

**Sea dynamics impacts on the macroaggregates:
a case study of the 1997 mucilage event in the northern Adriatic**

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ABSTRACT

The massive formations of mucilaginous macroaggregates of various forms and dimensions are typical for the northern Adriatic (i.e., mucilage phenomenon). In this work we analysed the 1997 mucilage event and showed that macroaggregates were formed in the upper layers of the water column in the central parts of large gyres and were dispersed from their origin by geostrophic currents. Beside *Cylindrotheca closterium*, an already established regular opportunistic species of the mucilage phenomenon, originating from the sediment, we detected another benthic species, *Thalassionema nitzschioides*, of a sporadic character. Both were present in scarce amounts in the water column at the time of sampling first macroaggregates, but reached high abundances after/close to the mucilage event ending. Several surface heat loss were documented during the period of investigation. The first one, and the most intense, occurred on 18 July (heat loss over 250 W m, during episode of NW wind), in time of the first macroaggregates formation (first macroaggregates sampled on 22–23 July), induced intense turbulent mixing near the bottom and introduced *C. closterium* from sediment into the water column. *T. nitzschioides* was already present in the water column in central part of the region on 8 July and was included in the first macroaggregates, which were dispersed westwards and eastwards after the 18 July heat loss episode which changed circulation pattern. The later, less intense, surface heat loss episodes, induced mostly by NE winds, were efficient in tearing already formed macroaggregates, inhabited with opportunistic species, and induced their release from a macroaggregate into the surrounding water column. The mucilage event drastically changed the phytoplankton composition in the water column with increased abundance of the opportunistic species at the time of macroaggregates decay. In addition, winds from NE were responsible for drawing lower salinity waters, with its specific species community and in which macroaggregates were already formed, towards eastern coast extending the event to large area. In addition to aforementioned findings, our investigation resulted in two hypothesis. First, summer surface heat loss episodes play a role in sustaining mucilage events by introduction of *C. closterium* from the sediment and/or decaying

macroaggregates in the water column. Second, geostrophic currents are important in spreading of various types of aggregates, including marine snow, with consequent spreading of species over the wider marine areas.

Keywords

mucilage event, macroaggregates, geostrophic currents, northern Adriatic, *Cylindrotheca closterium*, *Thalassionema nitzschioides*

1. INTRODUCTION

The mucilage phenomenon is an event typical of the northern Adriatic (further in text: NA), characterised by the massive formation of white to brownish, amorphous, sticky, mucilaginous macroaggregates (further in text: macroaggregates) of various forms and dimensions, ranging from 0.5 cm to several km in diameter. Small types of macroaggregates, flocs (0.5-1 cm) and macroflocs (1-5 cm) are ubiquitous and present in the water column year-round with extremely variable abundances and are commonly known as marine snow (Alldredge and Silver, 1988; Stachowitsch et al., 1990; Precali et al., 2005). Large type macroaggregates are present only during mucilage events in various abundances and include elongated types: stringers (few mm x 2 – 25 cm) and ribbons (few cm x 10/20 cm – 1 m), amorphous forms: clouds (diameter 0.5 – 3/4 m) and layers of various dimensions: cowebs, false bottoms, blankets, creamy or gelatinous surface layers. Large macroaggregates spread over wide areas of the sea surface or throughout the water column, even for as much as hundreds of kilometres (Stachowitsch et al., 1990; Degobbi et al., 1999; Precali et al., 2005).

Although occasional occurrences of the mucilage events were observed in Dalmatian, Greek, Tyrrhenian and Sicilian coastal areas (Stachowitsch et al., 1990; Calvo et al., 1995; Gotsis-Skretas, 1995; Innamorati, 1995), they were neither as frequent, nor of such an enormous scale, as are those occurring in the NA. According to the gathered observations from an assortment of popular and scientific publications, the first record of mucilage phenomenon in the NA dates back to 1729, followed by occurrences in 1872, 1880, 1891, 1903, 1905, 1920-1922, 1924, 1927, 1929, 1930, 1935, 1941, 1949, 1951, 1959, 1973, 1976 and 1983 (Fondammani et al., 1989; Danovaro et al., 2009). Based on available records, it seems that in the last period mucilage event recurred more often: in 1988 – 1991, 1997 an year by year in periods 2000 – 2004 and 2006 – 2008 (Degobbi et al., 1999; Precali et al., 2005; Danovaro et al., 2009).

Unusual bloom in the English Channel, massive foam production noticed in the North Sea or a mass accumulation of the mucilage in Tasman Bay of New Zealand have all been easily attributed to specific phytoplankton species such as *Chaetoceros wailesii*, *Phaeocystis* or *Gonyaulax hyaline*, respectively, due to coinciding massive abundances of the mentioned species (Fogg, 1995; Lancelot, 1995; MacKenzie et al., 2002). On the contrary, for the mucilage event occurring in the winter of 2007/08 in the Sea of Marmara, several diatoms and dinoflagellates were related to the event (Aktan, et al., 2008; Tüfekçi et al., 2010). In contrast, no specific species coinciding with the mucilage events occurring in the NA were ever identified (Pistocchi et al., 2005). During macroaggregates formation process, a wide range of organisms coincidentally inhabiting the ambient water (Revelante and Gilmartin, 1991; Flander-Putrlle and Malej, 2008) get entrapped in the newly formed matrix of the suspended organic and inorganic material, generated by gelling of mainly microphytoplankton exuded polysaccharides, primarily diatoms (Degobbis, 1989; Stachowitsch et al., 1990; Kaltenbock and Herndl, 1992; Kovac et al., 2002). Although the macroaggregates microphytoplankton composition varied from one event to another, the main contributors were recurrently diatoms (Herndl and Peduzzi, 1988; Stachowitsch et al., 1990; Rinaldi et al., 1995; Fonda Umani et al., 2005; Totti et al., 2005). Species present in macroaggregates are typical members of the spring/summer phytoplankton community of the NA (Revelante and Gilmartin, 1976; Revelante et al., 1984; Totti et al., 2005). In the newly formed macroaggregates, phytoplankton community and relative abundances of dominant species resemble those in the ambient water (Najdek et al., 2002, 2005; Flander-Putrlle and Malej, 2008). After the formation, the macroaggregate embodies a self-sustaining ecosystem with the internal primary production and organic matter recycling (Revelante and Gilmartin, 1991; Degobbis et al., 1995; Najdek, 1996), enabling pronounced inside transformations, alternations and species successions, generally dissimilar to the ones ongoing in the ambient water (Revelante and Gilmartin, 1991; Decho and Herndl, 1995; Najdek et al., 2002). It is hypothesised that macroaggregates have a relatively long residence time of one to three months as their species composition progressively changes with age and from the one characterising the ambient water (Fogg, 1995). With the macroaggregate age, abundance of all organisms comprised within was observed to increase for several orders of magnitudes (from 1 to 3, even up to 5) from their abundance in the seawater at the time of the macroaggregate formation (i.e., origin) and independently from the concurrent surrounding water (e.g., Alldredge and Silver, 1988; Revelante and Gilmartin, 1991; Del Negro et al., 2005; Totti et al., 2005). In addition, macroaggregates were found to differ from the ambient water in relative proportions of dominant species (Revelante and Gilmartin, 1991) or

to differ in species composition completely (Cataletto et al., 1996). Based on findings that seawater phytoplankton biomass during the mucilage event did not significantly differ from the years without the event (Revelante and Gilmartin, 1991; Flander Putrle et al., 2000; Najdek et al., 2002; Flander Putrle, 2003), it was suggested that macroaggregates development does not influence the phytoplankton biomass in the surrounding water (Flander-Putrle and Malej, 2008).

For some phytoplankton species macroaggregates represent a highly favourable microhabitat (Alldredge, 1976). *Cylindrotheca closterium* has been recognised as a recurrent, opportunistic diatom, colonising regularly already formed macroaggregates (Najdek et al., 2002; Totti et al., 2005). It seems that the macroaggregates, simulating nutrient rich and hard bottom, represent an optimal habitat for this benthic species. After colonisation of a macroaggregate (Najdek et al., 2002), follow an intense multiplication (Monti et al., 1995) and a consequent dominance of *C. closterium* in the phytoplankton community, often ending with monospecific populations (Najdek et al., 2002; Totti et al., 2005). This process was also observed in the laboratory conditions (Kraus et al., 2004). Hence, according to the percentage contribution of *C. closterium* in macroaggregates, it is feasible to deduce whether a macroaggregate is a freshly formed one, an aging or aged one (Najdek et al., 2002; Blažina et al., 2005; Totti et al., 2005). It appears that *C. closterium* has an enhanced potential of reproduction in the mucous compared to other species and that it consequently prevails over all other species initially inhabiting macroaggregates (Grossart, 1999; Degobbis et al., 1999). *C. closterium* prolonged existence in mucilage macroaggregates may also be related to a persistent nutrient input in macroaggregates, resulting from a continuous internal recycling of the organic matter (Kaltenböck and Herndl, 1992; Decho and Herndl, 1995). This species is also known for the lavishing exudation of mucilage matter (Monti et al., 1995; Alldredge, 1999), under controlled stress conditions, such as nutrient starvation (Monti et al., 1994; Welker and Monti, 1994). Under the specific conditions of marked stratification and P-limitation, *C. closterium* exudates are hypothesised to relevantly contribute to the accumulation and persistence of organic matter in the region (Urbani et al., 2005).

Abrupt changes of the physical and biogeochemical conditions in the basin seem to precede the development of mucilage event (Cozzi et al., 2004; De Lazzari et al., 2008; Blažina et al., 2011). The event seems to start usually in the upper layer of the extremely stratified seawater column (Alldredge and Crocker, 1995; Degobbis et al., 1999; Najdek et al., 2002; Precali et al., 2005) when the waters of the NA are in restricted contact with waters of the central

Adriatic (Degobbi et al., 2000). The formation of a usual phytoplankton bloom in a circulation system of the NA was already observed (Kraus and Supić, 2011). Macroaggregates are also held to accumulate within the isolated circulation cells, in which freshened waters from the Po River delta are kept for a longer time period, and whose position is well described by the distribution of geostrophic currents (Supić et al., 2000; Supić et al., 2001-2; Supić et al., 2003). Circulation patterns of the NA show the pronounced interannual variability (e.g., Supić et al., 2012). In general, the region is included in the large Adriatic-wide cyclonic meander during cold part of the year and gets separated from it in the warm part of the year, when several circulation cells become well developed (Krajcar, 2003). A cyclonic gyre typically appears north to the Po River delta and an anticyclonic in the eastern part off Rovinj. The anticyclonic is identified by the presence of a current in the eastern coastal zone, the Istrian Coastal Countercurrent (ICCC) which, according to Supić et al. (2003), was present in the entire period from March to September of 1997. Once formed, the macroaggregates distribute over the entire water column, with a tendency of accumulation around the pycnocline (Herndl, 1992; Alldredge and Crocker, 1995). Throughout the extended residing period at the pycnocline layer, the macroaggregates grow to larger forms (Precali et al., 2005). During the entire period of the mucilage event, all types of macroaggregates continuously form (Najdek et al., 2005; Precali et al., 2005). The small macroaggregates are paired with the large ones and it is supposed that they form concurrently and/or that the small ones are torn from the large ones (Najdek et al., 2002).

Macroaggregates buoyancy changes induce their vertical movements. They gain buoyancy from the numerous gas bubbles forming within, due to the constant inside microbacterial processes, which enable them to float towards the surface (Rinaldi et al., 1995). Correspondingly, the diffusion of more saline waters into macroaggregates induces their loss of buoyancy, which is followed by their sinking (Alldredge and Crocker, 1995). Conversely to macroaggregates vertical movements, their horizontal whereabouts remain rather unexplained. The concept that macroaggregates occurred at an earlier time and/or at the different site from their sampling location was already introduced (Revelante and Gilmartin, 1991). Yet, without any conclusive evidences, the assumption that macroaggregates can be dispersed from their origin by currents, remained only a hypothesis (Degobbi et al., 1995; Rinaldi et al., 1995; Cataletto et al., 1996; Danovaro et al., 2009).

Turbulence induced by weak winds, resulting with mixing in upper layers, is held to facilitate the aggregation processes (e.g., de Lazzari et al., 2008). On the other hand, in conditions of several days blowing of the NE weak winds the large aggregates can be dispersed and/or settled to the bottom (e.g., de Lazzari et al., 2008). Unusually persistent and intense wind

blowing, mainly bora, can regress or interrupt the phenomenon development (Russo et al., 2005), while the storms were attributed to have interrupted the mucilage event (Russo et al., 2005, Precali et al., 2005).

The aim of our work was to investigate the effects of the sea dynamics on the mucilage event basing our research on 1997 data. This was done by analysis of the microphytoplankton species composition in the macroaggregates and in surrounding waters, surface heat fluxes and mixing in the water column and geostrophic currents.

2. MATERIALS AND METHODS

2. 1. Data

We analyzed three types of data obtained at the oceanographic cruises in the NA during the summer and autumn of 1997: (a) phytoplankton data (128 seawater and 21 macroaggregate samples, sampling dates in Suppl. 1. and Table 4, respectively), (b) oceanographic data (temperature and salinity) and (c) meteorologic data and sea surface temperature data for computing surface fluxes. Temperature, salinity and seawater phytoplankton samples were collected during summer of 1997 in July (8, 22, 23 and 24), August (4, 11 and 12) and September (1 and 23) at the standard depths (0 m, 5 m, 10 m, 20 m, 25 m and near the bottom, approx. at 30 m) at nine stations in the NA (SJ001, SJ007, ZI012, SJ108, SJ101, SJ103, SJ105, SJ107 and ZI032; Fig. 1.). Stations SJ105 and SJ107 are deeper (35 m), so at SJ105 bottom layer was sampled at 35 m, however at SJ107, at 30 and 35 m. Oceanographic data were, during the 22-24 July and 11-13 August cruises, sampled at several additional stations in the region (Fig. 1.). To get a rough estimation of meteorological and hydrological conditions during and between the cruises in July and August we used in addition wind data measured three times a day at Pula station (at 6 h, 13 h and 20 h UTC) and data on the Po River daily discharge rates collected at the station Pontelagoscuro. Wind data were supplied by the Hydrometeorological Institute, Zagreb and by the “Assessorato della Programmazione, Pianificazione e Ambiente” of the Emilia Romagna region (Italy).

2.1.1. Phytoplankton data

2.1.1.1. Collection of samples and subsamples treatment

Five litre seawater samples were collected with Van Dorn bottles, immediately filtrated through 300 µm mesh plankton net and subsamples of 200 mL taken for phytoplankton determination.

Mucilage macroaggregates were sampled by scuba divers at various depths (7, 9, 10, 11, 12, 15, 16, 18 and 20 m). Depending on the macroaggregate size, samples were taken by 500 mL Plexiglas tubes (diameter 5.5 cm) closed by a rubber stopper at both ends (large macroaggregates, ranging approx. from 2 m to 5 m in length and from 0.5 m to 2 m in height) or by 20 mL polyethylene syringes with the forward end cut off (small macroaggregates, flocks of diameter up to 10 mm).

Subsamples of small macroaggregates (15 – 20 mL), large macroaggregates (20 – 200 mL) and seawater (200 mL) were immediately preserved with Lugol's solution prepared with sodium acetate, stored at + 4 °C and processed within a month. Prior to microscopic analysis, subsamples of macroaggregates were carefully shaken manually, to homogenize the sample, yet to prevent the deformation of cells. In cases of high mucosity of a sample, which could diminish the accuracy of cell count and determination, the sample was diluted with filtered seawater.

