

Spatial and seasonal variability of the macrobenthic fauna in Mediterranean solar saltworks ecosystems

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ABSTRACT

1. Solar saltworks are man-made systems for the production of salt and are characterized by high habitat heterogeneity owing to the existence of a strong salinity/confinement gradient. Although solar saltworks are considered to be artificial systems, they are also coastal aquatic ecosystems sharing common characteristics with natural transitional waters ecosystems, which are of special interest to the Water Framework Directive (2000/60/EC).

2. Spatial and seasonal distribution of macrobenthic invertebrates in relation to the abiotic environment of two Mediterranean solar saltworks ecosystems were assessed in this study. The spatial distribution investigated in this study ranged from the regional scale (Kalloni Saltworks, NE Aegean and Margherita di Savoia Saltworks, S. Adriatic) down to a local scale of 100s of metres in each ecosystem.

3. The macrobenthic community was variable at most spatial and seasonal scales examined in this study: spatial variability at the regional level was greater than seasonal variability within ecosystems and spatial variability among the salinity/confinement gradient levels was greater than within-level variability.

4. The abiotic environment, in both its spatial and seasonal aspects, was found to be crucial in determining the macrobenthic community structure. Biotic factors such as the life cycle of key species, the inter-specific competition as well as dispersion/colonization/extinction processes were also found to play an important role in structuring the macrobenthic fauna both in space and time.

5. A strong similarity of the macroinvertebrate faunal composition and community structure of the lower salinity ponds of solar saltworks with that of natural transitional waters ecosystems was observed. Therefore, solar saltworks ecosystems can be considered as important sites of study for the purposes of the Water Framework Directive.

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INTRODUCTION

Solar saltworks, also known as 'salinas' or 'salterns', are man-made systems where a wide salinity gradient is maintained along a series of interconnected ponds for the extraction of salt from sea water by means of solar evaporation (Korovessis and Lekkas, 2000). They are also coastal aquatic ecosystems of considerable heterogeneity, as they combine a spectrum of environmental types along the salinity gradient, starting with the lagoonal environment of the initial few evaporation ponds and ending in the harsh, extremely hyperhaline environment of the crystallizers (Davis, 2000). This physico-chemical diversity is reflected in the diverse flora and fauna that is adapted to and colonizes each environmental type in the saltworks system. Consequently, solar saltworks are considered to be habitats of great ecological importance and their proper management and conservation are necessary.

According to the Water Framework Directive (WFD) definition (EC, 2000, 2003), Transitional Waters (TW) are 'bodies of surface water in the vicinity of river mouths substantially influenced by freshwater flows'. Despite the fact that solar saltworks ecosystems are not directly influenced by freshwater flows (except for rainfall), they maintain the character of an artificial system that presents many affinities with natural TW ecosystems and especially coastal lagoons, regarding both the abiotic environment and biota, especially as many solar saltworks have been constructed at sites of former coastal lagoons (Britton and Johnson, 1987; Pavlova *et al.*, 1998; Zeno, 2006). Furthermore, the lower salinity ponds (salinity lower than 100 psu) of the solar saltworks usually retain the soft bottom and banks of the original coastal lagoon, since human intervention there is minimal, and this is also reflected in the biota. This biota, according to the few existing data, is very similar in structure to that distributed in natural coastal lagoons, occasionally displaying considerable diversity (Evangelopoulos and Koutsoubas, 2008). The solar saltworks ecosystems are also related to the natural wetland ecosystems of salt marshes, where areas covered by hyperhaline water known as 'salt pans' are the natural counterparts of the solar saltworks evaporation ponds, which are often also termed salt pans. Finally, the importance of solar saltworks ecosystems for water birds (Britton and Johnson, 1987; Sadoul *et al.*, 1998; Walmsley, 2000) is complementary to, and occasionally even greater than that of natural wetlands and other similar TW ecosystems (Korovessis and Lekkas, 2000).

Salinity has been considered as the critical factor determining the environmental conditions as well as the structure and distribution of the biological communities in Mediterranean TW ecosystems and has been used for the

classification of ecosystem types in a mono-factorial approach (Venice system; Battaglia, 1959). Alternative perspectives, such as the concept of 'confinement' (Guelorget and Perthuisot, 1992) commonly applied in the Mediterranean TW ecosystems and essentially multi-factorial, have been put forward. In solar saltworks, salinity and 'confinement' seem to play a leading role in determining the properties of the environmental gradient and the distribution of the biota (Britton and Johnson, 1987; Davis, 2000; Evangelopoulos and Koutsoubas, 2008). Therefore, solar saltworks can be used as model ecosystems for the investigation of the role of the above mentioned parameters as natural stressors structuring the biota of TW ecosystems.

Macrobenthic assemblages have been effectively utilized as descriptors of environmental health in several TW ecosystems (Dauer, 1993; Koutsoubas *et al.*, 2000; De Biasi *et al.*, 2003; Sconfiatti *et al.*, 2003; Reizopoulou and Nicolaidou, 2004; Rossi *et al.*, 2006). Published information considering the study of macrobenthic communities of solar saltworks is scarce (Vieira and Galhano, 1985; Britton and Johnson, 1987; Vieira and Amat, 1997; Pavlova *et al.*, 1998) and more comprehensive studies have only recently been published (Evangelopoulos *et al.*, 2006; Evangelopoulos and Koutsoubas, 2008).

This work is part of the EU funded INTERREG IIIB—CADSES project 'Management and sustainable development of protected transitional waters'. The main objective is to study the structure of the macrobenthic invertebrate communities in two Mediterranean solar saltworks ecosystems and to provide information for a certain type of TW ecosystem, which has generally been neglected so far. Macrobenthic communities of the two saltworks ecosystems are compared, to assess their seasonal variability associated with physico-chemical variables and at different spatial scales. The quantification at the appropriate ecological scales of natural variability in the TW ecosystems, as well as the elucidation of the ecological interactions between and within abiotic factors and the biotic components in these ecosystems, are essential for the development of plans for their management and conservation.

METHODS

Study areas

The two Mediterranean solar saltworks ecosystems studied were Kalloni Saltworks (KS), located on the south coast of the island of Lesbos, NE Aegean Sea, Greece (Figure 1(a)) and Margherita di Savoia Saltworks (MSS), on the S. Adriatic Sea

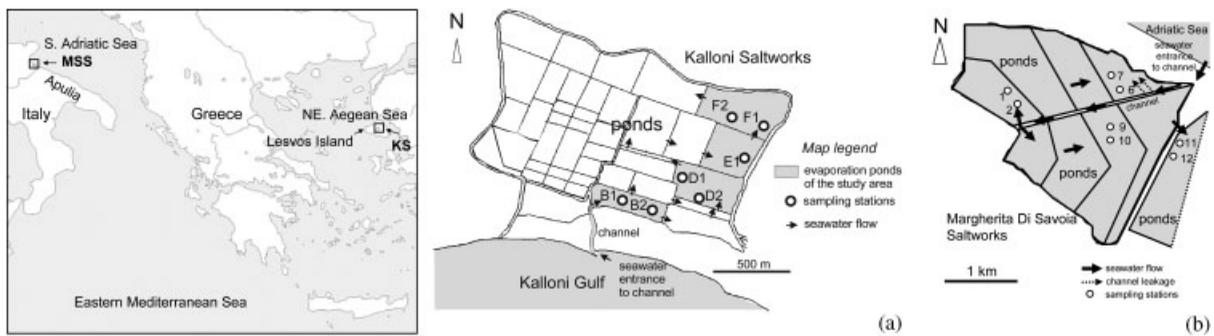


Figure 1. Maps of the study areas (a) Kalloni saltworks (KS) and (b) Margherita di Savoia saltworks (MSS), indicating the sampling stations.

coast of Italy (Figure 1(b)). KS (surface area 263 ha) are considered to be the most important of several wetlands on the coast of Kalloni Gulf (Dafis *et al.*, 1997) and are the third biggest solar saltworks in Greece (annual production over 40 000 tons of salt). MSS (surface area 4000 ha) are the most productive solar saltworks in Italy (annual production over 550 000 tons of salt) and comprise part of the Apulian wetlands, which are among the most diverse and important throughout Italy (Zeno, 2006). Both ecosystems play a very important role for migrating birds that cross the Mediterranean and have been proposed as Sites of Community Interest (KS: GR4110004; MSS: IT9110006) in the NATURA 2000 network (Dafis *et al.*, 1997; Zeno, 2006). In addition, MSS has been declared a wetland of international importance according to the Ramsar Convention (Zeno, 2006). Both saltworks, which have been constructed in areas of former coastal lagoons, consist of interconnected shallow ponds with levelled soft sediment bottoms. They are seasonal saltworks, producing salt only during the period April/May to September. The areas of the saltworks considered in this study were restricted to the lower salinity ponds (seawater salinity less than 100 psu), because in the higher salinity ponds the environmental conditions are too extreme to allow the survival of most macrobenthic invertebrates (Britton and Johnson, 1987).

