Long Term Biological Simulation Experiments of Mixing Red Sea Waters with Dead Sea Brine, and the Effect of Antiscalants on the Biology of the Dead Sea

Aharon Oren, Ittai Gavrieli, Jonah Gavrieli, Marco Kohen, Joseph Lati, Mordehay Aharoni

Cover design: Bat-Sheva Cohen

Cover:
Culture of a unicellular flagellate green alga *Dunaliella* isolated from the Dead Sea. Cells are approximately 8 µm in size.
Long Term Biological Simulation Experiments of Mixing Red Sea Waters with Dead Sea Brine, and the Effect of Antiscalants on the Biology of the Dead Sea

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ABSTRACT

To obtain a deeper understanding of the factors that determine the extent of blooms of algae (the unicellular green alga *Dunaliella*) and halophilic Archaea in the Dead Sea, and to predict the possible effects of the planned Red Sea – Dead Sea water carrier on the biology of the Dead Sea, we performed simulation experiments in 0.9 m³ experimental ponds on the grounds of the Dead Sea Works Ltd. at Sedom, as well as in the laboratory. Laboratory simulations showed that development of *Dunaliella* was possible only when Dead Sea water was diluted with minimally 10 percent (by volume) of Red Sea water. Addition of phosphate was essential for the algae to grow, and growth rates and yields increased with increasing phosphate concentration and decreasing salinity. Field simulations in outdoor ponds showed that development of algae was rapidly followed by development of dense blooms of red halophilic Archaea, which imparted an intensely red color to the ponds. While algal densities declined after the peak of the bloom had been reached, densities of halophilic Archaea and levels of archaeal pigments remained high for periods of at least two years. The results show that a microbial bloom, once formed, may remain present in the Dead Sea for long time periods. These observations are important when attempting to predict how the biological characteristics of the lake may change in the future, and they have important implications for the planning of the Red Sea – Dead Sea water carrier.

As the extent of availability of inorganic phosphate determines the occurrence of algal blooms in the Dead Sea, it is important to understand the possible impact of the addition of polyphosphate or phosphonate-based antiscalants to be considered for use in the seawater desalination plant planned in the framework of the “Peace Conduit” Red Sea – Dead Sea water carrier. We therefore tested the effects of a range of commercial antiscalant agents on the potential development of algal and archaeal blooms in Dead Sea – Red Sea or Dead Sea – freshwater mixtures. Two kinds of experiments were performed to test the effect of different antiscalants on the formation of microbial blooms in Dead Sea water: small-scale laboratory simulations and simulation experiments in mesocosms at Sedom. Phosphate-containing antiscalants were found to trigger rapid algal and/or bacterial growth, while most of the non-phosphate antiscalants did not significantly enhance the biological activity as compared to the control ponds with no additives. The finding that phosphate-containing antiscalants may lead to enhanced microbial bloomings should be considered when planning the Peace Conduit water desalination plant that will discharge its refuse brine into the Dead Sea.
1. OVERVIEW

This report summarizes experiments performed at the experimental ponds site on the grounds of the Dead Sea Works at Sedom from the beginning of 2003 until the end of 2005, as well as laboratory-scale experiments at the Institute of Life Sciences, The Hebrew University of Jerusalem. The research was initiated by the Geological Survey of Israel (GSI) in the framework of the preliminary feasibility studies of the planned Red Sea – Dead Sea water carrier (the “Peace Conduit”). It is part of a broader effort of the GSI toward the formulation of a dynamic limnological model for the Dead Sea that will model the mixing of seawater in Dead Sea brine.

The goals of the research described in this report were as follows:

1. It has been previously established that the main factors that determine the development of microbial blooms in the Dead Sea (the green alga *Dunaliella* and red halophilic Archaea of the family *Halobacteriaceae*) are the dilution of Dead Sea water with less saline water, as the Dead Sea in its present state is too saline to support microbial blooms. This conclusion was based on the monitoring of the biology of the Dead Sea in the last 25 years (massive blooms in 1980 and in 1992, which followed the formation of relatively diluted upper water layers in those particularly rainy winters), on laboratory simulations, and on simulations in mesocosms in Beth HaArava (1984-1985) and Sedom (from the summer of 2002 onwards). On the basis of the data collected thus far, it is predicted that the rate of microbial development and the extent of the microbial blooms to be expected in the future will depend on the final salinity of the upper water layers, as determined by the amounts of Red Sea water to enter the lake, the mode of mixing, and also any additional fresh water that may enter the lake in the future during winter rain floods. Therefore it is important to define the boundary conditions in terms of salinity and extent of dilution that determine whether or not microbial blooms may be expected to occur.

2. Analysis of the data collected during the natural microbial blooms (1980-1982 and 1992-1995) have shown that blooms of the unicellular green alga *Dunaliella* appear rapidly, but are also subject to rapid decline. However, the community of red halophilic Archaea that subsequently develops at the expense of organic material produced by the algae from inorganic carbon, using energy supplied by light energy from the sun, may remain present for long time periods. To examine the longevity of such blooms under controlled conditions we
have set up long-term experiments at the experimental mesocosm pond facility in Sedom to simulate such sustained blooms and to examine their properties.

3. An important part of the the “Peace Conduit” is the seawater desalination plant. To protect the expensive reverse-osmosis semipermeable membranes from clogging by crystallization of calcium salts, mainly calcium carbonate, reverse osmosis desalination plants generally add “antiscalant” agents to the water, which prevent damage to the membranes. These antiscalants end up in the reject brine from the desalination plant, which in the case of the Peace Conduit will flow into the Dead Sea. Many commercial antiscalants are based on polyphosphates or phosphonates. As it was earlier established that the extent of algal blooms in the Dead Sea is limited by the availability of inorganic phosphate, examination of the effect of different antiscalants in Dead Sea – Red Sea water mixtures is essential to assess the possible implications of the use of antiscalants in the water desalination operation to be planned, to avoid potential ecological adverse effects of the antiscalants on the entire Dead Sea ecosystem.

Much of the research presented in this report was performed at the experimental ponds facility at Sedom. The Dead Sea Works Ltd. kindly provided the site and manpower to maintain the experiments. We herewith thank the staff of the Dead Sea Works for setting up and maintaining the site, for routine sampling of the experimental ponds, and – last but not least – for their excellent hospitality during the many meetings held in Sedom.

The report is a sequel to an earlier GSI report on the microbiological aspects of the proposed Peace Conduit:

Much of our research in recent years on the biology of the Dead Sea and the possible implications of the planned Red Sea – Dead Sea water carrier on its biology has also been summarized in a number of publications that have appeared or are to appear in the international scientific literature:


2. THE UPPER SALINITY BOUNDARY FOR THE DEVELOPMENT OF MICROBIAL BLOOMS IN THE DEAD SEA, AND LONG-TERM SIMULATION OF MICROBIAL BLOOM AT THE EXPERIMENTAL MESOCOSM FACILITY IN SEDOM

The section below describes our long-term microbial bloom simulation experiments (2003-2005) in the experimental ponds at Sedom, as well as our attempts to define the minimum salinity above which no microbial blooms can be expected to occur in the lake. The text is to a large extent derived from a manuscript, based on a lecture given by Aharon Oren during the International Society for Salt Lake Research symposium held in Perth, Western Australia, in September 2005. This manuscript has been submitted for publication in the proceedings of the meeting, to appear in the future in a special volume of the journal Hydrobiologia. The text below is a slightly modified and extended version of the manuscript.

