



REVIEW

Saltern evaporation ponds as model systems for the study of primary production processes under hypersaline conditions

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ABSTRACT: Multi-pond solar salterns, which are used worldwide for salt production along tropical and subtropical coastal areas, present an environment with increasing salt concentrations, from seawater to NaCl saturation. Characteristic salt-adapted microbial communities are found along the salinity gradient. In ponds of intermediate salinity (100 to 250 g l⁻¹), most of the primary production occurs in benthic microbial mats dominated by different types of unicellular and filamentous Cyanobacteria (*Aphanothece*, *Microcoleus*, *Phormidium* and others), sometimes in association with diatoms. In crystallizer ponds, the unicellular green alga *Dunaliella* is the sole primary producer that lives in association with dense communities of heterotrophic halophilic Archaea that color the brines red. This basic pattern is common to all saltern systems, in spite of local variations in climate and nutrient availability. Photosynthetic activities of benthic cyanobacterial mats in the evaporation ponds and of endoevaporitic microbial communities within the gypsum crust that precipitates at intermediate salinities have been extensively studied in salterns at different locations, using oxygen microelectrodes and other techniques adapted to the study of benthic communities. These environments are generally highly productive, although most of the oxygen produced during daytime by the Cyanobacteria is recycled within the mats rather than exchanged with the overlying water and the atmosphere. Surprisingly few attempts have been made thus far to estimate the photosynthetic activity of *Dunaliella*, which is often present in numbers between 10³ and 10⁵ cells ml⁻¹ in the heavily salt-stressed environment of crystallizer ponds, so that the dynamics of the system is largely unknown.

KEY WORDS: Salterns · Cyanobacterial mats · *Dunaliella* · Primary production · Hypersaline

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INTRODUCTION

Along tropical and subtropical coasts worldwide, we find saltern systems in which seawater is evaporated for the commercial production of common salt (NaCl) and sometimes other salts as well. To obtain salt of high purity, such salterns are designed to consist of a series of shallow ponds in which the seawater is evaporated in stages, keeping the salinity of each pond within a narrow range. Calcium carbonate (calcite, aragonite) and calcium sulfate (gypsum) precipitate in the early stages of evaporation. Then sodium chloride (halite) precipitates in crystallizer ponds (total dissolved salt concentration ~300 to 350 g l⁻¹)¹. The

remaining brines (the 'bitterns') that contain high concentrations of magnesium, potassium, chloride and sulfate are generally returned to the sea. Some coastal salt production facilities are very large, such as the Exportadora de Sal, Guerrero Negro, Baja California, Mexico which extends over ~330 km²; others are much smaller, such as the well-studied salterns of the Israel

¹In the literature on which this review is based, different units are used to express salt concentrations: g l⁻¹, g kg⁻¹, mol l⁻¹ (for NaCl solutions), %, ‰, and °Bé [degrees Baumé; the density in g ml⁻¹ being 145 / (145 - degrees Baumé)]. To enable easy comparison, total dissolved salt concentrations were converted here to g l⁻¹ wherever possible

Salt Company near Eilat at the Red Sea coast of Israel, with a surface of only 2.3 km².

Solar salterns contain rich and varied communities of phototrophic microorganisms along the saltern gradient, and the photosynthetic primary production by these communities largely determines the properties of the saltern system. The study of the communities of phototrophs that inhabit salterns is not only of purely scientific interest: the benthic cyanobacterial mats that develop in saltern ponds of intermediate salinity effectively seal the bottom of these ponds and prevent leakage of brine; on the other hand, unicellular Cyanobacteria in these mats and in the brine itself sometimes produce massive amounts of polysaccharide slime that unfavorably affects the salt production process (Davis 1993, Davis & Giordano 1996). The red pigmentation of the dense microbial communities in crystallizer ponds is caused both by the β -carotene accumulated by the green alga *Dunaliella salina* which is the main or sole primary producer in these ponds, as well as by the carotenoid and retinal protein-based pigments of the heterotrophic community of prokaryotes that develop at the expense of photosynthetically fixed carbon derived from *Dunaliella* (Litchfield 1991). This red pigmentation increases light absorption by the brine and increases its temperature, thus enhancing the salt production process (Bonython 1966, Javor 1989, 2002). Even purely esthetic considerations have been used as incentive to study the highly diverse communities of phototrophic microorganisms in salterns (Gunde-Cimerman et al. 2005, Oren 2005a). Thus, saltern pond systems have been studied extensively, and this review on phototrophs and photosynthetic activities is based on research performed in salterns in Australia, Bulgaria, France, Greece, India, Italy, Israel, Mexico, Puerto Rico, Slovenia, Spain, and others countries.

Characteristic salt-adapted microbial communities are found along the salinity gradient in salterns. This basic pattern is common to all saltern systems despite local variations in climate and nutrient availability. In ponds of intermediate salinity (100 to 250 g l⁻¹), most of the primary production occurs in benthic microbial mats dominated by different types of unicellular and filamentous Cyanobacteria (*Aphanothece*, *Microcoleus*, *Oscillatoria*, *Phormidium* and others) (Oren 2000), sometimes in association with diatoms. Below the cyanobacterial layers, a purple layer of anoxygenic phototrophic sulfur bacteria (*Halochromatium*, *Ectothiorhodospira* and related organisms) is often found. Especially beautiful are the layered communities of orange and dark-green Cyanobacteria and purple sulfur bacteria found embedded within the gypsum layers that precipitate at intermediate salinities (Caumette et al. 1994, Oren et al. 1995, Sørensen et al. 2004). Due to the elevated salinity, grazing is reduced, enabling the

development of stratified benthic microbial mats that may often reach several centimeters in thickness.

