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Links between epibenthic community patterns and habitat characteristics in the Parila lagoon (Croatia)

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Abstract The aim of the study was to describe epibenthic macroinvertebrates and demersal fish and to identify links between spatial patterns and habitat characteristics (sediment, salinity, oxygen and temperature). The research was performed in the Parila lagoon in Croatia; an important nursery ground where conditions in epibenthic community can have significant direct and indirect impact on fisheries. Diversity (30 species) and abundance (from 0 to 7 ind m^{-2}) of epibenthic assemblages was low on all stations and showed remarkable seasonal changes. Such patchiness could support the estuarine quality paradox idea, i.e. the most abundant species are well adapted to changing conditions in a variable environment. The ecological analysis showed that total phosphorus concentration (TP), temperature (T) and salinity (S) were the main environmental parameters affecting community structure of the Parila lagoon. Six species listed as frequent (*Nerophis ophidion*, *Zosterisessor ophiocephalus*, *Syngnathus typhle*, *Cerastoderma glaucum*, *Palaemon adspersus* and *Carcinus aestuarii*) have a potential to be used as indicators of habitat quality. In terms of trophic diversity, community was dominated by carnivores and omnivores. Fish trophic index (above 2.7) indicated upward shift of the local food chain and towards mostly carnivorous species, while the absence of herbivorous species pointed to low environmental quality. The present study gives an insight into the current ecological conditions in the Parila lagoon. Further research about the sensitivity of these potential indicators to different types of pressure is necessary in order to select robust early-warning indicators framework.

Keywords Coastal lagoon · Epibenthic community · Epibenthic predation · Trophic guilds · Indicators · Ecological quality

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Introduction

Mediterranean coastal lagoons are valuable systems, providing an array of ecosystem services and thus having a crucial ecological and social relevance (Ferrarin et al. 2014). Although their importance has been widely recognized at the international level (Ramsar Convention; Natura 2000 ecological network), their ecological functioning is still not thoroughly studied. It is a known fact that lagoons are naturally stressed systems. Due to tight boundary to terrestrial ecosystems, lagoons are nowadays facing increasing impacts, including physical and chemical transformation, habitat destruction and changes in biodiversity (Acquavita et al. 2015; Newton et al. 2014; Pérez-Ruzafa et al. 2011; de Wit et al. 2017).

A wide array of indicators were developed and used to assess the Ecological Quality (EcoQ) status of a given water body, to detect the cause of environmental change and to forecast future changes in the environment (e.g. Dale and Beyeler 2001; Niemi and McDonald 2004; Van den Broeck et al. 2015). Pinpointing key indicators and relationship among them can help identify stressors and habitat change and represents an important link to detect large-scale changes (Stem et al. 2005). This approach is fundamental to good management practice.

Benthic and more specifically, epibenthic macroinvertebrates are considered as potentially powerful indicators of marine ecosystem health: they are situated at the sediment-water interface where they play an important role in recycling nutrients and materials and are excellent integrators of change of both systems (Dauvin 1993; Blanchet et al. 2008). In estuarine food webs, they represent a link between highly productive organic matter sources of the shallows and ecologically and economically important nekton that use such habitat during their lifecycle as nurseries (Gillet 2010). Nursery function of estuaries and lagoons is evidenced (as reported in O'Mara et al. 2016), but there are still knowledge gaps about how fish communities depict e.g. lagoon conditions. The ecological organization of fish communities is considered to be influenced primarily by environmental variables and habitat complexity. Soft bottoms, due to a high degree of physical disturbances, usually exhibit scarcely developed epibenthic fish species, favoring predators with sedentary habits and cryptic features (Farré et al. 2015). Epibenthic predation pressure has a structuring influence on the macrobenthos and is one of the major organizing forces within communities (van der Heide et al. 2014; Van Tomme et al. 2014). Since transitional waters often have crucial nursery function, epibenthic conditions can have significant direct and indirect impact on fisheries as an important ecosystem service (Van Tomme et al. 2014). Any change in composition and production in the macrobenthic community is expected to cascade through other trophic levels. Therefore, both epibenthic macroinvertebrates and fish and the relationship between them should be observed together when assessing ecosystem EcoQ status. Due to their different biological traits (e.g. life cycles, trophic levels and/or mobility), macroinvertebrates and fish are expected to have different responses to environmental parameters and disturbances (Henriques et al. 2014).

Parila lagoon in Croatia, remaining fragment of the old Mediterranean wetland, has crucial ecological role as an important feeding, spawning and nursery ground for fish, crustacean and bivalve species and coastal marine fisheries depend on the lagoon for stocks recruitment (Glamuzina 2010). Species utilising nurseries are constrained to a set of habitats during their juvenile life phase (Parsons et al. 2015). Changes in habitat quality due to multiple stressors may result in decrease in nursery function: a fish community facing severe anthropogenic disturbance will be characterized with lower diversity and complexity (Fonseca et al. 2013) and consequently affect adult abundance and connectivity between coastal and marine environments (Vasconcelos et al. 2011, 2014). Over the past decades, the lagoon has undergone many natural and anthropogenic changes (Dulčić et al. 2011; Sarà et al. 2014), and it is of paramount importance to better understand these habitats that serve as nurseries and the factors that create site-specific variability in order to improve conservation and management of this area.

The aim of the study was to describe the epibenthic macroinvertebrates and demersal fish community in the Parila lagoon and to identify links between spatial patterns and habitat characteristics through geochemical characteristics of the sediment, salinity, oxygen and temperature. In addition to contributing to a better understanding of the area, more information on the benthic environment in this lagoon is beneficial because of its aforesaid ecological role. Consequently, another objective of this research was to evaluate the potential of obtained data to describe EcoQ status of Mediterranean lagoons and the suitability to use both epibenthic macroinvertebrates and fish as indicators of habitat change.

Materials and methods

Study area

Parila lagoon is a brackish coastal lagoon connected to the Adriatic Sea (Fig. 1a). The lagoon has a total surface of 210 ha and is located north of Neretva River Delta, between the river mouth and Port of Ploče in Croatia. Neretva River Delta, together with the adjacent Parila lagoon, represents the largest area of brackish waters in Croatia, and the only fragments of the old Mediterranean wetlands that have survived extensive land reclamation projects (RIS, Ramsar Information Sheet 2012). The lagoon is characterized with sandy to muddy bottom, *Cymodocea nodosa* patches and a maximum depth of 50 cm (Glamuzina L. et al. 2014a). It is considered as an area of great biological diversity (RIS 2012), included in Natura 2000 ecological network.

Sampling and analysing biological and environmental descriptors

Sampling was carried out seasonally at 3 months interval from February to November 2011 (hereinafter February was considered as winter, May as spring, August as summer and November as autumn season). Samples were collected at 9 stations encompassing different depths and sediment types and different hydrological regimes inside the lagoon (Fig. 1b). The biological descriptors included epibenthic macroinvertebrates and benthic fish species. The environmental descriptors included sediment grain-size, organic matter, carbonate and phosphorus content, water salinity, dissolved oxygen and water temperature.

