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Abundance and growth response of microalgae at Megalon Embolon solar saltworks in northern Greece: An aquaculture prospect

Nicolas P. Dolapsakis[1,*], Triantafyllos Tafas[1], Theodore J. Abatzopoulos[2], Stephanie Ziller[1] and Athena Economou-Amilli[1]
[1]*Faculty of Biology, Department of Ecology and Systematics, University of Athens, Panepistimiopolis, Athens, Greece;* [2]*Faculty of Biology, Department of Genetics, Development & Molecular Biology, Aristotle University of Thessaloniki, Greece*

*Author for correspondence: e-mail: ndol@biol.uoa.gr; phone: +30-210-727-4042/4338; fax: +30-210-727-4885

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Abstract

There is continuous interest in many countries in maintaining and manipulating the rich ecological value of hypersaline ecosystems for aquaculture. The Megalon Embolon solar saltworks (northern Greece) were studied in sites of increasing salinity of 60–144 ppt to evaluate *Dunaliella salina* abundance and microalgal composition, in relation to physical and chemical parameters. Cluster and ordination analyses were performed based on the biotic and abiotic data matrices. Using fresh aliquots from 60 and 140 ppt salinity waters, phytoplankton performance was appraised with flask cultures in the laboratory by varying the inorganic PO_4-P concentration at 23 °C and 30 °C. At the saltworks, among the most abundant microalgae identified were species of the genera *Dunaliella*, *Chlamydomonas*, *Amphora*, *Navicula*, and *Nitzschia*. *Dunaliella salina* populations were predominant comprising 5–22% of the total microalgal assemblages during spring, but only 0.3–1.0% during the summer, when grazing by *Artemia parthenogenetica* and *Fabrea salina* was intense. *D. salina* cell density in April–July was in the range of 0.4–12.5 $\times$ 10^6 L^{-1} with typical densities of 1.5–4.5 $\times$ 10^6 L^{-1}. Overall, microalgal densities were high in salinities of $\geq$100 ppt when inorganic-P concentrations were $\geq$0.20 mg L^{-1} within saltworks waters. Multivariate analysis of species abundance showed that algal growth responses were primarily related to variation in salinity and inorganic-P concentrations, but also to NO_3-N concentration. In the laboratory, experiments indicated effective fertilization and denser microalgal growth under high inorganic PO_4-P applications (4.0 and 8.0 mg L^{-1}) at 60 ppt salinity and 23 °C. The lower PO_4-P applications (0.6–2.0 mg L^{-1}) were more effective at 60 ppt salinity and 30 °C. At 140 ppt salinity, microalgal growth response was less obvious at any of the corresponding phosphorus concentrations or temperatures. In both salinity experiments, *Dunaliella salina* bloomed easily and was predominant among the microalgae. Our observations indicate that *Dunaliella salina* populations and the overall rich microalgal profile of the saltworks, along with their performance in laboratory mono–and mixed cultures hold promise for mass cultivation within the M. Embolon saltworks basins.

Introduction

Hypersaline waters span large areas worldwide, not only in salt production areas (solar saltworks, salterns or salinas) but also in natural lakes and lagoons, and in tidal ponds (Javor, 1989). The ecological and productivity value of coastal hypersaline ecosystems is significant worldwide and in the Mediterranean in particular (Korovessis & Lekkas, 2000; see also RAMSAR's and UNEP's databases). There is continuous interest in many countries in maintaining such ecosystems while manipulating them to a small extent, making economic use of their rich biota (Cheng, 1991; Marian & Roy, 2001). Coastal shallow ponds and basins, such as those found at solar saltworks, are favored due to the controlled water exchange, ease of management, and

their large and robust phytoplankton and *Artemia* populations (Vos & De la Rosa, 1980; Sorgeloos, 1987). Saltwork waters have in general very high amounts of micro- and macronutrients, a fact compounded by evaporation (Javor, 1989; Wen & Zhi-Hui, 1999) which helps support high algal densities.

The planktonic algae in hypersaline waters are of interest because of growth capabilities and cultivation potential. The production of microalgal biomass is valuable and irreplaceable as aquaculture feed due the variety of cell sizes and the nutritional characteristics (Coutteau, 1996; Borowitzka, 1997). Hypersaline microalgae have already formed the focus of past and future research for aquaculture applications (e.g. Goldman, 1979; Borowitzka, 1988; Benemann, 1990; Borowitzka, 1997). Such microflora also have possible species-specific genetic and biochemical character implications for future research (Oren, 2002). The high carotenoid concentrations found in hypersaline microalgae are an advantage for aquaculture since they are necessary for pigmentation, vitamin activation, antioxidation, growth and possibly even reproduction of species (Ong & Tee 1992; Pfander 1992; Britton, 1995; Liñán-Cabello et al., 2002). High carotenoid concentrations are due to the stress imposed on the microalgae by environmental factors (Litchfield & Oren, 2001).

