



Microbial response to the presence of invasive ctenophore *Mnemiopsis leidyi* in the coastal waters of the Northeastern Adriatic

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ABSTRACT

Seasonal blooms of the invasive ctenophore *Mnemiopsis leidyi* have been recorded throughout the Adriatic Sea, affecting the ecosystem and its pelagic resources. Gelatinous organisms such as *M. leidyi* release significant quantities of excreta as labile and surface active dissolved organic carbon (DOC) that supports the microbial activity.

The effects of *M. leidyi*'s presence on heterotrophic prokaryotes (HP), cyanobacteria (CB) and heterotrophic nanoflagellates (HNF) were investigated at five locations in the coastal waters of Rovinj-Rovigno (northern Adriatic) during intensive blooms in September 2019. Additionally, circulation in the study area was examined using available current measurements and a hydrodynamic model. In September, low-pass filtered currents near the surface were mostly directed toward S-SE reaching 25 cm/s, indicating the presence of the Istrian Coastal Countercurrent (ICCC).

Ctenophores displayed a patchy spatial distribution from 0.05 to 100 *M. leidyi* individuals per m³. When ctenophore density in the swarms surpassed 1 individual per m³, the change in the abundances of microbial groups became evident. The lowest microbial densities and lowest concentrations of dissolved and particulate organic carbon (DOC and POC) and surface-active substances occurred in the absence of *M. leidyi*, while the highest were within the swarms.

We hypothesise that HP utilises the available DOC enhancing the remineralisation of organic matter. Finally, greater availability of HNF's prey (HP and CB) leads to an increase in their abundance.

This study illustrates how the release of surface-active DOC during intensive *M. leidyi*'s blooms can stimulate the microbial loop. The current system with the ICCC prevailing in September enables the spreading of *M. leidyi* from its hypothetical origin in an open sea gyre towards the coast. Bora wind episodes may initiate and favour this type of circulation. In these conditions, *M. leidyi* agglomerates in large numbers along the coast, enabling the accumulation of organic matter and altering the abundance of pico- and nanoplankton.

1. Introduction

The invasive ctenophore *Mnemiopsis leidyi* A. Agassiz, 1865, has successfully established in the Adriatic Sea since 2016 (Malej et al., 2017; Budiša et al., in press). *M. leidyi*'s swarms last usually from the summer to the end of the autumn, occupying vast coastal areas in

between the northwestern (NW) coast of Istria (Croatia and Slovenia) and through the Italian coast down to Ancona, mostly reaching their maximum density and extension in September (P. Paliaga, pers. comm.). Effects on both zooplankton and fish stocks in the Adriatic have been reported (Budiša et al., in press), raising concerns on the long-term consequences for the equilibria in the upper trophic levels in one of

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the most productive parts of the Mediterranean (Ben-Hamadou et al., 2012). Similar effects have been previously reported for other food webs in pelagic, coastal and estuarine ecosystems (Grosholz, 2002; Marambio et al., 2013), e.g. the Black Sea (Shiganova, 1997), the Sea of Azov (Studenikina et al., 1991) and the Caspian Sea (Ivanov et al., 2000) where *M. leidyi* depleted the zooplankton stocks and contributed to the fisheries collapse (Kideys et al., 2005).

Gelatinous zooplankton, e.g. *M. leidyi*, can also affect the lower trophic levels. Some members of this diverse group of pelagic taxa (i.e. cnidarians, ctenophores, chaetognaths and tunicates) are known to form blooms (Lucas et al., 2014; Lebrato et al., 2019; Sola et al., 2019; Luo et al., 2020). A *M. leidyi* bloom can reach enormous biomasses, i.e. 10^9 t of wet weight such as the one recorded in the Black Sea (summer-autumn 1988), exceeding even the world's annual fish landings (Ivanov et al., 2000; Sorokin, 2002).

In these events, substantial quantities of dissolved organic matter (DOM) and nutrients can be released via excretion, mucus production or body decomposition (Pitt et al., 2009; Condon et al., 2011; Hao et al., 2019; Tinta et al., 2020).

HP are the most abundant component of the marine microbial community (Azam, 1998) and the major consumers of DOM in the oceans (Williams and Gray, 1970; Azam and Hodson, 1977). Massive gelatinous organism blooms can be considered an ecosystem disturbance, and their effects on the dynamics of the microbial community are still unclear (Riemann et al., 2006; Titelman et al., 2006; Tinta et al., 2010, 2012 & 2016; Condon et al., 2011; Dinasquet et al., 2012a, b & 2013; Blanchet et al., 2015; Basso et al., 2019; Hao et al., 2019; Jaspers et al., 2019; Guy-Haim et al., 2020). In many marine systems, the majority of bacterial production is sustained by organic matter (OM) derived from primary production or phytoplankton exudates (del Giorgio and Cole, 1998; Hansell and Carlson, 2002). However, the bacterial carbon demand often exceeds the supply rates from primary production (del Giorgio and Peters, 1993; del Giorgio and Cole, 1998), and bulk organic pools contain large concentrations of refractory DOM (Hansell and Carlson, 2002). In those conditions, the DOM excreted by gelatinous zooplankton may be an important source of C, N and P for bacterioplankton. The predominant forms of DOM produced by jellyfish are labile, suggesting a tight coupling between DOM excretion and bacterioplankton production (Pitt et al., 2009; Condon et al., 2011; Hao et al., 2019; Tinta et al., 2020).

A field study by Riemann et al. (2006) found an increased bacterial activity where the biomass of jellyfish *Periphylla periphylla* (Péron & Lesueur, 1810) was the highest. *In situ* incubated jellyfish *Aurelia aurita* (Linnaeus, 1758) doubled bacterial biomass and production (Turk et al., 2008). Decaying jellyfish blooms also promote bacterial growth and biomass production (Titelman et al., 2006; Tinta et al., 2010; Blanchet, 2015; Blanchet et al., 2015). Laboratory-scale incubation experiments revealed the influence of *M. leidyi* on bacterioplankton activity and microbial community composition (Dinasquet et al., 2012a). Temporary aggregations of ctenophore locally affect bacterial growth and may significantly contribute to the spatial heterogeneity of bacterioplankton activity and community composition (Dinasquet et al., 2012a).

Relative to other marine phytoplankton, cyanobacteria (CB) have lower growth rates (Paerl and Millie, 1996). Nonetheless, it is estimated that $\frac{1}{4}$ of the ocean primary production is assigned to unicellular CB (Flombaum et al., 2013). Picocyanobacterial abundances (cells/mL) at their peak in the Adriatic are reported from 10^2 – 10^6 (del Negro, 2007).

HP and CB represent the main prey for heterotrophic nanoflagellates (HNF) (Caron et al., 1992). In ecosystems where CB are the dominant primary producers, e.g. oligotrophic Mediterranean waters, HNF preferably preyed on CB rather than HP (Hagström et al., 1988).

Primary and secondary production, including zooplankton grazing, directly reflects on DOM quantity and quality (Pettine et al., 2001; Fonda Umani et al., 2007). OM quality in seawater, i.e. reactivity, can be directly related to its hydrophobic and hydrophilic properties, measured under the term surface-active substances (SAS) (Čosović and Vojvodić, 1998; Croot et al., 2007; Sabbaghzadeh et al., 2017; Ciglenečki et al., 2020).

Our work presents a field study aiming to explain the ecological effects of the invasive *M. leidyi* presence on the microbial community, dissolved organic carbon (DOC), and its surface-active substances (SAS) fraction in the Adriatic Sea. We hypothesise that dense swarms will affect the abundance of the microbial community by providing readily available OM.

