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SEASONAL DYNAMICS OF MACROZOOBENTHIC COMMUNITY IN THE WETLAND OF THE NATURAL REGIONAL RESERVE OF THE ISONZO RIVER MOUTH, NORTHEAST ITALY: A THREE-YEARS ANALYSIS

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ABSTRACT

This study was seasonally performed over a three year period in a large freshwater temporary pond to investigate (a) seasonal trends of the main abiotic factors, (b) the most important abiotic drivers in shaping macrobenthic invertebrate communities and (c) the structures of these communities among different years. As the study area was placed within a Natural Reserve, the leaf bag technique was used as non invasive sampling method in order to reduce disturbance. The results of chemical and physical investigations point out a clear seasonal trend, while macrobenthic communities differ significantly from year to year, and their main shape drivers were identified to be conductivity, temperature and dissolved oxygen.

Key words: macrozoobenthos, leaf bag, wetland, temporary pool, seasonal dynamics

DINAMICA STAGIONALE DELLE COMUNITÀ MACROZOOBENTONICHE NELLA RISERVA NATURALE REGIONALE DELLA FOCE DELL'ISONZO, NORDEST ITALIA: ANALISI TRIENNALE

SINTESI

Il presente studio triennale è stato condotto stagionalmente in un ampio stagno temporaneo d'acqua dolce, allo scopo di (a) verificare la presenza di gradienti stagionali per i principali fattori abiotici, (b) identificare quali tra questi fattori abbiano il maggior peso nel plasmare le comunità macrozoobentoniche e (c) indagare la struttura di dette comunità su scala pluriennale. Poiché l'area di studio è situata all'interno di una Riserva Naturale, per i campionamenti è stata utilizzata la tecnica dei pacchi fogliari, allo scopo minimizzare l'impatto dovuto alle operazioni di raccolta. Per quanto concerne i parametri abiotici è stato individuato un chiaro trend stagionale, mentre le comunità macrobentoniche sono risultate differire significativamente di anno in anno ed i principali fattori abiotici che le condizionano sono stati identificati nella conduttività, nella temperatura e nell'ossigeno disciolto.

Parole chiave: macrozoobenthos, pacchi fogliari, zone umide, stagni temporanei, dinamica stagionale

INTRODUCTION

Wetlands are one of the most biologically productive ecosystems (Dixion & Wood, 2003; Rolon & Maltchik, 2006; Mereta *et al.*, 2012), which perform a wide variety of ecological functions, including nutrient cycling (Bunn *et al.*, 1999) and carbon storage (Adhikari & Bajracharaya, 2009). They are also main breeding and feeding grounds for many birds and other wildlife (Williams, 2006). Some temporary wetlands in the Mediterranean Region are considered as priority habitat under the Habitat Directive 92/43/CEE, according to the Natura 2000 network of the European Union (Natura code 3170, 92/43/CEE, 21 May 1992), wetland restoration becoming increasingly important to reverse habitat degradation, recover ecosystem services and maintain biodiversity (Sebastián González & Green, 2014).

Macrobenthic invertebrates have a central role in these ecosystems as they cover all trophic functions (Cummins, 1974; Metcalfe Smith, 1994) and are trophic resources for many species of fishes and birds (Pizzul *et al.*, 2008). Due to the severe conditions characterizing temporary habitats, morphological, physiological and/or behavioural adaptation is required for living organisms to survive (Wiggins *et al.*, 1980). Alternation of dry and wet phases, which vary from year to year in the Mediterranean Region makes hydroperiod one of the main challenging factors for macrobenthic community structures (Wellborn *et al.*, 1996; Spencer *et al.*, 1999). Drought in particular, due to its unpredictability, represents a major constraint for organisms inhabiting temporary waters (Grillas & Roche, 1997). In fact, the macroinvertebrate assemblage structures are deeply affected by droughts (Acuña *et al.*, 2004; Bonada *et al.*, 2006), though the extent of changes depends on the biological adaptations of the species within the community (Boulton & Lake, 1992). Annual and seasonal variations of macroinvertebrate assemblages have been reported for temporary ponds in wetlands of Massachusetts (Brooks, 2000) or in intermittent streams in Victoria Australia (Boulton & Lake, 1992) and have been associated with parallel changes in environmental conditions during the wet phase. Jeffries (1994) found differences in the macroinvertebrate assemblages of the same ponds in three different years, including a low rainfall year in which ponds did not fill. Similar studies have been also recently carried out, also in the Mediterranean area (Florencio *et al.*, 2009; Dìaz-Paniagua *et al.*, 2010).

Our investigation was carried out in the Regional Reserve of the Isonzo River Mouth (Northeast Italy), a marshy area including mainly freshwater environments alongside with brackish. The Reserve represents the northernmost wetland in the Mediterranean area and it is included in a Site of Community Importance (SCI IT3330005) and in a Special Protection Area (SPA IT3330005). Several studies on the macrobenthic fauna were conducted in the Reserve, both in freshwater and salt marshes (Stoch, 1995; Pizzul *et al.*, 2008; Boggero *et al.*, 2011; Ruzič *et al.*, 2013) to assess the taxonomic structure of the communities, but a long temporal scale (years) was yet never considered.

This study was performed within a large freshwater temporary pond over a three year period. Our aims were to investigate (a) the presence of seasonal trends for the main abiotic factors, (b) which are the most important chemical and physical drivers in shaping macrobenthic invertebrates communities, and (c) the presence of differences in macrobenthic invertebrate communities structure among different years in a temporary environment.

MATERIAL AND METHODS

Study area

The study was performed in an enclosed basin with a surface of about 30 ha (Fig. 1) and supplied mostly by rainwater and by an artesian well. The basin, enclosed by an embankment, looks like a large temporary pond, partially resulting from environmental recovery management. The western portion of this area is a damp pasture, while the eastern portion is a reed bed (*Phragmites*

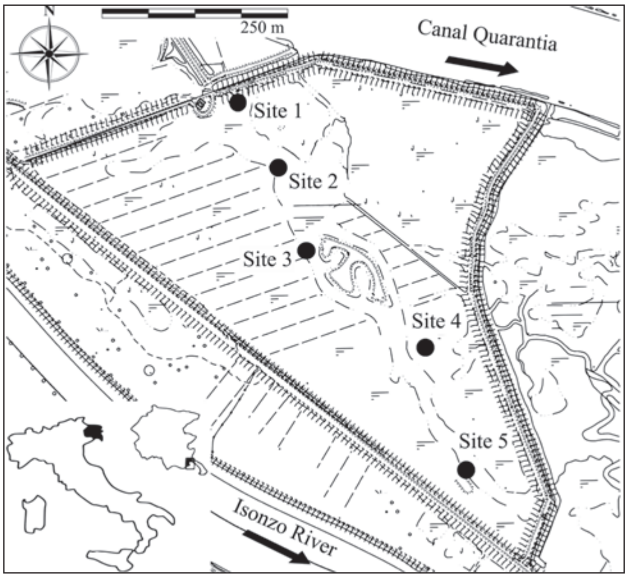

Fig. 1: Study area and sampling sites (UTM coordinates: site 1 N 33T5067699.47 – E 383832.23; site 2 N 33T5067605.64 – E 383897.48; site 3 N 33T5067474.89 – E 383955.56; site 4 N 33T5067349.38 – E 384063.44; site 5 N 33T5067140.24 – E 384187.07).