2.1.1.2. Phytoplankton determination

Phytoplankton abundance and composition were determined at 200 x magnification in 100 random vision fields (if necessary, 50, 200 or 400 depending on the sample density) after 40 hours sedimentation of a 50 mL subsample by a Zeiss inverted microscope, using the Utermöhl settling technique (1958). In our analyses we used phytoplankton data in the size range of 20 – 200 µm. Identification was carried out to the species or genus level.

2.1.2. Oceanographic data

Temperature and salinity were used to assess surface geostrophic currents relative to the 30 dbar fields, which derive from spatial distribution of dynamic depths of the corresponding surface. At each sampling station (s), for each separate cruise (c), the dynamic depths of the 30 dbar surface [$D_{30}(s,c)-D_0(s,c)$] were computed after:

$$D_{30}(s,c)-D_d(s,c)=\int_d^{30}\alpha(s,c)dp.$$

The specific volume α depends on sea density ρ and equals to $\alpha=1/\rho$. Sea density was computed from temperature and salinity data at standard depths using standard equation.

Each sampling station was, for each separate cruise, characterised with oceanographic parameters (OP) which are thermocline depth (assessed from the data at the standard sampling depths and amounting to 7.5 m, 15 m or 25 m), average temperature, salinity and density above and below the thermocline, density differences between layers above and below the thermocline, density differences between surface and 30 m layer, density differences between surface and 20 m layer, dynamic depth of the 30 dbar surface and dynamic depth of the 20 dbar surface.

2.1.3. Set of data for computing air-sea fluxes

The set of data consists of daily values of standard meteorological data (air pressure, air temperature, wind speed, fractional cloud cover, water vapour pressure and precipitation) measured at Pula Meteorological Station (44° 52' N, 13° 55' E; Fig. 1) and sea surface temperature (SST) data, measured at the lighthouse Sv. Ivan na Pučini, in the interval from 1 July to 30 September 1997. These data were provided by the Hydrometeorological Institute in Zagreb (meteorological data) and by the Maritime Meteorological Center in Split (sea surface temperature data). Surface fluxes, among which we present the surface loss due to evaporation Q_e and total heat air-sea heat flux Q , were computed using the standard set of equations proposed by Gill, as described in details by Supić and Orlić (1999).

2. 2. Data analysis

For statistical analyses of phytoplankton dataset PRIMER (Plymouth Routines In Multivariate Ecological Research) v5 software package was used. As a transformation method, we used contribution percentages of each species in the sample, to enhance the importance of qualitative and down-weight the quantitative aspect of the data, as the comparisons were performed between seawater samples and macroaggregate samples, which considerably differ in phytoplankton abundances.

Seawater phytoplankton dataset obtained during July and August 1997 was separated in 13 seawater phytoplankton groups (further in text: SPG) upon the cluster analysis (not shown). Groups were formed at the similarity level of 60 and above. The significance of the spatial and temporal variation between the SPGs obtained by a Cluster analysis was tested using a One-Way Analysis of Similarities (ANOSIM) routine (Table 1b). Multivariate routine SIMPER (similarity percentage) was used to calculate the average similarity percentage between at least two samples, based on the overall percentage contribution of each species in the sample. In our research, SIMPER was used to calculate similarities between the 13 determined SPGs (Table

1.a), within each SPG (Table 2.a) and between each of 21 macroaggregate samples and each of 13 SPGs (Table 4). The maximum similarity between the two samples (totally identical samples) would equal 100, while no similarity would equal 0. SIMPER was also used to quantify the overall species contributions to each SPG (Table 2a) and macroaggregate (Table 3). Similarities of 40 or higher were considered significant as they belong above upper quartile of all calculated similarities.

We calculated basic statistics of OP for each separate cruise (8 July, 22-24 July and 11-12 August; Suppl. 2) at stations of hypothetical macroaggregate formation (origin stations), stations where macroaggregates were found (sampling stations), stations where macroaggregates were supposed not to have been formed (non-origin stations) and complete dataset (all stations).

Additionally, sets of OP were, for each separate cruise (8 July, 22-24 July and 11-12 August; Table 6), were analysed by PCA to find out the main OP characterising the investigated stations. Note that input parameters for the PCA were sets of OP with parameter Origin. Origin is a parameter which resulted from our investigation on hypothetical locations of macroaggregates formation. Stations where macroaggregate/s were assumed to have formed was characterised by 1, while stations where macroaggregate/s were assumed not to have been formed were characterised by 0. All OP data were $\log(X+1)$ transformed.

The Distance-based linear model (DISTLM) and distance-based redundancy analysis (dbRDA) were used for determination of the relation between phytoplankton community at 0 m, 10 m or bottom and OP for each cruise separately (8 July, 22-24 July and 11-12 August; Suppl. 3). Analyses were performed (a) with information on origin (Origin included) and (b) without it (Origin excluded). For DISTLM we used Euclidean distance for creating the resemblance measure, forward selection as procedure and adjusted R^2 as criterion.

3. RESULTS

3.1. Phytoplankton community in the NA during the summer of 1997

3.1.1. Seawater phytoplankton community

Table 2a represents 13 SPGs, outlined by similarities between the seawater phytoplankton samples obtained in July and August of 1997, based on species percentage contributions to each sample. After establishing the statistical significance of difference between the determined SPGs with ANOSIM test ($R = 0,967$, significance level = 0,1 %), these SPGs were used in further analyses in our work. Based on similarities between the SPGs, the association

schematics of SPGs was created (Fig. 2). Horizontal and vertical distributions of the seawater phytoplankton community in the NA observed on 8 July, 22-24 July and 11-12 August 1997 are presented by means of determined SPGs in Fig. 3 and Fig. 4, respectively. In Table 2b we present distribution of SPGs with dominant species during July and August at the sampled stations and depths.

The situation in the NA on 8 July was rather complex (Figs. 7a,b and 8). The waters from the Po River delta, after a huge Po River discharge accumulated in the two low salinity surface pools, one around st. SJ108 and another one about st. SJ103 – st. SJ105. In both, SPG 2 with dominant diatom *Skeletonema costatum* was observed. Additionally, this riverine discharge induced a pronounced vertical gradient at st. SJ108, resulting in a stratified array of phytoplankton communities (SPGs 1, 2, 8 and 12, with diverse dominant diatoms: *Chaetoceros socialis*, *S. costatum*, *Leptocylindrus danicus* and *Pseudo-nitzschia delicatissima*, respectively). At the surface layer at st. SJ101 and st. SJ107, SPG 9 with dominant *P. delicatissima* was observed, which was an older water body according to the higher salinity at the locations. In the lower layer at the eastern part, a higher salinity waters with SPG 12, dominated by *P. delicatissima*, were spread between st. SJ101 and st. SJ107.

During the entire analysed period, diatoms dominated the NA microphytoplankton community. Later in July (22–24), at the surface of st. SJ001, SJ101, SJ103 and SJ007, SPG 4 with *Dactyliosolen fragilissimus* was detected, while at the rest of the region SPGs 12 and 13 with *P. delicatissima* were observed. Only at the bottom at st. SJ001, SPG 7 with *Nitzschia longissima* f. *tenuirostris* was found. On 11–12 August, *D. fragilissimus* was dominant species at the surface layer of the entire region (SPGs 4 and 5). At the lower layer, *P. delicatissima* was dominant (SPGs 10–13), with *Paralia sulcata*, *N. longissima* f. *tenuirostris* and *Cylindrotheca closterium* found respectively at st. SJ107, SJ007 and ZI012 in SPGs 3, 7 and 6.

Due to specific importance of *Thalassionema nitzschioides* and *Cylindrotheca closterium* during the 1997 mucilage event, we present vertical distribution of these two particular species in the period from July to September (Fig. 5 and Fig. 6, respectively). On 8 July 1997, *T. nitzschioides* was first noticed solely in the upper layer at st. SJ103. Over the following two weeks, it spread over the entire water column of st. SJ103, westwards in the surface layer to st. SJ101 and SJ108 and eastwards in the bottom layer to st. SJ105. Until mid August it was detected over the entire water column of st. SJ103 and SJ105 and further eastwards, in the surface layer of st. SJ107. In the beginning of September it was found in the upper 10 m at all stations of the Po River–Rovinj profile (except at st. RV001) and spread over the entire water

column later in the month. Similarly, distribution of *C. closterium* on 8 July started from one location, surface of st. SJ108. Later in the month, it was found additionally in the bottom layer of st. SJ103. During mid August, it was detected from 10 m towards bottom of st. SJ103, SJ105 and SJ107. Over the next month, it spread over the entire Po River–Rovinj profile (except at st. RV001), from 5 m depth towards bottom.

3.1.2. Mucilage macroaggregates phytoplankton community

List of all 21 sampled macroaggregates (10 large and 11 small ones) with sampling location (station and depth) and date, and phytoplankton composition is presented in Table 3. In Table 4 we present similarities between each macroaggregate and every SPG established during the period 8 July – 11 August as well as the hypothetical time and location of formation of each macroaggregate (i.e., origin water column). We hypothesized the most possible formation location/s and point/s in time for each macroaggregate considering several factors: similarities between the macroaggregate and SPGs (Table 4) and the distribution patterns of the two species, *Thalassionema nitzschioides* (Fig. 5) and *Cylindrotheca closterium* (Fig. 6). Macroaggregates that we believe have remained at the formation location until sampling are referred to as “residing”, while the ones which are supposed to have been transferred from the hypothetical formation location to a different, sampling location are referred to as “transient” macroaggregates.

3.1.2.1. Macroaggregates sampled on 22-23 July

Residing macroaggregates (3, 4, 6, 7 and 8)

Macroaggregates contained none or up to 18 % of *Cylindrotheca closterium*, indicating that probably none or only some succession changes occurred inside them. Data suggest that these macroaggregates most probably remained at the formation station, i.e., that the sampling station is also the (hypothetical) formation station, as the highest similarities of these macroaggregates (40 – 79 %) were with SPGs which were observed at the time of sampling at the sampling station.

Transient macroaggregates (1, 2 and 5)

Macroaggregates contained from 14 up to 52 % of *C. closterium*, indicating that probably some or even considerable succession changes occurred inside them. Generally, similarities with all SPG's were low (< 30). However, presence of *Thalassionema nitzschioides* in all three macroaggregates (13 – 67 %) implies that this diatom was obligatory present in the seawater at the locations and during moments when they formed, or later passed during their transport

by currents, and that during that time some succession changes did occur. As this diatom was detected only at st. SJ103 until 22 July, it is possible that these macroaggregates were formed at this station, wherefrom they were transferred by currents to their sampling stations, st. SJ107 and st. SJ108.

3.1.2.2. Macroaggregates sampled on 4 August

Unfortunately, no microphytoplankton was determined in the seawater samples on this date.

Resident or transient macroaggregates (11 and 12)

Macroaggregates contained none and 18 % of *C. closterium*, indicating that probably none or only some succession changes occurred inside them. Highest similarities of these two macroaggregates was with SPG 4 (59 – 74 %), which was noticed to have spread with currents from st. SJ101/SJ103 to almost the entire region during the period from 22–24 July until 11–12 August, indicating that this most probably occurred at st. ZI032 as well. Hence, we data suggest these macroaggregates could have formed anywhere in the area during late July, that they went through some succession changes and remained at/floated to st. ZI032 where they were sampled.

Transient macroaggregates (9 and 10)

Both macroaggregates contained high abundances of *C. closterium* (66 – 75 %), indicating occurrence of considerable succession changes. Moreover, *T. nitzschioides* was detected in both macroaggregates (8 – 17 %) and *Nitzschia longissima* f. *tenuirostris* only in the second one (8 %). A closest location where these diatoms were noticed was at the beginning of July in the upper layer at st. SJ103, wherefrom it spread vertically towards bottom and horizontally, westwards to st. SJ101 (5 m) and st. SJ108 (0 m) and eastwards to st. SJ105 (35 m) until 22 July. These data suggest that both macroaggregates formed in the area of st. SJ103 and floated to st. SJ107. Furthermore, as macroaggregate no. 9 comprised about 50 % more of *C. closterium* and 50 % less of *T. nitzschioides* than macroaggregate no. 5, and since data suggest that both macroaggregates originate from the same area (around st. SJ103), we suppose that macroaggregate no. 9 is an aged “macroaggregate np. 5 – type”, i.e., that macroaggregates after formation at st. SJ103 in July, floated to st. SJ107 and went through succession changes until 4 August.

3.1.2.3. Macroaggregates sampled on 11-12 August

Residing macroaggregates (13, 14, 15, 16, 17 and 20)

Macroaggregates contained various proportions of *C. closterium* (from none to 7 % and 88 %), indicating that none, some or even considerable succession changes occurred inside them. All macroaggregates except no. 16 showed highest similarities with the SPG 4 (40 – 80 %) which was at the time detected over the surface layer of the entire Po River profile. Data suggest that macroaggregates no. 13, 14 and 17 most probably remained at their formation stations, st. SJ108 and st. SJ107, where they were sampled. Macroaggregate no. 15 formed at the surface layer of st. SJ101 and sunk to 9 m depth where it was sampled. The no. 20 most probably formed at st. SJ001 where no surrounding water was sampled, but most probably SPG 4 had spread also to this station (Fig. 3). The remaining no. 16 comprised of 88 % of *C. closterium*, indicating substantial succession changes and resulting with low similarities with any SPG. Hence, we based our conclusion on presence of 7,20 % of *T. nitzschioides* in the macroaggregate. As *T. nitzschioides* on 22–24 July was observed at 5 m at st. SJ101 and at 0 m at st. SJ103 in SPG 4, and at 10 m and 30 m at st. SJ103 in SPG 12, it seems that no. 16 also remained in the same area in which it was formed, around st. SJ101– st. SJ103.

Transient macroaggregates (19 and 21)

Unfortunately, no microphytoplankton was determined in the seawater samples on this date at the sampling stations of these two macroaggregates. Macroaggregate no. 19 contained low proportion of *C. closterium* (4 %) and the other none, indicating scarce succession changes. The first one comprised 78,80 % of *T. nitzschioides* which was found at the closest at 0 m at st. SJ103 in late July and mid August and at 0 m in mid August at st. SJ105 in SPG 4, and at 10 m at mid August at st. SJ105 in SPG 5. Hence, it is possible that these macroaggregates could have formed there and circulating within a closed circulation cell got transferred to the sampling location, st. SJ001. During that period some succession changes occurred. Macroaggregate no. 21 showed highest similarity with the SPG 4 (69,3 %), which was detected in the upper layer of st. SJ007 on 11 August. Therefore, it is possible that it formed around st. SJ007 and was transported to sampling location, st. ZI012.

Resident or transient macroaggregate (18)

This macroaggregate contained no *C. closterium*, however, as it contained high amount of *T. nitzschioides* (63 %) we assume that some succession changes occurred within. Highest similarities (38 – 40 %) were noticed with SPGs 4 and 5. In both SPGs this diatom was observed. SPG 4 was detected at 5 m of the same station and SPG 5 at 10 m at st. SJ105, both belonging to the same circulation system (Fig. 8). Hence, we suppose it could have formed at either of the two stations.

