Chemical composition and egg production capacity throughout bloom development of ctenophore *Mnemiopsis leidyi* in the northern Adratic Sea

Kevin Rečnik [1] , **Katja Klun** [1] , **Lovrenc Lipej** [1] , **Alenka Malej** [1] , **Tinkara Tinta** [Corresp. 1]

[1] Marine Biology Station Piran, National Institute of Biology, Piran, Slovenia

Corresponding Author: Tinkara Tinta
Email address: TINKARA.TINTA@NIB.SI

High abundances of gelatinous zooplankton (GZ) can significantly impact marine ecosystem by acting as both sinks and sources of organic matter (OM) and nutrients. The decay of GZ bloom can introduce large amounts of OM into ocean interior. The variability in the quantity and quality of available GZ-OM is influenced by various life traits and environmental factors. These variations can have significant impacts on end-consumers, such as microbial communities, which play vital roles in marine biogeochemical cycles. The invasive ctenophores *Mnemiopsis leidyi* forms massive blooms in the northern Adriatic Sea since 2016. However, the variability in the chemical composition and egg production of blooming populations, as well as the role of environmental factors in governing this variability, remains largely unknown. Our analysis of biometry, chemical composition, and fecundity of *M. leidyi* sampled in the Gulf of Trieste in 2021 revealed stable carbon and nitrogen content throughout bloom development, with no significant correlation with ambient seawater temperature, salinity, oxygen, and chlorophyll *a* concentration. Although our studied population exhibited homogeneity in terms of biometry and chemical composition, the number of produced eggs varied substantially, showing no clear correlation with environmental variables and being somewhat lower than previously reported for our study area and other invaded Mediterranean systems. We observed a positive correlation between the wet weight of individuals and the percentage of hatched eggs, as well as a significant positive correlation between the percentage of hatched eggs and ambient seawater temperature. Additionally, we noted that the speed of hatching decreased with lowering ambient seawater temperature in autumn, towards end of *M. leidyi* bloom.

Manuscript Title:

Chemical composition and egg production capacity throughout bloom development of ctenophore *Mnemiopsis leidyi* in the northern Adratic Sea.

Authors:

Rečnik K.[1], Klun K.[1], Lipej L.[1], Malej A.[1], Tinta T.[1*]

Affiliations:

1 Marine Biology Station Piran, National Institute of Biology, Fornače 41, 6330 Piran

Corresponding author:

Tinkara Tinta

e-mail: tinkara.tinta@nib.si

Abstract

High abundances of gelatinous zooplankton (GZ) can significantly impact marine ecosystem by acting as both sinks and sources of organic matter (OM) and nutrients. The decay of GZ bloom can introduce large amounts of OM into ocean interior. The variability in the quantity and quality of available GZ-OM is influenced by various life traits and environmental factors. These variations can have significant impacts on end-consumers, such as microbial communities, which play vital roles in marine biogeochemical cycles. The invasive ctenophores *Mnemiopsis leidyi* forms massive blooms in the northern Adriatic Sea since 2016. However, the variability in the chemical composition and egg production of blooming populations, as well as the role of environmental factors in governing this variability, remains largely unknown. Our analysis of biometry, chemical composition, and fecundity of *M. leidyi* sampled in the Gulf of Trieste in 2021 revealed stable carbon and nitrogen content throughout bloom development, with no significant correlation with ambient seawater temperature, salinity, oxygen, and chlorophyll *a* concentration. Although our studied population exhibited homogeneity in terms of biometry and chemical composition, the number of produced eggs varied substantially, showing no clear correlation with environmental variables and being somewhat lower than previously reported for our study area and other invaded Mediterranean systems. We observed a positive correlation between the wet weight of individuals and the percentage of hatched eggs, as well as a significant positive correlation between the percentage of hatched eggs and ambient seawater temperature. Additionally, we noted that the speed of hatching decreased with lowering ambient seawater temperature in autumn, towards end of *M. leidyi* bloom.

Introduction

The successful spread of the notorious invasive ctenophore *Mnemiopsis leidyi* A. Agassiz, 1865 to various ecosystems worldwide (Costello et al., 2012; Jaspers et al., 2018; Jaspers, Bezio & Hinrichsen, 2021) hinges on its life history traits, *e.g.*, ecological plasticity regarding environmental conditions, continuous early production post-hatching, self-fertilization ability, and cannibalism (Baker & Reeve, 1974; Colin et al., 2010; Jaspers, Costello & Colin, 2015; Jaspers, Møller & Kiørboe, 2015; Shiganova et al., 2019b; Javidpour et al., 2020; Edgar, Miguel Ponciano & Martindale, 2022). When present in high abundances, this species can significantly impact local ecosystem by acting as nutrient sink, competing for food, and influencing interspecies predatory relationships (Oguz, Fach & Salihoglu, 2008; Shiganova et al., 2019a; Fiori et al., 2019; Schroeder et al., 2023). However, the role of this gelatinous invasive species as source of organic matter (OM) and nutrients for affected ecosystems has received less attention (Pitt, Welsh & Condon, 2009; Condon, Steinberg & Bronk, 2010; Dinasquet, Granhag & Riemann, 2012; McNamara, Lonsdale & Cerrato, 2013; Dinasquet et al., 2013). Particularly, the decay of ctenophore blooms can result in large influx of ctenophore-derived OM, disrupting the quality and quantity of the ambient OM reservoir (Fadeev et al., 2024). It has been demonstrated that the dissolved fraction of gelatinous zooplankton-derived OM, rich in proteins and characterized by low C:N ratio, can undergo rapid decomposition by opportunistic microbes with high growth efficiency (Tinta et al., 2020). This implies that significant portion of gelatinous-OM is swiftly assimilated into bacterial biomass, subsequently becoming accessible to bacterial grazers, and thereby not lost from the system *via* respiration (Tinta et al., 2020). Such dynamics can have critical implications for the fate and flux of gelatinous-derived OM and for the functioning and biogeochemical state of marine ecosystems (Dinasquet et al., 2013; Tinta et al., 2023; Fadeev et al., 2024). ~~However,~~ the composition of gelatinous-OM can vary between species and within population of the same species due to various factors such as individual organisms' properties (e.g., biometrics, fertility, age), seasonal environmental factors, prey type and availability, and possible parasites infestation (Condon, Steinberg & Bronk, 2010; McNamara, Lonsdale & Cerrato, 2013). This variability can significantly influence the dynamics of surrounding systems, particularly affecting end-consumers like microbial communities, which are true drivers of marine biogeochemical cycles (Azam & Malfatti, 2007). Understanding the factors governing the chemical composition and egg production of gelatinous-OM is therefore crucial for comprehending the interaction between microbes and gelatinous-OM and accurately integrating jelly-OM into oceanic biogeochemical budgets.

Since 2016, annual blooms of *M. leidyi* have also been observed from summer until late autumn in the northern Adriatic (Malej et al., 2017; Pestorić et al., 2021). While some aspects of the potential impact of these bloom formations on the local ecosystem have been studied (Fiori et al., 2019; Ciglenečki et al., 2021; Paliaga et al., 2021; Fadeev et al., 2024), there is limited data available on the chemical composition and egg production capacity of northern Adriatic *M. leidyi* populations (Malej et al., 2017; Fadeev et al., 2024). Our aim was to elucidate variability in biometry, chemical composition, and fecundity of the *M. leidyi* population throughout its annual bloom development and address our hypothesis that ambient environmental variables affect observed potential variations. To achieve this objective, *M. leidyi* individuals were sampled at different locations in the Gulf of Trieste throughout their blooming season from August to October 2021, measuring ~~biometric parameters (~~wet and dry mass~~)~~, ~~chemical composition (~~carbon and nitrogen content~~)~~ and conduct~~ed~~ egg production experiments. Concurrently, a set of environmental factors was monitored, and statistical analysis was applied to infer correlations between variables.