Hydrographic conditions alone, especially circulation dynamics in the Adriatic coastal area, are often held responsible for favouring gelatinous zooplankton aggregations by directing population variations, especially at local scales (Graham et al., 2001). Thus, we also examined the relation with the circulation properties during the sampling period. The end of summer circulation in the Istrian coastal zone in some years is characterised by the Istrian Coastal Countercurrent (ICCC), a surface geostrophic current following the coastal line, with a pronounced southeastern (SE) component and an average speed of 7 cm/s (Supić et al., 2000). The current is a part of an anticyclonic gyre located in open waters of the NE Adriatic and filled with lower salinity waters of Po River origin (Supić et al., 2003). This gyre is assumed to be an important seasonal spawning area for *M. leidyi* and the main origin for ctenophore along the coast of Istria (Budiša et al., in press). The SE motions, especially intense in September, as observed during several years of measurements in open waters off the Istrian coast (Brana and Krajcar, 1995), are presumably a part of the same gyre. Numerical modelling results for September 2019 show that the gyre and the ICCC were also present in 2019. The possibility of *M. leidyi*'s transport from the gyre to the coast by the ICCC is also investigated here.

2. Materials and methods

2.1. Sampling

The samplings were performed on 4 and September 21, 2019 at five locations in the coastal waters of Rovinj-Rovigno (Rv), NE Adriatic (Fig. 1, Table S1) during *M. leidyi*'s annual bloom peak. Four locations represented semi-enclosed accumulation areas for ctenophore and OM in contrast to the open sea area of Bagnole. *M. leidyi* was counted, and samples were collected for biological and chemical analysis. At each location, 4 to 12 sites were sampled along a grid distributed at distances from 50 to 200 m. Additional field observations on *M. leidyi* presence in relation to prevailing currents were carried out in the summer of 2020.

Water samples were collected at 0.5 m depth because *M. leidyi* individuals were concentrated close to the surface. At every location, water temperature and salinity were measured with a pIONeer 65 probe (Radiometer analytical, Copenhagen). Moreover, the relation between the invasive ctenophore, microbial community and OM was investigated.

2.2. Abundance and distribution of *M. leidyi*, pico- and nanoplankton

At the beginning of September, ctenophores were monitored at Valdibora Bay, Monsena Bay and the Southern Harbour on the same day, while at the end of the month, locations around islands St. Ivan and Bagnole were sampled. The density (ind./m³) was determined by visual census of specimens (length > 1 cm) by scuba divers with the help of a submerged cube frame (0.5 m x 0.5 m x 0.5 m) carried along the transects of the coastline. Samples for microbial community analysis were preserved with formaldehyde (2% final concentration) and stored at 4 °C. For determining HP and HNF abundances, 2 mL of fixed samples were stained with 4,6-diamidino-2-phenylindol (DAPI, 1 µg/mL final concentration) for 10 min, and then passed through 0.2 µm black polycarbonate filters (Whatman® Nuclepore™). HP abundance was determined by epifluorescence microscopy at a magnification of 1000x (Leitz Laborlux D) according to Porter and Feig (1980). For HP count, at least 500 cells per sample were counted. From the total number of prokaryotes, the number of CB was subtracted to obtain the number of HP. The HNF abundance was determined by counting a minimum of

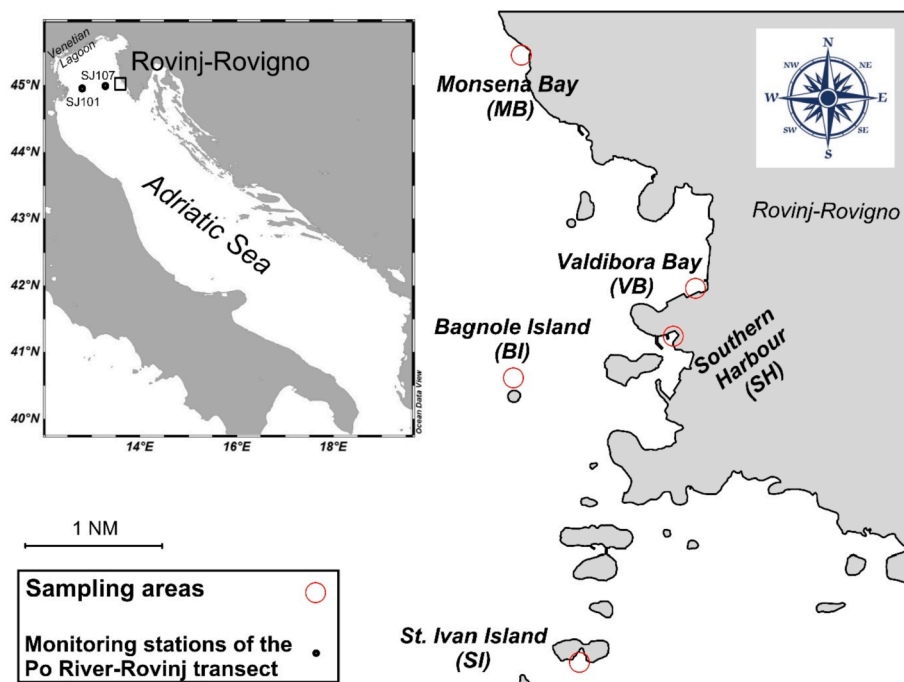


Fig. 1. Sampling locations in the coastal waters of Rv.

100 cells/sample (Sherr et al., 1993). The CB abundance was determined using green light excitation, with a minimum of 300 cells/sample counted (Takahashi et al., 1985).

Data sets were tested for normal distribution using the Shapiro-Wilk test and data homogeneity using Levene's test. For normally distributed ($p > 0.05$) homogeneous data ($p > 0.05$), One-Way ANOVA or two-tailed T-tests were performed. When logarithm transformation of data did not help attain a normal distribution, non-parametric two-tailed Mann-Whitney or Kruskal-Wallis tests were performed. For data that violated homogeneity of variances, the Games-Howell test was applied. In addition, plankton abundances were normalised and compared.

2.3. Organic matter content measured as surface-active substance (SAS), DOC and POC

For DOC and POC quantification, seawater was sampled in dark 1.3 L bottles and then filtered (1 L) through 0.7 μm pore size precombusted Whatman GF/F fibre filters for 4 h at 450 $^{\circ}\text{C}$. Sub-samples of the filtrate were collected in 20 mL glass vials, preserved with 100 μL of mercury (II)-chloride (10 mg/L), closed with Teflon-lined screw caps and stored in the dark until analysis. These filtrates were used for DOC analysis. Filters were stored in liquid nitrogen (-80°C) until TOC, i.e. POC analysis. DOC was determined by the high-temperature catalytic oxidation (HTCO) method at a TOC-VCPH instrument (Shimadzu, Japan) (Cauwet, 1994, 1999; Dafner and Wangersky, 2002; Dautović et al., 2017). The DOC measurement was performed by direct injection of the sample into a combustion furnace at a temperature of 680 $^{\circ}\text{C}$ through a sensitive Pt/silica catalyst. Organic carbon from the sample generated CO_2 that was directly measured using a non-dispersed infrared (NDIR) detector. Prior to the oxidation, the inorganic carbon was removed from the sample by acidification with HCl ($\sim 2\%$) to pH 2–3 and evaporation of the resulting CO_2 in the stream of pure air for 10 min. System calibration was performed with potassium hydrogen phthalate. POC was determined in filters with the calibration using glucose. A solid sample module SSM-5000A with TOC-VCPH carbon analyzer was used for measurements. Inorganic carbon was removed from samples prior to POC measurement by adding 2 mol/dm $^{-3}$ HCl. Oxidation of organic carbon in the flow of pure oxygen at 900 $^{\circ}\text{C}$

produced CO_2 that was detected using an NDIR detector. The quantification limit was 0.228 mg/dm 3 for DOC and 7.11 $\mu\text{g}/\text{dm}^3$ for POC. The precision of DOC and POC measurements, given as the RSD, was based on the analysis of selected samples and the reference materials; RSD never exceeded 6% and 5%, respectively. Both measurement procedures were validated through the international intercalibrations as a prerequisite for accreditation achieved in 2017 (HRN EN ISO/IEC 17025:2007).