3.2. Wind data, Po River discharge rates and oceanographic conditions

Daily values of the total surface heat fluxes in summer 1997 were highly dependent on evaporative fluxes and wind speed (Fig. 7a). In July and August, as well as in the first part of September, the total surface heat flux was generally positive with several episodes of surface heat losses, among which the one on the 18 July was the most intense (above 200 W m^{-2}) and followed by the ones on the 1 and 11 August, with surface heat losses of 160 W m^{-2} and 84 W m^{-2} , respectively. After the 10 September, the sea was generally losing heat during an intense heat loss episode (of 179 W m^{-2}), with maximal strength on 15 September (256 W m^{-2}). Wind data taken at 07, 14 and 21 h (not shown) suggest that the most intense heat loss episode on 18 July was induced by wind from N in evening of the day, which was preceded by winds from W and followed by winds from WNW, that winds from ENE played an important role in episodes of the 1 and 13 August and that winds from south induced the 27–28 August surface heat loss episode. Po River rates were high only in the first half of July and were low and constant afterwards (Fig. 7a).

According to hydrographic conditions, the water column across the Po River delta – Rovinj profile on 8 July was stratified (Fig. 7), with minimal density values in the two low salinity surface pools, one close to the delta at st. SJ108 (27) and another in the central part of the profile around st. SJ103 (30). Two weeks later, on 22 July, a change in hydrographic conditions was observed. The two lower density pools combined into one, extending from st. SJ108 to around st. SJ105. Density increased with respect to the previous cruise, hence documenting a strong mixing event after the 18 July surface heat loss episode. The common feature of the horizontal distribution of dynamic depths of the 30 dbar surface on 22–24 July and 11 August (Fig. 8) was the presence of the low salinity deep basin located south–east to Rovinj, which was the central part of an anticyclonic gyre. However, in central and eastern part of the investigated region different circulation patterns between the cruises were observed. On 22–24 July the motions in the western part of the Po River delta – Rovinj section were anticyclonic, while on 11 August the transversal motions spreading waters from the Po River delta towards east prevailed in the western and central part of the Po River delta – Rovinj section.

3.3. Environmental factors

Simple statistics (Suppl. 1), cluster analysis (not shown) and PCA (Table 6) performed on datasets of 8 July, 22–24 July and 11–13 August 1997 showed that differences between areas

in which macroaggregates were formed (hypothetically, according to our analysis of similarity between phytoplankton communities in macroaggregates and seawater) and other areas were not highly pronounced. It seems that origin water columns were generally of lower salinity and density in upper layers above the thermocline and were more stratified than surrounding areas in which macroaggregates were not formed. Interestingly, results of PCA analysis indicate that dynamic depths of origin columns were in July lower and in August higher than dynamic depths of non-origin columns.

A much more detailed analysis was performed by DISTLM, which in addition to water column parameters and information whether the water column was origin or non-origin one, included seawater phytoplankton community composition. It showed that one particular SPG can appear in various types of environmental conditions. The distribution of SPG's was mainly driven by distribution of salinity and stratification degree and/or dynamic depths of 20 dbar surface (Suppl. 2, Origin excluded). Similar as PCA, DISTLM pointed to salinity, density and dynamic depths as the main environmental parameters which characterise the origin water column. More specifically, it follows that macroaggregate formation, which as a rule occurs in 0 – 10 m layer, appeared in areas characterised by: (1) extremely low or high values of salinity in the overall water column on 8 July, (2) low dynamic depths of 20 dbar surface, stratified surface layer of 10 m and extreme low or high density values in the lower part of the water column on 22–24 July and (3) high dynamic depths of 20 dbar surface, extreme values of salinity in the overall water column and intensely stratification of the water column (Suppl. 2, origin included).

4. DISCUSSION

On locations of macroaggregates formation

Although the mucilage formation was observed in the eastern Gulf of Trieste during May 1997 (Flander-Putrlle and Malej, 2008), data suggest that massive mucilage event in the open NA actually started after 8 July as during the cruise on that day no macroaggregates were found. The first observed macroaggregates were found on 22–23 July (both large, no. 3 and 6, and small, no. 7) in the layer 12 – 15 m at st. SJ001 and SJ101, locations belonging at that moment to a cyclonic gyre (Fig. 8), and at 10 m at st. ZI012 (small, no. 8) to an anticyclonic one (Fig. 8). They had phytoplankton composition which was similar to the composition of the surrounding water. Thus we conclude that they remained at their formation location until the sampling, residing within a closed circulation cells of cyclonic or anticyclonic rotation sense.

There are more evidences that macroaggregates reside within gyres: the macroaggregate no. 16, found on 11 August, according to *T. nitzschioides* distribution, formed previously in July around st. SJ101 – st. SJ103, and remained in the same area for a longer time period, between the July and August cruises. According to our geostrophic circulation map, the macroaggregate no. 16 was kept in a cyclonic gyre, present around st. SJ101 – st. SJ103 on both 22–24 July and 11 August cruises (Fig. 8). Interestingly, the basic of NA circulation patterns, with an cyclonic gyre in western part of the region and anticyclonic in the eastern, as shown at Fig. 8 for July and August cruises, remained (at least) till September, which is visible also on one of the first satellite images of the NA taken on 16 and 17 September 1997 (Fig. 8; Mauri and Poulain, 2001). It was already hypothesized that gyres are favourable location for macroaggregate's formation and residing (e.g., Supić et al., 2001-2; 2003) and our findings support these previous assumptions.

Our results show that during the entire mucilage event new macroaggregates are constantly being formed and that at the same time in the same region old and fresh macroaggregates coexist (as already observed by Precali et al., 2005 and Najdek et al., 2002). Furthermore, data suggest that all macroaggregates during the 1997 mucilage event formed in the upper layer (0 – 15 m), substantiating the thesis that the formation of mucilage macroaggregates occurs predominantly above the pycnocline and in the upper layer (Degobbi et al., 1995; Precali et al., 2005). In contrast, the sampling depths ranged from 7 to 20 m indicating that some macroaggregates sink after the formation. These findings are in accordance with the previous observations that macroaggregates during their life span sink to deeper layers, usually accumulating around the pycnocline (Precali et al., 2005). We found no indication that any of the macroaggregates rose in the water column after its formation during the 1997 event.

On the macroaggregates transport by geostrophic currents

Several macroaggregates (no. 1, 2, 5, 9 and 10) seem to have formed at the st. SJ103, all of them at the beginning of the 1997 mucilage event, as each macroaggregate contained *Thalassionema nitzschioides*, a diatom which was at that time present only at this particular location. These macroaggregates were sampled on 22 July and 4 August, both in the western (at st. SJ108) and in the eastern part of the profile (at st. SJ107). Our idea that these macroaggregates were transported by circulation pattern from around st. SJ103 to these two sampling locations is sustained by the geostrophic currents patterns and velocities (Figs. 1 and 8). To reach these stations in three days the macroaggregate would travel with speed of 5 cm s^{-1} (distance between st. SJ103 and st. SJ101 is around 13 km) or 10 cm s^{-1} (distance between st. SJ103 and st. SJ107 is around 25 km). These values roughly coincide with speeds computed

for the alongshore section in eastern part of the region in summer 2003, and amounting up to 4 cm s⁻¹ in calm conditions and up to 10 cm s⁻¹ one day after an episode of surface heat loss (of around 140 W m⁻²) as presented by Lyons et al. (2007).

We assume that the circulation pattern observed on 22 – 24 July formed during the 18 July surface heat loss event, simultaneously with the changes in hydrographic conditions discussed above in Results section. Thus, the macroaggregates formed and resided in a low salinity surface pool at st. SJ103, before the surface loss of the 18 July event. After this date, macroaggregates have likely been transported both towards east and west, as presented schematically at Fig. 1, to st. SJ107 and st. SJ108. The idea that macroaggregates get transferred from one location to another was already introduced (Franco and Michelato, 1992; Revelante and Gilmartin, 1991; Cataletto et al., 1996; Russo et al., 2005; Danovaro et al., 2009). However, in this work we investigated the idea more closely and found relation between macroaggreagate and surrounding phytoplankton distribution and geostrophic circulation paths supporting this idea.

On air-sea influence on phytoplankton species distribution and mucilage events

Results of DISTLM analysis, relating salinity, and other parameters highly dependent on it, density and depth of 20 dbar surface, to phytoplankton distribution, imply that mechanism controlling the low salinity Po River waters spreading across the NA controls also distribution of phytoplankton species in the investigated region. Spreading of Po River waters across the NA is basically dependant on stratification degree, winds, and air-sea fluxes (e.g., Lyons et al., 2007). The first heat loss episode, on 18 July, changed existing density fields and circulation patterns, replaced the macroaggregates formed in central part of the Po River delta-Rovinj profile towards east and west, and altered the existing phytoplankton distribution in the investigated region. On 22-24 July cruise the number of SPG in upper layers was reduced with respect to the 8 July cruise, and some SPG documented previously (e.g., 2, 8 and 9) totally dissapered and were replaced by others (4, 12 and 13). The large expansion of low salinity waters towards east between 22-24 July cruise and 11-13 August cruises brought SPG 4 (Figs. 3, 4 and 8), which seems to be the origin water of several macroaggreagates (Table 4). Thus, SPG 4, residing in cyclonic gyre off Po River delta, was transported into the large anticyclonic off Istra around st. SJ107, location at which before 22 July (Table 4) no macroaggregates were formed. By introduction of new species with SPG 4, including the opportunistic *T. nitzschoides*, the gyre around st. SJ107 became a new location of macroaggregates formation. This explains the shift between July and August origin water columns position, which was

preferably in areas of low 20 dbar surface (cyclonic gyre) in July and in areas of high 20 dbar surface (anticyclonic gyres) in August, as indicated by PCA (Table 6).

Spreading of lower salinity Po River waters after summer bora events up to the eastern coastal zone and intensification of motions in the anticyclonic gyre off Rovinj, was previously documented (Lyons et al., 2007). Thus arrival of lower salinity waters with its specific SPG in the eastern NA with shift in macroaggregate formation areas in 1997 seems to be provoked by atmospheric forcing, namely by strong winds from ENE blowing during 1 August and/or 13 August surface heat loss episodes. The first and most intense surface loss episode of 18 July was induced by N wind, which was preceded by winds from W and followed by winds from WNW, although changed significantly density fields and circulation patterns and transporting around already formed macroaggregats, did not invoke lower salinity waters spreading to the eastern coast.

On *Cylindrotheca closterium* introduction in the water column

The question of how a benthic diatom, *C. closterium*, occasionally detected in the water column in the NA, enters the water column and subsequently colonises the macroaggregate, is still unanswered (Monti et al., 1995; Alcoverro et al., 2000; Najdek et al., 2005; Totti et al., 2005). Resuspension and redistribution of microphytobenthic species by water movements was repeatedly reported (Thornton, 2002; de Jonge and Van Beusekom, 1992, 1995). We assumed that this was the case with *C. closterium* and confirmed the assumption by analysing its abundance in relation of oceanographic and meteorologic data.

At the beginning of the 1997 mucilage event, *C. closterium* was generally absent from the investigated waters with the exception of surface layer at st. SJ108. The intense 18 July evaporation event, which induced turbulent mixing in the entire water column, could have provoked sediment resuspension and consequent *C. closterium* elevation from the sea bottom into lower layers of the water column (as observed at st. SJ103; Fig. 6). It was previously shown that the summer episodes of surface heat losses can reflect on dynamics of bottom layers in the region, inducing bottom currents and changes in hydrographic conditions (e.g., Vilibić et al., 2008). The intense episode on 18 July seems to be one of them. Namely, bottom salinity, at all stations, increased for 0.5-0.6 (not shown). Such a large salinity rise is rarely observed between the two subsequent cruises, as can be concluded from long term bottom salinity data (at st. SJ107; Supić and Vilibić, 2006). Thus we believe that 18 July surface heat loss episode was capable of introducing mixing in the upper sediment layers, able to elevate bottom species to upper parts of the water column, and induced *C. closterium*, which was absent previously

from deeper layers of the water column, as it was observed at 30 m depth at st. SJ103 on 22 July (Fig. 6). Other summer surface heat losses episodes, which occurred later, were less intense, inducing thus also less intense near bottom turbulent mixing. Namely the bottom layer density change (at 30 m) in the open NA (st. SJ101 – st. SJ107) was around 0.4 on 8 and 22 July and around only 0.04 between 11 August and 1 September. Thus these less intense surface heat loss episodes were less effective in the bottom sediment elevation. (The rise in abundance of opportunistic species as observed between 11 August and 1 September at Figs. 5 and 6 must therefore be invoked by other reasons and not from sediment resuspension.)

Once *C. closterium* was elevated from sediment and was introduced in the water column, it can easily reach and colonise existing macroaggregates.

On the opportunistic species Thalassionema nitzschioides

Just as *C. closterium*, *T. nitzschioides* is also a highly silicified, large pennate diatom, favouring nutrient enriched habitats (Najdek et al., 2002). Our findings indicate that *T. nitzschioides*, just as *C. closterium*, can prevail over other species inhabiting the macroaggregates by multiplying to high abundances, regardless of the small initial abundance of *T. nitzschioides* in the ambient seawater. Obviously, *T. nitzschioides* can successfully outcompete other species in a macroaggregate just as successfully as *C. closterium* does. Nevertheless, it seems that intense multiplication and dominance of *T. nitzschioides* in the macroaggregate does not reduce the probability of the following, final succession phase colonisation with *C. closterium* (see macroaggregate no. 9 in Results section). Consequently, we can conclude that both, *C. closterium* and *T. nitzschioides* are opportunistic species of the mucilage phenomenon. It is also interesting to notice that in one small macroaggregate (no. 8) neither *C. closterium* nor *T. nitzschioides* were observed. This shows that for macroaggregate formation, presence of the two opportunistic species is not essential.

Although 18 July heat loss episode induced sediment resuspension and elevation of *C. closterium* in the water column, the same cannot be presumed also for *T. nitzschioides* as it was already present in the upper layer of st. SJ103. Thus presence of *T. nitzschioides* in the bottom layer of st. SJ103 on 22 July might have occurred alternatively, from vertical spreading from the upper layer.

According to current state-of-the art, macroaggregate contains species which were present in the water column at the time of its formation and later undergoes species succession marked with colonisation and increase of *C. closterium* abundance, and microphytoplankton diversity decrease and abundance reduction of all other species. Hence, it follows that *T. nitzschioides* also entered macroaggregate during formation process. However, for our research

on macroaggregates transportation with currents it is irrelevant if the macroaggregate formed or just passed a location with *T. nitzschioides*.

On the macroaggregate fractioning after the surface heat loss episode

Depending on the wind intensity, mucilage event can get interrupted or macroaggregates dispersed without any major consequences on the event itself. The latter was the case which we document with calculated surface fluxes. Phytoplankton composition indicates that the small macroaggregate no. 4, found on 22 July at 10 m in SPG 12, was formed in SPG 9, i.e., in the surface waters of the same location (st. SJ101) or nearby, around 8 July. Sampled macroaggregate no. 4 was a small one. However, we are unsure whether this macroaggregate was initially small or if it was originally part of a larger one which fractured. As between 8 and 22 July (on 18 July) an evaporation event occurred (Fig. 7), inducing turbulent mixing in the water column, which could fraction a large, existing macroaggregate, the second hypothesis seems to be more plausible. Presence of *C. closterium* in this macroaggregate (18 %) points out that after its formation, some succession changes occurred inside this macroaggregate and thus confirming that the macroaggregate could had been formed about two weeks earlier and was a large one at the time.

On prolongation of macroaggregates life due to *Cylindrotheca closterium*

C. closterium exudates are hypothesised to relevantly contribute to the accumulation and persistence of organic matter in the region under the specific conditions of marked stratification and P-limitation (Urbani et al., 2005). As low P content and high C/P ratio were detected in macroaggregates (Giani et al., 2005), it is possible that *C. closterium* exudates within the macroaggregates might extend resident time of macroaggregates themselves.

