PERSEUS

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Environmental condition favoring gelatinous plankton outbreaks in the SES

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CONTENTS

EXECUTIVE SUMMARY / ABSTRACT

Jellyfish dynamics have been handled through data-driven process field and experimental work on observation of the life cycle of selected native (Aurelia sp.) and non-native bloom-forming gelatinous species common and not common to both seas species ctenophores *Mnemiopsis leidyi* and *Beroe ovata* that prevalent in the Black Sea and had been found in the Adriatic Sea proliferate in the Mediterranean and the Black Sea and jellyfish *Cotylorhiza tuberculata* and in the environmental control on a lobata ctenophore (as *Mnemiopsis leidyi*) *Bolinopsis vitrea* and Pelagia noctiluca in the western Mediterranean (CSIC). Most of results were obtained from specifically designed laboratory experiments and mesocosms carried out in WP1 (SIO RAS and IO BAS) in the Black Sea and SIO RAS and NIB MBS) in WP-4 with special observation of the effect of development of gelatinous plankton in relation to changing environmental conditions: hydro- and biochemical composition of dissolved and suspended organic matter, microplankton species diversity and abundance and phytoplankton species composition and biomass.

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SCOPE

The aims of this study were:

- Research carried out has been mainly focused to descriptor D4 (Food webs) but also to D2 (Non-indigenous species) and D5 (Human-induced eutrophication) which are relevant to MSFD.

INTRODUCTION

Study areas

Study areas are the northern Adriatic Sea, the Black Sea Southern branch SIO RAS Gelendzhik (northeastern Black Sea); in 2014 also Varna (IOBAS) (northwestern Black Sea) in collaboration with IO BAS and the western Mediterranean (Balearic Sea).

I. GENERAL SCIENTIFIC ACTIVITIES OF THE SIO RAS, NIB-MBS, AND CSIC

The NIB-MBS team and SIO-RAS team in the study of bloom-forming gelatinous species common to the Mediterranean and Black Seas (*Aurelia aurita* s.l., *Mnemiopsis leidyi* and *Noctiluca scintillans*) and with CSIC in study of holoplanktonic medusa *Pelagia noctiluca*.

We have carried out a comparative analysis of *Aurelia aurita* populations in the the northern Black seas with SIO-RAS team elucidating some common factors that govern accumulation and formation of red tides in the two systems. Experimental field work carried out in collaboration with SIO-RAS near Gelendzik (northern Black Sea) on degradation of moon jellyfish and the impact of this process on nutrients and microbial community has been summarized and presented at the 49th European Marine Biology Symposium and submitted paper T. Tinta, T. Kogovšek , V. Turk, T.A. Shiganova, A.S. Mikaelyan, A. Malej "Microbial transformation of jellyfish organic matter affects the nitrogen cycle in the marine water column - a Black Sea case study"

The increasing trend in jellyfish blooms that has been observed in some coastal areas around the world can have serious and long-term ecological consequences. In particular, the fate of jellyfish organic matter (jelly-OM), after the decay of jellyfish blooms, and the implications for marine biogeochemical cycles and ecosystem functioning, is still unclear. In order to study bacteria-jelly-OM interactions and the associated fate of the jelly-OM, we conducted two sets of short-term jelly-OM enrichment experiments using the coastal and offshore ambient pelagic bacterial assemblages from the Black Sea, where the scyphozoan medusa *Aurelia aurita* (Linnaeus, 1758) blooms seasonally. The microbial transformation of the jelly-OM was followed using stable $\delta^{13}C$ and $\delta^{15}N$ analysis of particulate jelly-OM together with standard organic and inorganic matter chemical analyses. The effect of the jelly-OM on the ambient bacterial community was investigated by following changes in bacterial abundance, growth rates and community structure. The Black Sea's pelagic surface bacterial assemblages from both systems, coastal and offshore, responded rapidly to the jelly-OM enrichment, preferentially utilizing nitrogen-rich constituents of the jelly-OM, leaving carbon-enriched particulate OM in the system. The end products of the bacteria-mediated jelly-OM degradation process—*i.e.* total dissolved nitrogen and ammonium, accumulated in the system, indicating possible implications for the nitrogen cycle. Despite the differences in the Black Sea's coastal and offshore seawater background nutrient concentrations and particulate OM quality, the nitrogen budget was very much the same in both studied systems, however there were differences in the bacterial community function/performance from these two environments. The addition of jelly-OM triggered different structural changes in the coastal and offshore ambient bacterial communities, suggesting that different bacterial groups are capable of utilizing jelly-OM. Comparison of the effect of jelly-OM degradation on the functioning of different marine ecosystem indicates that the degree of bacterial growth rate and the rate of ammonium accumulation depend on the incidence of jellyfish occurrence, physiochemical environmental conditions and possibly also on ambient bacterial community composition.

II. GELATINOUS PLANKTON EXPERIMENTAL STUDY IN THE SES

1. Experimental joint work WP 4.10, WP1: Black Sea experiment; and WP-2 IO BAS side (Gelendzhik site) and (Varna site) (SIO RAS; IOBAS)

– Participants

***Organisations*:** SIO-RAS (Tamara Shiganova, Alexander Mikaelyan, Sergey Mosharov, Irina Mosharova)

IO-BAS (Snejana Moncheva, Kremena Stefanova, Natalya Slabakova, Radka Mavrodieva, Boryana Dzurova, Elitza Stefanova, Valentina Doncheva)

Contact person(s): Tamara Shiganova (SIO-RAS), Snejana Moncheva (IO-BAS)

Objectives

The goal of the present study was to investigate the potential impact of excretion and mucus release by the invasive ctenophores *Mnemiopsis leidyi* and *Beroe ovata* on the low trophic levels of Black Sea ecosystem. Two types of experiments were conducted jointly by SIO- RAS and IO-BAS– lab aquaria and *in situ* mesocosms in the coastal area of Gelendzhik (2013) and Varna (2014).

Activities performed and methodology

The laboratory (aquaria) experiments were designed to analyze the effects of invasive ctenophores on hydrochemical parameters of the environment (pH, O_2, nutrients - N, P, Si and their ratios) and the structural and functional traits of food web lower trophic ecosystem components: bacteria, authotrophic/heterotrophic flagellates, nano-micro phytoplankton and microzooplankton communities taxonomic composition, abundance and biomass, chlorophyll a and Primary production. In the mesocosms experiments, one more trophic level - mesozooplankton as the main prey of *M. leidyi* was examined in addition to the same set of parameters (*here the preliminary results of Varna lab experimental phase are presented*).

Triplicates for each experimental treatment - Control (C), Menimiopsis (M) and Beroe (B) were prepared in 12 l aquaria and incubated under the same light-dark (12:12h) and temperature (~ 22 ^{o}C) regime for 8 days – Fig.1.

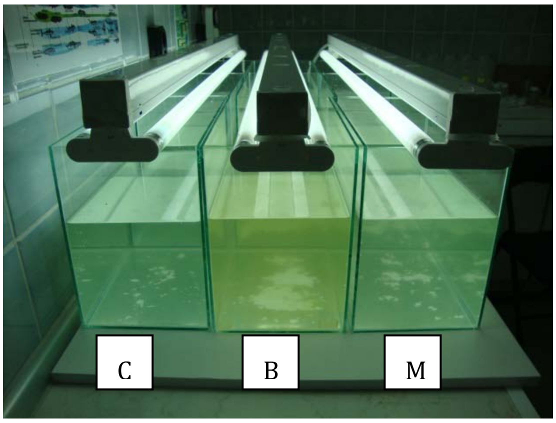


Fig. 1. View of experimental aquaria: C-control; B – Beroe; M - Mnemiopsis

Fresh animals and sea water were collected prior to the experiment from the area of Varna Bay. Equal aliquots of each replicate were pooled at the beginning of the experiment and on the 2nd, 4th and 8th day for analysis of the parameters listed above. The aquaria were inoculated with 7 individuals of Mnemiopsis of similar total length (16.3 mm in average) and 3 Beroe ovata of average length 25.5 mm for each replicate corresponding to an average densities found in field observations (Shiganova et all.,2014).

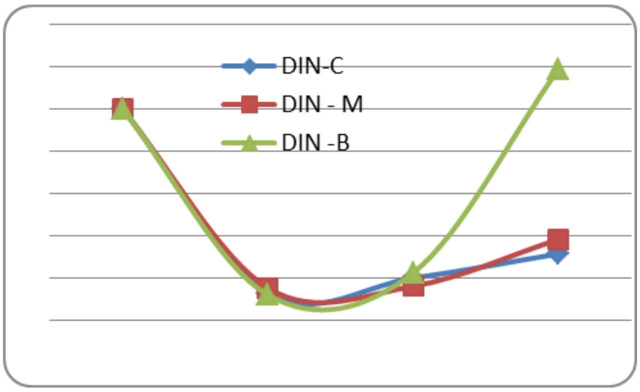
Synthesis of results and discussion

Changes in the measured parameters occurred in all experimental treatments while patterns varied among the different settings.

