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Distribution, diet and relationships of the invasive ctenophore *Mnemiopsis leidyi* with anchovies and zooplankton, in the northeastern Adriatic Sea

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Abstract

Blooms of invasive ctenophore *Mnemiopsis leidyi* can have massive consequences on fish stocks and marine food webs. The distribution, abundance and diet of this ctenophore were investigated in the northeastern (NE) Adriatic between 2016 and 2019. The abundance of *M. leidyi* was determined daily along the coast of Rovinj (Croatia), and its spatial distribution monitored by visual census from research vessels and by pelagic trawl during acoustic surveys in September of each year. Mesozooplankton samples were collected along the western coast of Istria by vertical tows from the bottom to the surface. Spatial distribution and abundance of anchovy (*Engraulis encrasicolus*) assemblages were determined by geo-referenced hydro-acoustic sampling using a scientific echosounder. Large swarms of *M. leidyi* covering several km^2 were regularly reported between July and November extending up to 25 NM from the western coast of the Istrian peninsula and reaching maximum offshore densities of 270 individuals per m^2. The abundance of anchovy in the areas where *M. leidyi* was present evidently decreased. The content of *M. leidyi*'s digestive tract and plankton samples consisted both mainly of cladocerans, copepods, pteropods, echinodermata and bivalvia larvae. Results indicate that *M. leidyi* may accumulate and increase its abundance in the stagnant and trophically rich areas of the northern Adriatic gyres, spreading to the surrounding larger areas with currents. We assume that *M. leidyi* presence correlates with a decrease in anchovy population due to competition for food (zooplankton). Our findings support the importance of implementing an international monitoring program throughout the Adriatic Sea and demonstrate the ability of current MEDIAS surveys to detect changes in the pelagic ecosystem throughout surveyed areas.

Keywords: *Mnemiopsis leidyi*; Adriatic Sea; zooplankton; *Engraulis encrasicolus*; acoustic survey.

Introduction

Swarms of gelatinous organisms are not an unusual phenomenon in the Mediterranean (Boero, 2013). However, over the past few decades, new invasive species such as the ctenophore *Mnemiopsis leidyi* (sea walnut) have appeared in many Mediterranean basins, raising concerns about the ecological impact they may have on those systems (Shiganova *et al.,* 2019a, b). In the Adriatic Sea, *M. leidyi* made its first appearance in 2005 (Shiganova & Malej, 2009), but it failed to establish a stable population. However, the situation changed since 2016, when *M. leidyi* started appearing in large swarms every year from early summer until early winter (Malej *et al.,* 2017). In the northern Adriatic, it exhibited high fecundity and appeared in a wide range of sizes indicating favourable conditions, e.g., sufficient food to reproduce successfully, grow and agglomerate in significant numbers (Malej *et al.,* 2017).

M. leidyi is a very successful and adaptable species, which was able to survive a voyage by ballast waters in the '80s from its native habitats – the Gulf of Mexico and the eastern coast of the USA, to the other side of the Atlantic Ocean, specifically to the Black Sea (Vinogradov *et al.,* 1989). From the Black Sea, it has spread to other coastal areas (e.g., Mediterranean, Caspian and Azov

Seas) (Shiganova, 1997; Shiganova *et al.,* 2019a) showing a wide range of tolerance to different temperatures and salinities (Costello *et al.,* 2012). *M. leidyi* arrived in the Adriatic most likely through ballast waters since its appearance was not recorded in the southern part of the basin. Moreover, the molecular analysis of *M. leidyi* individuals isolated from the Adriatic, based on the Cytochrome Oxidase, indicated relation to the ones from the Black Sea (A. Baričević, pers. comm.).

The presence of this invasive organism in the Adriatic could represent a potential threat considering it is a fast reproducing, voracious carnivore that preys mainly on zooplankton (Monteleone & Duguay, 1988; Purcell *et al.,* 2001; Costello *et al.,* 2012) and has a limited number of predators (Grosholz, 2002; Marambio *et al.,* 2013). *M. leidyi* has already disrupted various food webs affecting valuable pelagic, coastal and estuarine ecosystems (Grosholz, 2002; Marambio *et al.,* 2013), especially in the Black Sea (Shiganova, 1997), the Sea of Azov (Studenikina *et al.,* 1991) and the Caspian Sea (Ivanov *et al.,* 2000). In those areas *Mnemiopsis* blooms contributed to the collapse of local fisheries, depleting the available stock of zooplankton consumed by commercial fish such as anchovy (*Engraulis encrasicolus*) additionally feeding on fish eggs and larvae (Kideys *et al.,* 2005).

Anchovy's spawning season in the Adriatic Sea (April-October) (Morello & Arneri, 2009) unfortunately coincides with the appearance of ctenophore blooms. In the Adriatic, anchovies represent one of the most relevant fish stocks, and their populations over time have been subjected to many stressors such as climate change-driven shifts in biotic factors and overfishing (Grbec *et al.,* 2002). Specific autumn and winter conditions were invoked in the explanation of Adriatic anchovy stock changes (Santojanni *et al.,* 2006; Kraus & Supić, 2011; Kraus *et al.,* 2015). Moreover, the northern Adriatic is considered a vital nursery and foraging area for anchovy and sardine (*Sardina pilchardus*), which together account for about 40% of the total marine catch in the basin (Morello & Arneri, 2009).

We hypothesize that the presence of *M. leidyi* in the NE Adriatic has affected the native ecosystem, in particular, the components that can be preyed on or outcompeted by it. The main objective of this study is to investigate the relation between the abundance and the distributions of *M. leidyi* and those of zooplankton and anchovy from 2016 to 2019 in the NE Adriatic Sea.

Materials and Methods

Study area

The study area (Fig. 1) is defined as the eastern part of the northern Adriatic Sea that consists of Croatian territorial waters and the Ecological and Fisheries Protection

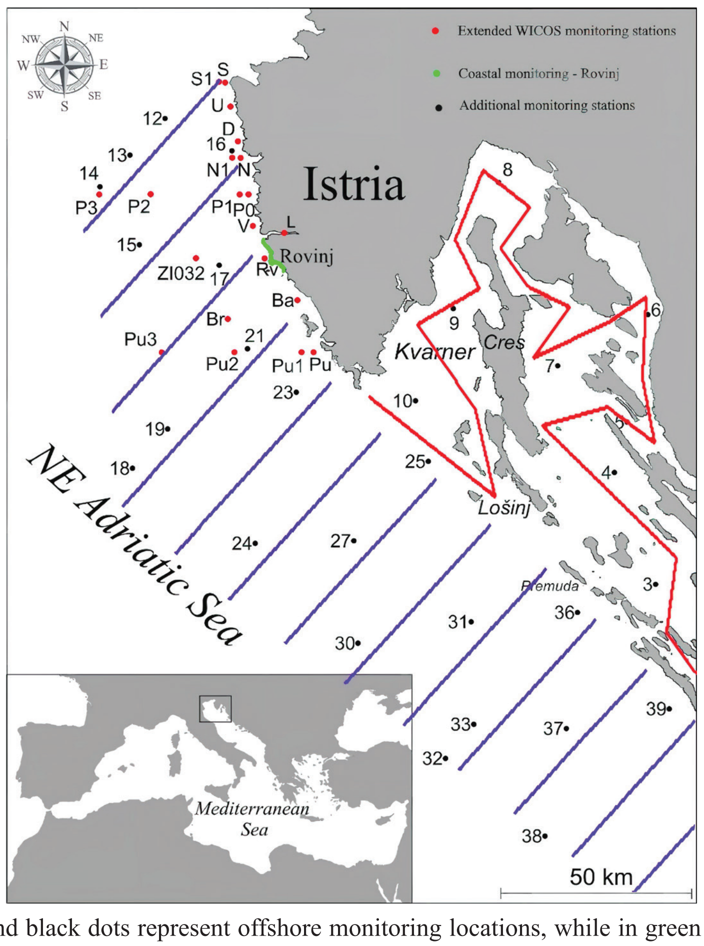

Fig. 1: The study area; red and black dots represent offshore monitoring locations, while in green stretch covers the location of the coastal monitoring; MEDIAS transects of acoustic sampling in September are marked by red lines for transects in the inner sea and blue lines for transects in the open Adriatic Sea.