Epibenthic macroinvertebrates were sampled to a depth of 20 cm using a small hand dredge (80 × 20 mm) equipped with a nylon net bag (100 cm length and a 5 mm mesh size). Fish were sampled using a 400 cm long beach seine with 200 cm long wings (mesh wings size 10 mm and mesh bag size 6 mm)

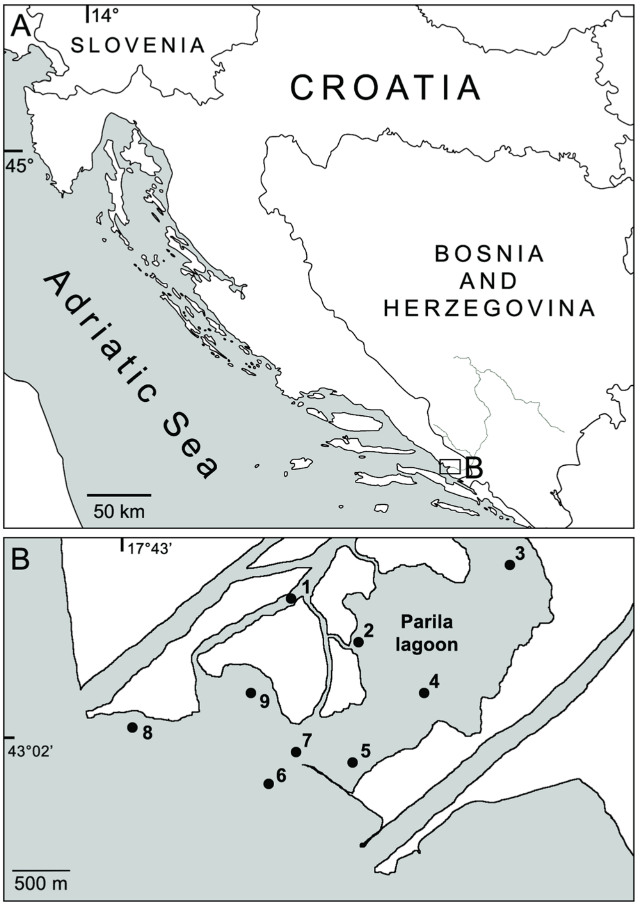

Fig. 1 Study area. **a** location of the Parila lagoon; **b** sampling stations encompassing different depths, sediment types and hydrological regimes inside the lagoon

which was dragged along the sea bottom. The dragged area for dredge and net was recorded each time to enable the expression of sample information per m^2 (ind m^{-2}). The data obtained with the dredge and the seine were analysed separately, as they sampled different components of the benthic community. Samples were preserved in 4% formalin solution in seawater until laboratory processing, after which they were sorted, identified to the species level (where possible), measured and counted.

Sediment was collected using an Uwitec gravity corer adapted for hand sampling with 9 cm diameter core. The sediment core was sliced into 1 cm thick sub-samples, frozen and freeze-dried until further analysis. In the present paper, mean values for the first 10 cm of cores were presented. The grain-size analysis in the sub-samples was determined by sieving (> 63 μm), and hydrometric method according to Casagrande (< 63 μm) (Strmac 1952). Sediment type was determined according to the Shepard classification (Shepard 1954). Granulometric parameters were calculated according to Folk and Ward (1957).

The organic matter content was determined by H_2O_2 treatment of the samples at 450 °C for 6 h. The carbonate content pronounced as $CaCO_3$ was determined as weight loss after treatment with 4 M HCl (Loring and Rantala 1992). Values for organic matter and carbonate contents were expressed in percentages (%). Freeze-dried sediment samples were grounded and sieved (ϕ <250 μm), and total phosphorus (TP) was determined using the method by Aspila et al. (1976). Phosphorus concentrations in extracted solutions were measured with a Shimadzu UV-VIS Spectrophotometer according to Strickland and Parsons (1972) and values were expressed as μmol g^{-1}. Standard sediment material PACS-2 (NRC-CNRC) was used for method evaluation.

Water temperature (°C), salinity (practical salinity scale) and dissolved oxygen (mg l^{-1}) were measured at each site during sampling with multiparametric probe (YSI Pro2030). It should be noted that some physico-chemical parameters were not measured on all stations during winter and autumn samplings due to inclement weather conditions.

Data analysis

The main structure parameters of the epibenthic macroinvertebrates and demersal fish measured at each station were the specific richness (S, number of species) and the abundance (A, number of individuals per m^2). In addition, the seasonal Shannon-Wiener index (H′) and interspecific species association were calculated in accordance to standard methods (Ludwig and Reynolkds 1988). Comparison between average values was conducted using standard ANOVA tests with significance level set at 0.05. Identified species were classified into trophic groups based on available databases (The European Register of Marine Species, ERMS; World Fish Database, FishBase; World Register of Marine Species, WoRMS) as follows: surface deposit feeders (SDF), sub-surface deposit feeders (SSDF), suspension feeders (SF), omnivores (O) and carnivores (C). Species belonging to more than one feeding guild were classified by their most common feeding mechanism. The Trophic Index for the sampled fish species was derived from FishBase.

Ecological analysis of the effects of the environmental parameters on the species assemblages on each of the 9 stations was conducted using the software Primer 6 (User 6981; Clarke and Gorley 2006). Monthly abundance data (ind m^{-2}) were used to standardize samples originating from the two gears. Due to high variability and seasonality of the samples, the raw abundance data were transformed based on the square root of the raw data, while the raw environmental data were normalized. Pairwise resemblance matrix of the abundance data was calculated based on the Bray-Curtis similarity index, while the resemblance matrix of the environmental data was calculated based on the Euclidean distances of the data (PCO mode). Firstly, a Principal Components Analysis (PCA, not shown here) was conducted to receive a first view of the monthly relations between stations (data) and environmental data (vectors) (PC1: 56.3%, PC2:22%, overall = 78.3%). In a second stage, the relate function (based on the pairwise Spearman analysis of the resemblance matrices of the environmental and biological data) was used in order to examine whether the variability of the environmental parameters was related to the variability of the abundance data i.e. to examine if there were statistically significant overall patterns between the two resemblance matrices. A step-wise Distance Linear Model (DistLM) analysis was performed between the two resemblance matrices in order to examine and identify in detail which environmental parameters were affecting significantly the community patterns in the Parila lagoon. Best model selection criterion used in this analysis was the small-sample-corrected Akaike Information Criterion (AICc) based on which the model with the least environmental parameters was selected (in order to minimize Akaike value; Burnham and Anderson 2002). Similar procedure was followed using a biological data set: the abundance data per feeding guild of the species in the samples. The species were grouped based on their basic feeding guild as Carnivores (C), Omnivores (O) and Organic Matter feeders (OM) and their abundance per month was averaged and then related to the environmental data matrix. Finally, a PERMANOVA design was created using 2 factors: the sampling month (temporal factor) and the station (location factor) in order to further analyse the spatio-temporal effects on the community structure.

Results

Spatial and temporal pattern of epibenthic macroinvertebrates and fish

A total of 30 species were found in the samples from Parila lagoon, belonging to the groups of fish (18 species), crustaceans (prawns and crabs; 6 species), gastropods (4 species) and bivalves (2 species). List of species and their feeding guilds is summarized in Table 1. Species diversity and abundance showed clear spatial and temporal fluctuations. Shannon-Wiener index (H′) was highly variable in all stations from the samples collected in winter and spring, with the highest values of diversity recorded during spring and autumn (H′ ± SD, 1.03 ± 0.30 and 1.09 ± 0.57, respectively). The diversity profile during summer was smoother than all the other seasons with the lowest observed values (0.87 ± 0.15), indicating a more uniform environment in the whole Parila lagoon area (Fig. 2). The species spatial appearance pattern was highly variable: while some species appeared at all stations in all seasons (i.e. crustaceans *Carcinus aestuarii*, *Palaemon adspersus* and fish *Zosterisessor ophiocephalus*), other were recorded on a few or one station only (e.g. bivalve *Chamelea gallina*, crustacean *Crangon crangon* or fish *Symphodus roissali*).