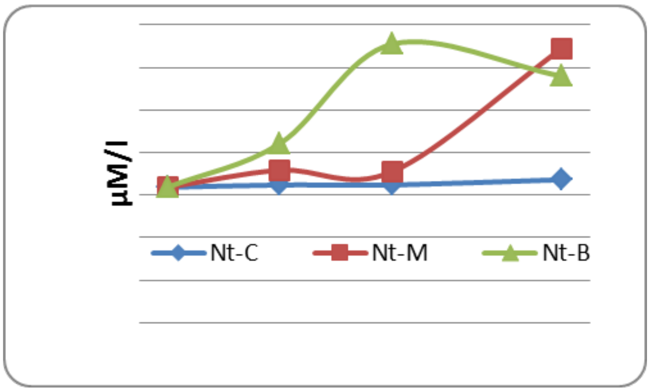
Both ctenophores *Beroe ovata* and *Mnemiopsis leidyi* induced changes in the chemical properties of the water, but they were more pronounced in B experiments – Fig. 2. Oxygen content increased on the second day, the highest (383 μM) measured in B experiments, while on the 4th dropped to 244 μM without significant changes by the end of the experiment in all treatments.

The inorganic N was almost depleted between 2nd-4th day, and increased sharply only in the B treatment, contrary to the organic N. NH_4 and IP showed the same rate of decrease in all treatments by the 4th day , and increased only in the B aquaria by the 6th day to 5.55 μM, and 2.78 μM respectively. Similarly, Ptot increase was more intensive in B treatments relative to C and M where significant changes were not found, that resulted in shifts in nutrient ratios. While there was almost no difference in the N/P variations in all the experimental settings, declining on the second day and increasing towards the end to levels lower than the initial concentrations, Si/N dropped to 0 in B replicates and to 10 in M, contrary to the 3 fold increase in C (45) – Fig. 2. Si/P exposed totally different pattern, altogether inducing “stoichiometric modulations” in the experimental environment (Mitra & Flin, 2007).

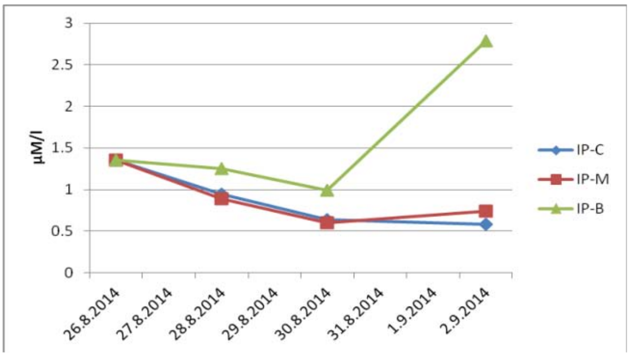
a)


DIN-C
DIN - M
DIN -B

b)


µM/l
Nt-C
Nt-M
Nt-B

c)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IP-C
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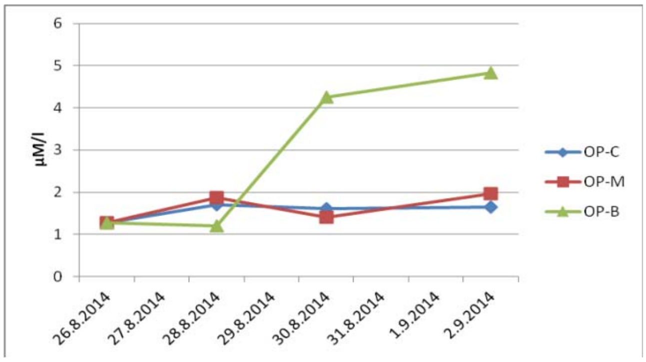
d)


µM/l
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OP-C
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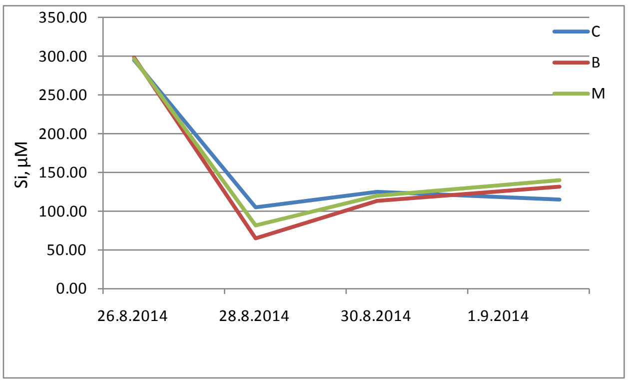
e)


Si, µM
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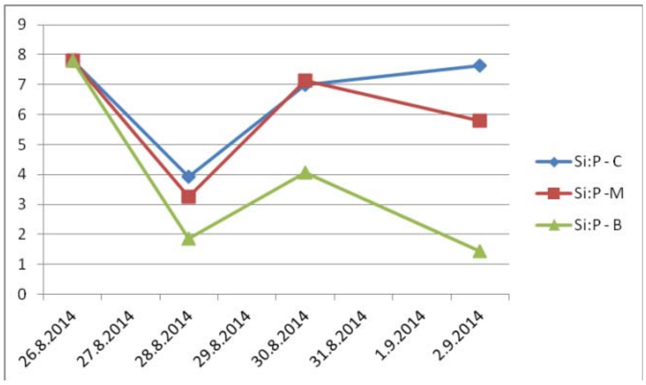
B
M

f)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Si:P - C
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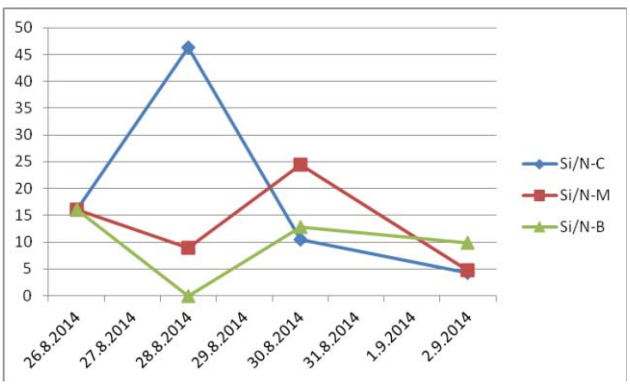
g)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Si/N-C
Si/N-M
Si/N-B

h)


4
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3
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26.8.2014
28.8.2014
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2.9.2014
N/P-C
N/P-M
N/P-B

Figure 2. Nutrients variations, μM/l: a) DIN (dissolved inorganic nitrogen), b) Nt-total nitrogen; c) IP (inorganic phosphorous), d) OP – organic phosphorous; e) Si (silicate); f) Si/P ratio; g) Si/N ratio and h) N/P ratio.

Phytoplankton (nano-micro microscope counts)

The initial concentration of phytoplankton community was high - average abundance 1300439 ± 368720 cells/l and biomass - 819.275 ± 217 mg/m3, composed of high diversity of species from 15 taxonomic classes. The high initial abundance was accounted mainly by Bacillariophycea (40% from the total abundance), Prasinophyceae (20%) and Dinophyceae species (15%), while in the biomass the contribution of diatoms (~40%) was shared by that of dinoflagellates (~50%), the remaining classess proportion bellow 10% due to the prevalence of small-size species.

While variations in the abundance and biomass occurred in all treatments the growth displayed uneven pattern. An increase was measured on the 2nd day in all treatments, declining sharply on the 4th day and maintained at almost the same level by the experiment termination. Even if both Mnemiopsis and Beroe stimulated phytoplankton growth the difference between the control was much higher in B setting, where the abundance and biomass increased more than 17 times during the first 48h (15890842 ± cells/l and 10596.989 ± 933 mg/m3) relative to about 7 times increase in the M enrichment (5278.609±1082 mg/m3) and 5 times in the control (5004.501±1071 mg/m3), ending with abundance 9 times higher than the initial density in B, as compared to ~ 5 times elevation in M and less than 3 times in C treatments – Fig.3. Diatoms total density accumulated from 207538± 520999 cells/l to a high of 11704905± 776612 cells/l in B setting (~ 56 times increase) , with lower growth in M (5653305±136063, e.g. ~12 fold increase) and lowest in C (4487745± 142983 cells/l, ~ 8 times increase), declining smoothly over the experimental incubation in all treatments, but similar to the total abundance, to levels several fold higher than the initial ones. Dinoflagellates showed a steady increase to a final density of 4134710 ± 379508 cells/l in B (~ 15 fold) , to 1043322±72076 cells/l in M (~6 fold) and about 3 fold in C (635124± 260234 cells/l). Euglenoidea growth was most intensively stimulated by Beroe manipulation especially after the 4th day of incubation (221563±6326 cells/l), and contrasting to M and C treatments where it dropped to the initial concentration on the 8th day, the final density exceeded the initial one by ~5 times- Fig.3.