Zone. Those areas have been monitored by acoustic surveys conducted in September each year (2015-2019) following MEDIAS working protocols (www.medias-project.eu). This part of the Adriatic extends to a bathymetry of approximately 80 m of depth to the south; and includes the western Istrian coastal waters and the Kvarner Bay.

Seawater temperature in the northern Adriatic shows pronounced seasonal cycles (Supić & Vilibić, 2006), fluctuating between around 10 °C (February) and 25 °C (July, August) while at the bottom temperature is around 10 °C (February) and 15 °C (in October).

The salinity seasonal cycle is highly pronounced in the surface layer only. The salinity of the lowermost layer is approximately 38 throughout the entire year, while the surface value changes depending on season and region, e.g., during winter, it is approximately 38; in the eastern part of the region, it is approximately 35; during June in the western part of the region, it is 32. The circulation in the northern Adriatic is part of the global Adriatic cyclonic gyre during the winter, while in spring, low-salinity waters start meandering toward the east. This anticyclonic meander reaches the Istrian coast in August and September (Krajcar, 2003). In general, the circulation is held to be "open" in the colder part of the year, allowing central Adriatic water to enter freely the northern Adriatic basin and "closed" in the warmer part of the year, implying that the northern Adriatic is separated from the rest of the basin with waters from the Po River spreading towards the east (e.g., Orlić *et al.,* 1992; Krajcar, 2003). However, there are winters in which waters from the Po River spread across the northern Adriatic (Supić *et al.,* 2012). The warm part of the year is generally characterised by the presence of gyres whose position and extent varies from year to year (Supić *et al.,* 2000, 2003; Orlić *et al.,* 2013; Djakovac *et al.,* 2013, 2015; Kuzmić *et al.,* 2007). An anticyclonic gyre, containing low salinity waters originating from the Po River, whose presence is indicated by the Istrian Coastal Countercurrent, frequently appears in the eastern part of the region, starting from early spring (or even earlier; the circumstances in which the gyre is formed were not yet investigated).

Mnemiopsis monitoring

Monitoring included the waters along the coast of Rovinj and a grid of 49 offshore stations along the western coast of Istria (large-scale monitoring) (Fig. 1).

The surveys were performed with the research vessels 'Vila Velebita', 'Burin', R/V 'BIOS DVA', and a private motorboat. The collected spatial distribution data were visualised using SURFER® 12 Golden Software.

In the coastal waters of Rovinj, the monitoring of *M. leidyi* was carried out from the summer of 2016 until the end of 2019 (Table 1). Ctenophora presence and abundance were surveyed daily along the coast (in a four-year period). Abundance was determined by visual census of individuals longer than 1 cm (the only observable *in situ* with a naked eye) and by scuba divers along transects parallel to the coast with the help of a cube frame of a known volume (cube dimensions: 1×1×1 m and 0.5×0.5×0.5 m) (Fig. S1) to facilitate the counting of the individuals. The average depth of the surveyed area along the coast was around 3 m. The coastal surveys were performed by determining the daily average number of ctenophores along 3 km of coastline.

The offshore monitoring was performed from the boat by visual census coupled with the WP2 plankton net which was towed (3 times) at the sea surface in stretches of 100 m for about 3 km and scuba divers. The densities of the individuals were counted per m^3 and expressed as the number of individuals per unit area (ind. m^{-2}) (Table S1, Supplementary Material).

The large-scale monitoring of the abundance and distribution of *M. leidyi* (49 stations), which covered a large portion of the northeastern (NE) Adriatic, was carried out only during September 2016, 2017, 2018 and 2019 respectively (Table 1). The stations along the western coast of Istria were disposed of two groups: the first one (20 stations) – the extended WICOS transect – included 14 stations (Pu, Pu1, Ba, Rv, V, P1, P_0, N, N1, D, U, S, S1, L) located at 1 NM and 2 NM from the coast and 6 stations (P2, P3, ZI032, Br, Pu2, Pu3) away from the coast (between 12 and 20 NM); the second group consisted of 29 additional stations covering the majority of the NE

Table 1. The spatial and temporal overview of sampling throughout the monitoring project, where environmental indices imply parameters describing hydrological conditions, and indicated locations correspond to those given in Figure 1.

Parameter		Sampling period	Location
M. leidyi	Abundance and distribution	September (2016-2019)	NE Adriatic (Extended WICOS and MEDIAS)
	Daily abundance	2016-2019	Rovinj coastal waters (3 km along the coast)
Zooplankton	Abundance and identification	August 2017-August 2018	WICOS and Rv station
Anchovy	Abundance and distribution	September (2015-2019)	NE Adriatic (MEDIAS)
Environmental indices	Temperature, salinity and density	September (2016-2019)	North Adriatic
	Seasonal temperature and seasonal salinity	2016-2019	Rv station

Adriatic (Fig. 1). *M. leidyi* presence and abundance were determined by visual census of individuals longer than 1 cm from the boat and by scuba divers.

The distribution of *M. leidyi* was plotted on the basis of the data collected on various days in September (1, 2, 6, 8, 28 and 29 in 2016; 4, 5, 6, 7, 13 and 21 in 2017; 10, 17, 21 and 29 in 2018, 7, 8, 9, 26, 27, 28 and 29 in 2019). Thus, the discrepancy in time intervals of sampling should be considered when analysing the data in regard to average means of current fields.

Hydrological parameters

Temperature and salinity were measured seasonally during the period 2016-2019 with multiparametric CTD (Sea Conductivity, Temperature, Depth) probe (SeaBird Electronic SBE 25) and an automated buoy system at the station Rv at 1 NM west of Rovinj (Fig. 1). The data were graphically displayed.

The atmosphere-ocean modelling system

The distribution of temperature, salinity and density in the northernmost part of the Adriatic was investigated in September of 2016, 2017, 2018 and 2019. Monthly fields of vertically averaged temperature, salinity and currents were obtained by the Regional Ocean Modelling System (ROMS) that was implemented over the Adriatic Sea at the spatial resolution of 2 km. Meteorological forcing was obtained from Weather Research and Forecasting (WRF) model run over the area at 8 km spatial resolution. Open boundary conditions at the Otranto strait were provided by the operational Adriatic REGional model (AREG) within the Adriatic Forecasting System (AFS). The climatological values for the Adriatic river run-offs were used. More details on the atmosphere-ocean modelling system can be found in Mihanović *et al.* (2018).

Zooplankton

Mesozooplankton was sampled monthly from August 2017 to August 2018 at the Rv station. At the other WICOS stations, sampling was carried out in August, September and October of 2017, and March, May and July of 2018 (Fig. 1, Table 1).

Samples were collected vertically from bottom to surface by WP2 plankton nets and preserved in 4% formaldehyde for microscopic inspection. Subsequently, the taxonomic level identification analysis was performed in a laboratory on a stereomicroscope with an 80x magnification. The results are shown as the number of individuals per cubic meter (ind. m^{-3}).

Mnemiopsis leidyi's *diet*

Samples for *M. leidyi*'s diet analysis were taken during 2017: in August, September and October at Rv station, and in September at stations Pu and P_0. In total, 155 individuals were examined. *M. leidyi* individuals were collected by a soft mesh landing net at a depth of 0-0.5 m and immediately placed with care in glass beakers with filtered seawater (0.22 µm pore size). Individuals were then exposed for 5 min to a range of stressful conditions such as vortexing the surrounding water and prodding them with tweezers to stimulate the excretion of their stomach contents, all performed in laboratory conditions. The expelled filamentous dark substance was collected with a pipette, transferred to a falcon tube (50 mL), and preserved by adding neutralized formaldehyde to a final concentration of 2%. Finally, samples were stored at 4 °C until further analysis of the diet composition by a stereomicroscope.

Based on the numbers of prey noted in September 2017 at the stations Rv, Pu and P_0 after analyses of 95 individuals, the Similarity Percentage (SIMPER) method was used to determine which taxa contribute to the similarity of prey. For the above-mentioned multivariate analyses, the statistical package PRIMER v6 was used.