Artemia is also a very valuable organism with about 40% of aquaculture's feed demand for early stages dependent on its availability, yet *Artemia* production is nearly entirely dependant on natural stocks that have a limited and nonincreasing annual turnover (Lavens & Sorgeloos, 2000; Sorgeloos et al., 2001). Rising demand has resulted in steadily increasing prices along with a decrease in quality and availability of this crustacean. Problems in cyst quality and supply of *Artemia* have been compounded by increasing land reclamation, pollution, drought and flood problems (Dolapsakis, 1997; Triantaphyllidis et al., 1998). The investigation of microalgae at saltworks and their potential use for phytoplankton culture to feed a second organism (e.g. *Artemia*) within the saltworks basins is, therefore, attractive. Algal cultivation at saltworks can also help achieve higher salinities that eventually assist higher production of salt (Javor, 1989; Davis, 2000). Therefore, aquaculture can be used either as a replacement of the salt-producing activities, or in combination (integration) by specific management of the saltworks activities to increase their efficiency and economic viability.

The availability of inorganic macronutrients, especially nitrogen and phosphorus, tends to restrict microalgal densities in natural waters, and microalgae assimilate inorganic forms for growth (Kaplan et al., 1986; Wetzel, 2001). Nitrate nitrogen (NO_3-N) is a readily available source of inorganic nitrogen, while it appears to perform very well for *Dunaliella* cultured from solar saltworks strains (Gibor, 1956). The concentration of inorganic phosphorus is more critical since its dissolution and availability is restricted in hypersaline waters, especially at lower water temperatures. This is partly due to Ca^{2+} and Mg^{2+} ions causing the formation of nearly insoluble phosphates at high salinity (Javor, 1989). Recent work has also shown that phosphorus may actually be more critical than nitrogen for primary productivity in seawater (Tyrrell, 1999; Sañudo-Wilhelmy et al., 2001). A high phosphorus content is, therefore, imperative in order to ensure high algal densities (Spectorova et al., 1982). As a result, seawater enrichment regimes using inorganic fertilization use a low N:P ratio of 1–5:1 with 1–10 mg-N L^{-1} and 0.3–3.3 mg-P L^{-1} applications (Davis, 1978; Tackaert & Sorgeloos, 1991; Baert et al.,1996).

The Megalon Embolon solar saltworks in northern Greece (Aggelohori, near Thessaloniki, 40:29:46N, 22:50:04E) are small at $\sim 350 \times 10^3$ m^2 (excluding the reservoir lake, see Figure 1). Their low requirements of land and water manipulation and small scale of operation could hold promise for the development of aquaculture activities. The partial use of the saltworks for aquaculture is possible without disturbing the local ecology and could contribute to the economic competitiveness of the salt production sector. The existing small and shallow basins allow significant diffusion of O_2 and CO_2 into and out of the water column and are

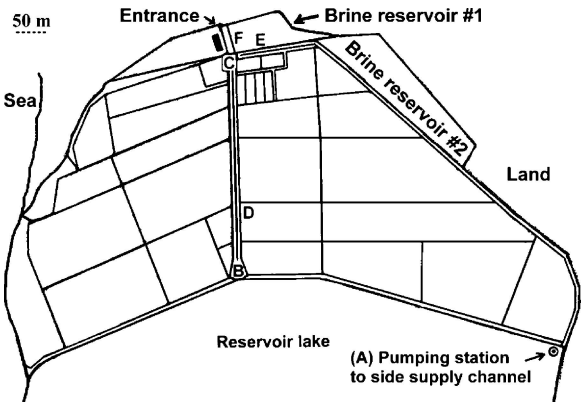

Figure 1. Areal view of the six sampling sites of the M. Embolon saltworks (April 2000). (A) Pumping outlet from the reservoir lake to side supply channel; (B) Central supply channel near the central gate of the reservoir lake; (C) Central supply channel near the enginehouse; (D) Salt pond; (E/F) Brine reservoir.

suitable for culturing mixed populations of *Dunaliella* with other phytoplankton as food for large local-based populations of parthenogenetic *Artemia*. An investigation of the physical, chemical and biological environment in relation to the microalgal community characteristics at various sites of the M. Embolon saltworks was conducted during spring and summer. An initial evaluation of enhancing production of planktonic microalgae was also carried out in the laboratory using inorganic fertilization. The objective was to gain information on the biotic and abiotic conditions existent at the saltworks and appraise the potential to enhance primary production, and especially *Dunaliella salina*, on site within the basins using information gained.