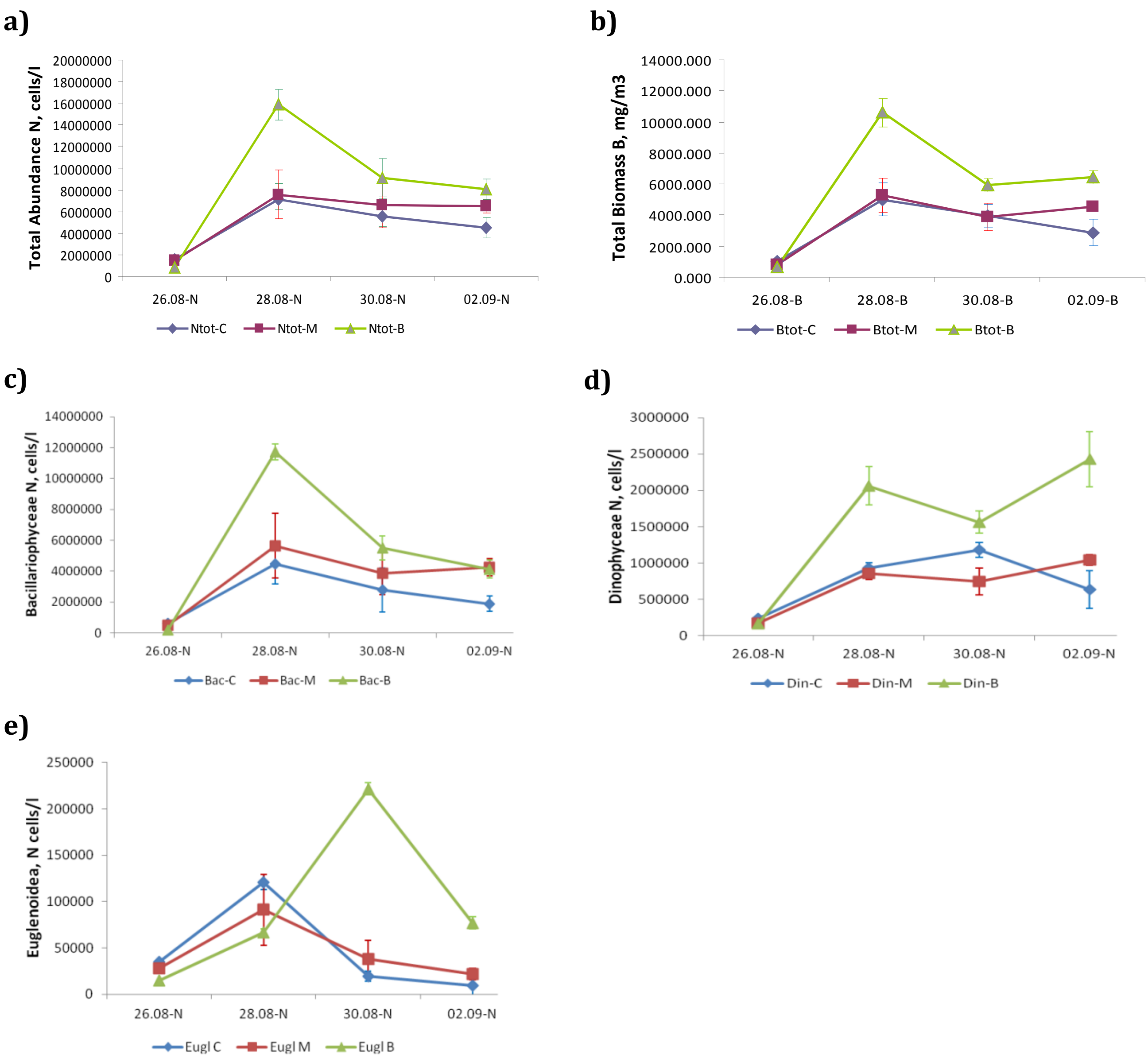


Figure 3. Variation in nano-microphytoplankton: a) Total abundance, N cells/l; b) Total biommss, B mg/m^3 , c) Bacillariophyceae abundance, N cells/l; d) Dinophyceae abundance, N cells/l; e) Euglenoidea abundance N, cells/l; C-control, M-Mnemiopsis, B - Beroe

Along with the quantitative variations, concomitant alteration in the taxonomic profile of phytoplankton community occurred particularly in Beroe treatment on the account of mainly mixotrophic Dinophyceae (their proportion increased from 18 to 30% in the total abundance) and Euglenophyceae species, even if diatoms also increased from 23 to 51%. The corresponding proportions were in favour of diatoms in M treatment (65% diatoms, 16% dinoflgagellates), without apparent change in the control – Fig.4.

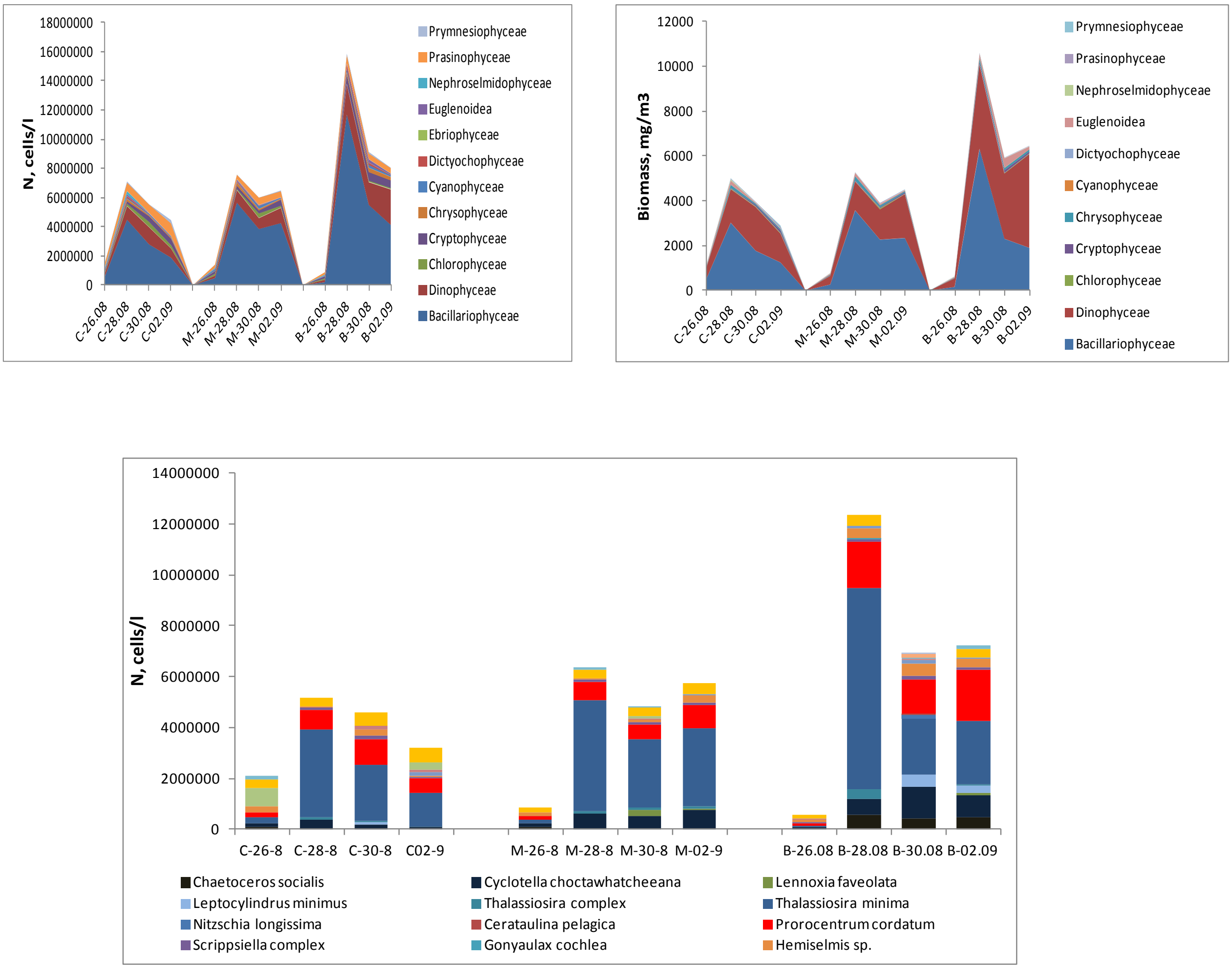
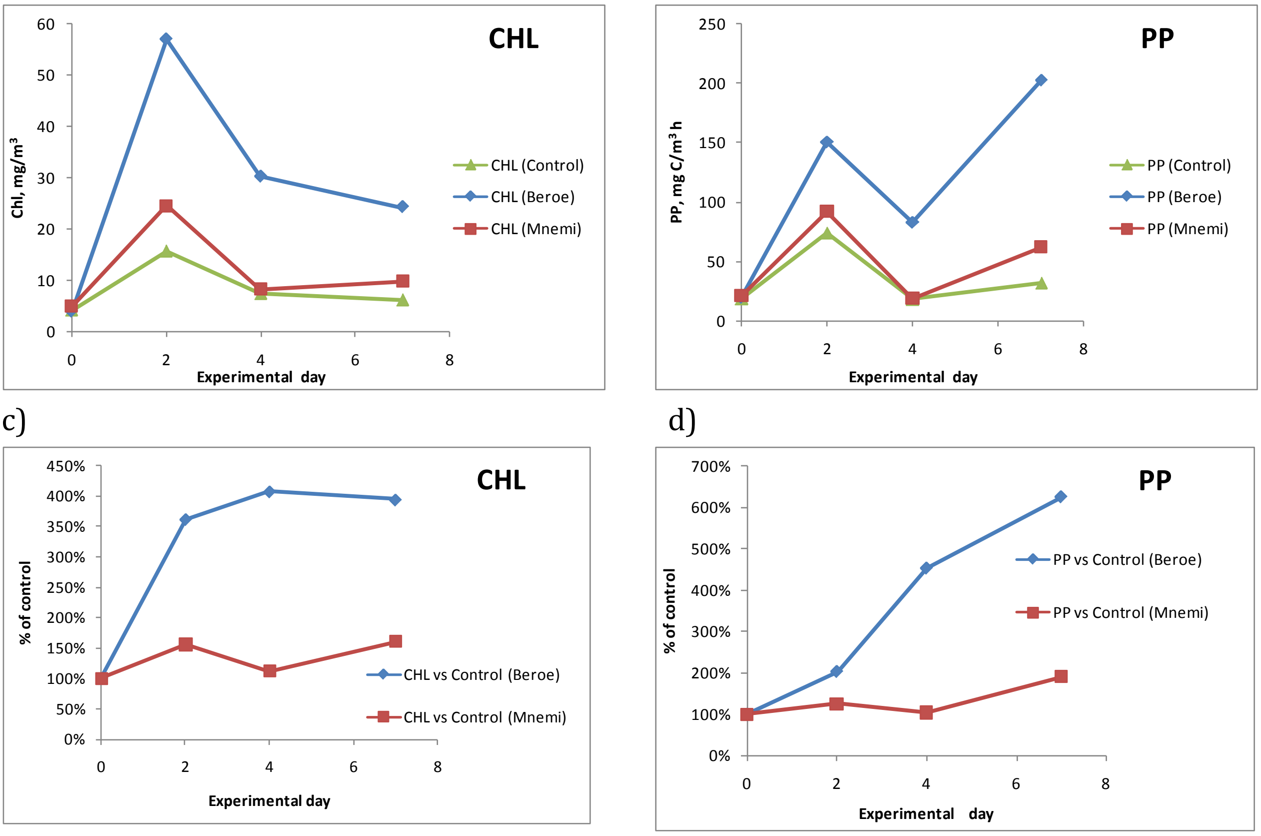


