Biological and chemical features associated with salt production in solar saltfields at Dry Creek, South Australia

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DEDICATED TO THE MEMORY OF MY FATHER

DR. G. H. GHASSEMZADEH

DECLARATION

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ABSTRACT

Sodium chloride is important in the manufacture of chlorine, caustic soda and soda ash. It is obtained from several sources, an important one being solar saltfields. One third of the world's sodium chloride is from solar salt fields. Solar salt fields at Dry Creek are the major site of salt production in South Australia. They comprise a series of interconnected evaporating and crystallising ponds where seawater is evaporated and the concentration of sodium chloride increases. Finally, water terminates in crystallisers where evaporation continues and sodium chloride precipitates and is then harvested. The aims of this research are to evaluate the biological and physico-chemical parameters and their interactions with the production of salt.

Salt production is strongly dependent upon biological, physical and chemical factors. These were investigated in eight ponds at Dry Creek solar saltfields. Nutrient and other chemical and physical parameters, including salinity, alkalinity, pH, dissolved oxygen, temperature, wind and rainfall, which control the composition of the biota and salt production in the solar saltfields, were measured. Salinity ranged from 46 to 243.5 g/L. The systems were alkaline and low in oxygen concentrations (0.4 - 9.5 mg/L). Ranges of nutrients were 7-22 and 4-19 μg/L for reactive phosphate and nitrate-nitrogen concentrations in the study ponds, respectively.

There are two sources of seawater intake, Middle Beach (low nutrient) and Chapman Creek (high nutrient). Seawater from Middle Beach with low salinity (38 g/L) and nutrients (SRP, 40 μg/L; total P, 52 μg/L; NO_3-N, 4.5 μg/L) is different from seawater from Chapman Creek with high salinity (47 g/L) and high nutrients (SRP, 250 μg/L; total P, 290 μg/L; NO_3-N, 133 μg/L). This excessive amount of nutrients that enter the ponds with intake seawater caused high biological productivity in several ponds. The major source of these nutrients is from the outfall of the nearby Bolivar sewage works.

Standard methods of analysis of plant nutrients (NO_3-N and PO_4-P) may lead to significant errors when applied to saline water. For this reason, analytical methods for the estimation of both NO_3-N and PO_4-P in saline water were re-examined. It was established that the ascorbic acid with antimony (III) phospho-molybdenum blue method is the most useful method for phosphate determination in saline water and shows minimal salinity interferences. For nitrate estimation it was shown that there are substantial salt errors when using the Cd column technique followed by colorimetric procedures. Methods involving dilution, standard addition or the application of a salt error correction are essential for accurate nitrate determination in saline waters.

The biota and biological communities in the solar saltfields at Dry Creek are similar to those found in other solar salt fields. Forty - two species of algae dominated by diatoms, and Cyanobacteria, and 10 species of zooplankton dominated by crustaceans, were identified. Marine brackish fauna and saline forms are the dominant groups of organisms in early stage ponds. As salinity increases through the series of ponds, species diversity falls and finally only halobiont species remain. In early ponds the fauna includes fishes, gastropods, isopods, amphipods and copepods (calanoids, cyclopoids and harpacticoids),

ostracods and insects (Trichoptera and Diptera). The fish, *Atherinosoma microstoma and Pseudogobious olorum*, have been recorded from study ponds with salinity 46-110 g/L. Two isopods, (*Exosphaeroma bicolor* and *Synischis* sp.), the amphipod, (*Parhyalella kunkel*), one gastropod (*Hydrododdus tasmanicus*) and *Palaemon serenus*, a large swimming prawn in pond XD1 at a salinity of 55 g/L, represent tolerant macrospecies. They may prevent the growth of benthic mats in this pond. *Acartia* is the main copepod and tolerates a salinity up to 110 g/L. As salinity increases through the series of ponds, species diversity falls and finally only halotolerant species remain. *Daicypris dictyote* and *Reticypris herbsti* were present at a salinity 55.5-137.5 g/L. They did not occur at a higher salinity probably not only due to high salinity but also due to deficiency of some ions, such as carbonates (see Chapter 2). The occurrence of *Symphitoneuria wheeleri* was notable because this insect is rare in saline water and does not belong to the Philanisidae, the family containing the only known marine Trichoptera. Two species of chironomids (*Cladotanytarsus* sp. and *Tanytarsus barbitarsis*) and one species of Ephydridae (?*Ephydra riparia*), occurred in most ponds. The planktonic community of these highly saline evaporating ponds consists mainly of *Artemia franciscana* and *Parartemia zietziana*. Seasonal variation in zooplankton populations reflects seasonal changes in temperature, light, nutrients and algal abundance. Thus, the presence of zooplankton is governed primarily by its salinity tolerance and its abundance by trophic conditions. At salinities above 180 g/L, brine shrimp (*Artemia franciscana* and *Parartemia zietziana*), hypersaline algae (*Dunaliella salina*), and a hypersaline Cyanobacterium (*Synechococcus*) are abundant.

At high salinity, *Synechococcus* excreted large amounts of extracellular material and this caused an additional increase in brine viscosity with effects on salt production. The effects of the extracellular material produced by *Synechococcus* were tested in a field experiment which used microcosms to investigate how *Synechococcus* affected the quantity and quality of salt produced. Five tanks were filled with brine from salt ponds and inoculated with different amounts of *Synechococcus*. The tanks were monitored for 12 weeks in two stages. In stage I, *Synechococcus* was present in evaporating tanks until the brine concentration increased to the point of sodium chloride crystallisation. Regular measurements were made of salinity and viscosity in different treatments. In stage II, *Synechococcus* was excluded and salt was allowed to crystallise in the tanks; deposited salt was harvested at the end of the experimental period. The values of pH in the brine ranged 7.3 to 9. The value for salinity ranged 210 to 326.5 g/L and correspondingly, for specific gravity, from 1.1495 to 1.2278 during the period of experiment. The relative viscosity increased throughout stage I; it ranged from 1.103 to 1.290, 1.301, 1.336, 1.346 and 1.353 in tanks 1, 2, 3, 4 and 5, respectively. The highest value occurred in tank #5 which contained the highest amounts of *Synechococcus*. The salt quality harvested from each treatments was evaluated by the dry sieve technique using a Scanning Electron Microscope (SEM) and Energy Dispersion X-Ray Analyser (EDX) was used to determine crystal form, size and elemental composition, thus to determine impurities in the harvested salt. The results showed that the size and shape of salt crystals harvested are affected by liberated organic material (ECP) produced by *Synechococcus*, and that more brine is retained in these salt crystals which affects the composition of the salt and leads to a decreased in the quality of salt crystals. Data on size distribution of salt crystals showed that it was unimodal for all tanks and ranged from 0.125 to 4 mm. Overall, the largest crystals were

from the control tanks (tank #1), and the smallest from the high *Synechococcus* tank (tank # 5). Moreover, the quantity of harvested salt decreased with increasing amounts of *Synechococcus* due to the decreasing percentage of evaporating water.

The effects of high salinity and high light intensity on the production of extracellular material were tested by an experiment using aquaria as microcosms. Twelve aquaria with the same amounts of *Synechococcus* standing crop were filled with low and high salinity brine from ponds PA7 (190.5g/L) and FA1 (285 g/L) and kept under covered and uncovered conditions. Viscosity values were 1.51340 and 1.5790 centistokes in ponds PA7 and FA1, respectively. Regular measurements were made of salinity and viscosity in different treatments. The highest value of viscosity occurred in high salinity-uncovered sample in week four with viscosity of 1.94342 centistokes and the lowest value was in low salinity-covered aquarium and in week one. The salinity was almost constant throughout the experiment. Samples from the aquaria were taken to assess the amount of extracellular material and to investigate its structure. The results of ash free dry weight (AFDW) of the *Synechococcus* standing crop showed that more organic material was produced under high salinity and high light intensity than at low salinity and low intensity of light. Part of this organic material produced by *Synechococcus* was released to the media and increased the viscosity of the brine. Thus, relative viscosity was higher in conditions of high salinity and high light intensity due to the ECP produced by *Synechococcus*. The chemical composition of extracellular material was investigated by solid state resolution 13C Nuclear Magnetic Resonance (NMR) with Cross Polarisation and Magnetic Angle Spinning technique. These technique indicated that the samples were mixture of glycoproteins and glycolipids.

The information collected from the physico-chemical, biological and experimental investigation is used to make appropriate recommendations about solar salt pond management. Proper management of biological systems is essential for production of high quality salt.

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CHAPTER ONE

INTRODUCTION

Salt, sodium chloride (NaCl), is used by every human in every country as a basic need for nutrition, health and hygenic purposes. The history of salt is synonymous with the history of mankind. Since earliest times, man's physiological need for salt has led him to the edges of the sea. He began the ancient business of removing salt from the ocean's waters by using the evaporating power of solar energy and dry wind. Today, although most of the world's supply of salt comes from other sources, this method is still used and its techniques have changed little.

The world population at the beginning of the 20th century was about 1.6 billion and the world salt production totalled 12.2 million tonnes per year (Kostick, 1993). Between 1950 and 1995, world salt production grew from 48.1 million to 200 million tonnes per year, an increase of 316 per cent (Fig. 1.1). Most growth in world salt consumption took place between 1950 and 1970. If the trend in production observed from 1970 to 1995 continues, the salt production to population ratio will have grown from one to three tons /100 individuals / year from the beginning to the end of the 20th century (Ayub and Bremer, 1993; Weeks, 1996). Figure 1.1 clearly shows that this trend in world salt consumption continues to grow.

Today, annual salt production is approximately 200 million tonnes per year; 10 million tons are produced by Oceania with 650,000 tons from Dry Creek Solar saltfield, the major producer in South Australia. The major producing countries are

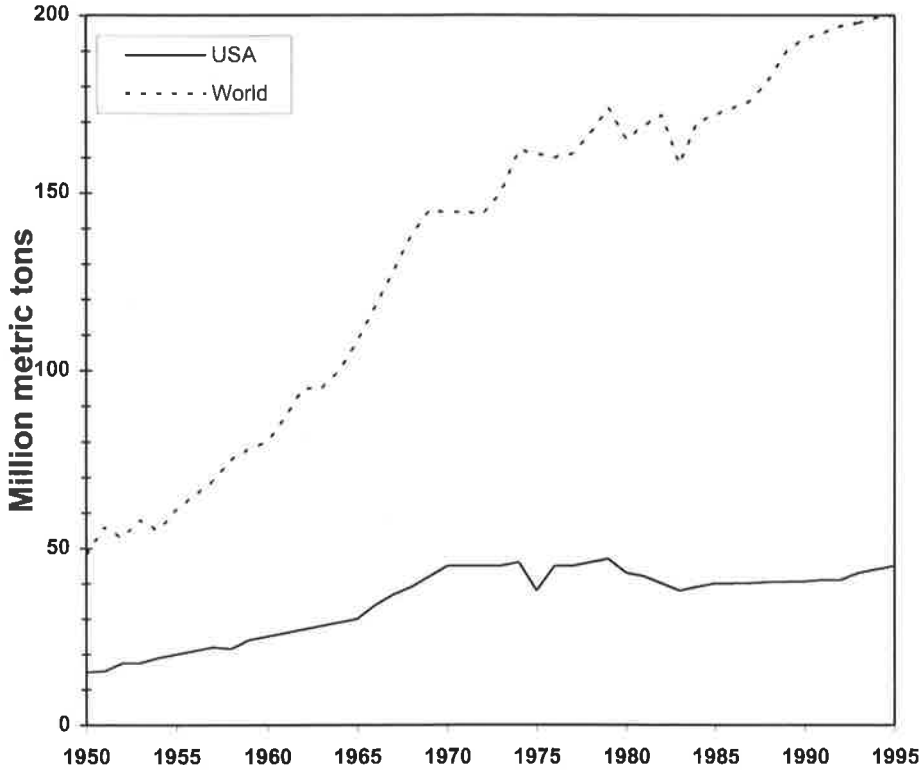

Figure 1.1. Salt consumption in the world and USA (modified from Kostick, 1993).

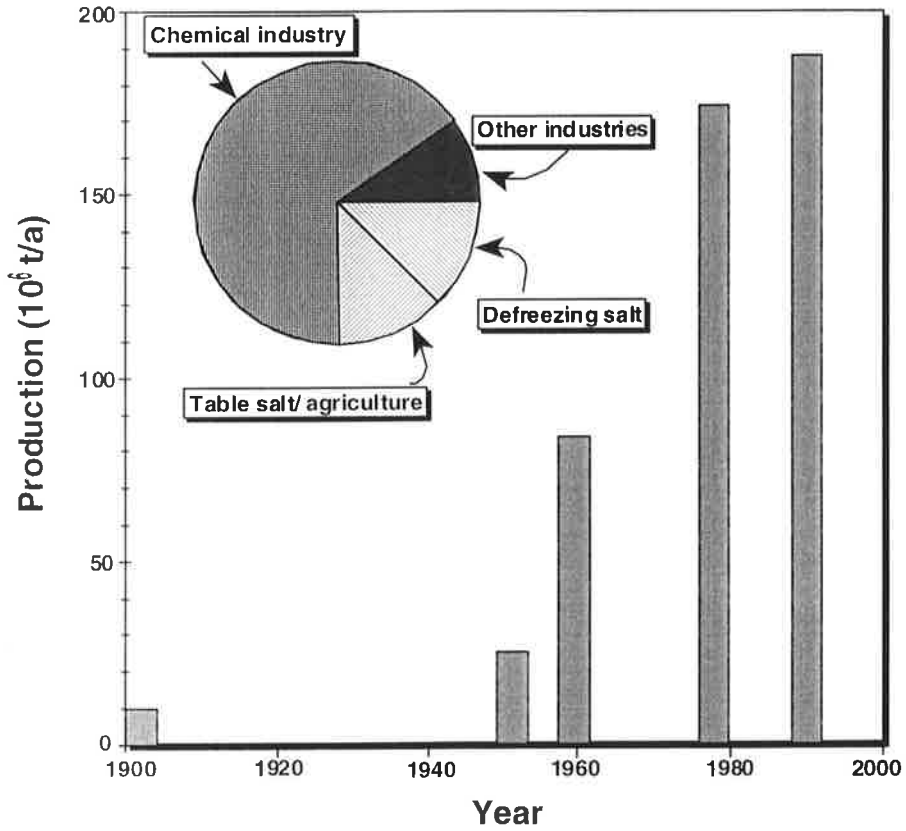

Figure 1.2. Salt production and use of salt world wide (from Schmitz and Wohlk, 1993).

the United States, England, France, Germany, India and the former USSR (Schmitz and Wohlk, 1993). The major salt producing countries in the pacific region are Australia and Mexico. They are the most important solar salt producers for the Japanese chloralkali industry which serves the pulp and paper industry of the northwest USA (Ayub and Bremer, 1993). Less than 10% of the annual salt production is used for human consumption; most is used by chemical industries (Tackaert and Sorgeloos, 1993). Figure 1.2 shows the major users of this salt.

Salt is the raw material for the manufacture of chlorine and caustic soda ash. The manufacture of these chemicals uses at least half the world's salt production. They are used extensively in the plastics, glass, and paper industries. The majority of all chemicals used in industry require salt for their manufacture either directly or indirectly. Some important factors that have influenced salt consumption per capita in the United States are shown in Figure 1.3. After 1880, the development of synthetic soda ash, chlorine facilities, water treatment, and highway de-icing resulted in salt becoming more important industrially. It will continue to be the most important mineral used by the world chemical industry for many years.

The greatest fraction of the world's salt is produced directly from natural resources. There are two main types of natural salt resource, salt from rock, and salt from saline water. Historically, seawater has been a convenient source for much of the world's needs because of its ready availability. Most countries adjacent to the ocean produce their salt from oceanic water through natural solar evaporation (solar saltfields). The production process, which depends on crystallisation over a range of brine densities, relies on the ocean's consistent chemical composition. Those countries not located near the ocean use other sources of salt - generally from mining of near surface and subsurface rock salt.

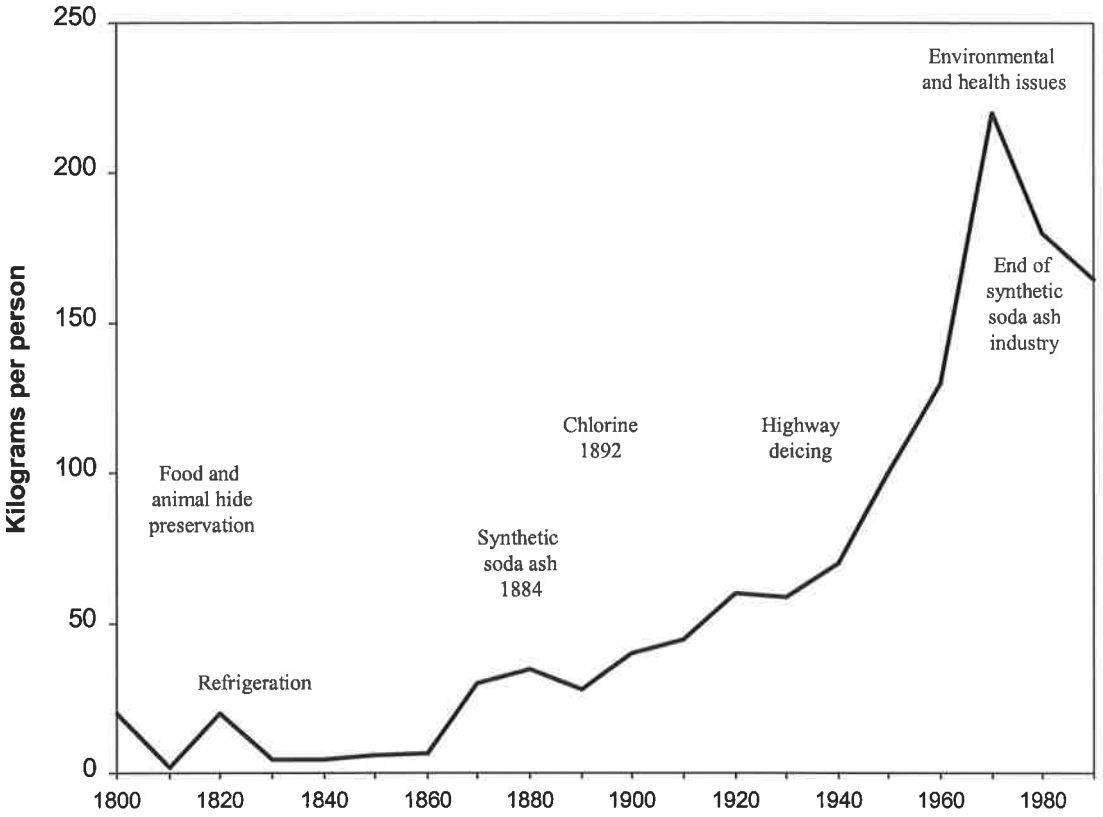

Figure 1.3. Factors that influenced per capita consumption of salt in USA, 1950-1990 (from Kostick ., 1993).

The world's ocean contains approximately $530x10^6$ km^3 of water, and one cubic kilometre of seawater weighs 2.6 billion tons (Kostick, 1993). Seawater from the open ocean contains 18.95g Cl^- and 10.48g Na^+ per 1000 grams of brine (Baseggio, 1974). Therefore, the world's ocean contains 78 million tons of salt per km^3. This is equal to about one half of the total annual world output of all forms of salt production (Kostick, 1993). There is enough salt in the oceans to last more than $22x10^{10}$ years if the annual rate of world salt consumption is about $183x10^6$ tons. In addition to sodium chloride, the sea is now also a valuable source of many other salts (Williams, 1981a). For example, seawater is the major source of bromine.

1.1 History of solar saltfields

Since early times, man has developed systems to concentrate seawater and to harvest sodium chloride as a basic need for nutrition and health. Over the centuries, thousands of hectares of salt ponds have been constructed worldwide in tropical and subtropical areas as so - called solar saltfields, solar salt ponds, or salinas (in this study, the terms solar saltfields or solar salt ponds will be used).

The concept of solar salt production has not changed significantly throughout history; only the details of harvesting techniques have changed. The first solar salt was probably produced along the margins of saline lakes and coastal areas from the white crusts of evaporated water (Kostick, 1993). Production of salt from oceanic water began in most countries about the same time. The Mayan civilisation in Mesoamerica along the Yucatan peninsula is one of the earliest solar salt activities recorded in the western hemisphere. Salt production has been economically important for the past 2,000 years in northern Yucatan. Although Yucatan had been the largest producer of 'sun salt ' in Mesoamerica, countries such as Guatemala and El Salvador were also historically important sources of salt production. However, they produced salt in a different manner; they filtered estuarine water through large

wooden canoes filled with salty marsh soil and through which a saline brine percolated. Then, the collected brine was boiled to yield salt.

In the first century B.C., salt production became a major industry in Europe. In England, the quality of salt was improved by slow rather than rapid boiling; this produced larger crystals. Today, many of the same principles are still used by small salt producers, such as those along the west coast of France (Kostick, 1993) or at the salt works of Secovje in Yugoslavia which have existed for more than 700 years without any major change in technique (Schneider and Herrmann, 1980)

In Asia, the Chinese used two different methods for collecting salt from coastal areas. In the first method, they collected seaweed and dried them in the sun. Then, the dried seaweed was boiled and the brine evaporated to recover the salt. The second method was the so-called winter-summer process: during the winter, salt workers dug pits about 2 metres deep over which bamboo poles were placed. The poles were covered with mats and sand: during tidal inundation, saline water filtered through the sand and mats and precipitated in the pits. The brine was removed with buckets from the pits and poured into clay-lined evaporating ponds. After evaporation, the salt was raked into one corner of the pond from where it was drained before use (Kostick, 1993).

Thus it is clear that the production of salt from seawater through natural solar evaporation is an ancient process and the technology is simple. It has been used in many parts of the world under a wide variety of conditions. As the world's sources of rock salt and other minerals become depleted, the importance of seawater as a source of various chemicals ions again will become more important (Lozano *et al.*, 1993). Seawater contains salts of almost every chemical element including gold in at least trace amounts (Tackaert and Sorgeloos, 1993). It contains a mixture of many compounds, including KCl, $MgCl_2$, $CaSO_4$, $CaCO_3$ and $MgSO_4$ as well as

NaCl. To obtain near pure NaCl, which makes up about 80% of the weight of the total mixture, precipitation of salts is carried out. Since various salts dissolved in sea water have characteristic solubilities, as the water evaporates and the salts become increasingly concentrated those that are least soluble and present in the greatest concentration relative to their solubility begin to precipitate. In natural, unmanaged systems such as the hypersaline lagoons of Texas and Mexico, this process may continue while many different salts become saturated and the bottom becomes covered with layers of salt arranged according to their solubility (Copeland and Nixon, 1974).

Solar salt fields are convenient systems to produce salt since their resources (seawater) are virtually inexhaustible. The system requires management such that the water that remains after salt compounds have precipitated is moved by pump or gravity to another section where the next salts may precipitate relatively free from contamination by less soluble ions. Thus, the ionic composition of the water above the precipitating salts progressively changes and becomes enriched with the more soluble ions. This continuously shifting chemical composition of evaporating seawater is shown in Figure 1.4. After precipitation of salts and transfers, the point is reached where NaCl, one of the more soluble salts, becomes saturated and precipitates strongly. The remaining liquid, now called a bittern and rich in Mg^{++} and K^{+}, is removed to reduce contamination of sodium chloride with Mg^{++} salts and other salts that begin to precipitate at these elevated salinities. The technique of solar salt production thus involves fractional crystallisation of the salts in different ponds to obtain the purest form of sodium chloride, up to 99.7% on a dry weight basis (Fig. 1.4). Only energy from the sun and wind is used in the evaporative process to produce commercially valuable salt. Solar saltfields are strongly dependent on climatic factors beyond human control such as sunshine, temperature, rainfall, humidity and wind velocity. Thus, a well-organised weather station is necessary at every solar salt field for effective management (Butts, 1993).

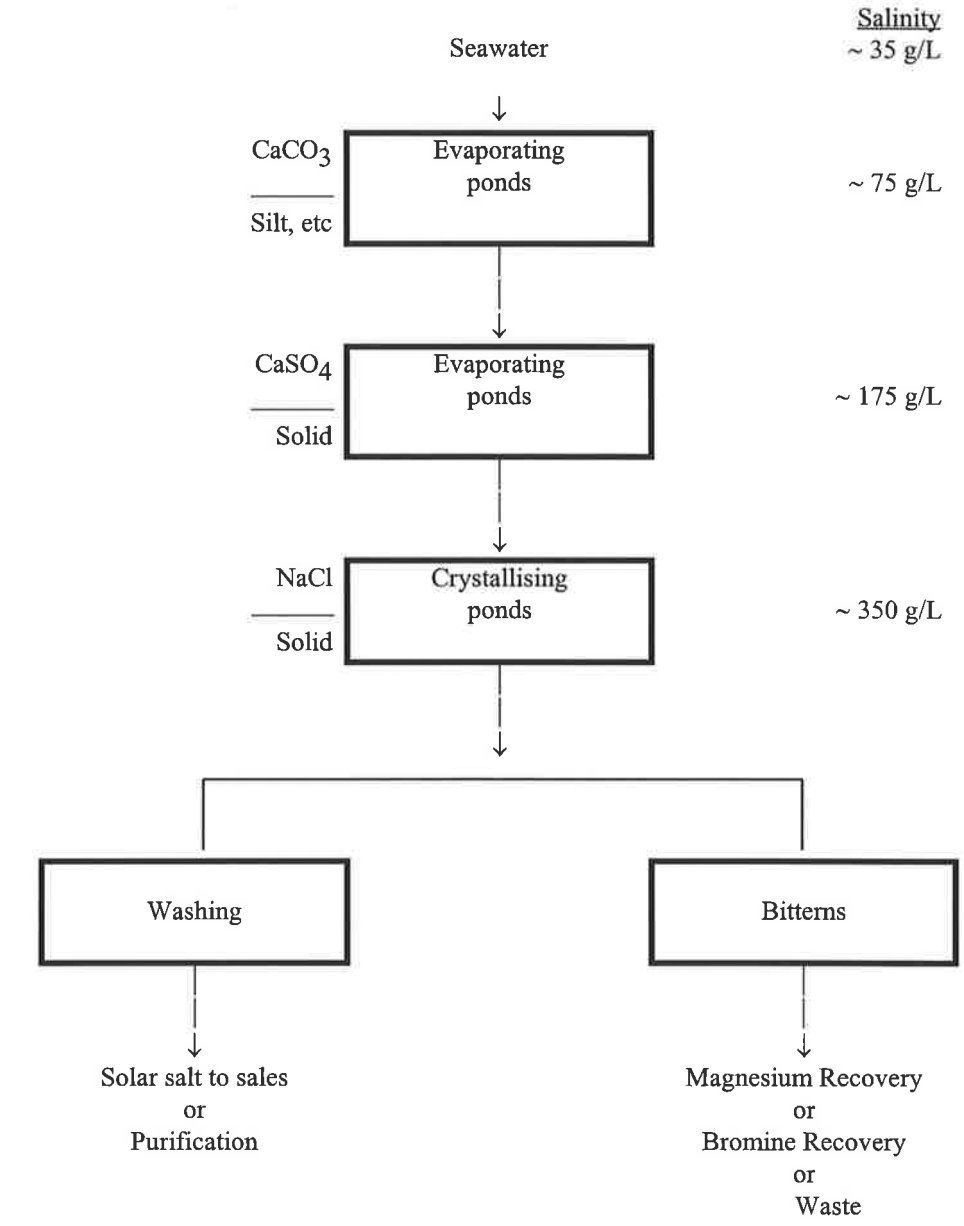

Figure 1.4. Typical processes for the production of solar salt from seawater.

Moreover, since the solar saltponds function as ecosystems, hydrobiological activities in solar salt ponds are important in determining the quality and quantity of salt that is produced from these systems.

1.2 Literature review

Although the hydrobiology of solar saltfields is an interesting area of research and important to the quality and quantity of salt production, it has been little studied compared with the hydrobiology of fresh, brackish and marine waters. This reflects the general paucity of studies on inland (non-marine) saline waters despite their many scientific and other values (Williams, 1981b).

Several papers have been published on the effect of biological conditions on salt production (Davis, 1975, 1978 and 1980a and b; Jones *et al.,* 1981; Sorgeloos, 1983; Sorgeloos *et al.*, 1986). These are all, however, broadly based on the pioneer model published by Davis (1978). Since 1979, there has been little addition to the fundamental published understanding of how the biology affects salt production and how it is important in solar salt fields management. Even so, there are numerous studies concerned with particular elements of the biota and other features in salt ponds.

Several investigations have shown that organisms in solar salt fields influence the quality and quantity of harvested salt (Davis, 1974, 1978, and 1980a, b; Schneider and Herrmann, 1980; Jones *et al.,* 1981). The importance of major dissolved nutrients, which determine the biological status of solar salt fields, was investigated by Javor (1983a and b), Davis (1978) and Jones *et al.* (1981). They compared low with high nutrient systems and the quality of salt production. In 1978, Davis demonstrated that a poorly developed biota in a solar salt field was responsible for a poor harvest of NaCL. Meredith (1992) made a comparison between artificial and

natural solar salt lakes in South Australia. Rahaman *et al.* (1993) have shown that photosynthetic algae in Indian solar salt fields accumulate large amounts of organic matter that retard the rate of evaporation and adversely affect salt crystallisation. In general, Davis (1990, 1991 and 1993) described some aspects of biological management important to salt production and demonstrated biological concepts necessary for design and operation of salt works. Tackaert and Sorgeloos (1993) also looked at the beneficial role of *Artemia* in balancing the hydrobiological activity of solar salt pond systems and they highlighted some of the critical aspects essential to proper management of *Artemia*. More comprehensive reviews of the biological and chemical literature are given in the following chapters.

The relationship of species diversity to salinity at Dry Creek saltfields was studied by Caon (1974). He found that the number of species diversity decreased as salinity increased and marine invertebrates did not penetrate beyond the salinity of 80 g/L. Some of the other biological studies conducted previously at solar salt fields in South Australia have dealt with the brine shrimps present (Mitchell and Geddes, 1977; Lea, 1978; Newton, 1980). Mitchell and Geddes (1977) studied the distribution of the brine shrimps *Paratemia zietziana* Sayce and *Artemia* (*salina)* along a salinity and oxygen gradient in Dry Creek solar salt fields of South Australia. They found that the two species overlapped in the range 214 to 285‰ salt and also indicated that *Artemia* (*salina*) had a lower critical oxygen concentration than *Parartemia zietziana*. Lea (1978) made an comparative studies on the brine shrimp *Artemia salina* and *Paratemia zietziana Sayce* in a salt evaporation pond at Dry Creek. He stated that the two species occur in similar niches, but possible competition between them is minimised by their asynchronous occurrence. Newton (1980), in her studies of the competition between *Parartemia zietziana* and *Artemia* species in Dry Creek solar salt fields, found that *Artemia* did best over the summer months, while *Parartemia zietziana* did better in winter. She

also stated that the distribution of these organisms during different seasons was not regulated by physico-chemical factors.

A number of papers have dealt with microbiological studies of solar salt fields. They includes Campbell and Golubic (1985), Jørgensen and De Marais (1986), Oren, 1990a, b, and 1994, Nakashizuka and Arita (1993) and Morishita and Kitano (1993). The zonal distribution and species composition of benthic cyanophyte mats was studied by Campell and Golubic (1985) in the hypersaline Solar Lake (Sinai, Israel), one of the most intensively studied saline water-bodies from a geological, geochemical and microbial point of view. Jørgensen and De Marais (1986) stated that there is competition between colourless and purple sulphur bacteria in cyanobacterial mats. Javor (1989) discussed the major features of the microbiology and biogeochemistry of solar salt ponds. The salt resistance and growth potential of halophilic bacteria isolated from solar salt fields were investigated by Javor (1984), Rodriguez-Valera (1985), Nakashizuk and Arita. (1993), Morishita and Kitano (1993), respectively.

Overall productivity and diurnal patterns of photosynthesis and respiration in solar salt fields systems were studied by Carpelan (1957 and 1964), and Copeland and Jones (1965). Carpelan's (1957) research was involved with lower salinity ponds in solar saltfields in California, where the major nutrients were concentrated by evaporation. He found a strong seasonal pulse of algal production following increasing inputs of solar energy in spring and summer. Copeland and Jones (1965) studied solar salt fields in Puerto Rico, and obtained data on diurnal changes in dissolved oxygen and carbon dioxide in systems operating at high concentrations of salt. Their studies indicated low productivity at high salinity. Nixon (1974) stated that when solar salt ponds are enriched with inorganic fertiliser they can become productive and maintain themselves at higher metabolic levels for long periods of time.

The sedimentology and geochemistry of sediments of solar salt lakes have been studied by several geologist (e.g. Herrmann *et al.*, 1973; Schneider and Herrmann, 1980; Mackenzie *et al.*, 1995) and will not be reviewed in this section, as these topics are beyond the scope of this research.

Finally, there have been several valuable investigations concerned with the operation and control of solar saltfields (Myers and Bonython, 1958; Garret, 1966a and b; McArthur, 1980; Joshi and Bhalt, 1985; Manner and Bradley, 1984; Boudet, 1993).

1.3 Study area

The Dry Creek solar salt fields of South Australia was established in 1940. Located approximately 30 km north of Adelaide along the St. Vincent's Gulf, they are built on old creek beds, mangroves and scrub land (Harbison, 1991). These solar ponds have now provide important habitats for some of Australia's rarest wading birds, as well as being economically important for salt production.

Dry Creek is in a temperate area with moderate evaporation (2032 mm/yr) and low rainfall (420 mm/yr). The climate is hot and dry from November to March and has normally a wet season from July to September. During the wet season, rainfall exceeds evaporation and the field is shut down for salt making.

The solar salt fields comprise 44 shallow evaporating ponds and 8 crystallisers, extending over 4040 ha (Fig. 1.5). This maintains stable ecosystems that are less susceptible to changes (De Medeiros Rocha and Camara, 1993). The shape of each pond is irregular, as determined by naturally occurring banks and high ground contours.

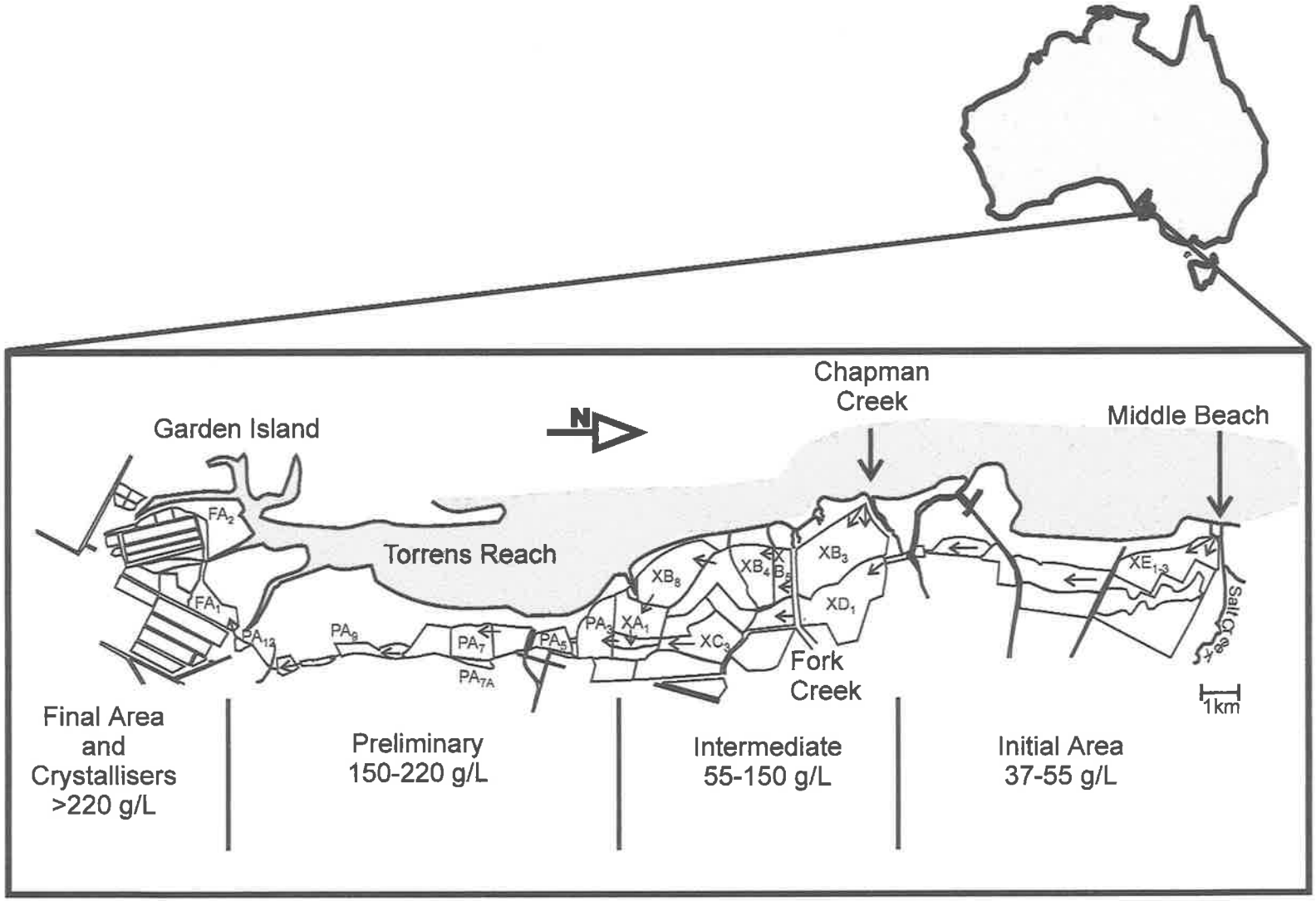

Figure 1.5. Dry Creek solar saltfields, South Australia. Arrows show the direction of brine flow.

The mean depth of the shallow evaporation ponds varies from 1.25 to 1.5 m, and the mean depth of the crystalliser ponds is approximately 15 cm. The evaporating ponds are relatively deep with respect to other salt fields in the world. Pond infiltration is a significant problem at Dry Creek because of the high permeability of the substratum, mainly clay to shell grit.

Sea water is pumped into two ponds at different locations, ponds XE1-3 at Middle Beach in the north, and pond XB3 at Chapman's Creek in the south (Fig. 1.5). The two sources mix in pond XA1. After seawater enters the system, evaporation acts along the length of the evaporation ponds until salinity reaches more than 300 g/L in the final pond (FA5). Then, the maiden brine is pumped into shallow open pan crystallisers. The water is moved by siphons or gravity or is pumped from one pond to another. Approximately 50 Mt /yr of seawater is pumped into the evaporation ponds and the retention time is up to two years, that is, up to two years may elapse from the time seawater enters until the saline body of water reaches the crystallisation pond. During evaporation, chemical minerals in different ponds are forming in ascending order from carbonate, sulphates and sodium chloride. To obtain the NaCl in as pure a condition as possible, differential ion precipitation occurs (Fig. 1.4).

The eight crystallising ponds are rectangular and cover a total area of 370 ha. They have been consolidated with clay floors that are suitable for the use of mechanical harvesting equipment. Each crystallising pond covers an area of about 43 ha (315 m wide and 2.5 km long). The brine feeds into them by underground siphon pipes and flow is controlled by wooden gates. Normally, saturated brine depth is about 15 cm above the deposited salt. The time when salt production commences depends upon the winter weather and the availability of good brine. Usually it starts in October. In many solar salt fields, NaCl of at least 99.8% purity is harvested throughout the year, but some like Dry Creek solar salt fields, are harvested during the dry season

only in order to protect the salt floors of the crystalliser ponds from dissolution due to rain (Javor, 1989). Harvesting is normally in the autumn of each year, starting in the second week of March and extending for 90 days. It is usually completed in early June before winter rains commence. Again, the harvest time is dependent upon the weather in each year. The salt at Dry Creek salt fields is mechanically harvested and the daily harvest is about 8,000 to 10,000 tons.

The solar salt fields at Dry Creek can be divided into initial areas (salinity ~ 37-55 g/L), intermediate areas (salinity ~ 55-150 g/L), preliminary areas (salinity ~ 150-220g/L) final areas (salinity ~ 220-320g/L), and crystallising areas (salinity >320g/L). The initial areas (initial evaporation) permits gradual adaptation of some of the estuarine species to the higher salinity water. In this area, most organisms are marine. A hypersaline flora and fauna develops in the intermediate areas and only halobiont species occur in the preliminary and final areas. The biology of these areas is indicated in the following chapters.

The fringing macrophyte community consisted chiefly of *Wilsonia numilis*, *Halosarcia flabelliformis* and S*alicornia* sp. These often grew on the pond banks. Occasional patches of *Wilsonia numilis*, *Halosarcia flabelliformis* and *Salicornia* sp. were extensive and with several other species helped stabilise dikes. Small islands in these ponds also supported these plants as well as other scattered specimens. *Wilsonia humilis* is a ground-hugging silver-grey creeper with imbricate leaves, and seems to tolerate both seawater and freshwater inundation (flood). It can also survive in a wide range of light conditions. Its ability to grow in the shelter of other plants makes this species difficult to detect. *Halosarcia flabelliformis* is easy to detect during spring and summer when it has a noticeably bright green appearance; this enables observers to identify it from a considerable distance away. However, it becomes dormant in colder months, shedding its spikes and so becomes almost invisible.

The fringe community of the study area consisted of *Avicennia marina* (grey mangrove). Mangrove forests are one of the most important and widespread coastal ecosystems. In Australia, mangrove forests occupy approximately 11600 km^2 of coastal foreshore and estuary areas and cover a total area of approximately 230 km^2 in South Australia (Edyvane, 1995).

Avicennia marina (grey mangrove) is the most widely distributed mangrove in southern Australia and it grows in disjunct pockets along the southern Australia coastline (Bridgewater, 1982). In South Australia, this species grows to about 3.5 to 5 metres high and is easily identified by its light green leaves with a silvery-grey undersurface and pencil-shaped aerial roots (pneumatophores) which project vertically from the sediment surface. The pneumatophores grow upwards from the trees network of shallow, radiating horizontal roots, sometimes referred to as cable roots. The viviparous appearance of a large cotyledons (seed leaves) gives it the appearance large lima beans.

According to salinity variations and the nutrient stream involved, eight ponds were selected for study from initial, intermediate, preliminary and final areas in Dry Creek solar saltfields (Figs. 1.5 and 1.6). In recent years, the Chapman's creek series of concentrating ponds has undergone considerable eutrophication. The major source of this nutrient input is from the outfall of the nearby Bolivar sewage treatment works. Once the seawater has entered Chapman's creek, it is pumped into the first pond of the Chapman's creek concentrating series (XB3) and extends along the salinity gradient. The inlet from Middle beach, further removed from the Bolivar outfall and lower in nutrient concentration, enters pond XE1 and extends along the salinity gradient. These low and high nutrient saline waters are mixed in pond XA1. Thus, pond XB3 and XB8 were selected from the high nutrient stream and pond XD1 and XC3 from the low nutrient stream. PA3 was selected because complete mixing has taken place there. PA7, PA9 and PA12 were three ponds in

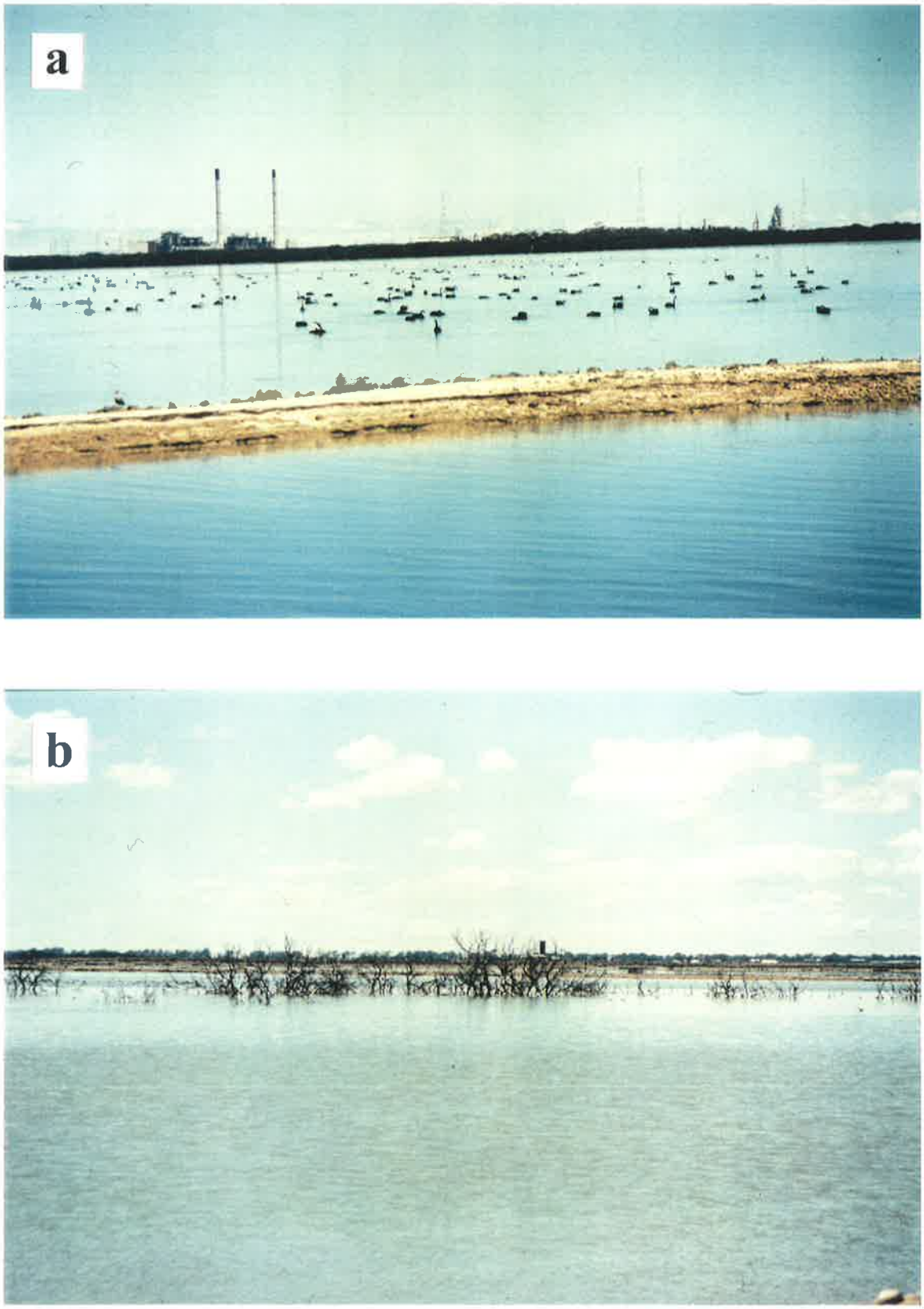

Figure 1.6. Views of study ponds at Dry Creek solar saltfields. (a) pond XB3; (b) pond XB8; (c) pond PA7 and (d) pond PA9.

Figure 1.6. continued

the preliminary area, where most gypsum has been precipitated, and also where *Synechococcus* sp. is abundant and may affect salt quality and quantity.

1.4 Aims of study

Saltmaking in solar saltfields is strongly dependent upon on environmental factors, including biological, physical and chemical ones. Some physical factors, such as solar radiation, temperature, rainfall, humidity and wind velocity are beyond human control. However, many other features, including biological ones, can be controlled by environmental management. The role of environmental parameters in the quality and quantity of salt production in solar saltfields, however, has not been extensively studied, as indicated. This study therefore aims to provide a comprehensive description of key biological and chemical features associated with salt production. The main aims are:

1. To document and evaluate the biological and physicochemical parameters and the spatial and temporal changes that occur annually in a salt field.
2. To determine how the chemical and biological factors are related to and interact with the production of salt.
3. To indicate how this information may be applied in the management of solar saltfields.
4. To evaluate by field and laboratory experiments the effect of *Synechococcus* sp., the dominant Cyanobacterium in highly saline water, on salt quality and the quantity of salt produced. Further, the effects of some environmental parameters on *Synechococcus* sp. will be investigated with a view of proposing how these results may be applied in the management of solar saltfields.
5. To evaluate the accuracy of commonly used methods of phosphate and nitrate measurement in highly saline water, the molybdenum blue spectrophotometric and the Cd column reduction method, given the possible error effect of salt on measurements.

To achieve these aims, studies were conducted at Dry Creek solar saltfields in South Australia over the period March 1994 to September 1995.

CHAPTER TWO

PHYSICO-CHEMICAL INVESTIGATIONS

2.1 Introduction

Organisms within solar salt fields are subject to a range of physico-chemical factors. Some factors - solar radiation, temperature, rainfall, humidity and wind velocity - are beyond human control, but others, such as salinity, water depth, or nutrient concentrations can be managed. This management is both an integral feature of salt production and a significant determinant of the ecology of each solar salt pond. A comprehensive description of these factors is therefore essential in any description of biological events that proceed in the ponds and relate to salt production. To that end, the aims of this part of the investigation were:

- to determine on both a temporal and spatial basis, changes in the major physicochemical parameters that occur annually in the salt field.
- to analyse how physico-chemical factors relate to and interact with the ecology of each pond, and how they may influence salt production.

Major physical factors studied were monthly maximum and minimum temperature, rainfall, gross and net evaporation (rainfall - gross evaporation = net evaporation), and light penetration. Major chemical factors studied were: salinity, dissolved oxygen concentration, alkalinity, pH and soluble reactive phosphorus and nitrate-nitrogen concentrations. Soluble reactive phosphorus and nitrate-nitrogen are the principal plant nutrients and together with chlorophyll *a* measurements indicated the trophic status of the ponds. All factors were monitored between April 1994 and

September 1995. Spatial changes were measured by monitoring throughout the salt field.

Meteorological information (maximum and minimum temperature, rainfall, evaporation and wind speed) was provided by the Penrice Company at the Dry Creek solar saltfield from its *in situ* meteorological station.

2.2 Methods and materials

The major physico-chemical parameters were measured regularly in the last week of each month for 18 months, commencing April 1994 until September 1995. Water samples were taken from eight selected ponds: XD1 and XC3 (low nutrient ponds), XB3, XB8 (high nutrient ponds), PA3, PA7, PA9 and PA12 (mixed ponds)(Fig. 2.1). The reasons for selecting these ponds are indicated in chapter one. Sampling sites were chosen near the outlet of each selected pond (Fig. 2.1).

2.2.1. Field methods

Air and water temperature and Secchi depth were measured *in situ*. Water samples for pH, alkalinity and salinity determination were collected in black polyethylene bottles at the same time. All samples were collected from 20 cm below the water surface.

Surface water temperature was measured with a mercury-in-glass thermometer. Light penetration was estimated by a Secchi disc (25 cm). Water samples for soluble reactive phosphorus, total phosphorus and nitrate measurements were also were collected in black polyethylene bottles. These had been acid-washed and were rinsed with pond water at the sampling sites prior to the collection of the samples. Samples were stored on ice before and during transfer to the laboratory.

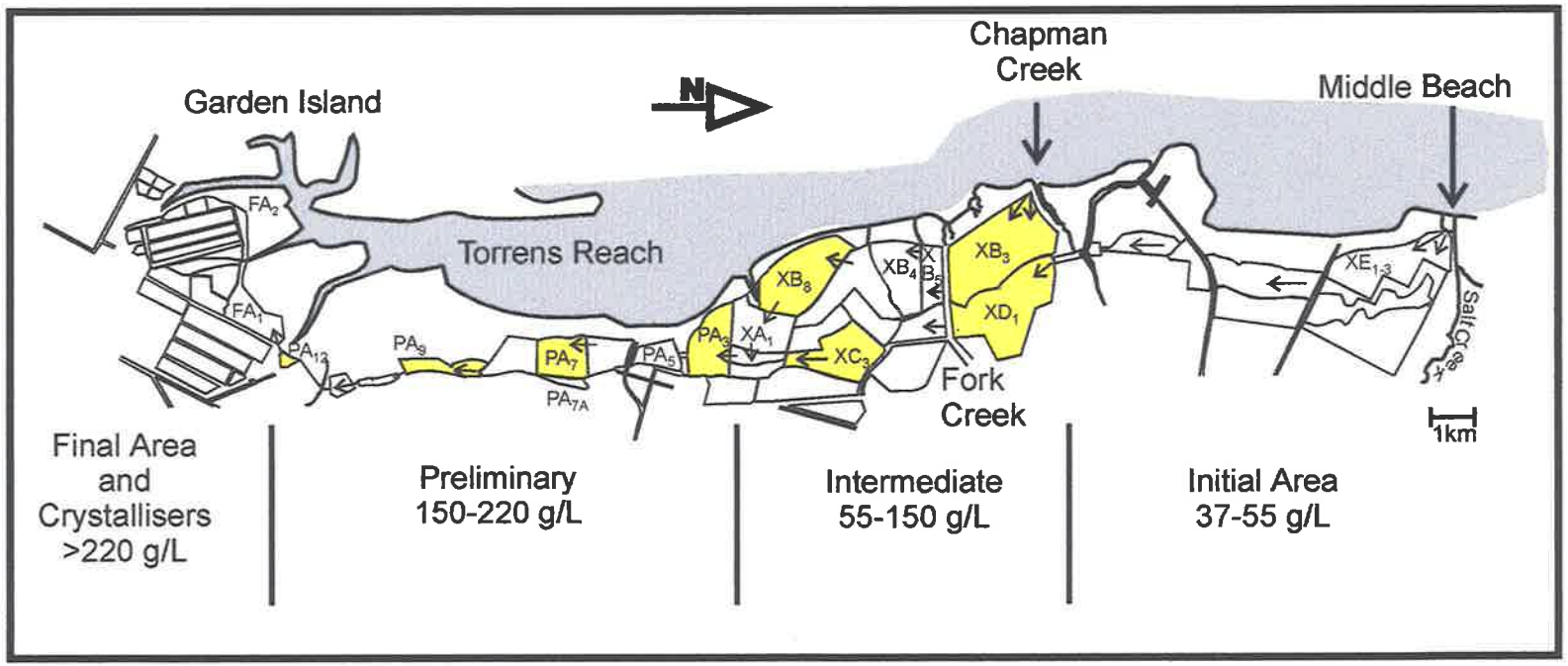

Figure 2.1. Dry Creek solar saltfields, South Australia. Arrows show the direction of brine flow. Ponds shown in yellow are those used in this study.

Water samples from the two different intake sites for the saltfields, Middle Beach and Chapman Creek, were also collected in black polyethylene bottles for salinity, pH, alkalinity, dissolved oxygen, soluble reactive phosphorus (SRP), total phosphorus and nitrate measurements in November and December 1994 and January 1995. A seawater sample from 200 m off-shore near Adelaide was collected for determination of chlorine, sodium, magnesium, sulphur, potassium, calcium and bromine the major ions in seawater.

2.2.2. Laboratory methods

Salinity, pH and alkalinity were determined from unfiltered subsamples as soon as possible on return to the laboratory (no later than 8 hrs after collection). The conductivity of water samples was measured with a conductivity meter (Hanna Instruments HI 8820 N meter) and converted to salinity using the regression equation of Williams (1986a). Samples with a salinity more of than 70 g/L were diluted. pH was determined by pH meter (Hanna instrument HI 9017). Total alkalinity was determined by titration with 0.01 N hydrochloric acid to the appropriate endpoint (pH 4.5) using BDH indicator (Mackereth *et al.,* 1989). The modified Winkler method was used to measure dissolved oxygen (Strickland and Parsons, 1972; APHA, 1992).

Concentration of the major elements in seawater was determined by induction coupled plasma (ICP) emission spectrometry by the CSIRO, Adelaide. ICP emission spectrometry is a 'flame' technique which is capable of measuring most elements with low detection limits and good precision (Rollinson, 1993).

Water for nutrient analyses was filtered (Gelman, TCLP glass fiber, 0.7 μm) as soon as possible on the same day of collection and analysis was completed within 24 hours. The filtered samples were kept at 4°C. All glass and plastic containers were

acid washed and rinsed with deionised water before use. Soluble reactive phosphorus (SRP) was determined using the molybdenum blue method of Murphy and Riley (1962). This method is the most useful for phosphate determinations in hypersaline water (see Chapter 4). For determining SRP, 15 mL of filtered sample was analysed in triplicate for each sample. Then a mixed reagent (1mL), which contained a molybdate antimony solution and ascorbic acid solution (Murphy and Riley, 1962), was added to the samples and shaken thoroughly. A blank of distilled water and 0.004, 0.02, 0.2 mg/L PO_4-P were used as phosphate standards. After 8 minutes - but before 30 - the absorbance of the samples and standards were read in a spectrophotometer at 705 nm using a 5 cm path. For details see Chapter 4. Total phosphorus (TP) was determined using the persulphate-sulphuric acid digestion method (APHA, 1992). Samples were cooled to room temperature before determination as for soluble reactive phosphorus.

NO_2/NO_3-N concentrations were determined according to the method of Morris and Riley (1963) and Wood *et al.* (1967). In this, nitrate was reduced to nitrite in a Cd column and then analysed for nitrite. Because of salt effects, the method must be applied cautiously and the standard addition method (Fortner *et al.*, 1976; Atkinson, 1987) and salt error corrections at different salinities were applied to estimate true nitrate concentration in highly saline samples (Ghassemzadeh *et al.*, 1996a and in review).

2.3. Results

Seawater is pumped into two ponds at different locations, pond XE1-3 at Middle Beach in the north, and pond XB3 at Chapman Creek further south (Fig. 2.1). Water flow from one pond to the next is shown in Figure 2.1. Water from initial ponds is transferred to pond XD1 and to pond XC3. The source of water in pond

XB3 is mainly from XD1, but during the summer the seawater from Chapman Creek is also added to this pond. Water from pond XB3 is transferred to ponds XB4-5, and several other ponds in that series and then XB8. These two sources leave ponds XB8 and XC3 and mix in pond XA1. After this pond, water is transferred to ponds PA3, PA4-5, PA6-7-8, PA9, PA10, PA11 and PA12 and finally to crystallising ponds. The volume of seawater entering the saltfields from the two different sources is shown in Table 2.1 and Figure 2.2. This Table also shows the volumes of seawater which entered at different seasons during 1994 and 1995. Intake rates in spring 1994 were about those of half of summer 1995 at Chapman Creek. The data was provided by the Penrice company at Dry Creek solar saltfields.

Table 2.1. The volume (ML) of seawater inflow into the Dry Creek saltfields during 1994-1995.

Seawater inflow	Middle Beach	Chapman Creek
Total volume (1994)	40657.4	2735.5
Summer	20645.0	1473.0
Autumn	5814.0	0
Winter	6011.2	0
Spring	8187.2	1262.5
Total volume (1995	46947.2	5033.1
Summer	21957.2	2935.5
Autumn	1808.8	0
Winter	3026.0	0
Spring	20155.2	2097.6

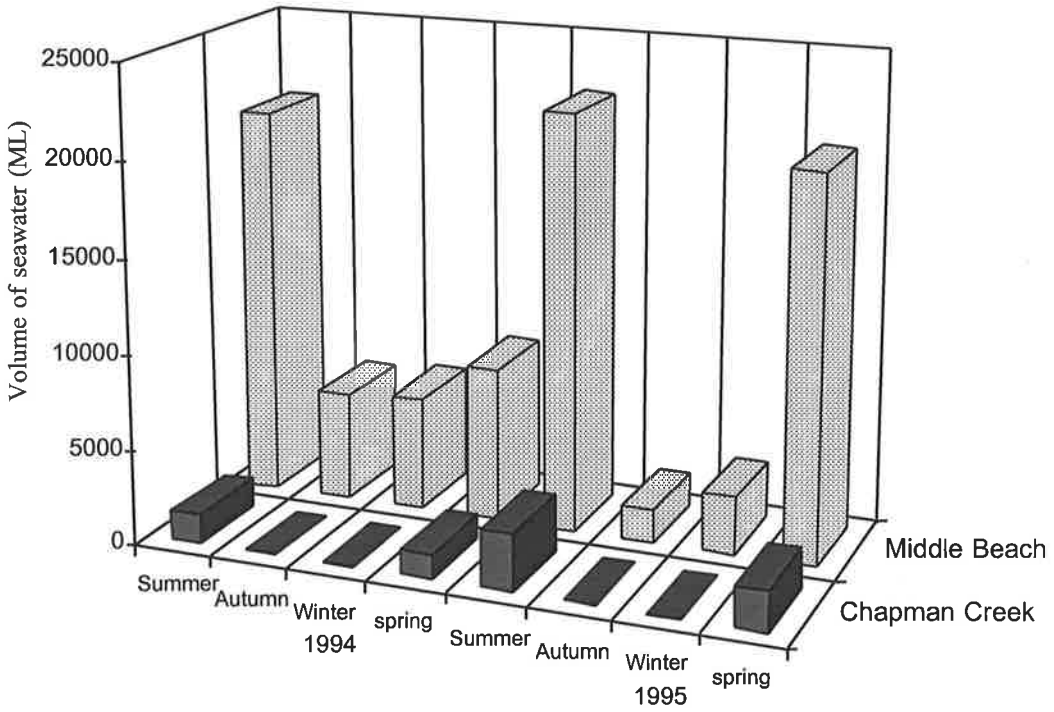

Figure 2.2. Seasonal seawater flow from Chapman creek and Middle Beach.

Mean salinity, pH, alkalinity, dissolved oxygen, soluble reactive phosphorus, total phosphorus and nitrate measurements in Middle Beach and Chapman Creek inflows are shown in Table 2.2. These data show that the salinity and nutrients are low at the Middle Beach inflow, while they are high at the Chapman Creek inflow.

Table 2.2. Major chemical features of seawater inflow to solar salt ponds. Data are mean values.

Parameters	Inflow	
	Middle Beach	**Chapman Creek**
Salinity (g/L)	38	47
pH	8.9	9.1
Alkalinity (meq/L)	4.9	5.2
D.O.(mg/L)	7.5	7.1
SRP (µg/L)	40	250
Total P (µg/L)	52	290
NO_3-N (µg/L)	4.5	133

2.3.1. Physical parameters

Mean monthly meteorological data are shown in Table 2.3 and Appendix 1.1. The mean monthly rainfall and evaporation, April 1994 - April 1995, were 25.73 and 162.72 mm, respectively. The lowest monthly rainfall was 2.4 mm in April 1994 and the highest 112.9 mm in July 1995. Average monthly rain in 1994-1995 was 35.02 and in 1995-1996 was 40.33 mm. Annual rain in 1994-1995 and 1995- 1996 was 420.2 and 483.9 mm, respectively. Rainfall exceeded evaporation in June 1994 and July 1995 when net evaporation was negative (Fig. 2.3). The average temperature ranged between 10.7 and 23.4 °C. The lowest and highest evaporation during the study period occurred in June 1994 (44.3 mm) and in January 1995 (299.6 mm), respectively.

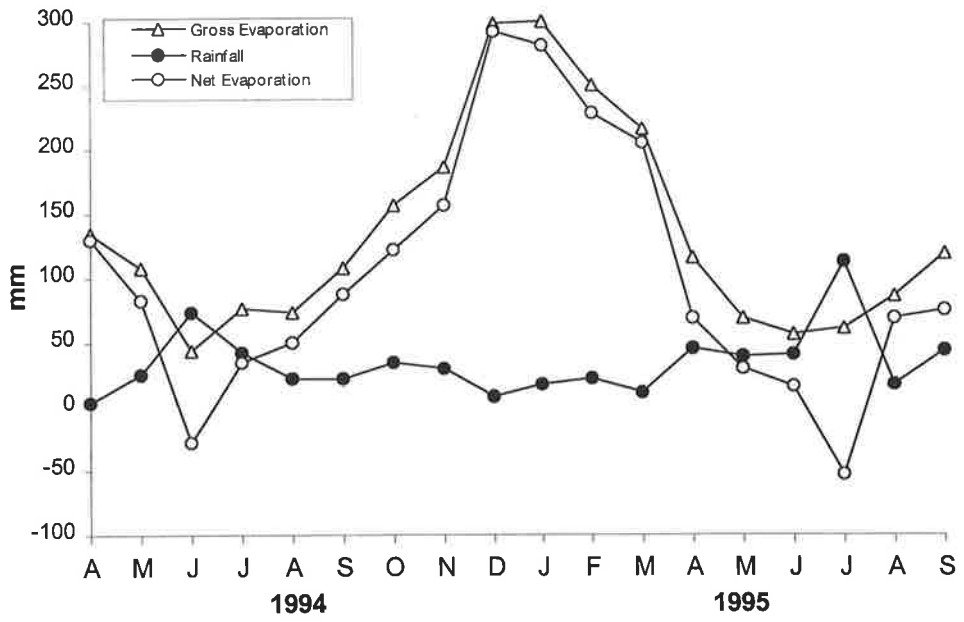

Figure 2.3. The seasonal variations in rainfall and gross evaporation. Net evaporation is also presented.

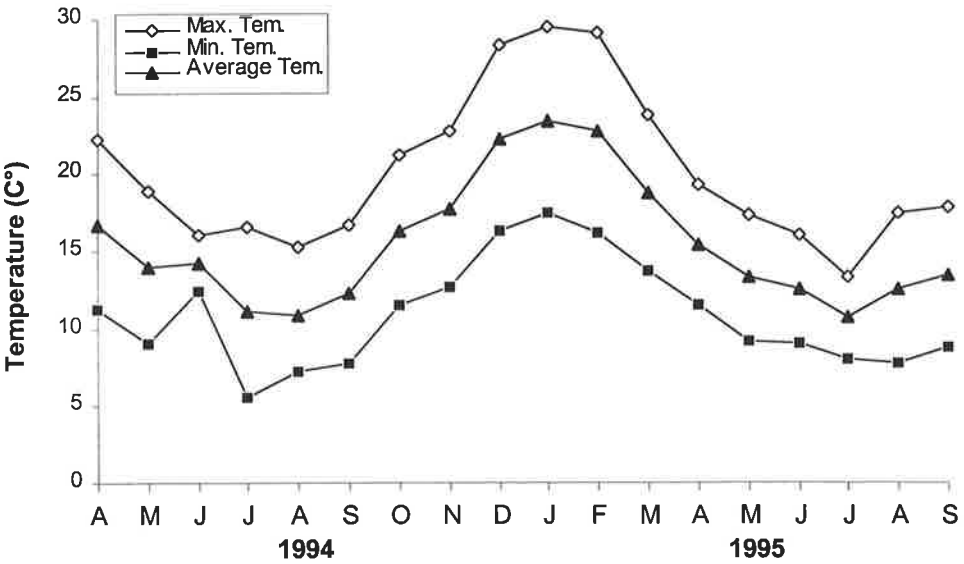

Figure 2.4. Maximum, minimum and mean air temperature during the study period.

Table 2.3. Mean meteorological data over 18 months. Detailed data (daily records) are given in Appendix 2.1.

Date	Temperature (C°)			Rainfall (mm)	Evaporation (mm)	
	Max.	Min.	Mean		Gross	Net
April 1994	22.2	11.2	16.7	2.4	135	132.6
May	18.9	9.1	14	25.6	108.2	82.6
June	16	12.4	14.2	72.9	44.3	-28.6
July	16.6	5.6	11.1	42.5	77.1	34.6
August	15.3	7.3	10.8	22.2	72.8	50.6
September	16.7	7.8	12.3	21.3	108.5	87.2
October	21.2	11.5	16.3	33.7	155.6	121.9
November	22.7	12.7	17.7	29.5	186.4	156.9
December	28.3	16.3	22.3	7.3	299.1	291.8
January 1995	29.5	17.4	23.4	17.9	299.6	281.7
February	29.1	16.2	22.7	21.9	249.9	228
March	23.8	13.7	18.7	11.6	216.2	204.6
April	19.3	11.5	15.4	45.9	115.4	69.5
May	17.3	9.2	13.3	39.6	68.7	29.1
June	16	9.1	12.6	39.9	56.2	16.3
July	13.3	8	10.7	112.9	60.5	-52.4
August	17.4	7.7	12.6	16.9	86.2	69.3
September	17.9	8.8	13.4	43.1	118.1	75

Monthly maximum, minimum and mean air temperatures during the study period are shown in Figure 2.4 and Appendix 1.2. The lowest average minimum temperature was 5.6 °C and was recorded in July 1994. The highest average maximum was 29.5 °C and was recorded in January 1995. Mean air and water temperatures and mean wind speed for individual ponds are indicated in Figure 2.5. Water temperatures varied in the study ponds between 10 and 32 C°. The values of wind speed on the day of sampling ranged from 0.1 to 75.5 km/h (Fig. 2.5 and Appendix 1.1).

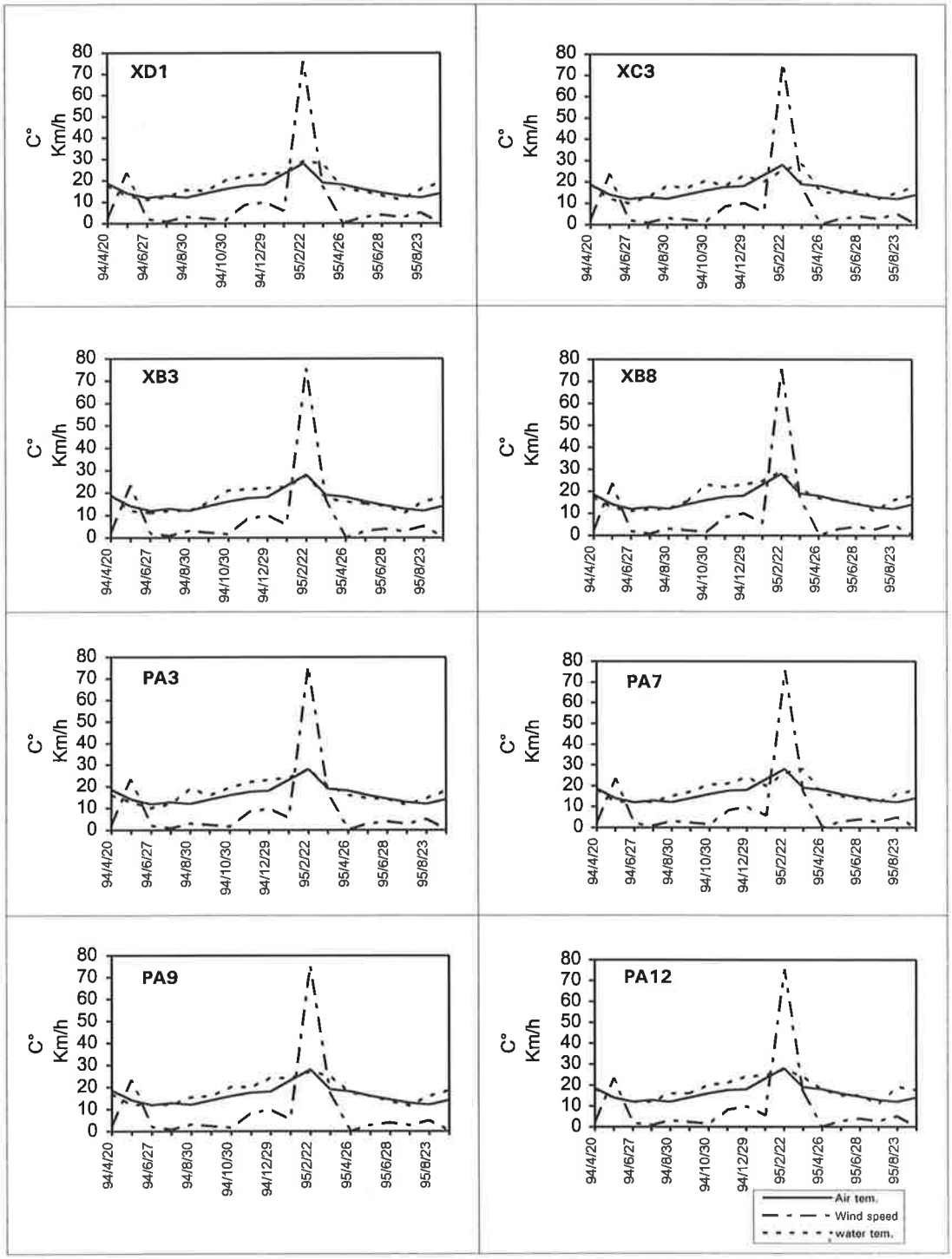

Figure 2.5. Air and water temperature and wind speed in study ponds.

Secchi disc values are indicated in Figure 2.6 and Appendix 1.2. The Secchi disc was always visible to the bottom in ponds XD1 and XC3. Secchi disc transparency in pond XB3 ranged from 15 to 34 cm during autumn and winter 1994 and it was more than pond depth in the rest of the study period. Pond XB8 was turbid during the study period and transparency ranged from 15 to 30 cm. In pond PA3 transparency depths were lowest (20 to 30 cm) during autumn and winter 1994, in late summer, late autumn and winter 1995. The Secchi disc was always visible to the bottom in ponds PA7, PA9 and PA12, except in pond PA12 in December 1994 (Secchi depth 20 cm).

2.3.2. Chemical parameters

The mean values for salinity, pH, alkalinity, dissolved oxygen and nutrients (SRP, NO_3-N) over the 18 months period of measurements are shown in Table 2.4. and graphically in Figure 2.7. Detailed results of the regular monitoring are also given in Appendix 1.2.

The mean values of salinity in the study ponds (Fig. 2.7a) ranged from 55.6 (XD1) to 243.5 g/L (PA12). Mean values for dissolved oxygen concentration are shown in Figure 2.7b. They ranged from 2.1 (PA9) to 4.9 mg/L (XD1). These values are compared in this figure to equilibrium standard atmospheric concentration (C°).

Mean pH and total alkalinity in the study ponds over 18 months are shown in Figure 2.7c. The pH of samples ranged from 7.84 to 8.26. The lowest value for alkalinity was 3.09 meq/L (pond XC3), the highest, 4.89 meq/L (ponds PA9 and PA12). These values indicate that all ponds were alkaline. A significant correlation between salinity and alkalinity ($r= 0.72$, $p<0.01$) is present.