2.1. Introduction

The Dead Sea presents fascinating challenges to the biologist who attempts to understand the biological processes and the limits of life in one of the most extreme environments on Earth. Its waters contain around 340 g l⁻¹ of salts, and have a highly unusual ionic composition: divalent cations (1.89 M Mg²⁺, 0.44 M Ca²⁺) dominate over monovalent cations (1.59 M Na⁺, 0.20 M K⁺). The anions are 99% Cl⁻ (6.34 M) and 1% Br⁻ (0.068 M) (values for 1996). Sulfate concentrations are low (0.005 M), and the brine has a pH of about 6.

Only few microorganisms can survive in such an environment. Quantitatively the most important inhabitants of the water column are the unicellular green alga Dunaliella – the sole primary producer in the lake, and red extremely halophilic Archaea of the family Halobacteriaceae. Species first reported from the Dead Sea include Haloferax volcanii (Mullakhanbhai & Larsen, 1975), Haloarcula marismortui (Oren et al., 1990), Halorubrum sodomense (Oren, 1983a), and Halobaculum gomorrense (Oren et al., 1995a). Other organisms have been isolated from the Dead Sea as well, including colorless members of the domain Bacteria, protozoa, and fungi (Oren, 1988; Oren, 2003). Their quantitative importance in governing the biological properties of the lake has never been ascertained.

Systematic monitoring of the algal and prokaryotic communities in the water column of the Dead Sea since 1980 has yielded the following general picture: undiluted Dead Sea water is a too harsh environment even for the best salt-adapted microorganisms. However, exceptionally rainy winters can turn the holomictic regime into a meromictic one with the formation of a pycnocline at depths varying between 5 and about 30 m just prior to overturn
(Gavrieli & Oren, 2004; Gavrieli et al., 1999). When the surface waters become sufficiently
diluted, dense blooms of algae and red Archaea develop in the upper meters of Dead Sea
water column. Such blooms were recorded in 1980 (lasting until a renewed mixing of the
water column in the end of 1982) and in 1992 (lasting until the end of 1995). During these
blooms the density of the biota reached very high values: up to $9 \times 10^3$ and $1.5 \times 10^4$ \textit{Dunaliella}
cells ml$^{-1}$, and up to $2 \times 10^7$ and $3.5 \times 10^7$ archaeal cells ml$^{-1}$ were counted in 1980 and 1992,
respectively (Oren, 1983a; Oren, 1985; Oren, 1993a; Oren, 1997; Oren, 1999a; Oren, 2000;
Oren & Gurevich, 1993; Oren & Gurevich, 1995; Oren & Shilo, 1982; Oren et al., 1995b).
These archaeal blooms imparted a red color to the entire lake. Field observations combined
with laboratory simulations have shown that two conditions must be fulfilled for a microbial
bloom to occur in the Dead Sea: the upper water layers must become diluted to a sufficient
extent, and phosphate, the limiting nutrient in the lake, must be available.

A thorough understanding of the biological phenomena in the Dead Sea and the factors
that determine the nature and extent of biological blooms in the lake is of great importance
when planning human interference in the properties of the lake. During the past decade the
level has dropped approximately one meter per year on the average (Gavrieli & Oren, 2004;
Oren & Gavrieli, 2002; Yechieli et al., 1998). This drop in water level is causing severe
problems in the area, most notably the development of sinkholes along the shores (Abelson et
al., 2003; Arkin and Gilat, 2000), which threatens and damages local infrastructure, tourism
and industrial activities. Currently a proposal is being investigated for the construction of the
"Peace Conduit", connecting the Dead Sea with the Gulf of Aqaba (Red Sea). This planned
water carrier is intended to counteract the drop in Dead Sea water level, mitigating the
damaging processes that currently occur in the Dead Sea and its surrounding area. The
difference in elevation between the Red Sea and the Dead Sea (current surface level: -418 m)
may be exploited for energy generation and seawater desalination (Gavrieli et al., 2005; Oren
et al., 2004; Oren et al., 2005).

The present study, combining laboratory model experiments with simulations in outdoor
ponds, was intended to provide answers to two basic questions: (1), what are the boundary
conditions with respect to dilution and phosphate concentrations that enable the development
of algae in the Dead Sea, and (2), how long may algal and archaeal blooms, once formed,
remain present in the lake if the limnological conditions will remain constant.
2.2. Methods

*Field-scale simulation experiments in the experimental ponds at Sedom*

Mixtures of Dead Sea water and Red Sea water were incubated in experimental ponds on the grounds of the Dead Sea Works Ltd. at Sedom (Fig. 1). The experimental setup consisted of white plastic tanks (1 x 1 x 1 m; Dolav, Kibbutz Dvir, Israel), buried for 75% in the ground.

These tanks were filled with 0.9 m$^3$ of mixtures of Dead Sea water (from the northern basin of the lake, sampled from the channel that feeds the evaporation ponds of the Dead Sea Works Ltd.) and water from the Gulf of Aqaba, purified through a filter of 60-70 cm sand, eliminating particles larger than 20-30 µm. The first set of experiments performed at the site, initiated in July 2002, as well as further details of the experimental setup, have been documented by Oren et al. (2004). The experiments documented in the present paper started in 2002-2003, and are based on two ponds. One pond (“no. 4”) was filled with a mixture of 80% Dead Sea water and 20% Red Sea water, amended with 1 µM KH$_2$PO$_4$ and inoculated with 50 ml of brine from a pond that had developed a bloom of *Dunaliella* and halophilic Archaea in a previous set of experiments. The second pond (“no. 9”) contained a 1:1 mixture of water from ponds no. 9 and 10 from the earlier 2002 experiment. Ponds 9 and 10 both contained a 70% Dead Sea water – 30% Red Sea mixture and 1 and 10 µM KH$_2$PO$_4$, respectively, thus the new experiment was based on brine that had received an equivalent concentration of 5.5 µM phosphate, and started at the high algal and archaeal densities that had developed in the first round of experiments as documented (Oren et al., 2004). The ponds were stirred daily, and their water level was kept constant by adding deionized water every 1-2 days, followed by thorough mixing. In November 2004, an additional...
portion of 1 µM KH$_2$PO$_4$ was added to pond no. 4. The ponds were sampled once every two weeks for the determination of the density of the *Dunaliella* population, the halophilic archaeal community density, and the content of algal chlorophyll and archaeal carotenoids. The table below presents the ion concentration analyses of samples withdrawn from the two ponds on March 31, 2004 and on November 24, 2004. It should be noted that the values measured for pond no. 4 in March 2004 are unexpectedly low, possibly due to incomplete mixing after addition of distilled water to compensate for water loss by evaporation.