All glassware used for organic matter analyses, including sampling and sample filtration and preservation till measurements, were washed with chromic-sulphuric acid and rinsed well with organic-free Milli-Q water (Millipore) and seawater samples. SAS measurements were done by the electrochemical method a.c. voltammetry based on the measurements of the SAS adsorption effects at the surface of the hanging mercury drop as a working electrode in the seawater sample (0.55 M NaCl as the model electrolyte) (Ćosović & Vojvodić, 1982, 1998; Ciglencečki et al., 2020). Selected adsorption potential conditions (-0.6 V by Ag/AgCl electrode) and adsorption time (15–120 s) with stirring were used. Changes of the working electrode double layer reflected on the capacitance current are approximately proportional to all (dissolved and particulate) SAS in the solution. The total SAS (i.e. NF, non-filtered) is separated into particulate and dissolved fractions (SAS F, filtered) by filtering through 0.7 μm GF/F Whatman® filters. SAS content is expressed in the equivalents of the selected model OM substance of Triton-X-100 (eq. T-X-100) – a good representative of the most reactive part of the SAS in natural waters (Ćosović and Vojvodić, 1982, 1998; Ciglencečki et al., 2020 and references therein). The detection limit (LoD) for voltammetric SAS determination is 0.02 mg/L eq. T-X-100, with the limit of quantification (LoQ) of 0.05 mg/L. If SAS is normalised to DOC, then the normalised surfactant activity value, NSA = [SAS (eq. T-X-100)/DOC], can be calculated. NSA can be compared with normalised surfactant activities of different model SAS substances: Triton-X-100 (1.54), protein albumin (0.20), fulvic acid (0.17), polysaccharides dextran T-500 (0.20) and xanthan (0.04), which are representatives for surface active organic matter composition of natural samples (Ćosović and Vojvodić, 1998; Ćosović et al., 2000; Ciglencečki et al., 2018).

In addition, the Pearson's 'r' correlation coefficients were calculated

(significance level, $\alpha = 0.05$) for *M. leidy* abundances and C-content.

2.4. Current measurements

Vertical profiles of currents were measured close to Bagnole Island (Fig. 1, Table S1). Teledyne RDI ADCP 600 kHz instrument (acoustic Doppler current profiler) was deployed at 29.5 m depth (bottom depth at the location was 31.5 m) between July 5 2019, and March 9 2020, enabling continuous high-resolution current profiling (1 m vertical resolution and 15 min sampling interval). Measurements were available at 3.4 m–27.4 m depths. Sea temperature data were collected at a depth of the instrument (29.5 m). Current data were quality checked. Data analysis included spectral analysis, low-pass filtering procedures (to exclude tidal and inertial motions and seiches) and daily averaging of current measurements in July–September 2019 period, covering the period before and during *M. leidy* sampling.

2.5. Numerical modelling

The ROMS model was used for the hydrodynamic simulation of the northern Adriatic system and is described in detail in Janeković et al. (2010). The surface forcing was obtained from the ECMWF forecasts and provides 2 m air temperature, cloud fraction, 2 m relative humidity, mean sea level pressure, 10 m wind, longwave and shortwave flux, rainfall rate. The boundary forcing at the Otranto strait was obtained from the ADAM-ADRIA project results (Janeković et al., 2020) and is

used as climatology in this run. The climatological river inflow data for 41 Adriatic rivers, provided by Vilibić et al. (2016), was also used in the model setup. Monthly averaged surface current fields and the results of a dispersion model coupled with the hydrodynamic model are presented in our study. The drifters in the lagrangian dispersion model were set to be purely isobaric (they follow currents but remain at the same depth) to model the behaviour of ctenophores. Starting on July 1, 2019, drifters were continuously released from the model grid point corresponding to the station SJ107 (Fig. 1) with a rate equalling 15 drifters per day (3 drifters for each of the 5 different depths: 0.2 m, 0.4 m, 0.6 m, 0.8 m, 1.0 m). Their average distribution for July, August and September 2019 is analysed and presented here.

3. Results

3.1. Temperature and salinity

Surface temperatures during the whole sampling period were similar at all locations (23.4 ± 1.2 °C). The water column was strongly stratified with the thermocline at 16 m depth. Surface salinities were also very similar at all the locations (37.1 ± 0.01) but were slightly higher at the end of the investigated month (37.4 ± 0.02).

3.2. ADCP currents

Stationary spectral analysis (Jenkins and Watts, 1968) showed that

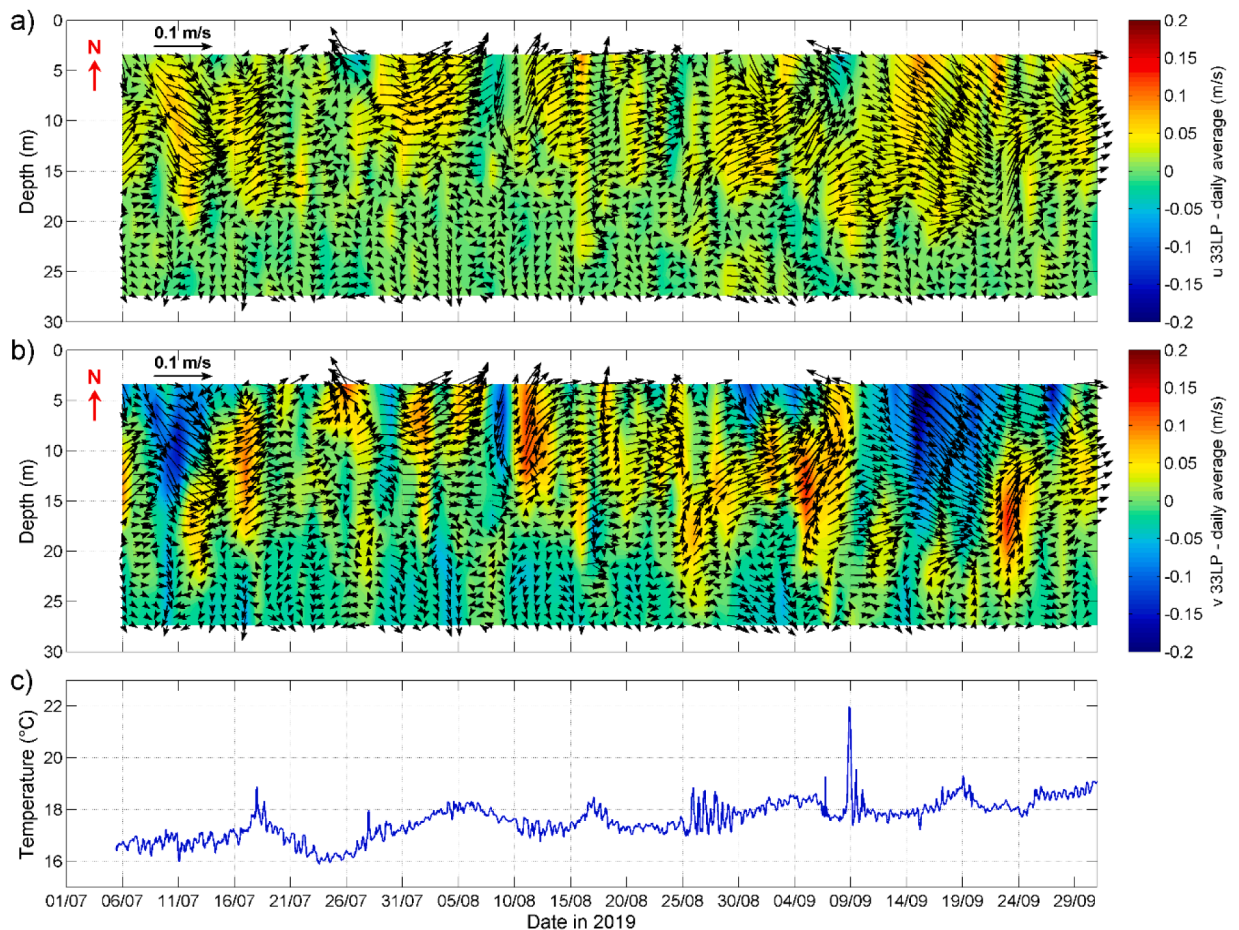


Fig. 2. a) Daily-averaged current vectors obtained by filtering original current measurements using a low-pass filter with a cut-off period of 33 h. The corresponding eastern current component is presented as a filled contour plot. b) As in a) except that the northern current component is presented as a filled contour plot. c) Near-bottom sea temperature measured every 15 min at the current meter location (sensor depth 29.5 m).

the most energetic oscillations in ADCP current data between July and September 2019 were at tidal frequencies (not shown), more specifically at diurnal (~ 24 h) and semi-diurnal period (~ 12 h). Moreover, inertial oscillations were also present, with a period of ~ 17 h.