Material and methods

Field sampling

Bi-monthly sampling of *M. leidyi* twice was conducted from August to October 2021 (Table 1) to encompass the period when *M. leidyi* specimens are most abundant in the northernmost part of the Adriatic Sea, namely the Gulf of Trieste (Fig. 1). To address the spatial heterogeneity of populations, sampling was conducted at various locations within our study area (Fig. 1). Ctenophores were collected directly from a boat or from the pier using a plastic bucket previously rinsed with ambient seawater. Subsequently, the collected ctenophores were transferred ~~in buckets~~ directly to the laboratory, maintaining *in situ* temperature and light conditions. Between 15 – 20 individuals were collected during each sampling ~~campaign around~~ (Table S1). We acknowledge that a larger ~~number of individuals per~~ sampling ~~day~~ would provide a more robust statistical analysis; however, we had to adjust the number of samples due to operational constraints. For each sampling ~~campaign~~, we obtained data on ~~ambient~~ seawater temperature, salinity, oxygen and chlorophyll a concentrations from a depth of 3 meters at our reference long-term sampling station - oceanographic buoy Vida (https://www.nib.si/mbp/en/oceanographic-data-and-measurements/buoy-2) (Fig. 1, Table S1).

Biometry and elemental composition analysis

First, the total body length (TBL) of each collected individual (*i.e.*, oral-aboral length including lobes) was measured and the wet mass of each ctenophore was determined using the calibrated scale Sartorius TE1502S. Subsequently, we placed each specimen into separate clean zip lock bag and stored them at -20 °C, for minimum of 24h until further processing. For elemental composition analysis, each specimen was freeze-dried at -45 °C for 3 days (as previously Tinta et al., 2020). The dry mass of each individual was determined using the calibrated scale Sartorius CP225D (d=0.01mg (80g), d=0.1mg (220g)). The dry material of each specimen was then homogenized with a pre-sterilized pestle and agate mortar and stored separately in sterile 15 mL grainer tubes at -20° C until further analysed. From each sample we weighed approximately 15 – 20 mg of dry homogenized matter into small capsules using a calibrated Micro scale (Mettler Toledo). Elemental composition of carbon (C) and nitrogen (N) was determined after combustion at 1150° C (Elementar, Vario Micro Cube elemental analyser) with 3 % accuracy. To minimize the risk of contamination and material degradation, care was taken to maintain sterile conditions throughout the process. This includes using combusted glassware, working on the ice at all intermediate steps and minimizing the number of freezing/thawing cycles to a bare minimum (as recommended (Kogovšek et al., 2014).

Reproduction experiment

~~During~~ each sampling c~~ampaign~~, a batch of 5 individuals, similar in size (averaging 6.9 ± 1.1 cm) and wet weight (averaging 24.1 ± 7.8 g), ~~was~~ selected to assess the reproduction capacity of the sampled ctenophores (Table 1). Each of the 5 selected specimens was placed individually into a 1L glass Erlenmeyer flask filled with 800mL of pre-filtered seawater (using GF/F Whatman filters) and covered with parafilm. Afterwards an incubation period of 20 hours at the *in situ* seawater temperature in darkness, seawater was examined for produced eggs and/or other developmental stage using an Olympus stereo microscope SZH. Initially, the volume of analysed seawater was reduced using mesh filter with pore size of 200 μm and then examined using a small container with grid-patterned bottom. All the eggs and other developmental phase in each sample were counted after 24h and 48h of the experiment. Once the experiment concluded, the ctenophores were sacrificed for further analysis of dry mass and elemental composition, as described above ~~(see *Biometrics and elemental composition analysis*~~).

Statistics

All statistical analysis and visualizations were conducted using specialized packages in R (https:// www.r-project. org/). The Pearson correlation coefficient and the Holm-Bonferroni p-value adjustment method was determined using R correlation package. Visualization we achieved using the ggplot2 package in R, and the figures we combined using Bio render.

Results

Environmental parameters

The ~~ambient~~ seawater temperature remained relatively stable around 22°C throughout ~~our~~ sampling period, peaking at 25°C in August and reaching a low of 17°C by the end of October in our study (Fig. 2A). Salinity levels ranged between 36-37, dropping to a minimum of 35 in August and rising to a maximum of 38 in October ~~during our sampling~~ (Fig. 2A). Chlorophyll concentration showed an increasing trend from 0.39 µg mL^{-1} in August to 1.19 µg mL^{-1} in the first half of October, with a slight decrease to 1 µg mL^{-1} observed in the second half of October (Fig. 2B). Oxygen concentration in ~~ambient~~ seawater was lowest in August (4.48 mg mL^{-1}) and highest in October (5.06 mg mL^{-1}) ~~during our sampling period~~ (Fig. 2B).

Biometric parameters and elemental composition of ctenophore populations

A total of 89 individuals was collected ~~in our study~~, ~~representing the ctenophore population blooming~~ between August and October 2021. The average total body length (*i.e.*, oral-aboral length including lobes) and width of individuals were 6.6 ±1.1 cm and 4.2 ± 0.6 cm, respectively (Table S2). The average wet weight (WW) was 30.8±13.6 g (Fig. 3A), while the average dry weight (DW) was 1.3 ± 0.6 g (Fig. S1), representing approximately 4.1 ± 0.2 % of wet weight (Fig. 3B). Both minimum and maximum wet and dry weights (*i.e.*, min WW = 4.96 g and max WW = 70.2 g; min DW = 0.2 g and max DW = 2.93 g) were observed within the population collected in the first half of September, which exhibited the greatest size heterogeneity overall (Table S1). To test our hypothesis that changes in environmental variables affect the biometric properties of ctenophore populations, we calculated the Pearson correlation coefficient between selected environmental variables (temperature, salinity, oxygen, and chlorophyll a concentration) and all biometric characteristics of ctenophores (wet weight, dry weight, and percentage of dry weight). We found significant correlation between salinity and the percentage of dry weight ($r=0.40$, $p < 0.001$**, p-value adjustment method: Holm-Bonferroni) (Fig. 3C). However, no other environmental variable correlated with the biometric characteristics of ctenophore populations (Table S3).

The average carbon and nitrogen percentage in the dry mass of all individuals of the studied ctenophore population were 1.59±0.29% and 0.42±0.08 %, respectively (Table S1). The minimum carbon and nitrogen percentage in the dry mass were recorded for the population collected in the second half of September, measuring at 1.03% and 0.28%, respectively (Table S1), while the maximum percentages were observed for the population in the second half of October, measuring at 2.73% and 0.71%, respectively (Table S1). However, neither carbon nor nitrogen content exhibited a significant trend throughout the study period or a significant correlation with environmental variables in our dataset (Fig. 4A, Table S3). The carbon-to-nitrogen (C:N) molar ratio remained relatively constant throughout the study period, averaging at 4.46±0.19:1, with the minimum ratio measured in the first half of August (3.81:1) and the maximum in the first half of September (4.95:1) (Table S1, Fig. 4). No significant correlation was found between C:N ratio and environmental variables in our dataset (Table S3).