Sampling design

The spatial distribution of the macrobenthic fauna in the solar saltworks was assessed at three different scales (Figure 1). (i) A regional scale: comparison of the ecosystems of KS and MSS located in the Aegean Sea and the Adriatic Sea, respectively, which belong to the same eco-region of the Mediterranean Sea. (ii) Along the salinity/confinement stressor gradient established along the course of seawater flow in the ponds of each ecosystem: comparison of four pairs of sampling stations (one pair of outermost stations (B1 and B2 in KS, 1 and 2 in MSS), two pairs of intermediate stations (D1 and D2,

E1 in KS, 6 and 7, 9 and 10 in MSS) and one pair of innermost stations (F1 and F2 in KS, 11 and 12 in MSS)) located at four different ponds representing successive levels of the salinity/confinement gradient in each ecosystem. (iii) At a local spatial scale at each salinity/confinement gradient level (i.e. pond): comparison of the sampling stations of each pair. All stations in both studied ecosystems were shallow (depth <0.5 m). The seasonal distribution of the macrobenthic fauna was assessed at two sampling periods, the autumn of 2004 and late spring of 2005.

Field and laboratory methods

Water samples (four replicates) for the analyses of inorganic nutrients were collected from only one sampling station at each of the four ponds in the study area of both KS (i.e. B1, D1, E1 and F1) and MSS (i.e. 1, 6, 9 and 11). Water column temperature and dissolved oxygen concentration were measured *in situ* by means of portable instruments (oxygen/temperature meter—OxyGuard Handy, pHmeter—OxyGuard Handy Birkerød, Denmark) at all the sampling stations of both solar saltworks. Sediment samples were also collected from all the sampling stations for determination of the organic matter and grain size analysis of the uppermost layer of the bottom sediment. Sediment grain size analysis in both saltworks and sediment organic matter in MSS were measured only during the spring sampling. Water samples were analysed for inorganic nutrients (phosphate phosphorus, ammonium and nitrate nitrogen concentration) according to standard spectro-photometric methods (Strickland and Parsons, 1968; Liddicoat *et al.*, 1975, 1976). Readily oxidizable sediment organic matter was analysed using the wet oxidation method of Walkey and Black (Walkey, 1947) as modified by Jackson (1958) and described by Loring and Rantala (1992). Finally, sediment grain size analysis was carried out using a Micrometrics Sedigraph 5100 (Norcross, USA) sediment grain size laser analyser.

Sediment samples (five replicates) were taken for analysis of the macrobenthic invertebrates from all sampling stations using a hand operated box-corer type sampler (0.03 m^2) and were subsequently sieved through a 0.5 mm mesh size net. The samples were fixed with 4% formaldehyde and stained with Rose Bengal. The macrobenthic invertebrates were sorted in the laboratory, identified to species level where possible and counted.

Data analysis

Alpha diversity of the macrobenthic communities was measured by the calculation of species richness, the Shannon heterogeneity index H' (Shannon and Weaver, 1949) and the Pielou evenness index J' (Pielou, 1969). The taxonomic indices examined included average taxonomic distinctness Δ^+ (Warwick and Clarke, 1995) and variation in taxonomic distinctness Λ^+ (Clarke and Warwick, 2001a; Warwick and Clarke, 2001). Differences in the univariate descriptors between the sampling stations were tested for significance with the Kruskal–Wallis non-parametric test, performed with SPSS 12 for Windows software. Among multivariate methods, hierarchical agglomerative clustering (CLUSTER) analysis using the group average linkage technique, was applied for the classification of samples into groups of similar community structure. One-way ANOSIM (Clarke and Green, 1988) was used to detect statistically significant differences between seasons in each ecosystem. Two-way crossed ANOSIM (Clarke, 1993) was used to detect statistically significant differences between groups of stations across seasons or between seasons across stations in each ecosystem. The contribution of each species to the similarity of the stations was investigated using SIMPER analysis (Clarke, 1993). Seriation in community change along the course of seawater flow in the ponds was tested with the Index of Multivariate Seriation (IMS) (Clarke *et al.*, 1993), by application of the RELATE analysis. The triangular similarity matrices required by the multivariate analyses were calculated using the Bray–Curtis similarity coefficient (Bray and Curtis, 1957). Density data were transformed before the analyses to $\log(x+1)$ in order to downweight the contribution of the dominant species. All the aforementioned univariate and multivariate methods were performed using the PRIMER 5.2.9 multivariate statistical software (Clarke and Gorley, 2001), as described and discussed by Clarke and Warwick (2001b).

Redundancy analysis (RDA), a linear eigenvector direct gradient analysis (Lepš and Šmilauer, 2003), was performed to investigate to what extent the spatial and temporal aspects of the sampling design were responsible for the variation in the environmental variables examined. In the RDA ordination, the environmental data served as the response variables, whereas the spatial and seasonal aspects of the sampling design

(coded as dummy variables) served as the explanatory variables. Canonical correspondence analysis (CCA) (ter Braak, 1986) was then applied to investigate whether any aspects of community structure (response variables) were related to the measured abiotic variables (explanatory variables). CCA is based on an underlying uni-modal species response model and constrains the axes to be linear combinations of the measured environmental variables (ter Braak, 1986). The salinity values used in the RDA and CCA ordinations were the salinity values known to be typical of each pond in the two solar saltworks during their operation. In the case of MSS, the inorganic nutrient concentrations measured at only one of the two stations in each pond were treated in the RDA and CCA ordinations as being representative of both stations in each pond. RDA and CCA ordinations were performed with the CANOCO for Windows 4.5.2 software for canonical community ordination (ter Braak and Šmilauer, 2002).

RESULTS

Abiotic environment

The RDA ordination (Figure 2) of the environmental data (Table 1), constrained by the sampling design, indicated that a

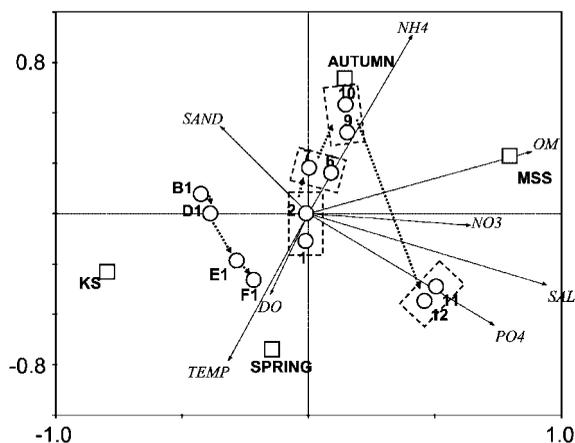


Figure 2. RDA ordination of the measured environmental variables, constrained by the spatial and temporal aspects of the sampling design (ecosystems, sampling stations, seasonal samplings). The sampling design variables are indicated by the respective centroids and the environmental variables by arrows (SAL = salinity, TEMP = temperature, DO = dissolved oxygen, PO₄ = phosphates, NO₃ = nitrates, NH₄ = ammonia, SAND = sediment sand %, OM = sediment organic matter %). The dotted arrows joining the sampling stations centroids indicate the course of seawater flow in the ponds. Dashed rectangles indicate the pairs of adjacent sampling stations in each pond in MSS.

Table 1. Environmental data (mean values) from the study areas (that were also used in the RDA and CCA ordinations)

	KS				MSS							
	B1	D1	E1	F1	1	2	6	7	9	10	11	12
Salinity/confinement gradient level	Level 1	Level 2	Level 3	Level 4	Level 1	Level 1	Level 2	Level 2	Level 3	Level 3	Level 4	Level 4
Salinity gradient (psu)	40	45	50	55	58	58	56	56	64	64	87	87
Sediment sand (%)	78	91	34	5	12	48	38	60	27	64	40	31
AUTUMN												
Temperature (°C)	8.2	7.5	11.6	12.8	14	14	14	14	8	9	10	11
Dissolved oxygen (mg L ⁻¹)	8.3	9.1	9.5	8.7	7.3	7.9	4.1	3.7	6.3	8.4	1.8	1.8
Phosphate (µmol L ⁻¹)	0.21	0.4	0.16	0.07	0.07	0.07	0.11	0.11	0.11	0.11	31.77	31.77
Ammonium (µmol L ⁻¹)	1.48	0.67	0.4	1.2	11.19	11.19	86.60	86.60	76.02	76.02	30.92	30.92
Nitrate (µmol L ⁻¹)	2.08	4.22	4.69	3.21	4.76	4.76	7.01	7.01	4.47	4.47	6.98	6.98
Sediment organic matter (%)	1.2	0.72	1.53	2.04	8.10	10.71	13.13	8.92	16.42	20.28	18.94	14.37
SPRING												
Temperature (°C)	26.8	32.3	30.4	29.9	19	19	23	24	21	21	22	22
Dissolved oxygen (mg L ⁻¹)	6.8	8.4	7.4	6.2	6.8	5.9	6.4	7.0	5.5	5.3	14.2	13.0
Phosphate (µmol L ⁻¹)	0.24	0.43	0.28	0.29	0.05	0.05	0.06	0.06	0.09	0.09	22.74	22.74
Ammonium (µmol L ⁻¹)	9.08	3.10	2.90	3.58	0.88	0.88	0.70	0.70	68.65	68.65	5.69	5.69
Nitrate (µmol L ⁻¹)	0.24	2.28	0.06	0.02	8.23	8.23	9.78	9.78	3.97	3.97	8.67	8.67
Sediment organic matter (%)	0.66	0.7	2.21	3.09	8.10	10.71	13.13	8.92	16.42	20.28	18.94	14.37

