

# MONITORING GELATINOUS ZOOPLANKTON AND ITS DYNAMICS IN THE EASTERN ADRIATIC (2018–2023)

Andrea Budiša, Neven Iveša, Petra Burić, Gioconda Millotti, Moira Buršić, Emina Pustijanac, Ante Žunec, Ines Kovačić, Mauro Štifanić, Nataly Milovan, Paolo Paliaga

**Abstract:** Gelatinous zooplankton, widespread in pelagic environments, pose a challenge for surveillance due to their delicate, see-through bodies. Their presence often seems irregular, with fluctuating populations occasionally forming dense swarms. These efficient and non-selective feeders compete with fish for resources, and their abundant proliferation can alter the marine food web and result in ecosystem degradation. Hence, monitoring gelatinous zooplankton is crucial. In this study, we document their encounters over six years along the northeastern Adriatic coast, an important spawning area for small pelagic fish now experiencing frequent gelatinous zooplankton blooms. We compare findings from the north to those in the southern Adriatic. Continuous monitoring revealed that the invasive ctenophore *Mnemiopsis leidyi*, predominantly present in the northern basin, significantly altered the taxonomic composition, temporal occurrence, and intensity of gelatinous zooplankton blooms, shifting dominance from native to introduced species.

**Keywords:** Eastern Adriatic, Gelatinous zooplankton, Istrian coast, Monitoring

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## Introduction

Gelatinous zooplankton (GZ) encompasses diverse organisms, including cnidarians, ctenophores, and tunicates [1], which pose a significant challenge for detection and enumeration due to their delicate and transparent bodies. Consequently, direct visual census methods are often employed in their monitoring efforts. GZ plays a crucial role in marine ecosystems, as they are voracious consumers of zooplankton and can have profound impacts on pelagic fish populations [2–4]. Moreover, many GZ species are not commonly preyed upon by other organisms, potentially leading to trophic dead-ends, which may alter the ecosystem's natural structure and energy flows [2].

Enclosed seas, such as the Adriatic Sea and, notably, its northern part (NAd), are particularly susceptible to GZ proliferation, often linked to anthropogenic influences [1, 4]. The NAd, renowned for its biological richness within the Mediterranean [3–5], has witnessed a notable increase in GZ populations attributed to overfishing, climate change, and ballast water discharge [5]. Given the ecological importance of the NAd and vulnerability to such perturbations, understanding the dynamics of GZ populations in this region is imperative. This study seeks to examine the temporal dynamics of GZ populations over a six-year period in the NAd, a region known for its moderate anthropogenic influence and its variable productivity marked by fluctuating freshwater inputs from the west and the presence of highly saline, oligotrophic waters from the south [6,7]. In addition, we contrast these findings to data collected from the southern part of the basin, distinguished by its oligotrophic nature, low primary production [4], and comparably exposed to less anthropogenic pressure. By examining trends in GZ abundance, taxonomic structure, and distribution, we seek to examine the underlying factors driving their proliferation in this biologically significant area. Ultimately, the presented data should contribute to management strategies to mitigate the negative impacts of GZ proliferation on marine ecosystems and implement sustainable fisheries in the NAd and beyond.

## Materials & Methods

Daily monitoring of gelatinous zooplankton (GZ) was conducted from 2018 to 2023 by applying a visual census technique in the coastal waters of the western part of the Istrian peninsula, i.e., 3 km along the coastline of the town Rovinj-Rovigno (~3000 m<sup>2</sup>). Specimens larger than 1 cm, discernible to the naked eye, were tallied on-site following the methodology outlined in the literature [3]. Daily observations were conducted from the shoreline, supplemented by snorkeling or scuba diving along transects 1 m wide parallel to the coast, varying according to seasonal conditions and available technical resources. When GZ abundance was high and posed a challenge for enumeration, a cube frame was utilized, as described in the literature [3]. Extreme GZ abundances (>3000 specimens spotted within 2 h), we scanned the rest of the area and gave an estimate of the remaining exact number of GZ. Data collection efforts included ~40 % of reports from local citizens who engage in year-round swimming activities and fishermen trained by the researchers

to recognize the species. They volunteered the information and regularly provided photographic documentation of their observations.