Sl. 1: Območje raziskave in vzorčna mesta (UTM koordinate: lokaliteta 1 N 33T5067699.47 – E 383832.23; lokaliteta 2 N 33T5067605.64 – E 383897.48; lokaliteta 3 N 33T5067474.89 – E 383955.56; lokaliteta 4 N 33T5067349.38 – E 384063.44; lokaliteta 5 N 33T5067140.24 – E 384187.07)

australis (Cav.) Trin ex Steud). The study pond was of autumnal origin (autumnal ponds, *sensu* Wiggins *et al*., 1980): the dry phase occurs in summer (from June to September) and the wet phase begins in early autumn. Basin waters are classified from limnic to oligohaline (Stoch, 1995) and maximum depth ranged from 1.7 to 2.0 m (Stoch, 1995; Perco *et al*., 2006).

As this area represents a wintering and stopover site for many bird species, management policy limits the human access only to the working personnel. However, some management actions are conducted: the vegetation growth control is performed both passively (grazing of Camargue horses and periodically cattle) and actively (using machines), while the water level is controlled only with a flap sluice gate placed at the northwest side of the basin. This gate is occasionally open in summer for a few days (usually late July/mid August, but not every year) to ease the up drying of the basin. The water level is controlled for ecological reasons (Pizzul *et al*., 2008), to avoid anoxic condition in the bottom sediments and to favour the nutrients remineralisation (Street, 1982).

Sampling design

The present study was carried out from October 2009 to July 2012 at five sampling sites (Fig. 1), which were chosen considering water depth, vegetation coverage on the bottom and presence of vegetation nearby. The first site was placed near the sluice side gate, the second beside an islet without vegetation and the third beside a wooded islet, the fourth site was placed at the centre of the basin and the fifth near the reeds, where vegetation cover was observed. Sampling campaigns were conducted in autumn, spring and early summer. Although planned, the winter campaigns were not performed, as the presence of ice prevented the access to the area.

Abiotic parameters

Depth was measured at each sampling time with a graduated rod. In water column, conductivity, temperature, dissolved oxygen (DO) and pH were recorded using field meters. Sediment samples were collected with a manual corer (6 cm internal diameter) in order to assess concentrations of NH_4^+, NO_2^-, and NO_3^- in the bottom interstitial water from October 2011 to July 2012. The corer was pushed into the surface sediment about 20-25 centimetres, then samples were frozen and brought to the laboratory, where concentrations of nutrients were measured using the methods reported by Solórzano (1969) and Presley (1971) for NH_4^+, IRSA CNR & APAT (2003) for NO_2^-, and APHA (1992) for NO_3^-. Seasonal rainfall data (mm of fallen rain) were obtained by a weather station placed nearby the Reserve.

Macrobenthic invertebrates

The leaf bag technique (Petersen & Cummins, 1974) was used to assess macrobenthic community structure.

Tab. 1: Mean seasonal values and standard deviations (in parenthesis) of the physical and chemical parameters for the whole study area.
Tab. 1: Srednje sezonske vrednosti in standardni odkloni (v oklepaju) fizikalnih in kemijskih parametrov za celotno območje raziskave

	2009-2010		2010-2011		2011-2012		
	Autumn	Spring	Autumn	Spring	Autumn	Spring	Summer
Depth (cm)	30.2 (9.2)	36.4 (11.1)	37.9 (11.4)	24.2 (14.6)	33.8 (10.4)	27.8 (10.7)	12.6 (11.5)
T (°C)	11.7 (3.4)	19.0 (4.2)	13.3 (1.9)	19.3 (2.5)	10.0 (5.2)	20.3 (2.6)	22.3 (1.6)
pH	7.2 (0.5)	7.8 (0.6)	7.7 (0.6)	8.5 (0.4)	7.7 (0.3)	7.8 (0.3)	8.3 (0.7)
DO (mg l^{-1})	4.5 (2.3)	4.0 (1.9)	5.1 (1.0)	2.3 (0.6)	8.5 (2.7)	2.4 (1.3)	3.2 (1.3)
Cond (mS cm^{-1})	2.6 (0.9)	2.7 (0.6)	2.1 (0.2)	3.0 (1.4)	3.7 (1.0)	4.9 (1.3)	6.5 (2.9)
Rainfall (mm)	185.3 (3.8)	62.4 (26.8)	172.2 (65.3)	51.8 (21.1)	96.9 (103.5)	104.6 (39.1)	31.5 (28.2)
NO_3^- (mg l^{-1})	-	-	-	-	0.74 (0.41)	0.55 (0.16)	0.52 (0.25)
NO_2^- (mg l^{-1})	-	-	-	-	0.06 (0.08)	1.11 (1.07)	1.38 (1.40)
NH_4^+ (mg l^{-1})	-	-	-	-	2.78 (1.85)	3.97 (1.91)	4.73 (1.93)

This method is commonly accepted as a quantitative approach to the study of both detritus processing and colonization by macrobenthic invertebrates and it was used in lotic environments (Robinson & Jolindon, 2005; Fenoglio *et al.*, 2006) as well as in lentic (Pope *et al.*, 1999) and transitional waters (Mancinelli *et al.*, 2005; Sangiorgio *et al.*, 2008). The technique was chosen in agreement with the management policies of the Reserve in order to reduce the disturbance and the impact of the sampling operations, because this method is less invasive than others (*e.g.* grabs and corers), it is faster to be performed on the field and therefore the disturbance to the present fauna (especially birds) was reduced. Leaves of *P. australis* were collected within the Reserve in early autumn 2009 and were air dried. The fragments of central leaf section (10 cm length) were oven dried to constant weight (60 °C for 72 h) and single lots of 3±0.001 g dry weight were placed in mesh bags (5×5 mm mesh size). Subsample units were set up connecting four leaf bags. Three subsamples were placed at each site in early October, early April and at the beginning of June for the collection in autumn, spring and summer, respectively. Subsamples were recovered after 15, 30 and 45 days of submersion. In the laboratory, leaf bags were opened and macrobenthic invertebrates were separated from the leaves and stored in a formaldehyde solution (final concentration 4%) until sorting operations. After washing, all invertebrates were counted and identified to family level. Chironomidae were identified until subfamily or tribe level. Percentage frequencies and seasonal mean number of observed taxa were calculated for each site.

Statistical analysis

Seasonal mean values of physical and chemical parameters were calculated for each sampling site and Principal Component Analysis (PCA) was performed using a three year data set to search for ecological gradients. Another PCA was carried out using only the third year data set, which includes also nutrients. Pearson's coefficient was used to seek correlation among abiotic variables and between variables and PCA axes. Two way ANOVA (factors: season, year) and LSD post hoc tests were performed to search for significant differences among the seasons and among same seasons of different years, while one way ANOVA was performed on the last year data set, to assess seasonal differences. Before analysis, all data were log(x+1) transformed.