**Laboratory-scale simulation experiments of microbial development in Dead Sea – Red Sea water mixtures**

To examine in further depth the effect of salinity and phosphate concentration on the development of *Dunaliella* in Dead Sea – Red Sea waters, we set up laboratory experiments in which 100-ml Erlenmeyer flasks were filled with 75 ml of mixtures of Dead Sea water (sampled in July 2005 from a depth of 10 m at the deepest point of the lake 8 km north-east of Ein Gedi) and filtered Red Sea water at different ratios. Different concentrations of KH$_2$PO$_4$ were added, and all flasks were inoculated with a culture of *Dunaliella* from the Dead Sea in 80% Dead Sea water – 20% Red Sea water to supply an inoculum of about 500 *Dunaliella* cells ml$^{-1}$. The flasks were incubated at 30°C under constant illumination (100 µM quanta m$^{-2}$ s$^{-1}$) by white fluorescent tubes. To prevent evaporation and to ensure constant salinity for the duration of the experiment, the flasks were closed with Parafilm. After 25 days samples were withdrawn for microscopic enumeration of *Dunaliella* cells and chlorophyll assay.

<table>
<thead>
<tr>
<th>Ion</th>
<th>Pond no. 4 (80% Dead Sea water - 20% Red Sea water)</th>
<th>Pond no. 9 (70% Dead Sea water - 30% Red Sea water)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>31.3.2004</td>
<td>24.11.2004</td>
</tr>
<tr>
<td>Na$^+$</td>
<td>22,400</td>
<td>27,900</td>
</tr>
<tr>
<td>K$^+$</td>
<td>4,900</td>
<td>6,100</td>
</tr>
<tr>
<td>Ca$^{2+}$</td>
<td>11,300</td>
<td>14,100</td>
</tr>
<tr>
<td>Mg$^{2+}$</td>
<td>28,500</td>
<td>35,300</td>
</tr>
<tr>
<td>Sr$^{2+}$</td>
<td>210</td>
<td>255</td>
</tr>
<tr>
<td>Cl$^-$</td>
<td>142,490</td>
<td>173,960</td>
</tr>
<tr>
<td>Br$^-$</td>
<td>3,190</td>
<td>3,898</td>
</tr>
<tr>
<td>SO$_4^{2-}$</td>
<td>416</td>
<td>685</td>
</tr>
<tr>
<td>Total dissolved salts</td>
<td>213,406</td>
<td>262,511</td>
</tr>
<tr>
<td></td>
<td>231,480</td>
<td>224,015</td>
</tr>
</tbody>
</table>

Ion concentrations in the experimental ponds (values in mg/l), as measured on March 31, 2004.
**Enumeration of algae and halophilic Archaea**

To count the density of the algal (*Dunaliella*) and halophilic archaeal communities, 50 ml of samples from the experimental ponds were fixed with 1 ml of 37% formaldehyde, previously cleared by filtration through a 0.2 µm pore size membrane filter. Samples were stored at room temperature until analysis, performed within 3-4 weeks.

For the enumeration of *Dunaliella* cells, 2.5-ml portions of fixed samples were supplemented with 0.1 ml of 0.1 N iodine to stain intracellular starch. The samples were then filtered through Millipore filters (25 mm diameter, 5 µm mean pore size, cat. no. SMWP-25). Filters were placed on microscope slides, and cells were counted under a 16x or a 40x objective. A similar procedure was followed for the enumeration of algal cells in laboratory experiments. No prior fixation was used in this case, and variable volumes of water filtered were filtered according to the density of *Dunaliella* in the flasks. Cell numbers were calculated from the average number of cells per field and the field diameter, calibrated with the aid of the grid of a Petroff-Hauser counting chamber (Oren and Shilo, 1982; Oren et al., 1995b). Prokaryotic cells (Archaea and Bacteria combined) were enumerated microscopically using a Petroff-Hauser counting chamber after 5-10-fold concentration by centrifugation (20 min, 12,000 x g). The relative accuracy of the algal and prokaryotic cell counts was estimated at ± 10 and 20%, respectively.

**Pigment determinations**

The content of chlorophyll and carotenoids in the experimental ponds was determined by filtering 50-ml sample portions through glass fiber filters (Whatman GF/C, 47 mm diameter) within 1 hour after sampling. Filters were kept at –10°C in the dark until further processing within 3-4 weeks. Filters were then extracted overnight in 5 ml methanol/acetone (1:1, by volume). For the determination of chlorophyll in laboratory cultures, 30 ml portions of liquid were filtered through 25 mm diameter GF/C filters, and the filters were extracted in 2.5 ml of methanol/acetone. The extracts were cleared of particles by centrifugation, and their absorption spectra (400-700 nm) were measured in a Cary Varian model E1 scanning spectrophotometer, using the solvent as a blank. Chlorophyll concentrations were calculated, assuming a specific absorption of 73.5 l mg⁻¹ cm⁻¹ at 665 nm.

Archaeal bacterioruberin pigments were quantified based on a specific absorption of 25.4 l mg⁻¹ cm⁻¹ at 496 nm for α-bacterioruberin. A correction was made for the contribution of algal pigments to the total absorbance at this wavelength, as outlined in Oren et al. (2004).
2.3. Results

Our outdoor simulation experiments in 0.9 m³ ponds show that once a microbial bloom has formed in a Dead Sea – Red Sea water mixture, it can persist for at least two years (Figs. 2 and 3).

Figure 2 documents a bloom of *Dunaliella* and halophilic Archaea in a mixture of 80% Dead Sea water and 20% Red Sea water, supplemented with 1 µM orthophosphate (pond no. 4 in the outdoor experiment). Algal numbers reached values of up to 12,000 cells ml⁻¹ (16 µg chlorophyll liter⁻¹) after 4 months, and then declined to low values. Mass development of algae was followed by the growth of halophilic Archaea, which are heterotrophic microorganisms that develop at the expense of organic compounds produced by the autotrophic algae. We counted up to 65x10⁶ prokaryotic cells ml⁻¹, and their bacterioruberin carotenoids (up to 18 µg liter⁻¹) imparted an intensely red color to the brine. This community remained present at 20-30x10⁶ cells ml⁻¹ throughout the experiment, without any major decline for more than two years. To prove that further algal development was limited by the availability of phosphate after the initially added phosphate had been taken up by the microbial community and was incorporated in the biomass, we added an additional 1 µM orthophosphate in November 2004. This addition quickly resulted in a renewed development of *Dunaliella*.

A similar long-living microbial bloom was obtained in pond no. 9 that had received a mixture of 70% Dead Sea water – 30% Red Sea and 5.5 µM phosphate (Fig. 3). This experiment was a continuation of an experiment set up in July 2002, documented earlier (Oren et al., 2004). It shows once more that such microbial blooms can be sustained for long periods, in this case for over two years.
Figure 2. Numbers of *Dunaliella* cells, concentrations of chlorophyll, numbers of archaeal cells, and concentration of bacterioruberin carotenoids in an outdoor pond filled with a mixture of 80% Dead Sea water and 20% Red Sea water, and amended with 1 µM KH$_2$PO$_4$ from the start of the experiment in March 2003 until August 2005. An additional portion of 1 µM KH$_2$PO$_4$ was added in November 2004 (arrow).