Materials and methods

The Megalon Embolon solar saltworks has a large reservoir lake which is filled with seawater during fall season, and its salinity allowed to increase until summer. Its waters are pumped to two large brine reservoirs ($\sim$30–35,000 m^3 combined capacity) and the rest of the saltwork basins from the pumping station A (see Figure 1 for details). The central and side channels are used for water manipulations. The salinity gradient is lower on the right half of the saltworks with salt production on the left half. The majority of the saltworks is filled with hypersaline water throughout the year. The basins are on average 20-cm deep, the channels 50-cm deep and the reservoirs 100-cm deep. The saltworks experience extensive climatic changes induced by heavy rainfall, gusting winds, and cloud cover throughout the year. During spring and early summer, the existing conditions are more representative of desired sampling conditions, as water manipulation for salt production is minimal, water temperatures are above 13 °C, and zooplankton grazing is minimal.

Sampling

Water samples were taken at midday from six stations of increasing salinity (see Figure 1 for details) of 60–144 ppt in mid-April of the year 2000 from the Megalon Embolon solar saltworks. Some comparative sampling was carried out in these and other stations during spring and summer of 2000 and 2001. One liter, acid-cleaned, plastic bottles were used to take two samples per sampling point, one for biological and one for chemical analysis. All the sampling was done near the land-water interface (approximately 0.75 m from the edge) and from the middle of the respective water column by hand, except in sites (B), (E) and (F), where a Ruttner sampler was used in the deeper water column. All samples were stored on ice. Salinity, pH, water temperature and depth were recorded on site using a portable conductivity meter (CO 150, HACH), a pH-meter (Sension 01, HACH), and a calibrated depth stick, respectively. For zooplankton, net tows and 1-L bottle samplings were carried out along the circumference of the ponds or during pond water outflow. Netted samples were immediately washed with freshwater and stored at -20 °C, while bottled samples were preserved with Lugol's solution and refrigerated for observation.

Algal cultures

Water samples containing live algae were introduced directly from the 1-L bottles into sterile cotton-plugged test tubes with Walne's growth medium (Walne, 1966; Laing, 1991) adjusted for salinity accordingly. The test-tube cultures were placed in the laboratory (at 23 °C, 16:8 h light:dark, illumination at 60–70 μmol m^{-2} s^{-1} with no aeration), and examined 5 days later for identification and isolation of algal taxa.

Algal enhancement experiments

Using 100-mL culture flasks, 50-mL volume aliquot of water, collected from sites of the lowest (60 ppt) and upper (140 ppt) salinity (A and E, respectively) and filtered with 55 μm Nitex-net, were enriched in the laboratory. Using 4 mg L^{-1} NO_3-N and increasing concentrations of inorganic PO_4-P (0.6, 1.0, 2.0, 4.0 and 8.0 mg L^{-1}). Trials were conducted under two temperature regimes of 23 ° and 30 °C, without stirring or aeration, at 60–70 μmol photon m^{-2} s^{-1} illumination in 16:8 h light:dark cycles for 10 days, in order to appraise the effectiveness of each recipe under different temperatures, water conditions and algal profiles. After 13 days of cultivation, algal cells were fixed with Lugol's solution and counted using a Neubauer improved haemacytometer.

Chlorophyll measurements and fixation of algal samples

An aliquot of the bottled samples was used to provide subsamples of 150–250 mL for chlorophyll filtration using Whatman (Kent, U.K.) GF/C filters. The remaining bottled algal samples were fixed with Lugol's solution. The foil envelopes containing the chlorophyll

half-folded filters were stored on ice packs for transportation and later stored at $-20\,^{\circ}$C. The chlorophyll and carotenoid content was measured using the method outlined in Parsons et al. (1984) and a Shimadzu (Kyoto, Japan) UV-120-02 spectrophotometer.

Diatom cleaning and preparation

Volume of 10-mL aloquots of Lugol-fixed samples from each sampling site were washed with distilled water and centrifuged at $300 \times$ g for 5 min. For diatom cleaning the method of Hasle and Fryxell (1970) was used.