The long-term data collection from the NAd was then compared with other studies but also to results gathered during a week-long monitoring cruise aboard a research vessel (Progetto M.A.R.E. 2023) in June 2023 in the archipelago of southern Croatian islands, i.e., the southern Adriatic (SAd), where the same monitoring approach was used to quantify the presence of GZ. Because the taxonomic GZ composition in that area often accounted for great numbers of specimens just over a cm in length, a zooplankton net (WP2) horizontally pulled to filter a known volume of surface water was employed for their exact enumeration.

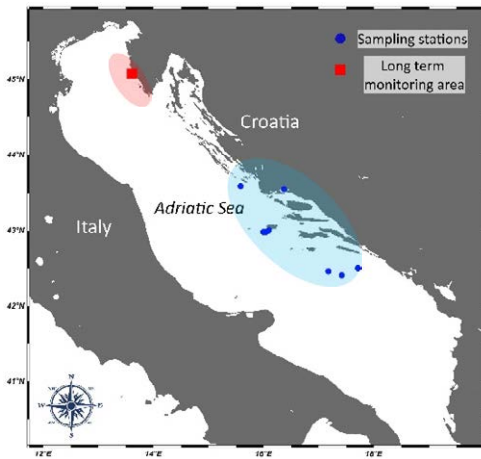


Figure 1 – Study area in the eastern Adriatic: i) long-term monitoring (daily, 2018–2023) along the western coast of the Istrian peninsula in NAd (red), and ii) blue) cruise (June 2023) in the southern part, SAd (blue) - as the data analysed and used for this part of the project have been collected with the contribution of One Ocean Foundation and Centro Velico Caprera within the framework of the M.A.R.E. Marine Adventure for Research and Education project.

In all our monitoring, we observed a particulate GZ on that day, the number of individuals reported, and the abundance. Thus, here we report on the frequency of occurrences of different GZ throughout the year and between years, trends in their numbers, and variability in shares of different taxa in different periods. We expressed the frequency of occurrence as the number (N) of days when GZ was present within a month, regardless of the number of specimens, i.e., GZ days/days in the month.

$$\text{Frequency (GZ)}[\%] = \left( \frac{N(\text{GZ days})}{N(\text{days in a month})} \right) \cdot 100 \quad (\text{Eq. 1}).$$

Statistical analyses on datasets were performed using R ver. 4.3.1 packages ‘stat’ and ‘FSA’ [8, 9]. We expressed the trends as means  $\pm$  standard deviation (SD) or standard error (SE) or as a confidence interval (CI). In addition, taxonomic diversity indices i.e. Shannon-Wiener (H'), Margalef H, Pielou evenness (J), and

dominance index (Y) were calculated applying formulas from the literature [10] while Simpson index of diversity (D) was calculated using the following equation:

$$D = 1 - \left( \frac{\sum n(n-1)}{N(N-1)} \right); \quad (\text{Eq. 2}).$$

where n is the number of specimens of one GZ taxa group per m<sup>2</sup> of the studied area, and N is the total number of all GZ per m<sup>2</sup> of the same area.