In the crystallizer ponds where halite precipitates, benthic microbial mats are usually absent. The occurrence of a leathery layer of *Microcoleus*, locally called 'petola', on the bottom of the salt production ponds in the ancient, traditionally operated salterns of Sečovlje, Slovenia (Schneider 1995, Gunde-Cimerman et al. 2005) is an exception rather than the rule. The 'petola' develops during the earlier evaporation stages and apparently survives even after the salinity of the ponds has reached halite saturation. Photosynthetic activity under these conditions is probably negligible, but quantitative data are not available. Primary production is mostly limited to the planktonic unicellular green alga *Dunaliella salina*, which is often found in dense populations together with heterotrophic red Archaea (the flat square *Haloquadratum walsbyi* and other members of the Halobacteriaceae) and the red-orange *Salinibacter* (Bacteroidetes branch of the Bacteria), which all contain different carotenoid pigments as well as retinal proteins that may also contribute to the pink-red color of the brines.

This review primarily deals with the oxygenic photosynthetic microbial communities in salterns worldwide and the primary production they contribute to the saltern ecosystem. Our understanding of the dynamics of anoxygenic photosynthesis (e.g. the CO₂ fixation activities of the purple sulfur bacteria that often occur in the benthic mats at lower salinity) and the contribution of chemoautotrophic bacteria to the overall carbon fixation in the ponds will also be discussed below, even though only limited data have been collected thus far.

Microbial processes in saltern ponds, including primary production by the halophilic algae and Cyanobacteria in the water and in the sediments, are largely comparable to processes occurring in natural salt lakes of similar salinity, from natural coastal lagoons to inland saline lakes. A study of the saltern ecosystem thus provides a convenient model for the understanding of the ecosystems in other seawater-derived and hypersaline environments. A discussion of primary production processes in such salt lakes and a comparison with phenomena observed in the artificial environment of the salterns may therefore be of interest. However, due to space limitation, this review will be restricted to data collected in coastal solar salterns, and no attempt is made to discuss the wealth of information on primary production in natural salt lakes of comparable salinity.

METHODOLOGICAL CONSIDERATIONS

The methods generally used to assess primary production in aquatic environments have been developed

and optimized for use in freshwater and marine environments. Hypersaline environments pose special methodological problems, as many of the standard protocols used for chemical analysis in freshwater or seawater do not function properly at elevated salinities.

Part of the methods for the measurement of primary production are based on the monitoring of oxygen concentrations, which are evaluated by chemical methods (the Winkler titration) or by means of oxygen microelectrodes. Such microelectrodes have been extensively used in photosynthesis studies of hypersaline microbial mats in saltern evaporation ponds (Canfield & Des Marais 1993, Caumette et al. 1994, Des Marais 1995). No special problems are reported to have been caused by the hypersaline nature of the medium that may necessitate special modifications in the construction or calibration of such electrodes. When used in studies of microbial activities in solid gypsum crusts, narrow holes may have to be drilled in the sediment to accommodate the electrodes, and the use of especially sturdy electrodes protected within a steel needle is recommended (Canfield et al. 2004, Sørensen et al. 2004). To stabilize the microbial community within the cyanobacterial mat to be used in oxygen microelectrode studies, it is possible to embed the mat in agar (Wieland & Kühl 2006).

The Winkler titration can be applied even in the salt-saturated brines of crystallizer ponds. Incubation of 'light' and 'dark' bottles for 4 h at mid-day, followed by standard oxygen titration has also been successfully applied for the evaluation of photosynthetic activity in Spanish saltern ponds over the whole salt concentration range (Pedrós-Alió et al. 2000) and for the determination of oxygen concentration in water of $\sim 200 \text{ g l}^{-1}$ salt overlying a gypsum crust (Canfield et al. 2004). In some cases, the amount of sulfuric acid needed to dissolve the precipitate formed after the addition of the reagents has to be increased compared to the standard protocol (Javor 2002). Some authors (Javor 1983a, Sammy 1983) recommended the use of the azide modification of the Winkler procedure (Wright 1983).

At elevated salt concentrations, the solubility of oxygen and other gases in water is strongly reduced. This reduction sometimes results in conditions in crystallizer ponds being close to anaerobic, especially when the little oxygen that either enters from the atmosphere by diffusion or is produced by *Dunaliella* is taken up for respiration by the dense community of heterotrophic Archaea and Bacteria (Javor 1989). The values of 0.24 and 0.08 mg l^{-1} oxygen (7.5 and 2.5 μM , respectively) reported from Australian crystallizer pond waters with densities of 1.224 and 1.235 g ml^{-1} (Sammy 1983) are probably the lowest values found in the literature. For comparison, air-saturated freshwater

and standard seawater at 25°C contain 8.22 and 6.79 mg l^{-1} oxygen, which are equivalent to 257 and 212 μM (Sherwood et al. 1991, 1992). A concentration of 1.87 mg l^{-1} (58 μM) oxygen was reported in the crystallizer brines of a Bulgarian salt works (Pavlova et al. 1998). Canfield et al. (2004) found saturation concentrations of 82 and 69 μM oxygen at 20°C in brines of 230 and 200 g l^{-1} total dissolved salts overlying a gypsum crust in the salterns of Eilat, Israel.

When using primary production assays that involve measurement of the incorporation of ^{14}C -labeled bicarbonate, considerable errors may be introduced since the carbonate system behaves differently in salterns than in seawater (Javor 2002). Estimates of total inorganic carbon based on standard alkalinity titrations need to be corrected using a salinity-dependent factor (Burke & Atkinson 1988). This approach has been used by Joint et al. (2002) in their study of primary production along the salt gradient in a Spanish saltern. An alternative is to determine the total inorganic carbon in the brines using a CO_2 analyzer (Wieland et al. 2005).

Specific problems may also arise in ^{14}C -labeling studies due to the special nature of some of the photosynthetic species responsible for the CO_2 fixation in salterns. The cells of *Dunaliella*, which lack a rigid cell wall, are very fragile and easily broken during filtration. Moreover, such cells transform a significant fraction of the carbon fixed into glycerol, which is accumulated inside the cell up to molar concentrations to provide osmotic balance of the cytoplasm with the salty brines. Glycerol may therefore be one of the key compounds used by the heterotrophic communities in such ponds (Oren 1993, 1995). A study of the marine species *D. tertiolecta* showed that up to half of the fixed carbon could be lost during filtration on polycarbonate filters, especially when the filters were left dry between rinses. The pressure difference applied to the vacuum filtration system was also found to influence the amount of label lost (Goldman & Dennett 1985). It may be assumed that the problem could be even more severe for *D. salina* in crystallizer brines, as they contain much higher glycerol concentrations than marine species, and a greater differential pressure is needed to filter the highly viscous saltern brines through small-pore membrane filters. The question 'To what extent are the very low primary production values reported by Joint et al. (2002) for the crystallizer ponds of a Spanish saltern underestimated relative to the true values as a result of massive cell lysis during vacuum filtration on 0.2 μm pore size polycarbonate filters?' should therefore be asked.

