

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

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ABSTRACT

High abundances of gelatinous zooplankton (GZ) can significantly impact marine ecosystem by acting as both sink and source of organic matter (OM) and nutrients. The decay of GZ bloom can introduce significant amount of OM to the ocean interior, with its variability influenced by GZ life traits and environmental factors, impacting microbial communities vital to marine biogeochemical cycles. The invasive ctenophores *Mnemiopsis leidyi* has formed 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 seawater temperature, salinity, oxygen, and chlorophyll *a* concentration. Although the 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 the study area and other Mediterranean areas. 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 decreasing seawater temperature in autumn, corresponding to the end of *M. leidyi* bloom.

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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, such as ecological plasticity regarding environmental conditions, self-fertilization ability, and cannibalism (*Baker & Reeve, 1974; Jaspers, Møller & Kiørboe, 2015; Javidpour et al., 2020*). 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). 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; Dinasquet, Granhag & Riemann, 2012). The decay of ctenophore blooms can lead to a large influx of ctenophore-derived OM, disrupting the quality and quantity of the surrounding seawater OM reservoir (Fadeev et al., 2024). The dissolved fraction of gelatinous zooplankton-derived OM, rich in proteins and characterized by low C:N ratio, is rapidly degraded by opportunistic microbes, swiftly assimilated into bacterial biomass, and is 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 (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). 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 (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 the 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 wet and dry mass, carbon and nitrogen content, and conducting 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 directly to the laboratory, maintaining *in situ* temperature and light conditions. Between 15–20 individuals were collected during each sampling survey (Table S1). We acknowledge that a larger sampling effort would provide a more robust statistical analysis; however, we had to adjust the number of samples due to operational constraints. For each sampling, we obtained data on seawater temperature, salinity, dissolved oxygen and chlorophyll a concentrations from a depth of 3 m 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 24 h 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 ($d = 0.01$ mg (80 g), $d = 0.1$ mg (220 g)). 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, Columbus, OH, USA). Elemental composition of carbon (C) and nitrogen (N) was determined after combustion at 1150°C (Vario Micro Cube elemental analyser; Elementar, Long Island, NY, USA) 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

For each sampling, a batch of five 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, Table S2). Each of the five selected specimens was placed individually into a 1 L glass Erlenmeyer flask filled with 800 mL of pre-filtered seawater (using GF/F Whatman filters) and covered with parafilm. Afterwards an incubation period of 20 h 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 $200\ \mu\text{m}$ and then examined using a small container with grid-patterned bottom. All the eggs and other developmental phase in each sample were counted after 24 h and 48 h of the 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 24 h per individual and average for each experiment.

Date	T (°C)	Sal	chl <i>a</i> (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
16.08.2021	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	
24.08.2021	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	

Notes.

Note that average percentage of hatched eggs is calculated by considering only those individuals that produced eggs.

T, temperature (°C); TLB, Chl *a*—chlorophyll *a* 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.



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. Map credit: Google.

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To determine the percentage of hatched eggs, we divided the number of hatched eggs after 24 h by the total number of eggs produced by each individual within that time frame. Once the experiment concluded, the ctenophores were sacrificed for further analysis of dry mass and elemental composition, as described above.

Statistics

All statistical analysis and visualizations were conducted using specialized packages in R (<http://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 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 (Fig. 2A). Chlorophyll concentration showed an increasing trend from 0.39 $\mu\text{g mL}^{-1}$ in August to 1.19 $\mu\text{g mL}^{-1}$ in the first half of October, with a slight decrease to 1 $\mu\text{g mL}^{-1}$ observed in the second half of October (Fig. 2B). Oxygen concentration in seawater was lowest in August (4.48 mg mL^{-1}) and highest in October (5.06 mg mL^{-1}) (Fig. 2B).