## Results

The taxonomical composition of the reported GZ taxa in the NAd included seven different pelagic cnidarians, one autochthonous and one allochthonous ctenophore, and a pelagic tunicate (Table 1). The overall frequency of GZ occurrences in the NAd remained consistent over the six-year monitoring period, showing no significant differences between years (Kruskal-Wallis test,  $H(5) = 5.4$ ,  $p > 0.05$ ) but varied significantly within a year (by months) (Kruskal-Wallis test,  $H(12) = 47.9$ ,  $p < 0.001$ ) (Fig. 2). The number of GZ reports was the highest in Oct (95 % CI [672, 2219]) and the lowest in Jan (90 % CI [0.5, 2.2]). That dynamic was heavily steered by the oscillations in *M. leidy* abundance (Fig. 3), on average, over  $10.5 \cdot 10^6$  ind. m<sup>-2</sup> per year and counted for >90 % of all individual GZ reported in NAd. That is 25× higher than the otherwise most abundant *Aurelia* ( $4.4 \cdot 10^5 \pm 0.6 \cdot 10^5$  (SD) ind. m<sup>-2</sup>). Moreover, year-to-year differences are clear when comparing total numbers of ind. revealing the lowest values in 2018 & 2023 ( $< 5 \cdot 10^5$ ) and the maximum in 2020 ( $> 4 \cdot 10^6$ ), dominated by *M. leidy* except for 2023 when *Aurelia* spp. took over the first position (Fig. 4). Based on the frequency of encounters over the six years, most GZ were categorized as incidental (Table 1). However, certain cnidarians, such as *Aurelia* spp. and *Cotylorhiza tuberculata*, could be considered as occasional encounters during spring and summer, respectively. The only GZ species with a consistent presence was *M. leidy* in autumn (Oct–Dec), remaining the sole GZ species present throughout the entire year, while cnidarian *Rhizostoma pulmo* was present for 11 months, albeit being present only ~10 % of the month (Jul, Oct). *Leucothea multicornis*, *Aurelia* spp., and *Chrysaora hysoscella* were present for 9, 8, and 6 months, respectively. Notably, a diverse array of taxa was present throughout most of the year, but only Ctenophora was present in Dec (Table 1). The appearance of *M. leidy* during the year differed significantly from the other GZ (Dunn test,  $p_{\text{adjusted}}=0.03$ ), with noticeably higher abundances recorded (Fig. 5). It was observed that in the first half of the year (January-July), *M. leidy* was present with a much lower frequency and lower numbers than the other GZ components (Fig. 3&5). For instance, the tunicate (*Salpa* spp.) was most numerous in March ( $54 \cdot 10^3$  ind. m<sup>-2</sup>) while cnidarians reached their maximum in May (*Aurelia* spp.  $\sim 147 \cdot 10^3$  ind. m<sup>-2</sup>) and June (*C. hysoscella*  $\sim 30 \cdot 10^3$  ind. m<sup>-2</sup>) but *C. tuberculata* although exhibiting its maximum in August ( $\sim 81 \cdot 10^3$  ind.) was consistently outnumbered by *M. leidy*.

Indeed, from August to December,  $95 \pm 10$  % (SD) of all GZ reported was *M. leidyi* (Fig. 6). This indicates a split in the taxonomic structure of GZ between autochthonous and allochthonous GZ in NAd and highlights a clear periodic dominance of the invasive ctenophore *M. leidyi*.

While non-native taxa largely influenced GZ dynamics in the NAd, in Sad, GZ was predominantly native (Fig. 7) and included the taxa absent in NAd, such as tunicate *Thalia democratica* and ctenophore *Bolinopsis vitrea*. In June 2023, cnidarians were among the most common (>50 %) in both NAd and SAD, similarly frequent as pelagic tunicates in SAD. Higher diversity indices (i.e., D, H', H, J) and dominance index (Y) were found for SAD (0.18, 0.3, 0.96, 0.24, and 0.84) rather than NAd (0.13, 0.3, 0.5, 0.22 and 0.5).

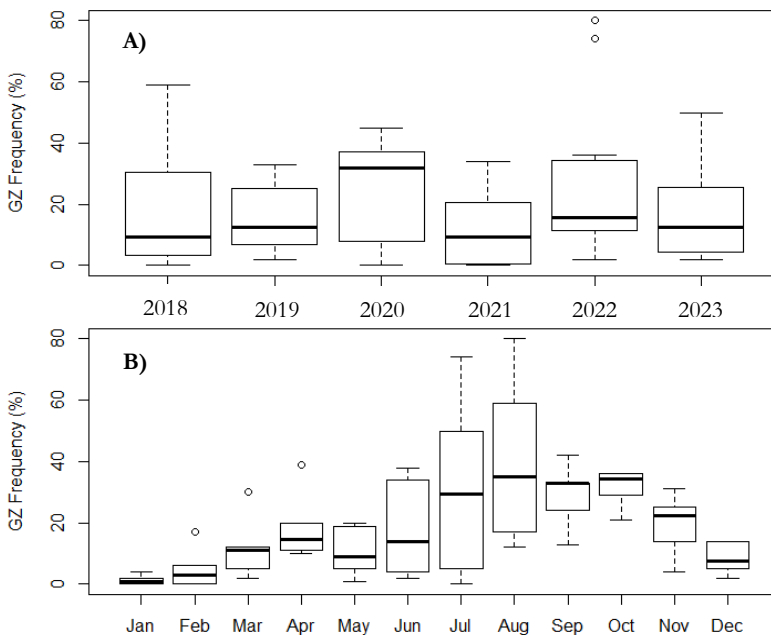


Figure 2 – Frequency of GZ reports in NAd (2018-2023):

A) per year and B) cumulatively within a year, boxes represent 25-75<sup>th</sup> percentile values, horizontal lines denote medians, whiskers extreme (adjacent) values within 1.5 interquartile range of the 25-75<sup>th</sup> percentile, and dots values outside the range of adjacent values.