The examples shown in Figs. 2 and 3 are part of a more extensive set of experiments in which we examined the effect of different parameters on the timing and extent of microbial development in Dead Sea – Red Sea mixtures. Some of the early experiments have been described in an earlier paper (Oren et al., 2004). Without added phosphate no significant algal and archaeal blooms were observed. Insoluble finely powdered rock phosphate (apatite) did not trigger blooms either (not shown).
Figure 3. Numbers of Dunaliella cells, concentrations of chlorophyll, numbers of archaeal cells, and concentration of bacterioruberin carotenoids in an outdoor pond filled with a mixture of 70% Dead Sea water and 30% Red Sea water. In March 2003 the pond was filled with a 1:1 mixture of water from ponds no. 9 and 10 from an earlier experiment, which contained 70% Dead Sea water – 30% Red Sea and 1 and 10 µM KH2PO4 (Oren et al., 2004), explaining the high initial values of biological parameters.

To further test the boundary conditions that enable the onset of an algal bloom in Dead Sea – Red Sea water mixtures, we set up a laboratory simulation experiment in which different mixtures were supplemented with different concentrations of orthophosphate and an inoculum of Dunaliella. After incubation in the light for four weeks at 30 °C, algae developed only when the concentration of Dead Sea water in the mixtures was below 90%, and the rate at which the cells multiplied increased with decreasing salinity of the water mixture. The extent of the algal growth obtained was a function of the concentration of phosphate added (Fig. 4). These results confirm and extend laboratory simulation
experiments performed in the early 1980s in which Dead Sea water was diluted with freshwater (Oren & Shilo, 1985).

Figure 4. Development of *Dunaliella* cells and chlorophyll in a laboratory simulation experiment in which Dead Sea – Red Sea water mixtures were incubated for 25 days at 30 °C in the light in the presence of 1 (▲), 2.5 (■) and 5 µM KH₂PO₄ (●) and an inoculum of 500 *Dunaliella* cells ml⁻¹, whereafter the density of *Dunaliella* cells (left panel) and the chlorophyll content of the water (right panel) were determined. Without added phosphate no *Dunaliella* growth was observed.

2.4. Discussion

Compared to all other aquatic environments of lower salinity, the Dead Sea is a very simple ecosystem. Higher animals are absent, and protozoa, if they are present at all, do not appear to play a significant role in regulating community densities of unicellular algae and heterotrophic prokaryotes. The main players are one type of primary producer – the alga *Dunaliella*, and several species of halophilic Archaea (Kaplan & Friedmann, 1970; Oren, 1988; Oren, 1997). Figure 5 presents a general model of the biological processes that occur in the aerobic water column of the Dead Sea, the organisms involved, and some of the interrelationships between the biota. This model is based on observations of the dynamics of algal and archaeal communities in the lake, laboratory simulations, as well as field-scale simulations such as documented in the present study.
Figure 5. Schematic representation of the processes that govern the development of algal and archaeal blooms in the Dead Sea water column.

Undiluted Dead Sea water is too harsh an environment even for *Dunaliella*, the best salt-adapted alga known. Therefore algal blooms, and subsequent mass development of Archaea that live at the expense of organic material produced by the algae, can only occur after dilution with less saline water. Analysis of the biological events following the rainy winters of 1979-1980 and 1991-1992 have provided ample illustration of this (Oren, 1983b; Oren, 1993a; Oren & Gurevich, 1995; Oren & Shilo, 1992; Oren et al., 1995b), as have simulation experiments (Oren & Shilo, 1985; Oren et al., 2003; Fig. 4 in the present study). One of the organic compounds produced in massive amounts by *Dunaliella* is glycerol, used by the algae to provide osmotic stabilization. Evidence has accumulated that this glycerol is probably one of the major nutrients used by the halophilic Archaea (Oren, 1993b; Oren, 1994).

Phosphate is clearly the limiting nutrient that governs the extent of microbial blooms in the lake. Inorganic nitrogen is plentifully available in the Dead Sea in the form of ammonium ions, but phosphate concentrations are low. Stiller & Nissenbaum (1999) and Nissenbaum et al. (1990) reported dissolved phosphate levels of about 1 µM. Due to the difficulty in performing high-precision chemical analyses in the presence of molar concentrations of other interfering salt it cannot be ascertained to what extent this value
indeed represents the actual concentration of biologically available phosphate. Furthermore, little information has been obtained on the spatial and temporal variation in the concentration of dissolved phosphate in the Dead Sea water column. In any case, the dramatic response of the *Dunaliella* community to phosphate addition in laboratory and field-scale simulation experiments unequivocally proves its importance as a key nutrient that determines the properties of the Dead Sea as an ecosystem. Following uptake by the algae, the phosphate becomes fixed in the algal and archaeal biomass. Addition of more phosphate provides the opportunity for renewed algal growth (Fig. 2). However, this was not followed by a rapid increase in bacterial numbers.

The simulation studies documented in Figs. 2 and 3, as well as in an earlier publication (Oren et al., 2004), show that mass development of *Dunaliella* is generally followed by a rapid decline. The causes of this decline are still poorly understood. Following the 1992 spring bloom of the alga, the cells were observed to form cyst-like structures, possibly zygotes, which sank to the bottom (Oren et al., 1995b). Evidence has been obtained that such thick-walled cysts serve as the inoculum that enables rapid development of *Dunaliella* in the Dead Sea as soon as the upper water layers become diluted by freshwater floods (Oren, 1999b; Oren & Ben-Yosef, 1997). We never observed formation of such cysts in the experimental outdoor ponds.

While algal blooms, both in the Dead Sea itself and in the pond simulation experiments, were always of limited duration, the Archaea remained present for very long times both in the lake (Oren, 1983b; Oren & Gurevich, 1995,) and in the experimental ponds which after 2-3 years remained as brightly red-colored as when the bloom first started (Figs. 2 and 3). It has been suggested that the halophilic Archaea in the Dead Sea can to some extent use light energy absorbed by the retinal pigment bacteriorhodopsin as an energy source for maintenance (Oren, 1983c; Oren & Shilo, 1981). Little is known about the factors that remove archaeal cells from the Dead Sea water column. Overturn of the water column with mixing of the Archaea-rich upper layer with the lower water masses has been a major factor in the decrease in prokaryote densities following the 1980-1982 and 1992-1995 blooms (Oren, 1985; Oren, 1988; Oren, 2000; Oren, 2003). In addition, in an experimental pond identical to pond no. 9, to which no water was added to compensate for evaporation, salinity increase was accompanied by rapid disappearance of the bacteria and of the red color. Thus, increased salinity is clearly an important factor controlling bacterial blooming in the lake. Bacteriophages may also be involved in regulating archaeal community densities in the lake, as direct electron microscopic examination revealed large numbers of phage-like particles
(Oren et al., 1997). However, their true impact on the community dynamics has never been ascertained (Oren, 1999b).