Flow cytometry has thus far been seldom used to estimate the numbers and biomass of planktonic phototrophs in saltern ponds, but a recent study shows that the technique can be employed in hypersaline systems (Estrada et al. 2004).

CYANOBACTERIA-DOMINATED MICROBIAL MATS IN THE EARLY CONCENTRATION PONDS

In the first sets of ponds in saltern operations, seawater is concentrated from ~35 to 100–120 g l⁻¹ total dissolved salts. In the process, small amounts of CaCO₃ precipitate to the bottom as calcite or aragonite, but the bottom layers are generally soft. Dense microbial mats consisting of Cyanobacteria, anoxygenic phototrophic bacteria, a variety of heterotrophic prokaryotes, diatoms, Protozoa, and other microorganisms often develop on the bottom of such ponds. Slimy polysaccharides excreted by the biota cause the formation of a cohesive microbial mass that may sometimes reach several centimeters in depth.

The filamentous cyanobacterium *Microcoleus chthonoplastes* is often one of the most prominent members of the phototrophic community in such benthic mats. In the Guerrero Negro salterns of Baja California, it dominated the ecosystem at salt concentrations of up to 110 g l⁻¹ (Des Marais 1995, Nübel et al. 2000, Decker et al. 2005). We find such mats of *Microcoleus*, often accompanied by *Lyngbya* species, also in salterns in the south of France (Fourçans et al. 2004), Spain (Thomas 1984), Puerto Rico (Casillas-Martinez et al. 2005) and elsewhere. At somewhat higher salt concentrations (140 g l⁻¹), the unicellular *Eubalthece* (*Aphanothece*) and the filamentous *Halospirulina* became dominant in the Guerrero Negro evaporation ponds (Nübel et al. 2000). Other Cyanobacteria reported to abound in such benthic mats include *Oscillatoria* sp., *Halomicronema*, *Microcystis*, *Chroococcus*, *Gloeocapsa*, *Synechocystis*, *Leptolyngbya*, *Phormidium*, *Pleurocapsa*, and *Calothrix* (Des Marais 1995, Oren 2000, Fourçans et al. 2004). There is often considerable confusion about the proper identification of the organisms present, and some of these Cyanobacteria are known under more than one name (Oren 2000). Diatoms such as *Nitzschia* are often present (Wieland et al. 2005), but the Cyanobacteria are quantitatively the main photosynthetic component of the system. Anoxygenic phototrophs (different types of Chromatiaceae and Ectothiorhodospiraceae, as well as green non-sulfur bacteria of the Chloroflexi group) and filamentous chemolithotrophs (*Beggiatoa* and relatives) abound as well in such mats.

Most attempts to estimate primary production in such benthic photosynthetic mat systems have employed oxygen microelectrodes, following changes in the ambient oxygen concentration at different depths during light–dark shifts. Such measurements yield information about both gross primary production at any defined depth and the community respiration and other oxygen sinks. Little information is obtained about the fluxes of oxygen from or into the system at

any time, and the different techniques and calculation methods used by different investigators make direct comparison of data often difficult. While attempting to also estimate the net evolution and/or uptake of oxygen by such mats, some studies have measured changes in oxygen concentration in the overlying water in cores upon illumination (Wieland et al. 2005), or used flow chambers to assess changes in oxygen concentration mediated by the microbial mat communities (Wieland & Kühl 2006). Table 1 summarizes the photosynthetic activities of such benthic photosynthetic mats as studied in saltern ponds at different locations. Because of the diversity of techniques and approaches used in the different studies, direct comparison of the primary production values quoted by the authors is not always possible.

Exposure of a *Microcoleus*-dominated mat from a 100 g l⁻¹ salinity pond of the Salin de Giraud (France) saltern to changes in salt concentration resulted in little change in net photosynthesis activity when salinity was decreased to 40 g l⁻¹, but rates decreased at elevated salt concentrations of up to 160 g l⁻¹ at all levels of irradiance (Wieland & Kühl 2006). This behavior is consistent with that of *Microcoleus* in laboratory culture (Oren 2000). Dark oxygen consumption rates and gross photosynthetic rates at light saturation were also relatively constant over a broad salinity range (60–100 g l⁻¹). Within the range of natural variation in the mat, temperature was more important than salinity in regulating photosynthesis and oxygen consumption (Wieland & Kühl 2006).

The in-depth studies of Canfield & Des Marais in the Guerrero Negro evaporation ponds (salinity 65 to 125 g l⁻¹) provide interesting information on the primary productivity of such mats. Monitoring of oxygen profiles and measurements of kinetic parameters during day–night cycles enabled the establishment of a detailed model. During daytime, most of the oxygen formed in the mat is recycled locally by respiration of organic carbon and by oxidation of sulfide that diffuses from the deeper layers. At night, oxidation of sulfide near the mat–water interface is the main oxygen-consuming process. Dissimilatory sulfate reduction is the principal source of dissolved inorganic carbon at night. The oxygen and dissolved inorganic carbon fluxes were found to balance over a 24 h cycle. However, a careful comparison of the oxygen and dissolved inorganic carbon fluxes across the mat–water interface revealed that during the day, there is more inorganic carbon diffusing into the mat than oxygen diffusing out; at night, more inorganic carbon diffuses out of the mat than oxygen that diffuses in. Thus, relatively oxidized carbon compounds appear to be incorporated into the mat during the day, and these oxidized compounds are respired by the mat at night. The chemical

