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Plankton community of trafficked ports as a baseline reference for Non Indigenous Species arrivals. Case study of the Port of Bar (South Adriatic Sea)

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Abstract

Plankton (ichthyoplankton, zooplankton and phytoplankton) communities were studied in the temperate, shallow waters of the Port of Bar, one of the main cargo ports on the south-eastern Adriatic coast. Sampling was undertaken in February, April, June and October of 2015 at 12 stations using the BALMAS Port Baseline Survey protocol. The research was conducted to determine the presence of invasive and potentially toxic plankton species in the port as a result of the discharge of ballast water by ships. The most dominant species of ichthyoplankton were the eggs and larvae of the families Engraulidae, Bothidae and Sparidae, with a dominance of *Engraulis encrasicolus*, *Arnoglossus laterna* and *Diplodus annularis*. In addition to ichthyoplankton, sampling of phytoplankton and zooplankton was performed to assess the abundance and diversity of the species.

The most numerous zooplankton species throughout the investigated period were *Penilia avirostris*, *Euterpina acutifrons*, *Oithona nana*, *Acartia clausi, Centropages kroyeri, Paracalanus parvus*, Oncaeidae and the larvae of Bivalvia. One very unusual occurrence was the spawning of parrotfish, *Sparisoma cretense* (Linnaeus, 1758), a species with Atlantic origins and tropical affinities, whose presence throughout the Mediterranean has shown an increasing trend over the last decade. The most dominant species of phytoplankton were the diatoms *Chaetoceros affinis* and *Chaetoceros* spp., *Asterionellopsis glacialis, Pseudo-nitzschia* spp*., Thalassionema nitzschioides*, and the dinoflagellates G*ymnodinium* spp. and *Prorocentrum triestinum*. Potentially toxic species from the genus *Pseudo-nitzschia* reached an abundance of 10^4 cells L^{-1}. The toxic dinoflagellates *Prorocentrum cordatum* and *P. micans* reached values of 10^3 cells L^{-1}.

Although there were no HAOP species found during the survey, the presence of several potentially toxic and toxic phytoplankton species, whose impact is not sufficiently known, indicates the necessity of introducing regular monitoring activities and defining preventive protection measures.

Keywords: Fish eggs and larvae; zooplankton; phytoplankton; ballast water management; Adriatic Sea.

Introduction

Qualitative and quantitative analysis of the composition and diversity of species assemblages in the marine ecosystem is the basis for understanding the quality of the environment and the establishment of possible measures for its protection and improvement. The research of plankton communities, as an essential component of the monitoring of the marine ecosystem in the cargo port, aids the assessment of the state of the marine environment. In addition, it allows for monitoring of the entry of non-indigenous species, since maritime traffic is one of the primary pathways for introducing non-indigenous marine organisms (Barnes, 2002; Davidson *et al*., 2009; Mineur *et al*., 2009; Wanless *et al*., 2010). The impact of maritime transport on marine ecosystems in ports includes changes in water quality, changes in coastal hydrology, elevated noise levels and benthic contamination (Walker *et al*., 2019).

With the aim of preventing, minimising and ultimately eliminating the risk to the environment, human health, property and resources from the transfer of harmful aquatic organisms and pathogens via ships' ballast waters and related sediments, the *International Convention for the Control and Management of Ships' Ballast Water and Sediments (BWM Convention)* was adopted in 2004. Based on the requirements of the Convention and its additional Guidelines, and within the framework of the

European project BALMAS (Ballast Water Management System for Adriatic Sea Protection) the BALMAS Port Baseline Survey protocol was drawn up (Ninčević *et al*., 2014). One of the main tasks of this research was to use the BALMAS Port Baseline Survey protocol to conduct research in the Port of Bar, which is defined as a particularly sensitive area for the introduction of harmful aquatic organisms and pathogens (HAOP). More than 1,000 alien species have been identified in Europe's seas (Werschkun *et al*., 2014). Vilà *et al.* (2009) identified a list of the 100 most impacting species introduced into European waters. The transfer of invasive species occurs not only over larger distances, between continents, but also as a secondary spread in regional seas (David *et al*., 2013). Few investigations regarding plankton communities have been conducted in the 12 ports of the Adriatic Sea, including the Port of Bar (Možetič *et al*., 2017; Vidjak *et al*., 2018).