Understanding the factors that trigger the development of microbial blooms and determining their longevity is important in the planning of the Red Sea – Dead Sea water carrier (Gavrieli et al., 2005; Oren et al., 2004; Oren et al., 2005). A permanent stratification is likely to develop as the upper layers of the lake will become diluted with much less dense Red Sea water, whether or not concentrated in a process of reverse osmosis desalination. Experiments such as those presented in Fig. 4 suggest that conditions will be established for the development of blooms as soon as the upper layers will become diluted by more than 10% by the waters from the Red Sea. The extent of these blooms will be a direct function of the availability of phosphate. The waters of the Gulf of Aqaba are very low in phosphate, but other sources (e.g., plankton in the Red Sea water, phosphate entering with flood waters from the catchment area, and runoff due to human activities) can be quantitatively far more important. It should be stressed here that the experiment shown in Fig. 4 was performed at 30 °C. These experiments will soon be extended to both lower and higher temperatures as may be relevant to the Dead Sea ecosystem (between 20-35 °C), to check for any temperature effects on the minimal salinity value that may support microbial blooms. It is well known that the salinity range (minimum, optimum and maximum) of certain halophilic or halotolerant microorganisms shifts toward higher values as the temperature is increased, see e.g. the case of the Dead Sea archaeon *Haloferax volcanii* as documented by Mullakhanbhai & Larsen (1975).

The results of the simulation experiments documented in this study show that the conditions that lead to the formation of a microbial bloom in the Dead Sea are now quite well understood. They also show that such blooms, once formed, can remain present for long periods and determine to a large extent the properties of the lake for many years. Yet, it should be pointed out that no reports are available from the first half of the 20th century, when the upper water column of the Dead Sea was relatively diluted, which describe blooming and red or green coloration of the surface water. Whether dense communities of microorganisms were present at the time can no longer be ascertained.
3. Simulation of the Effects of Antiscalant Compounds on the Microbiology of the Dead Sea

3.1. Introduction

The extent of algal blooms in the Dead Sea is determined not only by the degree of dilution of Dead Sea water, but also by the availability of inorganic phosphate. Therefore it is important to understand the possible impact of the addition of polyphosphate or phosphonate-based antiscalants to be considered for use in the planned seawater desalination plant. We therefore tested the effects of a range of commercial antiscalant agents on the potential development of algal and archaeal blooms in Dead Sea – Red Sea or Dead Sea – freshwater mixtures. The simplest such antiscalant, sodium hexametaphosphate (NaPO$_3$)$_6$ (“Calgone”) was not tested as we assumed that the phosphorus in this compound will easily be converted to biologically available orthophosphate.

Below follows a list of the antiscalants included in our studies. Their phosphorus content was assayed at the Geological Survey of Israel unless stated otherwise.

**PermaTreat PC-191** (Nalco)
- Described in the factory-supplied Material Safety Data Sheet as a phosphonate.
- Recommended concentration for use: 4 ppm.
- Phosphorus content: 7.9 ± 0.8%.

**SpectraGuard** (Professional Water Technologies Inc.)
- Recommended concentration for use with seawater: 2 ppm.
- Phosphorus content: < 10 ppm.

**Spectraguard/Bioguard** (Professional Water Technologies Inc.)
- This is a combination of the SpectraGuard antiscalant mentioned mixed at a 1:1 ratio with a biocidal compound intended to prevent development of microbial biofilms on the reverse osmosis membranes.
- Recommended concentration for use with seawater: 4 ppm.
- Phosphorus content: < 10 ppm.
Z-30 (AmGal)
Described in the factory-supplied Material Safety Data Sheet as containing organic
derivatives of phosphorus and nitrogen (organic phosphonates), polycarboxylic acids -
sodium salt, stabilizers and deionized water.
Recommended concentration: 2-5 ppm.
Phosphorus content: 9.5% ± 1%.

Aktiphos 641 (BK Giulini)
Described in the factory-supplied Material Safety Data Sheet as a mixture of
phosphorous sulfonate and polycarbonate.
Phosphorus content: 2.5% ± 0.1%.
Recommended concentration: 2-10 ppm.

EL-5600 (Nalco)
Described in the factory-supplied Material Safety Data Sheet as containing: “Water,
Polymer, Salts”.
Recommended concentration: 4 ppm.
Phosphorus content 1.6% ± 0.1%.

Turbodispin D-100 (BK Giulini)
Described in the factory-supplied Material Safety Data Sheet as a “sulfonized
polycarboxylic acid sodium salt in water”.
Recommended concentration: 4 ppm.
Phosphorus content: 22.2 ppm (<10 and 51 ppm in duplicate assays).

Aquarex 1211 (Tambour Ecology)
Described in the Material Safety Data Sheet as a multifunctional antiscalant for reverse
osmosis systems, highly effective in preventing calcium carbonate, calcium sulfate,
calcium fluoride and metal oxide scale formation. No details were given regarding its
chemical composition.
Recommended concentration: 2-6 ppm.
Phosphorus content: 5.77% (as analyzed by IMI, Haifa).

Two kinds of experiments were performed to test the effect of different antiscalants on the
formation of microbial blooms in Dead Sea water: small-scale laboratory simulations and
simulation experiments in mesocosms at Sedom. The methods employed for the quantitation
of the communities of *Dunaliella* and halophilic Archaea and their pigments are as described on pp. 13-14.

### 3.2 Laboratory simulations of the stimulating activity of different antiscalants on the growth of dunaliella in diluted Dead Sea water

We performed an experiment in which 100 ml portions of 70% Dead Sea water – 30% distilled water in 250 ml Erlenmeyer flasks were supplemented with different antiscalant compounds (PermaTreat PC-191, Turbodispin D-100, Aktiphos-641, Aquerex, Z-30, and EL-5600). The concentrations used of these compounds were taken so as to comply with the average usage concentrations as recommended by the manufacturers for the maintenance of reverse osmosis membrane systems. To simulate long-term degradation of the antiscalant compounds, some of the flasks received antiscalant that had been heated for 1 hour or for 20 hours at 80 °C in 70% Dead Sea water or in distilled water at a concentration 100x higher than the final concentration added in the experiment. All flasks were supplemented with 0.5 ml of a culture of *Dunaliella* grown in 70% Dead Sea water + 10 μM KH$_2$PO$_4$, and were incubated in an illuminated incubator room at 30 °C. The experiment was started on April 19, 2004, and the chlorophyll content of the cultures, as a measure of *Dunaliella* development, was assessed on May 9. The results are shown in Figs. 6-8. For each compound the extent of algal growth was given, as compared to a control experiment without additives and a control enriched with 10 μM KH$_2$PO$_4$. Results shown for pre-heated antiscalant samples refer to those systems in which the compounds investigated were heated in 70% Dead Sea water. Experiments in which the antiscalant compounds were pre-heated in distilled water gave qualitatively similar results.
Figure 6. Effect of the antiscalants PermaTreat PC-191 and Turbodispin D-100 on algal development in 70% Dead Sea water in a laboratory simulation experiment.

Figure 7. Effect of the antiscalants Aktiphos-641 and Aquerex on algal development in 70% Dead Sea water in a laboratory simulation experiment.
Figure 8. Effect of the antiscalants Z-30 and EL-5600 on algal development in 70% Dead Sea water in a laboratory simulation experiment.