Species abundance varied between 0 and 7 ind m^{-2} (minimum and maximum from all seasons and stations) with an overall average of 0.07 ± 0.4 in. m^{-2} (average ± SD). This high variance was caused with the seasonality of species appearance. In the samples from winter, spring and summer, only 5 ± 2 species per station appeared, while 7 ± 4 species per station appeared in autumn. The difference between the averages were not statistically significant (ANOVA test, $p = 0.58$). The overall number of species recorded in each sampling month was 12, 16, 19 and 24 during winter, spring, summer and autumn, respectively (all stations pooled). The seasonal appearance pattern per species is summarised in Table 2. The ten most abundant species from all samples pooled were (listed here based on taxonomic groups): bivalves, the lagoon cockle *Cerastoderma glaucum* and the striped venus clam *Chamelea gallina,* gastropods, the netted dog welk *Nassarius reticulatus* and the cyclops nassa *Cyclope pellucida,* crustaceans, the Baltic prawn *Palaemon adspersus* and the green crab *Carcinus aestuarii,* fish, the grass goby *Zosterissessor ophiocephalus,* grey wrasse *Symphodus cinereus,* the straightnose pipefish *Nerophis ophidion* and the broadnosed pipefish *Syngnathus typhle* (see Table 3 for details). Out of these ten species, only six of them were listed as frequent, i.e. appearing from 10 to 27 times throughout the year (*N. ophidion, Z. ophiocephalus, S. typhle, C. glaucum, P. adspersus* and *C. aestuarii,* respectively) while others were occasional (from 4 to 9 appearances during the study).

Trophic groups

Trophic groups showed spatial and temporal variations (Fig. 3). All trophic groups were present throughout the year, except sediment deposit feeders and sub-surface deposit feeders that were not observed during the winter. All stations pooled, carnivores (42%) and omnivores (39%) were at the leading positions all year round, followed by suspension feeders (12%), sediment deposit feeders (5%) and sub-surface deposit feeders (2%). The majority of the stations were dominated (50%) by carnivores and omnivores (in winter at station 3 and 5; in spring at stations 3, 5 and 9; in summer at stations 2, 3, 4 and 9 (Fig. 3a–c). Suspension-feeders were the only group present at stations 2 and 6 in winter (100%), while surface deposit feeders were dominant at stations 1 in summer (August, 50%). In autumn, all groups were more balanced, with omnivores dominating widely (100% at station 3), followed by carnivores and suspension feeders almost equally distributed through all stations (Fig 3d).

Table 1 List of recorded species in the Parila lagoon, Croatia

Group	Species	Length (mm)	Feeding type
Bivalves	*Cerastoderma glaucum*	27.67 ± 7.54	SF
	Chamelea gallina	23.05 ± 3.15	SF
Gastropods	*Cerithium vulgatum*	23.40 ± 10.75	SSDF
	Cyclope pellucida	5.99 ± 1.69	O
	Hexaplex trunculus	50.37 ± 14.11	C
	Nassarius reticulatus	22.34 ± 3.01	SDF
Crustaceans	*Carcinus aestuarii*	25.19 ± 8.67	O
	Crangon crangon	69.00	C
	Pagurus sp.	22.09 ± 4.52	SDF
	Palaemon adspersus	54.44 ± 12.81	O
	Palaemon serratus	54.44 ± 12.81	O
	Penaeus kerathurus	66.00 ± 13.53	O
Fish	*Aidablennius sphynx*	59.80 ± 9.22	O
	Atherina hepsetus	52.90 ± 9.00	C
	Atherina boyeri	59.55 ± 12.76	C
	Blennius sp.	54.00	O
	Callionymus risso	38.60 ± 5.68	C
	Diplodus annularis	50.75 ± 36.98	C
	Diplodus puntazzo	24.50 ± 6.36	O
	Gobius geniporus	73.31 ± 23.99	C
	Mullus barbatus	100.54 ± 23.50	C
	Nerophis ophidion	98.94 ± 11.84	C
	Platichthys flesus	70.60 ± 11.28	C
	Pomatoschistus marmoratus	43.43 ± 13.94	C
	Solea solea	61 ± 15.56	C
	Syngnathus acus	105.67 ± 39.13	C
	Syngnathus typhle	115.42 ± 35.37	C
	Symphodus cinereus	54.09 ± 12.18	C
	Symphodus roissali	59.74 ± 7.91	C
	Zosterisessor ophiocephalus	54.68 ± 26.27	C

For each species, length (average ± standard deviation) and feeding type is given. *O* omnivores, *C* carnivores, *SSDF* sub-surface deposit feeders, *SDF* surface deposit feeders, *SF* suspension feeders

Species association and niche overlap

The most abundant species association analysis based on species presence/absence data showed only a few significant associations (negative or positive). The overall species association score (VR) is estimated to be 2.26 (W statistic = 20.38) which indicates an overall positive association between the species (chi-square association test, df = 9, $p = 0.05$).

The observed Trophic Index (TI) of the sampled fish species (Fig. 4) ranged between 2.7 (*A. sphynx*) and 4.3 (*S. typhle*) with an average 3.39 ± 0.42 (average ± SD). This value is similar to the average TI for the Mediterranean Sea (3.26 ± 0.03) for the period 1950–2004 (European Environmental Agency accessed on March 16, 2016).

Characterization of the environmental descriptors

Sediment composition at each station in Parila lagoon is shown on Fig. 5. Stations close to the open sea (stations 5, 6, 7, 8; see Fig. 1b) exhibited a high percentage of sand moved by the sea tides and waves whereas others, located further inshore, are characterized by clayey silt of land runoff origin (stations 3, 4). Station 2, which was inshore but further from the land, showed rather high sand percentage than expected. Stations 9 had similar percentage of sand, silt and clay, while station 1, located at the area with higher flow (coming from the sea during dry season or from the catchment during wet season) showed similar percentages of sand, silt and clay, with sand being somewhat higher.

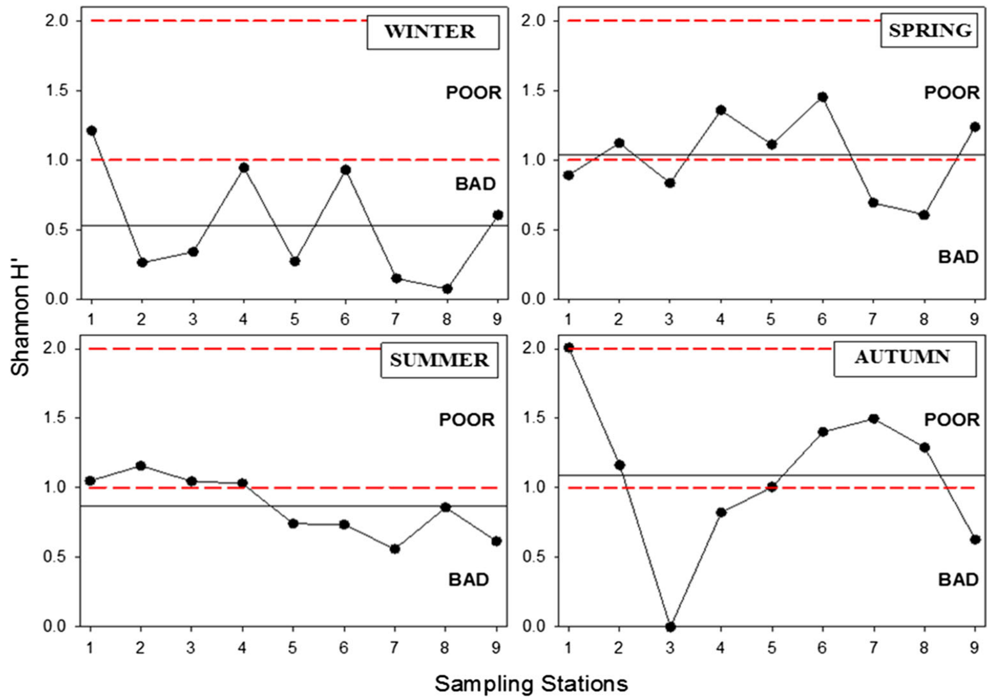

Fig. 2 Seasonal Shannon-Wiener diversity index (H′) recorded in the Parila lagoon, Croatia. Full horizontal lines indicate average values; dotted lines indicate ecosystem quality limits

Physico-chemical parameters showed spatial and temporal variations. Values of organic matter (Fig. 6a) ranged between 3.7 ± 3.1% in autumn to 4.4 ± 2.2% in winter (average ± SD), with the maximum value recorded at station 9 in autumn (11.3%). Carbonates (Fig. 6b) ranged between 35.6 ± 6.1% in winter to 40.5 ± 7.9% in summer, with minimum value recorded at station 9 in autumn (20.7%) and maximum at station 6 in summer (55.7%). Values of total phosphorus (Fig. 6c) ranged between 13.3 ± 4.2 μmol g^{-1} in autumn to 16.6 ± 4.1 μmol g^{-1} in winter.