The aim of this study is: to assess the biological status of plankton communities as an indicator of the health of the marine ecosystem; to determine the presence of non-indigenous, toxic and potentially toxic species in the water of the port as a consequence of ballast water discharge or pollution. The main goal is to contribute to the implementation of a monitoring system for ballast water and to propose adequate management measures for the protection and improvement of the quality of the marine ecosystem.

Study area

The Port of Bar (Fig. 1) is a moderately developed seaport located in the southern Adriatic Sea. It is a port of national importance and one of the most important cargo ports on the south-eastern Adriatic coast. It has a significant competitive advantage over the northern Adriatic ports, shortening the transit time and creating savings in the cost of maritime transport. It was established in 1909, while its present form was redeveloped in 1983, when a trans-shipment terminal was installed with a capacity of 4.5 million tons of cargo per year, representing about a third of the port's projected capacity (Feasibility Study for the Port of Bar, 28 June 2014). The Port of Bar is a joint stock company, whose main business is handling and storing goods. The long-term operations of the port and the significant number of ships have caused a relatively high risk of introducing non-indigenous species through ballast waters.

Material and Methods

The sampling of plankton was carried out during 2015 (February, April, June and October). The sampling methodology of ichthyoplankton required the use of a WP2 plankton net with a mesh size of 300 μm. According to the Protocol, the sampling was performed at four predetermined stations (Fig. 1, Table 1), with the proviso that from each main position another two samples were taken at a distance of 10–15 m (a total of 12 stations were analysed during each season). Conductivity, temperature and pressure were recorded from the water column profile at each of the investigated stations. Zooplankton was sampled with a 125-μm mesh Nansen plankton net (55 cm in diameter, 150 cm in length). At each station, ichthyoplankton and zooplankton samples were collected through vertical net hauls in order to analyse the qualitative and quantitative composition. After sampling, the ichthyoplankton and zooplankton material was preserved in a 4% buffered formaldehyde solution.

The ichthyoplankton material was sorted using

Table 1. Geographical coordinates of 4 main sampling stations (WGS system).

Station	Latitude [degrees north]	Longitude [degrees east]
P1	42.05551°	19.05384°
P2	42.05155°	19.05019°
P3	42.05621°	19.04734°
P4	42.05811°	19.04831°

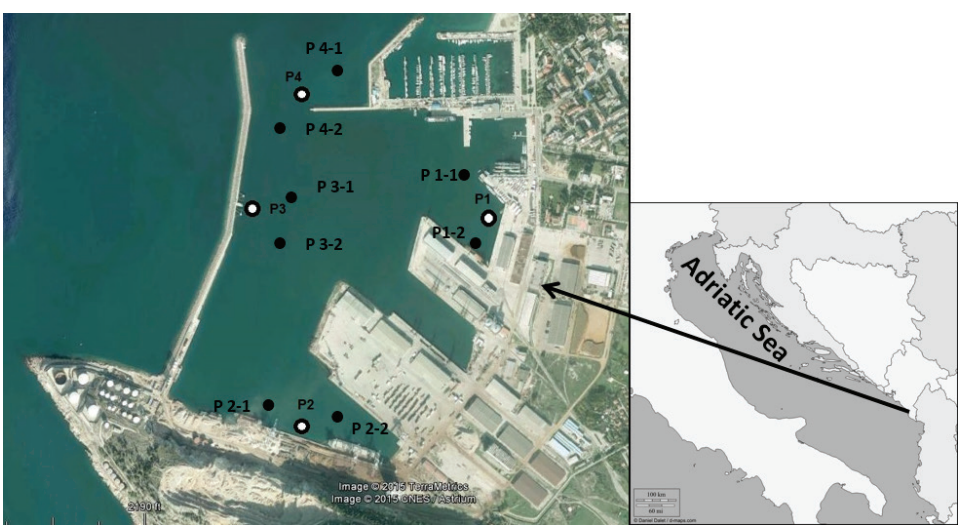

Fig. 1: Study area (Port of Bar) with 4 main sampling stations (white circles, P1-P4) and 8 additional stations (black circles, P1-1, P4-2).

