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.

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EXTENDED ABSTRACT

Phytoplankton and macrobenthic invertebrates are two important components of the biological system of solar saltworks. The spatial variations of both phytoplankton and macrobenthic invertebrates descriptors (composition, abundance, biomass) have been studied at 6 sampling sites in the low salinity ponds of Kalloni Saltworks, Hellas, in November 2004. In phytoplankton samples, a total of 48 taxa of Bacillariophyceae, Dinophyceae, Eugleonophyceae, Cryptophyceae, Cyanophyceae, Haptophyceae and Dictyochophyceae have been identified. The photoautotrophic ciliate *Mesodinium rubrum* has also been recorded. Phytoplankton diversity was lower in the low salinity ponds in comparison to the adjacent marine area of Kalloni Gulf, whereas abundance and biomass were higher in the initial ponds in comparison with the marine area and declined downstream the pond sequence. Euglena acusformis and Mesodinium rubrum comprised the bulk of the phytoplankton biomass in the study area and their high abundances suggest an eutrophic status for the low salinity area of Kalloni Saltworks. In macrobenthic invertebrates samples, a total of 54 taxa of Mollusca, Polychaeta, Crustacea, Insecta and Nemertea have been identified. Community structure is characterized by the dominance of lagoonal species and the gradual dissapearence of the marine fauna downstream the pond sequence. Species number is gradually decreasing as water flows downstream, whereas abundance and biomass are higher in the lowest salinity ponds in comparison to both the adjacent marine area and the higher salinity ponds. Finer spatial scaling of the sampling scheme has revealed that the phytoplankton and macrobenthic invertebrates communities in the low salinity area of Kalloni Saltworks are characterized by a significant diversity and spatial heterogeneity. The composition, abundance and biomass spatial variations of both phytoplankton and macrobenthic invertebrates in the study area are in many aspects similar to what has been observed in other solar saltworks and many lagoonal ecosystems around the Mediterranean but several differences found are also being discussed.

Keywords: saltworks, phytoplankton, macrobenthic invertebrates, community structure, confinement

INTRODUCTION

Solar saltworks are man-made systems for the salt extraction from seawater by means of solar evaporation. They are also coastal aquatic ecosystems of considerable heterogeneity as they combine a full spectrum of environmental types along a strong salinity gradient, starting with the lagoonal environment of the initial few evaporation ponds and ending in the harsh, extremely hypersaline environment of the crystallizers. This physical and chemical diversity is reflected in the diverse flora and fauna that is adapted to and colonises each environmental type in the saltworks system (Davis 2000b). Moreover, the biological system of the solar saltworks, through its diversity and multiplicity of functions is essential for the production of good quality salt (Davis 2000a; Korovessis & Lekkas 2000). As a consequence, solar saltworks are considered to be habitats of great ecological importance and their proper management and conservation are imperative, all the more as coastal aquatic ecosystems are shrinking at a worldwide scale (Primack 2000). Their conservation and management as valuable ecosystems are considered to be compatible and bound together with their primary use, the production of high quality salt (Davis 2000b; Korovessis 2000) as well as other side uses, i.e. aquaculture and biotechnology applications (Dolapsakis et al. 2005). Finally, solar saltworks are also recognized to be unique cultural landscapes that deserve conservation and also constitute destinations for eco/agrotourism (Petanidou 2000).

The importance of the biological system of solar saltworks has been appraised mainly in (1) the importance of planktonic organisms and benthic microorganisms for the salt production (e.g. Davis 2000a), (2) the rich waterfowl avifauna they usually support (e.g. Britton & Johnson 1987) and (3) the planktonic organisms they host that find applications in aquaculture and biotechnology (e.g. Dolapsakis *et al.* 2005). Macrobenthic invertebrates is a less appraised but equally important biotic component of a saltworks ecosystem as (1) they are a fundamental food source for the waterfowl, (2) they interact with the other biota through trophic relationships and (3) they have a considerable impact on the sediment properties e.g. by bioturbation. In biological assessment and monitoring of all aquatic ecosystems, phytoplankton and macrobenthic invertebrates are considered to be two fundamental quality elements of their ecological status (Gibson *et al.* 2000; EC 2000, 2003). Consequently, the assessment and monitoring of the status of macrobenthic invertebrates and phytoplankton should be applied in saltworks ecosystems as well.