high percentage (82%, $P=0.002$) of the total variance of the environmental data could be explained by the spatial and temporal aspects of the sampling design. There was an apparent differentiation between the two ecosystems along the first RDA axis (eigenvalue=0.379, $P=0.002$) and between the two seasons along the second RDA axis (eigenvalue=0.175, $P=0.014$). On a regional level, MSS was characterized by higher salinity and concentrations of phosphates, nitrates, ammonia and sediment organic matter in comparison with KS. On a seasonal level, autumn was characterized by a lower temperature in both ecosystems and a much higher concentration of ammonia in MSS in comparison with spring. The ordination of the stations along both ordination axes corresponded in both ecosystems to their relative positions along the course of seawater flow in the ponds (with the exception of the ordination of the innermost stations 11 and 12 in MSS along the second ordination axis).

Macrobenthic community

Taxonomic composition

In total, 22 704 individuals (20 404 and 2300 in autumn and spring, respectively) were collected in KS. These individuals were identified to 43 taxa (36 and 23 in autumn and spring, respectively), belonging to Mollusca (19), Polychaeta (10), Crustacea (10), Insecta (2), Anthozoa (1) and Phoronida (1) (Table 2). In total 9729 individuals (4459 and 5270 in autumn and spring, respectively) were collected in MSS. These individuals were identified to 37 taxa (16 and 31 in autumn

and spring respectively), belonging to Mollusca (10), Polychaeta (10), Crustacea (8), Insecta (6), Anthozoa (1), Oligochaeta (1) and Planariidae (1) (Table 2). The commonest taxa collected in the two ecosystems in both seasons are presented in Table 3. The most abundant species were the gastropod mollusc *Hydrobia acuta* and the larvae of the dipteran insect *Chironomus salinarius* in KS and in MSS, respectively. Only eight taxa were found to be common to both ecosystems. The only abundant one among them was the amphipod *Microdeutopus gryllotalpa*. Seasonal turnover of species was comparatively more important, as 16 species were common between seasons in KS and nine species in MSS.

Biocoenoses

According to the classification scheme of the benthic biocoenoses of the Mediterranean (Pères, 1967; Augier, 1982), species that are characteristic of or accompany the biocoenosis of superficial muddy sands in sheltered areas (SVMC; e.g. *Microdeutopus gryllotalpa*, *Pirenella conica*, *Heteromastus filiformis*) as well as the euryhaline and eurythermal biocoenosis in brackish waters (LEE; e.g. *Abra segmentum*, *Hydrobia acuta*) were abundant in KS. The presence of species characteristic of the biocoenosis of fine surface sands in very shallow waters (SFHN; e.g. *Tellina tenuis*) at the stations closer to the seawater inflow channel during autumn is also notable. A similar pattern was observed in MSS; at the innermost stations over both seasons, the dominant species was *Chironomus salinarius*, a species characteristic of the LEE biocoenosis, while species that are

Table 2. Distribution of the macrobenthic invertebrate fauna in the study areas

Taxon	Kallioni Saltworks												Margherita Di Savoia Saltworks															
	Autumn						Spring						Autumn						Spring									
	B1	B2	D1	D2	E1	F1	B1	B2	D1	D2	E1	F1	F2	1	2	6	7	9	10	11	12	1	2	6	7	9	10	11
Mollusca																												
<i>Abra segmentum</i> (Récluz, 1843)																												
<i>Anodonta fragilis</i> (Philippi, 1836)																												
<i>Bitium reticulatum</i> (Da Costa, 1778)																												
<i>Cerastoderma glaucum</i> (Poiret, 1789)																												
<i>Cerithium vulgatum</i> (Bruguere, 1792)																												
<i>Chamaelea gallina</i> (L., 1758)																												
<i>Cyclope neritica</i> (L., 1758)																												
Cypridae																												
<i>Hannaea</i> sp.																												
<i>Hydrobia acuta</i> (Drapparnaud, 1805)																												
<i>Helicobia stagnorum</i> (Gmelin 1791)																												
<i>Hydrobia ventrosa</i> (Montagu 1803)																												
<i>Loripes lacteus</i> (L., 1758)																												
<i>Monophiorus perversus</i> (L., 1758)																												
<i>Nassarius</i> sp.																												
<i>Pipirella conica</i> (Blainville, 1826)																												
<i>Rissoa guerinii</i> (Récluz, 1843)																												
<i>Rissoa monodonta</i> (Philippi, 1836)																												
<i>Rissoa splendida</i> (Eichwald, 1830)																												
<i>Solen marginatus</i> (Pennant, 1777)																												
<i>Tapes decussatus</i> (L., 1758)																												
<i>Tapes</i> sp.																												
<i>Tellina planata</i> (L., 1758)																												
<i>Tellina</i> sp.																												
<i>Tellina tenuis</i> (Poli, 1791)																												
<i>Trophon muricatus</i> (Montagu, 1803)																												
Crustacea																												
<i>Ampelisca</i> sp.																												
<i>Bathyporeia</i> sp.																												
<i>Corophium orientale</i> (Schellenberg, 1928)																												
<i>Corophium</i> sp.																												
Cumacea																												
Decapoda																												

Table 2 (continued)

Taxon	Kalloni Saltworks						Margherita Di Savoia Saltworks									
	Autumn			Spring			Autumn			Spring						
	B1	B2	D1	D2	E1	F1	F2	I	2	6	7	9	10	11	12	
Lumbricidae																
Oligochaeta																
Phoronida																
Planariidae																

Table 3. List of the commonest (>1% in any season) macrobenthic invertebrate taxa in the study areas, also indicating their percentage of the total number of individuals collected

Taxa	All seasons	Autumn	Spring
KS <i>Hydrobia acuta</i>	70	77	10
<i>Microdeutopus gryllotalpa</i>	7	7	9
<i>Capitella capitata</i>	6	5	15
<i>Hediste diversicolor</i>	4	1	30
<i>Malacoceros fuliginosus</i>	3	4	<1
<i>Pirenella conica</i>	2	2	4
<i>Gammarus aequicauda</i>	2	1	11
<i>Corophium orientale</i>	1	1	2
<i>Cyclope neritea</i>	1	1	3
<i>Cerastoderma glaucum</i>	1	<1	5
<i>Bittium reticulatum</i>	1	<1	4
Chironomidae sp. 1	<1	<1	2
Decapoda	<1	—	2
MSS <i>Chironomus salinarius</i>	48	91	12
<i>Hydrobia ventrosa</i>	17	—	32
<i>Corophium</i> sp.	11	4	17
<i>Gammarus insensibilis</i>	9	1	17
<i>Microdeutopus gryllotalpa</i>	4	<1	7
Oligochaeta	3	—	5
<i>Loripes lacteus</i>	2	<1	3

characteristic of or accompany the SVMC biocoenosis such as *Loripes lacteus* and *Microdeutopus gryllotalpa* were abundant at the outermost (closer to the seawater inflow channel) and intermediate stations. Finally, in autumn, species characteristic of the SFHN biocoenosis (e.g. *Idotea baltica*) were recorded only at the outermost stations.