Three antiscalants strongly stimulated development of *Dunaliella*: PermaTreat PC-191, Aquerex, and Z-30. Turbodispin D-100 and Aktiphos-641 had no significant effect. Pre-heating had no great effect on the outcome of these experiments: those compounds that did not increase algal growth also did not do so after the heating treatment; for PermaTreat PC-191 and Aquerex, growth of *Dunaliella* was somewhat faster in the bottles that had received pre-heated antiscalant than in the non-heated control, suggesting that the heat treatment may have accelerated the release of available orthophosphate from the compounds. The results for EL-5600 (Fig. 8) were somewhat less clear: some growth was observed following enrichment with untreated antiscalant, while addition of heat-treated EL-5600 did not lead to any significant stimulation of *Dunaliella* growth. Repetition of this experiment is therefore recommended.

In the second experiment, 100 ml portions of 70% Dead Sea water – 30% distilled water in 250 ml Erlenmeyer flasks were supplemented with Spectroguard and Spectroguard/Bioguard (= Spectroguard supplemented with a biocidal compound). Both compounds were used as a stock solution of 38.8 mg/ml, of which portions of 30 or 150 µl were added to the experimental systems, yielding final concentrations of 11.6 and 58.2 ppm., respectively. To simulate long-term degradation of the antiscalant compounds, some of the flasks received
antiscalant that had been heated for 1 hour at 80 °C. Control systems included bottles without added antiscalant and bottles enriched a control enriched with 10 µM KH$_2$PO$_4$. To assess the efficacy of the biocidal effect of the Spectroguard/Bioguard mixture, additional control systems were set that contained both 150 µl of the antiscalant mixture and 10 µM KH$_2$PO$_4$. All flasks were supplemented with 0.5 ml of a culture of Dunaliella grown in 70% Dead Sea water + 10 µM KH$_2$PO$_4$, and were incubated in an illuminated incubator room at 30 °C. The experiment was performed in November-December 2004. The chlorophyll content of the cultures, as a measure of Dunaliella development, was assessed after 13, 16 and 20 days. The results are shown in Figs. 9 and 10.

![Graph 1](image1)

**Figure 9.** Effect of the antiscalants Spectroguard and Spectroguard/Bioguard (final concentrations: 11.6 and 58.2 ppm) on algal development in 70% Dead Sea water in a laboratory simulation experiment.
Figure 10. Effect of the antiscalants Spectroguard and Spectroguard/Bioguard (final concentration: 58.2 ppm) after pre-heating on algal development in 70% Dead Sea water in a laboratory simulation experiment, and assessment of the biocidal effect of Bioguard on the development of *Dunaliella*.

Neither SpectraGuard nor the SpectraGuard/BioGuard cocktail caused any significant development of *Dunaliella* in these experiments. However, when heated for 1 hour at 80 °C, sufficient inorganic phosphate was released to support rapid growth of the alga. It should be noted that the presence of “BioGuard”, which supposedly acts as a biocidal compound, did not inhibit development of *Dunaliella* at the concentrations applied, as massive growth of *Dunaliella* was observed both in 70% Dead Sea water supplemented with both SpectraGuard/BioGuard and inorganic phosphate. Similarly, algal growth was similar in the
The experiment was repeated in December 2005. Erlenmeyer flasks (125 ml) were set up with 100 ml of 70% Dead Sea water (sampled 13.7.2004 from a depth of 10 m at station Ein Gedi 310. SpectraGuard and SpectraGuard/BioGuard (20 or 100 ppm) and KH$_2$PO$_4$ (10 µM) were added as specified in the table below. In some of the systems the antiscalants were first heated for 1 or 4 hours at 80°C, either as supplied by the manufacturer or following diluted 1:10 in Dead Sea water. All bottles received 0.2 ml inoculum from a Dunaliella culture grown in 70% Dead Sea water + 100 µM phosphate. The systems were incubated in the light at 30°C, and chlorophyll concentrations were determined after 13 and 20 days.

<table>
<thead>
<tr>
<th>No.</th>
<th>Additive</th>
<th>Chlorophyll (µg/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>13 days</td>
</tr>
<tr>
<td>1</td>
<td>None</td>
<td>&lt; 5</td>
</tr>
<tr>
<td>2</td>
<td>None</td>
<td>&lt; 5</td>
</tr>
<tr>
<td>3</td>
<td>10 µM phosphate</td>
<td>105</td>
</tr>
<tr>
<td>4</td>
<td>10 µM phosphate</td>
<td>134</td>
</tr>
<tr>
<td>5</td>
<td>20 ppm SpectroGuard</td>
<td>&lt; 5</td>
</tr>
<tr>
<td>6</td>
<td>20 ppm SpectroGuard</td>
<td>&lt; 5</td>
</tr>
<tr>
<td>7</td>
<td>20 ppm SpectroGuard /BioGuard</td>
<td>&lt; 5</td>
</tr>
<tr>
<td>8</td>
<td>100 ppm SpectroGuard</td>
<td>&lt; 5</td>
</tr>
<tr>
<td>9</td>
<td>100 ppm SpectroGuard</td>
<td>&lt; 5</td>
</tr>
<tr>
<td>10</td>
<td>100 ppm SpectroGuard /BioGuard</td>
<td>&lt; 5</td>
</tr>
<tr>
<td>11</td>
<td>100 ppm SpectroGuard, 1 h heated</td>
<td>&lt; 5</td>
</tr>
<tr>
<td>12</td>
<td>100 ppm SpectroGuard /BioGuard, 1 h heated</td>
<td>&lt; 5</td>
</tr>
<tr>
<td>13</td>
<td>100 ppm SpectroGuard, 1 h heated in Dead Sea water</td>
<td>&lt; 5</td>
</tr>
<tr>
<td>14</td>
<td>100 ppm SpectroGuard /BioGuard, 1 h heated in Dead Sea water</td>
<td>&lt; 5</td>
</tr>
<tr>
<td>15</td>
<td>100 ppm SpectroGuard, 4 h heated</td>
<td>&lt; 5</td>
</tr>
<tr>
<td>16</td>
<td>100 ppm SpectroGuard /BioGuard, 4 h heated</td>
<td>&lt; 5</td>
</tr>
<tr>
<td>17</td>
<td>100 ppm SpectroGuard, 4 h heated in Dead Sea water</td>
<td>&lt; 5</td>
</tr>
<tr>
<td>18</td>
<td>100 ppm SpectroGuard /BioGuard, 4 h heated in Dead Sea water</td>
<td>&lt; 5</td>
</tr>
<tr>
<td>19</td>
<td>10 µM phosphate + 100 ppm SpectroGuard /BioGuard</td>
<td>83</td>
</tr>
<tr>
<td>20</td>
<td>10 µM phosphate + 100 ppm SpectroGuard /BioGuard</td>
<td>124</td>
</tr>
<tr>
<td>21</td>
<td>10 µM phosphate + 100 ppm SpectroGuard /BioGuard</td>
<td>99</td>
</tr>
<tr>
<td>22</td>
<td>10 µM phosphate + 100 ppm SpectroGuard /BioGuard</td>
<td>125</td>
</tr>
</tbody>
</table>