Algal population analyses

Lugol-preserved aliquots of the bottled samples were diluted as necessary with hypersaline filtered water and counted in 25-mL Kolkwitz plankton counting chambers. Qualitative and quantitative analysis was done according to Utermöhl (1958) and Lund et al. (1958) using an inverted Zeiss optical microscope at $400\times$ magnification.

Chemical analyses

Analyses were done on-site at the saltworks. Large suspended solids and living matter were removed by two-stage-filtering aliquots from the bottled samples with Whatman GF/C filters and 0.2 μm Sartorius (Goettingen, Germany) membrane filters using a Nalgene (Rochester, NY) filter holder/receiver under vacuum; aliquots were stored on ice in acid-cleaned and rubber-stopped glass bottles for analyses within 3 h. Approximate chemical concentrations were measured using the DREL/2010 Portable Laboratory and the following standardized analytical methods (HACH, 1997; APHA, 1998) which account for hypersalinity interference: Total-P (organic + inorganic) converted to reactive orthophosphates using acid persulfate digestion method and sulfuric acid with heating, and inorganic-P (expressed as PO_4-P) converted to orthophosphates using only sulfuric acid and heating to hydrolyze sample to orthophosphate, followed by orthophosphate (reactive phosphorus) determination using ascorbic acid PhosVer® 3 powder pillows method for both total and inorganic methods; NO_3-N measured using the cadmium reduction method with powder pillows and chloride calibration; NH_4-N with the Nessler method using distillation and sample dilution with de-ionized water; Si using the heteropoly blue method; SO_4^{-2} using SulfaVer® 4 method using powder pillows and sample dilution with de-ionized water; total-Fe with the FerroVer® method using powder pillows; and total alkalinity with the phenolphthalein method using sulfuric acid and digital titration (expressed as mg L^{-1} $CaCO_3$). Inorganic N is here expressed as NO_3-N + NH_4–N. The ratio of inorganic-N to inorganic-P is here expressed by as NO_3-N + NH_4-N: PO_4-P.

Multivariate analysis

The microalgal data matrix analysis was performed using cluster analysis (Group Average Method) and ordination analysis (Non-metric Multidimensional Scaling, nMDS) (Field et al., 1982; Legendre & Legendre 1984; Pielou 1984; Clarke & Green 1988). Both analytical processes were based on a Bray-Curtis similarity index matrix (Bray & Curtis 1957) using data from Tables 1 and 2. Analyses were performed using the PRIMER multivariate analysis software package (Clarke 1993). Superimposition of eight environmental variables (pH, salinity, NH_4-N, NO_3-N, PO_4-P, Total-P, SiO_2-Si and SO_4-S) on the two-dimensional configurations of the microalgal MDS solutions was used to indicate possible variables that correlate with microalgal abundance (Field et al., 1982; Gray et al., 1988).

Results

Abiotic parameters

The recorded physical and chemical parameters for the sampling period of April 2000 are shown in Table 1. From early spring to late summer of 2000 and 2001, pH and total alkalinity levels were high at 7.8–8.4 and 140–250 units, respectively. The inorganic-N and -P concentrations during this period were found to be associated with salinity, high nutrient concentrations being found in high salinities. Inorganic-N and -P concentrations from April to June 2000 were found to be similar, but greatly increased by the end of July along with salinity and water temperature values at various sites. This was observed again by 2001 samplings which showed that in comparison to spring reading, summer concentrations of inorganic-P were more than 300% higher, inorganic-N was 40–50% higher, while salinities had increased by 40% and midday water temperatures by 85% indicating a concentration of nutrients due to evaporation.

Table 1. Physical, chemical and biological parameters at six sampling sites A–F (see details in Figure 1) at the saltworks in April 2000.