Univariate descriptors

On a seasonal basis, density values of the macrobenthic invertebrates in KS (Figure 3) were lower in spring than in autumn. On a spatial basis, the distributional pattern over seasons was partially different. In autumn, density increased from the outermost stations to its maximum at the intermediate stations (86 333 ind. m⁻²) and then decreased to its minimum at the innermost ones (Kruskal–Wallis: *P* < 0.05). In spring, density decreased from its maximum (4300 ind. m⁻²) at the outermost stations to similarly low levels at the intermediate and innermost ones (Kruskal–Wallis: *P* < 0.05). The seasonal pattern of macrobenthic distribution in MSS (Figure 3) was opposite from that observed in KS; density values were generally much higher in spring than in autumn (except at the innermost stations). On a spatial basis, the distributional pattern over seasons was partially different from that observed in KS (Figure 3). In autumn, density increased from similar levels at the outermost and intermediate stations to its maximum at the innermost ones (11 567 ind. m⁻²) (Kruskal–Wallis: *P* < 0.01). In spring, density decreased from similar levels at the outermost and intermediate stations

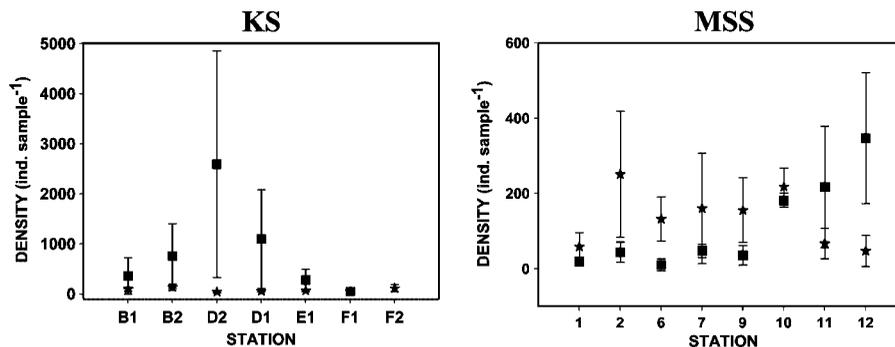


Figure 3. Variations of macrobenthic invertebrates density in the study areas (rectangles and asterisks indicate mean values in autumn and spring, error bars indicate standard error of the mean).

(maximum 7233 ind. m⁻²) to its minimum at the innermost ones (Kruskal–Wallis: $P < 0.01$).

Species richness in both KSS and MSS (Figure 4) was significantly lower (Kruskal–Wallis: $P < 0.05$) at the innermost stations than at the outermost ones in both seasons. In addition, generally higher values of species richness were measured in autumn than in spring in KS, while the opposite pattern was observed in MSS. Moreover, species richness was higher in KS than in MSS in autumn, while in spring species richness was similar in both areas.

In autumn, α diversity (as measured by the Shannon and Pielou indices—Figure 4) minimum was measured at the intermediate stations in KS (Kruskal–Wallis: $P < 0.05$) and at the innermost stations in MSS (Kruskal–Wallis: $P < 0.05$) owing to the high levels of dominance therein. In spring, α diversity was significantly higher at the outermost than at the innermost stations in both ecosystems (Kruskal–Wallis: $P < 0.05$). Additionally, α diversity at the intermediate and innermost stations was higher in spring than in autumn in both ecosystems.

Average taxonomic distinctness (as measured by the $\Delta+$ index—Figure 5) differed significantly among the sampling stations in KS only in spring (Kruskal–Wallis: $P < 0.05$) and in MSS only in autumn (Kruskal–Wallis: $P < 0.01$). Moreover, variation in taxonomic distinctness (as measured by the $\Lambda+$ index—Figure 5) differed significantly among the sampling stations only in MSS in autumn (Kruskal–Wallis: $P < 0.05$). In MSS in autumn, the differentiation observed among the sampling stations was due to the zero values measured in both $\Delta+$ and $\Lambda+$ at the innermost stations, where only *Chironomus salinarius* was recorded.

Multivariate analyses

The CLUSTER classification analysis (Figure 6) revealed a much better separation of ecosystems (two-way crossed ANOSIM (ecosystems averaged across seasons): global $R = 0.932$, $P = 0.1\%$) than of seasons (two-way crossed

ANOSIM (seasons averaged across ecosystems): global $R = 0.341$, $P = 0.1\%$). Seasons were weakly separated within both MSS (one-way ANOSIM: global $R = 0.409$, $P = 0.5\%$) and KS (one-way ANOSIM: global $R = 0.233$, $P = 4.1\%$). The sampling stations at each pond were in most cases grouped together in both ecosystems. Moreover, the calculation of the Index of Multivariate Seriation (IMS) revealed that there was a statistically significant serial pattern of community change along the course of seawater flow both in KS (autumn: IMS = 0.645, $P = 1.4\%$; spring: IMS = 0.661, $P = 0.3\%$) and MSS (autumn: IMS = 0.617, $P = 0.1\%$; spring: IMS = 0.844, $P = 0.1\%$) in both seasons.

According to the results of the SIMPER analysis, the most common species in KS in autumn were (in order of importance) *Hydrobia acuta*, *Capitella capitata*, *Pirenella conica*, *Gammarus aequicauda*, *Hediste diversicolor*, *Microdeutopus gryllotalpa*, *Cyclope neritea* and *Cerastoderma glaucum*, while in MSS the most important species were *Chironomus salinarius* and *Corophium* sp. The species that contributed most to the high dissimilarity (95%) between the two ecosystems during autumn were the gastropod *Hydrobia acuta*, which was present and numerically dominant only in KS, and *Chironomus salinarius*, which was numerically dominant and present only in MSS. During the spring sampling period, the most common species in KS were the same as in autumn (with the exception of *Capitella capitata*), while in MSS the most abundant species were *Gammarus insensibilis*, *Chironomus salinarius* and *Hydrobia ventrosa*. The species that contributed most to the high dissimilarity (95%) between the two ecosystems in spring were those present only (or mainly) in KS, such as *Hediste diversicolor*, *Gammarus aequicauda* and *Hydrobia acuta* or only in MSS, such as *Gammarus insensibilis*, *Chironomus salinarius* and *Hydrobia ventrosa*. However, it should be noted here that as it was not possible to cross-check the identification of some species, the dissimilarity between the two ecosystems may be slightly overestimated. The species that contributed most to the

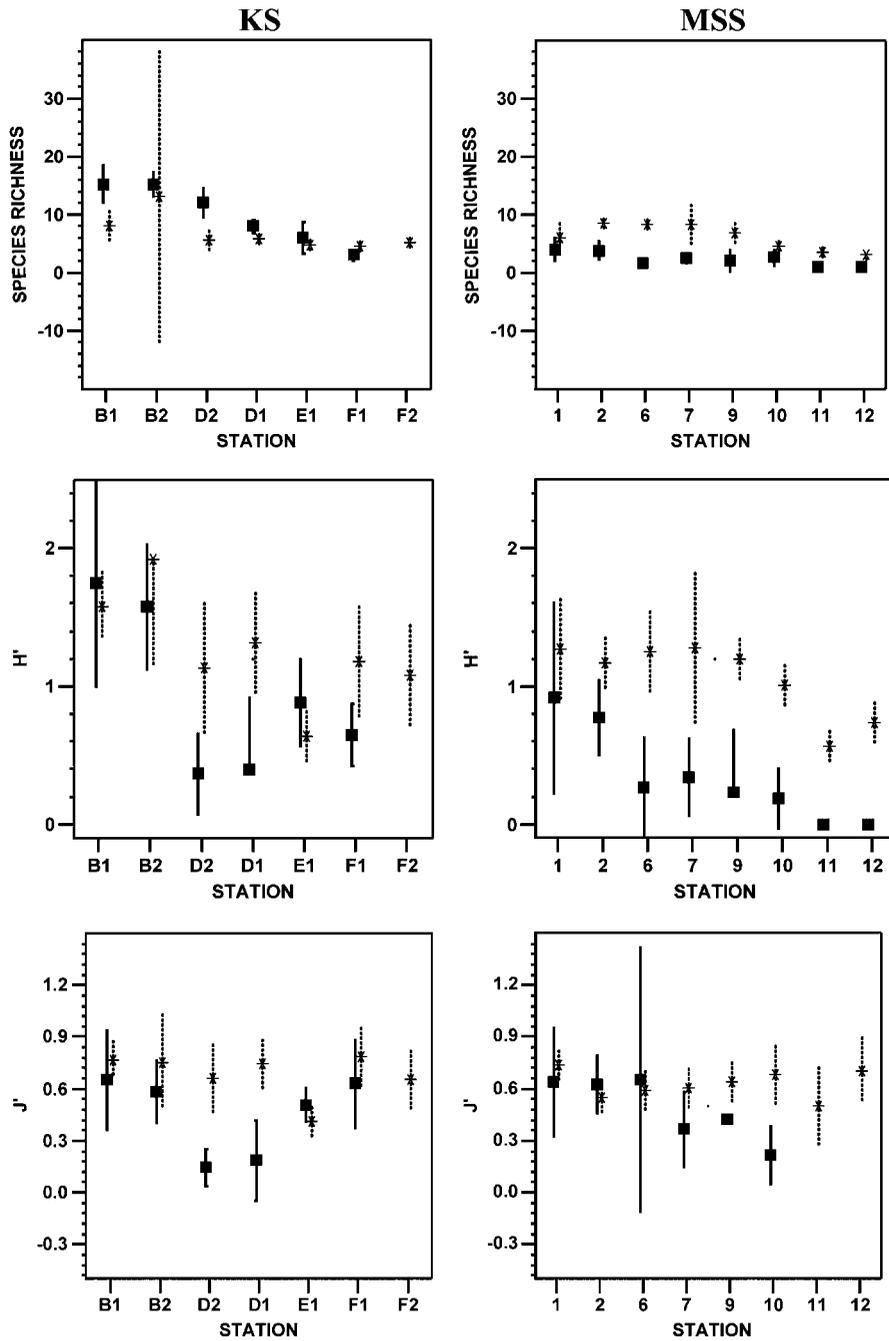


Figure 4. Variations of macrobenthic invertebrates α diversity indices in the study areas (rectangles and asterisks indicate mean values in autumn and spring, error bars indicate standard error of the mean).

dissimilarity between the two seasons in KS (60%) were *Hydrobia acuta* and *Capitella capitata* which were more abundant in autumn, while in MSS (77%) it was *Chironomus*

salinarius that was more abundant in autumn and *Gammarus aequicauda* along with *Hydrobia ventrosa* that were more abundant in spring.