Egg production

For the egg production experiment, individuals of similar size were selected, with an average body length of 6.6 ±1.1 cm and an average body width of 4.2 ± 0.6 cm (Table S2). Their average wet mass (24.1 ± 7.8 g), dry mass percentage of WW (4.1 ± 0.3%), carbon (1.57 ± 0.33%) and nitrogen (0.42 ± 0.09%) content, and C:N ratio (4.4 ± 0.2:1) fell within the range of values measured for the total collected population in our study (Table 1, Table S1, S2). Slightly over half (57 %) of individuals produced eggs in our study, with an average of 165 eggs per individual (Table 1). The percentage of individuals that did not produce any eggs was highest within the population collected in the second half of September and in the first half of October (Table 1). There was significant variability in the total number of eggs produced by individuals across the entire dataset and within specific experiments (Table 1, Fig. 5A). The largest number of eggs produced (638) was recorded in the second half of October, while the lowest (3) was recorded in the first half of September. However, no significant correlation between the total number of eggs produced and/or chemical characteristics of individuals or environmental variables was found (Table S4).

In our 48-hour fecundity experiment, most eggs had developed into cydippid larva stage by the end of the first 24 hours. To determine the percentage of hatched eggs, we divided the number of hatched eggs after 24 hours by the total number of eggs produced by each individual within that time frame. The average percentage of hatched eggs per experiment was calculated by considering only those individuals that produced eggs. The overall average percentage of hatched eggs across the entire dataset was 67% ± 32%, with a minimum of 13% observed in the second half of October and a maximum of 100% observed in the first half of August and the first half of September (Fig. 5B). Significant correlation between percentage of hatched eggs and ambient seawater temperature was found (r=0.36, $p < 0.05$*), which became even more significant when considering only individuals that produced eggs (r=0.72, $p < 0.01$**) (Fig. 5C, Table S4). Additionally, there was a significant positive correlation between the percentage of hatched eggs and the wet weight of ctenophores (r=0.38, $p < 0.05$*) (Fig. 5D, Table S4). Note that while these correlations were significant, they were not confirmed using Holm-Bonferroni method. However, no significant correlation between the percentage of hatched eggs and the chemical characteristics of individuals or environmental variables was found (Table S4).

Discussion

Variations in environmental parameters are not reflected in the chemical composition of blooming ctenophore population.

Mnemiopsis leydi populations in our study area begin to increase in late July, peaking between September and October, with intermediate massive blooms, while individuals are rarely observed during colder part of the year (Malej et al., 2017; Budiša et al., 2021). Hence, *M. leydi* was sampled from the second half of August until the end of October to capture the 2021 bloom in our study area. During this period physical, chemical, and biological factors in ambient seawater changed (Fig. 2), yet no significant temporal trend in wet weight, dry weight, percentage of carbon and nitrogen content or carbon to nitrogen molar ratio in the sampled population were observed (Fig. 3, Fig. 4). The carbon to nitrogen ratio remained relatively constant, averaging at 4.5:1, consistent with previous reports (Pitt, Welsh & Condon, 2009; Lucas et al., 2011) and showed no correlation with environmental variables (Table S3, Fig. 4). Thus, we rejected our hypothesis that changes in environmental variables would be reflected in the carbon to nitrogen ratio of ctenophore biomass. However, significant correlation between salinity and percentage of dry weight was found (r=0.40, $p < 0.001$**, p-value adjustment method: Holm-Bonferroni) (Fig. 3C, Table S3). The percentage of dry mass increased progressively during our sampling campaign, likely due to an increase of ambient seawater salinity, resulting in higher salt content in jellyfish due to osmoregulation (Hirst & Lucas, 1998). Additionally, the maximum percentage of carbon and nitrogen in the dry mass was measured for the population collected in the second half of October (Table S1), coinciding with the recorded peak of chlorophyll *a* concentration. A higher concentration of chlorophyll a in ambient seawater implies a more productive system and could be indicative of higher zooplankton prey abundance. Moreover, the annual pattern of zooplankton biomass in our study area typically exhibits a bimodal distribution with a peak in spring and secondary peak in autumn (Mozetič et al., 2012).

Egg production is highly variable within population.

The number of eggs produced varied greatly among individuals ~~in our fecundity experiments~~, with approximately half of them not producing any eggs despite being homogenous in biometric properties. The percentage of non-egg-producing individuals was higher in autumn compared to summer months, but no correlation with environmental variables was found. The average number of eggs produced was 165 ± 179, lower than previously reported for the northern Adriatic Sea (4320 ± 3980 eggs, (Malej et al., 2017) or other areas in Mediterranean Sea, where reproductive output of animals freshly collected from the natural environment was assessed (Table 2). ~~When comparing different studies, it's important to consider previous findings~~. For instance, it has been previously noted that laboratory-reared animals never reached the maximum daily egg production observed in animals collected from their natural environment (Baker &

Reeve, 1974). Egg production variability has been observed across different invaded and native areas; in the northern Europe (e.g., 3000 eggs ind^{-1} day^{-1}; (Javidpour et al., 2009); maximum rates of 11 232 eggs ind^{-1} day^{-1}, (Jaspers, Costello & Colin, 2015) or in the southern Europe (e.g., 12 000 eggs ind^{-1} day^{-1}, (Zaika & Revkov, 1994)). A study in the Black, Azov and Caspian Seas (Shiganova, 2020) found that the number of eggs laid per individual per day was related to salinity and temperature. The egg production of *M. leidyi* also varied greatly in the native areas, e.g. with 0 to 9910 eggs ind^{-1} day^{-1} (Baker & Reeve, 1974) and a maximum of up to 14 233 eggs ind^{-1} day^{-1} (Kremer, 1976).

Some hypothesize that the transition towards oligotrophy (Mozetič et al., 2012) in the northern Adriatic may negatively impact ctenophores fecundity (Ciglenečki et al., 2021)). However, the lower reproductive performance observed in our study compared to measurements in the initial year of *M. leidyi* colonization in the Northern Adriatic Sea (Malej et al., 2017) could be contributed to a decline in invasive opportunistic traits since the invasion. Nonetheless, Jaspers and her colleagues (Jaspers et al., 2018) observed no such effect and explained the persistent invasive traits in *Mnemiopsis* through multiple reinvasions and a large variation in reproductive traits in the (native) source population.

The observed variability can be due to varying size of individuals selected in those studies, as eggs produced generally increases with size (Sasson & Ryan, 2016). In our study individuals of specific size were deliberately selected to minimize the effect of body size. This allowed us to investigate how changing environmental conditions affect ctenophore reproductive potential, but no significant correlations were found between egg production, biometric/chemical characteristics, or environmental variables. There was significant correlation between the percentage of hatched eggs and ambient seawater temperature and wet weight of ctenophores

(Figure 5C, D). However, no significant correlation was found between the percentage of hatched eggs and individual chemical characteristics or environmental variables. Thus, our hypothesis that changing environmental variables and biometric/chemical characteristics affect ctenophore reproduction is partly supported. The positive correlation between wet weight and the percentage of hatched eggs aligns with the general trend of increased egg viability with individual size (Sasson & Ryan, 2016). The percentage of hatched eggs reached over 80 % at temperature around 25 °C in our study but dropped significantly in the late October when the seawater temperature decreased. It is possible that lower temperatures slowed embryo development, consistent with findings in other studies showing increased development speed with temperature up to an optimum of around 25°C (Sullivan & Gifford, 2004; Gambill, Møller & Peck, 2015).