NIKON SMZ 800 binocular equipped with a MOTIC camera. Determination of the material was made at the species level, and in cases when this was not possible, determination was done just at the genus level. The number of eggs, larvae and postlarvae was expressed as the number of individuals per m^2 of the sea surface, using the formula given by Tanaka (1973). Each sample of zooplankton was subsampled in the laboratory, depending on the abundance of individuals in the total sample. Zooplankton was counted from a representative sample of 1/64 of the total catch. After that, the entire material was carefully analysed in order to record any rare species. Zooplankton was presented as the number of individuals per m^3.

Phytoplankton was sampled with 5-litre Niskin bottles, at three depths: the surface, middle and bottom (0.5 m, 5 m and 10 m, respectively). The samples were preserved in 250-ml plastic bottles using a 3% neutralized formaldehyde solution.

At each station micro-phytoplankton (>20 μm) was collected by vertical tows using a phytoplankton net with a mesh size of 20 μm for qualitative analysis. Another net sample was obtained by a horizontal tow at a depth of approximately 2 m. Samples for the determination of chlorophyll *a* were also collected at three depths: the surface, middle and bottom at each position using a 5-litre Niskin bottle.

Phytoplankton cells were identified and counted using a Leica DMI4000 B inverted microscope (Heerbrugg, Switzerland) in subsamples of 25 ml after 24 hours of sedimentation, following Utermöhl (1958). Enumeration was carried out using phase contrast and bright field illumination at magnifications of 100, 200 and 630×. At a 100× magnification, the entire chamber bottom was scanned for taxa larger than 30 μm, while abundant micro-phytoplankton (>20 μm) was counted at two transects at a magnification of 200×. The cell abundance was expressed by the number of cells/L. Due to the very shallow waters in the port (6-15 m), all the samples were taken at a maximum depth of 10 m.

Data analysis

The similarity between the most dominant plankton species was analysed using clustering analyses based on the Bray-Curtis similarity metric, applying a comparison of the abundance data. The Spearman's rank correlation routine in the Primer 5.0 computer package was used to identify the species that contributed most to the Bray-Curtis similarities of stations within the identified station groupings. The plankton community structure was linked to environmental variables (temperature and salinity) using Spearman's Rank Order Correlation. This analysis compares ordinations from abiotic configurations and selects the subset of environmental variables that provides the best match with the species presence.

The diversity of plankton communities was analysed using the Shannon diversity index (H′). Diversity indices are measures of community attributes that are often used as indicators of the environmental conditions (Clarke & Warwick, 1994). Diversity analysis was carried out for each investigated position on the basis of the season. The Shannon diversity index allowed the individuation of the importance of rare species in the sample. It was calculated according to the following formula (Krebs, 1999):

$$H' = -\sum_{i=1}^{s} (p_i)(\log_2 p_i)$$

where p_i is the proportion of the i-th species in the sample, and s is the number of species in the sample. The value of the Shannon index increases with an increasing number of species. In practice it has been shown that for biological communities the value of H′ does not exceed 5.0 (Krebs, 1999).

Results

A total of 17 species of pelagic eggs and the larvae of fish belonging to 11 families were found during the investigated period (Table 2).

The dominant species of ichthyoplankton were the eggs and larvae of the families Engraulidae, Bothidae and Sparidae, with an abundance of 5–56 eggs m^{-2} for *Engraulis encrasicolus*, 5–41 eggs m^{-2} for *Diplodus annularis* and 5–36 eggs m^{-2} for *Arnoglossus laterna*.

In February 2015, from a total of 12 samples, the early development stages of fish were found in only two samples with a very low abundance (3.3 eggs/larvae m^{-2}). All the other samples were negative for ichthyoplankton,

Table 2. List of ichthyoplankton species by season (x, presence in the samples).

Species	February	April	June	October
Arnoglossus laterna	x	x	x	
Buglossidium luteum		x		
Callionymus pusillus			x	
Callionymus risso	x			
Centrolophus niger			x	
Diplodus annularis		x	x	
Engraulis encrasicolus			x	
Gobius sp.				x
Lithognathus mormyrus			x	
Mullus sp.			x	
Sciaena umbra			x	
Scomber japonicus				x
Scomber scombrus				x
Scophthalmus maximus		x		
Serranus scriba			x	
Sparisoma cretense				x
undetermined			x	

which was expected due to the relatively low depths at which sampling was carried out (6–10 m). In April 2015, eight stations tested positive for the presence of the early life stages of fish, while in July all the samples tested positive for ichthyoplankton with a higher degree of diversity and abundance in the range of 3.3–26.6 eggs/larvae m^{-2}. The most dominant species were *D. annularis* and *A. laterna*. In October, only six stations tested positive for ichthyoplankton with the presence of *Sparisoma cretense, Scomber scombrus, S. japonicus* and larvae of *Gobius sp*. During the October spawning the intensity was very low (3.3 eggs/larvae m^{-2}).