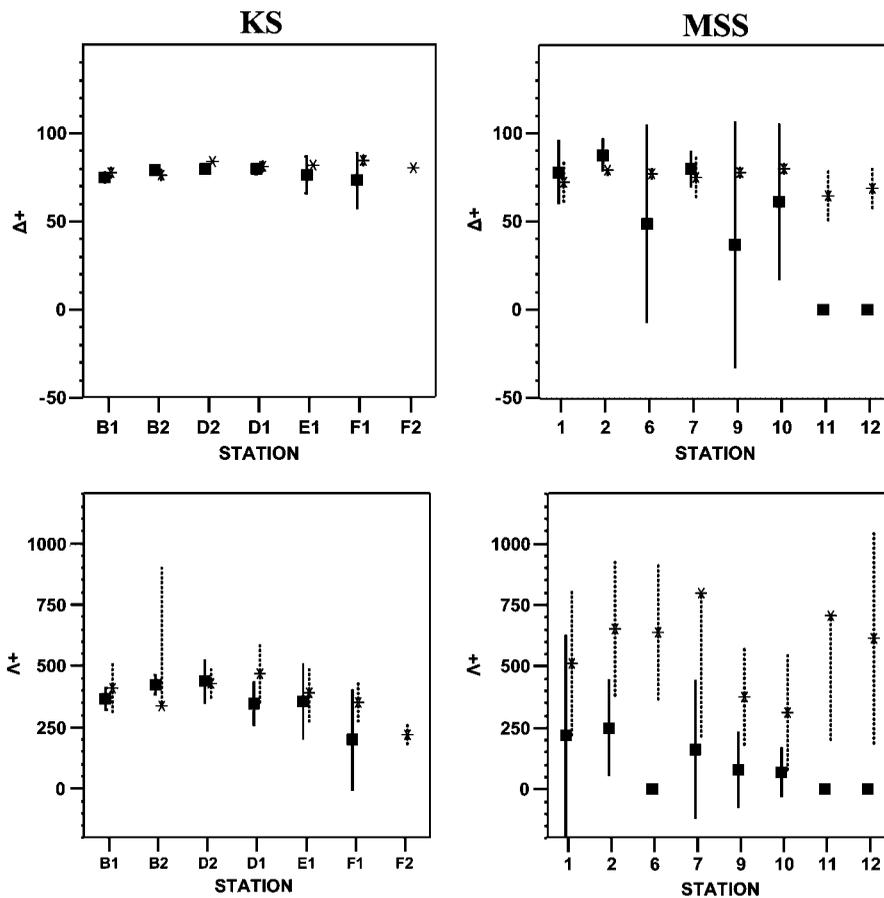


Figure 5. Variations of macrobenthic invertebrates taxonomic indices in the study areas (rectangles and asterisks indicate mean values in autumn and spring, error bars indicate standard error of the mean).

Linking macrobenthic community structure and the abiotic environment

The CCA ordinations of the biotic data of each ecosystem (Figure 7), constrained by the environmental data (Table 1), indicated that a significant part of the total inertia of the biotic data (59% in KS, 50% in MSS, $P=0.002$) could be explained by the environmental data examined in this study. Variability in the biotic data was much higher in MSS than in KS (total inertia = 3.234 in MSS and 1.662 in KS).

In KS, there was differentiation of the outermost station from the intermediate and innermost stations along the first ordination axis (eigenvalue = 0.346, $P=0.002$) in both seasons (Figure 7(a)). The intermediate and innermost stations were differentiated along the second ordination axis (eigenvalue = 0.256, $P=0.002$) in autumn but not in spring. The ordination of the stations along both axes generally corresponded to their relative positions along the course of

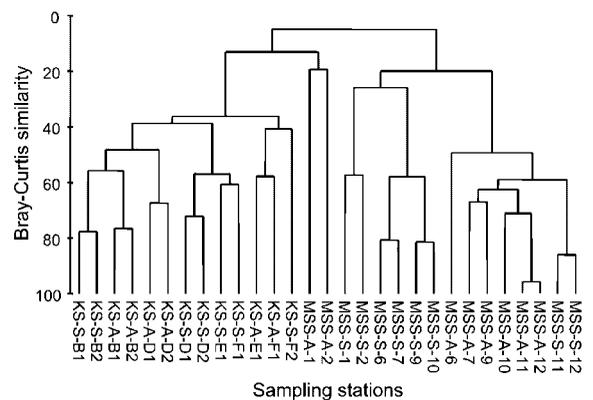


Figure 6. CLUSTER classification of the sampling stations in both study areas and seasons samplings (sampling stations prefixes: KS-A- = KS in autumn, KS-S- = KS in spring, MSS-A- = MSS in autumn, MSS-S- = MSS in spring).

seawater flow in the ponds. Seasons were well separated at all sampling stations, mainly along the second ordination axis. Among the environmental variables strongly correlated with either of the first two ordination axes (correlation > 0.6), salinity was more strongly correlated with the first ordination axis, whereas most of the other environmental variables were more strongly correlated with the second ordination axis.

In MSS during autumn, there was a differentiation of the pair of outermost stations from a group of the pairs of the intermediate and innermost stations along both the first (eigenvalue = 0.586, $P = 0.002$) and second (eigenvalue = 0.468, $P = 0.002$) ordination axes (Figure 7(b)). During spring, there was better differentiation of the pairs of stations along the first than along the second ordination axis. As in KS, the ordination of the stations in MSS along the axes generally corresponded to their relative positions along the course of seawater flow in the ponds. The seasons were well separated at all stations. The seasons at the outermost stations were separated mainly along the second ordination axis, whereas at the intermediate and innermost stations, the seasons are separated along both ordination axes. Among the environmental variables strongly correlated with either of the first two ordination axes (correlation > 0.6), salinity, phosphates and sediment organic matter were more strongly correlated with the first ordination axis, whereas temperature was more strongly correlated with the second ordination axis.

DISCUSSION

The results demonstrate the strong similarity of the macrobenthic fauna of the lower salinity ponds of solar saltworks with that of natural TW ecosystems. The major macrobenthic taxonomic groups, i.e. molluscs, polychaetes, crustaceans and chironomid larvae, distributed in both study areas, are typical of the macrobenthic fauna of TW ecosystems (Barnes, 1980, 1994a). Moreover, the macrobenthic species that were found to be abundant in the study areas are considered to be typical lagoonal species or marine/estuarine species commonly occurring in coastal lagoons (Barnes, 1980, 1994a,b; Guelorget and Perthuisot, 1992).

The dominance of opportunistic deposit feeders (*Hydrobia acuta*, *Hydrobia ventrosa*, *Capitella capitata*, *Chironomus salinarius*) in both study areas can be attributed to the high levels of organic matter, microphytobenthos and detritus in the bottom sediment. This phenomenon is known to occur not only in other solar saltworks (Davis, 2000), but also in various types of natural TW ecosystems (Barnes, 1980, 1994a; Little, 2000; McLusky and Elliott, 2004).

Two different species of hydrobiid snails, which are characteristic of soft substrate benthos in lagoonal ecosystems of the Mediterranean (Koutsoubas, 1992), were found in the two study areas. The two species, *Hydrobia acuta* in KS and *Hydrobia ventrosa* in MSS, have overlapping niches, as they are both epibenthic deposit feeders, feeding mainly on

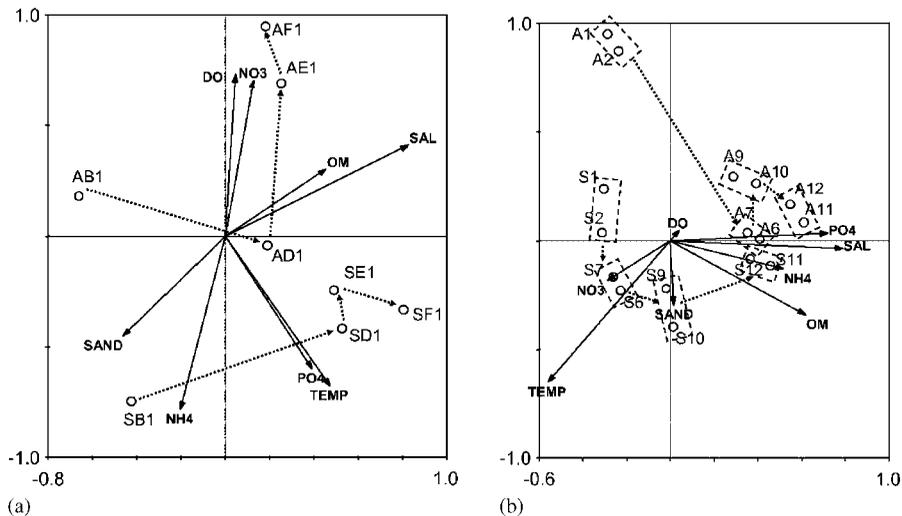


Figure 7. CCA ordinations of the biotic data in (a) KS and (b) MSS, constrained by the measured environmental variables. The sampling stations are indicated with the respective centroids and the environmental variables by arrows (sampling stations prefixes: A = autumn, S = spring; SAL = salinity, TEMP = temperature, DO = dissolved oxygen, PO₄ = phosphates, NO₃ = nitrates, NH₄ = ammonia, SAND = sediment sand %, OM = sediment organic matter %). The dotted arrows joining the sampling stations centroids indicate the course of seawater flow in the ponds.

