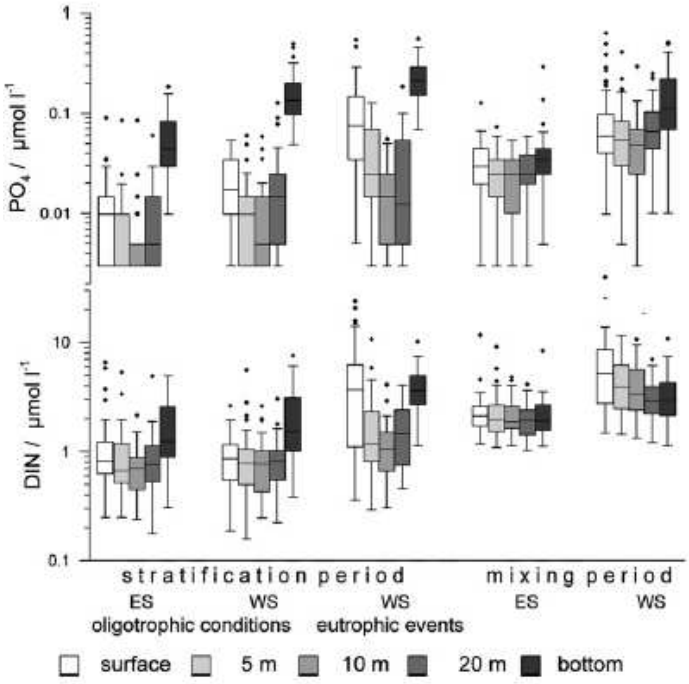

Fig. 3. Box–whisker plot of orthophosphate (PO4) and dissolved inorganic nitrogen (DIN) during the stratification and mixing period at the eastern (ES) and western (WS) stations in oligotrophic conditions and during eutrophic events for the 2003–2008 period. Vertical bars are referred to 95% of data.

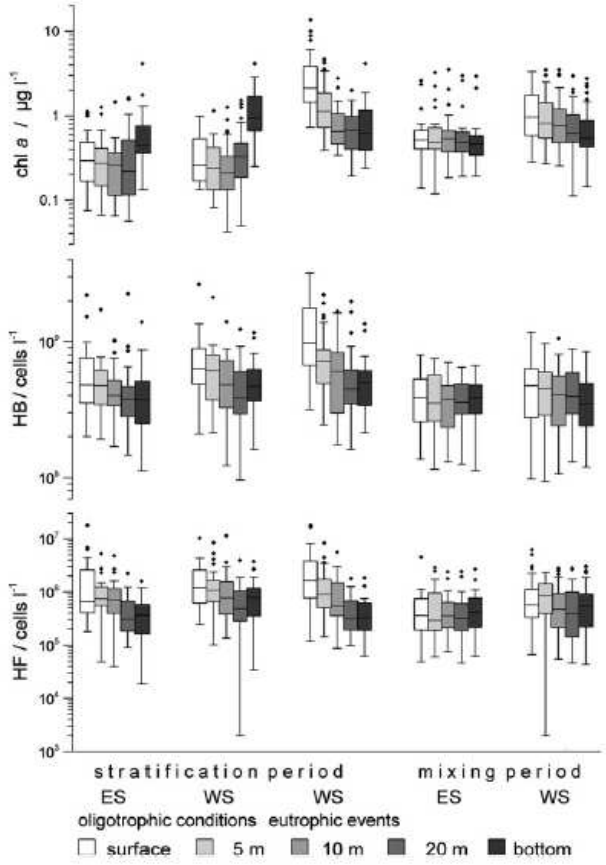

Fig. 4. Box–whisker plot of chlorophyll a (chl a), heterotrophic bacteria (HB) and heterotrophic flagellates (HF) during the stratification and mixing period at the eastern (ES) and western (WS) stations in oligotrophic conditions and during eutrophic events for the 2003–2008 period. Vertical bars are referred to 95% of data.