The qualitative and quantitative analyses of the zooplankton species composition showed the presence of 60 different species. The most abundant (more than 90% of the total) were *Penilia avirostris* (1–794 individuals m^{-3}), *Euterpina acutifrons* (4–368 ind. m^{-3}), *Oithona nana* (8–144 ind. m^{-3}), *Acartia clausi* (4–112 ind. m^{-3}), *Centropages kroyeri* (2–96 ind. m^{-3}), *Paracalanus parvus* (3–96 ind. m^{-3}), Onceaidae (13–341 ind. m^{-3}) and the larvae of Bivalvia (4–192 ind. m^{-3}) (Table 3).

The qualitative and quantitative analyses of the phytoplankton species composition showed the presence of 141 different species. The most dominant species of phytoplankton were the diatoms *Chaetoceros affinis* and *Chaetoceros* spp. which reached 10^5 cells L^{-1}. *Asterionellopsis glacialis, Pseudo-nitzschia* spp. and *Thalassionema nitzschioides* reached abundances of up to 10^4 cells L^{-1}. The dinoflagellates G*ymnodinium* spp. and *Prorocentrum triestinum* reached abundances of up to 10^4 cells L^{-1}. Potentially toxic species from the genus *Pseudo-nitzschia* reached an abundance of 10^4 cells L^{-1}. The toxic dinoflagellates *Prorocentrum cordatum* and *P. micans* reached values of 10^3 cells L^{-1} (Table 4). For phytoplankton and zooplankton, only the dominant species were presented.

Table 3. Dominant zooplankton species (in order of contribution, %).

Species	% of contribution	min (ind. m^{-3})	max (ind. m^{-3})
Penilia avirostris	16.77	1	793.6
Euterpina acutifrons	16.22	4	368
Oncaeidae	13.42	15	350
Bivalvia larvae	10.58	4	400
Acartia clausi	5.17	4	112
Oithona nana	4.67	24	144
Paracalanus parvus	4.18	4	96
Centropages kroyeri	4.07	2	140.8
Gastropoda larvae	3.64	4	85.3
Oithona similis	2.93	0.5	96

Environmental data

The hydrographical data was processed in the Ocean Data View software package (Schlitzer, 2018). Comparative analysis of the data for temperature and salinity showed no anomalies caused by the inflow of water from rivers, underground sources or changes in salinity and surface temperature caused by precipitation. The sea water temperature varied from 11.4°C to 23.3°C, depending