Conclusions and future perspectives

Our analysis of the chemical composition of the northern Adriatic *M. leidyi* population revealed stable carbon and nitrogen content throughout its bloom development, with no significant correlation observed with ambient seawater temperature, salinity, oxygen, and chlorophyll a concentration. However, maximum carbon and nitrogen content coincided with a shift towards a more productive system, possibly due to higher prey abundance. It's important to note that our study focused only on the blooming period of *M. leidyi* in the northern Adriatic, excluding individuals present during winter to spring. The number of eggs produced per individual was lower than previously reported for the same area and other invaded Mediterranean Sea basins and exhibited high variability despite the homogeneity of the studied population. This suggests the need to consider a broader range of environmental factors influencing ctenophore fecundity. We observed a positive correlation between the percentage of hatched eggs and ambient seawater temperature as well as between the wet weight of individuals and the percentage of developed eggs. By investigating the factors influencing the chemical composition and egg production of gelatinous zooplankton, we enhance our understanding of microbe-gelatinous-OM interactions, vital for accurately incorporating the gelatinous component into oceanic biogeochemical budgets. However, further examination at the individual molecular compound level is warranted to understand how ctenophores contribute to the ambient dissolved organic matter pool, which in turn shapes the structure and function of microbial communities and drives biogeochemical cycles in the marine food web.

Acknowledgment

We would like to thank to the staff of Marine Biology Station Piran and crew of RV Carolina for their help with sampling.

References

Azam F, Malfatti F. 2007. Microbial structuring of marine ecosystems. Nature Reviews Microbiology 5:782–791. DOI: 10.1038/nrmicro1747.

Baker LD, Reeve MR. 1974. Laboratory culture of the lobate ctenophore Mnemiopsis mccradyi with notes on feeding and fecundity. Marine Biology 26:57–62. DOI: 10.1007/BF00389086.

Budiša A, Paliaga P, Juretić T, Lučić D, Supić N, Pasarić Z, Djakovac T, Mladinić M, Dadić V, Tičina V. 2021. Distribution, diet and relationships of the invasive ctenophore Mnemiopsis leidyi with anchovies and zooplankton, in the northeastern Adriatic Sea. Mediterranean Marine Science 22:827–842. DOI: 10.12681/mms.23305.

Ciglenečki I, Paliaga P, Budiša A, Čanković M, Dautović J, Djakovac T, Dutour-Sikirić M, Kraus R, Kužat N, Lučić D, Pfannkuchen DM, Njire J, Pasarić Z, Supić N. 2021. Dissolved organic carbon accumulation during a bloom of invasive gelatinous zooplankton Mnemiopsis leidyi in the northern Adriatic Sea; case of the anomalous summer in 2017. Journal of Marine Systems 222:103599. DOI: https://doi.org/10.1016/j.jmarsys.2021.103599.

Colin SP, Costello JH, Hansson LJ, Titelman J, Dabiri JO. 2010. Stealth predation and the predatory success of the invasive ctenophore Mnemiopsis leidyi. Proceedings of the National Academy of Sciences 107:17223–17227. DOI: 10.1073/pnas.1003170107.

Condon RH, Steinberg DK, Bronk DA. 2010. Production of dissolved organic matter and inorganic nutrients by gelatinous zooplankton in the York River estuary, Chesapeake Bay. Journal of Plankton Research 32:153–170. DOI: 10.1093/plankt/fbp109.

Costello JH, Bayha KM, Mianzan HW, Shiganova TA, Purcell JE. 2012. Transitions of Mnemiopsis leidyi (Ctenophora: Lobata) from a native to an exotic species: a review. Hydrobiologia 690:21–46. DOI: 10.1007/s10750-012-1037-9.

Costello JH, Sullivan BK, Gifford DJ, Van Keuren D, Sullivan LJ. 2006. Seasonal refugia, shoreward thermal amplification, and metapopulation dynamics of the ctenophore Mnemiopsis leidyi in Narragansett Bay, Rhode Island. Limnology and Oceanography 51:1819–1831. DOI: https://doi.org/10.4319/lo.2006.51.4.1819.

Dinasquet J, Granhag L, Riemann L. 2012. Stimulated bacterioplankton growth and selection for certain bacterial taxa in the vicinity of the ctenophore Mnemiopsis leidyi. Frontiers in Microbiology 3. DOI: 10.3389/fmicb.2012.00302.

Dinasquet J, Kragh T, Schrøter ML, Søndergaard M, Riemann L. 2013. Functional and compositional succession of bacterioplankton in response to a gradient in bioavailable dissolved organic carbon. Environmental Microbiology 15:2616–2628. DOI: 10.1111/1462-2920.12178.

Edgar A, Miguel Ponciano J, Martindale MQ. 2022. Ctenophores are direct developers that reproduce continuously beginning very early after hatching. DOI: 10.1073/pnas.

Fadeev E, H HJ, Chie A, Zihao Z, Katja K, J HG, Tinkara T. 2024. Bacterial degradation of ctenophore Mnemiopsis leidyi organic matter. mSystems 0:e01264-23. DOI: 10.1128/msystems.01264-23.

Fiori E, Benzi M, Ferrari CR, Mazziotti C. 2019. Zooplankton community structure before and after Mnemiopsis leidyi arrival. Journal of Plankton Research 41:803–820. DOI: 10.1093/plankt/fbz060.

Gambill M, Møller LF, Peck MA. 2015. Effects of temperature on the feeding and growth of the larvae of the invasive ctenophore Mnemiopsis leidyi. Journal of Plankton Research 37:1001–1005. DOI: 10.1093/plankt/fbv039.

Hirst AG, Lucas CH. 1998. Salinity influences body weight quantification in the scyphomedusa Aurelia aurita: Important implications for body weight determination in gelatinous zooplankton. Marine Ecology Progress Series 165:259–269. DOI: 10.3354/meps165259.

Jaspers C, Bezio N, Hinrichsen H-H. 2021. Diversity and Physiological Tolerance of Native and Invasive Jellyfish/Ctenophores along the Extreme Salinity Gradient of the Baltic Sea. Diversity 13. DOI: 10.3390/d13020057.

Jaspers C, Costello JH, Colin SP. 2015. Carbon content of Mnemiopsis leidyi eggs and specific egg production rates in northern Europe. Journal of Plankton Research 37:11–15. DOI: 10.1093/plankt/fbu102.

Jaspers C, Huwer B, Antajan E, Hosia A, Hinrichsen H-H, Biastoch A, Angel D, Asmus R, Augustin C, Bagheri S, Beggs SE, Balsby TJS, Boersma M, Bonnet D, Christensen JT, Dänhardt A, Delpy F, Falkenhaug T, Finenko G, Fleming NEC, Fuentes V, Galil B, Gittenberger A, Griffin DC, Haslob H, Javidpour J, Kamburska L, Kube S, Langenberg VT, Lehtiniemi M, Lombard F, Malzahn A, Marambio M, Mihneva V, Møller LF, Niermann U, Okyar MI, Özdemir ZB, Pitois S, Reusch TBH, Robbens J, Stefanova K, Thibault D, van der Veer HW, Vansteenbrugge L, van Walraven L, Woźniczka A. 2018. Ocean current connectivity propelling the secondary spread of a marine invasive comb jelly across western Eurasia. Global Ecology and Biogeography 27:814–827. DOI: https://doi.org/10.1111/geb.12742.

Jaspers C, Møller LF, Kiørboe T. 2015. Reproduction rates under variable food conditions and starvation in Mnemiopsis leidyi: significance for the invasion success of a ctenophore. Journal of Plankton Research 37:1011–1018. DOI: 10.1093/plankt/fbv017.

Javidpour J, Molinero JC, Peschutter J, Sommer U. 2009. Seasonal changes and population dynamics of the ctenophore Mnemiopsis leidyi after its first year of invasion in the Kiel Fjord, Western Baltic Sea. Biological Invasions 11:873–882. DOI: 10.1007/s10530-008-9300-8.