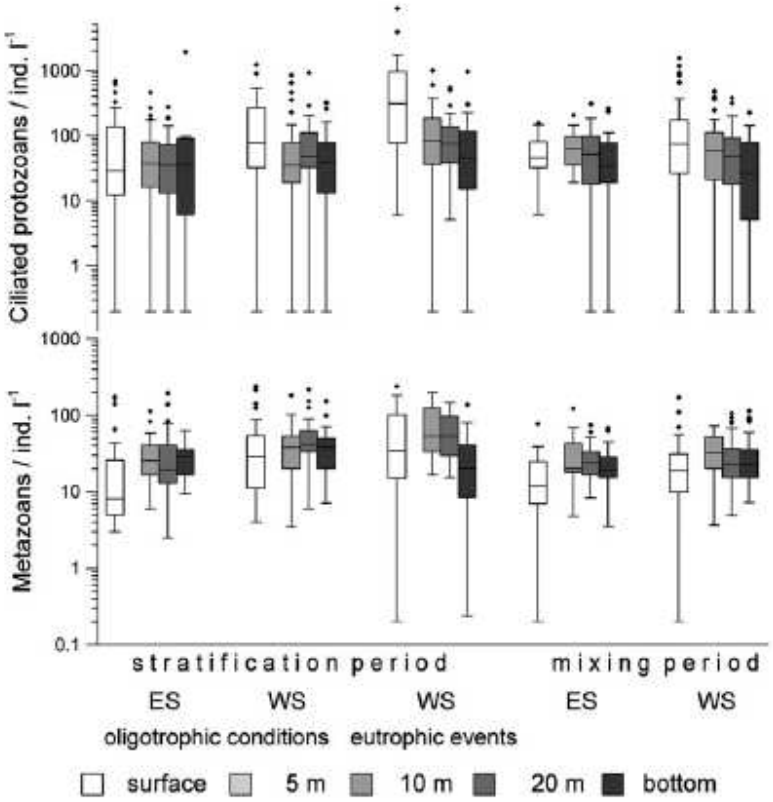

Fig. 5. Box–whisker plot of ciliated protozoans and metazoans during the stratification and mixing period at the eastern (ES) and western (WS) stations in oligotrophic conditions and during eutrophic events for the 2003–2008 period. Vertical bars are referred to 95% of data.

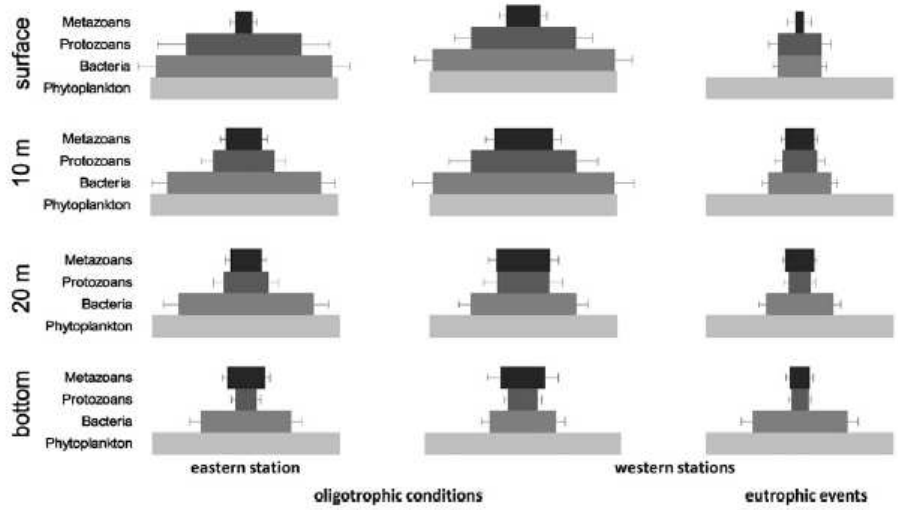

Fig. 6. Mean (plus standard error) biomasses of metazoans, protozoans and bacteria relative to that of autotrophic biomass for the stratification period at the eastern and western stations in oligotrophic conditions and during eutrophic events for the 2003–2008 period.