Table 1. Photosynthetic activities of benthic microbial mats in saltern evaporation ponds with up to 150 g l⁻¹ salts from different geographical locations. Wherever relevant, oxygen evolution rates were converted to nmol cm⁻³ min⁻¹

| Location | Salinity (g l ⁻¹) | Other environmental conditions | Photosynthesis and respiration activity | Comments | Source |
|---|--|---|--|--|---|
| Salin de Giraud, Camargue, France | 100 | 30°C | Oxygen evolution up to 900 nmol cm ⁻³ min ⁻¹ ; average 600 nmol cm ⁻³ min ⁻¹ in the photic zone | Values relate to the upper 0.6 mm of the mat; maximum activity at 0.3–0.6 mm depth | Fourçans et al. (2004) |
| Salin de Giraud, Camargue, France | 72–94 | 25°C (day)–11°C (night) | Up to 10.8 nmol O ₂ cm ⁻² min ⁻¹ | Net photosynthesis estimate from core incubations and microelectrode studies | Wieland et al. (2005) |
| Salin de Giraud, Camargue, France | 100 | 25°C | Up to 42 nmol O ₂ cm ⁻² min ⁻¹ | Microelectrode measurements | Wieland & Kühl (2006) |
| Cabo Rojo, Puerto Rico | 40–150 during the wet season (Aug–Dec) | | Oxygen evolution up to 48.9 ± 11.5 nmol cm ⁻³ min ⁻¹ , maximal oxygen consumption rate 29.1 ± 7.2 nmol cm ⁻³ min ⁻¹ | Chlorophyll content of the mat 340 ± 28 µg cm ⁻² . In the dry season (Jan–Apr), salinity increased to 150–265 g l ⁻¹ , chlorophyll decreased to 85 ± 24 µg cm ⁻² and maximal O ₂ production and consumption rates were 9.4 ± 3.3 and 7.1 ± 1.8 nmol cm ³ min ⁻¹ , respectively | Casillas-Martinez et al. (2005) |
| Guerrero Negro, Baja California, Mexico | 90–108 | Experiments at temperatures between 17 and 30°C | Daytime oxygen evolution 75 mmol m ⁻² (17°C); 235 mmol m ⁻² (30°C); daytime O ₂ respiration 53 mmol m ⁻² (17°C); 155 mmol m ⁻² (30°C) | Daytime carbon uptake by oxygenic photosynthesis 76 mmol m ⁻² (17°C); 236 mmol m ⁻² (30°C); carbon uptake by anoxygenic photosynthesis 6 mmol m ⁻² (17°C); 23 mmol m ⁻² (30°C); inorganic carbon release by aerobic respiration 54 mmol m ⁻² (17°C); 158 mmol m ⁻² (30°C) | Canfield & Des Marais (1993), Des Marais (1995) |

nature of this carbon is still unknown (Canfield & Des Marais 1993, Des Marais 1995). An alternative explanation for the unexpected carbon to oxygen ratios may be the underestimation of net photosynthesis by the measured oxygen flux due to consumption of oxygen by the oxidation of metal sulfides accumulated during the dark period (Wieland et al. 2005).

PHOTOSYNTHETIC PROCESSES IN BENTHIC GYPSUM CRUSTS

When the evaporation of seawater has proceeded so that salinity is 3 to 4× the original salinity, the solubility of calcium sulfate is exceeded, and gypsum (CaSO₄·2H₂O) precipitates at the bottom of the ponds. In some saltern systems, gypsum accumulates in ponds with a salinity of 150 to 250 g l⁻¹ in a crust of many centimeters thickness. Layered communities of phototrophic microorganisms are characteristically found in the gypsum: an upper layer of carotenoid-rich orange-colored Cyanobacteria of the *Eubalthece-Aphanothece* group (Garcia-Pichel et al. 1998), below which a dark-green layer of *Phormidium*-type filamentous Cyanobacteria occurs. Underneath, a purple layer of *Halochromatium* and *Ectothiorhodospira* performs anoxygenic photosynthesis, with sulfide derived from sulfate reduction in the anaerobic layers below as electron donor. The gypsum crystals are often arranged such that light penetrates deep into the crust, allowing for the possibility of active photosynthesis down to depths of similar centimeters. Such layered phototrophic communities have been described from salterns in the south of France (Cornée 1982, Caumette et al. 1994), in Spain (Orti Cabo et al. 1984, Thomas 1984) and Israel (Oren et al. 1995, Oren 1997, Canfield et al. 2004, Sørensen et al. 2004, 2005, Ionescu et al. 2007, Oren et al. 2009).

Oxygen microelectrodes have been used to estimate primary production and other photosynthesis-related parameters in the gypsum crusts of the Salin de Giraud, France (Caumette et al. 1994) and the Israel Salt Company in Eilat (Canfield et al. 2004). The results of these studies are summarized in Table 2. Overall, photosynthetic rates in the Eilat gypsum crust were much lower than those typically found in organic-rich microbial mats at lower salinities. However, on a per cell-volume basis, the rates were comparable. Microelectrode measurements also showed that the green layer, which receives ~0.1% of the light intensity at the surface of the crust (Oren et al. 1995), was adapted to function at extremely low light intensities, and was light-saturated during much of the day (Canfield et al. 2004). A large percentage of the oxygen produced during the day accumulated inside the crust and was internally recycled, and only a relatively