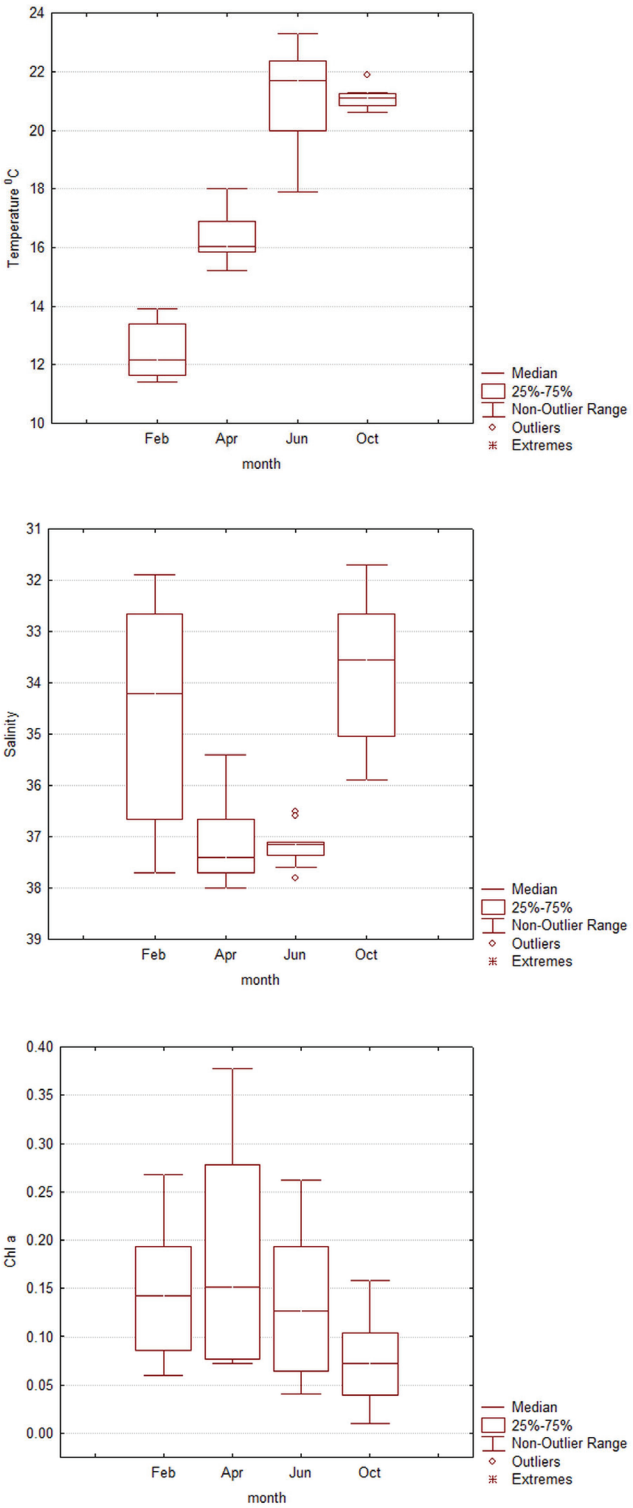

***Fig. 2**:* Box plot diagram – Temperature (T °C), Salinity (PSU) and Chlorophyll *a* (mg/m3) variations according to seasons.

Table 4. Dominant phytoplankton species (in order of contribution % and presence in different part of water column. surf – surface; midd – middle; bott – bottom).

Species	water column	% of contribution	min cells/L	max cells/L
Chaetoceros spp.	*surf*	17.14	3,140	154,645
Chaetoceros affinis	*surf*	8.69	1,570	100,480
Pseudo-nitzschia spp.	*surf*	5.26	706	22,765
Gymnodinium spp.	*surf*	3.70	600	41,605
Bacteriastrum hyalinum	*surf*	3.22	200	49,455
Asterionellopsis glacialis	*surf*	2.87	480	28,560
Prorocentrum triestinum	*surf*	1.94	40	32,185
Thalassionema nitzschioides	*surf*	0.85	640	7,080
Calyptrosphaera oblonga	*surf*	0.85	523	9,420
Chaetoceros danicus	*surf*	0.66	10,990	11,775
Chaetoceros spp.	*midd*	10.38	1,570	71,435
Chaetoceros affinis	*midd*	4.17	360	29,830
Pseudo-nitzschia spp.	*midd*	3.73	1,570	21,195
Asterionellopsis glacialis	*midd*	3.10	1,000	30,160
Bacteriastrum hyalinum	*midd*	2.28	440	32,185
Thalassionema nitzschioides	*midd*	1.05	600	7,720
Calyptrosphaera oblonga	*midd*	1.04	523	7,850
Gymnodinium spp.	*midd*	1.00	785	7,605
Chaetoceros danicus	*midd*	0.59	7,065	13,345
Rhabdosphaera tignifer	*midd*	0.43	785	7,065
Chaetoceros spp.	*bott*	6.80	1,570	65,155
Pseudo-nitzschia spp.	*bott*	4.45	785	33,755
Chaetoceros affinis	*bott*	4.15	120	51,025
Thalassionema nitzschioides	*bott*	0.87	520	4,640
Bacteriastrum hyalinum	*bott*	0.75	4,710	21,195
Asterionellopsis glacialis	*bott*	0.53	1,600	6,760
Gymnodinium spp.	*bott*	0.48	785	4,710
Calyptrosphaera oblonga	*bott*	0.36	1,570	3,140
Navicula spp.	*bott*	0.35	280	3,400
Syracosphaera pulchra	*bott*	0.22	785	3,140

on the investigated season, while the salinity ranged from 31.7 to 38.0 PSU (Fig. 2). Although the investigation was conducted in shallow water, fluctuations of salinity in the entire water column were observed, which extended from the surface down to a depth of 12 m.