On increased abundance of opportunistic species in the water column at the end of mucilage event

Less intense summer heat losses after 18 July did not induce so intense resuspension of sediment as the one on 18 July. Thus the increased abundance of opportunistic species *C. closterium* and *T. nitzschioides* came from another origin – which is, as we state here – from the macroaggregates at time of their decay. This process explains the increase of their abundance in the water column between 11–12 August and 1 September (Figs. 5 and 6). A futher, large increase of abundances of *C. closterium* and *T. nitzschioides* during September, a period following the 1997 mucilage event, originate from the macroaggregates from the locations where they were present in large amounts. Intense turbulent mixing in water column occurring during episodes of surface heat losses induced partial fragmentation and/or final

disintegration of macroaggregates during which species from the macroaggregates got released into the ambient water.

On changes in abundance of the opportunistic species after the mucilage event

Following the mucilage event, large abundances of opportunistic species remain in the region where the event had occurred. Thus pointing out that mucilage event can significantly impact phytoplankton composition in the NA, particularly concerning the presence and abundance of the opportunistic species.

On macroaggregates as vectors of microbial community over the marine realm

“The mucilage can act as a controlling factor of microbial diversity across wide oceanic regions and could have the potential to act as carrier of specific microorganisms, thereby increasing the spread of pathogenic bacteria” (Danovaro et al., 2009). It is reasonable to add that this applies to all microorganisms within the macroaggregates. Our investigation substantiates this statement by presenting strong indications that geostrophic currents actually do transport macroaggregates over wider areas. This implies that geostrophic transport of marine snow as small, universally present macroaggregates, is an important mode of transporting microorganisms to larger distances.

5. CONCLUSIONS

The hypothesis that microphytoplankton distribution is an useful tool in tracing macroaggregates transport paths is confirmed with the existing circulation fields. The macroaggregates get transported by the geostrophic currents and are most likely to appear in the central part of gyres, in the upper layers. Surface heat losses during several bora (NE wind) episodes at the beginning of August, close to the end of mucilage event, induced spreading of lower salinity waters, in which macroaggregates formed, from western and central parts of NA towards the east. These waters became part of an anticyclonic gyre which became in that way a new area of macroaggregates formation.

Thalassionema nitzschioides is an opportunistic species of the mucilage phenomenon, occurring occasionally (only if present in the water column during macroaggregate formation or during transfer of macroaggregates by currents over their lifespan) and temporary in the macroaggregate phytoplankton succession (followed by a succession climax with *C. closterium*).

We documented that increased mixing in the water column after an evaporation event can result with the increased abundances of the sediment origin species *Cylindrotheca closterium* in the water column.

The species inhabiting the macroaggregates can influence the water community after their release from the macroaggregates in the surrounding water due to partial fragmentation and/or final disintegration of the macroaggregates.

The similar approach to the one applied here can be used in tracing plankton paths via marine snow across the sea, also in other sea regions in which geostrophic currents play significant role as it is case for NA. This could be especially convenient as the density data used for computation of geostrophic current are easily available, and can be applied also for historic data, as they are in standard measured during oceanographic cruises.

FIGURES

Fig. 1. Map of the NA with sampling stations and schematic of macroaggregates transport from st. SJ103 towards st. SJ101 and st. SJ107, as indicated by geostrophic currents distribution on 21-24 July. Several stations at which only the OP data were taken are marked with black dots.

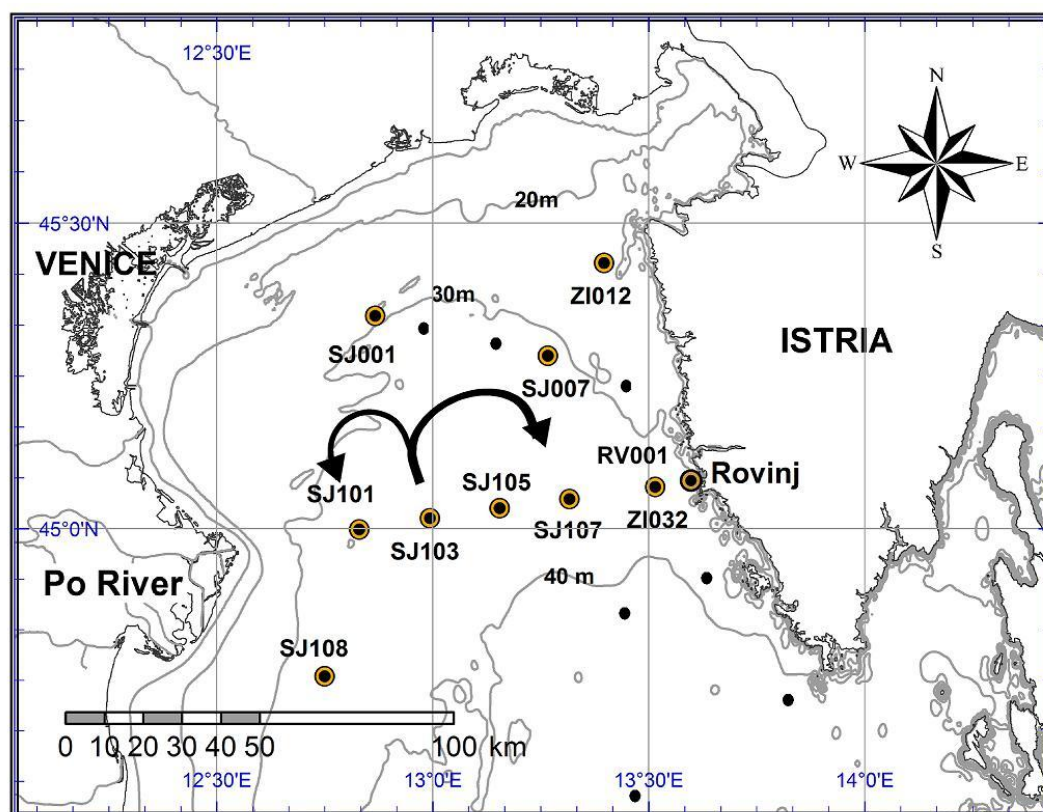


Table 1a. Average similarities between the 13 seawater phytoplankton groups (SPGs) calculated by SIMPER multivariate routine. Similarities of 40 and higher are considered significant as they belong above upper quartile of all calculated similarities (in bold on grey). Statistical significance of SPGs were tested with ANOSIM (Table 1b).

	1	2	3	4	5	6	7	8	9	10	11	12	13
1													
2	54												
3	21	24											
4	11	17	26										
5	4	11	17	52									
6	9	15	28	21	23								
7	18	21	26	25	27	54							
8	53	56	24	16	17	14	20						
9	31	35	29	23	21	16	27	56					
10	26	20	41	20	17	27	24	19	40				
11	23	23	32	28	28	37	54	24	43	59			
12	26	24	26	21	16	20	30	27	54	45	47		
13	23	26	32	49	39	30	40	25	51	47	56	53	

Table 1b. Statistical significance of SPGs tested with ANOSIM. Bold on grey - statistically significant results ($p < 5\%$); On white - statistical significance unreliable due to insufficient size of one or both groups included in analysis; "—" - Pairwise tests failed (at least one group must be larger than 1 in size). Size of groups presented in Table 3a.

	1	2	3	4	5	6	7	8	9	10	11	12	13
1													
2	0,96												
3	—	1											
4	1	1	1										
5	1	1	1	0,98									
6	—	1	—	1	1								
7	1	1	1	1	1	1							
8	1	0,94	1	1	1	1	1						
9	1	1	1	1	1	1	1	1					
10	—	1	—	1	1	—	1	1	1				
11	—	1	—	1	1	—	1	1	1	—			
12	1	1	1	1	1	1	1	1	0,90	0,99	0,98		
13	1	1	1	0,92	1	1	0,96	1	0,92	0,98	0,82	0,88	

Fig. 2. Association schematics of seawater phytoplankton groups (SPGs) based on significant similarities between the SPG (complete presentation of similarities in Table 2). Full line encircles five groups of SPGs based on presented similarities. Dashed line encircles SPGs in which macroaggregates were hypothetically formed.

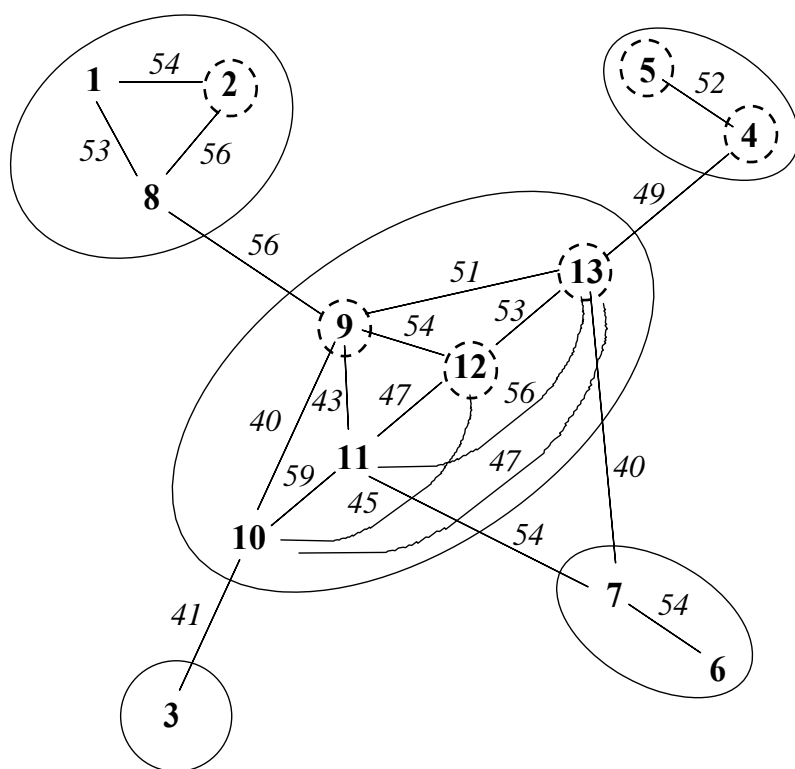


Table 2a. Phytoplankton community composition presented by average species contribution percentages and average similarity of 13 seawater phytoplankton groups (SPGs) calculated by SIMPER multivariate routine (in bold - highest species percentage contribution for each SPG). * For SPG consisting of one seawater sample similarity is impossible to calculate.

	Average contributions of species / %												
SPG	1	2	3	4	5	6	7	8	9	10	11	12	13
Species													
Bacillariophyceae													
<i>Cerataulina pelagica</i> (Cleve) Hendey	0	2,69	12,00	13,62	0	0	0	3,55	6,00	0	0	1,49	8,26
<i>Chaetoceros affinis</i> Lauder	0	0,03	0	0,01	<0,01	0	0	<0,01	0,37	0	0	0,03	0,20
<i>Chaetoceros curvisetus</i> Cleve	0	0,06	0	0	0	0	0	<0,01	0,11	0	0	0,04	0
<i>Chaetoceros insignis</i> Pr.-Lavr.	0	0	0	0	0	0	0	0	0	0	0	<0,01	<0,01
<i>Chaetoceros peruvianus</i> Brightwell	0	0	0	0	0	0	0	0	0	0	0	0,01	0
<i>Chaetoceros socialis</i> Lauder	43,62	9,56	0	<0,01	0	0	0	9,48	3	0	0	<0,01	0,04
<i>Chaetoceros</i> sp.	0	<0,01	0	<0,01	<0,01	0	0	<0,01	0,25	0	0	0,01	0
<i>Cylindrotheca closterium</i> (Ehrenberg) Reimann et	0	<0,01	4,00	<0,01	<0,01	40,38	0	0	0	0	0	0,03	0,01
<i>Dactyliosolen fragilissimus</i> (Bergon) Hasle	1,06	1,65	8,00	76,95	67,27	11,54	9,53	4,06	5,31	4,76	12,82	5,65	35,57
<i>Diploneis</i> sp.	0	0	0	0	0	0	0	0	0	4,76	0	0	0
<i>Guinardia flaccida</i> (Castracane) Peragallo	0	0,03	4,00	0,10	0	0	2,30	0,20	0,14	4,76	10,26	1,21	1,82
<i>Leptocylindrus danicus</i> Cleve	0	4,31	4,00	<0,01	<0,01	0	0	35,82	24	0	0	0,53	0,10
<i>Leptocylindrus mediterraneus</i> (Peragallo) Hasle	0	0	0	0	0	0	<0,01	0	<0,01	0	0	0,01	0
<i>Leptocylindrus</i> sp.	0	0	0	<0,01	<0,01	0	0	0	0	0	0	<0,01	0,02
<i>Navicula</i> sp.	3,19	0,02	12,00	<0,01	0	0	0	0	0	14,29	0	0,14	<0,01
<i>Nitzschia longissima</i> f. <i>tenuirostris</i> Mereschowsky	0	0,98	8,00	1,22	<0,01	32,69	55,28	0,22	<0,01	14,29	17,95	1,44	3,87
<i>Paralia sulcata</i> (Ehrenberg) Cleve	7,45	<0,01	28,00	0	0	0	0	0	0	0	0	0,02	0
<i>Pseudo-nitzschia delicatissima</i> (Cleve) Heiden	21,28	17,11	8,00	5,77	<0,01	7,69	19,06	16,04	49,93	33,33	30,77	86,10	46,62
<i>Pseudo-nitzschia seriata</i> (Cleve) Peragallo	0	0	0	0	0	0	0	0	0	9,52	0	0,01	0
<i>Rhizosolenia alata</i> f. <i>gracillima</i> (Cleve) Grunow	1,06	0,48	4,00	0,09	1,82	0	0	3,41	4,26	0	5,13	1,93	0,74
<i>Rhizosolenia</i> sp.	0	0	4,00	<0,01	<0,01	0	0	0	0	4,76	0	0,08	0,02
<i>Skeletonema costatum</i> (Greville) Cleve	22,34	62,87	0	<0,01	0	0	0	26,33	2,76	0	0	0,12	<0,01
<i>Thalassionema nitzschioides</i> (Grunow) Hustedt	0	0	0	0,17	3,64	0	<0,01	0	0	4,76	0	0,01	0,03
Bacillariophyceae (other)	0	0,04	0	0,07	1,82	1,92	<0,01	0,80	3,60	0	0	0,32	0,24
Dinophyceae													
<i>Ceratium fusus</i> (Ehrenberg) Dujardin	0	0	0	<0,01	<0,01	0	0	<0,01	0	0	12,82	0,03	0,02
<i>Ceratium</i> sp.	0	0,01	0	0,01	<0,01	0	0	0	0,04	0	2,56	0,01	0,04
<i>Gymnodinium</i> sp.	0	<0,01	0	0,52	3,64	1,92	9,21	0	0	0	0	0,09	0,25
<i>Gyrodinium</i> sp.	0	0,02	0	0,04	3,64	3,85	<0,01	<0,01	0	0	0	0,18	0,53
<i>Protoperidinium</i> sp.	0	0,06	4,00	0,01	0	0	<0,01	<0,01	0	0	0	0,05	<0,01
Dinophyceae (other)	0	0,06	0	1,35	18,18	0	4,61	0,10	0,32	4,76	7,69	0,47	1,32
Haptophyceae													
<i>Syracosphaera pulchra</i> Lohmann	0	0	0	0,07	0	0	0	0	0	0	0	<0,01	0,25
Haptophyceae (other)	0	<0,01	0	0	0	0	0	0	0	0	0	<0,01	0,02

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AVERAGE SPG SIMILARITY	*	74	*	78	61	*	72	85	76	*	*	76	70
No. of seawater samples in each SPG	1	6	1	21	2	1	2	2	5	1	1	31	15

Table 2b. Dominant phytoplankton species in the seawater phytoplankton groups (SPG) determined on 8 July, 22/23 July and 11/12 August at the sampling stations and depths.