	A	B	C	D	E	F
Physical parameters						
Salinity (ppt)	60	94	101	140	140	144
pH	8.1	7.8	7.8	7.8	7.9	7.9
Temperature (°C)	14.8	13.0	14.5	14.7	14.8	14.7
Depth (m)	0.5–1.0	0.5	0.5	0.2	1.0	1.0
Chemical parameters (mg L^{-1})						
NO_3-N	0.9	0.9	0.8	2.0	1.9	1.9
NH_4-N	6.0	13.3	16.2	11.5	11.5	11.5
PO_4-P	0.07	0.16	0.09	0.24	0.20	0.21
Total-P	0.12	0.26	0.20	1.08	0.24	0.25
SiO_2-Si	1.096	0.081	0.069	0.127	0.101	0.106
SO_4^{2-}	150	125	170	100	125	125
Total-Fe	0.04	0.15	0.12	0.17	0.03	0.03
NO_3-N + NH_4-N: PO_4-P	98.6:1	88.7:1	156.7:1	56.2:1	56.7:1	56.7:1
Biological parameters						
Microalgae (cells 10^6 L^{-1})	249.2	13.3	7.1	15.8	19.8	19.0
Pigments (μg L^{-1})						
Chlorophyll-α	28.5	12.9	16.2	15.1	12.8	14.6
Chlorophyll-*b*	5.4	3.2	4.0	5.2	4.7	5.0
Chlorophyll-*c* ($c_1 + c_2$)	13.5	6.9	10.4	9.6	8.2	9.0
Phaeo-pigments	3.2	4.8	9.0	4.1	4.3	11.8
Total carotenoids	5.8	3.1	4.4	14.0	12.0	18.9

Microalgal composition and abundance

There was a rich microalgal community observed at the saltworks. Among the most abundant microalgae identified were species of the genera *Dunaliella*, *Chlamydomonas*, *Amphora*, *Navicula*, and *Nitzschia*. The most commonly observed species of microalgae in the samples are depicted in Table 2. The highest number of algal species (25) was observed at the central channel (B) near the reservoir lake, and the lowest (only 12) at the brine reservoir (E/F) which had the highest salinity. *Dunaliella* spp. were found throughout the saltworks, especially in the lower and upper salinities. Apart from a bloom in the reservoir lake (A), cyanophytes (Cyanobacteria) diversity was large at very high salinities (D, E/F). Dinoflagellates were especially common during spring and at various points at the central channel near the reservoir lake (point B) and near the land-water interface of the reservoir lake (A), especially periphytically on dead and live macroalgae. *Gymnodinium* spp. were abundant throughout the salinity range examined. The highest number of diatom taxa (16) was observed at the salt pond (D), and the lowest (only 3 taxa) at the brine reservoir (E/F).

The chlorophytes *Dunaliella* spp. and *Chlamydomonas* sp. were among the most abundant taxa at the investigated sites that were of aquacultural interest, the ratio of *Dunaliella* reaching 5–22% of the total planktonic microalgal population in spring (Table 3). Total cell counts of *Dunaliella* in April–July 2000 ranged between 0.4×10^6 L^{-1} (C) and 12.5×10^6 L^{-1} (A) with typical densities of 1.5–4.5 $\times$ 10^6 L^{-1} (sites A, C, E, F). *Chlamydomonas* sp. densities ranged between 5.3 and 12.6 cells L^{-1}. Total phytoplankton surpassed 1.2×10^9 cells L^{-1} in salt ponds of 80–100 ppt during summer, a near 20-fold increase compared to the spring readings.

Nutrient availability and microalgal density

Overall, there was an apparent relationship between the high concentrations of PO_4-P and NO_3-N and large *Dunaliella* spp. densities, especially at high salinities. Furthermore, sampling points with higher inorganic

Table 2. Common microalgae identified at six sampling sites A–F (see details in Figure 1) at the saltworks in April 2000.

Taxon	A	B	C	D	E/F
Chlorophyta					
Ankistrodesmus acicularis (Braun) Korshikov		+			
Chlamydomonas sp.	+	+	+	+	+
Dunaliella salina Teodoresco	+	+	+	+	+
Dunaliella viridis Teodoresco	+	+	+	+	+
Micractinium pusillum Fresenius	+	+			
Pediastrum integrum Ehrenberg			+		
Prasinophyceae	+				
Cyanophyta					
Anabaena spp.					+
Lyngbya birgei G.M. Smith		+		+	+
Microcystis aeruginosa (Kützing) Lemmermann		+			+
Oscillatoria tenuis Agardh				+	+
Coccochloris elabens Brébisson					+
Spirulina subsalsa Oersted				+	
Dinophyta					
Gymnodinium spp.	+	+		+	+
Gyrodinium sp.	+	+			
Oxyrrhis marina Dujardin	+	+		+	
Bacillariophyta					
Amphora coffeaeformis (Agardh) Kützing	+	+	+	+	
A. delicatissima Krasske			+	+	
A. lineolata (Ehrenberg) Kützing			+		
A. ovalis Kützing	+	+	+		
Cocconeis pediculus Ehrenberg	+				
Cymbella pusilla Grunow				+	
Entomoneis paludosa (Smith) Reimer		+	+		
Fragilaria fasciculata (Agardh) Lange-Bertalot	+				
Mastogloia braunii Grunow	+				
M. cf. *smithii* Thwaites	+				
Navicula cincta (Ehrenberg) Kützing			+		+
Navicula clamans Hustedt	+			+	
Navicula cryptocephala Kützing		+			
Navicula halophila (Grunow) Cleve		+	+		
Navicula lanceolata (Agardh) Kützing				+	
Navicula salinarum Grunow			+		+
Navicula sp. 1		+			
Navicula sp. 2				+	
Navicula sp. 3				+	
Navicula sp. 4				+	
Nitzschia epithemioides Grunow		+	+	+	
Nitzschia longissima (Brébisson) Ralfs		+		+	
Nitzschia ovalis Arnott ex Grunow		+	+	+	+
Nitzschia cf. *pellucida* Grunow	+	+	+	+	
Nitzschia cf. *perspicua* Cholnoky		+	+	+	
Nitzschia scalpelliformis Grunow	+	+	+	+	
Nitzschia sigma Kützing	+	+		+	
Nitzschia sp.	+	+	+	+	
Synedra ulna (Nitzsch) Ehrenberg	+	+			
Total Bacillariophyta	12	15	14	16	3
Total algal taxa	20	25	18	24	12