Currents components were filtered using 33-h low-pass filter (e.g. Emery and Thomson, 1997) to remove higher frequency oscillations and analyse low-frequency changes in the current field. Filtered data were then averaged every 24 h. Daily-averaged current vectors obtained from such a procedure are presented in Fig. 2 at every depth between July and September 2019. Current vectors and the corresponding eastern and northern components are given in Fig. 2a and b, respectively. Additionally, original temperature data at 29.5 m depth, measured every 15 min, are shown in Fig. 2c.

Daily averaged low-pass filtered currents having S-SE direction, with speeds up to 25 cm/s, generally appeared in upper layers (0–15 m). S-SE circulation was first observed between 8 and 11 July, down to 15 m depth, with speeds reaching 12 cm/s. Another short episode with mainly E-SE currents occurred at the end of July. S-SE currents were again observed at the end of August/beginning of September in the uppermost layer (down to 8 m), with speeds reaching 10 cm/s. These S-SE motions could be attributed to the ICCG, as they are in its direction and corresponding range. In September 2019, they were more frequent and prevailed over motions with the N-NE component (typical for August). Sub-surface currents were more intense than the bottom ones. From 10–21 September, they were predominantly S-SE directed down to 15–20 m depth, reaching their maximum on 15 September (~ 25 cm/s).

A prominent change in current direction and magnitude occurred after September 9, 2019, when near-bottom temperature swiftly rose from ~ 18 °C to ~ 22 °C, possibly due to vertical mixing induced by surface cooling episodes within a period of strong winds over the northern Adriatic. Between 6 and 9 of September 2019, the wind changed from bora (N, NE) to sirocco (SE) (Croatian Meteorological and Hydrological Service, 2019). The change in the near-bottom sea temperature was followed by a change in current direction in upper layers, from NW (8 and 9 September) to SE (10 September onwards). After this bora-sirocco episode, currents in the upper layer were mostly in the SE direction, almost till the end of the month. From 10–21 September, sub-surface and intermediate low-pass filtered currents with SE direction were occasionally very strong (up to 25 cm/s close to the surface) and observed to 20 m depth.

3.3. Modelled currents and dispersion model results

Monthly averaged modelled surface currents in July–September period indicate the presence of an anticyclonic gyre in the northeastern Adriatic (to the southwest of Rv) and a large cyclonic gyre encompassing almost the entire area north of the Po delta-Rv transect (Fig. 3). These two gyres seem to be permanent features during this period, although the anticyclonic gyre slightly shifted towards the northwest. The resulting currents in the area around station SJ107 (which is at the rim of this anticyclonic gyre) were mostly directed eastward towards the western Istrian coast.

The dispersion of passive surface drifters originating at the station SJ107 (where a high accumulation of *M. leidyi* was observed during the summer of 2017; P. Paliaga, pers. comm.) was also examined, and nondimensional drifter concentrations were the highest between SJ107 and the coast (in August) and along the western Istrian coast (in July and September; Fig. 3). These results qualitatively confirm the hypothesis that *M. leidyi* seasonally accumulated in an anticyclonic gyre to the west-southwest of Rv could be advected towards the western Istrian coast, as we observed in September 2019.

3.4. *M. leidyi* abundance

Spatial distributions of *M. leidyi* are given in Fig. 4 and supplementary material (Fig. S1). At the beginning of September, the highest

average abundances were registered in Valdibora Bay (~ 59 ind./m³), reaching up to 100 ind./m³ accumulated along the SE coast and covering $1.3 \cdot 10^4$ m². At Monsena, abundance averaged to ~ 6 ind./m³, covering $2.7 \cdot 10^4$ m². The maximum (12 ind./m³) was found in the inner part due to wave transport. Northwards to the open sea, abundances dropped to zero. The lowest sampling average (~ 5 ind./m³) was found in the Southern Harbour. Abundances reached up to 10 ind./m³ and covered $1.6 \cdot 10^4$ m². Due to the current flow, ctenophore amassed at the SE coast. At St. Ivan, abundances averaged to 13 ind./m³ covering $3 \cdot 10^4$ m². The maximum recorded on the bay's NE side decreased progressively towards the outer sea, indicating accumulation by waves and currents. Around Bagnole Island, a relatively uniform distribution of 0.05 ind./m³ covered $\sim 10^5$ m². Ctenophora swarms remained approximately within the same areas for a couple of days before being redistributed to other locations.

3.5. Organic matter content measured as surface-active substance (SAS), DOC and POC

The lowest concentrations (mg/L) of DOC (1.35 ± 0.1), POC (0.112 ± 0.02), SAS NF and F (0.143 ± 0.01) were found outside of swarms, while the highest were measured in the presence of ctenophore (1.9 ± 0.64 for DOC, 1.3 ± 1.38 for POC, 0.2 ± 0.08 for SAS NF and F). Overall, DOC, POC and the total SAS (NF and F fractions) were higher in the denser swarms (Fig. S2a).

The values measured at Bagnole represented a background level (0.133 mg/L) (data not shown) for SAS concentration and were comparable to those measured where ≤ 1 ind./m³ (Fig. S2b). In Fig. 4, spatial distributions of plankton abundances and C-contents are given. A coupling between an increasing density of ctenophore and higher concentrations of SAS was more pronounced at the locations Valdibora and St. Ivan, where ctenophore density surpassed 10 ind./m³. When the abundance of *M. leidyi* per m³ was lower than 10, OM was dominantly in the surface-active DOC form, while the POC form increased with the higher abundance of *M. leidyi* (Fig. S2). The normalised surfactant activity of analysed samples to their DOC content (NSA) indicate the presence of more hydrophobic nature of OM with NSA values around 0.1.

3.6. Microbial abundance

During all samplings, the distribution of HP, HNF and CB indicated a visible coupling between them and increased ctenophore densities. The relation with SAS appeared to a lower extent (Fig. 4 & S1). The average abundances (cells/mL) of HP, HNF and CB were the highest at Valdibora Bay ($1.2 \pm 0.3 \cdot 10^6$, $18.9 \pm 9.2 \cdot 10^3$ and $7.4 \pm 1.1 \cdot 10^4$ respectively). The average HP abundance in swarms doubled ($1.4 \cdot 10^6$ cells/mL) with respect to the outside waters ($6.9 \cdot 10^5$ cells/mL) (Fig. 5a), HNF abundances ($7 \cdot 10^3$ cells/mL) increased 3.5x in swarms ($24 \cdot 10^3$ cells/mL) (Fig. 5c), while CB increased by 40% (Fig. 5b). In Monsena Bay, abundances (cells/mL) increased by 50% ($8.12 \cdot 10^5$) for HP, 2.5x ($5.1 \cdot 10^4$) for CB and 60% ($6 \cdot 10^3$) for HNF (Fig. 5). In harbour's waters, abundances of HP CB, and HNF, increased moderately, i.e. by 40%, 80% and $\frac{1}{3}$, respectively (Fig. 5).