In this experiment neither SpectroGuard nor SpectroGuard/BioGuard caused any stimulation of algal growth. As before, presence of the biocidal compound in the SpectraGuard/BioGuard mixture did not inhibit development of the algae.
3.3 Mesocosm simulations of the stimulating activity of different antiscalants on the growth of *Dunaliella* and halophilic Archaea in diluted Dead Sea water

The first series of mesocosm simulations with antiscalant compounds was set up in April 2004 and lasted until November 2004. The ponds contained 900 liter of a mixture of 80% Dead Sea water – 20% Red Sea water. The following antiscalants were included in the experiment:

<table>
<thead>
<tr>
<th>Antiscalant</th>
<th>Grams added to 900 liter pond</th>
<th>To yield antiscalant concentration in ppm</th>
</tr>
</thead>
<tbody>
<tr>
<td>PermaTreat PC-191</td>
<td>7.06</td>
<td>8</td>
</tr>
<tr>
<td>Z-30</td>
<td>5.87</td>
<td>6</td>
</tr>
<tr>
<td>Aktiphos 641</td>
<td>22.3</td>
<td>24</td>
</tr>
<tr>
<td>EL-5600</td>
<td>34.9</td>
<td>38</td>
</tr>
<tr>
<td>Turbodispin D-100</td>
<td>34.9</td>
<td>38</td>
</tr>
</tbody>
</table>

The concentrations were chosen so that for the phosphorus-rich antiscalants (PermaTreat PC-191, Z-30, Aktiphos 641, and EL-5600) the estimated phosphorus concentration added, as based on our analyses of the phosphorus content of the antiscalant preparations, was 20 milliequivalent P per liter. For the “phosphorus-free” antiscalant Turbodispin D-100 we used the same concentration (in ppm) as for EL-5600.

Control systems included in the experiment were a pond with 80% Dead Sea water – 20% Red Sea water without added antiscalant, as well as a pond with 80% Dead Sea water – 20% Red Sea water amended with 1 µM KH₂PO₄.

Figures 11-16 show the results of the experiments. In these figures the upper two panels (*Dunaliella* cells/ml and chlorophyll concentration in µg/l) are a measure of algal development; the lower two panels (bacteria – probably mostly red Archaea, in millions of cells/ml and bacterioruberin – the red carotenoid pigment of the Archaea, in µg/l) describe the extent of bacterial development. The solid lines with the solid symbols present the data for the pond supplemented with antiscalant or inorganic phosphate, the dashed lines connecting open symbols represent the control pond without additives.

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Figure 11. Effect of the addition of 1 µM KH$_2$PO$_4$ on the development of *Dunaliella* (cells/ml in the upper panel, chlorophyll in the second panel) and halophilic Archaea (cells/ml in the third panel, bacterioruberin pigments in the lower panel) in the experimental ponds at Sedom, April-November 2004.
Figure 12. Effect of the addition of Permatreat PC-191 (8 ppm) on the development of *Dunaliella* (cells/ml in the upper panel, chlorophyll in the second panel) and halophilic Archaea (cells/ml in the third panel, bacterioruberin pigments in the lower panel) in the experimental ponds at Sedom, April-November 2004.
Figure 13. Effect of the addition of Z-30 (6 ppm) on the development of *Dunaliella* (cells/ml in the upper panel, chlorophyll in the second panel) and halophilic Archaea (cells/ml in the third panel, bacterioruberin pigments in the lower panel) in the experimental ponds at Sedom, April-November 2004.
Figure 14. Effect of the addition of Aktiphos 641 (22.3 ppm) on the development of *Dunaliella* (cells/ml in the upper panel, chlorophyll in the second panel) and halophilic Archaea (cells/ml in the third panel, bacterioruberin pigments in the lower panel) in the experimental ponds at Sedom, April-November 2004.
Figure 15. Effect of the addition of EL-5600 (38 ppm) on the development of *Dunaliella* (cells/ml in the upper panel, chlorophyll in the second panel) and halophilic Archaea (cells/ml in the third panel, bacterioruberin pigments in the lower panel) in the experimental ponds at Sedom, April-November 2004.
Figure 16. Effect of the addition of Turbodispin D-100 (38 ppm) on the development of *Dunaliella* (cells/ml in the upper panel, chlorophyll in the second panel) and halophilic Archaea (cells/ml in the third panel, bacterioruberin pigments in the lower panel) in the experimental ponds at Sedom, April-November 2004.
The Sedom pond simulation experiments, which included monitoring of the bacterial communities as well, a parameter not measured in the short-term laboratory simulations described above, largely confirmed the results obtained in the laboratory. A summary of the results is presented in the table below.

<table>
<thead>
<tr>
<th>Antiscalant</th>
<th>Pond experiment</th>
<th>Short-term laboratory simulation</th>
</tr>
</thead>
<tbody>
<tr>
<td>PermaTreat PC-191</td>
<td>Strong stimulation of algal growth and concomitant bloom of red bacteria</td>
<td>Increase in algal growth</td>
</tr>
<tr>
<td>Z-30</td>
<td>Only moderate increase in algal growth. However, a strong increase in bacterial numbers and bacterial pigments was observed, suggesting that the bacteria may grow on the antiscalant compound</td>
<td>Increase in algal growth</td>
</tr>
<tr>
<td>Aktiphos 641</td>
<td>Significant stimulation of both algal and bacterial development</td>
<td>No stimulation of algal growth, also not after pre-heating</td>
</tr>
<tr>
<td>EL-5600</td>
<td>Strong increase in algal numbers, with only a moderate increase in bacterial development</td>
<td>Some increase in algal growth</td>
</tr>
<tr>
<td>Turbodispin D-100</td>
<td>Slight increase in algal growth</td>
<td>No effect on algal growth</td>
</tr>
</tbody>
</table>

The second series of mesocosm simulations with antiscalant compounds was set up in December 2004 and lasted until August 2005. The ponds again contained 900 l of a mixture of 80% Dead Sea water – 20% Red Sea water. The following antiscalants were included in the experiment:

<table>
<thead>
<tr>
<th>Antiscalant</th>
<th>Amount added to 900 ml pond</th>
<th>To yield concentration in ppm</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>SpectraGuard</td>
<td>17.5 g</td>
<td>19.5</td>
<td>Added on January 24, 2005</td>
</tr>
<tr>
<td>Turbodispin D-100</td>
<td>17.5 g</td>
<td>19.5</td>
<td>Added on November 4, 2004</td>
</tr>
</tbody>
</table>
Control systems included in the experiment were a pond with 80% Dead Sea water – 20% Red Sea water without added antiscalant, as well as two ponds with 80% Dead Sea water – 20% Red Sea water amended with 1 µM and 5 µM KH₂PO₄. The table below presents the chemical analysis of the water mixtures in the ponds at the end of the experiment.