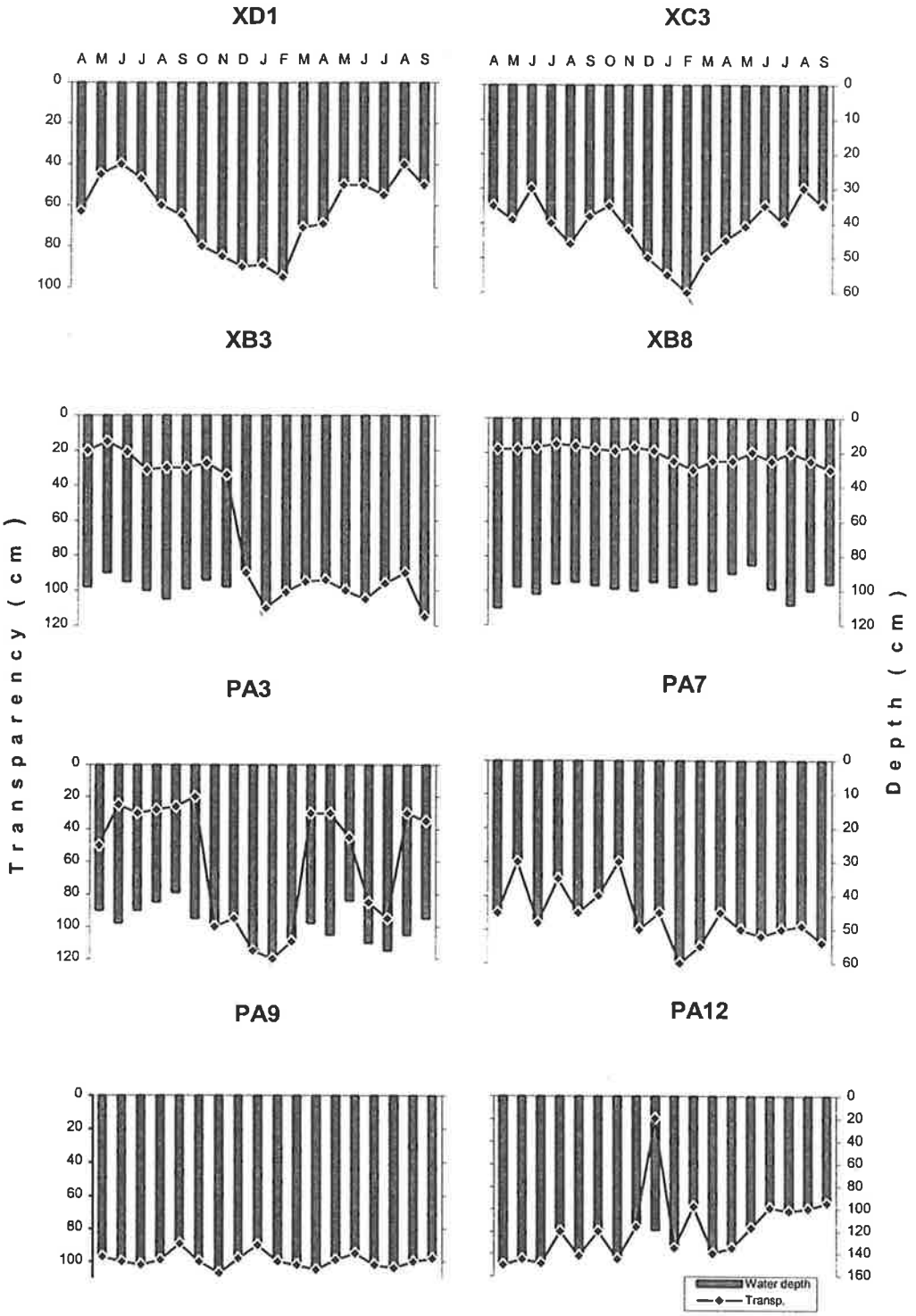

Figure 2.6. Secchi disc transparency in selected ponds. Scale is not the same.

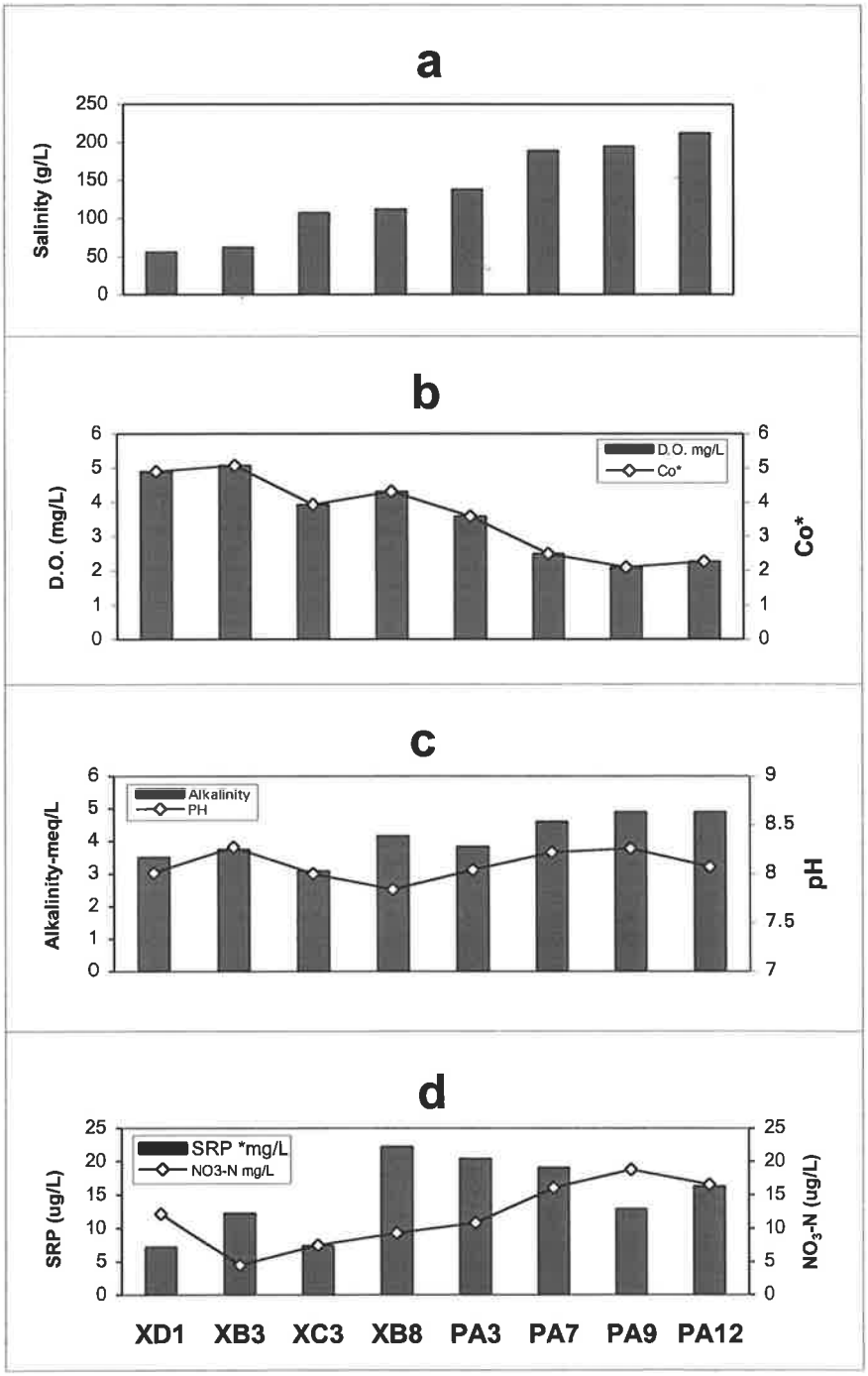

Figure 2.7. The mean of salinity (a), dissolved oxygen (b), pH and alkalinity (c), and nutrients (d) in study ponds.

Table 2.4. Maximum, minimum, mean and standard deviation for major chemical features for the period April 1994 - September 1995 at the Dry Creek saltfields.

Ponds	XD1	XC3	XB3	XB8	PA3	PA7	PA9	PA12
Salinity (g/L)								
max.	63.5	136.8	80.75	130.4	164.5	240	214	243.5
min.	46.1	89.8	53.4	93.6	113.4	155	168.5	189
mean	55.6	107.3	62.1	112	137.6	189	195	213
std.	4.4	12.4	5.8	8.9	13.4	20.7	13.2	17.8
pH								
max.	8.5	8.42	8.7	8.63	8.98	9.05	9.32	9.02
min.	7.7	7.6	7.9	7.5	7	7.3	7.5	7.5
mean	8.01	8.0	8.24	7.84	8.04	8.22	8.26	8.07
std.	0.32	0.23	0.23	0.39	0.45	0.52	0.46	0.52
Alkalinity(meq/L)								
max.	4.5	4.4	4.4	5.7	4.8	5.3	5.8	6.0
min.	2.05	1.7	2.1	2.44	2.33	3.2	4	3.4
mean	3.51	3.09	3.75	4.16	3.83	4.6	4.89	4.89
std.	0.52	0.48	0.49	0.84	0.52	0.55	0.44	0.62
D.O[1] (mg/L)								
max.	9.5	7	9.8	8.5	6.4	4.8	4.0	4.8
min.	1.6	1.4	2.2	1.2	1.2	1.1	1.0	0.9
mean	4.9	3.93	5.0	4.31	3.58	2.49	2.1	2.27
std.	2.7	2.0	2.5	2.2	1.5	1.1	0.86	1.1
SRP[2] (µg/L)								
max.	20	20	33	56	51	51	27	42
min.	<1	<1	0	3	4	4	2	1
mean	7	7	12	22	20	19	13	16
std.	4.3	4.8	9.4	14.1	12.1	12.6	8.09	11.9
NO_3-N (µg/L)								
max.	58	29	22	24	35	65	24	46
min.	1	0	<1	<1	<1	1	0	1
mean	12	7	5	9	11	16	19	16
std.	15.63	7.8	4.7	6.74	9.03	13.8	5.69	13.75

1-Dissolved oxygen, 2- Soluble reactive phosphorus

Mean concentrations of SRP and nitrate-nitrogen measurements are presented in Figure 2.7d. The ranges for SRP and nitrate nitrogen were 7 to 22 and 5 to19 µg/L, respectively.

The concentration of major elements in seawater, Middle Beach inflow, Chapman Creek inflow, concentrating ponds, crystallising ponds and final brine is shown in Table 2.5. Chloride and sodium have the highest values and magnesium, sulfur and calcium have, respectively, the next highest values in seawater. However, in the final brine at Dry Creek saltfields, chloride and magnesium have the highest value.

Table 2.5. Elemental analysis in different samples from Dry Creek solar saltfields. ND= Not determined

	Cl	Na	Mg	S	K	Ca
	mg/L					
Seawater	19336.6	10740	1273.3	925.3	447	446.3
Middle Beach	22,492	13,040	1,840	1,080	456	548
Chapman Creek	22,224	12,840	1,760	1,053	432	552
Concentrating ponds	ND*	ND*	13230	4970	ND*	1100
Crystallising ponds	187,925	97,392	15,156	6,650	3,860	490
Final Brine	264,013	19,800	95,500	18,950	21,900	150

The seasonal variations of salinity and dissolved oxygen during the study period are shown in Figure 2.8. The salinity values ranged from 46.1 to 63.5 g/L (pond XD1); 53.4 to 67.2 g/L (pond XB3); 92.4 to 136.8 g/L (pond XC3) and 93.6 to 130.4 g/L (pond XB8). The salinity values increased in ponds PA3, PA7, PA9 and PA12 and the values ranged from 113.4 to 164.5 g/L; 155 to 240 g/L, 179 to 214 g/L and 189 to 243.5 g/L, respectively. Values are low during winter and high during summer in most study ponds, but, the variations are greater at the lower salinity than at higher salinity. As shown in Figure 2.8, dissolved oxygen concentrations decreased with increasing salinity and they ranged from 1 (pond PA9 and PA12) to 9.8 mg/L (pond XD1).

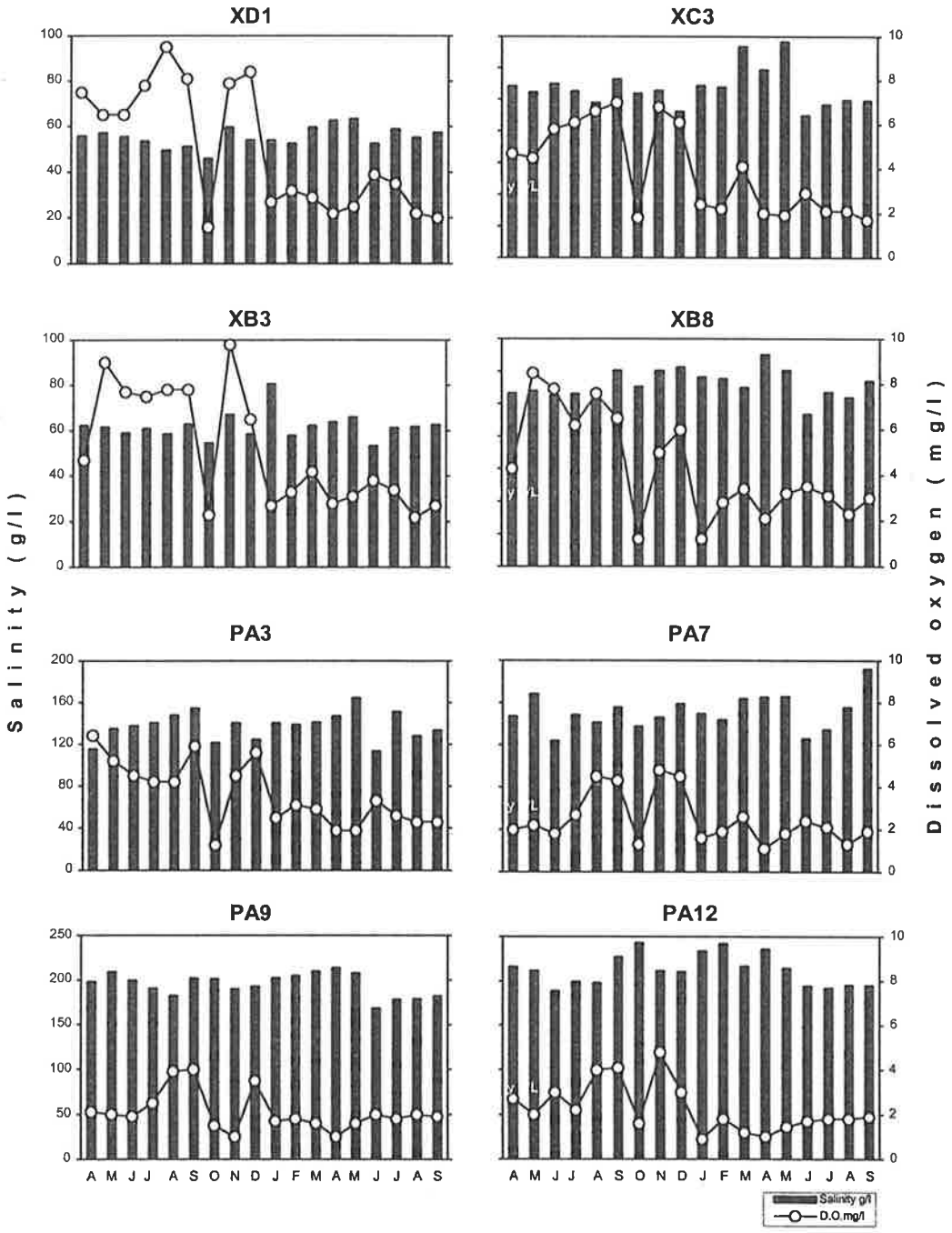

Figure 2.8 .The seasonal variations of salinity and dissolved oxygen in study ponds. Scale is not the same.

Seasonal variations of pH and alkalinity are presented in Figure 2.9. pH ranged from 6.9 (pond XB8) to 9.32 (PA9). The total alkalinity values ranged from 2.05 meq./L (pond XD1) to 5.8 meq./L (ponds PA9 and PA12).

There is a significant correlation between dissolved oxygen and salinity in the brine ($r = 0.98$, $p < 0.05$). However, the correlation between dissolved oxygen, pH and alkalinity is not significant ($r = 0.114$, $p > 0.05$; $r = 0.098$, $p > 0.05$), respectively.

Seasonal variations in nutrient concentrations (SRP and NO_2/NO_3-N) are indicated in Figure 2.10. The soluble reactive phosphorus concentration varied from 0 µg/L (pond XB3) to 56 µg/L (pond XB8). The ranges in ponds XD1, XC3, XB3 and XB8 were <1 to 20, <1 to 20, 0 to 33, and 3 to 56 µg/L, respectively. The ranges in ponds PA3, PA7, PA9 and PA12 were 4 to 51, 4 to 51, 2 to 27 and 1 to 42 µg/L, respectively. In most ponds, higher amounts of soluble reactive phosphate occurred in summer and winter seasons. However, the ranges of nitrate nitrogen concentration in selected ponds varied from 0 µg/L (pond XC3) to 65 µg/L (pond PA7). The concentrations in ponds XD1, XC3, XB3 and XB8 were 1 to 58, 0 to 29, <1 to 22 and <1 to 24, µg/L. The ranges in ponds PA3, PA7, PA9 and PA12 were <1 to 35, 1 to 65, 0 to 24, and 1 to 46 µg/L, respectively. The highest value occurred in pond XD1 during autumn 1994.

2.4. Discussion

The present section addresses the nature and variation in physico-chemical factors in the solar saltfield at Dry Creek, South Australia. Like most other saltfields, the study ponds are shallow and exposed to wind action. Physico-chemical factors vary between ponds across the salt gradient and with season. Physico-chemical factors and their relation to the biological conditions (Chapter 3) are important in the management of the saltfields.

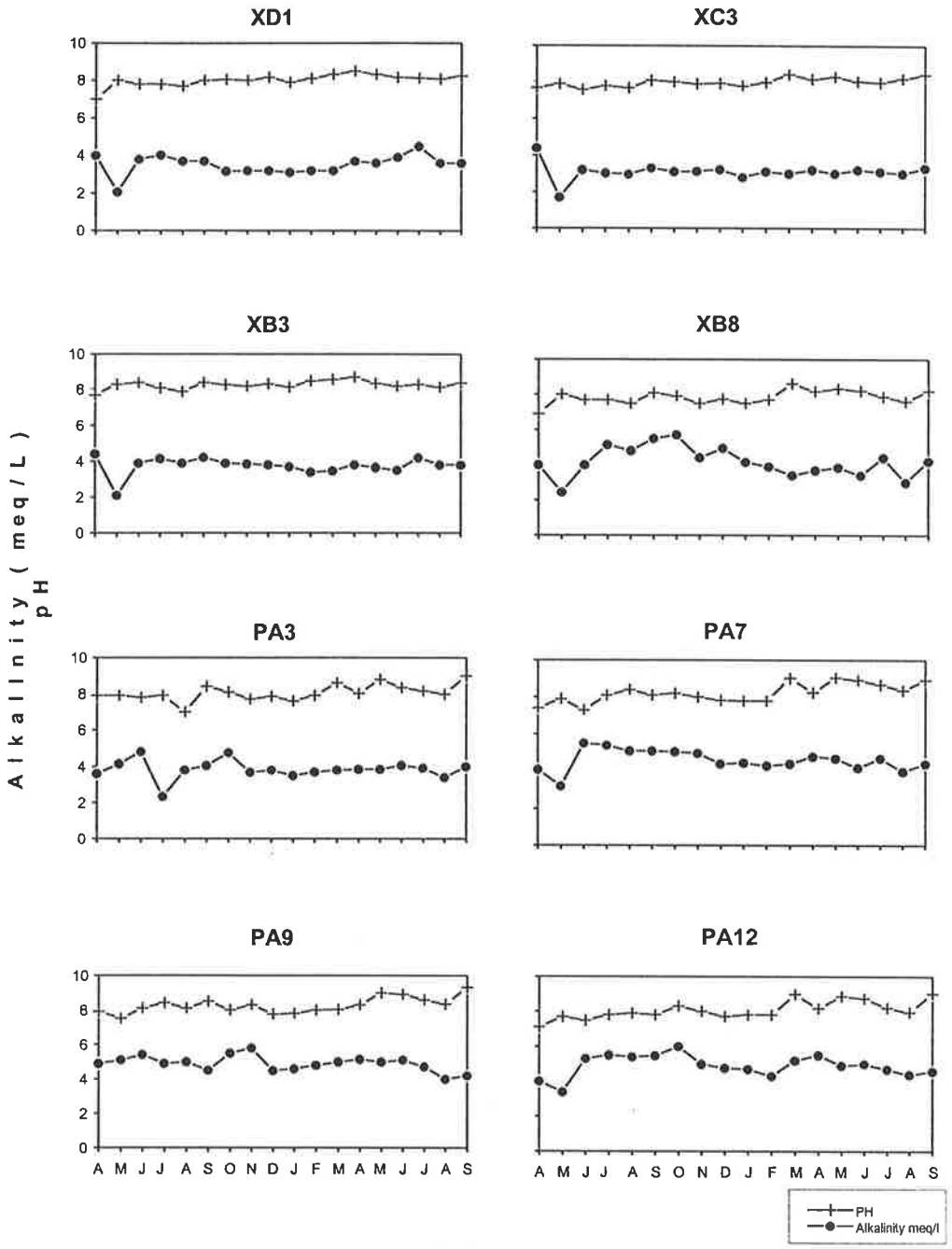

Figure 2.9. The seasonal variations of alkalinity and pH in study ponds.

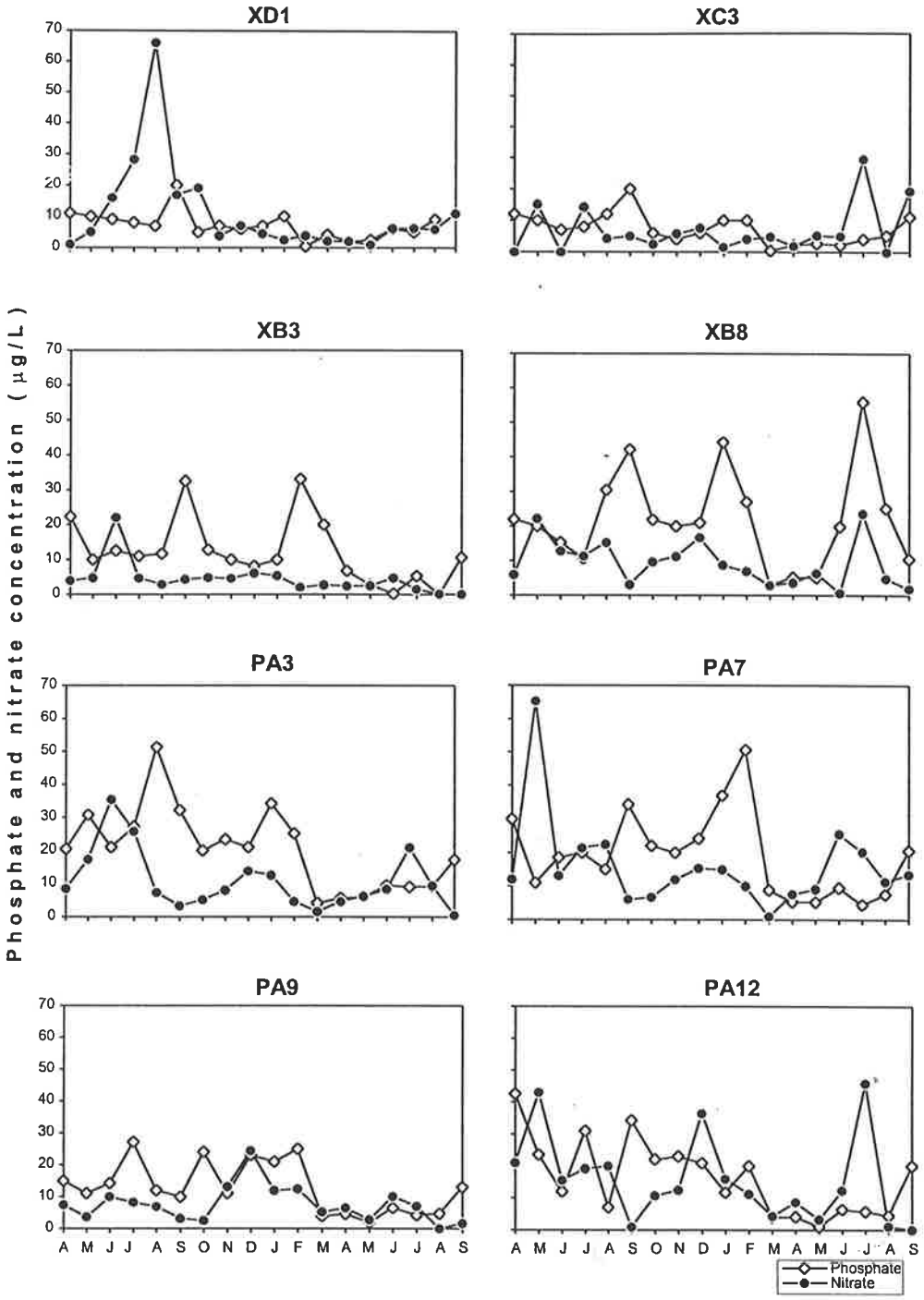

Figure 2.10. The seasonal variations in nutrient concentrations in study ponds.

The seawater inlet from Middle Beach is low in nutrient concentration, while the inlet from Chapman Creek is high in nutrient concentration and these differences are reflected in the series of ponds associated with each intake. In recent years, the Chapman creek series of concentrating ponds has undergone considerable eutrophication. The major source of nutrient input is from the outfall of the nearby E&WS Bolivar Sewage treatment works. The Bolivar Sewage treatment works pumps nutrient-rich, treated effluent into the ocean via a channel which runs through the saltfields. The effluent channel enters Fork Creek, a tidal creek 2 km south of the Chapman creek inlet, and the nutrient rich water is likely to move north (towards Chapman Creek) on a flood tide (Harbison, 1991). Several papers have dealt with the effects of this nutrient rich sewage output into the coastal waters of South Australia (Connolly, 1986; Neverauskas, 1988a and b). The management of the saltfields at Dry Creek has decided to use a small volume of this water in the saltfields (Fig. 2.2). This affects the trophic status of the ponds and will be discussed in Chapter 3. There are other minor nutrients inputs to the ponds, including storm water runoff from urban and rural development.

2.4.1. Physical parameters

The results of investigation of physical factors clearly indicate the strong seasonal climatic changes. Thus, evaporation rates varied by a factor of four between summer and winter. The amount of evaporation has a direct relation to high brine flow rate in summer compared with winter and clearly affects the amount of nutrient coming from seawater to the fields and the amount of brine passing from one pond to the next pond. This is important for distribution of biota in the saltfields (see Chapter 3).

Water temperature, reflecting seasonal climatic changes, varied seasonally but showed no differences between ponds. No stratification was evident in any pond,

due to both shallow depth and exposure to wind. At all seasons, water and air temperatures were similar but generally water temperature was slightly higher than air temperature. This can be explained on the basis of the specific heat of water and high salinity.

Wind speed was likely to have most affected nutrient recycling and sediment resuspension in shallow ponds. The wind also influenced both horizontal and vertical water movements in the ponds. Moreover, diurnal mixing patterns depend strongly on wind effects as well as on water intake from previous ponds. Evaporation is also controlled by the wind and vapor fluxes over the water - body (Winter, 1995).

Although, transparency was generally high in most ponds, the greater volume of seawater flow (with high nutrient) to pond XB3 from Chapman Creek in 1993 was the probable cause of high phytoplankton growth and low transparency in this pond. In 1995, the higher transparency in this pond was probably due to low phytoplankton standing crop and because of the small volume of seawater from Chapman Creek that had entered the field. Transparency in pond XB8 was low because water of high nutrient content entered the system and also because the sediment supplied nutrients for phytoplankton growth. The variation of transparency in pond PA3 was due to massive blooms of the aggregated form of algae that coated the surface of this pond in spring and summer. Its transparency was low due to algal blooms in autumn and winter, but was high during spring and summer due to the presence of large clumps of the aggregated form of algae. At the end of summer, these aggregated forms of algae accumulated in the pond and led to a decrease in the transparency once more.

2.4.2. Chemical parameters

The order of ionic dominance in seawater and the source inflow to the saltfields at Dry Creek was Na^{+}> Mg^{++}> $K^{+}\cong Ca^{++}$ and Cl^{-}> SO_4^{--} > $HCO3^{-}$> $CO3^{--}$ (pH of seawater was 8.2 - 9.1). This is the same as the order in most natural saline lakes and solar saltfields as reported by other researchers (Volcani, 1944; Bayly and Williams, 1966; Williams and Buckney, 1976; Oren and Shilo, 1982; Burk and Knott, 1989; Burk, 1990; Chitins and Sanghavi, 1993; Chambers *et al.,* 1993). This pattern of ionic dominance prevails in most of the study ponds at Dry Creek, at least until calcite ($CaCO_3$) and gypsum ($CaSO_4$, $2H_2O$) start to precipitate. Ionic proportions change after gypsum precipitation and also in the final brine which is rich in magnesium. A gradual decrease in calcium concentration in ponds PA3, PA7, PA9 and PA12 can be attributed to gypsum precipitation in these ponds. Gypsum precipitation did not take place until a salinity 151 g/L was reached. Note that the theoretically accepted density is 1.0894 g/cm^3 for gypsum precipitation as determined in concentrates of pure seawater (Baseggio, 1974). Using the conversion table of Bengtson *et al.* (1991) (see Appendix 1.3), the salinity is about 130 g/L. Gypsum precipitation also occurred at a higher salinity (169 g/L) in solar saltfield at Port Alma, Queensland (M. Colman, pers. comm. 1994). At Dampier saltfield in Western Australia, it occurred at a lower salinity, 123 g/L (Roux, 1996).

This precipitation of gypsum at Dry Creek at higher salinities than predicted is probably a consequence of a biological activity, and particularly the biogenic precipitation of $CaCO_3$. This activity modifies the relative concentrations of calcium and sulphate in the brine. As an example, the presence of a high density of phytoplankton in ponds PA3 (see Chapter 3) increases the pH and this in turn has an effect on carbonate precipitation. The increase in pH was due to the removal of carbon dioxide by algal photosynthesis during the day, causing a shift from

bicarbonate to carbonate (Amit and Bentor, 1971). In the presence of Ca^{2+}, carbonate precipitates under these conditions and Ca^{2+} decreases in the brine (Sawyer and McCarty, 1978; Manahan, 1993). The presence of phytoplankton in the brine therefore helps to precipitate calcite which are impurities in the sodium chloride crystals harvested from the saltfields and delay to precipitate gypsum due to the longer time to reach to the degree of saturation.(Susarla and Sanghavi, 1993). This is important in the determination of salt quality. The quality of salt produced from saltfields will be further discussed in Chapter 5.

According to the pattern of brine evolution predicted by Eugster and Hardie (1978) and Faure (1991), calcite precipitates first and carbonate ions are eliminated from solution before all Ca^{2+} has been removed and then, at this stage, Mg-silicate precipitates (Fig. 2.11A). As evaporation continues, the concentration of remaining Ca^{2+} rises until gypsum begins to precipitate. Continued precipitation of gypsum and Mg-silicate eventually depletes the water of Ca^{+2}, Mg^{+2} and silicon, leaving Na^{+} and K^{+} as the principal cations. The low silicon concentration in water affects diatom abundance in high salinity ponds (see Chapter 3). When gypsum precipitation began, the mole ratio of Ca^{+2} : SO_4^{-2} was less than one. Therefore, an excess of SO_4^{-2} remains after Ca^{+2} has been virtually eliminated from solution, leading to sulphate - rich and Ca - poor solutions (path 3). Depending on the ionic proportions of the brine, an alternative path may produce a Ca^{+2} and Mg^{+2} - rich but sulphate - poor brine (path 4). Path 3 leads to brines that are enriched in sulfate, and chloride, whereas path 4 produces chloride brines.

The evolutionary pathways in Figure 2.11A do not explain the origin of certain Mg-rich brines. For Mg^{2+}-rich brines to be produced, the formation of Mg-silicate must be inhibited (Faure, 1991). Two kinds of Mg^{2+}-rich brines may form, as shown in Figure 2.11B. Path 1 leads to gypsum precipitation and produces either a Mg

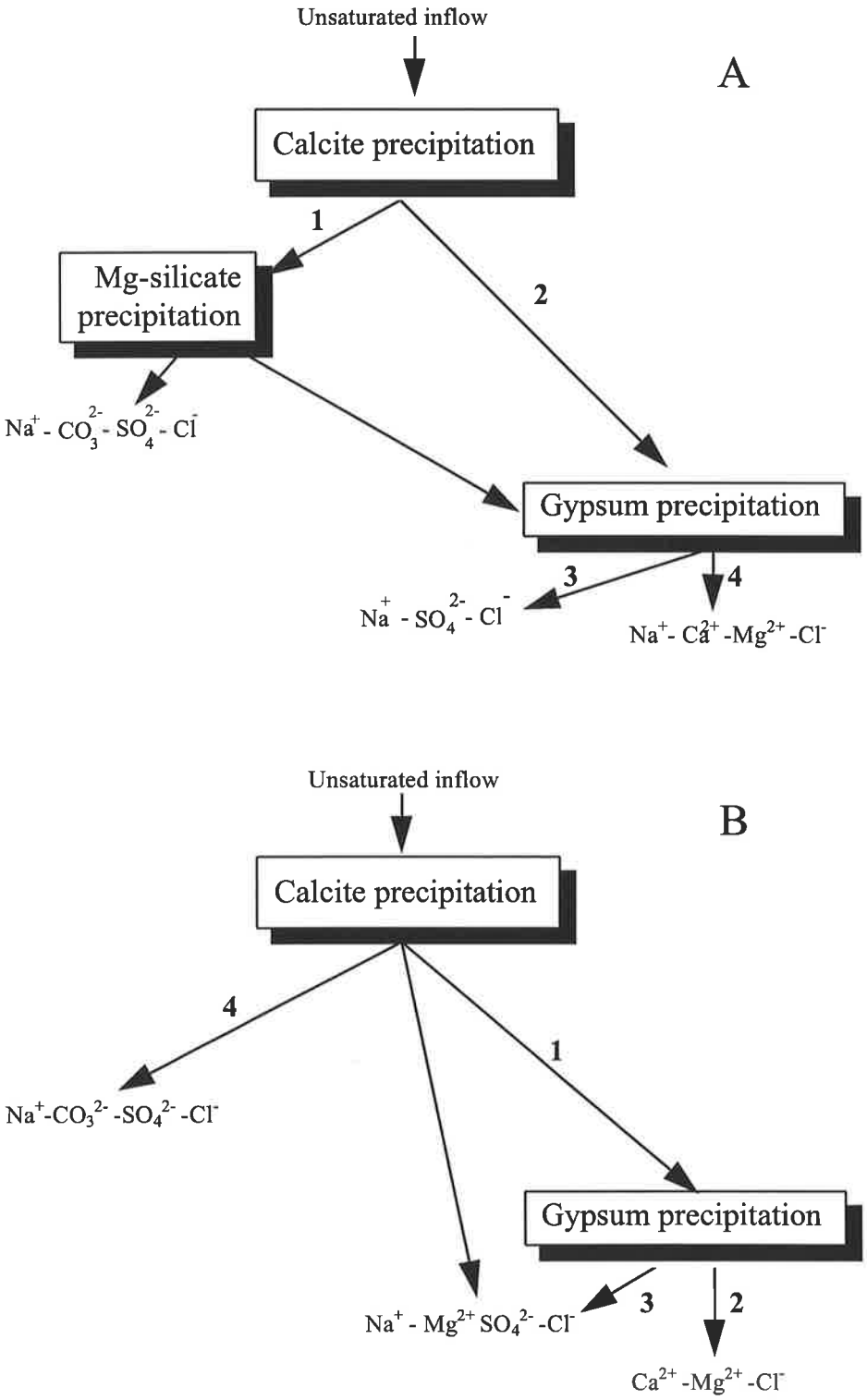

Figure 2.11. Chemical evolution of seawater in study area. See text for details.

sulfate (Ca-poor) brine (path 3) or a Mg^{2+} + Ca^{2+} + Cl^- (sulfate-poor) brine (path 2). Alternatively, gypsum precipitation may be bypassed and a Na^+ - Mg^{2+} Ca^{2+} - SO_4^{2-} -Cl^- brine may form directly after calcite precipitation (path 4). Final brine (bittern) in saltfields at Dry Creek is high in Na^+ - Mg^{2+} - SO_4^{2-} -Cl^-, so that path 3 in this model appears to hold true for the saltfield during this investigation.

The point of this discussion is to emphasize that a wide variety of precipitated salts may form by evaporative concentration of water in solar saltfields. Which salt form depend on the ionic systems that operate during the precipitation of compounds and which deplete particular cations or anions depending on their molecular ratio. Moreover, biological activities affect ionic equilibria. Further investigations are required to amplify the relationship between precipitation and biological events.

The small seasonal variation in salinity is a result of the complex interaction between temperature, evaporation, rainfall and the management of flow into the ponds. Increases in temperature during the summer cause evaporation to increase. This increases the concentration of ions in water. However, transferring brine from one pond to the next results in almost constant salinity with small variations. This is an important difference between natural saline lakes and saltfields ecosystems. In natural saline lakes the fluctuations in salinity are often high and often desiccation occurs (Williams, 1966; Hammer, 1986).

The total alkalinity of all natural saline waters with a salinity exceeding 10 g/L, results from the ionic concentrations of CO_3^{2-}, HCO_3^-, $H_2BO_3^-$ and OH^- (Hammer, 1986). But in high salinity brine, such as ponds PA3, PA7, PA9 and PA12, the CO_3^{2-} and HCO_3^- concentration was low and the brine contained a high concentration of chloride ions. Thus, part of the alkalinity variation at the Dry

Creek saltfields may be associated to the high chloride concentration in high salinity brine which has effect on the alkalinity determination (Caljon, 1983).

The occurrence of high oxygen values in ponds with a well-developed benthic vegetation (XD1 and XB3) is probably due to the photosynthetic activity of this vegetation, especially the green algae (*Chara*). Dissolved oxygen in ponds that are covered by a thick layer of organic mud (XB8 and XC3) show lower values in summer because mud consumes dissolved oxygen during decomposition of organic material. There are other fluctuations in dissolved oxygen concentration in the study ponds. It is not easy to explain these in very shallow ponds, because of the close relationship between free water and the influence of wind (Comin *et al.*, 1983). Moreover, the concentration of dissolved oxygen is not only dependent on the oxygen production and consumption by phytoplankton but also on oxygen consumption by zooplankton, zoobenthos and bacteria. Decreasing oxygen concentrations were apparent across the saltfield ponds and in different seasons associated with high temperature and salinity together with decomposition of settled organic matter (Wetzel and Likens, 1991).

Decreased nutrient concentration in pond XB3 was a result of recent inflows of lower nutrient water from Middle Beach compared to the earlier intake of Chapman Creek water, with high nutrients (from 1992, it was started to decrease the amount of seawater from Chapman Creek). The higher phosphate concentration in ponds XB8, PA3 and PA7 compared to ponds XD1, XB3 and XC3 may be related to phosphorus reserves that are present in the sediments and which can be regenerated (Andersen, 1975; Wetzel, 1983). This high concentration of phosphorus in sediments probably results from a history of high nutrients input from Chapman Creek (Table 2.2). It has been noted that ionic composition, pH, and high levels of dissolved organic carbon in saline waters play an important role in modifying the availability of phosphate concentration (Waiser and Robarts, 1995). Low phosphate

concentrations during spring, summer and autumn in ponds XD1, XB3, XB8 and PA3 correspond with the photosynthesis activity in these ponds (see Chapter 3) with uptake of phosphate. NO_3-N : PO_4-P ratio was low during the period of this study especially in higher salinity pond which was probably a reason for the abundant of cyanobacteria in these ponds (Paerl, 1996).

The soluble reactive phosphate and nitrate concentrations in the study ponds are similar to those reported from other solar salt systems (Carpelan, 1957; Jones *et al.*, 1981; Javor, 1983a and b; Rahaman *et al.,* 1993) (Table 2.6). They are also similar to those reported by Sammy (1985) in the concentrating ponds of Dampier in Western Australia. The ranges of phosphate concentration were 0.93-2.37and 1.48 to 3.63 μg/L at two different solar saltworks in India (Rahaman *et al.,* 1993). Much higher values have been reported by Jones *et al.* (1981) in the saltfields at Dry Creek, where the PO_4-P concentrations were up to 900 μg/L in the first concentrating ponds (Table 2.6). The brine there had a high density of phytoplankton and a high turbidity. The reason for this enrichment was a higher amount of seawater intake from Chapman Creek at that time. Total phosphate input to the saltfields is reported by Jones *et al.* (1981) as 15 x 10^3 kg (PO_4-P)/year. This value in the period of this research was 0.79 x 10^3 to 2.1 x 10^3 kg (PO_4-P)/year (1994) and 0.79 x 10^3 to 2.4 x 10^3 kg (PO_4-P)/year (1995) input from Middle Beach and Chapman Creek, respectively. Decreased nutrient concentration during 1994 - 1995 compared to 1981 is related to the smaller intake of seawater from the Chapman Creek area. In 1992, it was decided to decrease the amount of seawater from Chapman creek into the system because the Bolivar Sewage treatment works pumps nutrient-rich, treated effluent into the ocean nearby this area. It was also decided to add groundwater to the evaporating ponds after mid - summer 1996 (after the monitoring for this study had been completed). The groundwater is high in

Saltfields	Salinity (g/L)	Nutrient conc. ug/L		Nutrient status	Reference[a]
		PO_4^{3-}	NO_3^-		
Dry Creek (South Australia)	55.6-213	7-22	5-19	Oligotrophic	1
Vedaranyam (India)	57-297	1-3	<1-3	Oligotrophic	2
Kelambakkam, (India)	45-326	1-2	<1-1	Oligotrophic	3
Dry Creek (South Australia)	37-120	800-900	100-250	Eutrophic	4
Dampier (Western Australia)		3-25	ND[b]	Oligothrophic	5
Exportadora de sal (Mexico)		0	0-124	Oligotrophic	6
Western salt (California, USA)		0-4	0-2294	Eutrophic	7
Salin-de-Giraud (France)		1-6	25-886	Moderately eutrophic	8
Alviso (California, USA)		28-1235	31-2015	Eutrophic	9

Table 2.6: Nutrient status of solar saltfields
a (1) Ghassemzadeh *et al.* (1996a and b); (2 and 3) Rahaman *et al.* (1993); (4) Jones *et al.* (1981); (5) Sammy (1985); (6) Javor (1983b); (7) Javor (1983a); (8) Landry and Jaccard (1982); (9) Carpelan ((1957).
b Not detemined

salinity and silicon. Salinity and silicon concentration are 70 g/L and 8.5 mg/L, respectively (reported by Department Mines and Energy) which are higher than in seawater (Faure, 1991). The high concentration of silicon may affect and promote the growth of diatoms and may prevent the growth of cyanobacteria at high salinity ponds.

2.5. Conclusions

The investigations outlined above provide a basis for studies concerning the physico - chemical nature of man - made solar saltponds. Eight selected ponds at Dry Creek solar saltfields, Adelaide, South Australia were studied to provide information on various environmental factors which are biologically important and significant for salt production.

Nutrient and other basic chemical and physical parameters such as salinity, pH, alkalinity, dissolved oxygen, temperature, wind and rainfall control the composition of biota and salt production in the solar saltfields. The total variation in the salinity of the study ponds covers a range of 46 to over 243.5 g/L.

Physical parameters, reflecting seasonal climatic changes, varied seasonally. Salinity increases as the seawater evaporates as it flows through the ponds. Mean annual salinity ranges from 55.5 g/L in pond XD1 (evaporating ponds in initial areas) to 213 g/L in pond PA12 (evaporating ponds in preliminary areas). A well - developed benthic vegetation causes higher oxygen concentrations in most of the ponds at lower salinity. Relatively high concentrations of nutrients which enter with seawater from Chapman Creek and from reserves in sediments are assimilated by microscopic algae in ponds XB3, XB8 and PA3. Nutrients are not available to later ponds until bacterial action releases them from organic material, however the rate of its action is slow.

Higher temperatures in the higher salinity ponds, low oxygen concentrations, unusual buffer systems, varied ionic systems due to the differential precipitation and other factors all affect the biology of the pond. In turn this affects salt production in these high saline systems.

CHAPTER THREE

BIOLOGICAL INVESTIGATIONS

3.1. Introduction

The quality and quantity of salt produced in solar saltfields is partly determined by biological events within the ponds. The purpose of the present study was to provide further understanding of this. To that end, investigations were undertaken of the population structure and abundance of the zooplankton and benthic organisms; the type, composition and density of phytoplankton; the structure of the microbial mat on sediments; and the microbial density in the benthic mat.

3.1.1. Biological communities in natural saline waters

Saline lakes are widely distributed throughout the world and form a significant portion of the waters of almost every continent. Even so, little attention has been paid them and they have not been well studied. In part, this is because only a few salt lakes are located near centres of limnological activities in western Europe and North America. Most limnologists indeed, have tended to view salt lakes as aberrant, inaccessible and essentially insignificant bodies of water (Williams, 1986b).

The total volume of inland water that is saline (with > 3 g/L total salts) and the number of inland saline lakes is not much less than the volume of inland fresh water and the number of fresh water-bodies. The total volume of inland saline water forms 0.008% of biospheric water and for fresh water is 0.009% (Williams, 1993).

More than half of this saline water is within the Caspian sea, the largest lake (371,000 km^2) in the world (Aladin and Plotnikov, 1993). Other very large saline lakes include the Aral Sea (66,000 km^2) and Balkhash (22,000 km^2) in central Asia, and lake Urmia (5,000km^2) in Iran. A large number of salt lakes are not permanent, and also included are many small bodies of saline water in arid and semiarid areas.

The salinity of saline lakes ranges from 3 g/L total solids to more than 300 g/L. Remane and Schlieper (1958) stated that the salinity of hypersaline water should be regarded as more than 45 g/L, but according to Hammer (1986) the salinity of hypersaline waters is greater than 50 g/L. In the classification of saline lakes developed by the International Conference of Inland Waters in 1958, lakes with salinities of 40 ppt or higher are classified as highly saline (Beadle, 1958). Since these waters are often used as sources of raw materials by the chemical industry, and the brine and mud of certain lakes are also used for therapeutic purposes, hypersaline waters are of considerable interest to many researchers (Ivanova, 1990). Apart from the importance of highly saline waters to industry and health, they are also natural laboratories to investigate interspecies competition for food, natural mortality rates of fauna in the absence of predators and correlations of ecosystem stability and structure.

In addition to natural saline lakes, artificially constructed solar saltlakes situated in coastal areas in various places around the world are now common in suitable areas. These lakes have salinity ranges similar to natural hypersaline waters and can be colonised by a hypersaline fauna.

There is now a considerable body of published information on the fauna of salt lakes (Comin and Northcote, 1990; Hurlbert, 1993), and studies include many undertaken in North America (e.g. Moore, 1952; Scudder, 1969; Hammer *et al.*, 1975; Hammer, 1983, 1986) and Australia (Bayly and Williams 1966; Geddes, 1976; Bayly, 1967;

De Deckker and Geddes, 1980; Geddes *et al.*, 1981; Williams, 1981c). Fewer studies exist of the biology of solar salt fields in Australia.

Biological communities in hyposaline waters, i.e. at salinities between 3 to 20 g/L (Hammer, 1978, 1983 and 1986; Hammer *et al.*, 1983), are also often found in fresh waters. They essentially comprise halotolerant freshwater forms. The proportion of halotolerant freshwater forms is lower in mesosaline waters, i.e. at a salinity between 20 to 50 g/L (Hammer, 1986). Most of the biota of mesosaline waters consist of taxa restricted to inland saline waters of moderate salinity. In hypersaline waters, i.e. at a salinity of more than 50 g/L (Hammer, 1986), the biota is restricted to highly saline waters with only a few genera. Since salinity of some saline lakes, such as, the Dead Sea, Lake Urmia and artificial solar salt lakes, is well beyond 50 g/L (> 300 g/L), the biota of these hypersaline waters is quite different from that of fresh waters.

Salt lakes are less complex trophically than fresh water ecosystems, and, in general, with increasing salinity there is a decrease in species diversity (Williams, 1972). Typically, they are shallower and more exposed to wind action and generally less uniform in their chemical and physical features than fresh water. Saline lakes are thus stressful to biota because of fluctuating salinities, exposure to high light intensity, and ephemerality. Great seasonal variations in salinity, equally great variations in water-level, and a tendency to desiccate during the dry season were identified by Beadle (1958) as the most important physico-chemical features of highly saline waters. The development of a hypersaline biota is indicative of the success of various behavioural, anatomical and physiological adaptations to these stresses.

Studies on the limnology of saline lakes in western Victoria have been reviewed by

Williams (1981c). Few studies have been made of the plankton in Australian salt lakes, and these are mainly taxonomic, as outlined by Bayly and Williams (1966), Hammer, (1986), Bauld (1981) and De Deckker and Williams (1982). The phytoplankton communities of inland saline waters have been studied (Borowitzka, 1981; Hammer, 1986) but little emphasis has been given variation in the faunal components in relation to changing physico-chemical features of the environment (Bayly, 1970; Wongrat, 1986). On the international scale, many investigations have been made on the brine shrimp *Artemia* in saline water; most of these have investigated tolerances to environmental stresses. As an example, the effect of increasing salinity on *Artemia* populations of Mono lake, California (Dana and Lenz, 1986), and the effects of temperature and salinity on the biology and ecology of *Artemia fransciscana* have been studied (Wear and Haslett, 1987; Wear *et al.*, 1986a and b). The presence of predators and the absence of *Artemia* in saline waters was studied by Hammer and Hurlbert (1992).

A review of the literature (Bayly and Williams, 1966; Bayly, 1967 and 1972; Hammer, 1983; Melack, 1985; Hammer, 1986; Comin and Northcote, 1990; Hurlbert, 1993) suggests that the most distinctive elements of the biota of natural bodies of inland saline water are as follows.

Bacteria

- Archaeobacteria: six genera are presently recogonized: *Halobacterium*, *Haloferax, Haloarcula, Halococcus, Natrobacterium* and *Natronococcus*.
- Phototrophic purple bacteria: *Rhodospirillum* (a non-sulphur purple bacterium), *Chromatium* and *Ectothiorhodospira* (purple sulphur bacteria),
- Green Eubacteria: *Prosthecochloris* (green bacterium), non-phototrophic halotolerant Eubacteria,
- Cyanobacteria: *Synechococcus* (*Aphanothece), Spirulina, Oscillatoria, Microcoleus, Dactylococcopsis* and *Nodularia*.

Algae

- Bacillariophyta: e.g. *Amphora, Navicula, Nitzschia*
- Chlorophyta: e.g., *Dunaliella salina, Enteromorphora intestinalis, Ctenocladus circinatus*
- Charophyta: e.g., *Tolypella, Chara, Lamprothamnium papulosum*

Non algal macrophytes

- e.g. *Ruppia*, *Lepilaena*, *Scirpus maritimus*.

Crustacea

- Anostraca, *Artemia*, with at least 5 species (*A. persimilis, A. franciscana, A. tunisiana, A. urmia,* and *A. parthenogenetica*), and, in Australia only, *Parartemia* (with 8 species)
- Copepoda: e.g. *Arctodiaptomus salina, Calamoecia salina, Cletocamptus albuquerquensis*
- Cladocera: e.g., *Moina mongolica, Daphniopsis pusilla*
- Ostracoda: e.g., *Australocypris rectangularis, Diacypris compacta*
- Isopoda: *Haloniscus searlii,* an oniscoid isopod (endemic to Australia)

Insects

- Diptera: e.g., *Ephydra* (Ephydridae), *Tanytarsus* (Chironomidae), *Culicoides* (Ceratopogonidae), *Aedes* (Culicidae), and *Hydrophorus* (Dolichopodidae).
- Hemiptera: Corixidae
- Odonata
- Coleoptera
- Trichoptera: *Symphytoneuria wheeleri*

Non-arthropod invertebrates

- Rotifera: e.g., *Brachionus plicatilis*
- Cnidaria: e.g., *Cordylophora caspia*

- Mollusca: *Coxiella striata*
- Nematodes, turbellarians and protozoans

Fishes

- e.g., *Oreochromis alcalicus grahami, Cyprinodon* species, *Atherinosoma microstoma* (marine derived form in coastal salt lakes)

In summary, it may be said that the composition of biological communities in inland saline water is different from that of fresh waters, with differences becoming more pronounced as salinity increases.

The biological communities of hypersaline waters include primary producers, consumers and predators as in other ecosystems (Borowitzka, 1981). All elements of the biota, of course, must have the ability to live in an environment of high salinity (osmotic problems) , differential ion precipitation, varying pH, low dissolved oxygen and fluctuating temperature regimes (Copeland and Nixon, 1974). The hypersaline biota prefers or requires high salinity environments, ponds and lakes.

Comprehensive studies of salt lake communities are few. However, communities in the Great Salt Lake, Utah, have been relatively well - studied. In that lake, the dominant organisms were studied by Post (1977). He found that the brine shrimp (*Artemia* sp. and brine fly (*Ephydra* spp.) are organisms that occur during summer. *Dunaliella salina* and *D. viridis* are the dominant primary producers. Post (1977) also found that the halobacteria and halococci, along with *D. salina*, which is red in colour due to intracellular carotenoids, are present in the hypersaline brine in such quantities that they give a red colour to the brine. Cyanobacteria and protozoans have also been reported in this hypersaline water - body but in modest numbers.

The Dead Sea is another well studied hypersaline water. Its average salt concentration is about 340 g/L, with Mg^{2+} (about 1.86M), Na^{+} (about 1.60M), Ca^{2+} (about 0.44 M) and K^{+} (about 0.2M) as the dominant cations and chloride as the main anion (Oren and Gurevich, 1995). It differs from the Great Salt Lake in a number of respects. The total salinity is about the same but the dominant ions differ. There is a much lower concentration of Na^{+} and Ca^{++}, but Cl^{-} is still the dominant ion (Nissenbaum, 1975). The variety of life forms living in the lake is extremely limited. *Dunaliella parva* is responsible for all primary productivity (Oren, 1993). Red halophilic bacteria of the family Halobacteriaceae develop and use the organic material produced by the algae (Oren, 1983, 1988, 1990a and b, 1993). Protozoans are rare or absent, and no higher forms of life are present (Oren and Gurevich, 1995). Oren and Shilo (1985) studied the effect of salinity and the addition of glycerol, glucose and a different concentration of phosphate on the rate and extent of bacterial and algal growth. Addition of phosphate appeared to be essential for the development of mass blooms of both the green alga *Dunaliella* and of red halobacteria. Glucose, glycerol and a *Dunaliella* bloom all proved to be suitable carbon sources for mass development of halobacteria (Oren, 1993; Oren and Gurevich, 1995).

3.1.2. Biological communities in solar saltfields

In the first studies of solar salt fields, Carpelan (1957) described the physico-chemical and biological factors of Alviso salt ponds, California. He stated that the distribution of organisms within the ponds was related to environmental factors and mostly to the salinity of each pond. He also found an inverse relation between salinity and photosynthetic rates. Nixon (1974) reviewed three different salina systems in France, USA and Puerto Rico. He stated that the characteristic organisms of these systems appear wherever such systems are developed throughout

the world. He noted three main stages in the system with increasing salinity: the first, with blue-green algae (Cyanophyta) predominant in benthic mats, the second with the phytoplanktonic *Dunaliella*, and the third, by red and pink bacteria.

The biota in ponds of a solar saltfields in Long Island, Bahamas, was studied before and after the addition of fertilisers to allow plant growth and promote rapid succession (Davis, 1978). After fertilisation, most organisms were found in the thick, several-layered bottom mats that consisted mostly of blue-greens and other bacteria with lower numbers of ciliates, dinoflagellates and diatoms. The growth and succession of plants and the development of mats following fertilisation coincided with an increase in salinity and water colour compared with conditions before fertilisation. The importance of a balanced biological system in solar saltfields was noted by Davis (1980a). He reported that *Artemia* is essential to the proper operation of the biological system. Algal blooms are generally beneficial in that they increase solar heat absorption, resulting in an increase in evaporation and higher yields of salt. However, if they are not biodegraded in time, salt is contaminated by organic impurities that affect salt quality. The presence of *Artemia* in sufficient numbers is essential not only for controlling algal blooms (Davis, 1980a and b) but also for providing essential nutrients for the development of halobacteria in the crystallisation ponds (Jones *et al.*, 1981).

Javor (1983a) studied the differences in species composition at two different nutrient levels in solar saltfields: at the Exportadora de sal., South America (an oligotrophic system), and at the Guerrero Negro, Baja California, USA (a eutrophic system). She reported that the oligotrophic saltfields maintained sparser phytoplanktonic and *Artemia* populations in precrystallizer brines than the eutrophic saltfields. *Dunaliella* was present in eutrophic and absent in oligotrophic saltfields. Javor (1989) reported that the standing crop increased especially during the summer, while species diversity in general decreased with increasing salinity.

Solar salt fields in Bohai Bay (China) have been studied extensively. They are fed by highly enriched waters which cause an excessive accumulation of organic matter detrimental to salt production (Davis, 1991). Despite the abundance of unicellular algae, *Artemia* densities in these salt ponds remain limited, especially during spring, and are unable to remove excessive amounts of organic matter. The densities are limited by low reproductive rates of the local parthenogenetic strain at the lower temperature regimes, and the poor resistance of this strain to high salinity (Tackaert and Sorgeloos, 1991). A recent inoculation of a new strain, selected for its high reproductive capacity at relatively low temperature and with good resistance to high salinities, helped improve the system (Vanhaecke and Sorgeloos, 1989).

The biology of solar saltfields in relation to nutrients in Tamil Nadu at Vedaranyam and Kelambakkam in India was studied by Rahaman *et al.* (1993). They reported that the communities in Vedaranyam saltfields were more productive than Kelambakkam because of high nutrient concentrations. A negative relation between salinity and phytoplankton and zooplankton diversity was observed. They also reported blooms of *Dunaliella salina* at a salinity of 220 ppt in Kelambakkam. *Artemia* was present at both solar saltfields over a wide range of salinity, 42 to 187 g/L. Other zooplankton included Copepoda, Protozoa, and insect larvae.

High phytoplankton blooms was reported in solar saltfields in the state of Rio Grande do Norte (Brazil) due to the uptake of nutrient rich waters at high tides (De Mederios Rocha and Camara, 1993). These blooms have been managed and controlled by introducing brine shrimp to the ponds and macroalgae were also planted to trap nutrients.

The beneficial role of *Artemia* in balancing the biological activities in hypersaline waters, solar saltfields was suggested by Tackaert and Sorgeloos (1993). The inoculation of *Artemia* to ponds without brine shrimp and also where the local

Artemia population has a poor productivity and remained too small to control the algae bloom was recommended. They suggested that inoculation led to the improvement of salt production and also the opportunity for harvesting the valuable production of *Artemia* as cysts and biomass.

3.1.3. Aims

There has been only a limited number of biological studies on Australian solar saltfields. Solar saltfields at Dry Creek were established in 1940 for the purpose of salt production. Because the quality and quantity of the salt produced in them is partly determined by biological events within the ponds, the aims of this study were to identify the fauna and flora of the study ponds, and to provide further understanding of how biological events affected salt quality and quantity. To that end, regular monthly samples over 18 months were collected and investigations were undertaken of: the population structure and abundance of zooplankton and benthic organisms; the type, composition and density of phytoplankton; the structure of the microbial mat on sediments; and the microbial density in the benthic mat.

3.2. Methods and materials

In this study, the biota in solar salt fields at Dry creek was monitored by recording the abundance and distribution of zooplankton and benthic organisms, the population density and the distribution of phytoplankton, and the microbial density of benthic mats. Data were collected between February 1994 to September 1995.

The distribution of the fauna was investigated mainly in terms of its horizontal distribution throughout the field. The population density of zooplankton was recorded as the number of individuals per litre. The population density of *Artemia*

and *Parartemia,* as key organisms, was analysed as the number of males, females and subadaults in samples.

The population density of the phytoplankton was estimated by chlorophyll *a* measurement. Primary productivity at high salinity (>180g/L) in the solar saltfields occurs predominantly in the benthic mats, with limited algal production in the water column. This means that the main food source for *Artemia*, as a key organism in these ponds, is in the benthic microbial community. This is cohesive and not readily available to *Artemia*. The abundance of the benthic microbial and algal community in the benthic mat was estimated by chlorophyll *a* and carotenoid measurements.

Associated features of the physico-chemical environment and temporal changes that occurred annually in the ponds were also investigated and are discussed.

3.2.1 Description of sampling sites

Fourteen ponds at Dry Creek were selected for a pilot study. These ponds were XE1-3 from the initial area (salinity ~ 37-55 g/L), XD1, XB3, XB4-5, XB8, XC3 from the intermediate area (salinity ~ 55-150 g/L), PA3, PA5, PA7, PA7A, PA9, PA12 from the preliminary area (salinity ~ 150-200 g/L) and FA1 and FA2 from the final areas (salinity ~ 220-320 g/L) of the solar saltfields. Eight ponds were then selected for further study based on their salinity and the position of nutrient inputs (Fig. 2.1). The ponds selected were XD1, XB3, XB8, XC3 (representative of the intermediate area), PA3, PA7, PA9 (representative of the preliminary area), and PA12 (the last pond before the final area). Ponds XD1 and XC3 had low nutrient input, while XB3 and XB8 had high nutrient inputs. The waters with low and high nutrient inputs were mixed before reaching pond PA3, and this pond was selected because it contained mixed water. Ponds PA7, PA9 and PA12 were selected

because the salinity was relatively close to the point at which salt crystallisation occurs and also *Synechococcus* sp. is abundant there.

Pond XD1 is irregular in shape, 242 ha in area, its substratum comprises fine grained material (silt and clay), water depth is maintained at a level of 2.83m and mean salinity is ~55.5 g/L. (Table 2.4). Pond XB3 is subrectangular in shape, 344 ha in area, and the largest pond in the field. Its substratum comprises clay and fine grained material, water depth is maintained at a level of 2.20 m, and mean salinity is ~62 g/L (Table 2.4). Pond XB8 is subtriangular in shape and 219 ha in area. The pond bottom comprises fine grains of sediment and detrital material and water depth is maintained at a level of 2.40 m. Mean salinity is ~ 112 g/L (Table 2.4). Pond XC3 is irregular in shape, 163 ha in area, its substratum comprises clay and calcium carbonate precipitation, and water depth is maintained at a level of 2.35 m with an extensive shallow shoreline. Mean salinity is ~107 g/L (Table 2.4). Pond PA3 is subrectangular in shape, 81 ha in area, its substratum comprises fine and detrital sediment together with calcium carbonate sediment, and water depth is maintained at a level of 1.8 m. Mean salinity is ~137.5 g/L.(Table 2.4). Pond PA7 is subsquare in shape, 68 ha in area, and covered with a benthic mat and gypsum precipitate (Fig. 2.1). Benthic mats are defined as cohesive algal and microbial communities which are often laminated and found growing at solid-aqueous interfaces. These are discussed later. Its water depth is maintained at a level of 2.20 m. There is an extensive shallow shoreline and mean salinity is ~189 g/L (Table 2.4). Pond PA9 is 44 ha, subrectangular in shape and very narrow, and its width is almost 1/5 of its length (Fig. 2.1). Its floor is covered by gypsum precipitate and a benthic mat. Mean salinity is 195 g/L. Water depth is maintained at a level of 1.76 m. Pond PA12 is the smallest pond in the field, irregular in shape, 12 ha in area, and its substratum comprises a benthic mat and gypsum. Water depth is maintained at a level of 1.31 m. Mean salinity is ~213 g/L (Table 2.4).

Data from preliminary sampling (Smith, 1989) indicated that three sampling stations at each pond provided a representative sample for the pond; there were no appreciable differences in faunal distribution across the ponds and the ponds are small in area. Sampling stations were chosen along the main roads due to the road conditions during the wet season and to ensure sampling throughout the year. The sample sites at each pond included one near the outlet, and two between the outlet and inlet (Fig. 2.1).

3.2.2. Field methods

Sampling occurred regularly in the last week of each month for 18 months, commencing April 1994 and continuing to September 1995. Zooplankton was collected quantitatively by using a hand-held plankton net 30x20 cm wide, with a mesh aperture of 150 μm and with a flowmeter attached. Using this net, 50 L water was collected from 20 cm below the water surface and immediately above the pond bottom. Sampling was preferentially undertaken as early as possible in the morning when *Artemia* are apparently more uniformly distributed (Vu DO Quyh and Nguyen Ngoc Lam, 1987). Similar nets but with a mesh of 59 μm were used to collect phytoplankton. Zooplankton samples were preserved in the field with 5% formalin to prevent "clouding" of samples (the development of floc after the addition of alcohol at high salinities). Phytoplankton samples were preserved in Lugol's solution. Ostracods were not preserved in formalin because it is slightly acidic and softens or even dissolves ostracod valves. Ostracod examination is then difficult and soft valves became easily deformed when dried (De Deckker, 1995). Ostracods were transported to the laboratory live.

According to the nature of the bottom, three different methods for benthic sampling were employed. Three sites were selected at random at each station. A 50 mL syringe and a hand operated corer were used to collect material from ponds where

the bottom was soft sediment. These methods could not be used when bottoms comprised gravel and large particles; these were examined visually for benthic invertebrates. The hand-operated corer was an aluminium tube as described by Paterson and Walker (1974): 52 cm long, 38.5 cm^2 cross-sectional area, and with its lower edge tapered to assist penetration and collect loose sediment. The upper end of the tube was sealed by a rubber bung and this maintained a sufficient pressure to prevent the loss of material through the lower end of the corer. The samples were taken at the bottom of the pond, one metre from the shoreline. Samples were placed in plastic bags for transport to the laboratory.

Macroalgae and periphyton was scraped from substrates in ponds XD1, XB3 and XB8 and suspended in sample water for qualitative and quantitative measurements. Samples were collected in a manner that avoided bare patches created by previous sampling, but the same location was used on all dates.

Microbial mats were sampled by removing fragments of the solid matrix at 1 metre depth from the shoreline or by short plastic corer (length 25 cm, diameter 5 cm). It was used to penetrate the upper sedimentary layers and collect undisturbed cores about 10cm long. Both qualitative and quantitative measurements were undertaken at each station. The same sampling stations were used on all dates but, as before, samples were collected so as avoid bare patches created by previous samplings. Quantitative analysis of benthic mats involved the determination of chlorophyll *a* and carotenoid concentrations in 5 cm^2-segments of freshly collected cores. The green surface layer of the mat was carefully removed from the underlying sediment by means of a scalpel. Qualitative determination involved microscopic examination. Most samples were obtained by wading.

Brine samples were collected by water sampler at the same time and stored in black polyethylene bottles. From them, chlorophyll *a* and carotenoid concentrations and

phytoplankton biomass were estimated. 250 mL of water sample was also used to concentrate algae by sedimentation and to identify the phytoplankton.

Traps baited for fish, as described by Molsher (1991), were used in ponds XD1, XB3, XB8, XC3 and PA3, where the salinity was within the survival range of fish, in October, November and December 1994. According to Molsher (1991), the spawning of *Atherinosoma microstoma* occurs from September to December. The trap was triangular, of dimensions 36x36x68 cm. They were baited with chicken bones that had small amounts of flesh attached; this was found to be the preferred bait (Molsher, 1991). Three traps were set and left overnight at three sites. The fish were collected the next morning and preserved immediately in 5% formalin for later identification.

3.2.3. Laboratory processing

The fauna from the zooplankton collections was preserved in 70 % alcohol for identification and counting. For counting, three subsamples were collected from each sample by using a swirling flask and a graduated pipette (McCallum, 1979). These were identified and counted using a stereo-microscope. If the sample was too dense, it was further divided using a modified Motoda sampler (Motoda, 1956). Samples were continuously halved until an appropriate number of organisms (500) was present.

Fresh ostracod samples were kept in the refrigerator for three days. The lack of oxygen helped to keep valves open and thus made specimens easier to identify (De Deckker, 1995). Ostracods were later transferred into 70% ethanol for identification.

Benthic samples were emptied into sorting trays and species presence/absence recorded for each taxon. Organisms were identified to the lowest taxonomic level possible, usually to genus or species. Identification of some organisms was confirmed by specialist taxonomists.

Undisturbed samples of benthic mats were collected from each station for quantitative determination of chlorophyll *a* and carotenoid. This was determined by grinding 1 cm^2 of undisturbed sample in a teflon tissue grinder with 90% acetone until thoroughly macerated. The macerated sample was placed in a centrifuge tube together with acetone rinsed from the mortar and pestles, and the final volume was made up to 8 ml plus the volume of the sample. This was then sonicated for 2 min to disintegrated cellular material using an Ultrasonic Disintegrator (sonicator) made (Thomas optical & Scientific Co). Material was protected from light with aluminium foil to avoid the breakdown of chlorophylls and carotenoids when exposed to light, acids, or oxygen (Holm-Hansen, 1978). $MgCO_3$ was added to the sample prior to extract ion to neutralise any organic acids released when tissues were disrupted. The centrifuge tubes were kept in a refrigerator and were shaken once or twice during extraction. The analyses were completed within 24 to 36 hrs. Absorbances were read at 750, 665, 645 and 630 nm in a 1 cm cell using a Varian UV/visible spectrophtometer. The equation of Strickland and Parsons (1972) was used to calculate chlorophyll *a* concentration:

Chlorophyll *a* $\mu g/L = C (v / A \times 1^*)$

$C = 11.6\ E_{665} - 1.31\ E_{645} - 0.14\ E_{630}$

v = volume of acetone (mL)

$A = 1\ cm^2$

* 1 cm light path cuvette was used

Carotenoids were also measured using the equation of Strickland and Parsons (1972) and Parsons *et al.* (1984) to estimate algal biomass.

Carotenoid μg/L = C (v / A x 1*)

$C = 7.6 (E_{480}-1.49E_{510})$, Strickland and Parsons (1972)

and

$C = 4 (E_{480})$, Parsons *et al.* (1984)

v = volume of acetone (mL)

$A = 1\ cm^2$

* 1 cm light path cuvette was used

Water samples were filtered first through a 250 μm filter to remove zooplankton and floating algae and then through 0.45 μm millipore filters. The same processes for extraction and chlorophyll *a* determination previously described were applied for quantitative estimates of phytoplankton. The equation of Strickland and Parsons (1972) was also used to calculate chlorophyll *a* concentration:

Chlorophyll *a* μg/L = C (v / V x 1*)

$C = 11.6\ E_{665} - 1.31\ E_{645} - 0.14\ E_{630}$

v = volume of acetone (mL)

V = volume of sample (L)

*1 cm light path cuvette was used

Carotenoids were also measured using the equation of Strickland and Parsons (1972) and Parsons *et al.* (1984) to estimate phytoplankton biomass.

Carotenoid μg/L = C (v / V x 1*)

$C = 7.6 (E_{480}-1.49E_{510})$, Strickland and Parsons (1972)

and

C = 4 (E_{480}), Parsons *et al.* (1984)

v = volume of acetone (mL)

V = volume of sample (L)

*1 cm light path cuvette was used

Water samples were filtered first through a 250 μm filter to remove zooplankton. Then, phytoplankton was concentrated for microscopic examination by Lugol iodine-sedimentation (Britton and Greeson, 1989). 250 mL samples of water, fixed with Lugol iodine-solution, were sedimentation in sedimentation chamber and left overnight. These collections were used for supplementary phytoplankton examination.

3.3. Results

3.3.1. Flora

In general terms, significant differences in the composition of the biological and plant communities occurred in the pond series. Ponds XD1 and XC3 were clear with low concentrations of phytoplankton. Ponds XB3, XB8 and PA3 were turbid with high concentrations of phytoplankton. Ponds PA7, PA9 and PA12 were low in phytoplankton communities, but algae and a significant microbial community were present in the benthic mat.

3.3.1.1. Phytoplanktonic communities

The phytoplankton community was characterized by populations of green algae, diatoms and Cyanobacteria in ponds XB3, XB8, PA3, PA7, PA9 and PA12. Table 3.1 documents the species of algae and Cyanobacteria in the phytoplankton. Twenty-seven species of diatoms were present at various salinities. Planktonic

Table 3.1. Momentary species richness of phytoplankton at Dry Creek solar salt field, mean salinity, g/L.

Name of Taxon	XD1 (62)	XC3 (108)	XB3 (58)	XB8 (107)	PA3 (115)	PA7 (184)	PA9 (198)	PA12 (216)
Peridinium sp.	+		+					
Dunaliella viridis	+	+	+	+	+	+	+	
Dunaliella salina	+	+	+	+	+	+	+	+
Chlamydomonas sp.	+	+	+	+	+			
Gonium sp.		+	+	+				
Achanthes brevispes	+		+	+	+	+		+
Achanthes sp.	+	+	+		+	+	+	
Amphora angusta	+		+					
A. coffeaeformis	+	+	+	+				
A. holstica	+		+					
A. ventricosa	+		+					
Gomphonema sp.			+	+	+			
Gramatophora oceanica	+		+					
Gyrosigma scalproides	+		+					
Mastogloia exilis	+		+					
M. exigua	+		+					
M. pumila	+		+					
Microcoleus sp.				+	+	+		
Navicula salinarum	+		+					
N. stundlii	+		+					
N. dissipata	+		+					
Navicula sp.	+	+	+		+	+	+	+

Table 3.1. (continued)

Name of Taxon	XD1 (62)	XC3 (108)	XB3 (58)	XB8 (107)	PA3 (115)	PA7 (184)	PA9 (198)	PA12 (216)
N. marina	+	+						
Nitzschia obtusa	+	+						
N. dissipata	+	+						
N. hungarica	+	+						
N. sigma	+	+						
N. rostellata	+	+	+	+	+	+	+	+
Nitzschia sp.	+	+						
Rhopalodia musculus	+	+						
R. gibberula	+	+						
Synedra fasciculata	+	+						
Chroococus turgidous		+	+	+	+	+	+	+
C. montanus		+	+	+	+	+		
Gloecoapsa sp.	+	+	+	+	+			
Oscillatoria miniata	+	+	+	+	+			
O. salina			+	+	+			
Schizothrix sp.							+	+
Sirulina sp.					+			
S. labyrinthiformis					+	+	+	
Synechococcus sp.		+		+	+	+	+	+
micro algae	+	+	+	+	+	+	+	+
Species richness	32	23	28	16	18	12	10	8

flagellates and diatoms were the most characteristic group of algae in ponds XD1, XB3 and XB8, and diatoms usually represented more than 50% of the total biomass. In pond XB8, where mangroves occurred, there were few epiphytic diatoms; these grew on dead mangroves. Three species of diatoms were collected from the water column in ponds PA7, PA9 and PA12. Ponds XD1, XB3, XB8 and PA3 had the highest species richness and had some marine species. Ponds XC3, PA7, PA9 and PA12 were clear, and momentary species richness was lower than ponds XD1, XB3, XB8 and PA3. Figure 3.1 illustrates the most common species.