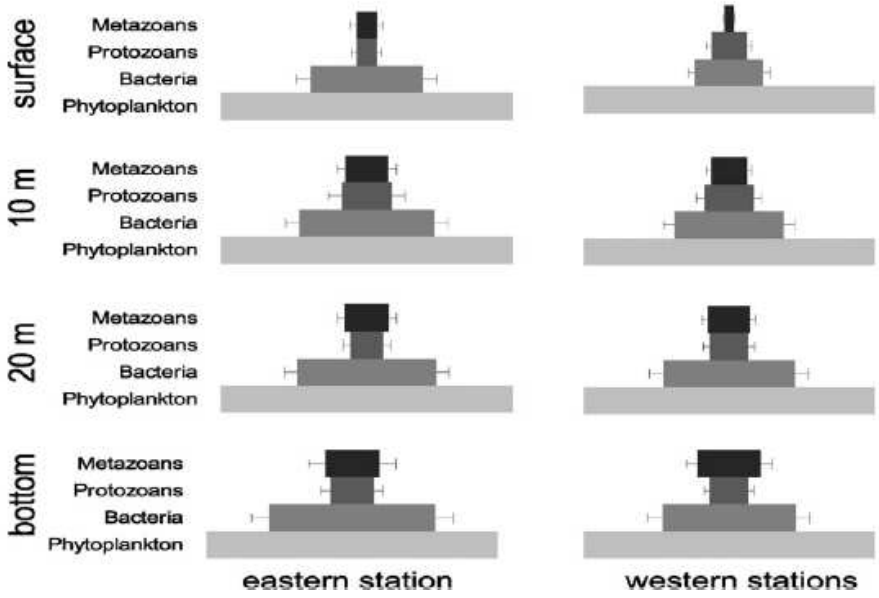

Fig. 7. Mean (plus standard error) biomasses of metazoans, protozoans and bacteria relative to that of autotrophic biomass for the mixing period at the eastern and western stations for the 2003–2008 period.

Table 1. Mean percentage (∀SE) of nonloricate ciliates in ciliated protozoans and copepods nauplii in metazoans and heterotrophic/autotrophic ratio (H/A) during the stratification and mixing period at the eastern and western stations in conditions with (freshets) and without freshwater input (steady) during 2003-2008.

		Stratification			Mixing	
		Eastern s.	Western s.		Eastern s.	Western s.
Parameter	depth		steady	freshets		
% Non-loricate ciliates	surf.	71.2∀5.4(33)	70.6∀5.4(34)	63.5∀6.4(35)	71.7∀6.5(26)	82.4∀3.6(52)
	10	80.8∀5.0(33)	74.8∀4.8(37)	68.8∀5.5(31)	82.6∀5.3(26)	80.0∀3.5(52)
	20	70.6∀5.3(33)	71.0∀5.1(37)	54.6∀5.6(31)	79.9∀5.8(26)	69.3∀4.8(51)
	Bott.	55.9∀6.9(31)	54.9∀6.3(37)	44.4∀6.3(31)	65. 0∀7.4(25)	61.9∀4.9(51)
% Nauplii	surf.	78.1∀3.6(33)	73.1∀4.1(34)	70.0∀3.1(34)	73.5∀4.8(26)	77.8∀2.8(52)
	10	69.5∀3.8(33)	64.5∀3.7(37)	60.3∀3.3(31)	66.8∀4.2(26)	66.4∀2.6(52)
	20	61.7∀4.2(33)	60.8∀3.8(37)	56.0∀3.3(31)	55.9∀3.2(26)	53.9∀2.9(51)
	Bott.	48.0∀4.1(31)	42.5∀3.6(37)	56.9∀5.2(31)	54.8∀4.3(25)	48.5∀3.8(51)
H/A	surf.	1.45∀0.13(33)	1.64∀0.14(34)	0.38∀0.03(35)	0.53∀0.08(26)	0.36∀0.03(52)
	10	1.44∀0.11(33)	1.88∀0.19(37)	0.62∀0.05(31)	0.49∀0.05(26)	0.43∀0.04(52)
	20	1.23∀0.12(33)	1.25∀0.16(37)	0.63∀0.06(31)	0.51∀0.05(26)	0.50∀0.05(51)
	bott.	0.58∀0.05(31)	0.44∀0.05(37)	0.50∀0.05(31)	0.61∀0.09(25)	0.56∀0.06(51)