Figure 4. Variation in the taxonomic profile of nano-micro phytoplankton: a) Abundance , N cells/l; b) Biomass, B mg/m^3; c) dominant species, N cells/l; C-control, M-Mnemiopsis, B - Beroe

The dominant complex of species that have accounted to the observed restructuring and disproportional growth of phytoplankton community was rather polyphyletic, composed of **diatoms** *Cyclotella choctawhatcheeana, Lennoxia faveolata,Leptocylindrus minimus, Thalassiosira complex, Thalassiosira minima, Nitzschia longissima, Cerataulina pelagica,* **dinoflagellates** *Prorocentrum cordatum, Scrippsiella complex, Gonyaulax cochlea,* **cryptophytes** *Hemiselmis sp., Hillea fusiformis, Plagioselmis sp.,* **prasinophytes** *Pyramimonas sp. and Pachysphaera sp* – Fig.4. Among diatoms *Thalassiosira minima* proliferated to blooming density in all treatments – in the control to 3.4x10^6 cells/l, in Mnemiopsis manipulation 4.3 x 10^6 cells/l and about twice higher in Beroe setting – 7.9 x10^6 cells/l, and in concert with several other diatom species intensive growth most likely caused the observed depletion of DIN and Si during the first 48 h. The peak of dinoflagellates was associated to *Prorocentrum cordatum* and *Scrippssiella complex*, whereas *P. minimum* attained blooming densities only in the B manipulations – 2.01 x10^6 cells/l. Concurrently euglenophytes (*Eutreptia globulifera, Eutreptia lanowii)* grew to sizable proportion in B experiments in particular after the first 48 hours in parallel to the increasing Norg and Porg concentrations – Fig 2, Fig.4.

Primary production, chlorophyll a and bacterioplankton mirrored the pattern of phytoplankton dynamic, again the highest intensity measured in *Beroe* manipulation – Fig.5.

a) b) c) d)



e)

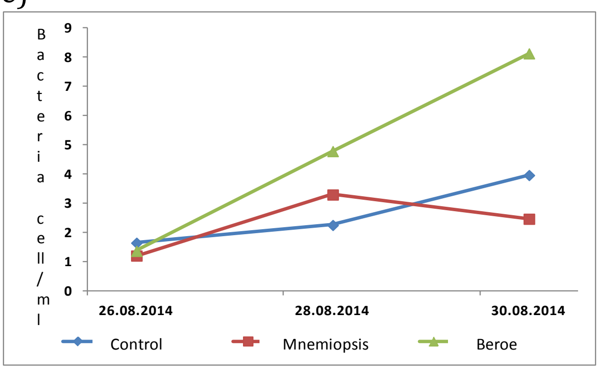


Figure 5. Variations of (a) Chlorophyll a [mg/m^3], (b) primary production [mgC/m^3h], (c) percent of chlorophyll a and primary production (d) in *Mnemiopsis* and *Beroe* treatments in comparison with control manipulation and (e) bacterioplankton [cell/ml].

Microzooplankton (MZP) Taxonomic composition

A total of 18 species/taxa of microzooplankton (size 20-200 µm) were identified from 6 taxonomic subclasses and a numerous ciliates (phylum Ciliophora) unidentified to lower taxonomic level (Table 1).

Table 1. Taxonomic composition of microzooplankton identified during the 7 days lab experiment

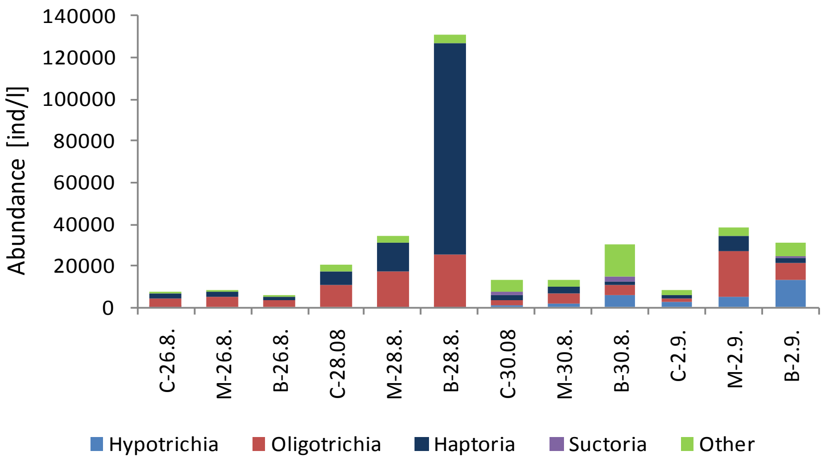
Subclass	Species/taxa
Hypotrichia	Euplotes sp
Oligotrichia	Eutintinnus sp
	Tintinnidium sp.
	Tintinnid ciliate
	Strobilidium sp.
	Strombidinopsis sp.
	Strombidium sp.
	Laboea strobila
	Tontonia sp.
Haptoria	Mesodinium sp.
Suctoria	Acineta sp.
Other	
- **Monogononta**	Trichocerca sp.
	Keratella sp.
- **Copepoda**	Nauplial larvae stage
- **Ciliophora**	unidentified

Initially (26 August) microzooplankton dominant complex in abundance structure consisted of Mesodinium sp. with 28 ± 4 % , Tintinnids - 29 ± 4% and Strombidium sp. (31 ± 1 %). Corresponding biomass figure demonstrated prevalence of Tintinnids (51 ± 24%) and Strombidium sp. (22 ± 9 %) (Fig 6. A, B). Generally, the three types of aquaria presented similar qualitative and quantitative attributes.

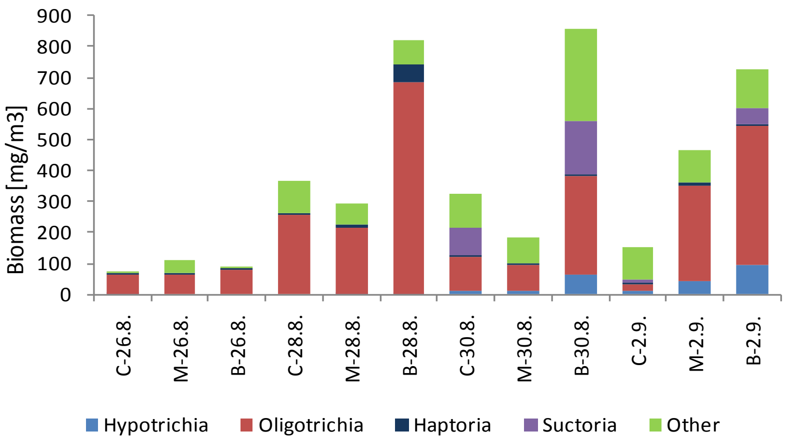
Two days after settlement (28 August) the growth of small size microzooplankton (15 - 40 µm) was stimulated by the ctenophores release better presented in Beroe aquaria. Community was dominated by Mesodinium sp. with 33 % in the control aquaria (C), 39 % in *Mnemiopsis leidyi* aquaria (M) and the highest percent (77 %) in *Beroe ovata* manipulation (B), followed by Strombidium sp. with 15 %, 21 % and 7 %, respectively. According to the biomass structure Strobilidium sp. contributed more to aquaria C and B with 37 and 38 %, respectively and Tintinnids in aquaria M (36 %). Other species/taxa such as *L. strobila*, Strombidium sp., Strombidinopsis sp., Eutintinnus sp., unidentified ciliates co-dominate the community ranging from 7 to 18%.

At the second sampling date (30 August), new taxa (Euplotida sp., Acineta sp. and a few unidentified ciliates), probably very rare initially, proliferated to higher levels reorganizing community structure. For example Euplotida was presented in the complex with 9 % (control aquaria), 12 % in Mnemiopsis and 21 % in Beroe aquaria while for ciliates corresponding figure was 38 % (C), 15 % (M), 52 % (B), respectively (figure 6 A, B). Mesodinium sp., starting with abundance 1974 ± 522 ind/l, increased 3 folds in control aquaria, 6 times in aquaria with *Mnemiopsis leidyi* and 73 folds in (B) after two days of experiment (28.08) and dropped to values comparable with the initial one during the followed measurements.