Cyanobacteria were found in large numbers and were one of the most abundant forms of primary producer in the water column and in benthic mats at a salinity of more than 150 g/L. Many localised algal blooms were found along the edge of ponds PA3, XC3 (pond XC3 less frequently), PA7, PA9 and PA12. Aggregated floating algae were present in pond PA3, and mostly consisted of diatoms and Cyanobacteria, and floated as pseudophytoplankton in visible masses (up to 1 cm diameter). In pond PA7, the blooms consisted of diatoms and non-heterocystous, filamentous and unicellular species of Cyanobacteria. In ponds PA9 and PA12, the phytoplankton consisted mainly of unicellular species of non-heterocystous Cyanobacteria *Synechococcus* sp. (Fig. 3.2a) and a few filamentous Cyanobacteria, *Oscillatoria* and *Spirulina*. As the brine approached saturation (pond FA5 and later ponds; Fig. 2.1), only a few species survived in the phytoplankton community. They included some residual Cyanobacteria, *Synechococcus*, *Duniella salina* (Fig. 3.2b), *Stephanoptera* sp., and a few halophilic bacteria. In the crystalliser ponds, *D.salina* and halobacteria imparted a strong colour to the brine. *D. salina* gave rise to orange to red colours, whereas pink colours arose from pigmented aerobic halobacteria and other aerobic halobacteria with a high content of bacterioruberin (Oren and Dubinsky, 1994)

Seasonal variations of chlorophyll *a* and carotenoid concentrations, indicative of

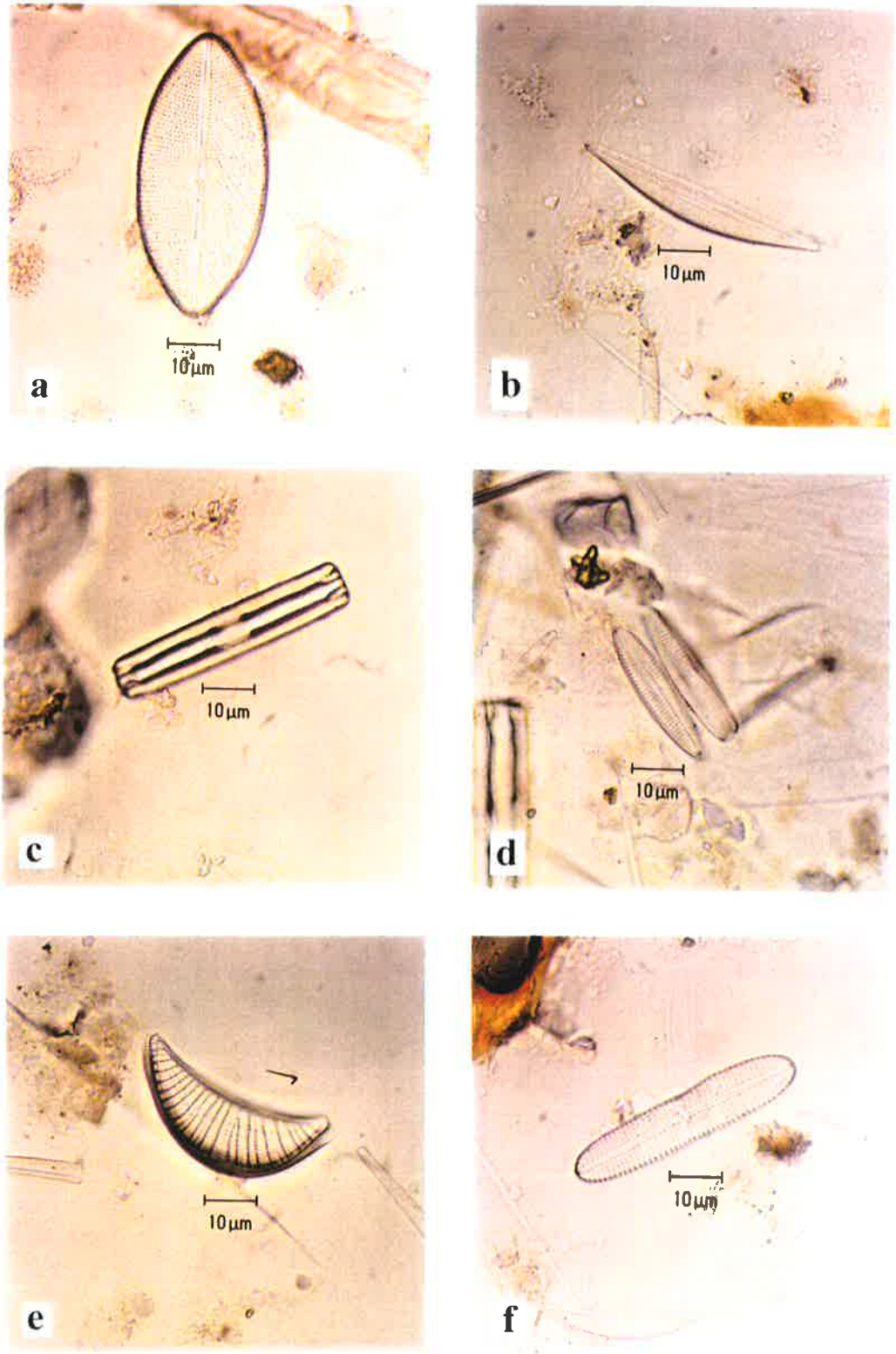

Figure 3.1. Most common species of diatom in the study ponds. (a) *Navicula marina* (b) *Amphora ventricosa* (c) *Gramatophora oceanica*; (d) *Navicula* sp.; (e) *Rhopalodia musculus*; (f) *Achnanthes brevipes*.

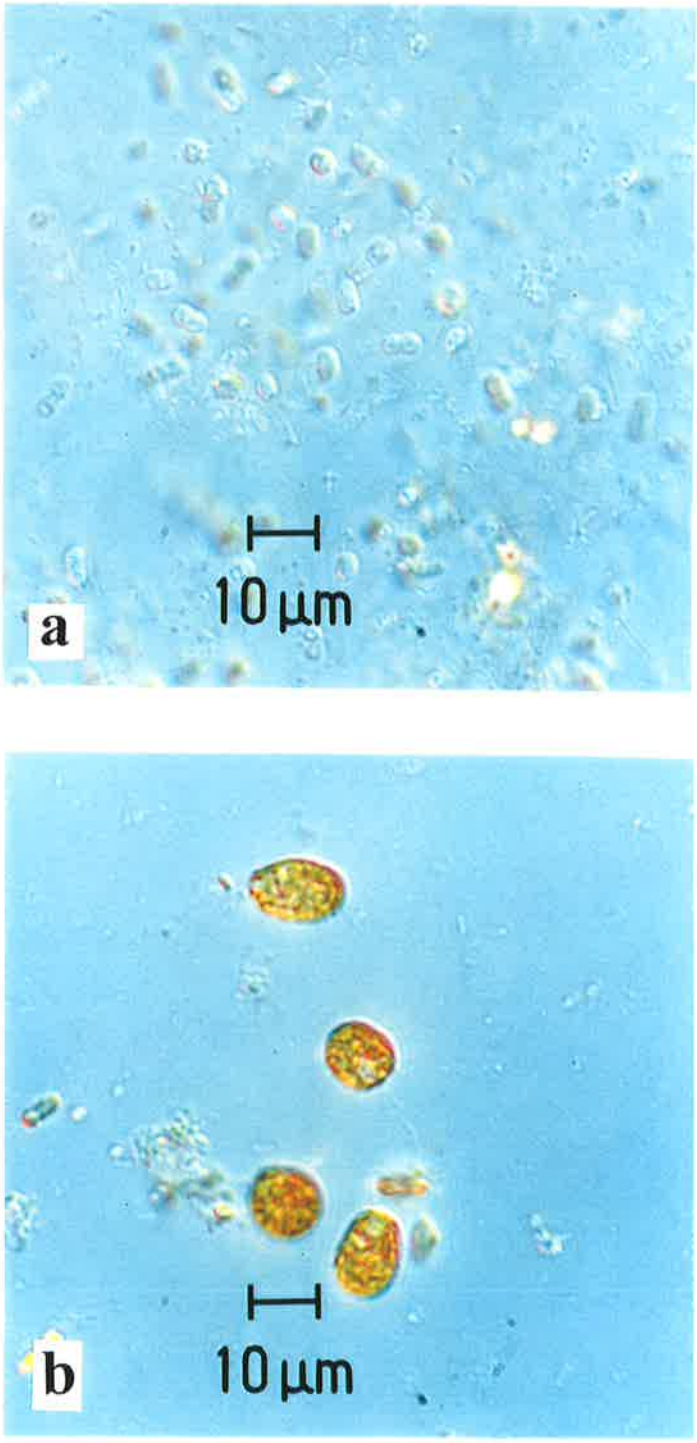

Figure 3.2. Most common species of algae in the study ponds. (a) *Synechococcus* sp.; (b) *Dunaliella salina*.

changes in phytoplankton density, are shown in Figure 3.3 and Appendices 2.1 and 2.2. The results from pond XD1 and XC3 show that the concentration of phytoplankton in these ponds was lower than in ponds XB3, XB8 and PA3. The bloom in ponds XB3 and XB8 started at a lower salinity during winter. The spring planktonic blooms in pond XB3 were initiated predominantly by algae other than diatoms. In pond XB8, dinoflagellates, *Dunaliella* and diatoms were dominant. Throughout the summer, there were also diatom blooms of *Nitzchia* species and Cyanobacteria (*Gleocapsa* spp.) in ponds XB8 and PA3. Pond XB8 was always turbid and had a high density of phytoplankton throughout the study period (Fig. 3.3). Massive blooms of blue-green algae coated the surface of pond PA3 in spring and early summer.

3.3.1.2. Macroalgae and periphyton communities

The macrophytes comprised extensive growths of seagrass, *Heterozostera,* and *Chara* sp., in pond XD1 and both *Ulva* sp. and *Chara* sp. in pond XB3. These were probably the main contributors to primary production in these ponds, where mean salinity ranged from 55.5 to 62.0 g/L. Chlorophyll *a* and carotenoid concentrations in 1 cm^2 where *Chara* and *Ulva* covered the bottom gravel are shown in Figure 3.4. The patchy distribution of green periphytic algae attached to the gravel occurred only in the shallow area near the edge at the bottom of pond XB8. *Ulva* and *Chara* were not present in this pond. Chlorophyll *a* and carotenoid concentrations in 1 cm^2 of periphytic algae on the bottom gravel in pond XB8 are shown in Figure 3.4.

3.3.1.3. Benthic mat communities

Non-planktonic algae occurred as a benthic mat which covered the bottom of high salinity ponds. The mat consisted of diatoms and Cyanobacteria. The algal and microbial benthic mat community was the main primary producer in ponds with a

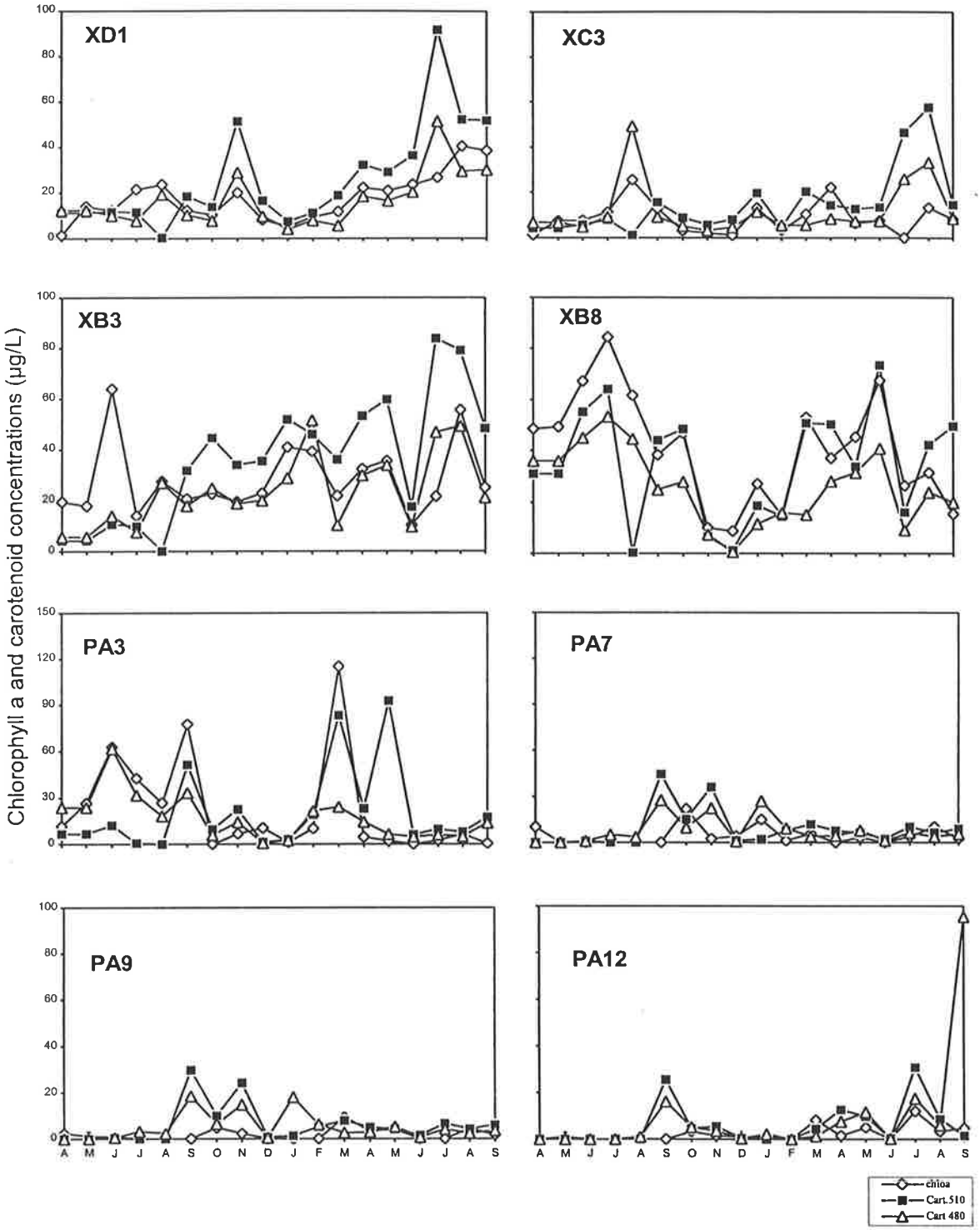

Figure 3.3. Chlorophyll *a* and carotenoid concentrations in study ponds.

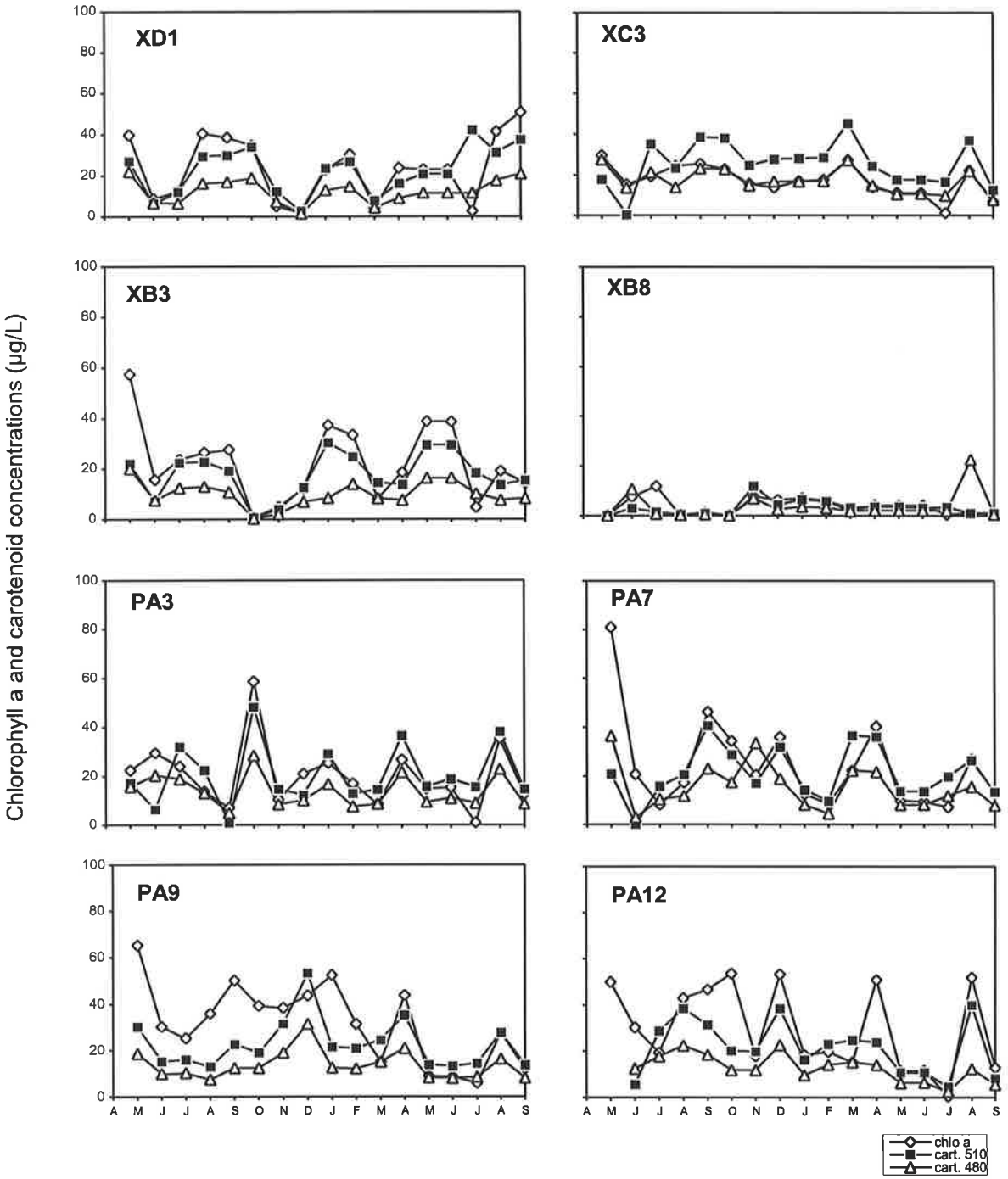

Figure 3.4. Chlorophyll *a* and carotenoid concentrations from macroalgae (ponds XD1 and XB3), periphytic algae (pond XB8) and algal microbial benthic mats (ponds PA3, PA7, PA9 and PA12).

salinity of more than 180g/L. The extent of seasonal variation of chlorophyll *a* and carotenoid obtained from microbial benthic mats is indicated in Figure 3.4. The chlorophyll *a* and carotenoid data are indicative of the density of algae and the autotrophic microbial community in the benthic mats of ponds XC3, PA3, PA7, PA9 and PA12. The results show that there were large variations in the density of micro-organisms over the study period. This figure indicates a high density of micro-organisms in samples except in winter 1995 when lower densities prevailed. The microbial benthic mat in the different ponds comprises several populations or communities of micro-organisms that occur vertically stratified as laminae which are readily recognised by striking colours differences caused by the pigments of concentrated organisms. Mats in Dry Creek saltfields are dominated by unicellular, colonial forms of *Synechococcus*. These forms are probably favoured by an environment of relatively constant salinity, but the amount of slime produced by *Synechococcus* varied enormously in different ponds especially during summer (see Chapter 6).

Ponds XC3, PA3, PA7, PA9 and PA12 were characterised by benthic mats. Mats were rare in pond XC3 and PA3, but extensive in ponds PA7, PA9 and PA12. The mats were covered by a layer of chemically precipitated minerals according to salinity. For example, in ponds XC3 and PA3, calcium carbonate was the usual precipitate, whilst in ponds PA7, PA9 and PA12, it was gypsum, and in ponds FA1-5 and the crystallisers it was sodium chloride. The distribution of benthic mats in the study ponds is shown in Figure 3.5. There were no benthic mats in ponds XD1, XB3 and XB8.

3.3.2. Fauna

The fauna recorded and the range of salinity at which it occurred are presented in Table 3.2 and Appendix 2.3. The numerically dominant species in each pond is also

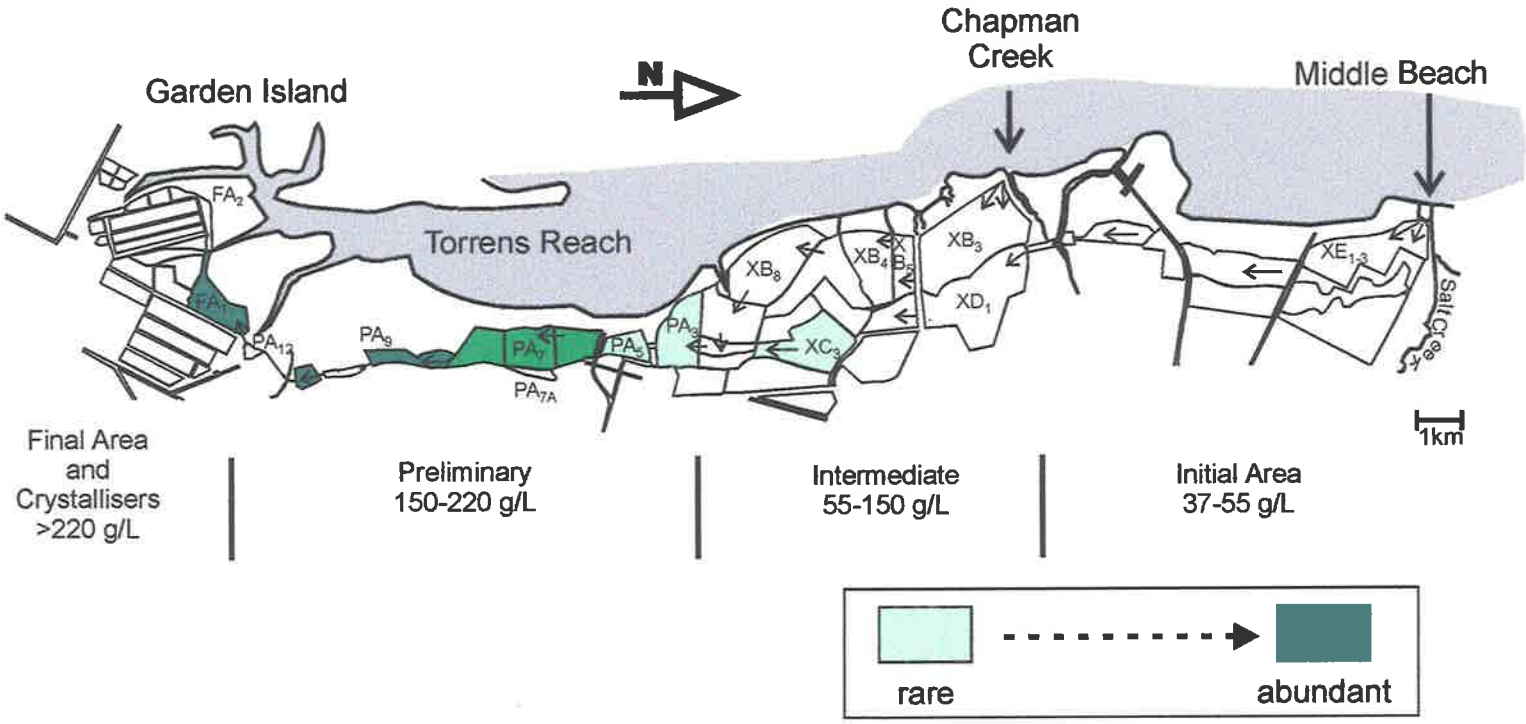

Figure 3.5. The distribution of algal benthic mats in the Dry Creek solar saltfields, South Australia.

Table 3.2: Momentary species diversity and momentary species richness of fauna at Dry Creek solar saltfields, salinity, g/L; species diversity, no/L; *, indicates dominant species.

Name of Taxon	XD1 (62)	XC3 (108)	XB3 (58)	XB8 (107)	PA3 (115)	PA7 (184)	PA9 (198)	PA12 (216)
Artemia franciscana		+			+	+*	+*	+*
Parartemia zietziana	+	+	+	+*	+*	+		
Diacypris dictyote	+	+*	+	+	+			
Reticypris herbsti	+	+	+	+	+			
Mesochra parva	+		+	+				
Harpacticoida	+		+	+				
Austracyclops. Australis	+		+	+	+			
Cyclopoid sp.			+					
Acartia clausi	+*		+*	+		+	+	
Tanytarsus barbitarsis	+		+	+	+	+	+	
Cladotanytarsus sp.	+	+	+	+	+	+	+	
Ephydra riparia	+	+	+	+	+			
Species diversity	**9.22**	**1.81**	**14.6**	**17.91**	**16.46**	**18.09**	**21.13**	**0.46**
Species richness	**10**	**6**	**11**	**10**	**8**	**5**	**4**	**1**

Artemia franciscana: Crustacea: Anostraca: Artemiidae; ***Parartemia zietziana:*** Crustacea: Anostraca: Branchipodidae; ***D. dictyote***: Ostracoda: Cypridacea: Cyprididae, *Diacypris* ***Reticypris herbsti***: Ostracoda: Cypridacea: Cyprididae; Harpacticoida: sp: Copepoda; ***Mesochra parva*:** Copepoda: Harpacticoida; ***Austracyclops australis:*** Copepoda, Cyclopoida; ***Acartia clausi:*** Copepoda, Calanoida; ***Tanytarsus barbitarsis:*** Insecta: Diptera: Chironomidae, Chironominae; ***Chironomus* sp**: Insecta: Diptera: Chironomidae; ***Ephydra riparia*** Insecta: Diptera: Ephydridae

indicated. The mean salinity, the abundance of microcrustacean zooplankters (number per litre), and the occurrence of other zooplanktonic and benthic organisms collected in a period of over 18 months in the study area are given in Appendix 3.1.

The data indicate that the fauna of the low salinity ponds (50-110 g/L) included fishes (2 species), decapods (1 species), gastropods (1 species), isopods (2 species), amphipoda (1 species), Anostraca (2 species), Ostracoda (2 species), Copepoda (4 species), and insects (3 species). Animals in the higher salinity ponds (> 110 g/L) were Anostraca (2 species), Ostracoda (2 species), and insects (3 species). Brine shrimps were the dominant organisms in ponds with a salinity of more than 180 g/L. *Parartemia zietziana* was found in ponds XB3, XC3 (rare), XB8, PA3 and PA7 (abundant), i.e. at a salinity of 50-180 g/L. *Artemia franciscana* occurred in pond XC3 once (September, 1994) and in ponds PA7, PA9 and PA12, i.e. at a salinity of 180-250 g/L. As the brine approached saturation (FA5 and further ponds), only a few species survived, with only *Artemia franciscana* at the highest salinity (Fig. 3.6).

3.3.2.1. Nekton

Two species of fish were recorded. *Pseudogobious olorum* was collected in pond XD1 and XB3 (salinity 46-63.5 g/L) and *Atherinosoma microstoma* was found in ponds XD1, XB3 and XB8 (salinity 46-130.5 g/L). Large numbers of *A. microstoma* larvae were collected from October to December from ponds XD1, XB3 and XB8.

Palaemon serenus, a large swimming prawn, was collected from pond XD1 where salinity was 46-63.5 g/L. This is the lowest salinity pond.

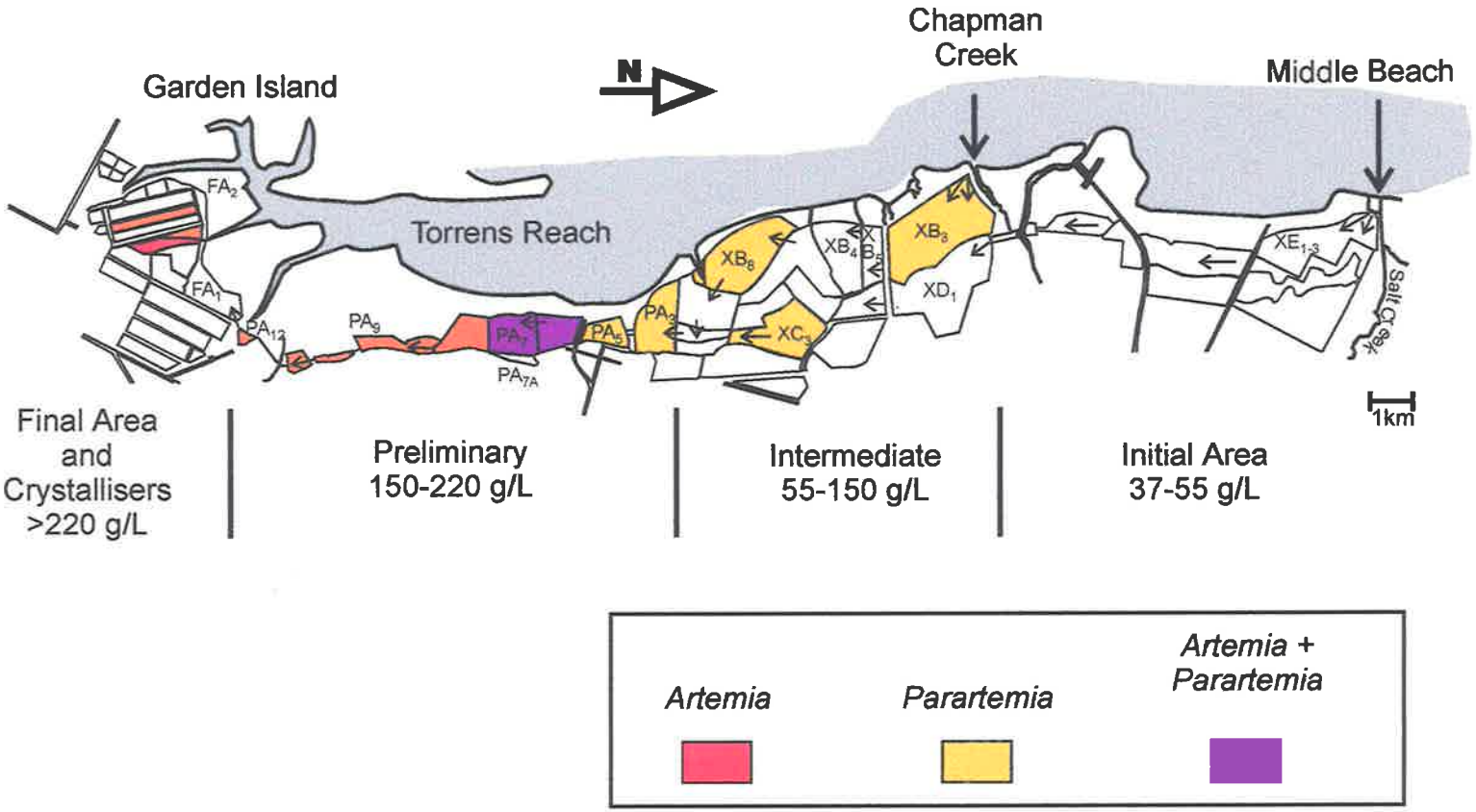

Figure 3.6. The distribution of *Artemia* and *Parartemia* in the Dry Creek solar saltfields, South Australia.

Parartemia zietziana was the characteristic anostracan of ponds XB3, XC3, XB8, PA3 and PA7. Seasonal patterns in the abundance of *P. zietziana* are shown in Figure 3.7. It was present in pond XB3 in May and December 1994 and in January, June and August 1995 and in pond XC3 in October and November 1994 and in March 1995. In pond XB8, *P. zietziana* was abundant in June and August 1994, and in January and August 1995. In pond PA3, it was abundant throughout the study period except that density was low in July, August and October 1994 and also in February, July and August 1995. *P. zietziana* was also abundant in October 1994 and January 1995 in pond PA7. The relative proportions of developmental categories of *P. zietziana*, as a percentage of the total population in each pond (male, female, female with eggs and sub-adult), are presented in Figure 3.8; detailed data are given in Appendix 2.4. Sub-adults were present in ponds XB8 and PA3 all year for most of the study period (not in August 1994 in pond PA3). The highest percentage of sub-adults in the total population occurred in pond PA7 in July 1994. Only one period of reproduction occurred in pond XC3, January to April 1995. Peaks of abundance of females with eggs occurred in pond XB3 in October 1994 and January 1995, in PA3 in winter and early spring 1994 and winter (July) 1995. PA7, the most saline pond with *P.zietziana*, showed only one period of reproduction, August to November 1994.

Artemia franciscana was found in ponds PA7, PA9 and PA12. Its distribution is shown in Figure 3.6. Figure 3.9. shows the seasonal variation in population density in these ponds. The species was abundant in pond PA7 in September 1994, February and March 1995. Its density in pond PA9 was low throughout the study period, except in September and November 1994, and September 1995. In pond PA12, it was relatively abundant in July 1994, but its density was otherwise low throughout the study period. The relative proportions of developmental categories of *A. franciscana* as a percentage of the total population in each pond are indicated in Figure 3.10; detailed data are given in Appendix 3.2. Sub-adults were present in

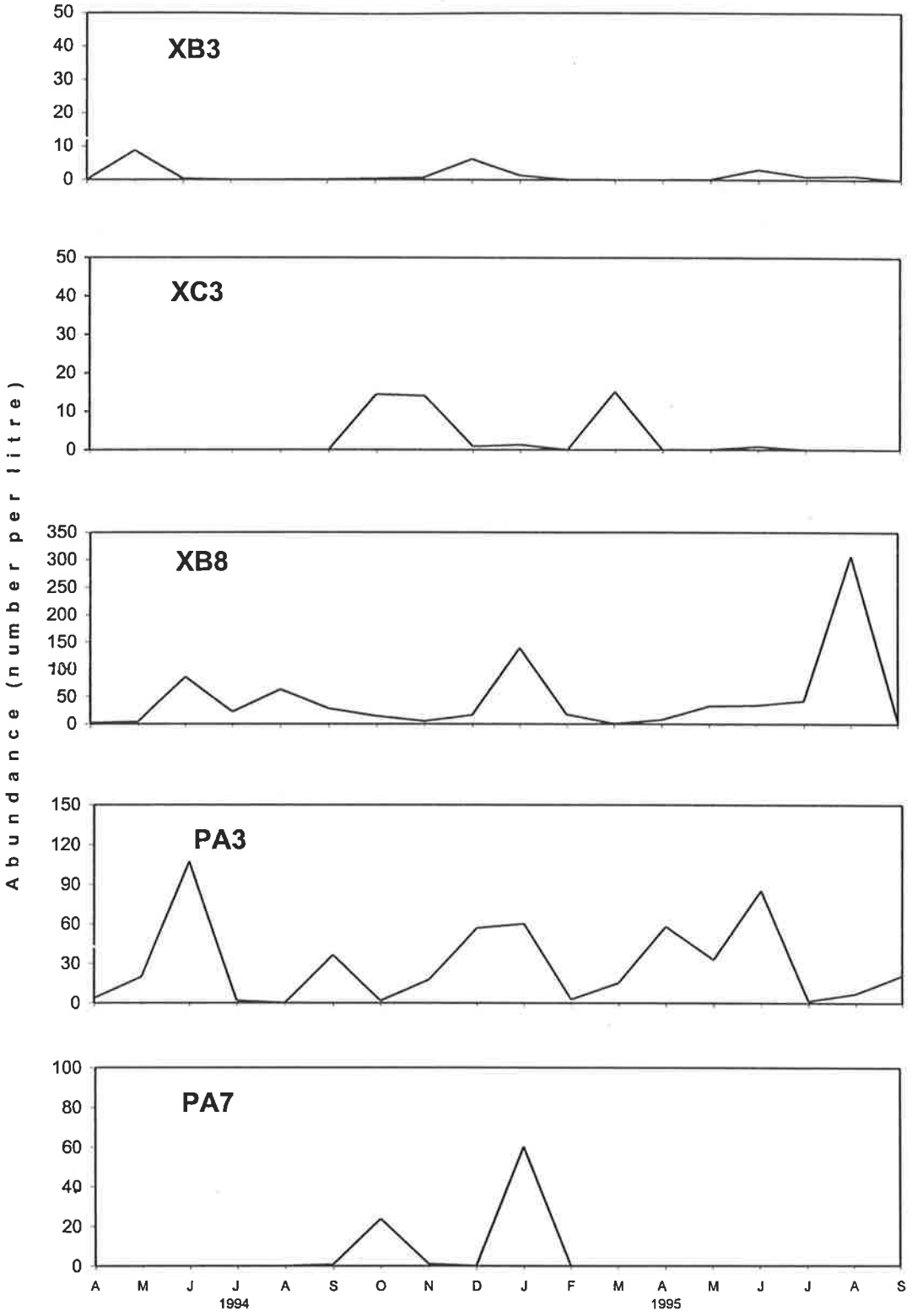

Figure 3.7. The seasonal patterns in abundance of *Parartemia zietziana* in study ponds.

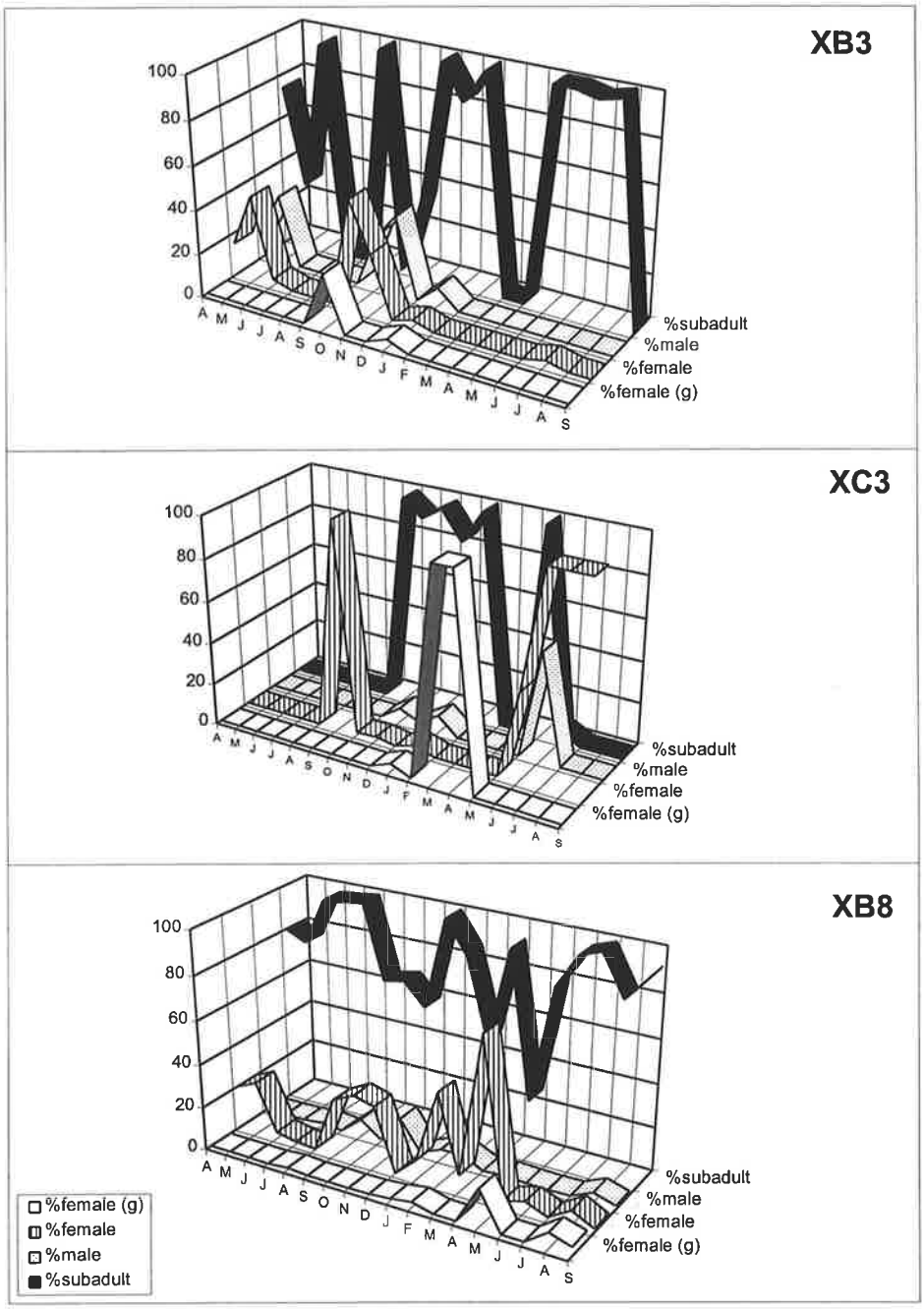

Figure 3.8. Percentage of male, female, gravid female and subadult of *Parartemia zietziana* in study ponds.

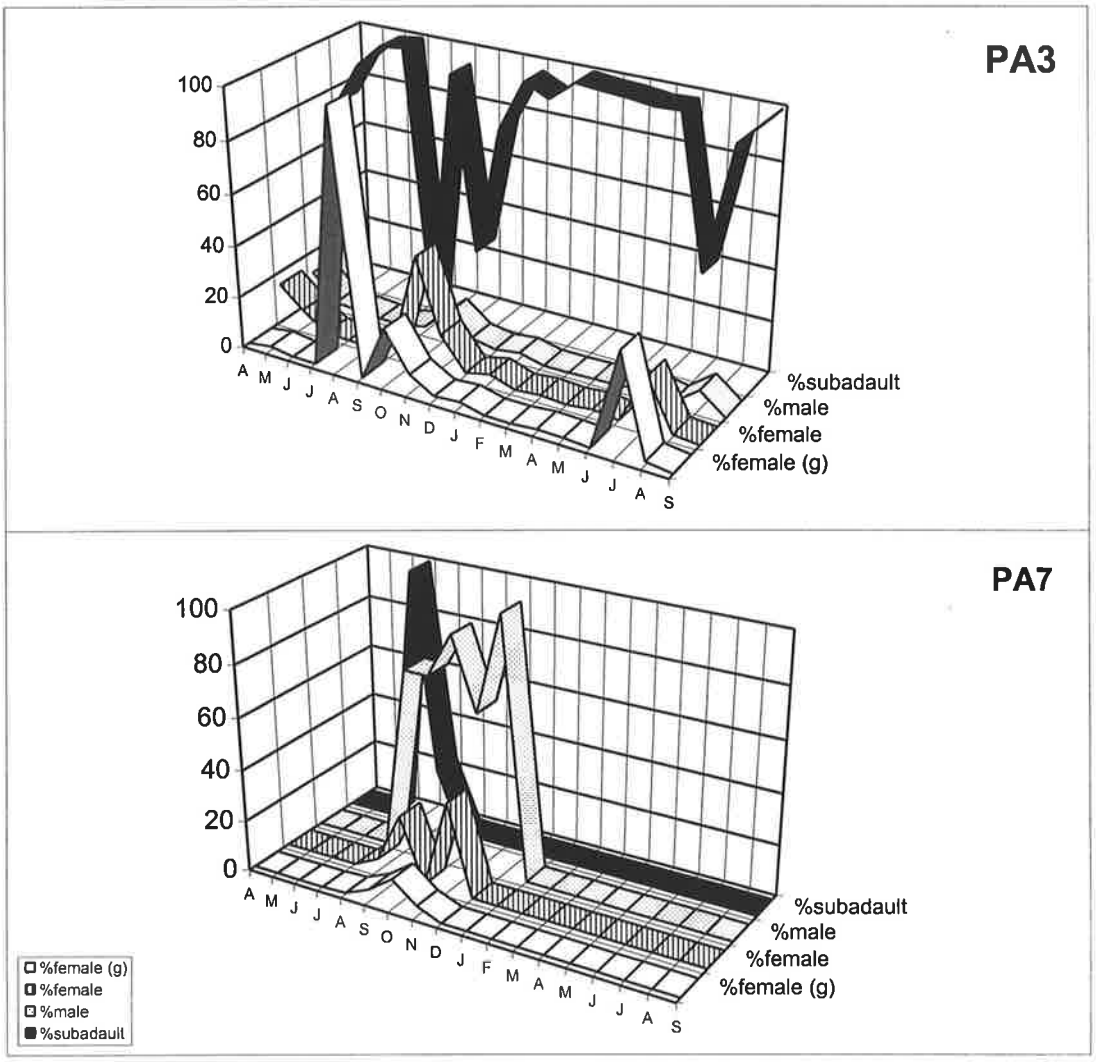

Figure 3.8. (continued)

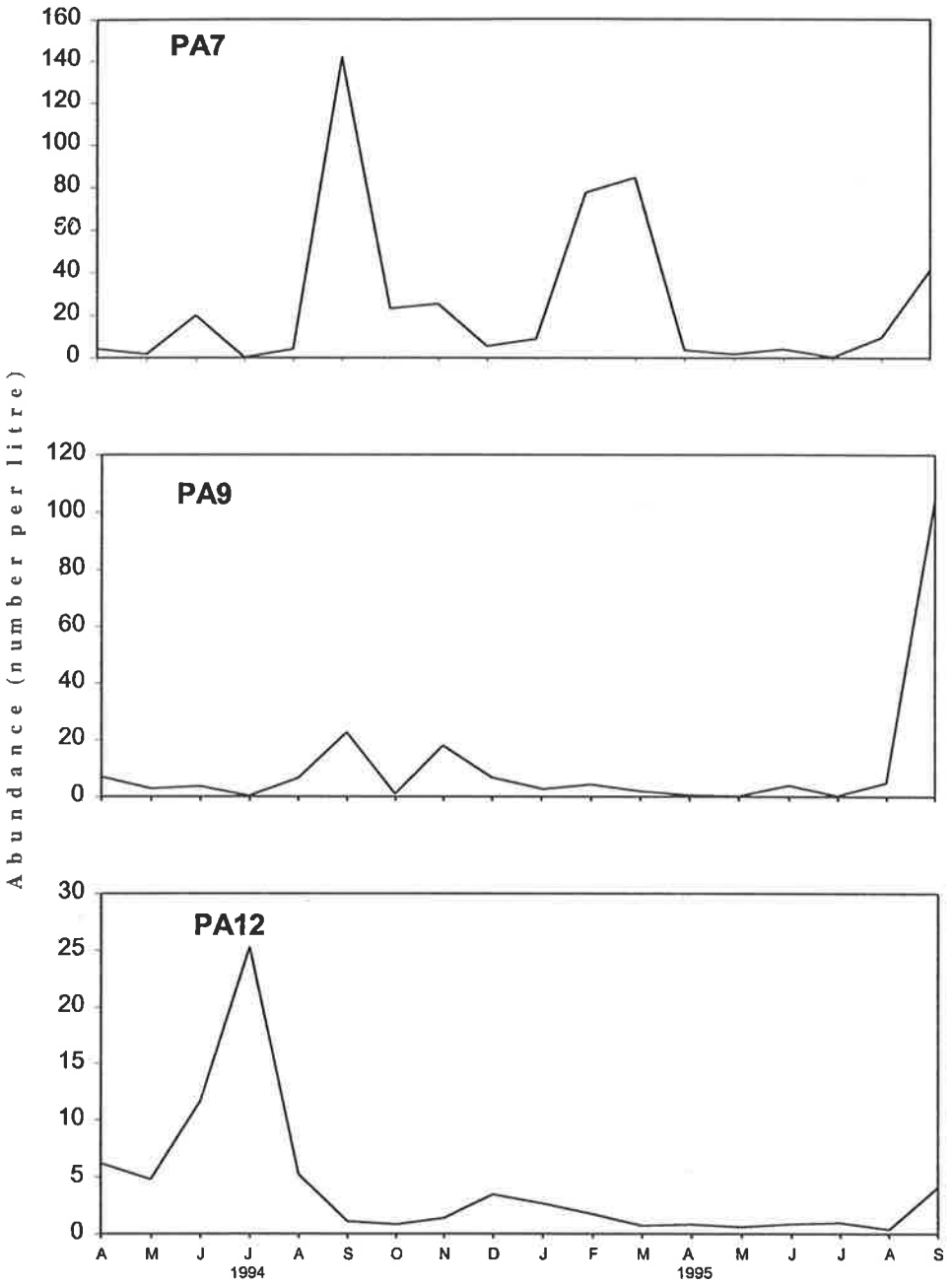

Figure 3.9. The seasonal patterns in abundance of *Artemia franciscana* in study ponds.

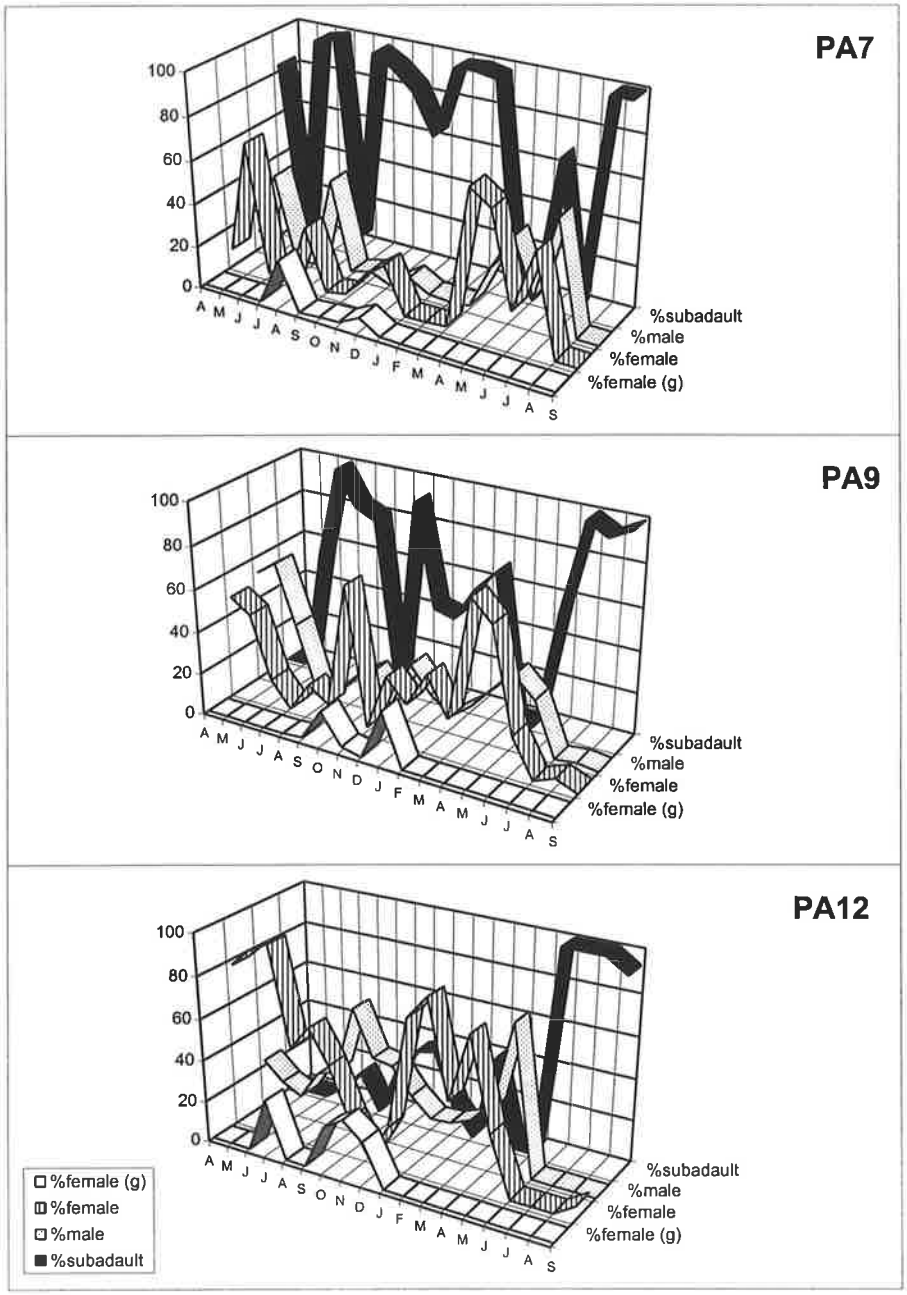

Figure 3.10. Percentage of male, femal, gravid female and sub-adult of *Artemia franciscana* in study ponds.

in these ponds. The species was abundant in pond PA7 in September 1994, February and March 1995. Its density in pond PA9 was low throughout the study period, except in September and November 1994, and September 1995. In pond PA12, it was relatively abundant in July 1994, but its density was otherwise low throughout the study period. The relative proportions of developmental categories of *A. fransciscana* as a percentage of the total population in each pond are indicated in Figure 3.10, detailed data are given in Appendix 3.2. Sub-adults were present in ponds PA7, PA9 and PA12 almost all year for most of the study period. Peaks in the abundance of females with eggs occurred in pond PA7 in August 1994, and PA9 in October 1994 and January 1995. The most saline pond with *A. franciscana* was pond PA12, where the reproductive period was July, and October to December 1994.

Both *Artemia fransciscana* and *Parartemia zietziana* were collected from pond PA7 in October (abundant), November, December (rare) 1994, and in January 1995 (abundant).

3.3.2.2. Zooplankton

Four species of copepods were collected. All occurred in ponds with a salinity of less than 110 g/L. *Acartia clausi*, (a euryhaline Calanoida, Bayly 1969 and 1978), was the most abundant copepod in low salinity ponds (50-80g/L). Its density was highest in June, July, August and September 1994 in pond XD1, and in May and November 1994 and June 1995 in pond XB3 (Fig. 3.11). *Austracyclops australis* (Cyclopoida) was rare and found only during a limited period (April, July 1994 and July 1995 in pond XD1 and January and September 1995 in pond XB3) (see Appendix 3.1). *Mesochra parva* and other harpacticoid species were rare and found in low density in ponds XD1, XB3 and XB8.

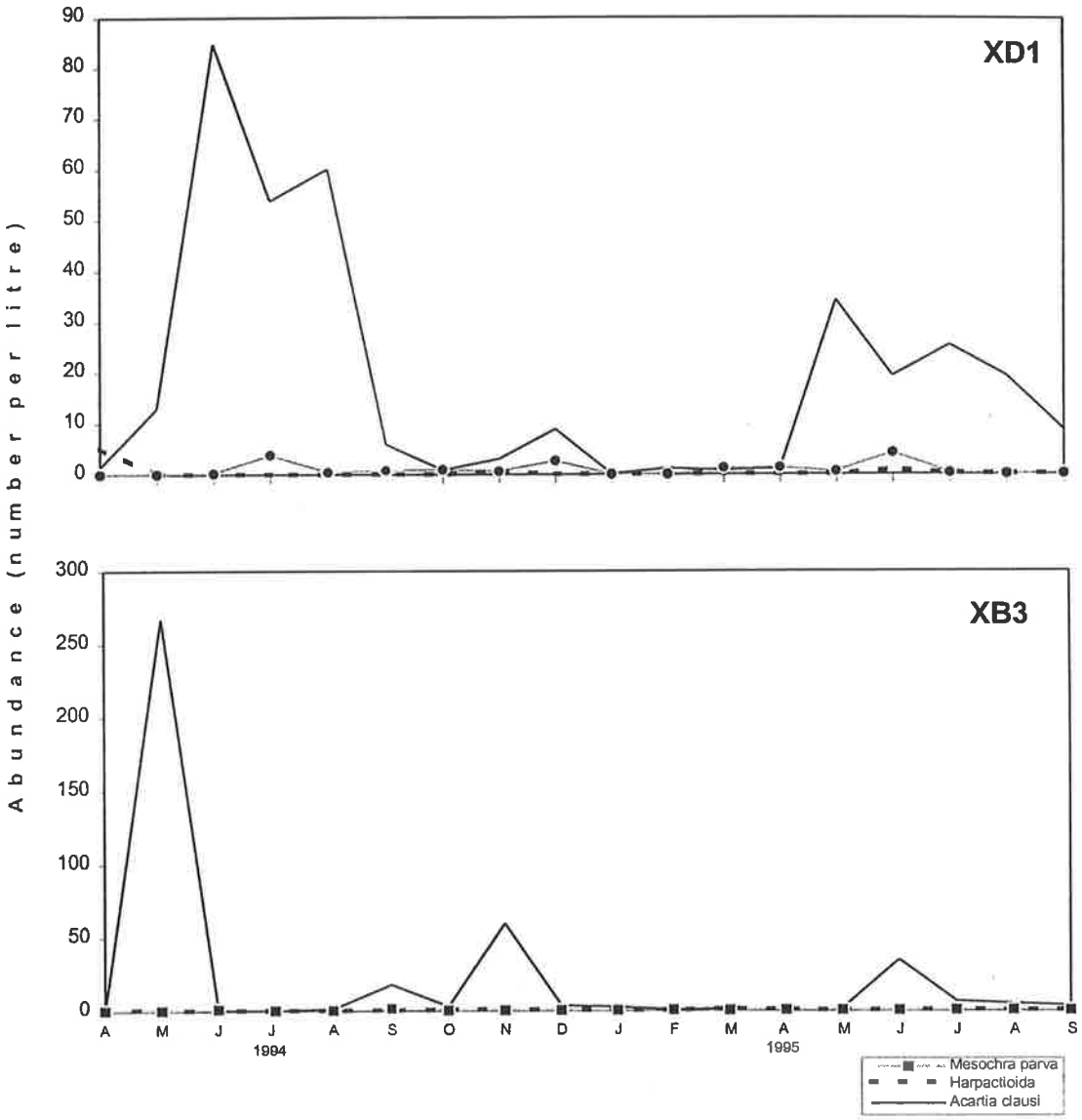

Figure 3.11. The seasonal patterns in abundance of copepods (*Acartia clausi, Mesochara parva* and Harpactioida) in ponds XD1 and XB3.

Two species of ostracod (*Diacypris dictyote* and *Reticypris herbsti*) occurred in ponds XD1, XB3, XB8, XC3 and PA3. These had a salinity of 55.5 - 137.5 g/L. They were rare in ponds XD1 and XB3 and low in density in ponds XC3 througout the study period, see Appendix 3.1. Seasonal patterns of their abundance in ponds XB8, XC3 and PA3 are presented in Figure 3.12. The density of *D. dictyote* was relatively high in October 1994 in ponds XB8 and XC3. In pond PA3, *D. dictyote* was present in relatively high densities in March and December 1994. *R. herbsti* was relatively abundant in April 1994 and June and August 1995 in pond XB8; however, its density was low in pond XC3 throughout the study period. It was relatively abundant in May, August 1994 and November 1995.

3.3.2.3. Benthos

The only gastropod collected was *Hydrococcus tasmanicus*. It was found in pond XD1 at a salinity of 55 g/L. Two species of isopods were also found in this pond. *Exosphaeroma bicolor* was most abundant and found in April and December 1994 and January, February, April, July and September 1995. *Synischia* sp. was collected in April 1994, January and September 1995. *Parhyalella* kunkel was the amphipoda that was also collected from this pond in April, October 1994 and December ,1995.

Larvae of *Symphytoneuria wheeleri* (Trichoptera) were collected from pond XD1 in January, February and March 1995. Three species of Diptera were recorded: two species of Chironomidae and one of Ephydridae. *Cladotanytarsus* sp. occurred in most ponds but mainly in spring, and *Tanytarsus barbitarsis* occurred in most ponds in almost all seasons (Fig. 3.13). These results shows only the number of the larva in the collected samples. Ephydrids (?*Ephydra riparia*) were collected from ponds with a salinity of more than 100 g/L, but were more abundant in the benthos of ponds with a salinity of more than 180 g/L.

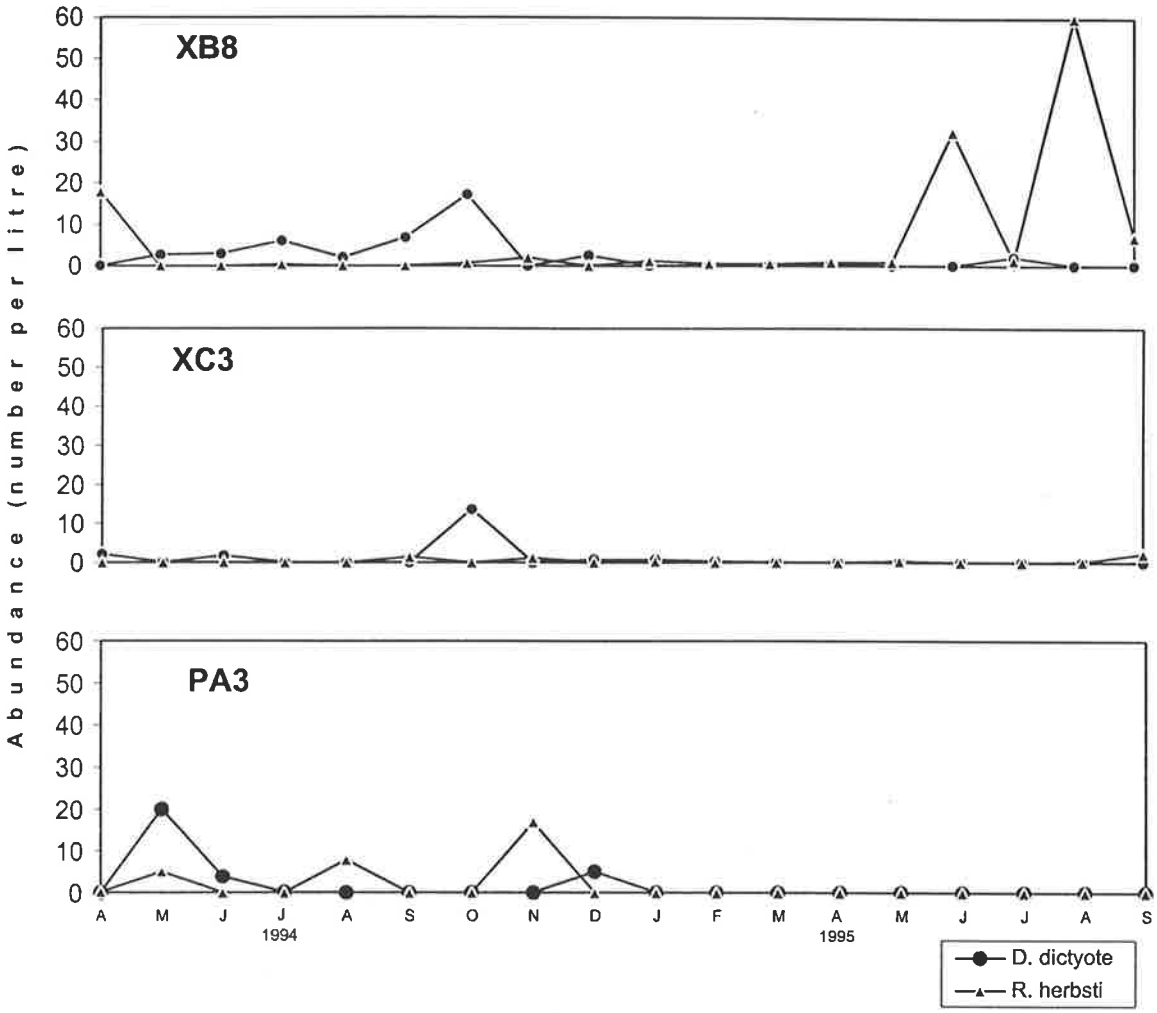

Figure 3.12. The seasonal patterns in abundance of ostracods (*Diacypris dictyote* and *Reticypris herbsti*) in study ponds.

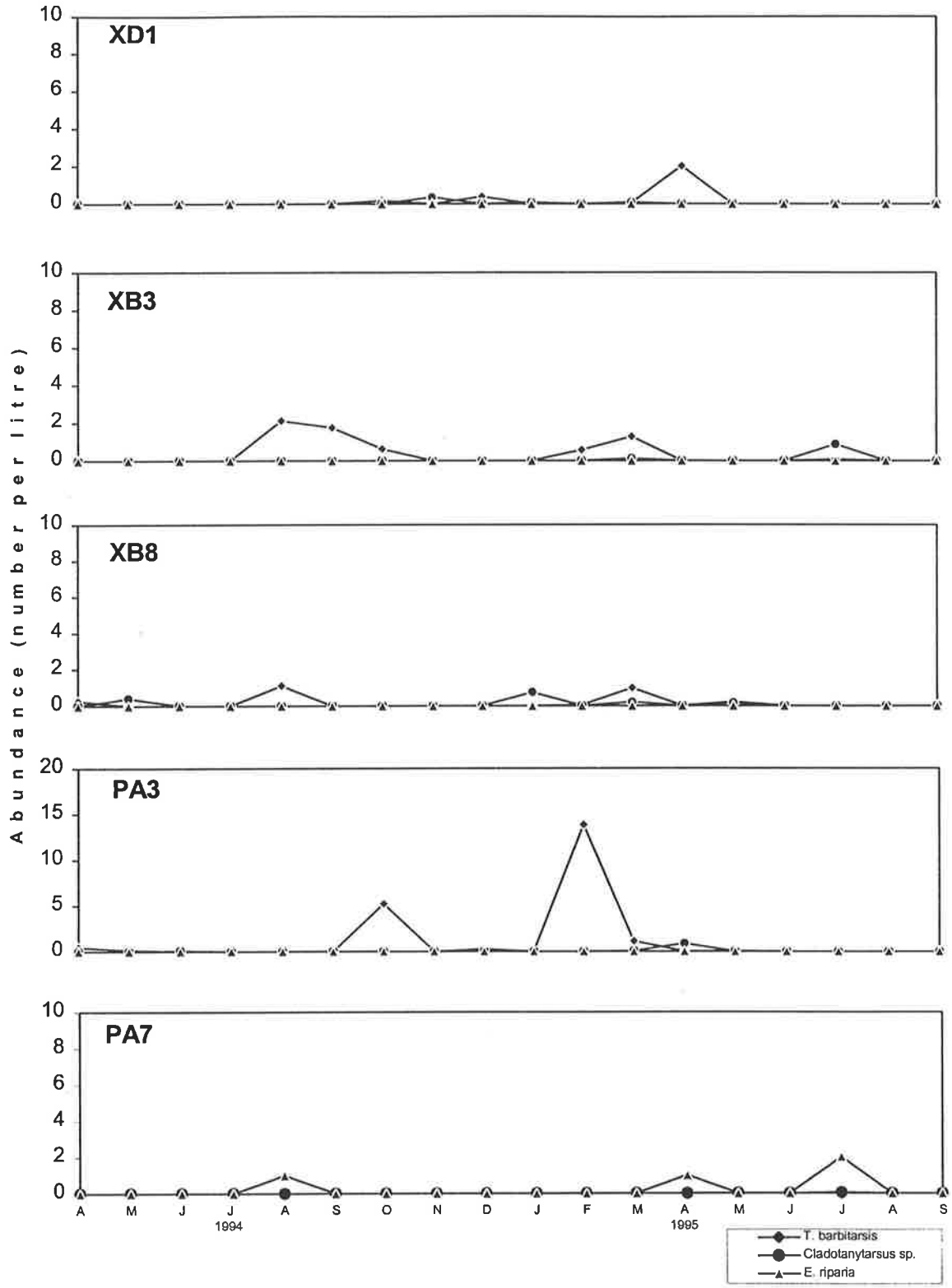

Figure 3.13. The seasonal patterns in abundance of (*Tanytarsus barbitarsis*, *Cladotanytarsus* sp. and *Ephydra riparia*) in study ponds.

3.4. Discussion

A balanced biological system in solar saltfields is important for the production of high quality salt (Davis and Giordano, 1996). Nutrients are essential to promote phytoplankton and benthic mat growth. Phytoplankton increases evaporation because it results in brine coloration and this increases light absorption. This is an advantage in the preliminary areas of the saltfield. Phytoplankton also provide food for other organisms in the salt ponds such as the brine shrimp *Artemia* and *Parartemia*. These anostracans are regarded as key organisms in salt ponds and control the planktonic population and favour benthic mat growth (Davis, 1990 and 1993). The maintenance of a clear brine thus requires a high brine shrimp population and this in turn requires a stable level of phytoplankton based upon consistent but not excessive levels of nutrient. Excess nutrients would produce algal blooms. In addition to nutrients, other basic chemical and physical parameters such as salinity, alkalinity and relative abundances of different ions, pH, dissolved oxygen, temperature, wind and rainfall may also control the composition of the biota of saltponds.

The source of seawater and the amount of nutrient present in it are important factors in maintaining a balanced biological system in solar saltfields. At Dry Creek there are two sources of seawater; Middle Beach, which has low nutrient concentrations, and Chapman Creek, with high nutrient concentrations (see Chapter 2). As a result, in recent years, ponds receiving water from Chapman Creek have undergone eutrophication. Consequently, it was decided to decrease the amount of seawater from Chapman Creek into the system. Now much of the import water comes from Middle Beach and recently, groundwater was also added to evaporating ponds (since mid summer 1996 and after the completion of the monitoring in this study). During hot weather in summer, however, when the rate of evaporation is high and the amount of water from Middle Beach is insufficient to compensate for the

evaporation, some water from Chapman Creek is added to the system. This amount, however, is still less than in previous years (see Chapter 2).

3.4.1. Flora

Although seawater from Chapman Creek during hot weather does contribute high nutrients to receiving ponds, the phytoplankton density still remains low. This maybe due to the assimilation of nutrients by the macroalgae (Pederson and Borum, 1996).

3.4.1.1. Phytoplankton

The spatial distribution of phytoplankton in individual ponds undoubtedly reflects variation in physico-chemical factors. Green algae and diatoms were major elements at a salinity < 150 g/L, and *Dunaliella* and Cyanobacteria at >150 g/L. The change from diatom dominance in ponds XB8 and PA3 to cyanobacterial dominance in ponds PA7, PA9, PA12 is probably not only due to the greater salinity tolerance of the Cyanobacteria alone, since diatoms are often abundant in hypersaline waters (Ehrlich, 1975, 1978; Ajaili *et al.*, 1986; Hammer, 1986; Compere, 1994; Blinn, 1993, 1995; Gell, in press). Nutrient concentrations, (especially low NO_3-N : PO_4-P ratio), or unstable hydrology may be also important for this changing in community (Turpin and Harrison 1979; Eppley *et al.*, 1978). Rhee (1972) also found that cyanobacteria were more effective than algae in using low PO4 concentrations. Furthermore, the amount of silicon in evaporating ponds is important for maintaining a high density of diatoms. Although all phytoplankton have a requirement for the small amounts of silicon involved in protein and carbohydrate synthesis, silicon as solid or colloidal silicate polymers (Reynolds, 1984) is especially important for diatoms. Diatoms react more immediately to external deficiency of silicon than any major nutrients such as phosphorus and

nitrogen (Werner, 1977). It was decided in mid summer 1996 to add groundwater to the evaporating ponds. This will probably help the growth of diatoms at higher salinity ponds and may prevent the growth of the cyanobacteria, namely *Synechococcus*. This widespread hypersaline cyanobacterium has been reported in many studies as Aphanothece or Coccochloris (e.g. Javor, 1989; Sammay, 1993; Davis and Giordano, 1996), see Chapter 5 for more details.

The diverse diatom flora at the lower salinities was dominated by marine and brackish water taxa. Most of the genera occurred have also been recorded from other saline lakes and saltfields: Subbaramaiah (1972, in Rajasthan, India), Jones *et al.* (1981, in Australia), Bauld (1981, in Australia) and Javor (1983a and b, in Mexico), Hammer *et al.* (1983, in Canada), Hammer (1986), Blinn, (1991, in South Australia), Tudor *et al.*, (1991, in Western Australia), Blinn, (1993 and 1995, in Australia) Gell and Gasse, (1994, in Australia).

3.4.1.2. Macroalgae and periphyton

Water from Chapman Creek (see chapter 2) gave rise to high nutrient levels in pond XB3 in the past and encouraged the growth of macrophyte algae such as *Ulva* sp. which then killed the surrounding seagrass. From 1991, a patchy distribution of *Chara* began to cover the bottom of pond XB3, following the decrease in the volume of high nutrient seawater (see chapter 2). The phytoplankton community decreased because of the lower nutrient concentration in pond XB3, and this helped the growth of macroalgae in this pond. However, such changes were not completely reflected in other ponds (such as XB8 and PA3), and these still had a high density of phytoplankton but no macroalgae (Figure 3.4). Jones *et al.* (1981) reported a high population of phytoplankton in pond XB3; this was probably because of the high volume of high nutrient water from Chapman Creek transferred to this pond.

3.4.1.3. Benthic mat

Decreasing phytoplankton populations due to high salinity led to greater illumination of the sediment surface. This allowed benthic mats to establish at the bottom of ponds with salinities >150 g/L. A similar association has been shown by Tuite (1981), Burk and Knott (1989) in saline lakes. The saline lakes where this has been happened include Lake Chilwa (Malawi), Lake Aranguadi (Ethiopia) and shallow lakes in Wadi Natrun (Egypt) (Moss and Moss, 1969; Talling *et al.,* 1973; Imhoff *et al.*, 1979). The inhibition of benthic mats by turbid brines in solar saltfields at Dry Creek has previously been reported by Jones *et al.* (1981). Stal (1995) and Bergman *et al.* (1997) reviewed the importance of non-heterocystous Cyanobacteria in benthic mats. They reported that *Synechococcus* is a nitrogen - fixing bacterium, with photosynthesis occurring in the day when sunlight is available, and nitrogen - fixation occurring at night when oxygen levels within the cells are lower. Thus, *Synechococcus* has advantages over other Cyanobacteria and algae in highly saline water with low oxygen concentrations and low nitrate concentrations (see Chapter 2, also Mague *et al.*, 1980; Mitsui *et al.*, 1986; Grobbelaar *et al.*, 1986; Arad *et al.*, 1988; Schneegurt *et al.*, 1994; Liu *et al.*, 1996).

The presence of grazers and burrowing animals also interfere with the development of benthic mats (Stal, 1995). For example, ostracods in ponds XB8, XC3 and PA3 are extensive bottom feeders and have been observed to graze and ingest algae. In pond XD1, the benthic mat was grazed by a gastropods, a phenomenon previously reported by Garett (1970), Pace *et al.* (1979), Grredes and Krumbien (1984), Javor and Castenholz (1984) for typical marine environments.

The presence of benthic mats in ponds with a salinity >150 g/L is important as they increase evaporation, oxygenate brine, oxidise and recycle organic matter, reduce permeability and prevent leakage. The presence of thick benthic mats in ponds PA7

to PA12 is likely to have reduced the permeability of the bottom there, so decreasing brine leakage. However, the large amount of mucilage produced by *Synechococcus* affects salt quality and quantity (Ghassemzadeh et al. 1996c and d; also see chapter 5). Jones *et al.*(1981) reported that the presence of a 1mm thick benthic mat over 1 m of shell grit reduced the permeability of the bed of shell grit by a factor of 10. The importance of benthic mats was recognised at a very early date by Bass-Becking (1931). Mats have been reported from other solar saltfields in the world by Carpelan (1957, in California, USA), Copeland and Jones (1965, in Texas, USA), Baha Al-Deen and Baha-Al-Deen (1972, in Venezuela), Subbaramaih (1972, in Rajasthan, India), Davis (1974, 1978, in USA), Schneider and Herrman (1980, in Australia), Jones *et al.* (1981, in Australia), Bauld (1981, in Australia), and Javor (1983a, in Mexico).

3.4.2. Fauna

3.4.2.1. Nekton

The occurrence of *Atherinosoma microstoma* and *Pesudogobious olorum* is not surprising for they have also been reported by others in coastal lagoons in South Australia (Paton, 1982; Geddes and Butler 1984; Molsher *et al.*, 1994). The large numbers of *A. microstoma* larvae from October to December in ponds XD1, XB3 and XB8 are probably due to the single annual breeding season of this fish. Molsher *et al.* (1994) noted that this season was from September to December in the Coorong, South Australia.

Marine species in ponds with a salinity of 50 - 100 g/L enter only when seawater enters. The occurrence of some marine species (such as *Palaemon serenus* in pond XD1) may also prevent the growth of a benthic mat.

Parartemia zietziana and *Artemia fransciscana* appear to have reduced phytoplankton populations in ponds with a salinity of >150 g/L, thus promoting the growth of the benthic mat. The spatial and temporal distribution of these anostracans is affected by both biological interactions and abiotic factors (Lenz, 1987, Hammer and Hurlbert, 1992). Biological interactions, however, are limited because of low species diversity in the hypersaline waters and the virtual absence of predators.

The population of *Artemia* at Dry Creek solar saltfields in the 1970s was parthenogenetic (Mitchell and Geddes, 1977; Geddes, 1979 and 1980 and 1981). A sexual strain *Artemia* was introduced at Dry Creek in 1980's and identified as *Artemia fransciscana* (pers. comm., Chesson and Geddes). Several factors may contribute to the explanation of the displacement of the parthenogenetic *Artemia*. The parthenogenetic *Artemia* may have been outcompeted for resources by the sexual species. Experiments conducted by Browne (1980) and Browne and Halanych (1989) and Triantaphyllidis *et al.* (1995) show that sexual *Artemia* species completely displace parthenogenetic species.