STATION	Depth/m	DATE					
		8 July		22-23 July		11-12 August	
		SPG	Dominant sp.	SPG	Dominant sp.	SPG	Dominant sp.
SJ108	0	2	<i>S. costatum</i>	13	<i>P. delicatissima</i>	4	<i>D. fragilissimus</i>
	5	12	<i>P. delicatissima</i>	12	<i>P. delicatissima</i>	4	<i>D. fragilissimus</i>
	10	8	<i>L. danicus</i>	12	<i>P. delicatissima</i>	4	<i>D. fragilissimus</i>
	20	2	<i>S. costatum</i>	12	<i>P. delicatissima</i>	13	<i>P. delicatissima</i>
	30	1	<i>C. socialis</i>	12	<i>P. delicatissima</i>	4	<i>D. fragilissimus</i>
SJ101	0	9	<i>P. delicatissima</i>	4	<i>D. fragilissimus</i>	4	<i>D. fragilissimus</i>
	5	8	<i>L. danicus</i>	4	<i>D. fragilissimus</i>	4	<i>D. fragilissimus</i>
	10	9	<i>P. delicatissima</i>	12	<i>P. delicatissima</i>	13	<i>P. delicatissima</i>
	20	2	<i>S. costatum</i>	12	<i>P. delicatissima</i>	12	<i>P. delicatissima</i>
	30	12	<i>P. delicatissima</i>	12	<i>P. delicatissima</i>	13	<i>P. delicatissima</i>
SJ103	0	2	<i>S. costatum</i>	4	<i>D. fragilissimus</i>	4	<i>D. fragilissimus</i>
	10	12	<i>P. delicatissima</i>	12	<i>P. delicatissima</i>	13	<i>P. delicatissima</i>
	30	2	<i>S. costatum</i>	12	<i>P. delicatissima</i>	13	<i>P. delicatissima</i>
SJ105	0	2	<i>S. costatum</i>	13	<i>P. delicatissima</i>	4	<i>D. fragilissimus</i>
	10	12	<i>P. delicatissima</i>	12	<i>P. delicatissima</i>	5	<i>D. fragilissimus</i>
	30	12	<i>P. delicatissima</i>	12	<i>P. delicatissima</i>	10	<i>P. delicatissima</i>
SJ107	0	9	<i>P. delicatissima</i>	13	<i>P. delicatissima</i>	4	<i>D. fragilissimus</i>
	5	9	<i>P. delicatissima</i>	12	<i>P. delicatissima</i>	4	<i>D. fragilissimus</i>
	10	9	<i>P. delicatissima</i>	12	<i>P. delicatissima</i>	4	<i>D. fragilissimus</i>
	20	12	<i>P. delicatissima</i>	12	<i>P. delicatissima</i>	11	<i>P. delicatissima</i>
	30	-	-	-	-	3	<i>P. sulcata</i>
ZI032	35	12	<i>P. delicatissima</i>	13	<i>P. delicatissima</i>	12	<i>P. delicatissima</i>
	0	-	-	13	<i>P. delicatissima</i>	-	-
	10	-	-	13	<i>P. delicatissima</i>	-	-
SJ001	30	-	-	13	<i>P. delicatissima</i>	-	-
	0	-	-	4	<i>D. fragilissimus</i>	-	-
	10	-	-	12	<i>P. delicatissima</i>	-	-
SJ007	30	-	-	7	<i>N. tenuirostris</i>	-	-
	0	-	-	4	<i>D. fragilissimus</i>	4	<i>D. fragilissimus</i>
	10	-	-	12	<i>P. delicatissima</i>	4	<i>D. fragilissimus</i>
ZI012	30	-	-	12	<i>P. delicatissima</i>	7	<i>N. tenuirostris</i>
	0	-	-	13	<i>P. delicatissima</i>	5	<i>D. fragilissimus</i>
	10	-	-	13	<i>P. delicatissima</i>	13	<i>P. delicatissima</i>
	30	-	-	13	<i>P. delicatissima</i>	6	<i>C. closterium</i>

727 Fig. 3. Horizontal distribution of seawater phytoplankton groups (SPGs) in the NA at 0 m, 10 m and 30 m (i.e., bottom) on 8 July, 22–24 July
728 and 11–12 August.

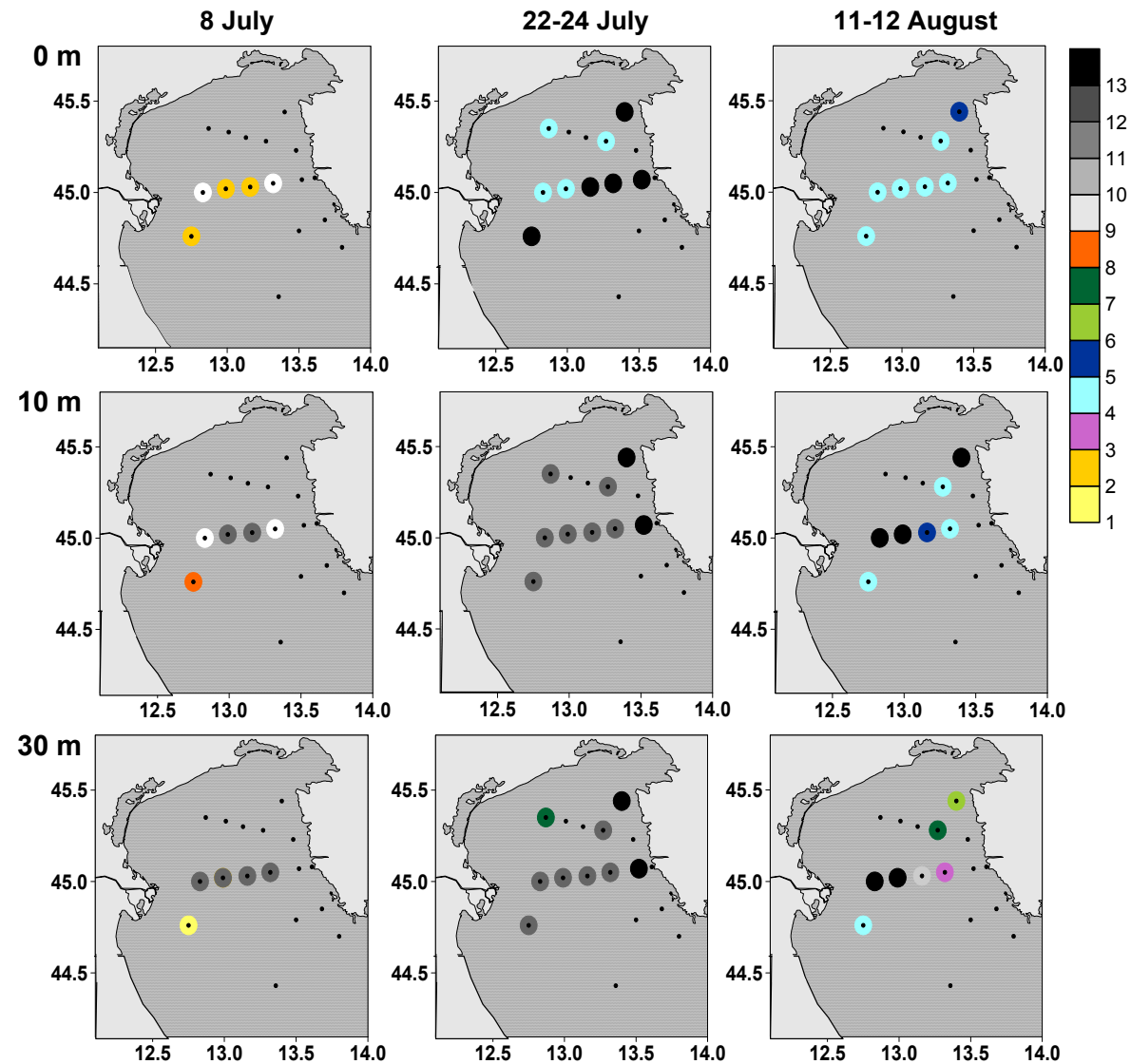
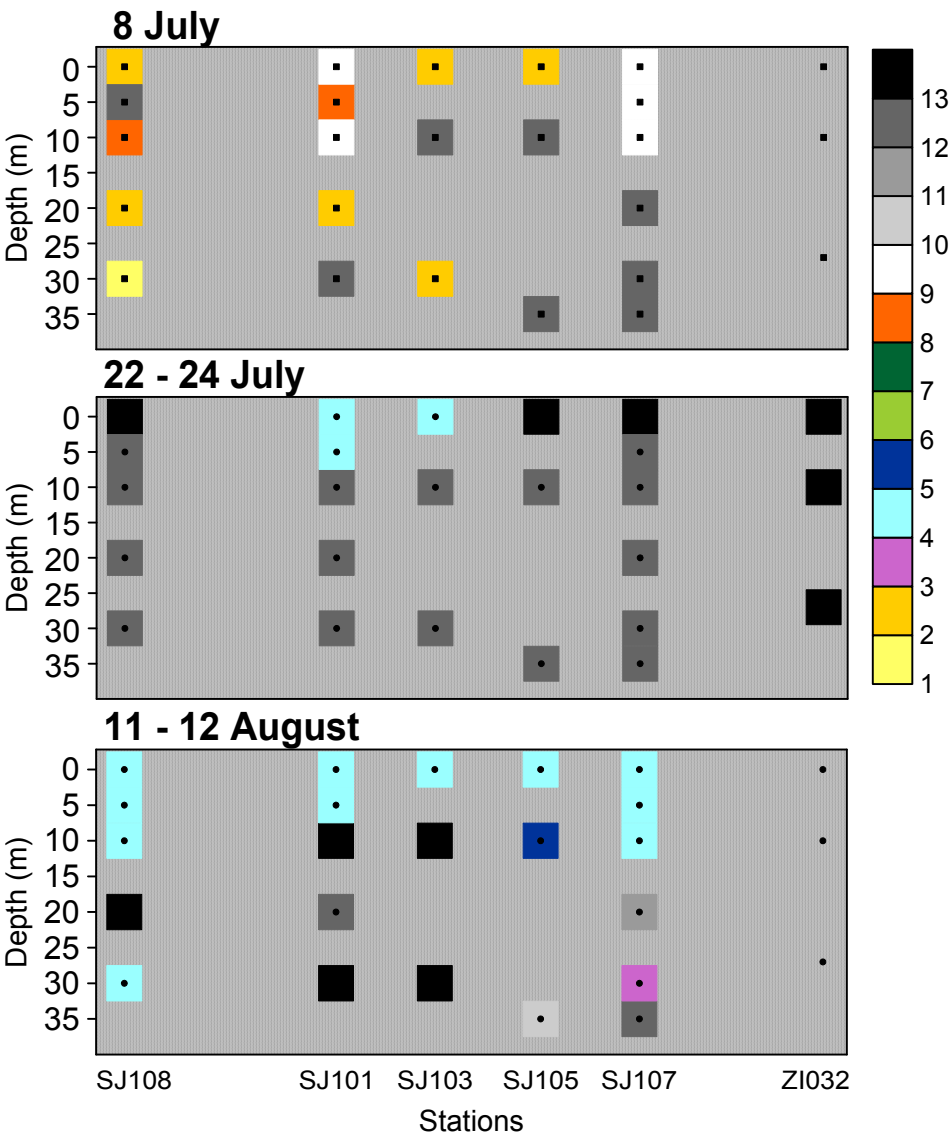


Fig. 4. Vertical distribution of seawater phytoplankton groups (SPGs) at the Po River delta – Rovinj profile at 0 m, 5 m, 10 m, 20 m, 30 m and 35 m on 8 July, 22–24 July and 11–12 August.



758 Table 3. Macroaggregates phytoplankton community composition presented by species contribution percentages with information about
759 macroaggregates dimension and sampling location (date, station and depth). L – large, S – small macroaggregates.

Date	22 July 2012					23 July 2012			04 August 2012				11 August 2012						12 August 2012		
Macroaggregate / No.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
Dimension	L	S	L	S	L	L	S	S	L	S	S	L	L	S	S	L	S	L	L	S	S
Station	SJ108		SJ101		SJ107	SJ001		ZI012	SJ107		ZI032		SJ108		SJ101		SJ107		SJ001		ZI012
Depth / m	11	11	12	12	16	15	15	10	10	10	7	18	12	12	9	15	10	20	20	12	10
Species																					
Bacillariophyceae																					
<i>Cerataulina pelagica</i>	2,72	1,46	2,64	0	0	4,51	2,00	4,08	0	0	39,14	17,91	16,79	29,58	21,21	0,49	45,25	0	0	47,62	0
<i>Chaetoceros affinis</i>	0,65	0	0	0	0	0	0	27,21	0	0	0	0	0	0	0	0	2,28	0	0	0	0
<i>Chaetoceros socialis</i>	0	0	0	0	0	1,20	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Cylindrotheca closterium</i>	51,59	15,20	4,34	17,91	13,66	2,67	0	0	66,03	75,36	0	11,55	6,96	1,41	0	87,46	1,90	0	4,40	0	0
<i>Dactyliosolen fragilissimus</i>	11,41	0	8,30	0	1,12	2,39	5,05	36,05	1,03	2,90	35,68	53,03	50,71	47,89	62,10	0,93	19,39	36,90	5,60	23,81	85,29
<i>Guinardia flaccida</i>	0	0	0,94	0	1,94	0	1,47	0,68	5,00	5,07	0,43	1,57	0,09	0	0,36	0	0	0	1,60	4,76	2,94
<i>Guinardia striata</i>	0	0	0,85	0	0	0	0	0	0	0	0	0,39	0	0	0	0	0	0	0	0	0,98
<i>Leptocylindrus danicus</i>	0	0	0,75	8,21	0,92	0	0	1,36	0	0	10,50	0	0	0	0	0	0	0	0	14,29	0
<i>Leptocylindrus mediterraneus</i>	0	0	0,38	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Nitzschia longissima</i> f. <i>tenuirostris</i>	0	0	2,26	1,49	0	1,57	0	0	0	7,97	0	0	10,80	12,68	9,43	2,16	25,48	0	0	0	0
<i>Pleurosigma normanii</i>	0	0	0	0	0,10	0	0	0	0	0	0	0	0,36	0	0	0	0	0	0	0	0
<i>Pleurosigma</i> sp.	0	0	0	0	0,20	0	0	0	0	0	0	0	0,18	0	0	0,05	0	0	0	0	0
<i>Pseudo-nitzschia delicatissima</i>	14,52	14,33	63,87	27,61	9,89	54,05	83,26	20,41	1,47	0	12,66	10,96	0,98	1,41	1,18	1,37	0,76	0	0	0	4,90
<i>Rhizosolenia alata</i> f. <i>gracillima</i>	5,70	0	3,11	6,72	0,61	1,93	0,74	2,04	4,12	2,17	0	0,49	0,18	0	0	0	0	0	0	0	1,96
<i>Rhizosolenia</i> sp.	0	0,29	0,09	0	0	0	0	0	5,00	1,45	0	0,39	0	0	0	0	0	0	0	0	0
<i>Thalassionema nitzschioides</i>	13,35	65,64	11,89	38,06	67,18	27,44	6,21	0	17,35	2,90	1,15	2,54	11,52	5,63	2,81	7,20	4,18	62,88	78,80	9,52	3,92
Bacillariophyceae (other)	0	0	0,09	0	1,63	3,41	0	6,80	0	0	0,29	0,68	0	0	0	0,29	0	0	0,40	0	0
Dinophyceae																					
<i>Ceratium</i> sp.	0	0,15	0	0	0,10	0,09	0,32	0	0	0	0	0	0	0	0,09	0	0	0,22	0,40	0	0
<i>Gymnodinium</i> sp.	0	0,44	0	0	0,41	0	0	0	0	0	0	0	0,54	0	2,54	0	0	0	0	0	0
<i>Protoperidinium</i> sp.	0	0	0,19	0	0,71	0	0,32	0	0	0	0	0,10	0	0	0	0	0	0	2,80	0	0
Dinophyceae (other)	0	2,49	0,28	0	1,53	0,74	0,63	1,36	0	2,17	0,14	0,39	0,71	1,41	0,27	0,05	0,76	0	6,00	0	0
Haptophyceae																					
<i>Syracosphaera pulchra</i>	0	0	0	0	0	0	0	0	0	0	0	0	0,18	0	0	0	0	0	0	0	0

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761

762 Table 4. Summarised presentation of macroaggregates information: actual sampling and hypothetical formation locations and times, hypothetical
763 types of macroaggregates and macroaggregates movement path after the formation and calculated similarities by SIMPER routine between each
764 macroaggregate and determined seawater phytoplankton group (SPG) in the investigated area of the NA during the investigation period, July and
765 August of 1997. Abbreviations indicate as follows; types of macroaggregates: R (residing), T (transient), R / T (either resident or transient) and
766 macroaggregates movements: S (sinking), F (floating), none (no movement), none / F (none or floating). In bold on grey – high similarity of
767 macroaggregate sample with SPG indicates noted SPG as the formation location of a particular macroaggregate. In bold with frame – assumption
768 that noted SPG is the formation location based on *T. nitzschoides* presence both in macroaggregate sample and in water sample at the specific
769 depth and station.