Differences about community composition were investigated with two way PERMANOVA (factors year and season; 999 permutations) (Anderson, 2001) and SIMPER test, which were carried out on the similarity matrix based on the Bray-Curtis coefficient. Finally, Canonical Correspondence Analysis (CCA) was adopted to correlate abiotic factors and taxa relative abundances. Taxa present only in a subsample and with very low occurrences (1 individual) were considered rare and excluded from the analysis. Relations between biotic and abiotic data were also investigated with Mantel test (Mantel & Valand, 1970) performed on Bray Curtis based similarity matrix. The normality of all datasets was verified with the Kolmogorov-Smirnov test and variance homogeneity was checked with Brown-Forsythe test. We used STATISTICA 7.1 and PAST 3.2 (Hammer *et al.*, 2001) for all analyses. Leaf bags for site 3 were lost in autumn 2009, because of the interference of animals living in the area (horses and/or coypus). Summer data were obtained only for the last year (summer 2012), but not for site 5 because it dries up before the end of the sampling operations. Therefore, data of sites 3 (autumn 2009) and 5 (summer 2012) were not included in the statistical analysis.

RESULTS

Abiotic parameters

Mean seasonal abiotic parameters are reported in Table 1. The first two components obtained with the PCA using the three year dataset, explain 70.2 % of the system variance (Fig. 2a): loadings of sampling stations groups indicate a seasonal gradient, identified by the first axis, which explains 53.0 % of the system variation. Total rainfall, dissolved oxygen, conductivity and temperature are correlated to the first axis. The second axis explains 17.2 % of variance and is correlated to depth and pH (Tab. 2) and likely identifies a spatial gradient. In particular, we observed that site 5 always displayed at the bottom of seasonal groups: this site shows lower depths than the others, it was the first site subjected to draining in late spring and the last site subjected to flooding in early autumn. Finally, it harbours rich cover vegetation during spring. Except for depth and pH, two way ANOVA showed significant differences for all abiotic parameters among seasons and confirmed the seasonal trend ($F_{5,23} > 3.13$, $p < 0.05$; LSD test: at least $p < 0.05$, except for dissolved oxygen and conductivity during the first year and for rainfall during the last year).

Temperature values differ significantly from year to year both in autumn and spring as dissolved oxygen levels do (except between first and second year in autumn) (LSD test: at least $p < 0.05$). Conductivity and rainfall values observed during the third year differ significantly from others (LSD test: at least $p < 0.05$). Positive correlations with rainfall were found for depth ($r = 0.42$, $p < 0.05$) and dissolved oxygen concentrations ($r = 0.45$, $p < 0.05$) while a negative correlation was found with water temperature ($r = -0.66$, $p < 0.05$), pH ($r = -0.46$, $p < 0.05$) and conductivity ($r = -0.54$, $p < 0.05$).

The seasonal trend was confirmed by the results of the PCA using the last year data set with an increased number of parameters (Fig. 2b). The first two components explain 71.3 % of the variability and the main seasonal gradient was identified by the first axis again: all

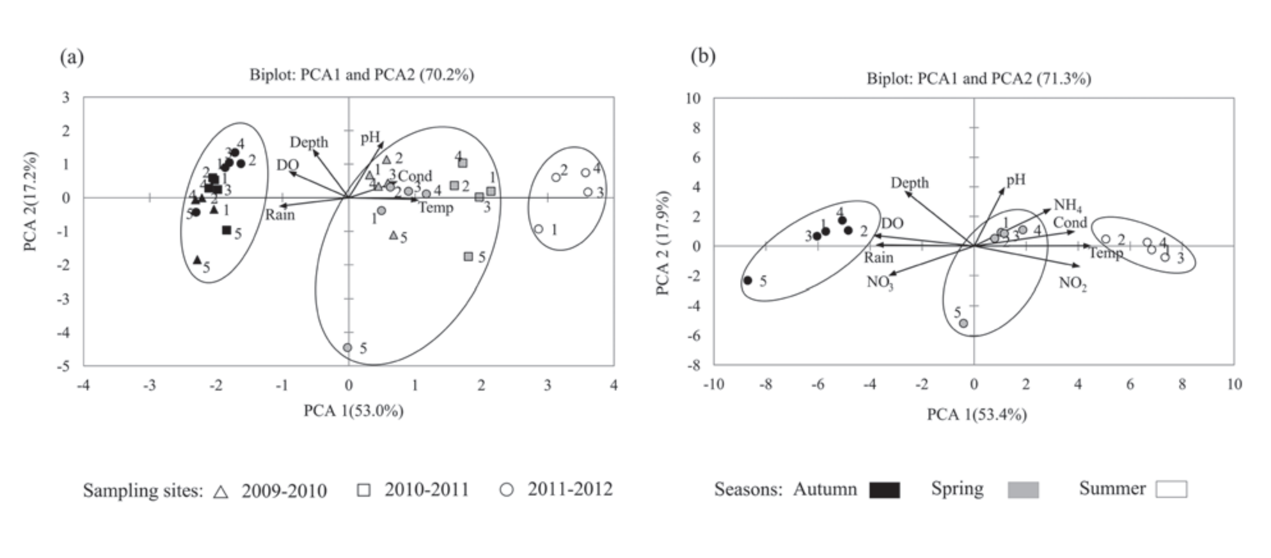

Fig. 2: (a) Principal Component Analysis (PCA) applied to the mean seasonal values of physical and chemical parameters for each sampling site to the three year data set; (b) PCA applied to the mean seasonal values of physical and chemical parameters (including nutrients) for each sampling site during the years 2011-2012. DO - dissolved oxygen, Cond - conductivity, Temp - temperature.

Sl. 2: (a) Analiza glavnih komponent (PCA) srednjih sezonskih vrednosti fizikalnih in kemijskih parametrov za vsako vzorčno mesto na triletnem podatkovnem nizu; (b) PCA analiza srednjih sezonskih vrednosti fizikalnih in kemijskih parametrov (vključno s hranili) za vsako vzorčno mesto v letih 2011 in 2012. DO - raztopljeni kisik, Cond - prevodnost, Temp - temperatura.

Tab. 2: Significant correlations (Pearson coefficient enlightened in bold, p < 0.001) among parameters and PCA factors (axes 1 and 2).

Tab. 2: Značilne korelacije (Pearsonov koeficient v krepkem tisku, p < 0,001) med parametri in PCA dejavniki (osi 1 in 2)

	PCA 1		**PCA 2**	
Axis	F1	F2	F1	F2
Depth	-0.445	**-0.664**	-0.538	**0.753**
Temperature	**0.879**	-0.022	**0.930**	0.004
pH	0.411	**-0.756**	0.235	-0.800
DO	**-0.724**	-0.359	**-0.776**	0.145
Cond	**0.602**	-0.220	**0.781**	0.198
Rainfall	**-0.903**	0.126	**-0.758**	0.015
NO_3^-	-	-	**-0.679**	-0.406
NO_2^-	-	-	**0.833**	-0.287
NH_4^+	-	-	**0.593**	0.502

monitored nutrients are correlated to the first axis (Tab. 2) and follow the seasonal trend with higher concentration of NO_3^- in autumn and higher concentrations of NO_2^- and NH_4^+ during the warmer seasons. One way ANOVA showed significant seasonal differences for depth, temperature, dissolved oxygen, conductivity and NO_2^- ($F_{5,23} > 4.66$, at least $p < 0.05$). Water temperature was positively correlated with NO_2^- and NH_4^+ concentrations (r = 0.83 and 0.59, respectively, $p < 0.05$) and negatively with NO_3^- (r = -0.69, $p < 0.05$).