Temperature appears to the most important physiological factor affecting production of *Parartemia zietziana* and *Artemia fransciscana*. Even so, *P. zietziana* reproduced all year for most of the study period, except in pond PA7. Females of *A. fransciscana* were in low densities during winter because they were not reproductively active and the population was dominated mostly by adults and juveniles. As temperature rose, reproduction began, and the remaining season was characterised by many overlapping generations. This population has a cycle similar that recorded in the South Pacific by Wear *et al.* (1986); Wear and Haslett, (1986 and 1987).

There are two modes of reproduction in *A. fransciscana*: oviparous and ovoviviparous. Females can produce either encysted embryos released in diapause (oviparous) or embryos which continue to develop in the ovisac (ovoviviparous) and resulting in the release of nauplius larvae (Drinkwater and Clegg, 1991; Savage, 1994). Ovoviviparity is typical of *Artemia* population with uninterrupted population cycles (Lenz and Browne, 1991). At Dry Creek, *A. fransciscana* hatched from overwintering cysts when the temperature was 10-17°C, and developed in slowly rising temperatures and high food conditions. In February to August, ovoviviparity developed at relatively high temperatures and under conditions of low food quality and quantity. Thus, low densities of *Artemia* and *Parartemia* are not only dependent upon temperature, but also relate to the quality and quantity of planktonic algae. As an example, green algae (e.g., *Dunaliella*) and diatoms (e.g., *Navicula*) are much better food for *Artemia* than blue-green algae (Javor, 1983a; Davis, 1978), which are more abundant in ponds PA7 than in PA9 and PA12. This may explain differences in population densities in these ponds. Other important factors may be illumination, oxygen concentration, as well as salinity. The high density of *A. fransciscana* in shallow ponds such as pond PA7 (see Chapter 2) may be the result of high illumination, considered one of a number of favourable conditions for maximum hatchability (Laven and Sorgeloos, 1987; Van der Linden *et al.*, 1986a, b and 1988). The presence of high numbers of brown eggs and diapause during summer and beginning of the autumn 1995 in pond PA12 was caused by high salinity and low oxygen, a phenomenon also observed by Lavens and Sorgeloos (1984 and 1987) and Sorgeloos (1989). These authors have also reported that salinity shocks are effective in causing the population to produce cysts. Low oxygen concentrations also induce oviparous reproduction in *Artemia* (Versichele and Sorgeloos, 1980; Lavens and Sorgeloos, 1984). Low oxygen stimulates the synthesis of haemoglobin and the excretion by the brown shell and of its metabolic product, haematin, which is the main constituent of the cyst shell of *Artemia* (Lavens and Sorgeloos, 1987).

The differences in the salinity ranges of *P. zietziana* and *A. fransciscana* from pre-1981 to 1995 are shown in Figure 3.14. This indicates that the distribution of *P. zietziana* and *A. fransciscana* at Dry Creek saltfields has changed during this period.

In general, as is shown in Figure 3.14, the salinity range for *A. fransciscana* and *P. zietziana* has changed throughout the years. Ecological isolation between these species may occur due to differences in physico-chemical and biological preferences. Studies by Mitchell and Geddes (1977) and Newton (1980) showed that the parthenogenetic *Artemia* species existed at salinities between 119 and 330 g/L and *P. zietziana* between 85 and 285 g/L. Overlap occurred in the range 119-285 g/L (Fig. 3.14). These figures are comparable with those obtained in the study by Meredith (1992) of 138 to 292 g/L for sexual *Artemia* species and 98 to 134 g/L for *P. zietziana*. In this study, the distribution of *A. fransciscana* is 98 to 330 g/L and for *P. zietziana* is 50 to 180g/L.

In terms of differential spatial shifts, studies by Mitchell and Geddes (1977), Geddes (1980) and Newton (1980) showed that *Artemia* was found between ponds PA12 and FA5, and sporadically in ponds PA6 to PA8, whilst *P. zietziana* was found in all ponds between PA5 and FA3. The Study by Meredith (1992) showed that sexual *Artemia* was found from ponds PA5 to FA5, but *P. zietziana* only in ponds XB8 to PA3. This change in both distribution and salinity range of *P. zietziana* and *A. fransciscana* is probably related to local extinction of the parthenogenetic *Artemia* sp. In the same way that the asexual *Artemia* is unable to compete with sexual *Artemia*, *P. zietziana* may also be unable to resist the competitive strength of *A. fransciscana* and is therefore currently being excluded from most ponds where *Artemia* is present and restricted to the lower salinity ponds. Moreover, the kind and availability of food, predators and substrate may affect the distribution of *A. fransciscana* and *P. zietziana*.

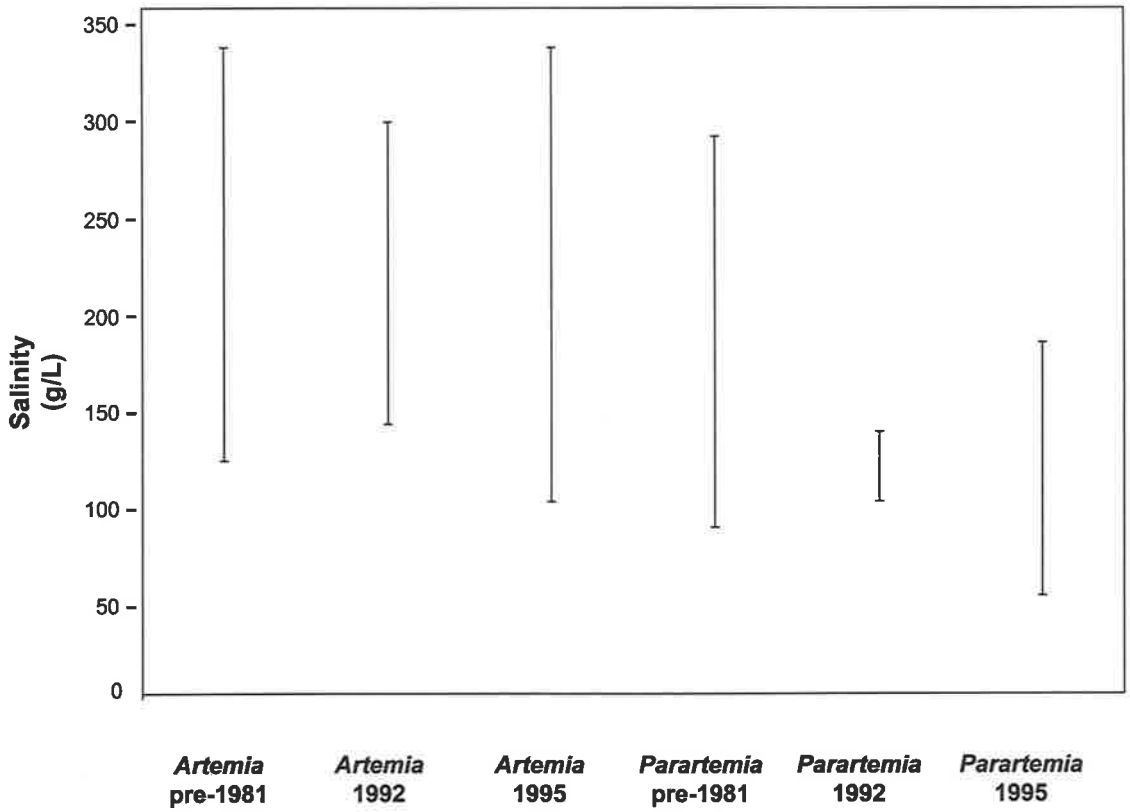

Figure 3.14. The distribution of *Parartemia* and *Artemia* at Dry Creek.

The large variation found between recent and past studies of brine shrimp suggests that the relationship between the two species involved is currently a dynamic system in which equilibrium has not yet been reached. It is not possible to predict the end of this competitive exclusion. *P. zietziana* is bounded by *A. fransciscana* at the higher end of salinity and fish at the lower end. Bird densities are also the main predator through the system for both *P. zietziana* and *A. fransciscana*.

3.4.2.2. Zooplankton

Zooplankton has received less attention than has the phytoplankton of solar saltfields. Nevertheless, some general factors displayed by the phytoplankton also apply to the zooplankton. The most obvious is the gradual decrease in the number of species with increasing salinity. The zooplankton are major primary and secondary consumers in ponds XD1, XB3, XC3 and XB8. Here, they feed on bacteria, phytoplankton and organic detritus, or on other zooplankton. Although several groups are represented, crustaceans are the major functional groups. High salinity was probably the major factor restricting most of them. For some, such as ostracods, not only salinity but probably also a deficiency in some ions may affect their distribution in ponds with a salinity of more than 100 g/L (e.g. carbonate, see Chapter 2).

High zooplankton biomass in ponds with a salinity of <120 g/L probably occurred not only because of the low salinity, but also because of the availability of food. Thus, increases in the diversity and density of zooplankton in ponds XB3, XB8, PA3 and PA7 was probably related to increases in the abundance of phytoplankton. There was, in any case, sufficient zooplankton to support significant populations of planktivorous adults and juveniles of *A. microstoma* and *P. olorum*.

Seasonal variation in zooplankton populations undoubtedly reflects seasonal changes in temperature, light, nutrients, and algal abundance. Thus, the presence of zooplankton is governed primarily by its salinity tolerance and its abundance by trophic conditions. Note, however, that trophic conditions may change within a few weeks, especially in saline and hypersaline waters.

3.4.2.3. Benthos

The occurrence of amphipods and isopods in the low salinity ponds (46-56 g/L) is not surprising as these are marine species and would have entered the system with seawater inflows. High salinity was probably the major feature restricting their distribution beyond the initial ponds.

Caddisflies (Trichoptera) are not common in saline water in Australia or elsewhere (Hammer, 1986). The occurrence of *Symphitoneuria wheeleri* (Trichoptera) is notable because this insect order occurs only rarely in saline waters (Schmitz, 1959). The species recorded has also been reported from saline lakes in Western Australia (Edward, 1983). Bayly and Williams (1966) noted that the species they found in Lake Coradgill was probably *S. wheeleri*, as indicated by Mosely and Kimmins (1953). It is interesting that the caddisfly in the Dry Creek ponds did not belong to the Philanisidae, the family containing the only known marine caddisfly (Mosely and Kimmins, 1953).

3.5. Summary and conclusion

Elements of marine brackish fauna provide the dominant groups of organisms in early stage ponds. These include fishes, gastropods, isopods and amphipods, and the the calanoid, cyclopoid and harpacticoid copepods. *Acartia* is the main copepod that tolerates salinity up to 110 g/L. In general, crustaceans are the main

component of the study ponds. The brine shrimps, *Artemia* and *Parartemia*, are the dominant filter-feeding plankton in high salinity ponds and are important for the production of high quality salt. The principal zoobenthos in ponds with a salinity of more than 150 g/L is an insect, Ephydridae.

Differences in phytoplankton density occurred in ponds XD1 and XC3 (low nutrient) and ponds XB3 and XB8 (high nutrient) and also in pond PA3 where mixing took place, reflecting nutrient concentrations in these ponds. XD1, receiving water from ponds XE1-5 which in turn received water from the Middle Beach intake, was clear and had abundant fish. High nutrient intakes in pond XB3 and nutrient reserves in the sediments of this pond and ponds XB8 and PA3 caused these ponds to become slightly eutrophic. The plant community was dominated by planktonic algae.

The phytoplankton community-algae and cyanobacteria-is the only source of primary production in high salinity water. Here, diatoms are dominant forms at a salinity < 150 g/L, and Cyanobacteria and green algae, *Dunaliella* and *Stephanoptera* at > 150 g/L. Phytoplankton densities increased in the water column because of high nutrients. Dense populations shaded the benthos. Benthic mat communities covered the bottom of higher salinity ponds (>150 g/L) and are the major source of primary production in these ponds. Benthic mats are important in increasing evaporation, oxygenating the brine and recycling organic matter, reducing permeability, and preventing leakage. Cyanobacteria, especially *Synechococcus*, is the dominant form in benthic mats at salinity > 150 g/L. *Synechococcus* has advantages over other Cyanobacteria and algae in highly saline water because low oxygen concentrations, low nitrate concentrations and relatively constant salinity is a favourable environment for this form of Cyanobacterium. *Synechococcus* produces significant amounts of extracellular material that may affect salt quality and quantity (see Chapter 5).

Seasonal variation in zooplankton populations undoubtedly reflects seasonal changes in temperature, light, nutrients, and algal abundance. Thus, the presence zooplankton is governed primarily by its salinity tolerance and its abundance by trophic conditions.

In general, the trophic structure of fauna in these saline ponds is very simple; aquatic predators are absent or rare, except in lower salinities ponds where fishes are present. However, bird densities are sometimes high and are a major non- aquatic predator through the system.

Comparison between solar saltfields at Dry Creek with other solar saltfields shows that, despite of the great similarity in biological composition in the ponds they differ in many quantitative and qualitative characteristics. World - wide, the planktonic community of highly saline concentrating ponds consists mainly of *Artemia* spp. (brine shrimp), *Ephydra* larvae, Chironomid larvae and *Dunaliella salina*, *Synechococcus* and species of other algae, species of aerobic halobacteria, flagellates and ciliates. The presence of *Parartemia zietzianz* at Dry creek, which is endemic to Australia, is a major fauna difference between Australian solar saltfield and saltfields elsewhere. Differences between solar saltfields world-wide relate to the status of nutrients in the ponds, which are important in determining the productivity flora and fauna in the fields.

Information from this biological investigation can be used to make appropriate decisions about pond management. Proper management of biological systems is essential for production of high quality salt.

CHAPTER FOUR

THE DETERMINATION OF PHOSPHATE AND NITRATE IN HIGHLY SALINE WATERS

4.1. Introduction

Compounds of nitrogen and phosphorus are major cellular components of organisms. Nitrates and phosphates are the principl reservoirs providing these essential elements in any aquatic ecosystem. Since the availability of these elements may be less than biological demand, shortage can regulate or limit the productivity of organisms in water. The measurement of these compounds, and the use of appropriate methods for their analysis, are therefore very important in the study of any aquatic ecosystem.

The determination of phosphate and nitrate in fresh water is contentious, their analysis in seawater is more difficult because of the salt effect of particular ions Fortner *et al.*, 1974). This is even more difficult in waters more saline than seawater. Thus, analytical procedures developed for fresh and marine waters must be applied cautiously to saline waters. For this reason, the salt effect on analyses of phosphate and nitrate by the most commonly used analytical technique was examined during this study.

4.1.1. Previous work

Despite an extensive literature dealing with phosphate analysis (e.g. Olsen, 1967; Griffith *et al.*, 1973; Broberg and Pettersson, 1988), little attention appears to have been paid to the possible influence of salinity (the salt effect) on phosphate analysis until the recent study of Sherwood *et al.* (1995). Most nutrient studies have been concerned with fresh and seawater, and the size of the salt effect in the analysis of phosphate in highly saline water had not been estimated.

Murphy and Riley (1958 and 1962) and Burton and Riley (1956) reported small salt effects, up to only 4 per cent in seawater, when antimony (III) was used with ascorbic acid in the phosphomolybdenum blue spectrophotometric method. Riley and Skirrow (1975) indicated that the major cations and anions of seawater did not have not any effect on the molybdenum blue method if ascorbic acid was used as reductant. Sherwood *et al.* (1995), after extensively studying the salt effect on phosphate measurement in high saline water, found that the phosphomolybdenum blue spectrophotometric method is most useful method for phosphate determinations in hypersaline water. They compared three methods of analysis of phosphate based on the formation of phosphomolybdenum blue complexes for hypersaline waters. They found that stannous chloride reduction in aqueous media exhibits a substantial salt effect and its use was not recommended. They also pointed out that stannous chloride reduction following extraction into non-aqueous solvents shows a significant salt effect (up to 30 per cent) in solutions of salinity > 100 g/L. They finally concluded that ascorbic acid reduction, catalysed by antimony (III) ions, appears to offer the most useful method for phosphate determinations in hypersaline water. In view of their studies, only some more confirmation of salt error in phosphate analysis was undertaken in the present study.

The most widely used method for analysis of nitrate in seawater involves the quantitative reduction of nitrate to nitrite. Mullin and Riley (1955) used hydrazine

in the presence of cupric ion as a catalyst for the reducing agents. Disadvantages of this method are the length of the time required for reduction, the sensitivity of the method to motion, and salt interferences (Riley and Skirrow, 1975). Chow and Johnson (1962) overcame first the two of these disadvantages by the use of zinc powder for reduction. However, the reduction is then sensitive to temperature and each sample must be filtered. The use of cadmium as the reducing agent was suggested by Potzl and Reiter (1960), and this method has subsequently proved to have many advantages in the routine analysis of nitrate in sea water. Morris and Riley (1963) also found that nitrate can be reduced to nitrite with a 91%±1% yield by passing samples through a column of amalgamated cadmium filings and reported that the results are free from salt error.

Grasshoff (1964) further refined the method of Morris and Riley (1963) with some modifications which included the addition of ammonium chloride. The procedure was again refined by Strickland and Parsons (1972), who replaced ammonium chloride with EDTA as the column conditioner. They also suggested that cadmium filings should be washed with nitric acid before amalgamation; this pits the surface of the filing, thus providing a greater surface area for the reduction process. Wood *et al.* (1967) suggested the use of copper rather than mercury as the reducing agent, and also that EDTA be used to complex the cadmium ion. Wetzel and Likens (1991) stated that the best methods for analysis of NO_3-N in fresh water is to reduce the nitrate in alkaline buffered solution to nitrite by passing the sample through a column of copperized cadmium metal filling. The nitrite is then measured by a sensitive diazotization method that results in stable pink azo dye whose absorbance obeys Beer's law up to about 500 μg NO_3-N or NO_2-N/L.

Yoshida (1967) has suggested a novel method for the determination of low concentrations of nitrate (0.06-60 μg/L NO_3-N). In this, nitrate is reduced to nitrite

by nitrate-reducing bacteria. However, despite the variety of methods available, the measurement of NO_3/NO_2-N by the Cd column technique followed by colorimetric procedure, as introduced by Morris and Riley (1963) and Wood *et al.* (1967), remains the most commonly used technique fresh to saline waters.

Although salt interferences in colorimetric analyses of phosphate and nitrate in saline waters have been reported (e.g. Cooper, 1938; Jones and Spencer, 1963; Jones, 1966; Sherwood *et al.*, 1995) for the most part, such interferences have been regarded unimportant in nitrate analyses. The standard method references (e.g. APHA, 1992) make no mention of possible salt effects in the use of the Cd column technique, the technique most used by researchers who have studied nitrate concentrations in saline water (e.g. Atkinson, 1987; Tominaga *et al.*, 1987; Howard-Williams *et al.*, 1989; Wurtsbough and Berry, 1990). Morris and Riley (1963) described that the Cd column technique is free from salt error. Mullin and Riley (1955) reported salt interferences in sea water using the hydrazine method. Indeed, Parsons *et al.* (1984) noted that NO_3 analyses in seawater were salt affected when using the cadmium reduction column technique. Fortner *et al.* (1976) reported that the reduction of nitrate to nitrite by Cd column was inhibited by salt, but salt did interfere with nitrite analysis. Although there has been concern about salt effects, most researchers who have reported nitrate in saline water have ignored salt interferences.

4.1.2. Aims of the present study

To overcome salt interference in phosphate and nitrate analysis, three methods have been suggested:

- Dilution with deionized water (e.g. Fortner *et al.*, 1976; Ashton and Schoeman, 1983). This will place samples well within the salinity range of seawater, thus enabling the use of established techniques used in sea water analysis.
- The use of standard phosphate and nitrate solutions made from synthetic seawater (Parsons *et al.,* 1984). This overcomes any salt effect from waters around marine salinities.
- The use of a technique of standard addition (e.g. Fortner *et al.,* 1976; Atkinson, 1987). This technique can compensate for salt error.

To determine the efficiency of the two widely used methods for phosphate and nitrate analysis in highly saline waters, these methods were examined further in this study. The ascorbic acid with antimony (III) in phosphomolybdenum blue method was used for phosphate, and the Cd column technique followed by colorimetric procedure was used for nitrate. Because Sherwood *et al.* (1995) have described in detail the measurement of phosphate in highly saline water, emphasis was on the effect of salt on nitrate measurements. For this investigation, saline water was made from AR salt and evaporated sea salt of different salinity. Saline water from different ponds at Dry Creek Saltfield was also used.

4.2. Methods and material

All glassware was washed twice in 10% hydrochloric acid and rinsed with distilled water three times prior to use.

4.2.1. Analysis of PO_4-P

a. Preparation of standard solution: 0.219 g AR KH_2PO_4 (dried for 1 hour at 110C°) was dissolved in distilled water and diluted to one litre. This solution can be

kept in the laboratory for at least several months when stored in the dark in a tightly stopped glass bottle. It contains 0.2 mg PO_4-P/mL and was used for the preparation of standard solutions.

b. Preparation of reagents: All reagents were prepared according to Murphy and Riley (1962) and were prepared using freshly deionised water.

c. Procedure: Triplicate standard samples were prepared from standard stock solution, diluting by appropriate amounts of the stock solution with saline water. One mL of mixture of ascorbic acid and antimony (III) solution was added to 15 mL of standard sample and mixed. After 8 minutes, the absorbance was measured in a 5 cm cuvette at 705 nm by a Varian UV/Visible spectrophotometer.

4.2.2. Analysis of NO_3-N

Standard stock of nitrate solution and reagents were prepared according to Morris and Riley (1963) and Wood *et al.* (1967).

a. Preparation of standard solution: 0.722g AR KNO_3 (dried for 1 hour at 110 C°) was dissolved in distilled water and diluted to one litre. This solution can be kept in the laboratory for at least several months when stored in the dark in a tightly stopped glass bottle. It contains 100µg NO_3-N/mL and was used for the preparation of standard solutions.

b. Preparation of reagents: All reagents and solutions were prepared from freshly deionised water.

- Buffer solution: 100g NH_4Cl, 20g $Na_2B_4O_7 \cdot 10H_2O$ and 1g EDTA were dissolved in 1 litre of deionised water. This solution is stable.

- Sulfanilamide solution: 5g of sulfanilamide was dissolved in a mixture of 50 mL of concentrated HCl and diluted with deionised water to 500 mL. This solution is stable for several months.

- Dihydrochloride solution: 0.10g N-(1-naphthylethylenediaminedihydro-chloride) was dissolved in 100 mL of deionised water. The solution was stored in a dark bottle.

c. Procedure: Triplicate standard samples were prepared from the standard stock solution by diluting appropriate amounts of stock standards in a volumetric flask. Saline water (made from NaCl, AR, or evaporated sea salt) and synthetic seawater was added to each flask up to the mark. Five mL of buffer solution and 50 mL of standards were added to containers. The standard samples were then passed through the Cd column (50 mL) to reduce nitrate to nitrite. Twenty mL of the reduced sample was collected after discarding the first 20-22 mL. Immediately after reduction, 1 mL of sulfanilamide solution was added to the samples and mixed. After 2 minutes, 1 mL of naphthylethylenediamine solution was added and mixed. Between 10 and 120 minutes afterwards, the absorption of the solution at 543 nm was measured in a 1 cm cuvette against saline water or seawater using a Varian UV/Visible spectrophotometer. Blanks samples having the same salinities but to which no nitrate had been added were used throughout the procedure.

4.2.3. Effect of salinity on the flow rate of samples through the nitrate reduction column

In order to determine the flow rate in two different columns, 50mL and 25mL burettes were prepared according to Wood *et al.* (1967) and Wetzel and Likens (1991). The flow rate between 100 µg/L standard samples made from distilled (DW) and saline water of different salinities (41-271 g/L) were compared by using these two columns. The same procedure, as described in 4.2.2.e, was applied except

that 2.5 mL of buffer solution and 25 mL of standard sample (instead of 5 mLof buffer solution and 50 mL of standard sample) were added to containers and then passed through 25 mL column. Ten mL of reduced sample was collected after discarding the first 10-12 mL.

In all subsequent work, a column of 50 mL in volume was used, but smaller columns would have been equally satisfactory. The length of amalgamated cadmium fillings was the same in both columns.

4.2.4. Effect of salinity on the estimation of PO_4-P and NO_3-N in saline water (NaCl) (Preliminary study)

As a preliminary study, three standard samples were prepared from a stock standard solution of PO_4-P in (1) deionised water (2) in 35 g/L, and (3) in 105 g/L saline water. Salinewater was made from AR salt (NaCl) in appropriate amounts of salt in deionised water. It was then filtered under vacuum to remove possible insoluble matter (0.2, 0.02 and 0.004 mg/L). The analytical procedure described for PO_4-P (4.2.1.c) was applied to demonstrate the salt effect on phosphate measurements.

Likewise, for nitrate measurement, nitrate standards were also prepared from deionised water, saline water 35 g/L and 105 g/L salt and nitrate stock solution in appropriate concentration (1, 10 and 100 μg/L).. The same procedure described for analysis of nitrate (4.2.2.c) was applied to demonstrate the salt effect on nitrate measurements

4.2.5. Nitrate estimation in saline water

All three methods suggested as measures to overcome salt interferences were studied.

a. Dilution method: Natural saline samples of different salinities from different ponds at Dry Creek Saltfields were diluted with deionised water. The initial salinities of these samples were 41, 57, 96, 128, 143, 218, and 271 g/L. All were diluted to salinity of seawater, is approximately 35 g/L.

b. Synthetic seawater method:

Preparation of synthetic seawater: Synthetic seawater was made from 310g NaCl (AR), 100g $MgSO_4 \cdot 7H_2O$ (AR) and 0.5g of $NaHCO_3 \cdot H_2O$ (AR) or 410 g of evaporated sea salt (Parsons et al., 1984). Different weights in the same portion of salts were added to 10 L of deionised water to produce waters to salinity of seawater and above. They were used to produce standard nitrate solutions. The synthetic sea water was filtered under vacuum to remove insoluble matter. The same procedure as described before was again applied (4.2.2.c). For this study, appropriate volumes of synthetic seawater and saline water were made.

c. Standard addition method: In this technique, a measured increments of nitrate added to a known portion of sample to obtain the standards. The sample absorbance at zero concentration of added nitrate is read and if it is assumed the system follows Beer's law, the unknown concentration can be readily calculated from absorbance measurement of the sample solution.

Standards were prepared from a standard stock solution and natural saline waters, the latter ranging from 23-271 g/L in salinity (10, 50, 100 and 200 μg/L NO_3-N). The saline waters were collected from different ponds at Dry Creek solar saltfields and had salinities ranging from 41-271 g/L. The sample with a salinity of 23 g/L was prepared by diluting the sample of salinity 41 g/L.

4.2.6. Statistical analysis

Simple linear regression was used to determine the strength of the relationship between the salt error and estimated nitrate in the samples and calculate the actual measurement for nitrate in the samples. In addition, the line of best fit was drawn through each association and the differences were also confirmed by analysis of covariance (ANCOVA).

4.3. Results

4.3.1. Effect of salinity on flow rate of samples through nitrate reduction column

Results from flow rate comparison between distilled water and saline water of different salinity (41-271 g/L) are shown in Table 4.1. The mean of three readings is presented for each sample. The flow rate decreased from 0.34 mL/sec in deionized to 0.18 mL/sec in highly saline using 50 mL column. The decrease was from 0.34 to 0.19 mL/sec using 25 mL column. As salinity increased, the time of flow taken for the sample to flow through the column was increased by about 80%, between deionised water and highly saline water. They also show that the time taken to pass through the reduction column is related to sample volume.

4.3.2. Estimation of PO_4-P and NO_3-N in saline water (NaCl) (preliminary studies)

The results of PO_4-P measurement at different standard samples of salinity 0, 35 and 105 g/L showed that there was no significant differences between phosphate measurement using the ascorbic acid with antimony (III) in phosphomolybdenum blue spectrophotometric method. The difference was confirmed by fitting simple

Table 4.1. Flow rate comparison in reduction of nitrate to nitrite in standard samples of 100 µg/L nitrate with two different columns (50 mL and 25 mL) and different salinities.

Samples	Salinity (g/L)	Time[1] (seconds)	Flow rate[1] (mL/sec)	Time[2] (seconds)	Flow rate[2] (mL/sec)
DW	0	59.11	0.34	29.55	0.34
1	41	65.42	0.30	32.68	0.30
2	57	67.04	0.29	33.59	0.30
3	96	72.00	0.28	35.49	0.28
4	128	75.58	0.26	36.69	0.27
5	143	77.18	0.26	37.19	0.27
6	179	85.15	0.23	41.51	0.24
7	195	95.78	0.21	46.19	0.22
8	218	98.94	0.21	47.63	0.21
9	271	106.22	0.18	49.92	0.19

1= 50 mL column, 2= 25 mL column

regression lines represent to the optical density data, and applying an analysis of covariance (ANCOVA: F (slopes)= 2.099, P> 0.05; F (intercepts)= 16.121, P> 0.05). The result is shown in Table 4.2. The details data are given in Appendices 3.1 and 3.2.

Table 4.2. Phosphate regression equation and r^2 (O.D., optical density; P, phosphate concentration).

salinity g/L	n	equation	r^2
0	3	OD = 0.0009 (P) + 0.0696	0.9753
35	3	OD = 0.0007 (P) + 0.0263	0.9974
105	3	OD = 0.0011 (P) + 0.0192	0.9953

The results from NO_3-N measurements at different standard samples of salinity (0, 35 and 105 g/L) showed that there was a differences between nitrate measurement when the Cd column technique followed by colorimetric method in deionised and saline water with different salinity was used. The difference was confirmed by fitting simple regression lines to represent the optical density data, and applying an analysis of covariance (ANCOVA: F (slopes)= 20.241, $P< 0.05$; F (intercepts)= 108.580, $P< 0.05$). This result is shown in Table 4.3.

Table 4.3 Nitrate regression equation and r^2 (O.D., optical density; N, nitrate concentration).

salinity g/L	n	equation	r^2
0	3	OD = 2.2911 (N) + 0.0124	0.9984
35	3	OD = 2.3477 (N) + 0.0039	0.9998
105	3	OD = 2.2868 (N) + 0.0047	0.9997

4.3.3. Nitrate estimation in saline water

a. Dilution method

The results of nitrate measurement in samples of original salinity 41, 57 and 96 g/L when diluted to 35 g/L were 7, 9 and 10 μg/L after the dilution method was applied. However, when samples with a salinity more than 120 g/L were diluted nitrate concentrations were so low that they could not be detected. Therefore, the salinity in some samples was too high to apply dilution method at the levels of nitrate concentration in the system..

b. Synthetic seawater

Results using synthetic seawater made from AR salts and evaporated sea salt indicated that salts were contaminated with nitrate. The results from the use of AR salt are shown in Table 4.4 and Appendix 3.3. Data for the blank (0 NO_2-N) show that the salt was contaminated with nitrate and as the salinity increased, so did the absorption, reflects increasing contamination. Similar results were obtained when other standards using synthetic saline water were used.

According to Wood *et al.* (1967), absorbance should be determined after the colour has developed for 10 minutes. But in this investigation, the colour of samples in highly saline water (five times or more as saline as seawater) changed after a shorter time due to the formation of dark violet particles. The results are presented in Table 4.5. Absorbance (optical density) after 2 minutes was 0.84, and as time passed this value decreased. After 2 hours, the absorbance was only 0.290. This was observed in other samples of different nitrate concentration but only in synthetic saline water and not in natural saline water. Only the results for 10 µg/L are presented in Table 4.5.

Different AR salt (Ajax chemicals, Chem-supply) and evaporated seawater were also used to make synthetic saline water. The same results were obtained.

c. Standard addition

The relationship between nitrate concentration and optical density (absorption) when known amounts of nitrate are added to deionised and natural waters at different salinities is shown in Figure 4.1 and Appendix 3.4. The mean value of

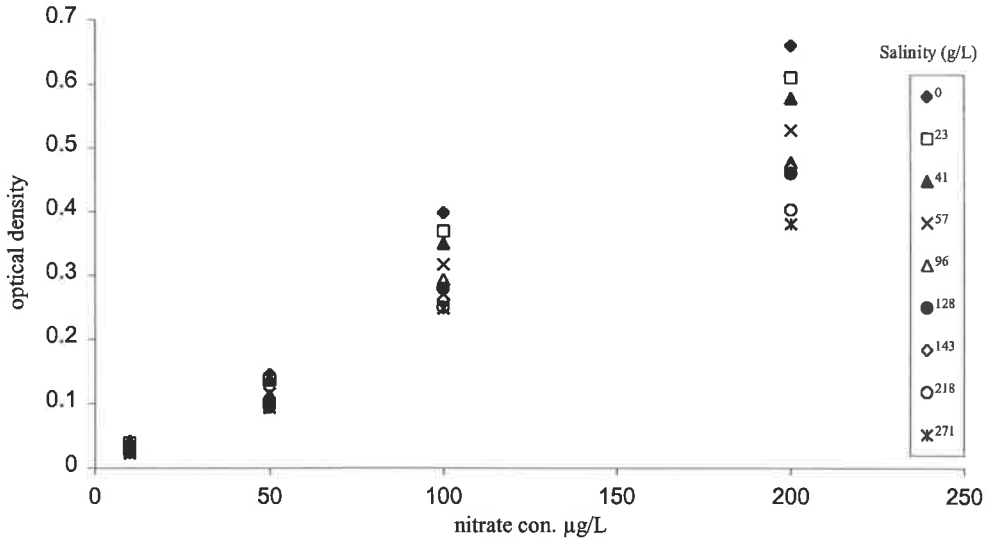

Figure 4.1. The relationship between nitrate concentration and optical density when known amounts of nitrate are added to deionised and saline waters.

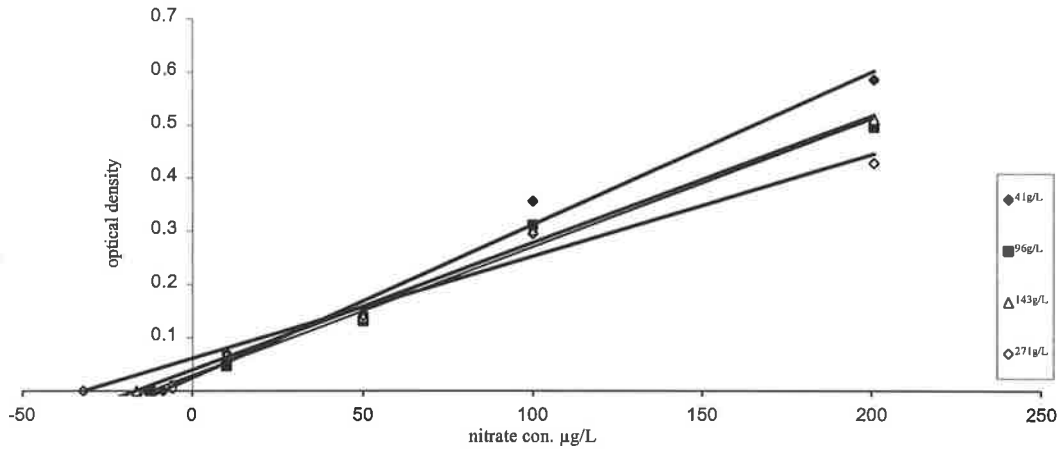

Figure 4.2. Nitrate analysis of saline waters using method of standard additions (each point is the mean of three optical density determinations).

three readings is represented by points on the graph and has been corrected for the blank.

Table 4.4. Effect of synthetic saline water of different salinity and different amount of added nitrate on the optical density for nitrate measurement.

NO_3-N conc. µg/L / Salinity of* samples g/L	0	1	5	10	25	50	75	100
0	0.007	0.016	0.026	0.034	0.045	0.161	0.240	0.299
17.5	0.085	0.098	0.099	0.111	0.123	0.237	0.308	0.414
35	0.269	0.270	0.286	0.311	0.315	0.409	0.478	0.546
70	0.484	0.500	0.545	0.527	0.544	0.602	0.683	0.751
105	0.589	0.613	0.616	0.623	0.649	0.719	0.736	0.840
140	0.708	0.718	0.724	0.732	0.746	0.819	0.873	0.920

(*These salinities chosen as multiplying of seawater salinities).

Table 4.5- Effects of time on optical density reading of 10µg/L NO3-N in synthetic saline water (210 g/L).

Time (min.)	2	5	15	30	120
Optical density	0.840	0.468	0.390	0.305	0.290

The results of standard additions are shown in Figure 4.2 where the intercept represents the concentration of NO_3-N in the sample solution. For simplicity, the representative optical density of four different nitrate concentration in samples with different salinities (41, 96, 143, 271 g/L) has been selected to allow calculation of nitrate concentration graphically or by the method of least squares.

The calculation of salt error in nitrate measurement for spiked samples with nitrate concentration (100µg/L) is presented in Table 4.6 and Appendix 3.5. These samples have different salinities and show the effect of salinity variation on nitrate measurement. Optical densities for the distilled water sample formed the basis for comparison with saline waters. A blank of distilled water gave an optical density of 0.0019. Optical densities of natural saline waters obtained from Dry creek ranged from 0.007 to 0.047. These levels (X) are related to the NO_3-N concentration in the original samples. X is the mean optical density for each sample (n = 3). Distilled water and saline water samples spiked with 100 µg/L NO_3-N showed variation in optical densities due to salt effects (Y). In order to calculate the salt error due to salinity increases, the blank optical density (X) was subtracted from spiked samples (Y). These numbers (Y') were then subtracted from Y' of distilled water (Y'DW) to calculate the % salt error. A similar procedure was used to calculate salt error for 10, 50, and 200 µg/L nitrate concentrations and the results are shown in Table 4.5 and Figure 4.3. In this figure, the percentage of salt error has been plotted against salinity. Salt error for a known salinity can then be determined graphically or from the equations given below:

% salt error = $-0.0007\text{salinity}^2 + 0.3579\text{salinity} - 2.5355$ (10 µg/L NO_3-N)

(r^2 =0.9754)

% salt error = $-0.0007\text{salinity}^2 + 0.336\text{salinity} - 0.9444$ (50 µg/L NO_3-N)

(r^2 =0.9444)

% salt error = $-0.0008\text{salinity}^2 + 0.3387\text{salinity} + 0.4366$ (100 µg/L NO_3-N)

(r^2 =0.9884)

% salt error = $-0.0006\text{salinity}^2 + 0.2946\text{salinity} + 1.6145$ (200 µg/L NO_3-N)

(r^2 =0.977)

Table 4.6. Salt error as indicated by addition of known amounts of NO_3-N (100 µg/L).

sample	salinity (g/L)	mean optical density (X)	mean optical density spiked with 100 µg/l NO_3-N (Y)	optical density for 100µg/l NO_3-N Y–X= Y'	salt error SE= (Y'DW) - (Y'S)	salt error % $\frac{SEx100}{Y'DW}$
DW[1]	0	0.019	0.416	0.397	0	0
1	23	0.016	0.387	0.371	0.026	6.5
2	41	0.007	0.357	0.350	0.047	11.8
3	57	0.015	0.332	0.317	0.08	20.1
4	96	0.018	0.311	0.293	0.104	26.1
5	128	0.023	0.301	0.278	0.119	29.9
6	143	0.041	0.302	0.261	0.136	34.2
7	218	0.018	0.268	0.250	0.147	37.0
8	271	0.047	0.288	0.241	0.156	39.2

1= Distilled water (DW)

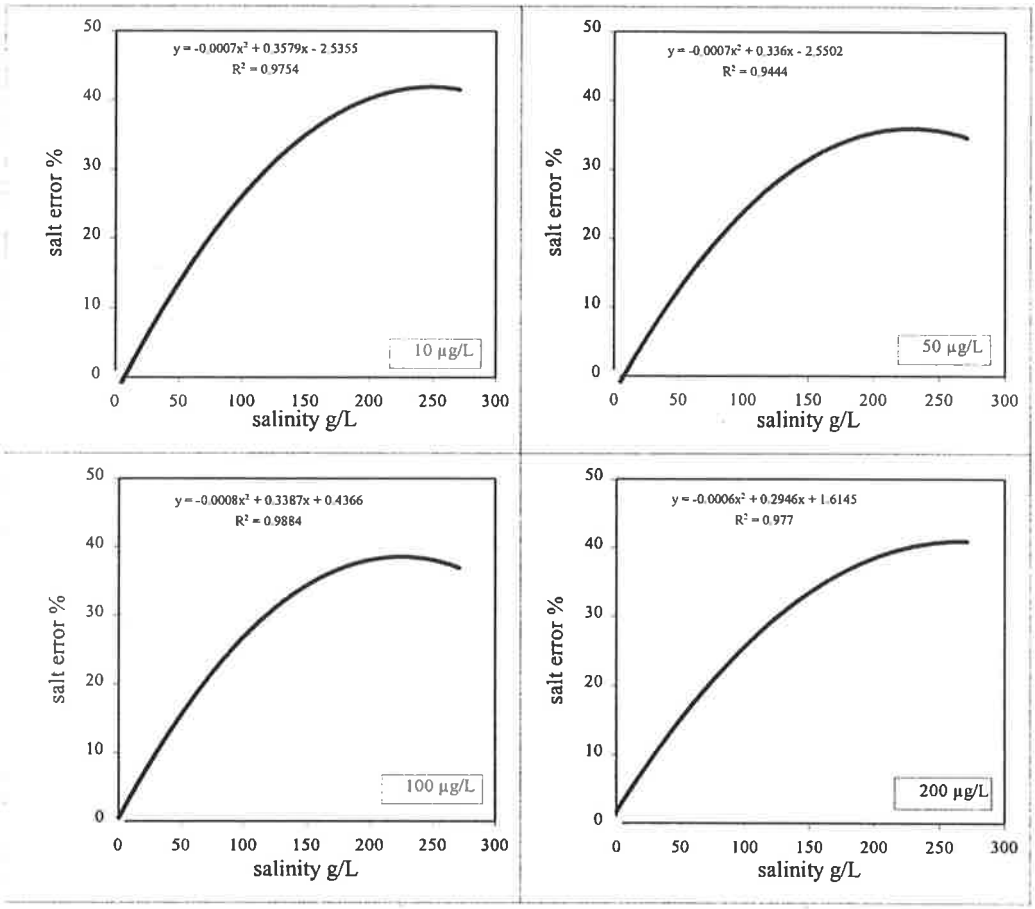

Figure 4.3. Salt error (%) at different salinities when known amounts of nitrate are added.

These data on salt error could be applied to nitrate estimation from routine methods of analysis without applying standard addition and used to determine more accurately nitrate concentration in saline waters.

4.5. Discussion

Comparison of sample absorbances of standards made from distilled and saline waters showed that ascorbic acid with antimony (III) in phosphomolybdenum blue spectrophotometric method would appear to offer the best option to estimate the phosphate concentration in saline water, as Sherwood *et al.* (1995) also have pointed out.

Comparison of sample absorbances of standards made from distilled and saline waters showed appreciable errors for nitrate measurement using the Cd column technique followed by colorimetric procedure.

The data of flow rate comparison of deionised and high saline samples in Cd column (25 and 50 mL) indicated that using 25 mL Cd column followed by washing the column by 10 % buffer helped to make the length of analysis considerably shorter. The 50 mL column, suggested by most researchers, took a longer time to reduce nitrate to nitrite in all samples, more than twice as long as the 25 mL column. With simple calculation, it could be shown that instead of spending 6.5 hours to reduce 120 samples by 50 mL column, it was possible to spend 3 hours to reduce the same number of samples. This is important when analysing many samples. This study also showed that if the column was washed with 10% buffer after each set of highly saline samples (in this study one set contained 16 samples), the total flow rate for each set was increased approximately 25%.

In this study, the amount of nitrate was low in highly saline waters (salinity more than 120 g/L) and the salinity itself too high for nitrate to be detected when applying the dilution method. There is a high variation in nitrate concentrations in highly saline waters (see Table 2.6).

This study also indicated that synthetic seawater should not be used in making standards for nitrate measurement in highly saline water because of nitrate contamination and the presence of impurities in grade AR salts. These impurities produced turbidity due to the formation of dark violet particles. Discussion of these particles and how they form are beyond the scope of this study.

The concentration of nitrate in saline water is considerably underestimated by the cadmium column reduction method using standards in deionized water, the method used in most studies. Moreover the salt error in nitrate determinations increases as salinity increases. Based on this study, two methods for estimating NO_3-N in saline waters are proposed. The first, involves the use of spiked NO_3-N in natural saline water, applying the standard addition method and generating a standard curve that allows estimation of nitrate in the samples. Alternatively the appropriate equation for salt error correction at different salinities can be used to estimate true nitrate concentrations in saline samples. Figure 4.3 can be used to determine graphically the amount of salt error in saline waters, such as those from Dry Creek solar saltfields, at different salinities and nitrate concentration. This calculated salt error correction should be applied to the routinely estimated nitrate concentration in samples to provide a more accurate figure for nitrate concentration in highly saline waters.

Salt error is relatively low at the salinity of seawater, and so has not previously been noted in nitrate estimation in sea water. However, for saline waters at salinities

above seawater, errors increase from 7 to 40% and these salt errors must be taken into account when analysing NO_3-N by using the Cd column technique.

4.6. Conclusion

Based on this study, the following conclusions were drawn.

- The ascorbic acid with antimony (III) in phosphomolybdenum blue spectrophotometric method is the most accurate method for phosphate determinations in saline water.
- In nitrate estimations, the Cd column technique, a 25 mL column, and followed by washing with 10% buffer solution, can be used for saline water to make the time of analysis shorter.
- Due to salt errors in nitrate estimation when using the Cd column technique, appropriate methods such as dilution, standard addition and salt error corection, must be apply.
- Synthetic seawater should not be used in making standards for NO3-N measurement in highly saline water, because of the possibility of nitrate contamination and impurities in grade AR salts and evaporated salt.

It is concluded that much previously published data for NO_3-N in saline water are probably are in error if appropriate corrections were not applied.

CHAPTER FIVE

THE IMPACT OF *SYNECHOCOCCUS* ON VISCOSITY AND SALT QUALITY AND QUANTITY

5.1. Introduction

5.1.1. Viscosity and its measurement

Viscosity of water is the property that resists orderly laminar flow of water through a capillary. The dynamic viscosity (η) of water is defined as the ratio between the force per area (shear stress, T) and the rate of shear for steady flow of the water. The SI unit of dynamic viscosity is the pascal second, and the millipascal second is equal to the unit centipoise (cP). The dynamic viscosity of air, fresh water and seawater is given in Table 5.1. (Denny, 1990). From 0-30°C, the viscosity of air increases 9%, while the viscosity of water decreases by 45%.

Table 5.1. The dynamic viscosity of air, fresh water and seawater (salinity 35 g/L) as a function of temperature. Viscosity data as kg/m/sec10^{-3} (from Vogel, 1994 and Denny, 1990).

Temperature °C	Air	Fresh water	Seawater
0	1.709	1.790	1.890
5		1.520	1.610
10		1.310	1.390
15		1.140	1.220
20	1.808	1.010	1.090
25		0.890	0.960
30		0.800	0.870
40	1.904		

The kinematic viscosity (υ) of water is the dynamic viscosity divided by the density of the water, both measured at the same temperature. The SI unit of kinematic viscosity is centimetre squared per second (cm^2 / s) and this is equal to the one stokes (St). One centistokes (cSt) is equal to 10^{-2} stokes. The kinematic viscosity is measured by glass capillary viscometer. Capillary viscometers are widely used for standard measurements and for industrial investigations of the viscosity of liquids at atmospheric pressure (Kawata *et al.*, 1991). In present study, reference to 'viscosity' means kinematic viscosity.

Viscosity increases with increasing salinity since dissolved salts themselves affect viscosity (Sonnenfeld, 1993). In this study, the term relative viscosity represents the ratio between (1) viscosity due to salinity alone and (2) viscosity of natural saline water where both salt and other products affect viscosity. Thus, relative viscosity is defined as the resistance of saline water to flow through a viscometer (kinematic viscosity) corrected for resistance from uncontaminated saline water of the same salinity; it is the difference between the viscosity of pure saline water and natural saline water of the same salinity.

The viscosity of pure water at 20°C as a standard for liquid viscosity is important because viscosity is a useful key for the measurement of flow rate, in calculating heat transfer coefficients, and during the control of chemical processes. Most countries have agreed to adopt 1.002 mPa (centipoise) for dynamic viscosity and 1.0038 mm^2 /s (centistokes) for kinematic viscosity of freshly distilled water at 20.00°C (BSI, 1977). These values were first measured by Swindells *et al.* (1952) at the U.S. National Bureau of Standards. These authors recommended that other laboratories adopt this value as the primary reference standard for comparative measurements of viscosity. Waekeam *et al.* (1991) also emphasised that this value remains valid for the calibration of viscometer standards.

Fluids differ in their ability to flow under a set of given environmental conditions (Weisberg and Parish, 1974). For water, as indicated, two important environmental factors in determining viscosity are temperature and salinity; viscosity decreases rapidly with increasing temperature and increases slowly with increasing salinity (Sverdrup *et al.*, 1942; Horne, 1969; Table 5.2).

Table 5.2. Relative viscosity of various concentrations of seawater at one atmosphere (η/η_o, where η_o is the viscosity of pure water at 0°C) (from Dorsey, 1940).

Temperature	Salinity (g/L)				
(°C)	5	10	20	30	40
0	1.009	1.017	1.032	0.056	1.054
5	0.855	0.863	0.877	0.891	0.905
10	0.738	0.745	0.785	0.772	0.785
15	0.643	0.649	0.662	.0.675	0.688
20	0.568	0.574	0.586	0.599	0.611
25	0.504	0.510	0.521	0.533	0.545
30	0.454	0.460	0.470	0.481	0.491

5.1.2. The Cyanobacterium *Synechococcus* and its extracellular products

Cyanobacteria are capable of growing in a wide range of environments. They include N_2-fixing bacteria that can live under both aerobic and anaerobic conditions. In saline water, the predominant forms are filamentous and single celled forms. One unicellular Cyanobacterium, *Synechococcus*, is found in saline waters with a salinity of more than 100 g/L and has been identified from the study area at the Dry creek solar salt ponds. This organism is a N_2-fixing and non-heterocystous Cyanobacterium that can fix free N_2 under anoxic conditions or in low oxygen concentrations (Stal, 1991). In culture, *Synechococcus* has a very variable size and shape, being 2-10 μm long and ellipsoidal, ovoid or cylindrical. It produces mucus as an extracellular material, and its cells are found embedded in this mucilaginous material (Borowitzka, 1981). *Synechococcus* usually takes the form of a slimy or gelatinous mat in the shallow and littoral zone of salt lakes (Bauld, 1981), but may

appear as single cells suspended in the water column. It was abundant at a salinity of 150-300 g/L in the study area, where cells are ovoid to cylindrical, 4-10 x 5 μm in size, and embedded in irregular lumps of mucus up to 75 cm across attached to rocks and sediment.

There has been some confusion over the taxonomy of *Synechococcus*. In many studies, this widespread hypersaline Cyanobacterium has been reported as *Aphanothece halophytica* (Borowitzka, 1981; Jones *et al.* 1981; Javor, 1989; Sammy, 1993). The taxon includes coccoid forms characterised by transversely-dividing cells, found singly or in pairs, or in sheathed colonies (Hof and Fremy, 1933, who first identified this organism; Eardley, 1938). Drouet and Daily (1956) later identified *Aphanothece halophytica* as a new taxon, *Coccochloris elabens*. More recently, Stainer *et al.* (1971) included *Aphanothece* in a typological group IA of the Chroococcales, while Rippka *et al.* (1979) and Rippka (1988) placed it among strains of *Synechococcus*. Padmaja (1972) and Krishnan (1991) differentiated between the genera *Aphanothece* and *Synechococcus* on the basis of mucilaginous colonies; they occur in *Aphanothece* but not in *Synechococcus*. However, Komarek (1983) stated that this feature is highly unstable and depends upon environmental conditions. Recently, Krüger *et al.* (1995) demonstrated that fatty acid composition is an effective taxonomic tool in clarifying taxonomical problems of coccoid Cyanobacteria.

In the light of these events, all coccoid forms characterised by traversely-dividing cells, found singly, paired, or in sheathed colonies and previous placed in the genera *Aphanothece* and *Coccochloris*, are now referred to as *Synechococcus* (Burnard and Tyler, 1993). It is important to recognise this synonymy. For simplicity, the name *Synechococcus* will be used here.

The occurrence of a great variety of extracellular product (ECP) by algae and bacteria is now well established (O' Colla, 1962; Fogg, 1962, 1966 and 1971; Hellebust, 1974; Arad *et al.*, 1985; De Philippis *et al.*, 1993). These ECPs are produced may subsequently be released in part into the medium. The ECP produced by algae and bacteria plays important roles in algal growth and physiology as well as more generally in food chains and ecosystems (Hellebust, 1974). The rate of production depends on internal physiological factors such as membrane permeability, intracellular concentration of ECP, and the ability to excrete this material to form cell - wall substances, gelatinous material, or directly into the medium. Environmental factors also effect the rate of ECP production; they include O_2 and CO_2 concentration, pH, light, salinity, nutrient and mineral deficiency (Hellebust, 1974).

Simple and complex polysaccharides are liberated as ECPs by a large number of taxonomically diverse algae and bacteria. They are produced during active or stationary growth phases. Jones and Yopp (1979) reported that the ECP produced by *Synechococcus* is a polysaccharide, containing glucose, fructose, mannose and galactose in a ratio of 1.00:0.60:0.32:0.23. These compounds are similar to those produced by other Cyanobacteria (Dunn and Wolk, 1970; Sangar and Dugan, 1972). Polysaccharides produced by microorganisms show predominantly hydrophilic characteristics, high viscosity at low concentration, a mucus form, and display an antifreeze behaviour (Lohmann, 1990). Three distinct types of polysaccharides are produced by Cyanobacteria: extracellular, structural, and intracellular storage forms. The extracellular polysaccharides can be further classified into two forms: (a) capsules that are an integral part of the cell wall, and (b) loose slime components that accumulate in large quantity outside the cell wall. Some of this extracellular polysaccharide is secreted and liberated to the medium (Guillard and Hellebust, 1971) and can increase its viscosity.

Golubic (1980) noted that *Synechococcus* [*Coccochloris*] is the most common genus that forms mucilaginous coatings in the benthos of many salt ponds. Seshadri and Buch (1958) observed that *Synechococcus* [*Aphanothece*] and other Cyanobacteria form a jelly which adheres to salt crystals, colouring the salt and imparting a foul smell to it, and may decrease the quality and quantity of harvested salt.

5.1.3. Salt quality

The industrial quality of salt is determined by the size, shape, specific gravity, percentage moisture, and Ca, Mg and SO_4 contamination in salt crystals (Coleman and White, 1993; Burnard and Tyler, 1993). Salt crystals (NaCl) grown slowly from pure solution are generally well - developed and cubic in shape. Mullin (1985) has demonstrated that impurities may be formed in a variety of ways: by fast and interrupted growth, by sudden changes in local conditions, by adsorption of impurities, or by temporal or concentration gradients in the crystalliser. According to Chernov' s interpretation (1989), temperature or fluctuations in concentration may cause the decomposition of smooth layers and their transformation into rough surfaces which may grow too quickly and thus lead to the formation of macro defects. Salt crystals grown from NaCl solution with some impurities are dendritic in shape and hold 5-30% of inclusions (Halasz and Tasuku, 1993). Halasz and Tasuku (1993) also reported that some crystals grown in impure NaCl solutions are micro-porous with a perfectly formed nucleus in their centre but covered by pitted layers of irregular growth. Burnard and Tyler (1993) also demonstrated that some impurities, such as long chain polysaccharides, can change salt crystal morphology from a solid cubic form to a hollow, skeletal 'hopper shaped' one. The overall effect of hopper crystal growth and adjacent growing crystals is to produce salt crystals of lower density (the density of pure sodium chloride salt is 2.16, Weast and Melvin, 1982). Hollow crystals may also trap long chain polysaccharides, gypsum and final

brine, thus causing an increase in the moisture of salt crystals and an increase in such impurities as Ca, Mg and SO_4 ions and organic material.

5.1.4. Experimental aims

An important feature associated with the excretion of large amounts of extracellular polysaccharides by *Synechococcus* is an additional increase in brine viscosity and a decrease in the quality and quantity of salt harvested. However, despite their obvious economic significance, rigorous evaluation of these effects is lacking. An experiment was therefore undertaken in late 1994 and early 1995 to relate (1) the presence of *Synechococcus* to an increase in water viscosity following the production of extracellular products, and (2) the quality and quantity of salt deposited with the viscosity of the water from which the salt had been precipitated.

5.2. Methods and materials

The experiment was conducted at Dry creek Solar Salt fields during the period 7 December 1994 to 3 March 1995.

5.2.1.Experimental design

Five circular outdoor fibreglass tanks, each 150 cm in diameter and 50 cm in depth (Fig. 5.1a), were located near pond PA9 (Fig. 2.1). The tanks were filled to a depth of 35 cm with water from pond PA9, of salinity 210g/L (s.g. 1.1495 at 20°C). The water was filtered through nylon mesh (mesh size ~ 50μm) to remove large organisms and debris. Long mucilaginous strands of *Synechococcus* material were then collected from pond PA9 with a triangular net to drain excess water and set amounts were added to each tank (except tank #1). This was done by transferring a

Figure 5.1. Experimental tanks near pond PA9 (a) stage I (evaporating tanks); (b) stage II (crystallising tanks covered by shading cloth).

standing crop of *Synechococcus* from an area equivalent to the tank area (1.76m^2) into tank #3 and from areas equivalent to half the tank area, twice and four times the tank area into tanks #2, #4 and #5, respectively. The tanks are considered as 0, +1/2, +1, +2, +4 *Synechococcus* treatments (Table 5.3).

Table 5.3. Relative amounts of *Synechococcus* material, phosphate and nitrate added to experimental tanks (see text for more detail).

Tanks	*Synechoccocus* added	Phosphate added	Nitrate added
# 1	0	-	-
# 2	+1/2	-	-
# 3	+1	-	-
# 4	+2	+	+
# 5	+4	+++	+++

To eliminate problems of nutrient depletion that might have caused a drop in the *Synechococcus* biomass, phosphorus (as K_2HPO_4) and nitrogen (as $NaNO_3$) were added weekly to Tanks #4 and #5 in set amounts (Table 5.3). The amount chosen (0.018 mg/L phosphate and 0.012 mg/L nitrate) approximated natural nutrient concentrations in pond PA9 (see Chapter 2). To compensate for water lost by evaporation, the water-level was kept relatively constant by twice weekly additions of water from pond PA9. This simulated conditions in the solar salt fields where brine is added to ponds as they evaporate - so providing an increasing salinity in the system. Before each addition of water, the decrease in water depth of each tank was measured to estimate evaporation. The evaporated water from each tank was summed to find the percent of total evaporated water. The addition of water continued until the salinity reached the crystallisation point of sodium chloride (Fig. 5.2). When the brine reached a point close to the crystallisation point of sodium chloride, it was transferred by electric pump from the evaporating tanks to clean and fresh tanks (crystallising tanks), simulating final salt production in crystalliser pond of the salt field. At this stage, *Synechococcus* was excluded by filtering the brine through a 150 μm mesh filter net. This occurred on January 12, 1995. Thus, the

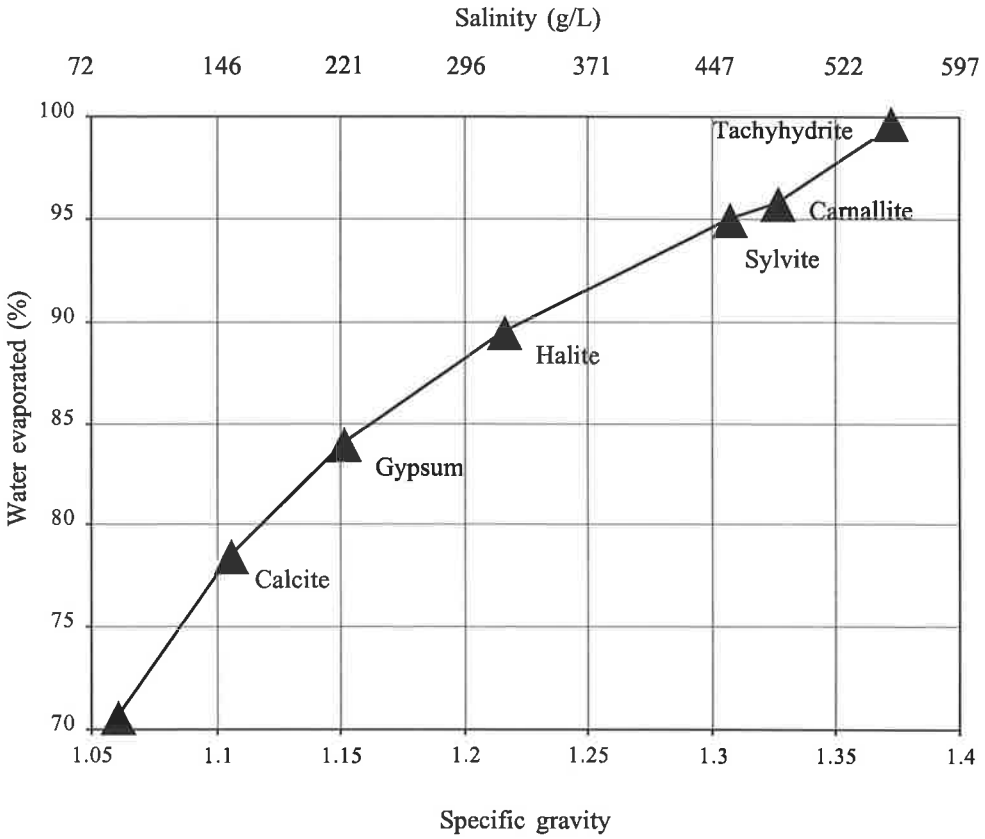

Figure 5.2. Primary precipitation curve (modified from Sonnenfeld, 1993).
Calcite ($CaCO_3$), Gypsum ($CaSO_4.2H_2O$), Halite (NaCl), Sylvite (KCl), Carnallite ($KMgCl3.\ 6H_2O$), Tachyhydrite ($CaMg_2Cl6.12H_2O$).

experiment was divided into two stages: stage I (when *Synechococcus* was present) and stage II (when *Synechococcus* was excluded). In stage II, nutrients and water were not added and tanks were allowed to evaporate with shade cloth covering each tank to decrease the evaporation rate so as to simulate natural conditions more closely (Fig. 5.1b). The volume of brine transferred from the evaporating tanks to the crystallising tanks was the same for all tanks and gave an initial water depth of 30 cm in each tank. On March 1995, salt deposited in each tank was harvested. At this stage, in the natural ponds the final brine is usually very high in magnesium and other elements (Fig. 5.2). This brine was pumped out and discarded before salt was harvested. The harvesting procedure again simulated the operation of the solar salt fields. Harvested salt from each tank was then mixed on a large nylon sheet and some of this mixed sample was homogenised by a cement mixer for later laboratory analysis.

5.2.2. Data collection in the field

Brine was sampled weekly from each tank. Specific gravity, pH and temperature were measured *in situ* using a hydrometer. Specific gravity was corrected for temperature (Table 5.4), and pH was determined by a Metrohm Herison E 488 meter. A mercury-in-glass thermometer was used to measure water temperature. Samples for the determination of conductivity, salinity and viscosity were collected in a 50 ml glass bottle and returned to the laboratory for analysis.

5.2.3. Laboratory measurement

Samples (50 mL) were collected weekly from each tank for determination of conductivity, salinity and viscosity.

Conductivity was measured in the laboratory using a conductimeter (Hanna instruments) on the day of sample collection. Conductivity measurements were converted to salinity using the regression equations of Williams (1986a).

Table 5.4. Correction factors for specific gravity of saline water according to temperature (Weast and Melvin, 1982).

Temperature ° C	Correction
9	-0.0055
10	-0.0050
11	-0.0045
12	-0.0040
13	-0.0035
14	-0.0030
15	-0.0025
16	-0.0020
17	-0.0015
18	-0.0010
19	-0.0005
20	0.0000
21	+0.0005
22	+0.0010
23	+0.0015
24	+0.0020
25	+0.0025
26	+0.0030
27	+0.0035
28	+0.0040
29	+0.0045
30	+0.0050

Samples with a salinity of more than approximately 70 g/L, when the relationship between conductivity and salinity diverges from linearity, were appropriately diluted.

Viscosity was measured with a capillary viscometer (PSL, Model C-3889, Type BS/U) in the laboratory (Fig. 5.3). As noted, capillary viscometers are widely used for standard measurements and for industrial investigations of the viscosity of liquids at atmospheric pressure (Kawata *et al.*, 1991). The viscosity of samples was

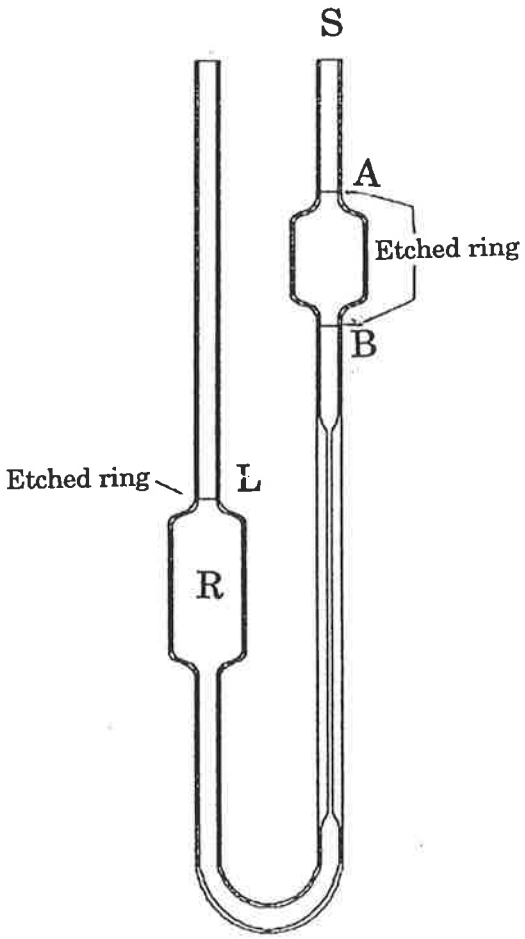

Figure 5.3. Diagram of viscometer (for further explanation see text).

determined by measuring the time that a fixed volume of brine took to flow between two known points under controlled conditions. A five - litre water bath was used to keep temperature constant (at 20°C). For each determination, the samples were filtered through a Whatman filter paper #1. The brine sample was poured through the inlet (I) until the reservoir (R) was full to level (L) (Fig. 5.3). A syringe and filter holder were used to fill the filter the sample in the capillary tube. After filling, the viscometer was immersed in the water bath and maintained vertically by a stand. Before measurement, brine was sucked into the capillary tube using the pipetting bulb through vent S to above level A (Fig. 5.3), then forced back to below level B. This process was repeated several times to adjust the brine temperature and fully lubricate the capillary tube. During actual measurements, as the brine level passed point A, a stop watch was used to time the interval until the brine reached point B. This time in seconds, multiplied by a calibrated constant number (specific to each viscometer), represents the viscosity in Centistokes (BSI, 1977). Five consecutive readings were taken for each sample. Generally, readings differed by 0.1 percent.

To determine the relative viscosity of natural saline water, it was necessary to determine the viscosity of synthetic saline water. Solutions of synthetic saline water (prepared from sodium chloride, A.R.), synthetic seawater (Parsons *et al.*, 1984), and evaporated seawater were prepared to provide solutions of different salinity and comparisons were made between the viscosity of these three different types of saline water. Seawater was filtered through 0.45 μm Millipore filters to remove cells and debris, autoclaved, and evaporated at 70°C to provide brines of different salinity. The viscosity of these solutions was measured and comparative curves made (Fig. 5.4). Data for the viscosity of evaporated seawater was used to prepare a calibration curve because of the chemical similarity of this evaporated seawater to the natural saline water at Dry Creek. Using calibrated values, the relative viscosity of the samples taken from the experimental tanks were obtained (see Appendix 4.1).

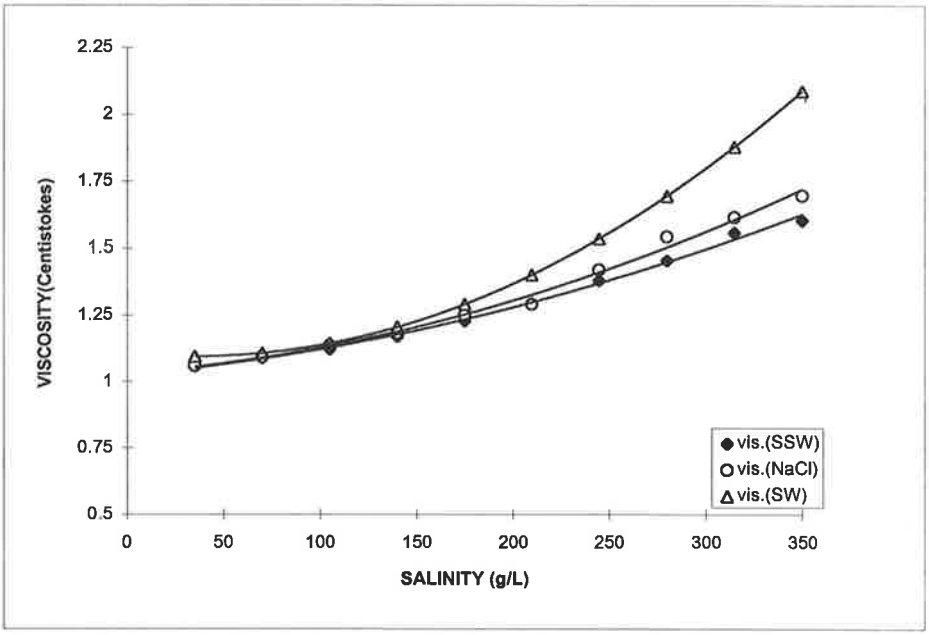

Figure 5.4. Relation between salinity and viscosity of synthetic seawater (SSW), sodium chloride (NaCl) and evaporated seawater (SW).

5.2.4. Salt quality

Salt crystals from an area of 25 cm^2 of the bottom and top layer of salt deposited in each tank were collected for size comparison and analysis of impurities. Six samples from each tank were collected.

Samples from homogenised harvested salt from each tank were used to determine crystal size. To do this, the dry sieve technique was used (Lewis and McConchie, 1994). First, a set of sieves (0.125, 0.25, 0.5, 1, 2, 4 mm) were selected to cover the range of crystal size in the sample. Before analysis, salt samples from each experimental tank were dried at 60°C for 24 hours. 500g of the dried salt was then poured into the coarsest sieve and covered. The sieve nest was secured in the sieve shaker and shaken for 10 minutes (as recommended by Lewis and McConchie, 1994). After shaking had been completed, the weight of salt crystals on each sieve was determined.

Six samples from each tank were analysed using a Scanning Electron Microscope (SEM) and Energy Dispersion X-Ray Analyser (EDX) to determine crystal form, size and elemental composition. Analyses were performed at the Centre for Electron Microscopy and Microstructure Analysis (CEMMSA) at the University of Adelaide. Before SEM analyses, samples were dried at 105°C for 48h in a desiccator. The samples were mounted on a stub (12.5 mm diameter) compatible with a microscope goniometer stage. Samples were coated by a conductive layer (carbon and gold / palladium) for SEM examination.

Samples from each tank also were sent to the Amdel (Australian Mineral Development Laboratory) to estimate salt quality according to several criteria:

specific gravity (by pycnometry), water content, calcium, magnesium and sulphate impurities.

5.2.5. Salt quantity

Deposited salt from each tank was harvested on 2 March 1995. Harvested salt was kept in perforated nylon bags and excess water left to drain from small holes in the corner of each bag. After two weeks, the bags were weighed to determine the quantity of the salt produced from each tank, simulating final salt production in the crystalliser ponds of the salt field.

5.3. Results

5.3.1. Physico-chemical parameters

Data for pH, specific gravity, salinity and the viscosity of brine in each tank during the experiment are presented in Table 5.5.

The values of pH range from 7.3 to 8.01 and 7.8 to 9.1 during stages I and II, respectively. A maximum pH occurred in tank #5 during both stages I and II as shown in Table 5.5. Overall, in most tanks pH values were higher during stage II than in stage I.

The values for specific gravity ranged from 1.1495 to 1.2278, and, correspondingly for salinity, from 210 to 326.5 g/L.

The values for viscosity show that as stage 1 of the experiment progressed viscosity increased in tanks containing *Synechococcus* (#2, #3, #4 and #5). A greater increase in viscosity was observed during stage I when compared to stage II (Table 5.5).

Viscosity values ranged from 1.5441 to 2.2326 centistokes at stage I. In this stage, brines in tank #1 showed the lowest and tank #5 the highest value for viscosity. In stage II, viscosity values ranged from 2.1576 to 2.4807 centistokes.

The relationship between salinity and viscosity in synthetic seawater, sodium chloride (A.R.) and evaporated seawater is shown in Figure 5.4. The viscosity of these different solutions was approximately equivalent at a salinity of 110 g/L. At higher salinities, viscosity increased to 1.605, 1.695 and 2.087, respectively (Appendix 4.1). Although it was easier to use synthetic saline water for calculating relative viscosity, evaporated seawater was used because of the similarity between this and brine in the experiment. Values for relative viscosity of brine from the experimental tanks are presented in Figure 5.5. The relative viscosity increased throughout stage I; it ranged from 1.103 to 1.290, 1.301, 1.336, 1.346 and 1.353 in tanks #1, 2, 3, 4 and 5, respectively. As shown in Figure 5.5 (stage I), the highest value for relative viscosity occurred in tank #5. In stage II, values decreased from week one to week five, but increased in the last two weeks of the experiment (Fig. 5.5). The details data are given in Appendix 4.1.

5.3.2. Salt quantity

The quantities of salt as estimated by salt weight and percentage of evaporated water from each tank at the two different stages are given in Table 5.6. This Table shows that tank #1 produced the highest amount of salt and tank #5 the lowest. Tank #1 produced 72.5 salt and tank #5, 60.5 kg. Moreover, the percentage of evaporated water from tank #1 at stage I and II was 35.4 and 56.5, respectively, and more than the other tanks. For tanks #4 and 5, the amounts were low, ranging from 30.6 to 46.8 per cent. The weight of harvested salt and the evaporated water are correlated. Note that, because of the summer season, the rate of precipitation in 1995, was low during the course of this experiment (see Appendix 1.1).