Anchovy abundance and distribution

Spatial distribution and abundance of anchovy assemblages within the study area have been determined by geo-referenced hydro-acoustic sampling (Dadić *et al.,* 2008) performed with the scientific echosounder SIMRAD EK60 operating at 38 kHz and using a split-beam transducer with 7° beam angle. The acoustic sampling equipment was installed on R/V 'BIOS DVA', and acoustic data were collected during the annual acoustic survey MEDIAS in September from 2015 to 2019 (Table 1), following common MEDIAS working protocol (MEDIAS, 2015). Nautical Area Scattering Coefficient (NASC, m^2/NM^2) data related to anchovy have been used as direct unbiased estimates of anchovy abundance. In addition to hydro-acoustic sampling performed by the echosounder SIMRAD EK60, a mid-water trawl with otter boards and small mesh size in the cod-end have been used in order to obtain information on assemblages of various pelagic organisms and characteristics (i.e. size) of different acoustic targets identified by hydro-acoustic sampling. Mid-water trawl samples were collected at trawling speed of 4 knots, with trawling duration of 30 min (i.e. along sampling trails of 2 NM).

Results

Mnemiopsis leidyi *abundance along the coast of Rovinj and the relation to thermohaline conditions at the Rv station (2016-2019)*

Daily average *M. leidyi* abundances along the coast of Rovinj are given in Figure 2, while the monthly averages are given in Figure S2 (Supplementary Material).

M. leidyi was recorded and identified for the first

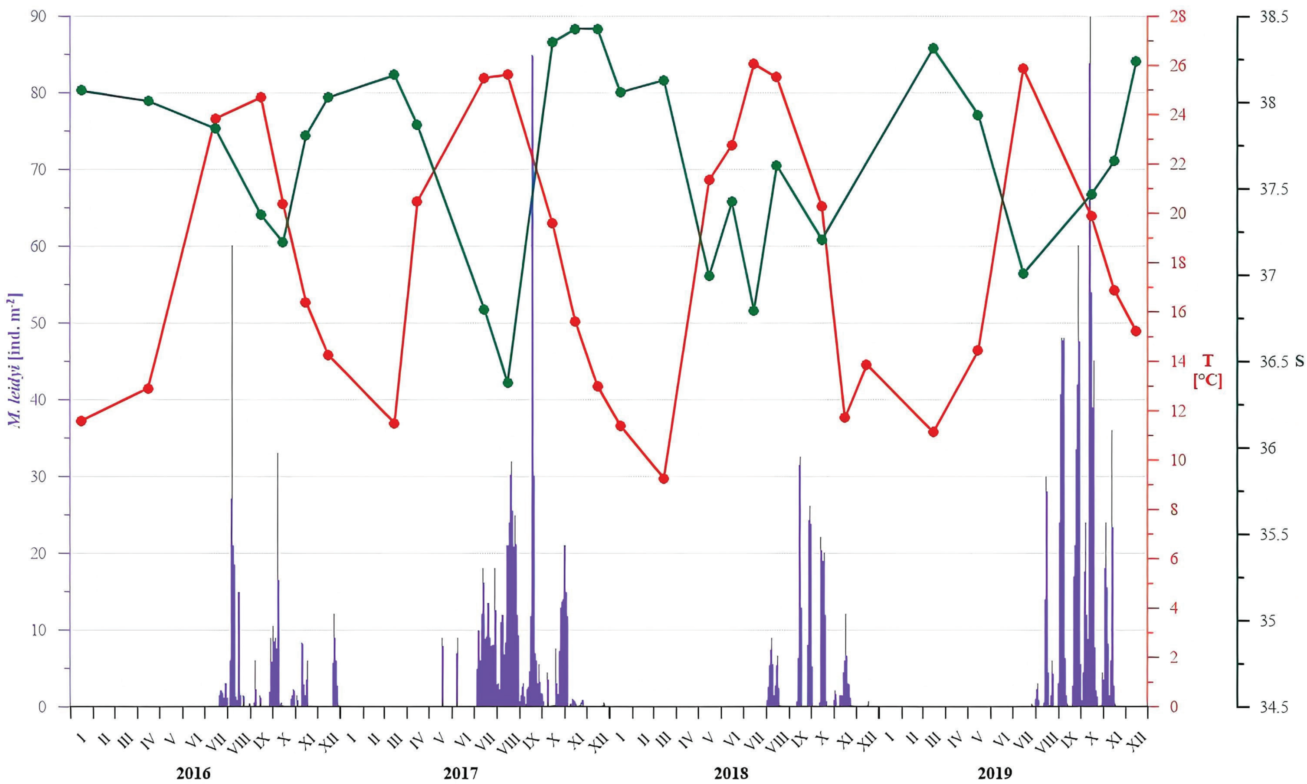

Fig. 2: Daily average abundance of *M. leidyi* (ind. m^{-2}) along the coast of Rovinj in the period from the summer of 2016 until the end of 2019 regarding the monthly sea surface temperatures (T) and salinities (S) at Rv station in the same period.

time along the coast of Rovinj at the end of July 2016 in moderate amounts (~1.5 ind. m^{-2}) (Fig.2). In the first half of August 2016, *M. leidyi* proliferated, reaching the highest average daily abundance of that year 60±45 ind. m^{-2}. This was also the month with the highest average abundance of *M. leidyi* in 2016 (5±12 ind. m^{-2}) (Fig. S2). Higher daily averages were registered again in the first half of October, reaching 33±30.9 ind. m^{-2}. After that, two minor swarming events were recorded, at the beginning of November, reaching 8.4±7.8 ind. m^{-2}, and at the end of December with 12±7.5 ind. m^{-2}.

M. leidyi was not present in the waters of Rovinj in the first three months of 2017. From the end of March until the beginning of July, records were infrequent. In contrast, from July to October 2017, individuals were present in considerable numbers almost continuously. Three distinct episodes with a significant increase in average daily abundances can be identified in Figure 2, i.e., mid-July (18±8.4 ind. m^{-2}), mid/end August (32.1±12 ind. m^{-2}), and mid/end September (84.9±60.6 ind. m^{-2}). The highest average monthly abundance in 2017 was measured in July (6.8±5.4 ind. m^{-2}) (Fig. S2). In August and September 2017, some parts of swarms confined within bays and harbours counted almost 900 ind. m^{-2} (Center for Marine Research database). During autumn, abundances were similar to those measured in July, but later, in December, records were scarce (Fig. 2).

In 2018, *M. leidyi* first appeared at the beginning of August (maximum daily average abundance 9±3 ind. m^{-2}), abundances remained low until the middle of September. Only a few episodes of relatively high daily average abundances were registered that year, i.e., 32.4±27.6 ind. m^{-2} and 26.1±22.5 ind. m^{-2} in September, 21.9±11.4 ind. m^{-2} in October and 12±4.3 ind. m^{-2} in November. Throughout December, abundances ranged between $3 \cdot 10^{-3}$ ind. m^{-2} and 0.6 ind. m^{-2} (Fig. 2).

In 2019, *M. leidyi* was first recorded at the end of July. In August, *M. leidyi* reached the maximum abundance of 30±25.8 ind. m^{-2}. In September and October, abundances increased, reaching the highest peak of the year in October (90±75.9 ind. m^{-2}) (Fig. 2). In the enclosed parts of bays and harbours, *M. leidyi* displayed the absolute highest abundances for the entire monitoring period (exceeding 1200 ind. m^{-2}) (Center for Marine Research database). In mid-November, the average daily abundance of *M. leidyi* reached up to 24±20.7 ind. m^{-2}, while in December, only a few individuals were detected (Fig. 2).

The data gathered over four years revealed that *M. leidyi* was present for the longest amount of time in 2017, counting 141 days in total. Populations were observed for a total of 79 days in 2016, 115 days in 2018, and 109 days in 2019. During 2018, the average daily abundances exhibited by *M. leidyi* were lower than those in other years (Fig. 2). The average monthly abundances in 2016 and 2018 never exceeded 6 ind. m^{-2}, while in 2017 and 2019 the values recorded were more than double (Fig. S2).

The oceanographic data gathered at the Rv station (1 NM west from Rovinj) allowed for a comparison of abundances of *M. leidyi* found in the coastal waters around Rovinj against sea surface temperature and salinity as shown in Figure 2. The sea temperatures follow seasonal changes which correlate with the air temperatures; whereas, salinity of the NE Adriatic is higher in the winter and generally lower in the summer/autumn due to the Po River inputs and the prevailing currents.