Javidpour J, Molinero J-C, Ramírez-Romero E, Roberts P, Larsen T. 2020. Cannibalism makes invasive comb jelly, Mnemiopsis leidyi, resilient to unfavourable conditions. Communications Biology 3:212. DOI: 10.1038/s42003-020-0940-2.

Kogovšek T, Tinta T, Klun K, Malej A. 2014. Jellyfish biochemical composition: Importance of standardised sample processing. Marine Ecology Progress Series 510:275–288. DOI: 10.3354/meps10959.

Kogovšek T, Vodopivec M, Raicich F, Uye S ichi, Malej A. 2018. Comparative analysis of the ecosystems in the northern Adriatic Sea and the Inland Sea of Japan: Can anthropogenic pressures disclose jellyfish outbreaks? Science of the Total Environment 626:982–994. DOI: 10.1016/j.scitotenv.2018.01.011.

Kremer P. 1976. POPULATION DYNAMICS AND ECOLOGICAL ENERGETICS OF A PULSED ZOOPLANKTON PREDATOR, THE CTENOPHORE MNEMIOPSIS LEIDYI. In: WILEY M ed. Estuarine Processes. Academic Press, 197–215. DOI: https://doi.org/10.1016/B978-0-12-751801-5.50024-1.

Lucas CH, Pitt KA, Purcell JE, Lebrato M, Condon RH. 2011. What's in a jellyfish? Proximate and elemental composition and biometric relationships for use in biogeochemical studies Ecological Archives E092-144.

Malej A, Tirelli V, Lučić D, Paliaga P, Vodopivec M, Goruppi A, Ancona S, Benzi M, Bettoso N, Camatti E, Ercolessi M, Ferrari CR, Shiganova T. 2017a. Mnemiopsis leidyi in the northern

Adriatic: here to stay? Journal of Sea Research 124:10–16. DOI: https://doi.org/10.1016/j.seares.2017.04.010.

Malej A, Tirelli V, Lučić D, Paliaga P, Vodopivec M, Goruppi A, Ancona S, Benzi M, Bettoso N, Camatti E, Ercolessi M, Ferrari CR, Shiganova T. 2017b. Mnemiopsis leidyi in the northern Adriatic: here to stay? Journal of Sea Research 124:10–16. DOI: https://doi.org/10.1016/j.seares.2017.04.010.

McNamara ME, Lonsdale DJ, Cerrato RM. 2013. Top-down control of mesozooplankton by adult Mnemiopsis leidyi influences microplankton abundance and composition enhancing prey conditions for larval ctenophores. Estuarine, Coastal and Shelf Science 133:2–10. DOI: https://doi.org/10.1016/j.ecss.2013.04.019.

Mozetič P, Francé J, Kogovšek TŠ, Talaber I, Malej A. 2012. Plankton trends and community changes in a coastal sea (northern Adriatic): Bottom-up vs. top-down control in relation to environmental drivers. Estuarine, Coastal and Shelf Science 115:138–148. DOI: 10.1016/j.ecss.2012.02.009.

Oguz T, Fach B, Salihoglu B. 2008. Invasion dynamics of the alien ctenophore Mnemiopsis leidyi and its impact on anchovy collapse in the Black Sea. Journal of Plankton Research 30:1385–1397. DOI: 10.1093/plankt/fbn094.

Paliaga P, Budiša A, Dautović J, Djakovac T, Dutour-Sikirić MA, Mihanović H, Supić N, Celić I, Iveša N, Buršić M, Balković I, Jurković L, Ciglenečki I. 2021. Microbial response to the presence of invasive ctenophore Mnemiopsis leidyi in the coastal waters of the Northeastern Adriatic. Estuarine, Coastal and Shelf Science 259:107459. DOI: https://doi.org/10.1016/j.ecss.2021.107459.

Pestorić B, Lučić D, Bojanić N, Vodopivec M, Kogovšek T, Violić I, Paliaga P, Malej A. 2021. Scyphomedusae and Ctenophora of the Eastern Adriatic: Historical Overview and New Data. Diversity 13. DOI: 10.3390/d13050186.

Pitt KA, Welsh DT, Condon RH. 2009. Influence of jellyfish blooms on carbon, nitrogen and phosphorus cycling and plankton production. In: Hydrobiologia. 133–149. DOI: 10.1007/s10750-008-9584-9.

Sasson DA, Ryan JF. 2016. The sex lives of ctenophores: the influence of light, body size, and self-fertilization on the reproductive output of the sea walnut, Mnemiopsis leidyi. PeerJ 4:e1846. DOI: 10.7717/peerj.1846.

Schroeder A, Camatti E, Pansera M, Pallavicini A. 2023. Feeding pressure on meroplankton by the invasive ctenophore Mnemiopsis leidyi. Biological Invasions 25:2007–2021. DOI: 10.1007/s10530-023-03023-5.

Shiganova TA. 2020. Adaptive strategies of Mnemiopsis leidyi A. Agassiz 1865 in different environments of the Eurasian seas. Marine Pollution Bulletin 161:111737. DOI: https://doi.org/10.1016/j.marpolbul.2020.111737.

Shiganova TA, Mikaelyan AS, Moncheva S, Stefanova K, Chasovnikov VK, Mosharov SA, Mosharova IN, Slabakova N, Mavrodieva R, Stefanova E, Zasko DN, Dzhurova B. 2019a. Effect of invasive ctenophores Mnemiopsis leidyi and Beroe ovata on low trophic webs of the Black Sea ecosystem. Marine Pollution Bulletin 141:434–447. DOI: https://doi.org/10.1016/j.marpolbul.2019.02.049.

Shiganova TA, Sommer U, Javidpour J, Molinero JC, Malej A, Kazmin AS, Isinibilir M, Christou E, Siokou- Frangou I, Marambio M, Fuentes V, Mirsoyan ZA, Gülsahin N, Lombard F, Lilley MKS, Angel DL, Galil BS, Bonnet D, Delpy F. 2019b. Patterns of invasive ctenophore Mnemiopsis leidyi distribution and variability in different recipient environments of the Eurasian seas: A review. Marine Environmental Research 152:104791. DOI: https://doi.org/10.1016/j.marenvres.2019.104791.

Shiganova Tamara A. and Christou ED and BJV and S-FI and ZS and SA. 2004. Distribution and biology of Mnemiopsis leidyi in the Northern Aegean Sea, and comparison with the indigenous Bolinopsis vitrea. In: Dumont Henri and Shiganova TA and NU ed. Aquatic Invasions in the Black, Caspian, and Mediterranean Seas. Dordrecht: Springer Netherlands, 113–135.

Stanlaw KA, Reeve MR, Walter MA. 1981. Growth, food, and vulnerability to damage of the ctenophore Mnemiopsis mccradyi in its early life history stages. Limnology and Oceanography 26:224–234. DOI: 10.4319/lo.1981.26.2.0224.

Sullivan LJ, Gifford DJ. 2004. Diet of the larval ctenophore Mnemiopsis leidyi A. Agassiz (Ctenophora, Lobata). Journal of Plankton Research 26:417–431. DOI: 10.1093/plankt/fbh033.

Tinta T, Zhao Z, Bayer B, Herndl GJ. 2023. Jellyfish detritus supports niche partitioning and metabolic interactions among pelagic marine bacteria. Microbiome 11:156. DOI: 10.1186/s40168-023-01598-8.

Tinta T, Zhao Z, Escobar A, Klun K, Bayer B, Amano C, Bamonti L, Herndl GJ. 2020. Microbial Processing of Jellyfish Detritus in the Ocean. Frontiers in Microbiology 11. DOI: 10.3389/fmicb.2020.590995.