The value of chlorophyll *a* ranged from 0 to 0.378 mg/m^3 with a fluctuation from the surface to the maximum sampling depth, without any regular changes in relation to depth. The box plot diagram showed no statistically significant differences in the values of temperature ($p = 0.9591$), salinity ($p = 0.8759$) and chlorophyll *a* ($p = 0.0511$) between stations, such differences were obvious between seasons ($p = 0.001$, $p = 0.00000003$ and $p = 0.0176$, respectively) (Fig. 2).

Data analysis

Analysis of the diversity index shows the average diversity during each season. The value of the Shannon index was 0.56–1.86, 1.79–2.45 and 1.32–2.18 for ichthyoplankton, zooplankton and phytoplankton, respectively (as an average value per station) (Fig. 3).

Analysis of Spearman's rank correlation (Table 5) between the species abundance and environmental factors (temperature and salinity) showed a positive correlation ($p < 0.01$) with temperature for *Chaetoceros* spp., *Pseudo-nitzschia* spp. and *P. avirostris*, while a positive correlation with salinity was evident only for *Gymnodinium* spp and *A. laterna*. The dendrogram of the Bray-Curtis similarity showed an important similarity between the samples for October and June and for February and April (Fig. 4).

Table 5. Spearman's Rank Order Correlation between dominant species and main environmental parameters (in order of correlation).

Spearman's Rank Order Correlation	Temperature (°C)	Salinity
Gymnodinium spp.	0.518*	0.655**
Engraulis encrasicolus	0.552*	0.599*
Diplodus annularis	0.522*	0.537*
Penilia avirostris	0.851***	0.176
Chaetoceros spp.	0.658**	-0.155
Chaetoceros affinis	0.634**	-0.160
Pseudo-nitzschia spp.	0.685**	-0.275
Arnoglossus laterna	0.072	0.625**
Euterpina acutifrons	0.497*	-0.109
Serranus scriba	0.345	0.161
Oithona nana	0.144	-0.179
Bacteriastrum hyalinum	0.138	-0.051
Oncaeidae	-0.215	0.258
Acartia clausi	-0.219	0.162
Callionymus risso	-0.431	0.239
Bivalvia larvae	-0.452	0.399

*p < 0.05; **p < 0.01; ***p < 0.001

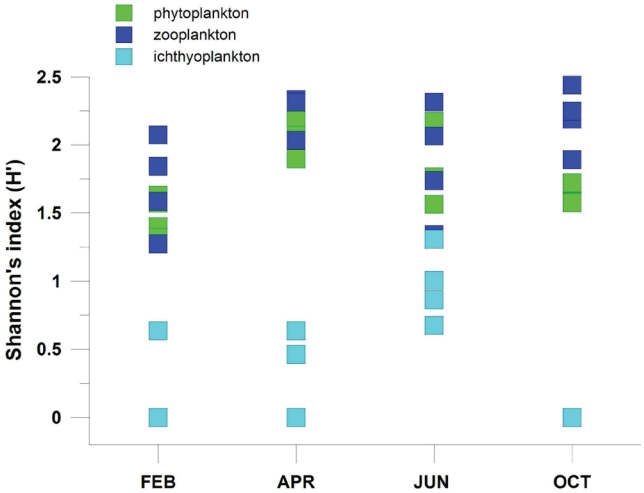

Fig. 3: Shannon index for ichthyoplankton, zooplankton and phytoplankton (average values for all investigated stations).

Discussion

This research was done in order to assess the presence of non-indigenous and/or potentially toxic species of ichthyoplankton, zooplankton and phytoplankton. Although it was conducted in a very small part of the Port of Bar and there were no occurrences of HAOP species in the investigated area, it provides quality recommendations for the improvement of the port's management measures in improving the ecological status, especially in the case of ballast water monitoring. Plankton samples were taken in order to form a baseline study of the communities before starting ballast water monitoring in ships' tanks.