In July 2016, when *M. leidyi* first appeared, sea surface temperatures were above 23 °C, and surface salinities began to decline seasonally from 37.9 in July to a minimum (37.2) in October. The average monthly abundance of *M. leidyi* was the highest in August (5±12 ind. m^{-2}) when temperatures were above 24 °C (Figs. 2 & S2). In the months following, the average abundances declined until December while the sea surface temperatures were below 15 °C and salinities rose back to 38.

In 2017 salinity was relatively high in March (38.1), then decreasing in April (37.9) and May when the temperature was around 20 °C. The highest average monthly abundances that year were recorded in August and coincided with the warmest sea surface temperature (25.5 °C) and the lowest salinity (36.4) (Fig. 2). *M. leidyi* completely disappeared in December when the sea had cooled to 14.3 °C (Fig. 2).

In 2018, salinity was high in March (38.1) and decreased towards the summer. *M. leidyi* appeared later that year (in August) when the sea surface temperature was above 25 °C. The maximum monthly average was recorded in September when salinity was around 37.3, and temperatures had already descended to 20 °C (Figs. 2 & S2). In November and December, temperatures oscillated between 11 and 14 °C and *M. leidyi* was not recorded.

In March 2019, salinity was 38.3 and decreased to a minimum of 37.1 in July. The sea surface warmed somewhat later, reaching the maximum in July (25.8 °C) when *M. leidyi* was first detected. Even higher monthly averages were recorded in September and October when the sea temperatures were ~20 °C, and salinity increased to 37.5. In November, temperatures were relatively high (16.9 °C), salinity continued to increase (37.7), and *M. leidyi* remained relatively abundant (3.2±10.7 ind. m^{-2}) (Figs. 2 & S2). In December, when salinity reached 38.2 and the temperature was 15.2 °C, *M. leidyi* were not found (Fig. 2).

Abundance and distribution of **Mnemiopsis leidyi** *in September (2016-2019) in the NE Adriatic*

Data presented from daily observations of the coastal waters of Rovinj and the monthly monitoring of the NE Adriatic during 2016-2019 (Center for Marine Research, Rovinj) showed that abundance of *M. leidyi* reached the peak of its expansion from the end of August until the end of October consecutively. In September 2016, *M. leidyi* was detected in a large area extending from the coast of the Istrian peninsula towards the west (Fig. 3A). The bloom was characterised by an almost continuous presence of *M. leidyi* with an average abundance of 0.3±0.8 ind. m^{-2}. Individuals were almost completely concentrated around the sea surface. The highest abundances were detected in the vicinity of the Istrian coast (P_0 and P1, 2-4 ind. m^{-2}). In the western Kvarner Bay, only a few individuals were found.

Extending in a wide stripe from west to south of Istria, *M. leidyi* blooms reached their widest spatial extension in 2017 (Fig. 3B) with an average number of individuals (14.5± 45.8 ind. m^{-2}) which were considerably higher (~50 times) than the previous year. In 2017, numerous *M. leidyi* were registered all the way to the thermocline (15 m) at Rv, P_0, and ZI032, with high abundances (99, 133 and 225 ind. m^{-2}, respectively). The area occupied by *M. leidyi* from the south to the east was considerably larger than previously seen, reaching the western coasts of the Kvarner islands for the first time.

In the 3rd year of *M. leidyi* invasion (2018), the bloom occupied a smaller area along the western Istrian coast compared to those measured in 2017 (Fig. 3C). *M. leidyi* was once again present only significantly in surface waters, and their average abundance (0.8±1.3 ind. m^{-2}) was a bit higher (2.6 times) than in 2016 but still much lower (~18 times) than the average in 2017. The highest concentrations were detected closer to the coast, with the maximum at Rv (~6 ind. m^{-2}) and N (~3 ind. m^{-2}). In 2018 no *individuals* were observed in Kvarner Bay.

During September 2019, we recorded the maximum average swarm abundance (27.7±65.8 ind. m^{-2}). *M. leidyi* was concentrated in the south-western Istrian waters with maxima at Pu, Pu1, Ba, Rv, and 23 (270, 195, 90, 105 and 90 ind. m^{-2} respectively) (Fig. 3D), where *M. leidyi was homogeneously distributed up to 15 m of depth. At greater depths, no individuals were recorded.* Along the eastern Istrian coast and the Kvarner Bay, no individuals were detected. However, a few individuals were recorded at 31 and 36, i.e. around the islands Premuda and Škarda, where *M. leidyi* had never been reported before (Fig. 3).

Numerical hydrodynamic model

The distribution of temperature, salinity and density indicates that the northernmost part of the Adriatic was affected by warmer, less salty and lower water density of the Po River origin (Figs. 4 & S3) in all the four investigated periods (September of 2016, 2017, 2018 and 2019). Circulation, represented by monthly means of vertically averaged currents, developed in gyres of various extent. An anticyclonic gyre containing Po affected waters of lower salinity was observed in all four years. The gyre was located north to the section Po delta-Rovinj in 2016, across the same line in 2017, south to it in 2018 and between lines Po delta-Rovinj and Pesaro-tip of Istria in 2019. In 2016, 2017, and 2018 salinity in the gyre region was below 38.1 and in 2019, over that value. In 2019 salinity of northern Adriatic was affected by very high salinity waters from the south, as indicated by the position of the 38.5 isolines, found to be more north than in other investigated years, stretching across the Pesaro-tip-of Istria line. Mixing of these very high salinity waters with freshened Po waters resulted in the water of higher salinity than in other investigated years. Model representation of high salinity values in 2019 is supported by observations performed in 2019 near the tip of Istria (38.5 in summer and 38.9 in autumn; N. Supić, pers. comm.). According to transversal currents along the Pesaro-tip-of- Istria line, which was pronounced in 2016, 2018 and