Zaika VE, Revkov NK. 1994. Anatomy of gonads and regime of spawning of of ctenophore Mnemiopsis sp. in the Black Sea. Zoologičeskij žurnal 73:5–10.

Figure Legends:

Figure 1: **Study area.** The Gulf of Trieste, located in the northernmost basin of the Adriatic Sea. Ctenophore sampling areas are highlighted in light red. Oceanographic buoy Vida is marked with red star.

Figure 2: **Dynamics of environmental parameters**. Dynamics of environmental parameters at 3 m depth at the reference station Oceanographic buoy Vida, located in the middle of the Gulf of Trieste, throughout our sampling campaign. **A** – ambient seawater temperature (°C, in red) and salinity (in blue); **B** – Chlorophyll *a* (µg mL^{-1}, in green) and oxygen (mg L^{-1}, in grey) concentration.

Figure 3: **Dynamics of wet and dry weight of studied ctenophore population**. Dynamics of **A** - Wet weight (WW in g), **B** - percentage of Dry Weight (%DW) and **C** - relationship between percentage of Dry Weight (%DW) and Salinity in the ctenophore population collected between August and October 2021 in the Gulf of Trieste, northern Adriatic Sea.

Figure 4: **Dynamic of carbon and nitrogen content of studied ctenophore population**. The percentage of carbon and nitrogen (**A**) and the carbon to nitrogen molar ratio (**B**) in the ctenophore population collected between August and October 2021 in the Gulf of Trieste, northern Adriatic Sea.

Figure 5: **Dynamic of produced and hatched eggs of studied ctenophore population** Total number of eggs produced (**A**) and percentage of hatched eggs (**B**) by ctenophores collected from August until October 2021 in the northern Adriatic Sea. Correlation between percentage of hatched eggs and (**C**) ambient seawater temperature and (**D**) wet weight of individuals. All individuals, also those that did not produce any eggs are considered.

Table Legends:

Table 1: **Biological and chemical characteristics of studied ctenophore population**. Biological and chemical characteristics of the subset of ctenophore samples selected for egg production experiments with total number of eggs produced and percentage of hatched eggs after 24h per individual and average for each experiment.

Table 2: **Egg production of field-collected *Mnemiopsis leidyi* from native areas and the Mediterranean Sea.**

Figure 1

Study area.

Study area, the Gulf of Trieste, located in the northernmost basin of the Adriatic Sea. Ctenophore sampling areas are highlighted in light red. Oceanographic buoy Vida is marked with red star.

https://about.google/brand-resource-center/products-and-services/geo-guidelines/#google-maps-google-earth-and-street-view

Figure 2

Dynamics of environmental parameters

Dynamics of environmental parameters at 3 m depth at the reference station Oceanographic buoy Vida, located in the middle of the Gulf of Trieste, throughout our sampling campaign. **A** – ambient seawater temperature (°C, in red) and salinity (in blue); **B** – Chlorophyll *a* (µg mL^{-1}, in green) and oxygen (mg L^{-1}, in grey) concentration.

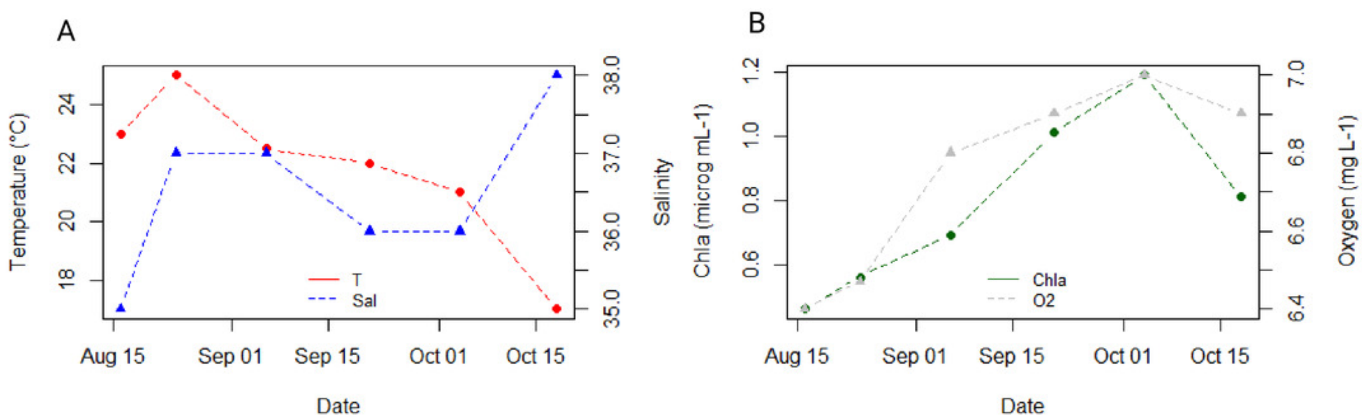

Figure 3

Dynamics of wet and dry weight of studied ctenophore population.

Dynamics of **A** - Wet weight (WW in g), **B** - percentage of Dry Weight (%DW) and **C** - relationship between percentage of Dry Weight (%DW) and Salinity in the ctenophore population collected between August and October 2021 in the Gulf of Trieste, northern Adriatic Sea.