Macrozoobenthic community

27,154 macrobenthic invertebrates, belonging to 23 taxa, were identified. Differences were detected among relative abundances during the three years but the organisms most frequently observed belonged to few taxonomical groups: Hexapoda, Oligochaeta, Ostracoda, Malacostraca and Nematoda (Tab. 3). Mean number of observed taxa was always significantly higher in spring (Fig. 3) ($F_{5,23} = 11.9$, $p < 0.001$; LSD test: $p < 0.001$ for all comparisons). The two way PERMANOVA (factors: year and season) was highly significant for both main effects (year: F = 6.67, $p < 0.001$; season: F = 2.18, $p < 0.001$) and also for the year × season interaction (F = -0.48, $p < 0.001$). SIMPER test showed that main contributes to the differences are related to Chironominae (30.8 %), Ostracoda (24.4 %), Nematoda (12.5 %) and

Tab. 3: Seasonal occurrence (%) of the observed taxa for all the study years. Codes used in the CCA analysis are reported for each taxon. Legend: Aut – autumn, Spr – spring, Sum – summer.
Tab. 3: Sezonsko pojavljanje (%) opazovanih taksonov v vseh letih raziskave. Za vsak takson je prikazana tudi koda, uporabljena pri CCA analizi. Legenda: Aut – jesen, Spr – pomlad, Sum – poletje.

Phylum	Class	Order	Family /Subfamily	CCA code	Aut 2009	Spr 2010	Aut 2010	Spr 2011	Aut 2011	Spr 2012	Sum 2012
Nematoda				Nem		1.61	0.87	5.00	32.76	11.94	1.49
Mollusca	Gastropoda	Pulmonata	Planorbidae	Pla	0.31	0.93	0.14	0.03		0.09	
			Physidae	Phy	0.10	0.03	1.92	0.13		0.03	
Anellida	Oligochaeta	Tubificida	Tubificidae	Tub	0.31	11.97	8.60	30.17	3.43	0.66	
			Naididae	Nai	0.31	1.83	6.40	16.89	0.53	0.02	
			Enchytraeidae	-				0.01			
		Opisthopora	Lumbricidae	-					0.20		
Arthropoda	Ostracoda			Ost	20.90	15.63	12.83	7.59	23.78	63.40	36.31
	Malacostraca		Asellidae	Ase	1.67	3.10	0.17	0.16	4.43	0.54	0.13
			Gammaridae	Gam	0.63	2.79	0.98	2.32	3.10	2.31	5.57
	Hexapoda	Odonata	Libellulidae	Lib		0.19		0.01			
			Corixidae	Cor	0.10					0.05	0.26
		Coleoptera	Haliplidae	Hal		0.16		0.03			
			Dytiscidae	Dyt		0.59	0.07	0.06		0.02	
			Hydrophilidae	Hyd	0.10	0.84	0.03	0.08		0.15	0.71
		Diptera	Ceratopogonidae	Cer	0.52	1.27		0.21	0.33	0.31	0.06
			Chironominae*	Chi	74.51	58.91	67.35	36.43	30.84	20.31	55.91
			Orthocladiinae*	Ort		0.03		0.09			
			Tanypodinae*	Tan	0.21	0.09	0.59	0.76	0.33	0.12	0.26
			Tabanidae	Tab	0.31	0.03	0.03	0.01	0.07	0.03	
			Dolichopodidae	-					0.07		
			Ephydridae	Eph					0.13	0.02	
			Muscidae	-				0.02			

* Subfamily

the Oligochaeta Tubificidae (12.4 %) and Naididae (7.1 %). Remaining taxa shows contributes less than 5 %. Diptera Chironomidae of the subfamily Chironominae (tribes Chironomini and Tanytarsini) represent the most abundant taxon (Tab. 3). The crustacean class Ostracoda showed percentage frequencies between 7.59 and 63.4 % and was the most abundant taxon during spring and summer of the third year (Tab. 3). Oligochaeta belonged almost exclusively to the families Tubificidae and Naididae and were more abundant in samples taken between spring 2010 and spring 2011 (Tab. 3), while a decline was observed during the third year. Finally, the Phylum Nematoda was one of the most abundant taxon in the last year, showing percentage frequencies significantly higher than in previous. Coleoptera (families Dytiscidae, Haliplidae, Hydrophilidae) and Gastropoda (families Planorbidae, Physidae) showed higher abundances in the sites near the reeds (sites 1 and 5).

Temperature, dissolved oxygen and conductivity were significantly correlated with biotic dataset (Mantel test: $p < 0.01$ for all cases). The relations among abiotic parameters and taxa are showed in the CCA graph (Fig. 4): the first two axes explain 74.8 % of the system variance; eigenvalues are reported in Table 4. All the sites

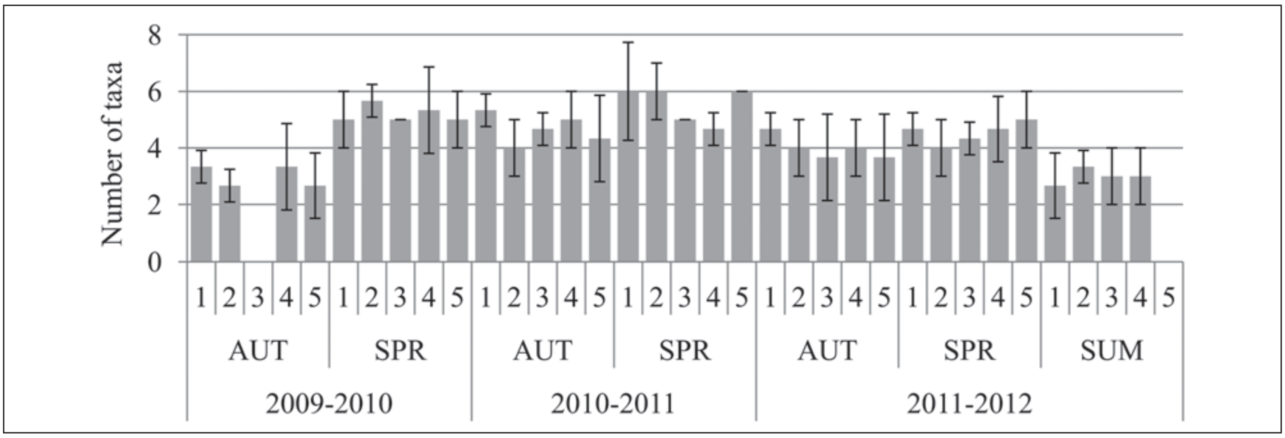

Fig. 3: Mean number of taxa ± SD observed in every site at each sampling season.
Sl. 3: Povprečno število taksonov ± SD za vsako vzorčno mesto, v vsaki sezoni vzorčenja

studied during the last year are placed at the left side of the graph, corresponding to significantly higher values of conductivity (Tab. 1) and to higher occurrences of Ostracoda (Tab. 3). Oligochaeta (families Tubificidae, Naididae) and Gastropoda (families Planorbidae and Physidae) showed negative preferences for higher conductivity values. Coleopterans Dytiscidae, Haliplidae and Hydrophilidae, as Gastropoda families Planorbidae and Physidae seem to be more related to the lower depths of site 5, where their occurrences were higher.