Biometric parameters and elemental composition of ctenophore populations

A total of 89 individuals was collected 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 $4.1 \pm 0.2\%$ of wet weight (Fig. 3B). Both minimum and

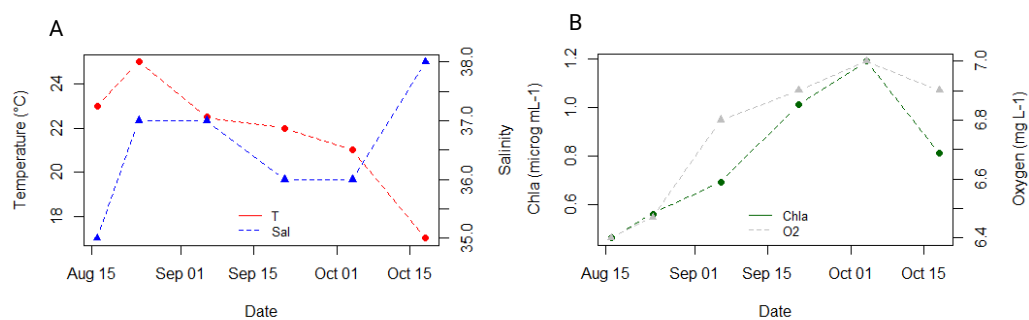


Figure 2 Dynamics of environmental parameters at 3 m depth at the reference station oceanographic buoy Vida, located in the middle of the Gulf of Trieste, throughout our sampling campaign. (A) Ambient seawater temperature (°C, in red) and salinity (in blue); (B) Chlorophyll *a* ($\mu\text{g mL}^{-1}$,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

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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 environmental variables affect the biometric properties of ctenophore populations, we calculated the Pearson correlation coefficient and found a significant correlation between salinity and the percentage of dry weight ($r = 0.40$, $p < 0.001^{**}$, Holm-Bonferroni adjustment), while no other environmental variable showed significant correlations (Fig. 3C, Table S3).

The average carbon and nitrogen percentage in the dry mass of all individuals of the studied ctenophore population were $1.59 \pm 0.29\%$ and $0.42 \pm 0.08\%$, 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 $4.46 \pm 0.19:1$, 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

The average wet mass (24.1 ± 7.8 g), dry mass percentage of WW ($4.1 \pm 0.3\%$), carbon ($1.57 \pm 0.33\%$) and nitrogen ($0.42 \pm 0.09\%$) content, and C:N ratio ($4.4 \pm 0.2:1$) of individuals selected for egg production experiment fell within the range of values measured for the total collected population in our study (Table 1, Tables 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

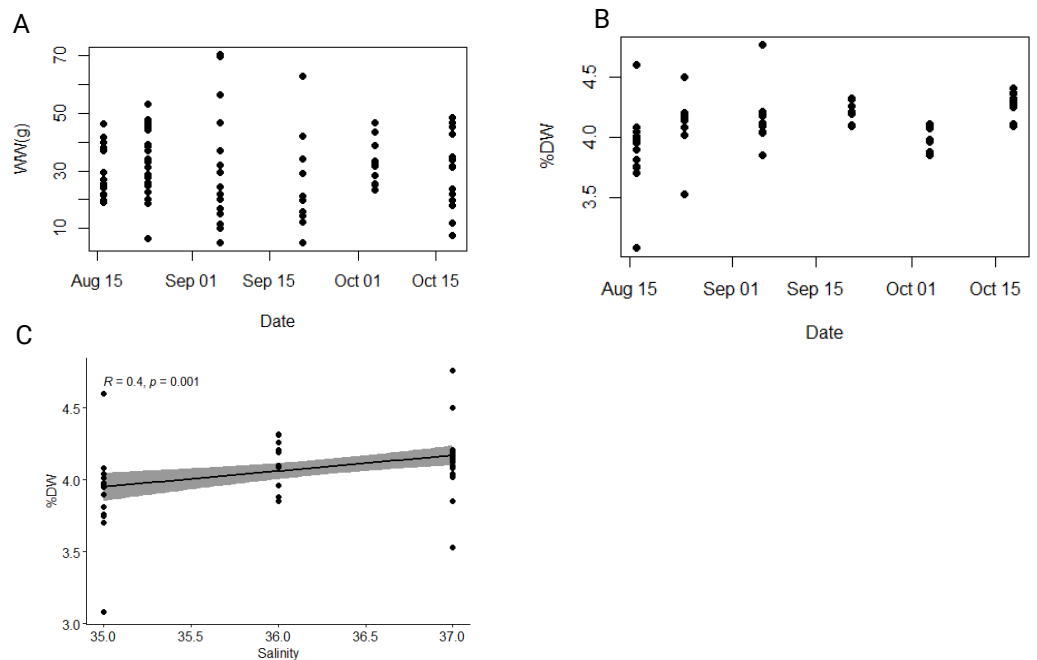


Figure 3 Dynamics of wet and dry weight of studied ctenophore population. Dynamics of (A) wet weight (WW in g), (B) percentage of dry weight (% DW) and (C) relationship between percentage of dry weight (% DW) and salinity in the ctenophore population collected between August and October 2021 in the Gulf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