Dashed rectangles indicate the pairs of adjacent sampling stations in each pond in MSS.

benthic diatoms and detritus (Little, 2000). *Hydrobia acuta* is known to occur in the ponds of solar saltworks (France: Britton, 1985; Britton and Johnson, 1987; Greece: Evagelopoulos and Koutsoubas, 2008). *Hydrobia ventrosa* has been reported to be more tolerant than *Hydrobia neglecta* (= *Hydrobia acuta neglecta*; Barnes, 2005) to increased levels of H₂S and anoxia (Fenchel, 1975). Therefore, *Hydrobia ventrosa* probably occurred in MSS owing to the high levels of organic matter in the sediment while *Hydrobia acuta* occurred in KS. However, the distribution patterns of hydrobiids cannot always be explained by ecological interactions, since populations of coastal hydrobiids are often ephemeral and episodes of colonization/extinction/recolonization by the same or different species are common (Fenchel, 1975; Barnes, 2005). In KS, the high abundance of *Hydrobia acuta* in autumn and the low abundance in spring, can probably be attributed to the life cycle of the species. *Hydrobia acuta* has been reported to be a strictly annual species in solar saltworks (Britton, 1985): reproduction occurs over a limited period in late spring (end of May) and then the adults die. The main period of growth is in autumn, when the juveniles attain their greatest size, before overwintering in the sediment when environmental conditions become adverse (low temperature, drainage of ponds).

Although chironomids are mostly freshwater species, larvae of *Chironomus salinarius* are among the predominant benthic macroinvertebrates living in shallow lagoons along the southern European coasts (Labourg, 1979; Ali and Majori, 1984; Ali *et al.*, 1985; Ceretti *et al.*, 1987; Drake and Arias, 1995). The abundance of *Chironomus salinarius* has been found to be the highest in these ecosystems, where water circulation is low and silt proportion in the sediment is high (Guelorget *et al.*, 1983; Ferrarese and Ceretti, 1986; Gamito, 1989; Drake and Arias, 1995). According to Guelorget and Perthuisot (1992), the distribution of chironomids is restricted to confined parts of coastal lagoons and have thus included them among the typical taxa of zone V of their confinement zonation scheme. Chironomid larvae can store oxygen and thus can live in areas where oxygen is limited, e.g. areas with high organic matter loads (Resh and Rosenberg, 1988). Therefore, the dominance of *Chironomus salinarius* in MSS in autumn is not surprising, since high levels of organic matter were found in the sediment and dissolved oxygen concentration was low at most of the stations. As the majority of chironomid species are opportunistic herbivores, feeding mainly on diatoms and detritus (Resh and Rosenberg, 1988), niches of *Chironomus salinarius* and *Hydrobia ventrosa* are apparently overlapping. These, low levels of dissolved oxygen in MSS in autumn coupled with the interspecific competition may have been the decisive factors in determining the dominance of *Chironomus salinarius* and the absence of *Hydrobia ventrosa* in MSS during that period.

The variations of several commonly applied univariate descriptors of the macrobenthic community were assessed in the present study. However, a consistent pattern of variation along the salinity gradient over ecosystems and seasons was identified only in density and species richness: density was higher at the outermost and/or the intermediate stations and decreased to low levels at the innermost stations. In addition, species richness decreased along the gradient in all cases. Decrease of density and species richness with increasing distance from the sea has also been reported to occur in many coastal lagoons (Guelorget and Perthuisot, 1992). This pattern has been attributed to the stress of increased salinity and high variations in temperature (Britton and Johnson, 1987) or increased confinement (Guelorget and Perthuisot, 1992) at the most remote parts of the ecosystems in relation to the sea.

On the other hand, the patterns of variation along the salinity gradient of α diversity were found to depend largely upon ecosystem and season. The influence of the distribution of the dominant species along the gradient was evident in the observed patterns. The dominant species, i.e. *Hydrobia acuta* in KS and *Chironomus salinarius* in MSS, are opportunistic herbivores/detritivores. The proliferation of opportunistic species is common in TW ecosystems, which are extremely variable environments and where the interplay of resources availability, limiting factors and biotic interactions, like dispersion processes and interspecific competition, determine the structure of biological communities both in space and time (Barnes, 1980, 1994a; Little, 2000; McLusky and Elliott, 2004).

Results from the application of rapid assessment techniques (i.e. average taxonomic distinctness and variation in taxonomic distinctness) also indicated the influence of the proliferation of opportunistic species (i.e. *Chironomus salinarius* in MSS, in autumn). Nonetheless, variations of the taxonomic diversity indices along the salinity gradient were, in most cases, not significant.

These results therefore indicate the significance of examining autecological aspects of the dominant macrobenthic species in order to achieve better insight into the dynamics of the saltworks communities, as has been suggested so far for natural TW ecosystems (Nicolaidou *et al.*, 2005b).

The RDA ordination revealed that the spatial variability of the measured abiotic parameters between ecosystems across seasons was higher than seasonal variability across ecosystems. Therefore, although the two study areas are considered to be of the same type (lower salinity ponds of Mediterranean solar saltworks), they were characterized by significantly different abiotic environmental conditions. The abiotic environment in MSS was characterized by higher salinity levels than KS owing to the different structuring and bigger sizes of the initial ponds in MSS. In addition, MSS is apparently also more eutrophic as evidenced by the higher water column inorganic nutrient

concentrations as well as the higher sediment organic matter concentration.

Seasonal variability in the abiotic environment is expected to play an important role in solar saltworks ecosystems for a variety of reasons, one of the most important being the shallowness of the water (<0.5 m), that leads to the quick heating and cooling of the water column. High temperatures during late spring and summer, when combined with adequate inorganic nutrient levels, may have a series of side effects, such as macroalgal blooms, a subsequent decay of the organic matter produced, increased metabolic rates, decreased oxygen levels in the water column and in bottom sediment, and consequently adverse effects on macrofauna. These phenomena are frequently met in coastal lagoons (Koutsoubas *et al.*, 2000; Nicolaidou *et al.*, 2005a). Therefore, the lower levels of dissolved oxygen and the high ammonium concentrations in KS during late spring should probably be attributed to the decomposition of the dead organic matter produced by the macroalgal bloom that had been observed in the initial two ponds just before the sampling took place (personal obs.). Both study areas were characterized by an abiotic environmental gradient that was generally structured along the course of seawater flow in the ponds, whereas variability of the abiotic environment within each pond in MSS was comparatively low. Salinity and confinement are two abiotic factors that gradually increase along the course of seawater flow in the ponds of solar saltworks. Both have been considered in the literature to be the primary components of the principal environmental gradient that structures the biotic communities in solar saltworks and TW ecosystems in general (Guelorget and Perthuisot, 1992; Davis, 2000).

Solar saltworks ecosystems are characterized by considerable habitat heterogeneity in response to an extreme salinity gradient (Davis, 2000). The gradual increase in salinity along the course of the seawater flow in the ponds causes changes in the ionic composition of the water and the structure of the bottom sediment. The biota responds to these alterations of the abiotic environment by a gradual change in the structure of the biological communities that inhabit the water column and the bottom sediment of the ponds (Davis, 2000).

However, the salinity gradient in solar saltworks is strongly correlated with the confinement gradient (Guelorget and Perthuisot, 1992). The confinement gradient affects benthic macrofauna by gradually changing composition (replacement of marine with 'paralic', e.g. typical lagoonal, and finally evaporitic fauna), density (initial increase in density is followed by a decrease where approaching the evaporitic pole) and α diversity (which decreases along the gradient) (Guelorget and Perthuisot, 1992).