Table 2. Primary production and related parameters of benthic gypsum crusts in saltern evaporation ponds, as measured using oxygen microelectrodes

| Location | Salinity (g l ⁻¹) | Other environmental conditions | Photosynthesis and respiration activity | Comments | Source |
|-----------------------------------|-------------------------------|--------------------------------|---|---|------------------------|
| Salin de Giraud, Camargue, France | Not reported | May–June | Up to 40 nmol O ₂ cm ⁻³ min ⁻¹ Overall production 48–50 mmol O ₂ m ⁻² d ⁻¹ | Maximum rate in the green layer | Caumette et al. (1994) |
| Eilat, Red Sea coast of Israel | 200 | Measured in December | Up to 12 nmol O ₂ cm ⁻³ min ⁻¹ in the brown layer; up to 6 nmol O ₂ cm ⁻³ min ⁻¹ in the green layer Daytime O ₂ production 1750 nmol cm ⁻² ; O ₂ diffusing from the crust 600 nmol cm ⁻² ; O ₂ respiration 1150 nmol cm ⁻² ; O ₂ diffusion deep into crust 150 nmol cm ⁻² Nighttime O ₂ diffusion into crust 650 nmol cm ⁻² | Up to 6 nmol O ₂ cm ⁻³ min ⁻¹ in the green layer Daytime O ₂ production 1750 nmol cm ⁻² ; O ₂ diffusing from the crust 600 nmol cm ⁻² ; O ₂ respiration 1150 nmol cm ⁻² ; O ₂ diffusion deep into crust 150 nmol cm ⁻² Nighttime O ₂ diffusion into crust 650 nmol cm ⁻² | Canfield et al. (2004) |
| Eilat, Red Sea coast of Israel | 230 | | Up to 2.4 nmol O ₂ cm ⁻³ min ⁻¹ in the brown layer; up to 1.0 nmol O ₂ cm ⁻³ min ⁻¹ in the green layer Daytime O ₂ production 370 nmol cm ⁻² ; O ₂ diffusing from the crust 47 nmol cm ⁻² ; O ₂ respiration 323 nmol cm ⁻² ; O ₂ diffusion deep into crust 41 nmol cm ⁻² . Nighttime O ₂ diffusion into crust 29 nmol cm ⁻² | Up to 1.0 nmol O ₂ cm ⁻³ min ⁻¹ in the green layer Daytime O ₂ production 370 nmol cm ⁻² ; O ₂ diffusing from the crust 47 nmol cm ⁻² ; O ₂ respiration 323 nmol cm ⁻² ; O ₂ diffusion deep into crust 41 nmol cm ⁻² . Nighttime O ₂ diffusion into crust 29 nmol cm ⁻² | Canfield et al. (2004) |

small percentage of the oxygen produced (16 to 34 %) escaped. For comparison, 30 % of the oxygen produced during the first half of the light cycle accumulated in the Eilat crust, while <1 % accumulated in the Guerrero Negro crust (Canfield & Des Marais 1993).

A prominent feature of the microbial mats within the gypsum crusts, as well as in the evaporation ponds of lower salinity, is the often copious amounts of polysaccharide slime associated with the growth of the unicellular *Eubalthece-Aphanothece* Cyanobacteria. These organisms also spread into the overlying water in some saltern systems. Massive slime formation can negatively affect the salt production process (De Medeiros Rocha & Camara 1993, Rahaman et al. 1993, Roux 1996). Lack of inorganic nutrients (nitrogen, phosphorus) may induce the cells to dispose of the photosynthetically fixed carbon in the form of these extracellular polysaccharides (Roux 1996).

The ambient salinity is one of the key factors determining the photosynthetic rates of the cyanobacterial community in the hypersaline gypsum crusts. Reduction of the salinity of such a crust in Eilat from 230 to 200 g l⁻¹ increased oxygen production, as measured by a microelectrode, from <2 to 8–9 μM min⁻¹ (Canfield et al. 2004). Such results are also consistent with pure culture studies showing that species of *Aphanothece/Eubalthece* that dominate the community have their salinity optimum far below that found in the gypsum crusts and the overlying brines (Garcia-Pichel et al. 1998, Oren 2000). However, ¹⁴C-bicarbonate uptake in slurries of gypsum crust (215 g l⁻¹ salt in the overlying brine) showed optimal photosynthetic activity between 180 and 240 g l⁻¹ salt in the upper brown-orange layer and between 80 and 230 g l⁻¹ salt in the green layer below (Sørensen et al. 2004).

The use of sulfide microelectrodes enabled the estimation of the photosynthetic rates of the purple sulfur bacteria community in the gypsum crust in the Salin de Giraud salterns. Monitoring of the changes in sulfide concentration in the red-purple layer of anoxygenic phototrophs following light–dark shifts yielded a sulfide oxidation rate of 12.7 μmol cm⁻³ min⁻¹ in the light, corresponding to 180–330 mmol sulfide m⁻² d⁻¹ oxidized photosynthetically (based on 8 to 10 h of daylight and a 3 mm thick zone of phototrophic sulfide oxidation). At a stoichiometry of 2 mol CO₂ fixed autotrophically mol⁻¹ of sulfide oxidized to sulfate, this would be equal to 360–660 mmol CO₂ m⁻² d⁻¹. It was estimated that between 65 and 95 % of the sulfide produced in the lower layers was oxidized phototrophically in the crust (Caumette et al. 1994). The remainder may be oxidized chemically as well as biologically by chemoautotrophic sulfur bacteria. Measurements of ¹⁴C-labeled bicarbonate uptake by slurries prepared from the purple layer of the Eilat gypsum crust in the light and in the presence

of sulfide showed optimal activity at 100 to 120 g l⁻¹ salt, which is far below concentrations in the crust in which the community had developed (215 g l⁻¹) (Sørensen et al. 2004). No further estimates of the contribution of chemoautotrophic sulfur bacteria to the CO₂ fixation process in the benthic microbial communities in saltern ponds could be found in the literature.

PLANKTONIC PRIMARY PRODUCERS AND PRIMARY PRODUCTION IN EVAPORATION PONDS

In contrast to the extensive studies of primary production in benthic cyanobacterial mats in saltern evaporation ponds of low and intermediate salinity, surprisingly little research has yet been devoted to primary production processes in the brines overlying these sediments. Joint et al. (2002) measured planktonic chlorophyll and 'potential' primary production (by ¹⁴C-bicarbonate uptake in 3 h laboratory incubations at a light intensity of 275 μmol quanta m⁻² s⁻¹) along the salinity gradient in the salterns of Alicante, Spain in May 1999. Chl *a* concentrations in the first ponds fed with Mediterranean water were ~4 μg l⁻¹, increasing to ~8 μg l⁻¹ at 54–102 g l⁻¹ salinity, then decreasing to low values with increasing salinity, to increase again in the crystallizer ponds at a salinity above 320 g l⁻¹. The highest potential photosynthetic rate (38.6 μg C l⁻¹ h⁻¹; 4.9 μg C μg chl a⁻¹ h⁻¹) was measured at 80 g l⁻¹ salinity. High activities were also measured at 54 and 110 g l⁻¹ salinity. In the first set of ponds (40 g l⁻¹ salt), activity was much lower (7.8 μg C l⁻¹ h⁻¹). Dark carbon fixation was high throughout the salt gradient: in most ponds the light:dark ratio of ¹⁴C fixation was ~1.3.