The qualitative and quantitative composition of ichthyoplankton showed a relatively low rate of diversity, except during June. By comparing the research with other Mediterranean areas, and taking into account the limitations of the investigated area – the very shallow waters and a small number of stations – it can be concluded that the general diversity of ichthyoplankton was high, although the spawning intensity was very low during the autumn and winter periods. In the Mar Menor lagoon in south-east Spain, a study of the qualitative and quantitative composition of ichthyoplankton at 20 positions during monthly sampling showed the presence of

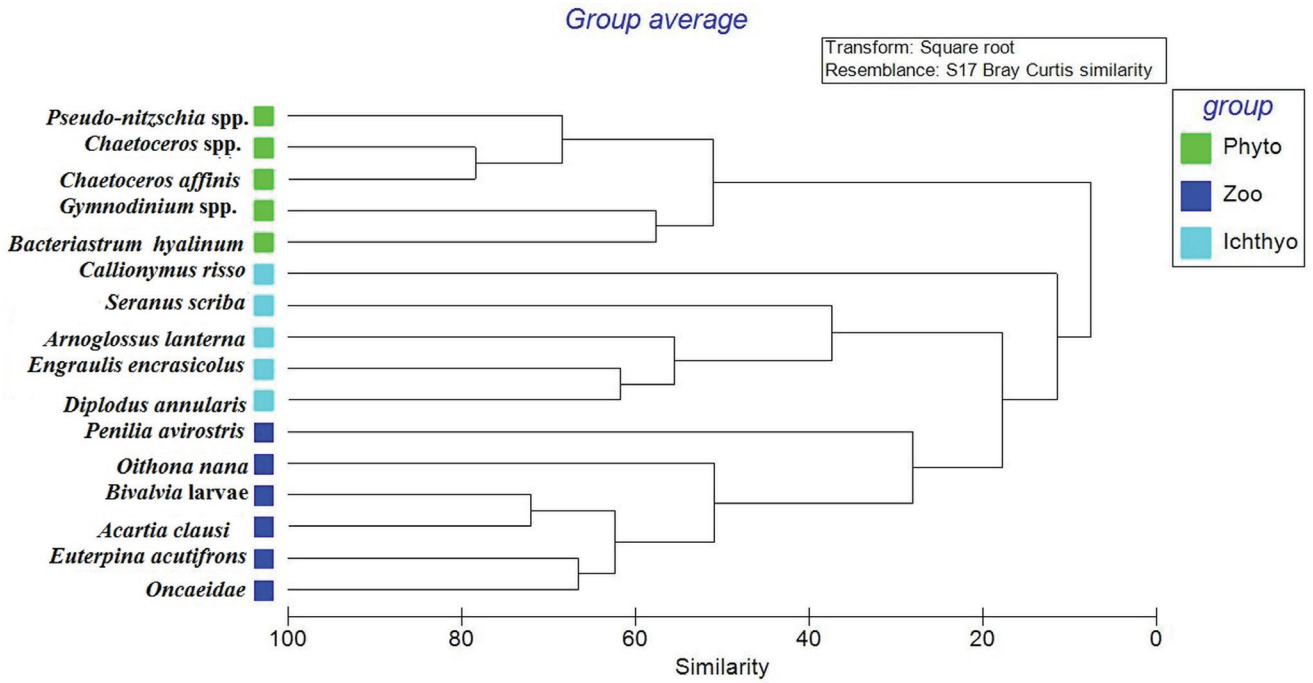

Fig. 4: Cluster analysis of all sampling seasons related to different plankton communities

36 species from 14 families in the lagoon's waters (Perez-Ruzafa *et al*, 2004). In the Boka Kotorska Bay, during a three-year seasonal research on the abundance and diversity of ichthyoplankton, the presence of 35 different species was determined (Mandić *et al*., 2014). In the harbour area of Porto Montenegro (Boka Kotorska Bay, south Adriatic Sea) a similar diversity of ichthyoplankton species was recorded (Pestorić *et al.,* 2018). Larval fish communities and their spatial distribution are the result of adult spawning strategies and environmental influences (Franco-Gordo *et al*., 2008; Sabatés *et al*., 2007). This can also be influenced by the specificity of the study area, i.e. the vicinity of the coast and continental runoff and upwelling areas (Matsuura, 1996; Lopes *et al*., 2006). It can be concluded that the small area of the Port of Bar and the protection from the wind play a significant role in the spatial distribution and retention of ichthyoplankton. Poor water circulation, the effect of the waves or winds (as important factors that influence the dispersion of the early development stages of fish) are limiting factors for the aggregation of adult fish and their spawning in the port area. Despite the low intensity and unfavourable conditions for spawning (different sources of pollution, noise and disturbance of the seafloor due to anchoring, as well as other port activities), there were still a significant number of different fish species found during the survey. The seasonal pattern of abundance and diversity of ichthyoplankton is consistent with the occurrence of seasonality in other (non-port) seas (Li *et al*., 2014).