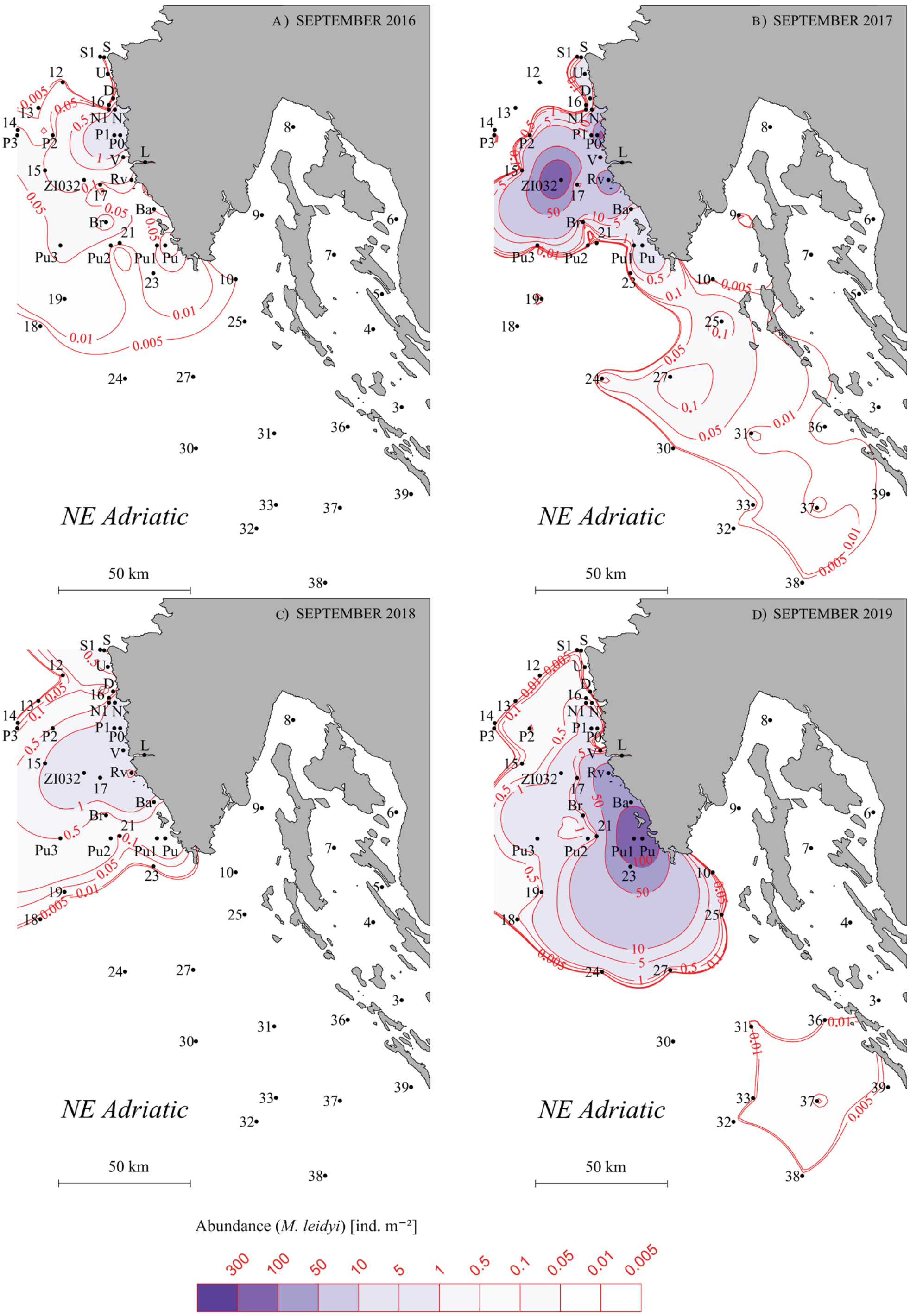

Fig. 3: Distribution and abundance of *M. leidyi* (ind. m^{-2}) in the NE Adriatic in Septembers 2016 (A), 2017 (B), 2018 (C) and 2019 (D); labels of stations are in black, while red numbers and lines refer to isolines of abundances.

2019, and not in 2017, the northern Adriatic was in these three years more isolated from the rest of the Adriatic than in 2017.

Mnemiopsis leidyi's *gut contents*

Food was found in 95 of the 155 ctenophores analysed. The amount of prey that was found in the digestive tract was relatively small, averaging between 1.2 and 2.5 zooplankton individuals per ctenophore. A total of 39 zooplankton taxa ranging from protists to fish eggs have been found in the digestive tract of *M. leidyi* (Table 2).

Annual distribution of zooplankton abundances at Rv station (Fig. S4) and along the west coast of Istria (Fig. S5) are given in the Supplementary Material.

At the Rv station, dominant prey taxa were bivalvia larvae, copepod *Temora stylifera* and cladocera *Penilia avirostris*. High contributions were annotated for calanoid copepodites, copepods (*Oithona plumifera* and *Clausocalanus furcatus*), and pteropods (*Limacina trochiformis* and *Creseis clava*), too.

At the Pu station, cladocerans, copepods, pteropods, and bivalvia larvae made up the majority of the prey. The

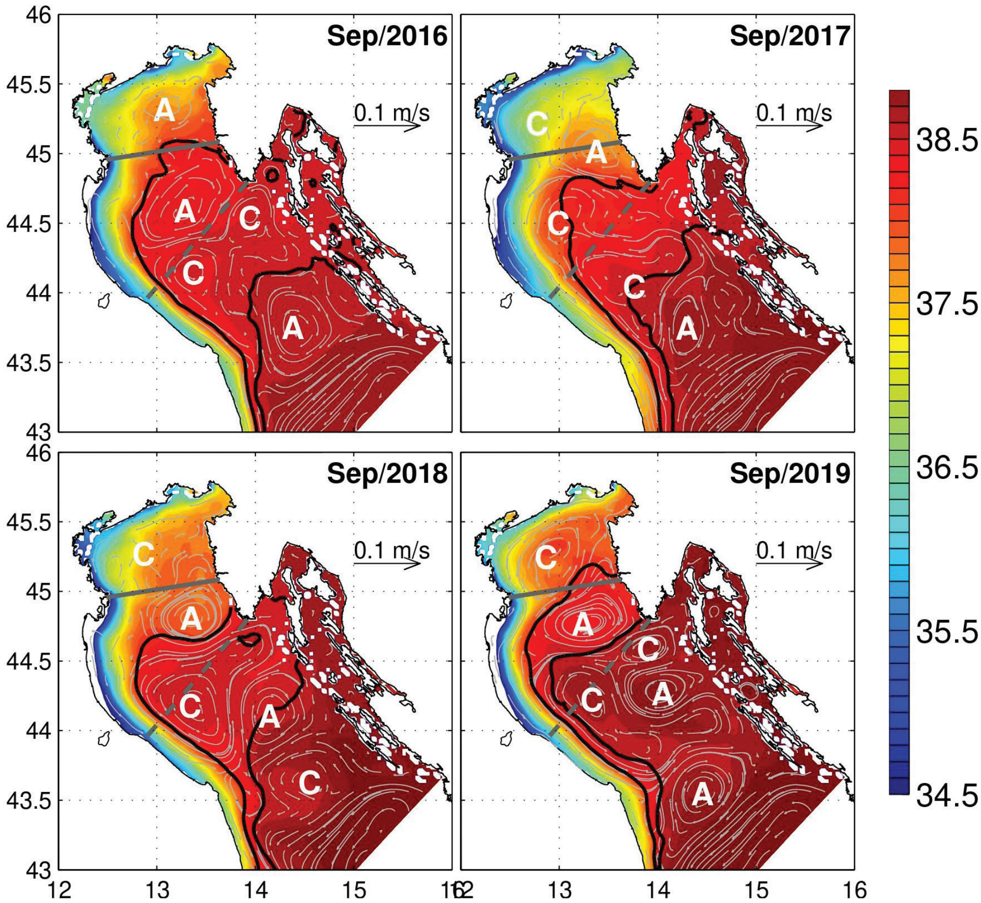

Fig. 4: Vertically averaged fields of salinity and currents in September 2016-2019, obtained by the numerical hydrodynamical model. The solid line is drawn between the Po River delta and Rovinj and dashed line between Pesaro (Italy) and the tip of Istria (Cape Kamenjak). Cyclonic gyres are marked with 'C' and anticyclonic with 'A'.

most dominant prey was the cladocera *Penilia avirostris*, followed by the copepod *Temora stylifera*. Additionally, the high contributions of other taxa included both *Evadne spinifera* and *Creseis virgula*. At the P_0 station, the dominant prey was the pteropod *Creseis clava* and bivalvia larvae.

A comparison in terms of percentages between prey specimens and abundances of zooplankton from net samples revealed noticeable differences (Table 2). Specifically, abundances of small copepods *Paracalanus parvus parvus* and *Oncaea* spp. were higher in net samples than in ctenophores gut contents. On the other hand, large copepods such as *Temora stylifera*, *Oithona plumifera* and *Clausocalanus furcatus* were dominant in the ctenophores' guts. This fact is especially relevant for pteropods which can reach a relatively large size of up to 4 mm. Moreover, prey analysis indicated that bivalve larvae prevailed in the gut contents but not in net samples. However, a similar relation was found for copepodites and cladocerans (*Evadne spinifera* and *Pseudevadne tergestina*). Based on SIMPER analysis of all zooplankton data obtained for September 2017 (n=95), ten taxa accounted for about 90% of *M. leidyi*'s prey (Table 3). Except for cladocerans, the majority of the other taxa were found to be more abundant in the gut contents rather than in net samples.

Distribution and abundance of anchovy from 2015-2019

Geo-referenced and log-transformed NASC data (Log values ranged 0-4) used as an unbiased proxy for abundance data, as shown in Figure 5 showed different patterns of anchovy distribution and abundance within the study area from 2015 to 2019.