Several studies have given information on the composition and ecology of phytoplankton in solar saltworks located in various countries (e.g. Davis 1990; Pedròs-Alió *et al.* 2000; Ayadi *et al.* 2004; Dolapsakis *et al.* 2005; Segal *et al.* 2006). However, the complete salinity range was usually examined and spatial resolution of information was consequently rather low. Recent publications that consider the macrobenthic invertebrates communities of the saltworks are comparatively very few, with the information given being in most cases only preliminary results (e.g. Vieira & Galhano 1985; Britton & Johnson 1987; Vieira & Amat 1997; Pavlova *et al.* 1998;). The phytoplankton flora of greek solar saltworks is poorly known whereas their macrobenthic invertebrates fauna has not been documented yet.

Solar saltworks have been considered by Guelorget & Perthuisot 1992 to be an artificial type of coastal aquatic or "paralic" ecosystems, their natural counterparts being coastal lagoons and estuaries. In "paralic" ecosystems, biological populations are characterised by the presence of strictly "paralic" (=lagoonal) species and a common qualitative and quantitative zonal species distribution that is relatively stable despite of fluctuations in the environment and is independent of the salinity gradient.

The present study consists a contribution to the better understanding of the biological system structure and functioning in Kalloni Saltworks (Lesvos Island, NE Aegean Sea, Hellas). This study focuses on the low salinity part of the saltworks (salinity range: seawater -70 %) that has affinities with the ecosystem of natural coastal lagoons as, in many saltworks, it was constructed in the place of preexisting lagoons with minimal

intervention (Britton & Johnson 1987; Pavlova *et al.* 1998). The specific aims of this study are (1) to provide comprehensive information on the spatial variations of phytoplankton and macrobenthic invertebrates descriptors (composition, abundance and biomass) along the salinity gradient at the low salinity ponds of Kalloni Saltworks at a finer scale than in studies of other saltworks, (2) to compare between the low salinity ponds of Kalloni Saltworks and the low salinity areas of other solar saltworks and (3) to assess whether the common zonal organization of flora and fauna observed in many coastal lagoons in the Mediterranean region also holds true in the study area.

MATERIALS AND METHODS

The study area (Figure 1) is located at Kalloni Saltworks (Lesvos Island, NE Aegean Sea, Hellas). Kalloni Saltworks is placed at the northeastern coast of Kalloni Gulf. Along the coastline of Kalloni Gulf several small wetlands exist, the largest of them being Kalloni and Polychnitos saltworks. Kalloni Gulf, its wetlands and its coastal zone constitute a proposed "Site of Community Interest" in the NATURA 2000 network for the conservation of the diversity of its habitats as well as for the preservation of the nesting, wintering and resting grounds it provides for its rich avifauna (Dafis et al. 1997).

Water column samples (four replicates) for the phytoplankton analyses as well as sediment samples (five replicates) for the analyses of the macrobenthic invertebrates were collected at six sampling stations along the salinity gradient (45-72%) at the low salinity ponds of Kalloni Saltworks in November 2004 (Figure 1). The sediment samples ($0.03~\text{m}^2$) were collected by use of a box-corer type sampler and were subsequently sieved through a 0.5~mm mesh size net. The samples were then fixed with 4% formaldehyde and stained with Rose Bengal. The invertebrates were sorted out in the laboratory, identified, counted and weighted (wet biomass including the shells in the case of molluscs). Phytoplankton species were identified and counted in Lugol solution fixed samples according to the Utermöhl method (Utermöhl 1958). Phytoplankton biomass was measured indirectly as chlorophyll a concentration, by use of the fluorometric method proposed by Neveaux & Panouse (1987), after filtration onto glass-fibre filters ($0.7~\text{\mu m}$ pore size Whatman GF/F) and extraction in 90% acetone (Edler 1979).