Table 3. Population density of *Dunaliella* spp., together with this value as % of the total algal population and values for three parameters in the water at the six sampling sites (A–F, Figure 1) at the saltworks in April 2000.

	A	B	C	D	E	F
Dunaliella spp. ($\times 10^6$ L^{-1})	12.5	1.7	0.4	2.5	4.4	1.3
Dunaliella spp. expressed as % other algae	5.0	12.9	5.5	12.8	22.0	7.0
NO_3-N (mg L^{-1})	0.9	0.9	0.8	2.0	1.9	1.9
PO_4-P (mg L^{-1})	0.07	0.16	0.09	0.24	0.20	0.21
Salinity (ppt)	65	94	101	140	140	144

P:N ratios had an overall larger microalgal density and higher *Dunaliella* spp. ratios.

Reservoir lake (A): low NO_3-N concentrations were recorded (0.9 mg L^{-1}) along with the lowest concentrations of NH_4-N and PO_4-P (6.0 and 0.07 mg L^{-1}, respectively). The total inorganic-N to -P ratio was the second lowest (99:1). Silica was measured to be the highest here. High microalgal densities (249 $\times$ 10^6 cells L^{-1}, of which 5% *Dunaliella* spp.) were observed due to the bloom of a small-celled coccoid cyanophyte (see Tables 1 and 3).

Central channel (B and C): PO_4-P and NO_3-N concentrations were between 0.16–0.09 and 0.9–0.8 mg L^{-1}, respectively. The NH_4-N concentrations were the highest at 13.3 and 16.2 mg L^{-1}, respectively, resulting in a low total inorganic N:P ratio (89:1 and 157:1, respectively). The lowest microalgal densities were found here (7–13 $\times$ 10^6 cells L^{-1}) with a comparatively low *Dunaliella* spp. ratio of 5–13%.

Brine reservoir (E and F) and salt pond (D): PO_4-P and NO_3-N concentrations ranged between 0.20–0.24 and 1.9–2.0 mg L^{-1}, respectively. The NH_4-N concentrations were average (11.5 mg L^{-1}). The total inorganic–N: P ratio was the lowest (57:1). The second highest microalgal densities ($\sim$16–20 $\times$ 10^6 cells L^{-1}) were observed here with a high *Dunaliella* spp. ratio of 7–22%.

Multivariate analysis

Multivariate analysis of species abundance data showed a distinction of sites on the basis of salinity variation, with the first group of sites (A) and (B) identified at a 64% Bray-Curtis similarity level, while (E) and (F) sites were linked at the last cluster at a 39% similarity level (Figure 2a). Salinity and nutrient availability affected the spatial arrangement of the stations as is displayed in the nMDS two-dimensional solution (Figure 2b). Superimposition of normalized physicochemical parameter values (displayed as increasing size geometrical shapes, Figure 2c–j) revealed that the microalgal population structure appeared to be largely