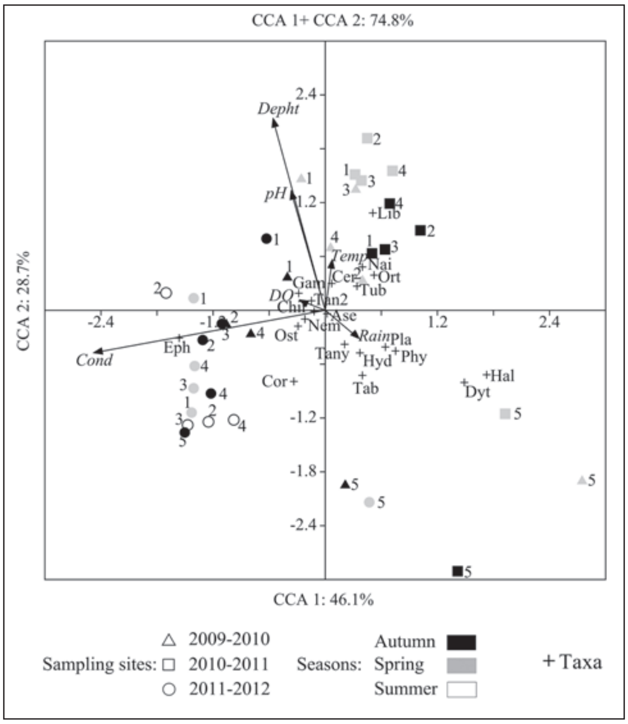

Fig. 4: Canonical Correspondence Analysis (CCA) biplot of environmental-taxa relationships. DO - dissolved oxygen, Cond - conductivity, Temp - temperature; taxa codes are reported in Table 3.
Sl. 4: Kanonična korespondenčna analiza (CCA): prikaz sestave taksonov v odnosu s spremenljivkami okolja. DO - raztopljeni kisik, Cond - prevodnost, Temp – temperatura; kratice taksonov so prikazane v tabeli 3.

DISCUSSION

The coupled effect of physical environmental factors and biotic interactions has been proposed as the mechanism for generating community structures in freshwater habitats (Wellborn *et al.*, 1996). Along a gradient from small ephemeral pools to larger semi-permanent and permanent ponds, periodic drying is recognized as a major constraint to invertebrate species composition (Jeffries, 1994; Schneider & Frost, 1996; Williams, 2006). The observed ranges for the values of dissolved oxygen concentrations, pH, water temperature and depth appear to be similar to what reported from other Authors in studies about Mediterranean temporary environments (Waterkeyn *et al.*, 2008; Bazzanti *et al.*, 2010; Dìaz-Paniagua *et al.*, 2010; Florencio *et al.*, 2013) and the results of chemical-physical investigations point out a seasonal trend, due to the partially natural and partially managed draining/flood cycle.

Conductivity shows a wide range among the years, probably because of infiltrations through the flap sluice gate but also because of the different rainfall observed. In fact, all the abiotic parameters appear to be related to rainfall, which can have great influence on many physical characteristics of temporary ponds, with important variation from dry to wet years. Consequently, macroinvertebrate assemblages may also differ among wet and dry periods (Jeffries, 1994).

Chemical and physical analyses point out a seasonal trend and the taxonomical richness (as number of taxa) was always significantly higher in spring. Nevertheless, the

Tab. 4: Summary of the CCA analysis performed on the observed taxa relative abundances and the physical-chemical parameters.
Tab. 4: Povzetek CCA analize, izvedene na podatkih o relativni številčnosti opazovanih taksonov in fizikalno-kemijskih parametrih

	Axis 1	Axis 2
Eigenvalue	0.109	0.068
Cumulative percentage variance of taxa-environment relationship	46.08	72.75
Significance to Montecarlo test (999 permutations), p	0.002	0.001

macrobenthic community was always dominated by few taxa which showed different occurrences from year to year.

Higher abundance of Ostracoda observed during the last year could be related to their wide ranges of ecological tolerance (Külköylüoğlu *et al.*, 2012). In particular, some species tolerate a wide range of salinity and/or temperature (Ghetti & Mc Kenzie, 1981). In addition a previous study within the study area (Stoch, 1995) reported the presence of *Cyclocypris ovum* and *Cypridopsis vidua*, able to resist to low concentrations of dissolved oxygen (respectively down to 2.0 and 1.6 mg l^{-1}) and to colonize both limnic and oligohaline environments. In this way temperature, dissolved oxygen and conductivity were found to be the main factors affecting the structure of macrobenthic invertebrates communities (Rossaro, 1991; Kagalou *et al.*, 2006; Gabriels *et al.*, 2007; Boets *et al.*, 2010), whereas the duration of the wet phase may have a particular impact on the number of present taxa (Schneider & Frost, 1996; Brooks, 2000; Batzer *et al.*, 2004; Della Bella *et al.*, 2005). The most abundant invertebrates collected during this study were Diptera Chironomidae (subfamily Chironominae), Oligochaeta, Ostracoda and Nematoda, which mainly contributed to the community differences among the years. The high abundance of these taxa is linked to their time of colonization strategies (Oter-