The turnover of the abundant species along the course of seawater flow in the ponds did not reveal the existence of a

strong gradient of confinement in either study area as all the ponds studied should be assigned to the zones of confinement IV–V (Guelorget and Perthuisot, 1992). A weak influence of the sea was detected only in the outermost ponds, where certain species typical of the confinement zones II or III (e.g. *Tellina tenuis*—SFHN biocoenosis and *Loripes lacteus*—SVMC biocoenosis) have been found to occur, although in low densities. A similar situation of dominance of a single zone of confinement (commonly IV–V) has also been recorded in many other TW ecosystems (Guelorget and Perthuisot, 1992).

The 'missing' confinement zones II–III have nevertheless been identified in the feeding channel and the adjacent marine area of Kalloni Gulf in previous research efforts in KS (Evangelopoulos and Koutsoubas, 2008). That study concerned the zone of transition between the marine and lagoonal environment that takes place between the adjacent marine area of Kalloni Gulf and the initial two ponds of KS. It was found that the strength of the confinement gradient and its importance for the structure of the molluscan community was greater than those of the salinity gradient. The present study adds that further downstream in the pond sequence, the confinement gradient becomes weaker and its importance for the structure of macrobenthic community is reduced.

Variability in the abiotic environment of the study areas was obviously manifested in the variability of the structure of the macrobenthic communities: similarly to the abiotic environment, their spatial variability between ecosystems across seasons was higher than seasonal variability across ecosystems. Moreover, macrobenthic communities at both ecosystems were structured along the salinity gradient, as evidenced by the CCA ordinations and the calculation of the IMS index, while seasonal variations within each ecosystem as well as variability within each pond were comparatively low. These results point out the significance for the biota of the salinity/confinement gradient in solar saltworks ecosystems, that is correlated, depending on the ecosystem, with other abiotic environmental factors, e.g. sediment structure (in KS), sediment organic matter concentration (in both KS and MSS) and inorganic nutrients concentrations (in MSS).

The salt pans of solar saltworks can be considered as microcosms that may help in understanding some aspects of mudflat ecology as many of the organisms that live in salt pans are typical of mudflats, e.g. *Corophium*, *Hediste* and *Hydrobia* (Little, 2000). Moreover, owing to their accessibility, salt pans have been regarded as miniature aquaria, allowing direct observations of the activities of their inhabitants (Steers, 1960). Solar saltworks can also be considered as systems insulated from land run-off and freshwater inputs (apart of course from rainfall) and allocthonous sources of chemical substances and aquatic organisms are limited principally to inputs from the sea. Furthermore, the structure of the biological community in

a salt pan is expected to be much simpler than in a typical estuarine zone (Comin *et al.*, 2004). The simplified nature of the saltworks' ecosystem can perhaps be utilized in ecological studies that focus on TW ecosystems, making more feasible studies regarding aquatic food webs and the development of ecological models.

Large natural spatio-temporal variability in both the abiotic environment and the biota of TW ecosystems are widely acknowledged to be intrinsic to this category of surface waters ecosystem (Barnes, 1980; McLusky and Elliott, 2004). Their quantification is of special interest to the WFD in relation to the development of a physical typology, as well as to the definition of type-specific biological reference conditions (EC, 2003). The present study investigates and quantifies at several ecological scales the causes of variability of the macrobenthic invertebrate fauna of solar saltworks, a particular type of TW ecosystem. It has been suggested that the study of hypersaline ecosystems such as salt lakes can play a substantial role in the debate concerning the ecological importance of biotic versus abiotic factors in aquatic ecosystems (Vareschi, 1987). The importance of scale for the interpretation of the relationships between abiotic factors and the biotic communities of saline lake ecosystems has also been stressed (Williams *et al.*, 1990). The present study suggests that the study of macrobenthic invertebrates of solar saltworks, when appropriate attention is paid to ecological scale, can play a similar role in elucidating the ecological interactions between and within abiotic factors (e.g. salinity and confinement) and biotic components in TW ecosystems. Finally, the present study contributes to the scientific knowledge that is needed to successfully assess or predict the impacts of salinization on the biota of TW ecosystems, for example, as a result of sea level rise. Comprehensive knowledge on the structure and function of TW ecosystems at the appropriate ecological scales is essential for the development of plans for their management and conservation, especially under the threat that global climate change poses for the coastal zone.

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REFERENCES

- Ali A, Majori G. 1984. A short-term investigation of chironomid midge (Diptera: Chironomidae) problem in saltwater lakes of Orbetello, Grosseto, Italy. *Mosquito News* **44**: 17–21.
- Ali A, Majori G, Ceretti G, Dandera F, Scattoini M, Ferrarese U. 1985. A chironomid (Diptera: Chironomidae) midge population study and laboratory evaluation of larvicides against midges inhabiting the lagoon of Venice, Italy. *Journal of the American Mosquito Control Association* **1**: 63–68.
- Augier H. 1982. Inventory and classification of marine benthic biocenoses of the Mediterranean. Council of Europe, Publications Section, Strasbourg.
- Barnes RSK. 1980. *Coastal Lagoons. The Natural History of a Neglected Habitat*. Cambridge Studies in Modern Biology 1. Cambridge University Press: Cambridge.
- Barnes RSK. 1994a. *The Brackish-water Fauna of Northwestern Europe*. Cambridge University Press: Cambridge.
- Barnes RSK. 1994b. A critical appraisal of the application of Guelorget and Perthuisot's concepts of the paralic ecosystem and confinement to macrotidal Europe. *Estuarine, Coastal and Shelf Science* **38**: 41–48.
- Barnes RSK. 2005. Interspecific competition and rarity in mudsnails: feeding interactions between and within *Hydrobia acuta neglecta* and sympatric *Hydrobia* species. *Aquatic Conservation: Marine and Freshwater Ecosystems* **15**: 485–493.
- Battaglia B. 1959. Final resolution of the symposium on the classification of brackish waters. *Archives Oceanography Limnology* **11**(suppl.): 243–248.
- Bray RJ, Curtis JT. 1957. An ordination of the upland forest communities of southern Wisconsin. *Ecological Monographs* **27**: 325–349.
- Britton RH. 1985. Life cycle and production of *Hydrobia acuta* Drap. (Gastropoda: Prosobranchia) in a hypersaline coastal lagoon. *Hydrobiologia* **122**: 219–230.
- Britton RH, Johnson AR. 1987. An ecological account of a Mediterranean salina: the Salin de Giraud, Camargue (S. France). *Biological Conservation* **42**: 185–230.
- Ceretti GU, Ferrarese A, Francescon A, Barbaro A. 1987. Chironomids (Diptera: Chironomidae) in the natural diet of gilthead seabream (*Sparus aurata* L.) farmed in the Venice lagoon. *Entomologica Scandinavica Supplement* **29**: 289–292.
- Clarke KR. 1993. Non-parametric multivariate analyses of changes in community structure. *Australian Journal of Ecology* **18**: 117–143.
- Clarke KR, Gorley RN. 2001. *PRIMER v.5: User Manual/Tutorial*. PRIMER-E: Plymouth.
- Clarke KR, Green RH. 1988. Statistical design and analysis for a 'biological effects' study. *Marine Ecology Progress Series* **46**: 213–226.
- Clarke KR, Warwick RM. 2001a. A further biodiversity index applicable to species lists: variation in taxonomic distinctness. *Marine Ecology Progress Series* **216**: 265–278.
- Clarke KR, Warwick RM. 2001b. *Change in Marine Communities: An Approach to Statistical Analysis and Interpretation* (2nd edn). PRIMER-E: Plymouth.