Incubation experiments of the above-mentioned samples were conducted for 24 h, with simultaneous measurement of nitrate and ammonium uptake. In these experiments, a dark period was included to estimate net rather than gross production. At salt concentrations below 110 g l⁻¹, the C:N uptake ratio was higher than the classic Redfield ratio of 16:1, but unexpectedly decreased to ~1.0 at 220 g l⁻¹ salinity and an even lower value (0.8) was estimated in a crystallizer pond at 370 g l⁻¹ salinity. If the analytical methods and calculations were indeed reliable, no obvious explanation can be brought forward for these observations.

PLANKTONIC PRIMARY PRODUCTION IN SALTERN CRYSTALLIZER PONDS

Michel Felix Dunal first saw the unicellular flagellate red-colored algae we know today as *Dunaliella salina* in saltern brines on the Mediterranean coast of France in 1838, and named the organism '*Haematococcus salina*' (Oren 2005b). This organism is the main phototrophic organism encountered in saltern crystallizer ponds all over the world, and is usually the only primary producer there. Large amounts of β-carotene are accumulated as globules between the thylakoids of the single chloroplast, and the pigment protects the cells from damage by high light intensities. *Dunaliella* is one of the causes of the pink-red coloration of saltern crystallizer brines; however, although β-carotene may be present in the biomass in amounts exceeding those of the bacterioruberin pigments of the halophilic Archaea, the algal pigment contributes only a minor part to the overall color of the brines because of its dense packaging (Oren et al. 1992, Oren & Dubinsky 1994).

Dunaliella salina has a broad salinity range, grows optimally at salt concentrations of 120 to 140 g l⁻¹ in the laboratory, but also tolerates 270 g l⁻¹ and above. At the highest salinities (>200 g l⁻¹), *D. salina* grows faster than smaller *Dunaliella* species such as *D. parva* and *D. viridis* (Gibor 1956, Margheri et al. 1987, Oren 2005b). At lower salt concentrations, *D. salina* is generally outcompeted by other phototrophs, but the alga was found in relatively low numbers (5 to 22% of the total cell counts) in the evaporation ponds of the Megalon Embolon solar salt works in northern Greece at 65 to 144 g l⁻¹ salt (Dolapsakis et al. 2005).

The population density of *Dunaliella* in crystallizer ponds varies greatly according to geographic location, nutrient status, and management of the salterns. Despite the fact that ecophysiological studies on *Dunaliella* were already started in the 1920s in attempts to understand its dynamics in salterns (Labbé 1921, Oren 2005b), we still know surprisingly little about its behavior in salt-saturated brines. In the Eilat salterns, the numbers of *D. salina* in the crystallizer ponds were found to vary between 160 and 2960 cells ml⁻¹ (Oren et al. 1992, Oren 1993, Oren & Dubinsky 1994, Oren 1995). Far larger numbers were observed in the crystallizer ponds of salterns on the Mediterranean coast of Spain: up to 100 000 cells ml⁻¹ at 300 g l⁻¹ salt, decreasing to ~5000 cells ml⁻¹ at 380 g l⁻¹ salt (Rodriguez-Valera et al. 1985). Patchy growth may sometimes lead to considerable spatial variation: on the 4 sides of a single pond of 370 g l⁻¹ salt, cell numbers varied between 5090 and 10 500 ml⁻¹, with chl *a* concentrations between 23.6 and 45.7 μg l⁻¹ (Joint et al. 2002).

Surprisingly few attempts have been made to assess the *in situ* photosynthetic activity of *Dunaliella* in saltern crystallizer ponds. In the salterns of Alicante, Spain, oxygen evolution/consumption was measured in light and dark bottles incubated for 4 h around noon. In ponds of intermediate (200 to 250 g l⁻¹) salinity, with 3.6 μg l⁻¹ chl *a*, and 74 to 96% of the biovolume being *Dunaliella* and 4 to 9% being Cyanobacteria, gross production was estimated at 0.07 to 1.27 mg C l⁻¹ d⁻¹. Sur-

prisingly, primary production in the crystallizer ponds ($>300 \text{ g l}^{-1}$ salt) was below the detection limit, despite the presence of $3.5 \mu\text{g l}^{-1}$ chl *a*, which was attributed entirely to *Dunaliella* (Pedrós-Alió et al. 2000). Also in a later study performed in the same ponds, this time measuring photoassimilation of ^{14}C -labeled bicarbonate during 3 h periods, very low rates of carbon fixation were found, despite the high chlorophyll concentrations present. Reported rates were as low as 27.5 to $56 \mu\text{g C l}^{-1} \text{ d}^{-1}$ in the light and 20.8 to $23.8 \mu\text{g C l}^{-1} \text{ d}^{-1}$ in the dark, or $0.1 \mu\text{g C} (\mu\text{g chl } a)^{-1} \text{ h}^{-1}$ in brine of 370 g l^{-1} salt (Joint et al. 2002). As discussed above, the filtration procedure used to separate the cells from the liquid may have caused considerable cell breakage, leading to a significant underestimation of the true rates. Monitoring diel changes in oxygen concentrations in mesocosms filled with brine from the crystallizer ponds in Eilat, Israel (1300 to 2100 *Dunaliella* cells ml^{-1}) gave net primary production rates of ~ 0.8 to $1.5 \mu\text{mol O}_2 \text{ l}^{-1} \text{ h}^{-1}$ (Elevi Bardavid et al. 2008). These rates of 120 to $220 \mu\text{g C l}^{-1} \text{ d}^{-1}$, based on a 12 h daylight time, are significantly higher than those quoted above from the study of Joint et al. (2002) in the Spanish saltern ponds populated by far denser *Dunaliella* communities. Collectively, these results show that the crystallizer brines are far from being an optimal environment for the alga, both because of supraoptimal salinities and high light intensities, but possibly also because of limitation of iron and other essential nutrients (see next section).