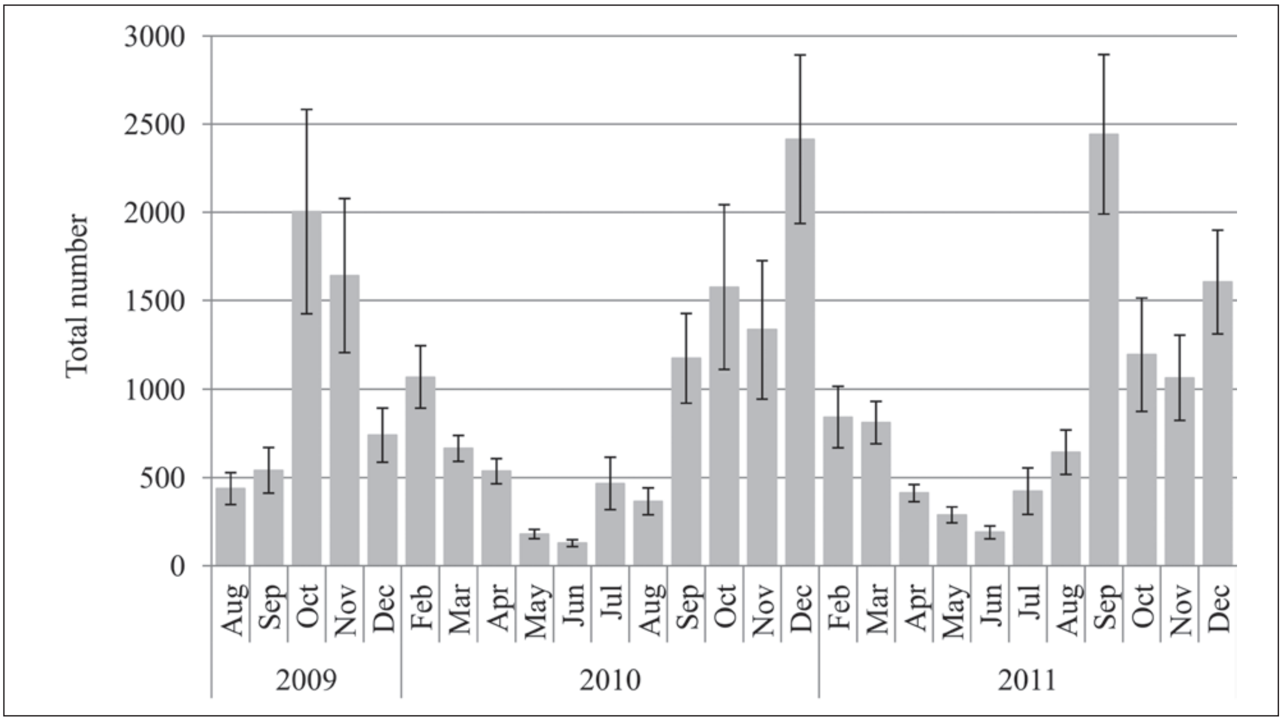

Fig. 5: Total number of bird specimens ± SD in the study area from August 2009 to December 2011. Considered species are: *Cygnus olor, Cygnus atratus, Anser fabalis, Anser albifrons, Anser erythropus, Anser anser, Tadorna tadorna, Anas penelope, Anas strepera, Anas crecca, Anas platyrhynchos, Anas acuta, Anas querquedula, Anas clypeata, Philomachus pugnax, Limosa limosa, Limosa lapponica, Anser indicus, Branta ruficollis, Branta leucopsis* **and** *Alopochen aegyptiaca* **(data provided by the Biological Station of Cona Island).**
Sl. 5: Skupno število osebkov ptičjih vrst ± SD na območju raziskave med avgustom 2009 in decembrom 2011. Upoštevane vrste so: *Cygnus olor, Cygnus atratus, Anser fabalis, Anser albifrons, Anser erythropus, Anser anser, Tadorna tadorna, Anas penelope, Anas strepera, Anas crecca, Anas platyrhynchos, Anas acuta, Anas querquedula, Anas clypeata, Philomachus pugnax, Limosa limosa, Limosa lapponica, Anser indicus, Branta ruficollis, Branta leucopsis* **in** *Alopochen aegyptiaca* **(podatke je posredovala Biološka postaja Isola della Cona).**

min *et al.*, 2002) and adaptations to overcome dry phases (Wiggins *et al.*, 1980). Furthermore, subfamily Chironominae can become numerically dominant in environments with features similar to those of the study area (Bazzanti *et al.*, 1997), whereas Oligochaeta and Nematoda can be found in the sediments, where they can feed on huge quantities of small sized organic matter (Heino, 2000). As observed by Ruzič *et al*. (2013), due to the water shallowness, the sediment probably greatly affects the water column processes. In fact, low concentrations of dissolved oxygen are likely influenced both by redox conditions and by deposition of an organic layer on the bottom sediment (Mereta *et al.*, 2012). In this work the nutrient load, in particular ammonium, was rather high and could be related to the massive presence of birds (Boros *et al.*, 2008). Indeed, during the study from August 2009 to December 2011 a higher number of individuals of main bird species always occurred between September and February (Fig. 5). Most likely, the sediment got a load of nutrients during autumn/winter whereas in summer the drainage of the area could have had an effect both on the substrate oxygenation and on the nutrients mineralization dynamics, which is dominated by aerobic processes (Reed *et al.*, 2011). Furthermore, also the management practices have to be considered: water level control can be implemented through the opening of the sluice gate during summer, which may have important consequences on the system, as observed, because dry phase enhances the mineralization of nutrients, avoiding hypoxia or anoxia conditions (Street, 1982; Pizzul *et al.*, 2008).

Finally, even though the leaf bag technique is known to be potentially selective for certain taxa (Basset *et al.*, 2006), for this long term study we preferred it instead of a quantitative approach with a box corer sampler, which was used by Boggero *et al*. (2011) in the same sites in a previous work. As shown by Quintino *et al*. (2011), box-corer and leaf bags could potentially lead to different results, but both techniques can disclose same ecological patterns in linkage between macrobenthic communities and abiotic descriptors. Furthermore, patterns of benthic communities variation present important similarities. As reported by Ruzič *et al*. (2013), data obtained for the spring communities appear to be comparable with those obtained by Boggero *et al*. (2011), and the impact of the sampling operations was considered greatly lower than a box-corer approach, which can be potentially unsustainable for a protected area, both in terms of human presence in the study site and environmental perturbation. Furthermore, the use of the box corer can be very time consuming.

The study area is included in a Natural Reserve, which is also a Site of Community Importance and a Special Protection Area and represent an example of ecological restoration, where biodiversity reaches high levels and where many microhabitats can be found (Perco *et al.*, 2006). The investigated system as the whole Natural Reserve could provide an excellent model to study succession and changes in macrobenthic invertebrate community structures (Boix *et al.*, 2012; Miguel Chinchilla *et al.*, 2014) and it represents a natural laboratory in a permanent re-colonization state (Matthaei *et al.*, 1996) in which the effect of conservation management strategies on communities of macrobenthic invertebrates could be directly observed (Ruzič *et al.*, 2013).

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SEZONSKA DINAMIKA MAKROZOOBENTOŠKIH SKUPNOSTI V REGIONALNEM NARAVNEM REZERVATU IZLIVA SOČE, SEVERNA ITALIJA: TRILETNA ANALIZA

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POVZETEK

Pričujoča triletna raziskava je bila izvedena sezonsko v veliki začasni sladkovodni mlaki, z namenom, da se: (a) preveri prisotnost sezonskih gradientov za glavne abiotske dejavnike, (b) ugotovi, kateri od teh dejavnikov imajo največjo težo pri oblikovanju makrobentoške skupnosti, in (c) razišče strukturo teh skupnosti v večletnem obdobju. Ker se območje raziskave nahaja v naravnem rezervatu, so avtorju uporabili tehniko »listnatih zavojev« za vzorčenje, da bi zmanjšali vpliv nabiranja vzorcev. Potrdili so sezonski trend abiotskih parametrov, medtem ko so se makrobentoške skupnosti bistveno razlikovale med leti. Avtorji so ugotovili, da med glavne abiotske dejavnike, ki vplivajo na makrobentoške skupnosti, lahko štejemo prevodnost, temperaturo in raztopljeni kisik.

Ključne besede: makrozoobentos, listnati zavoji, mokrišča, začasne mlake, sezonska dinamika

REFERENCES

Acuña, V., A. Giorgi, I. Munoz, U. Uehlinger & S. Sabater (2004): Flow extremes and benthic organic matter shape the metabolism of a headwater Mediterranean stream. Freshw. Biol., 49, 960–971.