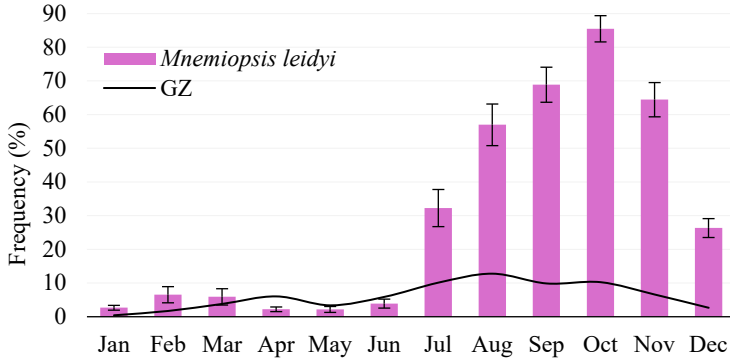


Figure 3 – Fluctuation in monthly frequencies of *M. leidyi* appearances expressed in categories as average  $\pm$  SE, in contrast to the average monthly frequency of appearance of GZ taxa (N=10) in NAd (2018-2023).

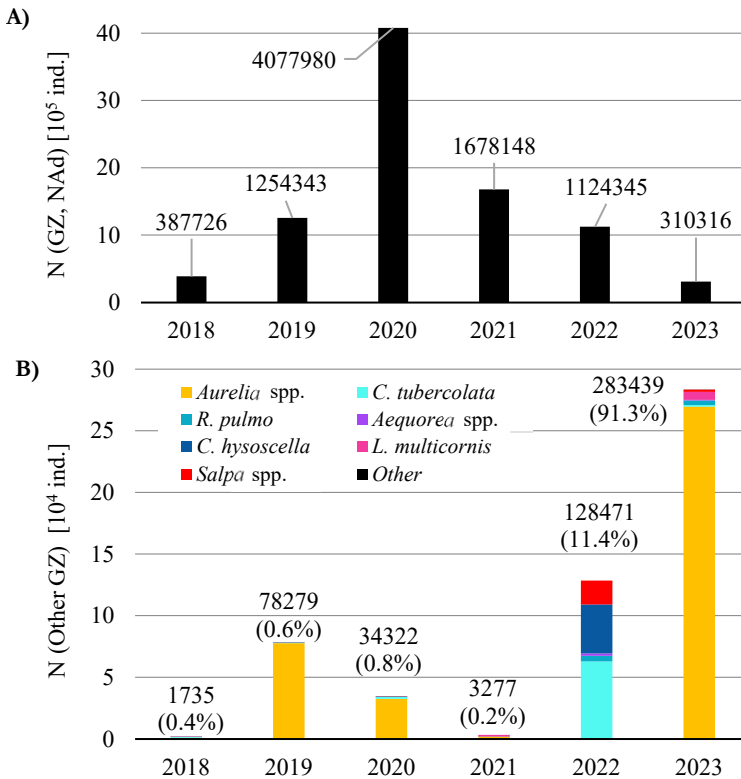


Figure 4 – A) Sum of all GZ ind. in the monitored area in NAd per year, and B) contribution to the total of other GZ aside of *M. leidyi*.

Table 1 – Monthly frequency (%) of encounters (expressed as average  $\pm$  SE) of various GZ taxa in NAd (2018–2023), i.e. A) cnidarians, and B) ctenophores and tunicates. Encounters with frequencies  $>50\%$  are labeled as constant (c) and  $25\div 50\%$  as accessory (a), while  $<25\%$  are considered incidental.