NEW No.	ACTUAL DATA	HYPOTETICAL DATA			CALCULATED DATA													
	Date, station and depth of macroaggregate sampling	Date, station and depth of macroaggregate formation	Type of macroaggregate	Movement of macroaggregate after the formation	Similarity between macroaggregate and SPG													
					1	2	3	4	5	6	7	8	9	10	11	12	13	
1	22 July, SJ108, 11 m	8 July, SJ103, 0–10 m	T	F	16,6	20,1	26,7	22,3	19,7	59,5	25,0	26,1	27,2	24,0	31,1	25,7	30,8	
2	22 July, SJ108, 11 m	after 8 July, SJ103, 0–10 m	T	F	14,3	14,9	13,8	11,1	8,8	23,3	18,7	15,5	16,4	21,9	17,0	17,1	18,2	
3	22 July, SJ101, 12 m	22 July, SJ101, 5–10 m	R	S	23,4	23,3	30,0	22,7	18,0	22,7	30,0	26,5	54,1	46,4	45,7	73,9	53,1	
4	22 July, SJ101, 12 m	8 July, SJ101, 0–10 m	R	S	22,3	21,0	21,5	11,2	12,7	27,1	20,1	27,7	40,6	33,9	34,2	33,1	31,0	
5	22 July, SJ107, 16 m	8 July, SJ103, 0–10 m	T	F	11,6	13,2	17,3	9,9	11,2	24,5	17,3	13,9	14,8	19,2	15,2	15,3	15,7	
6	23 July, SJ001, 15 m	23 July, SJ001, 10 m	R	none	24,6	22,8	21,1	18,4	13,4	16,3	25,0	24,6	54,3	42,8	37,5	61,9	48,4	
7	23 July, SJ001, 15 m	23 July, SJ001, 10 m	R	none	23,1	19,7	17,6	17,9	11,6	12,7	25,8	21,7	49,1	45,0	39,0	78,5	47,4	
8	23 July, ZI012, 10 m	23 July, ZI012, 10m	R	none	22,5	24,4	24,2	50,5	52,0	21,2	30,7	27,3	39,2	27,2	37,3	34,1	57,4	
9	4 August, SJ107, 10 m	8 July, SJ103, 0–10 m	T	F	3,6	3,2	18,5	3,8	8,0	42,9	7,6	6,2	6,2	16,8	11,6	7,4	7,2	
10	4 August, SJ107, 10 m	July, SJ103	T	F	2,1	5,3	22,5	8,2	12,1	51,3	17,9	5,8	7,2	22,2	20,3	10,4	12,3	
11	4 August, ZI032, 7 m	after 22 July, SJ107, 5–10 m or 22 July, ZI032, 10 m	R / T	none / F	13,7	25,4	32,4	59,1	44,7	19,5	23,2	31,7	36,5	19,2	26,1	24,8	51,0	
12	4 August, ZI032, 18 m	after 22 July, SJ107, 5–10 m or 22 July, ZI032, 10 m	R / T	none / F	12,5	20,0	34,5	73,9	51,6	31,5	23,8	20,4	25,4	20,6	26,2	23,4	51,8	
13	11 August, SJ108, 12 m	11 August, SJ108, 0–5 m	R	S	2,2	12,5	33,3	69,0	51,9	30,8	24,1	9,8	15,6	22,1	25,6	15,3	45,5	
14	11 August, SJ108, 12 m	11 August, SJ108, 10 m	R	none	2,5	12,7	30,8	69,9	51,7	27,0	26,5	9,9	15,9	25,0	28,3	15,5	46,2	
15	11 August, SJ101, 9 m	11 August, SJ101, 0 m	R	none	2,2	12,4	29,5	80,6	52,2	24,1	24,5	9,9	15,6	18,8	24,2	14,8	44,8	
16	11 August, SJ101, 15 m	22 July, SJ101, 5 m or SJ103, 0–10 m	R	S	2,3	4,0	9,0	4,5	6,4	45,1	6,4	3,6	3,5	9,3	4,5	4,5	5,1	
17	11 August, SJ107, 10 m	11 August, SJ107, 10 m	R	none	1,8	12,1	30,7	40,6	26,8	39,7	38,0	9,4	16,0	24,8	32,3	15,2	38,2	
18	11 August, SJ107, 20 m	11 August, SJ107, 5 m or SJ105, 10 m	R / T	none / F	1,1	3,9	8,0	37,5	39,8	11,5	11,0	4,9	6,0	9,5	13,0	7,5	29,1	
19	12 August, SJ001, 20 m	11 August, SJ105, 10 m	T	F	1,1	3,5	14,0	8,6	15,6	10,4	12,5	5,2	6,0	15,9	13,6	7,6	9,8	
20	12 August, SJ001, 12 m	after 23 July, SJ001, 0 m	R	S	1,1	13,5	28,0	40,6	32,8	11,5	14,2	22,5	27,3	14,3	17,6	13,4	34,7	

21	12 August, ZI012, 10 m	12 August, SJ007, 0m	T	F	7,0	9,7	17,8	69,3	51,3	16,4	18,2	11,9	13,1	16,5	22,6	15,4	37,5
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Fig. 5. Vertical distribution of *Thalassionema nitzschioides* at the Po River delta – Rovinj profile on 8 July, 22–24 July, 11–12 August, 1 September and 23 September.

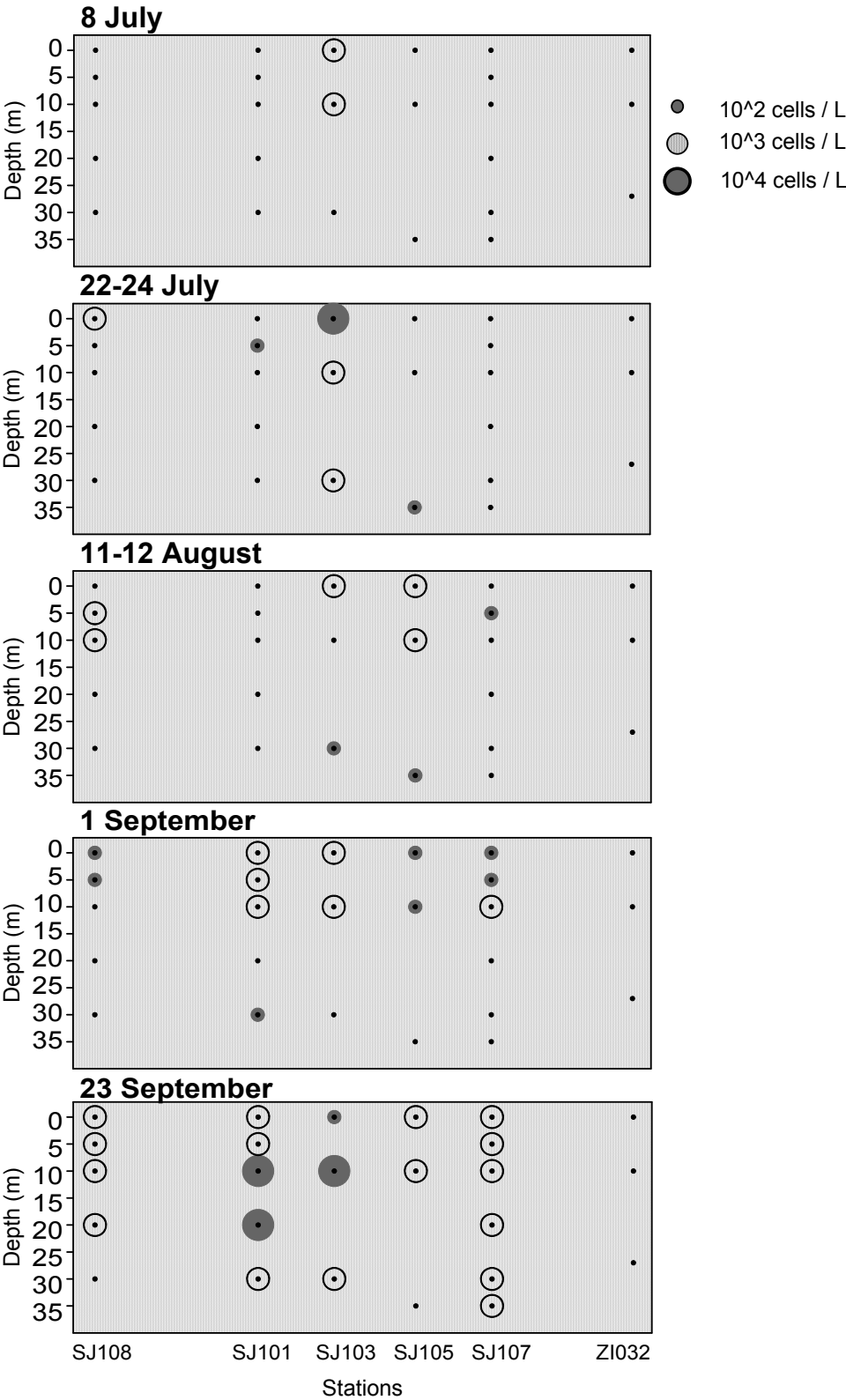


Fig. 6. Vertical distribution of *Cylindrotheca closterium* at the Po River delta – Rovinj profile on 8 July, 22–24 July, 11–12 August, 1 September and 23 September.

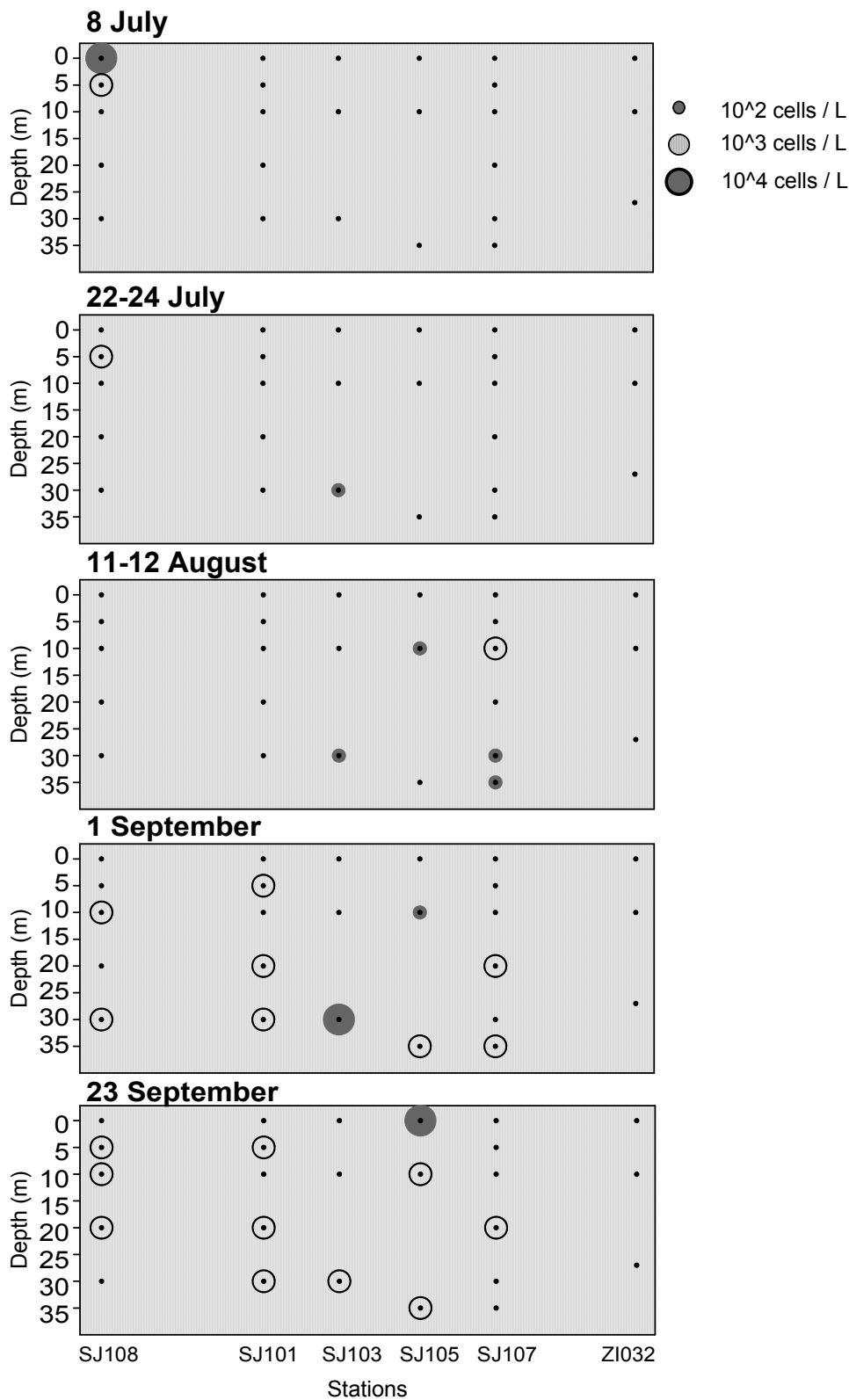


Fig. 7a. Total surface heat flux (Q) and flux due to evaporation (Q_e) along with wind speed (w) at Pula station and daily Po river rates in July-September 1997. Fluxes are positive when the sea gains heat. The grid lines coincide with sampling dates of the 8 July, the 22-24 July and the 11-13 August cruises.