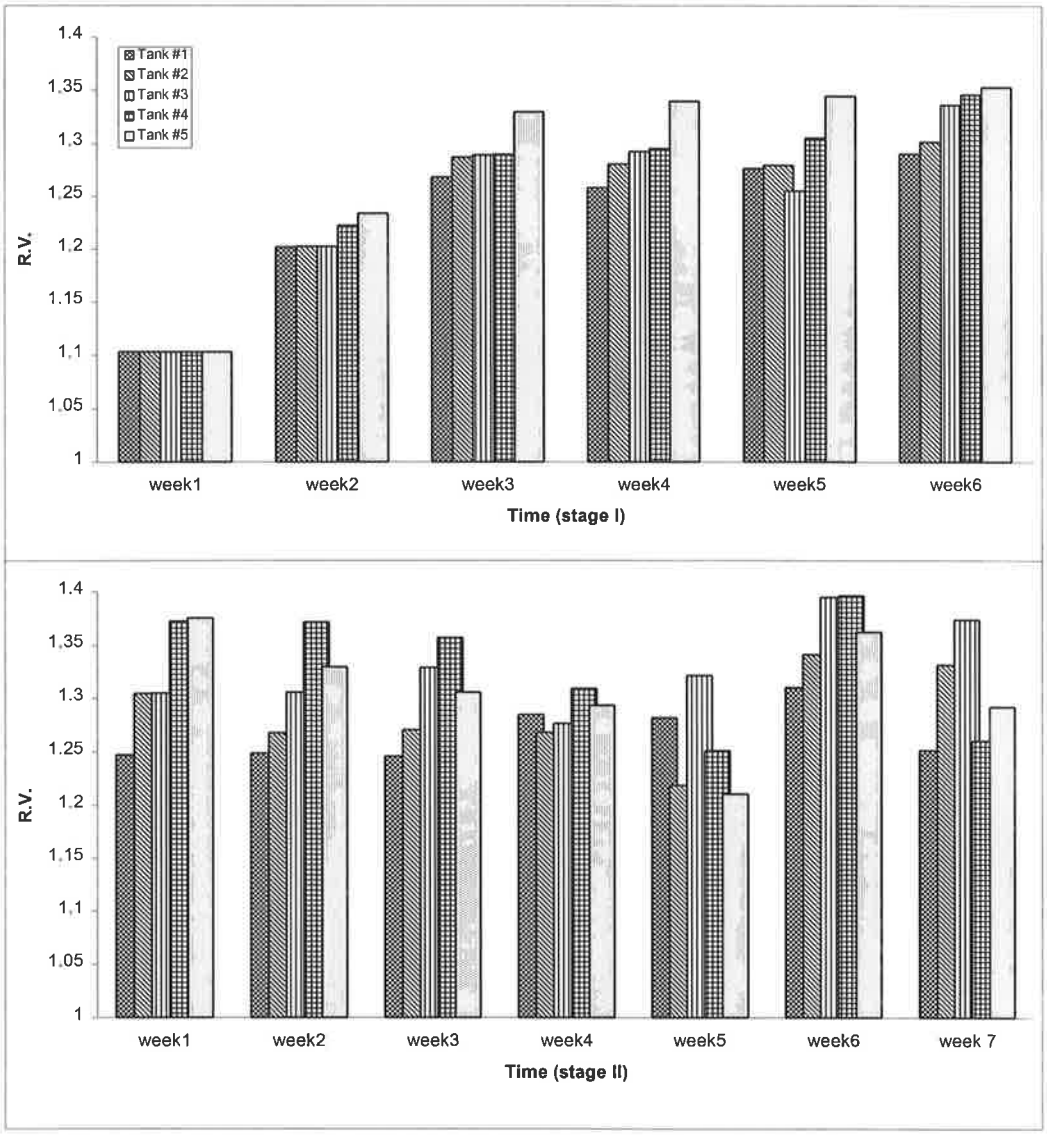

Figure 5.5. Relative viscosity (R.V.) of brines at stages I and II.

Table 5.5. Record of weekly measurement at two experimental stages:
Stage I: Long mucilaginous *Synechococcus* material included;
Stage II: Long mucilaginous *Synechococcus* material excluded.

Stage I

Date	Tanks	pH	Specific gravity	Salinity (g/L)	Viscosity (centistokes)
9/12/94					
	#1	7.6	1.1495	210	1.5441
	#2	7.75	1.1495	210	1.5441
	#3	7.7	1.1495	210	1.5441
	#4	7.85	1.1495	210	1.5441
	#5	7.85	1.1495	210	1.5441
15/12/94					
	#1	7.5	1.171	215	1.7066
	#2	7.55	1.171	215	1.7072
	#3	7.7	1.171	215	1.7071
	#4	7.95	1.171	215	1.7360
	#5	8.0	1.171	215	1.7524
22/12/94					
	#1	7.3	1.19	226	1.8518
	#2	7.3	1.1915	223	1.8659
	#3	7.5	1.1915	223	1.8694
	#4	7.6	1.1905	223	1.8700
	#5	8	1.1925	221	1.9147
29/12/94					
	#1	7.5	1.206	259	2.0113
	#2	7.6	1.2078	255	2.0236
	#3	7.65	1.208	255	2.0418
	#4	7.77	1.208	255	2.0464
	#5	7.9	1.21	250	2.0898
5/1/95					
	#1	7.6	1.22	271	2.1059
	#2	7.65	1.2197	270	2.1108
	#3	7.5	1.2232	280	2.1336
	#4	7.9	1.22	271	2.1535
	#5	8	1.218	269	2.2050
12/1/95					
	#1	7.6	1.223	273	2.1419
	#2	7.65	1.2197	270	2.1475
	#3	7.5	1.2218	269	2.1915
	#4	7.9	1.2218	269	2.2079
	#5	8.01	1.2197	270	2.2326

Table 5.5. continued

Stage II:

Date	Tanks	pH	Specific gravity	Salinity (g/L)	Viscosity (centistokes)
19/1/95	#1	7.8	1.2237	287	2.1576
	#2	7.95	1.2215	272	2.1659
	#3	8.2	1.2224	274	2.1790
	#4	8.25	1.211	259.5	2.1954
	#5	8.4	1.2220	264	2.2278
25/1/95	#1	8.1	1.2235	292.5	2.1979
	#2	8.0	1.222	290	2.2185
	#3	8.2	1.222	286	2.2462
	#4	8.2	1.2221	265	2.2213
	#5	8.5	1.215	275	2.2199
1/2/95	#1	8.65	1.2235	303.5	2.2609
	#2	8.8	1.2228	295	2.2490
	#3	8.69	1.223	280	2.2590
	#4	8.9	1.2215	272	2.2516
	#5	8.95	1.223	280	2.2201
8/2/95	#1	8.65	1.2232	301	2.3123
	#2	8.8	1.2252	305	2.3083
	#3	8.69	1.225	302	2.3110
	#4	8.9	1.2235	292	2.3042
	#5	9	1.2228	295	2.2894
15/2/95	#1	8.7	1.2252	305	2.3322
	#2	8.8	1.227	322	2.3396
	#3	8.75	1.2237	292.5	2.3256
	#4	8.95	1.2267	313.5	2.3396
	#5	9.0	1.2269	318	2.2994
22/2/95	#1	8.7	1.2252	305	2.3800
	#2	8.85	1.222	290.5	2.3531
	#3	8.8	1.223	281.5	2.3773
	#4	8.95	1.2235	284.5	2.3941
	#5	9.1	1.2233	280.5	2.3225
2/03/95	#1	8.75	1.228	326.5	2.4338
	#2	8.9	1.2255	310	2.4622
	#3	8.8	1.2245	299.5	2.4653
	#4	8.9	1.227	320	2.4069
	#5	9.0	1.2278	322	2.4807

Table 5.6. Weight of salt produced and evaporated water (%) from experimental tanks.

Tanks	Salt weight (kg ± 0.5)	evaporated water (%)	
		Stage I	Stage II
#1	72.5	35.4	56.5
#2	70.5	34.8	47.8
#3	69.5	33.7	47.6
#4	63.5	30.6	46.8
#5	60.5	32.0	42.5

5.3.3. Salt quality

The result of analyses for specific gravity, percentage of moisture content, and Ca, Mg, SO_4 ions as impurities in salt crystals is shown in Table 5.7.. The specific gravity of salt in salt harvested from tank #1 was 2.10 and higher than in others. Overall, the specific gravity of the harvested salt ranged from 2.06 to 2.10, and decreased from tank #1 to tank #5. The percentage of moisture content in salt crystals is related to the amount of moisture that is present in crystals. The percentage of moisture ranged from 3.28 to 3.88%. Salt from tank #1 had the highest and tank #3 the lowest percentage of moisture. However, data from tank #1, which was higher than the others, could indicate in consistent data. Samples for the percentage of moisture content were analysed twice with the same results. The trends across tanks were not consistent, but highest concentrations were in tank5 (CaO) and Tank 4 (MgO). The percentage of Ca and Mg in salt crystals ranged from 0.38 to 0.61% and 0.35 to 0.39% w/w, respectively.

Table 5.7. Specific gravity (S.G.), the amount of moisture and the percentage of CaO, MgO and SO_4 from salt crystals harvested from experimental tanks.

Tanks	S.G.of salt	H_2O %	CaO % (w/w)	MgO% (w/w)	SO_4 % (w/w)
#1	2.10	3.88	0.53	0.37	1.550
#2	2.09	3.33	0.38	0.36	1.117
#3	2.07	3.28	0.39	0.35	1.170
#4	2.08	3.32	0.41	0.39	0.798
#5	2.06	3.65	0.61	0.38	1.340

Data on the size distribution of salt crystals are shown in Figure 5.6. These data show that crystal size ranged from 0.125 to 4 mm. The size distribution of crystals is unimodal for all tanks, but in tank #1 (Fig. 5.6) the maximum percentage of size crystals is about 2 mm, while in tank #5, it is about 1 mm. Overall, the largest crystals were from tank #1, and the smallest from tank #5 (Appendix 5.3).

The results on SEM examination from salt crystals produced in tank #1 is shown in Figure 5.7. Comparison of salt crystals from bottom and top layers (Figs. 5.7a and b) showed that crystals from the bottom layers are smaller than those from the top layers. A magnified view of salt crystals from tank #1 (Fig. 5.7a) is shown in Figure 5.7c. Two points (X and Y) in part of the sample were analysed by EDX. The analysis indicated that the light colour (X) is mainly halite (NaCl), while darker spots are mainly gypsum ($CaSO_4$); this is shown in Figures 5.8a and b.

The results on SEM examination of salt crystals produced in tank #5 are shown in Figure 5.9. Figures 5.9a and b showed the comparison of salt crystals from top and bottom layers. These also show that crystals from the top layers are larger than those from bottom layers. A magnified view of salt crystals from tank #5 (Fig. 5.9a) is shown in Figure 5.9c. It revealed high carbon contamination. EDX analysis of

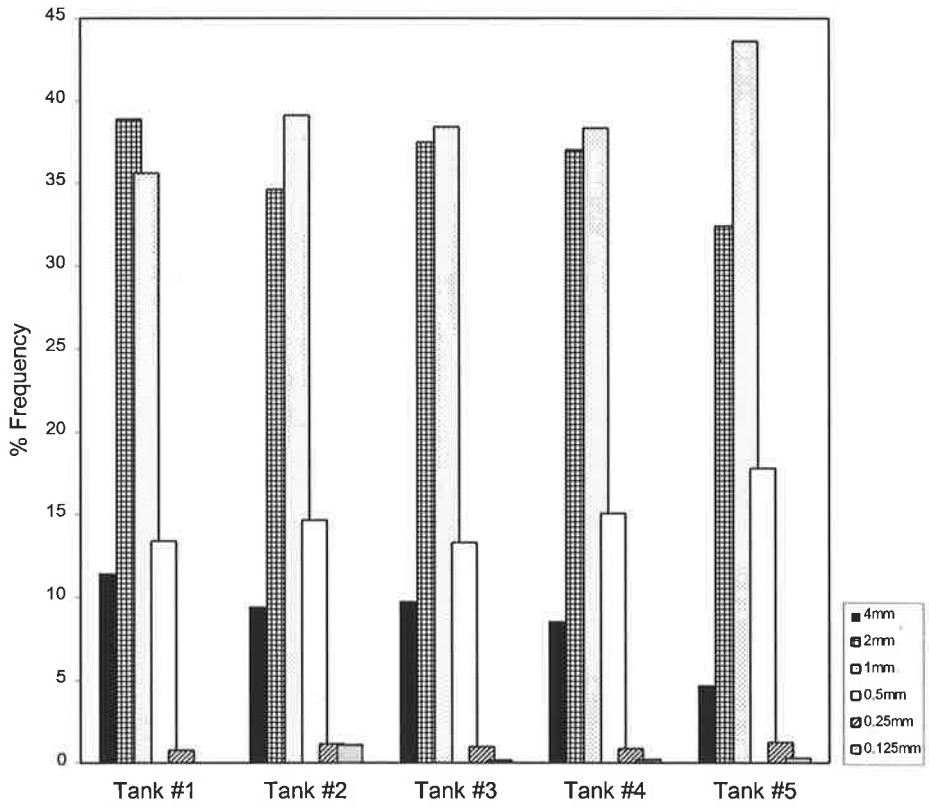

Figure 5.6. Size distribution of salt crystals produced in tanks # 1, 2, 3, 4 and 5.

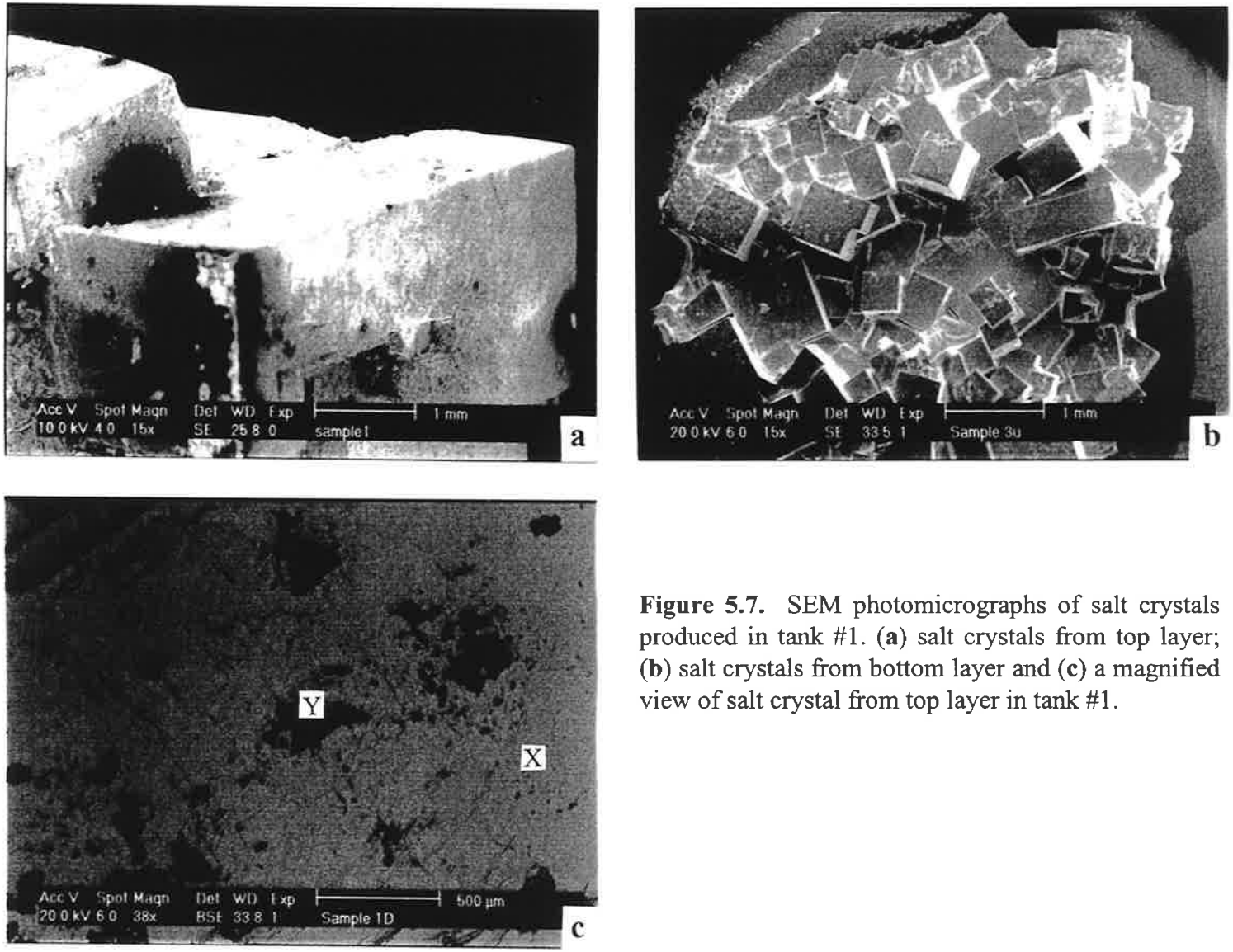

Figure 5.7. SEM photomicrographs of salt crystals produced in tank #1. (**a**) salt crystals from top layer; (**b**) salt crystals from bottom layer and (**c**) a magnified view of salt crystal from top layer in tank #1.

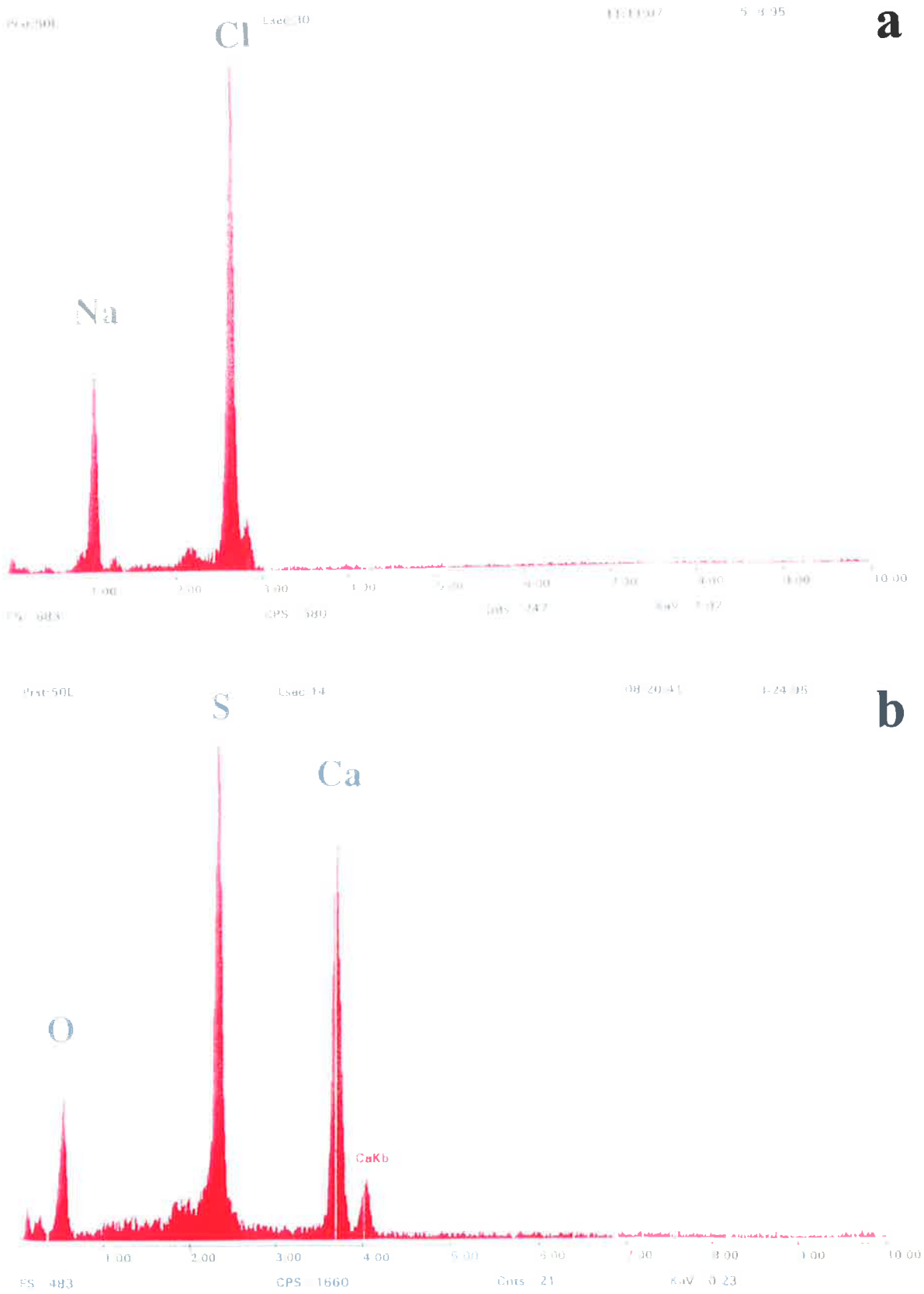

Figure 5.8. Analysis of elements in salt crystals from tank #1. (**a**) EDX from point X shown in Figure 5.7c; (**b**) EDX from point Y shown in Figure 5.7c.

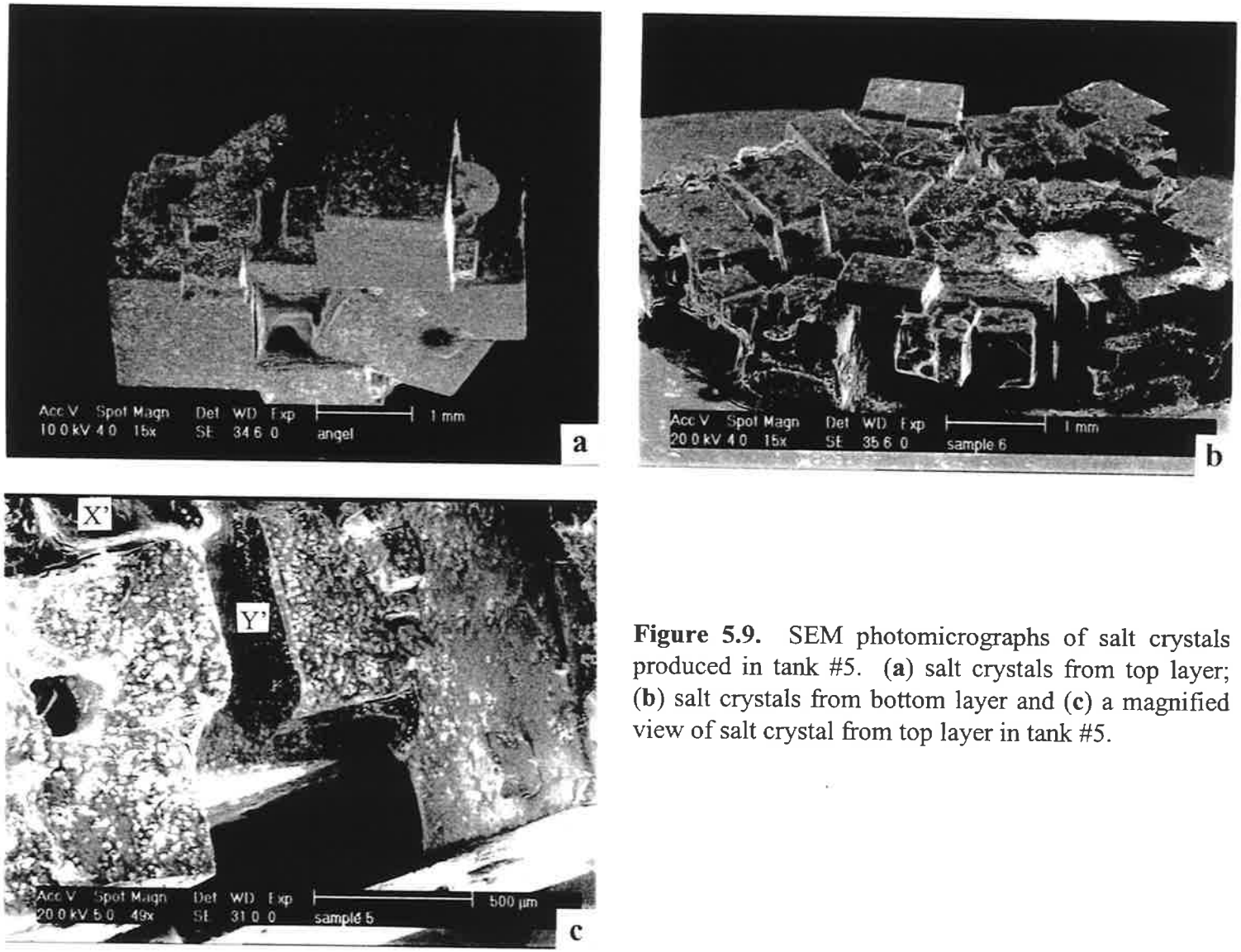

Figure 5.9. SEM photomicrographs of salt crystals produced in tank #5. (**a**) salt crystals from top layer; (**b**) salt crystals from bottom layer and (**c**) a magnified view of salt crystal from top layer in tank #5.

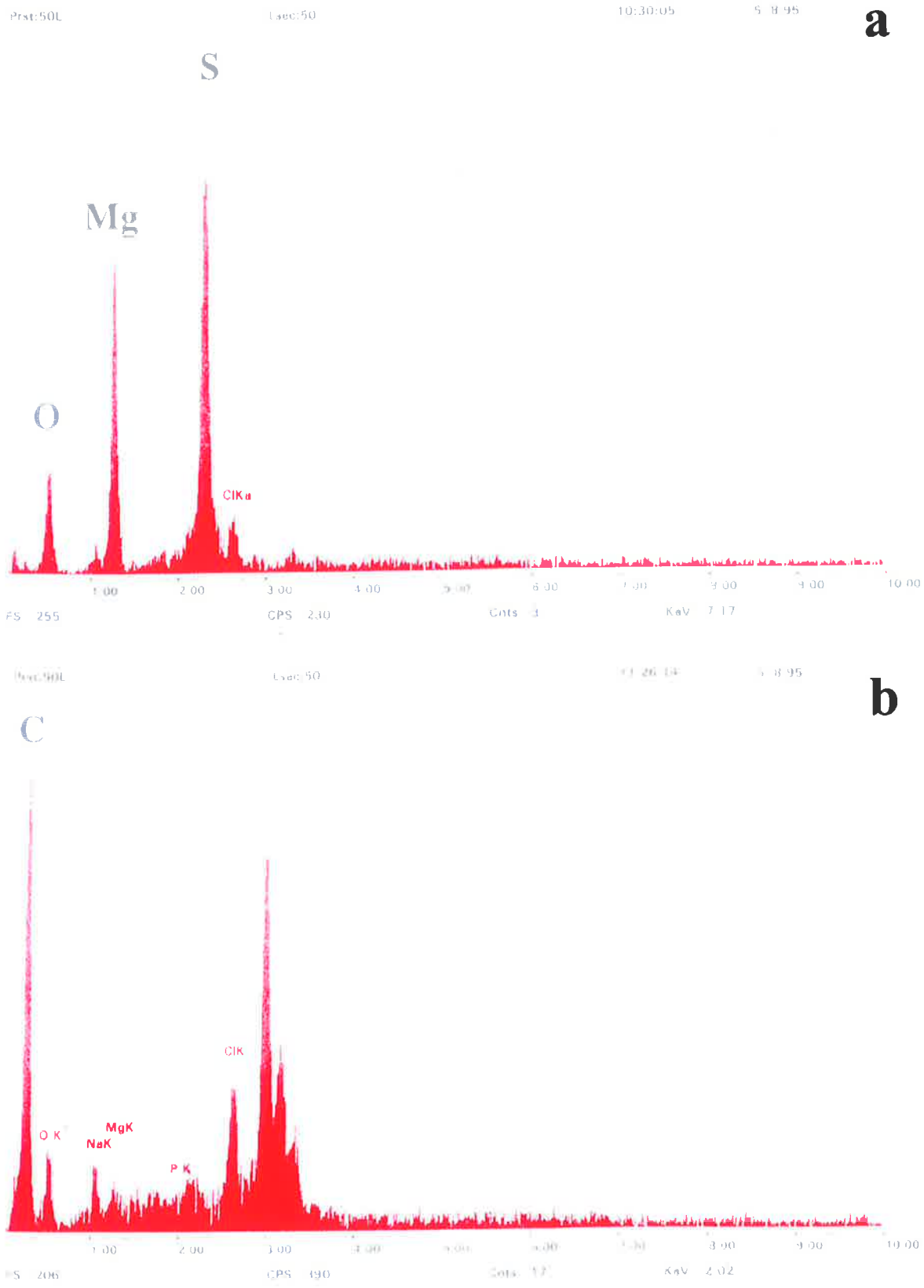

Figure 5.10. Analysis of elements in salt crystals from tank #5. (**a**) EDX from point X' shown in Figure 5.9c; (**b**) EDX from point Y' shown in Figure 5.9c.

material from upper left of salt crystals in Figure 10c (point X') is shown in Figure 5.10a. It indicated that most of the material was $MgSO_4$ (Fig. 5.10a). EDX analysis of material from the dark area in the centre of the SEM photomicrograph (Y') is shown in Figure 5.10b.

Examination of salt crystals from tanks #2, #3, #4 and #5 show that these crystals are hopper-shaped and easy to break. Hopper-shaped crystals are presented in Figure 5.9a and b, a photomicrograph of salt crystals produced in tank #5. Differences between crystal size and the extent of carbon and other impurities were also observed in samples from other tanks. Only the results of tank #1 (no *Synechococcus*) and tank #5 (high amount of *Synechococcus*), as key tanks, are presented here.

5.4. Discussion

Labiberte *et al.* (1994) stated that most Cyanobacteria are sensitive to low pH. However, during this experiment, pH was always >7. Thus, the *Synechococcus* lived in relatively optimal pH.

The results showed that relative viscosity increased during almost all phases of this experiment, i.e. at stage I and early stage II. At the time of salt crystal formation and precipitation (stage II) however, relative viscosity decreased in tanks with *Synechococcus* (tanks #2, 3, 4 and 5) due to the entrapment of mucilaginous material between salt crystals. After week 5 in stage II, relative viscosity- due to a high evaporation rate and ECP concentration in the brine- again increased.

The increase in brine viscosity led to additional effects, notably, hindrance to the circulation of water by wind. As a result, a density inversion effect occurred in the

tanks due to evaporation; the surface water was denser than the bottom water. With further increase in salinities, salt formed on the surface as a sheet. These salt crystals could be blown by the wind toward the margin of the ponds, a phenomenon referred to as 'salt drift' (Burnard and Tyler, 1993). During this experiment, the presence of drift salt caused the lowest amount of evaporation to occur in the tank with the higher amount of *Synechococcus*. As a result, the amount of salt harvested from this tank was the least.

The specific gravity of salt crystals produced from tank #1 was closer to the theoretical value for pure sodium chloride, viz. 2.165 (Weast and Melvin, 1982) than that from other tanks. This indicates that the salt produced from tank #1 was of higher quality than those produced from tanks with *Synechococcus*.

The crystals formed from brine with the highest viscosity were hollow and retained more brine. It is likely that there was ECP in the hollows of the salt crystals, and that this was hydrophilic (Lohmann, 1990) and absorbed the brine. Therefore the salt crystals contained more moisture and some impurities derived from the brine, especially Mg^{2+} and SO_4^{2-}.

EDX analysis from salt crystals produced in tank #1 showed that Ca and SO_4 ionic impurities were related to the presence of some small amounts of gypsum associated with salt. These impurities occurred because during the transference of brine from stage I to stage II the brine still contained some Ca and S_{O4} ions which were later precipitated as gypsum.

In tank 5 the presence of carbon in the salt crystals detected by EDX analysis indicated that the salt crystals examined were contaminated with organic matter (Fig. 5.10b). These contaminants clearly relate to the production of ECP by *Synechococcus*.

Moreover, the impurities of Mg and SO_4 in the salt crystals came from the final brine which contained high amount of Mg salts (Fig. 5.2). The comparison of salt crystals showed that crystals from the bottom layer formed first, and those of the top layer formed later; thus there was more concentrated brine and more time for larger, more cubic and solid crystals to grow in the top layer.

5.5. Conclusions

Based on this experiment, it was concluded that:

- The ECP produced by *Synechococcus* increased the viscosity of brine.
- The size and shape of salt crystals produced are affected by liberated organic material (ECP).
- The ECP caused more brine to be retained in salt crystals and which affects the composition of the salt and leads to a decrease in the quality of salt crystals.
- The quantity of harvested salt decreases with increasing amounts of *Synechococcus* due to the decreasing percentage of evaporated water.

CHAPTER SIX

THE EFFECT OF SALINITY AND LIGHT ON VISCOSITY AND THE PRODUCTION OF EXTRACELLULAR MATERIAL BY THE CYANOBACTERIUM, *SYNECHOCOCCUS*

6.1. Introduction

Cyanobacteria are an ancient group of bacterial organisms which, in evolution, connect heterotrophic bacteria to the higher plants. Various aspects of cyanobacterial physiology, biochemistry and ecology have been the subject of several reviews (Carr and Whitton, 1982; Stewart, 1974; Gallon, 1989; Stal and Caumette 1994; Stal, 1995). Like all bacteria, Cyanobacteria lack nuclei, mitochondria and a chloroplast. The O_2-evolving and CO_2-consuming photosynthetic system of green plants comprises two photosystems (PS I and PS II) connected in series and generate reductants from water (Waterbury and Stanier, 1981; Reed and Stewart, 1988; Stal, 1995), cyanobacteria also have these photosystems. Many cyanobacterial species are nitrogen-fixing bacteria and have the simplest nutritional requirements of all organisms. This is a major reason why they can grow photoautotrophically using only carbon dioxide, atmospheric nitrogen, water, and the simplest of inorganic nutrient supplies with light as the only energy source (Rothschild, 1994; Stal, 1995). Thus, Cyanobacteria are highly flexible in their metabolism and habitat.

The success of most Cyanobacteria in extreme and unstable hypersaline habitats, where excessive heating is naturally coupled with evaporative increases in salinity, explain the flexibility of this group of organisms (Dor and Hornoff, 1985). Most Cyanobacteria, such as *Synechococcus*, face a variety of biological, chemical and

been shown to be affected by numerous factors. These include competition (Orvos *et al.*, 1990), predation (Casida, 1980), starvation (George *et al.*, 1991), temperature (Wiebe *et al.*, 1992), light (Arana *et al.*, 1992), pH (McEldowney and Flectcher, 1988), N-limitation (De Philippis *et al.*, 1993) and other physico-chemical factors (Faust *et al.*, 1975; Henis *et al.*, 1989). The pressure of competition and predation is low in hypersaline water. However, among other factors, high salinity and high light intensity (usually coupled with high temperatures) affect the survival of *Synechococcus* in hypersaline waters. Even so, Cyanobacteria occur in a variety of environments, from fresh water to hypersaline systems. Moreover, they can survive in environments which show dramatic changes in salinity and are of diverse ionic composition (Fogg *et al.*, 1973; Golubic, 1980; Hagemann *et al.*, 1990). They can adapt to high or low concentrations of oxygen, or alternate between aerobic and anaerobic conditions because they possess photosystems I and II, and under conditions of low oxygen concentrations photosystem I can utilize hydrogen sulphide as the electron donor (e.g. Garlick *et al.*, 1977; Austin, 1990).

Unicellular Cyanobacteria constitute an important biological component of hypersaline habitats throughout the world (e.g. Bauld, 1981). They are characterised by cells surrounded by a mucilaginous envelope (Borowitzka, 1981; Campbell and Golubic, 1985) and so are capable of releasing large amounts of exopolysaccharide into the medium. High salinity and high light intensity are stressful factors or provide an extreme environment for Cyanobacteria. Stress has been defined as an external force, factor, or stimulus that causes changes in the ecosystem, or causes the ecosystem to respond (Rapport *et al.*, 1985); it usually creates instability, reduces species diversity, resets successional development, and causes a decline in productivity. One widespread sign of stress is the production of extracellular material (ECP) by Cyanobacteria (Vogal, 1994). Thus, gelatinous ECP produced by Cyanobacteria enable the cells to live in a self-made, possibly

controlled, microenvironment. This environment may be quite different from that of the surrounding water (Lang, 1976). Also, the presence of a mucilaginous polysaccharide layer around the cell may have significant effects on diffusion properties, both into and out of the cells and this could enhance nutrient uptake and increase metabolic activity (Dudman, 1977; Colwell *et al.*, 1985; Whitfield, 1988).

It has been suggested that soluble organic compounds, accumulated as internal osmolytes in response to salinity stress, may provide a major biochemical character distinguishing marine and freshwater forms of Cyanobacteria (Blumwald *et al.*, 1983; Mackay *et al.*, 1984; Reed *et al.*, 1984a and b). Three main salt-tolerant groups of Cyanobacteria are distinguished according to the osmoprotective compounds accumulated. Cyanobacteria of the lowest salt tolerance synthesise sucrose and trehalose; Cyanobacteria of intermediate salt tolerance use glycosylglycerol; and those of the highest salt tolerance accumulate glycine betaine and glutamate betaine (Reed *et al.*, 1986; Reed and Stewart, 1988). Richardson *et al.* (1983) have demonstrated that the unicellular Cyanobacteria *Synechocystic* can grow in both fresh and marine media and that, in response to osmotic stress, it produces glycosylglycerol as an internal osmoticum. Accumulation of glycosylglycerol is essential for the photosynthesis and growth of *Synechocystic* under high - salt conditions (Mikkat *et al.* 1996). Reed *et al.* (1984b) undertook a comprehensive survey of carbohydrate accumulation profiles of more than 70 strains of Cyanobacteria and identified three organic osmolytes (glycosylglycerol, sucrose and trehalose) in both freshwater and marine isolates under conditions of osmotic stress. Glycosylglycerol has been considered as unique to marine Cyanobacteria (Mackay *et al.*, 1983 and 1984), while sucrose has been reported to accumulate in response to osmotic stress in freshwater Cyanobacteria (Blumwald *et al.*, 1983).

6.1٦. Aims of the experiment

In this experiment, the aim was to investigate the effect of salinity and light on the amount of extracellular polysaccharide produced by *Synechococcus* together with its effect on brine viscosity. This effect is important because it affects the quality and quantity of produced salt (see Chapter 5), and is therefore important in the management of solar salt ponds. The experiment was undertaken under natural outdoor conditions during the period December, 11 1994, to January 22, 1995. A natural environment was used rather than a laboratory experiment, because higher amounts of extracellular material (ECP) are reported to be produced under natural conditions (Hellebust, 1974). Moreover, blue-green algae grow better in natural conditions than in the laboratory (Lang, 1976), and the outdoor experiment more closely simulated field conditions at Dry Creek.

6.2. Methods and Material

6.2.1.Experimental design

Twelve aquaria, each 40 x 30 x 50 cm (length, width, depth) were located on the roof of the Zoology Department, at the University of Adelaide (Fig. 6.1). Water from ponds PA7 and FA1 of salinity 190.5 g/L and 285 g/L, respectively, was filtered through a net (mesh size ~ 50 μm) to remove large organisms and debris. Ten litres of water, salinity 190.5 g/L, were added to six aquaria; they were designated as low salinity (LS) aquaria. The same amount of water, of salinity 285 g/L, was added to other six aquaria; these aquaria were designated high salinity (HS) aquaria. *Synechococcus* was collected from pond PA7, drained of excess water and stored in a large container, and then transferred to the laboratory. Approximately 700 g of *Synechococcus* standing crop was transferred to each

Figure 6.1. Experimental aquaria under natural conditions. The 12 aquaria at the beginning of the experiment: 6 covered (three high salinity and three low salinity) and 6 uncovered (three high salinity and three low salinity) aquaria.

aquarium. Before transference, as much as possible of the excess water was drained. Each aquarium was set on a white sheet of foam (25 mm thick) to protect it from additional heat. After 24 hrs, six aquaria, three each of LS and HS, were covered by shade cloth to decrease light intensity; these were designated as low-salinity-covered (LSC), and high-salinity-covered (HSC) aquaria. The other aquaria were designated as low-salinity-uncovered (LSU), and high-salinity-uncovered (HSU) aquaria. HSU, HSC, LSU and LSC aquaria were randomly arranged on the roof surface and every two days the position of each aquarium was randomly changed (Fig. 6.1).

To eliminate problems of nutrient depletion that might cause a drop in *Synechococcus* biomass, phosphorus (as K_2HPO_4) and nitrogen (as $NaNO_3$) were added daily to the aquaria. The amount chosen (0.019 mg/L phosphate and 0.016 mg/L nitrate) approximated natural nutrient concentrations in pond PA7 (see Chapter 2). To compensate for water lost by evaporation, the water-level was kept constant by daily additions of distilled water. Additionally, the aquaria walls about the level of the water were scrubbed daily with a plastic brush to prevent the build-up of salts on the walls. Before addition of water, the reduction of water in each tank was measured to estimate the volume of evaporated water.

Solar irradiance at 2.00pm varied from 1500 to 2500 $\mu Em^{-2} S^{-1}$ on cloudy and sunny days, respectively.

6.2.2. Sample collection

Three samples were collected randomly each week from each aquarium in a 30 ml glass bottle for analysis. Before sampling, the water was mixed slowly with glass

rod to homogenise it. Water and air temperatures were measured by a mercury-in-glass and minimum-maximum thermometers, respectively, daily at 2.00 p.m.

Some *Synechococcus* material was collected from each aquarium at the end of the first, third and last week of the experiment and transferred to the laboratory in order to measure chlorophyll *a*, carotenoids and estimate the amount of extracellular products (ECP). To do this, *Synechococcus* was collected on paper filter (Whatman #1) and drained by gentle suction for 2-3 minutes. The filter was then placed on a paper towel in a petri-dish to remove further brine. The filter paper was placed in a plastic bag and frozen until further analysis.

6.2.3. Laboratory measurements

pH and conductivity were measured from samples on the day of collection. Conductivity measurements were converted to salinity using the regression equations of Williams (1986a). Samples with a salinity of more than approximately 70 g/L were appropriately diluted. Viscosity was measured with a capillary viscometer (PSL, Model C-3889, Type BS/U). Relative viscosity was obtained from the viscosity of samples and the viscosity of evaporated seawater (see section 5.2.4.).

It is difficult to determine the amount of ECP per cell of *Synechococcus* due to the mucilaginous nature and filamentous shape of *Synechococcus* strands. Estimates were therefore made by measuring ECP in *Synechococcus* cells embedded in irregular masses. Quantitative estimates of this mucilaginous extracellular product were obtained by measuring chlorophyll *a*, carotenoid and ash-free dry weight (AFDW). A portion (1 gm) of *Synechococcus* standing crop that had been stored was used for chlorophyll *a* and carotenoid measurements. Chlorophyll *a* and

carotenoids was extracted using the methods of Krishman (1991). According to Krishman (1991), the most efficient extraction of chlorophyll *a* from *Synechococcus* is with 90% methanol. Pigments were extracted in 90% methanol by placing each sample with 5 ml of solvent at 4°C in the dark for 12-18 hours, and then transferring it to a 70°C water bath where it was boiled in methanol for 2 minutes. Short periods of boiling aided pigment extraction without converting significant amounts of chlorophyll *a* into phaeophytin (Tett *et al.,* 1975). First, the solvent reacted with the mucilage and white flakes appeared on the sample of *Synechococcus* cell surface; stirrers improved extraction. The solvent and extract were then poured into a centrifuge tube. After the first extraction, 5 ml of methanol was added to the sample, boiled for 2 minutes and added to the first extraction in a centrifuge tube. It was then centrifuged at 2000 RPM. Immediate centrifugation provided rapid cooling of the extracts, and aided prevention of pigment breakdown. Optical densities were measured against a methanol blank at 750, 665, and 750 nm in a 1 cm cell using a Varian UV/visible spectrophotometer. After the initial reading, extracts were acidified with two drops of 8% (2N) HCl and optical densities measured at the same wavelengths.

Chlorophyll *a* was estimated using the equation of Talling and Driver (1963):

Chlorophyll *a* (μg per g of sample) = 13.9 D665 [2.43 (D_b-D_a)] .V

Where:

D_a = optical density of methanol extract after acidification

D_b = optical density of methanol extract before acidification

V = volume of extract (mL).

In this equation, the factor 13.9 is the absorption coefficient for chlorophyll *a* in methanol, and the equation corrects for absorbency due to phaeophytin (Lorenzen, 1967). The readings at 750 nm were used to correct for absorption. As post-

acidification readings may increase with time, two background readings were used (Tett *et al.,* 1975).

Carotenoids was estimated by the methods of Strickland and Parsons (1972) and Parsons *et al.*, (1984) (see section 2.2.3).

The weight of extracellular material in 5 g of frozen *Synechococcus* material was first determined. Each sample was dried at 105° C for 24 hours (APHA, 1992), then ashed at 550°C for 4 hours in a muffle furnance (Aloi, 1990). AFDW gave an estimate of the amount of organic material in the sample, cyanobacterial cells and extracellular products. The cell biomass was estimated from chlorophyll *a* and carotenoid measurement. AFDW of the sample was calculated as the difference between dry weight and the weight of the ash after combustion.

Microscopic observation was carried out to confirm the identity of microorganisms and to estimate their density. *Synechococcus* material collected from each aquarium was placed in plastic bags and frozen until analysis for chlorophyll *a* and ECP estimation.

6.2.4 Determination of ECP composition by ^{13}C Nuclear Magnetic Spectroscopy

A portion of *Synechococcus* standing crop that had been stored frozen was freeze dried by Dynavac Freeze Drier, Model FD.5 for determination of ECP chemical composition. Solid state high resolution ^{13}C Nuclear Magnetic Resonance (NMR) with Cross Polarisation and Magic Angle Spinning (CP/MAS) techniques were used to determine the chemical composition of ECP produced by *Synechococcus*.

The 50.309 Mhz CP/MAS spectra of the samples (standing crop of *Synechococcus*) were obtained on a Varian Unity 200 spectrometer with a 4.7 T widebore Oxford superconducting magnet. Samples were spun at 5 kHz in 7 mm diameter zirconia rotors with Kel-F caps in a Doty Scientific MAS probe. All spectra were obtained with a 1 ms contact time and a 300 ms recycle time. The number of transients was 10000 for each sample. Using the standard Varian pulse sequence, the free induction decays were acquired over a sweep width of 40 kHz over an acquisition time of 15 ms in a 1216 point database. All spectra were obtained with 32 k zero filling and 50 Hz - Lorentzian line broadening and 0.005s gaussian broadening. Chemical shift assignments were externally referenced to the methyl resonance of hexamethyl benzene at 17.36 part per million (ppm).

6.2.5. Statistical analysis

The results are averages of several measurements ±SE. Statistical analysis of the data was performed using ANOVA (Zar, 1984). Multivariate statistical analysis was used to determine the nature of changes by salinity and light on relative viscosity measurements through time. This was analysed by ANOVA Multivariate Repeated Measure and was performed by using Systat software.

6.3. Results

6.3.1. Physico-chemical parameters

Table 6.1 shows air and water temperatures during the experimental period. The uncovered aquaria with higher salinities had slightly higher temperatures than uncovered lower salinity aquaria, and there was also a difference between high and low, covered and uncovered aquaria.

Table 6.1. Daily measurements of maximum, minimum air temperature (T_a) and water temperature (T_w) in experimental aquaria, Values as °C.

Date	T_a		T_w			
	max.	min.	HSU	HSC	LSU	LSC
(11/12/95)						
	24	12	25	21	23	20
W	29	18	29	25	27	25
E	39	23	41	38	39	37
E	43	23	40	39	38.5	38.5
K	43	23	39.5	34	35	33.5
	34	18	35	31.5	34	30
1	32	15	32	30	26	26
	39	12	40	35	38	35
W	42	10	40.5	39	40	38.5
E	40	16	39.5	34	35	33.5
E	39.5	14	38	33	37	33
K	36	13	40	34	38	33
	37	15	38	34.5	39	35
2	32	18	36	29	30	29
	39	19	40	33	37	31
W	39	14	38	33	34	34
E	36	16	40	36	38	36
E	35	15	41.5	34	40.5	33.5
K	41	20	39.5	35.5	37	35
	28	17	40	34	38	33
3	29	15	32	25	30	24
	35	15	38	30	36	30
W	30	14	28	25	26.5	22
E	33	15	35	31	32	30
E	34	18.5	42	36	36	34
K	38.5	18	40	34	39.5	33
	38	20	38	34	39	35
4	34	17	36	29	30	29
	32	18	35	30	34	30.5
W	36	18	36	31	33	31
E	35	19	34	32	34.5	30.5
E	36	17	34	31	35.5	31
K	44	29	39.5	35.5	36	35
	46	20	44	40	42	39.5
5	31	18	33	29	31	29
W	36	16	34	31	32	31
E	33	15	35	31	32.5	30
E	36	19	32	29	32	29
K	38	18	40	33.5	36.5	34
	35	15	36	32	34.5	32
6 (22/1/96)						

HSU, high salinity, uncovered; HSC, high salinity covered; LSU, low salinity, uncovered; LSC, low salinity, covered.

Weekly data for salinity, specific gravity, pH and viscosity of water from ponds PA7 and FA1 are presented in Table 6.2. These data are also presented for each experimental aquarium (Table 6.3). In the ponds, pH was 8.53 and 8.87 in ponds PA7 and FA1, respectively. The values for specific gravity were 1.14705 and 1.2132, and correspondingly, for salinity, 190.5 g/L and 285.0 g/L. The viscosity of pond FA1 water was higher than water in pond PA7. Viscosity values were 1.51340 and 1.5790 for ponds PA7 and FA1, respectively. For the high salinity aquaria, the values of pH ranged from 8.46 to 8.94, specific gravity from 1.1841 to 1.1929, and salinity from 243.5 - 255.5 g/L. For low salinity aquaria, the values were 8.38 to 8.88 for pH, 1.1432 to 1.1474 for specific gravity, and 185 - 191 g/L for salinity.

The values for viscosity in each aquarium are given in Table 6.3. This table shows that viscosity was higher in samples from higher salinity water. It also shows that viscosity increased in HSU and HSC samples up to week 4, ranging from 1.79036 to 1.94342 and 1.78616 to 1.86914 centistokes, respectively. However, it also shows some changes in LSU and LSC samples during the experimental periods. Viscosity ranged from 1.52368 to 1.58153 and 1.51764 to 1.55263 centistokes, respectively. The highest viscosity occurred in HSU samples in week four. The lowest viscosity was recorded in week one in an LSC aquarium.

Table 6.2. Chemical features of ponds PA7 and FA1 when used as a source of experimental water.

Date	Sample	pH	Specific gravity	Salinity (g/L)	Viscosity (centistokes)
(11/12/95)	PA7	8.53	1.14705	190.5	1.51340
	FA1	8.87	1.2132	285.0	1.5790

Each value is the mean of three measurements.

Table 6.3. Record of weekly measurements from experimental aquaria. Each value is the mean of three samples measurement.

Date	Aquarium	pH	Specific gravity	Salinity (g/L)	Viscosity (centistokes)
(12/12/95)					
	HSU	8.65	1.1908	253	1.82374
	HSU	8.69	1.1915	254	1.79289
	HSU	8.71	1.1916	254.2	1.79036
W	HSC	8.71	1.1912	253.6	1.78716
E	HSC	8.69	1.1910	253.3	1.79155
E	HSC	8.70	1.1908	253	1.78616
K	LSU	8.60	1.1470	190.5	1.52717
	LSU	8.58	1.1464	189.6	1.52471
1	LSU	8.59	1.1455	188.3	1.52368
	LSC	8.64	1.1461	189.2	1.5198
	LSC	8.67	1.1458	188.8	1.51764
	LSC	8.67	1.1462	189.3	1.52158
(19/12/95)					
	HSU	8.66	1.1908	253	1.87791
	HSU	8.59	1.1917	254.3	1.8897
	HSU	8.64	1.1918	254.5	1.89124
W	HSC	8.66	1.1912	253.6	1.80254
E	HSC	8.58	1.1929	256	1.86914
E	HSC	8.70	1.1841	243.5	1.80346
K	LSU	8.69	1.1460	189	1.52578
	LSU	8.69	1.1461	189.2	1.53214
2	LSU	8.72	1.1460	189	1.53319
	LSC	8.70	1.1474	191	1.5477
	LSC	8.69	1.1453	188	1.55263
	LSC	8.65	1.1467	190	1.54986
(26/12/95)					
	HSU	8.61	1.1903	252.3	1.94301
	HSU	8.62	1.1905	252.6	1.90797
	HSU	8.69	1.1882	249.3	1.84494
W	HSC	8.78	1.1888	249.3	1.81233
E	HSC	8.84	1.1901	250.2	1.80908
E	HSC	8.79	1.1903	252	1.82624
K	LSU	8.76	1.1453	188	1.52871
	LSU	8.79	1.1467	190	1.57692
3	LSU	8.83	1.1464	189.6	1.58153
	LSC	8.81	1.1446	187	1.53392
	LSC	8.76	1.1432	185	1.54097
	LSC	8.8	1.1458	188.8	1.55252

Table 6.3. continues:

Date	Aquarium	pH	Specific gravity	Salinity (g/L)	Viscosity (centistokes)
(3/1/95)					
	HSU	8.65	1.1919	254.6	1.94081
	HSU	8.80	1.1917	254.3	1.94342
	HSU	8.80	1.1920	254.8	1.90398
W	HSC	8.69	1.1910	253.3	1.86097
E	HSC	8.87	1.1918	254.5	1.83143
E	HSC	8.93	1.1891	250.6	1.82944
K	LSU	8.65	1.1465	189.8	1.5603
	LSU	8.70	1.1455	188.3	1.54344
4	LSU	8.61	1.1455	188.3	1.57704
	LSC	8.65	1.1446	187.0	1.52512
	LSC	8.56	1.1456	188.5	1.52104
	LSC	8.62	1.1458	188.8	1.51322
(10/1/95)					
	HSU	8.46	1.1919	254.6	1.86961
	HSU	8.81	1.1923	255.2	1.87238
	HSU	8.88	1.1894	251	1.85341
W	HSC	8.88	1.1925	255.5	1.89309
E	HSC	8.94	1.1923	255.2	1.84974
E	HSC	8.88	1.1925	255.5	1.81169
K	LSU	8.87	1.1464	189.6	1.56849
	LSU	8.88	1.146	189	1.54179
5	LSU	8.85	1.1468	190.2	1.56569
	LSC	8.71	1.1457	188.6	1.54971
	LSC	8.77	1.1454	188.2	1.5233
	LSC	8.77	1.1458	188.8	1.52409
(18/1/95)					
	HSU	8.48	1.1922	255	1.88314
	HSU	8.73	1.1917	254.3	1.86338
	HSU	8.78	1.1910	253.3	1.80867
W	HSC	8.90	1.1926	255.6	1.80423
E	HSC	8.80	1.1894	251	1.79733
E	HSC	8.82	1.1912	253.6	1.80049
K	LSU	8.70	1.1458	188.8	1.57454
	LSU	8.70	1.1461	189.2	1.5649
6	LSU	8.65	1.1449	187.5	1.56662
	LSC	8.38	1.1458	188.8	1.52156
	LSC	8.51	1.1457	188.6	1.52523
	LSC	8.47	1.1461	189.2	1.53669

Values for salinity and relative viscosity in the experimental aquaria are presented in Figure 6.2 (see also Appendix 4.2). This figure shows that the salinity in each aquarium was almost constant throughout the experimental period. The relative viscosity was higher in HS samples in weeks 2, 3 and 4. The effects of low-high salinity and cover-uncover status on the relative viscosity over a 12 weeks period can be examined from the two-factor analyses of variance (Table 6.4). This table shows that relative viscosity differed between samples from high and low salinity treatments ($p<0.01$) through time (salt factor). There was also a significant difference ($P<0.01$) between the relative viscosity in samples from covered and uncovered treatments. The interaction factor of the ANOVA is also significant indicating interaction between salinity and cover. This is shown diagramatically in Figure 6.3. Each diagram shows the values of relative viscosity, salinity and cover status measurements in a given week. Numbers 1 and 2 on X axes are low and high salinity and numbers 1 and 2 on Z axes are covered and uncovered treatments respectively. The plane shows the mean of three measurements for each treatment. Each corner of the plane shows the relative viscosity for a combination of the two factors, salinity and cover. The plane in weeks 1, 2 and 3 shows higher relative viscosity for high-uncovered treatments than other treatments, but the differences are small shown by the flat nature of the plane. During and after week 4, the plane is more steeply tilted and relative viscosity decreased in high salinity-uncovered treatments.

Table 6.4. ANOVA Multivariate Repeated Measures Analysis on relative viscosity under salinity (high and low salinity) and cover and uncovered treatments over 12 week period.

Source	SS	DF	MS	F	P
Salt	0.0056	1	0.0056	14.7853	0.0049
Cover	0.0085	1	0.0085	22.3396	0.0015
Salt*cover	0.0028	1	0.0028	7.4115	0.0262
Error	0.0030	8	0.0004		

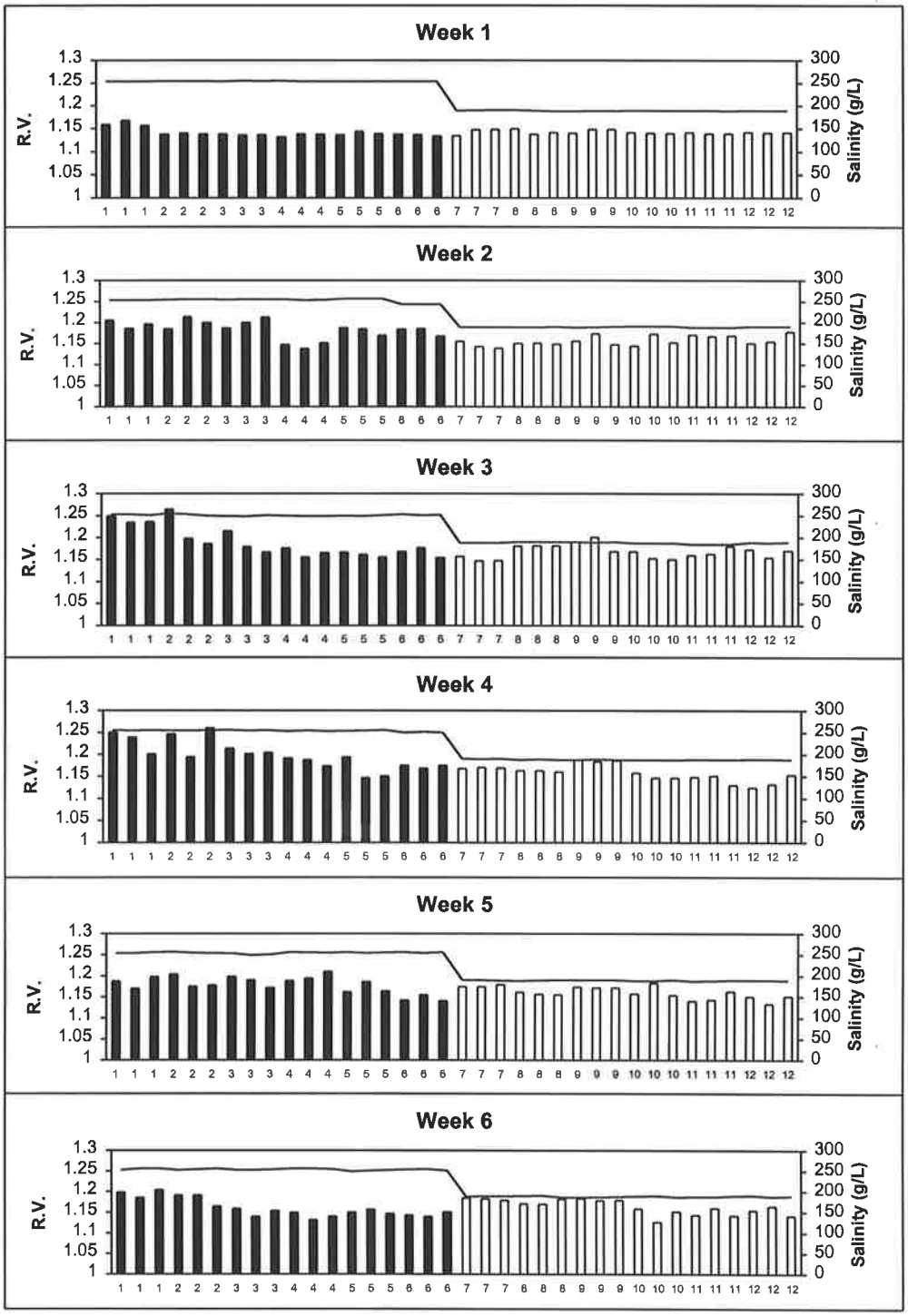

Figure 6.2. Salinity (g/L, solid line) and relative viscosity (RV, bars) of brine during the experimental period. 1-3, high salinity-uncovered; 4-6, high salinity-covered; 7-10, low salinity-uncovered; 10-12, low salinity-covered. Triplicate samples from each aquarium.

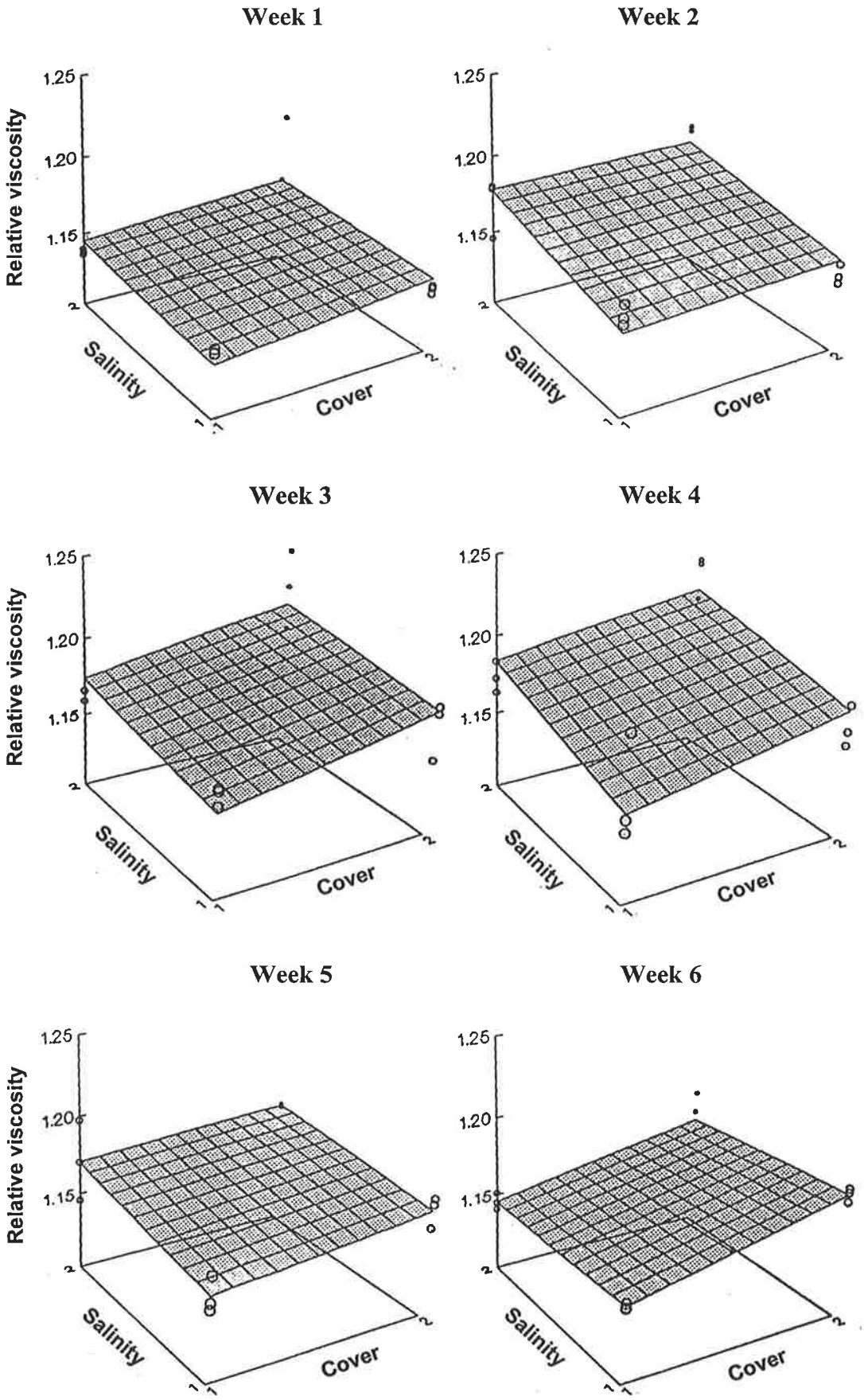

Figure 6.3. Diagrammatic representation of the relationship between salinity and cover condition. Each point is average of three measurements for viscosity from one treatment. Numbers 1 and 2 in X and Z axes are low and high salinity and covered and uncovered status respectively.

6.3.2. Identification of the chemical structure of ECP by ^{13}C NMR spectroscopy

Figure 6.4. shows natural-abundance ^{13}C NMR spectra of ECP in *Synechococcus* standing crop from four different treatments (HSU, HSC, LSU and LSC). The spectra were run under conditions which ensured that the peak heights accurately reflected the concentrations of various solutes in the extracts and peaks in these spectra show higher concentrations. The relative heights (or intensities) of their resonance reflect their relative concentrations in the samples. Therefore each sample displayed two or more well - resolved ^{13}C resonances which provided a useful check on relative comparisons between two samples and also provided a simple identification of the major organic composition in ECP.

All samples showed major signals at 175 ppm (carboxyl/ carboxyl), 129 ppm (aromatic protein residues/ lipid unsaturation), 103 ppm (anomeric carbon of sugars), 73 ppm (CHOH of carbohydrates other than anomeric), 65 ppm (carbohydrate CH_2OH), 56 ppm (protein alpha carbon), 31 ppm (lipid polymethylene), and at 24 ppm (protein methy groups). ^{13}C NMR studies confirmed the presence of glycoprotein and glycolipids in the samples. The relative amounts of organic materials in the samples from HSU, LSU, HSC and LSC treatments are shown in Figure 6.4. It shows higher amounts of organic matter in samples from high salinity with covered and uncovered (HSC - HSU) than in low salinity covered and uncovered (LSC and LSU) treatments. Some differences between uncovered and covered treatments, with peaks for HSU slightly greater than in HSC and for LSU slightly greater than LSC, can be seen by comparing Figure 6-4a and b.

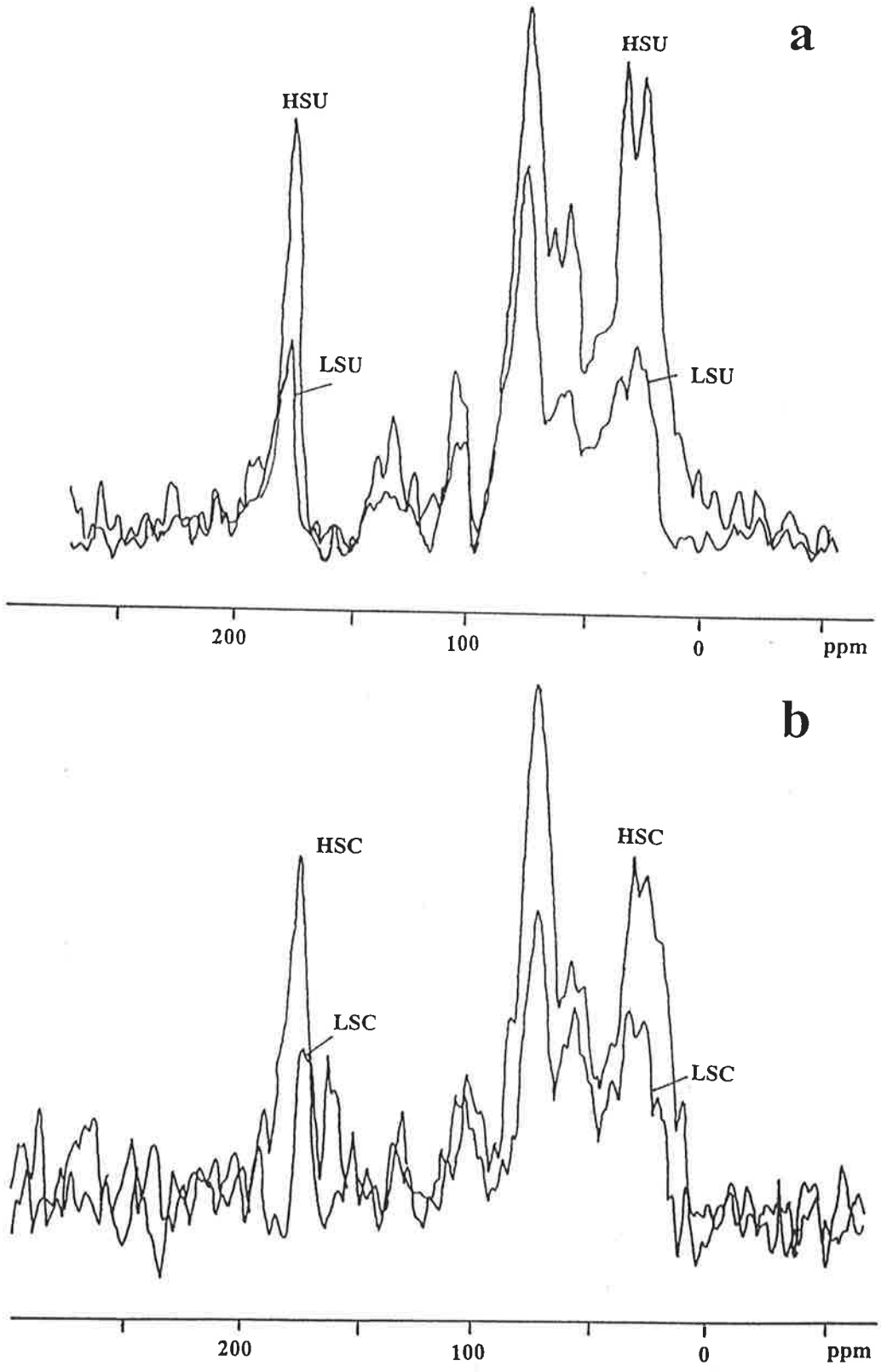

Figure 6.4. Relative amounts of ECP composition produced by *Synechococcus* standing crop. (a) samples from HSU (high salinity-uncovered) and LSU (low salinity-uncovered) aquaria; (b) samples from HSC (high salinity-covered) and LSC (low salinity-covered) aquaria.

6.3.3. Laboratory examinations

Data on the amount of chlorophyll *a*, the percentages of dry weight, the amount of water in standing crop of *Synechococcus* and the percentages of organic material (expressed as ash free dry weight, AFDW) in dry samples in the first and the last sampling are shown in Table 6.5. Chlorophyll *a* ranged from 0.20 mg/100g to 0.43mg/100g of wet sample in LSU and HSU aquaria in the first week samples. Dry weight ranged from 23.0 to 25.3 g/100g (%) of wet sample in LSU and HSC, respectively. There is no significant difference between the percentages of water in the samples; it ranged from only 74.93% to 76.20%. Percentages of organic material were higher in samples from the HSU aquaria than from HSC aquaria and higher in LSU than LSC aquaria, except for aquarium 9 (LSU). At the end of the experiment, chlorophyll *a* in the samples ranged from 0.13 mg/100g (HSU) to 0.51mg/100g (LSC) of wet sample. Dry weight ranged from 23.4 to 40.9 g/100g (%) of wet samples in LSU and HSC, respectively. The percentages of water in the different dry samples ranged from 59.06% (HSU) to 76.54%. (LSU). Percentages of organic material were higher in samples from high salinity than low salinity aquaria at the end of week one. Organic matter was the lowest in samples from HSU in the last week of the experiment.

Water in aquaria under low light intensity (HSC, LSC) developed a green colour, whereas aquaria under high light intensity (HSU, LSU) became yellowish or red. The comparison between chlorophyll *a* concentration and the percentages of organic material from *Synechococcus* standing crop samples collected directly from field (pond PA7), and in the samples from the first and the last samplings are presented in Figures 6.5 and 6.6. Data for the first and the last samplings in this figures are the mean of triplicate treatments. The amount of chlorophyll *a* in samples at the end of the experiment was higher in covered than uncovered aquaria, especially in LSC

Table 6.5. Chlorophyll *a*, percentages of dry weight, water and organic material from standing crop of *Synechococcus* (AFDW) at week 1 and week 6 of the experiment Each number is mean of three values.

Samples		Week 1				Week 6			
		Chlo.*a* mg/100g	Dry wt. %	H_2O %	AFDW %	Chlo.*a* mg/100g	Dry wt. %	H_2O %	AFDW %
1	HSU[1]	0.35	24.4	75.2	30.8	0.15	30.8	69.21	18.79
2	HSU	0.32	24.0	75.6	28.7	0.13	36.7	63.25	23.76
3	HSU	0.43	23.8	76.06	26.0	0.18	40.9	59.06	13.05
4	HSC[2]	0.25	23.5	76.2	25.4	0.25	40.7	59.28	17.61
5	HSC	0.25	23.6	75.46	23.8	0.18	33.4	66.53	22.80
6	HSC	0.27	25.3	74.93	23.3	0.20	33.4	66.58	27.81
7	LSU[3]	0.40	23.0	76.13	19.2	0.18	25.5	74.48	34.69
8	LSU	0.37	23.6	75.6	19.6	0.15	26.0	74.03	20.69
9	LSU	0.20	23.6	76.6	22.3	0.17	23.4	76.54	20.16
10	LSC[4]	0.26	23.7	75.86	22.0	0.51	33.2	66.81	21.25
11	LSC	0.28	23.9	75.47	20.6	0.25	26.2	73.80	20.9
12	LSC	0.26	24.7	75.27	20.1	0.23	30.2	69.79	21.19

(1) high salinity, uncoverd, (2) high salinity, covered, (3) low salinity, uncovered, (4) low salinity, covered

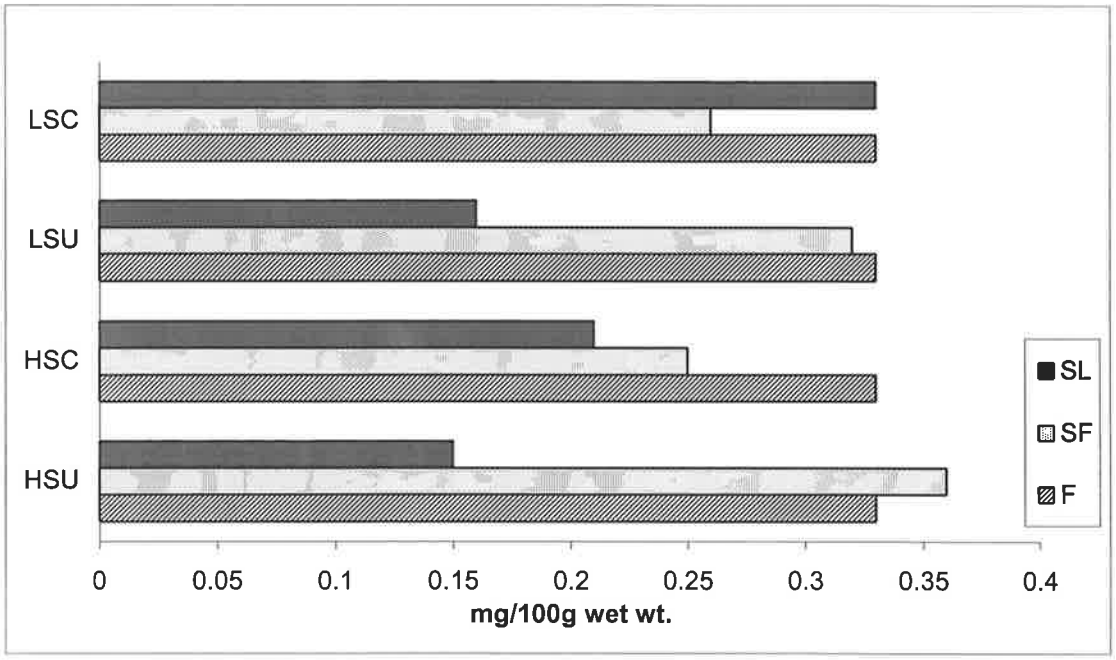

Figure 6.5. The comparison between the amount of chlorophyll *a* from *Synechococcus* standing crop: samples collected directly from field (pond PA7) (**F**), the first (**SF**), and the last (**SL**) samplings from each aquaria.