		<b>Cnidaria</b>					
A)	<i>Aurelia</i> spp.	<i>Cotylorhiza tuberculata</i>	<i>Rhizostoma pulmo</i>	<i>Aequorea</i> spp.	<i>Chrysaora hysoscella</i>	<i>Neoturris pileata</i>	<i>Pelagia noctiluca</i>
<b>Jan</b>			0.5 $\pm$ 0.2			1.1 $\pm$ 0.4	
<b>Feb</b>	4.2 $\pm$ 1.2		6.5 $\pm$ 1.7				
<b>Mar</b>	13.4 $\pm$ 1.9		7 $\pm$ 1.4	5.4 $\pm$ 0.7			
<b>Apr</b>	38.3 $\pm$ 4.7 <sup>a</sup>		2.2 $\pm$ 0.5	3.9 $\pm$ 0.9	5.6 $\pm$ 1.3		
<b>May</b>	16.1 $\pm$ 3.6	0.5 $\pm$ 0.2	6.5 $\pm$ 1.8		4.3 $\pm$ 0.8		
<b>Jun</b>	30.6 $\pm$ 6.3 <sup>a</sup>	0.6 $\pm$ 0.2	4.4 $\pm$ 1.1		13.9 $\pm$ 3.7		
<b>Jul</b>	10.2 $\pm$ 2.7	19.4 $\pm$ 5.5	10.2 $\pm$ 2.2	2.7 $\pm$ 1.1	10.8 $\pm$ 3.2		2.2 $\pm$ 0.9
<b>Aug</b>	1.1 $\pm$ 0.4	44.1 $\pm$ 6.6 <sup>a</sup>	5.9 $\pm$ 1.4		11.8 $\pm$ 3.8		
<b>Sep</b>	1.1 $\pm$ 0.5	21.1 $\pm$ 2.9	2.2 $\pm$ 0.7		0.6 $\pm$ 0.2		
<b>Oct</b>	1.6 $\pm$ 0.4	2.2 $\pm$ 0.7	10.8 $\pm$ 1.4		0.5 $\pm$ 0.2		1.1 $\pm$ 0.4
<b>Nov</b>	1.1 $\pm$ 0.5		0.6 $\pm$ 0.2				
<b>Dec</b>							

		<b>Ctenophora</b>		<b>Tunicata</b>
B)	<i>Mnemiopsis leidyi</i>	<i>Leucothea multicornis</i>	<i>Salpa</i> spp.	
<b>Jan</b>	2.7 $\pm$ 0.7			
<b>Feb</b>	6.6 $\pm$ 2.4			
<b>Mar</b>	5.9 $\pm$ 2.4	3.8 $\pm$ 1.1	2.7 $\pm$ 0.7	
<b>Apr</b>	2.2 $\pm$ 0.7	6.1 $\pm$ 1.2	2.2 $\pm$ 0.9	
<b>May</b>	2.2 $\pm$ 0.9	3.8 $\pm$ 0.9	0.5 $\pm$ 0.2	
<b>Jun</b>	3.9 $\pm$ 1.3	5.6 $\pm$ 1.1		
<b>Jul</b>	32.3 $\pm$ 5.5	9.1 $\pm$ 1.5	4.3 $\pm$ 1.3	
<b>Aug</b>	57 $\pm$ 6.2	5.4 $\pm$ 1.4	2.7 $\pm$ 1.1	
<b>Sep</b>	68.9 $\pm$ 5.2 <sup>c</sup>	5 $\pm$ 1.3		
<b>Oct</b>	85.5 $\pm$ 3.9 <sup>c</sup>	1.1 $\pm$ 0.4		
<b>Nov</b>	64.4 $\pm$ 5.1 <sup>c</sup>			
<b>Dec</b>	26.3 $\pm$ 2.8 <sup>a</sup>	0.5 $\pm$ 0.2		

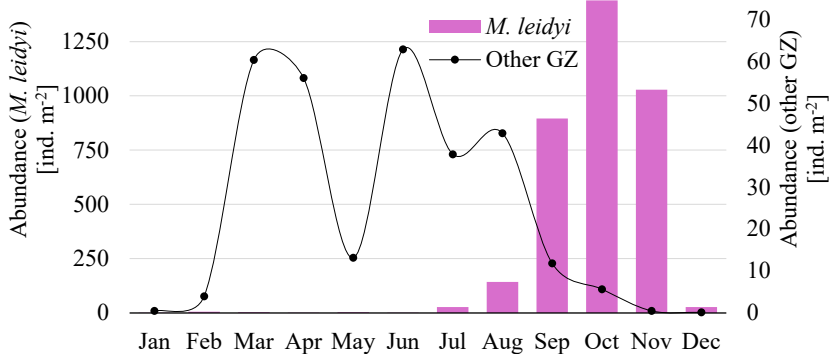


Figure 5 – Average monthly number of *M. leidyi* per m<sup>2</sup> and other GZ taxa through the year (NAd, 2018-2023). The dashed line separates the year to the 1<sup>st</sup> part when numbers of other GZ are greater from the 2<sup>nd</sup> part when *M. leidyi* numbers dominate in the GZ community.