Later in September, the average abundances (cells/mL) of HP, CB and HNF within the swarm at St. Ivan were $14.7 \cdot 10^5$, $12 \cdot 10^4$ and $21 \cdot 10^3$, respectively. The increase in abundance of microbial groups was the most pronounced in this location, where averages rose 3x for HP, 4x for CB and 5x for HNF (Fig. 5). HP displayed a strong coupling with *M. leidyi*. Their abundances rose from outer waters ($4.8 \cdot 10^5$ cells/mL) to maximums of $1.6 \cdot 10^6$. CB and HNF (cells/mL) displayed a trend similar to HP, reaching their peak concentrations ($41.3 \cdot 10^4$ and $26.5 \cdot 10^3$, respectively) in the densest part of the swarm and decreasing substantially to $3 \cdot 10^4$, and $4 \cdot 10^3$, respectively, in the open waters. Around Bagnole Island, ctenophores abundance was very low, and microbial abundances were comparable to the values registered at the outer parts

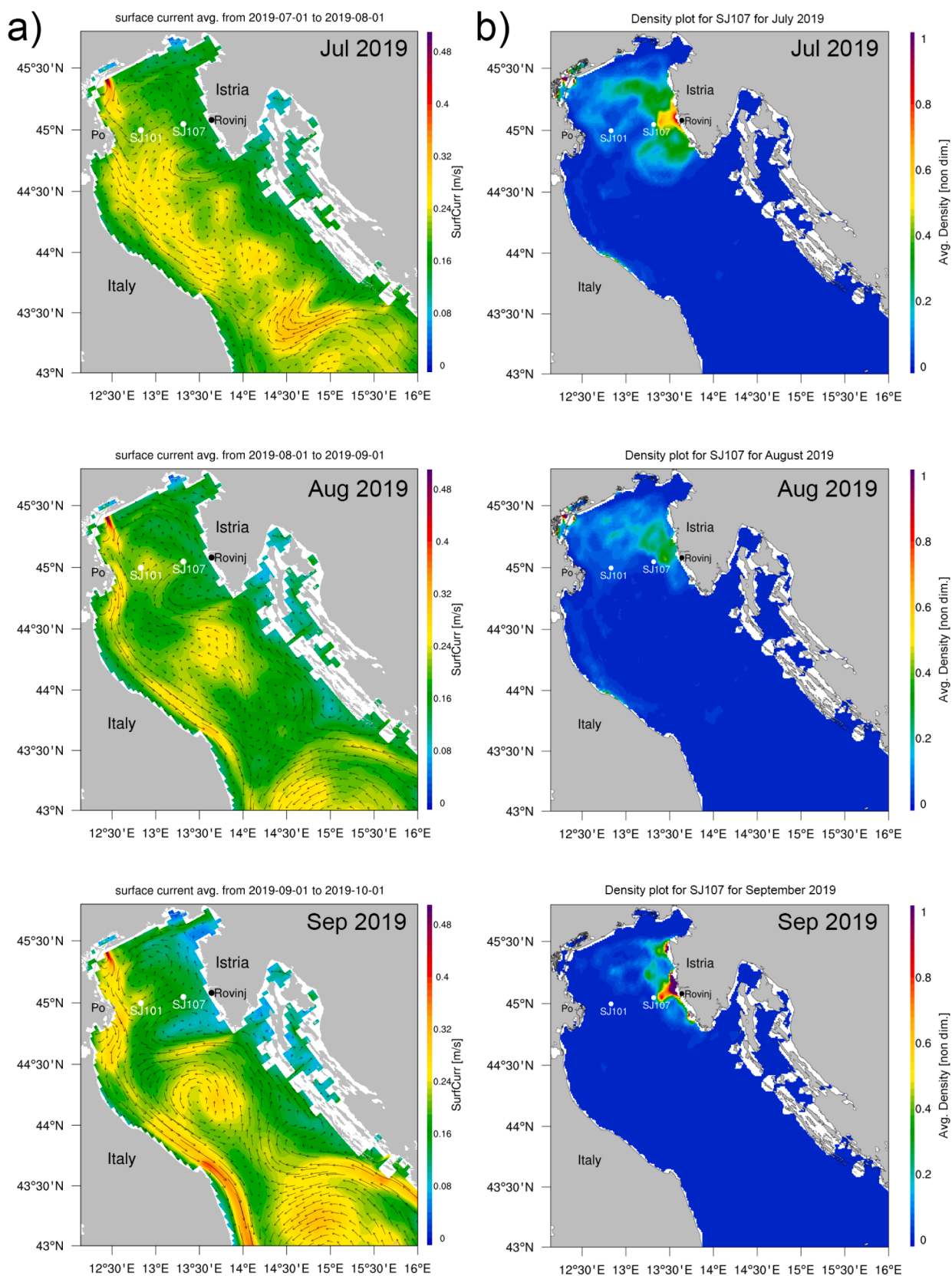


Fig. 3. a) Monthly averaged modelled surface currents for July (upper panel), August (middle panel) and September 2019 (lower panel), and b) monthly averaged density of drifters, released from 1 July 2019 in the amount of 15 drifters per day from the location corresponding to the station SJ107, for July (upper panel), August (middle panel) and September 2019 (lower panel).

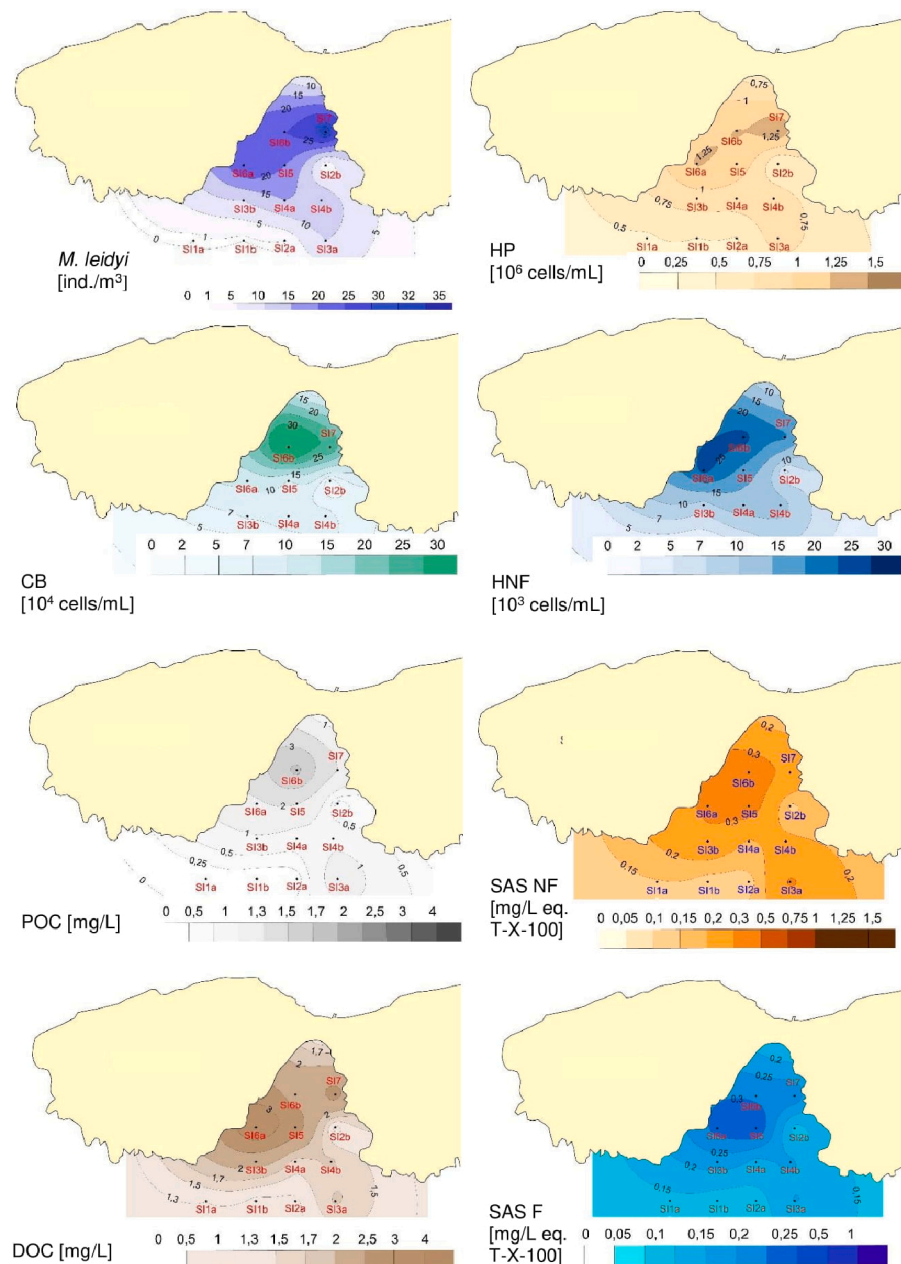


Fig. 4. Spatial distributions of plankton abundances (*M. leidy*, HP, HNF, CB) and C-contents (DOC, POC, SAS) at St. Ivan Island.