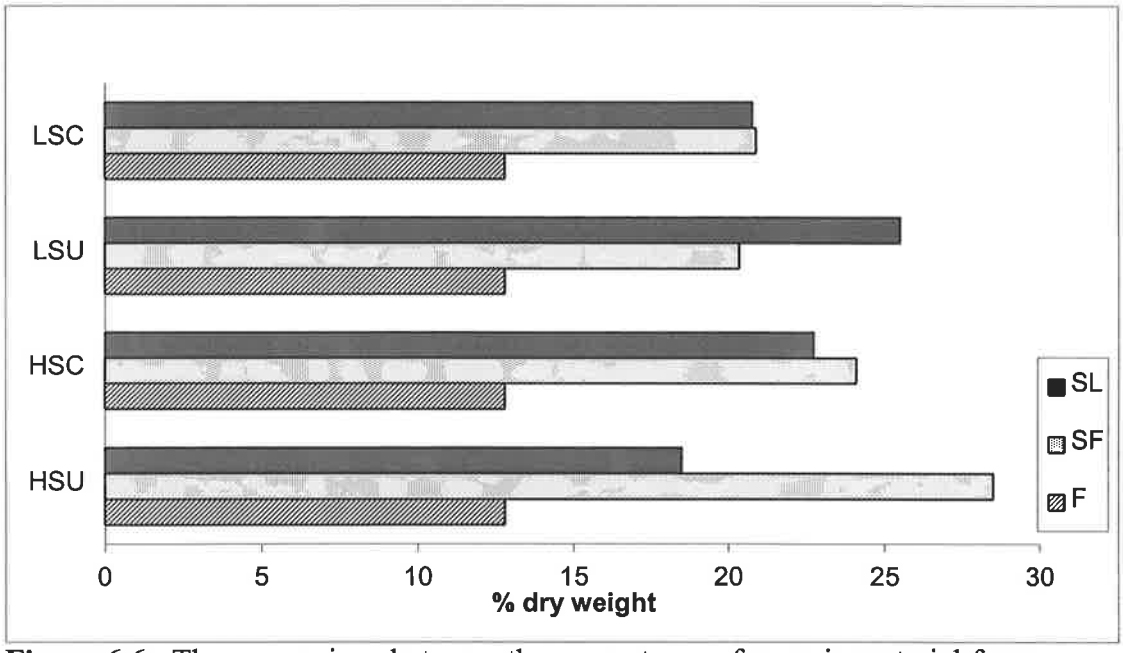

Figure 6.6. The comparison between the percentages of organic material from *Synechococcus* standing crop: samples collected directly from field (pond PA7) (**F**), the first (**SF**), and the last (**SL**) samplings from each aquaria.

Low salinity-covered and uncovered (LSC, LSU), high salinity-covered and uncovered (HSC, HSU).

treatment (Fig. 6.5). The organic content was higher in all samples from aquaria than from the field. There were no clear related to the salinity or covered treatments. Organic content was higher in uncovered - low salinity (LSU) aquaria than covered - low salinity aquaria (LSC). However, it was the lowest in uncovered - high salinity (HSU) aquaria from the last sampling.

The comparison between chlorophyll *a* and carotenoid concentrations was made between samples from different treatments at week 1, 3 and 6 as shown in Figure 6.7. Chlorophyll *a* concentration fall in week 1 to weeks 3 and 6. Carotenoid concentration increased in the samples from week 1 to week 3. At the last sampling carotenoid concentrations had decreased along with the decrease in chlorophyll *a*.

6.3.4. Microscopic observations

In all samples, microscopic examination revealed the presence of a unicellular green-coloured Cyanobacterium, 1-10 x 1-2 μm in size. This examination showed detectable morphological differences between organisms in the yellowish and green colonies in uncovered and covered aquaria, but both were referable to the genus *Synechococcus*. The cells from high salinity aquaria were different with respect to cell size, shape arrangement, the presence of a sheath, the amount of mucilage, and the degree of pigmentation. The spherical uni- and bicells, were embedded in a common mucilaginous material. In contrast, naked forms (such as spherical cells or short cylindrical groups of cells) grew in irregular masses, disassociated easily, and smeared when touched. They were present in samples from covered aquaria. Some unicells in samples from HSU aquaria, after week four were surrounded by a copious, reddish-granular slime; similar granular matter gave a red colour and accumulated in the brine in the solar fields (Fig. 6.7). In general, *Synechococcus* cells in LSC aquaria aggregated in short cylinders of large diameter (5-7 x 7-9 μm),

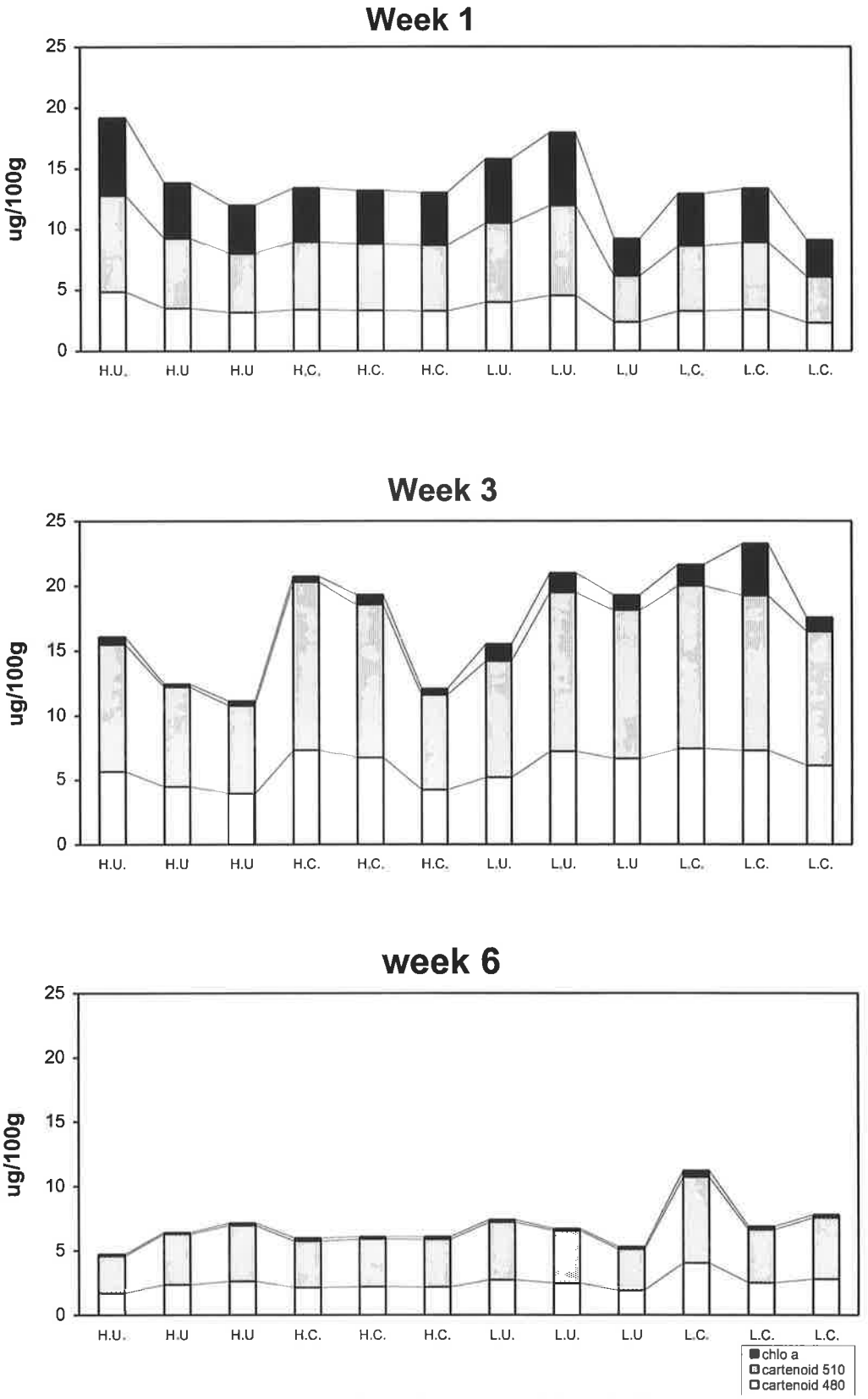

Figure 6.7. The comparison between chlorophyll *a* and carotenoid concentrations of samples from weeks 1, 3 and 6. Data are mean values of three measurements.

HU, high salinity-uncovered; HC, high salinity-covered; LU, low salinity-uncovered; LC, low salinity-covered.

while those which dominated in HSU aquaria were narrow cylinders of varying length (2-4 x 3-8 μm). Approximately 10 - 25% (in high and low salinity, respectively) of the *Synechococcus* comprised paired cells, showing cell division. In undisturbed samples, some cells were in the form of chains and embedded in the ECP produced by the cells. Two different colonies was observed in aquaria: yellowish and green colonies.

Microscopic examination of the samples indicated the presence of diatoms and *Dunaliella viridis* and *D. salina* as well as *Synechococcus* cells. Microscopic observation showed the high density of cocci and rods in HSU aquaria after week 4. These cocci and rods were probably colonies of Halobacteriaceae and imparted a red colour to the water. This was accompanied by parallel changes in the viscosity of the sample after week 4. *Dunaliella salina* and *Stephanoptera* sp. were abundant in high salinity aquaria in week 4, 5 and 6.

Microscopic observations also revealed that unicellular Cyanobacteria were more abundant in HSU and HSC aquaria up to week 4, that filamentous and unicellular Cyanobacteria and diatoms were present in LSC and LSU aquaria, and that diatoms were more abundant in those aquaria.

Microscopic observations provided estimates of the number of cells per 1 ml of *Synechococcus* standing crop in the sample. The densities of microorganisms in LSU and LSC samples from weeks 1, 2, 3, 4, 5, and 6 were approximately $1.2x10^3$, $1.5x10^3$, $1.8x10^3$, $1.4x10^3$, $2.1x10^3$, and $2.4x10^3$ individuals per ml, respectively. In the case of HSC and HSU, estimation was difficult due to the high amount of ECP produced by cells and the high viscosity of the medium.

6.4. Discussion

Most studies on coccoid Cyanobacteria inhabiting hypersaline waters are based on the examination of field material (e.g. studies reviewed by Bauld, 1981) or on laboratory studies of cultures in artificial media (e.g. Yopp *et al.*, 1978a and b). The present study was an attempt to simulate a natural field situation in easily controlled outdoor microcosms. Brine from ponds PA7 and FA1 supplied the low and high salinity water for experimental aquaria, respectively. The results on salinity and specific gravity show that these factors in the replicate low and high salinity aquaria were almost constant throughout the experimental period. Thus, there were no physiological shocks resulting from rapid salinity fluctuation in any aquarium. The results also showed that pH was always > 7, which is important in the survival of *Synechococcus* (Madigan, 1988). The microcosms were in high light environments, with shading providing filtered light to some aquaria. Appropriate light, salinity and temperature are required by halophilic Cyanobacteria (Dor and Hornoff, 1985) and the microcosms have provided suitable environments for *Synechococcus*. Some other Cyanobacteria have shown photoinhibition of productivity (Vonshak *et al.*, 1994; Rai, 1995; Vonshak, *et al.*, 1996)). They also reported that photosynthetic activity and responses to environmental stresses, such as salinity, light and temperature, varied in different strain of halophilic Cyanobacteria.

Synechococcus responded to the environmental stress of high salinity, light and temperature by the production of ECP. Increased production of ECP under stress is also reported by other studies on this taxon and other Cyanobacteria (Painter, 1983; Panoff *et al.*, 1988; Philips *et al.*, 1989; Vincenzini *et al.*, 1990a and b; De Philippis *et al.*, 1991 and 1993). *Synechococcus* is a nitrogen-fixing bacterium, with photosynthesis occurring in the day when sunlight is available, and nitrogen-fixation occurring at night when oxygen levels within cells are lower (Villbrandt *et al.*, 1990;

Stal, 1991). Thus, *Synechococcus* has advantages over other Cyanobacteria and algae in highly saline water with low oxygen concentration and low nitrate concentration (see Chapter 2, also Mague *et al.*, 1980; Mitsui *et al.*, 1986; Grobbelaar *et al.*, 1986; Arad, 1988; Schneegurt *et al.*, 1994; Liu *et al.*, 1996, Michard *et al.*, 1996).

The production of ECP is an osmotic response of *Synechococcus* in highly saline water; the ECP protect the cells from the stressful environment. The results demonstrate increasing relative viscosity due to the production of ECP by *Synechococcus* in hypersaline water and at high light intensity. They accord with published results for *Synechococcus* that show ECP increases at higher temperature, and in turn increases turbidity and viscosity (Yopp *et al.*, 1978a and b; Dor and Hornoff, 1985). The results from ^{13}C NMR spectroscopy indicate that the extracellular product was organic material, namely, glycoprotein and glycolipid. These molecules would have moderated the adjustment of the unicellular cyanobacterial cell to the hypersaline water: *Synechococcus* surrounds itself with material that can act as a barrier and may be important in (1) limiting desiccation of the cells under high salinity, (2) balancing ions, and (3) aiding the uptake of nutrients in highly saline water.

The relative amount of carotenoids in *Synechococcus* in the different treatments and the different colours of the *Synechococcus* material may be related to light intensity. *Synechococcus* had a green colour in the aquaria that were covered and a yellow to light brown colour in aquaria in direct sunlight. The yellow to brown colour in *Synechococcus* standing crop collected from HSU and LSU is probably related to the development of scytonemin. Garcia and Catenholz (1991) reported the presence of scytonemin in some Cyanobacteria found in habitats exposed to intensive solar radiation. They suggested that it is a protective mechanism in microalgae that

mitigates at least some of the harmful effects of UV. Both scytonemin and carotenoids in Cyanobacteria may act to decrease UV-produced toxic intermediates. The high carotenoids in uncovered aquaria were probably caused by the high UV under the experimental condition. Scytonemin (sheath pigments) absorbs strongly in the UV-A region, and its production is induced by UV-A and by carotenoids that may act to quench UV-produced toxic intermediates (Garcia-Pichel and Castenholz, 1991; Vincent and Roy, 1993). Thus, the yellow-brown colour in the standing crop of *Synechococcus* is the result of scytonemin production that is probably an adaptation (photoprotection) to high UV. This has previously has been proposed for Cyanobacteria in natural systems (Tripathi and Talpasayi, 1980; Muehlstein and Castenholz, 1983; Carr and Wyman, 1986) or others held experimentally (Garcia-Pichel and Castenholz, 1991). However, a more rigorous investigation of *Synechococcus* in stressful conditions of hypersaline water and high light intensity is needed. The absorption of light in the UV range increases as the salinity increases (Buch *et al.*, 1993). Phenotypic changes in the cell morphology of *Synechococcus* also were observed by microscopic examination. Morphological changes in *Synechococcus* from uncovered aquaria samples are probably also due to UV radiation (Van Baalen, 1973).

High salinity and Mg concentrations, and also high concentrations of ECP provide a suitable habitat for halobacteria. These therefore developed high densities and thus imparted a red colour to the brine (Fig. 6.8). Microscopic examinations revealed the presence of a great many red colonies of rod-shaped Halobacteria in the brine. These bacteria are chemoheterotrophs as reported by Nakashizuka and Arita (1993). Mg is a limiting factor for cell growth and the number of extremely halophilic bacteria cells increased with increasing amounts of Mg in high salinity media (4.0M NaCl). The brine from Dry Creek saltfield is high in Mg (see Chapter 1 and 5).

The density of halobacteria may have increased in samples from the high salinity aquaria in weeks 2, 3, and 4, due to the release of ECP into the brine, with the ECP a source of bacterial food. Interactions occurred after week 4 (Figure 6.2) because the community in the HSU and HSC aquaria then changed. Relative viscosity was lower in these samples suggesting that less ECP was available.

Dunaliella salina is abundant in highly saline samples in the saltfields at Dry Creek where it is the main primary producer. In these hypersaline brines, *Dunaliella* accumulates photosynthetically produced glycerol as an osmotic solute, so enabling the cell to withstand the high osmotic pressure of the external medium (Zamir, 1992). The chemoheterotrophic halophilic bacteria probably derive most of their carbon from ECP produced by *Synechococcus* and from glycerol produced by *Dunaliella salina* (Rodriguez-Valera *et al.,* 1980 and 1981; Borowitzka, 1981; Javor, 1984; Tindall, 1991).

6.5. Conclusion

The effect of light and salinity on ECP production has been experimentally investigated in twelve aquaria (high salinity uncovered and covered, low salinity uncovered and covered) for six weeks during summer 1996. The amount of ECP produced by S*ynechococcus* affects brine viscosity and this is important in determining salt quality and quantity in solar saltfields (see Chapter 5). Relative viscosity was higher in conditions of high salinity and high light intensity, because under these conditions S*ynechococcus* produced more ECP.

High salinity caused osmotic stress for S*ynechococcus* under the experimental conditions. The ECP thus provides a microenvironment that acts as a barrier and prevents cell desiccation. Moreover, it may to balance ions and promotes the uptake of nutrients in highly saline water. The results from ^{13}C NMR spectroscopy showed

that the composition of the ECP produced by *Synechococcus* under experimental conditions comprised glycolipid and glycoprotein.

The results of ash free dry weight (AFDW) determinations of *Synechococcus* standing crop showed that greater amounts of organic material were produced under high salinity and high light intensity than under low salinity and low light intensity. Part of the organic material produced by *Synechococcus* is released to the external medium and increases brine viscosity (see Chapter 5). Another part remains associated with the cells and thus contributes to AFDW.

Both high light intensity and temperature led to ECP production. It is probable that not only light intensity but rather; UV light also appears to be important in ECP production. The amount of ECP produced by *Synechococcus* was higher in the uncovered than covered treatments (covering decreased the amount of UV light). The cells of *Synechococcus* were yellow-light brown in uncovered conditions and cells showed developed scytonemin (sheath pigments) and carotenoids. Both of these products can reduce UV-produced toxic intermediates.

The organic material produced by *Synechococcus* also provided food for halobacteria that were abundant from week four in the high salinity treatments. The high density of halobacteria decreased the viscosity due to the consumption of ECP. Highly saline water at Dry Creek saltfields has a high concentration of Mg. Thus, the conditions of high salinity, high Mg concentration and high concentrations of ECP provide favourable conditions for halobacteria.

CHAPTER SEVEN

CONCLUSION

7.1. Summary

The solar salt fields at Dry Creek comprise a series of interconnected evaporating and crystallising ponds where seawater is evaporated and the concentration of sodium chloride increases until sodium chloride precipitates and is harvested. They comprise 44 shallow evaporating ponds and 8 crystallisers, extending over 4040 ha along the coast north of Adelaide. The shape of each pond is irregular, as determined by naturally occurring banks and high ground contours. The mean depth of the shallow evaporation ponds varies from 1.25 to 1.5 m, and the mean depth of the crystalliser ponds is approximately 15 cm. The evaporating ponds are relatively deep with respect to other salt fields in the world. Pond infiltration is a significant problem at Dry Creek because of the high permeability of the substratum, mainly clay to shell grit. Salinity of the ponds was between 40 to 350 g/L. Calcium carbonate begins to precipitate at salinity about 70 g/L while gypsum and other minerals precipitate as the salinity increases further. Thus the brine above the salinity of carbonate precipitation is not comparable to seawater, as the ionic composition as well as the osmotic pressure is quite different from seawater.

The aims of this research was to evaluate the biological and physicochemical parameters and their interactions with the production of salt at Dry Creek solar saltfields in South Australia.

7.1.1. Ecology of the ponds

Nutrient and other chemical and physical parameters, including salinity, alkalinity, pH, dissolved oxygen, temperature, wind and rainfall, which control the composition of the biota and salt production in the solar saltfields, were measured in eight ponds at Dry Creek solar saltfields over the period March 1994 to September 1995.

The averages during the study period for evaporation and rainfall in the area are 2032 and 420 mm; both were less than average and were 1952.9 and 286.6 mm, respectively. The climate is usually hot and dry from November to March with a wet season from July to September. In the study period, the mean monthly water temperature ranged from 10.7-22.7 C°. The lowest and the highest value were 5.6 and 29.5 in July 1994 and January 1995, respectively.

Ponds XD1, XC3, PA7, PA9 and PA12 were clear throughout the study period, except for pond PA12 in December 1994 due to the hot weather and dissolution of gypsum in the brine. Transparency varied in ponds XB3 and PA3 due to massive blooms of algae in these ponds. Pond XB8 was turbid through the study period due to persistently high algal biomass related to high nutrient content in the system.

Mean values of salinity in the ponds studied ranged from 55.5 to 243.5 g/L. The systems were alkaline and low in oxygen concentrations (0.4-9.5 mg/L). Significant correlations between salinity and dissolved oxygen ($r=0.98$, $p<0.05$) and salinity and alkalinity ($r=0.71$, $p< 0.05$) were present.

The mean values of soluble reactive phosphate and nitrate-nitrogen in the study ponds were 7-22 and 4-19 μg/L, respectively. Excessive nutrients that enter the ponds with intake seawater from Chapman Creek were rapidly taken up and caused

high biological productivity in several ponds. The major source of these nutrients is from the outfall of the nearby Bolivar sewage works. The accurate determination of phosphate and nitrate was essential in this investigation. However, standard methods of analysis lead to significant errors when applied to saline water. Salt effects for both phosphate and nitrate analyses have been reported. For this reason, analytical methods for estimation of both NO_3-N and PO_4-P in the saline water from solar saltfields at Dry Creek were critically evaluated. The ascorbic acid with antimony (III) phospho-molybdenum blue methods was used for phosphate, and the Cd column technique followed by colorimetric procedure was used for nitrate measurements. The phospho-molybdenum blue method is the most useful method for phosphate determination in saline water with minimal salinity interferences. There are substantial salt errors when using the Cd column technique followed by colorimetric procedure for nitrate estimation. Methods involving dilution, standard addition or applying a salt error correction are essential for accurate determination.

Three different areas, initial, preliminary and final areas, contain different organisms with those species less tolerant of high salinity being gradually replaced by more tolerant species. The initial ponds, near the area of seawater intakes have higher diversity and contain marine brackish species. The initial ponds are characterised mainly by benthic algae with extensive meadows of seagrass. The fauna include fishes, gastropods, isopods, amphipods, copepods (calanoids, cyclopoids and harpacticoids), ostracods and insects (Trichoptera and Diptera). The water typically is clear. The fish, *Atherinosoma microstoma* and *Pseudogobious olorum*, have been recorded from study ponds with salinity 46-110 g/L. Two isopods, (*Exosphaeroma bicolor* and *Synischis* sp.), one amphipod, (*Parhyalella* sp.), one gastropod (*Hydrododdus tasmanicus*) and *Palaemon serenus*, a large swimming prawn in pond XD1 at a salinity of 55 g/L, represent tolerant macrospecies. The abundance of large marine invertebrates and fishes may prevent the growth of benthic mats in pond XD1. *Acartia* is the main copepod and tolerates

salinity up to 110 g/L. As salinity increases through the series of ponds, species diversity falls and finally only halotolerant species remain. *Diacypris dictyote* and *Reticypris herbsti* were present at a salinity of 55.5-137.5 g/L. They did not occur at higher salinities probably not only due to high salinity but also due to deficiency of some ions, such as carbonates (see Chapter 2). The occurrence of *Symphitoneuria wheeleri* was notable because this insect is rare in saline water and did not belong to the Philanisidae, the family containing the only known marine Trichoptera. Two species of Chironomids (*Cladotanytarsus* sp. and *Tanytarsus barbitarsis*) and one species of Ephydridae (?*Ephydra riparia*), occurred in most ponds. The planktonic community of these highly saline evaporating ponds consists mainly of *Artemia fransciscana* and *Parartemia zietziana*. Seasonal variation in zooplankton populations reflects seasonal changes in temperature, light, nutrients and algal abundance. Thus, the presence of zooplankton is governed primarily by its salinity tolerance and its abundance by trophic conditions.

Forty two species of algae, dominated by diatoms and Cyanobacteria, were present in the evaporating areas. Differences in phytoplankton density occurred between ponds XD1 and XC3 (low nutrient) and ponds XB3 and XB8 (high nutrient), and also in pond PA3 where mixing took place. These ponds with high nutrients were slightly eutrophic and their community was dominated by planktonic algae.

The phytoplankton community, algae and cyanobacteria, is the only source of primary production in high salinity water. Here diatoms are the dominant forms at salinity below 150 g/L, and Cyanobacteria and green algae, *Dunaliella* and *Stephanoptera*, dominate at above 150 g/L. Benthic mat communities covered the bottom of higher salinity ponds (>150 g/L) and they are the major source of primary production in these ponds. Benthic mats are important in increasing evaporation, oxygenating the brine and recycling the organic matter, reducing permeability and preventing leakage. Cyanobacteria, especially *Synechococcus*, is the dominant form

in benthic mats at salinity > 180 g/L. *Synechococcus* produced extracellular material that affected salt quality and quantity.

7.1.2. *Synechococcus* and saltfield biology in salt production

The effects of the extracellular material produced by *Synechococcus* were tested in a field experiment which used microcosms to investigate how *Synechococcus* affected the quantity and quality of salt produced. Five tanks were filled with brine from salt ponds and inoculated with different amounts of *Synechococcus*. The tanks were monitored for 12 weeks in two stages. In stage I, *Synechococcus* was present in evaporating tanks until the brine concentration increased to the point of sodium chloride crystallisation. Regular measurements were made of salinity and viscosity in different treatments. In stage II, *Synechococcus* was excluded and salt was allowed to crystallise in the tanks; deposited salt was harvested at the end of the experimental period. The values of pH in the brine ranged from 7.3 to 9. The value for salinity ranged from 210 to 326.5 g/L and correspondingly, for specific gravity, from 1.1495 to 1.2278 during the period of experiment. The relative viscosity increased throughout stage I; it ranged from 1.103 to 1.290, 1.301, 1.336, 1.346 and 1.353 in tanks 1, 2, 3, 4 and 5, respectively. The highest value occurred in tank #5 which contained the highest amounts of *Synechococcus*. The salt quality harvested from each treatments was evaluated by the dry sieve technique and using a Scanning Electron Microscope (SEM) and Energy Dispersion X-Ray Analyser (EDX) to determine crystal form, size and elemental composition, thus to determine impurities in the harvested salt. The results showed that the size and shape of salt crystals harvested are affected by liberated organic material (ECP) produced by *Synechococcus*, and that more brine is retained in these salt crystals which affects the composition of the salt and leads to a decreased in the quality of salt crystals. Data on size distribution of salt crystals showed that it was unimodal for all tanks and ranged from 0.125 to 4 mm. Overall, the largest crystals were from the control

tanks (tank #1), and the smallest from the high *Synechococcus* tank (tank # 5). Moreover, the quantity of harvested salt decreased with increasing the amount of *Synechococcus* due to the decreasing percentage of evaporating water.

The effect of light and salinity on the production of extracellular material (ECP) by *Synechococcus* were tested using aquaria as microcosms in an experiment with high salinity uncovered and covered and low salinity uncovered and covered treatments. Twelve aquaria with salinity 190.5 g/L and 285 g/L were filled with brine from salt ponds and inoculated with the same amounts of *Synechococcus*. High salinity caused osmotic stress for S*ynechococcus* under experimental condition. The photosynthetic activity and its response to the environmental stresses, such as high salinity, high light intensity and high temperature, affected on the amount of ECP production by S*ynechococcus*. The ECP produced by *Synechococcus* built a microenvironment that may act as a barrier and be important in (1) limiting desiccation of the cells under high salinity (2) balancing ions (3) aiding the uptake of nutrients in highly saline water. The results of ash free dry weight (AFDW) of the S*ynechococcus* standing crop showed that more organic material was produced under high salinity and high light intensity than at low salinity and low intensity of light. Part of this organic material produced by S*ynechococcus* was released to the medium and increased the viscosity of the brine. Thus, relative viscosity was higher in the high salinity and high intensity of light due to the ECP produced by S*ynechococcus*.

The compositional analysis of ECP by ^{13}C NMR spectroscopy indicate that the extracellular material produced by S*ynechococcus* under experimental conditions was organic material, namely glycolipid and glycoprotein.

Not only the intensity of light but probably the UV wavelength of light is important in ECP production. The amount of ECP produced by S*ynechococcus* was higher in

the uncovered than covered treatments (covered status decreased the UV wavelength). The cells of *Synechococcus* were yellow-light brown colour when uncovered and green when covered. The yellow-brown colour in *Synechococcus* collected from high and low salinity uncovered was probably due to the presence of scytonemin (sheath pigments) and carotenoids in the cells. They can act to reduce the UV produced toxic intermediates. However, more research needs to be undertaken to clarify this.

The organic material produced (ECP) by *Synechococcus* also provides food for halobacteria that were abundant from week four in the high salinity treatments. Highly saline water at Dry Creek saltfields, which was the source of water for this experiment, is high in Mg. Therefore, high salinity, high Mg concentration, and also high concentration of ECP provide a suitable habitat for halobacteria and produce a red coloured brine at a salinity > 250 g/L.

It was concluded from this experiment that the amount of ECP produced by *Synechococcus* increased under natural condition in highly saline water (249-256 g/L) during the summer time. This affects the quality and quantity of salt produced at the solar saltfields.

7.2. Management

The information collected from the physicochemical, biological and experimental investigation can be used to make appropriate recommendations about solar salt pond management. Proper management of biological systems is essential for production of high quality salt.

A basic prerequisite for correct solar saltfields management is regular evaluation of the environmental conditions of different ponds. Thus, the physico-chemical

parameters, such as air and water temperature, water depth, transparency, water density, dissolved oxygen, pH and nutrients, must be regularly monitored.

Solar saltfields may be characterised by either low nutrients and low organic productivity or high nutrients and high organic productivity; both can produce high quality salt under good management practices. A base line study of the nutrients and organisms in solar saltfield indicates the health of the system. Since maintenance of a healthy phytoplankton population is considered to be one of the most important keys for a successful solar saltfields, regular monitoring of nutrient levels and associated standing crops of phytoplankton are important for proper saltfields management. A trained saltfield biologist can use these data to determine if and how changes can be made in solar saltfields to improve salt production. As an example at Dry Creek solar saltfields, the amount of incoming nutrient from the two sources of water, Chapman Creek and Middle Beach, is different. When transparency levels are high (> 30 cm) or pond nutrient levels become undetectable or fall below levels found in intake seawater from Middle Beach, intake of nutrient-rich seawater from Chapman Creek should be considered. Thus, the amount of seawater transfer to the field and the concentration of nutrients should be regularly controlled to ensure appropriate of nutrient levels in the fields which affect both biology and salt production (see Chapters 3 and 5).

Monitoring involves analysis of reactive phosphate (the major form of phosphorus required for algal cells) and reactive nitrate by standard colorimetric procedures; salt error correction is needed for nitrate analyses. (see Chapter 4). The phytoplankton population should be analysed more often in the production season. Phytoplankton densities may be determined from a representative sample by direct microscopic counting or by chlorophyll measurements. Whenever possible, species composition and cell size of the algal population should be determined, as the first may directly affect the nutritional value to the brine shrimp population which is important in salt

quality (see Chapter 3). This also determines whether the algal cells (especially when forming colonies) are small enough ($\leq$ 50 μm) for ingestion by brine shrimp. Other means of estimating phytoplankton densities are water turbidity, the concentration of chlorophyll, phytoplankton dry weight, or primary productivity. Estimation of *Artemia* densities, among other biological field data, may provide a valuable management tool for producing high quality salt. During temperature/salinity stratification (which can cause lethal high temperatures for *Artemia*) or when planktonic blue green algae become dominant, the bottom flow of water from pond to pond should be maximised breaking the stratification and increasing the nutrients. Encouragement or enhancement of desirable phytoplankton species and the prevention of the development of cyanophytes, which have the ability to adapt to high salinity and low nitrate concentration, may also be aided by addition of fertilisers during conditions where nitrogen is limiting but phosphorous is abundant. Application of silicate to enhance the growth of diatoms should be considered, as they do not produce high amounts of ECP and also are better food for brine shrimp. Some solar saltfields use groundwater as a part of their water source. Silicon concentration in groundwater is often higher than in seawater. Recently (from mid 1996 and after the completion of the monitoring in this study), groundwater was used at Dry Creek saltfields in the evaporating ponds. The higher silicon may help the growth of diatoms at higher salinity and prevent the growth of cyanobacteria, especially *Synechococcus*, which is abundant at high salinity and has deleterious effects on the salt quality and quantity harvested from the saltfields.

7.3. Final conclusion

It is concluded from this study that physico-chemical parameters are important factors in salt production at solar saltfields. High temperatures and salinity, lower oxygen concentrations and varied ionic systems due to the precipitation at different stages are factors that affect the biology and also salt production of these high saline

systems. The meteorological parameters and the amounts of nutrients control the productivity of the system and also the spatial and temporal changes that occur annually in the saltfields. High salinity in the systems may cause salt error in the determination of phosphate and nitrate in high saline water. The ascorbic acid with antimony (III) phospho-molybdenum blue method is the most useful method for phosphate determination. The routine Cd column technique followed by colorimetric procedure for nitrate determination produces salt errors; these can overcome by addition techniques or applying a salt error correction. The presence of *Synechococcus* in highly saline water affects the quality and quantity of harvested salt at the solar saltfields. Monitoring at solar saltfields could help their better management which should aim to enhance desirable phytoplankton species and to prevent the growth of *Synechococcus* and also to increase the population of brine shrimp. In future, computer models may become tools in management of solar saltfields by using long term data from such monitoring to predict biological outcomes and associated outcomes in the quality and quantity of salt produced.

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APPENDICES

APPENDIX 1

PHYSICO-CHEMICAL PARAMETERS

1.1. Meteorological measurements.

Apr-94

Day	Gross evaporation (mm)	Rain (mm)	Net evaporation (mm)	Max. Temp. (°C)	Min. Temp. (°C)	Wind speed (km/hr)
1	5.0	0.9	4.1	26.5	16.0	5.5
2	7.2	0.0	7.2	20.5	14.5	7.1
3	3.6	0.0	3.6	19.5	14.0	3.7
4	4.1	0.0	4.1	21.0	12.0	1.4
5	4.7	0.0	4.7	26.0	13.0	0.9
6	6.9	0.0	6.9	28.0	15.0	6.3
7	7.6	0.0	7.6	19.0	7.5	10.7
8	3.7	0.0	3.7	19.0	8.5	3.0
9	2.7	0.0	2.7	22.0	9.0	0.6
10	6.5	0.0	6.5	25.0	10.5	7.6
11	8.0	0.0	8.0	18.0	11.0	10.7
12	4.2	0.0	4.2	18.0	10.0	6.1
13	3.4	0.0	3.4	19.0	11.0	4.2
14	2.1	0.0	2.1	20.0	11.0	2.5
15	3.4	0.0	3.4	20.0	11.0	16.0
16	2.9	0.0	2.9	24.0	11.0	0.8
17	3.8	0.0	3.8	25.5	11.0	1.3
18	4.7	0.0	4.7	20.0	9.0	6.6
19	3.8	0.0	3.8	18.0	12.5	1.1
20	3.3	0.0	3.3	18.5	15.0	2.3
21	3.0	0.0	3.0	19.5	8.0	1.2
22	3.4	0.0	3.4	21.0	8.0	0.4
23	2.5	0.0	2.5	22.5	8.5	0.5
24	4.4	0.0	4.4	26.0	8.5	0.3
25	5.4	0.0	5.4	29.0	11.0	1.0
26	6.4	0.0	6.4	27.0	14.5	5.2
27	8.0	0.0	8.0	29.0	12.0	8.5
28	3.4	0.0	3.4	24.0	12.0	1.4
29	2.7	1.5	1.2	20.0	11.0	5.6
30	4.2	0.0	4.2	19.0	10.0	0.9
Total	135.0	2.4	132.6			
Average				22.2	11.2	3.6

May-94

1	3.7	0.0	3.7	23.0	10.0	2.3
2	4.4	0.0	4.4	20.0	11.0	2.8
3	3.4	0.0	3.4	19.0	9.5	2.9
4	3.2	0.0	3.2	18.0	7.0	2.0
5	4.5	0.0	4.5	19.5	5.0	1.1
6	3.1	0.0	3.1	22.5	5.0	0.1
7	2.6	0.0	2.6	22.0	8.0	0.3
8	3.5	0.0	3.5	20.0	8.0	2.2
9	3.4	0.8	2.6	17.0	7.0	2.4
10	2.9	0.5	2.4	8.0	9.0	3.2
11	2.7	1.1	1.6	15.5	8.0	2.5

May-94 (cont.)

Day	Gross evaporation (mm)	Rain (mm)	Net evaporation (mm)	Max. Temp. (°C)	Min. Temp. (°C)	Wind speed (km/hr)
12	1.8	0.0	1.8	15.7	7.0	1.0
13	3.1	0.0	3.1	17.0	6.5	2.3
14	5.4	7.7	-2.3	19.0	9.0	10.4
15	1.5	0.2	1.3	17.5	11.0	2.2
16	1.9	0.0	1.9	16.0	6.5	0.7
17	2.1	0.0	2.1	20.0	6.5	1.2
18	3.2	1.8	1.4	19.0	7.5	18.2
19	5.3	3.6	1.7	16.5	13.0	14.2
20	1.9	0.3	1.6	16.0	7.0	2.8
21	6.1	0.0	6.1	20.0	7.5	8.9
22	3.0	0.0	3.0	18.0	14.0	16.8
23	0.6	0.6	0.0	18.0	10.0	4.5
24	6.2	0.0	6.2	22.5	10.5	9.6
25	8.3	2.8	5.5	24.0	11.0	23.4
26	4.0	4.0	0.0	21.5	11.0	21.1
27	2.1	0.0	2.1	17.5	12.5	16.8
28	6.0	0.0	6.0	21.0	13.0	5.2
29	3.4	0.0	3.4	18.0	8.0	3.8
30	4.1	0.0	4.1	21.0	8.5	2.1
31	6.2	2.2	4.0	22.5	13.5	12.8
Total	108.2	25.6	82.6			
Average				18.9	9.1	6.4

Jun-94

1	0.8	0.0	0.8	19.0	8.0	NA
2	2.0	0.0	2.0	18.0	17.5	0.7
3	3.8	0.0	3.8	22.5	11.0	2.2
4	1.7	0.0	1.7	23.0	13.6	1.1
5	1.0	1.0	0.0	16.0	10.5	0.4
6	0.8	15.4	-14.6	13.0	10.0	0.7
7	1.0	8.6	-7.6	13.0	10.5	6.4
8	0.3	0.7	-0.4	16.0	7.0	0.1
9	1.4	0.0	1.4	14.5	5.5	0.1
10	1.2	0.0	1.2	16.5	5.0	0.1
11	0.9	0.2	0.7	17.1	6.0	0.3
12	1.1	2.0	-0.9	15.5	9.8	4.3
13	1.6	0.2	1.4	17.0	6.5	1.7
14	1.4	6.5	-5.1	15.5	10.0	7.6
15	1.3	5.0	-3.7	15.5	8.3	3.8
16	1.4	1.1	0.3	14.5	8.0	1.4
17	1.4	1.0	0.4	16.5	8.0	7.7
18	1.6	1.5	0.1	16.0	9.5	4.3
19	1.5	3.6	-2.1	15.5	8.5	16.5
20	2.0	0.1	1.9	14.6	8.0	3.6
21	1.5	0.0	1.5	16.5	8.0	1.1
22	2.8	0.0	2.8	17.0	8.5	7.7

Jun-94 (cont.)

Day	Gross evaporation (mm)	Rain (mm)	Net evaporation (mm)	Max. Temp. (°C)	Min. Temp. (°C)	Wind speed (km/hr)
23	2.6	0.0	2.6	16.0	9.0	5.9
24	0.9	12.8	-11.9	13.5	9.0	15.7
25	0.7	2.4	-1.7	15.0	11.0	8.3
26	3.5	9.0	-5.5	13.0	8.0	5.2
27	1.1	1.6	-0.5	14.0	3.0	1.9
28	0.8	0.2	0.6	14.5	3.5	0.5
29	1.0	0.0	1.0	14.8	4.2	0.5
30	1.2	0.0	1.2	15.5	5.0	1.0
Total	44.3	72.9	-28.6			
Average				16.0	12.4	3.8

Jul-94

1	1.3	0.0	1.3	16.0	5.0	0.2
2	2.1	0.0	2.1	14.5	5.0	4.0
3	3.0	0.0	3.0	16.0	7.0	5.3
4	2.5	0.0	2.5	17.0	7.5	1.1
5	1.5	0.0	1.5	19.0	7.5	0.8
6	1.3	0.0	1.3	20.1	4.5	0.8
7	1.5	0.0	1.5	20.0	5.0	1.3
8	1.5	0.0	1.5	21.2	6.1	0.3
9	2.8	0.0	2.8	20.5	6.5	1.0
10	2.1	0.0	2.1	21.0	10.0	1.1
11	1.6	1.1	0.5	22.0	8.0	5.8
12	2.5	1.6	0.9	16.0	8.0	8.5
13	1.9	1.0	0.9	14.0	9.6	7.3
14	1.7	0.0	1.7	16.2	7.8	3.1
15	2.3	0.0	2.3	17.0	6.0	4.9
16	1.5	2.1	-0.6	16.1	6.0	2.8
17	1.6	0.2	1.4	14.0	1.5	0.7
18	1.5	0.0	1.5	13.8	1.5	0.6
19	1.3	0.0	1.3	16.0	2.0	0.1
20	1.4	0.0	1.4	15.0	3.5	0.8
21	1.2	0.0	1.2	14.0	4.0	0.1
22	0.7	0.0	0.7	17.0	3.5	0.1
23	1.7	0.0	1.7	18.0	3.5	0.2
24	2.6	0.0	2.6	16.0	5.0	2.5
25	2.6	0.0	2.6	15.5	7.5	5.8
26	1.9	0.0	1.9	14.0	7.2	0.7
27	1.8	0.0	1.8	14.0	5.0	1.3
28	2.2	0.0	2.2	15.3	5.3	3.8
29	4.2	0.0	4.2	19.5	7.3	9.9
30	18.0	31.0	-13.0	12.8	4.5	4.5
31	3.3	5.5	-2.2	12.5	4.0	12.7
Total	77.1	42.5	34.6			
Average				16.6	5.6	3.0

Aug-94

Day	Gross evaporation (mm)	Rain (mm)	Net evaporation (mm)	Max. Temp. (°C)	Min. Temp. (°C)	Wind speed (km/hr)
1	3.8	1.4	2.4	13.0	10.0	6.4
2	1.1	1.6	-0.5	14.0	8.0	4.0
3	0.6	0.0	0.6	13.0	7.5	0.8
4	1.6	0.0	1.6	15.0	6.0	0.8
5	2.5	0.0	2.5	16.0	6.0	2.6
6	2.4	0.1	2.3	16.8	9.0	9.2
7	2.8	4.8	-2.0	15.0	9.5	10.4
8	2.7	2.7	0.0	12.0	3.5	15.5
9	2.4	2.0	0.4	12.5	3.5	5.1
10	1.1	0.5	0.6	11.5	2.0	1.8
11	2.5	0.0	2.5	13.5	2.5	3.7
12	4.6	0.0	4.6	14.5	8.0	7.4
13	0.1	5.5	-5.4	14.0	7.0	10.8
14	3.5	0.3	3.2	12.5	3.0	4.1
15	1.2	0.5	0.7	13.0	5.0	2.0
16	0.8	0.0	0.8	13.0	7.0	0.0
17	2.2	0.0	2.2	13.5	3.0	1.0
18	2.2	0.0	2.2	16.5	4.0	0.4
19	2.1	0.0	2.1	14.5	7.0	1.7
20	1.3	0.0	1.3	14.0	7.0	0.9
21	3.0	0.0	3.0	16.0	5.0	1.0
22	1.6	0.0	1.6	15.0	6.0	4.3
23	2.8	1.1	1.7	17.0	7.5	10.4
24	2.7	0.0	2.7	14.5	5.0	3.1
25	1.6	0.0	1.6	14.5	7.0	3.4
26	1.9	0.0	1.9	17.0	6.0	1.7
27	2.6	0.0	2.6	21.5	7.5	0.3
28	4.7	0.0	4.7	22.5	7.5	5.5
29	2.9	1.7	1.2	19.5	9.5	1.7
30	3.4	0.0	3.4	16.0	7.5	3.0
31	4.1	0.0	4.1	19.5	8.0	2.7
Total	72.8	22.2	50.6			
Average				15.3	6.3	3.8

Sep-94

1	3.9	0.0	3.9	23.2	11.0	2.8
2	5.6	0.0	5.6	25.0	9.5	5.5
3	1.3	5.2	-3.9	20.5	11.0	0.7
4	0.3	5.0	-4.7	18.0	11.0	5.0
5	2.3	0.1	2.2	16.5	6.0	1.4
6	2.9	0.4	2.5	13.5	8.5	5.9
7	1.3	0.0	1.3	15.0	8.5	2.4
8	3.7	0.0	3.7	18.0	9.0	2.1
9	2.7	0.0	2.7	19.0	5.5	2.9
10	1.6	0.0	1.6	16.2	5.5	1.2
11	3.5	0.0	3.5	18.8	6.0	2.4
12	8.6	3.3	5.3	13.0	12.0	12.0
13	3.8	1.0	2.8	15.5	10.5	10.9

Sep-94 (cont.)

Day	Gross evaporation (mm)	Rain (mm)	Net evaporation (mm)	Max. Temp. (°C)	Min. Temp. (°C)	Wind speed (km/hr)
14	3.1	0.0	3.1	14.0	4.0	2.1
15	1.9	0.4	1.5	17.0	3.0	3.8
16	5.4	0.2	5.2	17.0	6.0	7.6
17	4.0	0.0	4.0	15.0	6.5	4.1
18	3.5	0.0	3.5	16.0	8.0	7.2
19	4.0	0.0	4.0	15.0	5.0	6.1
20	5.2	0.0	5.2	15.0	7.5	8.2
21	4.3	0.4	3.9	13.5	10.0	8.1
22	3.8	0.0	3.8	17.0	7.0	1.5
23	3.3	0.0	3.3	19.5	6.5	8.7
24	3.5	0.8	2.7	16.0	9.0	7.4
25	4.4	0.0	4.4	15.8	9.0	4.0
26	3.5	0.0	3.5	16.0	9.0	2.3
27	4.6	7.0	-2.4	15.0	5.5	6.8
28	3.5	0.0	3.5	15.0	7.0	2.0
29	3.7	2.2	1.5	17.5	10.0	11.8
30	5.3	1.6	3.7	15.5	7.0	7.1
Total	108.5	21.3	87.2			
Average				16.7	7.8	5.0

Oct-94

1	5.3	0.0	5.3	20.5	9.5	11.5
2	1.2	5.6	-4.4	17.5	8.0	3.8
3	2.8	2.8	0.0	16.0	7.5	6.6
4	6.5	13.6	-7.1	19.0	8.0	7.6
5	3.0	3.4	-0.4	16.5	10.0	7.7
6	4.6	0.2	4.4	18.0	10.0	3.7
7	2.9	3.8	-0.9	17.5	8.0	3.5
8	4.3	1.0	3.3	14.0	10.0	12.8
9	4.1	0.1	4.0	16.0	12.0	6.5
10	4.2	0.0	4.2	17.5	7.5	13.5
11	4.4	0.0	4.4	23.5	9.5	4.9
12	4.7	0.0	4.7	20.0	10.0	2.8
13	3.6	0.0	3.6	22.5	10.5	4.0
14	4.3	0.0	4.3	29.0	15.0	7.8
15	7.2	0.0	7.2	34.0	18.0	4.6
16	8.2	0.0	8.2	33.9	17.5	2.6
17	6.0	0.0	6.0	21.0	12.2	2.8
18	5.5	0.0	5.5	17.0	11.0	5.9
19	4.0	0.0	4.0	18.5	12.0	15.5
20	5.6	0.0	5.6	18.0	11.0	4.7
21	5.2	0.0	5.2	18.0	7.5	3.2
22	5.4	0.0	5.4	19.2	6.5	1.2
23	5.1	0.0	5.1	24.5	8.5	2.4
24	7.8	0.0	7.8	29.0	17.0	13.2
25	7.1	0.0	7.1	29.0	21.0	0.1
26	5.1	1.2	3.9	26.0	15.0	46.1

Oct-94 (cont.)

Day	Gross evaporation (mm)	Rain (mm)	Net evaporation (mm)	Max. Temp. (°C)	Min. Temp. (°C)	Wind speed (km/hr)
27	4.1	0.0	4.6	22.0	11.0	65.2
28	6.8	0.0	6.8	19.0	13.5	15.1
29	7.4	1.1	6.3	17.0	12.5	6.7
30	3.0	0.9	2.1	17.0	12.5	1.5
31	5.7	0.0	5.7	22.0	13.5	5.0
Total	155.6	33.7	121.9			
Average				21.1	11.5	9.4

Nov-94

1	10.3	1.9	8.4	29.0	10.5	13.7
2	3.3	9.6	-6.3	13.0	11.0	10.2
3	2.1	1.7	0.4	15.0	11.0	7.7
4	1.7	1.3	0.4	14.5	10.0	12.5
5	3.3	4.6	-1.3	15.5	11.8	5.6
6	4.1	1.0	3.1	17.0	13.0	1.4
7	4.4	1.7	2.7	17.0	13.0	2.3
8	6.3	0.0	6.3	16.5	12.8	1.2
9	4.4	0.0	4.4	18.0	8.0	3.0
10	3.2	0.0	3.2	27.0	11.0	5.3
11	7.5	0.0	7.5	33.0	15.5	10.2
12	3.5	0.0	3.5	24.0	15.0	8.4
13	3.5	0.0	3.5	16.5	11.3	1.9
14	5.3	1.8	3.5	17.3	9.7	2.8
15	6.7	0.0	6.7	19.5	11.5	2.9
16	7.0	0.0	7.0	21.0	10.0	5.1
17	6.1	0.0	6.1	24.0	11.2	1.9
18	7.0	0.0	7.0	27.5	16.0	5.8
19	10.4	0.0	10.4	32.2	20.0	1.5
20	40.6	1.0	39.6	32.0	14.2	2.6
21	7.8	1.6	6.2	32.0	10.5	5.0
22	6.4	0.0	6.4	17.0	12.0	7.2
23	4.8	0.0	4.8	19.5	11.0	6.2
24	8.3	0.0	8.3	30.0	17.5	3.6
25	9.4	3.3	6.1	30.0	16.0	2.7
26	6.8	0.0	6.8	26.0	16.0	4.7
27	7.7	0.0	7.7	32.5	16.5	5.0
28	9.2	0.0	9.2	20.0	12.0	8.2
29	7.5	0.0	7.5	25.0	12.5	2.7
30	7.8	0.0	7.8	19.0	10.0	NA
Total	186.4	29.5	156.9			
Average				22.7	12.7	5.0

Dec-94

Day	Gross evaporation (mm)	Rain (mm)	Net evaporation (mm)	Max. Temp. (°C)	Min. Temp. (°C)	Wind speed (km/hr)
1	9.1	0.0	9.1	19.5	11.0	20.8
2	8.3	0.0	8.3	23.5	13.5	4.7
3	10.0	0.0	10.0	30.2	20.5	4.9
4	11.9	0.0	11.9	35.5	23.2	5.1
5	14.4	0.0	14.4	37.0	22.5	5.5
6	12.9	0.0	12.9	37.5	25.0	4.6
7	11.1	2.0	9.1	35.0	18.0	10.5
8	9.7	4.3	5.4	19.5	11.0	6.6
9	10.2	0.0	10.2	20.5	10.5	11.1
10	5.8	0.0	5.8	23.0	12.0	3.5
11	8.2	0.0	8.2	28.0	14.0	1.5
12	10.1	0.0	10.1	34.0	17.7	5.3
13	11.0	0.0	11.0	36.0	17.0	7.8
14	9.4	0.0	9.4	24.5	13.5	6.8
15	10.9	0.0	10.9	27.0	15.0	6.4
16	9.0	0.0	9.0	30.5	15.0	3.2
17	9.1	0.0	9.1	27.0	16.5	0.4
18	8.9	0.0	8.9	32.0	16.5	0.9
19	12.3	0.0	12.3	38.5	22.5	3.1
20	10.0	0.0	10.0	35.0	23.5	7.9
21	8.5	1.0	7.5	34.0	22.5	8.2
22	9.8	0.0	9.8	34.0	24.0	6.0
23	9.7	0.0	9.7	23.5	13.5	8.0
24	11.1	0.0	11.1	22.5	15.0	12.4
25	8.3	0.0	8.3	23.0	12.0	8.1
26	6.6	0.0	6.6	21.0	11.7	3.9
27	7.7	0.0	7.7	26.5	14.0	2.6
28	8.0	0.0	8.0	30.5	17.5	1.5
29	11.1	0.0	11.1	23.0	13.0	9.8
30	9.0	0.0	9.0	22.0	11.0	7.0
31	7.0	0.0	7.0	23.5	12.0	5.2
Total	299.1	7.3	291.8			
Average				28.3	16.3	6.2

Jan-95

1	10.2	0.0	10.2	30.0	14.0	67.2
2	10.6	0.0	10.6	26.0	12.0	8.3
3	10.6	0.0	10.6	27.0	12.5	4.7
4	10.1	0.0	10.1	32.5	20.4	6.0
5	10.5	0.0	10.5	33.5	20.0	4.8
6	9.7	0.0	9.7	33.0	27.0	7.8
7	11.6	0.0	11.6	21.5	14.0	10.2
8	10.2	0.0	10.2	25.0	14.5	6.4
9	9.7	0.0	9.7	27.0	14.5	3.1
10	9.3	0.0	9.3	33.5	20.5	1.4
11	9.1	0.0	9.1	34.0	19.5	4.3
12	10.7	0.0	10.7	36.0	20.5	4.3
13	14.0	0.0	14.0	37.5	23.0	6.4

Jan-95 (cont.)

Day	Gross evaporation (mm)	Rain (mm)	Net evaporation (mm)	Max. Temp. (°C)	Min. Temp. (°C)	Wind speed (km/hr)
14	8.2	5.8	2.4	37.0	21.0	6.9
15	2.9	1.2	1.7	27.0	21.0	3.7
16	8.8	0.0	8.8	30.0	18.0	2.2
17	9.6	0.0	9.6	32.0	16.5	3.4
18	10.8	0.0	10.8	29.5	16.5	4.8
19	8.4	0.0	8.4	31.5	17.5	3.8
20	12.1	0.0	12.1	34.0	18.5	10.3
21	12.8	0.0	12.8	24.0	14.0	14.7
22	11.4	0.0	11.4	21.5	13.5	11.3
23	10.4	0.0	10.4	27.0	13.5	3.1
24	8.9	0.0	8.9	31.0	17.5	4.1
25	11.0	0.0	11.0	31.0	18.0	5.0
26	8.0	0.9	7.1	31.0	18.0	4.4
27	8.1	6.3	1.8	32.5	18.5	7.1
28	7.9	0.0	7.9	24.0	18.0	5.6
29	6.9	0.0	6.9	23.0	16.0	6.5
30	8.7	3.7	5.0	27.0	16.5	2.4
31	8.4	0.0	8.4	24.0	13	NA
Total	299.6	17.9	281.7			
Average				29.5	17.4	7.8

Feb-95

1	7.1	0.0	7.1	25.0	15.0	3.0
2	6.5	0.0	6.5	30.0	16.5	0.9
3	7.4	0.0	7.4	27.0	17.0	7.5
4	8.0	0.0	8.0	23.0	12.5	5.3
5	7.8	7.8	0.0	30.5	13.2	10.8
6	11.7	0.2	11.5	19.7	12.0	15.3
7	7.9	0.0	7.9	21.0	12.0	3.0
8	7.1	0.0	7.1	24.0	12.5	3.1
9	7.6	0.0	7.6	25.0	13.5	5.2
10	8.8	0.0	8.8	22.5	13.0	7.4
11	7.0	0.0	7.0	26.0	13.5	3.6
12	8.1	0.0	8.1	31.0	16.0	3.0
13	11.1	0.0	11.1	37.0	17.5	4.1
14	11.3	0.0	11.3	36.5	25.0	4.8
15	14.7	0.0	14.7	37.0	26.0	8.6
16	7.2	13.9	-6.7	35.0	17.5	10.5
17	12.5	0.0	12.5	21.5	12.0	9.7
18	7.4	0.0	7.4	23.5	13.3	5.0
19	7.8	0.0	7.8	26.5	13.7	4.0
20	7.8	0.0	7.8	27.5	15.0	4.4
21	8.3	0.0	8.3	30.5	15.2	2.1
22	8.9	0.0	8.9	32.0	16.0	2.7
23	9.4	0.0	9.4	31.0	16.0	3.7
23	9.4	0.0	9.4	31.0	16.0	3.7
24	10.7	0.0	10.7	32.0	18.3	4.5

Feb-95 (cont.)

Day	Gross evaporation (mm)	Rain (mm)	Net evaporation (mm)	Max. Temp. (°C)	Min. Temp. (°C)	Wind speed (km/hr)
25	12.3	0.0	12.3	38.5	22.0	2.8
26	10.5	0.0	10.5	36.0	21.1	5.4
27	9.0	0.0	9.0	33.0	18.0	75.5
28	6.3	0.0	6.3	33.2	20.0	3.0
Total	249.9	21.9	228.0			
Average				29.1	16.2	7.7

Mar-95

1	4.5	0.1	4.4	24.5	20.2	20.8
2	2.2	6.5	-4.3	24.0	18.3	1.2
3	9.5	0.0	9.5	30.5	19.0	5.1
4	12.7	0.0	12.7	32.8	21.0	6.6
5	14.2	0.0	14.2	34.5	20.0	6.0
6	13.5	0.0	13.5	34.3	20.0	7.4
7	8.9	0.0	8.9	34.5	19.5	6.8
8	7.3	0.0	7.3	23.0	17.7	5.2
9	8.5	0.0	8.5	22.5	14.5	6.2
10	7.9	0.0	7.9	22.5	14.5	9.6
11	6.7	0.0	6.7	25.3	14.8	3.9
12	7.8	0.4	7.4	26.5	13.7	6.7
13	10.1	0.0	10.1	20.2	8.8	9.2
14	6.6	0.0	6.6	21.0	9.0	3.8
15	7.2	0.0	7.2	24.0	11.0	5.2
16	6.5	0.0	6.5	23.0	12.5	5.3
17	6.7	0.0	6.7	23.5	11.0	2.7
18	5.7	0.0	5.7	25.2	10.5	1.2
19	7.1	0.0	7.1	20.8	12.0	5.6
20	6.8	0.0	6.8	20.5	10.0	5.4
21	6.7	0.0	6.7	20.5	10.1	3.9
22	4.3	0.0	4.3	22.0	13.5	1.9
23	5.0	0.0	5.0	22.0	11.0	2.8
24	5.8	0.0	5.8	23.0	11.0	2.8
25	7.1	0.0	7.1	20.5	13.0	16.9
26	5.4	0.0	5.4	19.5	9.0	2.6
27	4.8	0.0	4.8	19.0	9.0	3.9
28	6.1	0.0	6.1	19.5	10.0	5.3
29	3.1	0.0	3.1	19.0	12.5	1.5
30	1.5	2.3	-0.8	19.5	13.0	3.4
31	6.0	1.7	4.3	19.0	13.5	NA
Total	216.2	11.6	204.6			
Average				23.8	13.7	5.9

Apr-95

Day	Gross evaporation (mm)	Rain (mm)	Net evaporation (mm)	Max. Temp. (°C)	Min. Temp. (°C)	Wind speed (km/hr)
1	6.2	0.0	6.2	19.0	11.8	5.5
2	3.3	4.8	-1.5	18.0	8.5	7.3
3	4.9	0.0	4.9	18.0	9.5	1.8
4	7.4	0.0	7.4	25.0	13.5	6.1
5	1.3	5.4	-4.1	19.5	12.0	5.2
6	4.7	3.2	1.5	17.0	12.0	5.6
7	1.1	6.5	-5.4	17.5	11.0	10.4
8	7.0	1.4	5.6	14.5	11.0	9.0
9	1.1	4.0	-2.9	17.0	12.5	8.3
10	5.1	1.0	4.1	17.0	11.5	7.7
11	1.8	0.1	1.7	16.2	9.0	3.7
12	4.2	0.0	4.2	20.0	11.0	1.1
13	1.7	0.0	1.7	20.0	11.5	0.4
14	4.5	0.0	4.5	23.0	12.0	3.1
15	3.1	0.0	3.1	25.5	13.2	0.4
16	7.0	6.0	1.0	26.0	12.5	10.3
17	7.7	0.3	7.4	15.0	13.0	9.8
18	2.9	0.0	2.9	19.0	10.5	0.9
19	4.8	0.0	4.8	20.0	11.0	8.2
20	4.0	0.0	4.0	17.5	11.5	1.2
21	2.0	0.5	1.5	18.5	11.0	3.1
22	2.2	0.0	2.2	20.0	9.2	0.5
23	2.3	0.0	2.3	23.0	10.5	2.1
24	4.9	0.5	4.4	21.5	12.5	4.8
25	4.4	0.0	4.4	17.0	10.0	0.1
26	1.6	0.8	0.8	18.0	10.5	1.4
27	2.1	1.2	0.9	18.0	11.5	1.5
28	5.3	0.0	5.3	20.0	11.5	3.5
29	6.6	0.0	6.6	23.5	14.5	7.6
30	0.2	10.2	-10.0	14.5	15.0	1.3
Total	115.4	45.9	69.5			
Average				19.3	11.5	4.6

May-95

1	1.8	4.0	-2.2	16.5	10.0	1.9
2	2.4	0.0	2.4	16.5	10.5	1.9
3	1.0	12.4	-11.4	15.0	11.0	4.7
4	4.1	0.0	4.1	15.0	9.0	5.7
5	1.4	0.4	1.0	15.5	7.5	0.3
6	0.6	0.0	0.6	14.0	8.0	2.5
7	3.6	0.0	3.6	17.0	10.5	2.3
8	2.0	0.0	2.0	17.0	10.0	0.4
9	1.5	0.0	1.5	20.0	11.0	1.5
10	2.0	0.0	2.0	21.0	8.0	0.2
11	1.2	0.0	1.2	21.0	10.0	0.9
12	4.3	0.2	4.1	23.0	13.5	7.2
13	3.4	1.6	1.8	22.0	13.5	14.3
14	1.7	2.0	-0.3	19.0	13.2	8.7

May-95 (cont.) Day	Gross evaporation (mm)	Rain (mm)	Net evaporation (mm)	Max. Temp. (°C)	Min. Temp. (°C)	Wind speed (km/hr)
15	4.2	0.1	4.1	16.0	9.5	2.6
16	2.0	0.0	2.0	15.0	11.0	0.4
17	1.5	0.0	1.5	16.0	6.5	0.1
18	2.2	0.0	2.2	15.0	4.5	0.4
19	1.9	0.0	1.9	17.0	7.0	1.6
20	0.6	0.0	0.6	17.5	9.5	0.9
21	4.1	0.0	4.1	17.5	5.0	1.5
22	0.3	0.0	0.3	17.0	4.0	0.3
23	2.8	0.0	2.8	19.0	5.0	1.3
24	3.8	0.0	3.8	21.5	10.0	7.3
25	3.8	5.3	-1.5	17.0	10.0	20.2
26	1.6	13.0	-11.4	15.5	10.5	22.1
27	4.3	0.0	4.3	15.0	11.0	10.0
28	0.6	0.6	0.0	15.5	11.0	1.6
29	0.6	0.0	0.6	14.5	10.0	0.3
30	1.2	0.0	1.2	17.0	9.5	2.8
31	2.2	0.0	2.2	18.0	6.5	NA
Total	68.7	39.6	29.1			
Average				17.3	9.2	4.2
Jun-95						
1	1.0	0.0	1.0	17.0	7.0	0.2
2	2.5	0.0	2.5	21.0	7.0	1.2
3	1.7	0.0	1.7	18.9	7.0	0.6
4	1.7	0.0	1.7	17.5	7.0	1.5
5	2.3	0.0	2.3	19.0	9.5	0.6
6	0.0	5.2	-5.2	17.5	11.0	3.5
7	1.2	1.2	0.0	18.5	13.5	11.3
8	1.3	9.8	-8.5	18.0	15.0	8.0
9	1.9	7.4	-5.5	22.0	16.0	13.2
10	3.1	0.2	2.9	20.2	13.0	16.0
11	2.9	2.2	0.7	14.5	12.5	19.9
12	1.3	0.6	0.7	14.5	10.5	4.3
13	2.5	0.0	2.5	12.5	10.0	0.7
14	0.8	1.1	-0.3	12.5	9.0	0.9
15	0.4	0.0	0.4	15.0	9.0	0.2
16	1.5	0.1	1.4	15.5	8.0	1.1
17	1.0	1.0	0.0	15.0	8.0	4.8
18	2.8	0.0	2.8	13.5	8.0	0.0
19	1.3	0.0	1.3	14.0	8.0	2.2
20	0.8	0.0	0.8	16.0	9.0	0.5
21	2.1	0.0	2.1	12.5	7.0	0.4
22	1.3	0.0	1.3	14.0	6.0	1.4
23	3.5	0.0	3.5	14.0	4.5	1.9
24	1.9	0.0	1.9	14.9	5.2	16.9
25	4.6	0.0	4.6	17.0	8.0	12.5
26	1.0	0.0	1.0	16.0	8.0	4.2

Jun-95 (cont.)						
Day	Gross evaporation (mm)	Rain (mm)	Net evaporation (mm)	Max. Temp. (°C)	Min. Temp. (°C)	Wind speed (km/hr)
27	3.7	4.6	-0.9	18.0	8.0	17.3
28	3.3	4.6	-1.3	12.5	8.0	3.9
29	1.7	1.7	0.0	14.5	11.0	13.7
30	1.1	0.2	0.9	14.0	10.0	8.6
Total	56.2	39.9	16.3			
Average				16.0	9.1	5.7
Jul-95						
1	2.3	3.7	-1.4	14.0	11.0	NA
2	1.5	1.6	-0.1	13.5	9.0	4.8
3	1.1	6.5	-5.4	15.0	9.0	10.9
4	3.3	0.1	3.2	14.0	6.0	4.0
5	0.5	0.0	0.5	11.5	7.0	0.2
6	1.4	0.0	1.4	11.5	7.5	0.5
7	2.1	0.0	2.1	13.0	8.0	0.3
8	0.9	0.0	0.9	12.0	2.5	0.5
9	1.6	0.0	1.6	13.0	2.5	2.2
10	3.1	0.7	2.4	14.0	5.8	7.7
11	1.6	7.0	-5.4	13.0	8.1	12.2
12	1.0	10.7	-9.7	13.6	10.5	15.9
13	0.7	6.7	-6.0	14.0	11.2	10.3
14	3.3	9.6	-6.3	14.5	10.0	23.1
15	3.2	3.2	0.0	13.5	10.0	7.0
16	5.3	4.0	1.3	15.0	10.0	55.4
17	0.0	9.6	-9.6	15.5	11.0	8.5
18	2.1	2.6	-0.5	12.5	10.0	11.3
19	2.4	3.3	-0.9	13.0	10.0	10.3
20	2.3	0.0	2.3	14.0	6.5	25.0
21	2.4	7.9	-5.5	14.2	6.9	12.6
22	0.6	7.1	-6.5	14.0	6.9	21.9
23	1.4	5.1	-3.7	11.0	7.0	18.5
24	4.4	0.1	4.3	13.5	7.0	5.5
25	0.4	0.0	0.4	13.0	8.0	1.6
26	2.6	1.4	1.2	13.5	8.0	2.7
27	0.5	1.2	-0.7	12.0	8.0	NA
28	3.7	9.4	-5.7	14.0	10.0	12.2
29	0.1	0.1	0.0	13.0	6.0	6.6
30	4.5	0.0	4.5	13.0	7.0	6.6
31	0.2	11.3	-11.1	12.0	8.5	NA
Total	60.5	112.9	-52.4			
Average				13.3	8.0	10.4

Aug-95

Day	Gross evaporation (mm)	Rain (mm)	Net evaporation (mm)	Max. Temp. (°C)	Min. Temp. (°C)	Wind speed (km/hr)
1	3.6	0.6	3.0	13.0	7.5	5.8
2	1.8	6.0	-4.2	13.5	9.0	16.5
3	1.8	2.1	-0.3	12.0	5.5	8.1
4	1.1	0.6	0.5	14.0	5.5	4.2
5	1.1	0.0	1.1	14.0	7.5	1.6
6	1.1	0.0	1.1	14.0	8.0	3.0
7	3.6	0.4	3.2	13.0	8.5	5.4
8	2.3	0.5	1.8	12.0	4.0	2.5
9	3.7	0.2	3.5	13.0	2.5	NA
10	1.0	0.0	1.0	14.0	14.0	7.0
11	1.8	0.0	1.8	15.0	5.5	4.2
12	3.2	0.0	3.2	16.5	3.8	3.6
13	2.3	0.0	2.3	19.1	4.0	0.3
14	3.9	0.0	3.9	20.5	4.0	6.8
15	3.6	0.0	3.6	18.0	7.5	1.5
16	0.8	0.0	0.8	15.5	7.5	0.3
17	3.0	0.0	3.0	18.0	8.0	3.0
18	3.5	0.0	3.5	22.5	7.5	5.0
19	0.6	0.0	0.6	21.0	7.5	0.2
20	4.4	0.0	4.4	24.0	8.0	4.8
21	4.0	0.0	4.0	26.5	12.5	11.0
22	2.8	0.0	2.8	17.0	7.0	1.2
23	3.2	0.0	3.2	22.5	70.0	5.0
24	3.9	0.0	3.9	18.0	11.0	2.5
25	3.1	6.5	-3.4	24.0	14.0	12.6
26	4.1	0.0	4.1	16.0	7.5	4.6
27	1.9	0.0	1.9	18.0	7.0	4.5
28	3.7	0.0	3.7	18.0	7.0	1.2
29	3.0	0.0	3.0	20.0	6.0	1.1
30	5.4	0.0	5.4	22.5	11.5	9.4
31	2.9	0.0	2.9	15.0	11.0	NA
Total	86.2	16.9	69.3			
Average				17.4	7.7	4.7

Sep-95

1	3.8	6.2	-2.4	14.5	8.0	8.7
2	2.3	0.0	2.3	14.2	6.2	1.3
3	3.0	0.0	3.0	16.2	6.0	1.6
4	4.3	1.6	2.7	19.5	6.5	7.5
5	1.9	10.4	-8.5	13.0	6.0	2.0
6	3.5	0.0	3.5	13.0	6.0	4.7
7	1.0	0.0	1.0	11.5	6.0	0.8
8	5.7	0.2	5.5	15.0	7.5	7.2
9	1.4	0.7	0.7	16.5	11.0	7.7
10	2.4	0.0	2.4	16.0	6.0	0.6
11	1.7	0.4	1.3	15.5	6.5	4.0
12	2.7	0.0	2.7	17.0	6.5	0.6
13	4.9	0.0	4.9	24.0	10.0	3.5

Sep-95 (cont.)