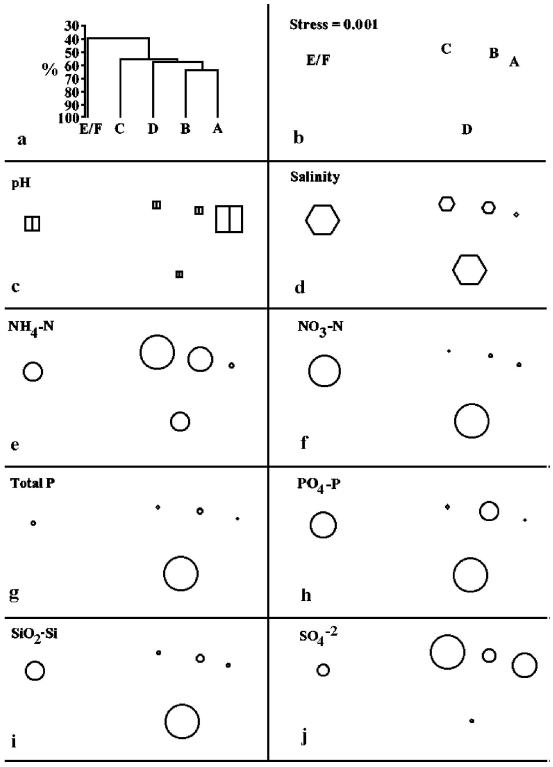

Figure 2. Cluster analysis and Multidimensional Scaling Ordination analysis of species data at six sampling sites A–F (see details in Figure 1) of the M. Embolon saltworks in April 2000, and the effect of physical/chemical parameters on microalgal abundance. (a) Bray-Curtis similarity (%) of samples based on microalgal taxa abundance; (b) nMDS two-dimensional configurations based on Bray-Curtis similarity index, and superimposition of normalized physical/chemical parameter values on nMDS two-dimensional solution, displayed as increasing size geometrical shapes: (c) pH, (d) salinity, (e) NH_4-N, (f) NO_3-N, (g) total-P, (h) inorganic PO_4-P, (i) SiO_2-Si, (j) SO_4^{-2}.

dependent on PO_4-P, NO_3-N and salinity, and to a lesser extent on silica.

Pigment measurements

Chlorophyll analysis at the corresponding sampling sites (see Table 1) did not reflect microalgal densities. In combination with the diversity and the size range of microalgal species in saltworks, the amount of degraded chlorophyll from dead or digested algae (expressed as phaeo-pigments) ranged from 36 to 56% of the Chl-a concentrations, except in the waters of the reservoir lake (A). Total amount of carotenoids were low in sites of low salinity (3.3–5.8 μg L^{-1} in 60–101 ppt), and high in sites of high salinity (12.0–18.9 μg L^{-1} in 140–144 ppt).

Enrichment cultures

Microalgal enhancement experiments with nitrogen and phosphorus enrichment showed that under laboratory conditions higher PO_4-P concentrations were required at 23 °C, especially at 140 ppt salinity (Table 4). Microalgal response to a ratio of high N:low P at 23 °C (e.g. 4:0.6–2 mg L^{-1} at 60 ppt salinity) was low. At high salinity there seemed to be a poorer general performance of the nutrients at either 23 or 30 °C, and total cell counts after 13 culture days increased 30–60-fold at 60 ppt salinity, whereas only 10-fold at 140 ppt. *Dunaliella salina* tended to grow faster and dominate over other species except for diatoms, and represented 38–53% of the total microalgae at the end of the experiment. Microalgal species easily flourished in Walne's medium and dense cell concentrations were attained, with *Dunaliella salina* surpassing 3×10^3 cells mL^{-1}.

Table 4. Microalgal response (+ + + is highest) to enrichment in the laboratory after 13 days of culture in flasks and addition of 4 mg L^{-1} NO_3-N and varying levels of inorganic-P to aliquots from sites A and E (see Figure 1).

4 mg L^{-1} NO_3-N added plus various PO_4-P concs.	60 ppt from (A)		140 ppt from (E)	
	23 °C	30 °C	23 °C	30 °C
0.6 mg L^{-1}	+	+ + +	+	+
1.0 mg L^{-1}	+	+ + +	+	+
2.0 mg L^{-1}	+	+ + +	+	+
4.0 mg L^{-1}	++	++	+	+
8.0 mg L^{-1}	+ + +	+ + +	++	+

Zooplankton and grazing

During the winter and early spring, populations of *Artemia parthenogenetica* (Crustacea, Branchiopoda, Anostraca) were restricted by low temperatures and salinities in the greater area of the saltworks. Biomass estimations indicated 13–15 metric tons (wet) in the brine reservoirs by the end of May 2000 (~40% adults as compared to ~10% in the first week of May). Populations grazed on microalgae during late spring and early summer and disappeared by the end of August, hampered by natural predation, high summer temperatures and salinities. During the summer period and up to October, planktonic grazing by the large (60–200 μm) ciliated zooplankter *Fabrea salina* Henneguy (Ciliphora, Heterotrichida) was also observed. During this time, *Fabrea* densities averaged about 10×10^3 cells L^{-1}, ranging 5–45 $\times$ 10^3 cells L^{-1}. *Dunaliella* densities were reduced significantly from June–October of 2000 and 2001 at salinities of 50–100 ppt due to grazing, with abundance measuring as low as 0.3–1.0% of the total microalgal composition.