Anchovy abundance data, collected during September 2015 acoustic survey, depicted a population whose spatial distribution showed a typical patchy pattern and occupied all parts of the study area (Fig. 5A). The following year (2016), acoustic recordings showed the absence of anchovy in the proximity of the western Istrian coast, which suggests no or very limited abundance of fish (Fig. 5B). Anchovy population in the rest of the studied area showed a patchy distribution with good coverage. A drastic change in its spatial distribution was noticed in data collected during September 2017. That year, anchovies were almost entirely absent from a large portion of the studied area (Fig. 5C). Anchovies were mostly present in the area furthest from the Istrian coast, along the mid-line, in the inner waters and further south. Data collected in September 2018 indicated a similar situation to that of 2017. Anchovy schools were again scarce in a large portion of the studied area (Fig. 5D), while the anchovies recorded alongside the mid-line of the Adriatic Sea in

Table 2. A comparison of zooplankton taxa (%) observed in *M. leidyi*'s gut contents and in net samples collected at the same time at the Rv (65 ctenophore analysed of which 27 with food), Pu (45 ctenophore analysed of which 15 with food) and P_0 (45 ctenophore analysed of which 18 with food) in September 2017. The prey is represented by the average values of the individuals found as a gut content of analysed specimens, while the "sample" as the average value of zooplankton for the water column, while '+' indicates the presence of a particular zooplankton when its share was under 1%.

	Rv prey	Rv sample	Pu prey	Pu sample	P_0 prey	P_0 sample
Prostista						
Tintinnina					1	
Foraminifera			+			
Radiolaria			1			
Hydrozoa						
Podocorynoides minima			+			
Clytia spp.			1		1	
Solmaris spp.			+			+
Muggiaea atlantica	+	+				
Ctenophora						
Cydipid larvae			+	+	+	
Cladocera						
Penilia avirostris	15	47	44	75	6	37
Evadne spinifera		4	8	2	6	+
Pseudevadne tergestina	5	2	3	2	7	1
Copepoda						
Nauplii			1	+	1	+
Copepodites	7	8	7	2	10	7
Calanus helgolandicus	+	+	+		1	+
Nannocalanus minor					+	
Paracalanus parvus	5	13	1	7	1	3
Clausocalanus arcuicornis			+	+	1	3
Clausocalanus furcatus	6	3	1	+	2	2
Pseudocalanus elongatus			1	+	1	1
Centopages kröyeri	1	+	+	+		
Temora stylifera	15	4	11	1	9	6
Acartia (Acartiura) clausi	3	+		+	+	
Oithona nana				+	1	
Oithona plumifera	7	2	1	1	1	1
Euterpina acutifrons			+	+		
Oncaeidae			1	3	4	8
Corycaeidae			1	+	+	
Pteropoda						
Limacina trochiformis	5	1				
Creseis clava	5	+	2		17	1
Creseis virgula			8	1	2	3
Appendicularia						
Oikopleura (Coecaria) longicauda	4	7	+	1	3	7
Oikopleura (Coecaria) fusiformis		+	+	2		
Chaetognatha						
Sagitta inflata			+	+		
Chaetognatha juv.		5	1	1		3
Other larvae						
Bivalvia larvae	21	+	4	1	22	16
Gastropoda larvae		+	+	+	+	
Polychaeta larvae	+	+	+	+		+
Decapoda larvae	+	1	+	+	1	+
Ophiopluteus larvae		2	2	+	1	
Fish eggs		+			+	

Table 3. Similarity of percentages (SIMPER, n=95) analysis shows species that account for the most similarity of *M. leidyi* prey between individuals collected along Istria coast during September 2017 (stations Rv, Pu and P_0).

Average similarity: 50.21	*Average abundance*	*Average similarity*	*Sim/SD*	*Share (%)*	*Cumulative (%)*
Penilia avirostris	19.89	10.01	0.86	25.01	48.58
Temora stylifera	6.72	6.12	1.43	15.31	40.32
Bivalvia larvae	6.39	5.16	0.98	12.90	53.22
Calanoida copepodites	5.06	4.90	1.40	12.25	65.47
Creseis clava	4.06	2.93	0.68	7.33	72.80
Pseudevadne tergestina	3.28	2.45	1.06	6.11	78.91
Evadne spinifera	4.06	1.96	0.87	4.90	83.18
Oikopleura (Coecaria) longicauda	1.17	0.95	0.72	2.38	86.18
Oncaeidae	1.61	0.94	0.66	2.36	88.54

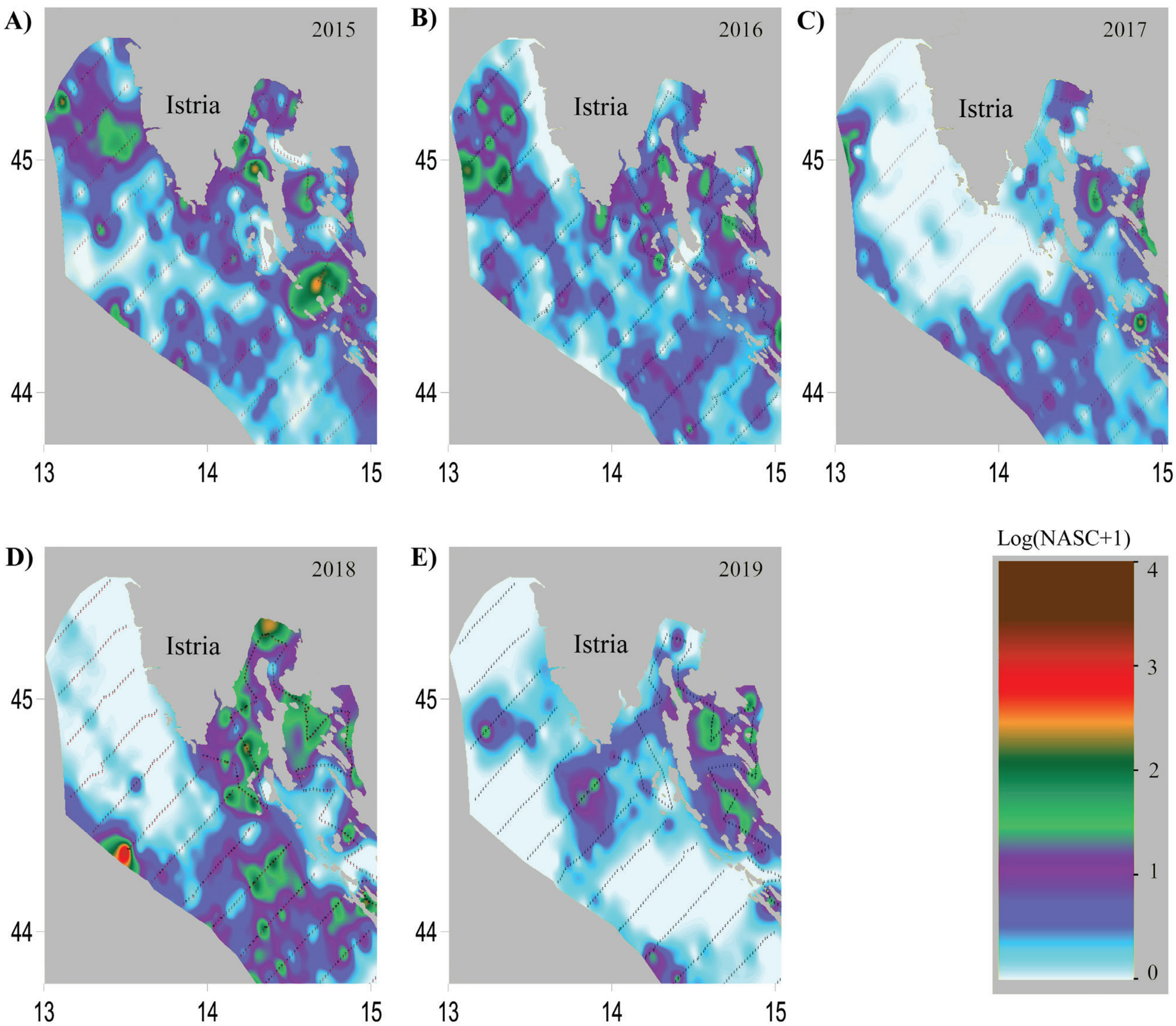

Fig. 5: Distribution and abundance of anchovy (*E. encrasicolus*) within the area covered by the acoustic survey in the NE Adriatic Sea in Septembers of following years: A) 2015, B) 2016, C) 2017, D) 2018 and E) 2019.

2018 were registered further south. The density of the anchovy in the inner waters, as well as the southern part of the studied area, was noticeably higher than the previous year. The most recent acoustic survey data collected in September 2019 showed a similar situation as in the period 2017-2018, indicating a low abundance of anchovy in the area close to western Istria shore. Some anchovy's schools were recorded in the open waters, distributed in several patches scattered throughout the northern Adriatic (Fig. 5E). The most northern part of the study area, zone along the mid-line, as well as areas further south, also showed very low anchovy abundance.