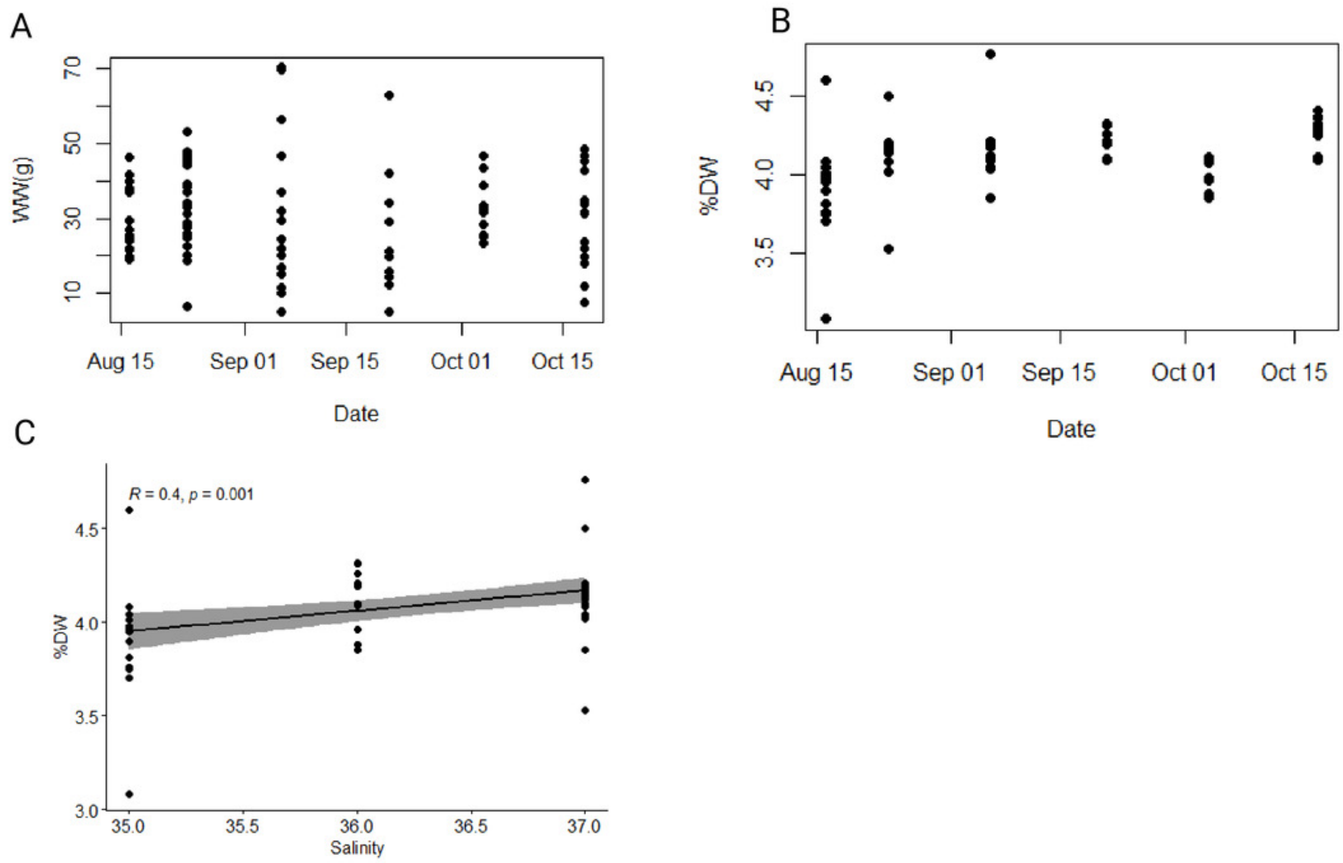

Figure 4

Dynamic of carbon and nitrogen content of studied ctenophore population

The percentage of carbon and nitrogen (**A**) and the carbon to nitrogen molar ratio (**B**) in the ctenophore population collected between August and October 2021 in the Gulf of Trieste, northern Adriatic Sea.

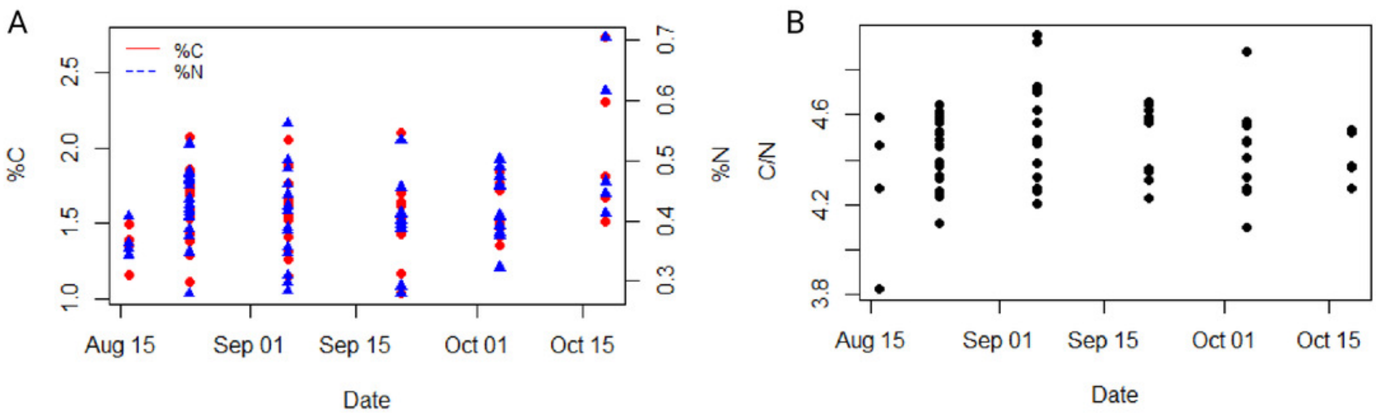

Figure 5

Dynamic of produced and hatched eggs of studied ctenophore population

Total number of eggs produced (**A**) and percentage of hatched eggs (**B**) by ctenophores collected from August until October 2021 in the northern Adriatic Sea. Correlation between percentage of hatched eggs and (**C**) ambient seawater temperature and (**D**) wet weight of individuals. All individuals, also those that did not produce any eggs are considered.

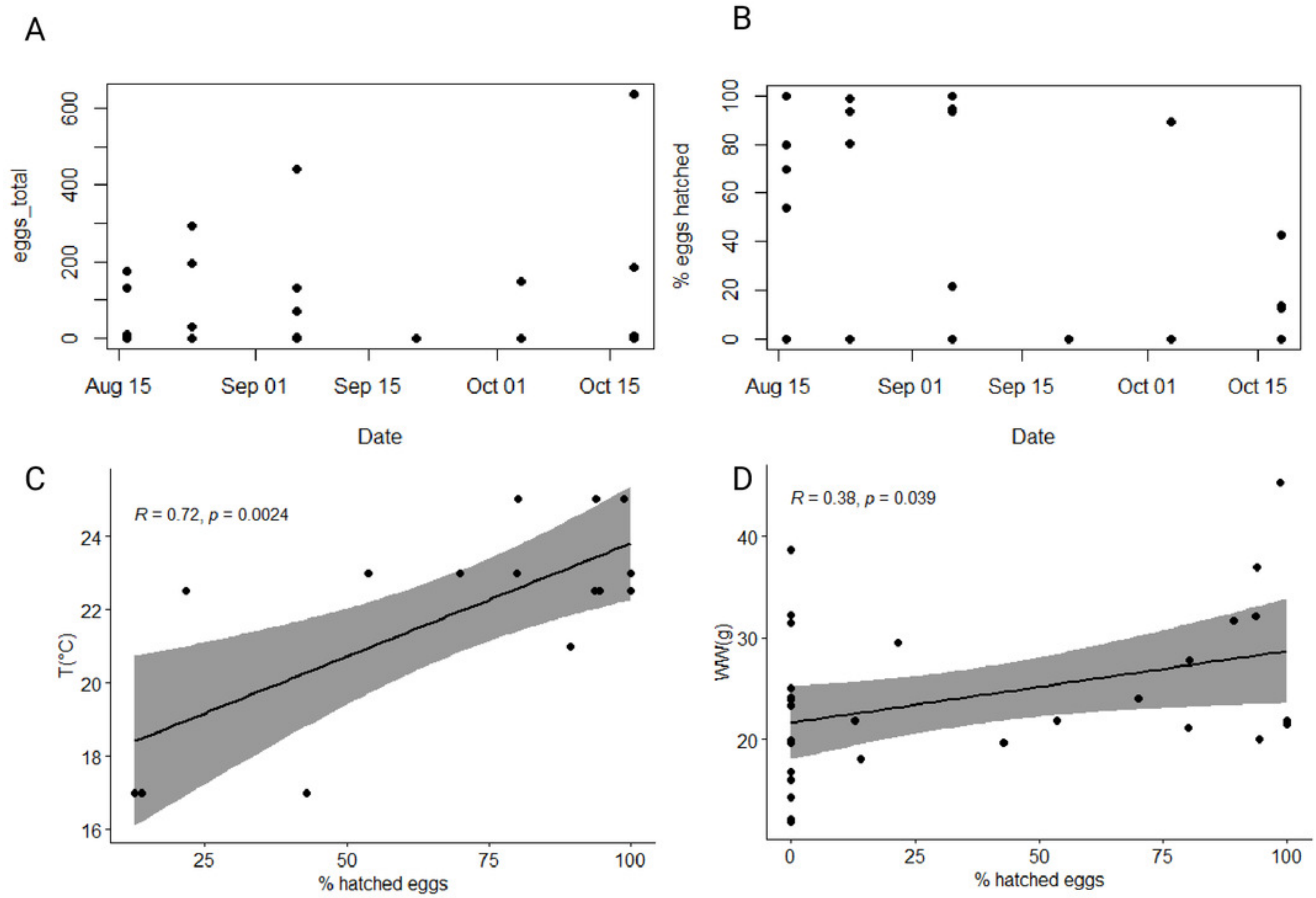

Table 1 (on next page)

Biological and chemical characteristics of studied ctenophore population

Biological and chemical characteristics of the subset of ctenophore samples selected for egg production experiments with total number of eggs produced and percentage of hatched eggs after 24h per individual and average for each experiment.