Discussion

The Megalon Embolon solar saltworks contain a rich and attractive microalgal reservoir and have a productive physicochemical environment, representing a potential location for investigating aquaculture applications. There are several microalgal species which could be used in large-scale algal polyculture for use as feed for *Artemia*. Specifically, *Dunaliella salina* thrives throughout the saltworks and can comprise up to a quarter of the natural algal population densities. Populations were observed to easily withstand high water temperatures (>32 °C) or salinity drops during heavy rainfall. Overall, chlorophyte and diatom populations were high in spring and throughout the summer. Higher concentrations of inorganic-P, resulting in higher P:N ratios (e.g. sampling points E/F vs. C), were accordingly accompanied by higher microalgal cell counts at the saltworks (excluding the reservoir lake), and higher *Dunaliella salina* ratios to total microalgae (see Tables 1 and 3). Although the water chemistry analyses methodology only gave approximate readings, it seemed to show that inorganic phosphorus played a significant role in restricting microalgal densities at the saltworks. It is, therefore, likely that hypersaline environments limited in inorganic-P will not favor large *Dunaliella* populations (see also Oren & Shilo, 1982;

Javor, 1983). Low concentrations of NO_3-N also exerted pressure on microalgal communities (see Figure 2f), showing this macronutrient's secondary importance. Our observations for the saltworks studied (e.g. in sites B and C, see Table 1) supported measurements by Dolapsakis et al. (2000) which showed that high concentrations of NH_4-N did not contribute to high *Dunaliella* densities, contrary to Post's (1977) findings. Rather, this form of nitrogen could support higher microalgal densities at M. Embolon only when found in combination with higher concentrations of NO_3-N and PO_4-P (0.20 mg L^{-1} or higher). This was confirmed by the comparatively higher microalgal densities found at points (D), (E) and (F) which had higher ratios of inorganic P:N. Most of the measured inorganic-N and -P concentrations at Megalon Embolon during spring and summer reflected an eutrophic environment (Por, 1980; Javor, 1989; Wetzel, 2001), favorable for microalgal growth.

Laboratory experiments indicated that a higher ratio of the PO_4-P: NO_3-N fertilizer application (e.g. 1:1–5 rather than 1:6–12) is necessary for enhancing microalgal growth in high salinity waters, whereas applications in 140 ppt salinity waters do not perform well. This disadvantage needs to be weighted with the fact that higher salinities favor *Dunaliella salina* and help restrict other microalgae during cultivation. Cultivation prospects for green algae that were abundant at the saltworks were reinforced by the fact that many populations bloomed easily in the laboratory. In enrichment cultures of algal polyculture, *Dunaliella salina* was able to increase its ratio to over 50% and displace other hypersaline microalgae, possibly as a better resource competitor.

Grazing was found to be the main cause of *Dunaliella* population declines from June to September due to the development of *Artemia parthenogenetica* and *Fabrea salina* populations. The contamination of algal cultures with these species, which are very adaptive and effective filter-feeders, seems to form the main phytoplankton culture obstacle at Megalon Embolon solar saltworks. *Fabrea* also produce mucilaginous substances which change the microplanktonic spectra and negatively impact *Dunaliella* and even young *Artemia* populations (De Simone & Repak, 1990; Capriulo & Degnan, 1991; Davis, 2000).

The major application of inorganic phosphorus along with smaller amounts of NO_3-N and some micronutrients could support higher microalgal densities at Megalon Embolon. Secondary nutrients measured in the basins, such as iron, sulphur and potassium, exist in ample amounts and, inorganic carbon and pH are in the range of algal optima, thus providing appropriate conditions for phytoplankton. The use of reservoir lake waters of 60 ppt salinity (A) which allow better fertilizer performance, mixed with brine reservoir waters of 140 ppt salinity (E/F) could offer the best salinity and *Dunaliella* profiles for phytoplankton cultivation.

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