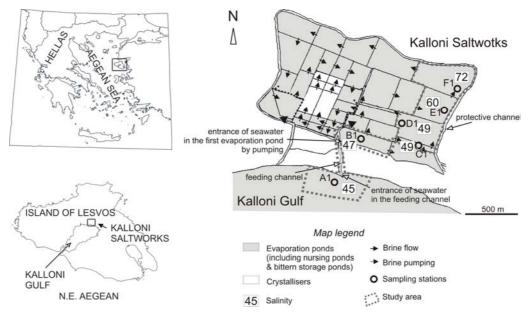


Figure 1. Map of the study area, indicating the salinity gradient.

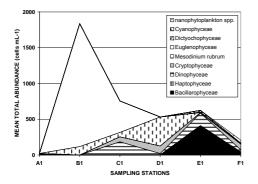
RESULTS

Phytoplankton

Phytoplankton in the Utermöhl samples were identified into a total of 48 taxa, belonging to 7 classes, i.e. Bacillariophyceae, Dinophyceae, Euglenophyceae, Cryptophyceae, Cyanophyceae, Haptophyceae, Dictyochophycea and a group of unidentified nanophytoplaktonic species. Most of the taxa identified in the study area belonged to Bacillariophyceae (23) and Dinophyceae (16). The photoautotrophic ciliate *Mesodinium rubrum* was also recorded in the study area.

The distribution of the phytoplankton classes as well as the abundant phytoplankton taxa (taxa that constituted collectively at least 75% of the total phytoplankton abundance) recorded at each station are presented in this study. Diatoms (Bacillariophyceae) were found at every station but were abundant primarily at the E1 station and secondly at the A1 and F1 stations. The most abundant diatom taxa were Thalassionema spp. at the A1 station and Cylindrotheca closterium at the E1 and F1 stations. Benthic pennate diatoms (order Bacillariales) were moderately abundant in the water column at the A1 station only. Less abundant diatom species were recorded mainly at the A1 station (e.g. Bacillaria paxillifera, Lithodesmium undulatum, Grammatophora marina). Dinophyceae taxa were present at all sampling stations but were abundant mainly at the C1 station (Oxyrrhis marina and Gymnodinium sanguineum) and E1 station (thecate dinophyceae spp.). Less abundant dinophycean species were recorded mainly at the A1 station (e.g Prorocentrum sigmoides, P. arcuatum, Ceratium kofoidii and Protoperidinium compressum) whereas Gymnodinium sanguineum was recorded at all stations except A1. Euglena acusformis (class Euglenophyceae) was very abundant at the B1 and C1 stations and was also present at the E1 and F1 stations. Cryptophycean sp. 1 was recorded at all sampling stations and was abundant at the F1 station. A second cryptophycean species (cryptophycean sp. 2) was also abundant at the F1 station. The abundance of cyanophycean taxa was higher at the E1 (Oscillatoriaceae spp.) and the F1 stations (Spirulina sp., Nostocaceae spp.). Unidentified nanophytoplanktonic species were abundant at the A1 and F1 stations. Taxa belonging to Haptophyceae (Syracosphaera pulchra) and Dictyochophyceae (Octactis octonaria var. pulchra) were recorded only at the A1 station in low abundances. Lastly, the photoautotrophic ciliate Mesodinium rubrum was recorded at all stations except A1 and E1 and was abundant at the D1 station.

Mean total phytoplankton abundance and biomass (measured as chl *a* concentration) maxima (1718 cells mL⁻¹ and 6.3 mg m⁻³ respectively) were recorded at the B1 station due to a *Euglena acusformis* bloom, whereas minima (27 cells mL⁻¹ and 0.7 mg m⁻³ respectively) were recorded at the A1 station (Figure 2). More phytoplankton taxa were identified at the marine area (i.e. A1 station) than at any other station in the saltworks ponds.