Minimum temperature was recorded at station 7 in winter (7.1 °C) and maximum at station 3 in summer (30.0 °C), while minimum salinity value was recorded at station 1 in autumn (12.2) and maximum at station 8 in summer (31.5). Minimum dissolved oxygen value was recorded at station 1 in summer (2.7 mg l^{-1}), and maximum at station 5 in autumn (11.9 mg l^{-1}). Maximum fluctuations of environmental parameters were predominant in autumn, and minimum in summer.

Effects of environmental parameters on the species assemblages

The overall relate function showed a rather small (Rho index = 0.21) but significant (Significance = 0.2% <5%) similarity between the two resemblance matrices (environmental and abundance data). The first level of selection (BEST function) showed that organic matter, carbonates, total phosphorus and salinity were the most important environmental parameters. Further analysis based on the DistLM method pointed out total phosphorus and temperature as the main environmental factors affecting the station communities (AIC = 284.5, correlation = 22%). An attempt to force the inclusion of salinity had very poor results (explained variation 3.2%). Finally, the use of all environmental variables in the same model gave the same AIC value for the combinations of sediment composition, organic matter, temperature, total phosphorus and salinity but the best relationship was estimated for the combination of total phosphorus and temperature. The results of the best model and the overall model (all parameters), as well as the intermediate combination models, are shown in Table 4.

The graphic illustration of the DistLM method results based on the most significant environmental parameters affecting the community structure in the Parila lagoon is shown in Fig. 7. There was a clear seasonal stratification between the stations along the axis of the temperature vector. From the higher temperature values to the lower, there was a group of station values for August (summer), followed by a group for May (spring) and then a mixed group of station values for November (autumn) and February (winter). Along the total phosphorus axis, group of stations 1 to 4 (inner stations in the Parila lagoon) were situated at high values, while stations 5 to 8 (closer to open sea) were grouped at low values of total phosphorus. Station 9 was located in the middle of the diagram showing an intermediate position in relation to total phosphorus values (see Fig. 7).

Furthermore, DistLM analysis showed that temperature, salinity and total phosphorus were the parameters that mostly affected the feeding guild composition of the community at studied stations (Fig. 8). At first, the data showed a rather uniform species community based on their feeding guild i.e. there was no specific distribution pattern. However, the results demonstrated that the location of each station in relation to the

Table 2 Seasonal species appearance pattern recorded in the Parila lagoon, Croatia

Species	Season				Statistics		Status
	Winter	Spring	Summer	Autumn	Average	*N*	
Carcinus aestuarii	5	9	5	8	7	27	Frequent
Palaemon adspersus	6	7	8	6	7	27	
Cerastoderma glaucum	6	4	3	7	5	20	
Syngnathus typhle	5	3	2	5	4	15	
Zosterisessor ophiocephalus		6	5	4	5	15	
Nerophis ophidion	2	5	3		3	10	
Palaemon serratus		4		4	4	8	Occasional
Nassarius reticulatus		2	3	3	3	8	
Diplodus annularis		1	4	2	2	7	
Symphodus cinereus		1	3	2	2	6	
Penaeus kerathurus			2	2	2	4	
Pomatoschistus marmoratus	4				4	4	
Gobius geniporus			1	3	2	4	
Chamelea gallina	1	1	1	1	1	4	
Atherina hepsetus			1	2	2	3	Rare
Mullus barbatus			2	1	2	3	
Cyclope pellucida		1	1	1	1	3	
Aidablennius sphynx	1	1	1		1	3	
Cerithium vulgatum		1	1	1	1	3	
Hexaplex trunculus	1		1	1	1	3	
Platichthys flesus		2			2	2	
Syngnathus acus				2	2	2	
Pagurus sp.	0		1	1	1	2	
Atherina boyeri	1	1			1	2	
Solea solea	1			1	1	2	
Blennius sp.				1	1	1	
Callionymus risso				1	1	1	
Crangon crangon				1	1	1	
Diplodus puntazzo	1				1	1	
Symphodus roissali				1	1	1	
TOTAL	12	16	19	24	192		

N total number of appearances. Based on this number, species status is determined: Frequent >9; Occasional, 4–9; Rare, 1–3

open sea has a changing community pattern during the year. In particular, stations located away from the open sea (e.g. stations 1–4) exhibited a changing pattern of species composition (depending on their feeding guild) throughout the year as the monthly values were located far apart (Fig. 8). The other stations close to the open sea (e.g. stations 5–9) showed more uniform and stable community structure per feeding guild as their values were more or less closely grouped (as seen in Fig. 8).

Finally, PERMANOVA design analysis (Table 5) showed an overall significant difference of the community structure between seasons and stations ($P < 0.001$). Pairwise analysis showed links between the community structure status on a seasonal and temporal scale. On a seasonal one, the similarities were shown between November and February and May and August and on a temporal scale, stations located adjacent to each other in the upper or middle parts of Parila, showed similar community structure.

Discussion

The study describes presence and abundance of epibenthic macroinvertebrates and demersal fish species associated with spatial and temporal variation in their habitat (sediment composition and environmental variables). Patterns in biotic responses to different parameters of habitat quality are assumed to concur; hence the observed trends are expected to reflect the

Table 3 Top 10 species with highest seasonal abundance (ind m^{-2}) recorded in Parila lagoon, Croatia

Species	Group	Winter	Spring	Summer	Autumn	Average	SD
Cerastoderma glaucum	Bivalves	1.639	1.094	0.889	0.875	1.124	0.36
Chamelea gallina	Bivalves	0.111	0.434	0.422	0.208	0.294	0.16
Palaemon adspersus	Crustaceans	0.309	0.275	0.389	0.05	0.256	0.15
Nasarius reticulatus	Gastropods		0.365	0.092	0.181	0.159	0.16
Cyclope pellucida	Gastropods		0.26	0.014	0.056	0.083	0.12
Carcinus aestuarii	Crustaceans	0.057	0.112	0.025	0.029	0.056	0.04
Zosterissessor ophiocephalus	Fish		0.025	0.026	0.005	0.014	0.01
Symphodus cinereus	Fish		0.001	0.019	0.027	0.012	0.01
Nerophis ophidion	Fish	0.005	0.034	0.004		0.011	0.16
Syngnathus typhle	Fish	0.008	0.018	0.003	0.007	0.011	0.01

SD standard deviation

real relationships in the ecosystem (Gillet 2010). It is important to underline that the sampling methods used in this study aimed to sample only large epibenthic invertebrates and demersal fish, thus excluding smaller infaunal invertebrates that would surely increase species abundance and diversity. The distribution of infaunal organisms have been typically related to sediment properties (Callaway et al. 2002; Van Tomme et al. 2014), but less is known about epibenthic communities and the trophic relations between epibenthic predators and macrofauna food web interactions. Although this study does not describe these interactions in details, it gives insight into the current ecological condition in the Parila lagoon.