Discussion

Even though *M. leidyi* was first spotted in the northern Adriatic (Gulf of Trieste) in 2005 (Shiganova & Malej, 2009), no individuals were observed in the following decade until 2016. The introduction of *M. leidyi* into the northern Adriatic most probably occurred by ballast waters, because in the southern and middle part the basin, this species has not been observed, except for the narrower part of the port of Ploče (south-eastern Adriatic) where individuals were found in September 2017 (D. Lučić, pers. comm.) and along the Apulian Coast (south-western Adriatic) where it was reported in 2019 (Dragičević *et al.,* 2019). Temporal trends collected over the four-year-long monitoring (2016-2019) showed that in the NE Adriatic *M. leidyi* tends to appear in higher numbers during summer and autumn. Conversely, in winter and spring, there were only scarce sightings of individuals or even no records at all. In the southern part of European waters, *M. leidyi* was found in a wide range of temperatures (3-30 °C) (Shiganova *et al.,* 2019a, b), while in the northern Adriatic, this species was observed in offshore, coastal, and lagoon systems at temperature ranging from 13 °C to 29 °C and salinity from 11 and 38 (Malej *et al.,* 2017). In the NE Adriatic (2016-2019), *M. leidyi* was present in the temperature range from 14.3-25.9 °C, while the highest abundances were detected at temperatures above 19 °C. This fits within the range of favourable temperatures for *M. leidyi*'s reproduction and population growth (12-27 °C), as previously reported (Shiganova *et al.,* 2019b). In 2017 when there was a warmer spring, ctenophores appeared earlier, while in 2019, when the spring was colder and the autumn warmer, the presence and the peak abundances were shifted to later in the year. The salinity range in which *M. leidyi* can successfully grow and reproduce is rather broad, ranging from 6 to 40 (Shiganova *et al.,* 2019b). In that regard, salinities found in the NE Adriatic are within that range and show minor seasonal oscillations. Our results are consistent with existing knowledge of the Mediterranean Sea, where *M. leidyi* abundance may be limited by low temperatures (Shiganova *et al.,* 2019b), while low salinity may be a limiting factor in the North Sea and the Baltic (Jaspers *et al.,* 2011).

The spatial distribution of *M. leidyi* during their maximum expansion in September (2016-2019) indicated that they were present on a large spatial scale. The area included mainly western Istrian coastal waters, from the shore to 25 NM offshore. Previously, large masses of *M. leidyi* have occurred in ecosystems that underwent extensive modifications due to overfishing and excess nutrient loading (Bodini et al., 2018). According to Shiganova et al. (2019a, b), global warming and other environmental disturbances in the marine environment facilitate the establishment of large *M. leidyi* populations. For example, large masses of Aurelia sp. disrupted relationships in the food web in the Black Sea and facilitated later the development of stable comb jelly populations (Shiganova et al., 2019a). The northern Adriatic, as a commercially important basin with a strong fishing effort (Bastardie et al., 2017) and intensive maritime traffic (Zupančič et al., 2015), in recent decades experienced ecological crises such as eutrophication (Degobbis et al., 2000; Giani et al., 2012; Marić et al., 2012) and a frequent mass occurrence of jellyfish (Kogovšek et al., 2010).

Both the maximum and the monthly average in the coastal waters of Rovinj (15.9±19.2 ind. m^{-2}) and the spatial average in the NE Adriatic in September (2016-2019) (10.8±13 ind. m^{-2}) were moderately higher than the values measured throughout four-years of monitoring in the NW Baltic (mean monthly values ranging from 0-4.3 ind. m^{-2} and maxima, generally oscillating between 10 and 20 ind. m^{-2}) (Schaber *et al.,* 2011). The lower averages of *M. leidyi* in the NE Adriatic during 2016 and 2018 were relatively close to those measured in the Dardanelli straight in September of 1998 (2.5 ind. m^{-2}) and the NE Aegean in June (1998) (1.5-2.5 ind. m^{-2}) (Shiganova *et al.,* 2001) while the higher ones (2017 and 2019) were comparable to the data registered in 1990 in the Saronikos Gulf in the W Aegean (45-75 ind. m^{-2}) (Shiganova *et al.,* 2001) and in a high-density patch (5-100 ind. m^{-2}) in the coastal waters of Denia (Israel) in July 2009 (Fuentes *et al.,* 2010). The distribution of *M. leidyi* in the NE Adriatic was not homogeneous and was characterised by high-density patches that could surpass even 100 ind. m^{-2}, mainly located between 1 and 10 NM off the western and southern coast of Istria. This increased abundance of *M. leidyi* in coastal waters was probably associated with favourably higher nutrient concentrations, greater density of phytoplankton, chlorophyll *a* concentration, and lastly, a greater zooplankton availability in accordance with previous observations in the Black Sea (Kamburska *et al.,* 2006; Shiganova *et al.,* 2019b).

High-density *M. leidyi* agglomerates in coastal areas were surrounded by large low-density areas where the abundances were comparable to the ones registered in oligotrophic seas such as the NW Mediterranean (Offshore Ligurian, Tirrenian and Sardinian waters) where scattered individuals were detected during the summer (Boero *et al.,* 2009; Diciotti *et al.,* 2016).

Furthermore, unlike in the Baltic Sea where *M. leidyi* appeared up to 60 m depth (Schaber *et al.,* 2011), in the NE Adriatic, this ctenophore was concentrated mainly along the sea surface with only a few findings during seasonal maxima, when it reached the thermocline at about 15 m of depth. Our findings are more in line with those published by Costello & Mianzan (2003) that have reported large clusters within a meter from the sea surface. Nonetheless, it has been reported that dense agglomerates of ctenophores can move through the water column from the top, just 0.5 m above the seafloor in shallow waters (<5 m depth) to depths of 70 m, within a single day (Costello & Mianzan, 2003).

The average *M. leidyi* densities in the NE Adriatic were much lower than the ones measured in the shelf area of the NE Black Sea, ranging from 4-11 ind. m^{-3}, or. 120-330 ind. m^{-2} respectively (given the average depth of 30 m), and those measured offshore in the Black Sea of 5-13 ind. m^{-3}, i.e., 150-390 ind. m^{-2} (Kamburska *et al.,* 2006). Only the highest peak abundances reported in the NE Adriatic were comparable to the NE shelf and offshore Black Sea averages.

The differences were even more pronounced between the NE Adriatic and the eutrophic shelf waters of the NW and W Black Sea (average abundances about 62 and 55 ind. m^{-3}, or about 1860 and 1650 ind. m^{-2} respectively) (Kamburska *et al.,* 2006). Similar results were also reported for the Caspian Sea, where the average abundances of *M. leidyi* were about 63 ind. m^{-3} (~1260 ind. m^{-2}), concentrated mostly in the top 20 m from the sea surface (Rostamian *et al.,* 2012).

However, some of the highest *M. leidyi* abundances reported indicating that the upper abundances in a system might reach up to 10^4 ind. m^{-2} surpassing the highest NE Adriatic abundances by two orders of magnitude. Such values were reported in 2001, in the central and N Caspian Sea in August and October (9103±5690 ind. m^{-2} and 5375±2145 ind. m^{-2}) (Shiganova *et al.,* 2004a) and during the most significant Black Sea outburst in the October of 1989 (~8000 ind. m^{-2}) (Shiganova, 1998).

Also, in the areas of the Black Sea where the natural predator of *M. leidyi – Beroe ovata* was present, the abundances (59.5±50 ind. m^{-2} inshore and 197±170 ind. m^{-2} offshore) (Shiganova *et al.,* 2004b) were well above the values found in the NE Adriatic. From the comparison with the most *M. leidyi*-impacted basins (Black and Caspian Sea), it emerges that the presence of this invasive Ctenophora in the NE Adriatic is still smaller by one-two orders of magnitude but perhaps not yet reached its full potential. In the Black Sea, *M. leidyi* was first observed at the beginning of the 1980s (Vinogradov & Tumantseva, 1993). It took several years after its initial accidental introduction to reach the extreme outbreaks of 1988-89. So far, the data on *M. leidyi* abundance and distribution in the NE Adriatic indicate that the changes between consecutive years can be substantial. There seems to be a trend of increasing *M. leidyi* overall abundance as well as affected areas becoming more widespread over the years. As noted in the Black Sea (Dumont *et al.,* 2004), the catastrophic consequences of *M. leidyi* were, in fact due to a combination of many different factors ranging from eutrophication (often exceeding chlorophyll *a* concentrations of 50 µg L^{-1} (ICPDR – ICPBS, 1999)), to overfishing. Thus, it is possible that the Adriatic, being less eutrophic (Djakovac *et al.,* 2012), might respond differently to the introduction of the invasive ctenophore *M. leidyi*.