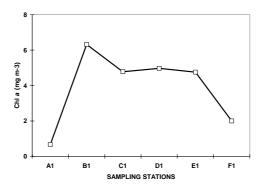


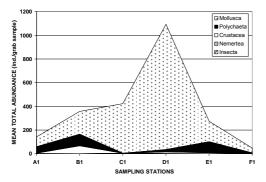
Figure 2. Spatial variations of (a) phytoplankton mean total abundance, indicating the contribution of each phytoplankton group and (b) water column chlorophyll *a* (Chl *a*) concentration in the study area.

Macrobenthic invertebrates

The macrobenthic invertebrates were identified into a total of 54 taxa, belonging to 5 groups, i.e. Mollusca, Polychaeta, Crustaceae, Insecta and Nemertea. Most of the taxa identified in the study area belonged to Mollusca (23), followed by Polychaeta (16) and Crustacea (13). Insecta and Nemertea were represented by a single species each.

The distribution of the macrobenthic invertebrates groups as well as the abundant macrobenthic invertebrates taxa (taxa that constituted collectively at least 75% of the total macrobenthic invertebrates abundance) recorded at each station are presented in this study. Molluscs, polychaetes and crustaceans were found at every station. Molluscs were more abundant at the D1 station and less so at the B1, C1 and E1 stations. The most abundant molluscan taxa were the gastropods *Hydrobia acuta* at the B1 – F1 stations and *Bittium reticulatum* at the A1 station. Less abundant molluscan species at the A1 station were the gastropods *Rissoa ventricosa* and *R. guerinii* as well as the bivalve *Tellina donacina*. The most abundant species of polychaetes were *Malacoceros fuliginosus* at the A1 and B1 stations and *Capitella capitata* at the A1, B1 and E1 stations. The amphipod *Microdeutopus gryllotalpa* was the only abundant crustacean species at the B1 station. Insecta were represented by the larvae of a single Chironomidae species at the C1, D1 and E1 stations whereas the Nemertea species was found only at the A1 station.

Mean total macrobenthic invertebrates abundance and biomass gradually increased downstream the pond sequence, starting from relatively low values at the A1 station (142 ind. and 7 g per grab sample respectively) and reaching their maxima (1094 ind. and 36 g per grab sample respectively) at the D1 station, mainly due to the high abundance of *Hydrobia acuta* and the high biomass of *Cerastoderma glaucum* at this station. After the D1 station, both mean total abundance and biomass gradually declined to their minima at the F1 station (47 ind. and 1 g per grab sample respectively). Both mean total abundance and biomass were dominated by mollusca at every station (Figure 3). Total number of macrobenthic invertebrates species gradually declined along the transect, from a maximum of 38 species at the A1 station to a minimum of 4 species at the F1 station.



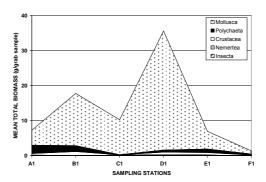


Figure 3. Spatial variations of (a) macrobenthic invertebrates mean total abundance and (b) biomass in the study area, indicating the contribution of each macrobenthic invertebrates group.

DISCUSSION

Phytoplankton

In many solar saltworks, the low salinity ponds are characterized by clear waters, as microalgae are present in low quantities (Davis 1978, 1990; Javor 1983; Britton & Johnson 1987), a fact that has been attributed to nutrient limitation (Javor 1983). The phytoplankton community has been reported to be composed mainly of benthic microalgae, i.e. benthic and epiphytic diatoms, dinoflagellates and cyanobacteria (Javor 1983; Davis 1990; Segal *et al.* 2006), although planktonic species such as various nanoflagellates (chlorophyceae, prasinophyceae, chrysophyceae and cryptophyceae) have also been reported (Dolapsakis *et al.* 2005; Segal *et al.* 2006). In Kalloni Saltworks,

planktonic diatom, dinophycean, cryptophycean as well as nanoflagellate species were found to be abundant in the low salinity ponds. On the other hand, benthic psammic, pelic and epiphytic diatoms as well as filamentous cyanobacteria were often observed in the water column samples but were not abundant.