Table 1: Biological and chemical characteristics of the subset of ctenophore samples selected for egg production experiments with total number of eggs produced and percentage of hatched eggs after 24h per individual and average for each experiment.

Date	**T (°C)**	**Sal**	**chl *a* (ug mL^{-1})**	**O_2 (mg L^{-1})**	**WW (g)**	**DW (g)**	**%DW**	**%N**	**mg N ind^{-1}**	**%C**	**mg C ind^{-1}**	**C:N**	**eggs_total**	**% hatched eggs in 24h**
16.08.2023	23	35	0.46	6.4	21.86	0.81	3.7	0.36	2.92	1.39	11.26	4.5	177	54
					21.57	0.81	3.75	0.34	2.75	1.35	10.94	4.6	9	100
					24.00	0.74	3.08	0.35	2.59	1.16	8.58	3.8	133	70
					19.76	1.53	4.6	0.42	6.43	1.58	24.17	4.4	0	n.a.
					21.16	1.02	3.76	0.41	4.18	1.50	15.30	4.3	5	80
avg±sd					**21.7±1.53**	**1.0±0.32**	**3.8±0.54**	**0.4±0.04**	**3.7±1.6**	**1.4±0.16**	**14.1±6.1**	**4.3±0.31**	**64.8±83.9**	**75.9±19.4**
24.08.2023	25	37	0.56	6.47	27.75	1.25	4.50	0.41	5.13	1.57	19.63	4.5	197	80
					36.89	1.53	4.15	0.44	6.73	1.69	25.86	4.5	32	94
					24.16	1.03	4.17	0.38	3.91	1.43	14.73	4.5	0	n.a.
					45.26	1.88	4.15	0.53	9.96	2.07	38.92	4.6	295	99
					31.44	1.11	3.53	0.41	4.55	1.43	15.87	4.1	0	n.a.
avg±sd					**33.1±8.27**	**1.4±0.35**	**4.1±0.35**	**0.4±0.06**	**6.1±2.4**	**1.6±0.26**	**23.0±9.9**	**4.4±0.19**	**104.8±134.1**	**90.9±9.6**
06.09.2021	22.5	37	0.69	6.8	16.85	0.71	4.21	0.39	2.77	1.52	10.79	4.6	0	n.a.
					21.84	1.04	4.76	0.28	2.91	1.15	11.96	4.7	3	100
					32.17	1.24	3.85	0.46	5.70	1.77	21.95	4.5	443	94
					29.56	1.21	4.09	0.43	5.20	1.53	18.51	4.2	130	22
					20.09	0.84	4.18	0.36	3.02	1.32	11.09	4.3	72	94
avg±sd					**24.1±6.49**	**1.0±0.23**	**4.2±0.33**	**0.4±0.07**	**3.9±1.4**	**1.5±0.23**	**14.9±5.1**	**4.5±0.21**	**129.6±183.3**	**77.4±37.4**
21.09.2021	22	36	1.01	6.9	16.00	0.69	4.31	0.39	2.69	1.54	10.63	4.6	1	0
					19.97	0.85	4.26	0.39	3.32	1.55	13.18	4.7	0	n.a.
					12.20	0.52	4.26	0.28	1.46	1.03	5.36	4.3	1	0
					19.72	0.84	4.26	0.41	3.44	1.61	13.52	4.6	0	n.a.
					14.38	0.62	4.31	0.46	2.85	1.7	10.54	4.4	0	n.a.
avg±sd					**16.5±3.38**	**0.7±0.14**	**4.3±0.03**	**0.4±0.07**	**2.8±0.8**	**1.5±0.26**	**10.6±3.3**	**4.5±0.16**	**0.4±0.5**	**0.0±0.0**
04.10.2021	21	36	1.19	7	32.28	1.28	3.96	0.41	5.25	1.51	19.33	4.3	0	n.a
					38.66	1.54	3.98	0.39	6.01	1.48	22.79	4.4	0	n.a
					31.66	1.3	4.11	0.38	4.94	1.41	18.33	4.3	149	89.26
					25.01	0.99	3.96	0.5	4.95	1.34	13.27	4.3	0	n.a
					23.4	0.93	3.97	0.41	3.81	1.5	13.95	4.3	0	n.a
avg±sd					**30.2±6.15**	**1.2±0.25**	**4.0±0.06**	**0.4±0.05**	**4.9±0.8**	**1.4±0.07**	**17.5±3.9**	**4.3±0.04**	**29.8±66.6**	**89.3**
18.10.2021	17	38	0.81	6.9	23.93	1.03	4.3	0.41	4.22	1.51	15.55	4.3	0	0
					11.92	0.52	4.36	0.71	3.69	2.73	14.20	4.5	0	0
					21.81	0.96	4.4	0.44	4.22	1.67	16.03	4.4	638	12.7

					18.09	0.79	4.37	0.62	4.90	2.31	18.25	4.4	187	13.9
					19.69	0.85	4.37	0.46	3.91	1.81	15.39	4.5	7	42.86
avg±sd					**19.1±4.57**	**0.8±0.20**	**4.4±0.04**	**0.5±0.13**	**4.2±0.5**	**2.0±0.50**	**15.9±1.5**	**4.4±0.08**	**166.4±275.5**	**23.2±17.1**

Note that average percentage of hatched eggs is calculated by considering only those individuals that produced eggs. T- temperature (°C); Chl a – chlorophyll a concentration; O_2 – oxygen concentration; WW – wet weight; DW – dry weight; mg N ind^{-1} – mg of nitrogen per individual specimen; mg C ind^{-1} – mg of carbon per individual specimen; C:N – carbon to nitrogen molar ratio.

Table 2(on next page)

Egg production of field-collected *Mnemiopsis leidyi* from native areas and the Mediterranean Sea

T = temperature, TLB = total body length, No. eggs = number of eggs produced by individual in 24 hours, % ind. eggs = percentage of individuals that produced eggs, % hatching = percentage of hatched eggs after 24 hours

Table 2: Egg production of field-collected *Mnemiopsis leidyi* from native areas and the Mediterranean Sea.

Native areas	T (^{0}C)	TBL (cm)	No. eggs	% ind. eggs	% hatching
Narragansett Bay (Costello et al., 2006)	6 – 25		0 – 3300	59	n.d.
Narragansett Bay (Kremer, 1976)	11 – 29		0 – 14 000		
Biscayne Bay (Baker & Reeve, 1974)	21 – 31	3.8 – 8.5	0 – 9990	90	n.d.
Biscayne Bay (Stanlaw, Reeve & Walter, 1981)	21		up to 10 000	most individuals	most eggs
Mediterranean Sea					
Aegean Sea (Shiganova et al., 2004)	21 – 25	1.7 – 3.4	0 – 448	75	82
Northern Adriatic (Malej et al., 2017)	20 – 22	5.4 – 11.5	136 – 13,512	100	n.d.
Northern Adriatic (Kogovšek et al., 2018)	20	4.1 – 9.8	0 – 1400	65	n.d.
This study	17 - 25	5 – 7	0 – 638	57	12 – 100

T = temperature, TLB = total body length, No. eggs = number of eggs produced by individual in 24 hours, % ind. eggs = percentage of individuals that produced eggs, % hatching = percentage of hatched eggs after 24 hours