What is very interesting is the occurrence of the larval stages of *Sparisoma cretense.* This species has an Atlantic origin and tropical affinities, with its presence in the Adriatic confirmed in 2000 (Dulčić & Pallaoro, 2001; Guidetti & Boero, 2001). Its growing presence along the entire Mediterranean coast during the last decade is considered as an indicator of tropicalisation (Kruschel *et al*., 2012; Astruch *et al*. 2016). It is very likely that this species has established its population and that spawning is only a confirmation of the favourable environmental conditions for growth, development, nutrition and reproduction in the area of the south Adriatic coast, and likely in the wider area. It is important to stress that, for the purpose of better comparability of the data with other similar research areas, it is necessary to standardise the sampling methodology of ichthyoplankton. This would require using a WP2 plankton net with a mesh size of 200 μm (instead of 300 μm), which is commonly used for vertical sampling of ichthyoplankton.

In the case of zooplankton, among the most dominant species during this investigation were species of the genera Cladocera and Copepoda. As in other coastal areas, the species from those genera are usually dominant in mesozooplankton (Vidjak *et al*., 2006; Vidjak *et al*., 2007; Pestorić *et al*., 2017). They play a very important trophodynamic role due to fact that they are food for carnivorous plankton (especially fish larvae and pelagic fish species) (Cheng & Chao, 1982; Beaugrand *et al*., 2003). Many studies have shown that zooplankton can be used as indicator for monitoring the state of a marine ecosystem during a period of climate change (Beaugrand *et al*., 2003; Eloire *et al*., 2010).

In the majority of plankton studies, salinity and temperature have been shown to be among the most important parameters affecting the distribution and abundance of plankton (Harris *et al*., 2000; Esteves *et al*., 2000; Mouny & Dauvin, 2002; Beaugrand *et al*., 2003). This study confirmed the correlation of certain species with temperature and salinity. Since it was a very shallow and confined area, variability of the environmental parameters was expected to be more intense than in the open sea (Belmonte *et al*., 2013).

Although there were no HAOP species found during the present survey, several phytoplankton genera (*Pseudo-nitzschia* spp., *Prorocentrum cordatum* and *P. micans*), which are toxic and/or potentially toxic, indicate the necessity of establishing measures for the regular monitoring of the port in order to define preventive protection measures. Those species were dominant throughout the water column (surface, middle and bottom) and their harmful effect is still not known. Potentially toxic diatom species from the genus *Pseudo-nitzschia* are permanently present in the phytoplankton community in the Mediterranean and in the Adriatic Sea (Orsini *et al*., 2002; Quiroga, 2006; Bosak *et al*., 2009; Drakulović *et al*. 2012; Drakulović *et al*. 2016; Drakulović *et al*., 2017). Species from this genus are able to produce domoic acid, which is responsible for amnesic shellfish poisoning (ASP) (Bates *et al*., 1998). The composition of domoic acid, its distribution and relation with physico-chemical parameters still needs to be clarified, and some domoic acid records have even been confirmed in the northern Adriatic (Marić *et al*., 2011).

In addition to the current proposed monitoring of the invasive and non-indigenous species, it is crucial to monitor the total diversity of species for the purpose of comparative analysis, preventive measures and improvement of the ecological status of the port. In order to prevent the risk of introducing non-indigenous, potentially toxic or toxic species through ballast water, it is important to regularly monitor plankton communities throughout the port and the wider area. Only a long-term data series, or a search for resting stages in the sediment, could indicate the real state of the diversity and abundance of species, as well as the connection between plankton communities in relation to the environmental conditions and possible sources of pollution.

Acknowledgements

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