- Clarke KR, Warwick RM, Brown BE. 1993. An index showing breakdown of seriation, related to disturbance, in a coral-reef assemblage. *Marine Ecology Progress Series* **102**: 153–160.
- Comín FA, Menéndez M, Herrera JA. 2004. Spatial and temporal scales for monitoring coastal aquatic ecosystems. *Aquatic Conservation: Marine and Freshwater Ecosystems* **14**: 5–17.
- Dafis S, Papastergiadou E, Georgiou K, Babalonas D, Georgiadis T, Papageorgiou M, Lazaridou T, Tsiaousi V. 1997. 92/43/EEC Directive: *The Habitats in Greece: Natura 2000 Network*. Contract number B4-3200/84/756. General Directorate XI, European Commission-Goulandri Museum of Natural History-Hellenic Center for Habitats and Wetlands.
- Dauer DM. 1993. Biological criteria, environmental health and estuarine community structure. *Marine Pollution Bulletin* **26**: 249–257.
- Davis JS. 2000. Structure, function and management of the biological system for seasonal solar saltworks. *Global Nest* **2**: 217–226.
- De Biasi AM, Benedetti-Cecchi L, Pacciardi L, Maggi E, Vaselli S, Bertocci I. 2003. Spatial heterogeneity in the distribution of plants and benthic invertebrates in the lagoon of Orbetello (Italy). *Oceanologica Acta* **26**: 39–46.
- Drake P, Arias AM. 1995. Distribution and production of *Chironomus salinarius* (Diptera: Chironomidae) in a shallow coastal lagoon in the Bay of Cádiz. *Hydrobiologia* **299**: 195–206.
- EC. 2000. Council directive for a legislative frame and action for the water policy, 2000/60/EC. Official Journal of the E.C., 22/12/2000, L 327.
- EC. 2003. Common Implementation Strategy for the Water Framework Directive (2000/60/EC). Guidance Document No 5. Transitional and Coastal Waters—Typology, Reference Conditions and Classification Systems. Office for Official Publications of the European Communities, Luxembourg.
- Evangelopoulos A, Koutsoubas D. 2008. Seasonal community structure of the molluscan macrofauna at the marine-lagoonal environmental transition at Kalloni solar saltworks (Lesvos Island, NE Aegean Sea, Greece). *Journal of Natural History* **42**(5): 597–618.
- Evangelopoulos A, Spyarakos E, Karydis M, Koutsoubas D. 2006. The biological system of Kalloni Saltworks (Lesvos Island, NE Aegean Sea, Hellas): variations of phytoplankton and macrobenthic invertebrate community structure along the salinity gradient in the low salinity ponds. In *Proceedings of the 1st International Conference on the Ecological Importance of Solar Saltworks (CEISSA 06)*, Santorini Island, Greece, 20–22 October 2006; 85–94.
- Fenchel T. 1975. Factors determining the distribution patterns of mud snails (Hydrobiidae). *Oecologia* **20**: 1–17.
- Ferrarese U, Ceretti G. 1986. Il problema dei chironomidi (Diptera, Chironomidae) in laguna di Venezia. *Annali dell'Istituto Superiore di Sanità* **22**: 129–136.
- Gamito GJ. 1989. The benthic macrofauna of some water reservoirs of salt pans from Ria Formosa (Portugal). *Scientia Marina* **53**: 639–644.
- Guelorget O, Perthuisot P. 1992. Paralic ecosystems. Biological organization and functioning. *Vie Millieu* **42**: 215–251.
- Guelorget O, Frisoni GF, Perthuisot P. 1983. La zonation biologique des milieux lagunaires: définition d'une échelle de confinement dans le domaine paralytique méditerranéen. *Journal de Recherche Océanographique* **3**: 15–35.
- Jackson ML. 1958. *Soil Chemical Analysis*. Prentice-Hall: New York.
- Korovessis NA, Lekkas TD. 2000. Solar saltworks production process evolution-wetland function. In *Saltworks: Preserving Saline Coastal Ecosystems. 6th Conference on Environmental Science and Technology*, Pythagorion, Samos, 1 September 1999, Korovessis NA, Lekkas TD (eds). GlobalNEST: Athens, 11–30.
- Koutsoubas D. 1992. Contribution in the study of the gastropod molluscs of the continental shelf of the North Aegean Sea. PhD thesis. Aristotle University of Thessaloniki, School of Applied Sciences, Department of Biology, Appendix No 43, Thessaloniki.
- Koutsoubas D, Dounas C, Arvanitidis C, Êornilios S, Petihakis G, Triantafyllou G, Eleftheriou A. 2000. Macrobenthic community structure and disturbance assessment in Gialova Lagoon, Ionian Sea. *ICES Journal of Marine Science* **57**: 1472–1480.
- Labourg JP. 1979. Structure et évolution de la macrofaune invertébrée d'un écosystème lagunaire aménagé (réservoirs à poissons de Certes). *CNEXO Actions Collection* **7**: 591–614.
- Lepš J, Šmilauer P. 2003. *Multivariate Analysis of Ecological Data Using CANOCO*. Cambridge University Press: Cambridge.
- Liddicoat MI, Tibbits S, Butler EI. 1975. The determination of ammonia in seawater. *Limnology and Oceanography* **20**: 131–132.
- Liddicoat MI, Tibbits S, Butler EI. 1976. The determination of ammonia in natural waters. *Water Research* **10**: 567–568.
- Little C. 2000. *The Biology of Soft Shores and Estuaries. Biology of Habitats*. Oxford University Press: Oxford.
- Loring DH, Rantala RTT. 1992. Manual for the geochemical analyses of marine sediments and suspended particle matter. *Earth Sciences Review* **32**: 235–283.
- McLusky DS, Elliott M. 2004. *The Estuarine Ecosystem. Ecology, Threats and Management* (3rd edn). Oxford University Press: Oxford.
- Nicolaidou A, Reizopoulou S, Koutsoubas D, Orfanidis S, Kevrekidis T. 2005a. Coastal lagoons, in *State of the Hellenic Marine Environment*, Papathanasiou E, Zenetos A (eds). HCMR Publications: Athens.
- Nicolaidou A, Reizopoulou S, Koutsoubas D, Orfanidis S, Kevrekidis T. 2005b. Biological components of Greek Lagoonal Ecosystems: An overview. *Mediterranean Marine Science*, **6**(2): 31–50.

- Pavlova P, Markova K, Tanev S, Davis JS. 1998. Observations on a solar saltworks near Burgas, Bulgaria. *International Journal of Salt Lake Research* **7**: 357–368.
- Pérès JM. 1967. The Mediterranean benthos. *Oceanography and Marine Biology. An Annual Review* **5**: 449–533.
- Pielou EC. 1969. *An Introduction to Mathematical Ecology*. Wiley: New York.
- Reizopoulou S, Nicolaidou A. 2004. Benthic diversity of coastal brackish-water lagoons in western Greece. *Aquatic Conservation: Marine and Freshwater Ecosystems* **14**: 93–102.
- Resh VH, Rosenberg DM. 1988. *The Ecology of Aquatic Insects*. Praeger Publishers Inc. New York.
- Rossi F, Castelli A, Lardicci C. 2006. Distribution of macrobenthic assemblages along a marine gradient in Mediterranean eutrophic coastal lagoons. *Marine Ecology* **27**: 66–75.
- Sadoul N, Walmsley JG, Charpentier B. 1998. *Salinas and Nature Conservation. Conservation of Mediterranean Wetlands 9*. Tour du Valat: Arles.
- Sconfiatti R, Marchini A, Occhipinti Ambrogi A, Sacchi CF. 2003. The sessile benthic community patterns on hard bottoms in response to continental vs. marine influence in northern Adriatic lagoons. *Oceanologica Acta* **26**: 47–56.
- Shannon CE, Weaver N. 1949. *The Mathematical Theory of Communication*. University of Illinois Press: Urbana.
- Steers JA. 1960. *Scot Head Island*. W. Heffer and Sons: Cambridge.
- Strickland JDH, Parsons R. 1968. A practical handbook of seawater analysis. *Fisheries Research Board of Canada Bulletin* **167**: 1–311.
- ter Braak CJF. 1986. Canonical correspondence analysis: a new eigenvector technique for multivariate direct gradient analysis. *Ecology* **67**: 1167–1179.
- ter Braak CJF, Šmilauer P. 2002. *CANOCO Reference Manual and CanoDraw for Windows User's Guide: Software for Canonical Community Ordination (version 4.5)*. Microcomputer Power: Ithaca, New York.
- Vareschi E. 1987. Saline lake ecosystems. In *Potentials and Limitations of Ecosystem Analysis*, Schultze E-D, Zwölfer H (eds). Springer Verlag: Berlin.
- Vieira MN, Amat F. 1997. The invertebrate benthic community of two solar salt ponds in Aveiro, Portugal. *International Journal of Salt Lake Research* **5**: 281–286.
- Vieira MN, Galhano MH. 1985. First data about the ecology of the Aveiro saltponds (Portugal). Publicações do Instituto de Zoologia 'Dr. Augusto Nobre' 109. Faculdade de Ciências do Porto, Porto.
- Walkey Á. 1947. A critical examination of a rapid method for determining organic carbon in soils: Effect of variations in digestion conditions and of inorganic soil constituents. *Soil Science* **63**: 251–263.
- Walmsley JG. 2000. The ecological importance of Mediterranean Salinas. In *Saltworks: Preserving Saline Coastal Ecosystems. 6th Conference on Environmental Science and Technology*, Pythagorion, Samos, 1 September 1999, Korovessis NA, Lekkas TD (eds). GlobalNEST: Athens; 81–95.
- Warwick RM, Clarke KR. 1995. New 'biodiversity' measures reveal a decrease in taxonomic distinctness with increasing stress. *Marine Ecology Progress Series* **129**: 301–305.
- Warwick RM, Clarke KR. 2001. A further biodiversity index applicable to species lists: variation in taxonomic distinctness. *Marine Ecology Progress Series* **216**: 265–278.
- Williams WD, Boulton AJ, Taaffe RG. 1990. Salinity as a determinant of salt lake fauna: a question of scale. *Hydrobiologia* **197**: 257–266.
- Zeno C. 2006. The ecological importance of the Margherita Di Savoia saltworks. *Proceedings of the 1st International Conference on the Ecological Importance of Solar Saltworks (CEISSA 06)*, Santorini Island, Greece, 20–22 October 2006; 15–24.