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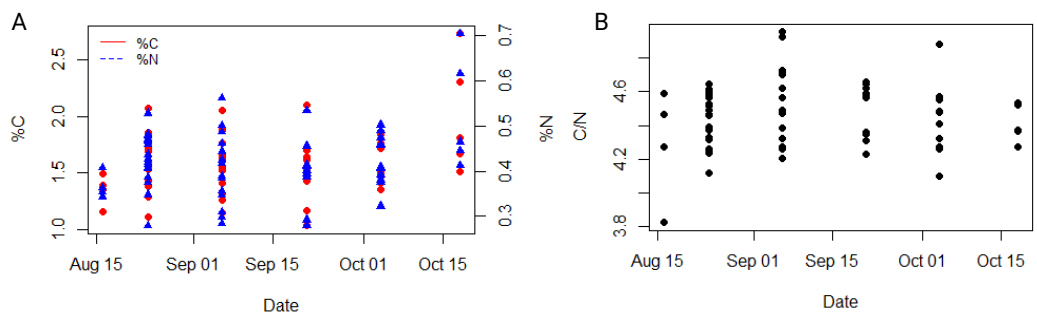


Figure 4 Dynamic of carbon and nitrogen content of studied ctenophore population. The percentage of carbon and nitrogen (A) and the carbon to nitrogen molar ratio (B) in the ctenophore population collected between August and October 2021 in the Gulf of Trieste, northern Adriatic Sea.

Full-size [DOI: 10.7717/peerj.17844/fig-4](https://doi.org/10.7717/peerj.17844/fig-4)

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).

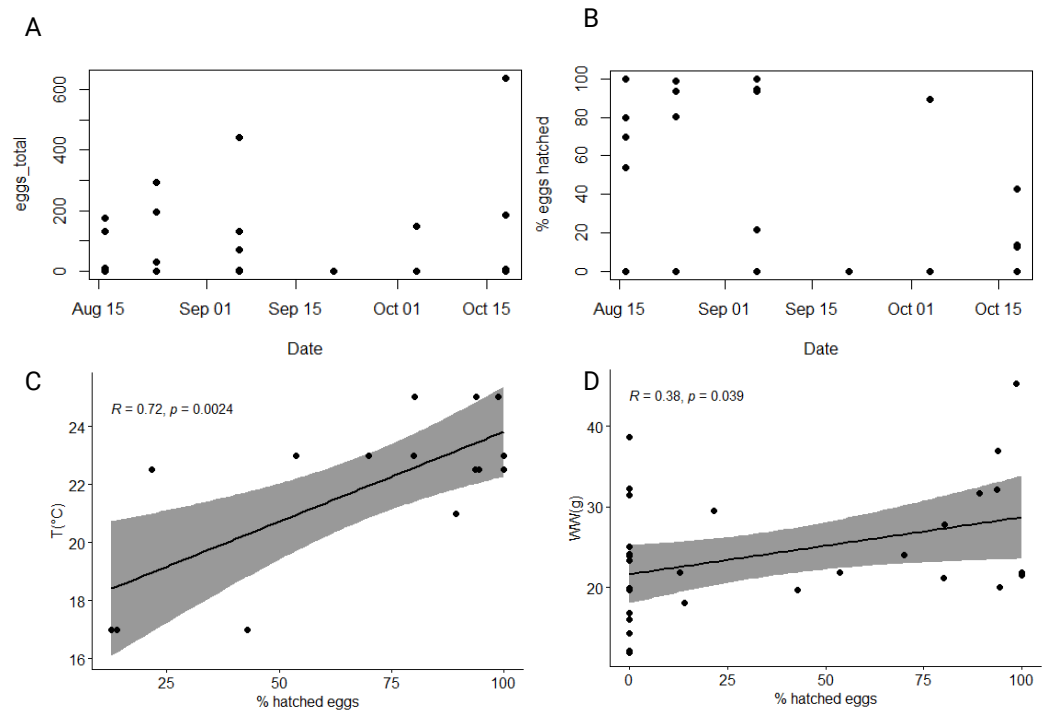


Figure 5 Dynamic of produced and hatched eggs of studied ctenophore population. Total number of eggs produced (A) and percentage of hatched eggs (B) by ctenophores collected from August until October 2021 in the northern Adriatic Sea. Correlation between percentage of hatched eggs and (C) ambient seawater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