AVAILABILITY OF INORGANIC NUTRIENTS TO THE PHOTOSYNTHETIC MICROBIAL COMMUNITY IN THE SALTERNS

Salterns worldwide vary greatly in the concentrations of inorganic nutrients of the brines. These variations depend on geographical location, season, management practices, and many other factors. The ambient nutrient concentrations within a single pond can also vary considerably even within a few days, as shown in the study of Joint et al. (2002) in the salterns of Santa Pola, Alicante, Spain. Nutrients (nitrogen and/or phosphorus) are sometimes added as fertilizers to enhance the development of benthic microbial mats or planktonic communities of light-absorbing microorganisms (Davis 1993). Table 3 summarizes data on ammonium, nitrate, and phosphate concentrations in saltern ponds of different salt concentrations at diverse locations.

When comparing the values presented in the table, it should be noted that different analytical methods have been used in the studies cited, and that the data may therefore not always be comparable. Standard methods for the analysis of seawater, including automated assays using autoanalyzers, can generally be used

after dilution of the samples with distilled water to seawater salinity (Pedrós-Alió et al. 2000). Such dilution necessarily reduces the sensitivity and the detection limit of the procedure, but this only rarely causes problems as the concentrations to be measured are often high. The phenol hypochlorite method for ammonia determination (Solórzano 1969) can be used in hypersaline solutions of up to 125 g l^{-1} salt (Javor 2002). To properly calibrate the analytical methods, it is good practice to spike the brine samples with known concentrations of the nutrient to be measured and to check recovery. Unfortunately, this is probably seldom done, except for the study of Coleman & White (1993) in the Cheetham salterns in Queensland, Australia.

In most saltern systems, nutrient concentrations increase with increasing salinity. This may be due both to the evaporative concentration of the water and to bacterial activity. Water birds are often found in great numbers near the evaporation ponds, and their droppings can also add to the nutrient load of the salterns. The benthic microbial mats can act as nutrient scavengers as well as nutrient sources. Thus, the sharp increase in ammonium concentration in the Cabo Rojo (Puerto Rico) saltern evaporation ponds during the wet season was tentatively explained by the microbial degradation of nitrogen-containing osmolytes (glycine betaine, ectoine, etc.) accumulated by the microbial community during the dry season (Casillas-Martinez et al. 2005). Phosphate concentrations were found to increase sharply with depth in the interstitial waters of the benthic microbial mat in an Australian saltern, from $<0.3 \mu\text{M}$ in the upper 10 mm to $15 \mu\text{M}$ at a depth of 82 mm, due to the anaerobic breakdown of organic matter (Coleman & White 1993). To what extent primary production in benthic cyanobacterial mats in the salterns may be limited by a lack of inorganic nutrients has never been ascertained. The effect of nutrient addition on gross photosynthesis and respiration has been investigated in similar mats in Lake Chiprana, an inland salt lake in Spain. The addition of ammonia or phosphate did not change net photosynthesis, but phosphate ($100 \mu\text{M}$) stimulated both gross photosynthesis and respiration, while ammonium at the high concentration of 3.5 mM inhibited both processes (Ludwig et al. 2006).

The fact that nitrate concentrations are often high in crystallizer ponds — often much higher than expected based on the passive concentration of seawater alone, and generally exceed the concentrations of ammonium, is still unexplained. In the evaporation ponds of the Santa Pola, Alicante salterns, turnover of nitrate (~ 100 d turnover time at salinities below 220 g l^{-1}) was much slower than that of ammonium (2 to 14 d turnover time). On the other hand, rapid light-dependent uptake of nitrate was found in the crystallizer ponds (6 to 12 d turnover time at 370 g l^{-1} salt).

Table 3. Nutrient concentrations reported from saltern evaporation and crystallizer ponds. If necessary, values reported were converted to μM

| Location | Salinity (g l^{-1}) | Ammonium-N (μM) | Nitrate-N (μM) | Phosphate (μM) | Comments (concentrations in μM) | Source |
|--|---|--|--------------------------------------|---|---|------------------------------------|
| Europe | | | | | | |
| Santa Pola, Alicante, Spain | 370 | 3.9 | 6.2 | | | Joint et al. (2002) |
| | 316 | 1–2 | 6.2 | | | |
| | 54–150 | 1.5–2.4 | 3–4 | | | |
| Bras del Port, Alicante, Spain | Crystallizer ponds (320–370) | 150–230 | 4–18 | 5–7 | Nitrite 8 | Pedros-Alió et al. (2000) |
| | Evaporation ponds (40–250) | 40–230 | 0–4 | 4–8 | Nitrite 8 | |
| La Trinitat, Ebro Delta, Spain | Crystallizer ponds (300–370) | 3–4 | 0–38 | 1–2 | Nitrite 0 | Pedros-Alió et al. (2000) |
| | Evaporation ponds (40–250) | 3–13 | 0–3 | 2–3 | | |
| Salin de Giraud, France | All salinities?? | | <2.0–25 | <0.01–0.02 | | Landry & Jaccard (1982) |
| | All salinities | Up to 500 near decomposing mats | Up to 25 in crystallizer ponds | < 0.04 | | Britton & Johnson (1987) |
| Burgas, Bulgaria | Crystallizer ponds | | | 8.6 | Total N (ammonium + nitrate) 2.2 | Pavlova et al. (1980) |
| Megalon Embolon salt works, Greece | 65–144 | 6–16 mg l^{-1} | 0.9– 1.9 mg l^{-1} | 0.07– 0.21 mg l^{-1} | | Dolapsakis et al. (2005) |
| Australia | | | | | | |
| Dampier salterns, W. Australia | Evaporation ponds | | | 0.05–0.13 | | Sammy (1983) |
| | Crystallizer ponds | | | Below detection limit | | |
| Cheetham salterns, Queensland | Different salinities | | | 0.16–0.48 | | Coleman & White (1993) |
| America | | | | | | |
| Exportadora de Sal, Baja California, Mexico | Evaporation ponds all salinities | < 5 | < 1 | < 0.2 | | Javor (1983a,b) |
| | Crystallizer ponds | 5–10 | 10–20 | 2–5 | In bittern ponds phosphate up to 10, nitrate up to 30, ammonium up to 50 | |
| Western Salt, Chula Vista, CA, USA | All salinities | 2–20 | 2–36 | 0–3.5 | | Javor (1983a,b) |
| Cabo Rojo, Puerto Rico | Evaporation ponds, wet season, 40–150; dry season 150–265 | >400 (wet season) <8 (dry season) | | < 0.2–3.5 | | Casillas-Martinez et al. (2005) |
| Worldwide | | | | | | |
| Worldwide average | Crystallizer ponds | 15 | 10 | 1 | | Davis & Giordano (1996) |
| Worldwide typical values | Gypsum ponds | 0–7 in oligotrophic salterns, 1–50 in eutrophic salterns | | <1 in oligotrophic salterns, 1–10 in eutrophic salterns | | Javor (2002) |
| | Crystallizer ponds | 0–7 in oligotrophic salterns, 5–50 in eutrophic salterns | Typically non- detectable | 0–2 in oligotrophic salterns, up to 2–60 in eutrophic salterns | | |