Effects of environmental parameters on the species assemblages

In terms of species richness, 30 identified species in Parila lagoon were similar to previously reported findings from most of the Mediterranean lagoons (Khedhri et al. 2014; Reizopoulou et al. 2014). When considering the relative importance of the most dominant macroinvertebrate taxa from this study (crustaceans and molluscs), it varied slightly throughout the Mediterranean lagoons: crustaceans were dominate in the lagoon of Smir (Morroco), followed by molluscs (Chaouati and Bayed 2005) and in the Tunisian lagoons, such as Bizerte, El Bibans and Boughrara (Afli et al. 2009; Khedhri et al. 2014) or that of Biguglia in Corsica (Clanzig 1991), the highest number of species were from molluscan taxon (especially gastropods) while crustaceans were subdominant. Macrofaunal diversity peaked in spring and autumn and this was in concordance with other Mediterranean lagoons (Reizopoulou et al. 2014).

In the Parila lagoon, 18 fish species divided in 10 families were recorded. The demersal fish fauna sampled in the Parila lagoon corresponds at generic or family level to other

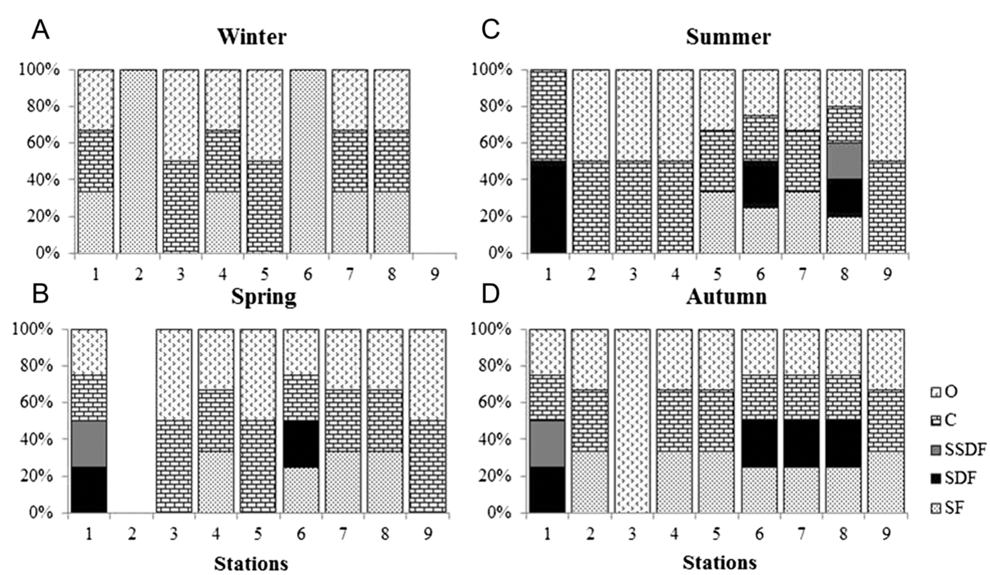

Fig. 3 Spatial-temporal variations of different trophic groups in the Parila lagoon, Croatia. **a** winter; **b** spring; **c** summer; **d** autumn. SDF, surface deposit feeders; SSDF, sub-surface deposit feeders; SF, suspension feeders; O, omnivores; C, carnivores. Species belonging to more than one feeding guild were classified by their most common feeding mechanism

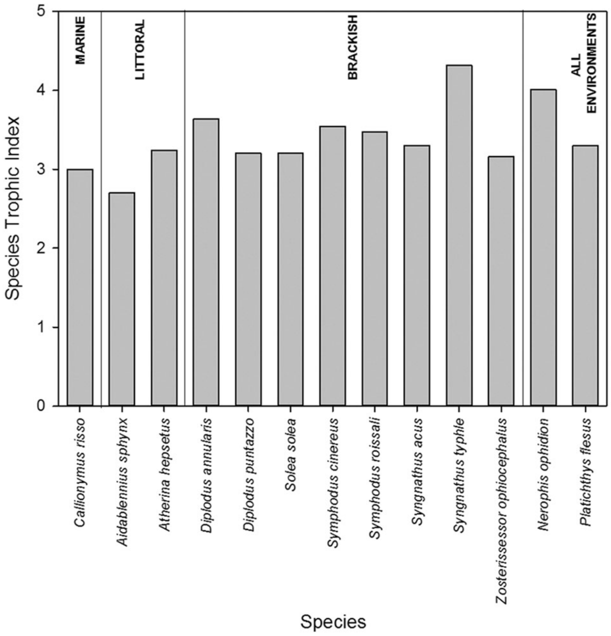

Fig. 4 Trophic Index (TI) and preferred habitats of fish species sampled in Parila lagoon, Croatia

Mediterranean coastal lagoons where Gobiidae, Blenniidae, Labridae or Sparidae were among the main families constituting the fish assemblages (Franco et al. 2006, 2012; Pérez-Ruzafa et al. 2006, 2007; Jaafour et al. 2015). Pérez-Ruzafa et al. (2007) reviewed data from 40 Atlanto-Mediterranean coastal lagoons and showed a high range of fish species richness, ranging between 6 and 48 with an average of 23 species. The difference in species diversity and richness among different lagoons can be contributed to different sampling methods. Methods used to sample fish in transitional waters are all selective to some degree. Seine net (used in this study) is considered to be a very effective method for sampling in shallow waters such as a lagoon, and efficient in sampling small lagoon resident species (e.g. *A. boyeri* or *P. marmoratus*) and juvenile marine migrants (as reviewed in Franco et al. 2012).

Several factors are likely to determine species richness and abundance inside a lagoon; the most important one being a degree of communication with the open sea and the size of a lagoon, while the phosphate concentration is usually negatively correlated with e.g. fish species richness (Pérez-Ruzafa et al. 2007). Ecological analysis performed in this study contributes to the latter and provides an interesting insight into the mechanisms that affect the community structure inside the Parila lagoon. Correlation coefficient value (cited in Table 4) is rather minimal in the final selection of the optimal and simplified linear model since statistically it tends to increase when more variables are added during the step-wise optimization. The overall low Rho index value indicated that the overall effect of the environmental data is weak. River delta systems are highly variable ecosystems in terms of hydrology and water quality and the variations usually occur very fast as the result of the simultaneous sea action and river flow. Such dynamics may hide the actual environmental parameters which govern community changes (Pérez-Ruzafa et al. 2007).

Further analysis showed that total phosphorus concentration and temperature were the main environmental parameters affecting species abundance in the Parila lagoon. The maximum number of species in autumn coincided with the minimum phosphorus values, while minimum species richness corresponded with maximum phosphorus values and minimum temperatures inside the lagoon. Moreover, total phosphorus concentration, temperature and salinity were the main environmental quality parameters affecting the community structure per feeding guild. The location of each station in relation to the open sea showed a changing pattern during the year, most likely related to the environmental quality variability. Such outcome reflects previously mentioned connection between highly variable, shallow deltaic ecosystems with pollution from river flow and other point or nonpoint run-offs, and highly variable physical water quality parameters. This was further supported with the PERMANOVA analysis that showed an overall significant difference of the community structure between seasons and stations.