<table>
<thead>
<tr>
<th></th>
<th>80% Dead Sea water control</th>
<th>Spectroguard 1 µM KH₂PO₄</th>
<th>5 µM KH₂PO₄</th>
</tr>
</thead>
<tbody>
<tr>
<td>Na⁺</td>
<td>31,230</td>
<td>31,330</td>
<td>31,750</td>
</tr>
<tr>
<td>K⁺</td>
<td>6,700</td>
<td>6,850</td>
<td>6,900</td>
</tr>
<tr>
<td>Ca²⁺</td>
<td>14,800</td>
<td>15,200</td>
<td>15,300</td>
</tr>
<tr>
<td>Mg²⁺</td>
<td>38,600</td>
<td>38,400</td>
<td>39,600</td>
</tr>
<tr>
<td>Sr²⁺</td>
<td>285</td>
<td>289</td>
<td>291</td>
</tr>
<tr>
<td>Cl⁻</td>
<td>193,540</td>
<td>192,000</td>
<td>198,450</td>
</tr>
<tr>
<td>Br⁻</td>
<td>4,826</td>
<td>4,798</td>
<td>4,830</td>
</tr>
<tr>
<td>SO₄²⁻</td>
<td>700</td>
<td>765</td>
<td>742</td>
</tr>
<tr>
<td>Total dissolved salts</td>
<td>290,681</td>
<td>289,632</td>
<td>297,863</td>
</tr>
<tr>
<td>Na/Cl ratio</td>
<td>0.249</td>
<td>0.252</td>
<td>0.247</td>
</tr>
</tbody>
</table>

Ion concentrations in the experimental ponds (values in mg/l), as measured on August 5, 2005.

Figures 17-20 show the results of the experiments. As in the earlier figures, the upper two panels represent growth of algae (Dunaliella cells/ml and chlorophyll concentration), the lower panels growth of red bacteria (cell numbers in millions of cells/ml and bacterioruberin content. The solid lines with the solid symbols again present the data for the pond supplemented with antiscalant or inorganic phosphate, the dashed lines connecting open symbols represent the control pond without additives. Note that the bacterial cell counts for the control – no additions experiment were lower than those for the summer 2004 experiment, but higher for the pond amended with 1 µM phosphate. Presently we have no explanation for this observation.
A summary of the results is presented in the table below.

<table>
<thead>
<tr>
<th>Antiscalant</th>
<th>Pond experiment</th>
<th>Short-term laboratory simulation</th>
</tr>
</thead>
<tbody>
<tr>
<td>SpectraGuard</td>
<td>No stimulation of algal and bacterial growth</td>
<td>No stimulation of algal growth in unheated antiscalant, but stimulation after heating</td>
</tr>
<tr>
<td>Turbodispin D-100</td>
<td>Slight increase in algal growth</td>
<td>No effect on algal growth</td>
</tr>
</tbody>
</table>

In the 2005 ponds experiment, SpectraGuard was examined as well (Fig. 19), and and Turbodispin D-100 was tested once more, with results (Fig. 20) similar to those obtained earlier (Fig. 16).

SpectraGuard had no significant effect on the biological communities in the eight-month experiment (Fig. 19). Earlier laboratory experiments had shown that heating of the compound increased its potential to trigger microbial blooms (Fig. 10). However, the compound appears to be sufficiently stable in the temperature range encountered in the Dead Sea and in the Sedom simulation ponds, so that no such a stimulation of algal and bacterial growth occurred, even over many months.
Figure 17. Effect of the addition of 1 µM KH$_2$PO$_4$ on the development of Dunaliella (cells/ml in the upper panel, chlorophyll in the second panel) and halophilic Archaea (cells/ml in the third panel, bacterioruberin pigments in the lower panel) in the experimental ponds at Sedom, December 2004 – August 2005.
Figure 18. Effect of the addition of 5 μM KH$_2$PO$_4$ on the development of *Dunaliella* (cells/ml in the upper panel, chlorophyll in the second panel) and halophilic Archaea (cells/ml in the third panel, bacterioruberin pigments in the lower panel) in the experimental ponds at Sedom, December 2004 – August 2005.
Figure 19. Effect of the addition of SpectraGuard (19.5 ppm) on the development of *Dunaliella* (cells/ml in the upper panel, chlorophyll in the second panel) and halophilic Archaea (cells/ml in the third panel, bacterioruberin pigments in the lower panel) in the experimental ponds at Sedom, December 2004 – August 2005.
Figure 20. Effect of the addition of Turbodispin D-100 (19.5 ppm) on the development of *Dunaliella* (cells/ml in the upper panel, chlorophyll in the second panel) and halophilic Archaea (cells/ml in the third panel, bacterioruberin pigments in the lower panel) in the experimental ponds at Sedom, December 2004 – August 2005.

Figures 21 and 22 summarize the mesocosm simulation studies with different antiscalant compounds performed in 2004 and 2005. They present the effect of the different compounds tested on the density of *Dunaliella* cells (Fig. 21) and of bacteria (Fig. 22) reached in the ponds.
Figure 21. The population density of *Dunaliella* cells reached in the experimental ponds in Sedom after addition of different antiscalant agents (2004 and 2005 experiments combined). The upper bars represent the algal densities obtained in control ponds of 80% Dead Sea water – 20% Red Sea water with and without 1 or 5 µM orthophosphate.

Figure 22. The community density of bacteria reached in the experimental ponds in Sedom after addition of different antiscalant agents (2004 and 2005 experiments combined). The upper bars represent the bacterial densities obtained in control ponds of 80% Dead Sea water – 20% Red Sea water with and without 1 or 5 µM orthophosphate.
Summarizing, it appears that of the antiscalants used in the experiments, some could be used in the Red Sea – Dead Sea water desalination plant without substantial danger of the rapid development of microbial blooms. The other antiscalant compounds we tested triggered rapid algal and/or bacterial growth, and thus we do not recommend their use in the framework of the Peace Conduit water desalination plant that will discharge its reject brine into the Dead Sea. We wish to emphasize that the choice of antiscalants used in the present experiment was based on their availability at the time of the setting up of the experiments. Clearly, if and when plans for the construction of the "Peace Conduit" materialize, similar experiments will have to be carried out with all the antiscalants that will be considered for the operation of the proposed desalination plant.
4. PLANS FOR FURTHER WORK

The following experiments have recently been set up or are being planned:

1. To answer the question whether plankton that may enter the Dead Sea by the Peace Conduit contains available phosphate in concentrations sufficient to trigger algal blooms in the Dead Sea, a series of outdoor pond experiments was initiated at Sedom in September 2005 in which different amounts of plankton collected from the Gulf of Aqaba were added to ponds filled with 80% Dead Sea water – 20% Red Sea water. The results of these experiments will be reported elsewhere.

2. We are planning a series of outdoor experiments during the winter months in which a number of ponds will be equipped with heating elements, so that the temperature can be maintained at levels common to the summer months. This will enable us to decide whether the low level of algal bloom development in the winter may be due to suboptimal temperatures or to the decreased availability of light.

3. Laboratory simulation experiments will be set up to refine the definition of the upper salinity boundary for the development of algal and archaeal blooms in the Dead Sea. These experiments will be performed at different temperatures, as the salt concentration optima, minima and maxima of many halophilic and halotolerant microorganisms are known to be temperature dependent. Higher temperatures often lead to increased salt tolerance.

4. The laboratory simulation experiments examining different commercial antiscalant preparations for their ability to trigger the development of algal blooms, based on release of available orthophosphate, will be extended.
5. REFERENCES