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In our 48-hour fecundity experiment, most eggs had developed into cydippid larva stage by the end of the first 24 h. 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 $67\% \pm 32\%$, 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

Mnemiopsis leidyi populations begin to increase in late July, peaking between September and October with periodic large blooms, while individuals are rarely observed during the

colder part of the year (Malej et al., 2017; Budiša et al., 2021). During this period physical, chemical, and biological factors in the surrounding seawater changed (Fig. 2); however, no significant temporal trends were observed in wet weight, dry weight, carbon and nitrogen content, or carbon-to-nitrogen molar ratio in the sampled population (Figs. 3, 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). Based on our current data, we reject our hypothesis that changes in environmental variables are reflected in the carbon-to-nitrogen ratio of ctenophore biomass; however, more experiments are needed to conclude definitively that environmental variables have no effect on the chemical composition of ctenophores biomass. Nevertheless, a significant correlation between salinity and percentage of dry weight was found ($r = 0.40$, $p < 0.001^{**}$, Holm-Bonferroni adjustment) (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).

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, but no correlation with environmental variables was found, necessitating further experiments and additional analysis to explain this observation. The average number of eggs produced was 165 ± 179 , which is lower than previously reported for the northern Adriatic Sea ($4,320 \pm 3,980$ eggs, (Malej et al., 2017) or other areas in the Mediterranean Sea, where the reproductive output of freshly collected animals from the natural environment was assessed (Table 2). For instance, it has been noted that laboratory-reared animals never achieved 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 (Shiganova et al., 2019b); in the northern Europe (e.g., 3,000 eggs $\text{ind}^{-1} \text{day}^{-1}$; (Javidpour et al., 2009); maximum rates of 11,232 eggs $\text{ind}^{-1} \text{day}^{-1}$, (Jaspers, Costello & Colin, 2015) or in the southern Europe (e.g., 12,000 eggs $\text{ind}^{-1} \text{day}^{-1}$, (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 9,910 eggs $\text{ind}^{-1} \text{day}^{-1}$ (Baker & Reeve, 1974) and a maximum of up to 14,233 eggs $\text{ind}^{-1} \text{day}^{-1}$ (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.,

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

<i>Native areas</i>	T (°C)	TBL (cm)	No. eggs	% ind. eggs	% hatching
Narragansett Bay (<i>Costello et al., 2006</i>)	6–25		0–3,300	59	n.d.
Narragansett Bay (<i>Kremer, 1976</i>)	11–29		0–14,000		
Biscayne Bay (<i>Baker & Reeve, 1974</i>)	21–31	3.8–8.5	0–9,990	90	n.d.
Biscayne Bay (<i>Stanlaw, Reeve & Walter, 1981</i>)	21		up to 10,000	most individuals	most eggs
<i>Mediterranean Sea</i>					
Aegean Sea (<i>Shiganova et al., 2004</i>)	21–25	1.7–3.4	0–448	75	82
Northern Adriatic (<i>Malej et al., 2017</i>)	20–22	5.4–11.5	136–13,512	100	n.d.
Northern Adriatic (<i>Kogovšek et al., 2018</i>)	20	4.1–9.8	0–1,400	65	n.d.
This study	17–25	5–7	0–638	57	12–100

Notes.

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

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 persistence of 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, as well as wet weight of ctenophores (Figs. 5C, 5D). However, no significant correlation was found between the percentage of hatched eggs and individual chemical characteristics or other environmental variables. Thus, our hypothesis that changing environmental variables and biometric/chemical characteristics affect ctenophore reproduction is only 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*). In our study, the percentage of hatched eggs exceeded 80% at around 25 °C but dropped significantly in 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 seawater temperature, salinity, oxygen, or 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 is important to note that our study focused only on the blooming period of *M. leidyi* in the northern Adriatic. 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 seawater temperature, as well as between the wet weight of individuals and the percentage of hatched eggs. Further examination at the individual molecular compound level is needed 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.

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Competing Interests

The authors declare there are no competing interests.