Total phosphorus concentration in the Parila lagoon can be explained with non-purified communal waste waters (further upstream) as well as with pesticides and fertilizers from surrounding arable land (RIS 2012). Leaching from chemicals used for the agriculture activities reaches the Delta with the river flow and is retained on the sediments affecting the benthos and demersal fish assemblages. Temperature, on the other hand, is known as the most important environmental parameter for the shallow transitional waters and usually shows a high annual variability (in the case of Parila lagoon between 7.1 and 30 °C). Since the work carried out in this paper was

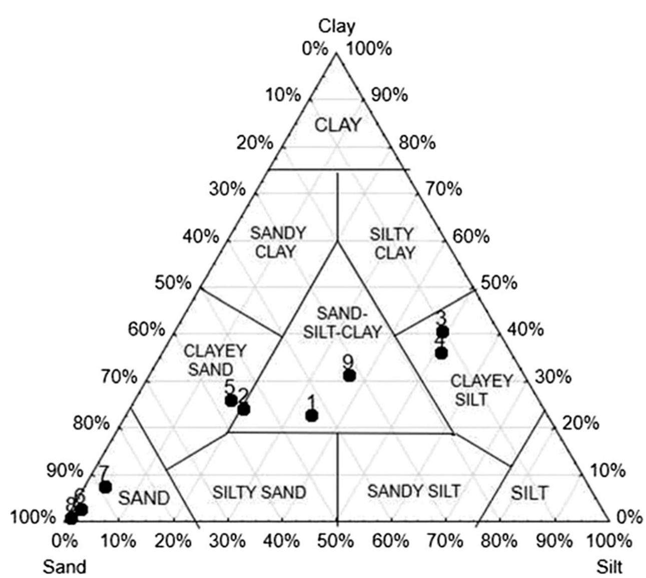

Fig. 5 Sediment type according to Shepard classification for average values of top 10 cm at sampling stations in Parila lagoon, Croatia

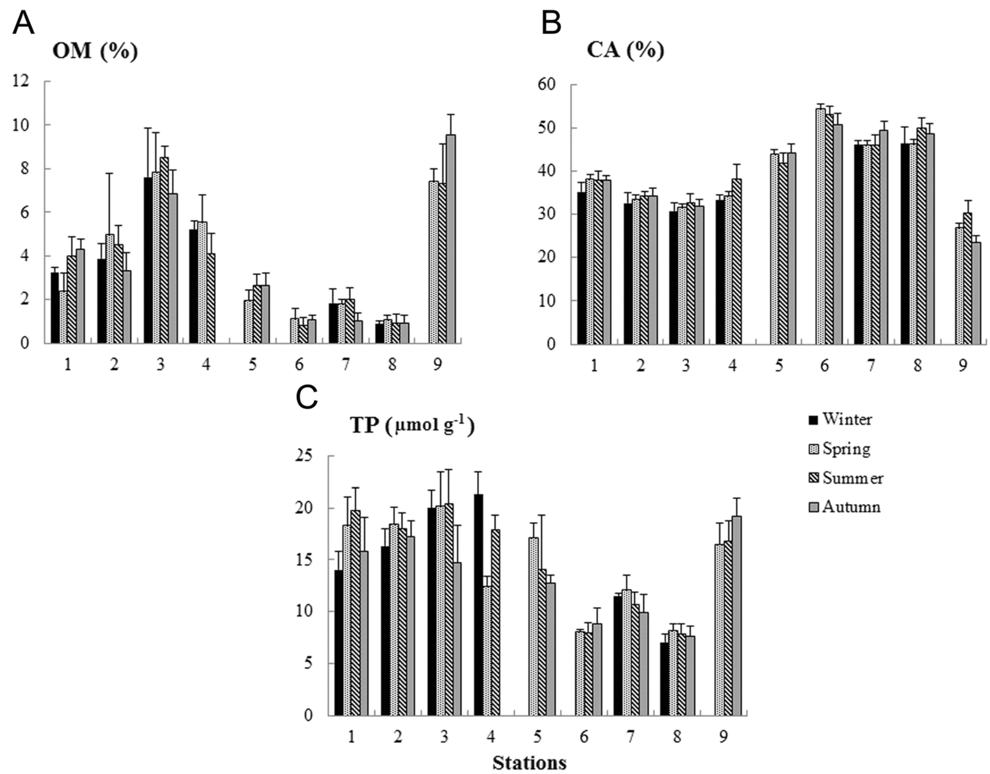

Fig. 6 Geochemical characteristics of the sediments in the surface 10 cm layer throughout Parila lagoon, Croatia. **a** organic matter content (OM); **b** carbonate content (CA); **c** total phosphorus concentrations (TP). Values for CA and OM are expressed in percentages (%), while values for TP are expressed as μmol g^{-1}

focused on epibenthic and demersal organisms, it was expected that the composition of the sediment would be an important factor for the observed community variability. AIC value for total phosphorus concentration and temperature model is very close to the AIC value of the model which included the sediment composition, organic matter and temperature (see Table 4), indicating that the actual environmental parameters affecting the community structure (apart from TP and T) could also include the sediment composition without the significant loss of statistical accuracy.

The connection between sediment and benthic fauna is complex and variable through space and time (Putro 2009; Khedhri et al. 2014). This complexity has been proven to be even greater (Mancinelli et al. 1998; Snelgrove and Butman 1994; Ysebaert and Herman 2002; Thrush et al. 2003) as many species have clear sediment preferences, while others show little affinity with any particular sediment type. Species abundance in the Parila lagoon (all seasons and stations pooled) was rather poor on all stations, varying from 0 to 7 ind m^{-2} and this high variance was caused with seasonality. At the stations with low salinity, species richness and diversity was lower compared to the stations with stronger marine influence that supported a higher number of species. Moreover, the species spatial appearance pattern was highly variable as mentioned earlier: while some species appeared at all stations in all seasons (i.e. crustaceans *C. aestuarii* and *P. adspersus* and fish *Z. ophiocephalus*), other were recorded on a few or one station only. The striped venus clam *Chamelea gallina* was present only at station 6 (at the entrance to the lagoon) but had high seasonal abundance.

Settlement of benthic communities is primarily influenced with food availability and feeding guilds, and this in turn is controlled with physico-chemical factors such as

Table 4 Summary of the ecological analysis performed on the species assemblages in Parila lagoon, Croatia

Parameters	AIC value	Correlation
Total phosphorus, Temperature	284.50	21.1%
Gravel (%), Sand (%), Silt (%), Clay (%), Organic Matter (%), Temperature	287.75	36.8%
Gravel (%), Sand (%), Silt (%), Clay (%), Total phosphorus, Temperature	289.17	34.2%
Gravel (%), Sand (%), Silt (%), Clay (%), Organic Matter (%), Total Phosphorus, Temperature	290.21	38.3%
All environmental parameters	296.94	46.2%

AIC Akaike Information Criterion

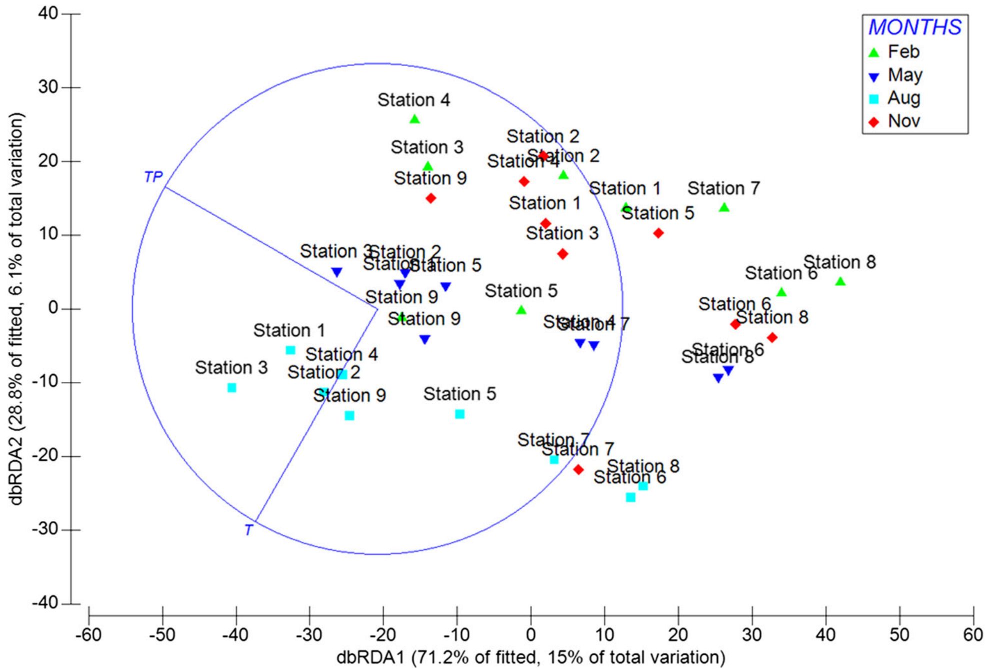

Fig. 7 Graphic illustration of the DistLM method results based on the most significant environmental parameters affecting the community structure per species abundance (ind m^{-2}) in Parila lagoon, Croatia. TP, total phosphorus; T, temperature

salinity, temperature, dissolved oxygen, geochemical characteristics of the sediment (Gaston et al. 1998; Putro 2009). Seasonal monitoring of these parameters in Parila lagoon showed a spatial heterogeneity linked to hydrodynamics and sedimentary composition. Summer season had the most uniform pattern of environmental parameters throughout the lagoon, where high organic matter content and high temperature and salinity led to oxygen depletion (minimum value of 2.7 mg l^{-1}). However, there was an overall increase from 12 species in winter, to 16 species in spring, 19 species in summer and 24 species in autumn when the highest value of dissolved oxygen was also recorded (11.9 mg l^{-1}). The highest species diversity in autumn is further supported with the maximum measured percentage value of organic matter, while maximum values of carbonates and phosphorus were recorded