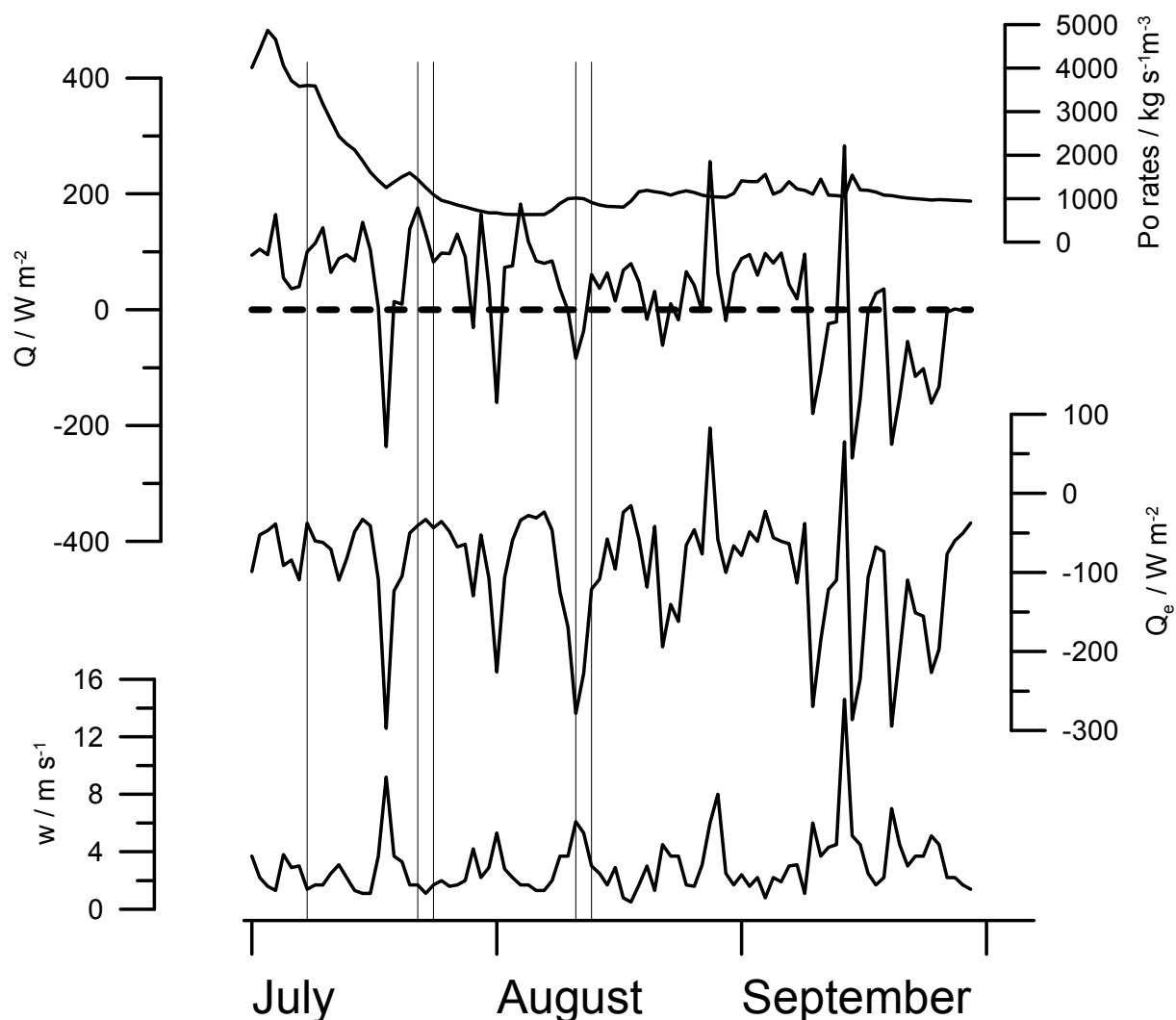


Fig. 7b. Sigma-t values at vertical section between SJ108 and RV001 on (a) 8 July, (b) 22-24 July and (c) 11-13 August.

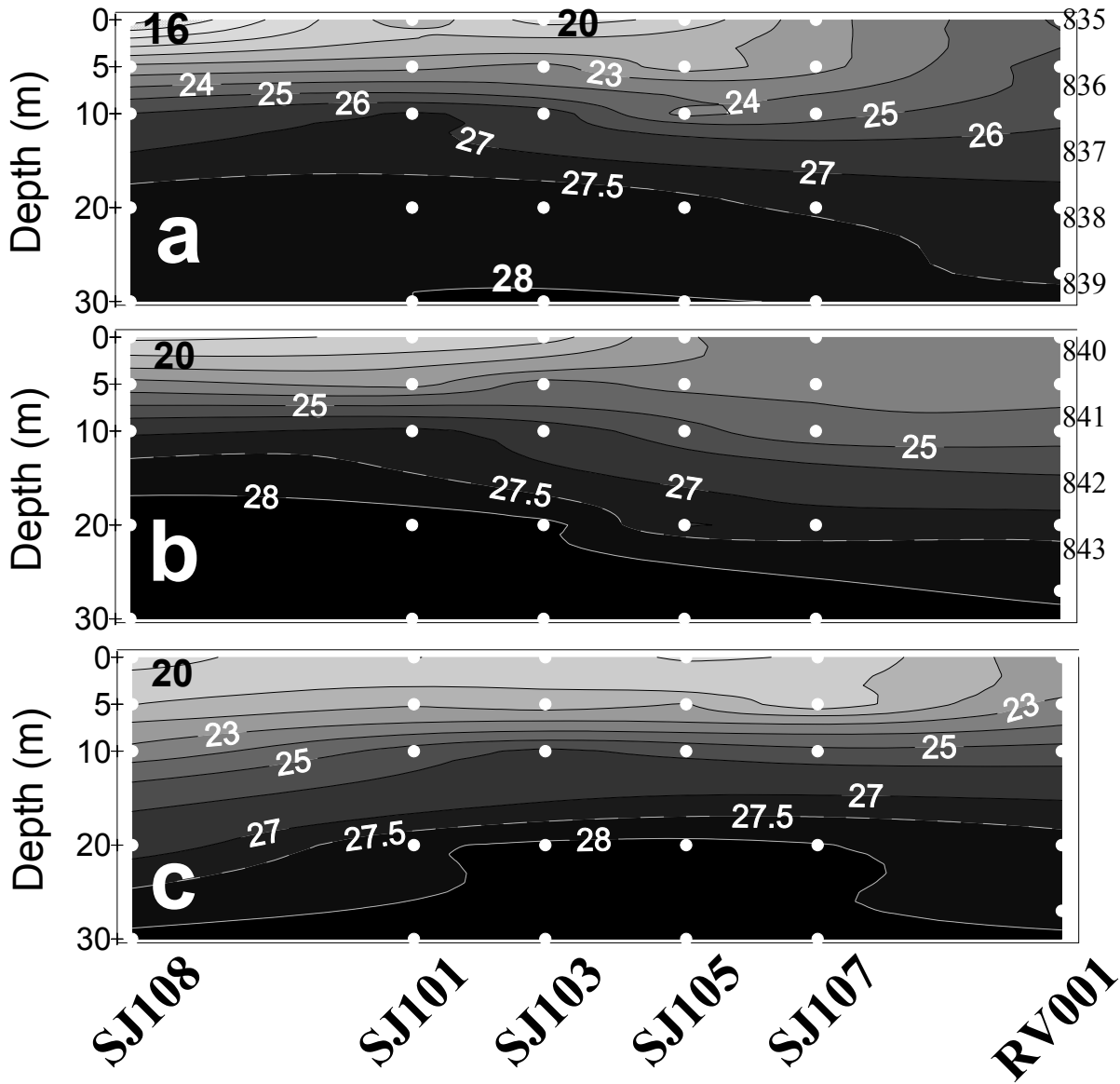
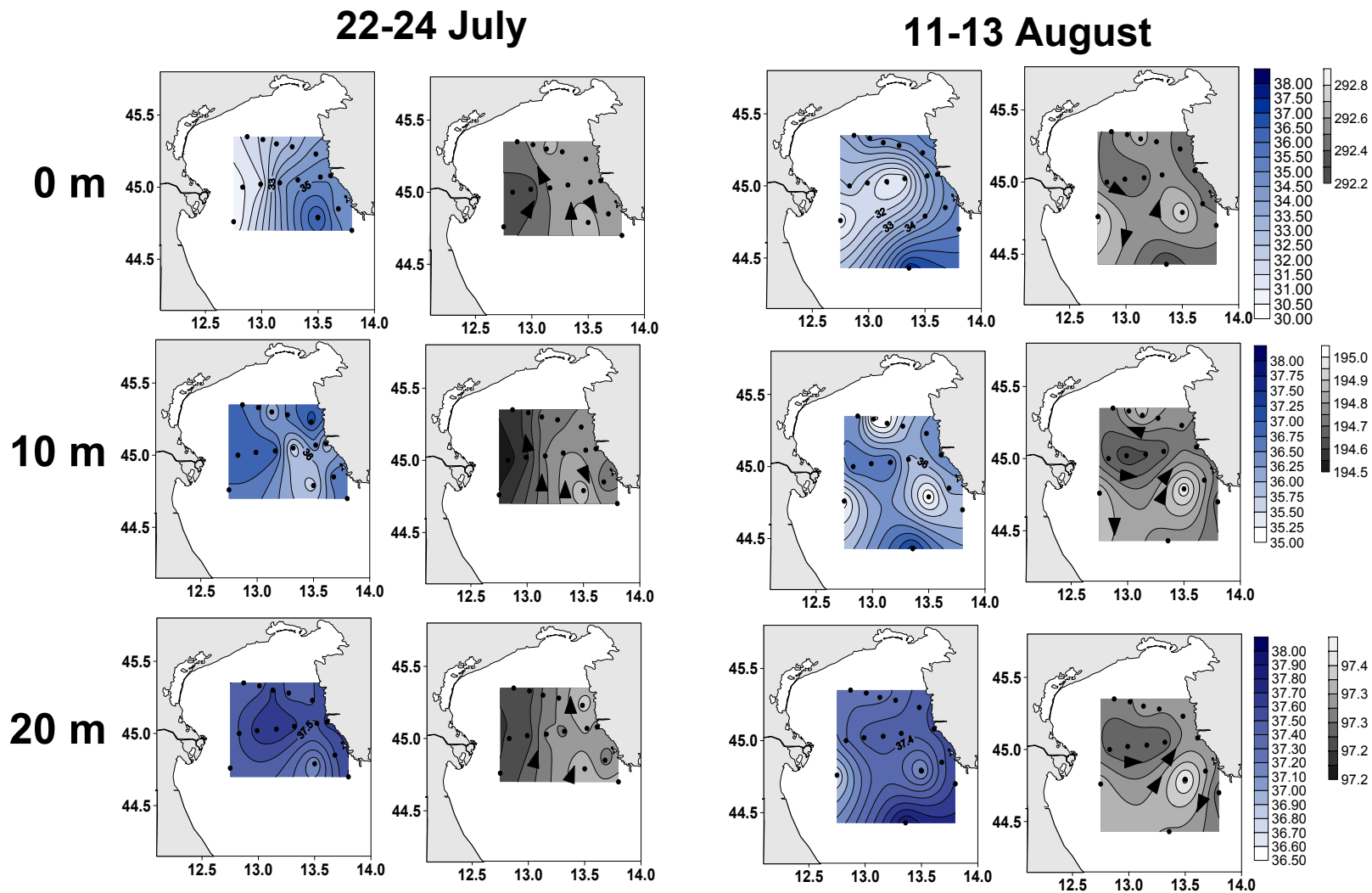


Fig. 8. Salinity (in blue) and dynamic depths of the 30-dbar surface (in grey) at 0 m, 10 m and 20 m on 22-24 July and 11-13 August.



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Table 6. Results of the PCA preformed on oceanographic parameters OP (Thermocline, Up temp – temperature above thermocline, Up psal – salinity above thermocline, Up dene – density above thermocline, Lo temp – temperature below thermocline, Lo psal – salinity below thermocline, Lo dene – density below thermocline, Dene (Up-Lo) – difference between densities above and below thermocline, Strat 30 – difference between densities in the 0 and 30 layers, Strat 10 – difference between densities in the 0 and 10 layers, Dyn dpth 30 – dynamic depth of the 30 dbar surface, Dyn dpth 20 – dynamic depth of the 20 dbar surface) for all stations with OP data sampled during cruises on 8 July, 22-24 July and 11-13 August 1997 with parameter Origin (can be 1 or 0) indicating whether the station is hypothetical origin of macroaggregate. Values in table represent contributions (loadings) of each investigated parameter to first (PC1) and second (PC2) principal component. Results of higher significancy are marked with bold or grey while n.s. means “not significant”.

	8 July		22-24 July		11-13 August	
	PC1	PC2	PC1	PC2	PC1	PC2
Thermocline	-0,69	n.s.	-0,84	n.s.	n.s.	n.s.
Up temp	0,94	n.s.	0,76	n.s.	-0,61	n.s.
Up psal	-0,99	n.s.	-0,98	n.s.	0,96	n.s.
Up dene	-0,99	n.s.	-0,97	n.s.	0,96	n.s.
Lo temp	-0,83	n.s.	-0,56	n.s.	0,79	n.s.
Lo psal	-0,80	n.s.	-0,89	n.s.	0,82	n.s.
Lo dene	n.s.	-0,52	n.s.	0,89	n.s.	0,89
Dene (Up-Lo)	1,00	n.s.	0,94	n.s.	-0,99	n.s.
Strat 30	0,97	n.s.	0,95	n.s.	-0,96	n.s.
Strat 10	0,91	n.s.	0,95	n.s.	-0,78	n.s.
Dyn dpth 30	0,46	n.s.	-0,78	n.s.	n.s.	n.s.
Dyn dpth 20	0,58	n.s.	-0,60	n.s.	-0,59	n.s.
Origin	n.s.	0,67	0,59	n.s.	-0,63	n.s.

Suppl. 1. List of 128 phytoplankton seawater samples collected during July, August and September 1997. Phytoplankton seawater samples from July and August are clustered into 13 SPGs (in brackets). For seawater samples in September SPGs were not determined.