The most important phytoplankton species in the study area at the time of sampling was the euglenophyceaen *Euglena acusformis*. This species was present in all ponds and constituted the bulk of phytoplankton biomass in the two initial ponds, especially in the first, where it formed a virtually monospecific algal bloom and colored the waters of the pond. High abundance of *Euglena acusformis* in the first pond of Kalloni Saltworks was also observed in the past in the study area (December 2003 - Evagelopoulos *et al.* 2005). As euglenoids are known to be favoured by organic enrichment (Reynolds 2006), the recurrent *Euglena acusformis* blooms may consist an indicator of eutrophication in the initial low salinity ponds of the saltworks in winter. This finding contrasts with the oligotrophic character that is generally expected in the low salinity ponds of a solar saltworks (Davis 1978, 1990; Javor 1983; Britton & Johnson 1987).

Mesodinium rubrum is a ubiquitous photosynthetic plankton ciliate that forms conspicuous red tides in estuaries and coastal waters (Williams 1996; Crawford *et al.* 1997; Hansen & Fenchel 2006). It was also recorded in the low salinity ponds at Kalloni Saltworks, where its abundance was high and its contribution to the total phytoplankton primary production is speculated to be significant as this species has an extremely high rate of primary production (Crawford *et al.* 1997). As Mesodinium rubrum blooms have been linked to coastal eutrophication (Williams 1996), the presence and high abundance of this ciliate species in the low salinity ponds of Kalloni Saltworks may consist another indicator of eutrophication during the time of sampling.

In the published studies of the spatial variations of phytoplankton flora in solar saltworks, the complete salinity range was usually examined (e.g. Davis 1978; Javor 1983; Britton & Johnson 1987; Dolapsakis *et al.* 2005; Segal *et al.* 2006) and as a consequence, the spatial resolution was rather low. The finer spatial scale of our sampling scheme enabled us to observe that although many of the abundant species were common to all or most of the ponds, different species were dominant in each individual pond. Moreover, the abundant species did not belong solely to diatoms, as was reported from other solar saltworks (e.g. Britton & Johnson 1987), but in several other classes as well. Phytoplankton spatial heterogeneity seems thus to be significant in the low salinity ponds at Kalloni Saltworks, at least in terms of the abundant species.

Water column chl *a* concentration levels in the low salinity ponds of Kalloni Saltworks were comparable to those reported from the low salinity areas of other saltworks (Javor 1983; Britton & Johnson 1987; Dolapsakis *et al.* 2005; Segal *et al.* 2006). The spatial variation of water column chl *a* concentration in the low salinity ponds of Kalloni Gulf, i.e. sharp increase in the first pond and then decrease along the pond sequence, was also observed in other saltworks (Segal *et al.* 2006).

The phytoplankton community structure was found to vary considerably between the low salinity ponds of the saltworks and the adjacent marine area: At the Kalloni Gulf station, the phytoplankton community was more diverse and consisted mainly by species that are typical of coastal marine phytoplankton, whereas in the low salinity pond stations, the phytoplankton assemblages were less species rich, dominance was stronger and the abundant species either did not occur at the marine or were present there in low abundances. On the other hand, total phytoplankton abundance and biomass were much higher in the pond stations. Most published studies do not present a comparative study of the phytoplankton community structure between the low salinity ponds of a solar saltworks and the adjacent nearshore marine area but similar observations to ours have been reported from natural coastal lagoons, a habitat type that has common features with solar saltworks (Guelorget & Perthuisot 1992; Segal *et al.* 2006): In natural coastal lagoons, as in the low salinity ponds of Kalloni Saltworks, phytoplankton diversity is reported to be lower than in neritic phytoplankton and is frequently enriched by benthic

diatoms (Nikolaidou *et al.* 2005). At the innermost parts of semi-enclosed lagoons, dinoflagellates have been reported to be the most important phytoplankton group (Dounas & Koutsoubas 1996), whereas in the most enclosed lagoons, cryptophytes have been found to be abundant (Kormas *et al.* 2001). Likewise, dinoflagellates and cryptophytes were also abundant in the low salinity ponds of Kalloni Saltworks.