Adhikari, S. & S. Bajracharaya (2009): A review of carbon dynamics and sequestration in wetlands. J. Wetl. Ecol., 2, 2–46.

APHA (1992): Standard methods for the examination of water and wastewater, 18th edition. American Public Health Association, Washington, DC.

Anderson, M. J. (2001): A new method for a non parametric multivariate analysis of variance. Aust. Ecol., 26, 32–46.

Basset, A., F. Sangiorgio & L. Sabetta (2006): Handbook for the application of body size descriptors to monitoring safety of transitional ecosystems. TW ReferenceNet - EU INTERREG III B Project 3B073. Management and sustainable development of protected transitional waters. University of Lecce, Lecce, 74 p.

Batzer, D. P., B. J. Palik & R. Buech (2004): Relationships between environmental characteristics and macroinvertebrate communities in seasonal woodland ponds of Minnesota. J. N. Am. Benthol. Soc., 23, 50–68.

Bazzanti, M., M. Seminara & S. Baldoni (1997): Chironomids (Diptera: Chironomidae) from three temporary ponds of different wet phase duration in Central Italy. J. Freshw. Ecol., 1, 89–99.

Bazzanti, M., C. Coccia & M. G. Dowgiallo (2010): Microdistribution of macroinvertebrates in a temporary pond of Central Italy: taxonomic and functional analyses. Limnologica, 50, 291–299.

Boets, P., K. Lock, M. Messiaen & P. L. M. Goethals (2010): Combining data driven methods and lab studies to analyze the ecology of *Dikerogammarus villosus*. Ecol. Inform., 5, 133–139.

Boggero, A., F. Vales, M. Bertoli & E. Pizzul (2011): Primi risultati riguardanti lo studio delle comunità macrozoobentoniche nella Riserva Regionale della Foce dell'Isonzo (Friuli Venezia Giulia, Nordest Italia). Studi Trent. Sci. Nat., 89, 69–74.

Boix, D., J. Biggs, R. Céréghino, A. P. Hull, T. Kalettka & B. Oertli (2012): Pond research and management in Europe: 'Small is Beautiful'. Hydrobiologia, 689, 1–9.

Bonada, N., M. Rieradevall, N. Prat & V. H. Resh (2006): Benthic macroinvertebrate assemblages and macrohabitat connectivity in Mediterranean-climate streams of northern California. J. N. Am. Benthol. Soc., 25, 32–43.

Boros, E., T. Nagy, C. Pigniczki, L. Kotymàn, K. V. Balogh & L. Voros (2008): The effect of aquatic birds on the nutrient load and water quality of soda pans in Hungary. Acta Zool. Hung., 54 (1), 207–224.

Boulton, A. J. & P. S. Lake (1992): The ecology of two intermittent streams in Victoria, Australia. II. Comparisons of faunal composition between habitats, rivers and years. Freshw. Biol., 27, 99–121.

Brooks, R. T. (2000): Annual and seasonal variation and the effects of hydroperiod on benthic macroinvertebrates of seasonal forest ('vernal') ponds in central Massachusetts, USA. Wetlands, 20, 707–715.

Bunn, S. E., P. M. Davies & T. D. Mosisch (1999): Ecosystem measures of river health and their response to riparian and catchment degradation. Freshw. Biol., 41, 333–345.

Cummins, K. W. (1974): Structure and function of stream ecosystems. Bioscience, 24, 631–641.

Della Bella, V., M. Bazzanti & F. Chiarotti (2005): Macroinvertebrate diversity and conservation status of Mediterranean ponds in Italy: water permanence and mesohabitat influence. Aquat. Conserv. Mar. Freshw. Ecosyst., 15, 583–600.

Díaz-Paniagua, C., R. Fernàndez-Zamudio, M. Florencio, P. Garcìa-Murillo, C. Gómez-Rodríguez, A. Portheault, L. Serrano & P. Siljeström (2010): Temporary ponds from Donana National Park: a system of natural habitats for the preservation of aquatic flora and fauna. Limnetica, 20 (1), 41–58.

Directive 92/43/EEC of the European Parliament and of the Council of 21 May 1992 on the conservation of natural habitats and of wild fauna and flora. OJ L 206, 22.7.1992, pp. 750.

Dixion, A. B. & A. P. Wood (2003): Wetland cultivation and hydrological management in Eastern Africa: matching community and hydrological needs through sustainable wetland use. Nat. Resour. Forum, 27, 117–129.

Fenoglio, S., T. Bo, M. Cucco & G. Malacarne (2006): Leaf break down patterns in a NW Italian stream: effect of leaf type, environmental conditions and patch size. Biol. Bratisl., 61 (5), 555–563.

Florencio, M., L. Serrano, C. Gomez-Rodriguez, A. Millan & C. Diaz-Paniagua (2009): Inter and intra-annual variations of macroinvertebrate assemblages are related to hydroperiod in Mediterranean temporary ponds. Hydrobiologia, 634, 167–183.

Florencio, M., C. Gomez-Rodriguez, L. Serrano, C. Diaz-Paniagua (2013): Competitive exclusion and habitat segregation in seasonal macroinvertebrate assemblages in temporary ponds. Freshw. Sci., 32 (2), 650–662.

Gabriels, W., P. L. M. Goethals, A. Dedecker, S. Lek & N. De Pauw (2007): Analysis of macrobenthic communities in Flanders, Belgium, using a stepwise input variable selection procedure with artificial neural networks. Aquatic Ecol., 41, 427–441.

Ghetti, P. F. & K. Mc Kenzie (1981): Ostracodi (Crustacea, Ostracoda). Guide per il riconoscimento delle specie animali delle acque interne italiane. Consiglio Nazionale delle Ricerche, Verona, 83 p.

Grillas, P. & J. Roche (1997): Vegetation of temporary marshes. Ecology and Management. Crivelli, A. J. & J. Jalbert (eds.): Conservation of Mediterranean wetlands no. 8. Station Biologique de la Tour du Valat, Arles (France), 86 p.

Hammer, Ø., D. A. T. Harper & P. D. Ryan (2001): PAST: Paleontological statistics software package for education and data analysis. Palaeontol. Electronica, 4 (1), 1–9.

Heino, J. (2000): Lentic macroinvertebrate assemblage structure along gradients in spatial heterogeneity, habitat size and water chemistry. Hydrobiologia, 418, 229–242.

IRSA CNR & APAT (2003): Metodi Analitici per le Acque, Metodo 4050. I.G.E.R. s.r.l., Roma, pp. 537–541.

Jeffries, M. (1994): Invertebrate communities and turnover in wetland ponds affected by drought. Freshw. Biol., 32, 603–612.

Kagalou, I., G. Economidis & I. Leonardos (2006): Assessment of a Mediterranean shallow lentic ecosystem (Lake Pamvotis, Greece) using benthic community diversity: Response to environmental. Limnologica, 36, 269–278.

Külköylüoğlu, O., D. Akdemir & R. Yuce (2012): Distribution, ecological tolerance and optimum levels of freshwater Ostracoda (Crustacea) from Diyarbakir, Turkey. Limnology, 13, 73–80.