Finalizing the experiment, microzooplankton complexes in abundance were dominated by Euplotes sp. (C - 34 %, M - 14 % and B - 42 %), Mesodinium sp. (C - 14 %, M - 18 % and B - 9 %), unidentified ciliates (C - 29 %, M - 9 % and B - 14 %). In the biomass also prevailed ciliates (57 % - C, 21 % - M, 12% - B) and Euplotes sp. (8 % - C, 10 % - M, 13 % - B) with some differences in separate aquaria - Strobilidium sp. (10 % - M, 37 % - B), Acineta sp. (8 % - C), tintinid ciliates (24 % - B), Strombidium sp. (17 % - M), Strombidinopsis sp. (18 % - M) and *L. strobila* (19 % - M).



a)



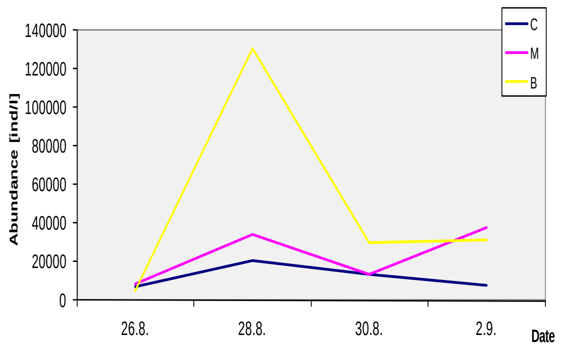
b)

Figure 6. Taxonomic structure by abundance (a) and biomass (b) in the control aquaria (C), aquaria with *M. leidyi* (M) and aquaria with *B. ovata* (B).

Abundance and biomass dynamic of microzooplankton

Microzooplankton abundance and biomass measured in the first day of the experiment ranged in very low levels from 5338 ind/l to 8396 ind/l and from 71.636 mg/m^3 to 109.084 mg/m^3 i.e 1.2-1.5 folds.

The average abundance and biomass estimated in 28.08 showed similar trends in the three types of aquaria but significantly increased in *Beroe* treatment – about 18 folds in abundance and 12 folds in biomass (130 761 ±56 687 ind/l, 823.25 ±362.15 mg/m^3) (figure 7). Much of the increase in microzooplankton abundance was associated with Mesodinium sp. (100 960 ±47 851 ind/l) while for the biomass with Strobilidium (309.34 ±71.20 mg/m^3). During the next measurements the peak in the Beroe aquaria biomass was still maintained in high values as a result of development of Strombidinopsis sp. and new taxa Acineta sp. and Euplotida sp.



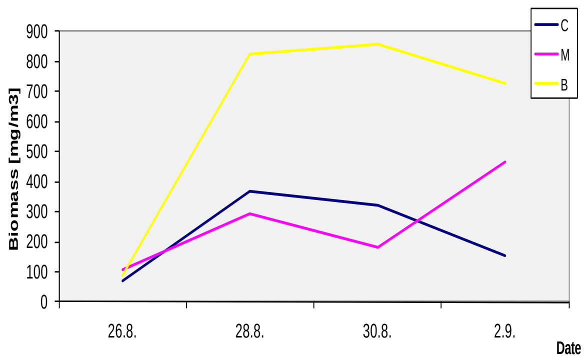


Figure 7. Microzooplankton average abundance (top) and biomass (bottom) in the control aquaria (C), aquaria with *M. leidyi* (M) and aquaria with *B. ovata* (B).

The estimate of the quantity of mucus released by ctenophora (both Mnemiopsis and Beroe) per inndividual for 24 h (in % of dry weght) show about 2 times higher amount in Beroe - 69.10% versus 39.3% in Mnemiopsis. The preliminary analysis show parallel trends in all treatments for the first 48 hours, the major differences being in the intensity of change in the different experimental settings. Most likely the high initial concentration of nutrients (inorganic and organic) were sufficient to support the growth of phytoplankton and bacterioplankton during the first 48 h in the control too, while the mucus released by the ctenophores contributed to the increased intensity of the processes. The decoupling of the growth patterns more obvious on the 4th day could be related to the altered chemical properties of the environment (habitat resource) and the associated restructuring of the biotic components and their interactions. Most likely the release of mucus trigger a cascade of processes that occur in parallel during the first 48 h - rapid increase of bacteria, phytoplankton abundance and biomass, chl. a and PP and microzooplankton with intensity corresponding to the intensity of mucus release, driving further changes in the strength and pattern of the biotic interactions.

Major highlights

A special measurement to estimate the quantity of mucus released by ctenophora (both Mnemiopsis and Beroe) per inndividual for 24 h (in % of dry weght) show about 2 times higher amount in Beroe - 69.10% versus 39.3% in Mnemiopsis. The natural components of mucus are macromolecular organic compounds of protein and polysaccharide, their complexes and derivatives. Hydrolytic enzymes (including proteases, and amylases) are natural catalysts for their degradation. In seawater extracellular enzymes are being generated through the activity of microorganisms (bacteria, microzooplankton and microphytoplankton).. They are playing a key role in the processes of transformation of organic matter and contribute to the rapid inclusion of suspended organic matter in the lower food chain, activating the microbial loop (Kornneva, Shiganova,1995,1998). In the case of *B.ovata* the amount of released mucus was about two times higher, which might explain the higher intensity of processes and related changes compare to *Mnemiopsis* and control.

The preliminary results (although in need of further cross-parameter analysis) give ground to conclude that Beroe act as a stronger agent in restructuring the lower pelagic food-web and the intensity of related processes. MZP consists of multiple populations each characterized by different resource use and adaptations, in addition due to the high presence of mixotrophic dinoflagellates, it is difficult to ascertain what nutritional mode they use at any given time based on structural analysis only, while exopolymeric (EPS) secretion from a species in a blooming density are known to deter MZP grazing, likely promoting bacterial production. So the complexity of microbial loop interactions do not allow a straight forward interpretation, but the experiments provide insight and expose interesting patterns of interactions not foreseen so far in field and experimental studies.

Contribution to filling gaps in understanding either the response of pelagic/benthic ecosystems to pressures or the impact of pollution

Plotting interactions promises a deeper understanding on the functioning of lower trophic food-web under the impact of ctenophores, which could be taken into consideration for further improvement of modelling in service of proper ecosystem management.

II. GELATINOUS PLANKTON EXPERIMENTAL STUDY IN THE SES (SIO RAS; NIB MBS)

1. Experimental joint work (Gelendzhik site)

Participants:

Organisations: SIO-RAS (Tamara Shiganova, Alexander Mikaelyan
NIB MDS (A.Malej, V.Turk, T. Tinta, T.Kogovosek)

Contact person(s): Alenka Malej (NIB MDS) , Tamara Shiganova (SIO-RAS)

Rationale

Recurrent blooms of different jellyfish species are spread worldwide and in some coastal areas they seem to be increasing (Kogovšek et al. 2010, Brotz et al. 2014). Due to very high numbers and their high protein content, jellyfish might represent high quality organic substrate for microbial community after the decay of blooms, and thus have the implications for marine biogeochemical cycles.

Objectives

In order to study the fate of *Aurelia* sp. blooms and the impact on the ambient microbial community and nutrients in the Black Sea, where this jellyfish reoccurs yearly, two set of short - term degradation experiments were conducted. One of the central objectives of our research was to examine the aspect of jellyfish substrate as selective force that either inhibits and/or stimulates the growth of specific bacterial groups. The microbial transformation of the *Aurelia*-derived organic matter (*Aurelia*-OM) was followed using stable $\delta^{13}C$ and $\delta^{15}N$ isotope ratios and organic and inorganic nutrient analyses.

Activities performed and methodology

We carried out two sets of short-term *Aurelia*-OM enrichment experiments using the coastal and offshore ambient pelagic bacterial assemblages from the north-eastern Black Sea. In both sets of experiments, the ambient microbial community was supplemented with the same amount of jellyfish biomass, however, in the first experiment the enclosures were filled with the coastal seawater (Blue Bay near Gelendzik), while in the second experiment the offshore seawater (location beyond continental shelf approx. 4 miles offshore) was used. Each experimental set up comprised of a triplicate of jellyfish-enriched bottles and a bottle without jellyfish addition that served as control. The experimental enclosures were incubated *in situ* to ensure natural conditions. The daily dynamic of bacterial community structure was followed using DNA fingerprinting technique – the *denaturing gradient gel electrophoresis* (DGGE). Furthermore, to obtain the information on the dynamics of the dominant bacterial families, the 16S rRNA bacterial gene clone libraries were constructed at the beginning and at the end of both experiments. These data were supplemented with particulate and dissolved organic and inorganic nutrient analyses as well as stable ^{13}C and ^{15}N isotope analyses of particulate organic matter (POM, ≥ 0.8 µm fraction), which provided an insight into the processes of organic matter transformations.

Synthesis of results and discussion

The jellyfish biomass addition triggered a rapid response of ambient bacterial community; during the exponential growth phase the bacterial community had considerably higher specific growth rates in the jellyfish-enriched bottles compared to the control. In the first 24 hours, the concentration of POM decreased for about 50% in both experiments, followed by an increase and accumulation of POM at the end of both experiments (day 3). As depicted from C to N ratio and stable isotope analysis the quality of POM changed over time in both experiments (Figure 1).