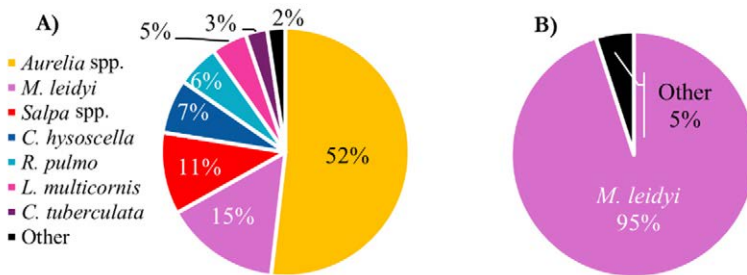


Figure 6 – Different taxa contribution (%) to average monthly GZ abundances (ind. m<sup>-2</sup>) in NAd, 2018-2023: A) Jan-Jul, and B) Aug-Dec.

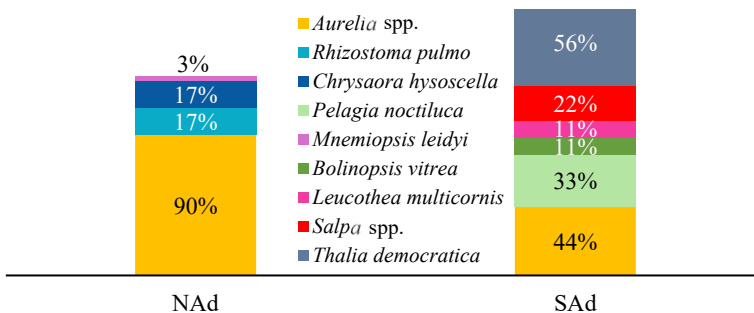


Figure 7 – Frequency of encounters of three GZ groups (Cnidaria – C<sup>n</sup>, Ctenophora – C<sup>t</sup>, and Tunicata – T) in June 2023 in NAd and SAd.



## Discussion

The alien invasive ctenophore *M. leidy* exerted significant dominance over the GZ community in NAd, both in abundance ( $>10^6$  ind.) and length of its presence – most of the year (Fig. 4–5) and frequency (constant presence Jul–Dec) (Table 1). Many adverse effects of *M. leidy* in NAd were reported, e.g., damaged fishing equipment [11], reduced zooplankton availability causing anchovy displacement [3], and impacts on nutrients, organic carbon fluxes, and microbial community [12,13]. The biggest proliferation of *M. leidy* recorded in NAd was reported in 2020 (Fig. 4A), with local maxima of  $\sim 450$  ind.  $m^{-2}$  (e.g., Nov 2020). Conversely, in 2023, *M. leidy* exhibited its minimum ( $14\times$  lower than the otherwise lowest in 2018) and has lost its dominant position in the GZ community. We assume that the extreme spring drought in 2023 caused the record reduction of River Po [14] and smaller streams that influenced circulation and nutrient loads in the NAd, affecting primary production and, consequently, higher trophic levels and GZ. Literature mentions incidental *M. leidy* presence in the Port of Ploče, Sep–Oct [3,4], which raised concern about their spread to nearby pristine areas of Mljet Island (SAd). However, we did not encounter *M. leidy* in the SAd, in accordance with previous studies [4], and assume morphologically similar *B. vitrea* could have caused confusion as, indeed, we encountered few specimens in the SAd but not in the NAd. However, this is a common spring/autumn species in NAd but rare in the SAd [4]. The presence of autochthonous Ctenophora *L. multicornis* was also incidental with low abundances in NAd, appearing before/together with *M. leidy* and lasting a few days or weeks. At *M. leidy* minimum in 2023, *L. multicornis* reached its maximum of 1 ind. per 100  $m^2$  ( $\sim 5\times$  higher than the otherwise maximum in 2021), indicating a preferred absence of food competition. In the SAd, *L. multicornis* was the prevalent Ctenophora, albeit in low abundance. These findings align with other reports as it is not a common species and, in SAd, appears in summer–autumn [4].