Day	Gross evaporation (mm)	Rain (mm)	Net evaporation (mm)	Max. Temp. (°C)	Min. Temp. (°C)	Wind speed (km/hr)
14	1.3	7.0	-5.7	23.0	12.5	6.9
15	4.9	0.0	4.9	15.5	6.5	4.5
16	2.1	0.0	2.1	17.0	8.0	1.5
17	3.2	0.0	3.2	18.5	10.0	2.8
18	3.1	0.0	3.1	20.0	10.2	0.6
19	5.1	0.0	5.1	23.5	11.5	7.5
20	1.0	0.2	0.8	17.5	8.5	2.8
21	5.5	0.0	5.5	16.9	10.5	10.0
22	3.2	0.0	3.2	16.8	10.2	5.1
23	1.2	0.4	0.8	16.9	10.2	1.7
24	18.1	12.0	6.1	15.8	10.0	5.4
25	7.4	0.2	7.2	17.0	8.0	8.0
26	3.9	0.0	3.9	17.0	4.5	2.3
27	2.6	0.0	2.6	20.5	8.0	0.2
28	5.7	0.0	5.7	23.5	12.0	3.0
29	5.8	0.0	5.8	26.5	17.0	3.9
30	5.4	3.8	1.6	25.0	14.5	8.8
Total	142.7	43.1	75.0			
Average				17.9	8.8	4.2

1.2. Physico-chemical measurements.

Pond XD1

	Transparency	Depth	Water	PH	Alkalinity	Salinity	D.O.	Phosphate	Nitrate	Salt error	Nitrate
	(cm)	(cm)	Temp. °C		meq/l	g/l	mg/l	µg/l	µg/l	(%)	µg/l
APR	63	63	17.50	7.00	4.00	55.90	7.50	11.00	0.84	15.28	2.82
MAY	45	45	13.00	8.00	2.05	57.10	6.50	10.00	4.30	15.62	6.15
JUN	40	40	11.00	7.80	3.80	55.60	6.50	9.00	13.50	15.20	15.11
JUL	47	47	12.20	7.80	4.02	53.60	7.80	8.00	24.13	14.64	25.50
AUG	60	60	15.50	7.70	3.70	49.70	9.50	7.00	57.14	13.52	58.23
SEP	65	65	15.00	8.00	3.70	51.30	8.10	20.00	14.50	13.98	17.75
OCT	80	80	20.00	8.05	3.15	46.10	1.60	5.00	16.70	12.48	17.41
NOV	85	85	22.00	8.00	3.20	59.80	7.90	7.00	3.20	16.36	4.57
DEC	90	90	23.00	8.17	3.19	54.10	8.40	6.00	6.00	14.78	7.04
JAN	89	89	23.50	7.90	3.10	54.10	2.70	7.00	3.80	14.78	5.01
FEB	95	95	29.00	8.10	3.20	52.70	3.20	10.00	2.15	14.38	3.83
MAR	71	71	28.00	8.32	3.20	59.80	2.90	0.40	3.22	16.36	3.30
APR	69	69	16.00	8.50	3.70	62.80	2.20	4.10	1.69	17.18	2.54
MAY	50	50	15.00	8.33	3.60	63.50	2.50	1.90	1.64	17.37	2.04
JUN	50	50	13.00	8.16	3.90	52.70	3.90	2.50	0.78	14.38	1.20
JUL	55	55	11.00	8.14	4.50	58.90	3.50	6.05	5.18	16.12	6.34
AUG	40	40	16.00	8.08	3.60	55.40	2.20	5.00	5.25	15.14	6.14
SEP	50	50	19.00	8.24	3.60	57.50	2.00	9.00	5.02	15.73	6.70
Average	63.56	63.56	17.76	8.02	3.51	55.59	4.94	7.16	9.39	15.18	10.65

Pond XC3

	Transparency	Depth	Tem. °C	PH	Alkalinity	Salinity	D.O.	Phosphate	Nitrate	Salt error	Nitrate
	(cm)	(cm)			meq/l	g/l	mg/l	µg/l	µg/l	(%)	µg/l
APR	35	35	19.00	7.70	4.40	108.60	4.70	12.00	0.02	28.08	0.03
MAY	39	39	12.00	7.90	1.70	104.70	4.50	10.00	11.00	27.26	15.12
JUN	30	30	9.90	7.60	3.20	110.00	5.80	7.00	0.02	28.36	0.02
JUL	40	40	12.80	7.80	3.02	105.40	6.10	8.00	10.33	27.41	14.23
AUG	46	46	18.00	7.70	2.95	98.00	6.60	12.00	3.16	25.82	4.26
SEP	38	38	16.50	8.10	3.30	113.00	7.00	20.00	3.60	28.97	5.07
OCT	35	35	20.50	8.02	3.10	103.80	1.80	6.00	1.81	27.07	2.48
NOV	42	42	18.00	7.90	3.12	105.60	6.80	4.00	4.21	27.45	5.80
DEC	50	50	23.00	7.94	3.21	92.40	6.10	6.00	5.68	24.56	7.53
JAN	55	55	20.00	7.80	2.80	108.80	2.40	10.00	1.10	28.12	1.53
FEB	60	60	25.50	8.00	3.10	107.80	2.20	10.00	2.91	27.91	4.04
MAR	50	50	28.00	8.42	2.98	133.60	4.10	0.40	3.22	32.79	4.79
APR	45	45	15.00	8.17	3.20	119.00	2.00	2.10	1.34	30.14	1.92
MAY	41	41	14.50	8.30	3.00	136.80	1.90	2.65	3.50	33.33	5.25
JUN	35	35	15.50	8.06	3.20	89.80	2.90	2.20	3.80	23.96	5.00
JUL	40	40	11.50	8.00	3.10	96.60	2.10	4.00	22.00	25.51	29.53
AUG	30	30	15.00	8.20	3.00	99.60	2.10	5.25	0.01	26.17	0.01
SEP	35	35	17.50	8.42	3.30	99.20	1.70	11.10	14.35	26.08	19.41
Average	41.44	41.44	17.34	8.00	3.09	107.37	3.93	7.37	5.11	27.82	7.00

Pond XB3

	Transparency	Depth	Tem. °C	PH	Alkalinity	Salinity	D.O.	Phosphate	Nitrate	Salt error	Nitrate
	(cm)	(cm)			meq/l	g/l	mg/l	µg/l	µg/l	(%)	µg/l
APR	20	20	19.00	7.70	4.40	62.40	4.70	22.30	3.30	17.07	3.98
MAY	15	15	12.00	8.30	2.10	61.70	9.00	10.00	4.00	16.88	4.81
JUN	21	21	11.00	8.40	3.90	59.10	7.70	12.50	18.50	16.17	22.07
JUL	31	31	11.60	8.10	4.15	61.00	7.50	11.00	3.90	16.69	4.68
AUG	30	30	12.00	7.90	3.90	58.60	7.80	11.70	2.49	16.03	2.97
SEP	30	30	15.50	8.40	4.22	63.00	7.80	32.50	3.60	17.23	4.35
OCT	27.3	27.3	21.00	8.28	3.90	54.60	2.30	12.80	4.13	14.92	4.85
NOV	34	34	21.50	8.20	3.85	67.20	9.80	10.00	3.76	18.35	4.61
DEC	90	90	22.00	8.32	3.79	58.60	6.50	8.20	5.16	16.03	6.15
JAN	110	110	22.50	8.15	3.70	80.75	2.70	10.00	4.20	21.80	5.37
FEB	101	101	27.50	8.50	3.40	58.00	3.30	33.10	1.74	15.87	2.07
MAR	95	95	17.50	8.57	3.45	62.50	4.20	20.00	2.32	17.10	2.80
APR	94	94	16.00	8.70	3.80	64.00	2.80	6.80	2.04	17.50	2.47
MAY	100	100	15.00	8.35	3.65	66.00	3.10	2.80	2.06	18.04	2.51
JUN	105	105	13.50	8.20	3.50	53.40	3.80	0.23	4.10	14.58	4.80
JUL	96	96	11.00	8.30	4.20	61.40	3.40	5.30	1.30	16.80	1.56
AUG	90	90	16.00	8.15	3.80	61.90	2.20	0.00	0.12	16.94	0.14
SEP	115	115	18.00	8.39	3.80	62.80	2.70	10.70	0.04	17.18	0.05
Average	66.91	66.91	16.81	8.27	3.75	62.05	5.07	12.22	3.71	16.98	4.46

Pond XB8

	Transparency	Depth	Tem. °C	PH	Alkalinity	Salinity	D.O.	Phosphate	Nitrate	Salt error	Nitrate
	(cm)	(cm)			meq/l	g/l	mg/l	µg/l	µg/l	(%)	µg/l
APR	18	110	17.50	6.90	4.00	106.80	4.30	22.00	4.29	27.70	5.93
MAY	18	98	12.50	8.00	2.44	108.00	8.50	20.00	16.00	27.95	22.21
JUN	17	102	11.00	7.70	4.00	105.00	7.80	15.30	9.30	27.33	12.80
JUL	15	96	11.90	7.70	5.15	106.20	6.20	10.70	8.25	27.58	11.39
AUG	16	95	12.00	7.50	4.80	103.20	7.60	30.50	11.17	26.94	15.29
SEP	18	97	15.50	8.10	5.50	120.80	6.50	42.20	2.15	30.48	3.09
OCT	19	99	23.00	7.91	5.70	110.60	1.20	21.90	6.90	28.49	9.65
NOV	17	100	22.00	7.50	4.40	120.40	5.00	20.00	7.82	30.41	11.24
DEC	19	95	23.00	7.74	4.94	122.60	6.00	21.00	11.57	30.82	16.72
JAN	25	98	24.50	7.50	4.15	116.60	1.20	44.30	6.15	29.68	8.75
FEB	30	96	29.00	7.70	3.90	115.40	2.80	27.10	4.90	29.44	6.94
MAR	25	100	21.00	8.63	3.40	110.00	3.40	3.00	2.05	28.36	2.86
APR	25	90	17.00	8.18	3.70	130.40	2.10	5.20	2.51	32.23	3.70
MAY	20	85	15.50	8.33	3.85	120.80	3.20	5.10	4.45	30.48	6.40
JUN	25	99	14.50	8.20	3.40	93.60	3.50	19.90	0.50	24.83	0.67
JUL	20	108	11.00	7.86	4.40	107.20	3.10	56.05	17.15	27.79	23.75
AUG	25	100	16.00	7.60	3.00	104.00	2.30	25.10	3.50	27.11	4.80
SEP	30	96	18.00	8.19	4.20	114.20	3.00	10.50	1.30	29.21	1.84
Average	21.22	98.00	17.49	7.85	4.16	111.99	4.32	22.21	6.66	28.71	9.34

Pond PA3

	Transparency	Depth	Tem. °C	PH	Alkalinity	Salinity	D.O.	Phosphate	Nitrate	Salt error	Nitrate
	(cm)	(cm)			meq/l	g/l	mg/l	µg/l	µg/l	(%)	µg/l
APR	50	90	16.00	7.90	3.60	115.40	6.40	20.50	6.01	29.44	8.52
MAY	25	98	12.90	7.90	4.15	135.00	5.20	30.70	11.61	33.02	17.33
JUN	30	90	10.00	7.80	4.80	138.00	4.50	21.00	23.50	33.52	35.35
JUL	28	85	12.00	7.90	2.33	141.00	4.20	27.20	17.00	34.01	25.76
AUG	26	79	19.00	7.00	3.80	148.00	4.20	51.30	4.78	35.10	7.37
SEP	20	95	16.00	8.40	4.04	154.50	5.90	32.10	2.15	36.05	3.36
OCT	100	100	19.50	8.09	4.75	121.60	1.20	20.00	3.60	30.63	5.19
NOV	95	95	22.00	7.70	3.68	140.60	4.50	23.30	5.32	33.95	8.05
DEC	115	115	23.00	7.85	3.80	124.60	5.60	21.00	9.49	31.19	13.79
JAN	120	120	24.00	7.60	3.50	140.60	2.50	34.20	8.30	33.95	12.57
FEB	109	109	28.00	7.90	3.70	139.00	3.10	25.10	3.15	33.69	4.75
MAR	30	98	20.50	8.60	3.80	141.20	2.90	4.20	1.06	34.04	1.61
APR	30	105	15.50	8.02	3.85	147.20	1.90	5.70	3.06	34.98	4.71
MAY	45	84	14.50	8.78	3.85	164.50	1.90	6.05	3.91	37.40	6.25
JUN	85	110	14.20	8.33	4.05	113.40	3.30	9.50	6.01	29.05	8.47
JUL	95	115	11.50	8.15	3.90	151.50	2.60	9.10	13.45	35.62	20.89
AUG	30	105	14.50	7.98	3.40	127.80	2.30	9.30	6.45	31.77	9.45
SEP	35	95	18.00	8.98	4.00	133.60	2.30	17.20	0.37	32.79	0.55
Average	59.33	99.33	17.28	8.05	3.83	137.64	3.58	20.41	7.18	33.34	10.78

Pond PA7

	Transparency	Depth	Tem. °C	PH	Alkalinity	Salinity	D.O.	Phosphate	Nitrate	Salt error	Nitrate
	(cm)	(cm)			meq/l	g/l	mg/l	µg/l	µg/l	(%)	µg/l
APR	45	45	18.00	7.40	4.10	184.00	2.00	30.00	7.24	39.62	11.99
MAY	30	30	12.50	7.90	3.20	210.50	2.20	11.00	38.00	41.79	65.28
JUN	48	48	11.90	7.30	5.50	155.00	1.80	18.50	8.40	36.12	13.15
JUL	35	35	12.20	8.10	5.40	185.50	2.70	20.00	12.90	39.77	21.42
AUG	45	45	15.00	8.40	5.10	177.00	4.50	15.00	13.73	38.88	22.46
SEP	40	40	16.50	8.10	5.10	194.50	4.30	34.30	3.61	40.59	6.08
OCT	30	30	20.50	8.20	5.05	172.50	1.30	22.00	4.13	38.37	6.70
NOV	50	50	21.00	8.00	4.98	182.50	4.80	20.00	7.25	39.47	11.98
DEC	45	45	24.00	7.82	4.40	199.00	4.50	24.10	9.03	40.97	15.30
JAN	60	60	20.00	7.80	4.45	187.00	1.60	37.00	8.95	39.91	14.90
FEB	55	55	26.00	7.80	4.30	180.00	1.90	50.70	6.11	39.21	10.05
MAR	45	45	28.00	9.01	4.40	205.00	2.60	8.90	0.57	41.42	0.97
APR	50	50	16.00	8.25	4.80	207.00	1.10	5.40	4.47	41.56	7.65
MAY	52	52	15.00	9.05	4.70	207.50	1.80	5.30	5.34	41.59	9.14
JUN	50	50	14.00	8.91	4.20	157.50	2.40	9.55	16.19	36.47	25.48
JUL	49	49	11.50	8.66	4.70	168.50	2.10	4.60	12.57	37.90	20.24
AUG	54	54	15.50	8.35	4.00	194.50	1.30	7.50	6.75	40.59	11.36
SEP	46.06	46.06	17.50	8.91	4.40	240.00	1.90	20.50	7.69	43.04	13.50
Average			17.51	8.22	4.60	189.31	2.49	19.13	9.61	39.85	15.98

Pond PA9

	Transparency	Depth	Tem. °C	PH	Alkalinity	Salinity	D.O.	Phosphate	Nitrate	Salt error	Nitrate
	(cm)	(cm)			meq/l	g/l	mg/l	µg/l	µg/l	(%)	µg/l
APR	97	97	17.00	7.90	4.90	198.00	2.10	15.00	4.40	40.89	7.44
MAY	100	100	12.00	7.50	5.10	209.00	2.00	11.00	2.10	41.69	3.60
JUN	102	102	11.90	8.10	5.40	200.00	1.90	14.20	5.90	41.04	10.01
JUL	99	99	12.00	8.40	4.90	190.80	2.50	27.10	4.90	40.27	8.20
AUG	89	89	15.50	8.10	5.00	182.50	3.90	12.00	4.20	39.47	6.94
SEP	100	100	16.00	8.50	4.50	202.00	4.00	9.90	1.90	41.20	3.23
OCT	107	107	20.00	8.00	5.50	201.50	1.50	24.00	1.50	41.16	2.55
NOV	98	98	20.00	8.30	5.80	190.00	1.00	11.00	7.90	40.20	13.21
DEC	90	90	24.00	7.75	4.48	193.00	3.50	23.00	14.50	40.46	24.36
JAN	100	100	24.00	7.80	4.60	202.50	1.70	21.00	7.04	41.23	11.98
FEB	102	102	27.00	8.00	4.80	205.00	1.80	25.00	7.30	41.42	12.46
MAR	105	105	25.00	8.02	5.00	210.00	1.60	4.00	3.05	41.75	5.24
APR	99	99	17.50	8.29	5.15	214.00	1.00	4.56	3.77	42.00	6.50
MAY	95	95	16.00	8.99	5.00	208.00	1.60	1.95	1.63	41.62	2.79
JUN	102	102	13.50	8.90	5.10	168.50	2.00	6.55	6.28	37.90	10.11
JUL	104	104	11.50	8.58	4.70	178.50	1.80	4.20	4.30	39.05	7.05
AUG	100	100	16.00	8.34	4.00	179.00	2.00	4.70	0.02	39.10	3.00
SEP	98	98	18.50	9.32	4.20	182.50	1.90	13.00	1.03	39.47	1.70
Average	99.28	99.28	17.63	8.27	4.90	195.27	2.10	12.90	4.54	40.55	19.00

Pond PA12

	Transparency	Depth	Tem. °C	PH	Alkalinity	Salinity	D.O.	Phosphate	Nitrate	Salt error	Nitrate
	(cm)	(cm)			meq/l	g/l	mg/l	µg/l	µg/l	(%)	µg/l
APR	150	150	18.00	7.10	4.00	216.00	2.70	42.50	12.16	42.11	21.01
MAY	145	145	14.00	7.70	3.40	211.50	2.00	23.50	25.00	41.85	42.99
JUN	149	149	12.00	7.50	5.30	189.00	3.00	12.00	9.31	40.10	15.54
JUL	120	120	12.00	7.80	5.50	199.50	2.20	31.00	11.35	41.01	19.24
AUG	142	142	16.00	7.90	5.40	198.00	4.00	7.05	11.84	40.89	20.03
SEP	120	120	16.00	7.80	5.46	227.50	4.10	34.20	0.51	42.66	0.89
OCT	145	145	20.00	8.30	6.00	243.50	1.60	22.10	6.10	43.11	10.72
NOV	116	116	21.00	8.00	4.98	211.50	4.80	23.00	7.25	41.85	12.47
DEC	19.6	119	24.00	7.71	4.76	210.50	3.00	20.90	21.20	41.79	36.42
JAN	135	135	24.50	7.80	4.70	234.00	0.90	11.70	9.17	42.88	16.06
FEB	98	98	27.50	7.80	4.30	242.50	1.80	20.00	6.42	43.09	11.28
MAR	140	140	24.00	8.97	5.20	217.00	1.20	4.00	2.55	42.17	4.41
APR	135	135	17.50	8.18	5.50	236.00	1.00	4.10	4.94	42.94	8.66
MAY	117	117	15.00	8.87	4.90	214.50	1.45	1.05	1.92	42.03	3.31
JUN	99	99	14.50	8.74	5.00	194.50	1.70	6.40	7.38	40.59	12.42
JUL	102	102	11.00	8.21	4.70	192.00	1.80	5.70	27.35	40.38	45.87
AUG	100	100	19.00	7.96	4.40	195.50	1.80	4.50	0.65	40.68	1.10
SEP	95	95	17.50	9.02	4.60	195.00	1.90	20.10	0.04	40.64	0.06
Average	118.20	123.72	17.97	8.08	4.89	212.67	2.28	16.32	9.17	41.71	15.69

1.3. Conversion factor for salinity and specific gravity

Density (g/ml)	Degree Beaume (Be)	Salinity (g/L)
1.02	2.8	25.54138
1.021	3	27.045899
1.022	3.1	28.550418
1.023	3.3	30.054937
1.024	3.4	31.559456
1.025	3.6	33.063975
1.026	3.7	34.568494
1.027	3.8	36.073013
1.028	4	37.577532
1.029	4.1	39.082051
1.03	4.2	40.58657
1.031	4.4	42.091089
1.032	4.5	43.595608
1.033	4.7	45.100127
1.034	4.8	46.604646
1.035	4.9	48.109165
1.036	5	49.613684
1.037	5.1	51.118203
1.038	5.3	52.622722
1.039	5.4	54.127241
1.04	5.5	55.63176
1.041	5.7	57.136279
1.042	5.8	58.640798
1.043	6	60.145317
1.044	6.1	61.649836
1.045	6.2	63.154355
1.046	6.4	64.658874
1.047	6.5	66.163393
1.048	6.6	67.667912
1.049	6.7	69.172431
1.05	6.8	70.67695
1.051	7	72.181469
1.052	7.2	73.685988
1.053	7.3	75.190507
1.054	7.5	76.695026
1.055	7.6	78.199545
1.056	7.7	79.704064
1.057	7.9	81.208583
1.058	8	82.713102
1.059	8.1	84.217621
1.06	8.2	85.72214
1.061	8.4	87.226659
1.062	8.5	88.731178
1.063	8.7	90.235697
1.064	8.8	91.740216

Density (g/ml)	Degree Beaume (Be)	Salinity (g/L)
1.065	8.9	93.244735
1.066	9	94.749254
1.068	9.3	97.758292
1.069	9.4	99.262811
1.07	9.5	100.76733
1.071	9.6	102.271849
1.072	9.7	103.776368
1.073	9.9	105.280887
1.074	10	106.785406
1.075	10.1	108.289925
1.076	10.2	109.794444
1.077	10.3	111.298963
1.078	10.5	112.803482
1.079	10.6	114.308001
1.08	10.7	115.81252
1.081	10.8	117.317039
1.082	11	118.821558
1.083	11.1	120.326077
1.084	11.2	121.830596
1.085	11.3	123.335115
1.086	11.5	124.839634
1.087	11.6	126.344153
1.088	11.7	127.848672
1.089	11.8	129.353191
1.09	11.9	130.85771
1.091	12	132.362229
1.092	12.1	133.866748
1.093	12.3	135.371267
1.094	12.4	136.875786
1.095	12.5	138.380305
1.096	12.6	139.884824
1.097	12.7	141.389343
1.098	12.8	142.893862
1.099	13	144.398381
1.1	13.1	145.9029
1.101	13.2	147.407419
1.102	13.4	148.911938
1.103	13.5	150.416457
1.104	13.6	151.920976
1.105	13.7	153.425495
1.106	13.8	154.930014
1.107	14	156.434533
1.108	14.2	157.939052
1.109	14.3	159.443571
1.11	14.4	160.94809

Density (g/ml)	Degree Beaume (Be)	Salinity (g/L)
1.111	14.5	162.452609
1.112	14.6	163.957128
1.113	14.7	165.461647
1.114	14.9	166.966166
1.115	15	168.470685
1.116	15.1	169.975204
1.117	15.2	171.479723
1.118	15.3	172.984242
1.119	15.4	174.488761
1.119	15.4	174.488761
1.12	15.5	175.99328
1.121	15.6	177.497799
1.122	15.7	179.002318
1.123	15.8	180.506837
1.124	15.9	182.011356
1.125	16	183.515875
1.126	16.2	185.020394
1.127	16.3	186.524913
1.128	16.4	188.029432
1.129	16.5	189.533951
1.13	16.6	191.03847
1.131	16.7	192.542989
1.132	16.8	194.047508
1.133	16.9	195.552027
1.134	17	197.056546
1.135	17.1	198.561065
1.136	17.3	200.065584
1.137	17.4	201.570103
1.138	17.5	203.074622
1.139	17.6	204.579141
1.14	17.7	206.08366
1.141	17.8	207.588179
1.142	17.9	209.092698
1.143	18	210.597217
1.144	18.1	212.101736
1.145	18.2	213.606255
1.146	18.3	215.110774
1.147	18.5	216.615293
1.148	18.6	218.119812
1.149	18.7	219.624331
1.15	18.8	221.12885
1.151	19	222.633369
1.152	19.1	224.137888
1.153	19.2	225.642407
1.154	19.3	227.146926
1.155	19.4	228.651445
1.156	19.5	230.155964
1.157	19.6	231.660483

Density (g/ml)	Degree Beaume (Be)	Salinity (g/L)
1.158	19.7	233.165002
1.159	19.8	234.669521
1.16	19.9	236.17404
1.161	20	237.678559
1.162	20.2	239.183078
1.163	20.3	240.687597
1.164	20.4	242.192116
1.165	20.5	243.696635
1.166	20.6	245.201154
1.167	20.7	246.705673
1.168	20.8	248.210192
1.169	20.9	249.714711
1.17	21	251.21923
1.171	21.1	252.723749
1.172	21.2	254.228268
1.173	21.3	255.732787
1.174	21.4	257.237306
1.175	21.5	258.741825
1.176	21.6	260.246344
1.177	21.7	261.750863
1.178	21.8	263.255382

APPENDIX 2

BIOLOGICAL PARAMETERS

2.1. Chlorophyll *a* measurement during the study period.

	A	M	J	J	A	S	O	N	D	J	F	M	A	M	J	J	A	S
a.Chlorophyll *a* concentration (ug/L) in water column.																		
XD1	1.31	14	12.1	21.45	23.45	12.25	10.2	19.95	8.05	5.27	9.15	11.75	22.17	20.8	23.5	26.6	40.5	30
XC3	1.31	7.35	7.6	11.19	25.51	12.85	3.08	2.1	1.15	13.05	3.3	10.35	22.17	6.38	7.51	0	13.27	29.8
XB3	19.45	17.9	63.8	14.05	27.46	20.75	22.5	19.5	22.78	40.9	39.2	21.8	32.26	33.54	10.25	21.5	55.4	21
XB8	121.4	123.2	167.9	211	154	95.95	116.8	25.04	21.84	67.75	37.15	132.3	92.58	113.26	168.5	66	78.6	49.6
PA3	11.46	26.32	62.9	42.6	26.71	77.65	0	6.74	10.5	1.3	10.1	115.1	4.65	2.17	0.01	2.5	6.5	13.75
PA7	6.7	0	0	0.03	0	0	14.5	1.5	2.68	9.9	0.75	3.9	0	2.17	0	2.53	6.75	7.69
PA9	2.25	1.12	0.78	0.01	0	0	4.8	2.32	0	0.01	0	9.3	3.45	4.04	0	0.85	4.4	2.95
PA12	0.005	1.01	0	0	0.09	0	3.2	1.5	1.1	1.01	0	8.1	1.5	5.08	0	11.96	3.3	3.5
b.Chlorophyll *a* concentration (ug/L) in benthic mat.																		
XD1	ND	39.72	8.64	12.26	40.41	38.5	34.74	5.18	1.67	22.78	30.3	4.32	23.68	23	23	2.85	41.51	50.74
XC3	ND	29.54	15.42	19.22	24.29	25.49	22.8	15.49	13.71	16.9	17.58	27.24	13.77	11.14	11	1.2	22	6.88
XB3	ND	57.44	15.61	23.61	26.36	27.47	0.46	4.8	12.04	37.24	33.3	8.19	18.54	38.72	38.57	4.71	19.03	14.28
XB8	ND	0	7.54	11.77	0	1.24	0	7.62	6.35	6.92	5.96	1.78	4.17	4.03	4.08	0.44	0.96	0.96
PA3	ND	22.36	29.37	24	13.7	6.97	58.59	10.47	20.97	25.38	16.93	8.57	26.67	14.83	15.35	0.97	35.33	12.64
PA7	ND	80.85	20.66	8.11	17.22	46.2	34.26	20.39	35.89	12.43	8.53	22.2	40.18	9.74	9.37	7.05	26.72	13
PA9	ND	65.22	30.19	25.18	35.78	50.12	39.2	38.28	43.7	52.42	31.32	14.96	43.72	8.8	8.62	5.79	27.74	11.99
PA12	ND	49.87	30.19	19.29	42.94	46.6	53.6	17.72	53.26	18.3	19.62	15.18	50.72	11.34	11.4	0.4	51.89	12.73

2.2. Carotenoid measurements during the study period.

a.Carotenoid concentration (ug/L) in water column.

	XD1	XD1	XC3	XC3	XB3	XB3	XB8	XB8	PA3	PA3	PA7	PA7	PA9	PA9	PA12	PA12
	510	480	510	480	510	480	510	480	510	480	510	480	510	480	510	480
APR	10.35	11.85	5.2	3.3	3.8	4.6	57.9	97.1	12.57	0.86	0	0	0	0	0	0
MAY	10.35	11.85	5.2	3.3	3.8	4.6	57.9	97.1	12.57	0.86	0	0	0	0	0	0
JUN	14.1	9.1	5.3	6.1	12.2	9.2	143.3	132.4	18.8	5.4	1.14	0	1	0.2	0	0
JUL	14.1	8.8	5.4	10.4	9.1	10.6	157.9	162.5	0	1.32	0	0	0.15	0	0	0
AUG	0.36	0.32	1.2	1.3	0.2	0.01	0.7	0.7	0.04	0.03	0	0	0	0	0	0
SEP	18.2	18.7	0	31.29	32.2	31.4	108.6	111.6	48.7	54.06	12.86	46.47	11.25	8.5	25.4	25.71
OCT	14.2	13.4	7.4	10.3	37.7	51.4	116.6	125.4	8.64	10.68	0	20.14	10.3	7	3.25	6
NOV	46.5	56.2	8.3	3.04	31.8	36.2	32.7	0	24.2	21.1	23.2	25	4.26	3.9	6.4	4.6
DEC	16.45	16.6	8.5	7.6	35.7	35	3	2.9	1.4	1.3	0.9	0.6	0.3	0.2	0.6	0.5
JAN	7.6	7.2	19.8	19.7	54.1	49.3	46.2	47.3	2.16	3.8	1.26	1.4	0.6	0.03	0.02	0.02
FEB	15.97	6.3	4.5	4.7	49.2	42.7	37.4	34.7	19.6	18.6	4.4	6	0	0	0	0
MAR	17.2	20.4	19.5	21.2	34.5	37.6	131.6	122.2	73.2	93.4	8.5	7.3	0.6	0.5	4.4	4.5
APR	33.6	30.7	14.9	13.8	54.2	52	125.4	126.3	23.8	22.3	4.3	5.8	12.4	5.8	11.2	14.2
MAY	30.4	27.7	12.2	13.2	57	62.2	80.8	88.2	10.2	175.1	3.9	4.5	9.7	4.4	10.2	9.2
JUN	35.4	37.4	13	13.9	16.8	18.2	184.1	183.2	6.4	6.2	1.5	1.5	0.7	0	0.6	0
JUL	93.7	89.9	50.9	42.3	84.5	83.2	39.7	40.9	9.6	9.7	10.2	3.2	5.7	3.8	30.4	31
AUG	52	52.5	57.2	58.1	75.7	82.4	108.8	102.6	6.3	9.4	5.5	3	6.6	9.7	9.6	7.6
SEP	51.6	51.9	13.24	15.9	36.7	59.9	48.1	199.7	19.3	15.5	6.6	5.3	5.5	3.8	1.5	1.5

2.2. (continued)

b. Carotenoid concentration (ug/L) in benthic mat.

	XD1	XD1	XC3	XC3	XB3	XB3	XB8	XB8	PA3	PA3	PA7	PA7	PA9	PA9	PA12	PA12
	510	480	510	480	510	480	510	480	510	480	510	480	510	480	510	480
APR	11.1	12.3	4.25	6.7	4.2	5.7	77.5	90	6.715	23.8	0	0	0	0	0	0
MAY	11.1	12.3	4.25	6.7	4.2	5.7	77.5	90	6.715	23.8	0	0	0	0	0	0
JUN	11.6	10	5.7	4.8	10.7	14	137.85	112.4	12.1	61.8	0.57	0.6	0.6	1.2	0	0
JUL	11.45	7.6	7.9	9.1	9.85	7.6	160.2	133.2	0.66	31.8	0	3.3	0.075	0.12	0	0
AUG	0.34	19.2	1.25	49.2	0.105	26.9	0.7	111.3	0.035	18.2	0	2.3	0	0.05	0	1
SEP	18.45	10.2	15.645	9	31.8	17.9	110.1	61.9	51.38	33.2	29.665	18.4	9.875	10.7	25.555	16.1
OCT	13.8	7.6	8.85	5.1	44.55	24.8	121	69.6	9.66	5.9	10.07	6.4	8.65	5.2	4.625	5.2
NOV	51.35	28.8	5.67	3.2	34	18.8	16.35	18.6	22.65	14.3	24.1	14.8	4.08	2.4	5.5	3.6
DEC	16.525	9.6	8.05	4.6	35.35	20	2.95	1.2	1.35	0.8	0.75	0.4	0.25	0.17	0.55	0.33
JAN	7.4	4.1	19.75	11.4	51.7	28.8	46.75	29	2.98	2.4	1.33	18	0.315	1	0.02	2.2
FEB	11.135	7.8	4.6	5.6	45.95	51.4	36.05	39.6	19.1	21.4	5.2	6.2	0	0	0	0
MAR	18.8	5.6	20.35	5.6	36.05	10.2	126.9	37.9	83.3	24	7.9	2.6	0.55	0.2	4.45	1.3
APR	32.15	18.4	14.35	8.4	53.1	29.8	125.85	70.2	23.05	14.4	5.05	2.9	9.1	5.3	12.7	7.6
MAY	29.05	16.4	12.7	7.2	59.6	33.8	84.5	78.6	92.65	6.4	4.2	5.2	7.05	8.8	9.7	11.6
JUN	36.4	20.2	13.45	7.4	17.5	9.6	183.65	102	6.3	4.8	1.5	0.8	0.35	0.2	0.3	0.2
JUL	91.8	51.4	46.6	26	83.85	46.8	40.3	22.8	9.65	6	6.7	4.2	4.75	3	30.7	17.4
AUG	52.25	29.4	57.65	33.3	79.05	49.2	105.7	59.6	7.85	5	4.25	2.5	8.15	5.5	8.6	6
SEP	51.75	30	14.57	8.5	48.3	21	123.9	49.6	17.4	13.76	5.95	3.6	4.65	3	1.5	95.3

2.3. The faunal recorded and the range of salinity at Dry Creek saltfields.

Date:	April 1994								
Code	Taxon / Ponds	XD1	XB3	XC3	XB8	PA3	PA7	PA9	PA12
		Salinity (g/L)							
		56	62.5	107	108.5	115.5	184	198	216
Ghh	*H.tasmanicus*	*	*						
Nem	Nematoda	*	*						
Aaa	*A.franciscana*						2.6 5.0 5.2	2.4 0.78 17.73	7.2 7.2 4.2
Abp	*P. zietziana*			0.8 4.4 0.8		3.2 5.2 2.4			
Ocd	*D. dictyote*	0.4 0.8 0.4	0.4 0.8 0.4		1.6 2.8 2.0				
Occ	*Reticypris herbsti*			16.8 18.0 18.8		0.2 0.2 N.P			
Chm	*Mesochra parva*								
Chs	Harpacticoida	4.4 7.2 4.0	0.4 1.6 0.4						
Cca	*A. australis*	1.6 1.16 1.7							
Ccc	*Acartia clausi*	1.6 1.16 1.7							
Iss	*E.bicolor*	*							
Iis	*Synischia* sp.	*							
Ahp	*Parhyalella* sp.	*							
Dct	*T. barbitarsis*			0.4 N.P 0.4	0.4 0.8 N.P				
Dcc	*Cladotanytarsus* sp.			*	*				
Dee	*E. riparia*				0.02 N.P. N.P.	*			
Tls	*S. wheeleri*								

Data as ind./L, * = Uncounted samples, **N.P.** = not present in samples

Ghh- Mollusca: Gastropoda, Mesogastropoda, Hydrococcidae, *Hydrococcus tasmanicus*.

Nem. Nematoda, sp.

Aaa- Crustacea: Anostraca: Artemiidae, *Artemia franciscana*; **Abp**- Crustacea: Anostraca: Branchipodidae, *Parartemia zietziana*

Ocd- Ostracoda: Cypridacea: Cyprididae, *Diacypris*; **Occ**- Ostracoda: Cypridacea: Cyprididae *Reticypris* herbsti

Chs- Copepoda: Harpacticoida: sp.; **Chm**- Copepoda: Harpacticoida, *Mesochra parva*; **Cca**- Copepoda: Cyclopoida: *Austracyclops australis*; **Ccc**- Copepoda: Calanoida, *Acartia clausi*

Iss- Isopoda: Sphaeromatidae: Exosphaeroma; E.bicolor; **Iis**- Isopoda: Idoteidae, Synischia Sp.,

Ahp. Gammaridean Amphipoda; Talitroidea, Hyalidae, *Parhyalella* sp.

Dct- Insecta: Dipetra: Chironomidae, Chironominae, *Tanytarsus barbitarsus*; **Dcc**- Insecta: Dipetra: Chironomidae *Cladotanytarsus sp.*; **Dee**- Insecta: Dipetra: Ephydridae, *Ephydra riparia*; **Tls**- Trichoptera: Leptoceridae, *Symphitoneuria wheeleri*

Date:	May 1994								
Code	Taxon	Ponds	XD1	XB3	XC3	XB8	PA3	PA7	PA9	PA12
			Salinity (g/L)							
			57	61.5	108	104.5	135	210.5	209	211.5
Ghh	*H.tasmanicus*			*	*					
Nem	Nematoda									
Aaa	*A.franciscana*							1.2 1.8 1.6	3.4 2.3 3.2	3.6 6.8 4.0
Abp	*P. zietziana*		0.2 0.1 N.P.	5.9 15.5 5.1	5.2 2.4 4.0		20.8 21.2 18.2			
Ocd	*D. dictyote*				3.2 2.4 2.4		1.2 12 2.0			
Occ	*Reticypris herbsti*									
Chm	*Mesochra parva*						*			
Chs	Harpacticoida									
Cca	*A. australis*									
Ccc	*Acartia clausi*		17.8 11.8 9.0	51.2 737.1 13.1		0.2 0.4 0.2				
Iss	*E.bicolor*									
Iis	*Synischia* sp.									
Ahp	*Parhyalella* sp.									
Dct	*T. barbitarsis*					0.2 0.2 N.P.				
Dcc	*Cladotanytarsus* sp.				0.8 0.4 N.P.		0.4 N.P. N.P.	*		
Dee	*E. riparia*						*			
Tls	*S. wheeleri*									

Data as ind./L, * = Uncounted samples, **N.P.** = not present in samples

Ghh- Mollusca: Gastropoda, Mesogastropoda, Hydrococcidae, *Hydrococcus tasmanicus*.

Nem. Nematoda, sp.

Aaa- Crustacea: Anostraca: Artemiidae, *Artemia franciscana*; **Abp**- Crustacea: Anostraca: Branchipodidae, *Parartemia zietziana*

Ocd- Ostracoda: Cypridacea: Cyprididae, *Diacypris*; **Occ**- Ostracoda: Cypridacea: Cyprididae *Reticypris* herbsti

Chs- Copepoda: Harpacticoida: sp.; **Chm**- Copepoda: Harpacticoida, *Mesochra parva*; **Cca**- Copepoda: Cyclopoida: *Austracyclops australis*; **Ccc**- Copepoda: Calanoida, *Acartia clausi*

Iss- Isopoda: Sphaeromatidae: Exosphaeroma; E.bicolor; **Iis**- Isopoda: Idoteidae, Synischia Sp.,

Ahp. Gammaridean Amphipoda; Talitroidea, Hyalidae, *Parhyalella* sp.

Dct- Insecta: Dipetra: Chironomidae, Chironominae, *Tanytarsus barbitarsus*; **Dcc**- Insecta: Dipetra: Chironomidae *Cladotanytarsus sp.*; **Dee**- Insecta: Dipetra: Ephydridae, *Ephydra riparia*; **Tls**- Trichoptera: Leptoceridae, *Symphitoneuria wheeleri*

Date:	June 1994								
Code	Taxon / Ponds	XD1	XB3	XC3	XB8	PA3	PA7	PA9	PA12
		Salinity (g/L)							
		55.5	59	105	110	138	155	169.5	189
Ghh	*H.tasmanicus*	*							
Nem	Nematoda	*							
Aaa	*A.franciscana*						1.82 0.68 57.44	4.3 4.5 2.15	17.2 15.6 2.23
Abp	*P. zietziana*		0.3 N.P. 0.66	30.26 170 57.8		82.82 102.06 137.02			
Ocd	*D. dictyote*			2.5 3.0 2.6	1.0 3.0 1.2	2.6 5.7 3.2			
Occ	*Reticypris herbsti*				N.P. N.P. 0.12				
Chm	*Mesochra parva*	0.24 0.24 N.P.	0.3 1.8 0.32						
Chs	Harpacticoida								
Cca	*A. australis*								
Ccc	*Acartia clausi*	103 64.8 86.5		N.P. N.P. 0.9	0.12 N.P. 0.12				
Iss	*E.bicolor*								
Iis	*Synischia* sp.								
Ahp	*Parhyalella* sp.								
Dct	*T. barbitarsis*								
Dcc	*Cladotanytarsus* sp.				0.13 0.06 0.06	0.12 0.12 0.12	*		
Dee	*E. riparia*								
Tls	*S. wheeleri*								

Data as ind./L, * = Uncounted samples, **N.P.** = not present in samples

Ghh- Mollusca: Gastropoda, Mesogastropoda, Hydrococcidae, *Hydrococcus tasmanicus*.

Nem. Nematoda, sp.

Aaa- Crustacea: Anostraca: Artemiidae, *Artemia franciscana*; **Abp**- Crustacea: Anostraca: Branchipodidae, *Parartemia zietziana*

Ocd- Ostracoda: Cypridacea: Cyprididae, *Diacypris*; **Occ**- Ostracoda: Cypridacea: Cyprididae *Reticypris* herbsti

Chs- Copepoda: Harpacticoida: sp.; **Chm**- Copepoda: Harpacticoida, *Mesochra parva*; **Cca**- Copepoda: Cyclopoida: *Austracyclops australis*; **Ccc**- Copepoda: Calanoida, *Acartia clausi*

Iss- Isopoda: Sphaeromatidae: Exosphaeroma; E.bicolor; **Iis**- Isopoda: Idoteidae, Synischia Sp.,

Ahp. Gammaridean Amphipoda; Talitroidea, Hyalidae, *Parhyalella* sp.

Dct- Insecta: Dipetra: Chironomidae, Chironominae, *Tanytarsus barbitarsus*; **Dcc**- Insecta: Dipetra: Chironomidae *Cladotanytarsus sp.*; **Dee**- Insecta: Dipetra: Ephydridae, *Ephydra riparia*; **Tls**- Trichoptera: Leptoceridae, *Symphitoneuria wheeleri*

Date:	July 1994								
Code	Taxon	Ponds	XD1	XB3	XC3	XB8	PA3	PA7	PA9	PA12
		Salinity (g/L)	53.5	61	106	105.5	141	185.5	191	199.5
Ghh	*H.tasmanicus*		*							
Nem	Nematoda									
Aaa	*A.franciscana*							0.04 0.06 0.11	0.6 0.4 0.3	20.0 0.18 5.08
Abp	*P. zietziana*				32.06 15.0 19.36		1.0 3.0 1.4			
Ocd	*D. dictyote*		N.P. 0.32 N.P.		1.3 9.9 7.0		N.P. 0.24 0.12			
Occ	*Reticypris herbsti*				1.0 0.06 1.2					
Chm	*Mesochra parva*		0.72 0.92 N.P.	0.2 0.09 N.P.						
Chs	Harpacticoida									
Cca	*A. australis*		*							
Ccc	*Acartia clausi*		66.8 15.6 79.3	0.6 0.3 4.8		0.2 0.9 0.3				
Iss	*E.bicolor*		*							
Iis	*Synischia* sp.		*							
Ahp	*Parhyalella* sp.									
Dct	*T. barbitarsis*									
Dcc	*Cladotanytarsus* sp.					*	*	*	*	*
Dee	*E. riparia*						*	*		
Tls	*S. wheeleri*									

Data as ind./L, * = Uncounted samples, **N.P.** = not present in samples

Ghh- Mollusca: Gastropoda, Mesogastropoda, Hydrococcidae, *Hydrococcus tasmanicus*.

Nem. Nematoda, sp.

Aaa- Crustacea: Anostraca: Artemiidae, *Artemia franciscana*; **Abp**- Crustacea: Anostraca: Branchipodidae, *Parartemia zietziana*

Ocd- Ostracoda: Cypridacea: Cyprididae, *Diacypris*; **Occ**- Ostracoda: Cypridacea: Cyprididae *Reticypris* herbsti

Chs- Copepoda: Harpacticoida: sp.; **Chm**- Copepoda: Harpacticoida, *Mesochra parva*; **Cca**- Copepoda: Cyclopoida: *Austracyclops australis*; **Ccc**- Copepoda: Calanoida, *Acartia clausi*

Iss- Isopoda: Sphaeromatidae: Exosphaeroma; E.bicolor; **Iis**- Isopoda: Idoteidae, Synischia Sp.,

Ahp. Gammaridean Amphipoda; Talitroidea, Hyalidae, *Parhyalella* sp.

Dct- Insecta: Dipetra: Chironomidae, Chironominae, *Tanytarsus barbitarsus*; **Dcc**- Insecta: Dipetra: Chironomidae *Cladotanytarsus sp.*; **Dee**- Insecta: Dipetra: Ephydridae, *Ephydra riparia*; **Tls**- Trichoptera: Leptoceridae, *Symphitoneuria wheeleri*

Date:	August 1994							
Code	Taxon / Ponds	XD1	XB3	XC3	XB8	PA3	PA7	PA9	PA12
		Salinity (g/L)							
		49.5	58.5	103	98	148	177	182.5	198
Ghh	*H.tasmanicus*	*	*	*					
Nem	Nematoda								
Aaa	*A.franciscana*						3.58 4.84 4.02	7.7 7.4 4.57	4.0 0.24 1.0
Abp	*P. zietziana*			172.6 6.8 9.54					
Ocd	*D. dictyote*			3.72 1.4 1.4.					
Occ	*Reticypris herbsti*					0.48 9.9 12.9			
Chm	*Mesochra parva*	1.2 N.P. N.P.							
Chs	Harpacticoida								
Cca	*A. australis*								
Ccc	*Acartia clausi*	82.5 30.3 67.5	0.64 1.64 0.52						
Iss	*E.bicolor*	*							
Iis	*Synischia* sp.	*							
Ahp	*Parhyalella* sp.								
Dct	*T. barbitarsis*		2.2 N.P. 4.1	0.2 1.2 1.9	*	0.2 1.2 1.9			
Dcc	*Cladotanytarsus* sp.				*	0.9 0.9 0.9	*	*	*
Dee	*E. riparia*						*	*	
Tls	*S. wheeleri*								

Data as ind./L, * = Uncounted samples, **N.P.** = not present in samples

Ghh- Mollusca: Gastropoda, Mesogastropoda, Hydrococcidae, *Hydrococcus tasmanicus*.

Nem. Nematoda, sp.

Aaa- Crustacea: Anostraca: Artemiidae, *Artemia franciscana*; **Abp**- Crustacea: Anostraca: Branchipodidae, *Parartemia zietziana*

Ocd- Ostracoda: Cypridacea: Cyprididae, *Diacypris*; **Occ**- Ostracoda: Cypridacea: Cyprididae *Reticypris* herbsti

Chs- Copepoda: Harpacticoida: sp.; **Chm**- Copepoda: Harpacticoida, *Mesochra parva*; **Cca**- Copepoda: Cyclopoida: *Austracyclops australis*; **Ccc**- Copepoda: Calanoida, *Acartia clausi*

Iss- Isopoda: Sphaeromatidae: Exosphaeroma; E.bicolor; **Iis**- Isopoda: Idoteidae, Synischia Sp.,

Ahp. Gammaridean Amphipoda; Talitroidea, Hyalidae, *Parhyalella* sp.

Dct- Insecta: Dipetra: Chironomidae, Chironominae, *Tanytarsus barbitarsus*; **Dcc**- Insecta: Dipetra: Chironomidae *Cladotanytarsus sp.*; **Dee**- Insecta: Dipetra: Ephydridae, *Ephydra riparia*; **Tls**- Trichoptera: Leptoceridae, *Symphitoneuria wheeleri*

Date:	September 1994								
Code	Taxon / Ponds	XD1	XB3	XC3	XB8	PA3	PA7	PA9	PA12
		Salinity (g/L)							
		51.5	63	120.5	113	154.5	194.5	202	227.5
Ghh	*H.tasmanicus*	*	*	*					
Nem	Nematoda	*	*		*				
Aaa	*A.franciscana*				0.12 0.26 N.P.		26.91 188.24 211.72	23.1 19.9 25.1	0.3 1.44 1.6
Abp	*P. zietziana*		0.12 N.P. N.P.	8.3 34.54 37.2		62.1 25.04 21.92	N.P. 1.32 1.18		
Ocd	*D. dictyote*			1.6 10 8.8					
Occ	*Reticypris herbsti*				2.0 0.92 1.2				
Chm	*Mesochra parva*	0.4 1.6 0.4	N.P. 4.7 3.0						
Chs	Harpacticoida								
Cca	*A. australis*		0.12 N.P. N.P.						
Ccc	*Acartia clausi*	8.0 6.2 9.6	5.8 23.8 23.2						
Iss	*E.bicolor*	*							
Iis	*Synischia* sp.	*							
Ahp	*Parhyalella* sp.								
Dct	*T. barbitarsis*		3.44 0.8 1.0		0.12 N.P. N.P.		*		*
Dcc	*Cladotanytarsus* sp.		*	*	0.12 N.P. N.P.		*		*
Dee	*E. riparia*						*	*	
Tls	*S. wheeleri*								

Data as ind./L, * = Uncounted samples, **N.P**. = not present in samples

Ghh- Mollusca: Gastropoda, Mesogastropoda, Hydrococcidae, *Hydrococcus tasmanicus*.

Nem. Nematoda, sp.

Aaa- Crustacea: Anostraca: Artemiidae, *Artemia franciscana*; **Abp**- Crustacea: Anostraca: Branchipodidae, *Parartemia zietziana*

Ocd- Ostracoda: Cypridacea: Cyprididae, *Diacypris*; **Occ**- Ostracoda: Cypridacea: Cyprididae *Reticypris* herbsti

Chs- Copepoda: Harpacticoida: sp.; **Chm**- Copepoda: Harpacticoida, *Mesochra parva*; **Cca**- Copepoda: Cyclopoida: *Austracyclops australis*; **Ccc**- Copepoda: Calanoida, *Acartia clausi*

Iss- Isopoda: Sphaeromatidae: Exosphaeroma; E.bicolor; **Iis**- Isopoda: Idoteidae, Synischia Sp.,

Ahp. Gammaridean Amphipoda; Talitroidea, Hyalidae, *Parhyalella* sp.

Dct- Insecta: Dipetra: Chironomidae, Chironominae, *Tanytarsus barbitarsus*; **Dcc**- Insecta: Dipetra: Chironomidae *Cladotanytarsus sp.*; **Dee**- Insecta: Dipetra: Ephydridae, *Ephydra riparia*; **Tls**- Trichoptera: Leptoceridae, *Symphitoneuria wheeleri*

Date:	October 1994								
Code	Taxon	Ponds	XD1	XB3	XC3	XB8	PA3	PA7	PA9	PA12
			Salinity (g/L)							
			46	54	110	103	121.5	172.5	201.5	243.5
Ghh	*H.tasmanicus*		*							
Nem	Nematoda		*	*						
Aaa	*A.franciscana*							15.54 7.34 46.54	0.76 1.88 0.24	0.36 0.15 1.96
Abp	*P. zietziana*			N.P. 0.48 0.48	26.11 8.78 7.98	13.5 14.6 15.2	2.74 1.16 1.15	1.22 0.52 0.36		
Ocd	*D. dictyote*				6.6 23.2 22.0	6.2 19.6 15.2	*			
Occ	*Reticypris herbsti*			N.P. 0.24 0.64	1.4 0.24 0.24	*	*			
Chm	*Mesochra parva*		0.6 1.2 1.06							
Chs	Harpacticoida			1.04 N.P. N.P.						
Cca	*A. australis*									
Ccc	*Acartia clausi*		1.84 0.42 0.44	3.44 1.44 2.12						
Iss	*E.bicolor*		*							
Iis	*Synischia* sp.		*							
Ahp	*Parhyalella* sp.		*							
Dct	*T. barbitarsis*		N.P. 0.32 0.2	N.P. 0.8 1.04		2.1 7.04 6.4				
Dcc	*Cladotanytarsus* sp.		*	*						
Dee	*E. riparia*							*	*	
Tls	*S. wheeleri*									

Data as ind./L, * = Uncounted samples, **N.P.** = not present in samples

Ghh- Mollusca: Gastropoda, Mesogastropoda, Hydrococcidae, *Hydrococcus tasmanicus*.

Nem. Nematoda, sp.

Aaa- Crustacea: Anostraca: Artemiidae, *Artemia franciscana*; **Abp**- Crustacea: Anostraca: Branchipodidae, *Parartemia zietziana*

Ocd- Ostracoda: Cypridacea: Cyprididae, *Diacypris*; **Occ**- Ostracoda: Cypridacea: Cyprididae *Reticypris* herbsti

Chs- Copepoda: Harpacticoida: sp.; **Chm**- Copepoda: Harpacticoida, *Mesochra parva*; **Cca**- Copepoda: Cyclopoida: *Austracyclops australis*; **Ccc**- Copepoda: Calanoida, *Acartia clausi*

Iss- Isopoda: Sphaeromatidae: Exosphaeroma; E.bicolor; **Iis**- Isopoda: Idoteidae, Synischia Sp.,

Ahp. Gammaridean Amphipoda; Talitroidea, Hyalidae, *Parhyalella* sp.

Dct- Insecta: Dipetra: Chironomidae, Chironominae, *Tanytarsus barbitarsus*; **Dcc**- Insecta: Dipetra: Chironomidae *Cladotanytarsus sp.*; **Dee**- Insecta: Dipetra: Ephydridae, *Ephydra riparia*; **Tls**- Trichoptera: Leptoceridae, *Symphitoneuria wheeleri*

Date:	November 1994								
Code	Taxon	Ponds	XD1	XB3	XC3	XB8	PA3	PA7	PA9	PA12
		Salinity (g/L)	59.5	67	120.5	105.5	140.5	182.5	190	211.5
Ghh	*H.tasmanicus*		*	*						
Nem	Nematoda									
Aaa	*A.franciscana*							38.09 18.82 19.73	20.86 13.16 20.06	2.2 1.44 0.52
Abp	*P. zietziana*			1.2 0.66 0	3.0 9.96 1.88	36.46 1.46 4	45.3 3.3 3.8	0.92 1.56 0.66		
Ocd	*D. dictyote*				*					
Occ	*Reticypris herbsti*				3.3 1.3 1.3	0.46 1.66 1.46	13.2 16.9 20.6			
Chm	*Mesochra parva*		1.6 0.13 0							
Chs	Harpacticoida		0 0.2 1.7	0 0.52 0.52						
Cca	*A. australis*									
Ccc	*Acartia clausi*		1.39 5.12 2.52	42.2 59.3 76.4						
Iss	*E.bicolor*									
Iis	*Synischia* sp.									
Ahp	*Parhyalella* sp.									
Dct	*T. barbitarsis*									
Dcc	*Cladotanytarsus* sp.			0.26 0.52 0.26	0.32 0.06 0.32		*			
Dee	*E. riparia*									
Tls	*S. wheeleri*									

Data as ind./L, * = Uncounted samples, **N.P.** = not present in samples

Ghh- Mollusca: Gastropoda, Mesogastropoda, Hydrococcidae, *Hydrococcus tasmanicus*.

Nem. Nematoda, sp.

Aaa- Crustacea: Anostraca: Artemiidae, *Artemia franciscana*; **Abp**- Crustacea: Anostraca: Branchipodidae, *Parartemia zietziana*

Ocd- Ostracoda: Cypridacea: Cyprididae, *Diacypris*; **Occ**- Ostracoda: Cypridacea: Cyprididae *Reticypris* herbsti

Chs- Copepoda: Harpacticoida: sp.; **Chm**- Copepoda: Harpacticoida, *Mesochra parva*; **Cca**- Copepoda: Cyclopoida: *Austracyclops australis*; **Ccc**- Copepoda: Calanoida, *Acartia clausi*

Iss- Isopoda: Sphaeromatidae: Exosphaeroma; E.bicolor; **Iis**- Isopoda: Idoteidae, Synischia Sp.,

Ahp. Gammaridean Amphipoda; Talitroidea, Hyalidae, *Parhyalella* sp.

Dct- Insecta: Dipetra: Chironomidae, Chironominae, *Tanytarsus barbitarsus*; **Dcc**- Insecta: Dipetra: Chironomidae *Cladotanytarsus sp.*; **Dee**- Insecta: Dipetra: Ephydridae, *Ephydra riparia*; **Tls**- Trichoptera: Leptoceridae, *Symphitoneuria wheeleri*

Date:	December 1994								
Code	Taxon / Ponds	XD1	XB3	XC3	XB8	PA3	PA7	PA9	PA12
		Salinity (g/L)							
		54	58.5	122.5	92.5	124.5	199.0	193.0	210.5
Ghh	*H.tasmanicus*	*							
Nem	Nematoda	*	*						
Aaa	*A.franciscana*						4.16 6.14 5.98	8.78 5.15 6.15	2.38 4.62 3.66
Abp	*P. zietziana*		0 11.72 6.9	171.69 144.52 174.08	1.7 0.52 0.26	2.04 82.12 86.46	0.06 0.01 0.04		
Ocd	*D. dictyote*			1.64 2.9 2.82	1.2 0.52 0.52	0.4 5.2 9.32			
Occ	*Reticypris herbsti*			*	0.26 N.P. N.P.				
Chm	*Mesochra parva*	1.0 1.46 0.54							
Chs	Harpacticoida								
Cca	*A. australis*								
Ccc	*Acartia clausi*	17.46 0.92 8.32	3.34 2.5 3.2						
Iss	*E.bicolor*	*							
Iis	*Synischia* sp.	*							
Ahp	*Parhyalella* sp.	*							
Dct	*T. barbitarsis*	0.13 1.0 0.06			0.64 0.12 N.P.				
Dcc	*Cladotanytarsus* sp.	*		*		*			
Dee	*E. riparia*			*					
Tls	*S. wheeleri*								

Data as ind./L, * = Uncounted samples, **N.P.** = not present in samples

Ghh- Mollusca: Gastropoda, Mesogastropoda, Hydrococcidae, *Hydrococcus tasmanicus*.

Nem. Nematoda, sp.

Aaa- Crustacea: Anostraca: Artemiidae, *Artemia franciscana*; **Abp**- Crustacea: Anostraca: Branchipodidae, *Parartemia zietziana*

Ocd- Ostracoda: Cypridacea: Cyprididae, *Diacypris*; **Occ**- Ostracoda: Cypridacea: Cyprididae *Reticypris* herbsti

Chs- Copepoda: Harpacticoida: sp.; **Chm**- Copepoda: Harpacticoida, *Mesochra parva*; **Cca**- Copepoda: Cyclopoida: *Austracyclops australis*; **Ccc**- Copepoda: Calanoida, *Acartia clausi*

Iss- Isopoda: Sphaeromatidae: Exosphaeroma; E.bicolor; **Iis**- Isopoda: Idoteidae, Synischia Sp.,

Ahp. Gammaridean Amphipoda; Talitroidea, Hyalidae, *Parhyalella* sp.

Dct- Insecta: Dipetra: Chironomidae, Chironominae, *Tanytarsus barbitarsus*; **Dcc**- Insecta: Dipetra: Chironomidae *Cladotanytarsus sp.*; **Dee**- Insecta: Dipetra: Ephydridae, *Ephydra riparia*; **Tls**- Trichoptera: Leptoceridae, *Symphitoneuria wheeleri*

Date:	January 1995								
Code	Ponds / Taxon	XD1	XB3	XC3	XB8	PA3	PA7	PA9	PA12
		Salinity (g/L)							
		54	81	116.5	109	140.5	187	202.5	234.0
Ghh	*H.tasmanicus*	*							
Nem	Nematoda	*	*	*	*				
Aaa	*A.franciscana*						1.18 12.36 12.98	3.38 2.4 2.66	2.8 4.12 1.12
Abp	*P. zietziana*		0 2.06 1.83	224 98.64 93.9	4.33 0.2 0.33	26.42 83.4 70.41			
Ocd	*D. dictyote*				0.72 0.92 0.6				
Occ	*Reticypris herbsti*		2.16 2.06 1.86	1.7 0.8 0.92	0.52 0.2 0.33				
Chm	*Mesochra parva*								
Chs	Harpacticoida	0.06 0.06 0.12							
Cca	*A. australis*		1.06 0.4 0.66						
Ccc	*Acartia clausi*	0.04 0 0.06	6.5 0 0.06						
Iss	*E.bicolor*	*							
Iis	*Synischia* sp.	*							
Ahp	*Parhyalella* sp.								
Dct	*T. barbitarsis*								
Dcc	*Cladotanytarsus* sp.	0 0.13 0.13		1.8 0.13 0.33		*			
Dee	*E. riparia*				*	*			
Tls	*S. wheeleri*	*							

Data as ind./L, * = Uncounted samples, **N.P**. = not present in samples

Ghh- Mollusca: Gastropoda, Mesogastropoda, Hydrococcidae, *Hydrococcus tasmanicus*.

Nem. Nematoda, sp.

Aaa- Crustacea: Anostraca: Artemiidae, *Artemia franciscana*; **Abp**- Crustacea: Anostraca: Branchipodidae, *Parartemia zietziana*

Ocd- Ostracoda: Cypridacea: Cyprididae, *Diacypris*; **Occ**- Ostracoda: Cypridacea: Cyprididae *Reticypris* herbsti

Chs- Copepoda: Harpacticoida: sp.; **Chm**- Copepoda: Harpacticoida, *Mesochra parva*; **Cca**- Copepoda: Cyclopoida: *Austracyclops australis*; **Ccc**- Copepoda: Calanoida, *Acartia clausi*

Iss- Isopoda: Sphaeromatidae: Exosphaeroma; E.bicolor; **Iis**- Isopoda: Idoteidae, Synischia Sp.,

Ahp. Gammaridean Amphipoda; Talitroidea, Hyalidae, *Parhyalella* sp.

Dct- Insecta: Dipetra: Chironomidae, Chironominae, *Tanytarsus barbitarsus*; **Dcc**- Insecta: Dipetra: Chironomidae *Cladotanytarsus sp.*; **Dee**- Insecta: Dipetra: Ephydridae, *Ephydra riparia*; **Tls**- Trichoptera: Leptoceridae, *Symphitoneuria wheeleri*

Date:	February 1995								
Code	Taxon / Ponds	XD1	XB3	XC3	XB8	PA3	PA7	PA9	PA12
		Salinity (g/L)							
		52.5	58.0	115.5	108	139	180	205	242.5
Ghh	*H.tasmanicus*	*							
Nem	Nematoda	*							
Aaa	*A.franciscana*						6.08 131.6 95.7	9.16 1.78 2.52	2.36 0.8 2.12
Abp	*P. zietziana*		0.12 0 0	24.84 2.04 24.04	*	3.6 2.0 2.24			
Ocd	*D. dictyote*		0.12 N.P. N.P.	0.04 0 0.02	0.52 0.2 0.2				
Occ	*Reticypris herbsti*			N.P. 1.72 N.P.					
Chm	*Mesochra parva*		0.12 N.P. 0.64						
Chs	Harpacticoida		N.P. 0.12 N.P.						
Cca	*A. australis*	*							
Ccc	*Acartia clausi*	1.99 0.69 0.85	0.26 N.P. 0.4						
Iss	*E.bicolor*	*							
Iis	*Synischia* sp.	*							
Ahp	*Parhyalella* sp.								
Dct	*T. barbitarsis*		N.P. 0.4 1.3	N.P. 0.12 N.P.	15.04 2.8 23.6	0.4 N.P. N.P.			
Dcc	*Cladotanytarsus* sp.		*	*	*	0.26 0.12 0.26			
Dee	*E. riparia*		*		*	*			
Tls	*S. wheeleri*	*							

Data as ind./L, * = Uncounted samples, **N.P**. = not present in samples

Ghh- Mollusca: Gastropoda, Mesogastropoda, Hydrococcidae, *Hydrococcus tasmanicus*.

Nem. Nematoda, sp.

Aaa- Crustacea: Anostraca: Artemiidae, *Artemia franciscana*; **Abp**- Crustacea: Anostraca: Branchipodidae, *Parartemia zietziana*

Ocd- Ostracoda: Cypridacea: Cyprididae, *Diacypris*; **Occ**- Ostracoda: Cypridacea: Cyprididae *Reticypris* herbsti

Chs- Copepoda: Harpacticoida: sp.; **Chm**- Copepoda: Harpacticoida, *Mesochra parva*; **Cca**- Copepoda: Cyclopoida: *Austracyclops australis*; **Ccc**- Copepoda: Calanoida, *Acartia clausi*

Iss- Isopoda: Sphaeromatidae: Exosphaeroma; E.bicolor; **Iis**- Isopoda: Idoteidae, Synischia Sp.,

Ahp. Gammaridean Amphipoda; Talitroidea, Hyalidae, *Parhyalella* sp.

Dct- Insecta: Dipetra: Chironomidae, Chironominae, *Tanytarsus barbitarsus*; **Dcc**- Insecta: Dipetra: Chironomidae *Cladotanytarsus sp.*; **Dee**- Insecta: Dipetra: Ephydridae, *Ephydra riparia*; **Tls**- Trichoptera: Leptoceridae, *Symphitoneuria wheeleri*

Date:	March 1995								
Code	Taxon / Ponds	XD1	XB3	XC3	XB8	PA3	PA7	PA9	PA12
		Salinity (g/L)							
		60	62.5	110	133.5	141	205	210	217
Ghh	*H.tasmanicus*	*							
Nem	Nematoda								
Aaa	*A.franciscana*						78.77 86.24 89.56	1.92 2.14 1.86	1.18 0.59 0.46
Abp	*P. zietziana*			0.66 0.06 0.08	1.74 N.P. N.P.	19.52 13.46 12.26			
Ocd	*D. dictyote*			0.2 0.2 N.P.					
Occ	*Reticypris herbsti*			0.22 0.4 0.4					
Chm	*Mesochra parva*	0.13 0.86 0.72							
Chs	Harpacticoida		N.P 1.2 1.4						
Cca	*A. australis*								
Ccc	*Acartia clausi*	1.32 0.38 0.64	1.6 0.64 0.52						
Iss	*E.bicolor*	*							
Iis	*Synischia* sp.	*							
Ahp	*Parhyalella* sp.								
Dct	*T. barbitarsis*	N.P. 0.06 N.P.	1.6 0.8 1.4	N.P. 1.46 1.46	1.44 0.52 1.44	0.12 N.P. 0.02			
Dcc	*Cladotanytarsus* sp.	0.06 0.12 0.08	0.1 0.08 0.12	0.18 0.32 0.08	0.12 0.02 0.02	0.08 0.02 N.P.			
Dee	*E. riparia*				*	*	*		
Tls	*S. wheeleri*								

Data as ind./L, * = Uncounted samples, **N.P.** = not present in samples

Ghh- Mollusca: Gastropoda, Mesogastropoda, Hydrococcidae, *Hydrococcus tasmanicus*.

Nem. Nematoda, sp.

Aaa- Crustacea: Anostraca: Artemiidae, *Artemia franciscana*; **Abp**- Crustacea: Anostraca: Branchipodidae, *Parartemia zietziana*

Ocd- Ostracoda: Cypridacea: Cyprididae, *Diacypris*; **Occ**- Ostracoda: Cypridacea: Cyprididae *Reticypris* herbsti

Chs- Copepoda: Harpacticoida: sp.; **Chm**- Copepoda: Harpacticoida, *Mesochra parva*; **Cca**- Copepoda: Cyclopoida: *Austracyclops australis*; **Ccc**- Copepoda: Calanoida, *Acartia clausi*

Iss- Isopoda: Sphaeromatidae: Exosphaeroma; E.bicolor; **Iis**- Isopoda: Idoteidae, Synischia Sp.,

Ahp. Gammaridean Amphipoda; Talitroidea, Hyalidae, *Parhyalella* sp.

Dct- Insecta: Dipetra: Chironomidae, Chironominae, *Tanytarsus barbitarsus*; **Dcc**- Insecta: Dipetra: Chironomidae *Cladotanytarsus sp.*; **Dee**- Insecta: Dipetra: Ephydridae, *Ephydra riparia*; **Tls**- Trichoptera: Leptoceridae, *Symphitoneuria wheeleri*

Date:	April 1995								
Code	Taxon / Ponds	XD1	XB3	XC3	XB8	PA3	PA7	PA9	PA12
		Salinity (g/L)							
		63	64.0	130.4	119	147	207	214	236
Ghh	*H.tasmanicus*	*							
Nem	Nematoda								
Aaa	*A.franciscana*						2.04 8.28 0.98	0.72 0.66 0.63	0.96 1.14 0.45
Abp	*P. zietziana*			21.04 0.65 0.64		27.70 81.80 64.85			
Ocd	*D. dictyote*			0.13 1.0 0.13					
Occ	*Reticypris herbsti*		*	1.0 1.2 0.5					
Chm	*Mesochra parva*	0.2 3.2 0.4	0.6 N.P. N.P.						
Chs	Harpacticoida		0.13 0.4 0.2						
Cca	*A. australis*								
Ccc	*Acartia clausi*	1.6 0.72 1.06							
Iss	*E.bicolor*								
Iis	*Synischia* sp.								
Ahp	*Parhyalella* sp.								
Dct	*T. barbitarsis*	0.6 N.P. N.P.							
Dcc	*Cladotanytarsus* sp.	*	*	*	0.06 0.13 0.13	0.72 1.6 0.2			
Dee	*E. riparia*					*			
Tls	*S. wheeleri*								

Data as ind./L, * = Uncounted samples, **N.P.** = not present in samples

Ghh- Mollusca: Gastropoda, Mesogastropoda, Hydrococcidae, *Hydrococcus tasmanicus*.

Nem. Nematoda, sp.

Aaa- Crustacea: Anostraca: Artemiidae, *Artemia franciscana*; **Abp**- Crustacea: Anostraca: Branchipodidae, *Parartemia zietziana*

Ocd- Ostracoda: Cypridacea: Cyprididae, *Diacypris*; **Occ**- Ostracoda: Cypridacea: Cyprididae *Reticypris* herbsti

Chs- Copepoda: Harpacticoida: sp.; **Chm**- Copepoda: Harpacticoida, *Mesochra parva*; **Cca**- Copepoda: Cyclopoida: *Austracyclops australis*; **Ccc**- Copepoda: Calanoida, *Acartia clausi*

Iss- Isopoda: Sphaeromatidae: Exosphaeroma; E.bicolor; **Iis**- Isopoda: Idoteidae, Synischia Sp.,

Ahp. Gammaridean Amphipoda; Talitroidea, Hyalidae, *Parhyalella* sp.

Dct- Insecta: Dipetra: Chironomidae, Chironominae, *Tanytarsus barbitarsus*; **Dcc**- Insecta: Dipetra: Chironomidae *Cladotanytarsus sp.*; **Dee**- Insecta: Dipetra: Ephydridae, *Ephydra riparia*; **Tls**- Trichoptera: Leptoceridae, *Symphitoneuria wheeleri*

Date:	May 1995								
Code	Taxon / Ponds	XD1	XB3	XC3	XB8	PA3	PA7	PA9	PA12
		Salinity (g/L)							
		63.5	66	121	137	164.5	207.5	208	214.5
Ghh	*H.tasmanicus*	*							
Nem	Nematoda	*	*						
Aaa	*A.franciscana*						3.9 0.98 N.P.	0.12 0.52 0.64	0.86 0.52 0.45
Abp	*P. zietziana*		N.P. 0.12 N.P.	41.2 21.7 34.6	0.12 0.06 0.06	18.34 4.63 76.38			
Ocd	*D. dictyote*								
Occ	*Reticypris herbsti*			0.32 1.32 1.06	0.2 0.46 0.2				
Chm	*Mesochra parva*	0.6 1.04 N.P.	0.2 0.06 0.13						
Chs	Harpacticoida								
Cca	*A. australis*								
Ccc	*Acartia clausi*	24.9 30.68 47.2	0.12 3.12 1.18						
Iss	*E.bicolor*	*							
Iis	*Synischia* sp.	*							
Ahp	*Parhyalella* sp.								
Dct	*T. barbitarsis*	*		0.06 0.4 0.2	N.P. 0.13 0.06	N.P. 0.64 N.P.			
Dcc	*Cladotanytarsus* sp.	*		0.13 0.06 0.26	*	*	N.P. 0.02 0.02		
Dee	*E. riparia*			*	*	*	*	*	
Tls	*S. wheeleri*								

Data as ind./L, * = Uncounted samples, **N.P**. = not present in samples

Ghh- Mollusca: Gastropoda, Mesogastropoda, Hydrococcidae, *Hydrococcus tasmanicus*.

Nem. Nematoda, sp.

Aaa- Crustacea: Anostraca: Artemiidae, *Artemia franciscana*; **Abp**- Crustacea: Anostraca: Branchipodidae, *Parartemia zietziana*

Ocd- Ostracoda: Cypridacea: Cyprididae, *Diacypris*; **Occ**- Ostracoda: Cypridacea: Cyprididae *Reticypris* herbsti

Chs- Copepoda: Harpacticoida: sp.; **Chm**- Copepoda: Harpacticoida, *Mesochra parva*; **Cca**- Copepoda: Cyclopoida: *Austracyclops australis*; **Ccc**- Copepoda: Calanoida, *Acartia clausi*

Iss- Isopoda: Sphaeromatidae: Exosphaeroma; E.bicolor; **Iis**- Isopoda: Idoteidae, Synischia Sp.,

Ahp. Gammaridean Amphipoda; Talitroidea, Hyalidae, *Parhyalella* sp.

Dct- Insecta: Dipetra: Chironomidae, Chironominae, *Tanytarsus barbitarsus*; **Dcc**- Insecta: Dipetra: Chironomidae *Cladotanytarsus sp.*; **Dee**- Insecta: Dipetra: Ephydridae, *Ephydra riparia*; **Tls**- Trichoptera: Leptoceridae, *Symphitoneuria wheeleri*

Date:	June 1995								
Code	Taxon / Ponds	XD1	XB3	XC3	XB8	PA3	PA7	PA9	PA12
		Salinity (g/L)							
		52.5	53.5	93.5	90	113.5	157.5	168.5	194.5
Ghh	*H.tasmanicus*	*							
Nem	Nematoda		*						
Aaa	*A.franciscana*						6.64 3.63 1.2	7.62 1.62 3.04	1.26 0.46 0.86
Abp	*P. zietziana*		3.0 2.6 3.6	53.2 46.8 2.8	1.19 0.39 1.0	90.4 80.9 84.73			
Ocd	*D. dictyote*								
Occ	*Reticypris herbsti*			37.2 29.0 30.4					
Chm	*Mesochra parva*	4.4 4.0 4.3							
Chs	Harpacticoida	2.6 0 0							
Cca	*A. australis*								
Ccc	*Acartia clausi*	25.3 18.1 14.5	20.7 25.2 30.8	0.13 0 0					
Iss	*E.bicolor*								
Iis	*Synischia* sp.								
Ahp	*Parhyalella* sp.								
Dct	*T. barbitarsis*								
Dcc	*Cladotanytarsus* sp.			*					
Dee	*E. riparia*		*	*	*	*		*	
Tls	*S. wheeleri*								

Data as ind./L, * = Uncounted samples, **N.P.** = not present in samples

Ghh- Mollusca: Gastropoda, Mesogastropoda, Hydrococcidae, *Hydrococcus tasmanicus*.

Nem. Nematoda, sp.

Aaa- Crustacea: Anostraca: Artemiidae, *Artemia franciscana*; **Abp**- Crustacea: Anostraca: Branchipodidae, *Parartemia zietziana*

Ocd- Ostracoda: Cypridacea: Cyprididae, *Diacypris*; **Occ**- Ostracoda: Cypridacea: Cyprididae *Reticypris* herbsti

Chs- Copepoda: Harpacticoida: sp.; **Chm**- Copepoda: Harpacticoida, *Mesochra parva*; **Cca**- Copepoda: Cyclopoida: *Austracyclops australis*; **Ccc**- Copepoda: Calanoida, *Acartia clausi*

Iss- Isopoda: Sphaeromatidae: Exosphaeroma; E.bicolor; **Iis**- Isopoda: Idoteidae, Synischia Sp.,

Ahp. Gammaridean Amphipoda; Talitroidea, Hyalidae, *Parhyalella* sp.

Dct- Insecta: Dipetra: Chironomidae, Chironominae, *Tanytarsus barbitarsus*; **Dcc**- Insecta: Dipetra: Chironomidae *Cladotanytarsus sp.*; **Dee**- Insecta: Dipetra: Ephydridae, *Ephydra riparia*; **Tls**- Trichoptera: Leptoceridae, *Symphitoneuria wheeleri*

Date: July 1995									
Code	Taxon / Ponds	XD1	XB3	XC3	XB8	PA3	PA7	PA9	PA12
		Salinity (g/L)							
		59	61.5	107	96.5	151.5	168.5	178.5	195.5
Ghh	*H.tasmanicus*								
Nem	Nematoda								
Aaa	*A.franciscana*						0.99 N.P. N.P.	0.66 0.26 0.26	1.73 0.73 0.46
Abp	*P. zietziana*		2.52 N.P. N.P.	43.34 38.86 43.52	0.02 N.P. 0.06	3.03 0.93 0.26			
Ocd	*D. dictyote*			1.53 2.13 2.4	N.P. 0.06 N.P.				
Occ	*Reticypris herbsti*			0.06 1.5 2.1					
Chm	*Mesochra parva*	0.4 0.4 N.P	0.06 0.33 0.13						
Chs	Harpacticoida	0.06 0.13 0.13	0.13 0.6 0.4						
Cca	*A. australis*	0.02 N.P. 0.06							
Ccc	*Acartia clausi*	12.4 35.06 28.42	2.99 8.06 7.19						
Iss	*E.bicolor*	*							
Iis	*Synischia* sp.	*							
Ahp	*Parhyalella* sp.								
Dct	*T. barbitarsis*		0.2 N.P. N.P.	.02 N.P. N.P.	N.P. 0.02 N.P.				
Dcc	*Cladotanytarsus* sp.		0.2 N.P. 0.06	*	*	1.06 1.6 1.86			
Dee	*E. riparia*		*		*	*	*		
Tls	*S. wheeleri*								

Data as ind./L, * = Uncounted samples, **N.P.** = not present in samples

Ghh- Mollusca: Gastropoda, Mesogastropoda, Hydrococcidae, *Hydrococcus tasmanicus*.

Nem. Nematoda, sp.

Aaa- Crustacea: Anostraca: Artemiidae, *Artemia franciscana*; **Abp**- Crustacea: Anostraca: Branchipodidae, *Parartemia zietziana*

Ocd- Ostracoda: Cypridacea: Cyprididae, *Diacypris*; **Occ**- Ostracoda: Cypridacea: Cyprididae *Reticypris* herbsti

Chs- Copepoda: Harpacticoida: sp.; **Chm**- Copepoda: Harpacticoida, *Mesochra parva*; **Cca**- Copepoda: Cyclopoida: *Austracyclops australis*; **Ccc**- Copepoda: Calanoida, *Acartia clausi*

Iss- Isopoda: Sphaeromatidae: Exosphaeroma; E.bicolor; **Iis**- Isopoda: Idoteidae, Synischia Sp.,

Ahp. Gammaridean Amphipoda; Talitroidea, Hyalidae, *Parhyalella* sp.