8.7.	0	SJ108, SJ103 and SJ105 (2), SJ101 and SJ107 (9)
	5	SJ108 (12), SJ101 (8), SJ107 (9)
	10	SJ108 (8), SJ101 and SJ107 (9), SJ103 and SJ105 (12)
	20	SJ108 and SJ101 (2), SJ107 (12)
	bottom	SJ108 (1), SJ103 (2), SJ101, SJ105 and SJ107 (12)
22.7.	0	SJ101 and SJ103 (4), SJ108, SJ105 and SJ107 (13)
	5	SJ101 (4), SJ108 and SJ107 (12)
	10	SJ108, SJ101, SJ103, SJ105 and SJ107 (12)
	20	SJ108, SJ101 and SJ107 (12)
	bottom	SJ108, SJ101, SJ103, SJ105 and SJ107 (12)
23.7.	0	SJ001 and SJ007 (4), ZI012 (13)
	10	SJ001 and SJ007 (12), ZI012 (13)
	bottom	SJ001 (7) SJ007 (12), ZI012 (13)
24.7.	0	ZI032 (13)
	10	ZI032 (13)
	bottom	ZI032 (13)
11.8.	0	SJ108, SJ101, SJ103, SJ105 and SJ107 (4)
	5	SJ108, SJ101 and SJ107 (4)
	10	SJ108 and SJ107 (4), SJ105 (5), SJ101 and SJ103 (13)
	20	SJ107 (11), SJ101 (12), SJ108 (13)
	bottom	SJ107, 30m (3), SJ108 (4), SJ105 (10), SJ107, 35m (12), SJ101 and SJ103 (13)
12.8.	0	SJ007 (4), ZI012 (5)
	10	SJ007 (4), ZI012 (13)
	bottom	ZI012 (6), SJ007 (7)
1.9.	0	SJ108, SJ101, SJ103, SJ105, SJ107
	5	SJ108, SJ101, SJ107
	10	SJ108, SJ101, SJ103, SJ105, SJ107
	20	SJ108, SJ101, SJ107
	bottom	SJ108, SJ101, SJ103, SJ105, SJ107
23.9.	0	SJ108, SJ101, SJ103, SJ105, SJ107
	5	SJ108, SJ101, SJ107
	10	SJ108, SJ101, SJ103, SJ105, SJ107
	20	SJ108, SJ101, SJ107
	bottom	SJ108, SJ101, SJ103, SJ105, SJ107

891 Suppl. 2. Basic statistics (average, standard deviation, min and max) of the OP (Thermocline – thermocline depth, Up temp – temperature above
892 thermocline, Up psal – salinity above thermocline, Up dene – density above thermocline, Lo temp – temperature below thermocline, Lo psal –
893 salinity below thermocline, Lo dene – density below thermocline, Dene (Up-Lo) –above and below thermocline, Strat 30 – difference between
894 densities in the 0 and 30 layers, Strat 10 – difference between densities in the 0 and 10 layers, Dyn dpth 30 – dynamic depth of the 30 dbar surface,
895 Dyn dpth 20 – dynamic depth of the 20 dbar surface) for all stations with OP data sampled during cruises on 8 July, 22-24 July and 11-13 August
896 1997 calculated for: all stations (all available data), origin stations (stations where macroaggregates hypothetically formed), sampling stations
897 (stations where macroaggregates were sampled) and non-origin stations (stations where hypothetically no macroaggregates were formed).

Date		All stations	Origin stations	Sampling stations	Non-origin stations
8 July	Thermocline	11,25±4,11 (7,50-15,00)	7,50±0,00 (7,50-7,50)	no macroaggregates were sampled on 8 July	13,10±3,80 (7,50-15,00)
	Up temp	23,02±0,62 (21,87-23,58)	23,23±0,29 (23,03-23,44)		22,92±0,76 (21,87-23,58)
	Up psal	32,80±2,51 (29,13-36,64)	32,30±0,82 (31,72-32,88)		33,06±3,17 (29,13-36,64)
	Up dene	22,26±2,06 (19,33-25,50)	21,82±0,54 (21,44-22,20)		22,49±2,60 (19,33-25,50)
	Lo temp	14,50±1,12 (13,46-16,65)	14,01±0,77 (13,46-14,55)		14,75±1,29 (13,93-16,65)
	Lo psal	36,93±0,28 (36,42-37,17)	36,89±0,11 (36,81-36,97)		36,95±0,35 (36,42-37,17)
	Lo dene	27,56±0,24 (27,27-27,83)	27,64±0,08 (27,58-27,70)		27,52±0,29 (27,27-27,83)
	Dene (Up-Lo)	5,29±2,08 (1,77-7,94)	5,82±0,46 (5,50-6,14)		5,03±2,62 (1,77-7,94)
	Strat 30	6,95±3,17 (2,17-11,53)	7,45±1,63 (6,29-8,60)		6,70±3,95 (2,17-11,53)
	Strat 10	4,71±3,35 (0,62-9,61)	6,31±1,25 (5,42-7,19)		3,91±3,96 (0,62-9,61)
	Dyn dpth 30	292,44±0,16 (292,28-292,67)	292,32±0,03 (292,30-292,34)		292,50±0,06 (292,28-292,67)
	Dyn dpth 20	195,14±0,16 (194,93-195,38)	195,04±0,04 (195,02-195,07)		195,19±0,19 (194,93-195,38)
22-24 July	Thermocline	13,75±4,47 (7,50-25,00)	10,50±4,10 (7,50-15,00)	10,50±4,11 (7,50-15,00)	15,20±3,90 (7,50-25,00)
	Up temp	24,11±0,48 (23,47-25,33)	24,51±0,55 (23,96-25,33)	24,53±0,60 (23,63-25,33)	23,92±0,33 (23,47-24,50)
	Up psal	34,35±1,22 (32,05-35,52)	33,58±1,50 (32,05-35,44)	33,30±1,45 (32,05-35,04)	34,70±0,96 (32,50-35,52)
	Up dene	23,12±1,03 (21,02-24,10)	22,42±1,26 (21,02-23,99)	22,20±1,24 (21,02-23,78)	23,44±0,78 (21,61-24,10)
	Lo temp	15,53±1,50 (13,17-17,93)	15,52±1,31 (13,95-16,98)	15,00±1,22 (13,95-16,73)	15,54±1,64 (13,17-17,93)
	Lo psal	37,50±0,20 (37,18-37,80)	37,35±0,16 (37,18-37,59)	37,31±0,18 (37,18-37,63)	37,57±0,18 (37,19-37,80)
	Lo dene	27,75±0,31 (27,24-28,37)	27,65±0,26 (27,30-27,99)	27,73±0,26 (27,30-27,99)	27,80±0,33 (27,24-28,37)
	Dene (Up-Lo)	4,63±1,15 (3,29-6,69)	5,23±1,46 (3,51-6,69)	5,54±1,38 (4,02-6,69)	4,36±0,93 (3,29-6,27)
	Strat 30	6,12±1,52 (4,22-8,59)	7,15±1,68 (4,66-8,27)	7,27±1,87 (4,52-8,59)	5,74±1,35 (4,22-8,59)
	Strat 10	3,31±2,31 (0,17-7,17)	4,32±2,93 (0,63-7,17)	4,20±3,51 (0,17-7,17)	2,85±1,96 (0,17-7,08)
	Dyn dpth 30	292,42±0,10 (292,23-292,62)	292,34±0,10 (292,23-292,46)	292,35±0,10 (292,27-292,48)	292,45±0,09 (292,23-292,62)
	Dyn dpth 20	195,14±0,08 (194,99-195,27)	195,11±0,10 (194,99-195,25)	195,13±0,09 (195,03-195,25)	195,15±0,07 (195,03-195,27)
11-13 August	Thermocline	15,16±3,22 (7,50-25,00)	15,00±0,00 (15,00-15,00)	15,00±0,00 (15,00-15,00)	15,30±4,20 (7,50-25,00)
	Up temp	24,95±0,84 (22,90-26,32)	25,19±0,59 (24,52-26,19)	24,76±1,21 (22,90-26,19)	24,81±0,96 (22,90-26,32)
	Up psal	34,76±1,38 (32,39-37,38)	33,90±0,95 (32,71-34,97)	34,33±1,78 (32,71-37,10)	35,27±1,37 (32,39-37,38)
	Up dene	23,17±1,23 (20,97-25,54)	22,44±0,76 (21,25-23,18)	22,90±1,65 (21,25-25,54)	23,60±1,29 (20,97-25,54)
	Lo temp	15,47±1,23 (13,56-17,66)	14,34±0,87 (13,56-15,50)	14,62±1,09 (13,56-16,03)	16,15±0,87 (14,49-17,66)
	Lo psal	37,42±0,23 (37,02-37,91)	37,30±0,16 (37,02-37,46)	37,28±0,17 (37,02-37,46)	37,49±0,24 (37,16-37,91)
	Lo dene	27,72±0,23 (27,39-28,17)	27,89±0,30 (27,39-28,17)	27,89±0,32 (27,39-28,14)	27,62±0,10 (27,50-27,80)
	Dene (Up-Lo)	4,55±1,29 (2,03-6,55)	5,44±0,71 (4,53-6,30)	4,90±1,72 (2,03-6,30)	4,02±1,29 (2,03-6,55)
	Strat 30	5,96±1,66 (2,87-8,59)	7,26±1,35 (5,28-8,59)	7,48±1,13 (6,07-8,59)	5,09±1,24 (2,87-7,64)
	Strat 10	2,41±1,97 (0,12-5,77)	3,88±1,60 (1,38-5,76)	3,48±1,49 (1,29-5,14)	1,53±1,67 (0,12-5,77)
	Dyn dpth 30	292,53±0,11 (292,30-292,79)	292,54±0,06 (292,49-292,65)	292,54±0,07 (292,49-292,65)	292,53±0,14 (292,30-292,79)
	Dyn dpth 20	195,20±0,15 (194,89-195,49)	195,27±0,12 (195,19-195,49)	195,22±0,22 (194,89-195,49)	195,17±0,15 (194,89-195,40)

Suppl. 3a,b,c. Running cumulative total (Cumul.) of the sequential tests on variables and dbRDA coordinate scores (dbRDAX; x - depending on number of identified variables in sequential tests) of the DISTLM performed with (1) seawater phytoplankton samples from the stations SJ001, SJ007, SJ101, SJ103, SJ105, SJ107, SJ108, ZI012 and ZI032 sampled at 0 m, 10 m and bottom and (2) variables: Thermocline, Up temp – temperature above thermocline, Up psal – salinity above thermocline, Up dene – density above thermocline, Lo temp – temperature below thermocline, Lo psal – salinity below thermocline, Lo dene – density below thermocline, Dene (Up-Lo) – difference between densities above and below thermocline, Strat 10 – difference between densities in the 0 and 10 layers, Dyn dpth 20 – dynamic depth of the 20 dbar surface with or without parameter Origin (can be 1 or 0) indicating whether the station is hypothetical origin of macroaggregate on 8 July (Date 1), 22-24 July (Date 2) and 11-13 August 1997 (Date 3). Unless specified, results were the same with or without parameter Origin (origin included or origin excluded, respectively) from the analysis. Marked + in column Origin denotes the location of the hypothetical origin of macroaggregate/s. Variable on grey points out those that are found most important for analysed samples. dbRDA coordinate score on grey indicates the highest value for each sample included in a DISTLM analysis for a specific depth and date.

Suppl. 3a.

Date 1									
		0 m			10 m			Bottom	
		Variable	Cumul.		Variable	Cumul.		Variable	Cumul.
		Up psal	0,46		Lo psal	0,73		Strat 10	0,69
		Lo psal	0,88		Strat 10	0,88		Lo psal	0,93
		Lo temp	0,96		Up psal	0,97			
Origin	Sample	dbRDA1	dbRDA2	dbRDA3	dbRDA1	dbRDA2	dbRDA3	dbRDA1	dbRDA2
+	SJ101-1	-37,92	-1,82	5,41	11,49	9,77	1,69	-13,77	3,87
+	SJ103-1	25,19	-0,16	-4,12	-23,80	5,01	-5,99	36,93	-18,74
	SJ105-1	18,55	-16,95	1,58	-21,16	-12,01	1,24	no data	no data
	SJ107-1	-28,49	3,51	-6,74	-7,25	2,80	5,43	-50,19	-4,44
	SJ108-1	22,67	15,41	3,87	40,72	-5,57	-2,37	27,03	19,32

918 Suppl. 3b.

Date 2																			
		0 m (origin excluded)						0 m (origin included)						10 m		Bottom			
		Variable	Cumul.					Variable	Cumul.					Variable	Cumul.	Variable	Cumul.		
		Strat 10	0,48					Strat 10	0,48					Lo dene	0,68	Lo dene	0,47		
		Dyn dpth 20	0,61					Origin	0,62							Up temp	0,76		
		Lo temp	0,77					Dyn dpth 20	0,75							Lo psal	0,85		
		Lo dene	0,86					Up temp	0,86										
		Thermocline	0,92					Lo psal	0,91										
		Lo psal	0,95																
Origin	Sample	dbRDA1	dbRDA2	dbRDA3	dbRDA4	dbRDA5	dbRDA6	dbRDA1	dbRDA2	dbRDA3	dbRDA4	dbRDA5	dbRDA1		dbRDA1	dbRDA2	dbRDA3		
+	SJ001-2	30,02	2,30	2,74	-2,10	1,08	-0,37	20,90	-2,60	2,06	0,43	-1,01	0,28		-36,70	8,44	-2,33		
	SJ007-2	15,11	3,23	-3,38	1,23	-1,09	-0,79	15,01	-2,51	-6,56	0,93	0,77	1,52		22,67	-5,37	0,10		
+	SJ101-2	20,63	-0,69	-5,40	-0,10	0,16	0,10	31,28	3,78	1,09	0,06	-0,02	21,48		13,95	7,90	-1,62		
+	SJ103-2	12,21	-7,16	2,19	-0,29	-0,20	0,89	10,21	4,82	2,24	-1,09	0,55	4,02		15,11	-1,71	0,13		
	SJ105-2	-24,54	-9,95	0,91	1,62	0,78	-0,66	-23,31	8,95	-0,28	0,57	-0,52	-0,97		no data	no data	no data		
	SJ107-2	-33,10	2,16	-5,90	-3,15	-0,09	0,12	-33,66	0,12	-0,98	0,16	0,19	7,76		22,73	2,00	4,55		
	SJ108-2	-2,89	4,83	-3,36	3,01	0,45	0,64	-4,18	-1,65	-6,77	-1,11	-0,47	12,75		21,44	-2,48	-5,29		
+	ZI012-2	-16,86	10,56	7,51	0,38	-0,01	0,02	-10,79	-13,24	3,60	-0,09	0,04	-30,91		-40,36	-8,79	-1,25		
+	ZI032-2	-0,57	-5,26	4,70	-0,60	-1,07	0,05	-5,47	2,33	5,61	0,13	0,48	-15,94		-18,85	0,00	5,72		

919

920 Suppl. 3c.

Date 3

0 m		10 m (origin excluded)				10 m (origin)		Bottom			
Variable	Cumul.	Variable	Cumul.	Variable	Cumul.	Variable	Cumul.	Variable	Cumul.		
Dene (Up-Lo)	0,50	Dyn dpth 20	0,34	Origin	0,42	Lo psal	0,44				
Up psal	0,78	Lo psal	0,56			Dene (Up-Lo)	0,64				
Lo temp	0,92	Lo temp	0,73			Up temp	0,84				
Thermocline	0,95	Lo dene	0,85			Strat 10	0,94				
Up dene	0,98										

Origin	Sample	dbRDA1	dbRDA2	dbRDA3	dbRDA4	dbRDA5	dbRDA1	dbRDA2	dbRDA3	dbRDA4	dbRDA1	dbRDA1	dbRDA2	dbRDA3	dbRDA4
+	SJ007-3	15,35	17,60	3,04	-0,94	0,28	-20,79	11,80	7,32	1,73	-10,23	30,09	-10,56	-19,03	10,45
+	SJ101-3	-6,49	6,19		2,29	-0,80	10,89	-13,84	11,42	2,91	-10,23	0,73	1,25	2,08	-7,97
	SJ103-3	-10,19	-1,96	-2,07	-1,88	-1,57	19,55	-0,14	3,08	-2,71	25,58	-8,70	-3,29	-10,02	-17,51
+	SJ105-3	-4,12	8,58	-2,54	0,43	0,55	-0,58	-7,48	-15,34	1,88	-10,23	no data	no data	no data	no data
+	SJ107-3	-10,99	-6,06	-2,52	-0,61	1,73	-27,16	-1,15	-2,13	3,20	-10,23	6,02	34,67	2,85	4,36
+	SJ108-3	-7,22	-9,55	8,11	0,37	-0,01	-10,17	-1,51	-0,61	-8,93	-10,23	-50,75	-7,89	1,07	9,93
	ZI012-3	23,65	-14,81	-2,44	0,34	-0,18	28,26	12,32	-3,73	1,92	25,58	22,62	-14,18	23,05	0,73

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