Among the cnidarians, the incidental *R. pulmo*, accessory *C. tuberculata*, and *Aurelia* spp. encounters were the most common and numerous. *Aurelia* was particularly numerous in 2023 (summer), dominating the GZ community in the NAd, presumably because *M. leidy* bloom was not particularly extensive. We also encountered *Aurelia* spp. in the SAd, representing the most abundant cnidarian although listed as a rare spring-occurring species in the area (except for the year-round presence of *A. relicta* endemic to Mljet Island [15]). *Aurelia* is a common species in the rest of the basin, particularly abundant in NAd along the Istrian coast and Gulf of Trieste (GoT), where its mass proliferations appear in spring [4]. In 2022, abundance of another cnidarian, *C. tuberculata*, increased  $60\times$  with respect to earlier while regularly cooccurring with *M. leidy* blooms as it was reported most frequently in Aug – when *M. leidy* becomes constantly present (Table 1). We assume that the different trophic requirements and autotrophic symbiotic zooxanthellae [16] enable it to depend less on food competition with *M. leidy*. Although listed as a common species in warmer months in the central part of the Adriatic with limited presence in SAd [4], it was not encountered during our cruise. *R. pulmo* was detected in NAd before and during *M. leidy* blooms, increasing substantially in presence in 2022 and 2023, while was not observed in the SAd. Literature reports on its sporadic summer–autumn presence in SAd, with large

blooms reported only in the GoT [4]. Moreover, incidentally present hydromedusa *Aequorea* spp. (in NAd), reached its highest abundance in spring 2022 (1 ind. per 2 m<sup>2</sup>) but was not recorded in the SAd, which is in accordance with the literature, as its spring blooms in the GoT decrease in abundance southwards [17].

The most frequent stinging jellyfish in the NAd was *C. hysoscella*, which appeared in high numbers only in the summer of 2022, just before massive *M. leidy* blooms. The species was not found in the SAd, consistently to studies listing it as sporadic, while common (Feb–Sep), albeit seldom in large numbers in the GoT [4]. But decades ago, another stinging Cnidaria – *P. noctiluca*, would periodically dominate harmful GZ in the NAd [18]. Its reappearance happened in 2023, firstly by accessory encounters south (Fig. 7), and progressed northwards to Istria in 2<sup>nd</sup> half of summer. Indeed, the SAd is known as the area with the most frequent blooms of *P. noctiluca* [4], while its further spread north could have been stopped by the presence of a food competitor – *M. leidy*, which appeared in that period.

Moreover, pelagic tunicates in the NAd represented only a marginal GZ component, reported as shorth lasting chain-forming *Salpa* spp. encounters (spring 2022 & 2023) along with other typical seasonal GZ. Different feeding patterns, e.g., filtration and diet consisting of protists and phytoplankton [19], rather than zooplankton, probably limited their dependency on competition with other GZ. However, we noticed a higher abundance of pelagic tunicates in SAd compared to the NAd, consistent with other findings mentioning occasional tunicate spring blooms [20]. In addition to sporadic findings, *Salpa* spp. encounters in both areas, in SAd, we witnessed a bloom of *T. democratica*, otherwise absent in the north. The rarest observations in the NAd regarded the newly discovered species: *M. benovici* – not recorded in the SAd, and *N. pileata*, which occasionally appears in the Adriatic and western Mediterranean [21]. Overall, the compared regions were characterized by consistently higher GZ diversity indices in the SAd.

## Conclusion

The pelagic coastal waters of western Istria (NAd) are significantly altered by *M. leidy* which dominated the GZ community 2018–2023, shifting the peak of most GZ occurrences from spring to autumn. Other GZ components (Cnidaria and Tunicata) exhibited accessory/incidental occurrence with an exception in 2023, as a likely consequence of reduced river supplies on the marine food web, resulting in a drastic drop of *M. leidy* abundance but an increase in native GZ taxa (*Aurelia* spp.). In addition, in the SAd, conditions appear suboptimal for *M. leidy*, leaving room for more diverse and mainly native GZ taxa dominated by Tunicata and Cnidaria. Moreover, in 2023, *P. noctiluca* reappeared in the NAd, possibly also due to the absence of food competitors such as *M. leidy*.

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