The $\delta^{15}N$ of POM gradually increased in both experiments, suggesting that the lighter isotope was taken up and transformed by bacteria thus either becoming volatile or dissolved and therefore transferred into the <0.8µm fraction, leading to the accumulation of the heavier isotope in the particulate (>0.8µm) fraction.

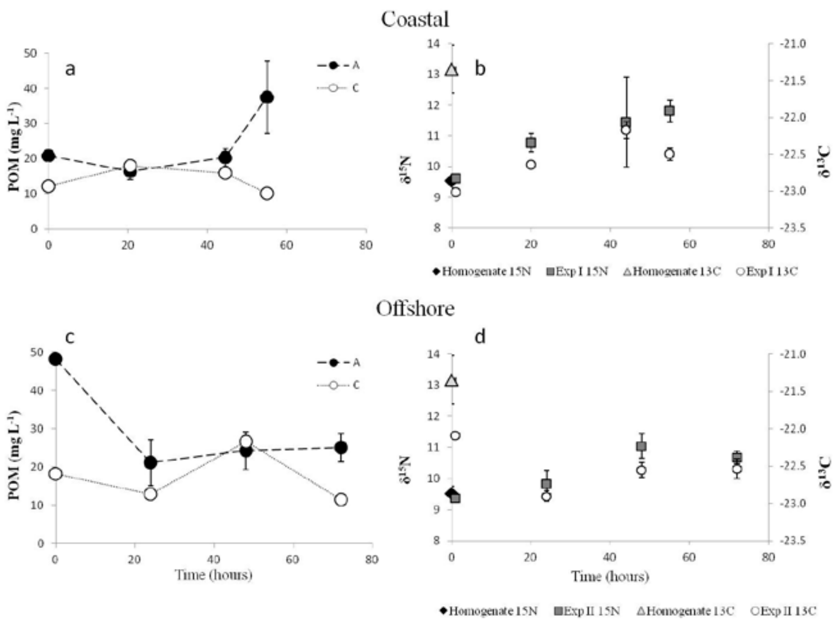


Figure 1: The dynamic of particulate organic matter (POM) (a, c) , $\delta^{15}N$ and $\delta^{13}C$ (b, d) over time in the coastal (a, b) and offshore (c, d) experiment conducted in the Black Sea (A – *Aurelia*-OM amended, C – control enclosures)

This was further supported by the measurements of nutrients (Figure 2) that showed the accumulation of dissolved nitrogen and ammonium (NH_4^+) in the < 0.8µm fraction at the end of the experiments.

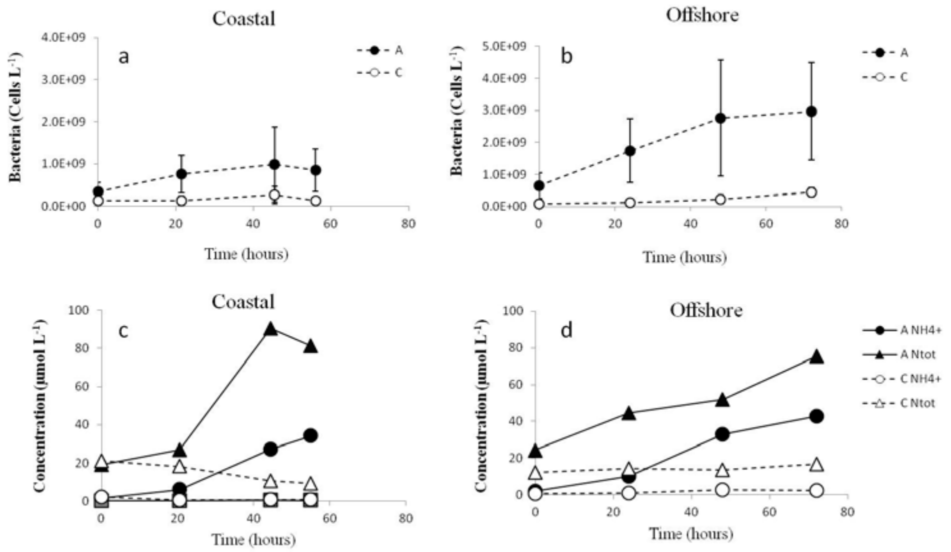


Figure 2: The dynamic of bacterial abundance (a, b), total dissolved nitrogen and ammonium (c, d) over time in the coastal (a, c) and offshore (b, d) experiment conducted in the Black Sea. (A – *Aurelia*-OM amended, C – control enclosures)

Thus, bacterial assemblages from both systems, coastal and offshore, responded rapidly to the *Aurelia*-OM preferentially utilizing nitrogen-rich constituents and leaving carbon-enriched particulate OM while total dissolved nitrogen and ammonium, accumulated in the system, indicating possible implications for the nitrogen cycle.

The analyses of bacterial community using DGGE fingerprinting method (Figure 3) revealed that the structure has changed after 24 hours in all experimental enclosures, which could be due to the bottle effect. However, already after 48 hours, the jellyfish-enriched and the control communities formed distinct clusters, in both experimental set ups. The analyses of bacterial 16S rRNA gene clone libraries showed that coastal and offshore communities were different at the start. The coastal community was prevailed by *Alphaproteobacteria* (which were dominated by SAR11 clade) and *Cyanobacteria*, followed by approx. equal contribution of *Gammaproteobacteria* and *Bacteroidetes* (from which only *Flavobacteriales*) and some minor and unclassified taxa. On the other hand, the offshore community comprised mostly of *Gammaproteobacteria*, followed by *Bacteroidetes* (from which only *Flavobacteriales*) and *Alphaproteobacteria* (dominated by SAR11) as well as some minor taxa and lower percentage of unclassified groups. The community composition within the jellyfish-enriched treatment was largely altered at the end of both experiments. A common response of both coastal and offshore community to jellyfish substrate was a decrease of *Alphaproteobacteria* (within which SAR11 clade diminished the most). In the coastal community the prevailing *Alphaproteobacteria* were outnumbered by known degraders of complex organic substrates - *Gammaproteobacteria* that constituted more than half of the total community at the end of the experiment. On the other hand, in the offshore community we recordered a shift from prevailing *Gammaproteobacteria* to *Flavobacteriales* (*Bacteroidetes*), which are also known to be capable of degrading high molecular weight organic compounds. In general, the jellyfish substrate inhibited the growth of *Alphaproteobacteria* (in particularly SAR11 clade) and stimulated the growth of

Gammaproteobacteria in the coastal and *Flavobacteriales* in the offshore experimental enclosures.

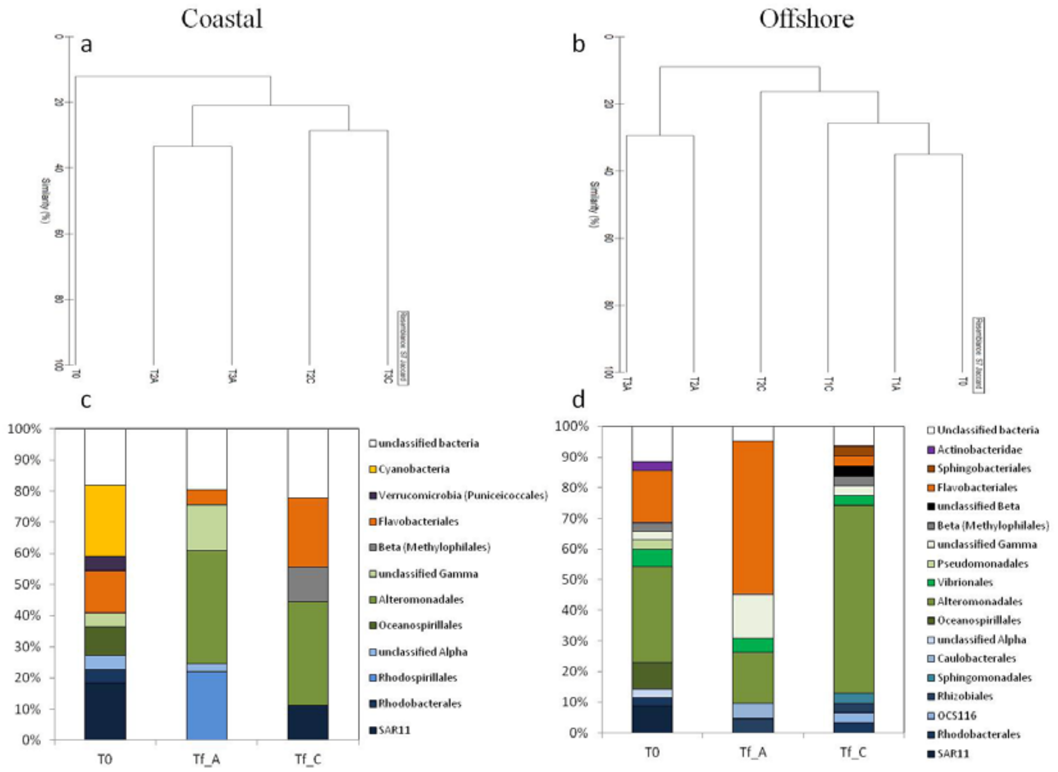


Figure 3: The dynamic of bacterial community structure as revealed by denaturing gradient gel electrophoresis (a, b) and 16S rRNA bacterial gene clone libraries (c, d) over time in the coastal (a, b) and offshore (c, d) experiment conducted in the Black Sea.