Dct- Insecta: Dipetra: Chironomidae, Chironominae, *Tanytarsus barbitarsus*; **Dcc**- Insecta: Dipetra: Chironomidae *Cladotanytarsus sp.*; **Dee**- Insecta: Dipetra: Ephydridae, *Ephydra riparia;* **Tls**- Trichoptera: Leptoceridae, *Symphitoneuria wheeleri*

Date:	August 1995								
Code	Taxon / Ponds	XD1	XB3	XC3	XB8	PA3	PA7	PA9	PA12
		Salinity (g/L)							
		55.5	62	104	99.5	128	194.5	179	195.5
Ghh	*H.tasmanicus*	*							
Nem	Nematoda					*	*	*	
Aaa	*A.franciscana*						1.3 25.3 2.2	3.13 6.3 5.2	0.46 6.0 4.6
Abp	*P. zietziana*	0.52 1.04 1.0	0.5 1.0 1.9	570.42 267.7 81.82		1.4 9.94 8.96			
Ocd	*D. dictyote*			0.01 0 0.06	0.06 0.1 0.08				
Occ	*Reticypris herbsti*			42.1 80.4 57.6	0.12 0.26 0.2				
Chm	*Mesochra parva*		0.8 0 0						
Chs	Harpacticoida								
Cca	*A. australis*								
Ccc	*Acartia clausi*	13.4 20.2 25.0	0.12 1.28 11.84						
Iss	*E.bicolor*								
Iis	*Synischia* sp.								
Ahp	*Parhyalella* sp.								
Dct	*T. barbitarsis*								
Dcc	*Cladotanytarsus* sp.	*	*	*	*				
Dee	*E. riparia*				*	*	*		
Tls	*S. wheeleri*								

Data as ind./L, * = Uncounted samples, **N.P.** = not present in samples

Ghh- Mollusca: Gastropoda, Mesogastropoda, Hydrococcidae, *Hydrococcus tasmanicus*.

Nem. Nematoda, sp.

Aaa- Crustacea: Anostraca: Artemiidae, *Artemia franciscana*; **Abp**- Crustacea: Anostraca: Branchipodidae, *Parartemia zietziana*

Ocd- Ostracoda: Cypridacea: Cyprididae, *Diacypris*; **Occ**- Ostracoda: Cypridacea: Cyprididae *Reticypris* herbsti

Chs- Copepoda: Harpacticoida: sp.; **Chm**- Copepoda: Harpacticoida, *Mesochra parva*; **Cca**- Copepoda: Cyclopoida: *Austracyclops australis*; **Ccc**- Copepoda: Calanoida, *Acartia clausi*

Iss- Isopoda: Sphaeromatidae: Exosphaeroma; E.bicolor; **Iis**- Isopoda: Idoteidae, Synischia Sp.,

Ahp. Gammaridean Amphipoda; Talitroidea, Hyalidae, *Parhyalella* sp.

Dct- Insecta: Dipetra: Chironomidae, Chironominae, *Tanytarsus barbitarsus*; **Dcc**- Insecta: Dipetra: Chironomidae *Cladotanytarsus sp.*; **Dee**- Insecta: Dipetra: Ephydridae, *Ephydra riparia*; **Tls**- Trichoptera: Leptoceridae, *Symphitoneuria wheeleri*

Date:	September 1995								
Code	Taxon / Ponds	XD1	XB3	XC3	XB8	PA3	PA7	PA9	PA12
		Salinity (g/L)							
		57.5	63	114	99	133.5	240	182.5	195
Ghh	*H.tasmanicus*	*							
Nem	Nematoda								
Aaa	*A.franciscana*						64.4 34.9 25.4	127.6 114.04 69.8	6.2 3.06 2.92
Abp	*P. zietziana*			2.18 3.16 9.06		9.32 19.6 33.6			
Ocd	*D. dictyote*				*				
Occ	*Reticypris herbsti*		0.64 0.06 0.12	0.92 7.5 12.0	0.52 3.2 2.8				
Chm	*Mesochra parva*	0.04 0.02 0.06							
Chs	Harpacticoida								
Cca	*A. australis*		0.92 0.92 0.06						
Ccc	*Acartia clausi*	2.54 8.32 15.16	1.28 3.84 3.8						
Iss	*E.bicolor*	*							
Iis	*Synischia* sp.	*							
Ahp	*Parhyalella* sp.								
Dct	*T. barbitarsis*								
Dcc	*Cladotanytarsus* sp.	*	*	*	*	*	*	*	*
Dee	*E. riparia*			*			*	*	
Tls	*S. wheeleri*								

Data as ind./L, * = Uncounted samples, **N.P.** = not present in samples

Ghh- Mollusca: Gastropoda, Mesogastropoda, Hydrococcidae, *Hydrococcus tasmanicus*.

Nem. Nematoda, sp.

Aaa- Crustacea: Anostraca: Artemiidae, *Artemia franciscana*; **Abp**- Crustacea: Anostraca: Branchipodidae, *Parartemia zietziana*

Ocd- Ostracoda: Cypridacea: Cyprididae, *Diacypris*; **Occ**- Ostracoda: Cypridacea: Cyprididae *Reticypris* herbsti

Chs- Copepoda: Harpacticoida: sp.; **Chm**- Copepoda: Harpacticoida, *Mesochra parva*; **Cca**- Copepoda: Cyclopoida: *Austracyclops australis*; **Ccc**- Copepoda: Calanoida, *Acartia clausi*

Iss- Isopoda: Sphaeromatidae: Exosphaeroma; E.bicolor; **Iis**- Isopoda: Idoteidae, Synischia Sp.,

Ahp. Gammaridean Amphipoda; Talitroidea, Hyalidae, *Parhyalella* sp.

Dct- Insecta: Dipetra: Chironomidae, Chironominae, *Tanytarsus barbitarsus*; **Dcc**- Insecta: Dipetra: Chironomidae *Cladotanytarsus sp.*; **Dee**- Insecta: Dipetra: Ephydridae, *Ephydra riparia*; **Tls**- Trichoptera: Leptoceridae, *Symphitoneuria wheeler*

2.4. Numbers and percentages of developmental groups in *Parartemia* and *Artemia.*

XC3 (*Parartemia*)

Date	female (g)	female	male	subadult	sum	female (g) %	female %	male %	subadult %
APR	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
MAY	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
JUN	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
JUL	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
AUG	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
SEP	0.00	0.12	0.00	0.00	0.12	0.00	100.00	0.00	0.00
OCT	0.00	0.12	0.00	10.30	10.42	0.00	1.15	0.00	98.85
NOV	0.00	0.15	0.62	8.33	9.10	0.00	1.65	6.81	91.54
DEC	0.00	0.00	0.04	1.57	1.61	0.00	0.00	2.48	97.52
JAN	0.08	0.06	0.17	1.70	2.01	3.98	2.99	8.46	84.58
FEB	0.00	0.00	0.00	0.42	0.42	0.00	0.00	0.00	100.00
MAR	0.02	0.00	0.00	0.00	0.02	100.00	0.00	0.00	0.00
APR	0.57	0.00	0.00	0.00	0.57	100.00	0.00	0.00	0.00
MAY	0.00	0.00	0.00	0.08	0.08	0.00	0.00	0.00	100.00
JUN	0.00	0.17	0.21	0.02	0.40	0.00	42.50	52.50	5.00
JUL	0.00	0.02	0.00	0.00	0.02	0.00	100.00	0.00	0.00
AUG	0.00	0.10	0.00	0.00	0.10	0.00	100.00	0.00	0.00
SEP	0.00	0.17	0.00	0.00	0.17	0.00	100.00	0.00	0.00

XB3 (*Parartemia*)

APR	0.00	0.53	0.40	2.60	3.53	0.00	15.01	11.33	73.65
MAY	0.00	3.33	2.66	2.50	8.49	0.00	39.22	31.33	29.45
JUN	0.00	2.56	0.17	83.40	86.13	0.00	2.97	0.20	96.83
JUL	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
AUG	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
SEP	0.00	0.00	0.00	0.04	0.04	0.00	0.00	0.00	100.00
OCT	0.08	0.16	0.08	0.00	0.32	25.00	50.00	25.00	0.00
NOV	0.00	0.50	0.66	0.64	1.80	0.00	27.78	36.67	35.56
DEC	0.00	0.00	0.00	6.20	6.20	0.00	0.00	0.00	100.00
JAN	0.08	0.06	0.17	1.70	2.01	3.98	2.99	8.46	84.58
FEB	0.00	0.00	0.00	0.04	0.04	0.00	0.00	0.00	100.00
MAR	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
APR	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
MAY	0.00	0.00	0.00	0.04	0.04	0.00	0.00	0.00	100.00
JUN	0.00	0.00	0.00	3.06	3.06	0.00	0.00	0.00	100.00
JUL	0.00	0.02	0.00	0.82	0.84	0.00	2.38	0.00	97.62
AUG	0.00	0.00	0.00	0.85	0.85	0.00	0.00	0.00	100.00
SEP	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00

XB8 (*Parartemia*)

APR	0	0.4	0	1.6	2	0.00	0.00	0.00	80.00
MAY	0	0.93	0	2.9	3.83	0.00	0.00	0.00	75.72
JUN	0	2.56	0.17	83.4	86.13	0.00	0.00	0.20	96.83
JUL	0	0.25	0.25	21.6	22.1	0.00	0.00	1.13	97.74
AUG	0.46	0.21	0	62.3	62.97	0.73	0.74	0.00	98.94
SEP	0	7.4	2.24	18.5	28.14	0.00	0.00	7.96	65.74

XB8 (*Parartemia*) continued

Date	female (g)	female	male	subadult	sum	female (g) %	female %	male %	subadult %
OCT	0	4.43	0.17	9.61	14.21	0.00	0.00	1.20	67.63
NOV	0	1.68	0.82	3.44	5.94	0.00	0.00	13.80	57.91
DEC	0.35	0.17	0.52	162.7	163.74	0.21	0.22	0.32	99.36
JAN	1.44	12.8	6.4	122.8	143.44	1.00	1.17	4.46	85.61
FEB	0.21	3.6	0.4	4.25	8.46	2.48	4.94	4.73	50.24
MAR	0	0.02	0	0.24	0.26	0.00	0.00	0.00	92.31
APR	0	19.3	0.23	7	26.53	0.00	0.00	0.87	26.39
JUN	0	2.1	0	43.23	45.33	0.00	0.00	0.00	95.37
JUL	0.02	0.1	0.04	13.98	14.14	0.14	0.14	0.28	98.87
AUG	22.61	21.1	14.5	248.4	306.61	7.37	9.10	4.73	81.01
SEP	0.76	0.28	0.3	16.2	17.54	4.33	4.69	1.71	92.36

PA3 (*Parartemia*)

APR	0	0.53	0.4	2.6	3.53	0.00	15.01	11.33	73.65
MAY	0.33	1.7	0.33	21.3	23.66	1.39	7.19	1.39	90.03
JUN	0.08	0.44	0.8	102.7	104.02	0.08	0.42	0.77	98.73
JUL	0	0	0	1.8	1.8	0.00	0.00	0.00	100.00
AUG	0.06	0	0	0	0.06	100.00	0.00	0.00	0.00
SEP	0	1.6	0.68	34	36.28	0.00	4.41	1.87	93.72
OCT	0.26	0.48	0.12	0.31	1.17	22.22	41.03	10.26	26.50
NOV	0.35	0.65	0.13	3.77	4.9	7.14	13.27	2.65	76.94
DEC	0.87	0.54	0.55	54.41	56.37	1.54	0.96	0.98	96.52
JAN	1.37	1.94	1.35	54.9	59.56	2.30	3.26	2.27	92.18
FEB	0	0	0	2.68	2.68	0.00	0.00	0.00	100.00
MAR	0	0.14	0	14.9	15.04	0.00	0.93	0.00	99.07
APR	0.54	0.14	0.21	56.9	57.79	0.93	0.24	0.36	98.46
MAY	0.52	0.98	0.52	71	73.02	0.71	1.34	0.71	97.23
JUN	0.04	0.08	1.77	82.7	84.59	0.05	0.09	2.09	97.77
JUL	0.86	0.44	0	0.88	2.18	39.45	20.18	0.00	40.37
AUG	0.08	0.05	0.48	5.56	6.17	1.30	0.81	7.78	90.11
SEP	0	0	0	14.1	14.1	0.00	0.00	0.00	100.00

PA7 (*Artemia*)

APR	0	0.33	0.33	3.2	3.86	0.00	8.55	8.55	82.90
MAY	0	1.6	1	0	2.6	0.00	61.54	38.46	0.00
JUN	0	0.29	0.12	19.63	20.04	0.00	1.45	0.60	97.95
JUL	0	0	0	0.07	0.07	0.00	0.00	0.00	100.00
AUG	0.6	0.78	1.16	0.16	2.7	22.22	28.89	42.96	5.93
SEP	0.12	1.13	2.27	138.4	141.92	0.08	0.80	1.60	97.52
OCT	0.2	0.55	0.91	20.5	22.16	0.90	2.48	4.11	92.51
NOV	0.38	3.66	0.06	21.7	25.8	1.47	14.19	0.23	84.11
DEC	0.38	1.3	0.39	3.9	5.97	6.37	21.78	6.53	65.33
JAN	0.06	0.09	0.5	38.9	39.55	0.15	0.23	1.26	98.36
FEB	0	0.3	0.46	77.31	78.07	0.00	0.38	0.59	99.03
MAR	0.04	1.05	1.26	82.8	85.15	0.05	1.23	1.48	97.24
APR	0	2.6	0.88	0.38	3.86	0.00	67.36	22.80	9.84
MAY	0	0.98	0.64	0	1.62	0.00	60.49	39.51	0.00

PA7 (*Artemia*) continued

Date	female (g)	female	male	subadult	sum	emale (g)	emale	ale	ubadult
JUN	0	0.43	0.35	1.71	2.49	0.00	17.27	14.06	68.67
JUL	0	0.15	0.17	0	0.32	0.00	46.88	53.13	0.00
AUG	0	0	0	9.6	9.6	0.00	0.00	0.00	100.00
SEP	0	0	0	73.86	73.86	0.00	0.00	0.00	100.00

PA9 (*Artemia*)

APR	0	3.31	3.61	0	6.92	0.00	47.83	52.17	0.00
MAY	0	1.2	1.7	0	2.9	0.00	41.38	58.62	0.00
JUN	0	0.43	1.12	2.1	3.65	0.00	11.78	30.68	57.53
JUL	0	0	0	0.43	0.43	0.00	0.00	0.00	100.00
AUG	0	0.9	0.07	5.29	6.26	0.00	14.38	1.12	84.50
SEP	0.2	1.7	2.6	18.2	22.7	0.88	7.49	11.45	80.18
OCT	0.13	0.54	0.14	0	0.81	16.05	66.67	17.28	0.00
NOV	0.39	0.34	0.65	16.6	17.98	2.17	1.89	3.62	92.32
DEC	0	0.41	0.37	0.64	1.42	0.00	28.87	26.06	45.07
JAN	0.73	0.55	0.42	1.26	2.96	24.66	18.58	14.19	42.57
FEB	0	2.02	0.36	3.38	5.76	0.00	35.07	6.25	58.68
MAR	0	0.37	0.3	1.48	2.15	0.00	17.21	13.95	68.84
APR	0	1.22	0.4	0	1.62	0.00	75.31	24.69	0.00
MAY	0	0.23	0.12	0	0.35	0.00	65.71	34.29	0.00
JUN	0	0.72	1.02	2.34	4.08	0.00	17.65	25.00	57.35
JUL	0	0	0	0.39	0.39	0.00	0.00	0.00	100.00
AUG	0	0.2	0.06	4.63	4.89	0.00	4.09	1.23	94.68
SEP	0	0.13	0.026	97.4	97.556	0.00	0.13	0.03	99.84

PA12 (*Artemia*)

APR	0	0.33	0.33	3.2	3.86	0.00	8.55	8.55	82.90
MAY	0	1.6	1	0	2.6	0.00	61.54	38.46	0.00
JUN	0	0.29	0.12	19.63	20.04	0.00	1.45	0.60	97.95
JUL	0	0	0	0.07	0.07	0.00	0.00	0.00	100.00
AUG	0.6	0.78	1.16	0.16	2.7	22.22	28.89	42.96	5.93
SEP	0.12	1.13	2.27	138.4	141.92	0.08	0.80	1.60	97.52
OCT	0.2	0.55	0.91	20.5	22.16	0.90	2.48	4.11	92.51
NOV	0.38	3.66	0.06	21.7	25.8	1.47	14.19	0.23	84.11
DEC	0.38	1.3	0.39	3.9	5.97	6.37	21.78	6.53	65.33
JAN	0.06	0.09	0.5	38.9	39.55	0.15	0.23	1.26	98.36
FEB	0	0.3	0.46	77.31	78.07	0.00	0.38	0.59	99.03
MAR	0.04	1.05	1.26	82.8	85.15	0.05	1.23	1.48	97.24
APR	0	2.6	0.88	0.38	3.86	0.00	67.36	22.80	9.84
MAY	0	0.98	0.64	0	1.62	0.00	60.49	39.51	0.00
JUN	0	0.43	0.35	1.71	2.49	0.00	17.27	14.06	68.67
JUL	0	0.15	0.17	0	0.32	0.00	46.88	53.13	0.00
AUG	0	0	0	9.6	9.6	0.00	0.00	0.00	100.00
SEP	0	0	0	73.86	73.86	0.00	0.00	0.00	100.00

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APPENDIX 3

PHOSPHATE AND NITRATE DETERMINATION

3.1. Optical density reading for PO_4-P measurements at different salinities.

PO_4-P	Salinity 35		
(mg/L)	0 (g/L)	(g/L)	105 (g/L)
0.2	0.458	0.466	0.445
0.2	0.465	0.468	0.447
0.2	0.464	0.464	0.448
0.02	0.046	0.04	0.024
0.02	0.049	0.039	0.024
0.02	0.046	0.039	0.021
0.004	0.02	0.01	0.007
0.004	0.016	0.01	0.006
0.004	0.015	0.007	0.006

3.2. Optical density reading for NO_3-N measurements at different salinities.

NO_3-N	Salinity 35		
(ug/L)	0 (g/L)	(g/L)	105 (g/L)
100	0.128	0.098	0.141
100	0.122	0.099	0.142
100	0.123	0.099	0.142
10	0.036	0.032	0.089
10	0.029	0.036	0.085
10	0.033	0.036	0.086
1	0.019	0.024	0.064
1	0.017	0.027	0.058
1	0.017	0.027	0.069

3.3. Optical density for nitrate concentration in synthetic saline waters.

Distilled water (0 g/L)

NO3-N con. (ug/L)	sample 1	sample 2	sample 3	Mean
0	0.007	0.006	0.007	0.00667
1	0.016	0.017	0.016	0.01633
5	0.026	0.26	0.26	0.182
10	0.034	0.034	0.034	0.034
25	0.045	0.045	0.046	0.04533
50	0.157	0.167	0.16	0.16133
75	0.241	0.239	0.24	0.24
100	0.23	0.231	0.23	0.23033

1/2 seawater (17.5 g/L)

NO3-N con. (ug/L)	sample 1	sample 2	sample 3	Mean
0	0.085	0.084	0.085	0.08467
1	0.104	0.1	0.091	0.09833
5	0.1	0.1	0.097	0.099
10	0.113	0.111	0.109	0.111
25	0.127	0.12	0.121	0.12267
50	0.235	0.237	0.239	0.237
75	0.316	0.305	0.305	0.30867
100	0.301	0.301	0.301	0.301

Seawater (35 g/L)

NO3-N con. (ug/L)	sample 1	sample 2	sample 3	Mean
0	0.27	0.268	0.269	0.269
1	0.269	0.27	0.272	0.27033
5	0.284	0.285	0.29	0.28633
10	0.307	0.31	0.317	0.31133
25	0.317	0.314	0.314	0.315
50	0.408	0.409	0.409	0.40867
75	0.478	0.478	0.479	0.47833
100	0.545	0.546	0.547	0.546

2 x seawater (70 g/L)

NO3-N con. (ug/L)	sample 1	sample 2	sample 3	Mean
0	0.485	0.483	0.483	0.48367
1	0.498	0.5	0.51	0.50267
5	0.566	0.556	0.514	0.54533
10	0.532	0.529	0.525	0.52867
25	0.546	0.545	0.54	0.54367
50	0.622	0.6	0.584	0.602
75	0.685	0.682	0.682	0.683
100	0.74	0.755	0.76	0.75167

3 x seawater (105 g/L)

NO3-N con. (ug/L)	sample 1	sample 2	sample 3	Mean
0	0.589	0.588	0.589	0.58867
1	0.612	0.612	0.614	0.61267
5	0.617	0.616	0.616	0.61633
10	0.621	0.623	0.625	0.623
25	0.657	0.659	0.633	0.64967
50	0.716	0.72	0.721	0.719
75	0.7	0.75	0.773	0.741
100	0.8	0.87	0.85	0.84

4 x seawater (140 g/L)

NO3-N con. (ug/L)	sample 1	sample 2	sample 3	Mean
0	0.708	0.707	0.708	0.70767
1	0.719	0.718	0.716	0.71767
5	0.732	0.724	0.716	0.724
10	0.732	0.732	0.732	0.732
25	0.738	0.74	0.761	0.74633
50	0.818	0.819	0.82	0.819
75	0.874	0.874	0.872	0.87333
100	0.92	0.92	0.919	0.91967

3.4. The optical density when known amounts of nitrate are added to deionised and natural waters at different salinities.

salinity	10	10	10	Mean	50	50	50	Mean	100	100	100	Mean	200	200	200	Mean
(g/L)	(ug/L)	(ug/L)	(ug/L)		(ug/L)	(ug/L)	(ug/L)		(ug/L)	(ug/L)	(ug/L)		(ug/L)	(ug/L)	(ug/L)	
0	0.05	0.05	0.05	0.05	0.16	0.15	0.15	0.15	0.40	0.40	0.41	0.40	0.67	0.67	0.67	0.67
23	0.05	0.05	0.05	0.05	0.15	0.15	0.14	0.14	0.38	0.38	0.38	0.38	0.62	0.62	0.62	0.62
41	0.05	0.05	0.05	0.05	0.15	0.15	0.15	0.15	0.35	0.36	0.36	0.36	0.59	0.59	0.59	0.59
57	0.04	0.05	0.05	0.05	0.13	0.13	0.13	0.13	0.33	0.33	0.33	0.33	0.54	0.55	0.54	0.54
96	0.05	0.05	0.05	0.05	0.13	0.13	0.13	0.13	0.31	0.31	0.31	0.31	0.50	0.49	0.49	0.50
128	0.05	0.05	0.05	0.05	0.13	0.13	0.13	0.13	0.30	0.31	0.31	0.30	0.48	0.49	0.48	0.48
143	0.07	0.07	0.07	0.07	0.14	0.14	0.14	0.14	0.30	0.31	0.30	0.30	0.51	0.51	0.51	0.51
218	0.05	0.04	0.05	0.05	0.12	0.12	0.11	0.11	0.27	0.27	0.27	0.27	0.42	0.43	0.42	0.42
271	0.07	0.07	0.07	0.07	0.14	0.14	0.14	0.14	0.30	0.29	0.30	0.30	0.43	0.43	0.43	0.43

3.5. The optical density when known amounts of nitrate are added to deionised and natural waters at different salinities and corrected for blank (X).

sample	salinity	X*	10	10	10	Mean	50	50	50	Mean	100	100	100	Mean	200	200	200
	(g/L)		(ug/L)	(ug/L)	(ug/L)		(ug/L)	(ug/L)	(ug/L)		(ug/L)	(ug/L)	(ug/L)		(ug/L)	(ug/L)	(ug/L)
0	0.01	0.04	0.04	0.04	0.04	0.15	0.14	0.14	0.14	0.40	0.40	0.40	0.40	0.66	0.66	0.66	0.66
23	0.01	0.04	0.04	0.04	0.04	0.14	0.14	0.13	0.14	0.37	0.37	0.37	0.37	0.61	0.61	0.61	0.61
41	0.01	0.04	0.04	0.04	0.04	0.14	0.14	0.14	0.14	0.34	0.35	0.35	0.35	0.58	0.58	0.58	0.58
57	0.02	0.03	0.03	0.03	0.03	0.12	0.12	0.12	0.12	0.31	0.32	0.32	0.32	0.53	0.53	0.52	0.53
96	0.02	0.03	0.03	0.03	0.03	0.11	0.12	0.11	0.11	0.29	0.29	0.29	0.29	0.48	0.48	0.47	0.48
128	0.02	0.03	0.03	0.02	0.03	0.11	0.11	0.10	0.11	0.28	0.28	0.28	0.28	0.46	0.46	0.46	0.46
143	0.04	0.03	0.02	0.03	0.03	0.10	0.10	0.10	0.10	0.26	0.26	0.26	0.26	0.47	0.47	0.47	0.47
218	0.02	0.03	0.02	0.03	0.03	0.10	0.10	0.09	0.10	0.25	0.25	0.25	0.25	0.40	0.41	0.40	0.40
271	0.05	0.03	0.03	0.02	0.02	0.09	0.10	0.09	0.09	0.25	0.25	0.25	0.25	0.38	0.38	0.39	0.38

X*= nitrate concentration in original sample without spiking

APPENDIX 4

EXPERIMENTAL MEASUREMENTS

4.1. Experiment I

a. Viscosity of synthetic seawater (SSW), sodium chloride (NaCl) and evaporated seawater (SW).

Salinity (g/L)	Viscosity (SSW)	(NaCl)	(SW)
35	1.0567116	1.0603208	1.09495
70	1.0890648	1.092014	1.1072
105	1.1218284	1.1359425	1.14395
140	1.1699136	1.1827277	1.2052
175	1.2277116	1.2507495	1.29095
210	1.2928968	1.2915518	1.4012
245	1.3786704	1.4198338	1.53595
280	1.4537052	1.5438577	1.6952
315	1.558152	1.6158142	1.87895
350	1.60512	1.6965564	2.0872

b. Viscosity and relative viscosity determinations.

Stage I

sample	time (sec.)	time (sec.)	time (sec.)	time (sec.)	time (sec.)	AVERAGE	viscosity (cSt)	Relative viscosity
9/12/95								
Tank #1	45.15	45.1	45.14	45.21	45.15	45.15	1.5441	1.1029
Tank #2	45.15	45.1	45.14	45.21	45.15	45.15	1.5441	1.1029
Tank #3	45.15	45.1	45.14	45.21	45.15	45.15	1.5441	1.1029
Tank #4	45.15	45.1	45.14	45.21	45.15	45.15	1.5441	1.1029
Tank #5	45.15	45.1	45.14	45.21	45.15	45.15	1.5441	1.1029
15/12/95								
Tank #1	49.94	49.89	49.97	49.8	49.9	49.90	1.7066	1.2018
Tank #2	49.88	49.96	49.95	49.9	49.9	49.92	1.7072	1.2022
Tank #3	49.88	49.95	49.92	49.9	49.93	49.92	1.7071	1.2022
Tank #4	50.75	50.68	50.78	50.79	50.8	50.76	1.7360	1.2225
Tank #5	51.21	51.3	51.23	51.25	51.21	51.24	1.7524	1.2341
22/12/94								
Tank #1	68.73	68.75	68.65	68.7	68.73	68.71	1.8518	1.2683
Tank #2	69.25	69.2	69.2	69.27	69.25	69.23	1.8659	1.2868
Tank #3	69.39	69.34	69.37	69.36	69.36	69.36	1.8694	1.2892
Tank #4	68.6	69.58	69.62	69.59	69.55	69.39	1.8700	1.2897
Tank #5	71.24	71.5	70.5	71	71	71.05	1.9147	1.3297
29/12/94								
Tank #1	74.75	74.65	74.65	74.7	74.74	74.70	2.0131	1.2582
Tank #2	75.12	75.19	74.98	74.95	75.2	75.09	2.0236	1.2808
Tank #3	75.79	75.77	75.77	75.72	75.76	75.76	2.0418	1.2923
Tank #4	75.95	75.95	75.9	75.95	75.92	75.93	2.0464	1.2952
Tank #5	77.6	77.5	77.47	77.54	77.6	77.54	2.0898	1.3396
5/01/95								
Tank #1	78.19	78.22	78.08	78.1	78.12	78.14	2.1059	1.2763
Tank #2	78.29	78.34	78.33	78.34	78.32	78.32	2.1108	1.2793
Tank #3	79.22	79.15	79.19	79.12	79.16	79.17	2.1336	1.2551
Tank #4	79.92	79.93	79.9	79.93	79.85	79.91	2.1535	1.3051
Tank #5	81.85	81.82	81.8	81.75	81.88	81.82	2.2050	1.3445
12/01/95								
Tank #1	79.45	79.55	79.5	79.45	79.43	79.48	2.1419	1.2903
Tank #2	79.65	79.73	79.65	79.7	79.69	79.68	2.1475	1.3015
Tank #3	81.3	81.28	81.35	81.3	81.35	81.32	2.1915	1.3363
Tank #4	81.88	81.97	81.95	81.94	81.88	81.92	2.2079	1.3463
Tank #5	82.8	82.88	82.85	82.89	82.8	82.84	2.2326	1.3531

b. (continued)

Stage II

sample	time (sec.)	time (sec.)	time (sec.)	time (sec.)	time (sec.)	AVERAGE	viscosity (cSt)	Relative viscosity
19/01/95								
Tank #1	80.07	80	80.1	80.05	80.07	80.058	2.1576	1.2471
Tank #2	80.45	80.35	80.39	80.3	80.35	80.368	2.1659	1.3047
Tank #3	80.84	80.85	80.83	80.88	80.87	80.854	2.1790	1.3048
Tank #4	81.46	81.44	81.46	81.5	81.45	81.462	2.1954	1.3721
Tank #5	82.66	82.67	82.65	82.7	82.65	82.666	2.2278	1.3752
25/01/95								
Tank #1	81.55	81.58	81.56	81.55	81.53	81.554	2.1979	1.2488
Tank #2	82.3	82.29	82.3	82.33	82.37	82.318	2.2185	1.2677
Tank #3	83.38	83.4	83.34	83.27	83.34	83.346	2.2462	1.3059
Tank #4	82.45	82.4	82.42	82.41	82.44	82.424	2.2213	1.3712
Tank #5	82.33	82.4	82.39	82.35	82.38	82.37	2.2199	1.3293
1/02/95								
Tank #1	83.9	83.88	83.88	83.89	83.92	83.894	2.2609	1.2457
Tank #2	83.45	83.46	83.44	83.5	83.4	83.45	2.2490	1.2706
Tank #3	83.66	83.73	83.7	83.72	83.74	83.71	2.2560	1.3288
Tank #4	83.55	83.55	83.6	83.48	83.56	83.548	2.2516	1.3564
Tank #5	82.36	82.4	82.41	82.35	82.38	82.38	2.2201	1.3059
8/02/95								
Tank #1	85.81	85.82	85.78	85.8	85.79	85.8	2.3123	1.2846
Tank #2	85.65	85.67	85.74	85.6	85.6	85.652	2.3083	1.2683
Tank #3	85.75	85.78	85.7	85.75	85.78	85.752	2.3110	1.2768
Tank #4	85.45	85.5	85.55	85.46	85.54	85.5	2.3042	1.3092
Tank #5	84.99	84.89	84.9	85	84.97	84.95	2.2894	1.2934
15/02/95								
Tank #1	86.56	86.5	86.6	86.53	86.5	86.538	2.3322	1.2815
Tank #2	86.78	86.78	86.82	86.85	86.83	86.812	2.3396	1.2185
Tank #3	86.32	86.3	86.26	86.3	86.29	86.294	2.3256	1.3214
Tank #4	86.78	86.78	86.82	86.85	86.83	86.812	2.3396	1.2511
Tank #5	85.34	85.38	85.35	85.29	85.25	85.322	2.2994	1.2102
22/02/95								
Tank #1	88.24	88.35	88.33	88.28	88.35	88.31	2.3800	1.3100
Tank #2	87.32	87.32	87.31	87.39	87.23	87.314	2.3531	1.3408
Tank #3	88.23	88.15	88.23	88.25	88.2	88.212	2.3773	1.3943
Tank #4	88.8	88.86	88.85	88.8	88.86	88.834	2.3941	1.3960
Tank #5	86.2	86.12	86.18	86.14	86.25	86.178	2.3225	1.3622
2/03/95								
Tank #1	90.07	90.09	90.1	90.16	91.12	90.308	2.4338	1.2513
Tank #2	91.32	91.4	91.37	91.35	91.37	91.362	2.4622	1.3309
Tank #3	91.5	91.47	91.45	91.47	91.49	91.476	2.4653	1.3734
Tank #4	89.33	89.3	89.29	89.3	89.33	89.31	2.4069	1.2602
Tank #5	92.08	92.05	92.08	92.04	92	92.05	2.4807	1.2921

c. Viscosity of evaporated seawater at different salinities.

Samples	Salinity (g/L)	Viscosity (centistokes)	Samples	Salinity (g/L)	Viscosity (centistokes)	Samples	Salinity (g/L)	Viscosity (centistokes)
1	150	1.2272	48	173.5	1.2868	95	197	1.3574
2	150.5	1.2284	49	174	1.2882	96	197.5	1.3590
3	151	1.2295	50	174.5	1.2896	97	198	1.3606
4	151.5	1.2307	51	175	1.2910	98	198.5	1.3623
5	152	1.2318	52	175.5	1.2924	99	199	1.3639
6	152.5	1.2330	53	176	1.2938	100	199.5	1.3656
7	153	1.2342	54	176.5	1.2952	101	200	1.3672
8	153.5	1.2354	55	177	1.2966	102	200.5	1.3689
9	154	1.2366	56	177.5	1.2980	103	201	1.3705
10	154.5	1.2378	57	178	1.2994	104	201.5	1.3722
11	155	1.2390	58	178.5	1.3009	105	202	1.3738
12	155.5	1.2402	59	179	1.3023	106	202.5	1.3755
13	156	1.2414	60	179.5	1.3038	107	203	1.3772
14	156.5	1.2426	61	180	1.3052	108	203.5	1.3789
15	157	1.2438	62	180.5	1.3067	109	204	1.3806
16	157.5	1.2450	63	181	1.3081	110	204.5	1.3823
17	158	1.2462	64	181.5	1.3096	111	205	1.3840
18	158.5	1.2475	65	182	1.3110	112	205.5	1.3857
19	159	1.2487	66	182.5	1.3125	113	206	1.3874
20	159.5	1.2500	67	183	1.3140	114	206.5	1.3891
21	160	1.2512	68	183.5	1.3155	115	207	1.3908
22	160.5	1.2525	69	184	1.3170	116	207.5	1.3925
23	161	1.2537	70	184.5	1.3185	117	208	1.3942
24	161.5	1.2550	71	185	1.3200	118	208.5	1.3960
25	162	1.2562	72	185.5	1.3215	119	209	1.3977
26	162.5	1.2575	73	186	1.3230	120	209.5	1.3995
27	163	1.2588	74	186.5	1.3245	121	210	1.4012
28	163.5	1.2601	75	187	1.3260	122	210.5	1.4030
29	164	1.2614	76	187.5	1.3275	123	211	1.4047
30	164.5	1.2627	77	188	1.3290	124	211.5	1.4065
31	165	1.2640	78	188.5	1.3306	125	212	1.4082
32	165.5	1.2653	79	189	1.3321	126	212.5	1.4100
33	166	1.2666	80	189.5	1.3337	127	213	1.4118
34	166.5	1.2679	81	190	1.3352	128	213.5	1.4136
35	167	1.2692	82	190.5	1.3368	129	214	1.4154
36	167.5	1.2705	83	191	1.3383	130	214.5	1.4172
37	168	1.2718	84	191.5	1.3399	131	215	1.4190
38	168.5	1.2732	85	192	1.3414	132	215.5	1.4208
39	169	1.2745	86	192.5	1.3430	133	216	1.4226
40	169.5	1.2759	87	193	1.3446	134	216.5	1.4244
41	170	1.2772	88	193.5	1.3462	135	217	1.4262
42	170.5	1.2786	89	194	1.3478	136	217.5	1.4280
43	171	1.2799	90	194.5	1.3494	137	218	1.4298
44	171.5	1.2813	91	195	1.3510	138	218.5	1.4317
45	172	1.2826	92	195.5	1.3526	139	219	1.4335
46	172.5	1.2840	93	196	1.3542	140	219.5	1.4354
47	173	1.2854	94	196.5	1.3558	141	220	1.4372

Samples	Salinity (g/L)	Viscosity (centistokes)	Samples	Salinity (g/L)	Viscosity (centistokes)	Samples	Salinity (g/L)	Viscosity (centistokes)
142	220.5	1.4391	191	245	1.5360	240	269.5	1.6449
143	221	1.4409	192	245.5	1.5381	241	270	1.6472
144	221.5	1.4428	193	246	1.5402	242	270.5	1.6496
145	222	1.4446	194	246.5	1.5423	243	271	1.6519
146	222.5	1.4465	195	247	1.5444	244	271.5	1.6543
147	223	1.4484	196	247.5	1.5465	245	272	1.6566
148	223.5	1.4503	197	248	1.5486	246	272.5	1.6590
149	224	1.4522	198	248.5	1.5508	247	273	1.6614
150	224.5	1.4541	199	249	1.5529	248	273.5	1.6638
151	225	1.4560	200	249.5	1.5551	249	274	1.6662
152	225.5	1.4579	201	250	1.5572	250	274.5	1.6686
153	226	1.4598	202	250.5	1.5594	251	275	1.6710
154	226.5	1.4617	203	251	1.5615	252	275.5	1.6734
155	227	1.4636	204	251.5	1.5637	253	276	1.6758
156	227.5	1.4655	205	252	1.5658	254	276.5	1.6782
157	228	1.4674	206	252.5	1.5680	255	277	1.6806
158	228.5	1.4694	207	253	1.5702	256	277.5	1.6830
159	229	1.4713	208	253.5	1.5724	257	278	1.6854
160	229.5	1.4733	209	254	1.5746	258	278.5	1.6879
161	230	1.4752	210	254.5	1.5768	259	279	1.6903
162	230.5	1.4772	211	255	1.5790	260	279.5	1.6928
163	231	1.4791	212	255.5	1.5812	261	280	1.6952
164	231.5	1.4811	213	256	1.5834	262	280.5	1.6977
165	232	1.4830	214	256.5	1.5856	263	281	1.7001
166	232.5	1.4850	215	257	1.5878	264	281.5	1.7026
167	233	1.4870	216	257.5	1.5900	265	282	1.7050
168	233.5	1.4890	217	258	1.5922	266	282.5	1.7075
169	234	1.4910	218	258.5	1.5945	267	283	1.7100
170	234.5	1.4930	219	259	1.5967	268	283.5	1.7125
171	235	1.4950	220	259.5	1.5990	269	284	1.7150
172	235.5	1.4970	221	260	1.6012	270	284.5	1.7175
173	236	1.4990	222	260.5	1.6035	271	285	1.7200
174	236.5	1.5010	223	261	1.6057	272	285.5	1.7225
175	237	1.5030	224	261.5	1.6080	273	286	1.7250
176	237.5	1.5050	225	262	1.6102	274	286.5	1.7275
177	238	1.5070	226	262.5	1.6125	275	287	1.7300
178	238.5	1.5091	227	263	1.6148	276	287.5	1.7325
179	239	1.5111	228	263.5	1.6171	277	288	1.7350
180	239.5	1.5132	229	264	1.6194	278	290	1.7452
181	240	1.5152	230	264.5	1.6217	279	290.5	1.7478
182	240.5	1.5173	231	265	1.6240	280	291	1.7503
183	241	1.5193	232	265.5	1.6263	281	291.5	1.7529
184	241.5	1.5214	233	266	1.6286	282	292	1.7554
185	242	1.5234	234	266.5	1.6309	283	292.5	1.7580
186	242.5	1.5255	235	267	1.6332	284	293	1.7606
187	243	1.5276	236	267.5	1.6355	285	293.5	1.7632
188	243.5	1.5297	237	268	1.6378	286	294	1.7658
189	244	1.5318	238	268.5	1.6402	287	294.5	1.7684
190	244.5	1.5339	239	269	1.6425	288	295	1.7710

Samples	Salinity (g/L)	Viscosity (centistokes)	Samples	Salinity (g/L)	Viscosity (centistokes)	Samples	Salinity (g/L)	Viscosity (centistokes)
289	295.5	1.7736	338	320	1.9072	387	344.5	2.0529
290	296	1.7762	339	320.5	1.9101	388	345	2.0560
291	296.5	1.7788	340	321	1.9129	389	345.5	2.0591
292	297	1.7814	341	321.5	1.9158	390	346	2.0622
293	297.5	1.7840	342	322	1.9186	391	347	2.0653
294	298	1.7866	343	322.5	1.9215	392	347.5	2.0684
295	298.5	1.7893	344	323	1.9244	393	348	2.0715
296	299	1.7919	345	323.5	1.9273	394	348.5	2.0746
297	299.5	1.7946	346	324	1.9302	395	349	2.0778
298	300	1.7972	347	324.5	1.9331	396	349.5	2.0809
299	300.5	1.7999	348	325	1.9360	397	350	2.0841
300	301	1.8025	349	325.5	1.9389	398	350.5	2.0872
301	301.5	1.8052	350	326	1.9418	399	351	2.0904
302	302	1.8078	351	326.5	1.9447	400	351.5	2.0935
303	302.5	1.8105	352	327	1.9476	401	352	2.0967
304	303	1.8132	353	327.5	1.9505	402	352.5	2.0998
305	303.5	1.8159	354	328	1.9534	403	353	2.1030
306	304	1.8186	355	328.5	1.9564	404	353.5	2.1062
307	304.5	1.8213	356	329	1.9593	405	354	2.1094
308	305	1.8240	357	329.5	1.9623	406	354.5	2.1126
309	305.5	1.8267	358	330	1.9652	407	355	2.1158
310	306	1.8294	359	330.5	1.9682	408	355.5	2.1190
311	306.5	1.8321	360	331	1.9711	409	356	2.1222
312	307	1.8348	361	331.5	1.9741	410	356.5	2.1254
313	307.5	1.8375	362	332	1.9770	411	357	2.1286
314	308	1.8402	363	332.5	1.9800	412	357.5	2.1318
315	308.5	1.8430	364	333	1.9830	413	358	2.1350
316	309	1.8457	365	333.5	1.9860	414	358.5	2.1382
317	309.5	1.8485	366	334	1.9890	415	359	2.1415
318	310	1.8512	367	334.5	1.9920	416	359.5	2.1447
319	310.5	1.8540	368	335	1.9950	417	360	2.1480
320	311	1.8567	369	335.5	1.9980	418	360.5	2.1512
321	311.5	1.8595	370	336	2.0010	419	361	2.1545
322	312	1.8622	371	336.5	2.0040	420	361.5	2.1577
323	312.5	1.8650	372	337	2.0070	421	362	2.1610
324	313	1.8678	373	337.5	2.0100	422	362.5	2.1642
325	313.5	1.8706	374	338	2.0130	423	363	2.1675
326	314	1.8734	375	338.5	2.0161	424	363.5	2.1708
327	314.5	1.8762	376	339	2.0191	425	364	2.1741
328	315	1.8790	377	339.5	0.0222	426	364.5	2.1774
329	315.5	1.8818	378	340	2.0252	427	365	2.1807
330	316	1.8846	379	340.5	2.0283	428	365.5	2.1840
331	316.5	1.8874	380	341	2.0313	429	366	2.1873
332	317	1.8902	381	341.5	2.0344			
333	317.5	1.8930	382	342	2.0374			
334	318	1.8958	383	342.5	2.0405			
335	318.5	1.8987	384	343	2.0436			
336	319	1.9015	385	343.5	2.0467			
337	319.5	1.9044	386	344	2.0498			

4.2. Experiment II

a. Viscosity and relative viscosity of the samples.

sample	Salinity (g/L)	time (sec.)	time (sec.)	time (sec.)	time (sec.)	time (sec.)	Average	Viscosity (cSt)	Viscosity (S/W)	Relative viscosity
Pond PA7	190.5	56.12	56.16	56.25	56.2	56.16	56.178	1.5140	1.3368	1.1326
Pond FA1	255	65.29	65.19	65.2	65.2	65.22	65.22	1.7577	1.57895	1.1132
Week 1										
1	253	67.58	67.49	67.6	67.59	67.47	67.546	1.8204	1.57019	1.1593
1	253	68.08	68	68.07	68.05	68.07	68.054	1.8341	1.57019	1.1680
1	253	67.43	67.4	67.45	67.39	67.4	67.414	1.8168	1.57019	1.1571
2	254	66.39	66.43	66.55	66.42	66.54	66.466	1.7913	1.57456	1.1376
2	254	66.87	66.45	66.63	66.55	66.5	66.6	1.7949	1.57456	1.1399
2	254	66.54	66.5	66.49	66.55	66.49	66.514	1.7926	1.57456	1.1384
3	253.5	66.39	66.41	66.39	66.42	66.45	66.412	1.7898	1.57237	1.1383
3	255	66.49	66.59	66.49	66.5	66.54	66.522	1.7928	1.57895	1.1354
3	254	66.34	66.39	66.39	66.4	66.3	66.364	1.7885	1.57456	1.1359
4	255	66.33	66.38	66.33	66.32	66.33	66.338	1.7878	1.57895	1.1323
4	253	66.31	66.35	66.35	66.2	66.38	66.318	1.7873	1.57019	1.1383
4	253	66.29	66.25	66.29	66.33	66.27	66.286	1.7864	1.57019	1.1377
5	253.5	66.35	66.26	66.36	66.26	66.29	66.304	1.7869	1.57237	1.1364
5	253	66.68	66.7	66.6	66.66	66.7	66.668	1.7967	1.57019	1.1443
5	253.5	66.47	66.49	66.4	66.43	66.5	66.458	1.7910	1.57237	1.1391
6	253	66.28	66.23	66.2	66.25	66.28	66.248	1.7854	1.57019	1.1370
6	253	66.17	66.25	66.17	66.27	66.25	66.222	1.7847	1.57019	1.1366
6	253	66.33	66.34	66.39	66.4	66.34	66.36	1.7884	1.57019	1.1390
7	190	56.17	56.19	56.2	56.22	56.17	56.19	1.5143	1.3352	1.1342
7	190.5	56.78	56.79	56.8	56.77	56.76	56.78	1.5302	1.3367	1.1448
7	191	57.03	57.05	57	57.01	57.06	57.03	1.5370	1.33831	1.1484
8	191	57.05	57.2	56.95	57.1	57.12	57.084	1.5384	1.33831	1.1495
8	190	56.29	56.37	56.37	56.34	56.38	56.35	1.5186	1.3352	1.1374

sample	Salinity (g/L)	time (sec.)	time (sec.)	time (sec.)	time (sec.)	time (sec.)	Average	Viscosity (cSt)	Viscosity (S/W)	Relative viscosity
8	188	56.27	56.29	56.3	56.27	56.33	56.292	1.5171	1.32904	1.1415
9	188	56.16	56.29	56.17	56.2	56.29	56.222	1.5152	1.32904	1.1401
9	188.5	56.71	56.64	56.7	56.69	56.7	56.688	1.5277	1.33057	1.1482
9	188.5	56.7	56.75	56.69	56.6	56.77	56.702	1.5281	1.33057	1.1485
10	189	56.47	56.43	56.4	56.37	56.43	56.42	1.5205	1.33211	1.1414
10	189	56.32	56.37	56.32	56.4	56.35	56.352	1.5187	1.33211	1.1401
10	189.5	56.35	56.41	56.41	56.4	56.47	56.408	1.5202	1.33365	1.1399
11	189	56.4	56.41	56.4	56.45	56.38	56.408	1.5202	1.33211	1.1412
11	189	56.29	56.32	56.28	56.32	56.33	56.308	1.5175	1.33211	1.1392
11	188.5	56.19	56.26	56.26	56.22	56.19	56.224	1.5152	1.33057	1.1388
12	189.5	56.57	56.56	56.49	56.47	56.4	56.498	1.5226	1.33365	1.1417
12	189.5	56.42	56.44	56.5	56.52	56.4	56.456	1.5215	1.33365	1.1408
12	189	56.39	56.4	56.44	56.44	56.45	56.424	1.5206	1.33211	1.1415
Week 2										
1	253	70.03	70.04	70.07	70.99	70.04	70.234	1.8928	1.57019	1.2055
1	253	69.13	69.08	69.05	69.1	69.07	69.086	1.8619	1.57019	1.1858
1	253	69.68	69.66	69.64	69.63	70.01	69.724	1.8791	1.57019	1.1967
2	254	69.21	69.15	69.21	69.19	69.21	69.194	1.8648	1.57456	1.1843
2	254.5	70.91	71.03	71.02	70.98	70.98	70.984	1.9130	1.57675	1.2133
2	254.5	70.14	70.35	70.1	70.2	70.1	70.178	1.8913	1.57675	1.1995
3	254	69.33	69.26	69.42	69.4	69.33	69.348	1.8689	1.57456	1.1870
3	254.5	70.14	70.21	70.2	70.19	70.15	70.178	1.8913	1.57675	1.1995
3	255	71.06	70.99	70.95	71.01	71	71.002	1.9135	1.57895	1.2119
4	254.5	67.13	67.1	67.15	67.1	67.19	67.134	1.8093	1.57675	1.1475
4	253	66.39	66.23	66.3	66.33	66.3	66.31	1.7871	1.57019	1.1381
4	253.5	67.25	67.2	67.19	67.19	67.22	67.21	1.8113	1.57237	1.1520
5	256	69.74	69.69	69.79	69.78	69.81	69.762	1.8801	1.58336	1.1874
5	256	69.56	69.6	69.61	69.59	69.57	69.586	1.8753	1.58336	1.1844
5	256	68.69	68.65	68.7	68.78	68.78	68.72	1.8520	1.58336	1.1697

sample	Salinity (g/L)	time (sec.)	time (sec.)	time (sec.)	time (sec.)	time (sec.)	Average	Viscosity (cSt)	Viscosity (S/W)	Relative viscosity
6	243.5	67.23	67.24	67.15	67.18	67.2	67.2	1.8110	1.52967	1.1839
6	243.5	67.25	67.31	67.3	67.24	67.29	67.278	1.8131	1.52967	1.1853
6	243.5	66.27	66.27	66.29	66.31	66.25	66.278	1.7862	1.52967	1.1677
7	189	57.1	57.07	57.13	57.06	57.06	57.084	1.5384	1.33211	1.1549
7	189	56.47	56.51	56.44	56.55	56.41	56.476	1.5220	1.33211	1.1426
7	189	56.25	56.29	56.33	56.35	56.21	56.286	1.5169	1.33211	1.1387
8	189	56.84	56.83	56.8	56.85	56.86	56.836	1.5317	1.33211	1.1499
8	189	56.82	56.87	56.93	56.83	56.89	56.868	1.5326	1.33211	1.1505
8	189.5	56.85	56.87	56.85	56.82	56.86	56.85	1.5321	1.33365	1.1488
9	188	56.94	56.99	57.01	56.97	57.03	56.988	1.5358	1.32904	1.1556
9	189	56.84	56.8	56.86	56.85	56.82	56.834	1.5317	1.3052	1.1735
9	190	56.84	56.87	56.88	56.8	56.85	56.848	1.5321	1.3352	1.1474
10	191	56.78	56.79	56.8	56.85	56.85	56.814	1.5311	1.33831	1.1441
10	191	58.09	58.98	58.03	58.06	58.03	58.238	1.5695	1.33831	1.1728
10	191	57.22	57.19	57.24	57.25	57.27	57.234	1.5425	1.33831	1.1525
11	188	57.64	57.7	57.72	57.7	57.68	57.688	1.5547	1.32904	1.1698
11	188	57.51	57.49	57.59	57.58	57.55	57.544	1.5508	1.32904	1.1669
11	188	57.61	57.64	57.59	57.59	57.58	57.602	1.5524	1.32904	1.1680
12	190	56.98	56.94	57.01	57	56.99	56.984	1.5357	1.3352	1.1502
12	190	57.24	57.16	57.16	57.2	57.25	57.202	1.5416	1.3352	1.1546
12	190	58.37	58.29	58.39	58.35	58.3	58.34	1.5723	1.3352	1.1775
Week 3										
1	253	72.78	72.75	72.78	72.8	72.79	72.78	1.9614	1.57019	1.2492
1	253	71.9	71.89	71.91	71.91	71.95	71.912	1.9380	1.57019	1.2343
1	251	71.58	71.6	71.62	71.58	71.61	71.598	1.9296	1.56151	1.2357
2	255	76.41	73.42	73.5	73.49	73.5	74.064	1.9960	1.57895	1.2641
2	253	69.78	69.8	69.81	69.79	69.8	69.796	1.8810	1.57019	1.1979
2	250	68.54	68.53	68.52	68.5	68.56	68.53	1.8469	1.5572	1.1860

sample	Salinity (g/L)	time (sec.)	time (sec.)	time (sec.)	time (sec.)	time (sec.)	Average	Viscosity (cSt)	Viscosity (S/W)	Relative viscosity
3	249	70.02	70.01	69.98	70.05	69.99	70.01	1.8868	1.55291	1.2150
3	248	67.75	67.77	67.8	67.76	67.81	67.778	1.8266	1.54864	1.1795
3	251	66.96	66.98	67.01	66.98	70	67.586	1.8214	1.56151	1.1665
4	250	67.94	67.96	67.99	68	68.01	67.98	1.8321	1.5572	1.1765
4	249	66.58	66.6	66.61	66.59	66.62	66.6	1.7949	1.55291	1.1558
4	249	67.15	67.22	67.2	67	67.25	67.164	1.8101	1.55291	1.1656
5	250	67.46	67.38	67.4	67.42	67.4	67.412	1.8168	1.5572	1.1667
5	249.5	66.96	67.08	67.04	66.98	67.05	67.022	1.8062	1.55505	1.1615
5	251	66.95	66.9	66.94	66.95	67	66.948	1.8042	1.56151	1.1555
6	253	68.08	68.1	68.1	68	68.06	68.068	1.8344	1.57019	1.1683
6	251	68.11	68.15	68.17	68.24	68.2	68.174	1.8373	1.56151	1.1766
6	252	67.05	67.03	67.09	67.03	67.05	67.05	1.8070	1.56584	1.1540
7	188	57.03	57.05	57.06	57.09	57.01	57.048	1.5374	1.32904	1.1568
7	188	56.53	56.55	56.57	56.54	56.6	56.558	1.5242	1.32904	1.1469
7	188	56.6	56.59	56.53	56.59	56.52	56.566	1.5245	1.32904	1.1470
8	190	58.49	58.48	58.51	58.5	58.52	58.5	1.5766	1.3352	1.1808
8	190	58.48	58.56	58.49	58.5	58.56	58.518	1.5771	1.3352	1.1811
8	190	58.55	58.5	58.48	58.58	58.49	58.52	1.5771	1.3352	1.1812
9	190	58.98	58.99	58.97	58.99	58.95	58.976	1.5894	1.3352	1.1904
9	189	59.39	59.32	59.34	59.35	59.34	59.348	1.5994	1.33211	1.2007
9	190	57.74	57.7	57.72	57.78	57.7	57.728	1.5558	1.3352	1.1652
10	187	57.43	57.53	57.42	57.5	57.48	57.472	1.5489	1.32599	1.1681
10	187	56.65	56.74	56.66	56.7	56.63	56.676	1.5274	1.32599	1.1519
10	187	56.53	56.71	56.57	56.57	56.64	56.604	1.5255	1.32599	1.1504
11	185	56.83	56.75	56.78	56.8	56.76	56.784	1.5303	1.31995	1.1594
11	185	56.92	56.98	56.97	56.99	57	56.972	1.5354	1.31995	1.1632
11	185	57.79	57.75	57.77	57.8	57.79	57.78	1.5572	1.31995	1.1797
12	189	57.93	57.97	57.02	57.95	59	57.974	1.5624	1.33211	1.1729
12	188	56.95	56.92	56.93	56.9	56.97	56.934	1.5344	1.32904	1.1545
12	189.5	57.9	57.89	57.92	57.95	57.91	57.914	1.5608	1.33365	1.1703

sample	Salinity (g/L)	time (sec.)	time (sec.)	time (sec.)	time (sec.)	time (sec.)	Average	Viscosity (cSt)	Viscosity (S/W)	Relative viscosity
Week 4										
1	255	73.33	73.25	73.28	73.25	73.3	73.282	1.9749	1.57895	1.2508
1	254	72.4	72.42	72.39	72.35	72.35	72.382	1.9507	1.57456	1.2389
1	255	70.41	70.4	70.34	70.34	70.42	70.382	1.8968	1.57895	1.2013
2	254	72.86	72.8	72.79	72.86	72.8	72.822	1.9626	1.57456	1.2464
2	254	69.72	69.72	69.75	69.76	69.71	69.732	1.8793	1.57456	1.1935
2	255	73.75	73.78	73.79	73.8	73.79	73.782	1.9884	1.57895	1.2593
3	256	71.25	71.37	71.27	71.25	71.24	71.276	1.9209	1.58336	1.2132
3	254	70.23	70.18	70.21	70.2	70.23	70.21	1.8922	1.57456	1.2017
3	254.5	70.47	70.46	70.44	70.45	70.48	70.46	1.8989	1.57675	1.2043
4	253	69.07	69.05	69.99	69.99	69.01	69.422	1.8709	1.57019	1.1915
4	254	69.3	69.5	69.7	69.3	69	69.36	1.8693	1.57456	1.1872
4	253	68.39	68.39	68.4	68.35	68.35	68.376	1.8427	1.57019	1.1736
5	253.5	69.67	69.64	69.63	69.61	69.64	69.638	1.8767	1.57237	1.1936
5	254	67.09	67.08	67.08	66.99	66.99	67.046	1.8069	1.57456	1.1476
5	256	67.15	67.19	67.18	67.21	67.2	67.186	1.8107	1.57336	1.1508
6	250	67.94	67.87	67.84	67.86	67.94	67.89	1.8296	1.5572	1.1750
6	252	67.88	67.86	67.89	67.89	67.85	67.874	1.8292	1.56584	1.1682
6	250	67.91	67.88	67.89	67.84	67.9	67.884	1.8295	1.5572	1.1748
7	190	57.85	57.95	57.85	57.8	57.84	57.858	1.5593	1.3352	1.1678
7	189.5	57.94	57.93	57.89	57.95	57.89	57.92	1.5609	1.33365	1.1704
7	190	57.96	57.96	57.89	57.87	57.87	57.91	1.5607	1.3352	1.1689
8	188	57.5	56.51	57.62	57.59	57.59	57.362	1.5459	1.32904	1.1632
8	189	57.46	57.48	57.5	57.5	57.52	57.492	1.5494	1.3321	1.1631
8	188	56.99	56.98	56.91	56.97	56.94	56.958	1.5350	1.32904	1.1550
9	188	58.5	58.51	58.62	58.59	58.59	58.562	1.5782	1.32904	1.1875
9	189	58.46	58.48	58.5	58.5	58.52	58.492	1.5764	1.3321	1.1834
9	188	58.49	58.48	58.51	58.47	58.54	58.498	1.5765	1.32904	1.1862
10	187	55.96	55.95	55.95	56	60.95	56.962	1.5351	1.32599	1.1577
10	187	56.34	56.35	56.4	56.42	56.44	56.39	1.5197	1.32599	1.1461

sample	Salinity (g/L)	time (sec.)	time (sec.)	time (sec.)	time (sec.)	time (sec.)	Average	Viscosity (cSt)	Viscosity (S/W)	Relative viscosity
10	187	56.4	56.42	56.43	56.47	56.38	56.42	1.5205	1.32599	1.1467
11	188.5	56.68	56.78	56.7	56.68	56.69	56.706	1.5282	1.33057	1.1486
11	188.5	56.82	56.88	56.85	56.8	56.79	56.828	1.5315	1.33057	1.1510
11	188.5	55.79	55.76	55.78	55.79	55.8	55.784	1.5034	1.33057	1.1299
12	189	55.01	55.91	55.99	55.98	55	55.578	1.4978	1.33211	1.1244
12	189	55.92	55.94	55.97	55.99	55.9	55.944	1.5077	1.33211	1.1318
12	188.5	56.9	56.89	56.94	56.94	56.96	56.926	1.5342	1.33057	1.1530
Week 5										
1	254	69.41	69.32	69.35	69.38	69.32	69.356	1.8691	1.57456	1.1871
1	254	68.36	68.35	68.35	68.4	68.42	68.376	1.8427	1.57456	1.1703
1	256	70.38	70.37	70.38	70.4	70.41	70.388	1.8970	1.58336	1.1981
2	256.5	70.76	70.77	70.79	70.8	70.89	70.802	1.9081	1.58557	1.2034
2	255	68.88	68.89	68.78	68.75	68.78	68.816	1.8546	1.57895	1.1746
2	254	68.83	68.79	68.8	68.81	68.82	68.81	1.8544	1.57456	1.1777
3	253	69.86	69.81	69.83	69.83	69.85	69.836	1.8821	1.57019	1.1986
3	249.5	68.87	68.6	68.69	68.67	68.62	68.69	1.8512	1.5555	1.1901
3	250.5	67.78	67.79	67.8	67.81	67.77	67.79	1.8269	1.55935	1.1716
4	256	69.77	69.78	69.85	69.8	69.84	69.808	1.8813	1.58336	1.1882
4	255.5	70.12	70.08	70.01	70.02	70.05	70.056	1.8880	1.58115	1.1941
4	255	70.89	70.84	70.9	70.85	70.87	70.87	1.9099	1.57895	1.2096
5	256	68.3	68.29	68.32	68.31	68.27	68.298	1.8406	1.58336	1.1625
5	254	69.34	69.33	69.29	69.29	69.28	69.306	1.8678	1.57456	1.1862
5	255.5	68.3	68.29	68.32	68.31	68.3	68.304	1.8408	1.58115	1.1642
6	256	67.07	67.17	67.1	67.12	67.15	67.122	1.8089	1.58336	1.1425
6	254.5	67.57	67.51	67.53	67.59	67.6	67.56	1.8207	1.57675	1.1547
6	256	66.96	67.02	67.01	67	66.96	66.99	1.8054	1.58336	1.1402
7	190	58.2	58.15	58.19	58.15	58.14	58.166	1.5676	1.3352	1.1740
7	190.5	58.24	58.23	58.25	58.19	58.23	58.228	1.5692	1.33675	1.1739
7	188.5	58.2	58.19	58.22	58.24	58.18	58.206	1.5687	1.33057	1.1789

sample	Salinity (g/L)	time (sec.)	time (sec.)	time (sec.)	time (sec.)	time (sec.)	Average	Viscosity (cSt)	Viscosity (S/W)	Relative viscosity
8	188	57.27	57.25	57.27	57.24	57.21	57.248	1.5428	1.32904	1.1609
8	189	57.09	57.19	57.18	57.15	57.19	57.16	1.5405	1.33211	1.1564
8	190	57.21	57.25	57.26	57.2	57.18	57.22	1.5421	1.3352	1.1549
9	190	58.22	58.13	58.1	58.09	58.1	58.128	1.5665	1.3352	1.1733
9	190.5	58.08	58.1	58.09	58.08	58.15	58.1	1.5658	1.33675	1.1713
9	190	58.01	58.09	58.1	58.05	58.05	58.06	1.5647	1.3352	1.1719
10	188	57.01	57.1	57.09	57.09	57.05	57.068	1.5380	1.32904	1.1572
10	188	58.3	58.29	58.27	58.3	58.33	58.298	1.5711	1.32904	1.1822
10	190	57.12	57.18	57.2	57.11	57.11	57.144	1.5400	1.3352	1.1534
11	187.5	56.13	56.19	56.15	56.15	56.14	56.152	1.5133	1.32751	1.1400
11	188	56.41	56.39	56.38	56.3	56.35	56.366	1.5191	1.32904	1.1430
11	189	57.07	57.07	57.05	57.04	57.03	57.052	1.5376	1.32211	1.1630
12	189	56.84	56.84	56.9	56.82	56.83	56.846	1.5320	1.33211	1.1501
12	189	55.99	55.99	55.95	55.97	55.99	55.978	1.5086	1.33211	1.1325
12	188.5	56.8	56.81	56.81	56.87	56.88	56.834	1.5317	1.33057	1.1511
Week 6										
1	253	69.81	69.79	69.82	69.79	69.83	69.808	1.8813	1.57019	1.1982
1	256	69.11	69.09	69.05	69.04	69.04	69.066	1.8613	1.57019	1.1854
1	256	70.96	70.95	70.94	70.9	70.01	70.752	1.9068	1.58336	1.2043
2	253	69.4	69.41	69.39	69.37	69.39	69.392	1.8701	1.57019	1.1910
2	254	69.6	69.67	69.61	69.59	69.58	69.61	1.8760	1.57456	1.1914
2	256	68.45	68.43	68.42	68.39	68.43	68.424	1.8440	1.58336	1.1646
3	253	67.51	67.5	67.49	67.59	67.51	67.52	1.8197	1.57019	1.1589
3	253	66.36	66.43	66.4	66.39	66.38	66.392	1.7893	1.57019	1.1395
3	254	67.35	67.49	67.48	67.4	67.4	67.424	1.8171	1.57456	1.1540
4	256	67.49	67.58	67.5	67.52	67.55	67.528	1.8199	1.58336	1.1494
4	256	66.54	66.5	66.5	66.49	66.51	66.508	1.7924	1.58336	1.1320
4	255	66.8	66.78	66.78	66.82	66.85	66.806	1.8004	1.57895	1.1403

sample	Salinity (g/L)	time (sec.)	time (sec.)	time (sec.)	time (sec.)	time (sec.)	Average	Viscosity (cSt)	Viscosity (S/W)	Relative viscosity
5	249	66.24	66.22	66.27	66.29	66.24	66.252	1.7855	1.55291	1.1498
5	251	67	67.01	67.04	67.03	67.04	67.024	1.8063	1.56151	1.1568
5	253	60.45	68.37	68.39	68.4	68.38	66.798	1.8002	1.57019	1.1465
6	254	66.79	66.8	66.75	66.74	66.78	66.772	1.7995	1.57456	1.1429
6	255	66.74	66.76	66.79	66.8	66.8	66.778	1.7997	1.57895	1.1398
6	252	66.82	66.89	66.9	66.9	66.87	66.876	1.8023	1.56584	1.1510
7	188.5	58.48	58.5	58.55	58.52	58.49	58.508	1.5768	1.33057	1.1850
7	189	58.29	58.29	59.2	58.28	58.2	58.452	1.5753	1.33211	1.1825
7	189	58.29	58.32	58.34	58.32	58.3	58.314	1.5716	1.33211	1.1798
8	190	57.94	58.01	58.01	57.99	58.02	57.994	1.5629	1.3352	1.1706
8	190.5	58.05	58.01	58.02	58.06	58.06	58.04	1.5642	1.33675	1.1701
8	187	57.79	58.03	58.01	58.99	58.01	58.166	1.5676	1.32599	1.1822
9	187	58.24	58.22	58.2	58.25	58.2	58.222	1.5691	1.32599	1.1833
9	187.5	57.97	58.05	58.08	58.08	58	58.036	1.5641	1.32751	1.1782
9	188	58.12	58.1	58.1	58.17	58.18	58.134	1.5667	1.32904	1.1788
10	189	56.85	56.81	56.82	56.85	56.89	56.844	1.5319	1.32211	1.1587
10	190	55.83	55.79	55.78	55.8	55.8	55.8	1.5038	1.3352	1.1263
10	187.5	56.69	56.78	56.75	56.7	56.74	56.732	1.5289	1.32751	1.1517
11	188	56.46	56.37	56.39	56.41	56.39	56.404	1.5201	1.32904	1.1437
11	188	57.25	57.2	57.19	57.27	57.25	57.232	1.5424	1.32904	1.1605
11	190	56.1	56.15	56.17	56.2	56.12	56.148	1.5132	1.3252	1.1419
12	190.5	57.24	57.22	57.27	57.29	57.24	57.252	1.5429	1.33675	1.1542
12	188	57.49	57.41	57.4	57.39	57.4	57.418	1.5474	1.32904	1.1643
12	189	56.37	56.39	56.4	56.38	56.41	56.39	1.5197	1.33211	1.1408

b. Organic material (AFDW) and pigments determinations.
First sampling

samples	dry wt (%)	H2O (%)	AFDW %	cartenoids 480 (mg/100g)	cartenoids 510 (mg/100g)	chlo *a* (mg/100g)
PA7	23.6	82.2	13.23	2.60	2.56	0.38
PA7	21.8	79.8	16.01	1.71	2.57	0.31
PA7	24.2	65.2	9.15	2.73	2.97	0.22
H.U.						
1	25.6	74.2	37.65	4.35	5.51	0.34
2	23.6	75.2	20.63	5.23	7.21	0.42
3	24.0	76.2	34.27	4.98	6.85	0.30
4	24.0	75.8	39.38	3.48	4.74	0.32
5	24.0	75.6	20.73	3.59	4.89	0.31
6	23.8	75.6	26.11	3.40	4.73	0.32
7	24.4	75.6	20.20	3.72	5.00	0.38
8	23.4	76.0	15.97	1.48	1.16	0.51
9	23.8	76.6	41.94	4.30	5.89	0.41
H.C.						
10	23.4	76.4	34.21	4.12	5.76	0.26
11	23.4	76.2	21.62	2.93	4.11	0.21
12	23.6	76.0	20.43	3.09	4.34	0.28
13	23.8	75.6	38.78	3.37	4.64	0.23
14	24.2	75.4	16.93	3.18	4.31	0.24
15	23.0	75.4	15.89	3.40	4.94	0.30
16	24.4	74.4	23.34	4.05	5.43	0.30
17	25.6	75.6	23.42	2.70	3.47	0.23
18	25.8	74.8	23.37	3.08	3.97	0.30
L.U.						
19	24.4	78.4	20.39	4.26	5.75	0.47
20	21.6	77.0	10.73	3.74	5.66	0.37
21	23.0	76.0	8.77	3.94	5.61	0.37
22	24.0	75.4	20.77	4.91	6.67	0.41
23	24.4	75.6	40.19	3.70	4.97	0.22
24	22.4	75.8	9.84	5.03	7.25	0.47
25	24.2	76.8	20.04	2.61	3.50	0.23
26	23.2	76.6	24.44	1.71	2.41	0.18
27	23.6	76.4	22.67	2.73	3.69	0.21
L.C.						
28	23.6	75.8	23.17	4.24	5.93	0.34
29	24.2	75.8	20.76	2.57	3.47	0.20
30	23.4	76.0	22.32	2.94	4.19	0.24
31	24.0	76.2	19.29	3.05	4.20	0.25
32	23.8	74.8	20.82	3.90	5.39	0.32
33	24.0	75.4	21.87	3.14	4.30	0.27
34	25.2	75.4	19.02	2.41	3.16	0.21
35	24.4	75.2	19.24	2.29	3.08	0.35
36	24.6	75.2	22.06	2.16	2.94	0.22

Last sampling

samples	dry wt (%)	H2O (%)	AFDW %	cartenoids 480 (mg/100g)	cartenoids 510 (mg/100g)	chlo *a* (mg/100g)
H.U.						
1	31.34	68.66	17.31	2.05	2.18	0.20
2	28.70	71.30	19.79	1.50	1.75	0.10
3	32.32	67.68	19.27	1.60	1.65	0.15
4	36.45	63.55	29.09	2.13	1.95	0.11
5	32.19	67.81	22.43	3.04	3.13	0.17
6	41.62	58.38	19.76	1.92	1.53	0.10
7	42.15	57.85	13.77	2.55	2.02	0.16
8	37.34	62.66	10.57	2.61	2.32	0.20
9	43.32	56.68	14.83	2.67	2.05	0.20
H.C.						
10	50.28	49.72	13.94	2.32	1.55	0.29
11	34.83	65.17	18.76	1.92	1.87	0.20
12	37.03	62.97	20.14	2.15	1.96	0.26
13	32.09	67.91	17.64	2.05	2.15	0.19
14	33.88	66.12	22.44	2.20	2.19	0.17
15	34.42	65.58	28.34	2.33	2.28	0.19
16	33.20	66.80	22.91	1.94	1.98	0.16
17	34.55	65.45	25.25	2.15	2.11	0.22
18	32.49	67.51	35.29	2.44	2.55	0.24
L.U.						
19	25.78	74.22	46.37	2.98	3.84	0.20
20	25.54	74.46	46.59	2.32	3.02	0.14
21	25.22	74.78	51.72	2.83	3.71	0.21
22	25.60	74.40	20.10	2.18	2.83	0.13
23	25.56	74.44	20.95	2.51	3.25	0.16
24	26.76	73.24	21.02	2.70	3.34	0.19
25	24.18	75.82	20.47	1.98	2.74	0.17
26	23.40	76.60	20.03	1.53	2.20	0.15
27	22.79	77.21	20.00	2.22	3.22	0.21
L..C.						
28	33.59	66.41	21.19	4.29	4.25	0.55
29	32.23	67.77	21.24	3.99	4.12	0.55
30	33.73	66.27	21.33	3.75	3.70	0.44
31	26.03	73.97	20.89	2.15	2.76	0.21
32	26.05	73.95	20.88	2.77	3.56	0.30
33	26.50	73.50	20.93	2.52	3.15	0.26
34	30.78	69.22	21.36	1.69	1.84	0.18
35	29.73	70.27	21.30	2.89	3.37	0.25
36	30.12	69.88	20.91	3.69	4.30	0.25

APPENDIX 5

PUBLICATIONS

The following is a list of publications either published, submitted for publication, or in press. They are arranged according to the chapters indicated.

1. A.1 Chapter 2.

Ghassemzadeh, F., Williams, W.D., and Geddes, M.C. (1996d). Physico-chemical factors in a solar saltfield at Dry Creek, Adelaide - with special reference to plant nutrient (abstract), 35th Congress of the Australian Society for Limnology, Berri South Australia, Programme and Abstracts,.63.

2. A.2 Chapter 3.

Ghassemzadeh, F., Williams, W.D., and Geddes, M.C. (1996c). Biology of solar saltfields in relation to salt quality and quantity (abstract), 35th Congress of the Australian Society for Limnology, Berri South Australia, Programme and Abstracts, 62.

3. A.3 Chapter 4.

Ghassemzadeh, F., Williams, W.D., and Geddes, M.C. (1996b). The determination of nitrate in highly salinewater (abstract), INTECOL V Internationl Wetlands Conference 22-28 September 1996, Perth, Western Australia, Programme and Abstracts, 129.

4. Ghassemzadeh, F., Williams, W.D., and Geddes, M.C. (in review), The determination of nitrate in highly salinewater. *International Journal of Salt Lake Research*

A.4 Chapter 5.

5. Ghassemzadeh, F., Williams, W.D., and Geddes, M.C. (1996a) The effects of *Synechococcus* on salt quality and quantity (abstract), INTECOL V Internationl Wetlands Conference 22-28 September 1996, Perth, Western Australia, Programme and Abstracts, 72.