of St. Ivan's swarm. HP varied in a narrow range ($3.7\text{--}4.1 \cdot 10^5$ cells/mL) and averaged to $\sim 3.8 \cdot 10^5$ cells/mL. CB and HNF (cells/mL), too, did not show significant variations ($2.5\text{--}3.3 \cdot 10^4$ and $2.4\text{--}3.4 \cdot 10^3$, respectively). Statistical tests for differences among groups revealed significance in microbial abundances and *M. leidy*'s presence at all the locations except at Bagnole (Table 1). Overall, statistically significant ($p < 0.01$, $N = 135$) positive correlations were obtained between *M. leidy*, HP ($r_{HP} = 0.72$) and HNF ($r_{HNF} = 0.78$), while a weak one was found for CB ($r_{CB} = 0.35$, $N = 133$). Overall averages ctenophore abundances and POC correlated strongly ($r_{POC} = 0.88$, $p < 0.01$, $N = 16$). HNF was strongly correlated with HP, $r_{HNF-HP} = 0.78$, and moderately with CB ($r_{HNF-CB} = 0.59$, $p < 0.01$, $N = 133$). Picoplankton abundances also strongly correlated among themselves ($r_{HP-CB} = 0.80$, $p < 0.01$, $N = 133$). At St. Ivan, where the best data distribution was available, swarms strongly correlated also with DOC and SAS F ($r_{DOC} = 0.89$, $r_{SAS F} = 0.79$, $p < 0.01$, $N = 7$) but not SAS NF. However, the latter is represented by a small data pool; thus, all the correlations should be considered just as an indication

of a trend that might be confirmed in future. In addition, regression lines between the averaged, normalised plankton abundances at four potential OM accumulation areas (St. Ivan, Bagnole, Valdibora, Monsena and South Harbour) were compared (Fig. S3) and revealed linearity ($R = \sim 0.8$) up to 35 ind./m³. In denser swarms (100 ind./m³), microbial abundances did not follow linearity.

4. Discussion

The average concentrations of ctenophores registered at the sampling locations ($0.05\text{--}59$ ind./m³) were comparable to those previously reported for September (2016–2019) for Rv coastal waters, i.e. 2.5 ± 2.1 ind./m³ (Budiša et al., in press) except in Valdibora Bay, where abundances were higher. Ctenophore (ind./m³) enumerated during the monitoring were comparable to those measured in other areas such as the Berre Lagoon (Mediterranean coast of France) ~ 2 (Marchessaux et al., 2020), 2–7 in the NW Baltic (Schaber et al., 2011), 4–11 the NE

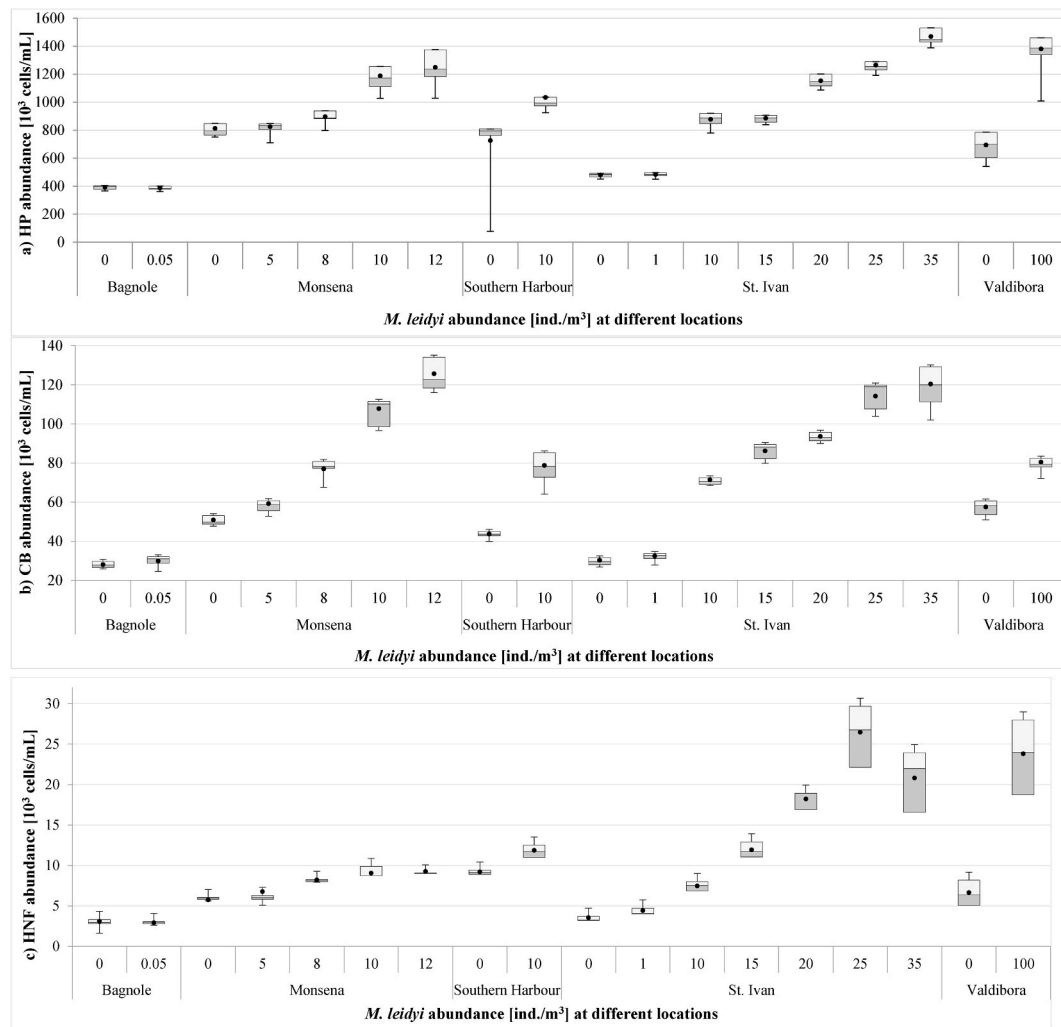


Fig. 5. The abundances of microbial community: a) HP, b) CB, and c) HNF in relation to the swarm density. The median is shown as a line inside the rectangle and the average (mean) as a dot, for each sample. The rectangle itself is an Inter-Quartile Range (IQR). The error bars represent the most extreme points, i.e. the maximum and minimum value of the group within the 1.5 IQR.

Table 1

Statistical differences between the microbial abundances in the swarms and when *M. leidyi* was absent (significance level $\alpha = 0.05$). The statistically non-significant values are marked in red. A small pool of data for the Mann-Whitney rank test allowed only for a comparison of calculated $U_{\text{statistical}}$ values with the table of critical values, and thus is marked with an asterisk (*).