Our results suggest that the jellyfish biomass stimulated the growth of bacterial community, which efficiently re-mineralized this rich organic substrate what led to the accumulation of dissolved inorganic nutrients indicating possible implications for the nitrogen cycle.

References

Brotz L.., W.L. Cheung, K. Kleisner, E. Pakhomov, D. Pauly. 2012. Increasing jellyfish populations: trends in Large Marine Ecosystems. Hydrobiologia 690: 3-20

Kogovšek T., B. Bogunović, A. Malej. 2010. Recurrence of bloom-forming scyphomedusae: wavelet analysis of a 200-year time series. Hydrobiologia 645: 81-96

VI. ACTIVITIS OF CSIC

The scientific work performed by the CSIC team within this subtask was as expected during this period report. In the first period report, the researchers centered their effort in the population dynamics of *Cotylorhiza tuberculata* and in the environmental control on a lobata ctenophore (as *Mnemiopsis leidyi*) *Bolinopsis vitrea*. The outcome of the work carried out during that period was mirrored in two scientific articles, five communications in scientific meetings (Spain and Japan) and 14 interviews to media (of national, regional and local coverage). In the second period report, the researchers had extend their experiment experience, field data and modeling effort to the other key species of jellyfish. In this sense, and in close connection with the work performed in WP1, Subtask 1.3.1. (ALBOREX field study), the researchers had published one research paper and one book. Apart from the ALBOREX field work, the researchers had a Master Thesis and now there is another paper that is under revision. Besides, there are five communications in scientific meetings (France, Greece and Morocco) and two interviews to media. During this last period (from January to April 2015), part of the work is the result of the CSIC team solely, but another part is the result of the collaboration with the other researchers participants from the other two institutes (SIO-RAS and NIB). In the work performed together, the researchers of the three institutes had organized a PERSEUS International Workshop named "Coming to grips with the jellyfish phenomenon in the Southern European and other Seas: research to the rescue of coastal managers" which was held in Cadiz, Spain, from 2-3 March, 2015. In this workshop around 50 researchers got together for two full days and the main focus of the Workshop was to review progress in our understanding of jellyfish blooms and their dynamics, and to discuss the development of observational systems that will eventually enable better management of their impacts. The workshop was mainly funded by PERSEUS, but also was co-funded by IOC-UNESCO and CEIMAR. Also, the workshop obtained the endorsement from IMBER. The agenda of the workshop included four sessions, each of them initiated by a Keynote speaker and guided it by a chairman. The last day of the workshop, four different teams worked separately on the same several questions regarding the cutting edge research (field, modeling and management) of the jellyfish phenomenon. Each of these four working groups were leaded by a Working Group Leader, which afterwards presented in plenary the outcomes of their teams.

Another activity performed during this period was analyzing all the information obtained from the oceanographic cruise of May 2014 leaded by the CSIC and where the SOCIB/IMEDEA-CSIC team also participated, as well as researchers from the NIB. As *Pelagia noctiluca* is the specie most frequent in the Mediterranean Sea and in order to model its dynamics is necessary to take into account the fact that this organism performs vertical migrations, an oceanographic cruise was carried in the Balearic Sea. This cruise (from 13- 15 May 2014), on board the R/V *SOCIB*, searched and "hunted" a swarm of *Pelagia* and followed their daily vertical migration using different techniques (ROVs, laser and video camera counting device from the NIB, plankton nets, Lagrangian buoys drifters) and the physical environment by CTD. A total of 69 stations were sampled during night and day following a transect from coast to shelf (up to 200 m depth) and viceversa. The first results from this cruise, showed that the Lagragian buoys drifters (anchored to different water masses) followed first the main current parallel to the west coast of Mallorca Island (Fig. 7) and once in the north of the Island, followed different trajectories.

Figure 7: Trayectories of the 5 Lagragian drifters

The daily migration of the *Pelagia noctiluca* is observed in the following Figures 8 and 9, where the position of the swarm of jellyfish differs from the night (Fig. 8), at surface and close to the shore, to the day (Fig. 9), at deeper depths and further from the coast.

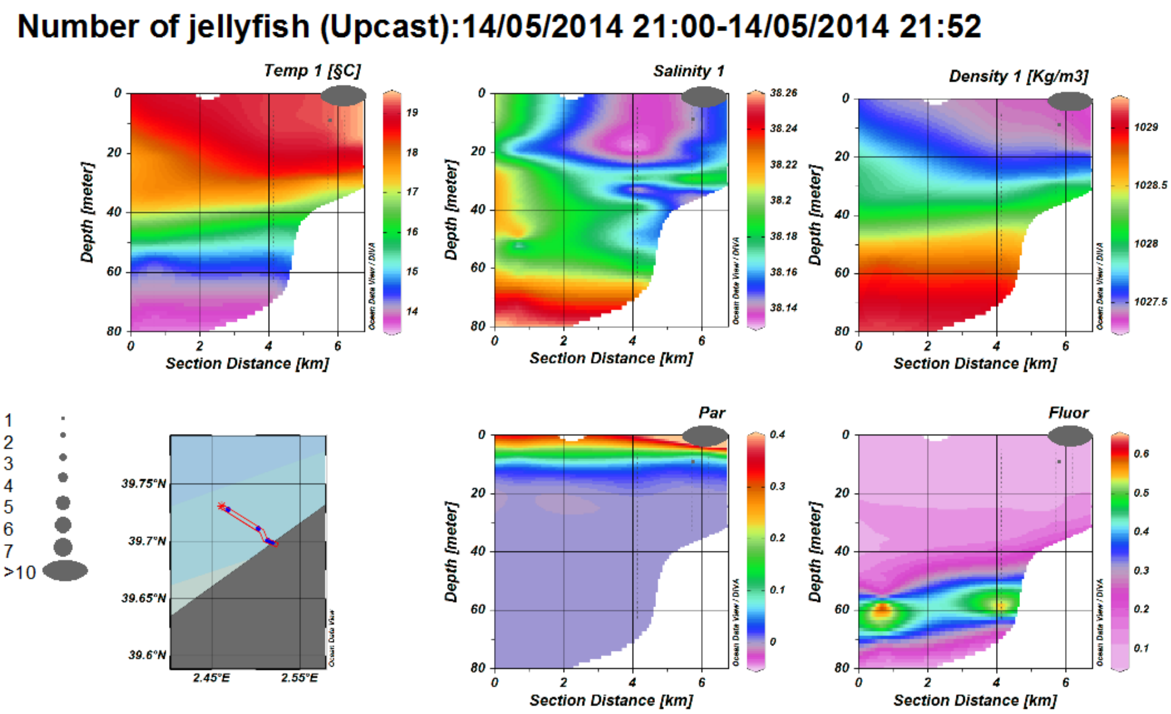


Figure 8. Transect at night showing the distribution of jellyfish (grey dots) with temperature, salinity, density, PAR and fluorescence due to chlorophyll.

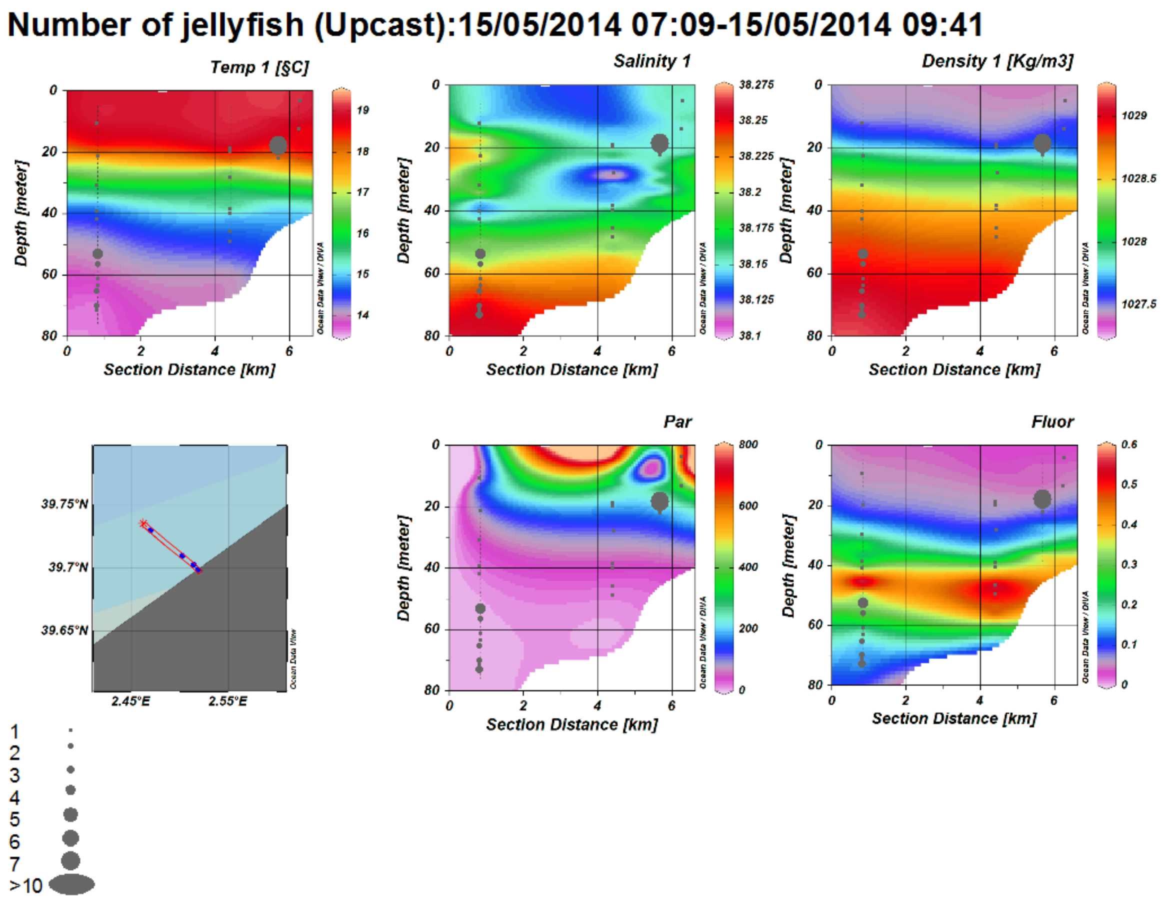


Figure 9. Transect during the day showing the distribution of jellyfish (grey dots) with temperature, salinity, density, PAR and fluorescence due to Chlorophyll.