According to Guelorget & Perthuisot (1992), phytoplankton species richness in "paralic" ecosystems decreases with distance from the sea, whereas biomass peaks are due to dinophyceaen and nanophytoplanktonic species and occur in areas away from the direct influence of the adjacent marine area. In the study area, the spatial variation of species richness is similar but the biomass spatial variation is different, as the peak is in the first pond.

Macrobenthic invertebrates

The spatial pattern of macrobenthic invertebrates composition, abundance and biomass in the low salinity ponds of Kalloni Saltworks is similar to what has been described in other solar saltworks (e.g. Britton & Johnson 1987) as well as natural coastal lagoons (e.g. Nikolaidou et al. 1988; Koutsoubas et al. 2000a, 2000b; Reizopoulou & Nikolaidou 2004): Community structure is characterized by the dominance of "paralic" (=lagoonal) species (e.g. Hydrobia acuta, Abra segmentum, Cerastoderma glaucum) and the gradual dissapearence of the marine fauna downstream the pond sequence. Species number is gradually decreasing as water flows downstream, whereas abundance and biomass are higher in the lowest salinity ponds in comparison to both the adjacent marine area and the higher salinity ponds. At the higher salinity ponds, that correspond to the "evaporitic pole" in natural costal lagoons, salinity levels and ionic composition of the water are rapidly becoming prohibitive for the existence of macrobenthic invertebrates (Britton & Johnson 1987; Guelorget & Perthuisot 1992). However, in coastal lagoons the gradual increase in the abundance of macrobenthic invertebrates is expected to coexist with a progressive decrease in biomass because there is a decrease in size of the organisms ("lagoonal dwarfism") (Guelorget & Perthuisot 1992). In Kalloni Saltworks this has not been observed, as the spatial variations of biomass reflect those of abundance and the higher values of biomass recorded in the lowest salinity ponds were due to the large body size of Cerastoderma glaucum and Abra segmentum individuals.

In the scientific literature, many environmental variables have been correlated with the spatial and temporal distribution patterns of the macrobenthic invertebrates in coastal aquatic ecosystems. These are either physical variables, like temperature and salinity (Barnes 1980) or factors associated with food supply (Gray 1981). According to Guelorget & Perthuisot (1992), the factor responsible for the observed zonation of the macrobenthic invertebrates fauna, as well as the abundance and biomass distribution patterns, is not simply salinity but a composite factor termed "confinement". "Confinement" sensu Guelorget & Perthuisot (1992) represents the time of renewal of elements of marine origin at any given point at the ecosystem and thus is stronger where the distance from the link of communication with the sea is greater. "Confinement" has been considered to be expressed in lagoonal ecosystems by a variety of environmental variables and, depending on season, some of them may become more critical than others in controlling the distribution of biota in the ecosystem (Koutsoubas et al. 2000). However, Britton & Johnson (1987) assumed that, in the low salinity ponds of a saltworks, it is the wide daily and annual fluctuation in water temperature and not confinement the factor which differentiates them from the sea and leads to the impoverishment of the fauna. In the study area, there is a zonal organization of macrobenthic invertebrates compatible to that described by Guelorget & Perthuisot (1992) and other authors to exist in "paralic" ecosystems and the sequence of zones of confinement II (Kalloni Gulf station) to VI (last pond downstream the pond sequence) has been recognised. Confinement may thus be considered to be a major factor controlling the macrobenthic fauna distribution in the study area.

Synthesis & conclusions

Conclusively, the composition, abundance and biomass spatial variations of both phytoplankton and macrobenthic invertebrates along the salinity/"confinement" gradient in the study area are in many aspects similar to what has been observed in other solar saltworks and many lagoonal ecosystems in the Mediterranean, but several differences have also been observed. The finer spatial scale of the sampling scheme has revealed that the phytoplankton and macrobenthic invertebrates communities in the low salinity area of Kalloni Saltworks are characterized by a significant diversity and spatial heterogeneity. The *Euglena acusformis* and *Mesodinium rubrum* high abundances recorded in the study area suggest an eutrophic status for the low salinity area of Kalloni Saltworks.

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