	Location	Monsena Bay	Southern Harbour	Valdibora Bay	St. Ivan Island	Bagnole Island
	N (samples)	30	20	35	40	10
HP	Normal distribution	yes	No	yes	yes	yes
	Equality of variances	Homogeneous	Homogeneous	Homogeneous	Homogeneous	Homogeneous
	Difference	One way ANOVA	Mann-Whitney test	T test	One way ANOVA	T test
	p-value	$<10^{-4}$	* $U_{\text{statistical}}(0) < U_{\text{critical}}(23)$	$<10^{-4}$	$<10^{-4}$	0.72
CB	Distribution	yes (Log)	Yes	Yes	yes	yes
	Equality of variances	Homogeneous	Homogeneous	Homogeneous	Homogeneous	Homogeneous
	Difference	One way ANOVA	T test	T test	One way ANOVA	T test
	p-value	$<10^{-4}$	$<10^{-4}$	$<10^{-4}$	$<10^{-4}$	0.33
HNF	Distribution	no	Yes	yes (Log)	yes	Yes
	Equality of variances	Homogeneous	Nonhomogeneous	Homogeneous	Nonhomogeneous	Homogeneous
	Difference	Kruskal-Wallis	Games-Howell	T test	Games-Howell	T test
	p-value	$<10^{-4}$	$<10^{-4}$	$<10^{-4}$	0.033	0.51

Black Sea (Kamburska et al., 2006) and ~55 the western Black Sea, (Kamburska et al., 2006). The lowest concentrations measured at the swarm edges and in the open sea were close to the ones registered in oligotrophic environments, such as by the coast of Israel (0.1 ind./m³) (Shiganova et al., 2019) and the Aegean Sea (0.3–0.5 ind./m³)

(Shiganova et al., 2001). *M. leidyi* swarms in the NE Adriatic appear in large swarms from May to November, with higher densities along the western coast of Istria and patches, covering several km² in the open sea (Budiša et al., in press).

In winter, *M. leidyi* is almost absent from the NE Adriatic and

essentially relegated to the waters in front of and within the Italian lagoons of the NW Adriatic with a few specimens reaching the monitoring station SJ101 (Fig. 1) (CMR – Center for Marine Research database and V. Tirelli, pers. comm.). From those areas, by the end of winter and in early spring, *M. leidy* can spread towards the NE Adriatic, as it occurred in 2017 (Ciglenečki et al., in press). *M. leidy* increased in number within an anticyclonic gyre around the station SJ107 (Fig. 1) between May and July 2017, indicating it as an accumulation area.

The anticyclonic gyre also appeared in summer 2019 and included an area around SJ107, as observed earlier in 2017. According to the numerical model, station SJ107 was at the northern rim of the anticyclonic gyre in July–September 2019 period (Fig. 3). By hypothesizing that the gyre acts as an accumulation place for *M. leidy*, we investigated its possible spreading paths using a lagrangian dispersion model, coupled with the results of the hydrodynamic model (Fig. 3). The results confirmed that the modelled circulation patterns could result in the advection of drifters from the gyre to the western Istrian coast. Further spreading of drifters along the coast is related to the coastal dynamic. Abiotic factors, temperature and salinity that can impact the abundance of picoplankton in the northern Adriatic (Fuks et al., 2005) were similar at all the sampling stations. Therefore, the described patterns of microbial distribution in this investigation cannot be explained by those conditions.

Low-pass filtered near-surface currents with mostly S-SE direction were observed during sampling (4 and 21 September) and could be responsible for transporting *M. leidy* along the coast (Fig. 2). The intensification of eastward transports towards the Istrian coast in relation to bora events (e.g. the one around September 6, 2019) was documented in several research papers for data collected in different time periods and based on models, satellite data and geostrophic currents computation (Kuzmić et al., 2007; Lyons et al., 2007; Kraus and Supić, 2015). Lyons et al. (2007) analysed a large number of weekly measured temperatures and salinities along the western Istrian coast with a high spatial resolution during the warm part of 2003. For most of the investigated period, geostrophic circulation implied both large cyclonic (located north of the line Po delta-Rv) and anticyclonic (positioned to the south) gyres off the Istrian coast. A similar pattern was obtained for the 2003 winter by Kuzmić et al. (2007), based on models and measurements. Changes in geostrophic circulation were found to be primarily related to changes in the thermal structure of the water column in the area, e.g. very strong eastward motions along the 'frontal' transect parallel to the western Istrian coast, induced by bora (September 2003), were related to the increase in average temperature in the southern part of the section, due to the formation of a deep warm water pool in open waters east of Pula (Lyons et al., 2007).

The change in current direction from NW to SE coinciding with a large temperature increase at the bottom (September 2019) may support the idea that changes in the thermal structure of the water column, reflecting on geostrophic components of the current field, were also observed directly from ADCP measurements. Finally, the intensification of eastward transports towards the western Istrian coast in September 2019 was also confirmed by the modelling results (Fig. 3).

The spatial distribution of the studied microbial components displayed a remarkably similar pattern to the swarms, indicating a coupling between them (Figs. 4 and S1). The abundances of HP, HNF and CB (cells/mL) outside the swarms (i.e. $6 \pm 2 \cdot 10^5$, $5.8 \pm 2.6 \cdot 10^3$, and $4.3 \pm 1.2 \cdot 10^3$, respectively) were close to the average, typical summer values at the sea surface of the northern Adriatic ($4.7 \cdot 10^5$ – $6.3 \cdot 10^5$, $0.5 \cdot 10^3$ – $1.2 \cdot 10^3$, and $40 \cdot 10^5$, respectively) (Fuks et al., 2005 & 2012). Moreover, by comparing our data to the observations for the microbial community in coastal waters of Rv registered in September (2000–2014) by the CMR database, which can be considered as a background level with an average HP of $6.9 \pm 3.4 \cdot 10^5$, HNF of $3.2 \pm 3 \cdot 10^3$ and CB of $5.5 \pm 5.4 \cdot 10^4$ cells/mL it emerges that outside the swarms, HP and HNF were very similar to the long-term averages while CB was quite smaller but not so unusual due to the high oscillations of this parameter in various

years. Where ctenophore densities were ≥ 1 ind./m³, the average HP abundance ($1.1 \pm 0.3 \cdot 10^6$ cells/mL) was around twice as high as the usual values, HNF concentration ($15 \pm 8.4 \cdot 10^3$ cells/mL) of an order of magnitude higher than the long-term maxima, and CB concentration ($8.3 \pm 2.3 \cdot 10^4$ cells/mL) close to the upper values previously registered in the basin and the long-term values in Rv (Fig. 5). Similar HP abundances were reported during phytoplankton blooms ($1.24 \cdot 10^6$ cells/mL) in the NW Adriatic, linked to the nutrient enrichment by the Po River inflow when HNF reached their annual maximum of $1.6 \cdot 10^3$ cells/mL (Fuks et al., 2012) – considerably lower than the values in swarms. Comparable HP abundances ($1.4 \cdot 10^6$ cells/mL) nearby the studied locations were reported around sewage (Paliaga et al., 2017) and industrial outfalls ($3.9 \cdot 10^6$ – $5.8 \cdot 10^6$ cells/mL) (Paliaga, 2015) and during mucilage events (summers of 1999–2002) when they coincided with higher DOC and more hydrophilic nature of SAS with NSA values around 0.05 (Dautović et al., 2017; Dautović, 2019; Ciglenečki et al., 2020). Microbial abundances (cells/mL) during mucilage events averaged to $\sim 1.1 \cdot 10^6$ (from $3 \cdot 10^5$ to $3.8 \cdot 10^6$) for HP, $\sim 3.5 \cdot 10^4$ (from 10^3 to $16.6 \cdot 10^4$) for CB, and to $\sim 2.4 \cdot 10^3$ (from 10^2 to $24 \cdot 10^3$) for HNF (Fuks et al., 2005). In general, CB develop faster with increased eutrophication and due to the shifts in the N:P ratio affecting the ecosystem's equilibrium (Stal et al., 2003). It seems that *M. leidy*'s effects on picoplankton resemble the effects of anthropogenic organic pollution, phytoplankton blooms or mucilage events.