Also, the connectivity of the population of this jellyfish in the SES was analyzed by genetic procedures considering samples form this cruise, together with samples from other sites of the SES. These analyses have been performed by the NIB-MBS team.

V. Communications in Congress, Symposiums, Meetings:

1. **Organization of workshop ICES/CIESM Mnemiopsis Sciences (JWMS**) chaired by Tamara Shiganova from PERSEUS and CIESM in A Coruna (Spain). The **ICES-CIESM workshop on 'Latest advances regarding the ecology and impact of *Mnemiopsis leidyi'*, including its associated alien predatory ctenophore *Beroe* spp. and economic aspects'**, chaired by Sophie Pitois, UK (ICES) and Tamara Shiganova, Russia (CIESM), was conducted in Spain, September 2014, back-to-back with the ASC. Joint Workshop to discuss the latest advances regarding the biogeography, ecology, impact and economic aspects of the invasive ctenophore *Mnemiopsis leidyi* and its *Beroe* predators (both native and invasive). The JWMS was attended by 20 scientists from 14 countries. The main objective of the workshop was to provide a forum for scientists to present and discuss the results of ongoing relevant research projects in the Black Sea and Mediterranean and the North Atlantic. The 13 presentations stimulated wide ranging discussions relevant to *Mnemiopsis* science.

Highlights:

- The latest knowledge regarding the biogeography and ecology of *Mnemiopsis leidyi* and of its *Beroe* predators were reviewed, as well as the spatial-and temporal occurrences across the ICES-CIESM sea basins. It was recognized that *Beroe* spp. are not the only predators of *M. leidyi* and that the various interactions of *M. leidyi* with the different components of the ecosystem, as well as mechanisms involved, are too complex to be comprehensively understood.
- *Mnemiopsis leidyi* is most likely exhibiting source-sink population dynamics within its European range.
- European seas are warming at present, and temperature is likely to affect the timing and distribution of both *M. leidyi* and *Beroe* spp. in those areas. However, the link with temperature was questioned, and the hypothesis was put forward, that food web pertubations from overfishing combined with eutrophication in coastal areas and major influx of invasive aliens, have permitted the establishment of *Mnemiopsis* propagules in some areas.

- *M. leidyi* has a wide environmental tolerance and phenotypic variability. Environmental conditions influence the development of *M. leidyi*, however it can establish in highly contrasting ecosystems. Its morphological features, growth rate, metabolism, size of maturity, fecundity differ according to environmental conditions particularly considering salinity, temperature, productivity and prey concentration.
- Modelling tools, based on experimental physiological knowledge, help to predict/understand the aforementioned processes and interactions. Several approaches were presented and discussed.
- At the same time it was acknowledged that there is not enough information on *M. leidyi* occurrence, seasonal and interannual variability in some areas, particularly in northern Europe and specific areas of the Mediterranean Sea. Improved monitoring and more comprehensive coverage of investigated areas are therefore deemed necessary, in particular field investigations of overwintering areas are needed.
- A key parameter to understand population dynamics is the winter biology of *M. leidyi*. Therefore more research effort should be devoted (i) to understand the low temperature/low food environmental interactions with *M. leidyi* and its survival under these conditions; (ii) to identify sites of overwintering populations.
- Citizen science may provide a valuable tool to improve our knowledge on poorly monitored areas, and should be encouraged.

Abstract booklet was published and put at web site ICES and CIESM: ICES. 2014. “Joint CIESM/ICES Workshop on *Mnemiopsis* Science (JWMS)”, co-editors Sophie Pitois, and Tamara Shiganova, 18–20 September 2014, A Coruña, Spain. ICES CM 2014/SSGHIE: 14. 80 pp.

2. PERSEUS International Jellyfish Workshop Cadiz (Spain), 2-3 March 2015

“Coming to grips with the jellyfish phenomenon in the Southern European and other Seas: research to the rescue of coastal managers.” *Co-funded by IOC-UNESCO and CEIMAR*

Abstract

The overall scientific objectives of PERSEUS are to identify the interacting patterns of natural and human-derived pressures on the Mediterranean and Black Seas, assess their impact on marine ecosystems and, using the objectives and principles of the Marine Strategy Framework Directive as a vehicle, to design an effective and innovative research governance framework based on sound scientific knowledge. Well-coordinated scientific research and socio-economic analysis will be applied at a wideranging scale, from basin to coastal. The new knowledge will advance our understanding on the selection and application of the appropriate descriptors and indicators of the MSFD. New tools will be

developed in order to evaluate the current environmental status, by way of combining monitoring and modelling capabilities and existing observational systems will be upgraded and extended. Moreover, PERSEUS will develop a concept of an innovative, small research vessel, aiming to serve as a scientific survey tool, in very shallow areas, where the currently available research vessels are inadequate.
In view of reaching Good Environmental Status (GES), a scenario-based framework of adaptive policies and management schemes will be developed. Scenarios of a suitable time frame and spatial scope will be used to explore interactions between projected anthropogenic and natural pressures. A feasible and realistic adaptation policy framework will be defined and ranked in relation to vulnerable marine sectors/groups/regions in order to design management schemes for marine governance. Finally, the project will promote the principles and objectives outlined in the MSFD across the SES.
Leading research Institutes and SMEs from EU Member States, Associated States, Associated Candidate countries, non-EU Mediterranean and Black Sea countries, will join forces in a coordinated manner, in order to address common environmental pressures, and ultimately, take action in the challenge of achieving GES.

VI. Published relevant papers:

Bella s. Galil, Shevy b.s. rothman, Roy gevili3 and Tamara Shiganova 2014
First record of Leucothea multicornis (Ctenophora: Lobata: Leucothidae) in the
eastern Mediterranean. Marine Biodiversity Records, page 1 of 3. # Marine Biological Association of the United Kingdom, 2014 doi:10.1017/S1755267214000979; Vol. 7; e89; 2014 Published online

Shiganova T. A., Riisgård H. U., Ghabooli S. and Tendal O. S.2014. First report on *Beroe ovata* in an unusual mixture of ctenophores in the Great Belt (Denmark) Aquatic Invasions. V. 9:1-6

Mikaelyan A S, Malej A, Shiganova T A, Turk V, Sivkovitch A E, Musaeva E I, Kogovsek T, Lukasheva T A. 2014. Populations of the red tide forming dinfl agellate *Noctiluca scintillans* (Macartney): A comparison between the Black Sea and the northern Adriatic Sea. *Harmful Algae*, Vol.33: 29–40.

Shiganova T. A., Riisgård H. U., Ghabooli S. and Tendal O. S.2014. First report on *Beroe ovata* in an unusual mixture of ctenophores in the Great Belt (Denmark) Aquatic Invasions. V. 9:1-6

Tamara A. Shiganova, Louis Legendre, Alexander S. Kazmin, Paul Nival 2014.
Interactions between invasive ctenophores in the Black Sea: assessment of control mechanisms based on long-term observations. Marine ecology Prog.Ser. Vol. 507: 111–123

Tinta T., T. Kogovšek, V. Turk, T.A. Shiganova, A.S. Mikaeliyan, A. Malej. 2014. The impact of *Aurelia* sp. degradation on the microbial community dynamics in the Black Sea. Abstracts 49th European Marine Biology Symposium, Saint Petersburg, Russia, 8-12 September 2014, p. 28

Tinta T., T. Kogovšek, V. Turk, T.A. Shiganova, A.S. Mikaeliyan, A. Malej. Microbial transformation of jellyfish organic matter affects the nitrogen cycle in the marine water column - a Black Sea case study. Journal of Experimental Marine Biology and Ecology (IN REVISION)

Prieto L., D. Macías, A. Peliz & J. Ruiz (under revision). Portuguese Man-of-War (*Physalia physalis*) in the Mediterranean: A killer jellyfish invasion? *Submitted to Scientific Reports.*

Media Interviews:

The impact of jellyfish blooms in the human activities that occur in the shore demands from the media the appearance of the researchers to inform the society about the subject. During this Period Report, Dr. Laura Prieto gave the following interviews, acknowledging PERSEUS (correlative numbers from Second Period Report)

PERSEUS

POLICY-ORIENTED MARINE ENVIRONMENTAL RESEARCH IN THE SOUTHERN EUROPEAN SEAS