Mancinelli, G., L. Sabetta & A. Basset (2005): Short term patch dynamics of macroinvertebrate colonization on decaying reed in a Mediterranean lagoon (Lake Alimni Grande, Apulia, SE Italy). Mar. Biol., 148, 271–283.

Mantel, N. & R. S. Valand (1970): A technique of nonparametric multivariate analysis. Biometrics, 26, 547–558.

Matthaei, C. D., U. Uehlinger, E. I. Meyer & A. Frutiger (1996): Recolonization by benthic invertebrates after experimental disturbance in a Swiss prealpine river. Freshw. Biol., 35, 233–248.

Metcalfe Smith, J. L. (1994): Biological Water Quality Assesment of Rivers: Use of Macroinvertebrate Communities. In: Calow, P. & G. E. Petts (eds.): The Rivers Handbook, Vol. 2. Blackwell Science, London, pp. 144–170.

Mereta, S. T., P. Boets, A. A. Bayih, A. Malu, Z. Ephrem, A. Sisay, H. Endale, M. Yitbarek, A. Jemal, L. De Meester & P. L. M. Goethals (2012): Analysis of environmental factors determining the abundance and diversity of macroinvertebrate taxa in natural wetlands of Southwest Ethiopia. Ecol. Inform., 7, 52–61.

Miguel Chinchilla, L., D. Boix, S. Gascòn & F. A. Comìn (2014): Macroinvertebrate biodiversity patterns during primary succession in manmade ponds in northeastern Spain. J. Limnol., 73 (3), 428–440.

Otermin, A., A. Basaguren & J. Pozo (2002): Recolonization by the macroinvertebrate community after a drought period in a first order stream (Agüera Basin, Northern Spain). Limnetica, 21 (1-2), 117–128.

Perco, F., P. Merluzzi & K. Kravos (2006): La foce dell'Isonzo e l'Isola della Cona. Edizioni della Laguna, Mariano del Friuli (GO), 145 p.

Petersen, R. C. & K. W. Cummins (1974): Leaf processing in a woodland stream. Freshw. Biol., 4, 343–368.

Pizzul, E., S. Guiotto & G. A. Moro (2008): Osservazioni sulle comunità macrozoobentoniche dell'Isola della Cona (Friuli Venezia Giulia, Nordest Italia). Annales, Ser. Hist. Nat., 18 (2), 79–90.

Pope, R. J., A. M. Gordon & N. K. Kaushik (1999): Leaf litter colonization by invertebrates in the littoral zone of a small oligotrophic lake. Hydrobiologia, 392, 99–112.

Presley, B. J. (1971): Techniques for analyzing interstitial water samples, part I: determination of selected minor and major inorganic constituents. In: Winterer, E. L., W. R. Riedel, P. Brönnimann, E. Gealy, G. Heath, L. Kroenke, E. Martini, R. Moberly Jr., J. Resig & T. Worsley (eds.): Initial Reports of the Deep Sea Drilling Project, Vol. 7. U.S. Government Printing Office, Washington, D,C., pp. 1749–1755.

Quintino, V., F. Sangiorgio, R. Mamede, F. Ricardo, L. Sampaio, R. Martins, R. Freitas, A. M. Rodrigues & A. Basset (2011): The leaf bag and the sediment sample: two sides of the same ecological quality story? Est. Coast. Shelf Sci., 95, 326–337.

Reed, D. C., C. P. Slomp & B. G. Gustafsson (2011): Sedimentary phosphorus dynamics and the evolution of bottom water hypoxia: A coupled benthic-pelagic model of a costal system. Limnol. Oceanogr., 56 (3), 1075–1092.

Robinson, C. T. & C. Jolindon (2005): Leaf breakdown and the ecosystem functioning of alpine streams. J. N. Am. Benthol. Soc., 24, 495–507.

Rolon, A. S. & L. Maltchik (2006): Environmental factors as predictors of aquatic macrophyte richness and composition in wetlands of southern Brazil. Hydrobiologia, 556, 221–231.

Rossaro, B. (1991): Factors that determine Chironomidae species distribution in fresh waters. Boll. Zool., 58, 281–286.

Ruzič, M., M. Bertoli, E. Pizzul, F. Vignes & A. Basset (2013): Macrozoobenthic communities in the Regional Natural Reserve of Isonzo River Mouth (Northeast Italy): first results of a leaf bag technique study. Annales, Ser. Hist. Nat., 23 (1), 7–16.

Sangiorgio, F., A. Basset, M. Pinna, L. Sabetta, M. Abbiati, M. Ponti, M. Minocci, S. Orfanidis, A. Nicolaiou, S. Moncheva, A. Trayanova, L. Georgescu, S. Dragan, S. Beqiraj, D. Koutsoubas, A. Evagelopoulos & S. Reizopoulou (2008): Environmental factors affecting *Phragmites australis* litter decomposition in Mediterranean and Black Sea transitional waters. Aquat. Conserv. Mar. Freshw. Ecosyst., 18, 16–26.

Schneider, D. W. & T. M. Frost (1996): Habitat duration and community structure in temporary ponds. J. N. Am. Benthol. Soc., 15, 64–86.

Sebastián González, E. & A. J. Green (2014): Habitat Use by Waterbirds in Relation to Pond Size, Water Depth, and Isolation: Lessons from a Restoration in Southern Spain. Restor. Ecol., 22, 311–318.

Solórzano, L. (1969): Determination of ammonia in natural waters by the phenolhypochlorite method. Limnol. Oceanogr., 14, 799–801.

Spencer, M., L. Blaustein, S. S. Schwartz & J. E. Cohen (1999): Species richness and the proportion of predatory animal species in temporary freshwater pools: relationships with habitat size and permanence. Ecol. Lett., 2 (3), 157–166.

Stoch, F. (1995): Indagine ecologico faunistica sui popolamenti ad entomostraci di alcuni stagni di acqua salmastra dell'Isola della Cona (foce del Fiume Isonzo, Italia nordorientale). Gortania, Atti Mus. Friul. St. Nat. Udine, 16, 151–173.

Street, M. (1982): The use of waste straw to promote the production of invertebrate foods for waterfowl in manmade wetlands. In: Scott, D. A. (ed.): Managing wetlands and their birds. Proceedings of the Third Technical Meeting on Western Palearctic Migratory Bird Management. International Waterfowl Research Bureau, Slimbridge, pp. 98–103.

Waterkeyn, A., P. Grillas, B. Vanshoenwinkel & L. Brendonck (2008): Invertebrate community patterns in Mediterranean temporary wetlands along Hydroperiod and salinity gradients. Freshw. Biol., 53, 1808–1822.

Wellborn, G. A., D. K. Skelly & E. E. Werner (1996): Mechanisms creating community structure across a freshwater habitat gradient. Annu. Rev. Ecol. Evol. Syst., 27, 337–363.

Wiggins, G. B., R. J. Mackay & I. M. Smith (1980): Evolutionary and ecological strategies of animals in annual temporary pools. Arch. Hydrobiol., Suppl. 58, 97–206.

Williams, D. D. (2006): The Biology of Temporary Waters. Oxford University Press, Oxford, 347 p.