Ctenophores (and gelatinous organisms) release OM in the surrounding waters in different ways. One way of releasing DOC to the surrounding waters is through excretion and mucus production. In Condon et al. (2010), the estimate for *M. leidy* is given between 0.02 and 8.86 mmol DOC (i.e. 0.24–106.4 µg C) per ind./h and increases at higher temperatures. By applying the expected higher excretion rates due to higher sea temperatures present in our research and considering an average residence time of *M. leidy* of 48 h (as it was observed on the field) to the average abundances found in here analysed swarms, we calculated that our swarms could release between 0.026 and 0.301 mg/L of DOC. This input could only partially explain the *in situ* differences between the swarms and the surrounding waters with no Ctenophora. Within every swarm, we observed damaged and dying individuals decomposing relatively quickly. Thus, it is reasonable to assume that they have been another significant source of DOC as it is the case in other gelatinous species such as *A. aurita*, where rates of release of TOC can be by order of magnitude greater than those produced by living medusa (Hansson and Norrman, 1995; Pitt et al., 2009).

Only a part of the ingested material by *M. leidy* is assimilated and incorporated in their biomass, while the rest is egested as faeces. This OM highly contributes to the SAS pool of DOC (Croot et al., 2007) – potentially as a significant source of utilisable substrate for HP, which is found here to be more hydrophobic OM type, with NSA around 0.1 (Ćosović et al., 2000; Ciglenečki et al., 2018, 2020). These substances contain hydrophobic (e.g. fatty acid chains, aromatic rings, or hydrocarbons) and hydrophilic functional groups (e.g. NH₂, COOH, OH, or SH) that enable the accumulation of SAS at different marine interfaces (seawater boundaries with living cells, with dispersed and particulate matter and with the atmosphere). By adsorption and desorption processes, SAS influence oceanographic properties and the structure of the natural interfaces, moderating transfer processes between different phases. Aside from zooplankton grazing, bacterial activities also contribute to the SAS pool (Kurata et al., 2016). Long-term data from the northern Adriatic demonstrated an increase in SAS concentrations, especially in its dissolved fraction (Ciglenečki et al., 2020). In part, that could be explained by recently recurring gelatinous blooms – for instance, the SAS concentration (mg/L eq. T-X-100) at the surface along the Po River-Rv transect (1989–2017) ranged from 0.015 to 0.417 (average 0.116) for SAS F, and 0.015–1.22 for SAS NF (average 0.137), being the lowest at the easternmost point – Bagnole Island (Dautović, 2019; Ciglenečki et al., 2020). There, from 1989 to 2020 averaged concentrations (mg/L eq. T-X-100) were 0.097 (in the range

0.015–0.272) for SAS F and 0.105 (in the range 0.016–0.273) for SAS NF, being lower than reported in this study (0.131–0.360 SAS NF and 0.127–0.352 SAS F). The average DOC concentration in the surface waters for 1989–2016 was 1.2 mg/L in the same area.

The total released OM by, e.g. ctenophore decomposition, may support microbial production, whilst nutrients (N, P) may support algal production (Pitt et al., 2009). Indeed, *M. leidyi*'s abundance, microbial assemblages and organic material (DOC) < 0.45 µm, e.g. living and dead cells, faecal pellets (Kharbush et al., 2020) positively correlated, pointing to enhanced microbial loop activities. The dissolved C fraction was probably readily assimilated. Expectedly, the picoplankton bloom correlated with higher abundances of its predator (HNF). Another correlation (i.e. between CB and HP) pointed to a mutual stimulus of picoplankton groups, as shown for mucilage events (Fuks et al., 2005). Up to 35 ind./m³ a linear regression to microbial groups was noticed (Fig. S3). Higher values (100 ind./m³) probably indicate the upper limits of the microbial response. However, further examinations of high-density swarms should be performed to prove the latter.

From late spring to the end of autumn, populations of invasive ctenophores in the northern Adriatic display substantial fluctuations in their presence (from few days to a month) (Budiša et al., in press). At the same time, maximal concentrations of DOC (up to 3.2 mg/L) overlapped with the presence of *M. leidyi* (Ciglencčki et al., in press). Previous studies showed that gelatinous zooplankton is a small DOC source compared to exudates from primary producers (Hansson and Norrman, 1995; Condon et al., 2010, 2011) but can be significant in oligotrophic environments (Guy-Haim et al., 2020) such as the northern Adriatic (Dautović et al., 2017; Ciglencčki et al., 2020) where their local abundance can be very high (Olesen et al., 1994; Benović et al., 2000). In oligotrophic ecosystems, DOM released by jellyfish is enough to sustain a high bacterial production (Turk et al., 2008). By the end of the summer in the northern Adriatic, the water column displays a strong vertical stratification and depletion of nutrients and OM in the top layer. When other sources are scarce, labile and highly reactive OM (more hydrophobic SAS) and nutrients released by the ctenophore seems to boost the growth of picoplankton that is being preyed on by HNF. The seasonal presence of *M. leidyi* in the NE Adriatic is starting to affect many components of the pelagic system that might lead to deleterious environmental alterations requiring further attentive monitoring.

5. Conclusions

The present study provides insights unravelling the effects of high densities aggregations of gelatinous zooplankton on the organic matter (SAS, DOC and POC) and the pelagic ecosystem. Intensive blooms of *M. leidyi* accumulated in the coastal waters of Rovinj-Rovigno change the content and type of OM (with more hydrophobic SAS) in the surrounding water, which exceeded SAS and DOC long-term values and caused a trophic cascade on the microbial community. The ctenophore release dissolved, highly surface-active OM that tends to be absorbed in different surfaces or interfaces or degraded by microorganisms. Moreover, OM accumulates only in bays with reduced current flow and the available C fuels the local pico- and nanoplankton microbial loop. In the open sea area with intense current dynamics, no accumulation of ctenophores, OM and microorganisms were found.

ADCP measurements of coastal currents and model results indicate that a specific circulation system of eastward transports and pronounced ICCZ could advect *M. leidyi* from its hypothesized spring/summer nursery areas towards and along the coast, where their number can significantly increase. The eastward motions of *M. leidyi* could be initiated by vertical instability events, altering thermal fields and reflecting on the geostrophic component of currents.

The *M. leidyi* biomass in the Adriatic Sea is higher than any other gelatinous zooplankton so far in the basin, suggesting the need to follow its effects on the pelagic system. In addition, other long-term effects of the invasive *M. leidyi* on the whole ecosystem, the fisheries could be

studied by ecosystem modelling approaches. To the best of our knowledge, this study is the first that attempted to connect and correlate *M. leidyi* and surface-active DOC.

CRediT authorship contribution statement

Paolo Paliaga: Conceptualization, Methodology, Validation, Visualization, Project administration, Writing – review & editing, Investigation. **Andrea Budiša:** Conceptualization, Project administration, Formal analysis, Investigation, Data curation, Writing – original draft, Visualization, Methodology, Validation, Writing – review & editing, Investigation, Writing – original draft. **Jelena Dautović:** Investigation. **Tamara Djakovac:** Funding acquisition, Supervision, Resources, Data curation, Writing – original draft, Writing – review & editing. **Mathieu Andre Dutour-Sikirić:** Writing – review & editing, Methodology, Software, Validation, Formal analysis. **Hrvoje Mihanović:** Conceptualization, Visualization, Writing – review & editing, Writing – original draft, Methodology, Software, Validation, Formal analysis. **Nastjenska Supić:** Conceptualization, Funding acquisition, Supervision, Writing – review & editing, Visualization, Methodology, Software, Resources, Formal analysis, Validation, Data curation, Writing – original draft. **Igor Celić:** Methodology, Validation, Formal analysis, Writing – original draft. **Neven Iveša:** Writing – review & editing. **Mojira Bursić:** Writing – original draft, Writing – review & editing. **Ivan Balković:** Writing – original draft, Writing – review & editing. **Lara Jurković:** Writing – original draft, Writing – review & editing. **Irena Ciglencčki:** Conceptualization, Funding acquisition, Project administration, Methodology, Writing – review & editing, Writing – original draft, Validation, Resources, Data curation.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.ecss.2021.107459>.

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