Author Contributions

- Kevin Rečnik conceived and designed the experiments, performed the experiments, analyzed the data, prepared figures and/or tables, authored or reviewed drafts of the article, and approved the final draft.
- Katja Klun conceived and designed the experiments, performed the experiments, analyzed the data, authored or reviewed drafts of the article, and approved the final draft.
- Lovrenc Lipej conceived and designed the experiments, authored or reviewed drafts of the article, and approved the final draft.

- Alenka Malej conceived and designed the experiments, analyzed the data, prepared figures and/or tables, authored or reviewed drafts of the article, and approved the final draft.
- Tinkara Tinta conceived and designed the experiments, performed the experiments, analyzed the data, prepared figures and/or tables, authored or reviewed drafts of the article, and approved the final draft.

Data Availability

The following information was supplied regarding data availability:

The data is available in the [Supplementary Files](#).

Supplemental Information

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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.
- Ciglencečki I, Paliaga P, Budiša A, Čanković M, Dautović J, Djakovac T, Dutour-Sikirić M, Kraus R, Kužat N, Lučić D, Pfanckuchen 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 10.1016/j.jmarsys.2021.103599.
- 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 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* 3:302 DOI [10.3389/fmicb.2012.00302](https://doi.org/10.3389/fmicb.2012.00302).
- Fadeev E, HJ H, Chie A, Zihao Z, Katja K, HG J, Tinta T. 2024.** Bacterial degradation of ctenophore *Mnemiopsis leidyi* organic matter. *MSystems* 0:e01264-23 DOI [10.1128/msystems.01264-23](https://doi.org/10.1128/msystems.01264-23).
- 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](https://doi.org/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](https://doi.org/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(2):57 DOI [10.3390/d13020057](https://doi.org/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](https://doi.org/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, Falkenhaus 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, Vansteenkotte 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 [10.1111/geb.12742](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](https://doi.org/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](https://doi.org/10.1007/s10530-008-9300-8).
- Javidpour J, Molinero J-C, Ramirez-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](https://doi.org/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](https://doi.org/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](https://doi.org/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*. New York: Academic Press, 197–215 DOI [10.1016/B978-0-12-751801-5.50024-1](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. *Ecology* **92**(8):1704–1704.
- 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. 2017.** *Mnemiopsis leidyi* in the northern Adriatic: here to stay? *Journal of Sea Research* **124**:10–16 DOI [10.1016/j.seares.2017.04.010](https://doi.org/10.1016/j.seares.2017.04.010).
- 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](https://doi.org/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](https://doi.org/10.1093/plankt/fbn094).
- Paliaga P, Budiša A, Dautović J, Djakovac T, Dutour-Sikirić MA, Mihanović H, Supić N, Celic I, Iveša N, Buršić M, Balković I, Jurković L, Ciglencečki 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 [10.1016/j.ecss.2021.107459](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**(5):186 DOI [10.3390/d13050186](https://doi.org/10.3390/d13050186).
- Pitt KA, Welsh DT, Condon RH. 2009.** Influence of jellyfish blooms on carbon, nitrogen and phosphorus cycling and plankton production. *Hydrobiologia* **616**:133–149 DOI [10.1007/s10750-008-9584-9](https://doi.org/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](https://doi.org/10.7717/peerj.1846).
- Shiganova TA. 2020.** Adaptive strategies of *Mnemiopsis leidyi* A. Agassiz 1865 in different environments of the Eurasian seas. *Marine Pollution Bulletin* **161**:111737 DOI [10.1016/j.marpolbul.2020.111737](https://doi.org/10.1016/j.marpolbul.2020.111737).
- Shiganova TA, Christou ED, Siokou-Frangou I, Bulgakova JV. 2004.** Distribution and biology of *Mnemiopsis leidyi* in the Northern Aegean Sea, and comparison with the indigenous *Bolinopsis vitrea*. In: Dumont H, Shiganova TA, Niermann U, eds. *Aquatic invasions in the Black, Caspian, and Mediterranean seas*. Dordrecht: Springer Netherlands, 113–135.

- 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 [10.1016/j.marpolbul.2019.02.049](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 [10.1016/j.marenvres.2019.104791](https://doi.org/10.1016/j.marenvres.2019.104791).
- 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](https://doi.org/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](https://doi.org/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](https://doi.org/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**:590995 DOI [10.3389/fmicb.2020.590995](https://doi.org/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.