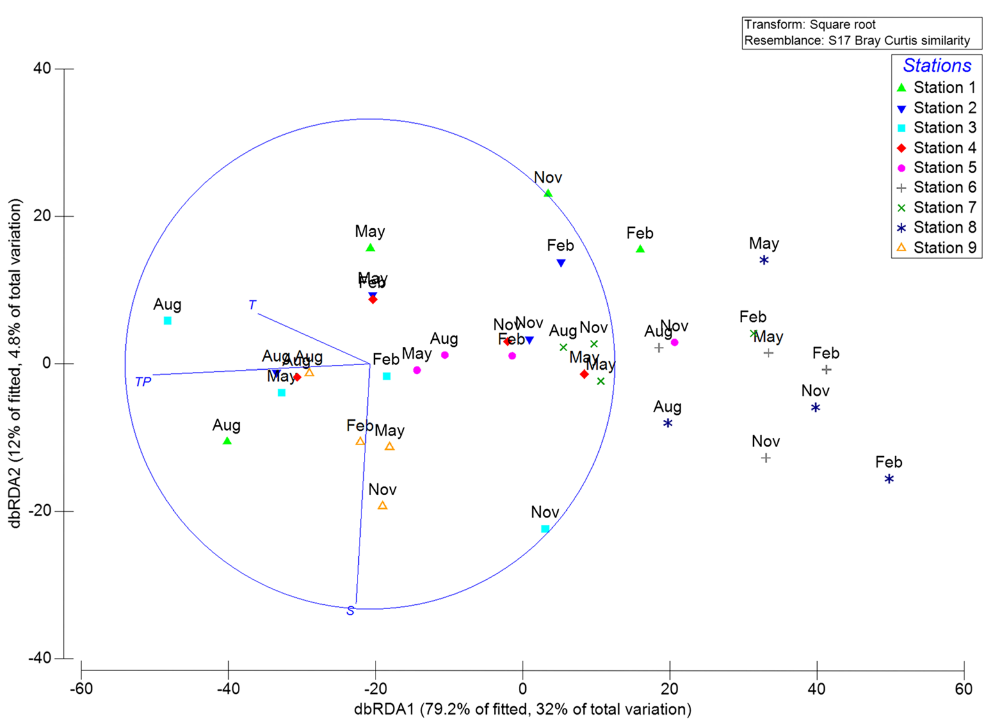

Fig. 8 Graphic illustration of the DistLM method results based on the most significant environmental parameters affecting the community structure per species feeding feeding guild (omnivores, carnivores and organic matter feeders) in Parila lagoon, Croatia. TP, total phosphorus; T, temperature; S, salinity

Table 5 PERMANOVA design analysis of the effects of season and location on the community structure in Parila lagoon, Croatia

Source	df	SS	MS	F	*P*
Months	3	15,421	5140.2	26.654	0.0004
Station	8	40,544	5068	2.628	0.0001
Residuals	24	46,283	1928.4		
Pairwise Groupings (months)					
Groups	t-test	*P*			
Feb, May	1.49	0.029			
Feb, Aug	1.38	0.050			
Feb, Nov	1.08	0.307*			
May, Aug	1.06	0.330*			
May, Nov	1.66	0.008			
Aug, Nov	1.52	0.017			
Pairwise Groupings (stations)					
Groups	t-test	*P*			
Station 1, Station 2	1.27	0.190*			
Station 1, Station 3	1.26	0.088			
Station 1, Station 4	1.83	0.027			
Station 1, Station 5	1.68	0.005			
Station 1, Station 6	2.32	0.004			
Station 1, Station 7	2.22	0.003			
Station 1, Station 8	2.09	0.018			
Station 1, Station 9	0.93	0.695*			
Station 2, Station 3	1.16	0.288*			
Station 2, Station 4	0.39	0.935*			
Station 2, Station 5	0.57	0.757*			
Station 2, Station 6	2.59	0.003			
Station 2, Station 7	1.17	0.329*			
Station 2, Station 8	1.76	0.049			
Station 2, Station 9	1.03	0.356*			
Station 3, Station 4	1.19	0.206*			
Station 3, Station 5	1.26	0.081			
Station 3, Station 6	1.90	0.020			
Station 3, Station 7	1.87	0.011			
Station 3, Station 8	1.93	0.018			
Station 3, Station 9	1.02	0.407*			
Station 4, Station 5	1.14	0.315*			
Station 4, Station 6	2.73	0.001			
Station 4, Station 7	1.46	0.085			
Station 4, Station 8	1.87	0.031			
Station 4, Station 9	1.09	0.285*			
Station 5, Station 6	2.34	0.019			
Station 5, Station 7	1.85	0.023			
Station 5, Station 8	2.29	0.004			
Station 5, Station 9	1.32	0.122*			
Station 6, Station 7	2.70	0.010			
Station 6, Station 8	2.91	0.004			
Station 6, Station 9	1.84	0.004			
Station 7, Station 8	1.49	0.156*			
Station 7, Station 9	1.45	0.028			
Station 8, Station 9	1.55	0.005			

Pairs with *P* values marked with * follow the hypothesis of similarity ($P = 5\%$)

during summer. As the sampling in this study was performed seasonally, the variability of some environmental parameters cannot be strictly attributed to seasonal variability of benthic communities, since species have biological cycles that are also linked to fluctuations of numerous environmental parameters (Khedhri et al. 2014).

Out of the ten most abundant species in the Parila lagoon, six of them were listed as frequent, i.e. appearing from 10 to 27 times throughout the year (*N. ophidion, Z. ophiocephalus, S.typhle, C. glaucum, P. adspersus* and *C. aestuarii,* respectively), while other were considered to be occasional (from 4 to 9 appearances during the study). This species spatial patchiness in appearance and abundance support the estuarine quality paradox idea (Elliott et al. 2007) stating that the most abundant benthic species living in a highly variable environment are usually well adapted to changing environmental conditions (Dauvin and Desroy 2005; Dauvin and Ruellet 2009). In addition, species with a wider niche are considered to be able to tolerate a wider set of physico-chemical conditions which in turns enable them to become locally abundant and widespread (as reported in Verberk et al. 2010). We could therefore, argue that these six frequent species in the Parila lagoon are well adapted to present environmental conditions and have potential to be used as indicators of habitat quality. Consequently, under favourable conditions (i.e. an increase of organic matter), they can rapidly increase their populations and give information about the quality of benthic environment, since increased organic matter input is usually reflected in reduced species and trophic guild diversity (Pearson and Rosenberg 1978; Gray et al. 2002). This seems to be the case with the opportunistic Baltic prawn that increased in abundance in the extreme season (summer). Although this is highly plausible scenario, such conclusion cannot be made with certainty for two obvious reasons: 1) no research was performed to test species specific response to change of environmental parameters and, 2) a longer period of monitoring is required. However, it does provide impetus for further research development.