Dunaliella salina can use both ammonium and nitrate as nitrogen source (Gibor 1956, Giordano & Beardall 2006). An unexpected and still unexplained finding in these studies was that far more nitrate and ammonium were taken up than could have been predicted from the carbon fixation rate: instead of the classic C:N Redfield ratio of 16:1, values found were as low as 1.0 at 220 g l⁻¹ salt and even 0.8 at 370 g l⁻¹ salt (Joint et al. 2002). The source of the often high nitrate concentrations in the salterns, however, is far from clear: autotrophic nitrification, which in normal environments is responsible for the oxidation of ammonium through nitrite to nitrate, does not operate at salt concentrations above 100 to 150 g l⁻¹ (Oren 1999, 2001).

An essential nutrient generally neglected in studies of saltern ecosystems is iron. Laboratory experiments with *Dunaliella salina* have shown that exposure to increasing salt concentrations triggers the induction of a membranal transferrin-like protein that is most probably involved in the scavenging of iron. The possibility has therefore been raised that salt concentrations may affect Fe³⁺ solubility or interfere with the iron uptake machinery (Fisher et al. 1997, 1998). Studies on the presence and availability of iron and its impact on the dynamics of primary production and other microbial processes in the salterns are therefore long overdue.

Inorganic carbon can also become a limiting factor for photosynthesis, especially in microbial mats with dense cyanobacterial communities. Carbon limitation can often be recognized by the enrichment in ¹³C in the organic matter formed, following depletion of ¹²C that is preferentially used in photosynthesis. Thus, high ‰¹³C values in specific lipids and other compounds extracted from the microbial mats in the La Trinitat and the Ebro Delta salterns in Spain were presented as evidence that the system was CO₂-limited (Schouten et al. 2001). When carbon limitation becomes even more extreme, invasion of isotopically light CO₂ from the atmosphere (the 'Baertschi effect') may, on the other hand, cause ¹³C depletion rather than ¹³C enrichment, as shown in the brines overlying the gypsum crusts of the Eilat, Israel salterns (Lazar & Erez 1990, 1992).

FINAL COMMENTS

On a global scale, solar salterns are not a major ecosystem that contributes much to primary production. However, the highly diverse biological system of the salterns, with evaporation and crystallizer ponds of different salinities, and with often high densities of planktonic as well as benthic phototrophic microorganisms, makes salterns excellent model systems for the study of primary production under a variety of conditions. Moreover, their generally good accessibility

and the relatively constant conditions maintained in each pond make the salterns a suitable environment for the study of fundamental questions about the activities of phototrophic microbial communities and the way these communities respond to their environment.

The potential that salterns thus offer to biologists has in many cases been extensively exploited. This is especially true for the benthic microbial mats that develop on the bottom of evaporation ponds of salterns worldwide. An in-depth understanding of the functioning of such mats has been obtained, including that on the tight coupling of primary production processes with other biogeochemical cycling processes operative in the system, often resulting in an efficient recycling of carbon through the mats (Canfield & Des Marais 1993, Des Marais 1995). The layered microbial communities are often embedded within a crust of gypsum that stabilizes the spatial arrangement of the organisms, making manipulation relatively easy. Other aspects of the biology of the phototrophs in salterns are still relatively neglected. For example, only few studies have addressed the planktonic microorganisms in the lower and intermediate salinity evaporation ponds and their activities. A notable exception is the recent study by Estrada et al. (2004) that examined the diversity of planktonic photoautotrophic microorganisms along a salinity gradient by microscopy, flow cytometry, pigment analysis, and DNA-based methods. Another neglected aspect is the possible importance of organic osmotic solutes that have to be produced in large amounts and accumulated in the cells to provide osmotic balance according to the salinity of the brines (Oren 2000, 2006). To what extent the biosynthesis of compounds like glycerol (made by *Dunaliella* in the crystallizer ponds), and glycine betaine, glucosylglycerol and others (accumulated by halophilic Cyanobacteria) affects primary production processes and to what extent such compounds may later be key substrates for heterotrophic microorganisms present in the system is still incompletely known (Oren 1993, 1995). We are still far removed from an understanding of the true activities of *Dunaliella salina* in saltern crystallizer ponds, where this alga is always present in large numbers. On the other hand, attempts to estimate its photosynthetic activities invariably yielded low values. As discussed above, the problem may partly be due to methodological problems, as techniques routinely applied in freshwater and marine phytoplankton studies may not always be suitable for the study of microorganisms that live in salt-saturated brines.

To answer these fundamental questions in primary production research, salterns present us with a convenient experimental system, and the results obtained in such studies are important not only for our understanding of photosynthetic processes in hypersaline lakes, but also in the marine and freshwater environments.

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