Expectedly, in the most oligotrophic parts of the northern Adriatic, where food could be scarce, jelly combs did not thrive. Their presence remained mainly restricted to the area that extends from the north of the Cape Kamenjak to the south part of the Po River delta (Malej *et al.,* 2017; Fiori *et al.,* 2019). *M. leidyi* thrived much more in the Black Sea than it ever did in the eastern Mediterranean, possibly due to high salinity (Shiganova, 2001). Similarly, the lower salinity and eutrophic conditions of the NW Adriatic could favour *M. leidyi* blooms, while the oligotrophic conditions in the east (Kvarner Bay) (Degobbis, 1983; Vukić Lušić *et al.,* 2013) might have limited its spread.

Areas of increased *M. leidyi* concentration were located near (2016) or within the low salinity anticyclonic gyres (2017, 2018). Since gyres are considered stagnant areas in which organic matter and food can accumulate, it can be hypothesised that the growth and reproduction of *M. leidyi* took place within them. Being multiplied in gyres, the population may spread either towards the coastal zone or towards the south. In 2017, when transversal currents along the Pesaro-tip of Istria line were less pronounced than in 2016 and 2018, the population of *M. leidyi* reached the far south. In 2019 the maximal concentration of *M. leidyi* corresponded roughly to a large anticyclonic gyre between the lines Po delta-Rovinj and Pesaro-tip of Istria in an area of salinity over 38.1. We suppose that the gyre area was affected by lower salinity and food rich waters from Po, which when mixed with central Adriatic waters of especially high salinity, resulted in waters of salinity over 38.1.

M. leidyi's gut content analysis revealed a diversified diet in terms of taxonomic composition and prey size. The dominant prey taxa were representatives of summer zooplankton. Copepods were the most diverse group in the gut content. Calanidae copepods were found among the dominant prey taxa in *Mnemiopsis*' gut. According to Borme *et al.* (2009), they are the main prey for anchovy, which indicates resource competition between *M. leidyi* and *E. encrasicolus.* Literature data also confirms that planktonic shrimps, mainly copepods, are the main prey for *M. leidyi*, followed by meroplankton, eggs and juvenile fish (Purcell & Arai, 2001; Hamer *et al.,* 2011; Costello *et al.,* 2012; Tiselius & Møller, 2017). We noted that large zooplanktons (>2 mm) were more abundant in the digestive system than the surrounding sea. Examples of the "prey selectivity" of *M. leidyi* are known from other seas. The main prey in the Black Sea and French lagoons were invasive alien species of Atlantic origin, such as the copepod *Acaria (Acanthacartia) tonsa* and nauplii of barnacle *Amphibalanus improvisus* (Gubanova, 2000; Delpy *et al.,* 2012). In the Gulf of Kiel (Baltic Sea), *A. improvisus* formed 82% of the *Mnemiopsis* preys during the winter, while in September and October 52-72% of prey composition was represented by the planulae of the schyphomedusa *Aurelia aurita* (Javidpour *et al.,* 2009).

A relatively small amount of prey found in the digestive tract (1.2-2.5 ind. per ctenophore) differs from considerably higher prey quantities reported in several studies (Reeve *et al.,* 1989; Mutlu, 1999; Costello *et al.,* 2012). Nevertheless, the average zooplankton abundances measured at stations along the Istrian coast during 2017 and 2018 were lower compared to those observed in the northern Adriatic survey over the last two decades, using the same sampling method (Fonda Umani *et al.,* 2005; Camatti *et al.,* 2008; Bernardi Aubry *et al.,* 2012; Bojanić Varezić *et al.,* 2015, Vidjak *et al.,* 2019).

The northernmost part of the Adriatic Sea is characterized by shallow waters (<50 m), and it is recognized as an important ground for the development of early stages of small pelagic fish (Giannoulaki *et al.,* 2013). Since the detection of swarms of *M. leidyi*, changes in the spatial distribution of anchovy were recorded in the same area; Starting in 2016 and becoming more noticeable from 2017 onward, a decrease in abundance was observed. In 2016, the decrease was assumed to cover coastal waters along the west side of the Istrian peninsula. From 2017, the affected area spread westwards, almost to the midline of the basin, which was previously rich in anchovy schools. A similar situation continued in 2018. This effect could have appeared due to a reduction in the number of fish (heightened mortality), or more likely, due to anchovy's migration to another zone within the Adriatic basin. The overlap in timing of the appearance of *M. leidyi* swarms with the change in the distribution of anchovy indicates a possible relation between the two.

In the Black Sea, negative correlations were reported between the abundance of early development stages of summer-spawning zooplanktivorous fish species (like anchovy) and ctenophores. These correlations were also recorded in winter-spawning species, but not to the same extent (Shiganova, 1998). If an event similar to the one in the Black Sea is presumed to have happened in the Adriatic, it would potentially explain why the anchovy population (which spawns during the peak of *M. leidyi* abundance) was so affected.

The influence of *M. leidyi* swarms on the distribution of anchovy could be due to strong competition for food resources as in the Adriatic Sea *E. encrasicolus* feeds on similar zooplanktonic taxa than this ctenophore (Borme *et al.,* 2009). *M. leidyi*'s predation on fish eggs was also suspected, but in this study, we rarely found fish eggs in *Mnemiopsis'*s gut contents, probably due to the low abundance of spawning fish in the area and the consequent absence of fish eggs at sea (Table 2). Local changes in the spatial distribution of anchovy are indeed evident, but the implications on their entire stock and the ecosystem of the Adriatic Sea remain unclear. Thus, further monitoring of the Adriatic Sea is recommended.

Conclusions

The simultaneous monitoring of *M. leidyi* and anchovy in the NE Adriatic revealed that *M. leidyi*'s presence coincided with a reduction in anchovy abundance. It can be assumed that the reduction in anchovy is a consequence of competition for food with the invasive generalist feeder – *M. leidyi*.

The spatial distribution *M. leidyi*'s appearance overlapped with large seasonal anticyclonic gyres, which indicated that within those systems, conditions suitable for its proliferation could be found. Further research on *M. leidyi* within gyres of NE Adriatic is expected to unravel the roles of those systems as their nurseries and conveyors.

The crisis in fish stock that occurred in the Black Sea during the '80s, together with our findings, serves as a warning. An attentive and focused international monitoring program should be implemented in the Adriatic with the aim to understand the changes occurring in the basin and to find possible solutions to contain the problem.

Although MEDIAS surveys are designed specifically to monitor small pelagic fish stocks; these surveys could be a valuable source of information on large-scale changes in macro and mesozooplankton communities and other ecosystem's components.

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Supplementary Data

The following supplementary information is available online for the article:

Figure S1: A cube frame used for *in situ* observations (dimensions: 0.5ˣ0.5ˣ0.5 m).

Figure S2: Monthly average abundances of *M. leidyi* along the coast of Rovinj for months when its presence was recordes during the four-year monitoring.

Figure S3: Monthly means of vertically averaged temperatures and sigma-t values for September 2016, 2017, 2018 and 2019, obtained by the numerical hydrodinamical model. Solid line is drawn between the Po River delta and Rovinj, and dashed line between Pesaro (Italy) and the tip of Istria (Cape Kamenjak). Isolines 38.1 and 38.5 are depicted in black.

Figure S4: Annual distribution of zooplankton abundances at Rv station.

Figure S5: Zooplankton abundances (ind. m^{-3}) at stations along the western Istrian coast from Agust 2017 to July 2018.

Table S1. *M. leidyi*'s abundance (ind. m^{-3}) at the sea surface measured in September (from 2016 to 2019) at each of the stations accompanied with the depth at the location.