Trophic groups and species associations

Researchers have recently focused on the relationship between diversity and function, since the structure and interaction within each functional group contribute to the productivity of the community, and in turn stability of the ecosystem (as reviewed in Putro et al. 2014). Epibenthic community from Parila lagoon has been grouped into functional groups (guilds) based on their most common feeding habit. Species responses to sediment grain size from this study generally concur well with the relationships found by other authors, implicating that fine grained sediments with high organic content are mostly dominated by deposit-feeding organisms, whereas coarse-sandy sediments with low organic content and high energy environments are dominated by suspension feeders and carnivores (Diaz and Rosenberg 1995; Gaston et al. 1998; Rakocinski et al. 2000, Putro et al. 2014).

In terms of trophic diversity, community was primarily dominated by carnivores and omnivores all year round, while reduction of dissolved oxygen, decomposition of organic matter and lower energy environment in summer increased the number of surface and sub-surface deposit feeders. In benthic communities that are depicted with a small number of species (such as Parila lagoon), trophic composition of the communities is determined by the abundance of the variations evident in the very few species. Moreover, this trophic composition is in concordance with majority of the Mediterranean lagoons where only one or two trophic groups dominate (Khedhri et al. 2014). Rakocinski (2012) stated that higher biodiversity usually relates with a more even distribution of trophic groups and their production potential, which explains the findings from this study where highest species diversity in autumn correlated with almost equally distributed trophic groups throughout the lagoon.

Our results showed relatively small abundance of commercially important bivalve species, such as *C. gallina* or *C. glaucum*, and high abundance of crustacean opportunistic species, such as *P. adspersus* and *C. aestuarii*. The long-term decline of the *C. gallina* clam in the Adriatic Sea has been already documented and reviewed (Romanelli et al. 2009), and previous bivalve survey in the wider Neretva River estuary (Bratoš Cetinić 2012) indicate decline in the total abundance when compared to the present results. Natural recovery of epibenthic bivalves is directly related to changes in abiotic conditions, as well as altered biotic interactions (Van der Heide et al. 2014). One of those potentially important biotic factors is outbreak of crustaceans due to climate change or overfishing of predatory fish that feed on crustaceans, which can in turn increased predation by crustaceans on bivalve spat (Van der Heide et al. 2014). Similar example of adverse effects of increased predation is recently recorded for the local population of gilthead seabream, *Sparus aurata*, in three areas along the SE Adriatic Sea: Mali Ston Bay (Croatia and Bosnia and Herzegovina), Neretva River estuary (Croatia) and Boka Kotorska Bay (Montenegro) on shellfish culture of Mediterranean mussels, *Mytilus galloprovincialis* and the European flat oyster *Ostrea edulis* (Glamuzina et al. 2014). The impact of such increased predation on the ecosystem is not yet known, but the socio-economic impact is already visible in the closure of local shellfish farms due to damages reaching over 90% of the production (Glamuzina et al. 2014).

Species interactions are important to study within a certain geographic area in order to understand the processes which affect the ecological balance. There are a number of environmental as well as biological factors within a certain community which affect the presence, absence and coexistence of certain species. This way, it appears that certain species show preference or avoidance for the same habitat, *inter alia*, as a result of predator-prey relationships or food and/or niche competition. The interspecific association analysis performed in this study showed that the species in the Parila lagoon had an overall positive association. This particular analysis does not provide information regarding the exact causes of such association pattern. However, it leads to the generation of hypotheses to explain the pattern. According to Schluter (1984) there are several possibilities of association (positive, negative or neutral), even in the case of no actual interaction between species. Among those possibilities and considering the feeding habits of the species collected from Parila lagoon, it is possible to assume that the positive association between species is based on prey-predator relationships between the associated species. This is further supported from the fact that there are very few omnivorous and no explicitly herbivorous species in the samples. The latter obviously do not appear in the lagoon due to the limited availability of plant materials and the low environmental quality of the lagoon. For the same reason, it is safe to assume that the carnivorous species identified in this study appear almost randomly in the various stations (for feeding purposes) and some of them, only during certain periods of the year. It is known that epibenthic predation pressure could influence structure of epibenthic communities (Van Tomme et al. 2014) but in order to elucidate these relations additional field experiments are necessary.

Trophic index and ecological quality

In addition to trophic guilds and species associations, trophic index of fish species is also a good measure of the structure of a community in relation to the state of the local food chain. As the most abundant (diversified) species in the Parila lagoon, they exhibited trophic levels above 2.7, indicating that the local food chain is shifted upwards and towards mostly carnivorous species. The absence of abundant herbivorous species (as stated above) could indicate the lower environmental quality, where the lagoon natural functions (extremes in salinity and temperature and probably intensive geomorphological changes due to waves and currents), hinder the development of marine flora which could support such species (benthos and/or plankton eating species).

An extensive list of diverse biotic indicators emerged over the last two decades in order to evaluate ecosystem status and condition under the scope of WFD (as reviewed in Pinto et al. 2009; Borja et al. 2013). The biotic indices in transitional ecosystems tend to either underestimate, as do BENTIX and M-AMBI, or overestimate the ecological status, such as AMBI (Simboura and Reizopoulou 2008; Reizopoulou et al. 2014). The assessment of fish fauna is a part of the WFD obligations to assess GES of European water bodies, and majority of the fish based methods use species richness, abundance and trophic or ecological guilds as metrics (Franco et al. 2012). This study points out the importance of connecting epibenthic macrocommunity patterns with prey-predator links, since any change in composition and production is expected to cascade through other trophic levels.

Conclusions

Taken into account that species living in highly variable environment tend to adapt and become more resilient to change (Dauvin 2007), a functional based approach compared to the structural approach has been proposed to assess ecological quality (de Jonge et al. 2006). Epibenthic macrofauna and related processes reflect the variability of ecosystem functions and this may be more useful than indicators species and biotic indices (Rakocinski and Zapfe 2005). Change in epibenthic community structure is the most relevant ecological measurement of environmental disturbances that reflects in the ecological functioning, which in turn will be realized throughout the ecosystem via shifts in trophic relationships, nutrient cycling, and other key processes, and eventually provisioning of ecosystem services (Gillet 2010).

Since Parila lagoon is considered as a nursery habitat for several economically important species (Dulčić et al. 2007), it is essential to further elaborate the EcoQ of the lagoon in the light of these species, which locally significantly contribute to fishery and socio-economy (Dulčić et al. 2007; Glamuzina 2010). It is well known that most abundant crustaceans in Parila lagoon, such as *C. aestuarii* and *P. adspersus* (Glamuzina L. et al. 2014) are important part of the sparid species diet (Arias 1980; Ramírez et al. 2015), which in turn are of great economical value for the entire Croatian fishery. These findings point to the need of more complex research of indirect impacts of Parila lagoon on adjacent waters. There are still knowledge gaps in quantifying ecosystem functions (e.g. nursery function), and more importantly, in choosing and using indicators as proxies of change in habitat quality, function and finally, provision of services (e.g. fisheries). Linking EcoQ status of the lagoon with increase in abundance of commercially important fish species and local artisanal fishery should provide more arguments to the better protection and management of Parila lagoon and other nurseries in the Neretva River Delta.

The present paper provides important findings in this direction. Moreover, the study showed that both epibenthic macroinvertebrates and fish should be used together as indicators of EcoQ due to their predator-prey relationship as the major organizing force within benthic communities. Further research about the epibenthic invertebrates and fish as indicators of sensitivity to different types of pressures is urgent in order to select robust indicators for early-warning framework. Without information on state of the invertebrate community in Parila lagoon, it is not possible to assess how fisheries or other types of human activities can affect the benthic invertebrates in the lagoon, as well as the other way around. The present study provides baseline for future research in order to detect the effects of activities that may occur around the lagoon in the future.

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